US 20070066524A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2007/0066524 A1**

Korneluk et al. (43) **Pub. Date: Mar. 22, 2007**

(54) **MAMMALIAN IAP GENE FAMILY, PRIMERS, PROBES AND DETECTION METHODS**

(76) Inventors: **Robert G. Korneluk**, Ottawa (CA); **Alexander E. MacKenzie**, Ottawa (CA); **Stephen Baird**, Ottawa (CA); **Peter Liston**, Ottawa (CA)

Correspondence Address:
PHILIP SWAIN, PHD
C/O GOWLING LAFLEUR HENDERSON
1 PLACE VILLE MARIE,
37TH FLOOR
MONTREAL, QC H3B 3P4 (CA)

(21) Appl. No.: **11/498,897**

(22) Filed: **Aug. 4, 2006**

Related U.S. Application Data

(63) Continuation of application No. 11/316,539, filed on Dec. 22, 2005, which is a continuation of application No. 10/600,272, filed on Jun. 20, 2003, now Pat. No. 7,067,281, which is a continuation of application No. 09/011,356, filed on Sep. 14, 1998, now Pat. No. 6,656,704, filed as 371 of international application No. PCT/IB96/01022, filed on Aug. 5, 1996, which is a continuation-in-part of application No. 08/576,956, filed on Dec. 22, 1995, now Pat. No. 6,156,535, which is a continuation-in-part of application No. 08/511,485, filed on Aug. 4, 1995, now Pat. No. 5,919,912.

Publication Classification

(51) **Int. Cl.**
A61K 38/17 (2006.01)
A61K 48/00 (2006.01)

(52) **U.S. Cl.** **514/12**; 514/44

(57) **ABSTRACT**

Disclosed is substantially pure DNA encoding mammalian IAP polypeptides; and methods of using such DNA to express the IAP polypeptides in cells and animals to inhibit apoptosis. Also disclosed are conserved regions characteristic of the IAP family and primer and probes for the identification and isolation of additional IAP genes. In addition, methods for treating diseases and disorders involving apoptosis are provided.

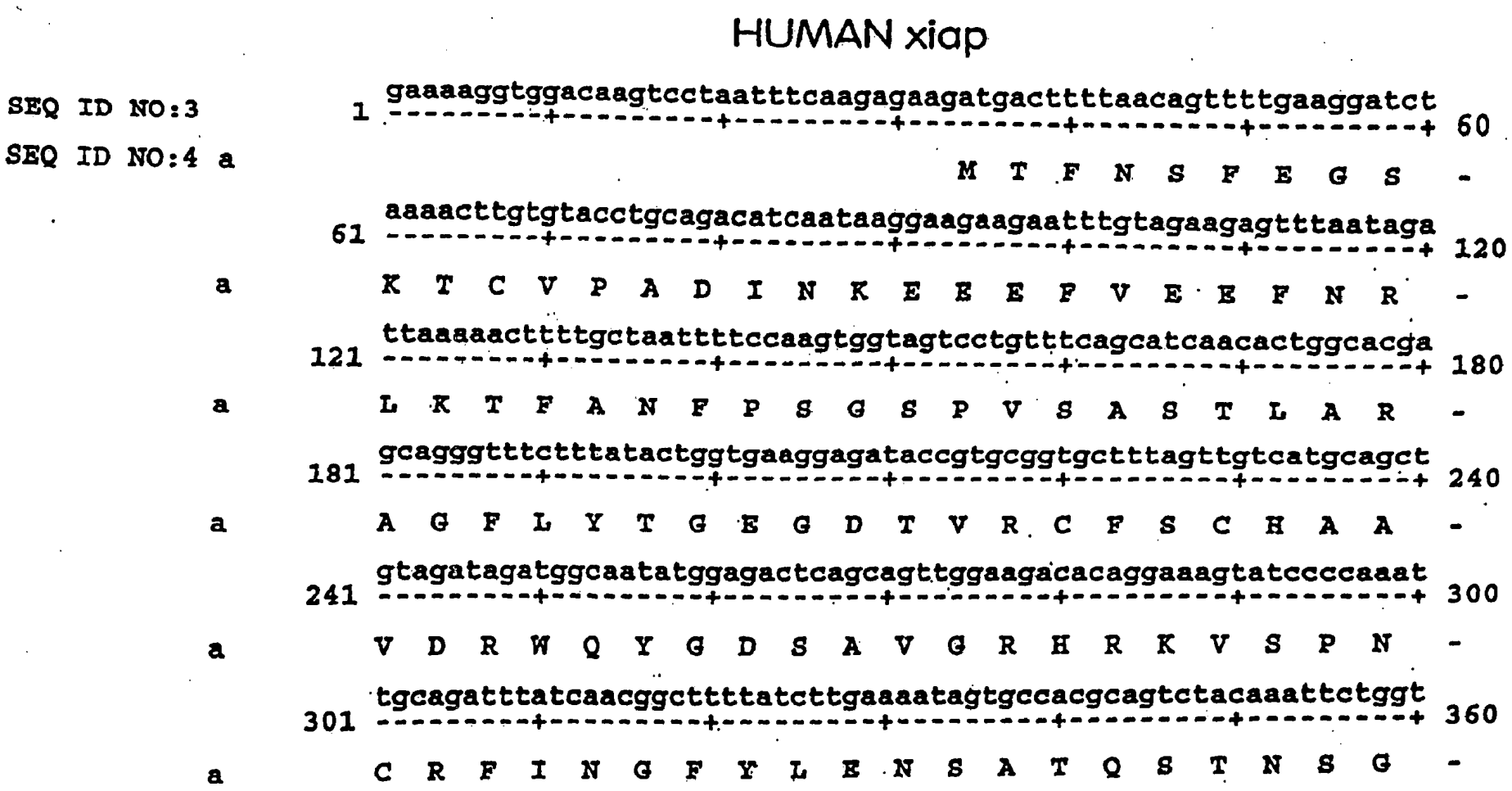
HUMAN xiap
SEQ ID NO:3
SEQ ID NO:4 a
1 gaaaaggtggacaagtcctaatttcaagagaagatgacttttaacagttttgaaggatct 60
M T F N S F E G S -
61 aaaacttgtgtacctgcagacatcaataaggaagaagaatttgtagaagagtttaataga 120
K T C V P A D I N K E E E F V E E F N R -
121 ttaaaaacttttgctaattttccaagtggtagtcctgtttcagcatcaacactggcacga 180
L K T F A N F P S G S P V S A S T L A R -
181 gcagggtttctttatactggtgaaggagataccgtgcggtgctttagttgtcatgcagct 240
A G F L Y T G E G D T V R C F S C H A A -
241 gtagatagatggcaatatggagactcagcagttggaagacacaggaaagtatccccaaat 300
V D R W Q Y G D S A V G R H R K V S P N -
301 tgcagatttatcaacggcttttatcttgaaaatagtgccacgcagtctacaaattctggt 360
C R F I N G F Y L E N S A T Q S T N S G -

Fig. 1A

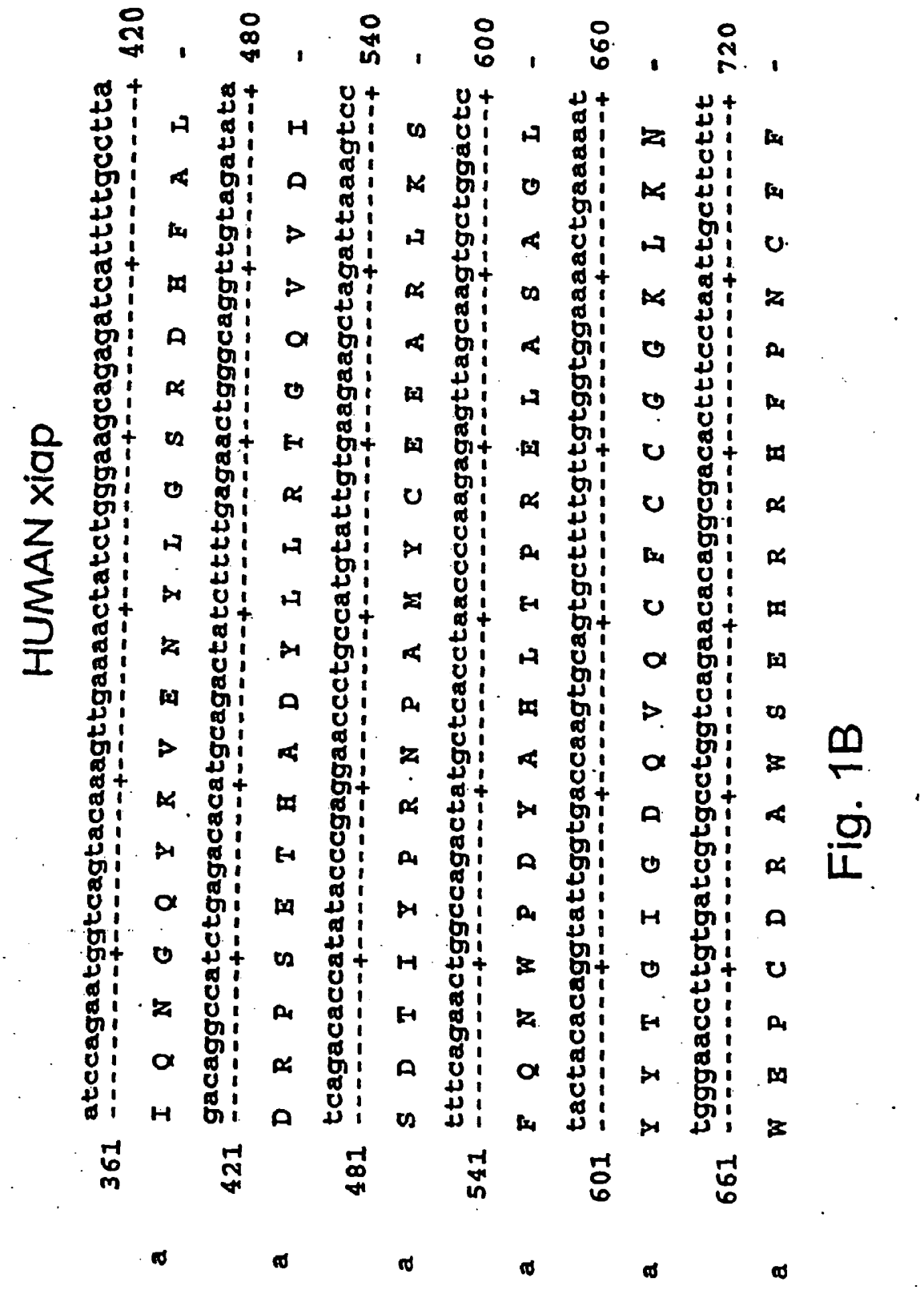
HUMAN xiap
361 atccagaatggtcagtacaaagttgaaaactatctgggaagcagagatcatttgcctta 420
a I Q N G Q Y K V E N Y L G S R D H F A L -
421 gacaggccatctgagacacatgcagactatctttgagaactgggcaggttgtagatata 480
a D R P S E T H A D Y L L R T G Q V V D I -
481 tcagacaccatatacccgaggaaccctgccatgtattgtgaagaagctagattaaagtcc 540
a S D T I Y P R N P A M Y C E E A R L K S -
541 ttcagaactggccagactatgctcacctaaccccaagagagttagcaagtgctggactc 600
a F Q N W P D Y A H L T P R E L A S A G L -
601 tactacacaggtattggtgaccaagtgcagtgctttttgttgtggtggaaaactgaaaaat 660
a Y Y T G I G D Q V Q C F C C G G K L K N -
661 tgggaaccttgtgatcgtgcctggtcagaacacaggcgacactttcctaattgcttcttt 720
a W E P C D R A W S E H R R H F P N C F F -

Fig. 1B

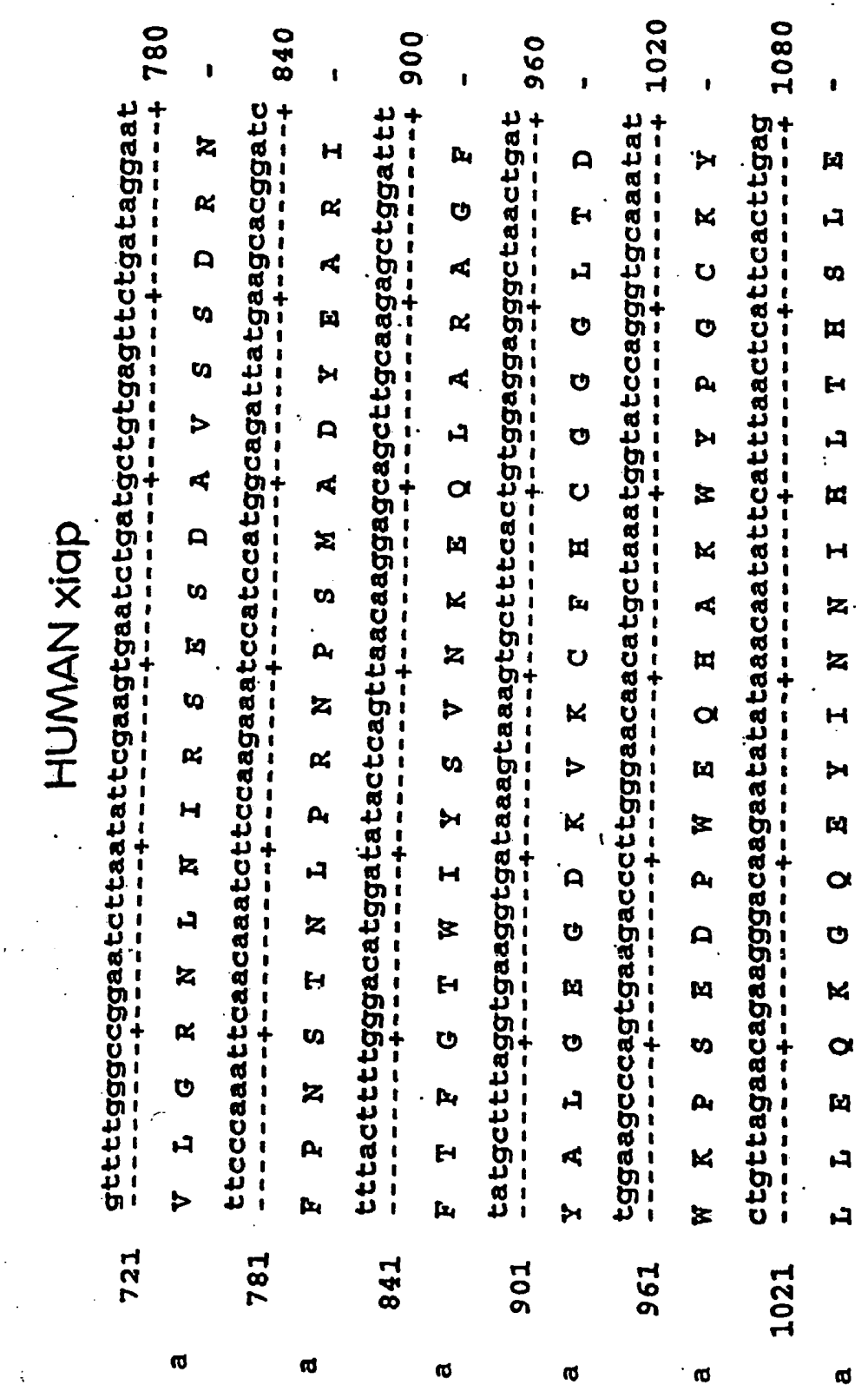
HUMAN xiap
721 gttttgggccggaatcttaatattcgaagtgaatctgatgctgtgagttctgataggaat
a V L G R N L N I R S E S D A V S S D R N -
781 ttcccaaattcaacaaatcttccaagaaatccatccatggcagattatgaagcacggatc
a F P N S T N L P R N P S M A D Y E A R I -
841 tttacttttgggacatggatactcagttaacaaggagcagcttgcaagagctggattt
a F T F G T W I Y S V N K E Q L A R A G F -
901 tatgctttaggtgaaggtgataaagtgctttcactgtggagggctaactgat
a Y A L G E G D K V K C F H C G G G L T D -
961 tggaagcccagtgaagaccccttgggaacaacatgctaaatggtatccagggtgcaaatat
a W K P S E D P W E Q H A K W Y P G C K Y -
1021 ctgttagaacagaagggacaagaatatataaacaaatattcatttaactcattcacttgag
a L L E Q K G Q Q E Y I N N I H L T H S L E -

Fig. 1C

HUMAN xiap

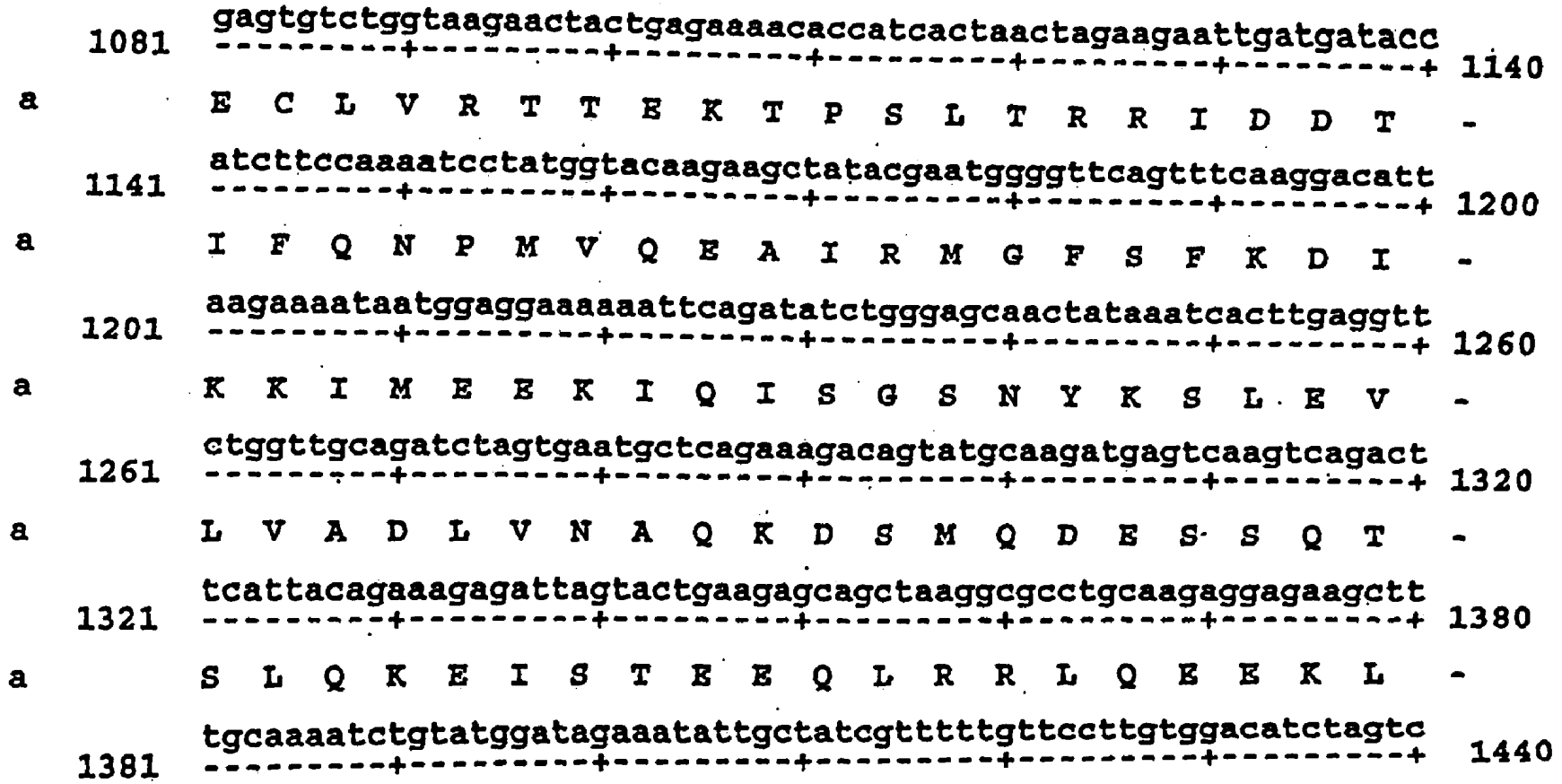

Fig. 1D

HUMAN xiap

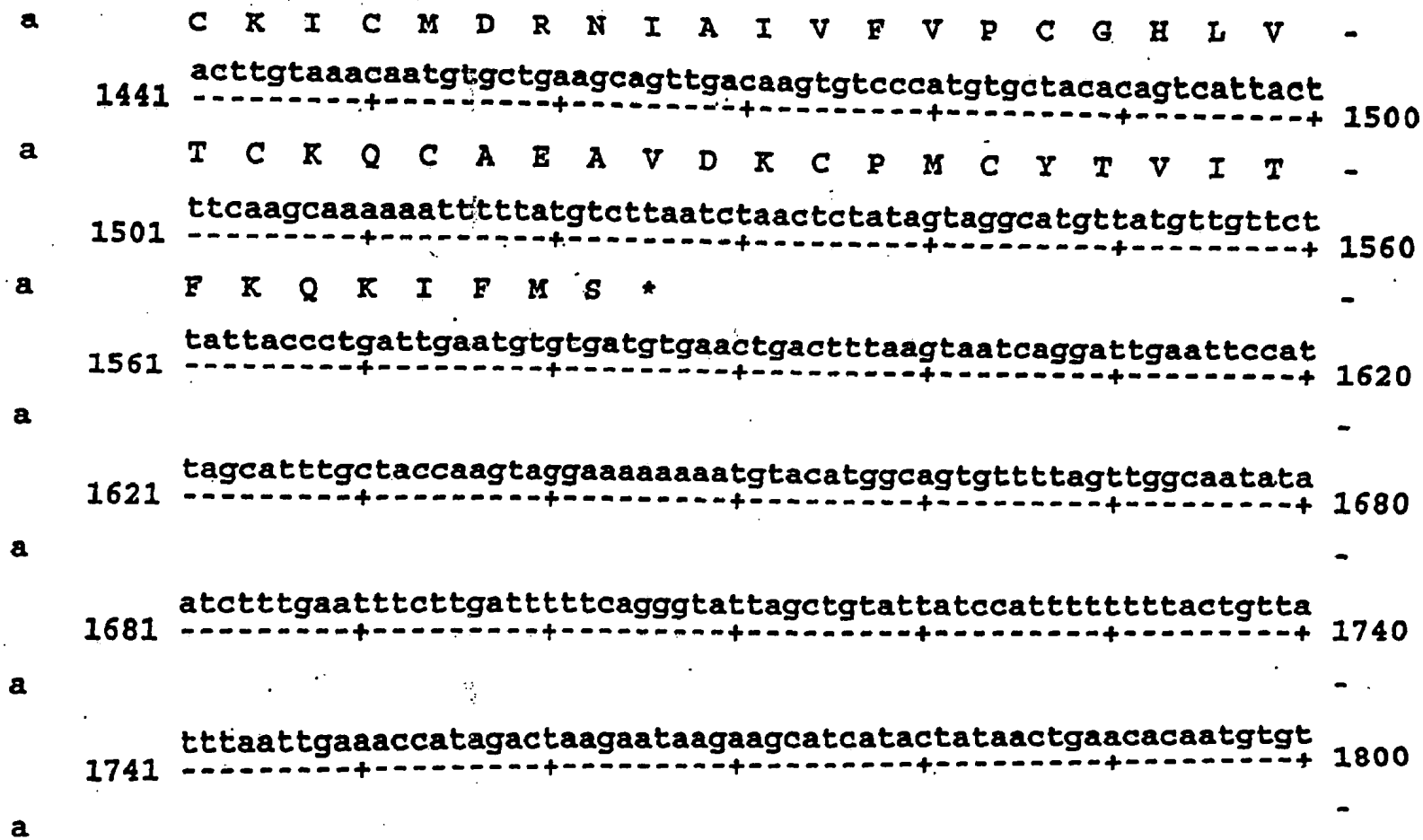

Fig. 1E

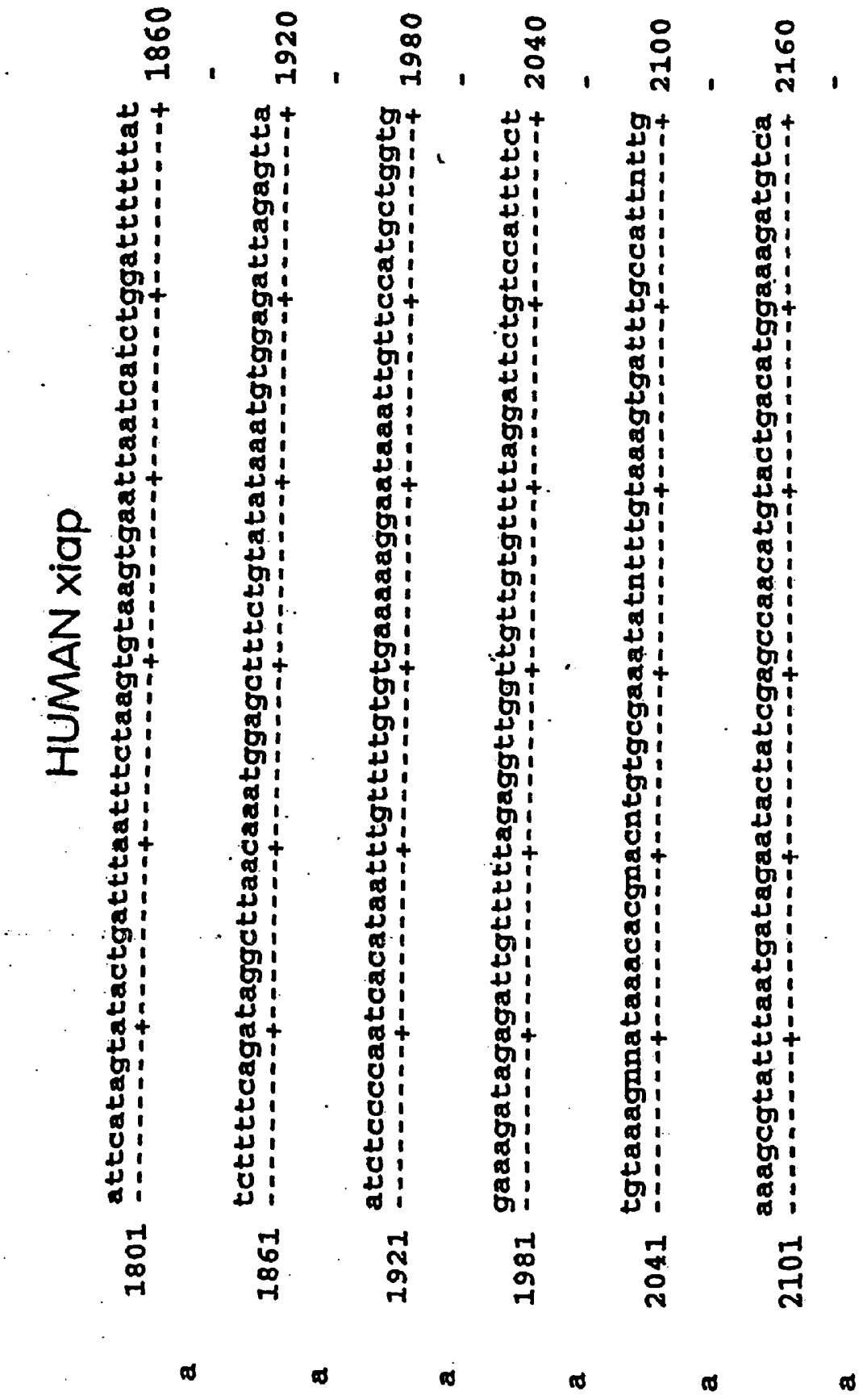
HUMAN xiap
1801 attcatagtatactgatttaatttctaagtgtaagtgaattaatcatctggatttttttat 1860
a - -
1861 tcttttcagataggcttaacaaatggagctttctgtatataaatgtggagattagagtta 1920
a - -
1921 atctccccaatcacataatttgtttttgtgtgaaaaaggaataaattgttccatgctgggtg 1980
a - -
1981 gaaagatagagattgttttagaggttggttgttgtttaggattctgtccatttttct 2040
a - -
2041 tgtaaagmnataaacacgmacntgtgcgaaatatntttgtaaagtgatttgccattnttg 2100
a - -
2101 aaagcgtatttaatgatagaatactatcgagccaacatgtactgacatggaaagatgtca 2160
a - -

Fig. 1F

HUMAN xiap

```
2161 nagatatgttaagtgtaaaatgcaagtggcnnnacactatgtatagtctgagccagatca 2220
a                                                                  -
2221 aagtatgtatgttnttaatatgcatagaacnanagatttggaaagatatacaccaaactg 2280
a                                                                  -
2281 ttaaatgtggtttctcttcggggaggggggattgggggaggggccccagaggggtttta 2340
a                                                                  -
2341 naggggccttttcactttcnacttttttcattttgttctgttcgnatttttataagtat 2400
a                                                                  -
2401 gtanaccccnaagggttttatggnaactaacatcagtaacctaaccccgtgactatcct 2460
a                                                                  -
2461 gtnctcttcctagggagctgtnttgtttcccacccaccaccccttccctctgaacaaatgc 2520
a                                                                  -
2521 ctgagtgctggggcactttn 2540
a
```

Fig. 1G

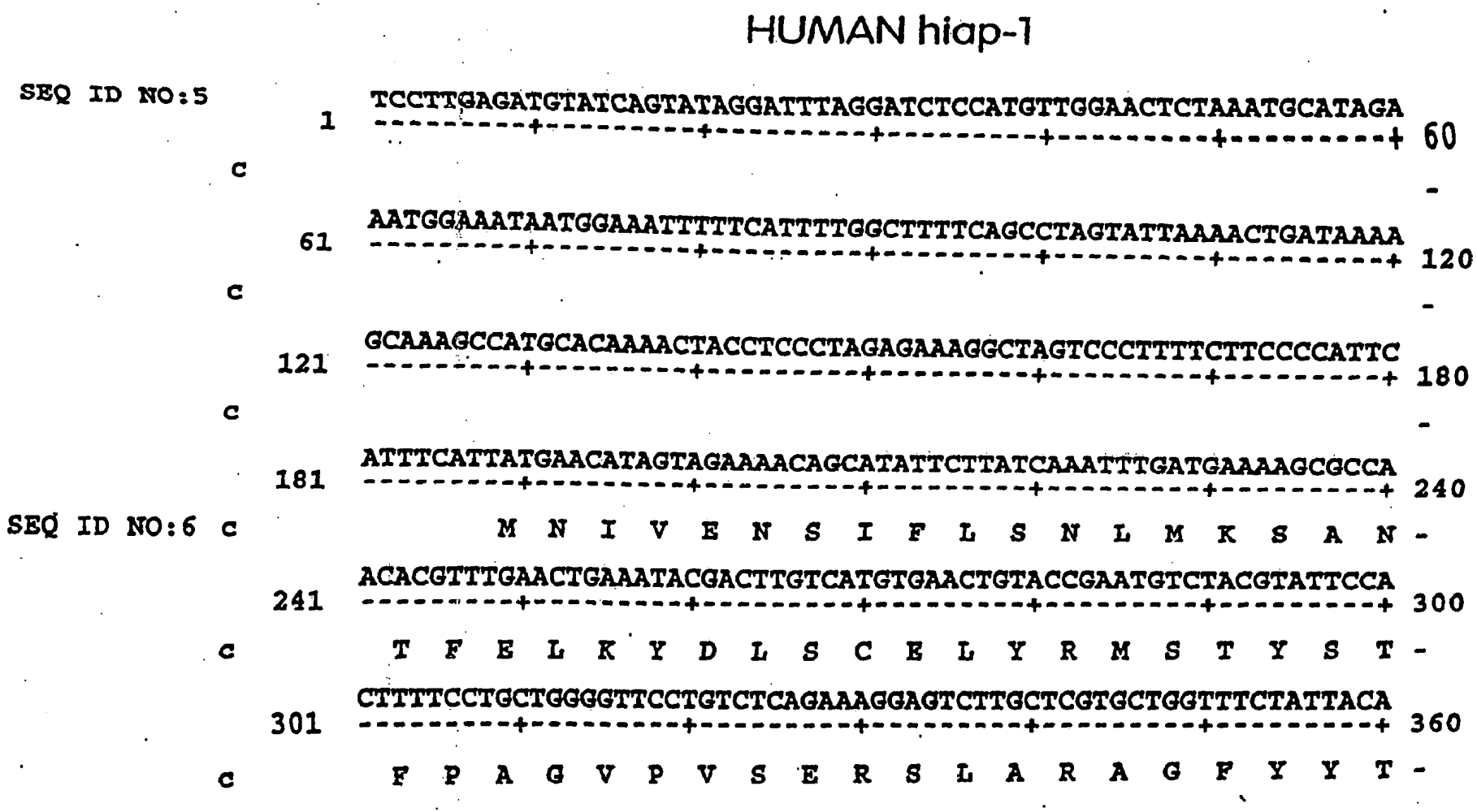
HUMAN hiap-1
SEQ ID NO:5
1
TCCTTGAGATGTATCAGTATAGGATTTAGGATCTCCATGTTGGAACTCTAAATGCATAGA
60
c
-
61
AATGGAAATAATGGAAATTTTTCATTTTGGCTTTTCAGCCTAGTATTAAAACTGATAAAA
120
c
-
121
GCAAAGCCATGCACAAAACTACCTCCCTAGAGAAAGGCTAGTCCCTTTTCTTCCCCATTC
180
c
-
181
ATTTCATTATGAACATAGTAGAAAACAGCATATTCTTATCAAATTTGATGAAAAGCGCCA
240
SEQ ID NO:6
c
M N I V E N S I F L S N L M K S A N -
241
ACACGTTTGAACTGAAATACGACTTGTCATGTGAACTGTACCGAATGTCTACGTATTCCA
300
c
T F E L K Y D L S C E L Y R M S T Y S T -
301
CTTTTCCTGCTGGGGTTCCTGTCTCAGAAAGGAGTCTTGCTCGTGCTGGTTTCTATTACA
360
c
F P A G V P V S E R S L A R A G F Y Y T -

Fig. 2A

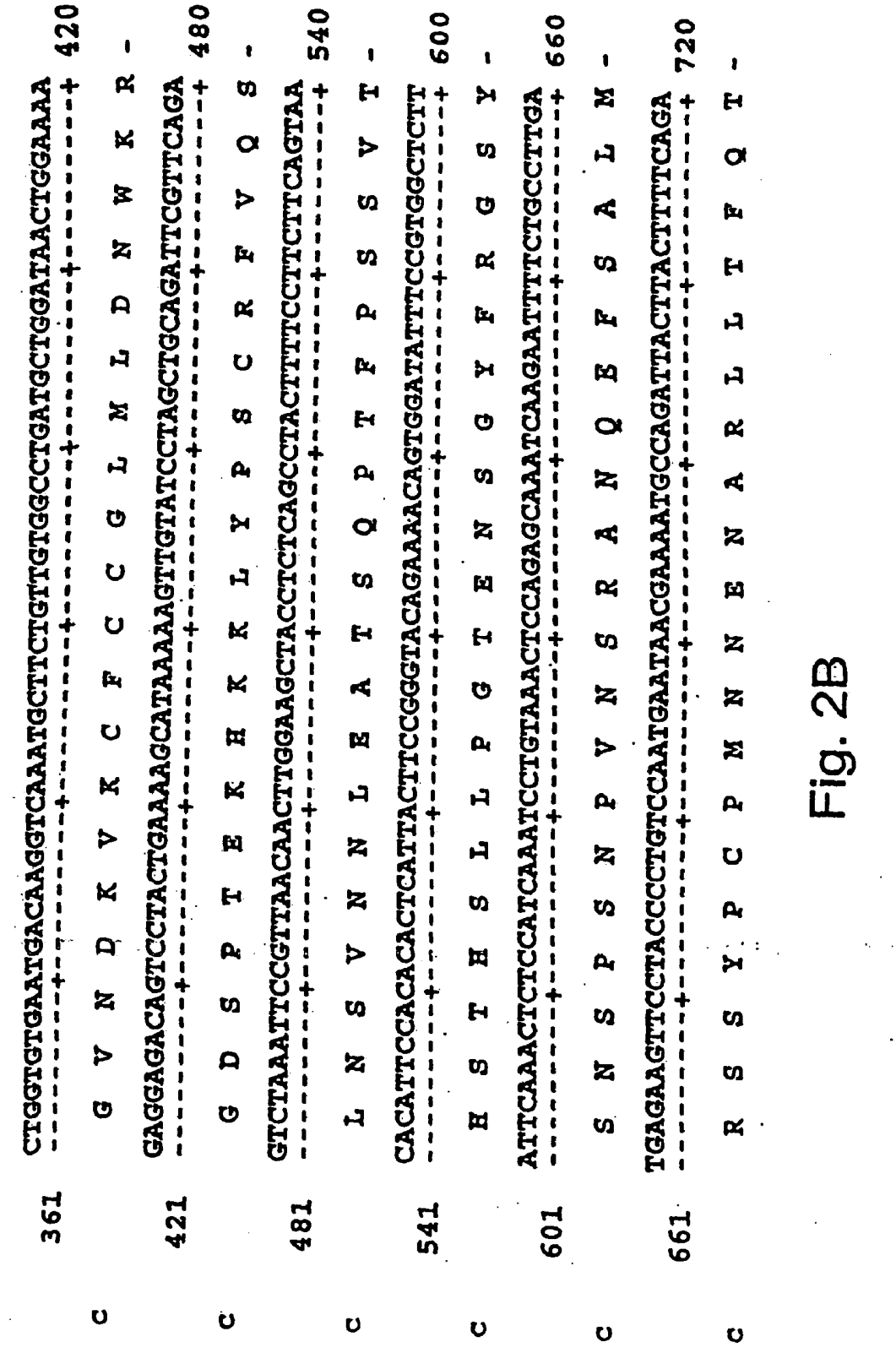
HUMAN hiap-1
361
CTGGTGTGAATGACAAGGTCAAATGCTTCTGTTGTGGCCTGATGCTGGATAACTGGAAAA
420
C G V N D K V K C F C C G L M L D N W K R -
421
GAGGAGACAGTCCTACTGAAAAGCATAAAAGTTGTATCCTAGCTGCAGATTCGTTCAGA
480
C G D S P T E K H K K L Y P S C R F F V Q S -
481
GTCTAAATTCCGTTAACAACTTGGAAGCTACCTCCTCAGCCTACTTTTCCTTCTTCAGTAA
540
C L N S V N N L E A T S Q P T F P S S S V T -
541
CACATTCCACACACTCATTACTTCCGGGTACAGAAAACAGTGGATATTTCCGTGGCTCTT
600
C H S T H S L L P G T E N S G Y Y F R G G S Y -
601
ATTCAAACTCTCCATCAAATCCTGTAAACTCCAGAGCAAATCAAGAATTTTCTGCCTTGA
660
C S N S P S N P V N S R A N Q E F S A L M -
661
TGAGAAGTTCCTACCCCTGTCCAATGAATAACGAAAATGCCAGATTACTTACTTTTCAGA
720
C R S S Y P C P M N N E N A R L L T F Q T -

Fig. 2B

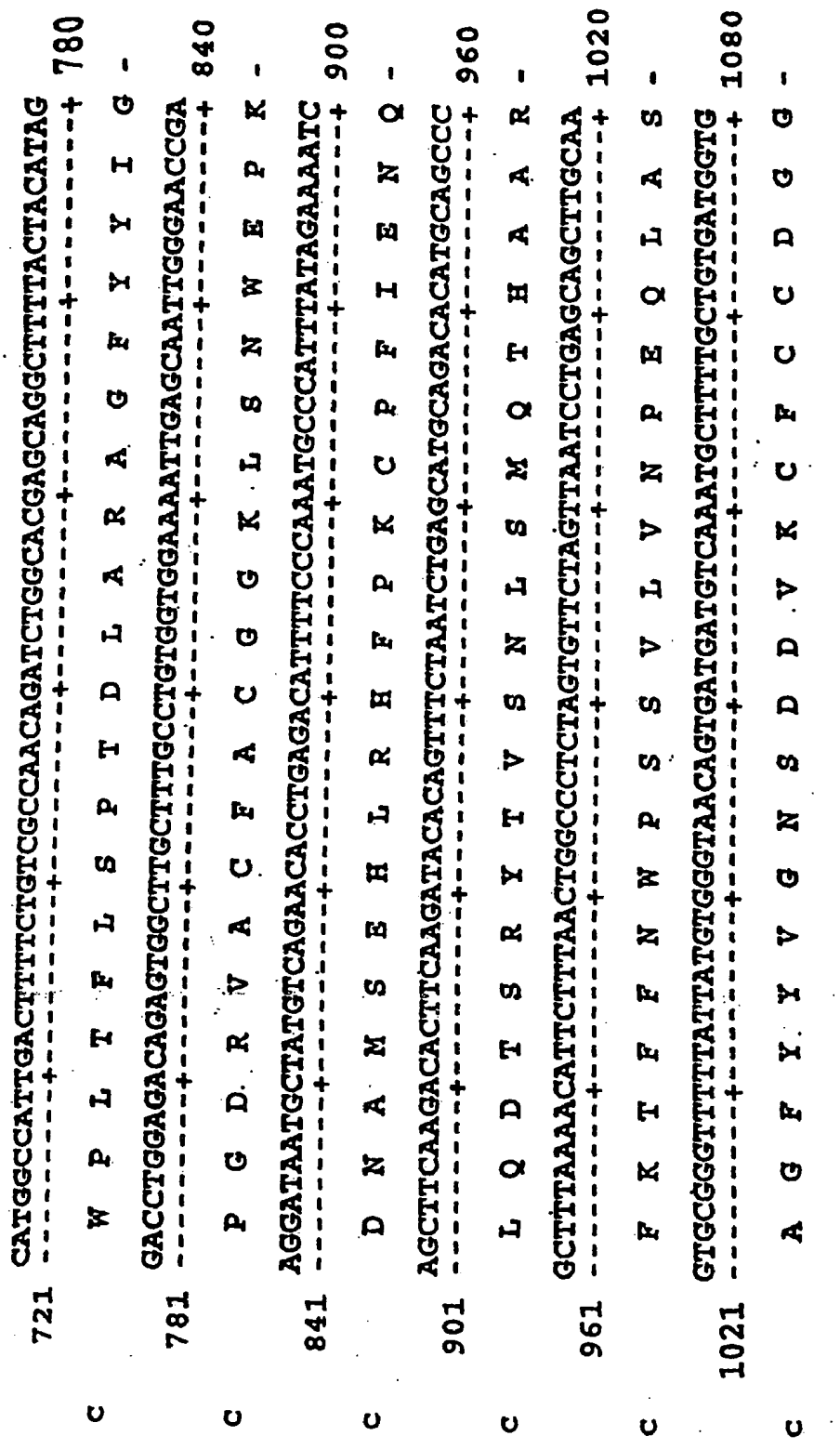
HUMAN hiap-1
721 CATGGCCATTGACTTTTCTGTCGCCAACAGATCTGGCACGAGCAGGCTTTTACTACATAG
780
C W P L T F L S P T D L A R A G F Y Y I G -
781 GACCTGGAGACAGAGTGGCTTTGCTTTGCCTGTGGTGGAAAATTGAGCAATTGGGAACCGA
840
C P G D R V A C F A C G G K L S N W E P K -
841 AGGATAATGCTATGTCAGAACACCTGAGACATTTTCCCAAATGCCCATTTATAGAAAATC
900
C D N A M S E H L R H F P K C P F I E N Q -
901 AGCTTCAAGACACTTCAAGATACACAGTTTCTAATCTGAGCATGCAGACACATGCAGCCC
960
C L Q D T S R Y T V S N L S M Q T H A A R -
961 GCTTTAAAACATTCTTTAACTGGCCCTCTAGTGTTCTAGTTAATCCTGAGCAGCTTGCAA
1020
C F K T F F N W P S S V L V N P E Q L A S -
1021 GTGCGGGTTTTTATTATGTGGGTAACAGTGATGATGTCAAATGCTTTTGCTGTGATGGTG
1080
C A G F Y Y V G N S D D V K C F C C D G G -

Fig. 2C

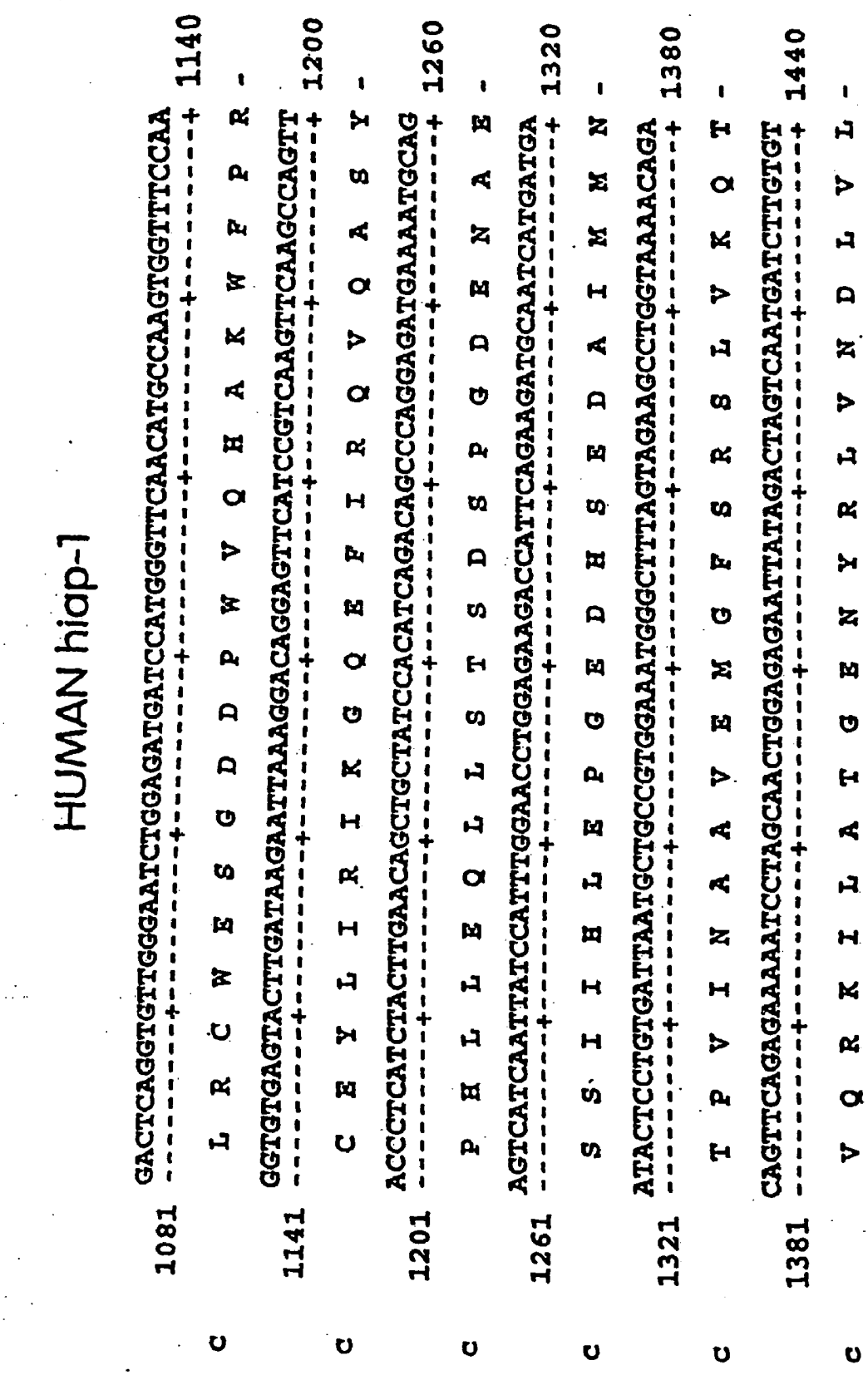
HUMAN hiap-1
1081 GACTCAGGTGTTGGGAATCTGGAGATGATCCATGGGTTCAACATGCCAAGTGGTTTCCAA 1140
c L R C W E S G D D P W V Q H A K W F P R -
1141 GGTGTGAGTACTTGATAAGAATTAAAGGACAGGAGTTCATCCGTCAAGTTCAAGCCAGTT 1200
c C E Y L I R I K G Q E F I R Q V Q A S Y -
1201 ACCCTCATCTACTTGAACAGCTGCTATCCACATCAGACAGCCCAGGAGATGAAAATGCAG 1260
c P H L L E Q L L S T S D S P G D E N A E -
1261 AGTCATCAATTATCCATTTGGAACCTGGAGAAGACCATTCAGAAGATGCAATCATGATGA 1320
c S S I I H L E P G E D H S E D A I M M N -
1321 ATACTCCTGTGATTAATGCTGCCGTGGAAATGGGCTTTAGTAGAAGCCTGGTAAAACAGA 1380
c T P V I N A A V E M G F S R S L V K Q T -
1381 CAGTTCAGAGAAAAATCCTAGCAACTGGAGAGAATTATAGACTAGTCAATGATCTTGTGT 1440
c V Q R K I L A T G E N Y R L V N D L V L -

Fig. 2D

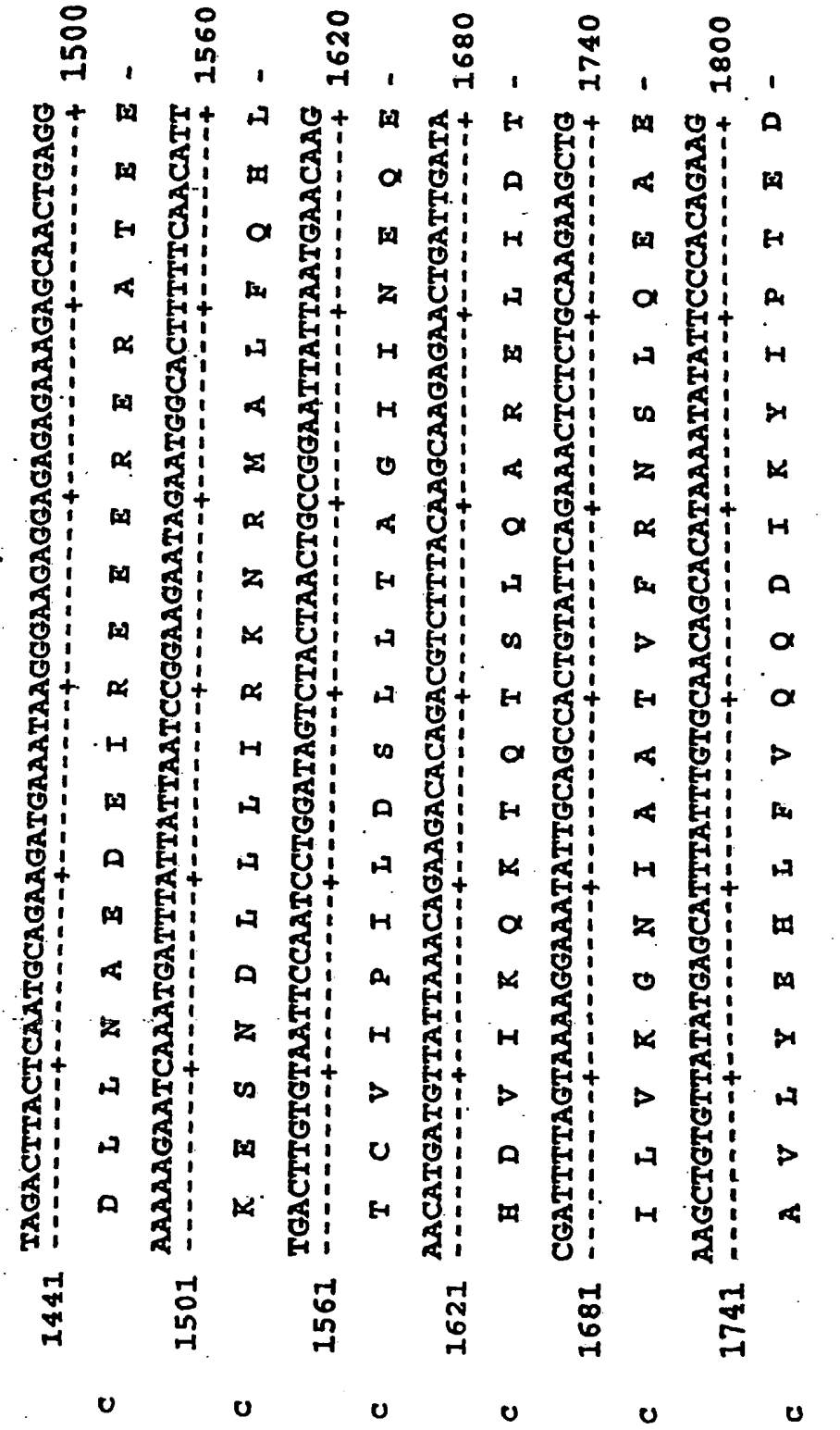
HUMAN hiap-1
1441 TAGACTTTACTCAATGCAGAAGATGAAATAAGGGAAGAGGAGAGAAAGAGCAACTGAGG 1500
C D L L N A E E D E E I R E E E E E R E R A T E E E -
1501 AAAAAGAATCAAATGATTTATTAATTAATCCGGAAGAATAGAATGGCACTTTTTCAACATT 1560
C K E S N D L L L I R K N R M A L L F Q H H L -
1561 TGACTTTGTGTAATTCCAATCCTGGATAGTCTACTAACTGCCGGAATTATTAATGAACAAG 1620
C T C V I P I L D S L L T A G I I I N E Q E -
1621 AACATGATGTTATTAAACAGAAGACACAGACGTCTTTACAAGCAAGAGAACTGATTGATA 1680
C H D V I K Q K T Q T S L Q A R E L I D T -
1681 CGATTTTAGTAAAAGGAAATATTGCAGCCACTGTATTCAGAAACTCTCTGCAAGAAGCTG 1740
C I L V K G N I A A T V F R N S L Q E A E -
1741 AAGCTGTGTTATATGAGCATTTATTTTGTGCAACAGCACATAAAATATATTCCCACAGAAG 1800
C A V L Y E H L F V Q Q D I K Y I P T E D -

Fig. 2E

HUMAN hiap-1

```
      1801 ATGTTTCAGATCTACCAGTGGAAGAACAATTGCGGAGACTACCAGAAGAAAGAACATGTA 1860
c            V  S  D  L  P  V  E  E  Q  L  R  R  L  P  E  E  R  T  C  K  -
      1861 AAGTGTGTATGGACAAAGAAGTGTCCATAGTGTTTATTCCTTGTGGTCATCTAGTAGTAT 1920
c            V  C  M  D  K  E  V  S  I  V  F  I  P  C  G  H  L  V  V  C  -
      1921 GCAAAGATTGTGCTCCTTCTTTAAGAAAGTGTCCTATTTGTAGGAGTACAATCAAGGGTA 1980
c            K  D  C  A  P  S  L  R  K  C  P  I  C  R  S  T  I  K  G  T  -
      1981 CAGTTCGTACATTTCTTTCATGAAGAAGAACCAAAACATCGTCTAAACTTTAGAATTAAT 2040
c            V  R  T  F  L  S  *                                          -
      2041 TTATTAAATGTATTATAACTTTAACTTTTATCCTAATTTGGTTTCCTTAAAATTTTTATT 2100
c                                                                         -
      2101 TATTTACAACTCAAAAAACATTGTTTTGTGTAACATATTTATATATGTATCTAAACCATA 2160
c                                                                         -
```

Fig. 2F

HUMAN hiap-1

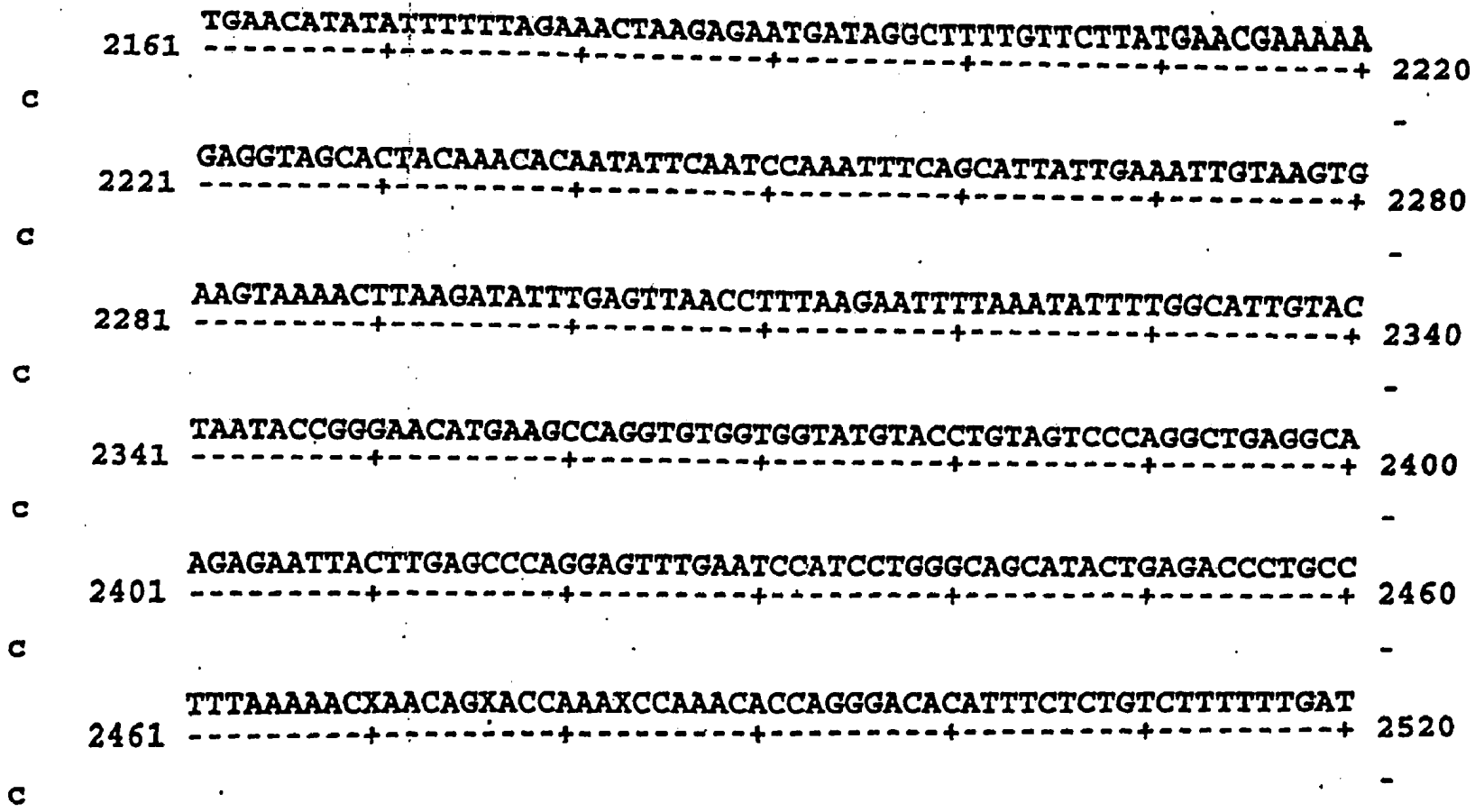

Fig. 2G

HUMAN hiap-1

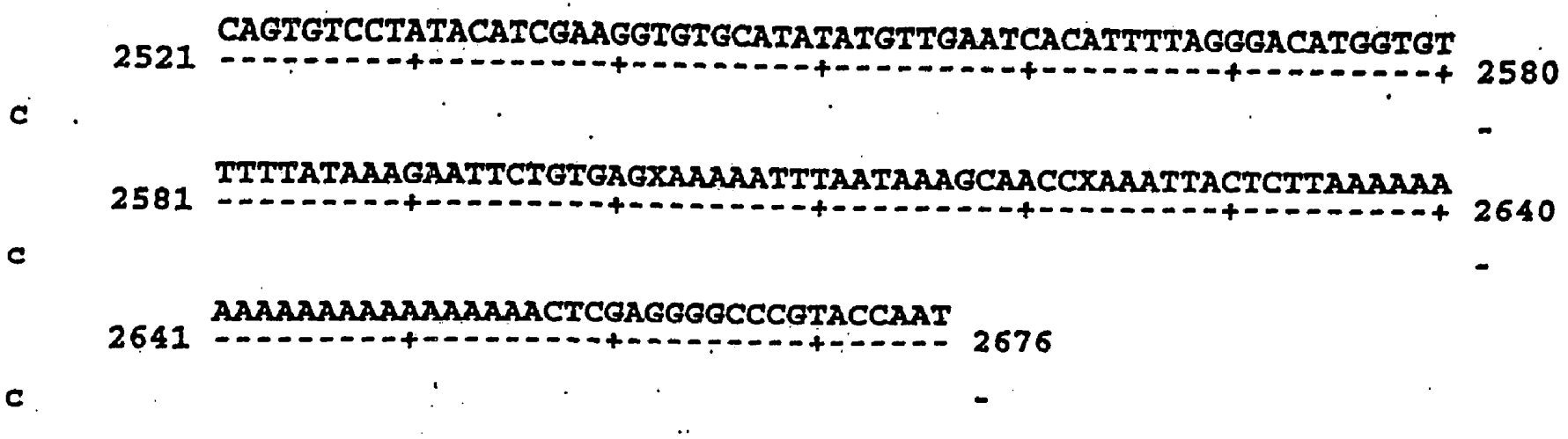

Fig. 2H

HUMAN hiap-2

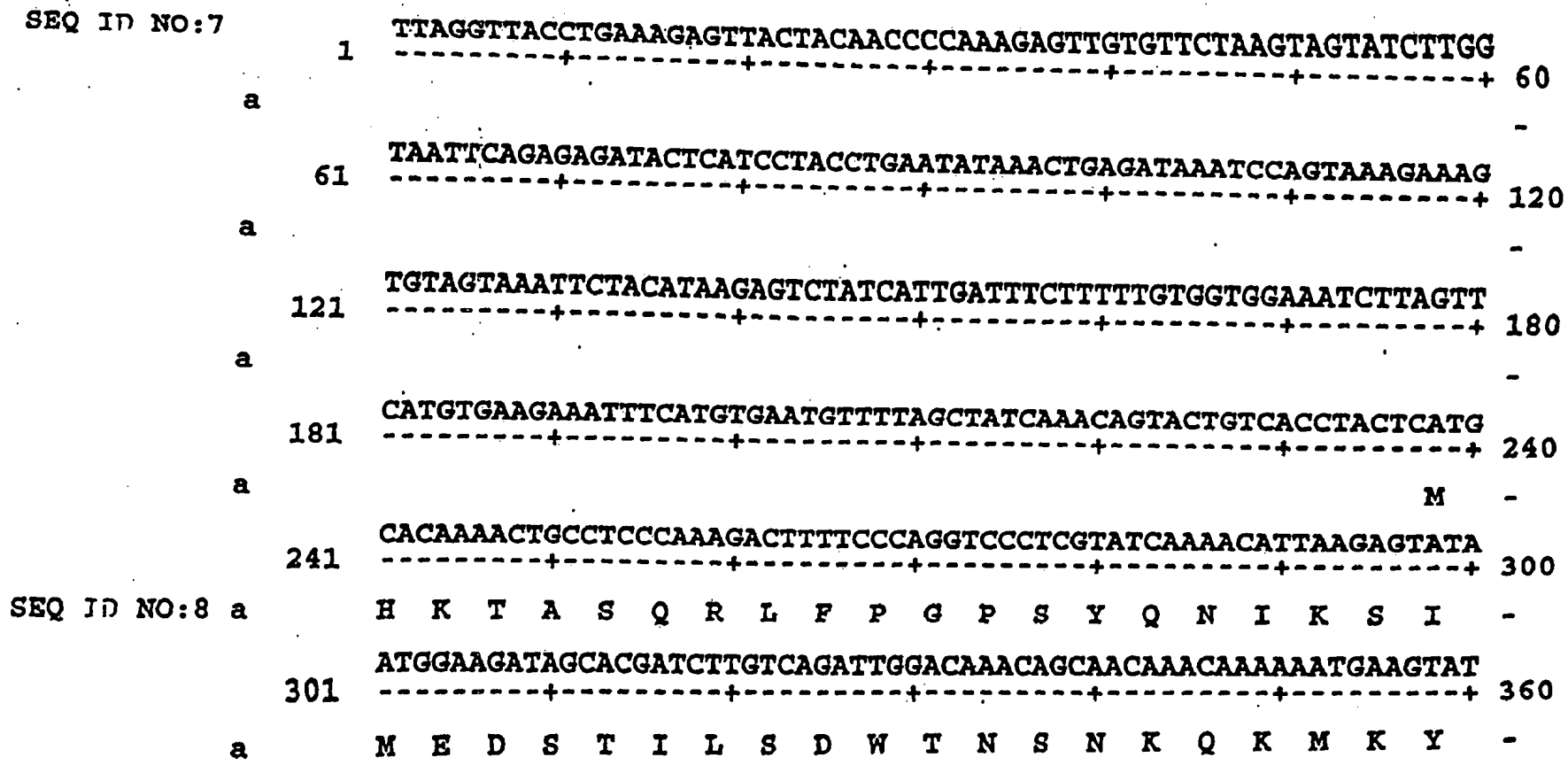

Fig. 3A

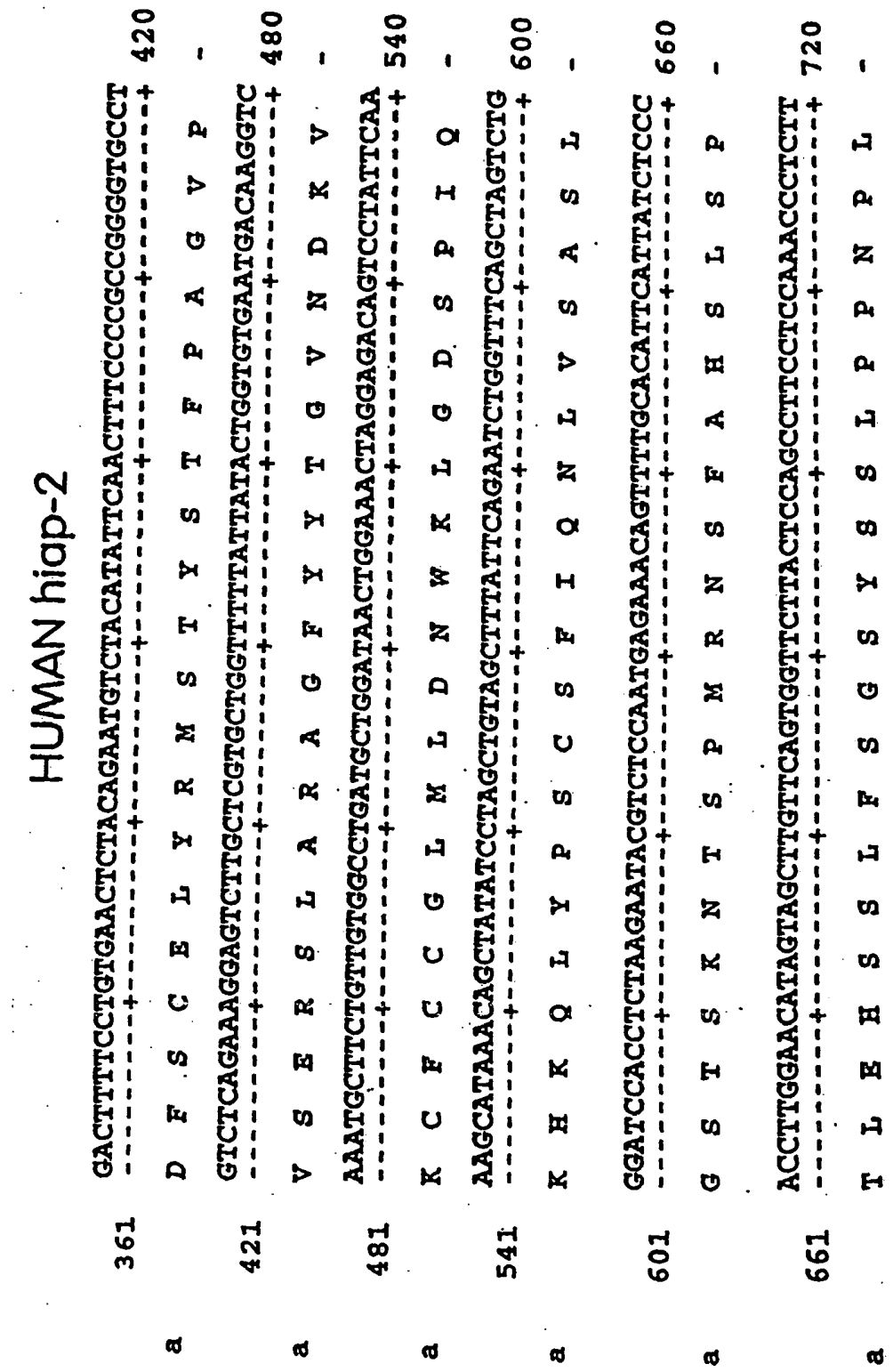
HUMAN hiap-2
361
GACTTTTCCTGTGAACTCTACAGAATGTCTACATATTCAACTTTCCCCGCCGGGGTGCCT
a D F S C E L Y R M S T Y S T F P A G V P -
421
GTCTCAGAAAGGAGTCTTGCTCGTGCTGGTTTTTATTATACTGGTGTGAATGACAAGGTC
a V S E R S L A R A G F Y Y T G V N D K V -
481
AAATGCTTCTGTTGTGGCCTGATGCTGGATAACTGGAAACTAGGAGACAGTCCTATTCAA
a K C F C C G L M L D N W K L G D S P I Q -
541
AAGCATAAACAGCTATATCCTAGCTGTAGCTTTATTCAGAATCTGGTTTCAGCTAGTCTG
a K H K Q L Y P S C S F I Q N L V S A S L -
601
GGATCCACCTCTAAGAATACGTCTCCAATGAGAAACAGTTTTGCACATTCATTATCTCCC
a G S T S K N T S P M R N S F A H S L S P -
661
ACCTTGGAACATAGTAGCTTGTTCAGTGGTTCTTACTCCAGCCTTCCTCCAAACCCTCTT
a T L E H S S L F S G S Y S S L P P N P L -

Fig. 3B

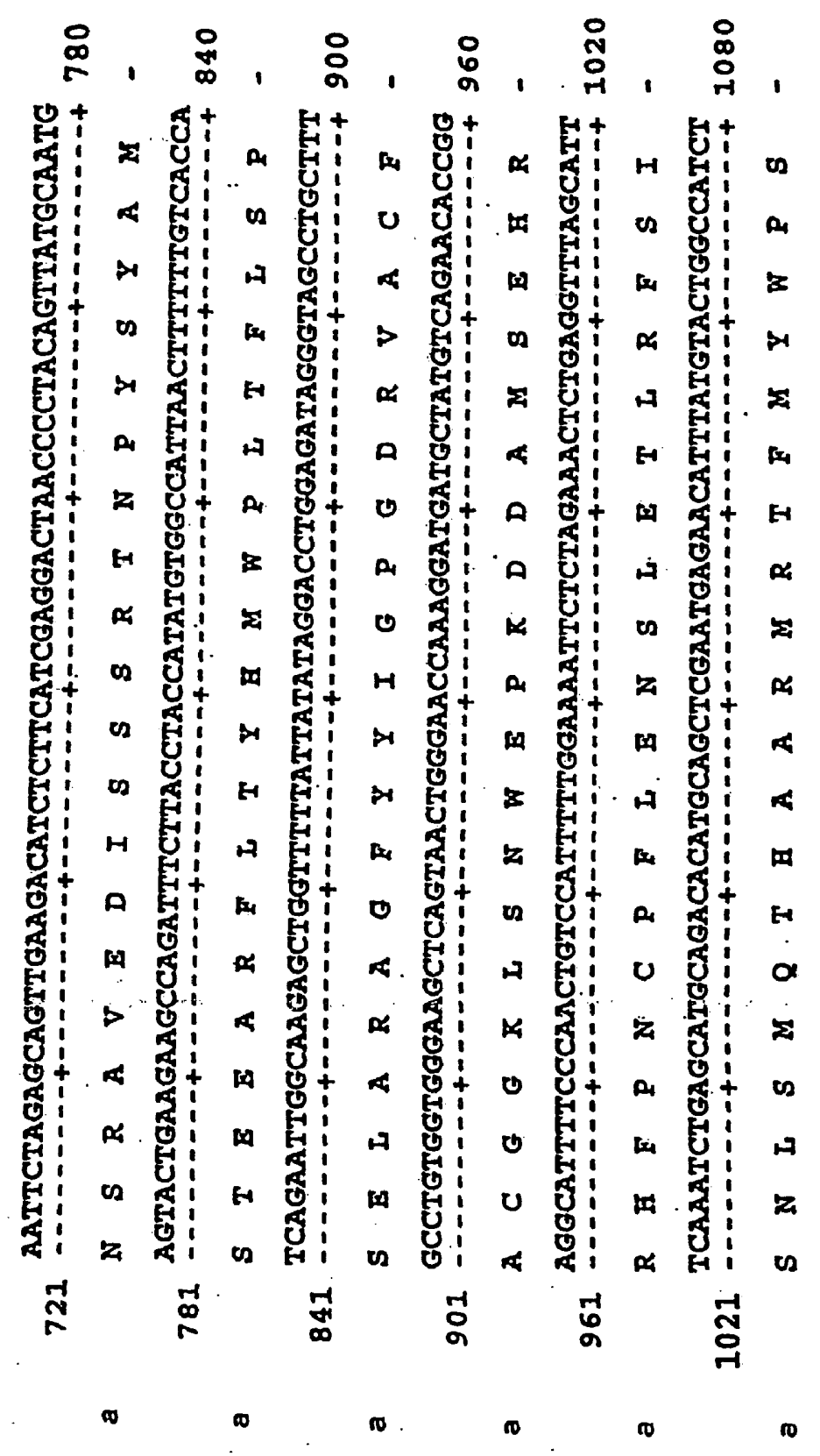
HUMAN hiap-2
721 AATTCTAGAGCAGTTGAAGACATCTCTTCATCGAGGACTAACCCCTACAGTTATGCAATG 780
a N S R A V E D I S S S R T N P Y S Y A M -
781 AGTACTGAAGAAGCCAGATTTCTTACCTACCATATGTGGCCATTAACTTTTTTGTCACCA 840
a S T E E A R F L T Y H M W P L T F L S P -
841 TCAGAATTGGCAAGAGCTGGTTTTTATTATATAGGACCTGGAGATAGGGTAGCCTGCTTT 900
a S E L A R A G F Y Y I G P G D R V A C F -
901 GCCTGTGGTGGGAAGCTCAGTAACTGGGAACCAAAGGATGATGCTATGTCAGAACACCGG 960
a A C G G K L S N W E P K D D A M S E H R -
961 AGGCATTTTCCCAACTGTCCATTTTTGGAAAATTCTCTAGAAACTCTGAGGTTTAGCATT 1020
a R H F P N C P F L E N S L E E T L R F S I -
1021 TCAAATCTGAGCATGCAGACACATGCAGCTCGAATGAGAACATTTATGTACTGGCCATCT 1080
a S N L S M Q T H A A R M R T F M Y W P S -

Fig. 3C

HUMAN hiap-2

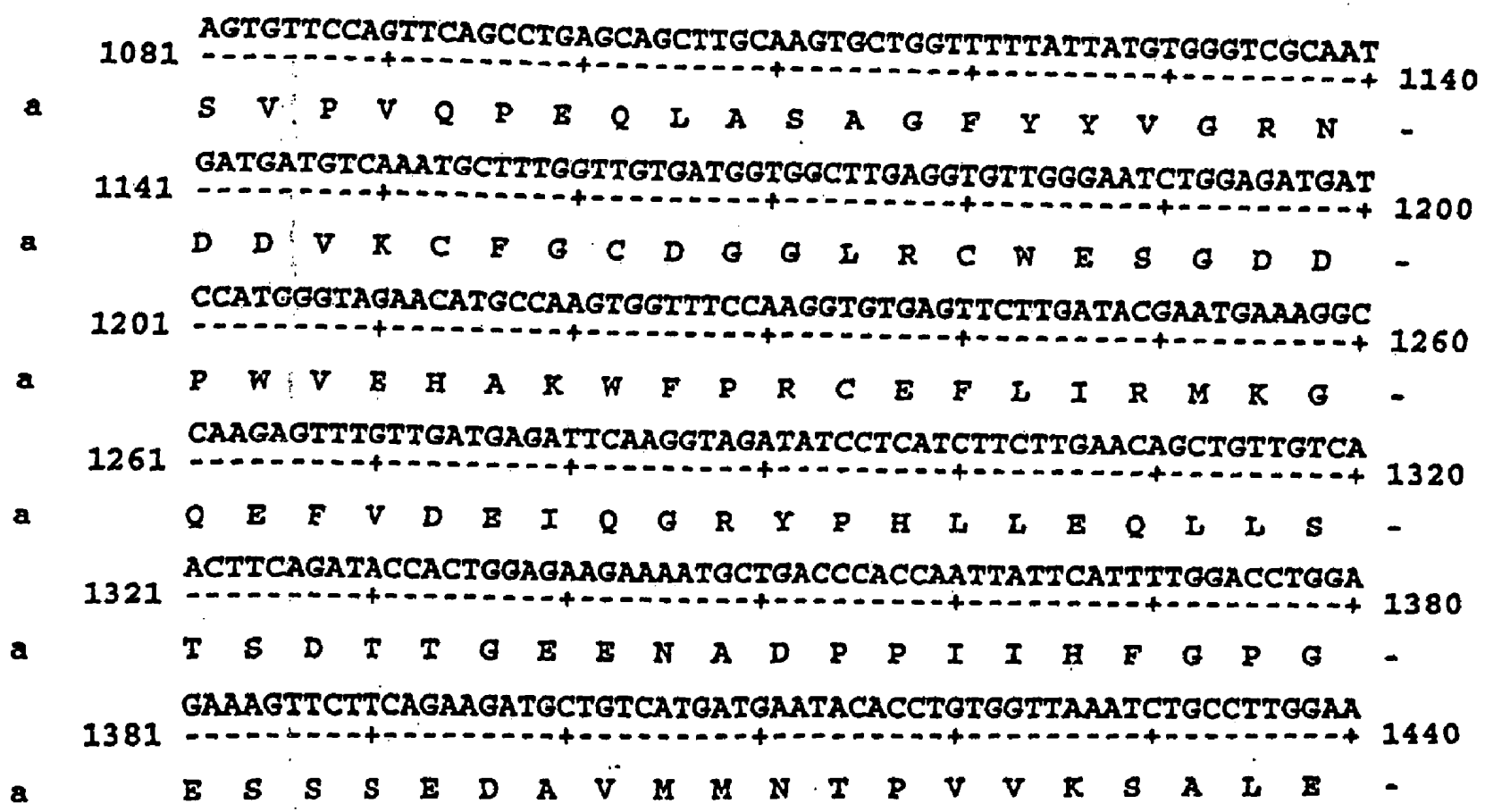

Fig. 3D

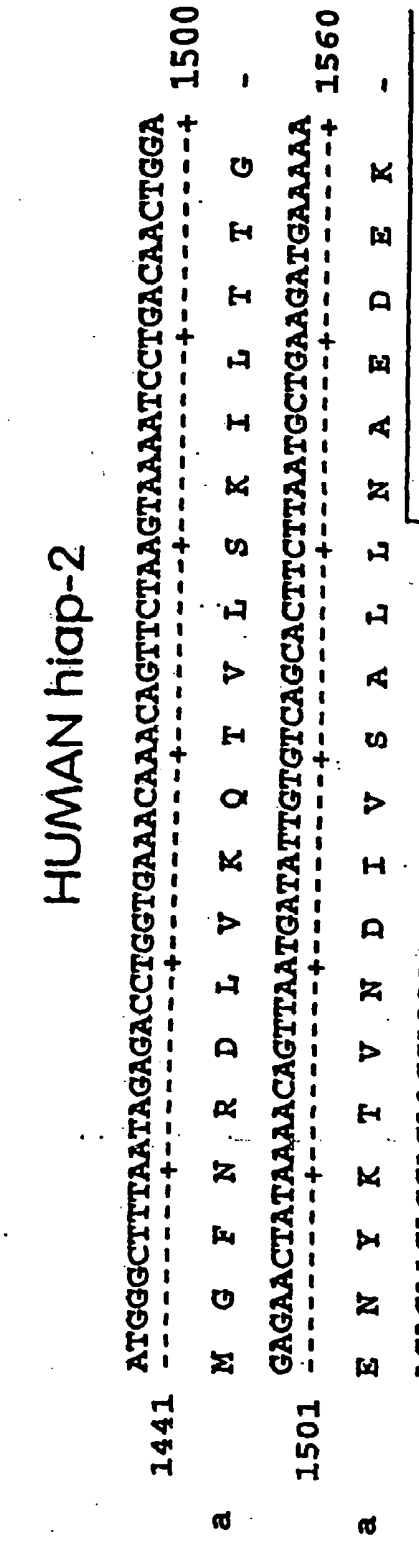
HUMAN hiap-2
1441 ATGGGCTTTAATAGAGACCTGGTGAAACAAACAGTTCTAAGTAAAATCCTGACAACTGGA 1500
a M G F N R D L V K Q T V L S K I L T T G -
1501 GAGAACTATAAAACAGTTAATGATATTGTGTCAGCACTTCTTAATGCTGAAGATGAAAAA 1560
a E N Y K T V N D I V S A L L N A E D E K -
1561 AGAGAAGAGGAGGAAGGAAAACAAGCTGAAGAAATGGCATCAGATGATTTGTCATTAATT 1620
a R E E E K E K Q A E E M A S D D L S L I -
1621 CGGAAGAACAGAATGGCTCTCTTTCAACAATTGACATGTGTGCTTCCTATCCTGGATAAT 1680
a R K N R M A L F Q Q L T C V L P I L D N -
1681 CTTTTAAAGGCCAATGTAATTAATAAACAGGAACATGATATTATTAAACAAAAAACACAG 1740
a L L K A N V I N K Q E H D I I K Q K T Q -
1741 ATACCTTTACAAGCGAGAGGAACTGATTGATACCATTTGGGTTAAAGGAAATGCTGCGGCC 1800
a I P L Q A R E E L I D T I W V K G N A A A -

Fig. 3E

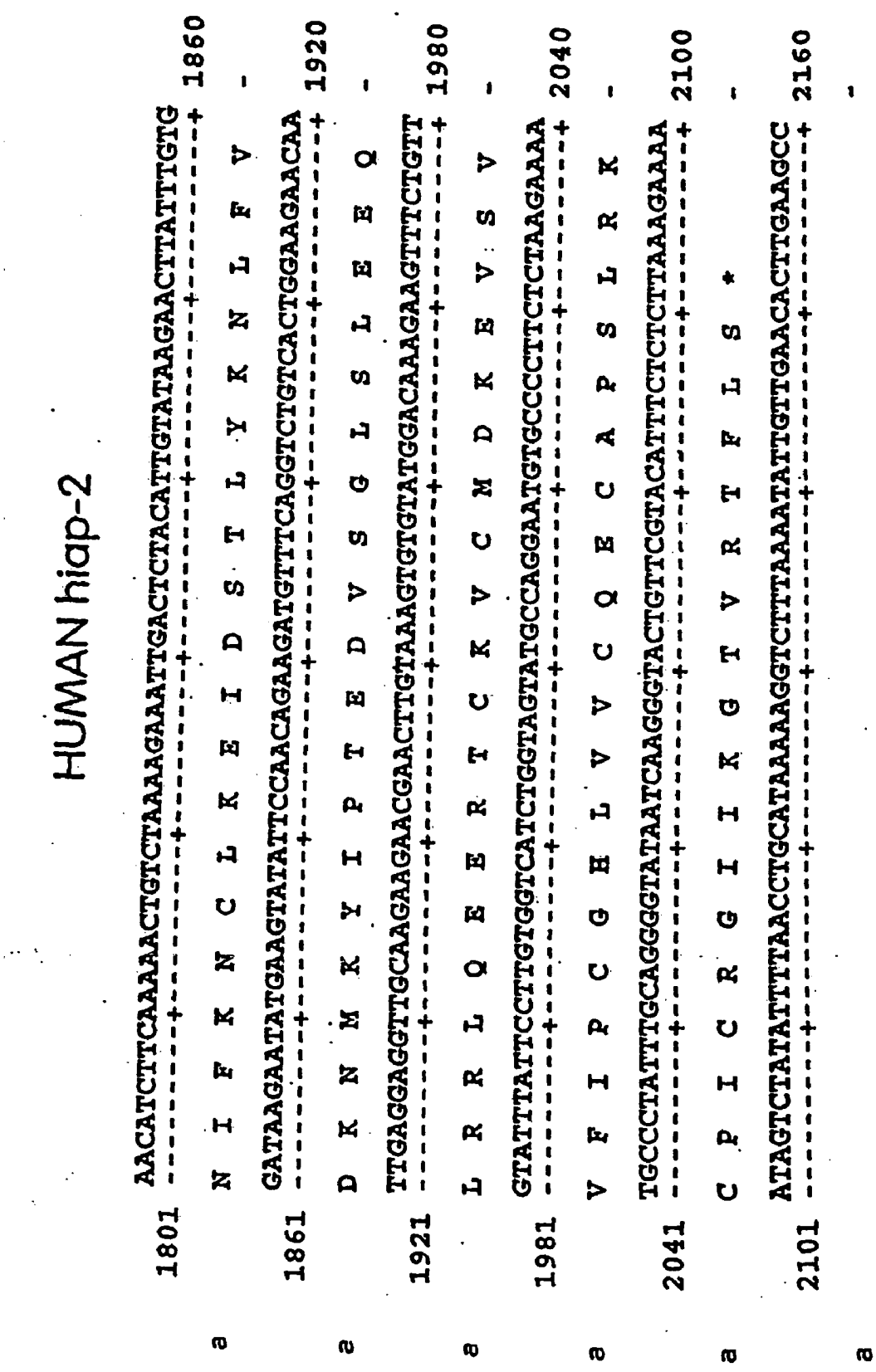
HUMAN hiap-2
1801 AACATCTTCAAAAACTGTCTAAAAGAAATTGACTCTACATTGTATAAGAACTTATTTGTG 1860
a N I F K N C L K E I D S T L Y K N L F V -
1861 GATAAGAATATGAAGTATATTCCAACAGAAGATGTTTCAGGTCTGTCACTGGAAGAACAA 1920
a D K N M K Y I P T E D V S G L S L E E Q -
1921 TTGAGGAGGTTGCAAGAAGAACGAACTTGTAAAGTGTGTATGGACAAAGAAGTTTCTGTT 1980
a L R R L Q E E R T C K V C M D K E V S V -
1981 GTATTTATTCCTTGTGGTCATCTGGTAGTATGCCAGGAATGTGCCCCTTCTCTAAGAAAA 2040
a V F I P C G H L V V C Q E C A P S L R K -
2041 TGCCCTATTTGCAGGGGTATAATCAAGGGTACTGTTCGTACATTTCTCTCTTAAAGAAAA 2100
a C P I C R G I I K G T V R T F L S * -
2101 ATAGTCTATATTTTAACCTGCATAAAAAGGTCTTTAAAATATTGTTGAACACTTGAAGCC 2160
a -

Fig. 3F

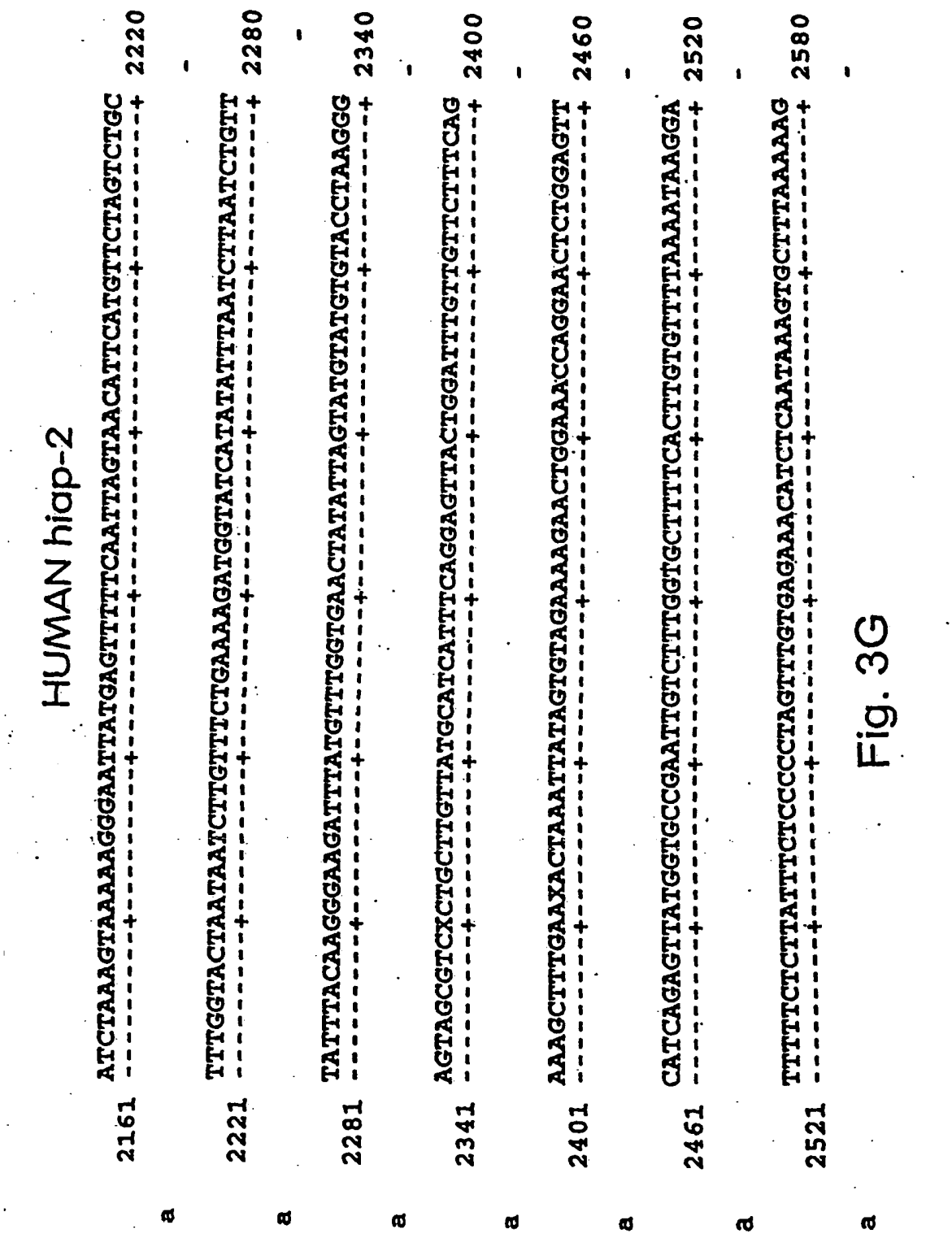
HUMAN hiap-2
2161 ATCTAAAGTAAAAAGGGAATTATGAGTTTTTCAATTAGTAACATTCATGTTCTAGTCTGC 2220
a
2221 TTTGGTACTAATAATCTTGTTTCTGAAAAGATGGTATCATATATTTAATCTTAATCTGTT 2280
a
2281 TATTTACAAGGGAAGATTTATGTTTGGTGAACTATATTAGTATGTATGTGTACCTAAGGG 2340
a
2341 AGTAGCGTCXCTGCTTGTTATGCATCATTTCAGGAGTTACTGGATTTGTTGTTCTTTCAG 2400
a
2401 AAAGCTTTGAAXACTAAATTATAGTGTAGAAAAGAACTGGAAACCAGGAACTCTGGAGTT 2460
a
2461 CATCAGAGTTATGGTGCCGAATTGTCTTTGGTGCTTTTCACTTGTGTTTTAAAATAAGGA 2520
a
2521 TTTTTCTCTTATTTCTCCCCCTAGTTTTGTGAGAAACATCTCAATAAAGTGCTTTAAAAAG 2580
a

Fig. 3G

MOUSE xiap

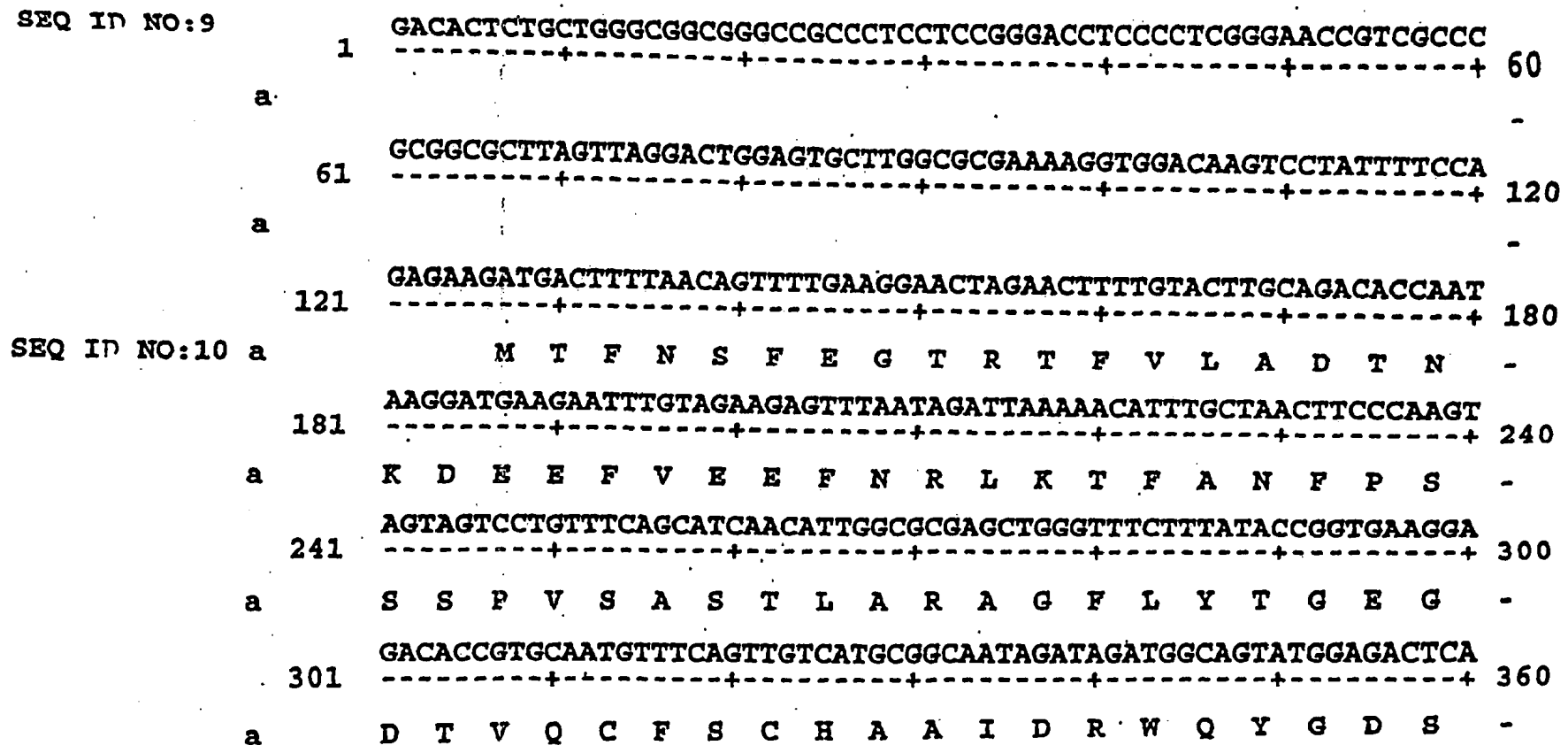

Fig. 4A

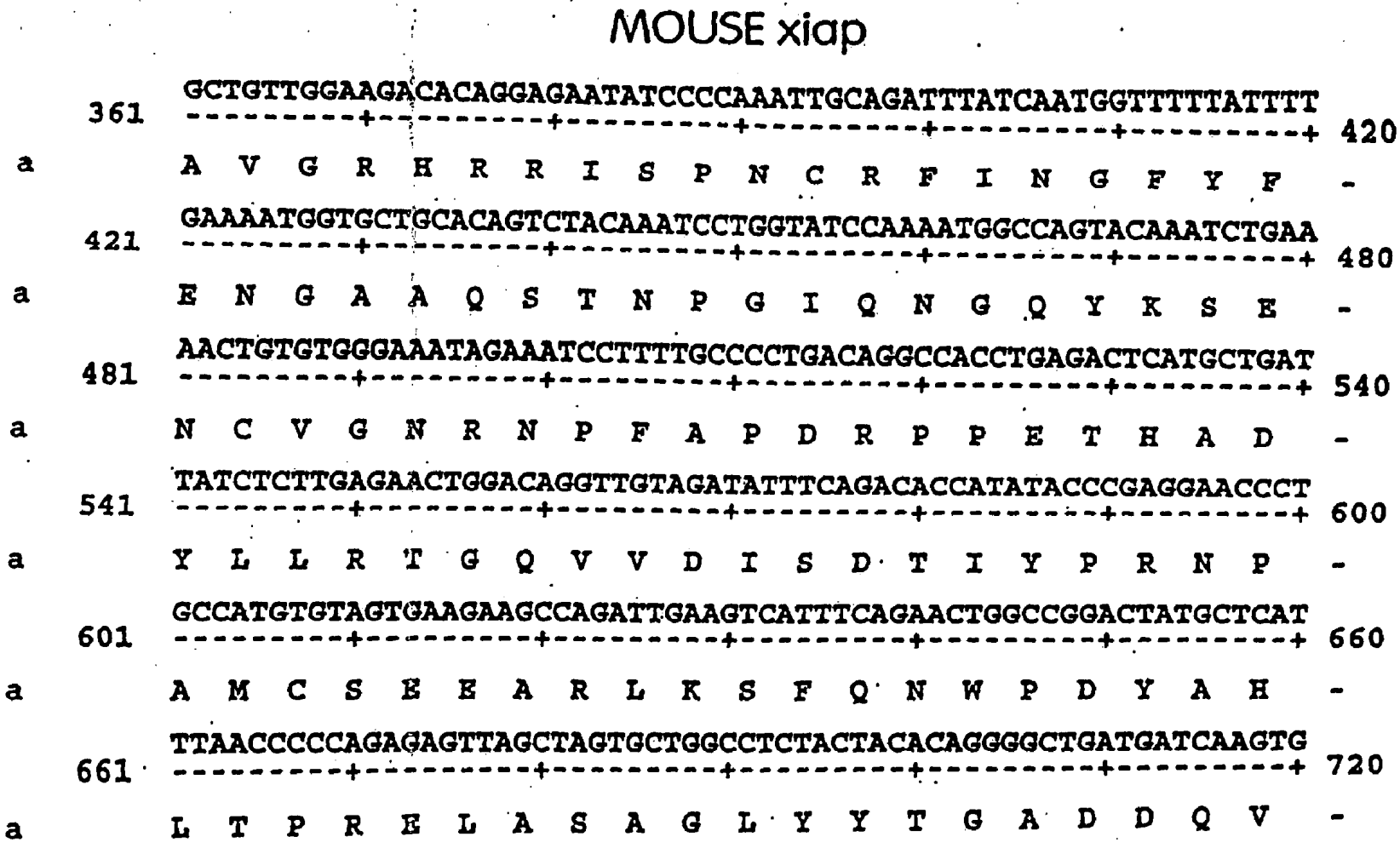
MOUSE xiap
361 GCTGTTGGAAGACACAGGAGAATATCCCCAAATTGCAGATTTATCAATGGTTTTTATTTT 420
a A V G R H R R I S P N C R F I N G F Y F -
421 GAAAATGGTGCTGCACAGTCTACAAATCCTGGTATCCAAAATGGCCAGTACAAATCTGAA 480
a E N G A A Q S T N P G I Q N G Q Y K S E -
481 AACTGTGTGGGAAATAGAAATCCTTTTGCCCCTGACAGGCCACCTGAGACTCATGCTGAT 540
a N C V G N R N P F A P D R P P E T H A D -
541 TATCTCTTGAGAACTGGACAGGTTGTAGATATTTCAGACACCATATACCCGAGGAACCCT 600
a Y L L R T G Q V V D I S D T I Y P R N P -
601 GCCATGTGTAGTGAAGAAGCCAGATTGAAGTCATTTCAGAACTGGCCGGACTATGCTCAT 660
a A M C S E E A R L K S F Q N W P D Y A H -
661 TTAACCCCCAGAGAGTTAGCTAGTGCTGGCCTCTACTACACAGGGGCTGATGATCAAGTG 720
a L T P R E L A S A G L Y Y T G A D D Q V -

Fig. 4B

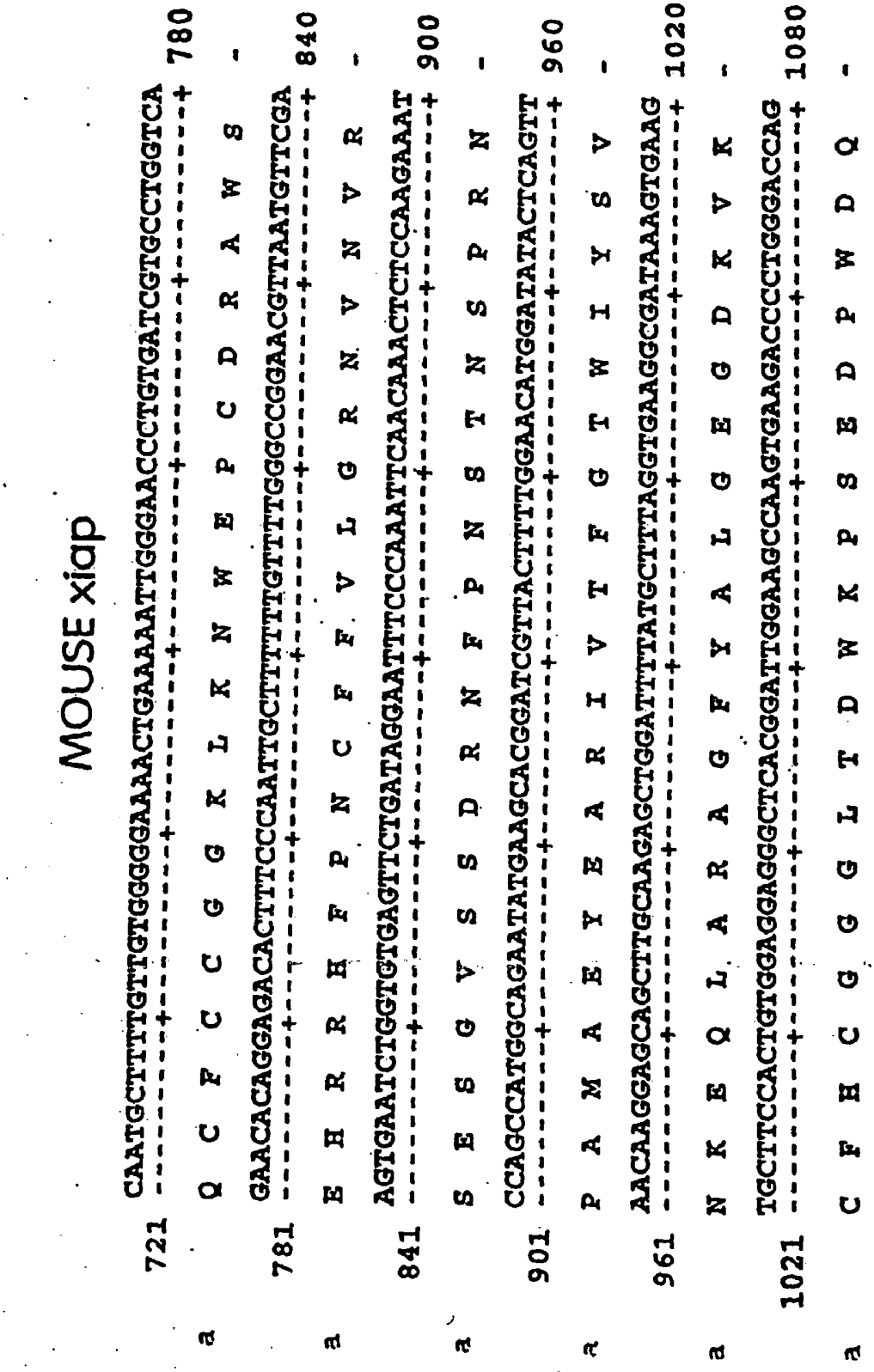
MOUSE xiap
721 CAATGCTTTTTGTTGTGGGGGAAAACTGAAAAATTGGGAACCCTGTGATCGTGCCTGGTCA 780
a Q C F C C G G K L K N W E P C D R A W S -
781 GAACACAGGAGACACTTTCCCAATTGCTTTTTTGTTTTGGGCCGGAACGTTAATGTTCGA 840
a E H R R H F P N C F F V L G R N V N V R -
841 AGTGAATCTGGTGTGAGTTCTGATAGGAATTTCCCAAATTCAACAAACTCTCCAAGAAAT 900
a S E S G V S S D R N F P N S T N S P R N -
901 CCAGCCATGGCAGAATATGAAGCACGGATCGTTACTTTTGGAACATGGATATACTCAGTT 960
a P A M A E Y E A R I V T F G T W I Y S V -
961 AACAAGGAGCAGCTTGCAAGAGCTGGATTTTATGCTTTAGGTGAAGGCGATAAAGTGAAG 1020
a N K E Q L A R A G F Y A L G E G D K V K -
1021 TGCTTCCACTGTGGAGGAGGGCTCACGGATTGGAAGCCAAGTGAAGACCCCTGGGACCAG 1080
a C F H C G G G L T D W K P S E D P W D Q -

Fig. 4C

MOUSE xiap

```
      1081 CATGCTAAGTGCTACCCAGGGTGCAAATACCTATTGGATGAGAAGGGGCAAGAATATATA
                ---------+---------+---------+---------+---------+---------+ 1140
a               H  A  K  C  Y  P  G  C  K  Y  L  L  D  E  K  G  Q  E  Y  I  -
      1141 AATAATATTCATTTAACCCATCCACTTGAGGAATCTTTGGGAAGAACTGCTGAAAAAACA
                ---------+---------+---------+---------+---------+---------+ 1200
a               N  N  I  H  L  T  H  P  L  E  E  S  L  G  R  T  A  E  K  T  -
      1201 CCACCGCTAACTAAAAAAATCGATGATACCATCTTCCAGAATCCTATGGTGCAAGAAGCT
                ---------+---------+---------+---------+---------+---------+ 1260
a               P  P  L  T  K  K  I  D  D  T  I  F  Q  N  P  M  V  Q  E  A  -
      1261 ATACGAATGGGATTTAGCTTCAAGGACCTTAAGAAAACAATGGAAGAAAAAATCCAAACA
                ---------+---------+---------+---------+---------+---------+ 1320
a               I  R  M  G  F  S  F  K  D  L  K  K  T  M  E  E  K  I  Q  T  -
                TCCGGGAGCAGCTATCTATCACTTGAGGTCCTGATTGCAGATCTTGTGAGTGCTCAGAAA
      1321 ---------+---------+---------+---------+---------+---------+---------+ 1380
a               S  G  S  S  Y  L  S  L  E  V  L  I  A  D  L  V  S  A  Q  K  -
                GATAATACGGAGGATGAGTCAAGTCAAACTTCATTGCAGAAAGACATTAGTACTGAAGAG
      1381 ---------+---------+---------+---------+---------+---------+---------+ 1440
a               D  N  T  E  D  E  S  S  Q  T  S  L  Q  K  D  I  S  T  E  E  -
```

Fig. 4D

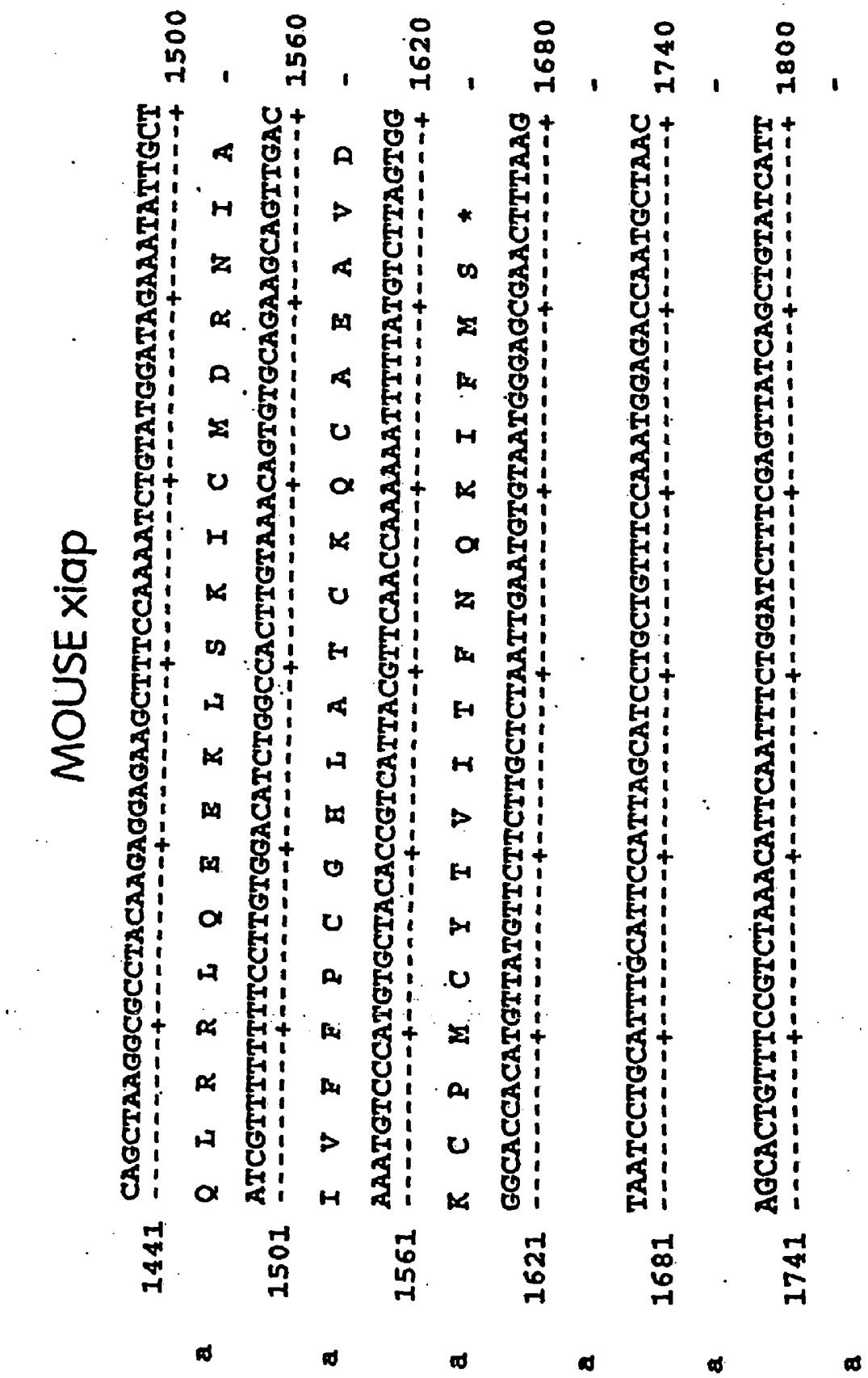
MOUSE xiap
1441 CAGCTAAGGCGCCTACAAGAGGAGAAGCTTTCCAAAATCTGTATGGATAGAAATATTGCT 1500
Q L R R L Q E E K L S K I C M D R N I A -
1501 ATCGTTTTTTTTTCCTTGTGGACATCTGGCCACTTGTAAACAGTGTGCAGAAGCAGTTGAC 1560
I V F F P C G H L A T C K Q C A E A V D -
1561 AAATGTCCCATGTGCTACACCGTCATTACGTTCAACCAAAAAATTTTTATGTCTTAGTGG 1620
K C P M C Y T V I T F N Q K I F M S * -
1621 GGCACCACATGTTATGTTCTTCTTGCTCTAATTGAATGTGTAATGGGAGCGAACTTTAAG 1680
1681 TAATCCTGCATTTGCATTCCATTAGCATCCTGCTGTTTCCAAATGGAGACCAATGCTAAC 1740
1741 AGCACTGTTTCCGTCTAAACATTCAATTTCTGGATCTTTCGAGTTATCAGCTGTATCATT 1800

Fig. 4E

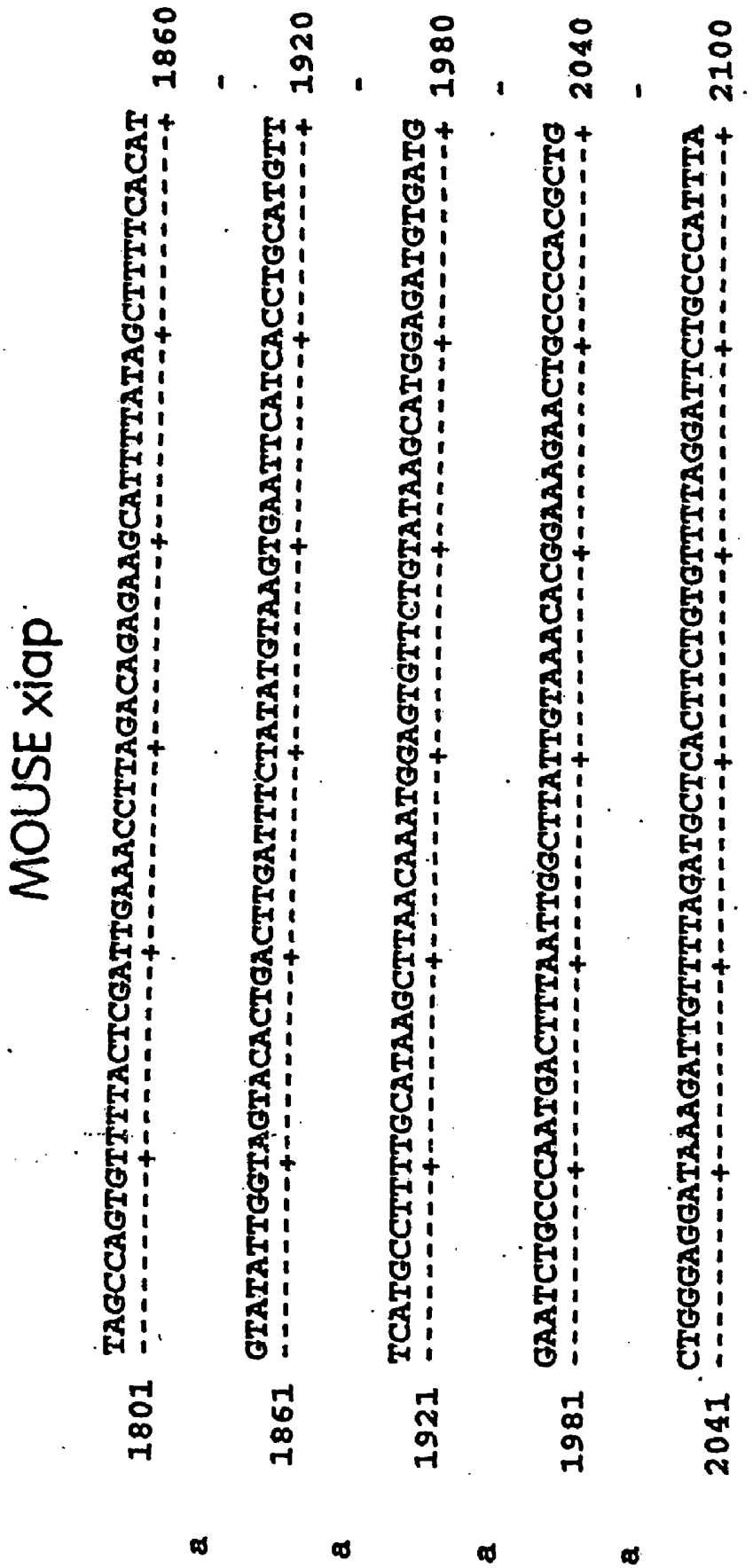
MOUSE xiap
1801 TAGCCAGTGTTTTACTCGATTGAAACCTTAGACAGAGAAGCATTTTATAGCTTTTTCACAT 1860
1861 GTATATTGGTAGTACACTGACTTGATTTCTATATGTAAGTGAATTCATCACCTGCATGTT 1920
1921 TCATGCCTTTTTGCATAAGCTTAACAAATGGAGTGTTCTGTATAAGCATGGAGATGTGATG 1980
1981 GAATCTGCCCAATGACTTTAATTGGCTTATTGTAAACACGGAAAGAACTGCCCCACGCTG 2040
2041 CTGGGAGGATAAAGATTGTTTTAGATGCTCACTTCTGTGTTTTTAGGATTCCTGCCCATTTA 2100

Fig. 4F

M-hiap-1

```
SEQ ID NO:39      GAATTCCGGGAGACCTACACCCCCGGAGATCAGAGGTCATTGCTGGCGTTCAGAGCCTAG
                1 ---------+---------+---------+---------+---------+---------+ 60
                  GAAGTGGGCTGCGGTATCAGCCTAGCAGTAAAACCGACCAGAAGCCATGCACAAAACTAC
               61 ---------+---------+---------+---------+---------+---------+ 120
                  ATCCCCAGAGAAAGACTTGTCCCTTCCCCTCCCTGTCATCTCACCATGAACATGGTTCAA
              121 ---------+---------+---------+---------+---------+---------+ 180
SEQ ID NO:40                                                  M  N  M  V  Q   -

                  GACAGCGCCTTTCTAGCCAAGCTGATGAAGAGTGCTGACACCTTTGAGTTGAAGTATGAC
              181 +---------+---------+---------+---------+---------+---------+ 240
                  D  S  A  F  L  A  K  L  M  K  S  A  D  T  F  E  L  K  Y  D   -

                  TTTTCCTGTGAGCTGTACCGATTGTCCACGTATTCAGCTTTTCCCAGGGGAGTTCCTGTG
              241 +---------+---------+---------+---------+---------+---------+ 300
                  F  S  C  E  L  Y  R  L  S  T  Y  S  A  F  D  R  G  V  P  V   -

                  TCAGAAAGGAGTCTGGCTCGTGCTGGCTTTTACTACACTGGTGCCAATGACAAGGTCAAG
              301 +---------+---------+---------+---------+---------+---------+ 360
                  S  E  R  S  L  A  R  A  G  F  Y  Y  T  G  A  N  D  K  V  K   -

                  TGCTTCTGCTGTGGCCTGATGCTAGACAACTGGAAACAAGGGGACAGTCCCATGGAGAAG
              361 +---------+---------+---------+---------+---------+---------+ 420
                  C  F  C  C  G  L  M  L  D  N  W  K  Q  G  D  S  P  M  E  K   -
```

Fig. 5A

M-hiap-1

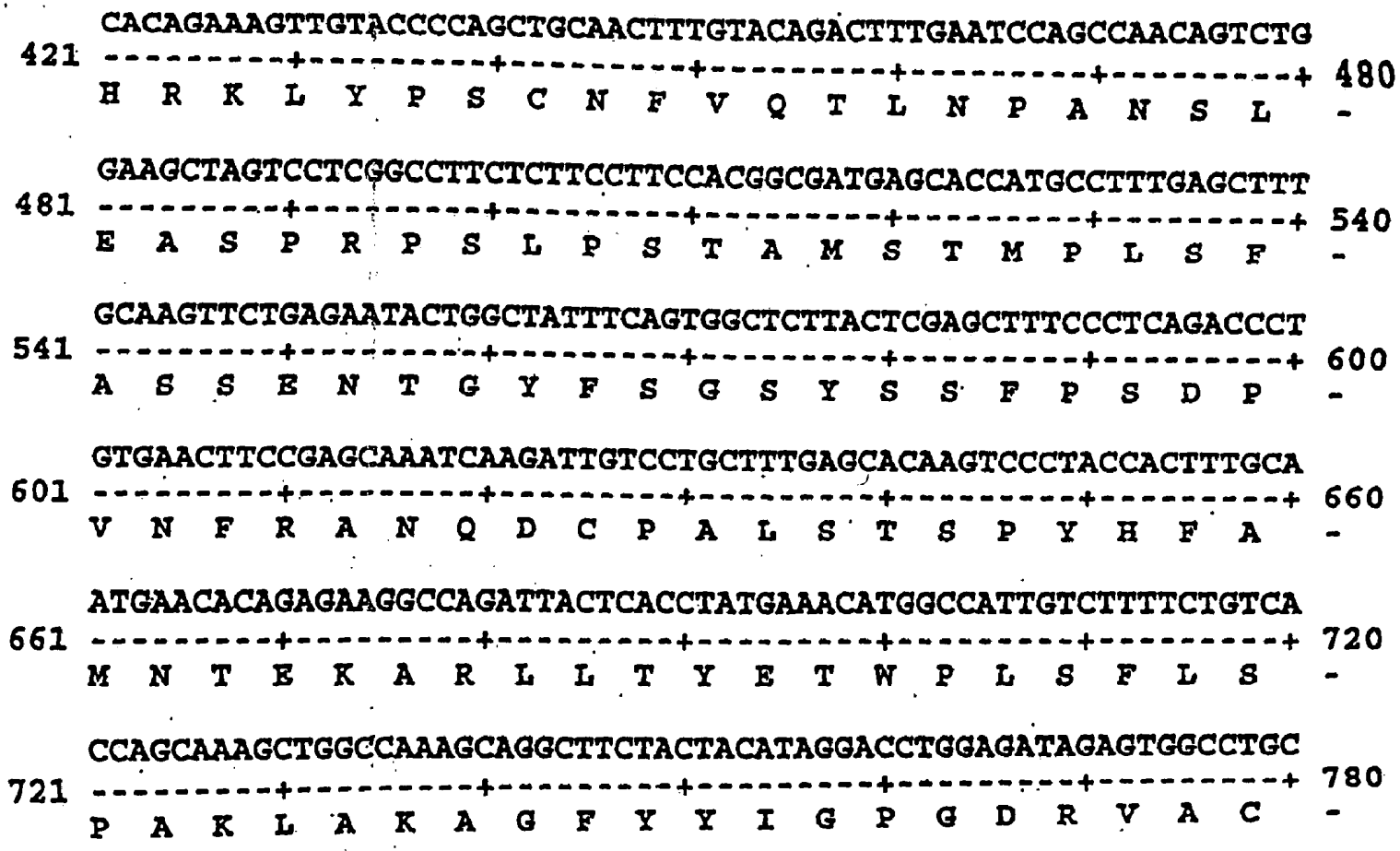

Fig. 5B

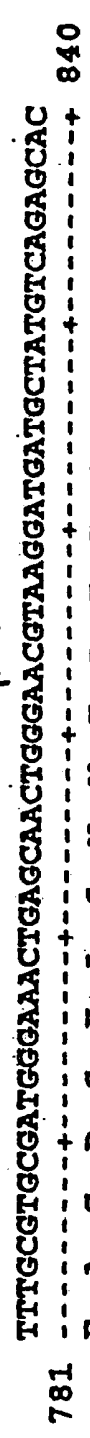
M-hiap-1
TTTGCGTGCGATGGGAAACTGAGCAACTGGGAACGTAAGGATGATGCTATGTCAGAGCAC
781 ----------+----------+----------+----------+----------+----------+ 840
F A C D G K L S N W E R K D D A M S E H -
CAGAGGCATTTCCCCAGCTGTCCGTTCTTALLkGACTTGGGTCAGTCTGCTTCGAGATAC
841 ----------+----------+----------+----------+----------+----------+ 900
Q R H F P S C P F L K D L G Q S A S R Y -
ACTGTCTCTAACCTGAGCATGCAGACACACGCAGCCCGTATTAGAACATTCTCTAACTGG
901 ----------+----------+----------+----------+----------+----------+ 960
T V S N L S M Q T H A A R I R T F S N W -
CCTTCTAGTGCACTAGTTCATTCCCAGGAACTTGCAAGTGCGGGCTTTTATTATACAGGA
961 ----------+----------+----------+----------+----------+----------+ 1020
P S S A L V H S Q E L A S A G F Y Y T G -
CACAGTGATGATGTCAAGTGTTTATGCTGTGATGGTGGGCTGAGGTGCTGGGAATCTGGA
1021 ----------+----------+----------+----------+----------+----------+ 1080
H S D D V K C L C C D G G L R C W E S G -
GATGACCCCTGGGTGGAACATGCCAAGTGGTTTCCAAGGTGTGAGTACTTGCTCAGAATC
1081 ----------+----------+----------+----------+----------+----------+ 1140
D D P W V E H A K W F P R C E Y L L R I -
AAAGGCCAAGAATTTGTCAGCCAAGTTCAAGCTGGCTATCCTCATCTACTTGAGCAGCTA
1141 ----------+----------+----------+----------+----------+----------+ 1200
K G Q E F V S Q V Q A G Y P H L L E Q L -

Fig. 5C

M-hiap-1

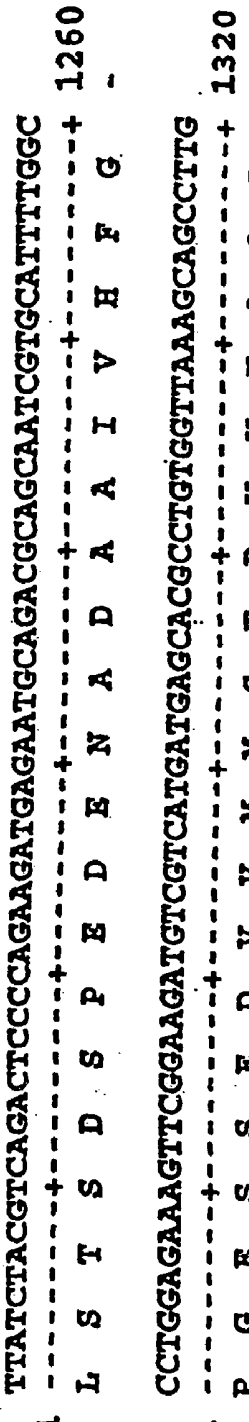

Fig. 5D

M-hiap-1

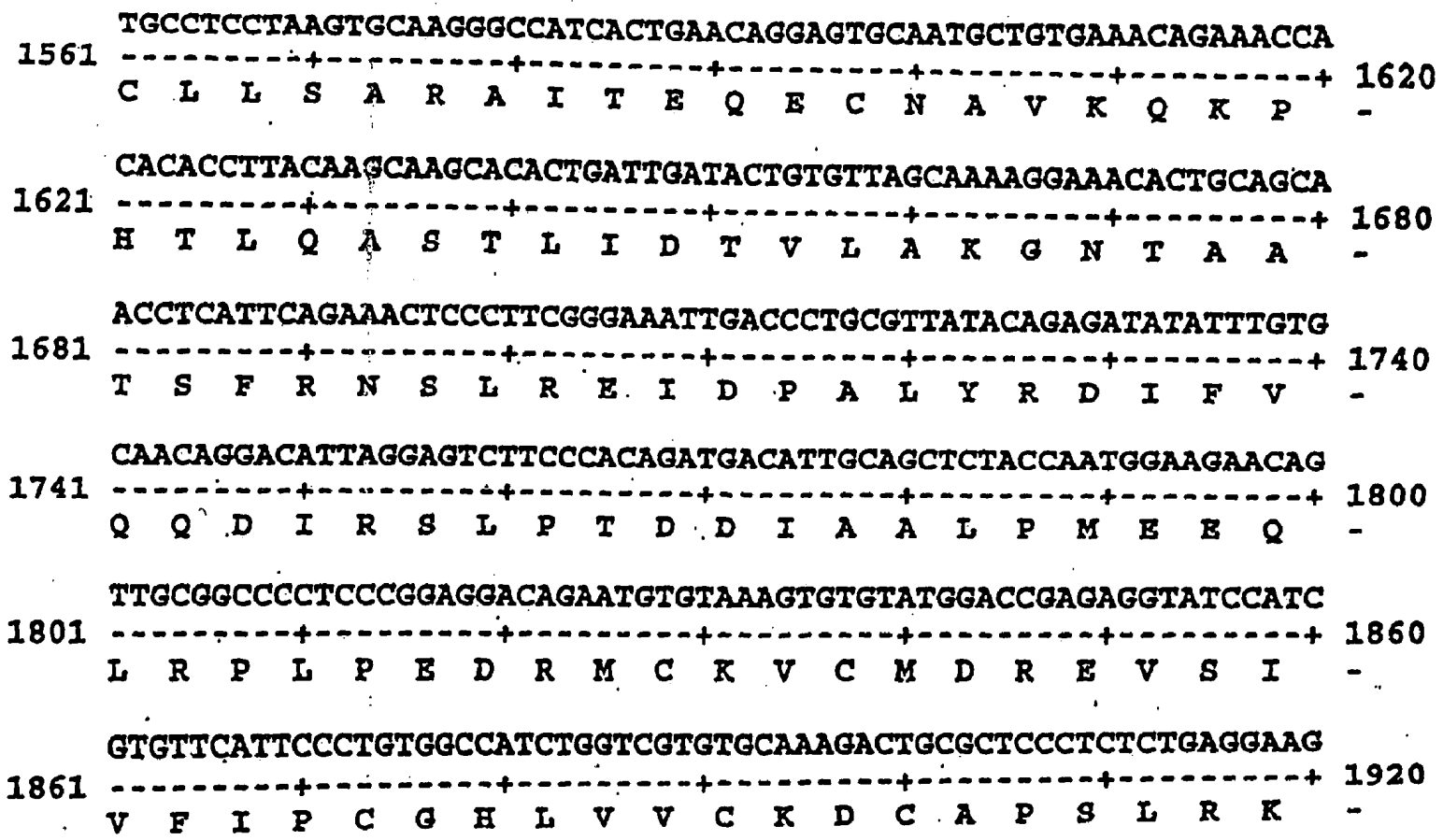

Fig. 5E

M-hiap-1

```
     TGTCCCATCTGTAGAGGGACCATCAAGGGCACAGTGCGCACATTTCTCTCCTGAACAAGA
1921 ---------+---------+---------+---------+---------+---------+ 1980
     C  P  I  C  R  G  T  I  K  G  T  V  R  T  F  L  S  *           -

     CTAATGGTCCATGGCTGCAACTTCAGCCAGGAGGAAGTTCACTGTCACTCCCAGTTCCAT
1981 ---------+---------+---------+---------+---------+---------+ 2040
     TCGGAACTTGAGGCCAGCCTGGATAGCACGAGACACCGCCAAACkCACAAATATAAACAT
2041 ---------+---------+---------+---------+---------+---------+ 2100
     GAAAAACTTTTGTCTGAAGTCAAGAATGAATGAATTACTTATATAATAATTTTAATTGGT
2101 ---------+---------+---------+---------+---------+---------+ 2160
     TTCCTTAAAAGTGCTATTTGTTCCCAACTCAGAAAATTGTTTTCTGTAAACATATTTACA
2161 ---------+---------+---------+---------+---------+---------+ 2220
     TACTACCTGCATCTAAAGTATTCATATATTCATATATTCAGATGTCATGAGAGAGGGTTT
2221 ---------+---------+---------+---------+---------+---------+ 2280
     TGTTCTTGTTCCTGAAAAGCTGGTTTATCATCTGATCAGCATATACTGCGCAACGGGCAG
2281 ---------+---------+---------+---------+---------+---------+ 2340
     GGCTAGAATCCATGAACCAAGCTGCAAAGATCTCACGCTAAATAAGGCGGAAAGATTTGG
2341 ---------+---------+---------+---------+---------+---------+ 2400
     AGAAACGAAAGGAAATTCTTTCCTGTCCAATGTATACTCTTCAGACTAATGACCTCTTCC
2401 ---------+---------+---------+---------+---------+---------+ 2460
     TATCAAGCCTTCTA
2461 ----------+---- 2474
```

Fig. 5F

M-hiap-2

SEQ ID NO:41

```
   CTGTGGTGGAGATCTATTGTCCAAGTGGTGAGAAACTTCATCTGGAAGTTTAAGCGGTCA
 1 ---------+---------+---------+---------+---------+---------+ 60
   GAAATACTATTACTACTCATGGACAkRACTGTCTCCCAGAGACTCGCCCAAGGTACCTTA
61 ---------+---------+---------+---------+---------+---------+ 120
   CACCCRAAAACTTAAACGTATAATGGAGAAGAGCACAATCTTGTCAAATTGGACAAAGGA
121 ---------+---------+---------+---------+---------+---------+ 180
SEQ ID NO:42                        M  E  K  S  T  I  L  S  N  W  T  K  E  -

   GAGCGAAGAAAAAATGAAGTTTGACTTTTCGTGTGAACTCTACCGAATGTCTACATATTC
181 ---------+---------+---------+---------+---------+---------+ 240
    S  E  E  K  M  K  F  D  F  S  C  E  L  Y  R  M  S  T  Y  S  -

   AGCTTTTCCCAGGGGAGTTCCTGTCTCAGAGAGGAGTCTGGCTCGTGCTGGCTTTTATTA
241 ---------+---------+---------+---------+---------+---------+ 300
    A  F  P  R  G  V  P  V  S  E  R  S  L  A  R  A  G  F  Y  Y  -

   TACAGGTGTGAATGACAAAGTCAAGTGCTTCTGCTGTGGCCTGATGTTGGATAACTGGAA
301 ---------+---------+---------+---------+---------+---------+ 360
    T  G  V  N  D  K  V  K  C  F  C  C  G  L  M  L  D  N  W  K  -

   ACAAGGGGACAGTCCTGTTGAAAAGCACAGACAGTTCTATCCCAGCTGCAGCTTTGTACA
361 ---------+---------+---------+---------+---------+---------+ 420
    Q  G  D  S  P  V  E  K  H  R  Q  F  Y  P  S  C  S  F  V  Q  -
```

Fig. 6A

M-hiap-2

```
    GACTCTGCTTTCAGCCAGTCTGCAGTCTCCATCTAAGAATATGTCTCCTGTGAAAAGTAG
421 ---------+---------+---------+---------+---------+---------+ 480
     T  L  L  S  A  S  L  Q  S  P  S  K  N  M  S  P  V  K  S  R  -

    ATTTGCACATTCGTCACCTCTGGAACGAGGTGGCATTCACTCCAACCTGTGCTCTAGCCC
481 ---------+---------+---------+---------+---------+---------+ 540
     F  A  H  S  S  P  L  E  R  G  G  I  H  S  N  L  C  S  S  P  -

    TCTTAATTCTAGAGCAGTGGAAGACTTCTCATCAAGGATGGATCCCTGCAGCTATGCCAT
541 ---------+---------+---------+---------+---------+---------+ 600
     L  N  S  R  A  V  E  D  F  S  S  R  M  D  P  C  S  Y  A  M  -

    GAGTACAGAAGAGGCCAGATTTCTTACTTACAGTATGTGGCCTTTAAGTTTTCTGTCACC
601 ---------+---------+---------+---------+---------+---------+ 660
     S  T  E  E  A  R  F  L  T  Y  S  M  W  P  L  S  F  L  S  P  -

    AGCAGAGCTGGCCAGAGCTGGCTTCTATTACATAGGGCCTGGAGACAGGGTGGCCTGTTT
661 ---------+---------+---------+---------+---------+---------+ 720
     A  E  L  A  R  A  G  F  Y  Y  I  G  P  G  D  R  V  A  C  F  -

    TGCCTGTGGTGGGAAACTGAGCAACTGGGAACCAAAGGATTATGCTATGTCAGAGCACCG
721 ---------+---------+---------+---------+---------+---------+ 780
     A  C  G  G  K  L  S  N  W  E  P  K  D  Y  A  M  S  E  H  R  -
```

Fig. 6B

M-hiap-2

```
    CAGACATTTTCCCCACTGTCCATTTCTGGAAAATACTTCAGAAACACAGAGGTTTAGTAT
781 ---------+---------+---------+---------+---------+---------+ 840
     R  H  F  P  H  C  P  F  L  E  N  T  S  E  T  Q  R  F  S  I  -

    ATCAAATCTAAGTATGCAGACACACTCTGCTCGATTGAGGACATTTCTGTACTGGCCACC
841 ---------+---------+---------+---------+---------+---------+ 900
     S  N  L  S  M  Q  T  H  S  A  R  L  R  T  F  L  Y  W  P  P  -

    TAGTGTTCCTGTTCAGCCCGAGCAGCTTGCAAGTGCTGGATTCTATTACGTGGATCGCRA
901 ---------+---------+---------+---------+---------+---------+ 960
     S  V  P  V  Q  P  E  Q  L  A  S  A  G  F  Y  Y  V  D  R  N  -

    TGATGATGTCAAGTGCCTTTGTTGTGATGGTGGCTTGAGATGTTGGGAACCTGGAGATGA
961 ---------+---------+---------+---------+---------+---------+ 1020
     D  D  V  K  C  L  C  C  D  G  G  L  R  C  W  E  P  G  D  D  -

    CCCCTGGATAGAACACGCCAAATGGTTTCCAAGGTGTGAGTTCTTGATACGGATGAAGGG
1021 ---------+---------+---------+---------+---------+---------+ 1080
     P  W  I  E  H  A  K  W  F  P  R  C  E  F  L  I  R  M  K  G  -

    TCAGGAGTTTGTTGATGAGATTCAAGCTAGATATCCTCATCTTCTTGAGCAGCTGTTGTC
1081 ---------+---------+---------+---------+---------+---------+ 1140
     Q  E  F  V  D  E  I  Q  A  R  Y  P  H  L  L  E  Q  L  L  S  -
```

Fig. 6C

M-hiap-2

```
     CACTTCAGACACCCCAGGAGAAGAAAATGCTGACCCTACAGAGACAGTGGTGCATTTTGG
1141 ---------+---------+---------+---------+---------+---------+ 1200
      T  S  D  T  P  G  E  E  N  A  D  P  T  E  T  V  V  H  F  G  -

     CCCTGGAGAAAGTTCGAAAGATGTCGTCATGATGAGCACGCCTGTGGTTAAAGCAGCCTT
1201 ---------+---------+---------+---------+---------+---------+ 1260
      P  G  E  S  S  K  D  V  V  M  M  S  T  P  V  V  K  A  A  L  -

     GGAAATGGGCTTCAGTAGGAGCCTGGTGAGACAGACGGTTCAGCGGCAGATCCTGGCCAC
1261 ---------+---------+---------+---------+---------+---------+ 1320
      E  M  G  F  S  R  S  L  V  R  Q  T  V  Q  R  Q  I  L  A  T  -

     TGGTGAGAACTACAGGACCGTCAATGATATTGTCTCAGTACTTTTGAATGCTGAAGATGA
1321 ---------+---------+---------+---------+---------+---------+ 1380
      G  E  N  Y  R  T  V  N  D  I  V  S  V  L  L  N  A  E  D  E  -

     GAGAAGAGAAGAGGAGAAGGAAAGACAGACTGAAGAGATGGCATCAGGTGACTTATCACT
1381 ---------+---------+---------+---------+---------+---------+ 1440
      R  R  E  E  E  K  E  R  Q  T  E  E  M  A  S  G  D  L  S  L  -

     GATTCGGAAGAATAGAATGGCCCTCTTTCAACAGTTGACACATGTCCTTCCTATCCTGGA
1441 ---------+---------+---------+---------+---------+---------+ 1500
      I  R  K  N  R  M  A  L  E  Q  Q  L  T  H  V  L  P  I  L  D  -
```

Fig. 6D

M-hiap-2

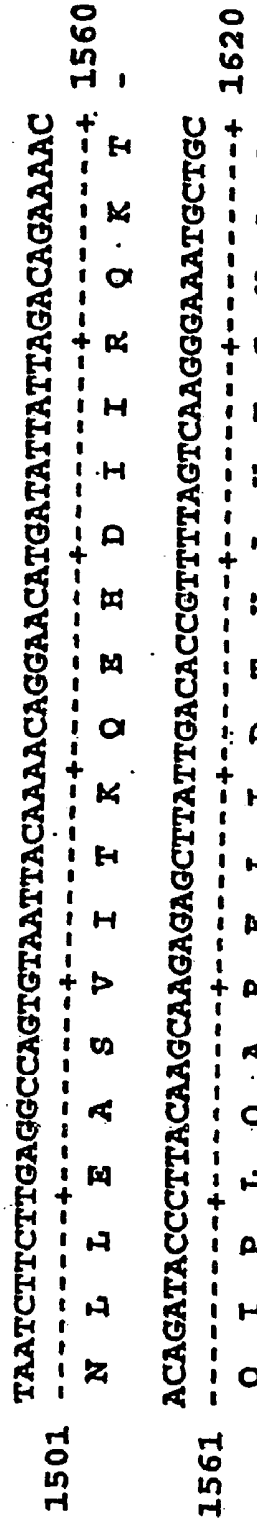

Fig. 6E

M-hiap-2

```
    GAAGTGCCCCATCTGCAGGGGGACAATCAAGGGGACTGTGCGCACATTTCTCTCATGAGT
1861 ---------+---------+---------+---------+---------+---------+ 1920
      K  C  P  I  C  R  G  T  I  K  G  T  V  R  T  F  L  S  *     -

    GAAGAATGGTCTGAAAGTATTGTTGGACATCAGAAGCTGTCAGAACAAAGAATGAACTAC
1921 ---------+---------+---------+---------+---------+---------+ 1980
    TGATTTCAGCTCTTCAGCAGGACATTCTACTCTCTTTCAAGATTAGTAATCTTGCTTTAT
1981 ---------+---------+---------+---------+---------+---------+ 2040
    GAAGGGTAGCATTGTATATTTAAGCTTAGTCTGTTGCAAGGGAAGGTCTATGCTGTTGAG
2041 ---------+---------+---------+---------+---------+---------+ 2100
    CTACAGGACTGTGTCTGTTCCAGAGCAGGAGTTGGGATGCTTGCTGTATGTCCTTCAGGA
2101 ---------+---------+---------+---------+---------+---------+ 2160
    CTTCTTGGGATTTGGGAATTTGGGGAAAGCTTTGGAATCCAGTGATGTGGAGCTCAGAAA
2161 ---------+---------+---------+---------+---------+---------+ 2220
    TCCTGGAACCAGTGACTCTGGTACTCAGTAGATAGGGTACCCTGTACTTCTTGGTGCTTT
2221 ---------+---------+---------+---------+---------+---------+ 2280
    TCCAGTCTGGGAAATAAGGAGGAATCTGCTGCTGGTAAAAATTTGCTGGATGTGAGAAAT
2281 ---------+---------+---------+---------+---------+---------+ 2340
    AGATGAAAGTGTTTCGGGTGGGGGCGTGCATCAGTGTAGTGTGTGCAGGGATGTATGCAG
2341 ---------+---------+---------+---------+---------+---------+ 2400
    GCCAAACACTGTGTAG
2401 ---------+------ 2416
```

Fig. 6F

Alignment of BIR (Baculoviral IAP Repeats) Domains

Baculovirus
- Cp_iap — Cydia pomonella
- Op_iap — Orgyia pseudotsugata

Human
- xiap — IAP on X chromosome
- hiap1, hiap2 — two different human IAP genes

Mouse
- m-xiap — mouse homologue of human xiap gene

Insect
- diap — Drosophilia IAP gene, not clearly a homologue of xiap or hiap

note on consensus: The consensus line represents amino acids or very similar amino acids which are present in 14 of the 19 BIR sequences at each position. Capitalized residues are those that are in the consensus sequence.

Fig. 7

		1	68
SEQ ID NO:11	Op_iap-1	kaaRLgTYtn WPvqf.leps rMAasGFYYl GrgDeVrCaf CkveitnWvr gDdpetdHkr waPqCpFV
SEQ ID NO:14	Cp_iap-1	eevRLnTFek WPvsf.lspe tMAknGFYYl GrsDeVrCaf CkveimrWke gEdpaadHkk waPqCpFV
SEQ ID NO:15	diap-2	eanRLvTFkd WPnpn.iLpq aLAkAGFYYl nrlDhVkCvw CngviakWek nDnafeeHkr ffPqCprV
SEQ ID NO:16	m-xiap-1	efnRLkTFan FPsspvsas tLArAGFLYt GegDtVqCFs ChaaidrWqy gDsavgrHrr isPnCrFI
SEQ ID NO:17	xiap-1	efnRLkTFan FPsgspvsas tLArAGFLYt GegDtVrCFs ChaavdrWqy gDsavgrHrk vsPnCrFI
SEQ ID NO:18	hiap1-1	elyRMsTYst FPagvpvser sLArAGFYYt GvnDkVkCFc CglmldnWkr gDsptekHkk lyPsCrFV
SEQ ID NO:19	hiap2-1	elyRMsTYst FPagvpvser sLArAGFYYt GvnDkVkCFc CglmldnWkl gDspiqkHkq lyPsCsFI
SEQ ID NO:20	m-xiap-2	eeaRLksFqn WPdyahltpr eLAsAGLYYt GadDqVqCFc CggklknWep cDrawseHrr hfPnCfFv
SEQ ID NO:21	xiap-2	eeaRLksFqn WPdyahltpr eLAsAGLLYt GigDqVqCFc CggklknWep cDrawseHrr hfPnCfFV
SEQ ID NO:22	hiap1-2	enaRLlTFqt WP.lLflspt dLArAGFYYi GpgDrVaCFa CggklsnWep kDnamseHlr hfPkCpFI
SEQ ID NO:23	hiap2-2	eeaRFlTYhm WP.lLflsps eLArAGFYYi GpgDrVaCFa CggklsnWep kDdamseHrr hfPnCpFl
SEQ ID NO:24	m-xiap-3	yeaRivTFgt Wiysv..nke qLArAGFYal GegDkVkCFh CgggltdWkp sEdpwdqHak cyPgCkYl
SEQ ID NO:25	xiap-3	yeaRifTFgt Wiysv..nke qLArAGFYal GegDkVkCFh CgggltdWkp sEdpweqHak wyPgCkYl
SEQ ID NO:26	hiap1-3	haaRFkTFfn WPssvlvnpe qLAsAgFYYv GnsDdVkCFc CdgglrcWes gDdpwvqHak wfPrC Yl
SEQ ID NO:27	hiap2-3	haaRMrTFmy WPssvpvqpe qLAsAGFYYv GrnDdVkCFg CdgglrcWes gDdpwveHak wfPrCeFl
SEQ ID NO:28	Op_iap-2	eaaRLrTFae WPrglkqrpe eLAeAGFFYt GqgDktrCFc CdgglkdWep dDapwqqHar wydrCeYV
SEQ ID NO:29	Cp_iap-2	eaaRvksFhn WPrcmkqrpe qMAdAGFFYt GygDntkCFy CdgglkdWep eDvpweqHvr widrCaYV
SEQ ID NO:30	diap-3	vdaRLrTFtd WPisniqpas aLAqAGLYYq kigDqVrCFh CniglrsWqk eDepwieHak wsPkCqFV
SEQ ID NO:31	diap-1	esvRLaTFge WPlnapvsae dLvanGFF.. GtwmeaeCdf ChvridrWey gDlvaerHrr ssPiCsmV
SEQ ID NO:2	Consensus	---RL-TF-- WP-------- -LA-AGFYY- G--D-V-CF- C------W-- -D-----H-- --P-C-FV

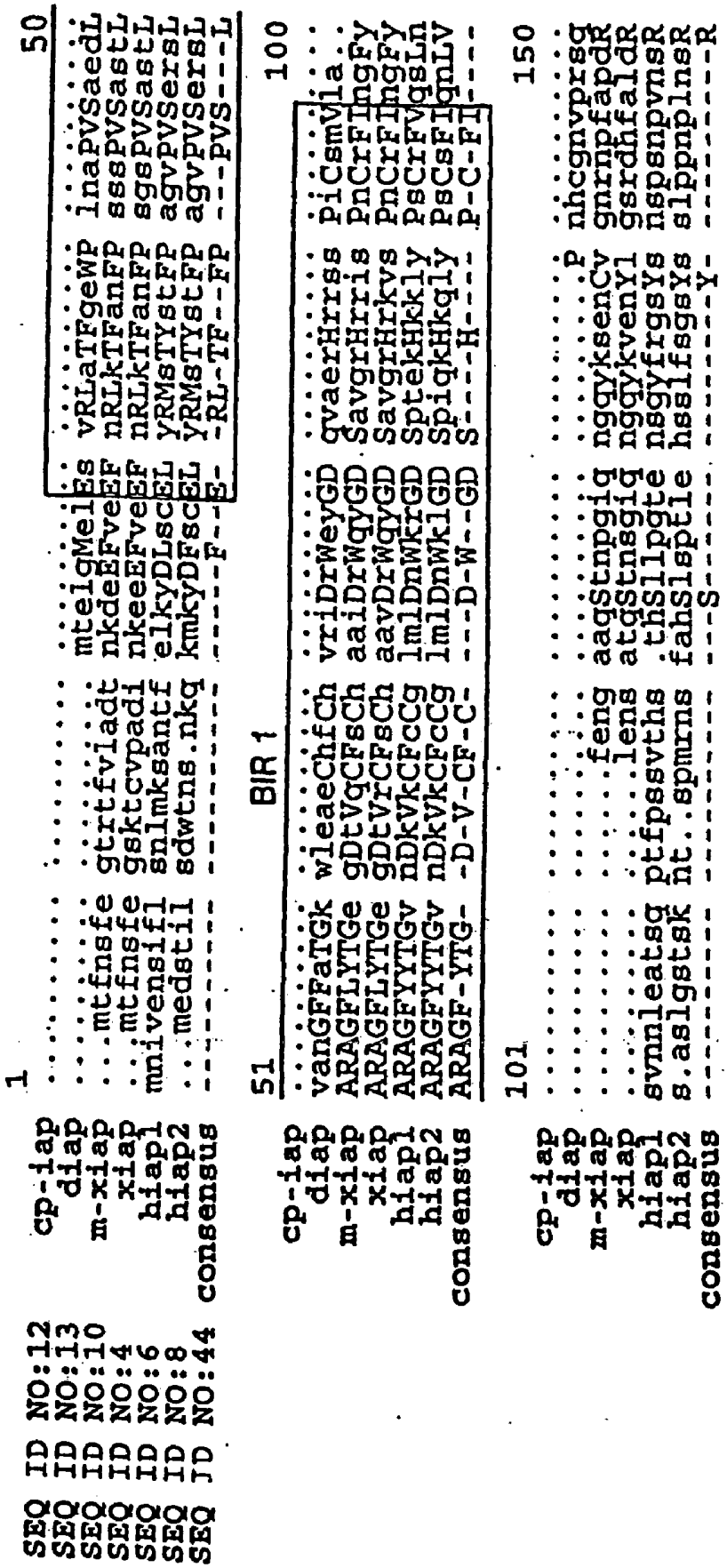
SEQ ID NO:12
SEQ ID NO:13
SEQ ID NO:10
SEQ ID NO:4
SEQ ID NO:6
SEQ ID NO:8
SEQ ID NO:44
cp-iap
diap
m-xiap
xiap
hiap1
hiap2
consensus
BIR 1

Fig. 8A

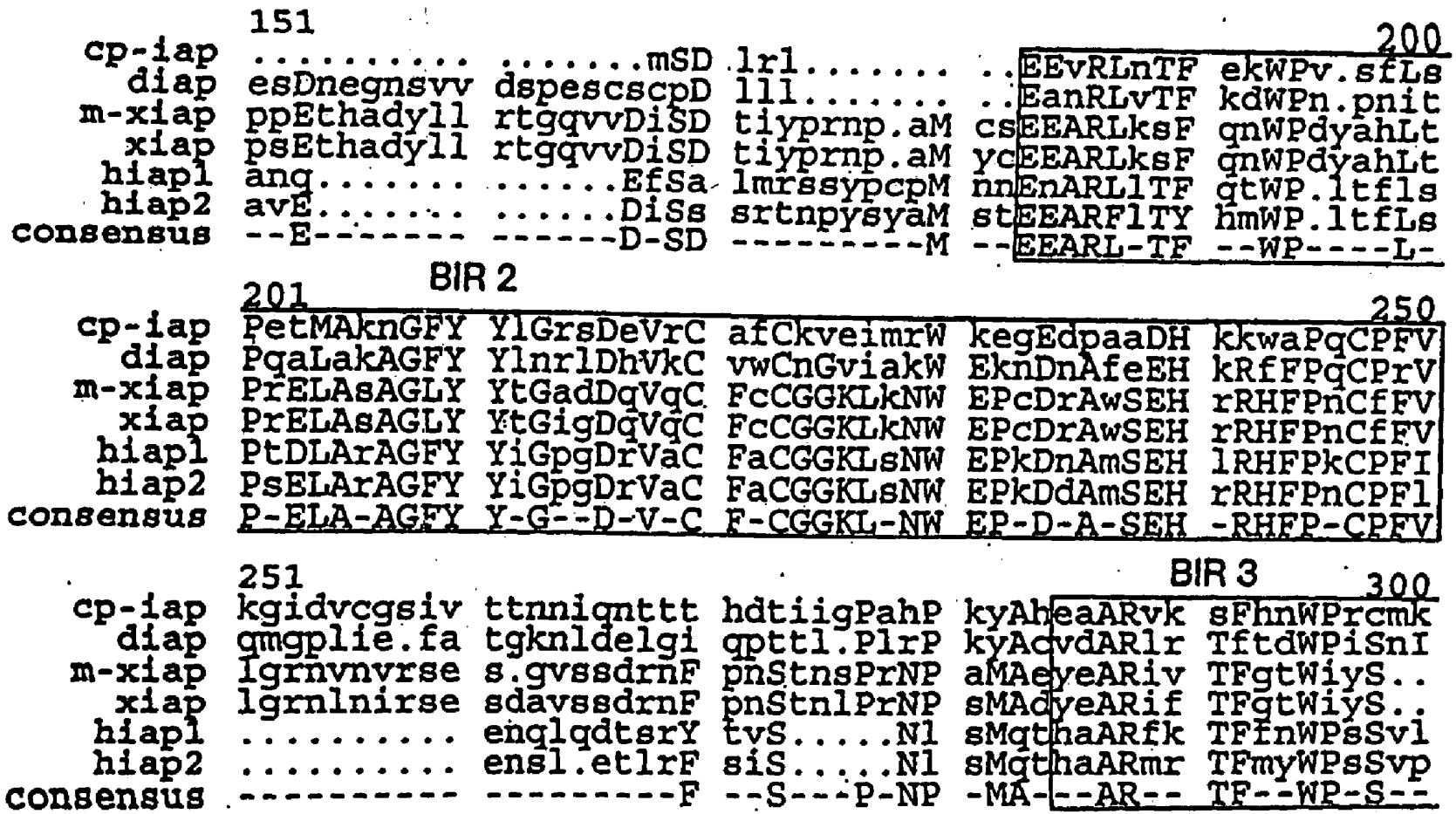
151
200
cp-iap ....mSD lrl..... ..EEvRLnTF ekWPv.sfLs
diap esDnegnsvv dspescscpD lll..... ..EanRLvTF kdWPn.pnit
m-xiap ppEthadyll rtgqvvDiSD tiyprnp.aM csEEARLksF qnWPdyahLt
xiap psEthadyll rtgqvvDiSD tiyprnp.aM ycEEARLksF qnWPdyahLt
hiap1 anq..... .....EfSa lmrssypcpM nnEnARL1TF qtWP.ltf1s
hiap2 avE..... .....DiSs srtnpysyaM stEEARF1TY hmWP.ltfLs
consensus --E------- ------D-SD ----------M --EEARL-TF --WP----L-
BIR 2
201
250
cp-iap PetMAknGFY Y1GrsDeVrC afCkveimrW kegEdpaaDH kkwaPqCPFV
diap PqaLakAGFY Y1nr1DhVkC vwCnGviakW EknDnAfeEH kRfFPqCPrV
m-xiap PrELAsAGLY YtGadDqVqC FcCGGKLkNW EPcDrAwSEH rRHFPnCfFV
xiap PrELAsAGLY YtGigDqVqC FcCGGKLkNW EPcDrAwSEH rRHFPnCfFV
hiap1 PtDLArAGFY YiGpgDrVaC FaCGGKLsNW EPkDnAmSEH 1RHFPkCPFI
hiap2 PsELArAGFY YiGpgDrVaC FaCGGKLsNW EPkDdAmSEH rRHFPnCPF1
consensus P-ELA-AGFY Y-G--D-V-C F-CGGKL-NW EP-D-A-SEH -RHFP-CPFV
251
BIR 3
300
cp-iap kgidvcgsiv ttnniqnttt hdtiigPahP kyAheaARvk sFhnWPrcmk
diap qmgplie.fa tgknldelgi qpttl.PlrP kyAcvdAR1r TftdWPiSnI
m-xiap lgrnvnvrse s.gvssdrnF pnStnsPrNP aMAeyeARiv TFgtWiyS..
xiap lgrn1nirse sdavssdrnF pnStn1PrNP sMAdyeARif TFgtWiyS..
hiap1 ..... enq1qdtsrY tvS.....N1 sMqthaARfk TFfnWPsSv1
hiap2 ..... ens1.et1rF siS.....N1 sMqthaARmr TFmyWPsSvp
consensus ---------- ---------F --S---P-NP -MA-__AR__ TF__WP-S__

Fig. 8B

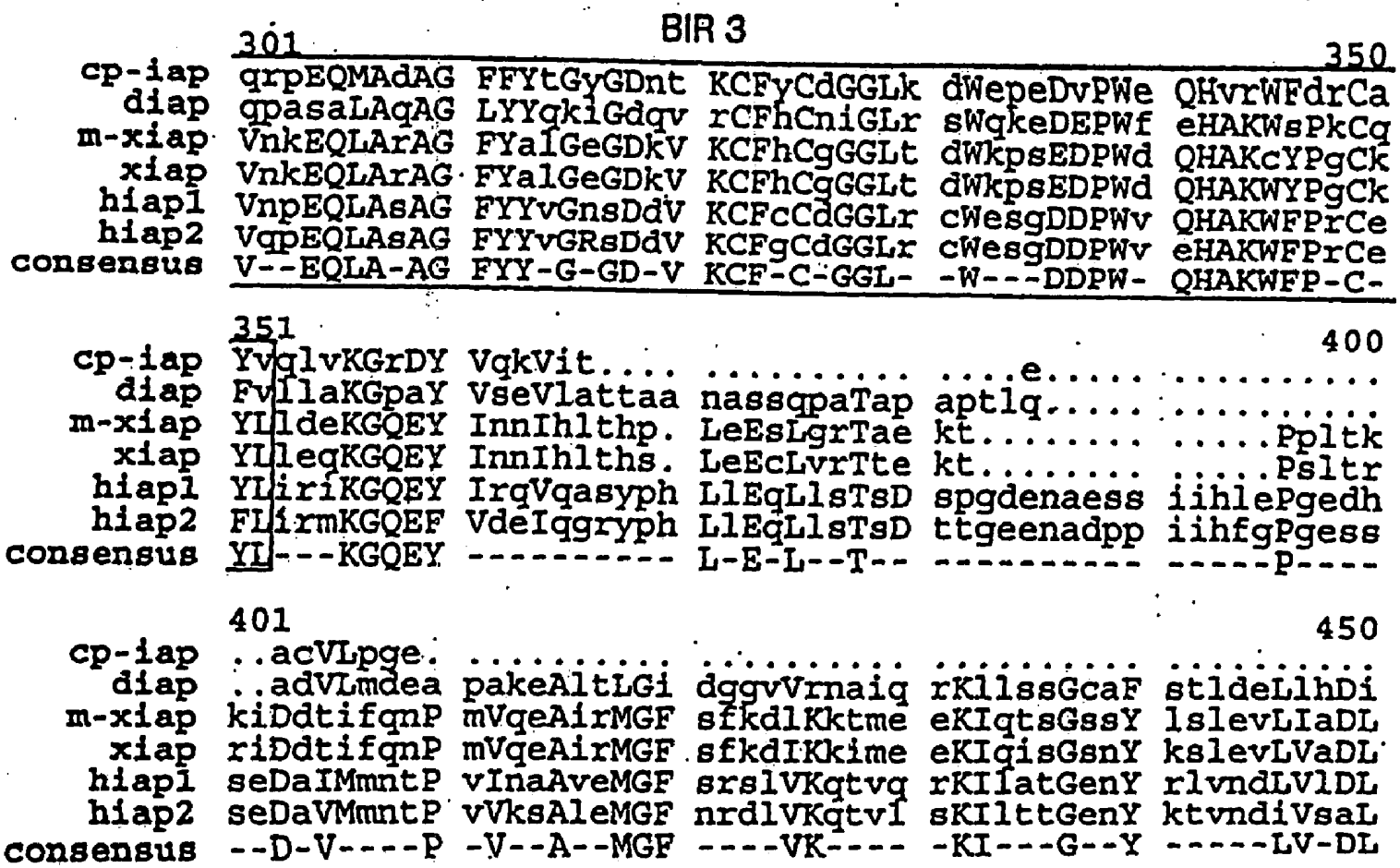
BIR 3
301
350
cp-iap qrpEQMAdAG FFYtGyGDnt KCFyCdGGLk dWepeDvPWe QHvrWFdrCa
diap qpasaLAqAG LYYqkiGdqv rCFhCniGLr sWqkeDEPWf eHAKWsPkCq
m-xiap VnkEQLArAG FYalGeGDkV KCFhCgGGLt dWkpsEDPWd QHAKcYPgCk
xiap VnkEQLArAG FYalGeGDkV KCFhCqGGLt dWkpsEDPWd QHAKWYPgCk
hiap1 VnpEQLAsAG FYYvGnsDdV KCFcCdGGLr cWesgDDPWv QHAKWFPrCe
hiap2 VqpEQLAsAG FYYvGRsDdV KCFgCdGGLr cWesgDDPWv eHAKWFPrCe
consensus V--EQLA-AG FYY-G-GD-V KCF-C-GGL- -W---DDPW- QHAKWFP-C-
351
400
cp-iap YvqlvKGrDY VqkVit.... ....e..... ....
diap FvllaKGpaY VseVlattaa nassqpaTap aptlq..... ....
m-xiap YLldeKGQEY InnIhlthp. LeEsLgrTae kt..... .....Ppltk
xiap YLleqKGQEY InnIhlths. LeEcLvrTte kt..... .....Psltr
hiap1 YLiriKGQEY IrqVqasyph LlEqLlsTsD spgdenaess iihlePgedh
hiap2 FLirmKGQEF VdeIqgryph LlEqLlsTsD ttgeenadpp iihfgPgess
consensus YL---KGQEY ---------- L-E-L--T-- ---------- -----P----
401
450
cp-iap ..acVLpge. ....
diap ..adVLmdea pakeAltLGi dggvVrnaiq rKllssGcaF stldeLlhDi
m-xiap kiDdtifqnP mVqeAirMGF sfkdlKktme eKIqtsGssY lslevLIaDL
xiap riDdtifqnP mVqeAirMGF sfkdIKkime eKIqisGsnY kslevLVaDL
hiap1 seDaIMmntP vInaAveMGF srslVKqtvq rKIlatGenY rlvndLVlDL
hiap2 seDaVMmntP vVksAleMGF nrdlVKqtvI sKIlttGenY ktvndiVsaL
consensus --D-V----P -V--A--MGF ----VK---- -KI---G--Y -----LV-DL

Fig. 8C

```
                451                                                500
cp-iap          ..........  ..........  ..........  ..........  ..........
diap            fddagagaal  Evreppe...  ..........  ..........  ..........
m-xiap          vsAqkDnteD  E.......... ..........  ..........  ..........
xiap            vnAqkDsmqD  E.......... ..........  ..........  ..........
hiap1           lnAedEireE  Ererateeke  sndlliirkn  rmalfqhltc  vipiidslit
hiap2           lnAedEkreE  Ekekqaeema  sddlslirkn  rmalfqglte  vlpildnllk
consensus       --A-------  E---------  ----------  ----------  ----------

                501                                                550
cp-iap          ..........  ..........  ..nttvgtaa  pvsepipe..  ..........
diap            ..........  ...psapfie  pcqattskaa  svpipvadsi  pakpqaaeav
m-xiap          ..........  .....ssQtsL  Q.........  ..........  ..........
xiap            ..........  .....ssQtsL  Q.........  ..........  ..........
hiap1           agiineqehd  vikqktQtsL  Qarelidtil  vkgmiaatvf  rnslqeaeav
hiap2           anvinkqehd  iikqktQipL  Qarelidtiw  vkgmaaanif  knclkeidst
consensus       ----------  ------Q--L  Q---------  ----------  ----------
```

Fig. 8D

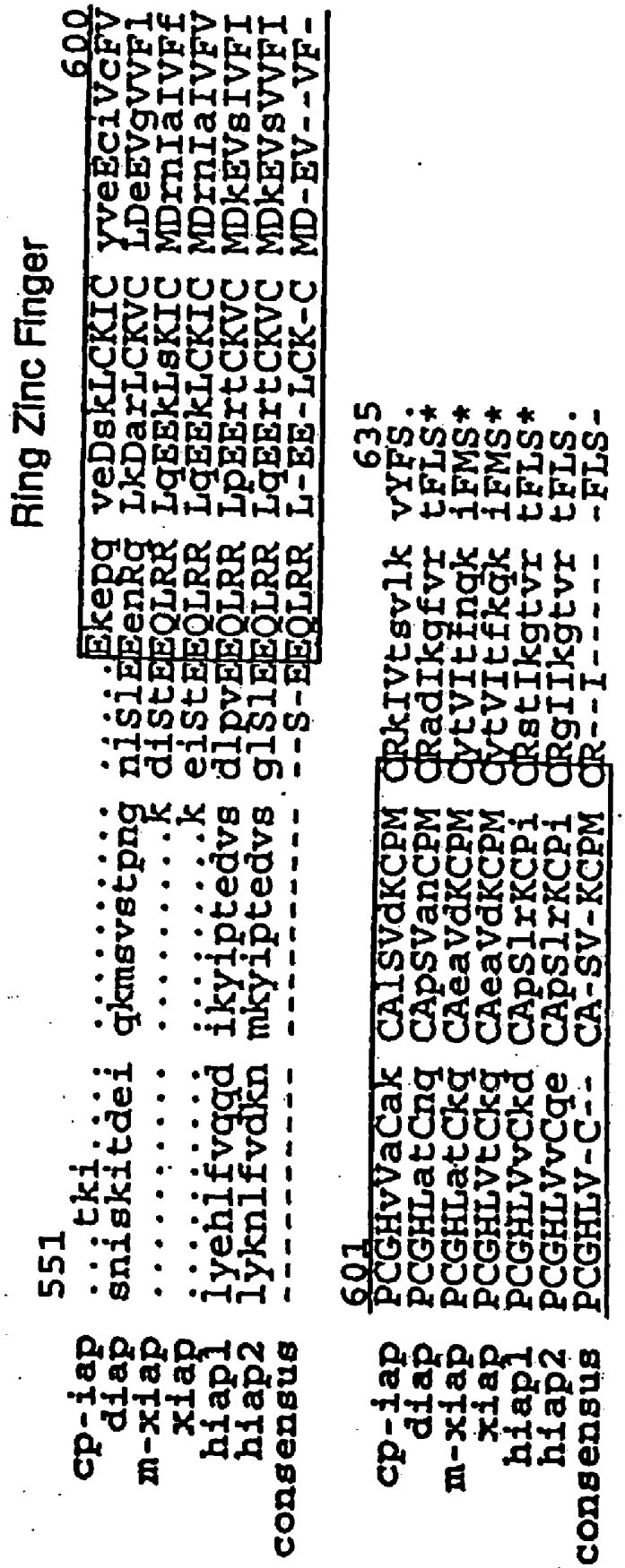
Ring Zinc Finger
551
600
cp-iap ...tki..... .....Ekepq veDskLCKIC yveEc1VcFV
diap snisKitdei qkmsvstpng nisiEEenRq LkDarLCKVC LDeEVgVVF1
m-xiap ....k diSteEQLRR LqEEkLsKIC MDmn1aIVFf
xiap ....k eiSteEQLRR LqEEkLCKIC MDmn1aIVFV
hiap1 iyehlfvgqd ikyiptedvs dlpvEEQLRR LpEErtCKVC MDkEVsIVFI
hiap2 lyknlfvdkn mkyiptedvs glS1EEQLRR LqEErtCKVC MDkEVsVVFI
consensus ---------- --S-EEQLRR L-EE-LCK-C MD-EV--VF-
601
635
cp-iap PCGHvVaCak CA1SVdKCPM CRkIVtsvlk vYFS.
diap PCGHLatCnq CApSVanCPM CRadIkqfvr tFLS*
m-xiap PCGHLatCkq CAeaVdKCPM CytVItfnqk iFMS*
xiap PCGHLVtCkq CAeaVdKCPM CytVItfkqk iFMS*
hiap1 PCGHLVvCkd CApS1rKCPi CRstIkgtvr tFLS*
hiap2 PCGHLVvCqe CApS1rKCPi CRgIIkgtvr tFLS.
consensus PCGHLV-C-- CA-SV-KCPM CR--I----- -FLS-

Fig. 8E

Alignment of RZF (Ring Zinc Finger) Domains

Baculovirus
- Cp_iap — Cydia pomonella
- Op_iap — Orgyia pseudotsugata

Human
- xiap — IAP on X chromosome
- hiap1, hiap2 — two different human IAP genes

Mouse
- m-xiap — mouse homologue of human xiap gene

Insect
- diap — Drosophilia IAP gene, not clearly a homologue of xiap or hiap

note on consensus: The consensus line represents amino acids or very similar amino acids which are present in 6 of the 7 RZF sequences at each position. Capitalized residues are those that are in the consensus sequence.

```
                                1                                          46
SEQ ID NO:32 hiap2      EqlrrlqEer tCKVCMdkev sVvFiPCGHl vvCqeCApel rkCPiC
SEQ ID NO:33 hiap1      EqlrrlpEer tCKVCMdkev sIvFiPCGHl w CkdCApsl rkCPiC
SEQ ID NO:34 m-xiap     EqlrrlqEek lsKICMdrni aIvFfPCGHl atCkqCAeav dkCPmC
SEQ ID NO:35 xiap       EqlrrlqEek lCKICMdrni aIvFvPCGHl vtCkqCAeav dkCPmC
SEQ ID NO:36 diap       EenrqlkDar lCKVCLdeev gVvFlPCGHl atCnqCApev anCPmC
SEQ ID NO:37 Cp_iap     EkepgveDsk lCKICyveec lVcFvPCGHv vaCakCAlsv dkCPmC
SEQ ID NO:38 Op_iap     aveaevaDdr lCKICLgack tVcFvPCGHv vaCgkCAagv ttCPvC
SEQ ID NO:1  consensus  E------E-- -CKICM---- -V-F-PCGH- --C--CA--- --CP-C
```

Fig. 9

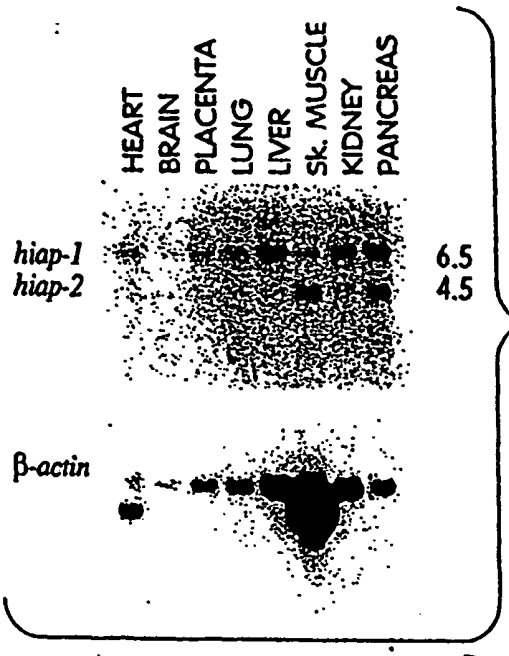

Fig. 10A

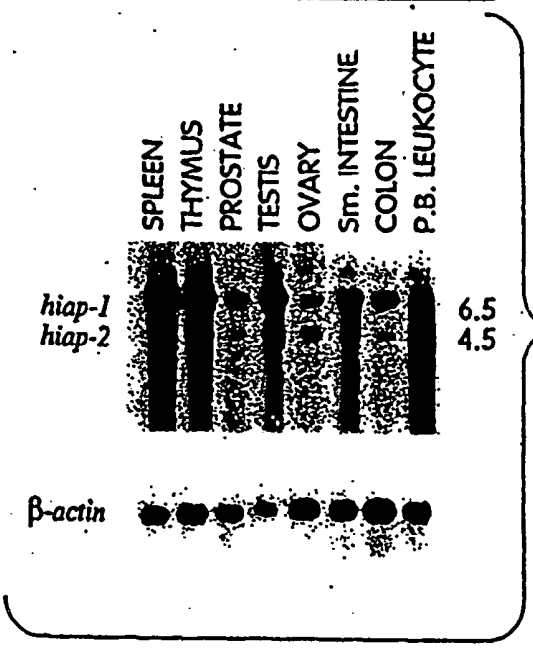

Fig. 10B

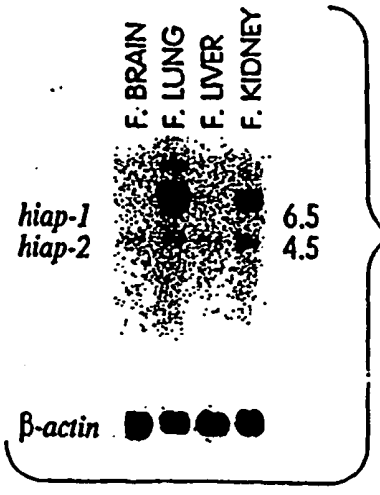

Fig. 10C

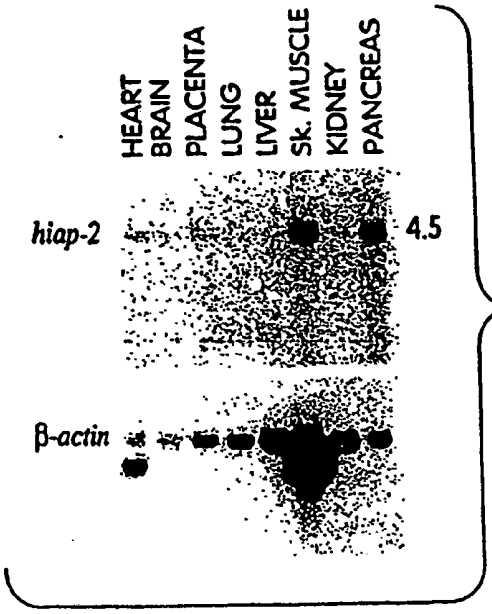

Fig. 11A

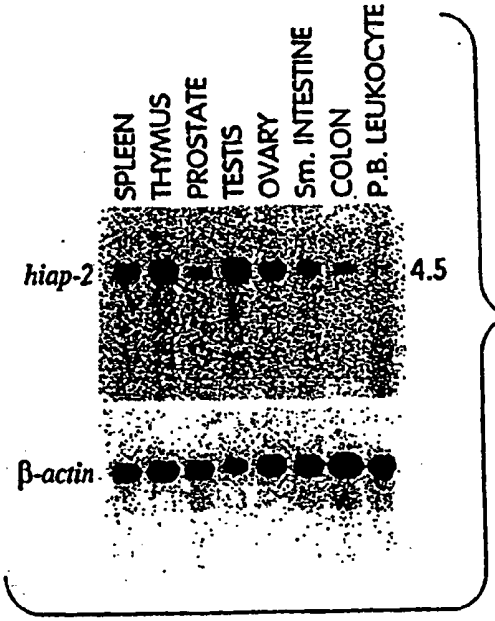

Fig. 11B

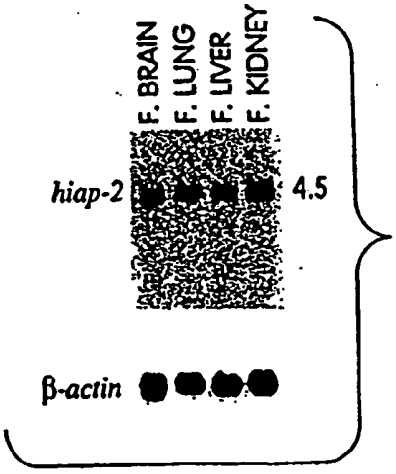

Fig. 11C

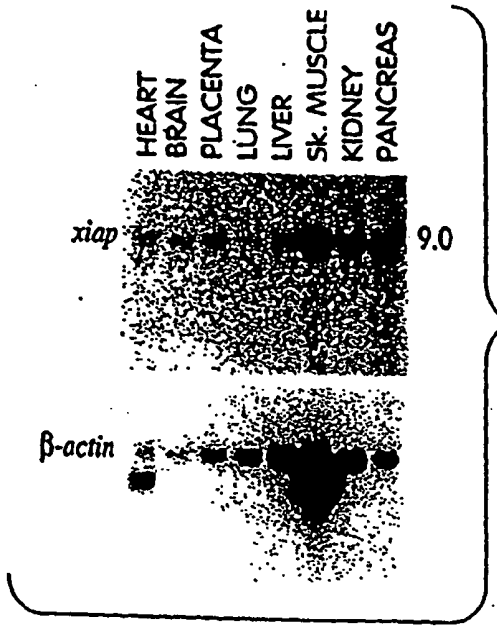

Fig. 12A

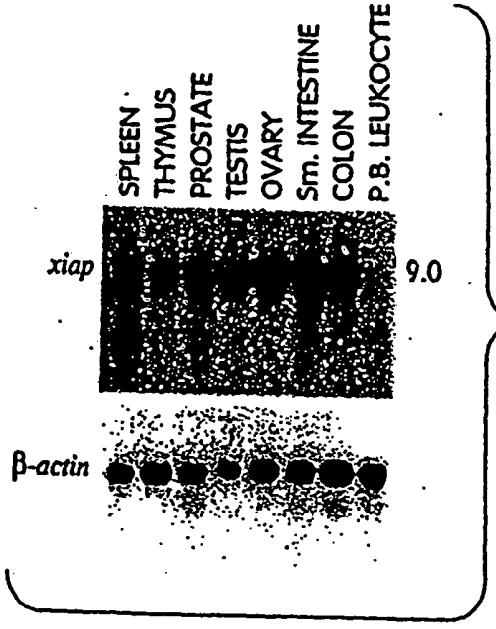

Fig. 12B

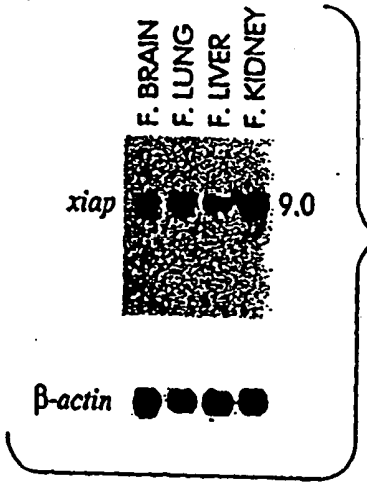

Fig. 12C

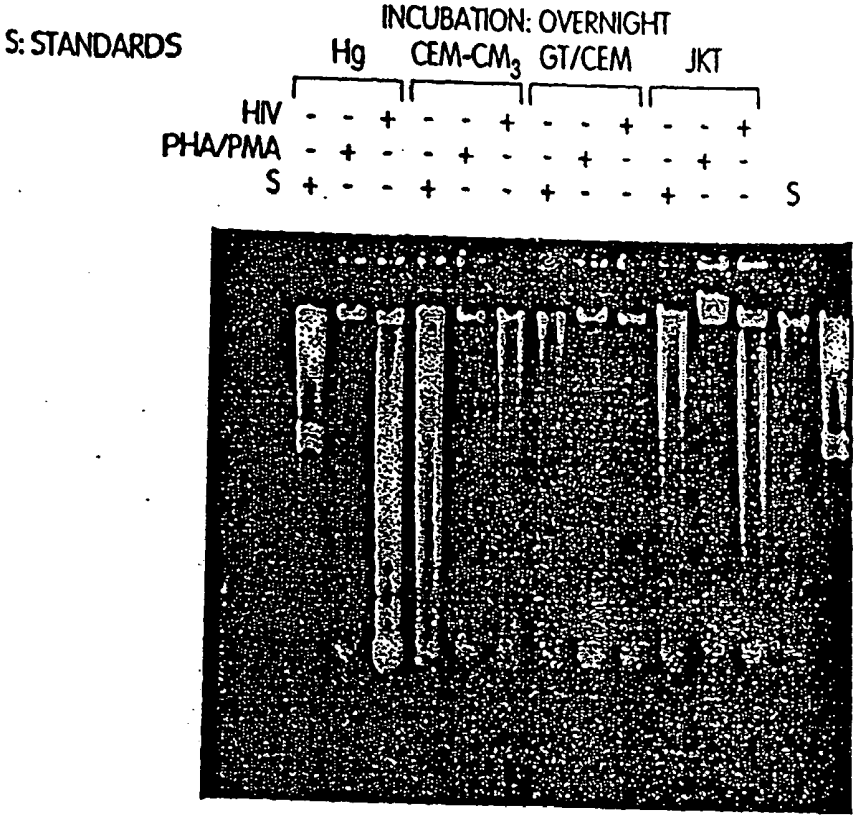

Fig. 13A

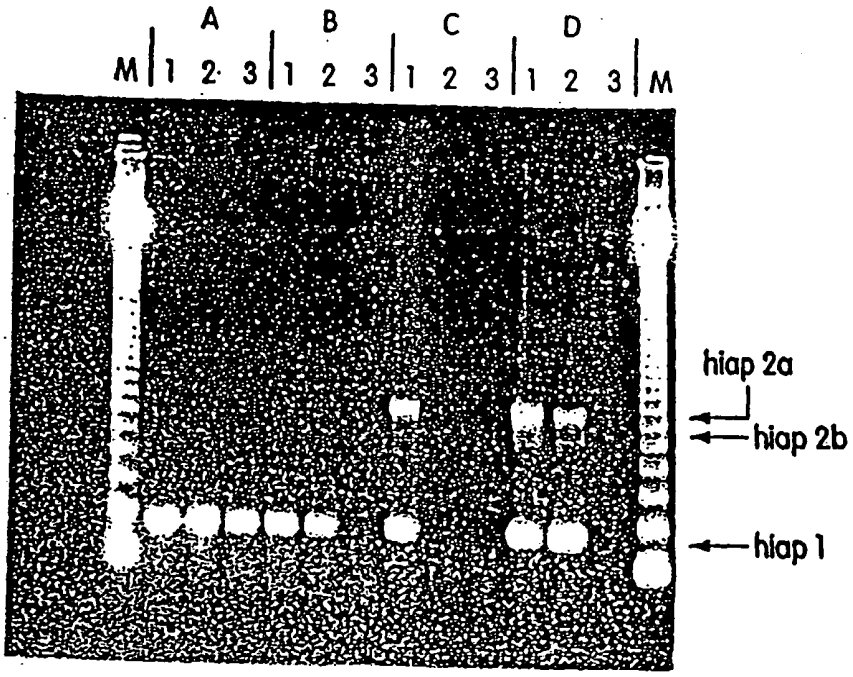

Fig. 13B

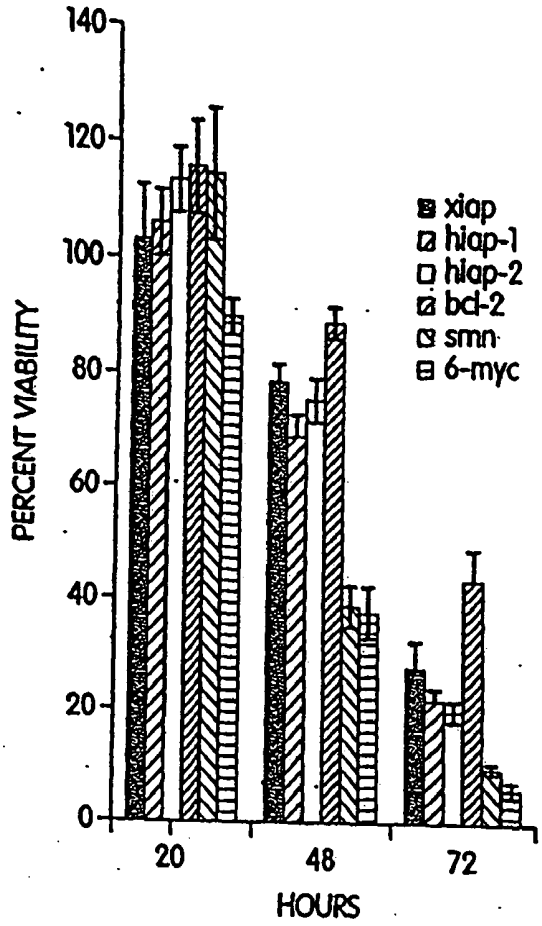

Fig. 14A

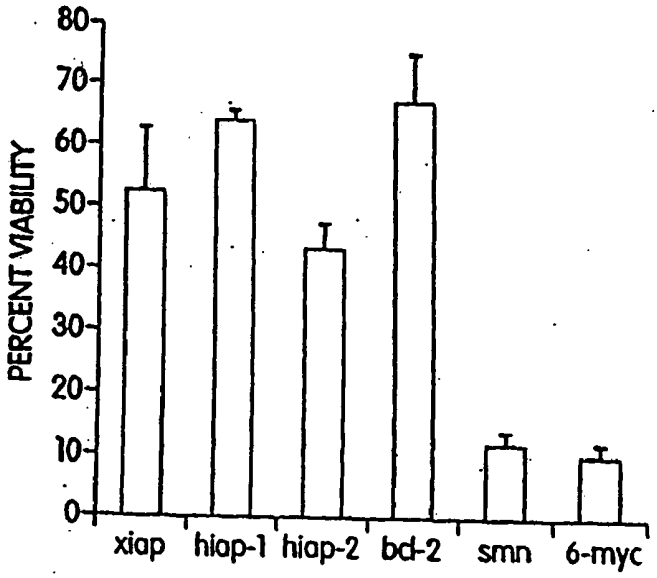

Fig. 14B

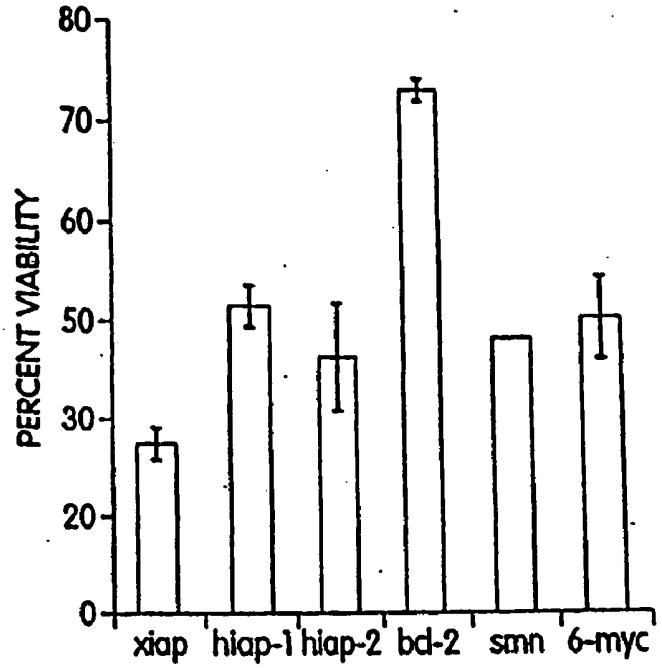

Fig. 14C

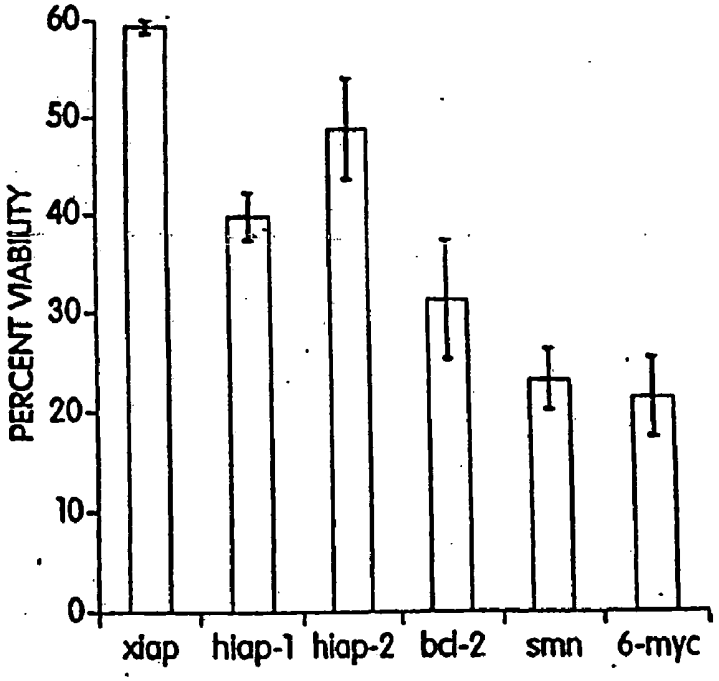

Fig. 14D

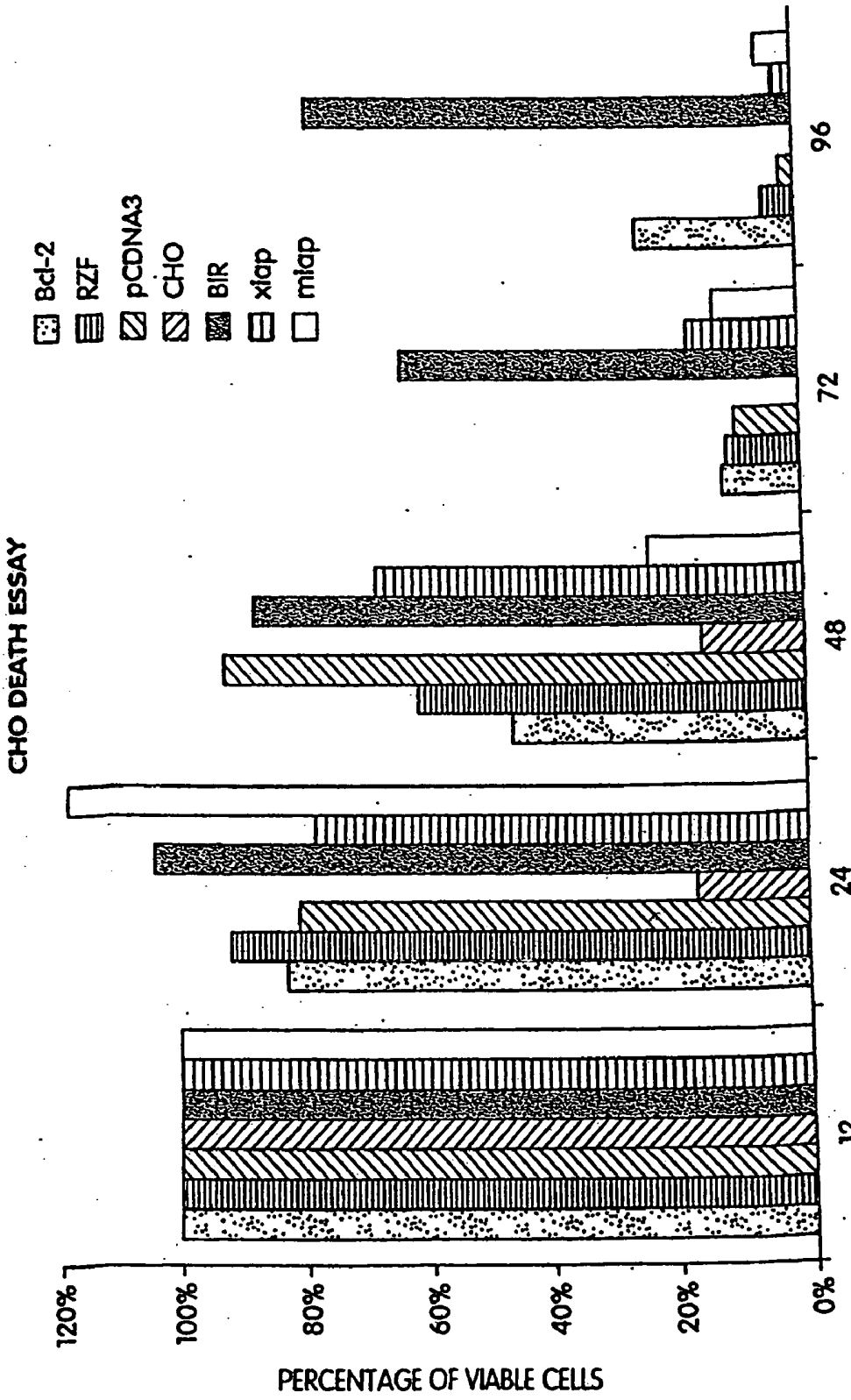
CHO DEATH ESSAY
Bcl-2
RZF
pCDNA3
CHO
BIR
xiap
miap
96
72
48
24
12
HOURS
120%
100%
80%
60%
40%
20%
0%
PERCENTAGE OF VIABLE CELLS

Fig. 15A

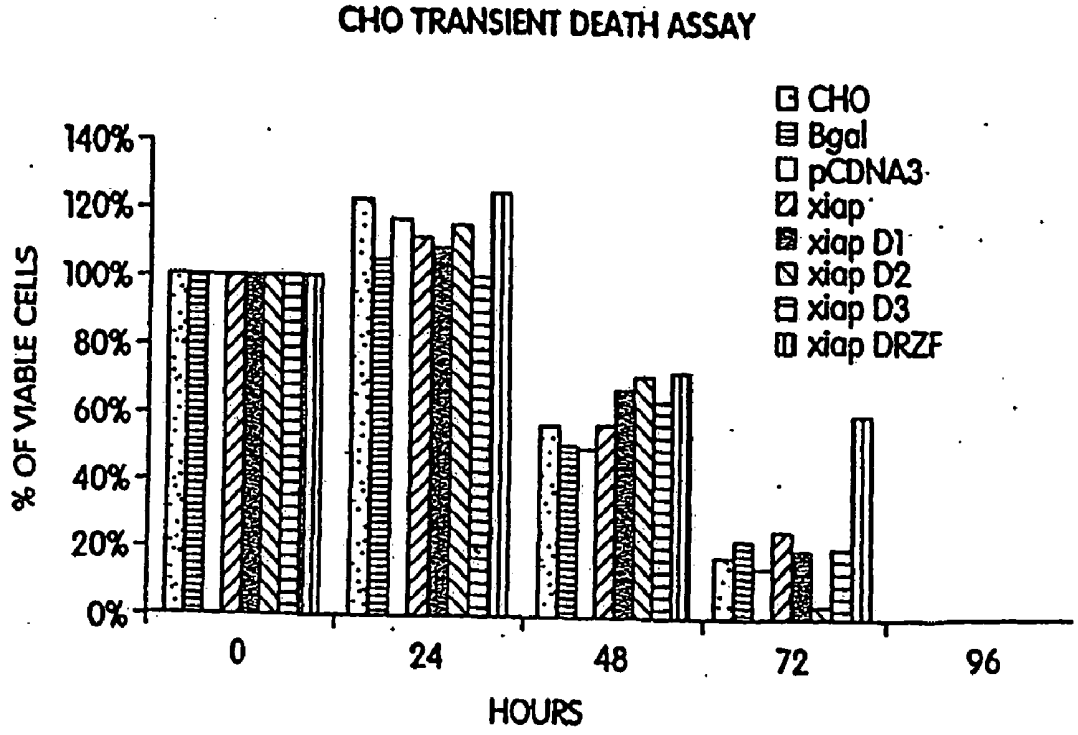
CHO TRANSIENT DEATH ASSAY
CHO
Bgal
pCDNA3
xiap
xiap D1
xiap D2
xiap D3
xiap DRZF
140%
120%
100%
80%
60%
40%
20%
0%
% OF VIABLE CELLS
0
24
48
72
96
HOURS

Fig. 15B

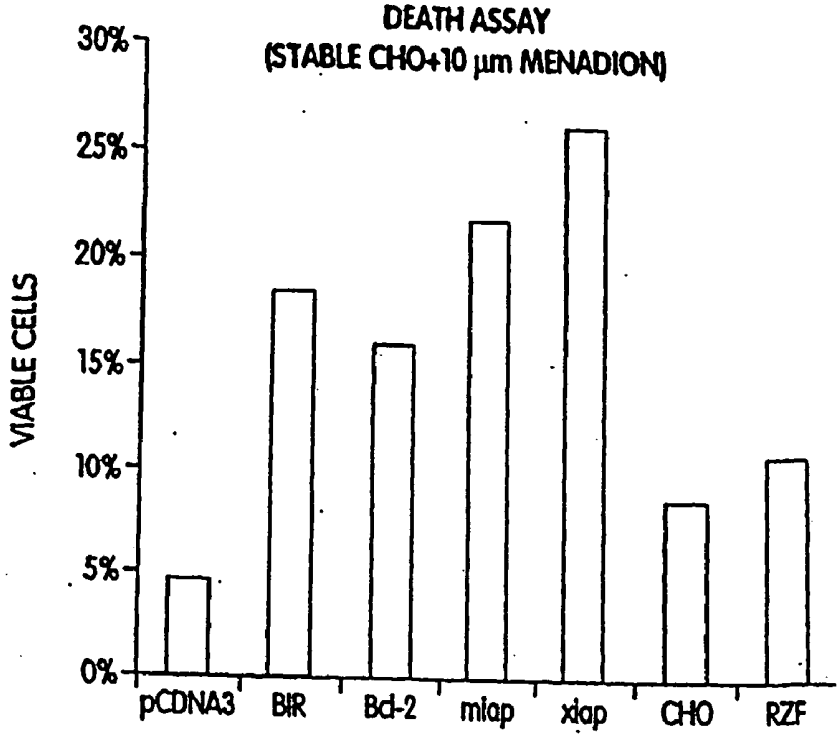

Fig. 16A

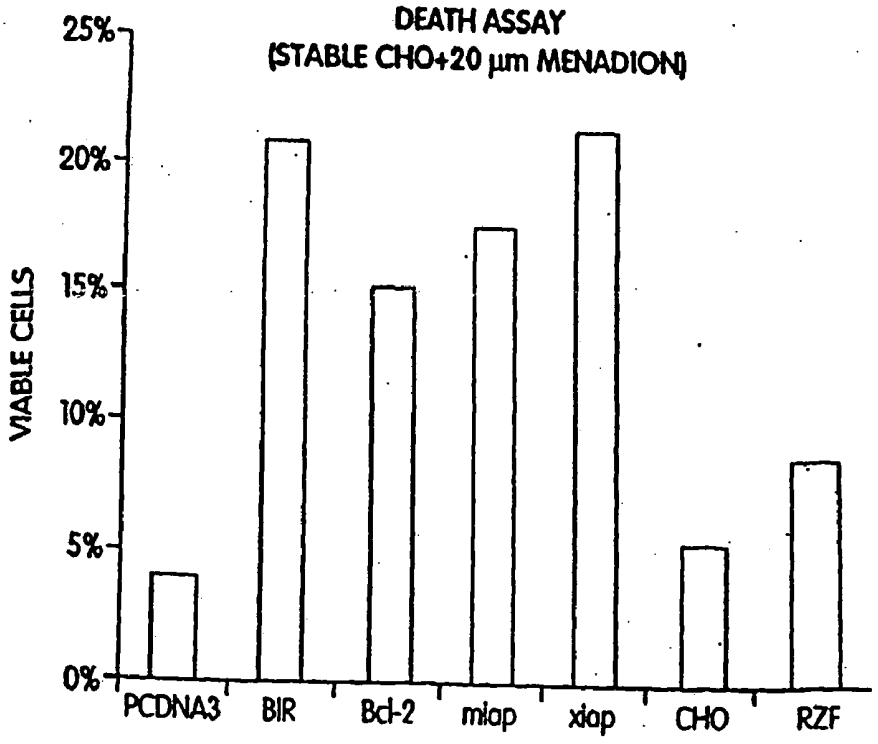

Fig. 16B

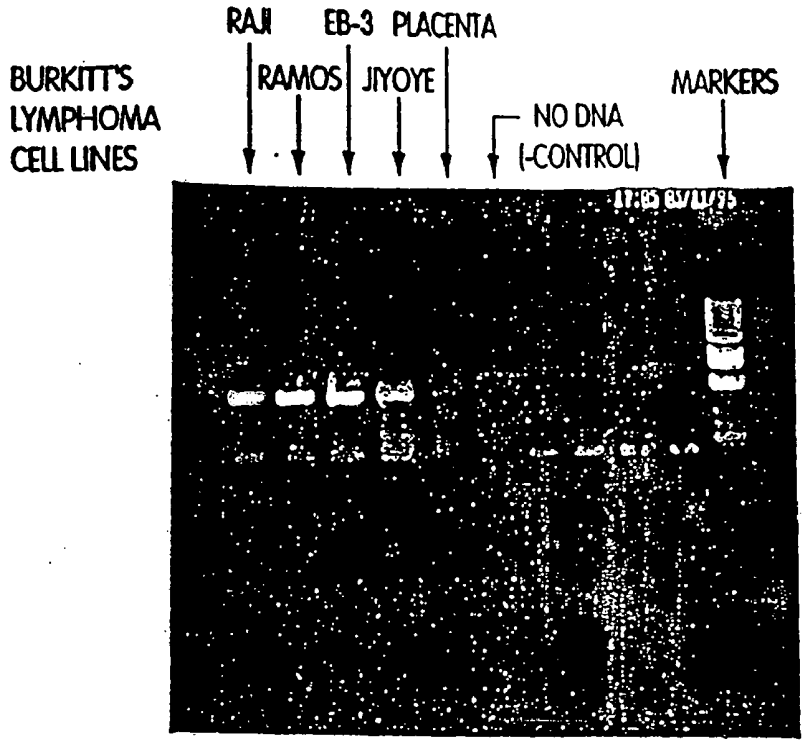
BURKITT'S
LYMPHOMA
CELL LINES
RAJI
RAMOS
EB-3
JIYOYE
PLACENTA
NO DNA
(-CONTROL)
MARKERS

Fig. 17

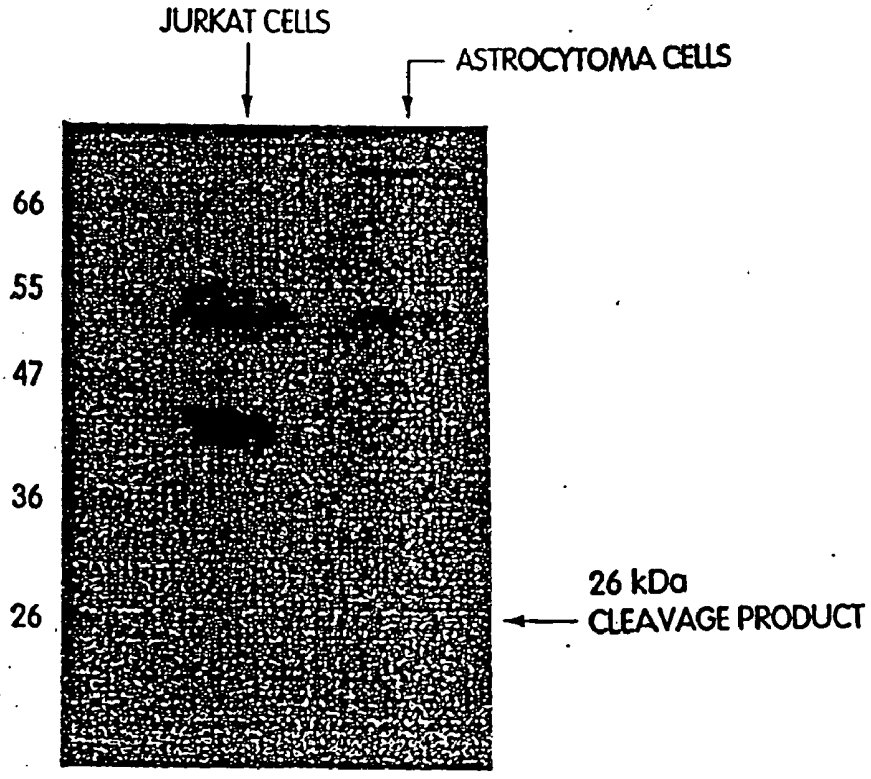
JURKAT CELLS
ASTROCYTOMA CELLS
66
55
47
36
26
26 kDa
CLEAVAGE PRODUCT

Fig. 18

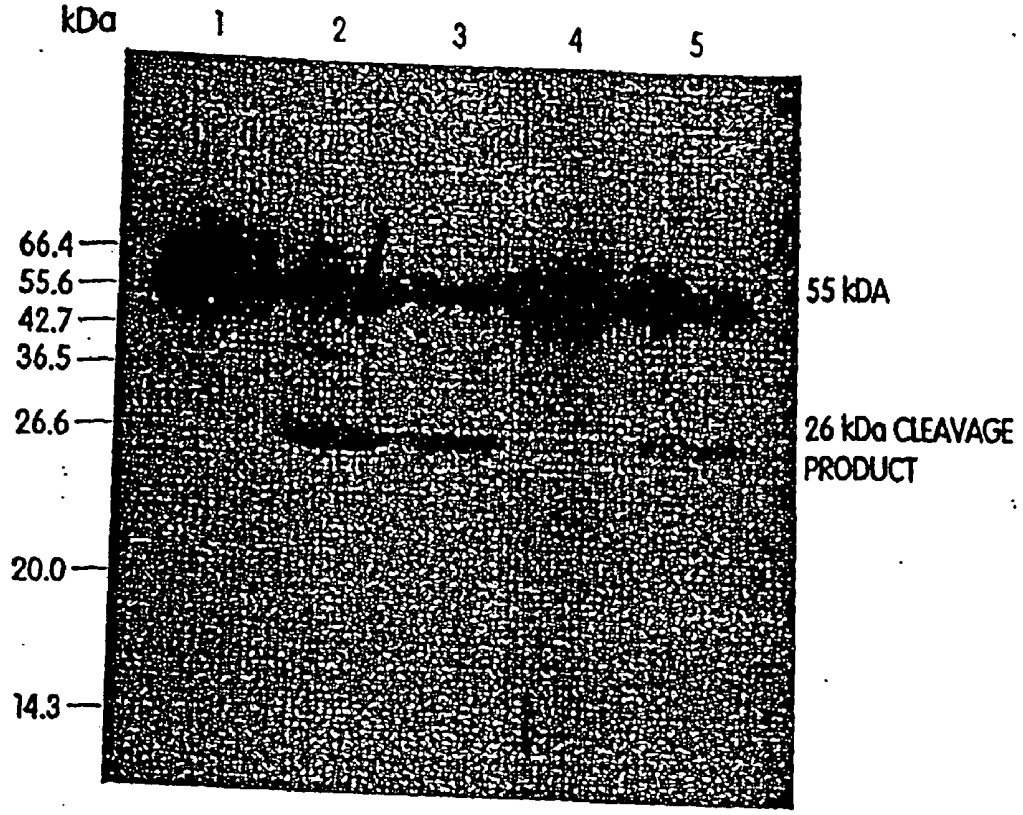
kDa
1
2
3
4
5
66.4
55.6
42.7
36.5
26.6
20.0
14.3
55 kDA
26 kDa CLEAVAGE PRODUCT

Fig. 19

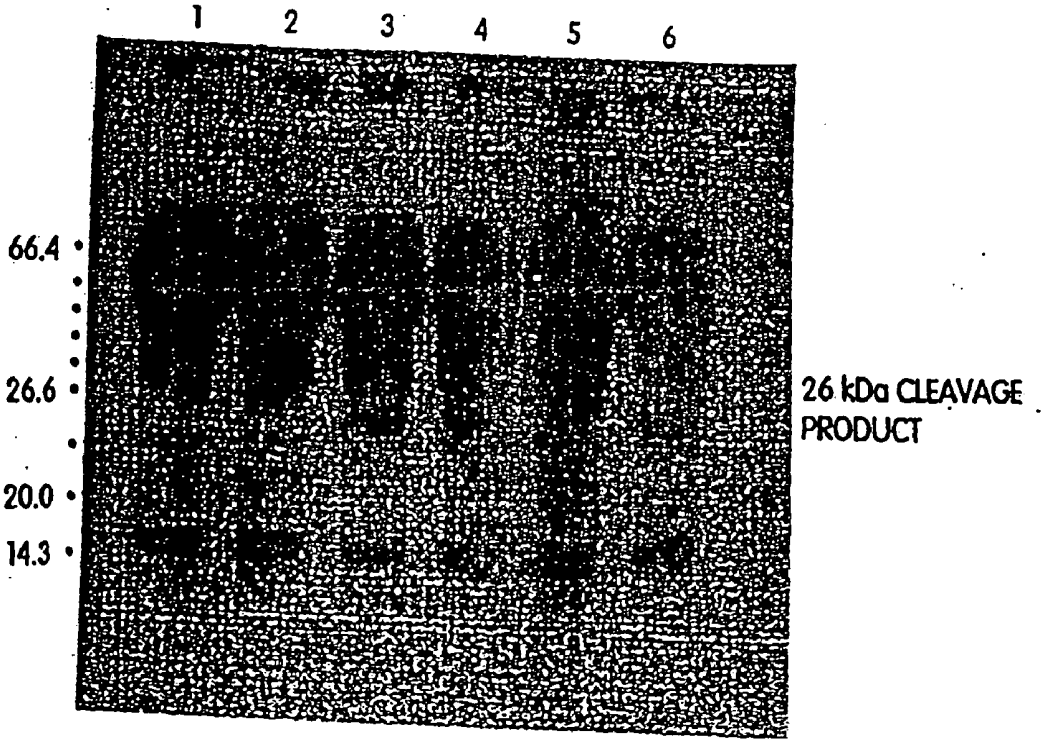
1
2
3
4
5
6
66.4
26.6
20.0
14.3
26 kDa CLEAVAGE PRODUCT

Fig. 20

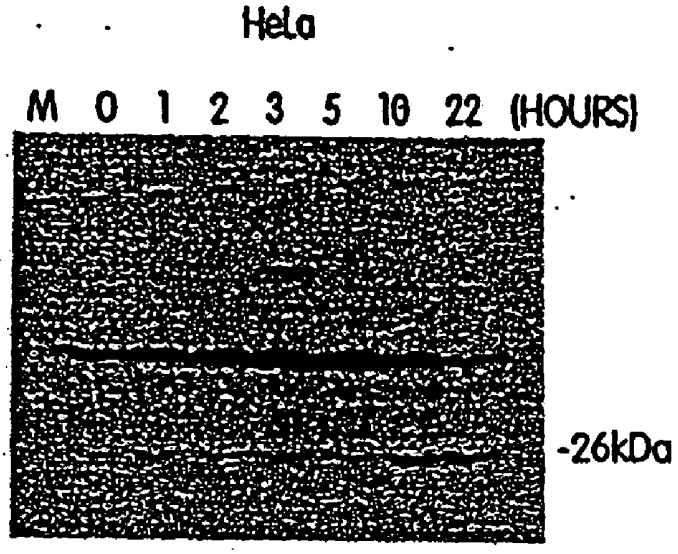

Fig. 21A

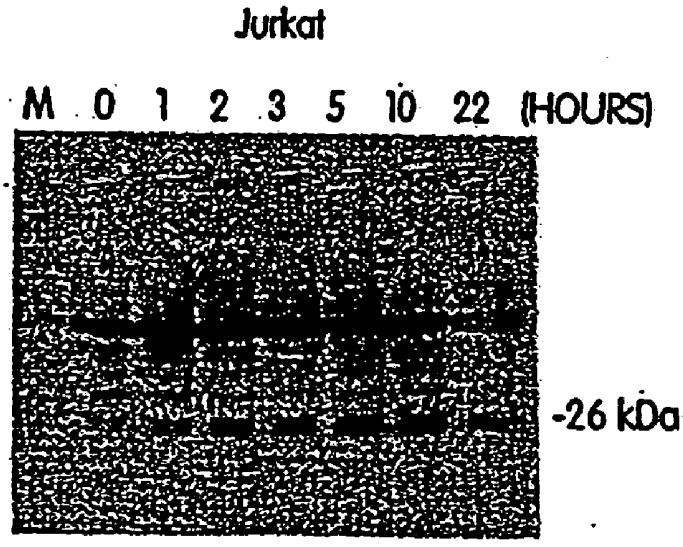

Fig. 21B

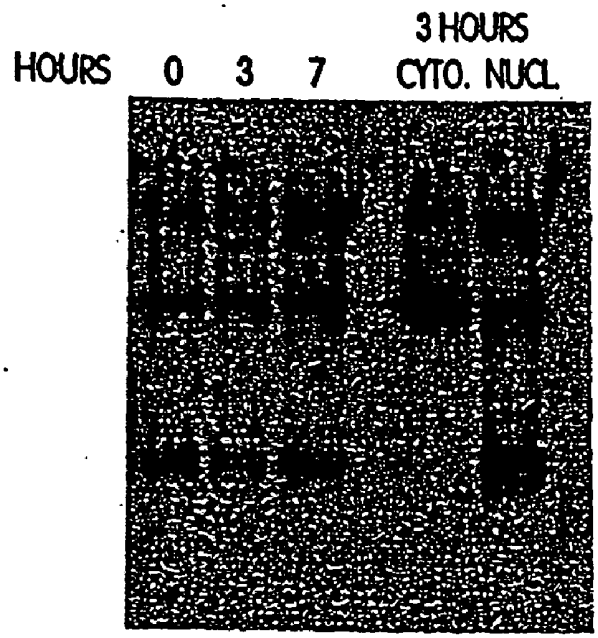

Fig. 22A

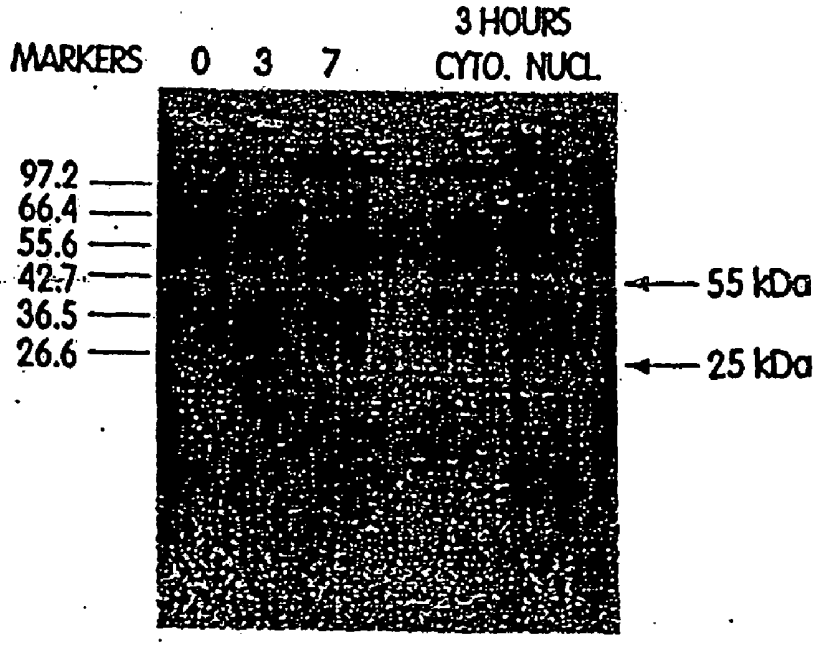

Fig. 22B

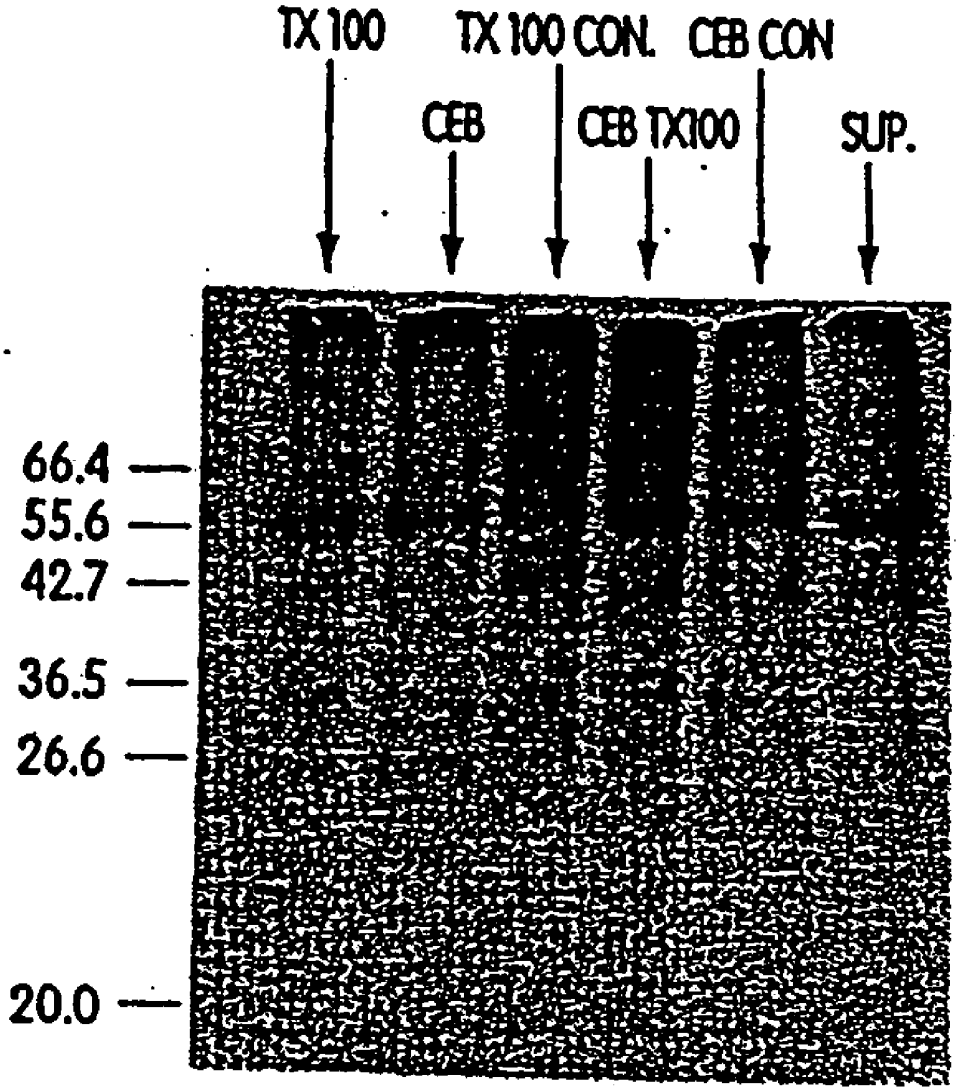
TX 100
TX 100 CON.
CEB CON
CEB
CEB TX100
SUP.
66.4
55.6
42.7
36.5
26.6
20.0

Fig. 23

MAMMALIAN IAP GENE FAMILY, PRIMERS, PROBES AND DETECTION METHODS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. Ser. No. 09/011,356, filed Feb. 4, 1998 (now pending), which is a U.S. National Phase application of PCT/IB/96/01022, filed Aug. 5, 1996, and published in English under PCT article 21(2), which claims benefit from U.S. Ser. No. 08/576,956, filed Dec. 22, 1995 (now U.S. Pat. No. 6,156,535), which is a continuation-in-part of U.S. Ser. No. 08/511,485, filed Aug. 4, 1995 (now U.S. Pat. No. 5,919,912), all of which are hereby incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] The invention relates to apoptosis.

[0003] There are two general ways by which cells die. The most easily recognized way is by necrosis, which is usually caused by an injury that is severe enough to disrupt cellular homeostasis. Typically, the cell's osmotic pressure is disturbed and, consequently, the cell swells and then ruptures. When the cellular contents are spilled into the surrounding tissue space, an inflammatory response often ensues.

[0004] The second general way by which cells die is referred to as apoptosis, or programmed cell death. Apoptosis often occurs so rapidly that it is difficult to detect. This may help to explain why the involvement of apoptosis in a wide spectrum of biological processes has only recently been recognized.

[0005] The apoptosis pathway has been highly conserved throughout evolution, and plays a critical role in embryonic development, viral pathogenesis, cancer, autoimmune disorders, and neurodegenerative disease. For example, inappropriate apoptosis may cause or contribute to AIDS, Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), retinitis pigmentosa and other diseases of the retina, myelodysplastic syndrome (e.g. aplastic anemia), toxin-induced liver disease, including alcoholism, and ischemic injury (e.g. myocardial infarction, stroke, and reperfusion injury). Conversely, the failure of an apoptotic response has been implicated in the development of cancer, particularly follicular lymphoma, p53-mediated carcinomas, and hormone-dependent tumors, in autoimmune disorders, such as lupus erythematosis and multiple sclerosis, and in viral infections, including those associated with herpes virus, poxvirus, and adenovirus.

[0006] In patients infected with HIV-1, mature $CD4^+$ T lymphocytes respond to stimulation from mitogens or superantigens by undergoing apoptosis. However, the great majority of these cells are not infected with the virus. Thus, inappropriate antigen-induced apoptosis could be responsible for the destruction of this vital part of the immune system in the early stages of HIV infection.

[0007] Baculoviruses encode proteins that are termed inhibitors of apoptosis proteins (IAPs) because they inhibit the apoptosis that would otherwise occur when insect cells are infected by the virus. These proteins are thought to work in a manner that is independent of other viral proteins. The baculovirus IAP genes include sequences encoding a ring zinc finger-like motif (RZF), which is presumed to be directly involved in DNA binding, and two N-terminal domains that consist of a 70 amino acid repeat motif termed a BIR domain (Baculovirus IAP Repeat).

SUMMARY OF THE INVENTION

[0008] In general, the invention features a substantially pure DNA molecule, such as a genomic, cDNA, or synthetic DNA molecule, that encodes a mammalian IAP polypeptide. This DNA may be incorporated into a vector, into a cell, which may be a mammalian, yeast, or bacterial cell, or into a transgenic animal or embryo thereof. In preferred embodiments, the DNA molecule is a murine gene (e.g., m-xiap, m-hiap-1, or m-hiap-2) or a human gene (e.g., xiap, hiap-1, or hiap-2). In most preferred embodiments the IAP gene is a human IAP gene. In other various preferred embodiments, the cell is a transformed cell. In related aspects, the invention features a transgenic animal containing a transgene that encodes an IAP polypeptide that is expressed in or delivered to tissue normally susceptible to apoptosis, i.e., to a tissue that may be harmed by either the induction or repression of apoptosis. In yet another aspect the invention features DNA encoding fragments of IAP polypeptides including the BIR domains and the RZF domains provided herein.

[0009] In specific embodiments, the invention features DNA sequences substantially identical to the DNA sequences shown in FIGS. **1-6**, or fragments thereof. In another aspect, the invention also features RNA which is encoded by the DNA described herein. Preferably, the RNA is mRNA. In another embodiment the RNA is antisense RNA.

[0010] In another aspect, the invention features a substantially pure polypeptide having a sequence substantially identical to one of the IAP amino acid sequences shown in FIGS. **1-6**.

[0011] In a second aspect, the invention features a substantially pure DNA which includes a promoter capable of expressing the IAP gene in a cell susceptible to apoptosis. In preferred embodiments, the IAP gene is xiap, hiap-1, or hiap-2. Most preferably, the genes are human or mouse genes. The gene encoding HIAP-2 may be the full-length gene, as shown in FIG. **3**, or a truncated variant, such as a variant having a deletion of the sequence boxed in FIG. **3**.

[0012] In preferred embodiments, the promoter is the promoter native to an IAP gene. Additionally, transcriptional and translational regulatory regions are, preferably, those native to an IAP gene. In another aspect, the invention provides transgenic cell lines and transgenic animals. The transgenic cells of the invention are preferably cells that are altered in their apoptotic response. In preferred embodiments, the transgenic cell is a fibroblast, neuronal cell, a lymphocyte cell, a glial cell, an embryonic stem cell, or an insect cell. Most preferably, the neuron is a motor neuron and the lymphocyte is a $CD4^+$ T cell.

[0013] In another aspect, the invention features a method of inhibiting apoptosis that involves producing a transgenic cell having a transgene encoding an IAP polypeptide. The transgene is integrated into the genome of the cell in a way that allows for expression. Furthermore, the level of expression in the cell is sufficient to inhibit apoptosis.

[0014] In a related aspect, the invention features a transgenic animal, preferably a mammal, more preferably a

rodent, and most preferably a mouse, having either increased copies of at least one IAP gene inserted into the genome (mutant or wild-type), or a knockout of at least one IAP gene in the genome. The transgenic animals will express either an increased or a decreased amount of IAP polypeptide, depending on the construct used and the nature of the genomic alteration. For example, utilizing a nucleic acid molecule that encodes all or part of an IAP to engineer a knockout mutation in an IAP gene would generate an animal with decreased expression of either all or part of the corresponding IAP polypeptide. In contrast, inserting exogenous copies of all or part of an IAP gene into the genome, preferably under the control of active regulatory and promoter elements, would lead to increased expression or the corresponding IAP polypeptide.

[0015] In another aspect, the invention features a method of detecting an IAP gene in a cell by contacting the IAP gene, or a portion thereof (which is greater than 9 nucleotides, and preferably greater than 18 nucleotides in length), with a preparation of genomic DNA from the cell. The IAP gene and the genomic DNA are brought into contact under conditions that allow for hybridization (and therefore, detection) of DNA sequences in the cell that are at least 50% identical to the DNA encoding HIAP-1, HIAP-2, or XIAP polypeptides.

[0016] In another aspect, the invention features a method of producing an IAP polypeptide. This method involves providing a cell with DNA encoding all or part of an IAP polypeptide (which is positioned for expression in the cell), culturing the cell under conditions that allow for expression of the DNA, and isolating the IAP polypeptide. In preferred embodiments, the IAP polypeptide is expressed by DNA that is under the control of a constitutive or inducible promotor. As described herein, the promotor may be a heterologous promotor.

[0017] In another aspect, the invention features substantially pure mammalian IAP polypeptide. Preferably, the polypeptide includes an amino acid sequence that is substantially identical to all, or to a fragment of, the amino acid sequence shown in any one of FIGS. **1-4**. Most preferably, the polypeptide is the XIAP, HIAP-1, HIAP-2, M-XIAP, M-HIAP-1, or M-HIAP-2 polypeptide. Fragments including one or more BIR domains (to the exclusion of the RZF), the RZF domain (to the exclusion of the BIR domains), and a RZF domain with at least one BIR domain, as provided herein, are also a part of the invention.

[0018] In another aspect, the invention features a recombinant mammalian polypeptide that is capable of modulating apoptosis. The polypeptide may include at least a RZF domain and a BIR domain as defined herein. In preferred embodiments, the invention features (a) a substantially pure polypeptide, and (b) an oligonucleotide encoding the polypeptide. In instances were the polypeptide includes a RZF domain, the RZF domain will have a sequence conforming to: Glu-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa2-Xaa1-Xaa1-Xaa1-Cys-Lys-Xaa3-Cys-Met-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa3-Xaa1-Phe-Xaa1-Pro-Cys-Gly-His-Xaa1-Xaa1-Xaa1-Cys-Xaa1-Xaa1-Cys-Ala-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Cys-Pro-Xaa1-Cys, where Xaa1 is any amino acid, Xaa2 is Glu or Asp, Xaa3 is Val or Ile (SEQ ID NO: 1); and where the polypeptide includes at least one BIR domain, the BIR domain will have a sequence conforming to: Xaa1-Xaa1-Xaa1-Arg-Leu-Xaa1-Thr-Phe-Xaa1-Xaa1-Trp-Pro-Xaa2-Xaa1-Xaa1-Xaa2-Xaa2-Xaa1-Xaa1-Xaa1-Xaa1-Leu-Ala-Xaa1-Ala-Gly-Phe-Tyr-Tyr-Xaa1-Gly-Xaa1-Xaa1-Asp-Xaa1-Val-Xaa1-Cys-Phe-Xaa1-Cys-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Trp-Xaa1-Xaa1-Xaa1-Asp-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-His-Xaa1-Xaa1-Xaa1-Xaa1-Pro-Xaa1-Cys-Xaa1-Phe-Val, where Xaa1 may be any amino acid and Xaa2 may be any amino acid or may be absent (SEQ ID NO: 2).

[0019] In various preferred embodiments the polypeptide has at least two or, more preferably at least three BIR domains, the RZF domain has one of the IAP sequences shown in FIG. **6**, and the BIR domains are comprised of BIR domains shown in FIG. **5**. In other preferred embodiments the BIR domains are at the amino terminal end of the protein relative to the RZF domain, which is at or near the carboxyl terminus of the polypeptide.

[0020] In another aspect, the invention features an IAP gene isolated according to the method involving: (a) providing a sample of DNA; (b) providing a pair of oligonucleotides having sequence homology to a conserved region of an IAP disease-resistance gene; (c) combining the pair of oligonucleotides with the cell DNA sample under conditions suitable for polymerase chain reaction-mediated DNA amplification; and (d) isolating the amplified IAP gene or fragment thereof.

[0021] In preferred embodiments, the amplification is carried out using a reverse-transcription polymerase chain reaction, for example, the RACE method. In another aspect, the invention features an IAP gene isolated according to the method involving: (a) providing a preparation of DNA; (b) providing a detectably labelled DNA sequence having homology to a conserved region of an IAP gene; (c) contacting the preparation of DNA with the detectably-labelled DNA sequence under hybridization conditions providing detection of genes having 50% or greater nucleotide sequence identity; and (d) identifying an IAP gene by its association with the detectable label.

[0022] In another aspect, the invention features an IAP gene isolated according to the method involving: (a) providing a cell sample; (b) introducing by transformation into the cell sample a candidate IAP gene; (c) expressing the candidate IAP gene within the cell sample; and (d) determining whether the cell sample exhibits an altered apoptotic response, whereby a response identifies an IAP gene.

[0023] In another aspect, the invention features a method of identifying an IAP gene in a cell, involving: (a) providing a preparation of cellular DNA (for example, from the human genome or a cDNA library (such as a cDNA library isolated from a cell type which undergoes apoptosis); (b) providing a detectably-labelled DNA sequence (for example, prepared by the methods of the invention) having homology to a conserved region of an IAP gene; (c) contacting the preparation of cellular DNA with the detectably-labelled DNA sequence under hybridization conditions providing detection of genes having 50% nucleotide or greater sequence identity; and (d) identifying an IAP gene by its association with the detectable label.

[0024] In another aspect, the invention features a method of isolating an IAP gene from a recombinant library, involving: (a) providing a recombinant library; (b) contacting the

library with a detectably-labelled gene fragment produced according to the PCR method of the invention under hybridization conditions providing detection of genes having 50% or greater nucleotide sequence identity; and (c) isolating an IAP gene by its association with the detectable label. In another aspect, the invention features a method of identifying an IAP gene involving: (a) providing a cell tissue sample; (b) introducing by transformation into the cell sample a candidate IAP gene; (c) expressing the candidate IAP gene within the cell sample; and (d) determining whether the cell sample exhibits inhibition of apoptosis, whereby a change in (i.e. modulation of) apoptosis identifies an IAP gene. Preferably, the cell sample is a cell type that may be assayed for apoptosis (e.g., T cells, B cells, neuronal cells, baculovirus-infected insect cells, glial cells, embryonic stem cells, and fibroblasts). The candidate IAP gene is obtained, for example, from a cDNA expression library, and the response assayed is the inhibition of apoptosis.

[0025] In another aspect, the invention features a method of inhibiting apoptosis in a mammal wherein the method includes: (a) providing DNA encoding at least one IAP polypeptide to a cell that is susceptible to apoptosis; wherein the DNA is integrated into the genome of the cell and is positioned for expression in the cell; and the IAP gene is under the control of regulatory sequences suitable for controlled expression of the gene(s); wherein the IAP transgene is expressed at a level sufficient to inhibit apoptosis relative to a cell lacking the IAP transgene. The DNA integrated into the genome may encode all or part of an IAP polypeptide. It may, for example, encode a ring zinc finger and one or more BIR domains. In contrast, it may encode either the ring zinc finger alone, or one or more BIR domains alone. Skilled artisans will appreciate that IAP polypeptides may also be administered directly to inhibit undesirable apoptosis.

[0026] In a related aspect, the invention features a method of inhibiting apoptosis by producing a cell that has integrated, into its genome, a transgene that includes the IAP gene, or a fragment thereof. The IAP gene may be placed under the control of a promoter providing constitutive expression of the IAP gene. Alternatively, the IAP transgene may be placed under the control of a promoter that allows expression of the gene to be regulated by environmental stimuli. For example, the IAP gene may be expressed using a tissue-specific or cell type-specific promoter, or by a promoter that is activated by the introduction of an external signal or agent, such as a chemical signal or agent. In preferred embodiments the cell is a lymphocyte, a neuronal cell, a glial cell, or a fibroblast. In other embodiments, the cell in an HIV-infected human, or in a mammal suffering from a neurodegenerative disease, an ischemic injury, a toxin-induced liver disease, or a myelodysplastic syndrome.

[0027] In a related aspect, the invention provides a method of inhibiting apoptosis in a mammal by providing an apoptosis-inhibiting amount of IAP polypeptide. The IAP polypeptide may be a full-length polypeptide, or it may be one of the fragments described herein.

[0028] In another aspect, the invention features a purified antibody that binds specifically to an IAP family protein. Such an antibody may be used in any standard immunodetection method for the identification of an IAP polypeptide. Preferably, the antibody binds specifically to XIAP, HIAP-1, or HIAP-2. In various embodiments, the antibody may react with other IAP polypeptides or may be specific for one or a few IAP polypeptides. The antibody may be a monoclonal or a polyclonal antibody. Preferably, the antibody reacts specifically with only one of the IAP polypeptides, for example, reacts with murine and human XIAP, but not with HIAP-1 or HIAP-2 from other mammalian species.

[0029] The antibodies of the invention may be prepared by a variety of methods. For example, the IAP polypeptide, or antigenic fragments thereof, can be administered to an animal in order to induce the production of polyclonal antibodies. Alternatively, antibodies used as described herein may be monoclonal antibodies, which are prepared using hybridoma technology (see, e.g., Kohler et al., Nature 256:495, 1975; Kohler et al., Eur. J. Immunol. 6:511, 1976; Kohler et al., Eur. J. Immunol. 6:292, 1976; Hammerling et al., In *Monoclonal Antibodies and T Cell Hybridomas*, Elsevier, N.Y., 1981). The invention features antibodies that specifically bind human or murine IAP polypeptides, or fragments thereof. In particular the invention features "neutralizing" antibodies. By "neutralizing" antibodies is meant antibodies that interfere with any of the biological activities of IAP polypeptides, particularly the ability of IAPs to inhibit apoptosis. The neutralizing antibody may reduce the ability of IAP polypeptides to inhibit polypeptides by, preferably 50%, more preferably by 70, and most preferably by 90% or more. Any standard assay of apoptosis, including those described herein, may be used to assess neutralizing antibodies.

[0030] In addition to intact monoclonal and polyclonal anti-IAP antibodies, the invention features various genetically engineered antibodies, humanized antibodies, and antibody fragments, including F(ab')2, Fab', Fab, Fv and sFv fragments. Antibodies can be humanized by methods known in the art, e.g., monoclonal antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland; Oxford Molecular, Palo Alto, Calif.). Fully human antibodies, such as those expressed in transgenic animals, are also features of the invention (Green et al., Nature Genetics 7:13, 1994).

[0031] Ladner (U.S. Pat. Nos. 4,946,778 and 4,704,692) describes methods for preparing single polypeptide chain antibodies. Ward et al. (Nature 341:544, 1989) describe the preparation of heavy chain variable domains, which they term "single domain antibodies," which have high antigen-binding affinities. McCafferty et al. (Nature 348:552, 1990) show that complete antibody V domains can be displayed on the surface of fd bacteriophage, that the phage bind specifically to antigen, and that rare phage (one in a million) can be isolated after affinity chromatography. Boss et al. (U.S. Pat. No. 4,816,397) describe various methods for producing immunoglobulins, and immunologically functional fragments thereof, which include at least the variable domains of the heavy and light chain in a single host cell. Cabilly et al. (U.S. Pat. No. 4,816,567) describe methods for preparing chimeric antibodies.

[0032] In another aspect, the invention features a method of identifying a compound that modulates apoptosis. The method includes providing a cell expressing an IAP polypeptide, contacting the cell with a candidate compound, and monitoring the expression of an IAP gene. An alteration in the level of expression of the IAP gene indicates the presence of a compound which modulates apoptosis. The

compound may be an inhibitor or an enhancer of apoptosis. In various preferred embodiments, the cell is a fibroblast, a neuronal cell, a glial cell, a lymphocyte (r cell or B cell), or an insect cell; the polypeptide expression being monitored is XIAP, HIAP-1, HIAP-2, M-XIAP, M-HIAP-1, or M-HIAP-2 (i.e., human or murine).

[0033] In a related aspect, the invention features methods of detecting compounds that modulate apoptosis using the interaction trap technology and IAP polypeptides, or fragments thereof, as a component of the bait. In preferred embodiments, the compound being tested as a modulator of apoptosis is also a polypeptide.

[0034] In another aspect, the invention features a method for diagnosing a cell proliferation disease, or an increased likelihood of such a disease, using an IAP nucleic acid probe or antibody. Preferably, the disease is a cancer. Most preferably, the disease is selected from the group consisting of promyelocytic leukemia, a HeLa-type carcinoma, chronic myelogenous leukemia (preferably using xiap or hiap-2 related probes), lymphoblastic leukemia (preferably using a xiap related probe), Burkitt's lymphoma (preferably using an hiap-1 related probe), colorectal adenocarcinoma, lung carcinoma, and melanoma (preferably using a xiap probe). Preferably, a diagnosis is indicated by a 2-fold increase in expression or activity, more preferably, at least a 10-fold increase in expression or activity.

[0035] Skilled artisans will recognize that a mammalian IAP, or a fragment thereof (as described herein), may serve as an active ingredient in a therapeutic composition. This composition, depending on the IAP or fragment included, may be used to modulate apoptosis and thereby treat any condition that is caused by a disturbance in apoptosis.

[0036] In addition, apoptosis may be induced in a cell by administering to the cell a negative regulator of the IAP-dependent anti-apoptotic pathway. The negative regulator may be, but is not limited to, an IAP polypeptide that includes a ring zinc finger, and an IAP polypeptide that includes a ring zinc finger and lacks at least one BIR domain. Alternatively, apoptosis may be induced in the cell by administering a gene encoding an IAP polypeptide, such as these two polypeptides. In yet another method, the negative regulator may be a purified antibody, or a fragment thereof, that binds specifically to an IAP polypeptide. For example, the antibody may bind to an approximately 26 kDa cleavage product of an IAP polypeptide that includes at least one BIR domain but lacks a ring zinc finger domain. The negative regulator may also be an IAP antisense mRNA molecule.

[0037] As summarized above, an IAP nucleic acid, or an IAP polypeptide may be used to modulate apoptosis. Furthermore, an IAP nucleic acid, or an IAP polypeptide, may be used in the manufacture of a medicament for the modulation of apoptosis.

[0038] By "IAP gene" is meant a gene encoding a polypeptide having at least one BIR domain and a ring zinc finger domain which is capable of modulating (inhibiting or enhancing) apoptosis in a cell or tissue when provided by other intracellular or extracellular delivery methods. In preferred embodiments the IAP gene is a gene having about 50% or greater nucleotide sequence identity to at least one of the IAP amino acid encoding sequences of FIGS. **1-4** or portions thereof. Preferably, the region of sequence over which identity is measured is a region encoding at least one BIR domain and a ring zinc finger domain. Mammalian IAP genes include nucleotide sequences isolated from any mammalian source. Preferably, the mammal is a human.

[0039] The term "IAP gene" is meant to encompass any member of the family of apoptosis inhibitory genes, which are characterized by their ability to modulate apoptosis. An IAP gene may encode a polypeptide that has at least 20%, preferably at least 30%, and most preferably at least 50% amino acid sequence identity with at least one of the conserved regions of one of the IAP members described herein (i.e., either the BIR or ring zinc finger domains from the human or murine xiap, hiap-1 and hiap-2). Representative members of the IAP gene family include, without limitation, the human and murine xiap, hiap-1, and hiap-2 genes.

[0040] By "IAP protein" or "IAP polypeptide" is meant a polypeptide, or fragment thereof, encoded by an IAP gene.

[0041] By "BIR domain" is meant a domain having the amino acid sequence of the consensus sequence: Xaa1-Xaa1-Xaa1-Arg-Leu-Xaa1-Thr-Phe-Xaa1-Xaa1-Trp-Pro-Xaa2-Xaa1-Xaa1-Xaa2-Xaa1-Xaa1-Xaa1-Xaa1-Leu-Ala-Xaa1-Ala-Gly-Phe-Tyr-Tyr-Xaa1-Gly-Xaa1-Xaa1-Asp-Xaa1-Val-Xaa1-Cys-Phe-Xaa1-Cys-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Trp-Xaa1-Xaa1-Xaa1-Asp-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-His-Xaa1-Xaa1-Xaa1-Xaa1-Pro-Xaa1-Cys-Xaa1-Phe-Val, wherein Xaa1 is any amino acid and Xaa2 is any amino acid or is absent (SEQ ID NO: 2). Preferably, the sequence is substantially identical to one of the BIR domain sequences provided herein for XIAP, HIAP-1, or HIAP-2.

[0042] By "ring zinc finger" or "RZF" is meant a domain having the amino acid sequence of the consensus sequence: Glu-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa2-Xaa1-Xaa1-Xaa1-Cys-Lys-Xaa3-Cys-Met-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa3-Xaa1-Phe-Xaa1-Pro-Cys-Gly-His-Xaa1-Xaa1-Xaa1-Cys-Xaa1-Xaa1-Cys-Ala-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Cys-Pro-Xaa1-Cys, wherein Xaa1 is any amino acid, Xaa2 is Glu or Asp, and Xaa3 is Val or Ile (SEQ ID NO: 1).

[0043] Preferably, the sequence is substantially identical to the RZF domains provided herein for the human or murine XIAP, HIAP-1, or HIAP-2.

[0044] By "modulating apoptosis" or "altering apoptosis" is meant increasing or decreasing the number of cells that would otherwise undergo apoptosis in a given cell population. Preferably, the cell population is selected from a group including T cells, neuronal cells, fibroblasts, or any other cell line known to undergo apoptosis in a laboratory setting (e.g., the baculovirus infected insect cells). It will be appreciated that the degree of modulation provided by an IAP or modulating compound in a given assay will vary, but that one skilled in the art can determine the statistically significant change in the level of apoptosis which identifies an IAP or a compound which modulates an IAP.

[0045] By "inhibiting apoptosis" is meant any decrease in the number of cells which undergo apoptosis relative to an untreated control. Preferably, the decrease is at least 25%, more preferably the decrease is 50%, and most preferably the decrease is at least one-fold.

[0046] By "polypeptide" is meant any chain of more than two amino acids, regardless of post-translational modification such as glycosylation or phosphorylation.

[0047] By "substantially identical" is meant a polypeptide or nucleic acid is exhibiting at least 50%, preferably 85%, more preferably 90%, and most preferably 95% homology to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids. For nucleic acids, the length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

[0048] Sequence identity is typically measured using sequence analysis software with the default parameters specified therein (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705). This software program matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

[0049] By "substantially pure polypeptide" is meant a polypeptide that has been separated from the components that naturally accompany it. Typically, the polypeptide is substantially pure when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the polypeptide is an IAP polypeptide that is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, pure. A substantially pure IAP polypeptide may be obtained, for example, by extraction from a natural source (e.g. a fibroblast, neuronal cell, or lymphocyte) by expression of a recombinant nucleic acid encoding an IAP polypeptide, or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

[0050] A protein is substantially free of naturally associated components when it is separated from those contaminants which accompany it in its natural state. Thus, a protein which is chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. Accordingly, substantially pure polypeptides include those derived from eukaryotic organisms but synthesized in *E. coli* or other prokaryotes. By "substantially pure DNA" is meant DNA that is free of the genes which, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

[0051] By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding (as used herein) an IAP polypeptide.

[0052] By "transgene" is meant any piece of DNA which is inserted by artifice into a cell, and becomes part of the genome of the organism which develops from that cell. Such a transgene may include a gene which is partly or entirely heterologous (i.e., foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the organism.

[0053] By "transgenic" is meant any cell which includes a DNA sequence which is inserted by artifice into a cell and becomes part of the genome of the organism which develops from that cell. As used herein, the transgenic organisms are generally transgenic mammalian (e.g., rodents such as rats or mice) and the DNA transgene) is inserted by artifice into the nuclear genome.

[0054] By "transformation" is meant any method for introducing foreign molecules into a cell. Lipofection, calcium phosphate precipitation, retroviral delivery, electroporation, and biolistic transformation are just a few of the teachings which may be used. For example, biolistic transformation is a method for introducing foreign molecules into a cell using velocity driven microprojectiles such as tungsten or gold particles. Such velocity-driven methods originate from pressure bursts which include, but are not limited to, helium-driven, air-driven, and gunpowder-driven techniques. Biolistic transformation may be applied to the transformation or transfection of a wide variety of cell types and intact tissues including, without limitation, intracellular organelles (e.g., and mitochondria and chloroplasts), bacteria, yeast, fungi, algae, animal tissue, and cultured cells.

[0055] By "positioned for expression" is meant that the DNA molecule is positioned adjacent to a DNA sequence which directs transcription and translation of the sequence (i.e., facilitates the production of, e.g., an IAP polypeptide, a recombinant protein or a RNA molecule). By "reporter gene" is meant a gene whose expression may be assayed; such genes include, without limitation, glucuronidase (GUS), luciferase, chloramphenicol transacetylase (CAT), and lacZ.

[0056] By "promoter" is meant minimal sequence sufficient to direct transcription. Also included in the invention are those promoter elements which is are sufficient to render promoter-dependent gene expression controllable for cell type-specific, tissue-specific or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the native gene.

[0057] By "operably linked" is meant that a gene and one or more regulatory sequences are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins are bound to the regulatory sequences).

[0058] By "conserved region" is meant any stretch of six or more contiguous amino acids exhibiting at least 30%, preferably 50%, and most preferably 70% amino acid sequence identity between two or more of the IAP family members, (e.g., between human HIAP-1, HIAP-2, and XIAP). Examples of preferred conserved regions are shown (as boxed or designated sequences) in FIGS. **5-7** and Tables 1 and 2, and include, without limitation, BIR domains and ring zinc finger domains.

[0059] By "detectably-labelled" is meant any means for marking and identifying the presence of a molecule, e.g., an oligonucleotide probe or primer, a gene or fragment thereof, or a cDNA molecule. Methods for detectably-labelling a molecule are well known in the art and include, without limitation, radioactive labelling (e.g., with an isotope such as ^{32}P or ^{35}S) and nonradioactive labelling (e.g., chemiluminescent labelling, e.g., fluorescein labelling).

[0060] By "antisense," as used herein in reference to nucleic acids, is meant a nucleic acid sequence, regardless of length, that is complementary to the coding strand of a gene.

[0061] By "purified antibody" is meant antibody which is at least 60%, by weight, free from proteins and naturally occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight, antibody, e.g., an IAP specific antibody. A purified antibody may be obtained, for example, by affinity chromatography using recombinantly-produced protein or conserved motif peptides and standard techniques.

[0062] By "specifically binds" is meant an antibody that recognizes and binds a protein but that does not substantially recognize and bind other molecules in a sample, e.g., a biological sample, that naturally includes protein.

[0063] Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0064] FIG. **1** is the human xiap cDNA sequence (SEQ ID NO: 3) and the XIAP polypeptide sequence (SEQ ID NO: 4).

[0065] FIG. **2** is the human hiap-1 cDNA sequence (SEQ ID NO: 5) and the HIAP-1 polypeptide sequence (SEQ ID NO: 6).

[0066] FIG. **3** is the human hiap-2 cDNA sequence (SEQ ID NO: 7) and the HIAP-2 polypeptide sequence (SEQ ID NO: 8). The sequence absent in the hiap-2-Δ variant is boxed.

[0067] FIG. **4** is the murine xiap cDNA sequence (SEQ ID NO: 9) and encoded murine XIAP polypeptide sequence (SEQ ID NO: 10).

[0068] FIG. **5** is the murine hiap-1 cDNA sequence (SEQ ID NO: 39) and the encoded murine HIAP-1 polypeptide sequence (SEQ ID NO: 40).

[0069] FIG. **6** is the murine hiap-2 cDNA sequence (SEQ ID NO: 41) and the encoded murine HIAP-2 polypeptide (SEQ ID NO: 42).

[0070] FIG. **7** is a representation of the alignment of the BIR domains of IAP proteins (SEQ ID NOs: 11 and 14-31).

[0071] FIG. **8** is a representation of the alignment of human IAP polypeptides with diap, cp-iap, and the IAP consensus sequence (SEQ ID NOs: 4, 6, 8, 10, 12, and 13).

[0072] FIG. **9** is a representation of the alignment of the ring zinc finger domains of IAP proteins (SEQ ID NOs: 32-38).

[0073] FIG. **10** is a photograph of a northern blot illustrating human hiap-1 and hiap-2 mRNA expression in human tissues.

[0074] FIG. **11** is a photograph of a northern blot illustrating human hiap-2 mRNA expression in human tissues.

[0075] FIG. **12** is a photograph of a northern blot illustrating human xiap mRNA expression in human tissues.

[0076] FIGS. **13**A and **13**B are photographs of agarose gels illustrating apoptotic DNA ladders and RT-PCR products using hiap-1 and hiap-2 specific probes in HIV-infected T cells.

[0077] FIG. **14**A-**14**D are graphs depicting suppression of apoptosis by XIAP, HIAP-1, HIAP-2, Bcl-2, smn, and 6-myc.

[0078] FIGS. **15**A and **15**B are bar graphs depicting the percentage of viable CHO cells following transient transfection with the cDNA constructs shown and subsequent serum withdrawal.

[0079] FIGS. **16**A and **16**B are bar graphs depicting the percentage of viable CHO cells following transient transfection with the cDNA constructs shown and subsequent exposure to menadione (FIG. **16**A=10 μM menadione; FIG. **16**B=20 μM menadione).

[0080] FIG. **17** is a photograph of an agarose gel containing cDNA fragments that were amplified, with hiap-1-specific primers, from RNA obtained from Raji, Ramos, EB-3, and Jiyoye cells, and from normal placenta.

[0081] FIG. **18** is a photograph of a western blot containing protein extracted from Jurkat and astrocytoma cells stained with an anti-XIAP antibody. The position and size of a series of marker proteins is indicated.

[0082] FIG. **19** is a photograph of a western blot containing protein extracted from Jurkat cells following treatment as described in Example XII. The blot was stained with a rabbit polyclonal anti-XIAP antibody. Lane 1, negative control; lane 2, anti-Fas antibody; lane 3, anti-Fas antibody and cycloheximide; lane 4, TNF-α; lane 5, TNF-α and cycloheximide.

[0083] FIG. **20** is a photograph of a western blot containing protein extracted from HeLa cells following exposure to anti-Fas antibodies. The blot was stained with a rabbit polyclonal anti-XIAP antibody. Lane 1, negative control; lane 2, cycloheximide; lane 3, anti-Fas antibody; lane 4, anti-Fas antibody and cycloheximide; lane 5, TNF-α; lane 6, TNF-α and cycloheximide.

[0084] FIGS. **21**A and **21**B are photographs of western blots stained with rabbit polyclonal anti-XIAP antibody. Protein was extracted from HeLa cells (FIG. **21**A) and Jurkat cells (FIG. **21**B) immediately, 1, 2, 3, 5, 10, and 22 hours after exposure to anti-Fas antibody.

[0085] FIGS. **22**A and **22**B are photographs of western blots stained with an anti-CPP32 antibody (FIG. **22**A) or a rabbit polyclonal anti-XIAP antibody (FIG. **22**B). Protein was extracted from Jurkat cells immediately, 3 hours, or 7 hours after exposure to an anti-Fas antibody. In addition to total protein, cytoplasmic and nuclear extracts are shown.

[0086] FIG. **23** is a photograph of a polyacrylamide gel following electrophoresis of the products of an in vitro XIAP cleavage assay.

DETAILED DESCRIPTION

I. IAP Genes and Polypeptides

[0087] A new class of mammalian proteins that modulate apoptosis (IAPs) and the genes that encode these proteins have been discovered. The IAP proteins are characterized by the presence of a ring zinc finger domain (RZF; FIG. **9**) and at least one BIR domain, as defined by the boxed consensus sequences shown in FIGS. **7** and **8**, and by the sequence domains listed in Tables 1 and 2. As examples of novel IAP genes and proteins, the cDNA sequences and amino acid sequences for human IAPs (HIAP-1, HIAP-2, and XIAP) and a new murine inhibitor of apoptosis, XIAP, are provided. Additional members of the mammalian IAP family (including homologs from other species and mutant sequences) may be isolated using standard cloning techniques and the conserved amino acid sequences, primers, and probes provided herein and known in the art. Furthermore, IAPs include those proteins lacking the ring zinc finger, as further described below.

TABLE 1

NUCLEOTIDE POSITION OF CONSERVED DOMAINS*

	BIR-1	BIR-2	BIR-3	Ring Zinc Finger
h-xiap	109–312	520–723	826–1023	1348–1485
m-xiap	202–405	613–816	916–1113	1438–1575
h-hiap-1	273–476	693–893	951–1154	1824–1961
m-hiap-1	251–453	670–870	928–1131	1795–1932
h-hiap-2	373–576	787–987	1042–1245	1915–2052
m-hiap-2	215–418	608–808	863–1066	1763–1876

*Positions indicated correspond to those shown in FIGS. 1–4.

[0088]

TABLE 2

AMINO ACID POSITION OF CONSERVED DOMAINS*

	BIR-1	BIR-2	BIR-3	Ring Zinc Finger
h-XAIP	26–93	163–230	265–330	439–484
m-XIAP	26–93	163–230	264–329	438–483
h-HIAP1	29–96	169–235	255–322	546–591
m-HIAP1	29–96	169–235	255–322	544–589
h-HIAP2	46–113	184–250	269–336	560–605
m-HIAP2	25–92	156–222	241–308	541–578

*Positions indicated correspond to those shown in FIGS. 1–4.

[0089] Recognition of the mammalian IAP family has provided an emergent pattern of protein structure. Recognition of this pattern allows proteins having a known, homologous sequence but unknown function to be classified as putative inhibitors of apoptosis. A *Drosophila* gene, now termed diap, was classified in this way (for sequence information see Genbank Accession Number M96581 and FIG. **6**). The conservation of these proteins across species indicates that the apoptosis signalling pathway has been conserved throughout evolution.

[0090] The IAP proteins may be used to inhibit the apoptosis that occurs as part of numerous disease processes or disorders. For example, IAP polypeptides or nucleic acid encoding IAP polypeptides may be administered for the treatment or prevention of apoptosis that occurs as a part of AIDS, neurodegenerative diseases, ischemic injury, toxin-induced liver disease and myelodysplastic syndromes. Nucleic acid encoding the IAP polypeptide may also be provided to inhibit apoptosis.

II. Cloning of IAP Genes

A. Human xiap

[0091] The search for human genes involved in apoptosis resulted in the identification of an X-linked sequence tag site (STS) in the GenBank database, which demonstrated strong homology with the conserved RZF domain of CpIAP and OpIAP, the two baculovirus genes known to inhibit apoptosis (Clem et al., Mol. Cell. Biol. 14:5212, 1994; Birnbaum et al., J. Virol. 68:2521, 1994). Screening a human fetal brain ZapII cDNA library (Stratagene, La Jolla, Calif.) with this STS resulted in the identification and cloning of xiap (for X-linked Inhibitor of Apoptosis Protein gene). The human gene has a 1.5 kb coding sequence that includes three BIR domains (Crook et al., J. Virol. 67:2168, 1993; Clem et al., Science 254:1388, 1991; Birnbaum et al., J. Virol. 68:2521, 1994) and a zinc finger. Northern blot analysis with xiap revealed message greater than 7 kb, which is expressed in various tissues, particularly liver and kidney (FIG. **12**). The large size of the transcript reflects large 5' and 3' untranslated regions.

B. Human hiap-1 and hiap-2

[0092] The hiap-1 and hiap-2 genes were cloned by screening a human liver library (Stratagene Inc., La Jolla, Calif.) with a probe including the entire xiap coding region at low stringency (the final wash was performed at 40° C. with 2×SSC, 10% SDS; FIGS. **2** and **3**). The hiap-1 and hiap-2 genes were also detected independently using a probe derived from an expressed sequence tag (EST; GenBank Accession No. T96284), which includes a portion of a BIR domain. The EST sequence was originally isolated by the polymerase chain reaction; a cDNA library was used as a template and amplified with EST-specific primers. The DNA amplified probe was then used to screen the human liver cDNA library for full-length hiap coding sequences. A third DNA was subsequently detected that includes the hiap-2 sequence but that appears to lack one exon, presumably due to alternative mRNA splicing (see boxed region in FIG. **3**). The expression of hiap-1 and hiap-2 in human tissues as assayed by northern blot analysis is shown in FIGS. **8** and **9**.

C. m-xiap

[0093] Fourteen cDNA and two genomic clones were identified by screening a mouse embryo λgt11 cDNA library (Clontech, Palo Alto, Calif.) and a mouse FIX II genomic library with a xiap cDNA probe, respectively. A cDNA contig spanning 8.0 kb was constructed using 12 overlapping mouse clones. Sequence analysis revealed a coding sequence of approximately 1.5 kb. The mouse gene, m-xiap, encodes a polypeptide with string homology to human XIAP at and around the initiation methionine, the stop codon, the three BIR domains, and the RZF domain. As with the human gene, the mouse homologue contains large 5' and 3' UTRs, which could produce a transcript as large as 7-8 kb.

[0094] Analysis of the sequence and restriction map of m-xiap further delineate the structure and genomic organization of m-xiap. Southern blot analysis and inverse PCR techniques (Groden et al., Cell 66:589, 1991) can be employed to map exons and define exon-intron boundaries.

[0095] Antisera can be raised against a M-XIAP fusion protein that was obtained from, for example, *E. coli* using a

bacterial expression system. The resulting antisera can be used along with northern blot analysis to analyze the spatial and temporal expression of m-xiap in the mouse.

D. m-hiap-1 and m-hiap-2

[0096] The murine homologs of hiap-1 and hiap-2 were cloned and sequenced in the same general manner as m-xiap using the human hiap-1 and hiap-2 sequences as probes. Cloning of m-hiap-1 and m-hiap-2 further demonstrate that homologs from different species may be isolated using the techniques provided herein and those generally known to artisans skilled in molecular biology.

III. Identification of Additional IAP Genes

[0097] Standard techniques, such as the polymerase chain reaction (PCR) and DNA hybridization, may be used to clone additional human IAP genes and their homologues in other species. Southern blots of human genomic DNA hybridized at low stringency with probes specific for xiap, hiap-1 and hiap-2 reveal bands that correspond to other known human IAP sequences as well as additional bands that do not correspond to known IAP sequences. Thus, additional IAP sequences may be readily identified using low stringency hybridization. Examples of murine and human xiap, hiap-1, and hiap-2 specific primers, which may be used to clone additional genes by RT-PCR, are shown in Table 5.

IV. Characterization of IAP Activity and Intracellular Localization Studies

[0098] The ability of putative IAPs to modulate apoptosis can be defined in in vitro systems in which alterations of apoptosis can be detected. Mammalian expression constructs carrying IAP cDNAs, which are either full-length or truncated, can be introduced into cell lines such as CHO, NIH 3T3, HL60, Rat-1, or Jurkat cells. In addition, Sf21 insect cells may be used, in which case the IAP gene is preferentially expressed using an insect heat shock promotor. Following transfection, apoptosis can be induced by standard methods, which include serum withdrawal, or application of staurosporine, menadione (which induces apoptosis via free radial formation), or anti-Fas antibodies. As a control, cells are cultured under the same conditions as those induced to undergo apoptosis, but either not transfected, or transfected with a vector that lacks an IAP insert. The ability of each IAP construct to inhibit apoptosis upon expression can be quantified by calculating the survival index of the cells, i.e., the ratio of surviving transfected cells to surviving control cells. These experiments can confirm the presence of apoptosis inhibiting activity and, as discussed below, can also be used to determine the functional region(s) of an IAP. These assays may also be performed in combination with the application of additional compounds in order to identify compounds that modulate apoptosis via IAP expression.

A. Cell Survival Following Transfection with Full-Length IAP Constructs and Induction of Apoptosis

[0099] Specific examples of the results obtained by performing various apoptosis suppression assays are shown in FIGS. **14**A to **14**D. For example, CHO cell survival following transfection with one of six constructs and subsequent serum withdrawal is shown in FIG. **14**A. The cells were transfected using Lipofectace™ with 2 μg of one of the following recombinant plasmids: pCDNA3-6myc-xiap (xiap), pCDNA3-6myc-hiap-1 (hiap-1), pCDNA3-6myc-hiap-2 (hiap-2), pCDNA3-bcl-2 (bcl-2), pCDNA3-HA-smn (smn), and pCDNA3-6myc (6-myc). Oligonucleotide primers were synthesized to allow PCR amplification and cloning of the xiap, hiap-1, and hiap-2 ORFs in pCDNA3 (Invitrogen). Each construct was modified to incorporate a synthetic myc tag encoding six repeats of the peptide sequence MEQKLISEEDL (SEQ ID NO: 43), thus allowing detection of myc-IAP fusion proteins via monoclonal anti-myc antiserum (Egan et al., Nature 363:45, 1993). Triplicate samples of cell lines in 24-well dishes were washed 5 times with serum free media and maintained in serum free conditions during the course of the experiment. Cells that excluded trypan blue, and that were therefore viable, were counted with a hemocytometer immediately, 24 hours, 48 hours, and 72 hours after serum withdrawal. Survival was calculated as a percentage of the initial number of viable cells. In this experiment, as well as those presented in FIGS. **14**B and **14**D, the percentage of viable cells shown represents the average of three separate experiments performed in triplicate, ±standard deviation.

[0100] The survival of CHO cells following transfection (with each one of the six constructs described above) and exposure to menadione is shown in FIG. **14**B. The cells were plated in 24-well dishes, allowed to grow overnight, and then exposed to 20 μM menadione (Sigma Chemical Co., St. Louis, Mo.) for 1.5 hours. Triplicate samples were harvested at the time of exposure to menadione and 24 hours afterward, and survival was assessed by trypan blue exclusion.

[0101] The survival of Rat-1 cells following transfection (with each one of the six constructs described above) and exposure to staurosporine is shown in FIG. **14**C. Rat-1 cells were transfected and then selected in medium containing 800 μg/ml G418 for two weeks. The cell line was assessed for resistance to staurosporine-induced apoptosis (1 μM) for 5 hours. Viable cells were counted 24 hours after exposure to staurosporine by trypan blue exclusion. The percentage of viable cells shown represents the average of two experiments, ±standard deviation.

[0102] The Rat-1 cell line was also used to test the resistance of these cells to menadione (FIG. **14**D) following transfection with each of the six constructs described above. The cells were exposed to 10 μM menadione for 1.5 hours, and the number of viable cells was counted 18 hours later.

B. Comparison of Cell Survival Following Transfection with Full-Length vs. Partial IAP Constructs

[0103] In order to investigate the mechanism whereby human IAPs, including XIAP, HIAP-1, and HIAP-2, afford protection against cell death, expression vectors were constructed that contained either: (1) full-length IAP cDNA (as described above), (2) a portion of an IAP gene that encodes the BIR domains, but not the RZF, or (3) a portion of an IAP gene that encodes the RZF, but not the BIR domains. Human and murine xiap or m-xiap cDNAs were tested by transient or stable expression in HeLa, Jurkat, and CHO cell lines. Following transfection, apoptosis was induced by serum withdrawal application of menadione, or application of an anti-Fas antibody. Cell death was then assessed, as described above, by trypan blue exclusion. As a control for transfection efficiency, the cells were co-transfected with a β-gal expression construct. Typically, approximately 20% of the cells were successfully transfected.

[0104] When CHO cells were transiently transfected, constructs containing full-length xiap or m-xiap cDNAs conferred modest protection against cell death (FIG. **15**A). In contrast, the survival of CHO cells transfected with con-

structs encoding only the BIR domains (i.e., lacking the RZF domain; see FIG. **15**A) was markedly enhanced 72 hours after serum deprivation. Furthermore, a large percentage of cells expressing the BIR domains were still viable after 96 hours, at which time no viable cells remained in the control, i.e. non-transfected, cell cultures (see "CHO" in FIG. **15**A), and less than 5% of the cells transfected with the vector only, i.e., lacking a cDNA insert, remained viable (see "pcDNA3" in FIG. **15**A). Deletion of any of the BIR domains results in the complete loss of apoptotic suppression, which is reflected by a decrease in the percentage of surviving CHO cells to control levels within 72 hours of serum withdrawal (FIG. **15**B; see "xiapΔ1" (which encodes amino acids 89-497 of XIAP (SEQ ID NO.:4)), "xiapΔ2" (which encodes amino acids 246-497 of XIAP (SEQ ID NO.:4)), and "xiapΔ3" (which encodes amino acids 342-497 of XIAP (SEQ ID NO.:4)) at 72 hours).

[**0105**] Stable pools of transfected CHO cells, which were maintained for several months under G418 selection, were induced to undergo apoptosis by exposure to 10 μM menadione for 2 hours. Among the CHO cells tested were those that were stably transfected with: (1) full-length m-xiap cDNA (miap), (2) full-length xiap cDNA (xiap), (3) full-length bcl-2 cDNA (Bcl-2), (4) cDNA encoding the three BIR domains (but not the RZF) of M-XIAP (BIR), and (5) cDNA encoding the RZF (but not BIR domains) of M-XIAP (RZF). Cells that were non-transfected (CHO) or transfected with the vector only (pcDNA3), served as controls for this experiment. Following exposure to 10 μM menadione, the transfected cells were washed with phosphate buffered saline (PBS) and cultured for an additional 24 hours in menadione-free medium. Cell death was assessed, as described above, by trypan blue exclusion. Less than 10% of the non-transfected or vector-only transfected cells remained viable at the end of the 24 hour survival period. Cells expressing the RZF did not fare significantly better. However, expression of full-length m-xiap, xiap, or bcl-2, and expression of the BIR domains, enhanced cell survival (FIG. **16**A). When the concentration of menadione was increased from 10 μM to 20 μm (with all other conditions of the experiment being the same as when 10 μM menadione was applied), the percentage of viable CHO cells that expressed the BIR domain cDNA construct was higher than the percentage of viable cells that expressed either full-length m-xiap or bcl-2 FIG. **16**B).

C. Analysis of the Subcellular Location of Expressed RZF and BIR Domains

[**0106**] The assays of cell death described above indicate that the RZF may act as a negative regulator of the anti-apoptotic function of IAPs. One way in which the RZF, and possibly other IAP domains, may exert their regulatory influence is by altering the expression of genes, whose products function in the apoptotic pathway.

[**0107**] In order to determine whether the subcellular locations of expressed RZF and BIR domains are consistent with roles as nuclear regulatory factors, COS cells were transiently transfected with the following four constructs, and the expressed polypeptide was localized by immunofluorescence microscopy: (1) pcDNA3-6myc-xiap, which encodes all 497 amino acids of SEQ ID NO: 4, (2) pcDNA3-6myc-m-xiap, which encodes all 497 amino acids of mouse xiap (SEQ ID NO: 10), (3) pcDNA3-myc-mxiap-BIR, which encodes amino acids 1 to 341 of m-xiap (SEQ ID NO: 10), and (4) pcDNA3-6myc-mxiap-RZF, which encodes amino acids 342-497 of m-xiap (SEQ ID NO: 10). The cells were grown on multi-well tissue culture slides for 12 hours, and then fixed and permeabilized with methanol. The constructs used (here and in the cell death assays) were tagged with a human Myc epitope tag at the N-terminus. Therefore, a monoclonal anti-Myc antibody and a secondary goat anti-mouse antibody, which was conjugated to FITC, could be used to localize the expressed products in transiently transfected COS cells. Full-length XIAP and MIAP were located in the cytoplasm, with accentuated expression in the perinuclear zone. The same pattern of localization was observed when the cells expressed a construct encoding the RZF domain (but not the BIR domains). However, cells expressing the BIR domains (without the RZF) exhibited, primarily, nuclear staining. The protein expressed by the BIR domain construct appeared to be in various stages of transfer to the nucleus.

[**0108**] These observations are consistent with the fact that, as described below, XIAP is cleaved within T cells that are treated with anti-Fas antibodies (which are potent inducers of apoptosis), and its N-terminal domain is translocated to the nucleus.

D. Examples of Additional Apoptosis Assays

[**0109**] Specific examples of apoptosis assays are also provided in the following references. Assays for apoptosis in lymphocytes are disclosed by: Li et al., Science 268:429, 1995; Gibellini et al., Br. J. Haematol. 89:24, 1995; Martin et al., J. Immunol. 152:330, 1994; Terai et al., J. Clin. Invest. 87:1710, 1991; Dhein et al., Nature 373:438, 1995; Katsikis et al., J. Exp. Med. 1815:2029, 1995; Westendorp et al., Nature 375:497, 1995; DeRossi et al., Virology 198:234, 1994.

[**0110**] Assays for apoptosis in fibroblasts are disclosed by: Vossbeck et al., Int. J. Cancer 61:92, 1995; Goruppi et al., Oncogene 9:1537, 1994; Fernandez et al., Oncogene 9:2009, 1994; Harrington et al., EMBO J., 13:3286, 1994; Itoh et al., J. Biol. Chem. 268:10932, 1993.

[**0111**] Assays for apoptosis in neuronal cells are disclosed by: Melino et al., Ann. Neurol. 36:864, 1994; Sato et al., J. Neurobiol. 25:1227, 1994; Ferrari et al., J. Neurosci. 1516:2857, 1995; Talley et al., Mol. Cell. Biol. 1585:2359, 1995; Talley et al., Mol. Cell. Biol. 15:2359, 1995; Walkinshaw et al., J. Clin. Invest. 95:2458, 1995.

[**0112**] Assays for apoptosis in insect cells are disclosed by: Clem et al., Science 254:1388, 1991; Crook et al., J. Virol. 67:2168, 1993; Rabizadeh et al., J. Neurochem. 61:2318, 1993; Birnbaum et al., J. Virol. 68:2521, 1994; Clem et al., Mol. Cell. Biol. 14:5212, 1994.

V. Construction of a Transgenic Animal

[**0113**] Characterization of IAP genes provides information that is necessary for an IAP knockout animal model to be developed by homologous recombination. Preferably, the model is a mammalian animal, most preferably a mouse. Similarly, an animal model of IAP overproduction may be generated by integrating one or more IAP sequences into the genome, according to standard transgenic techniques.

[**0114**] A replacement-type targeting vector, which would be used to create a knockout model, can be constructed using an isogenic genomic clone, for example, from a mouse strain such as 129/Sv (Stratagene Inc., La Jolla, Calif.). The targeting vector is introduced into a suitably-derived line of embryonic stem (ES) cells by electroporation to generate ES cell lines that carry a profoundly truncated form of an IAP. To generate chimeric founder mice, the targeted cell lines are

injected into a mouse blastula stage embryo. Heterozygous offspring are interbred to homozygosity. Knockout mice would provide the means, in vivo, to screen for therapeutic compounds that modulate apoptosis via an IAP-dependent pathway.

VI. IAP Protein Expression

[0115] IAP genes may be expressed in both prokaryotic and eukaryotic cell types. If an IAP modulates apoptosis by exacerbating it, it may be desirable to express that protein under control of an inducible promotor.

[0116] In general, IAPs according to the invention may be produced by transforming a suitable host cell with all or part of an IAP-encoding cDNA fragment that has been placed into a suitable expression vector.

[0117] Those skilled in the art of molecular biology will understand that a wide variety of expression systems may be used to produce the recombinant protein. The precise host cell used is not critical to the invention. The IAP protein may be produced in a prokaryotic host (e.g., *E. coli*) or in a eukaryotic host (e.g., *S. cerevisiae*, insect cells such as Sf21 cells, or mammalian cells such as COS-1, NIH 3T3, or HeLa cells). These cells are publicly available, for example, from the American Type Culture Collection (ATCC), Rockville, Md.; see also Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y., 1994. The method of transduction and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described, e.g., in Ausubel et al. (supra), and expression vehicles may be chosen from those provided, e.g. in *Cloning Vectors: A Laboratory Manual* (P. H. Pouwels et al., 1985, Supp. 1987).

[0118] A preferred expression system is the baculovirus system using, for example, the vector pBacPAK9, which is available from Clontech (Palo Alto, Calif.). If desired, this system may be used in conjunction with other protein expression techniques, for example, the myc tag approach described by Evan et al. (Mol. Cell. Biol. 5:3610, 1985).

[0119] Alternatively, an IAP may be produced by a stably-transfected mammalian cell line. A number of vectors suitable for stable transfection of mammalian cells are available to the public, (e.g., see Pouwels et al., supra), as are methods for constructing such cell lines (e.g., see Ausubel et al., supra). In one example, cDNA encoding an IAP is cloned into an expression vector that includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, integration of the IAP-encoding gene into the host cell chromosome is is selected for by inclusion of 0.01-300 μM methotrexate in the cell culture medium (as described in Ausubel et al., supra). This dominant selection can be accomplished in most cell types. Recombinant protein expression can be increased by DHFR-mediated amplification of the transfected gene.

[0120] Methods for selecting cell lines bearing gene amplifications are described in Ausubel et al. (supra). These methods generally involve extended culture in medium containing gradually increasing levels of methotrexate. The most commonly used DHFR-containing expression vectors are pCVSEII-DHFR and pAdD26SV(A) (described in Ausubel et al., supra). The host cells described above or, preferably, a DHFR-deficient CHO cell line (e.g., CHO DHFR cells, ATCC Accession No. CRL 9096) are among those most preferred for DHFR selection of a stably-transfected cell line or DHFR-mediated gene amplification.

[0121] Once the recombinant protein is expressed, it is isolated by, for example, affinity chromatography. In one example, an anti-IAP antibody, which may be produced by the methods described herein, can be attached to a column and used to isolate the IAP protein. Lysis and fractionation of IAP-harboring cells prior to affinity chromatography may be performed by standard methods (see e.g., Ausubel et al., supra). Once isolated, the recombinant protein can, if desired, be purified further by e.g., by high performance liquid chromatography (HPLC; e.g., see Fisher, *Laboratory Techniques In Biochemistry And Molecular Biology*, Work and Burdon, Eds., Elsevier, 1980).

[0122] Polypeptides of the invention, particularly short IAP fragments, can also be produced by chemical synthesis (e.g., by the methods described in *Solid Phase Peptide Synthesis,* 2nd ed., 1984 The Pierce Chemical Co., Rockford, Ill.). These general techniques of polypeptide expression and purification can also be used to produce and isolate useful IAP fragments or analogs, as described herein.

VII. Anti-IAP Antibodies

[0123] In order to generate IAP-specific antibodies, an IAP coding sequence (i.e., amino acids 180-276) can be expressed as a C-terminal fusion with glutathione S-transferase (GST; Smith et al., Gene 67:31, 1988). The fusion protein can be purified on glutathione-Sepharose beads, eluted with glutathione, and cleaved with thrombin (at the engineered cleavage site), and purified to the degree required to successfully immunize rabbits. Primary immunizations can be carried out with Freund's complete adjuvant and subsequent immunizations performed with Freund's incomplete adjuvant. Antibody titres are monitored by western blot and immunoprecipitation analyses using the thrombin-cleaved IAP fragment of the GST-IAP fusion protein. Immune sera are affinity purified using CNBr-Sepharose-coupled IAP protein. Antiserum specificity is determined using a panel of unrelated GST proteins (including GSTp53, Rb, HPV-16 E6, and E6-AP) and GST-trypsin (which was generated by PCR using known sequences).

[0124] As an alternate or adjunct immunogen to GST fusion proteins, peptides corresponding to relatively unique hydrophilic regions of IAP may be generated and coupled to keyhole limpet hemocyanin (KLH) through an introduced C-terminal lysine. Antiserum to each of these peptides is similarly affinity purified on peptides conjugated to BSA, and specificity is tested by ELISA and western blotting using peptide conjugates, and by western blotting and immunoprecipitation using IAP expressed as a GST fusion protein.

[0125] Alternatively, monoclonal antibodies may be prepared using the IAP proteins described above and standard hybridoma technology (see, e.g., Kohler et al., Nature 256:495, 1975; Kohler et al., Eur. J. Immunol. 6:511, 1976; Kohler et al., Eur. J. Immunol. 6:292, 1976; Hammerling et al., In *Monoclonal Antibodies and T Cell Hybridomas*, Elsevier, New York, N.Y., 1981; Ausubel et al., supra). Once produced, monoclonal antibodies are also tested for specific IAP recognition by western blot or immunoprecipitation analysis (by the methods described in Ausubel et al., supra).

[0126] Antibodies that specifically recognize IAPs or fragments of IAPs, such as those described herein containing

one or more BIR domains (but not a ring zinc finger domain), or that contain a ring zinc finger domain (but not a BIR domain) are considered useful in the invention. They may, for example, be used in an immunoassay to monitor IAP expression levels or to determine the subcellular location of an IAP or IAP fragment produced by a mammal. Antibodies that inhibit the 26 kDa IAP cleavage product described herein (which contains at least one BIR domain) may be especially useful in inducing apoptosis in cells undergoing undesirable proliferation.

[0127] Preferably, antibodies of the invention are produced using IAP sequence that does not reside within highly conserved regions, and that appears likely to be antigenic, as analyzed by criteria such as those provided by the Peptide structure program (Genetics Computer Group Sequence Analysis Package, Program Manual for the GCG Package, Version 7, 1991) using the algorithm of Jameson and Wolf (CABIOS 4:181, 1988). Specifically, these regions, which are found between BIR1 and BIR2 of all IAPs, are: amino acid 99 to amino acid 170 of HIAP-1, amino acid 123 to amino acid 184 of HIAP-2, and amino acid 116 to amino acid 133 of either XIAP or M-XIAP. These fragments can be generated by standard techniques, e.g., by PCR, and cloned into the pGEX expression vector (Ausubel et al., supra). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel et al. (supra). In order to minimize the potential for obtaining antisera that is non-specific, or exhibits low-affinity binding to IAP, two or three fusions are generated for each protein, and each fusion is injected into at least two rabbits. Antisera are raised by injections in series, preferably including at least three booster injections.

VIII. Identification of Molecules that Modulate IAP Protein Expression

[0128] Isolation of IAP cDNAs also facilitates the identification of molecules that increase or decrease IAP expression. In one approach, candidate molecules are added, in varying concentration, to the culture medium of cells expressing IAP mRNA. IAP expression is then measured, for example, by northern blot analysis (Ausubel et al., supra) using an IAP cDNA, or cDNA fragment, as a hybridization probe (see also Table 5). The level of IAP expression in the presence of the candidate molecule is compared to the level of IAP expression in the absence of the candidate molecule, all other factors (e.g. cell type and culture conditions) being equal.

[0129] The effect of candidate molecules on IAP-mediated apoptosis may, instead, be measured at the level of translation by using the general approach described above with standard protein detection techniques, such as western blotting or immunoprecipitation with an IAP-specific antibody (for example, the IAP antibody described herein).

[0130] Compounds that modulate the level of IAP may be purified, or substantially purified, or may be one component of a mixture of compounds such as an extract or supernatant obtained from cells (Ausubel et al., supra). In an assay of a mixture of compounds, IAP expression is tested against progressively smaller subsets of the compound pool (e.g., produced by standard purification techniques such as HPLC or FPLC) until a single compound or minimal number of effective compounds is demonstrated to modulate IAP expression.

[0131] Compounds may also be screened for their ability to modulate IAP apoptosis inhibiting activity. In this approach, the degree of apoptosis in the presence of a candidate compound is compared to the degree of apoptosis in its absence, under equivalent conditions. Again, the screen may begin with a pool of candidate compounds, from which one or more useful modulator compounds are isolated in a step-wise fashion. Apoptosis activity may be measured by any standard assay, for example, those described herein.

[0132] Another method for detecting compounds that modulate the activity of IAPs is to screen for compounds that interact physically with a given IAP polypeptide. These compounds may be detected by adapting interaction trap expression systems known in the art. These systems detect protein interactions using a transcriptional activation assay and are generally described by Gyuris et al. (Cell 75:791, 1993) and Field et al. (Nature 340:245, 1989), and are commercially available from Clontech (Palo Alto, Calif.). In addition, PCT Publication WO 95/28497 describes an interaction trap assay in which proteins involved in apoptosis, by virtue of their interaction with Bcl-2, are detected. A similar method may be used to identify proteins and other compounds that interact with IAPs.

[0133] Compounds or molecules that function as modulators of IAP-mediated cell death may include peptide and non-peptide molecules such as those present in cell extracts, mammalian serum, or growth medium in which mammalian cells have been cultured.

[0134] A molecule that promotes an increase in IAP expression or IAP activity is considered particularly useful in the invention; such a molecule may be used, for example, as a therapeutic to increase cellular levels of IAP and thereby exploit the ability of IAP polypeptides to inhibit apoptosis.

[0135] A molecule that decreases IAP activity (e.g., by decreasing IAP gene expression or polypeptide activity) may be used to decrease cellular proliferation. This would be advantageous in the treatment of neoplasms (see Table 3, below), or other cell proliferative diseases.

TABLE 3

NORTHERN BLOT IAP RNA LEVELS IN CANCER CELLS*

	xiap	hiap1	hiap2
Promyelocytic Leukemia HL-60	+	+	+
Hela S-3	+	+	+
Chronic Myelogenous Leukemia K-562	+++	+	+++
Lymphoblastic Leukemia MOLT-4	+++	+	+
Burkitt's Lymphoma Raji	+	+(x10)	+
Colorectal Adenocarcinoma SW-480	+++	+++	+++
Lung Carcinoma A-549	+	+	+
Melanoma G-361	+++	+	+

*Levels are indicated by a (+) and are the approximate increase in RNA levels relative to northern blots of RNA from non-cancerous control cell lines. A single plus indicates an estimated increase of at least 1-fold

[0136] Molecules that are found, by the methods described above, to effectively modulate IAP gene expression or polypeptide activity may be tested further in animal models. If they continue to function successfully in an in vivo setting, they may be used as therapeutics to either inhibit or enhance apoptosis, as appropriate.

IX. IAP Therapy

[0137] The level of IAP gene expression correlates with the level of apoptosis. Thus, IAP genes also find use in anti-apoptosis gene therapy. In particular, a functional IAP gene may be used to sustain neuronal cells that undergo apoptosis in the course of a neurodegenerative disease, lymphocytes (i.e., T cells and B cells), or cells that have been injured by ischemia.

[0138] Retroviral vectors, adenoviral vectors, adeno-associated viral vectors, or other viral vectors with the appropriate tropism for cells likely to be involved in apoptosis (for example, epithelial cells) may be used as a gene transfer delivery system for a therapeutic IAP gene construct. Numerous vectors useful for this purpose are generally known (Miller, Human Gene Therapy 15, 1990; Friedman, Science 244:1275, 1989; Eglitis and Anderson, Biotechniques 6:608, 1988; Tolstoshev and Anderson, Curr. Opin. Biotechnol. 1:55, 1990; Sharp, Lancet 337:1277, 1991; Cornetta et al., Nucleic Acid Research and Molecular Biology 36:311, 1987; Anderson, Science 226:401, 1984; Moen, Blood Cells 17:407, 1991; Miller et al., Biotechniques 7:980, 1989; La Salle et al., Science 259:988, 1993; Johnson, Chest 107:77S, 1995). Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg et al., N. Engl. J. Med. 323:370, 1990; Anderson et al., U.S. Pat. No. 5,399,346). Non-viral approaches may also be employed for the introduction of therapeutic DNA into cells otherwise predicted to undergo apoptosis. For example, IAP may be introduced into a neuron or a T cell by lipofection (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413, 1987; Ono et al., Neurosci. Lett. 117:259, 1990; Brigham et al., Am. J. Med. Sci. 298:278, 1989; Staubinger et al., Meth. Enzymol. 101:512, 1983), asialorosonucoid-polylysine conjugation (Wu et al., J. Biol. Chem. 263:14621, 1988; Wu et al., J. Biol. Chem. 264:16985, 1989); or, less preferably, microinjection under surgical conditions (Wolff et al., Science 247:1465, 1990).

[0139] For any of the methods of application described above, the therapeutic IAP DNA construct is preferably applied to the site of the predicted apoptosis event (for example, by injection). However, it may also be applied to tissue in the vicinity of the predicted apoptosis event or to a blood vessel supplying the cells predicted to undergo apoptosis.

[0140] In the constructs described, IAP cDNA expression can be directed from any suitable promoter (e.g., the human cytomegalovirus (CMV), simian virus 40 (SV40), or metallothionein promoters), and regulated by any appropriate mammalian regulatory element. For example, if desired, enhancers known to preferentially direct gene expression in neural cells, T cells, or B cells may be used to direct IAP expression. The enhancers used could include, without limitation, those that are characterized as tissue- or cell-specific in their expression. Alternatively, if an IAP genomic clone is used as a therapeutic construct (for example, following its isolation by hybridization with the IAP cDNA described above), regulation may be mediated by the cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, including any of the promoters or regulatory elements described above.

[0141] Less preferably, IAP gene therapy is accomplished by direct administration of the IAP mRNA or antisense IAP mRNA to a cell that is expected to undergo apoptosis. The mRNA may be produced and isolated by any standard technique, but is most readily produced by in vitro transcription using an IAP cDNA under the control of a high efficiency promoter (e.g., the 17 promoter). Administration of IAP mRNA to malignant cells can be carried out by any of the methods for direct nucleic acid administration described above.

[0142] Ideally, the production of IAP protein by any gene therapy approach will result in cellular levels of IAP that are at least equivalent to the normal, cellular level of IAP in an unaffected cell. Treatment by any IAP-mediated gene therapy approach may be combined with more traditional therapies.

[0143] Another therapeutic approach within the invention involves administration of recombinant IAP protein, either directly to the site of a predicted apoptosis event (for example, by injection) or systemically (for example, by any conventional recombinant protein administration technique). The dosage of IAP depends on a number of factors, including the size and health of the individual patient, but, generally, between 0.1 mg and 100 mg inclusive are administered per day to an adult in any pharmaceutically acceptable formulation.

X. Administration of IAP Polypeptides, IAP Genes, or Modulators of IAP Synthesis or Function

[0144] An IAP protein, gene, or modulator may be administered within a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer IAP to patients suffering from a disease that is caused by excessive apoptosis. Administration may begin before the patient is symptomatic. Any appropriate route of administration may be employed, for example, administration may be parenteral, intravenous, intraarterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration. Therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

[0145] Methods well known in the art for making formulations are found, for example, in "Remington's Pharmaceutical Sciences." Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for IAP modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

[0146] If desired, treatment with an IAP protein, gene, or modulatory compound may be combined with more tradi-

tional therapies for the disease such as surgery, steroid therapy, or chemotherapy for autoimmune disease; antiviral therapy for AIDS; and tissue plasminogen activator (TPA) for ischemic injury.

XI. Detection of Conditions Involving Altered Apoptosis

[0147] IAP polypeptides and nucleic acid sequences find diagnostic use in the detection or monitoring of conditions involving aberrant levels of apoptosis. For example, decrease expression of IAP may be correlated with enhanced apoptosis in humans (see section XII, below). Accordingly, a decrease or increase in the level of IAP production may provide an indication of a deleterious condition. Levels of IAP expression may be assayed by any standard technique. For example, IAP expression in a biological sample (e.g., a biopsy) may be monitored by standard northern blot analysis or may be aided by PCR (see, e.g., Ausubel et al., supra; *PCR Technology: Principles and Applications for DNA Amplification*, H. A. Ehrlich, Ed. Stockton Press, NY; Yap et al. Nucl. Acids. Res. 19:4294, 1991).

[0148] Alternatively, a biological sample obtained from a patient may be analyzed for one or more mutations in the IAP sequences using a mismatch detection approach. Generally, these techniques involve PCR amplification of nucleic acid from the patient sample, followed by identification of the mutation (i.e., mismatch) by either altered hybridization, aberrant electrophoretic gel migration, binding or cleavage mediated by mismatch binding proteins, or direct nucleic acid sequencing. Any of these techniques may be used to facilitate mutant IAP detection, and each is well known in the art; examples of particular techniques are described, without limitation, in Orita et al., Proc. Natl. Acad. Sci. USA 86:2766, 1989; Sheffield et al., Proc. Natl. Acad. Sci. USA 86:232, 1989).

[0149] In yet another approach, immunoassays are used to detect or monitor IAP protein in a biological sample. IAP-specific polyclonal or monoclonal antibodies (produced as described above) may be used in any standard immunoassay format (e.g., ELISA, western blot, or RIA) to measure IAP polypeptide levels. These levels would be compared to wild-type IAP levels, with a decrease in IAP production indicating a condition involving increased apoptosis. Examples of immunoassays are described, e.g., in Ausubel et al., supra. Immunohistochemical techniques may also be utilized for IAP detection. For is example, a tissue sample may be obtained from a patient, sectioned, and stained for the presence of IAP using an anti-IAP antibody and any standard detection system (e.g., one which includes a secondary antibody conjugated to horseradish peroxidase). General guidance regarding such techniques can be found in, e.g., Bancroft and Stevens (*Theory and Practice of Histological Techniques*, Churchill Livingstone, 1982) and Ausubel et al. (supra).

[0150] In one preferred example, a combined diagnostic method may be employed that begins with an evaluation of IAP protein production (for example, by immunological techniques or the protein truncation test (Hogerrorst et al., Nat. Gen. 10:208, 1995)) and also includes a nucleic acid-based detection technique designed to identify more subtle IAP mutations (for example, point mutations). As described above, a number of mismatch detection assays are available to those skilled in the art, and any preferred technique may be used. Mutations in IAP may be detected that either result in loss of IAP expression or loss of IAP biological activity. In a variation of this combined diagnostic method, IAP biological activity is measured as protease activity using any appropriate protease assay system (for example, those described above).

[0151] Mismatch detection assays also provide an opportunity to diagnose an IAP-mediated predisposition to diseases caused by inappropriate apoptosis. For example, a patient heterozygous for an IAP mutation may show no clinical symptoms and yet possess a higher than normal probability of developing one or more types of neurodegenerative, myelodysplastic or ischemic diseases. Given this diagnosis, a patient may take precautions to minimize their exposure to adverse environmental factors (for example, UV exposure or chemical mutagens) and to carefully monitor their medical condition (for example, through frequent physical examinations). This type of IAP diagnostic approach may also be used to detect IAP mutations in prenatal screens. The IAP diagnostic assays described above may be carried out using any biological sample (for example, any biopsy sample or bodily fluid or tissue) in which IAP is normally expressed. Identification of a mutant IAP gene may also be assayed using these sources for test samples.

[0152] Alternatively, an IAP mutation, particularly as part of a diagnosis for predisposition to IAP-associated degenerative disease, may be tested using a DNA sample from any cell, for example, by mismatch detection techniques. Preferably, the DNA sample is subjected to PCR amplification prior to analysis.

[0153] In order to demonstrate the utility of IAP gene sequences as diagnostics and prognostics for cancer, a Human Cancer Cell Line Multiple Tissue Northern Blot (Clontech, Palo Alto, Calif.; #7757-1) was probed. This northern blot contained approximately 2 μg of poly A^+ RNA per lane from eight different human cell lines: (1) promyelocytic leukemia L60, (2) HeLa cell S3, (3) chronic myelogenous leukemia K-562, (4) lymphoblastic leukemia MOLT-4, (5) Burkitt's lymphoma Raji, (6) colorectal adenocarcinoma SW480, (7) lung carcinoma A549, and (8) melanoma G361. As a control, a Human Multiple Tissue Northern Blot (Clontech, Palo Alto, Calif.; #7759-1) was probed. This northern blot contained approximately 2 μg of poly A^+ RNA from eight different human tissues: (1) spleen, (2) thymus, (3) prostate, (4) testis, (5) ovary, (6) small intestine, (7) colon, and (8) peripheral blood leukocytes.

[0154] The northern blots were hybridized sequentially with: (1) a 1.6 kb probe to the xiap coding region, (2) a 375 bp hiap-2 specific probe corresponding to the 3' untranslated region, (3) a 1.3 kb probe to the coding region of hiap-1, which cross-reacts with hiap-2, (4) a 1.0 kb probe derived from the coding region of bcl-2, and (5) a probe to β-actin, which was provided by the manufacturer. Hybridization was carried out at 50° C. overnight, according to the manufacturer's suggestion. The blot was washed twice with 2×SSC, 0.1% SDS at room temperature for 15 minutes and then with 2×SSC, 0.1% SDS at 50° C.

[0155] All cancer lines tested showed increased IAP expression relative to samples from non-cancerous control tissues (Table 3). Expression of xiap was particularly high in HeLa (S-3), chronic myelogenous leukemia (K-562), colorectal adenocarcinoma (SW-480), and melanoma (G-361)

lines. Expression of hiap was extremely high in Burkitt's lymphoma, and was also elevated in colorectal adenocarcinoma. Expression of hiap-2 was particularly high in chronic myelogenous leukemia (K-562) and colorectal adenocarcinoma (SW-480). Expression of bcl-2 was upregulated only in HL-60 leukemia cells.

[0156] These observations suggest that upregulation of the anti-apoptotic IAP genes may be a widespread phenomenon, perhaps occurring much more frequently than upregulation of bcl-2. Furthermore, upregulation may be necessary for the establishment or maintenance of the transformed state of cancerous cells.

[0157] In order to pursue the observation described above, i.e., that hiap-1 is overexpressed in the Raji Burkitt's lymphoma cell line, RT-PCR analysis was performed in multiple Burkitt's lymphoma cell lines. Total RNA was extracted from cells of the Raji, Ramos, EB-3, and Jiyoye cell lines, and as a positive control, from normal placental tissue. The RNA was reverse transcribed, and amplified by PCR with the following set of oligonucleotide primers: 5'-AGTGCGGGTTTTTATTATGTG-3' (SEQ ID NO: 44) and 5'-AGATGACCACAAGGAATAAACACTA-3' (SEQ ID NO: 45), which selectively amplify a hiap-1 cDNA fragment. RT-PCR was conducted using a is Perkin Elmer 480 Thermocycler to carry out 35 cycles of the following program: 94° C. for 1 minute, 50° C. for 1.5 minutes, and 72° C. for a minute. The PCR reaction product was electrophoresed on an agarose gel and stained with ethidium bromide. Amplified cDNA fragments of the appropriate size were clearly visible in all lanes containing Burkitt's lymphoma samples, but absent in the lanes containing the normal placental tissue sample, and absent in lanes containing negative control samples, where template DNA was omitted from the reaction (FIG. **17**).

XII. Accumulation of a 26 kDa Cleavage Protein in Astrocytoma Cells

A. Identification of a 26 kDa Cleavage Protein

[0158] A total protein extract was prepared from Jurkat and astrocytoma cells by sonicating them (×3 for 15 seconds at 4° C.) in 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM PMSF, 1 μg/ml aprotinin, and 5 mM benzamidine. Following sonication, the samples were centrifuged (14,000 RPM in a microfuge) for five minutes. Twenty μg of protein was loaded per well on a 10% SDS-polyacrylamide gel, electrophoresed, and electroblotted by standard methods to PVDF membranes. Western blot analysis, performed as described previously, revealed that the astrocytoma cell-line (CCF-STTG1) abundantly expressed an anti-xiap reactive band of approximately 26 kDa, despite the lack of an apoptotic trigger event (FIG. **18**). In fact, this cell line has been previously characterized as being particularly resistant to standard apoptotic triggers.

[0159] A 26 kDa XIAP-reactive band was also observed under the following is experimental conditions. Jurkat cells (a transformed human T cell line) were induced to undergo apoptosis by exposure to an anti-Fas antibody (1 μg/ml). Identical cultures of Jurkat cells were exposed either to: (1) anti-Fas antibody and cycloheximide (20 μg/ml), (2) tumor necrosis factor alpha (TNF-α, at 1,000 U/ml), or (3) TNF-α and cycloheximide (20 μg/ml). All cells were harvested 6 hours after treatment began. In addition, as a negative control, anti-Fas antibody was added to an extract after the cells were harvested. The cells were harvested in SDS sample buffer, electrophoresed on a 12.5% SDS polyacrylamide gel, and electroblotted onto PVDF membranes using standard methods. The membranes were immunostained with a rabbit polyclonal anti-XIAP antibody at 1:1000 for 1 hour at room temperature. Following four 15 minute washes, a goat anti-rabbit antibody conjugated to horse-radish peroxidase was applied at room temperature for 1 hour. Unbound secondary antibody was washed away, and chemiluminescent detection of XIAP protein was performed. The western blot revealed the presence of the full-length, 55 kDa XIAP protein, both in untreated and treated cells. In addition, a novel, approximately 26 kDa XIAP-reactive band was also observed in apoptotic cell extracts, but not in the control, untreated cell extracts (FIG. **19**).

[0160] Cleavage of XIAP occurs in a variety of cell types, including other cancer cell lines such as HeLa. The expression of the 26 kDa XIAP cleavage product was demonstrated in HeLa cells as follows. HeLa cells were treated with either: (1) cyclohexamide (20 μg/ml), (2) anti-Fas antibody (1 μg/ml), (3) anti-Fas antibody (1 μg/ml) and cyclohexamide (20 μg/ml), (4) TNFα (1,000 U/ml), or (5) TNFα (1,000 U/ml) and cyclohexamide (20 μg/ml). All cells were harvested 18 hours after treatment began. As above, anti-Fas antibody was added to an extract after the cells were harvested. HeLa cells were harvested, and the western blot was probed under the same conditions as used to visualize xiap-reactive bands from Jurkat cell samples. A 26 kDa XIAP band was again seen in the apoptotic cell preparations (FIG. **20**). Furthermore, the degree of XIAP cleavage correlated positively with the extent of apoptosis. Treatment of HeLa cells with cycloheximide or TNFα alone caused only minor apoptosis, and little cleavage product was observed. If the cells were treated with the anti-Fas antibody, a greater amount of cleavage product was apparent. These data indicate that XIAP is cleaved in more than one cell type and in response to more than one type of apoptotic trigger.

B. Time Course of Expression

[0161] The time course over which the 26 kDa cleavage product accumulates was examined by treating HeLa and Jurkat cells with anti-Fas antibody (1 μg/ml) and harvesting them either immediately, or 1, 2, 3, 5, 10, or 22 hours after treatment. Protein extracts were prepared and western blot analysis was performed as described above. Both types of cells accumulated increasing quantities of the 26 kDa cleavage product over the time course examined (FIGS. **21**A and **21**B).

C. Subcellular Localization of the 26 kDa XIAP Cleavage Product

[0162] In order to determine the subcellular location of the 26 kDa cleavage product, Jurkat cells were induced to undergo apoptosis by exposure to anti-Fas antibody (1 μg/ml) and were then harvested either immediately, 3 hours, or 7 hours later. Total protein extracts were prepared, as described above, from cells harvested at each time point. In order to prepare nuclear and cytoplasmic cell extracts, apoptotic Jurkat cells were washed with isotonic Tris buffered saline (pH 7.0) and lysed by freezing and thawing five times in cell extraction buffer (50 mM PIPES, 50 mM KCl, 5 mM EGTA, 2 mM $MgCl_2$, 1 mM DTT, and 20 μM cytochalasin B). Nuclei were pelleted by centrifugation and

resuspended in isotonic Tris (pH 7.0) and frozen at −80° C. The cytoplasmic fraction of the extract was processed further by centrifugation at 60,000 RPM in a TA 100.3 rotor for 30 minutes. Supernatants were removed and frozen at −80° C. Samples of both nuclear and cytoplasmic fractions were loaded on a 12.5% SDS-polyacrylamide gel, and electroblotted onto PVDF membranes. Western blot analysis was then performed using either an anti-CPP32 antibody (Transduction Laboratories Lexington, Ky.; FIG. **22**A) or the rabbit anti-XIAP antibody described above (FIG. **22**B).

[**0163**] The anti-CPP32 antibody, which recognizes the CPP32 protease (also known as YAMA or apopain) partitioned almost exclusively in the cytoplasmic fraction. The 55 kDa XIAP protein localized exclusively in the cytoplasm of apoptotic cells, in agreement with the studies presented above, where XIAP protein in normal, healthy COS cells was seen to localize, by immunofluoresence microscopy, to the cytoplasm. In contrast, the 26 kDa cleavage product localized exclusively to the nuclear fraction of apoptotic Jurkat cells. Taken together, these observations suggest that the anti-apoptotic component of XIAP could be the 26 kDa cleavage product, which exerts its influence within the nucleus.

D. In Vitro Cleavage of XIAP Protein and Characterization of the Cleavage Product

[**0164**] For this series of experiments, XIAP protein was labeled with ^{35}S using the plasmid pcDNA3-6myc-xiap, T7 RNA polymerase, and a coupled transcription/translation kit (Promega) according to the manufacturer's instructions. Radioactively labeled XIAP protein was separated from unincorporated methionine by column chromatography using Sephadex G-50™. In addition, extracts of apoptotic Jurkat cells were prepared following treatment with anti-Fas antibody (1 μg/ml) for three hours. To prepare the extracts, the cells were lysed in Triton X-100 buffer (1% Triton X-100, 25 mM Tris HCl) on ice for two hours and then microcentrifuged for 5 minutes. The soluble extract was retained (and was labelled "TX100"). Cells were lysed in cell extraction buffer with freeze/thawing. The soluble cytoplasmic fraction was set aside (and labelled "CEB"). Nuclear pellets from the preparation of the CEB cytoplasmic fraction were solubilized with Triton X-100 buffer, microcentrifuged, and the soluble fractions, which contains primarily nuclear DNA, was retained (and labelled "CEB-TX100"). Soluble cell extract was prepared by lysing cells with NP-40 buffer, followed by microcentrifugation for 5 minutes (and was labeled NP-40). In vitro cleavage was performed by incubating 16 μl of each extract (CEB, TX-100, CEB-TX100, and NP-40) with 4 μl of in vitro translated XIAP protein at 37° C. for 7 hours. Negative controls, containing only TX100 buffer or CEB buffer were also included. The proteins were separated on a 10% SDS-polyacrylamide gel, which was then dried and exposed to X-ray film overnight.

[**0165**] In vitro cleavage of XIAP was apparent in the CEB extract. The observed molecular weight of the cleavage product was approximately 36 kDa (FIG. **23**). The 10 kDa shift in the size of the cleavage product indicates that the observed product is derived from the amino-terminus of the recombinant protein, which contains six copies of the myc epitope (10 kDa). It thus appears that the cleavage product possesses at least two of the BIR domains, and that it is localized to the nucleus.

XIII. Treatment of HIV Infected Individuals

[**0166**] The expression of hiap-1 and hiap-2 is decreased significantly in HIV-infected human cells. Furthermore, this decrease precedes apoptosis. Therefore, administration of HIAP-1, HIAP-2, genes encoding these proteins, or compounds that upregulate these genes can be used to prevent T cell attrition in HIV-infected patients. The following assay may also be used to screen for compounds that alter hiap-1 and hiap-2 expression, and which also prevent apoptosis.

[**0167**] Cultured mature lymphocyte $CD4^+$ T cell lines (H9, labelled "a"; CEM/CM-3, labelled "b"; 6T-CEM, labelled "c"; and Jurkat, labelled "d" in FIGS. **13**A and **13**B), were examined for signs of apoptosis (FIG. **13**A) and hiap gene expression (FIG. **13**B) after exposure to mitogens or HIV infection. Apoptosis was demonstrated by the appearance of DNA "laddering" upon gel electrophoresis and gene expression was assessed by PCR. The results obtained from normal (non-infected, non-mitogen stimulated) cells are shown in each lane labelled "1" in FIGS. **13**A and **13**B. The results obtained 24 hours after PHA/PMA (phytohemagglutinin/phorbol ester) stimulation are shown in each lane labelled "2". The results obtained 24 hours after HIV strain III_B infection are shown in each lane labelled "3". The "M" refers to standard DNA markers (the 123 bp ladder in FIG. **13**B, and the lambda HindIII ladder in FIG. **13**A (both from Gibco-BRL)). DNA ladders (Prigent et al., J. Immunol. Meth., 160:139, 1993), which indicate apoptosis, are evident when DNA from the samples described above are electrophoresed on an ethidium bromide-stained agarose gel (FIG. **13**A). The sensitivity and degree of apoptosis of the four T cell lines tested varies following mitogen stimulation and HIV infection.

[**0168**] In order to examine hiap gene expression, total RNA was prepared from the cultured cells and reverse transcribed using oligo-dT priming. The RT cDNA products were amplified by PCR using specific primers (as shown in Table 5) for the detection of hiap-2a, hiap-2b and hiap-1. The PCR was conducted using a Perkin Elmer 480 thermocycler with 35 cycles of the following program: 94° C. for one minute, 55° C. for 2 minutes and 72° C. for 1.5 minutes. The RT-PCR reaction products were electrophoresed on a 1% agarose gel, which was stained with ethidium bromide. Absence of hiap-2 transcripts is noted in all four cell lines 24 hours after HIV infection. In three of four cell lines (all except H9), the hiap-1 gene is also dramatically down-regulated after HIV infection. PHA/PMA mitogen stimulation also appears to decrease hiap gene expression; particularly of hiap-2 and to a lesser extent, of hiap-1. The data from these experiments is summarized in Table 5. The expression of β-actin was consistent in all cell lines tested, indicating that there is not a flaw in the RT-PCR assay that could account for the decrease in hiap gene expression.

TABLE 4

OLIGONUCLEOTIDE PRIMERS FOR THE SPECIFIC RT-PCR AMPLIFICATION OF UNIQUE IAP GENES

IAP Gene	Forward Primer (nucleotide position*)	Reverse Primer (nucleotide position*)	Size of Product (bp)
h-xiap	p2415 (876–896)	p2449 (1291–1311)	435
m-xiap	p2566 (458–478)	p2490 (994–1013)	555

TABLE 4-continued

OLIGONUCLEOTIDE PRIMERS FOR THE SPECIFIC RT-PCR AMPLIFICATION OF UNIQUE IAP GENES

IAP Gene	Forward Primer (nucleotide position*)	Reverse Primer (nucleotide position*)	Size of Product (bp)
h-hiap1	p2465 (827–847)	p2464 (1008–1038)	211
m-hiap1	p2687 (747–767)	p2684 (1177–1197)	450
hiap2	p2595 (1562–1585)	p2578 (2339–2363)	801[a] 618[b]
m-hiap2	p2693 (1751–1772)	p2734 (2078–2100)	349

*Nucleotide position as determined from FIGS. 1–4 for each IAP gene
[a]PCR product size of hiap2a
[b]PCR product size of hiap2b

[0169]

TABLE 5

APOPTOSIS AND HIAP GENE EXPRESSION IN CULTURED T-CELLS FOLLOWING MITOGEN STIMULATION OR HIV INFECTION

Cell Line	Condition	Apoptosis	hiap1	hiap2
H9	not stimulated	–	+	±
	PHA/PMA stimulated	+++	+	±
	HIV infected	++	+	–
CEM/CM-3	not stimulated	–	+	±
	PHA/PMA stimulated	±	+	–
	HIV infected	±	–	–
6T-CEM	not stimulated	–	+	+
	PHA/PMA stimulated	±	–	–
	HIV infected	+	–	–
Jurkat	not stimulated	–	+	++
	PHA/PMA stimulated	+	+	+
	HIV infected	±	–	–

XIV. Assignment of xiap, hiap-1, and hiap-2 to Chromosomes Xq25 and 11q22-23 by Fluorescence In Situ Hybridization (FISH)

[0170] Fluorescence in situ hybridization (FISH) was used to identify the chromosomal location of xiap, hiap-1 and hiap-2. The probes used were cDNAs cloned in plasmid vectors: the 2.4 kb xiap clone included 1493 bp of coding sequence, 34 bp of 5' UTR (untranslated region) and 913 bp of 3'UTR; the hiap-1 cDNA was 3.1 kb long and included 1812 bp coding and 1300 bp of 3' UTR; and the hiap-2 clone consisted of 1856 bp of coding and 1200 bp of 5' UTR. A total of 1 μg of probe DNA was labelled with biotin by nick translation (BRL). Chromosome spreads prepared from a normal peripheral blood culture were denatured for 2 minutes at 70° C. in 50% formamide/2×SSC and subsequently hybridized with the biotin labelled DNA probe for 18 hours at 37° C. in a solution consisting of 2×SSC/70% formamide/10% dextran sulfate. After hybridization, the spreads were washed in 2×SSC/50% formamide, followed by a wash in 2×SSC at 42° C. The biotin labelled DNA was detected by fluorescein isothiocyanate (FITC) conjugated avidin antibodies and anti-avidin antibodies (ONCOR detection kit), according to the manufacturer's instructions. Chromosomes were counterstained with propidium iodide and examined with a Olympus BX60 epifluorescence microscope. For chromosome identification, the slides with recorded labelled metaphase spreads were destained, dehydrated, dried, digested with trypsin for 30 seconds and stained with 4% Giemsa stain for 2 minutes. The chromosome spreads were relocated and the images were compared.

[0171] A total of 101 metaphase spreads were examined with the xiap probe, as described above. Symmetrical fluorescent signals on either one or both homologs of chromosome Xq25 were observed in 74% of the cells analyzed. Following staining with hiap-1 and hiap-2 probes, 56 cells were analyzed and doublet signals in the region 11q22-23 were observed in 83% of cells examined. The xiap gene was mapped to Xq25 while the hiap- and hiap-2 genes were mapped at the border of 11q22 and 11q23 bands.

[0172] These experiments confirmed the location of the xiap gene on chromosome Xq25. No highly consistent chromosomal abnormalities involving band Xq25 have been reported so far in any malignancies. However, deletions within this region are associated with a number of immune system defects including X-linked lymphoproliferative disease (Wu et al., Genomics 17:163, 1993).

[0173] Cytogenetic abnormalities of band 11q23 have been identified in more than 50% of infant leukemias regardless of the phenotype (Martinez-Climet et al., Leukaemia 9:1299, 1995). Rearrangements of the MLL Gene (mixed lineage leukemia or myeloid lymphoid leukemia; Ziemin Van der Poel et al., Proc. Natl. Acad. Sci. USA 88:10735, 1991) have been detected in 80% of cases with 11q23 translocation, however patients whose rearrangements clearly involved regions other than the MLL gene were also reported (Kobayashi et al., Blood 82:547, 1993). Thus, the IAP genes may follow the Bcl-2 paradigm, and would therefore play an important role in cancer transformation.

XV. Preventive Anti-Apoptotic Therapy

[0174] In a patient diagnosed to be heterozygous for an IAP mutation or to be susceptible to IAP mutations (even if those mutations do not yet result in alteration or loss of IAP biological activity), or a patient diagnosed as HIV positive, any of the above therapies may be administered before the occurrence of the disease phenotype. For example, the therapies may be provided to a patient who is HIV positive but does not yet show a diminished T cell count or other overt signs of AIDS. In particular, compounds shown to increase IAP expression or IAP biological activity may be administered by any standard dosage and route of administration (see above). Alternatively, gene therapy using an IAP expression construct may be undertaken to reverse or prevent the cell defect prior to the development of the degenerative disease.

[0175] The methods of the instant invention may be used to reduce or diagnose the disorders described herein in any mammal, for example, humans, domestic pets, or livestock. Where a non-human mammal is treated or diagnosed, the IAP polypeptide, nucleic acid, or antibody employed is preferably specific for that species.

OTHER EMBODIMENTS

[0176] In other embodiments, the invention includes any protein which is substantially identical to a mammalian IAP polypeptides (FIGS. **1-6**; SEQ ID NOs: 1-42); such homologs include other substantially pure naturally-occurring mammalian IAP proteins as well as allelic variants;

natural mutants; induced mutants; DNA sequences which encode proteins and also hybridize to the IAP DNA sequences of FIGS. **1-6** (SEQ ID NOS: 1-42) under high stringency conditions or, less preferably, under low stringency conditions (e.g., washing at 2×SSC at 40° C. with a probe length of at least 40 nucleotides); and proteins specifically bound by antisera directed to a IAP polypeptide. The term also includes chimeric polypeptides that include a IAP portion.

[**0177**] The invention further includes analogs of any naturally-occurring IAP polypeptide. Analogs can differ from the naturally-occurring IAP protein by amino acid sequence differences, by post-translational modifications, or by both. Analogs of the invention will generally exhibit at least 85%, more preferably 90%, and most preferably 95% or even 99% identity with all or part of a naturally occurring IAP amino acid sequence. The length of sequence comparison is at least 15 amino acid residues, preferably at least 25 amino acid residues, and more preferably more than 35 amino acid residues. Modifications include in vivo and in vitro chemical derivatization of polypeptides, e.g., acetylation, carboxylation, phosphorylation, or glycosylation; such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. Analogs can also differ from the naturally-occurring IAP polypeptide by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by site-specific mutagenesis as described in Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual* (2d ed.), CSH Press, 1989, or Ausubel et al., supra). Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amino acids, e.g., D-amino acids or nonnaturally occurring or synthetic amino acids, e.g., β or γ amino acids. In addition to full-length polypeptides, the invention also includes IAP polypeptide fragments. As used herein, the term "fragment," means at least 20 contiguous amino acids, preferably at least 30 contiguous amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least 60 to 80 or more contiguous amino acids. Fragments of IAP polypeptides can be generated by methods known to those skilled in the art or may result from normal protein processing (e.g., removal of amino acids from the nascent polypeptide that are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events).

[**0178**] Preferable fragments or analogs according to the invention are those which facilitate specific detection of a IAP nucleic acid or amino acid sequence in a sample to be diagnosed. Particularly useful IAP fragments for this purpose include, without limitation, the amino acid fragments shown in Table 2.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 45

<210> SEQ ID NO 1
<211> LENGTH: 46
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic based on Homo sapiens, Mus musculus, Drosophila melanogaster, Cydia pomonella, and Orgyia pseudotsugata
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)...(45)
<223> OTHER INFORMATION: Xaa at positions 2, 3, 4, 5, 6, 7, 9, 10, 11, 17, 18, 19, 20, 21, 23, 25, 30, 31, 32, 34, 35, 38, 39, 40, 41, 42, and 45 may be any amino acid.
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (8)...(8)
<223> OTHER INFORMATION: Xaa at position 8 is Glu or Asp.
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (14)...(14)
<223> OTHER INFORMATION: Xaa at position 14 is Val or Ile.
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (22)...(22)
<223> OTHER INFORMATION: Xaa at position 22 is Val or Ile.

<400> SEQUENCE: 1

Glu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Lys Xaa Cys Met
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Phe Xaa Pro Cys Gly His Xaa Xaa Xaa
20 25 30

Cys Xaa Xaa Cys Ala Xaa Xaa Xaa Xaa Xaa Xaa Cys Pro Xaa Cys

-continued

35 40 45

<210> SEQ ID NO 2
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(66)
<223> OTHER INFORMATION: Xaa at positions 1, 2, 3, 6, 9, 10, 14, 15, 18, 19, 20, 21, 24, 30, 32, 33, 35, 37, 40, 42, 43, 44, 45, 46, 47, 49, 50, 51, 53, 54, 55, 56, 57, 59, 60, 61, 62, 64 and 66 may be any amino acid.
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (13)...(17)
<223> OTHER INFORMATION: Xaa at positions 13, 16 and 17 may be any amino acid or may be absent.
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic based on Homo sapiens, Mus musculus, Drosophila melanogaster, Cydia pomonella, and Orgyia pseudotsugata

<400> SEQUENCE: 2

Xaa Xaa Xaa Arg Leu Xaa Thr Phe Xaa Xaa Trp Pro Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Leu Ala Xaa Ala Gly Phe Tyr Tyr Xaa Gly Xaa
20 25 30

Xaa Asp Xaa Val Xaa Cys Phe Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Trp
35 40 45

Xaa Xaa Xaa Asp Xaa Xaa Xaa Xaa Xaa His Xaa Xaa Xaa Xaa Pro Xaa
50 55 60

Cys Xaa Phe Val
65

<210> SEQ ID NO 3
<211> LENGTH: 2540
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: variation
<222> LOCATION: (2540)...(2540)
<223> OTHER INFORMATION: N may be any nucleotide

<400> SEQUENCE: 3

gaaaaggtgg acaagtccta ttttcaagag aagatgactt ttaacagttt tgaaggatct 60

aaaacttgtg tacctgcaga catcaataag gaagaagaat ttgtagaaga gtttaataga 120

ttaaaaactt ttgctaattt tccaagtggt agtcctgttt cagcatcaac actggcacga 180

gcagggtttc tttatactgg tgaaggagat accgtgcggt gctttagttg tcatgcagct 240

gtagatagat ggcaatatgg agactcagca gttggaagac acaggaaagt atccccaaat 300

tgcagattta tcaacggctt ttatcttgaa aatagtgcca cgcagtctac aaattctggt 360

atccagaatg gtcagtacaa agttgaaaac tatctgggaa gcagagatca ttttgcctta 420

gacaggccat ctgagacaca tgcagactat cttttgagaa ctgggcaggt tgtagatata 480

tcagacacca tatacccgag gaaccctgcc atgtattgtg aagaagctag attaaagtcc 540

tttcagaact ggccagacta tgctcaccta accccaagag agttagcaag tgctggactc 600

tactacacag gtattggtga ccaagtgcag tgcttttgtt gtggtggaaa actgaaaaat 660

tgggaacctt gtgatcgtgc ctggtcagaa cacaggcgac actttcctaa ttgcttcttt 720

-continued

gttttgggcc ggaatcttaa tattcgaagt gaatctgatg ctgtgagttc tgataggaat 780

ttcccaaatt caacaaatct tccaagaaat ccatccatgg cagattatga agcacggatc 840

tttacttttg ggacatggat atactcagtt aacaaggagc agcttgcaag agctggattt 900

tatgctttag gtgaaggtga taaagtaaag tgctttcact gtggaggagg gctaactgat 960

tggaagccca gtgaagaccc ttgggaacaa catgctaaat ggtatccagg gtgcaaatat 1020

ctgttagaac agaagggaca agaatatata aacaatattc atttaactca ttcacttgag 1080

gagtgtctgg taagaactac tgagaaaaca ccatcactaa ctagaagaat tgatgatacc 1140

atcttccaaa atcctatggt acaagaagct atacgaatgg ggttcagttt caaggacatt 1200

aagaaaataa tggaggaaaa aattcagata tctgggagca actataaatc acttgaggtt 1260

ctggttgcag atctagtgaa tgctcagaaa gacagtatgc aagatgagtc aagtcagact 1320

tcattacaga aagagattag tactgaagag cagctaaggc gcctgcaaga ggagaagctt 1380

tgcaaaatct gtatggatag aaatattgct atcgtttttg ttccttgtgg acatctagtc 1440

acttgtaaac aatgtgctga agcagttgac aagtgtccca tgtgctacac agtcattact 1500

ttcaagcaaa aaatttttat gtcttaatct aactctatag taggcatgtt atgttgttct 1560

tattaccctg attgaatgtg tgatgtgaac tgactttaag taatcaggat tgaattccat 1620

tagcatttgc taccaagtag gaaaaaaaat gtacatggca gtgttttagt tggcaatata 1680

atctttgaat ttcttgattt ttcagggtat tagctgtatt atccattttt tttactgtta 1740

tttaattgaa accatagact aagaataaga agcatcatac tataactgaa cacaatgtgt 1800

attcatagta tactgattta atttctaagt gtaagtgaat taatcatctg gatttttat 1860

tcttttcaga taggcttaac aaatggagct ttctgtatat aaatgtggag attagagtta 1920

atctccccaa tcacataatt tgttttgtgt gaaaaaggaa taaattgttc catgctggtg 1980

gaaagataga gattgttttt agaggttggt tgttgtgttt taggattctg tccattttct 2040

tgtaaaggga taaacacgga cgtgtgcgaa atatgtttgt aaagtgattt gccattgttg 2100

aaagcgtatt taatgataga atactatcga gccaacatgt actgacatgg aaagatgtca 2160

gagatatgtt aagtgtaaaa tgcaagtggc gggacactat gtatagtctg agccagatca 2220

aagtatgtat gttgttaata tgcatagaac gagagatttg gaaagatata caccaaactg 2280

ttaaatgtgg tttctcttcg gggaggggggg gattgggggga ggggccccag aggggtttta 2340

gaggggcctt ttcactttcg actttttca ttttgttctg ttcggatttt ttataagtat 2400

gtagaccccg aagggtttta tgggaactaa catcagtaac ctaaccccg tgactatcct 2460

gtgctcttcc tagggagctg tgttgtttcc cacccaccac ccttccctct gaacaaatgc 2520

ctgagtgctg gggcactttn 2540

<210> SEQ ID NO 4
<211> LENGTH: 497
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met Thr Phe Asn Ser Phe Glu Gly Ser Lys Thr Cys Val Pro Ala Asp
 1   5   10   15

Ile Asn Lys Glu Glu Glu Phe Val Glu Glu Phe Asn Arg Leu Lys Thr
            20   25   30

-continued

```
Phe Ala Asn Phe Pro Ser Gly Ser Pro Val Ser Ala Ser Thr Leu Ala
        35                  40                  45

Arg Ala Gly Phe Leu Tyr Thr Gly Glu Gly Asp Thr Val Arg Cys Phe
    50                  55                  60

Ser Cys His Ala Ala Val Asp Arg Trp Gln Tyr Gly Asp Ser Ala Val
65                  70                  75                  80

Gly Arg His Arg Lys Val Ser Pro Asn Cys Arg Phe Ile Asn Gly Phe
                85                  90                  95

Tyr Leu Glu Asn Ser Ala Thr Gln Ser Thr Asn Ser Gly Ile Gln Asn
            100                 105                 110

Gly Gln Tyr Lys Val Glu Asn Tyr Leu Gly Ser Arg Asp His Phe Ala
        115                 120                 125

Leu Asp Arg Pro Ser Glu Thr His Ala Asp Tyr Leu Leu Arg Thr Gly
    130                 135                 140

Gln Val Val Asp Ile Ser Asp Thr Ile Tyr Pro Arg Asn Pro Ala Met
145                 150                 155                 160

Tyr Cys Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr
                165                 170                 175

Ala His Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr
            180                 185                 190

Gly Ile Gly Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys
        195                 200                 205

Asn Trp Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe
    210                 215                 220

Pro Asn Cys Phe Phe Val Leu Gly Arg Asn Leu Asn Ile Arg Ser Glu
225                 230                 235                 240

Ser Asp Ala Val Ser Ser Asp Arg Asn Phe Pro Asn Ser Thr Asn Leu
                245                 250                 255

Pro Arg Asn Pro Ser Met Ala Asp Tyr Glu Ala Arg Ile Phe Thr Phe
            260                 265                 270

Gly Thr Trp Ile Tyr Ser Val Asn Lys Glu Gln Leu Ala Arg Ala Gly
        275                 280                 285

Phe Tyr Ala Leu Gly Glu Gly Asp Lys Val Lys Cys Phe His Cys Gly
    290                 295                 300

Gly Gly Leu Thr Asp Trp Lys Pro Ser Glu Asp Pro Trp Glu Gln His
305                 310                 315                 320

Ala Lys Trp Tyr Pro Gly Cys Lys Tyr Leu Leu Glu Gln Lys Gly Gln
                325                 330                 335

Glu Tyr Ile Asn Asn Ile His Leu Thr His Ser Leu Glu Glu Cys Leu
            340                 345                 350

Val Arg Thr Thr Glu Lys Thr Pro Ser Leu Thr Arg Arg Ile Asp Asp
        355                 360                 365

Thr Ile Phe Gln Asn Pro Met Val Gln Glu Ala Ile Arg Met Gly Phe
    370                 375                 380

Ser Phe Lys Asp Ile Lys Lys Ile Met Glu Glu Lys Ile Gln Ile Ser
385                 390                 395                 400

Gly Ser Asn Tyr Lys Ser Leu Glu Val Leu Val Ala Asp Leu Val Asn
                405                 410                 415

Ala Gln Lys Asp Ser Met Gln Asp Glu Ser Ser Gln Thr Ser Leu Gln
            420                 425                 430
```

-continued

Lys Glu Ile Ser Thr Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys
435 440 445

Leu Cys Lys Ile Cys Met Asp Arg Asn Ile Ala Ile Val Phe Val Pro
450 455 460

Cys Gly His Leu Val Thr Cys Lys Gln Cys Ala Glu Ala Val Asp Lys
465 470 475 480

Cys Pro Met Cys Tyr Thr Val Ile Thr Phe Lys Gln Lys Ile Phe Met
485 490 495

Ser

<210> SEQ ID NO 5
<211> LENGTH: 2676
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: variation
<222> LOCATION: (2470)...(2470)
<223> OTHER INFORMATION: N may be any nucleotide
<220> FEATURE:
<221> NAME/KEY: variation
<222> LOCATION: (2476)...(2476)
<223> OTHER INFORMATION: N may be any nucleotide
<220> FEATURE:
<221> NAME/KEY: variation
<222> LOCATION: (2483)...(2483)
<223> OTHER INFORMATION: N may be any nucleotide
<220> FEATURE:
<221> NAME/KEY: variation
<222> LOCATION: (2602)...(2602)
<223> OTHER INFORMATION: N may be any nucleotide

<400> SEQUENCE: 5

tccttgagat gtatcagtat aggatttagg atctccatgt tggaactcta aatgcataga 60

aatggaaata atggaaattt ttcattttgg cttttcagcc tagtattaaa actgataaaa 120

gcaaagccat gcacaaaact acctccctag agaaaggcta gtccctttc ttccccattc 180

atttcattat gaacatagta gaaaacagca tattcttatc aaatttgatg aaaagcgcca 240

acacgtttga actgaaatac gacttgtcat gtgaactgta ccgaatgtct acgtattcca 300

cttttcctgc tggggttcct gtctcagaaa ggagtcttgc tcgtgctggt ttctattaca 360

ctggtgtgaa tgacaaggtc aaatgcttct gttgtggcct gatgctggat aactggaaaa 420

gaggagacag tcctactgaa aagcataaaa agttgtatcc tagctgcaga ttcgttcaga 480

gtctaaattc cgttaacaac ttggaagcta cctctcagcc tacttttcct tcttcagtaa 540

cacattccac acactcatta cttccgggta cagaaaacag tggatatttc cgtggctctt 600

attcaaactc tccatcaaat cctgtaaact ccagagcaaa tcaagaattt tctgccttga 660

tgagaagttc ctacccctgt ccaatgaata acgaaaatgc cagattactt acttttcaga 720

catggccatt gacttttctg tcgccaacag atctggcacg agcaggcttt tactacatag 780

gacctggaga cagagtggct tgctttgcct gtggtggaaa attgagcaat tgggaaccga 840

aggataatgc tatgtcagaa cacctgagac attttcccaa atgcccattt atagaaaatc 900

agcttcaaga cacttcaaga tacacagttt ctaatctgag catgcagaca catgcagccc 960

gctttaaaac attctttaac tggccctcta gtgttctagt taatcctgag cagcttgcaa 1020

gtgcgggttt ttattatgtg ggtaacagtg atgatgtcaa atgcttttgc tgtgatggtg 1080

gactcaggtg ttgggaatct ggagatgatc catgggttca acatgccaag tggtttccaa 1140

-continued

```
ggtgtgagta cttgataaga attaaaggac aggagttcat ccgtcaagtt caagccagtt   1200

accctcatct acttgaacag ctgctatcca catcagacag cccaggagat gaaaatgcag   1260

agtcatcaat tatccatttg gaacctggag aagaccattc agaagatgca atcatgatga   1320

atactcctgt gattaatgct gccgtggaaa tgggctttag tagaagcctg gtaaaacaga   1380

cagttcagag aaaaatccta gcaactggag agaattatag actagtcaat gatcttgtgt   1440

tagacttact caatgcagaa gatgaaataa gggaagagga gagagaaaga gcaactgagg   1500

aaaaagaatc aaatgattta ttattaatcc ggaagaatag aatggcactt tttcaacatt   1560

tgacttgtgt aattccaatc ctggatagtc tactaactgc cggaattatt aatgaacaag   1620

aacatgatgt tattaaacag aagacacaga cgtctttaca agcaagagaa ctgattgata   1680

cgattttagt aaaaggaaat attgcagcca ctgtattcag aaactctctg caagaagctg   1740

aagctgtgtt atatgagcat ttatttgtgc aacaggacat aaaatatatt cccacagaag   1800

atgtttcaga tctaccagtg gaagaacaat tgcggagact accagaagaa agaacatgta   1860

aagtgtgtat ggacaaagaa gtgtccatag tgtttattcc ttgtggtcat ctagtagtat   1920

gcaaagattg tgctccttct ttaagaaagt gtcctatttg taggagtaca atcaagggta   1980

cagttcgtac atttctttca tgaagaagaa ccaaaacatc gtctaaactt tagaattaat   2040

ttattaaatg tattataact ttaactttta tcctaatttg gtttccttaa aatttttatt   2100

tatttacaac tcaaaaaaca ttgttttgtg taacatattt atatatgtat ctaaaccata   2160

tgaacatata ttttttagaa actaagagaa tgataggctt ttgttcttat gaacgaaaaa   2220

gaggtagcac tacaaacaca atattcaatc caaatttcag cattattgaa attgtaagtg   2280

aagtaaaact taagatattt gagttaacct ttaagaattt taaatatttt ggcattgtac   2340

taataccggg aacatgaagc caggtgtggt ggtatgtacc tgtagtccca ggctgaggca   2400

agagaattac ttgagcccag gagtttgaat ccatcctggg cagcatactg agaccctgcc   2460

tttaaaaacn aacagnacca aanccaaaca ccagggacac atttctctgt ctttttgat   2520

cagtgtccta tacatcgaag gtgtgcatat atgttgaatc acattttagg gacatggtgt   2580

ttttataaag aattctgtga gnaaaaattt aataaagcaa ccaaattact cttaaaaaaa   2640

aaaaaaaaaa aaaaaactcg aggggcccgt accaat                             2676
```

<210> SEQ ID NO 6
<211> LENGTH: 604
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

```
Met Asn Ile Val Glu Asn Ser Ile Phe Leu Ser Asn Leu Met Lys Ser
 1               5                  10                  15

Ala Asn Thr Phe Glu Leu Lys Tyr Asp Leu Ser Cys Glu Leu Tyr Arg
            20                  25                  30

Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro Val Ser Glu Arg
        35                  40                  45

Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys Val
    50                  55                  60

Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Arg Gly Asp
65                  70                  75                  80

Ser Pro Thr Glu Lys His Lys Lys Leu Tyr Pro Ser Cys Arg Phe Val
```

-continued

```
                85                  90                  95

Gln Ser Leu Asn Ser Val Asn Asn Leu Glu Ala Thr Ser Gln Pro Thr
            100                 105                 110

Phe Pro Ser Ser Val Thr His Ser Thr His Ser Leu Leu Pro Gly Thr
        115                 120                 125

Glu Asn Ser Gly Tyr Phe Arg Gly Ser Tyr Ser Asn Ser Pro Ser Asn
    130                 135                 140

Pro Val Asn Ser Arg Ala Asn Gln Glu Phe Ser Ala Leu Met Arg Ser
145                 150                 155                 160

Ser Tyr Pro Cys Pro Met Asn Asn Glu Asn Ala Arg Leu Leu Thr Phe
                165                 170                 175

Gln Thr Trp Pro Leu Thr Phe Leu Ser Pro Thr Asp Leu Ala Arg Ala
            180                 185                 190

Gly Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys
        195                 200                 205

Gly Gly Lys Leu Ser Asn Trp Glu Pro Lys Asp Asn Ala Met Ser Glu
    210                 215                 220

His Leu Arg His Phe Pro Lys Cys Pro Phe Ile Glu Asn Gln Leu Gln
225                 230                 235                 240

Asp Thr Ser Arg Tyr Thr Val Ser Asn Leu Ser Met Gln Thr His Ala
                245                 250                 255

Ala Arg Phe Lys Thr Phe Phe Asn Trp Pro Ser Ser Val Leu Val Asn
            260                 265                 270

Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Asn Ser Asp
        275                 280                 285

Asp Val Lys Cys Phe Cys Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser
    290                 295                 300

Gly Asp Asp Pro Trp Val Gln His Ala Lys Trp Phe Pro Arg Cys Glu
305                 310                 315                 320

Tyr Leu Ile Arg Ile Lys Gly Gln Glu Phe Ile Arg Gln Val Gln Ala
                325                 330                 335

Ser Tyr Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Ser Pro
            340                 345                 350

Gly Asp Glu Asn Ala Glu Ser Ser Ile Ile His Leu Glu Pro Gly Glu
        355                 360                 365

Asp His Ser Glu Asp Ala Ile Met Met Asn Thr Pro Val Ile Asn Ala
    370                 375                 380

Ala Val Glu Met Gly Phe Ser Arg Ser Leu Val Lys Gln Thr Val Gln
385                 390                 395                 400

Arg Lys Ile Leu Ala Thr Gly Glu Asn Tyr Arg Leu Val Asn Asp Leu
                405                 410                 415

Val Leu Asp Leu Leu Asn Ala Glu Asp Glu Ile Arg Glu Glu Glu Arg
            420                 425                 430

Glu Arg Ala Thr Glu Glu Lys Glu Ser Asn Asp Leu Leu Leu Ile Arg
        435                 440                 445

Lys Asn Arg Met Ala Leu Phe Gln His Leu Thr Cys Val Ile Pro Ile
    450                 455                 460

Leu Asp Ser Leu Leu Thr Ala Gly Ile Ile Asn Glu Gln Glu His Asp
465                 470                 475                 480

Val Ile Lys Gln Lys Thr Gln Thr Ser Leu Gln Ala Arg Glu Leu Ile
                485                 490                 495
```

-continued

```
Asp Thr Ile Leu Val Lys Gly Asn Ile Ala Ala Thr Val Phe Arg Asn
            500                 505                 510

Ser Leu Gln Glu Ala Glu Ala Val Leu Tyr Glu His Leu Phe Val Gln
        515                 520                 525

Gln Asp Ile Lys Tyr Ile Pro Thr Glu Asp Val Ser Asp Leu Pro Val
    530                 535                 540

Glu Glu Gln Leu Arg Arg Leu Pro Glu Glu Arg Thr Cys Lys Val Cys
545                 550                 555                 560

Met Asp Lys Glu Val Ser Ile Val Phe Ile Pro Cys Gly His Leu Val
                565                 570                 575

Val Cys Lys Asp Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg
            580                 585                 590

Ser Thr Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
        595                 600


<210> SEQ ID NO 7
<211> LENGTH: 2580
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: variation
<222> LOCATION: (2412)...(2412)
<223> OTHER INFORMATION: N may be any nucleotide

<400> SEQUENCE: 7

ttaggttacc tgaaagagtt actacaaccc caaagagttg tgttctaagt agtatcttgg     60

taattcagag agatactcat cctacctgaa tataaactga gataaatcca gtaaagaaag    120

tgtagtaaat tctacataag agtctatcat tgatttcttt ttgtggtgga aatcttagtt    180

catgtgaaga aatttcatgt gaatgtttta gctatcaaac agtactgtca cctactcatg    240

cacaaaactg cctcccaaag acttttccca ggtccctcgt atcaaaacat taagagtata    300

atggaagata gcacgatctt gtcagattgg acaaacagca acaaacaaaa aatgaagtat    360

gacttttcct gtgaactcta cagaatgtct acatattcaa ctttccccgc cggggtgcct    420

gtctcagaaa ggagtcttgc tcgtgctggt ttttattata ctggtgtgaa tgacaaggtc    480

aaatgcttct gttgtggcct gatgctggat aactggaaac taggagacag tcctattcaa    540

aagcataaac agctatatcc tagctgtagc tttattcaga atctggtttc agctagtctg    600

ggatccacct ctaagaatac gtctccaatg agaaacagtt ttgcacattc attatctccc    660

accttggaac atagtagctt gttcagtggt tcttactcca gccttcctcc aaaccctctt    720

aattctagag cagttgaaga catctcttca tcgaggacta acccctacag ttatgcaatg    780

agtactgaag aagccagatt tcttacctac catatgtggc cattaacttt tttgtcacca    840

tcagaattgg caagagctgg tttttattat ataggacctg gagatagggt agcctgcttt    900

gcctgtggtg ggaagctcag taactgggaa ccaaaggatg atgctatgtc agaacaccgg    960

aggcattttc ccaactgtcc atttttggaa aattctctag aaactctgag gtttagcatt   1020

tcaaatctga gcatgcagac acatgcagct cgaatgagaa catttatgta ctggccatct   1080

agtgttccag ttcagcctga gcagcttgca agtgctggtt tttattatgt gggtcgcaat   1140

gatgatgtca aatgctttgg ttgtgatggt ggcttgaggt gttgggaatc tggagatgat   1200

ccatgggtag aacatgccaa gtggtttcca aggtgtgagt tcttgatacg aatgaaaggc   1260
```

-continued

caagagtttg ttgatgagat tcaaggtaga tatcctcatc ttcttgaaca gctgttgtca 1320

acttcagata ccactggaga agaaaatgct gacccaccaa ttattcattt tggacctgga 1380

gaaagttctt cagaagatgc tgtcatgatg aatacacctg tggttaaatc tgccttggaa 1440

atgggcttta atagagacct ggtgaaacaa acagttctaa gtaaaatcct gacaactgga 1500

gagaactata aaacagttaa tgatattgtg tcagcacttc ttaatgctga agatgaaaaa 1560

agagaagagg agaaggaaaa acaagctgaa gaaatggcat cagatgattt gtcattaatt 1620

cggaagaaca gaatggctct ctttcaacaa ttgacatgtg tgcttcctat cctggataat 1680

cttttaaagg ccaatgtaat taataaacag gaacatgata ttattaaaca aaaaacacag 1740

ataccttac aagcgagaga actgattgat accatttggg ttaaaggaaa tgctgcggcc 1800

aacatcttca aaaactgtct aaaagaaatt gactctacat tgtataagaa cttatttgtg 1860

gataagaata tgaagtatat tccaacagaa gatgtttcag gtctgtcact ggaagaacaa 1920

ttgaggaggt tgcaagaaga acgaacttgt aaagtgtgta tggacaaaga agtttctgtt 1980

gtatttattc cttgtggtca tctggtagta tgccaggaat gtgccccttc tctaagaaaa 2040

tgccctattt gcaggggtat aatcaagggt actgttcgta catttctctc ttaaagaaaa 2100

atagtctata ttttaacctg cataaaaagg tctttaaaat attgttgaac acttgaagcc 2160

atctaaagta aaaagggaat tatgagtttt tcaattagta acattcatgt tctagtctgc 2220

tttggtacta ataatcttgt ttctgaaaag atggtatcat atatttaatc ttaatctgtt 2280

tatttacaag ggaagattta tgtttggtga actatattag tatgtatgtg tacctaaggg 2340

agtagcgtcn ctgcttgtta tgcatcattt caggagttac tggatttgtt gttctttcag 2400

aaagctttga anactaaatt atagtgtaga aaagaactgg aaaccaggaa ctctggagtt 2460

catcagagtt atggtgccga attgtctttg gtgcttttca cttgtgtttt aaaataagga 2520

tttttctctt atttctcccc ctagtttgtg agaaacatct caataaagtg ctttaaaaag 2580

<210> SEQ ID NO 8
<211> LENGTH: 618
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met His Lys Thr Ala Ser Gln Arg Leu Phe Pro Gly Pro Ser Tyr Gln
 1   5   10   15

Asn Ile Lys Ser Ile Met Glu Asp Ser Thr Ile Leu Ser Asp Trp Thr
            20   25   30

Asn Ser Asn Lys Gln Lys Met Lys Tyr Asp Phe Ser Cys Glu Leu Tyr
        35   40   45

Arg Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro Val Ser Glu
    50   55   60

Arg Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys
65   70   75   80

Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Leu Gly
                85   90   95

Asp Ser Pro Ile Gln Lys His Lys Gln Leu Tyr Pro Ser Cys Ser Phe
            100   105   110

Ile Gln Asn Leu Val Ser Ala Ser Leu Gly Ser Thr Ser Lys Asn Thr
        115   120   125

-continued

```
Ser Pro Met Arg Asn Ser Phe Ala His Ser Leu Ser Pro Thr Leu Glu
    130                 135                 140

His Ser Ser Leu Phe Ser Gly Ser Tyr Ser Ser Leu Pro Pro Asn Pro
145                 150                 155                 160

Leu Asn Ser Arg Ala Val Glu Asp Ile Ser Ser Ser Arg Thr Asn Pro
                165                 170                 175

Tyr Ser Tyr Ala Met Ser Thr Glu Glu Ala Arg Phe Leu Thr Tyr His
            180                 185                 190

Met Trp Pro Leu Thr Phe Leu Ser Pro Ser Glu Leu Ala Arg Ala Gly
        195                 200                 205

Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys Gly
    210                 215                 220

Gly Lys Leu Ser Asn Trp Glu Pro Lys Asp Asp Ala Met Ser Glu His
225                 230                 235                 240

Arg Arg His Phe Pro Asn Cys Pro Phe Leu Glu Asn Ser Leu Glu Thr
                245                 250                 255

Leu Arg Phe Ser Ile Ser Asn Leu Ser Met Gln Thr His Ala Ala Arg
            260                 265                 270

Met Arg Thr Phe Met Tyr Trp Pro Ser Ser Val Pro Val Gln Pro Glu
        275                 280                 285

Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Arg Asn Asp Asp Val
    290                 295                 300

Lys Cys Phe Gly Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser Gly Asp
305                 310                 315                 320

Asp Pro Trp Val Glu His Ala Lys Trp Phe Pro Arg Cys Glu Phe Leu
                325                 330                 335

Ile Arg Met Lys Gly Gln Glu Phe Val Asp Glu Ile Gln Gly Arg Tyr
            340                 345                 350

Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Thr Thr Gly Glu
        355                 360                 365

Glu Asn Ala Asp Pro Pro Ile Ile His Phe Gly Pro Gly Glu Ser Ser
    370                 375                 380

Ser Glu Asp Ala Val Met Met Asn Thr Pro Val Val Lys Ser Ala Leu
385                 390                 395                 400

Glu Met Gly Phe Asn Arg Asp Leu Val Lys Gln Thr Val Leu Ser Lys
                405                 410                 415

Ile Leu Thr Thr Gly Glu Asn Tyr Lys Thr Val Asn Asp Ile Val Ser
            420                 425                 430

Ala Leu Leu Asn Ala Glu Asp Glu Lys Arg Glu Glu Glu Lys Glu Lys
        435                 440                 445

Gln Ala Glu Glu Met Ala Ser Asp Asp Leu Ser Leu Ile Arg Lys Asn
    450                 455                 460

Arg Met Ala Leu Phe Gln Gln Leu Thr Cys Val Leu Pro Ile Leu Asp
465                 470                 475                 480

Asn Leu Leu Lys Ala Asn Val Ile Asn Lys Gln Glu His Asp Ile Ile
                485                 490                 495

Lys Gln Lys Thr Gln Ile Pro Leu Gln Ala Arg Glu Leu Ile Asp Thr
            500                 505                 510

Ile Trp Val Lys Gly Asn Ala Ala Ala Asn Ile Phe Lys Asn Cys Leu
        515                 520                 525

Lys Glu Ile Asp Ser Thr Leu Tyr Lys Asn Leu Phe Val Asp Lys Asn
```

-continued

```
    530                 535                 540

Met Lys Tyr Ile Pro Thr Glu Asp Val Ser Gly Leu Ser Leu Glu Glu
545                 550                 555                 560

Gln Leu Arg Arg Leu Gln Glu Glu Arg Thr Cys Lys Val Cys Met Asp
                565                 570                 575

Lys Glu Val Ser Val Val Phe Ile Pro Cys Gly His Leu Val Val Cys
            580                 585                 590

Gln Glu Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg Gly Ile
        595                 600                 605

Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
    610                 615
```

<210> SEQ ID NO 9
<211> LENGTH: 2100
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 9

```
gacactctgc tgggcggcgg gccgccctcc tccgggacct cccctcggga accgtcgccc     60

gcggcgctta gttaggactg gagtgcttgg cgcgaaaagg tggacaagtc ctattttcca    120

gagaagatga cttttaacag ttttgaagga actagaactt ttgtacttgc agacaccaat    180

aaggatgaag aatttgtaga agagtttaat agattaaaaa catttgctaa cttcccaagt    240

agtagtcctg tttcagcatc aacattggcg cgagctgggt ttctttatac cggtgaagga    300

gacaccgtgc aatgtttcag ttgtcatgcg gcaatagata gatggcagta tggagactca    360

gctgttggaa gacacaggag aatatcccca aattgcagat ttatcaatgg tttttatttt    420

gaaaatggtg ctgcacagtc tacaaatcct ggtatccaaa atggccagta caaatctgaa    480

aactgtgtgg gaaatagaaa tccttttgcc cctgacaggc cacctgagac tcatgctgat    540

tatctcttga gaactggaca ggttgtagat atttcagaca ccatataccc gaggaaccct    600

gccatgtgta gtgaagaagc cagattgaag tcatttcaga actggccgga ctatgctcat    660

ttaaccccca gagagttagc tagtgctggc ctctactaca caggggctga tgatcaagtg    720

caatgctttt gttgtggggg aaaactgaaa aattgggaac cctgtgatcg tgcctggtca    780

gaacacagga gacactttcc caattgcttt tttgttttgg gccggaacgt taatgttcga    840

agtgaatctg gtgtgagttc tgataggaat ttcccaaatt caacaaactc tccaagaaat    900

ccagccatgg cagaatatga agcacggatc gttacttttg gaacatggat atactcagtt    960

aacaaggagc agcttgcaag agctggattt tatgctttag gtgaaggcga taaagtgaag   1020

tgcttccact gtggaggagg gctcacggat tggaagccaa gtgaagaccc ctgggaccag   1080

catgctaagt gctacccagg gtgcaaatac ctattggatg agaaggggca agaatatata   1140

aataatattc atttaaccca tccacttgag gaatctttgg gaagaactgc tgaaaaaaca   1200

ccaccgctaa ctaaaaaaat cgatgatacc atcttccaga atcctatggt gcaagaagct   1260

atacgaatgg gatttagctt caaggacctt aagaaaacaa tggaagaaaa aatccaaaca   1320

tccgggagca gctatctatc acttgaggtc ctgattgcag atcttgtgag tgctcagaaa   1380

gataatacgg aggatgagtc aagtcaaact tcattgcaga aagacattag tactgaagag   1440

cagctaaggc gcctacaaga ggagaagctt tccaaaatct gtatggatag aaatattgct   1500

atcgtttttt ttccttgtgg acatctggcc acttgtaaac agtgtgcaga agcagttgac   1560
```

-continued

aaatgtccca tgtgctacac cgtcattacg ttcaaccaaa aaatttttat gtcttagtgg 1620

ggcaccacat gttatgttct tcttgctcta attgaatgtg taatgggagc gaactttaag 1680

taatcctgca tttgcattcc attagcatcc tgctgtttcc aaatggagac caatgctaac 1740

agcactgttt ccgtctaaac attcaatttc tggatctttc gagttatcag ctgtatcatt 1800

tagccagtgt tttactcgat tgaaacctta gacagagaag cattttatag cttttcacat 1860

gtatattggt agtacactga cttgatttct atatgtaagt gaattcatca cctgcatgtt 1920

tcatgccttt tgcataagct taacaaatgg agtgttctgt ataagcatgg agatgtgatg 1980

gaatctgccc aatgacttta attggcttat tgtaaacacg gaaagaactg ccccacgctg 2040

ctgggaggat aaagattgtt ttagatgctc acttctgtgt tttaggattc tgcccattta 2100

<210> SEQ ID NO 10
<211> LENGTH: 496
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 10

Met Thr Phe Asn Ser Phe Glu Gly Thr Arg Thr Phe Val Leu Ala Asp
 1   5   10   15

Thr Asn Lys Asp Glu Glu Phe Val Glu Glu Phe Asn Arg Leu Lys Thr
            20   25   30

Phe Ala Asn Phe Pro Ser Ser Ser Pro Val Ser Ala Ser Thr Leu Ala
        35   40   45

Arg Ala Gly Phe Leu Tyr Thr Gly Glu Gly Asp Thr Val Gln Cys Phe
    50   55   60

Ser Cys His Ala Ala Ile Asp Arg Trp Gln Tyr Gly Asp Ser Ala Val
65   70   75   80

Gly Arg His Arg Arg Ile Ser Pro Asn Cys Arg Phe Ile Asn Gly Phe
                85   90   95

Tyr Phe Glu Asn Gly Ala Ala Gln Ser Thr Asn Pro Gly Ile Gln Asn
            100   105   110

Gly Gln Tyr Lys Ser Glu Asn Cys Val Gly Asn Arg Asn Pro Phe Ala
        115   120   125

Pro Asp Arg Pro Pro Glu Thr His Ala Asp Tyr Leu Leu Arg Thr Gly
    130   135   140

Gln Val Val Asp Ile Ser Asp Thr Ile Tyr Pro Arg Asn Pro Ala Met
145   150   155   160

Cys Ser Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr
                165   170   175

Ala His Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr
            180   185   190

Gly Ala Asp Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys
        195   200   205

Asn Trp Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe
    210   215   220

Pro Asn Cys Phe Phe Val Leu Gly Arg Asn Val Asn Val Arg Ser Glu
225   230   235   240

Ser Gly Val Ser Ser Asp Arg Asn Phe Pro Asn Ser Thr Asn Ser Pro
                245   250   255

Arg Asn Pro Ala Met Ala Glu Tyr Glu Ala Arg Ile Val Thr Phe Gly

-continued

```
           260                 265                 270

Thr Trp Ile Tyr Ser Val Asn Lys Glu Gln Leu Ala Arg Ala Gly Phe
        275                 280                 285

Tyr Ala Leu Gly Glu Gly Asp Lys Val Lys Cys Phe His Cys Gly Gly
    290                 295                 300

Gly Leu Thr Asp Trp Lys Pro Ser Glu Asp Pro Trp Asp Gln His Ala
305                 310                 315                 320

Lys Cys Tyr Pro Gly Cys Lys Tyr Leu Leu Asp Glu Lys Gly Gln Glu
                325                 330                 335

Tyr Ile Asn Asn Ile His Leu Thr His Pro Leu Glu Glu Ser Leu Gly
            340                 345                 350

Arg Thr Ala Glu Lys Thr Pro Pro Leu Thr Lys Lys Ile Asp Asp Thr
        355                 360                 365

Ile Phe Gln Asn Pro Met Val Gln Glu Ala Ile Arg Met Gly Phe Ser
    370                 375                 380

Phe Lys Asp Leu Lys Lys Thr Met Glu Glu Lys Ile Gln Thr Ser Gly
385                 390                 395                 400

Ser Ser Tyr Leu Ser Leu Glu Val Leu Ile Ala Asp Leu Val Ser Ala
                405                 410                 415

Gln Lys Asp Asn Thr Glu Asp Glu Ser Ser Gln Thr Ser Leu Gln Lys
            420                 425                 430

Asp Ile Ser Thr Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys Leu
        435                 440                 445

Ser Lys Ile Cys Met Asp Arg Asn Ile Ala Ile Val Phe Phe Pro Cys
    450                 455                 460

Gly His Leu Ala Thr Cys Lys Gln Cys Ala Glu Ala Val Asp Lys Cys
465                 470                 475                 480

Pro Met Cys Tyr Thr Val Ile Thr Phe Asn Gln Lys Ile Phe Met Ser
                485                 490                 495


<210> SEQ ID NO 11
<211> LENGTH: 67
<212> TYPE: PRT
<213> ORGANISM: Orgyia pseudotsugata

<400> SEQUENCE: 11

Lys Ala Ala Arg Leu Gly Thr Tyr Thr Asn Trp Pro Val Gln Phe Leu
 1               5                  10                  15

Glu Pro Ser Arg Met Ala Ala Ser Gly Phe Tyr Tyr Leu Gly Arg Gly
            20                  25                  30

Asp Glu Val Arg Cys Ala Phe Cys Lys Val Glu Ile Thr Asn Trp Val
        35                  40                  45

Arg Gly Asp Asp Pro Glu Thr Asp His Lys Arg Trp Ala Pro Gln Cys
    50                  55                  60

Pro Phe Val
65


<210> SEQ ID NO 12
<211> LENGTH: 275
<212> TYPE: PRT
<213> ORGANISM: Cydia pomonella

<400> SEQUENCE: 12

Met Ser Asp Leu Arg Leu Glu Glu Val Arg Leu Asn Thr Phe Glu Lys
```

-continued

```
 1               5                  10                  15

Trp Pro Val Ser Phe Leu Ser Pro Glu Thr Met Ala Lys Asn Gly Phe
            20                  25                  30

Tyr Tyr Leu Gly Arg Ser Asp Glu Val Arg Cys Ala Phe Cys Lys Val
        35                  40                  45

Glu Ile Met Arg Trp Lys Glu Gly Glu Asp Pro Ala Ala Asp His Lys
    50                  55                  60

Lys Trp Ala Pro Gln Cys Pro Phe Val Lys Gly Ile Asp Val Cys Gly
65                  70                  75                  80

Ser Ile Val Thr Thr Asn Asn Ile Gln Asn Thr Thr Thr His Asp Thr
                85                  90                  95

Ile Ile Gly Pro Ala His Pro Lys Tyr Ala His Glu Ala Ala Arg Val
            100                 105                 110

Lys Ser Phe His Asn Trp Pro Arg Cys Met Lys Gln Arg Pro Glu Gln
        115                 120                 125

Met Ala Asp Ala Gly Phe Phe Tyr Thr Gly Tyr Gly Asp Asn Thr Lys
    130                 135                 140

Cys Phe Tyr Cys Asp Gly Gly Leu Lys Asp Trp Glu Pro Glu Asp Val
145                 150                 155                 160

Pro Trp Glu Gln His Val Arg Trp Phe Asp Arg Cys Ala Tyr Val Gln
                165                 170                 175

Leu Val Lys Gly Arg Asp Tyr Val Gln Lys Val Ile Thr Glu Ala Cys
            180                 185                 190

Val Leu Pro Gly Glu Asn Thr Thr Val Ser Thr Ala Ala Pro Val Ser
        195                 200                 205

Glu Pro Ile Pro Glu Thr Lys Ile Glu Lys Glu Pro Gln Val Glu Asp
    210                 215                 220

Ser Lys Leu Cys Lys Ile Cys Tyr Val Glu Glu Cys Ile Val Cys Phe
225                 230                 235                 240

Val Pro Cys Gly His Val Val Ala Cys Ala Lys Cys Ala Leu Ser Val
                245                 250                 255

Asp Lys Cys Pro Met Cys Arg Lys Ile Val Thr Ser Val Leu Lys Val
            260                 265                 270

Tyr Phe Ser
        275


<210> SEQ ID NO 13
<211> LENGTH: 498
<212> TYPE: PRT
<213> ORGANISM: Drosophila melanogaster

<400> SEQUENCE: 13

Met Thr Glu Leu Gly Met Glu Leu Glu Ser Val Arg Leu Ala Thr Phe
 1               5                  10                  15

Gly Glu Trp Pro Leu Asn Ala Pro Val Ser Ala Glu Asp Leu Val Ala
            20                  25                  30

Asn Gly Phe Phe Ala Thr Gly Lys Trp Leu Glu Ala Glu Cys His Phe
        35                  40                  45

Cys His Val Arg Ile Asp Arg Trp Glu Tyr Gly Asp Gln Val Ala Glu
    50                  55                  60

Arg His Arg Arg Ser Ser Pro Ile Cys Ser Met Val Leu Ala Pro Asn
65                  70                  75                  80
```

-continued

```
His Cys Gly Asn Val Pro Arg Ser Gln Glu Ser Asp Asn Glu Gly Asn
                85                  90                  95

Ser Val Val Asp Ser Pro Glu Ser Cys Ser Cys Pro Asp Leu Leu Leu
            100                 105                 110

Glu Ala Asn Arg Leu Val Thr Phe Lys Asp Trp Pro Asn Pro Asn Ile
        115                 120                 125

Thr Pro Gln Ala Leu Ala Lys Ala Gly Phe Tyr Tyr Leu Asn Arg Leu
    130                 135                 140

Asp His Val Lys Cys Val Trp Cys Asn Gly Val Ile Ala Lys Trp Glu
145                 150                 155                 160

Lys Asn Asp Asn Ala Phe Glu Glu His Lys Arg Phe Phe Pro Gln Cys
                165                 170                 175

Pro Arg Val Gln Met Gly Pro Leu Ile Glu Phe Ala Thr Gly Lys Asn
            180                 185                 190

Leu Asp Glu Leu Gly Ile Gln Pro Thr Thr Leu Pro Leu Arg Pro Lys
        195                 200                 205

Tyr Ala Cys Val Asp Ala Arg Leu Arg Thr Phe Thr Asp Trp Pro Ile
    210                 215                 220

Ser Asn Ile Gln Pro Ala Ser Ala Leu Ala Gln Ala Gly Leu Tyr Tyr
225                 230                 235                 240

Gln Lys Ile Gly Asp Gln Val Arg Cys Phe His Cys Asn Ile Gly Leu
                245                 250                 255

Arg Ser Trp Gln Lys Glu Asp Glu Pro Trp Phe Glu His Ala Lys Trp
            260                 265                 270

Ser Pro Lys Cys Gln Phe Val Leu Leu Ala Lys Gly Pro Ala Tyr Val
        275                 280                 285

Ser Glu Val Leu Ala Thr Thr Ala Ala Asn Ala Ser Ser Gln Pro Ala
    290                 295                 300

Thr Ala Pro Ala Pro Thr Leu Gln Ala Asp Val Leu Met Asp Glu Ala
305                 310                 315                 320

Pro Ala Lys Glu Ala Leu Thr Leu Gly Ile Asp Gly Gly Val Val Arg
                325                 330                 335

Asn Ala Ile Gln Arg Lys Leu Leu Ser Ser Gly Cys Ala Phe Ser Thr
            340                 345                 350

Leu Asp Glu Leu Leu His Asp Ile Phe Asp Asp Ala Gly Ala Gly Ala
        355                 360                 365

Ala Leu Glu Val Arg Glu Pro Pro Glu Pro Ser Ala Pro Phe Ile Glu
    370                 375                 380

Pro Cys Gln Ala Thr Thr Ser Lys Ala Ala Ser Val Pro Ile Pro Val
385                 390                 395                 400

Ala Asp Ser Ile Pro Ala Lys Pro Gln Ala Ala Glu Ala Val Ser Asn
                405                 410                 415

Ile Ser Lys Ile Thr Asp Glu Ile Gln Lys Met Ser Val Ser Thr Pro
            420                 425                 430

Asn Gly Asn Leu Ser Leu Glu Glu Glu Asn Arg Gln Leu Lys Asp Ala
        435                 440                 445

Arg Leu Cys Lys Val Cys Leu Asp Glu Glu Val Gly Val Val Phe Leu
    450                 455                 460

Pro Cys Gly His Leu Ala Thr Cys Asn Gln Cys Ala Pro Ser Val Ala
465                 470                 475                 480

Asn Cys Pro Met Cys Arg Ala Asp Ile Lys Gly Phe Val Arg Thr Phe
```

-continued

485 490 495

Leu Ser

<210> SEQ ID NO 14
<211> LENGTH: 67
<212> TYPE: PRT
<213> ORGANISM: Cydia pomonella

<400> SEQUENCE: 14

Glu Glu Val Arg Leu Asn Thr Phe Glu Lys Trp Pro Val Ser Phe Leu
1 5 10 15

Ser Pro Glu Thr Met Ala Lys Asn Gly Phe Tyr Tyr Leu Gly Arg Ser
20 25 30

Asp Glu Val Arg Cys Ala Phe Cys Lys Val Glu Ile Met Arg Trp Lys
35 40 45

Glu Gly Glu Asp Pro Ala Ala Asp His Lys Lys Trp Ala Pro Gln Cys
50 55 60

Pro Phe Val
65

<210> SEQ ID NO 15
<211> LENGTH: 67
<212> TYPE: PRT
<213> ORGANISM: Drosophila melanogaster

<400> SEQUENCE: 15

Glu Ala Asn Arg Leu Val Thr Phe Lys Asp Trp Pro Asn Pro Asn Ile
1 5 10 15

Thr Pro Gln Ala Leu Ala Lys Ala Gly Phe Tyr Tyr Leu Asn Arg Leu
20 25 30

Asp His Val Lys Cys Val Trp Cys Asn Gly Val Ile Ala Lys Trp Glu
35 40 45

Lys Asn Asp Asn Ala Phe Glu Glu His Lys Arg Phe Phe Pro Gln Cys
50 55 60

Pro Arg Val
65

<210> SEQ ID NO 16
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 16

Glu Phe Asn Arg Leu Lys Thr Phe Ala Asn Phe Pro Ser Ser Ser Pro
1 5 10 15

Val Ser Ala Ser Thr Leu Ala Arg Ala Gly Phe Leu Tyr Thr Gly Glu
20 25 30

Gly Asp Thr Val Gln Cys Phe Ser Cys His Ala Ala Ile Asp Arg Trp
35 40 45

Gln Tyr Gly Asp Ser Ala Val Gly Arg His Arg Arg Ile Ser Pro Asn
50 55 60

Cys Arg Phe Ile
65

<210> SEQ ID NO 17
<211> LENGTH: 68

-continued

<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Glu Phe Asn Arg Leu Lys Thr Phe Ala Asn Phe Pro Ser Gly Ser Pro
1 5 10 15

Val Ser Ala Ser Thr Leu Ala Arg Ala Gly Phe Leu Tyr Thr Gly Glu
20 25 30

Gly Asp Thr Val Arg Cys Phe Ser Cys His Ala Ala Val Asp Arg Trp
35 40 45

Gln Tyr Gly Asp Ser Ala Val Gly Arg His Arg Lys Val Ser Pro Asn
50 55 60

Cys Arg Phe Ile
65

<210> SEQ ID NO 18
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Glu Leu Tyr Arg Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro
1 5 10 15

Val Ser Glu Arg Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val
20 25 30

Asn Asp Lys Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp
35 40 45

Lys Arg Gly Asp Ser Pro Thr Glu Lys His Lys Lys Leu Tyr Pro Ser
50 55 60

Cys Arg Phe Val
65

<210> SEQ ID NO 19
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Glu Leu Tyr Arg Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro
1 5 10 15

Val Ser Glu Arg Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val
20 25 30

Asn Asp Lys Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp
35 40 45

Lys Leu Gly Asp Ser Pro Ile Gln Lys His Lys Gln Leu Tyr Pro Ser
50 55 60

Cys Ser Phe Ile
65

<210> SEQ ID NO 20
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 20

Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr Ala His
1 5 10 15

-continued

Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr Gly Ala
20 25 30

Asp Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys Asn Trp
35 40 45

Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe Pro Asn
50 55 60

Cys Phe Phe Val
65

<210> SEQ ID NO 21
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr Ala His
1 5 10 15

Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr Gly Ile
20 25 30

Gly Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys Asn Trp
35 40 45

Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe Pro Asn
50 55 60

Cys Phe Phe Val
65

<210> SEQ ID NO 22
<211> LENGTH: 67
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

Glu Asn Ala Arg Leu Leu Thr Phe Gln Thr Trp Pro Leu Thr Phe Leu
1 5 10 15

Ser Pro Thr Asp Leu Ala Arg Ala Gly Phe Tyr Tyr Ile Gly Pro Gly
20 25 30

Asp Arg Val Ala Cys Phe Ala Cys Gly Gly Lys Leu Ser Asn Trp Glu
35 40 45

Pro Lys Asp Asn Ala Met Ser Glu His Leu Arg His Phe Pro Lys Cys
50 55 60

Pro Phe Ile
65

<210> SEQ ID NO 23
<211> LENGTH: 67
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

Glu Glu Ala Arg Phe Leu Thr Tyr His Met Trp Pro Leu Thr Phe Leu
1 5 10 15

Ser Pro Ser Glu Leu Ala Arg Ala Gly Phe Tyr Tyr Ile Gly Pro Gly
20 25 30

Asp Arg Val Ala Cys Phe Ala Cys Gly Gly Lys Leu Ser Asn Trp Glu
35 40 45

-continued

```
Pro Lys Asp Asp Ala Met Ser Glu His Arg Arg His Phe Pro Asn Cys
    50                  55                  60

Pro Phe Leu
65


<210> SEQ ID NO 24
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 24

Tyr Glu Ala Arg Ile Val Thr Phe Gly Thr Trp Ile Tyr Ser Val Asn
 1               5                  10                  15

Lys Glu Gln Leu Ala Arg Ala Gly Phe Tyr Ala Leu Gly Glu Gly Asp
            20                  25                  30

Lys Val Lys Cys Phe His Cys Gly Gly Gly Leu Thr Asp Trp Lys Pro
        35                  40                  45

Ser Glu Asp Pro Trp Asp Gln His Ala Lys Cys Tyr Pro Gly Cys Lys
    50                  55                  60

Tyr Leu
65


<210> SEQ ID NO 25
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

Tyr Glu Ala Arg Ile Phe Thr Phe Gly Thr Trp Ile Tyr Ser Val Asn
 1               5                  10                  15

Lys Glu Gln Leu Ala Arg Ala Gly Phe Tyr Ala Leu Gly Glu Gly Asp
            20                  25                  30

Lys Val Lys Cys Phe His Cys Gly Gly Gly Leu Thr Asp Trp Lys Pro
        35                  40                  45

Ser Glu Asp Pro Trp Glu Gln His Ala Lys Trp Tyr Pro Gly Cys Lys
    50                  55                  60

Tyr Leu
65


<210> SEQ ID NO 26
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

His Ala Ala Arg Phe Lys Thr Phe Phe Asn Trp Pro Ser Ser Val Leu
 1               5                  10                  15

Val Asn Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Asn
            20                  25                  30

Ser Asp Asp Val Lys Cys Phe Cys Cys Asp Gly Gly Leu Arg Cys Trp
        35                  40                  45

Glu Ser Gly Asp Asp Pro Trp Val Gln His Ala Lys Trp Phe Pro Arg
    50                  55                  60

Cys Glu Tyr Leu
65
```

-continued

<210> SEQ ID NO 27
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 27

His Ala Ala Arg Met Arg Thr Phe Met Tyr Trp Pro Ser Ser Val Pro
 1   5   10   15

Val Gln Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Arg
            20   25   30

Asn Asp Asp Val Lys Cys Phe Gly Cys Asp Gly Gly Leu Arg Cys Trp
        35   40   45

Glu Ser Gly Asp Asp Pro Trp Val Glu His Ala Lys Trp Phe Pro Arg
    50   55   60

Cys Glu Phe Leu
65

<210> SEQ ID NO 28
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Orgyia pseudotsugata

<400> SEQUENCE: 28

Glu Ala Ala Arg Leu Arg Thr Phe Ala Glu Trp Pro Arg Gly Leu Lys
 1   5   10   15

Gln Arg Pro Glu Glu Leu Ala Glu Ala Gly Phe Phe Tyr Thr Gly Gln
            20   25   30

Gly Asp Lys Thr Arg Cys Phe Cys Cys Asp Gly Gly Leu Lys Asp Trp
        35   40   45

Glu Pro Asp Asp Ala Pro Trp Gln Gln His Ala Arg Trp Tyr Asp Arg
    50   55   60

Cys Glu Tyr Val
65

<210> SEQ ID NO 29
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Cydia pomonella

<400> SEQUENCE: 29

Glu Ala Ala Arg Val Lys Ser Phe His Asn Trp Pro Arg Cys Met Lys
 1   5   10   15

Gln Arg Pro Glu Gln Met Ala Asp Ala Gly Phe Phe Tyr Thr Gly Tyr
            20   25   30

Gly Asp Asn Thr Lys Cys Phe Tyr Cys Asp Gly Gly Leu Lys Asp Trp
        35   40   45

Glu Pro Glu Asp Val Pro Trp Glu Gln His Val Arg Trp Phe Asp Arg
    50   55   60

Cys Ala Tyr Val
65

<210> SEQ ID NO 30
<211> LENGTH: 68
<212> TYPE: PRT
<213> ORGANISM: Drosophila melanogaster

<400> SEQUENCE: 30

-continued

```
Val Asp Ala Arg Leu Arg Thr Phe Thr Asp Trp Pro Ile Ser Asn Ile
 1               5                  10                  15

Gln Pro Ala Ser Ala Leu Ala Gln Ala Gly Leu Tyr Tyr Gln Lys Ile
            20                  25                  30

Gly Asp Gln Val Arg Cys Phe His Cys Asn Ile Gly Leu Arg Ser Trp
        35                  40                  45

Gln Lys Glu Asp Glu Pro Trp Phe Glu His Ala Lys Trp Ser Pro Lys
    50                  55                  60

Cys Gln Phe Val
65
```

<210> SEQ ID NO 31
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Drosophila melanogaster

<400> SEQUENCE: 31

```
Glu Ser Val Arg Leu Ala Thr Phe Gly Glu Trp Pro Leu Asn Ala Pro
 1               5                  10                  15

Val Ser Ala Glu Asp Leu Val Ala Asn Gly Phe Phe Gly Thr Trp Met
            20                  25                  30

Glu Ala Glu Cys Asp Phe Cys His Val Arg Ile Asp Arg Trp Glu Tyr
        35                  40                  45

Gly Asp Leu Val Ala Glu Arg His Arg Arg Ser Ser Pro Ile Cys Ser
    50                  55                  60

Met Val
65
```

<210> SEQ ID NO 32
<211> LENGTH: 46
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

```
Glu Gln Leu Arg Arg Leu Gln Glu Glu Arg Thr Cys Lys Val Cys Met
 1               5                  10                  15

Asp Lys Glu Val Ser Val Val Phe Ile Pro Cys Gly His Leu Val Val
            20                  25                  30

Cys Gln Glu Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys
        35                  40                  45
```

<210> SEQ ID NO 33
<211> LENGTH: 46
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 33

```
Glu Gln Leu Arg Arg Leu Pro Glu Glu Arg Thr Cys Lys Val Cys Met
 1               5                  10                  15

Asp Lys Glu Val Ser Ile Val Phe Ile Pro Cys Gly His Leu Val Val
            20                  25                  30

Cys Lys Asp Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys
        35                  40                  45
```

<210> SEQ ID NO 34
<211> LENGTH: 46
<212> TYPE: PRT

-continued

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 34

Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys Leu Ser Lys Ile Cys Met
1 5 10 15

Asp Arg Asn Ile Ala Ile Val Phe Phe Pro Cys Gly His Leu Ala Thr
20 25 30

Cys Lys Gln Cys Ala Glu Ala Val Asp Lys Cys Pro Met Cys
35 40 45

<210> SEQ ID NO 35
<211> LENGTH: 46
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 35

Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys Leu Cys Lys Ile Cys Met
1 5 10 15

Asp Arg Asn Ile Ala Ile Val Phe Val Pro Cys Gly His Leu Val Thr
20 25 30

Cys Lys Gln Cys Ala Glu Ala Val Asp Lys Cys Pro Met Cys
35 40 45

<210> SEQ ID NO 36
<211> LENGTH: 46
<212> TYPE: PRT
<213> ORGANISM: Drosophila melanogaster

<400> SEQUENCE: 36

Glu Glu Asn Arg Gln Leu Lys Asp Ala Arg Leu Cys Lys Val Cys Leu
1 5 10 15

Asp Glu Glu Val Gly Val Val Phe Leu Pro Cys Gly His Leu Ala Thr
20 25 30

Cys Asn Gln Cys Ala Pro Ser Val Ala Asn Cys Pro Met Cys
35 40 45

<210> SEQ ID NO 37
<211> LENGTH: 46
<212> TYPE: PRT
<213> ORGANISM: Cydia pomonella

<400> SEQUENCE: 37

Glu Lys Glu Pro Gln Val Glu Asp Ser Lys Leu Cys Lys Ile Cys Tyr
1 5 10 15

Val Glu Glu Cys Ile Val Cys Phe Val Pro Cys Gly His Val Val Ala
20 25 30

Cys Ala Lys Cys Ala Leu Ser Val Asp Lys Cys Pro Met Cys
35 40 45

<210> SEQ ID NO 38
<211> LENGTH: 46
<212> TYPE: PRT
<213> ORGANISM: Orgyia pseudotsugata

<400> SEQUENCE: 38

Ala Val Glu Ala Glu Val Ala Asp Asp Arg Leu Cys Lys Ile Cys Leu
1 5 10 15

Gly Ala Glu Lys Thr Val Cys Phe Val Pro Cys Gly His Val Val Ala

-continued

20 25 30

Cys Gly Lys Cys Ala Ala Gly Val Thr Thr Cys Pro Val Cys
35 40 45

<210> SEQ ID NO 39
<211> LENGTH: 2474
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 39

gaattccggg agacctacac ccccggagat cagaggtcat tgctggcgtt cagagcctag 60

gaagtgggct gcggtatcag cctagcagta aaaccgacca gaagccatgc acaaaactac 120

atccccagag aaagacttgt cccttccccct ccctgtcatc tcaccatgaa catggttcaa 180

gacagcgcct ttctagccaa gctgatgaag agtgctgaca cctttgagtt gaagtatgac 240

ttttcctgtg agctgtaccg attgtccacg tattcagctt ttcccagggg agttcctgtg 300

tcagaaagga gtctggctcg tgctggcttt tactacactg gtgccaatga caaggtcaag 360

tgcttctgct gtggcctgat gctagacaac tggaaacaag gggacagtcc catggagaag 420

cacagaaagt tgtaccccag ctgcaacttt gtacagactt tgaatccagc caacagtctg 480

gaagctagtc ctcggccttc tcttccttcc acggcgatga gcaccatgcc tttgagcttt 540

gcaagttctg agaatactgg ctatttcagt ggctcttact cgagctttcc ctcagaccct 600

gtgaacttcc gagcaaatca agattgtcct gctttgagca caagtcccta ccactttgca 660

atgaacacag agaaggccag attactcacc tatgaaacat ggccattgtc ttttctgtca 720

ccagcaaagc tggccaaagc aggcttctac tacataggac ctggagatag agtggcctgc 780

tttgcgtgcg atgggaaact gagcaactgg gaacgtaagg atgatgctat gtcagagcac 840

cagaggcatt tccccagctg tccgttctta aaagacttgg gtcagtctgc ttcgagatac 900

actgtctcta acctgagcat gcagacacac gcagcccgta ttagaacatt ctctaactgg 960

ccttctagtg cactagttca ttcccaggaa cttgcaagtg cgggctttta ttatacagga 1020

cacagtgatg atgtcaagtg tttatgctgt gatggtgggc tgaggtgctg ggaatctgga 1080

gatgacccct gggtggaaca tgccaagtgg tttccaaggt gtgagtactt gctcagaatc 1140

aaaggccaag aatttgtcag ccaagttcaa gctggctatc ctcatctact tgagcagcta 1200

ttatctacgt cagactcccc agaagatgag aatgcagacg cagcaatcgt gcattttggc 1260

cctggagaaa gttcggaaga tgtcgtcatg atgagcacgc ctgtggttaa agcagccttg 1320

gaaatgggct tcagtaggag cctggtgaga cagacggttc agtggcagat cctggccact 1380

ggtgagaact acaggaccgt cagtgacctc gttataggct tactcgatgc agaagacgag 1440

atgagagagg agcagatgga gcaggcggcc gaggaggagg agtcagatga tctagcacta 1500

atccggaaga acaaaatggt gcttttccaa catttgacgt gtgtgacacc aatgctgtat 1560

tgcctcctaa gtgcaagggc catcactgaa caggagtgca atgctgtgaa acagaaacca 1620

cacaccttac aagcaagcac actgattgat actgtgttag caaaaggaaa cactgcagca 1680

acctcattca gaaactccct tcgggaaatt gaccctgcgt tatacagaga tatatttgtg 1740

caacaggaca ttaggagtct tcccacagat gacattgcag ctctaccaat ggaagaacag 1800

ttgcggcccc tcccggagga cagaatgtgt aaagtgtgta tggaccgaga ggtatccatc 1860

gtgttcattc cctgtggcca tctggtcgtg tgcaaagact gcgctccctc tctgaggaag 1920

-continued

tgtcccatct gtagagggac catcaagggc acagtgcgca catttctctc ctgaacaaga 1980

ctaatggtcc atggctgcaa cttcagccag gaggaagttc actgtcactc ccagttccat 2040

tcggaacttg aggccagcct ggatagcacg agacaccgcc aaacacacaa atataaacat 2100

gaaaaacttt tgtctgaagt caagaatgaa tgaattactt atataataat tttaattggt 2160

ttccttaaaa gtgctatttg ttcccaactc agaaaattgt tttctgtaaa catatttaca 2220

tactacctgc atctaaagta ttcatatatt catatattca gatgtcatga gagagggttt 2280

tgttcttgtt cctgaaaagc tggtttatca tctgatcagc atatactgcg caacgggcag 2340

ggctagaatc catgaaccaa gctgcaaaga tctcacgcta aataaggcgg aaagatttgg 2400

agaaacgaaa ggaaattctt tcctgtccaa tgtatactct tcagactaat gacctcttcc 2460

tatcaagcct tcta 2474

<210> SEQ ID NO 40
<211> LENGTH: 602
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 40

Met Asn Met Val Gln Asp Ser Ala Phe Leu Ala Lys Leu Met Lys Ser
 1   5   10   15

Ala Asp Thr Phe Glu Leu Lys Tyr Asp Phe Ser Cys Glu Leu Tyr Arg
            20   25   30

Leu Ser Thr Tyr Ser Ala Phe Pro Arg Gly Val Pro Val Ser Glu Arg
        35   40   45

Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Ala Asn Asp Lys Val
    50   55   60

Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Gln Gly Asp
65   70   75   80

Ser Pro Met Glu Lys His Arg Lys Leu Tyr Pro Ser Cys Asn Phe Val
                85   90   95

Gln Thr Leu Asn Pro Ala Asn Ser Leu Glu Ala Ser Pro Arg Pro Ser
            100   105   110

Leu Pro Ser Thr Ala Met Ser Thr Met Pro Leu Ser Phe Ala Ser Ser
        115   120   125

Glu Asn Thr Gly Tyr Phe Ser Gly Ser Tyr Ser Ser Phe Pro Ser Asp
    130   135   140

Pro Val Asn Phe Arg Ala Asn Gln Asp Cys Pro Ala Leu Ser Thr Ser
145   150   155   160

Pro Tyr His Phe Ala Met Asn Thr Glu Lys Ala Arg Leu Leu Thr Tyr
                165   170   175

Glu Thr Trp Pro Leu Ser Phe Leu Ser Pro Ala Lys Leu Ala Lys Ala
            180   185   190

Gly Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys
        195   200   205

Asp Gly Lys Leu Ser Asn Trp Glu Arg Lys Asp Asp Ala Met Ser Glu
    210   215   220

His Gln Arg His Phe Pro Ser Cys Pro Phe Leu Lys Asp Leu Gly Gln
225   230   235   240

Ser Ala Ser Arg Tyr Thr Val Ser Asn Leu Ser Met Gln Thr His Ala
                245   250   255

-continued

```
Ala Arg Ile Arg Thr Phe Ser Asn Trp Pro Ser Ser Ala Leu Val His
            260                 265                 270

Ser Gln Glu Leu Ala Ser Ala Gly Phe Tyr Tyr Thr Gly His Ser Asp
        275                 280                 285

Asp Val Lys Cys Leu Cys Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser
    290                 295                 300

Gly Asp Asp Pro Trp Val Glu His Ala Lys Trp Phe Pro Arg Cys Glu
305                 310                 315                 320

Tyr Leu Leu Arg Ile Lys Gly Gln Glu Phe Val Ser Gln Val Gln Ala
                325                 330                 335

Gly Tyr Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Ser Pro
            340                 345                 350

Glu Asp Glu Asn Ala Asp Ala Ala Ile Val His Phe Gly Pro Gly Glu
        355                 360                 365

Ser Ser Glu Asp Val Val Met Met Ser Thr Pro Val Val Lys Ala Ala
    370                 375                 380

Leu Glu Met Gly Phe Ser Arg Ser Leu Val Arg Gln Thr Val Gln Trp
385                 390                 395                 400

Gln Ile Leu Ala Thr Gly Glu Asn Tyr Arg Thr Val Ser Asp Leu Val
                405                 410                 415

Ile Gly Leu Leu Asp Ala Glu Asp Glu Met Arg Glu Glu Gln Met Glu
            420                 425                 430

Gln Ala Ala Glu Glu Glu Glu Ser Asp Asp Leu Ala Leu Ile Arg Lys
        435                 440                 445

Asn Lys Met Val Leu Phe Gln His Leu Thr Cys Val Thr Pro Met Leu
    450                 455                 460

Tyr Cys Leu Leu Ser Ala Arg Ala Ile Thr Glu Gln Glu Cys Asn Ala
465                 470                 475                 480

Val Lys Gln Lys Pro His Thr Leu Gln Ala Ser Thr Leu Ile Asp Thr
                485                 490                 495

Val Leu Ala Lys Gly Asn Thr Ala Ala Thr Ser Phe Arg Asn Ser Leu
            500                 505                 510

Arg Glu Ile Asp Pro Ala Leu Tyr Arg Asp Ile Phe Val Gln Gln Asp
        515                 520                 525

Ile Arg Ser Leu Pro Thr Asp Asp Ile Ala Ala Leu Pro Met Glu Glu
    530                 535                 540

Gln Leu Arg Pro Leu Pro Glu Asp Arg Met Cys Lys Val Cys Met Asp
545                 550                 555                 560

Arg Glu Val Ser Ile Val Phe Ile Pro Cys Gly His Leu Val Val Cys
                565                 570                 575

Lys Asp Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg Gly Thr
            580                 585                 590

Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
        595                 600
```

<210> SEQ ID NO 41
<211> LENGTH: 2416
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 41

```
ctgtggtgga gatctattgt ccaagtggtg agaaacttca tctggaagtt taagcggtca     60
```

-continued

gaaatactat tactactcat ggacaaaact gtctcccaga gactcgccca aggtacctta 120

cacccaaaaa cttaaacgta taatggagaa gagcacaatc ttgtcaaatt ggacaaagga 180

gagcgaagaa aaaatgaagt ttgacttttc gtgtgaactc taccgaatgt ctacatattc 240

agcttttccc aggggagttc ctgtctcaga gaggagtctg gctcgtgctg gcttttatta 300

tacaggtgtg aatgacaaag tcaagtgctt ctgctgtggc ctgatgttgg ataactggaa 360

acaaggggac agtcctgttg aaaagcacag acagttctat cccagctgca gctttgtaca 420

gactctgctt tcagccagtc tgcagtctcc atctaagaat atgtctcctg tgaaaagtag 480

atttgcacat tcgtcacctc tggaacgagg tggcattcac tccaacctgt gctctagccc 540

tcttaattct agagcagtgg aagacttctc atcaaggatg gatccctgca gctatgccat 600

gagtacagaa gaggccagat ttcttactta cagtatgtgg cctttaagtt ttctgtcacc 660

agcagagctg gccagagctg gcttctatta catagggcct ggagacaggg tggcctgttt 720

tgcctgtggt gggaaactga gcaactggga accaaaggat tatgctatgt cagagcaccg 780

cagacatttt ccccactgtc catttctgga aaatacttca gaaacacaga ggtttagtat 840

atcaaatcta agtatgcaga cacactctgc tcgattgagg acatttctgt actggccacc 900

tagtgttcct gttcagcccg agcagcttgc aagtgctgga ttctattacg tggatcgcaa 960

tgatgatgtc aagtgccttt gttgtgatgg tggcttgaga tgttgggaac ctggagatga 1020

cccctggata gaacacgcca aatggtttcc aaggtgtgag ttcttgatac ggatgaaggg 1080

tcaggagttt gttgatgaga ttcaagctag atatcctcat cttcttgagc agctgttgtc 1140

cacttcagac accccaggag aagaaaatgc tgaccctaca gagacagtgg tgcattttgg 1200

ccctggagaa agttcgaaag atgtcgtcat gatgagcacg cctgtggtta aagcagcctt 1260

ggaaatgggc ttcagtagga gcctggtgag acagacggtt cagcggcaga tcctggccac 1320

tggtgagaac tacaggaccg tcaatgatat tgtctcagta cttttgaatg ctgaagatga 1380

gagaagagaa gaggagaagg aaagacagac tgaagagatg gcatcaggtg acttatcact 1440

gattcggaag aatagaatgg ccctctttca acagttgaca catgtccttc ctatcctgga 1500

taatcttctt gaggccagtg taattacaaa acaggaacat gatattatta gacagaaaac 1560

acagataccc ttacaagcaa gagagcttat tgacaccgtt ttagtcaagg gaaatgctgc 1620

agccaacatc ttcaaaaact ctctgaaggg aattgactcc acgttatatg aaaacttatt 1680

tgtggaaaag aatatgaagt atattccaac agaagacgtt tcaggcttgt cattggaaga 1740

gcagttgcgg agattacaag aagaacgaac ttgcaaagtg tgtatggaca gagaggtttc 1800

tattgtgttc attccgtgtg gtcatctagt agtctgccag gaatgtgccc cttctctaag 1860

gaagtgcccc atctgcaggg ggacaatcaa ggggactgtg cgcacatttc tctcatgagt 1920

gaagaatggt ctgaaagtat tgttggacat cagaagctgt cagaacaaag aatgaactac 1980

tgatttcagc tcttcagcag gacattctac tctctttcaa gattagtaat cttgctttat 2040

gaagggtagc attgtatatt taagcttagt ctgttgcaag ggaaggtcta tgctgttgag 2100

ctacaggact gtgtctgttc cagagcagga gttgggatgc ttgctgtatg tccttcagga 2160

cttcttggga tttgggaatt tggggaaagc tttggaatcc agtgatgtgg agctcagaaa 2220

tcctggaacc agtgactctg gtactcagta gatagggtac cctgtacttc ttggtgcttt 2280

tccagtctgg gaaataagga ggaatctgct gctggtaaaa atttgctgga tgtgagaaat 2340

-continued

agatgaaagt gtttcgggtg ggggcgtgca tcagtgtagt gtgtgcaggg atgtatgcag 2400

gccaaacact gtgtag 2416

<210> SEQ ID NO 42
<211> LENGTH: 591
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 42

Met Glu Lys Ser Thr Ile Leu Ser Asn Trp Thr Lys Glu Ser Glu Glu
1 5 10 15

Lys Met Lys Phe Asp Phe Ser Cys Glu Leu Tyr Arg Met Ser Thr Tyr
20 25 30

Ser Ala Phe Pro Arg Gly Val Pro Val Ser Glu Arg Ser Leu Ala Arg
35 40 45

Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys Val Lys Cys Phe Cys
50 55 60

Cys Gly Leu Met Leu Asp Asn Trp Lys Gln Gly Asp Ser Pro Val Glu
65 70 75 80

Lys His Arg Gln Phe Tyr Pro Ser Cys Ser Phe Val Gln Thr Leu Leu
85 90 95

Ser Ala Ser Leu Gln Ser Pro Ser Lys Asn Met Ser Pro Val Lys Ser
100 105 110

Arg Phe Ala His Ser Ser Pro Leu Glu Arg Gly Gly Ile His Ser Asn
115 120 125

Leu Cys Ser Ser Pro Leu Asn Ser Arg Ala Val Glu Asp Phe Ser Ser
130 135 140

Arg Met Asp Pro Cys Ser Tyr Ala Met Ser Thr Glu Glu Ala Arg Phe
145 150 155 160

Leu Thr Tyr Ser Met Trp Pro Leu Ser Phe Leu Ser Pro Ala Glu Leu
165 170 175

Ala Arg Ala Gly Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys
180 185 190

Phe Ala Cys Gly Gly Lys Leu Ser Asn Trp Glu Pro Lys Asp Tyr Ala
195 200 205

Met Ser Glu His Arg Arg His Phe Pro His Cys Pro Phe Leu Glu Asn
210 215 220

Thr Ser Glu Thr Gln Arg Phe Ser Ile Ser Asn Leu Ser Met Gln Thr
225 230 235 240

His Ser Ala Arg Leu Arg Thr Phe Leu Tyr Trp Pro Pro Ser Val Pro
245 250 255

Val Gln Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Asp Arg
260 265 270

Asn Asp Asp Val Lys Cys Leu Cys Cys Asp Gly Gly Leu Arg Cys Trp
275 280 285

Glu Pro Gly Asp Asp Pro Trp Ile Glu His Ala Lys Trp Phe Pro Arg
290 295 300

Cys Glu Phe Leu Ile Arg Met Lys Gly Gln Glu Phe Val Asp Glu Ile
305 310 315 320

Gln Ala Arg Tyr Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp
325 330 335

-continued

```
Thr Pro Gly Glu Glu Asn Ala Asp Pro Thr Glu Thr Val Val His Phe
            340                 345                 350

Gly Pro Gly Glu Ser Ser Lys Asp Val Val Met Met Ser Thr Pro Val
        355                 360                 365

Val Lys Ala Ala Leu Glu Met Gly Phe Ser Arg Ser Leu Val Arg Gln
    370                 375                 380

Thr Val Gln Arg Gln Ile Leu Ala Thr Gly Glu Asn Tyr Arg Thr Val
385                 390                 395                 400

Asn Asp Ile Val Ser Val Leu Leu Asn Ala Glu Asp Glu Arg Arg Glu
                405                 410                 415

Glu Glu Lys Glu Arg Gln Thr Glu Glu Met Ala Ser Gly Asp Leu Ser
            420                 425                 430

Leu Ile Arg Lys Asn Arg Met Ala Leu Phe Gln Gln Leu Thr His Val
        435                 440                 445

Leu Pro Ile Leu Asp Asn Leu Leu Glu Ala Ser Val Ile Thr Lys Gln
    450                 455                 460

Glu His Asp Ile Ile Arg Gln Lys Thr Gln Ile Pro Leu Gln Ala Arg
465                 470                 475                 480

Glu Leu Ile Asp Thr Val Leu Val Lys Gly Asn Ala Ala Ala Asn Ile
                485                 490                 495

Phe Lys Asn Ser Leu Lys Gly Ile Asp Ser Thr Leu Tyr Glu Asn Leu
            500                 505                 510

Phe Val Glu Lys Asn Met Lys Tyr Ile Pro Thr Glu Asp Val Ser Gly
        515                 520                 525

Leu Ser Leu Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Arg Thr Cys
    530                 535                 540

Lys Val Cys Met Asp Arg Glu Val Ser Ile Val Phe Ile Pro Cys Gly
545                 550                 555                 560

His Leu Val Val Cys Gln Glu Cys Ala Pro Ser Leu Arg Lys Cys Pro
                565                 570                 575

Ile Cys Arg Gly Thr Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
            580                 585                 590


<210> SEQ ID NO 43
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic based on viral sequence

<400> SEQUENCE: 43

Met Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
 1               5                  10


<210> SEQ ID NO 44
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer based on Homo sapiens

<400> SEQUENCE: 44

agtgcgggtt tttattatgt g                                             21


<210> SEQ ID NO 45
<211> LENGTH: 25
```

-continued

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer based on Homo sapiens

<400> SEQUENCE: 45

agatgaccac aaggaataaa cacta 25

What is claimed is:

1. A method of treating a patient diagnosed with cancer, the method comprising: administering to the patient an effective amount of a compound that inhibits the biological activity of XIAP polypeptide.

2. The method, according to claim 1, in which the compound is a negative regulator of XIAP anti-apoptotic pathway.

3. The method, according to claim 1, in which the compound is a compound that prevents cleavage of the XIAP polypeptide.

4. The method, according to claim 1, in which the biological activity is the level of expression of the XIAP polypeptide.

5. The method, according to claim 1, in which the biological activity is the level of expression of an mRNA molecule encoding the XIAP polypeptide.

6. The method, according to claim 1, in which the biological activity is an apoptosis-inhibiting activity.

7. The method, according to claim 1, in which the patient is a human.

8. The method, according to claim 1, in which the cancer is selected from the group consisting of: promyelocytic leukemia, a HeLa-type carcinoma, chronic myelogenous leukemia, lymphoblastic leukemia, Burkitt's lymphoma, colorectal adenocarcinoma, lung carcinoma, and melanoma.

9. The method, according to claim 1, in which the cancer is in a tissue selected from the group consisting of: ovary, lymph node. Skin, blood, lung, prostate, colon, rectum, testes, and small intestine.

10. The method, according to claim 1, in which the XIAP polypeptide comprises a domain having at least 80% sequence identity to a BIR domain selected from the group consisting of amino acids 26-93 of human X-linked IAP (XIAP, SEQ ID NO: 4), amino acids 163-230 of human X-linked IAP (XIAP, SEQ ID NO: 4), and amino acids 265-330 of human X-linked IAP (XIAP, SEQ ID NO: 4).

11. The method, according to claim 10, in which the polypeptide comprises a domain having at least 85% sequence identity to the BIR domain.

12. The method, according to claim 10 in which the polypeptide comprises a domain having at least 90% sequence identity to the BIR domain.

13. The method, according to claim 10 in which the polypeptide comprises a domain having at least 95% sequence identity to the BIR domain.

14. The method, according to claim 10, in which the polypeptide comprises a BIR domain selected from the group consisting of amino acids 26-93 of human X-linked IAP (XIAP, SEQ ID NO: 4), amino acids 163-230 of human X-linked IAP (XIAP, SEQ ID NO: 4), and amino acids 265-330 of human X-linked IAP (XIAP, SEQ ID NO: 4).

15. A method of treating a patient diagnosed with cancer, the method comprising: administering to the patient an effective amount of a compound that binds XIAP polypeptide.

16. The method, according to claim 15, in which the compound is a negative regulator of XIAP anti-apoptotic pathway.

17. The method, according to claim 15, in which the compound is a compound that prevents cleavage of the XIAP polypeptide.

18. The method, according to claim 15, in which the biological activity is the level of expression of the XIAP polypeptide.

19. The method, according to claim 15, in which the biological activity is the level of expression of an mRNA molecule encoding the XIAP polypeptide.

20. The method, according to claim 15, in which the biological activity is an apoptosis-inhibiting activity.

21. The method, according to claim 15, in which the patient is a human.

22. The method, according to claim 15, in which the cancer is selected from the group consisting of: promyelocytic leukemia, a HeLa-type carcinoma, chronic myelogenous leukemia, lymphoblastic leukemia, Burkitt's lymphoma, colorectal adenocarcinoma, lung carcinoma, and melanoma.

23. The method, according to claim 15, in which the cancer is in a tissue selected from the group consisting of: ovary, lymph node. Skin, blood, lung, prostate, colon, rectum, testes, and small intestine.

24. The method, according to claim 15, in which the XIAP polypeptide comprises a domain having at least 80% sequence identity to a BIR domain selected from the group consisting of amino acids 26-93 of human X-linked IAP (XIAP, SEQ ID NO: 4), amino acids 163-230 of human X-linked IAP (XIAP, SEQ ID NO: 4), and amino acids 265-330 of human X-linked IAP (XIAP, SEQ ID NO: 4).

25. The method, according to claim 24, in which the polypeptide comprises a domain having at least 85% sequence identity to the BIR domain.

26. The method, according to claim 24 in which the polypeptide comprises a domain having at least 90% sequence identity to the BIR domain.

27. The method, according to claim 24 in which the polypeptide comprises a domain having at least 95% sequence identity to the BIR domain.

28. The method, according to claim 24, in which the polypeptide comprises a BIR domain selected from the group consisting of amino acids 26-93 of human X-linked IAP (XIAP, SEQ ID NO: 4), amino acids 163-230 of human X-linked IAP (XIAP, SEQ ID NO: 4), and amino acids 265-330 of human X-linked IAP (XIAP, SEQ ID NO: 4).

* * * * *

专利名称(译)	哺乳动物IAP基因家族，引物，探针和检测方法		
公开(公告)号	US20070066524A1	公开(公告)日	2007-03-22
申请号	US11/498897	申请日	2006-08-04
[标]申请(专利权)人(译)	Korneluk罗伯特G MACKENZIE ALEXANDERê BAIRD STEPHEN LISTON PETER		
申请(专利权)人(译)	Korneluk罗伯特G MACKENZIE ALEXANDERê BAIRD STEPHEN LISTON PETER		
当前申请(专利权)人(译)	Korneluk罗伯特G MACKENZIE ALEXANDERê BAIRD STEPHEN LISTON PETER		
[标]发明人	KORNELUK ROBERT G MACKENZIE ALEXANDER E BAIRD STEPHEN LISTON PETER		
发明人	KORNELUK, ROBERT G. MACKENZIE, ALEXANDER E. BAIRD, STEPHEN LISTON, PETER		
IPC分类号	A61K38/17 A61K48/00 A01K67/027 A61K31/00 A61K38/00 A61K39/395 A61P1/00 A61P1/16 A61P9/00 A61P9/10 A61P25/00 A61P31/00 A61P31/18 A61P43/00 C07K14/47 C07K16/18 C12N5/10 C12N15/09 C12N15/12 C12P21/02 C12P21/08 C12Q1/68 C12R1/91 G01N33/15 G01N33/50 G01N33/53 G01N33/566		
CPC分类号	A01K2217/05 G01N2510/00 A61K38/00 A61K48/00 C07K14/4747 C12Q1/6883 C12Q1/6886 C12Q2600/118 C12Q2600/136 C12Q2600/156 C12Q2600/158 G01N33/5008 G01N33/502 G01N33/57407 G01N33/57419 G01N33/57423 G01N33/57426 G01N33/5743 G01N33/68 G01N33/6872 G01N2500/00 A01K2217/075 A61P1/00 A61P1/16 A61P25/00 A61P31/00 A61P31/18		
优先权	09/011356 1998-09-14 US PCT/IB1996/001022 1996-08-05 WO		
外部链接	Espacenet USPTO		

摘要(译)

公开了编码哺乳动物IAP多肽的基本上纯的DNA;以及使用这种DNA在细胞和动物中表达IAP多肽以抑制细胞凋亡的方法。还公开了IAP家族特有的保守区域和用于鉴定和分离另外的IAP基因的引物和探针。此外，提供了治疗涉及细胞凋亡的疾病和病症的方法。

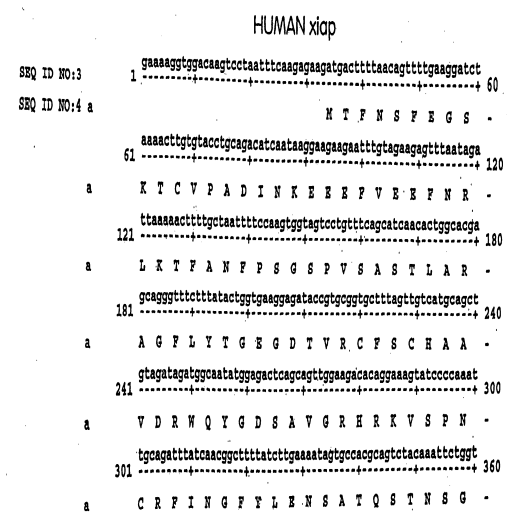
HUMAN xiap
SEQ ID NO:3
SEQ ID NO:4 a
1 gaaaaggtggacaagtcctaatttcaagagaagatgacttttaacagttttgaaggatct 60
M T F N S F E G S -
61 aaaacttgtgtacctgcagacatcaataaggaagaagaatttgtagaagagtttaataga 120
K T C V P A D I N K E E E F V E E F N R -
121 ttaaaaacttttgctaattttccaagtggtagtcctgtttcagcatcaacactggcacga 180
L K T F A N F P S G S P V S A S T L A R -
181 gcagggtttctttatactggtgaaggagataccgtgcggtgctttagttgtcatgcagct 240
A G F L Y T G E G D T V R C F S C H A A -
241 gtagatagatggcaatatggagactcagcagttggaagacacaggaaagtatccccaaat 300
V D R W Q Y G D S A V G R H R K V S P N -
301 tgcagatttatcaacggcttttatcttgaaaatagtgccacgcagtctacaaattctggt 360
C R F I N G F Y L E N S A T Q S T N S G -

Fig. 1A