PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 1/00, 14/00, 17/00, C12N 15/00, 15/09, 15/63, 15/70, 15/74, C12P 21/04, 21/06, C12Q 1/68, 1/70, G01N 33/566

A1

(11) International Publication Number: WO 96/11207

(43) International Publication Date: 18 April 1996 (18.04.96)

(21) International Application Number: PCT/US95/13426

(22) International Filing Date: 5 October 1995 (05.10.95)

(30) Priority Data:
08/319,376 6 October 1994 (06.10.94) US

(71) Applicant: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US).

(72) Inventors: LIANG, Tsanyang, Jake; 77 Pond Avenue C805, Brookline, MA 02146 (US). HUANG, Jiakang; Apartment 1, 7 Mullins Court, Cambridge, MA 02141 (US).

(74) Agents: WILLIAMS, Kathleen, A. et al.; Banner & Allegretti, Ltd., 10 South Wacker Drive, Chicago, IL 60606 (US).

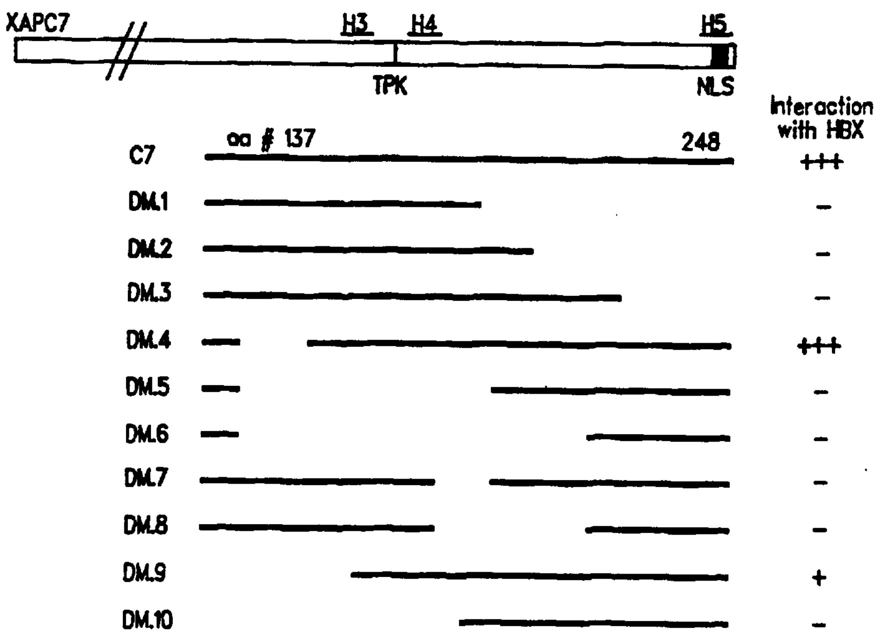
(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: COMPOSITIONS AND METHODS FOR INTERFERING WITH HEPATITIS B VIRUS INFECTION

Structural Mapping of XAPC7 Domain Interacting with HBX



(57) Abstract

The invention provides for compositions and methods for interfering with Hepatitis B viral infection that are based on the interaction of Hepatitis B virus X protein with two different proteasome subunits.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgystan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SI	Slovenia
SK	Slovakia
SN	Senegal
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

COMPOSITIONS AND METHODS FOR INTERFERING WITH HEPATITIS B VIRUS INFECTION

This invention was made in the course of work under a grant or award from the U.S. government, and therefore the U.S. government has rights in the invention.

BACKGROUND OF THE INVENTION

Hepatitis B virus (HBV) infection leads to a wide spectrum of liver injury varying from acute self-limited infection, fulminant hepatitis, asymptomatic healthy carrier state, to chronic hepatitis with progression to cirrhosis and liver failure. Moreover, chronic HBV infection has been linked to the subsequent development of hepatocellular carcinoma, a major cause of death from cancer worldwide. The pathogenesis of liver injury due to acute and chronic HBV infection is not well understood at present. The virus does not exert a cytopathic effect on hepatocytes; hepatotoxicity is likely a sequela of host immune responses to HBV antigens. In addition to hepatotropism, HBV infection has been associated with many other systemic immunological diseases such as polyarteritis nodosa, serum sickness-like syndrome, glomerulonephritis, and aplastic anemia. Therefore, the host immune responses to HBV-related antigens plays a major role in the pathogenesis of disease associated with HBV infection.

HBV is a partially double-stranded circular genome enclosed in a core structure surrounded by a lipid bilayer envelope containing Hepatitis B surface protein. HBV has a unique fourth open reading frame coding for a 16 kDa protein known as HBX. HBX appears to possess multiple functions. It activates a variety of viral and cellular promoters in diverse cell types (Colgrove et al., J. Virol. 63:4109-4026, 1989; Seto et al., Nature 344:72-74, 1990; Maguire et al., Science 252:842-844, 1991; Cross et al., Proc. Natl. Acad. Sci. USA 90:8078-8082, 1993), and therefore is a transactivator. Although the X protein does not bind to DNA directly, it activates transcription when it is targeted to a promoter by fusion to a heterologous DNA binding domain (Seto et al., supra; Maguire et al., supra; Cross et al., supra; Unger et al., The Eur. Mol. Biol. Org. J. 9:1889-1895, 1990). The protein has also been shown to function through AP-1 and AP-2 (Seto et al., supra) and to interact directly with members of CREB/ATF transcription factor family (Maguire et al., supra). Furthermore, a "Kunitz domain," characteristic of kunitz-type serine protease inhibitors, is present in HBX, and mutation of this consensus sequence inactivates the transactivation function of HBX (Takada et al., Jpn. J. Cancer Res. 81:1191-1194, 1990). In a transgenic mouse model, HBX has been shown to induce development of hepatocellular carcinoma (Kim et al., Nature 315:317-320, 1991). HBX also has been shown to play an essential role in HBV infection *in vivo* (Chen et al., J. Virol. 67:1218-1226, 1993; Zoulim et al., J. Virol. 68:2026-2030, 1994).

Eucaryotic cells contain multiple proteolytic systems, including the lysosomal proteases, calpains, the ATP-ubiquitin-proteasome-dependent pathway, and an ATP-independent nonlysosomal process. The major neutral proteolytic activity in the cytosol and nucleus is the proteasome, a 20S (700 Kda) particle with multiple peptidase activities. The function of the proteasome *in vivo* is not fully understood. However, the processing of protein antigens is believed to be accomplished by proteins of the proteasome complex. That is, small peptides are generated via proteolysis of large antigens, and are presented in the context of multiple histo- compatibility complex (MHC) molecules to T lymphocytes to initiate an immune response.

One object of the invention is the identification and characterization of cellular targets of HBX.

Another object of the invention is to provide a novel cellular protein target of HBX.

Yet another object of the invention is to provide compounds that interfere with Hepatitis B virus infection by preventing productive interaction between HBX and a cellular protein target.

SUMMARY OF THE INVENTION

The invention is based on the discovery of interaction between Hepatitis B virus X (HBX) protein and protein subunits of the proteasome complex. One subunit of the proteasome complex, XAPC7 (C7), has been isolated and characterized, and it has been discovered that C7 interacts with HBX protein. It also has been discovered that subunit 4 (S4) of the proteasome complex interacts with HBX protein.

The invention therefore features polypeptides corresponding to regions of interaction between the HBX and C7 proteins whose presence results in disruption of interaction between the two proteins.

The invention also therefore features polypeptides corresponding to regions of interaction between the HBX and S4 proteins whose presence results in disruption of interaction between the two proteins.

As used herein, "interaction" refers to the binding of HBX and a proteasome subunit to form a complex and/or to subunit-dependent transactivation of a viral or cellular promoter by HBX; thus, "prevents" or "disrupts" interaction refers to the ability to disrupt binding and/or transactivation. An "isolated" polypeptide refers to a recombinant polypeptide, a chemically synthesized polypeptide, or to a highly purified (i.e., greater than 90% by weight in a mixture of polypeptides) polypeptide.

In preferred embodiments, the polypeptide may be selected from those amino acid sequences that are based on the interacting regi s of HBX and C7, and HBX and S4. That is, the claimed polypeptide will be encompassed by an amino acid sequence of Hepatitis B virus X protein that interacts with C7 or S4, or an amino acid sequence encompassed by an amino acid sequence of C7 or S4 protein that interacts with Hepatitis B virus X protein. A polypeptide of the invention will be at least 5 amino acids in length within the encompassing sequence parameters, and may be any length within the encompassing sequence sufficient to prevent interaction of HBX and C7 or HBX and S4, e.g., 7 amino acids, 10 amino acids, 15, 20, 40, 50, 100 amino acids, etc. Preferably, the polypeptide prevents binding between HBX and C7 or HBX and S4.

As used herein, "encompassed by" is interpreted with reference to stated amino and carboxy terminal parameters and refers to a reference sequence that contains the claimed amino acid sequence in its entirety, and also includes an amino acid sequence that is identical to the stated reference sequence.

In other preferred embodiments, the isolated polypeptide comprises or consists essentially of (with respect to its ability to interfere with HBX and C7 interaction) an amino acid sequence encompassed by amino acids 220 - 248 of XAPC7 presented in Fig. 1; an amino acid sequence encompassed by amino acids 198 - 248 of XAPC7 presented in Fig. 1; an amino acid sequence encompassed by the 163-198 amino acid sequence presented in Fig. 1; an amino acid sequence encompassed by the XAPC7 carboxy terminal amino acids 171-248 and/or 188-198 presented in Fig. 1; and an amino acid sequence encompassed by residues 184-320 of S4 presented in Fig. 8A.

The invention also encompasses an isolated polypeptide comprising or consisting essentially of (with respect to its ability to interfere with binding between HBX and S4) an amino acid sequence encompassed by amino acids 184-320 of S4 presented in Fig. 8A.

The invention also includes an isolated polypeptide comprising an amino acid sequence encompassed by amino acids 132 - 145 of Hepatitis B virus X protein presented in Fig. 4.

Preferably, the polypeptide comprises an amino acid sequence encompassed by the amino acid sequence of Hepatitis B virus X protein presented in Fig. 4.

The invention also includes an isolated polypeptide selected from the group consisting of: amino acids 220 - 248 presented in Fig. 1; and amino acids 163 - 198 presented in Fig. 1; and particulary amino acids 171-248 presented in Fig. 1 and 188-198 presented in Fig 1; and also may consist of amino acids 132 - 145 presented in Fig. 4; and the amino acid sequence presented in Fig. 4.

Polypeptides of the invention are useful for interfering with Hepatitis B infection, when administered as taught herein, and are also useful as antigens to generate polyclonal or monoclonal antibodies specific for HBX , C7 or S4. These antibodies may be used as a diagnostic to detect the HBX/XAPC7 complex or the HBX/S4 complex in cells from an infected mammal as indicative of Hepatitis B growth and propagation, or to detect the individual proteins or the complex on a polyacrylamide gel, e.g., in a Western blot. Detection of the complex is critical in view of the proposed interference by HBX with proteasome function in antigen presentation.

The invention also features a recombinant nucleic acid comprising a nucleotide sequence encoding any one of the isolated polypeptides disclosed herein.

Preferably, the recombinant nucleic acid comprises the nucleotide sequence shown in Fig. 1.

Nucleic acids of the invention are useful for producing polypeptides of the invention, or as probes for detecting HBX mRNA or DNA as indicative of viral infection, or as probes for detecting C7 or S4 mRNA as indicative of expression of the gene. Alternatively, the nucleic acids may be used as primers for DNA amplification of a selected segment of HBX, C7 or S4 DNA.

The invention also features a host cell containing a recombinant nucleic acid as described above.

The invention also features a method of interfering with Hepatitis B virus infection in a mammal, comprising administering to a mammal suspected of harboring Hepatitis B virus a therapeutically effective amount of an isolated polypeptide, as described above. As used herein, "mammal" refers to any animal that is susceptible to Hepatitis B virus, and includes both non-primates and primates; "therapeutically effective" refers to an amount of polypeptide that results in reduction of detectable virus or virus-specific antibodies in the mammal's serum, or that results in reduction in symptoms associated with Hepatitis B virus infection. As used herein, "interfere" with HBV infection refers to significantly reducing (i.e., by 50% or more) the production of new HBV virions, as measured intra-cellularly or in serum, or to significantly reducing the titer of HBV-specific antibodies in serum, or to preventing growth of the virus, i.e., preventing replication of the viral genome. As used herein, "hepatitis B virus" refers to a family of viruses called hepadnaviruses, the family including but not limited to human HBV, woodchuck HBV, ground squirrel HBV, tree squirrel HBV, duck HBV, and heron HBV.

The invention also features a method of screening for a polypeptide that interferes with interaction of Hepatitis B virus X protein and proteasome C7 or S4 proteins, comprising providing Hepatitis B virus X protein, proteasome C7 protein or S4 protein, and a candidate polypeptide suspected of inhibiting interaction of X protein and XAPC7 protein or X protein and S4 protein; and combining the proteins under conditions sufficient to allow for interaction of X protein with C7 or S4 to form a complex, wherein the failure to form a complex in the presence of the candidate polypeptide is indicative of inhibition by the candidate polypeptide of protein/protein (i.e., HBX/C7 or HBX/S4) interaction.

In preferred embodiments of this method, the steps may involve *in vitro* or *in vivo* methods of providing and combining the X and XAPC7 or S4 proteins and the candidate polypeptide. For example, the proteins and candidate polypeptide may be provided and combined *in vitro* using synthesized or recombinant versions as described herein. Binding may be assessed using a labeled X, XAPC7, or S4 protein and conventional protein analysis, e.g., polyacrylamide gel separation followed by autoradiography. Alternatively, the X, XAPC7, and S4 proteins and the polypeptide may be provided *in vivo* using recombinant DNA encoding the proteins and the polypeptide and a host cell that allows for expression of the amino acid sequences. Binding and/or transactivation may be assessed as described herein, e.g., using a two-hybrid yeast system or an animal host, using conventional protein detection techniques.

Preferably, the candidate polypeptide comprises an amino acid sequence encompassed by the XAPC7 carboxy terminal amino acids 198-248 presented in Fig. 1; an amino acid sequence encompassed by the 163-198 amino acid sequence presented in Fig. 1; an amino acid sequence encompassed by the XAPC7 carboxy terminal amino acids 171-248 and/or 188-198 presented in Fig. 1; an amino acid sequence encompassed by the Hepatitis B virus X protein amino acids 132-145 presented in Fig. 4; an amino acid sequence encompassed by the Hepatitis B virus X protein amino acid sequence presented in Fig. 4; and an amino acid sequence encompassed by residues 184-320 of S4 presented in Fig. 8A.

The invention also includes a kit for interfering with Hepatitis B virus infection, the kit containing any one of the polypeptides described above that interferes with HBX/XAPC7 or HBX/S4 interaction, and container means therefore.

In an especially preferred embodiment of a kit of the invention, the kit may include a combination of two polypeptides, one polypeptide that interferes with HBX/C7 interaction and another polypeptide that interferes with HBX/S4 interaction.

In another preferred embodiment of the kit of the invention, the kit may include a consensus polypeptide which is an amino acid sequence based on sequence similarities between the regions of C7 and S4 that interact with HBX, which consensus polypeptide inhibits interaction of both HBX with C7 and HBX with S4.

The kit may further include written instructions to the effect that the polypeptide is to be administered to a mammal suspected of or known to harbor the Hepatitis B virus, preferably in a dosage that is effective to interfere with the life-cycle of the virus.

The invention also includes a kit for screening for compounds that interfere with interaction between HBX and XAPC7 or HBX and S4, the kit containing the XAPC7 and/or S4 proteins and container means therefore. The kit may further include the HBX protein. Preferably, one or more of the XAPC7, S4, and HBX proteins are labeled; if more than one protein is labeled, then it will be differentially labeled with respect to the other labeled proteins.

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

DETAILED DESCRIPTION

Before describing the invention in detail, the drawings will be briefly described.

DRAWINGS

Fig. 1 is the nucleotide sequence of the full-length cDNA of XAPC7 (SEQ ID NO: 1), along with the predicted amino acid sequence of the encoded protein (SEQ ID NO: 2).

Fig. 2 is a Northern blot analysis of several human cell lines probed with an XAPC7 DNA probe.

Fig. 3 is an autoradiogram of a polyacrylamide gel of HBX bound to XAPC7.

Fig. 4 presents a partial amino acid sequence of HBVX (top line) and WHVX (second line) in the putative Kunitz Domains (underlined) and the residues where site-directed mutations were introduced (third line).

Fig. 5 shows results of transactivation and binding between XAPC7 and HBX mutants.

Fig. 6 is a schematic illustration of a transactivation system for testing candidate compounds of the invention.

Fig. 7 is a schematic illustration of various C7 deletion mutants and results of binding experiments.

Fig. 8A is the amino acid sequence of the 26S protease S4 regulatory subunit (SEQ ID NO: 4).

Fig. 8B is the nucleotide sequence of the full-length cDNA of S4 (SEQ ID NO: 3).

Fig. 9 shows a sequence alignment of XAPC7 and XAPP13.

DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

The invention is based on the discovery of interaction between Hepatitis B virus X protein and a newly isolated and characterized protein of the proteasome complex, XAPC7. The invention also is based on the discovery of interaction between HBX protein and another proteasome subunit S4 (also referred to herein as XAPP13 or P13). The invention features polypeptides corresponding to regions of interaction between the HBX and C7, and HBX and S4 proteins, whose presence results in disruption of interaction between the proteins. Example I provides a description of the identification, cloning, and sequence determination of one cellular target of HBX, i.e., the proteasome subunit XAPC7, and provides the complete nucleotide sequence of XAPC7 and its predicted amino acid sequence. Example II provides a description of another cellular target of HBX, i.e., the proteasome subunit S4. Example III provides methods of testing of interaction between HBX and other proteins of the proteasome complex. Example IV provides methods for localization of the region of the Hepatitis B virus X protein that interacts with a proteasome subunit, e.g., the XAPC7 molecule, using HBX mutants. Example V provides methods of testing candidate polypeptides that interfere with the interaction between HBX and a cellular target such as XAPC7, and thus interfere with the HBV life-cycle. Example VI provides methods for making polypeptides of the invention. Example VII provides results of *in vivo* testing of candidate polypeptides for interference with HBX and XAPC7 interaction. Example VIII provides for methods of making nucleic acids encoding polypeptides of the invention and antibodies specific for such polypeptides.

Two proteasome subunits have been identified and characterized that interact specifically with HBX, and therefore appears to be functional targets of HBX *in vivo*. These proteasome subunits, XAPC7 and S4, were identified and characterized as follows.

EXAMPLE I

HBX appears to function as a transactivator in a variety of cell types including Hela cells. Thus, a Hela cell cDNA library containing cDNAs fused to the B42 activation domain in the yeast vector JG4-5 was used to screen for clones interacting with HBX. Four independent clones were identified. One clone strongly reacted (XAPC7) and was selected for further analysis. The screening system was as follows.

The HBX gene derived from an HBV adw strain (ATCC Nos. 45020 and 67193, or Robinson, in Fields, Virology, 2d ed., Raven Press, 1990, hereby incorporated by reference) was fused to the lexA DNA binding domain (pEG202) through the Nco 1 site (nucleotide 1375), which contains the AUG start codon of HBX. Using the yeast two hybrid system (Fields et al., Nature 340:245-246, 1989 and Gyuris et al., Cell 75:791-803, 1993, hereby incorporated by reference; see also Fig. 6), two reporter constructs were used in selection of the candidate clones; one contains the lexAop-Leu2 gene which allows for cell growth in the absence of leucine, the other has the lexAop-lacZ gene which permits selection based on ß-gal activity (pSH18-34). For screening and in initial experiments, the pSH18-34 lacZ reporter which contains eight LexA binding sites was used. In subsequent experiment, where the interaction is expected to be strong, such as the one between HBX and XAPC7, a less sensitive JK103 which contains two copies of LexA binding sites was used as the lacZ reporter. The cDNA library was transfected along with the X expression construct into EGY48 harboring both reporters.

Thus, the EGY48 yeast strain containing the LexAop-Leu2 and JK103 reporters was transformed with (1) pEG202HBX and JG4-5, (2) pEG202HBX and JG4-5XAPC7, (3) pEG202HBXRsr and JG4-5XAPC7, (4) pEG202HBXEn and JG4-5XAPC7, (5) pEG202XAPC7 and JG4-5HBX, (6) pEG202- and JG4-5HBX, (7) pEG202- and JG4-5XAPC7, and (8) pEG202XAPC7 and JG4-5. Transformants were spotted on 4 types of plates as indicated at the left and top, and the plates were photographed 3 days later. The sample positions are identical on each plate.

A pool of cells containing 2 X 10^6 primary library transformants were plated onto galactose-Leu^- selection plates. The leu^+ clones were patch-transferred to galactose-Leu^- selection plates containing X-gal. Approximately 200 clones showed detectable blue color. They were streaked out on 4 types of plates, glu-leu^-, gal-leu^-, glu-leu^+-X-gal, and gal-leu^+-X-gal. Nine clones showed definitive galactose-inducible leucine dependence and LacZ activity. Plasmids isolated from these clones were transfected into E. coli K-12 strain KC8 which allows for selection of the Trp marker carried by the library vector JG4-5. All 9 clones have cDNA inserts ranging from 0.5 to 1.5 kbp. Cross- hybridization demonstrated that some of them were derived from the same cDNA, resulting in a total of 4 groups. These clones were re-transfected back into EGY48 with HBX and the reporter constructs and they again demonstrated galactose-inducible leucine dependence and LacZ activity. In addition, transfection of these cDNA clones with pEG202 that contains the LexA DNA binding domain only (pEG202-) did not result in any transactivation of the reporter genes, indicating that specific interaction with the HBX domain of the lexA-HBX fusion protein is required. One of the cDNA clones (XAPC7) showing strong interaction was selected for further analysis.

Two HBX mutants were generated for initial analysis. One contains an amino acid (aa) insertion of arginine after aa #68 (HBXRsr); this was constructed by digesting the HBX sequence with Rsr II and then filling in the 3 nucleotide overhang with Klenow enzyme. The other contains a C-terminally truncated HBX gene ending at aa #90 (HBXEn), which was generated by truncating the HBX gene at EcoN1 restriction site (nucleotide 1645). To switch the interaction domains, HBX was fused in frame to the B42 acid patch transactivation domain of JG4-5 and XAPC7 to the lexA DNA binding domain of pEG202.

The two HBX mutants described above, one containing amino acid insertion of arginine after aa #68 (HBXRsr) and the other being a truncated N-terminal HBX (aa #1-90) (HBXEn) were included as controls. HBXRsr appeared to interact with XAPC7 but HBXEn was totally nonreactive. To demonstrate that this particular interaction between HBX and XAPC7 is not peculiar to the fusion proteins (lexA or B42), the reverse constructs were generated - HBX fused to the B42 activation domain in JG4-5 and XAPC7 fused to the lexA DNA binding domain. The result showed that this interaction between HBX and XAPC7 is specific regardless of the backbone of the fusion protein.

The full-length cDNA of XAPC7 was subsequently obtained from a λGT11 Hela cDNA library (Fig. 1). Fig. 1 shows the complete nucleotide and predicted amino acid sequence of XAPC7, based on sequencing of the full-length XAPC7 cDNA clone isolated from a λGT11 Hela cDNA library. The initiation AUG codon was identified at the 5′ end with appropriate Kozak consensus sequence. The entire ORF codes for 248 amino acids with an Mr of 27,896 kDa. Sequencing analysis of the XAPC7 clone (Fig. 1) reveals that it encodes for a polypeptide with high sequence homology to the PROS-28.1 subunit of proteasome (multicatalytic proteinase complex) of Drosophila melanogaster (Haass et al., Gene 90:235-241, 1990) and the α proteasome subunit of Arabidopsis thaliana (Genschik et al., J. FEBS Letters 309:311-315, 1992). It shows weaker but significant sequence homology to the other members of the proteasome family (Tanaka et al., The New Biologist 4:173-187, 1992). Sequence alignment between XAPC7 and its closest human relative HC8 is also shown (33% identity and 57% similarity). The fact that XAPC7 represents a highly conserved proteasome subunit with > 65% amino acid identity among members of both animal and plant kingdoms, supports the functional importance of this subunit. The domain interacting with HBX resides in the C terminus of the XAPC7 protein (i.e., encompassed by amino acids 137-248). The full-length XAPC7 was also shown to interact with HBX in the yeast (data not shown).

Using the XAPC7 cDNA as hybridization probe and washing under stringent condition, Northern blot analysis of RNA from several human and mouse cell lines, and human placenta (Clonetech) was performed and revealed a 1.0 kb XAPC7 transcript (Fig. 2). In Fig. 2, lane 1, human hepatoma cell line HepG2; lane 2, human hepatoma cell line HuH-7; lane 3, COS7; lane 4, mouse lymphoma cell line EL4, lane 5, human placenta RNA. The positions of 28S and 18S are indicated. A similar-size RNA species was also identified in RNA from a mouse lymphoma cell line.

EXAMPLE II

The 26S proteasome complex is a larger structure composed of the 20S proteasome core and several other components. In addition to the multiple peptidase activities of 20S, the 26S complex is the predominant cellular structure that degrades ubiquitin-conjugated proteins by an ATP-dependent process. S4, a 51 kDa polypeptide, belongs to a family of ATPase-related proteins that have recently been shown to confer ATP-dependent proteolytic function to the 20S proteasome core. This family of proteins is characterized by a highly conserved 300 amino acid domain containing an ATP-binding module. It has been discovered that S4 specifically interacts with HBX. The X-interacting region of S4 was mapped to within residues 184 and [illegible]20 by deletion and binding analysis in the yeast, two-hybrid system, as described herein. The S4 domain that interacts specifically with HBX contains or overlaps with the ATP-binding module.

The interaction between the C7 and S4 subunits of the proteasome complex was assessed using the yeast two-hybrid system, described herein. The results demonstrated specific interaction between XAPC7 and XAPP13 (S4). Thus, these two subunits naturally associate with each other as part of interaction between the 20S core and the S4 component of the 26S complex. XAPC7 and XAPP13 (P13 or S4) appear to interact with the same structural domain of HBX, though the affinity of C7 for HBX is much higher than that of P13 for HBX.

Sequence comparison of C7 and P13 reveals a domain with moderate homology in the C-terminus of both proteins (Fig. 9). Fig. 9 shows a sequence alignment of XAPC7 and XAPP13 using the GCG Gap program. A region of moderate homology (49% similarity and 25% identity) was identified in the C-terminal sequences of both proteins. Aligned sequences of this region are shown. Vertical lines and dots between residues represent identity and similarity, respectively. Interestingly, this P13 (S4) domain C-terminal to the ATPase module, is conserved (50-60%) identity among all the ATPase family. HBX may interfere with proteasome functions by interrupting the natural physiological interactions between the C7 and S4 proteasome subunits. Therefore, the invention also encompasses a consensus sequence polypeptide having a sequence based on the sequence homology between the C7 and S4 C-terminal sequences. This polypeptide may inhibit interaction between HBX and both C7 and S4.

EXAMPLE III

Other subunits of the proteasome family were also tested for interaction with HBX. cDNAs of five other human proteasome subunits (LMP-2, HC2, HC3, HC5, and HC8) (Brown et al., Nature 353:355-357, 1991; Glynne et al., Nature 353:357-360, 1991; Tamura et al., Biochimica et Biophysica Acta 1089:95-102, 1991) were cloned into yeast JG4-5 vector for interaction studies in yeast.

The carboxy-terminal portion of each cDNA for LMP-2, HC2, HC3, HC5, and HC8 proteasome subunits was inserted into JG4-5 to form a fusion protein with the B42 activation domain. Using PCR, the C-terminal portions of each of LMP-2 at amino acid (aa) 117, HC2 at aa 134, HC3 at aa 136, HC5 at aa 145, and HC8 at aa 138, which corresponds in sequence homology alignment to the original XAPC7 clone (aa #137-248), were inserted into JG4-5 to form fusion proteins with the B42 activation domain. The X gene of WHV derived from WHV81 strain was fused to the lexA DNA binding domain through the Nco 1 site of WHV (nt 1501), which contains the AUG start codon of WHVX. The resulting constructs by themselves did not activate the reporter (not shown). The constructs were then tested for transactivation of the reporter construct, as described above. Sample 1 is pEG202HBX and JG4-5XAPC7; sample 2, pEG202WHVX and JG4-5XAPC7; sample 3, pEG202HBX and JG4-5LMP-2; sample 4, pEG202HBX and JG4-5HC2; sample 5, pEG202HBX and JG4-5HC3; sample 6, pEG202HBX and JG4-5HC5; sample 7, pEG202HBX and JG4-5HC8.

The results showed that no interaction was observed between HBX and the proteasome subunits tested other than XAPC7. Since woodchuck hepatitis B virus also encodes an X protein (WHVX) with transactivation function and significant homology to HBX, WHVX was analyzed for its interaction with XAPC7 in yeast. The results showed that WHVX also interacts specifically with XAPC7. Experiments were as follows.

In order to demonstrate interaction of HBX and XAPC7 proteasome subunit *in vitro*, we constructed two GST-fusion expression plasmids, one with HBX and the other with the XAPC7 protein. The GST-HBX and GST-XAPC7 fusion proteins expressed in bacteria were purified with glutathione beads and then incubated with *in vitro* translated, [^{35}S]-Met labeled full-length XAPC7 and HBX polypeptides, respectively. Binding reactions with GST only were performed as controls in each binding assay.

In vitro binding experiments were performed as follows. HBX and full-length XAPC7 were cloned into pGEM11Zf(+) vector (Promega, Madison, Wi) and their transcripts were produced using *in vitro* transcription kit (Promega). Rabbit reticulocytelysates from Promega were used to generate [^{35}S]-Met labeled proteins, which were used immediately for binding studies. HBX (full-length) and XAPC7 (aa #137-248) were cloned separately into the pGEX-KG vector (Pharmacia, Piscataway, NJ) to be expressed as a fusion protein with the glutathione-S transferase. The binding reactions were performed in NETN buffer (0.5% of NP-40, 20 mM Tris pH 8.0, EDTA 1 mM, 100 mM NaCl) at room temperature for 1 hour with constant mixing. The beads were washed extensively with the same buffer and the bound proteins were subjected to 15% SDS-PAGE analysis.

In Fig. 3, the *in vitro* translated protein products are shown in lane 1 as HBX and lane 4 as XAPC7. The other lanes are as indicated. The background labeling of a 35 kDa is often seen in this preparation of rabbit reticulocytelysates (Promega). This irrelevant protein product represents an internal control for binding to the GST fusion protein-containing beads.

The results, shown in Fig. 3, demonstrate that HBX bound specifically to GST-XAPC7 and not GST alone; similar binding was also seen between XAPC7 and GST-HBX.

EXAMPLE IV

Structural and functional mapping of HBX have defined two domains that are crucial for the transactivation function of HBX (Takada et al., supra; Arii et al., Oncogene 7:397-

403, 1992; Runkel et al., Virology 197:529-536, 1993). These two domains appear to overlap with the putative "Kunitz-type" domain of protease inhibitor that are present in both HBX and WHVX. Several key residues in these two domains were mutated and studied with respect to the transactivation function of HBX and interaction between HBX and XAPC7 in the yeast two-hybrid system. The glycine and cysteine residues were mutated in both domains (Fig. 4); several other conserved residues were also mutated. In Fig. 4, amino acid sequences of HBX and WHVX around the putative Kunitz Domains (underlined) are shown. Amino acid numbers of the HBX protein are shown at the top. Site-directed mutations (shown at the bottom of the amino acid residues) were introduced and are numbered as MT1 to 10 sequentially: MT1 with Cys to Ser mutation at aa #61, MT2 with Gly to Ala mutation at aa #67, MT3 with Pro to Ala at aa #68, MT4 with Cys to Ser at aa #69, MT5 with Trp to Arg at aa #120, MT6 with Phe to Tyr at aa #132, MT7 with Gly to Val at aa #136, MT8 with Cys to Ser at aa #137, MT9 with Arg to Gln at aa #138, MT10 with His to Asp at aa #139. An additional mutant, MT11, was generated by replacement of aa residues 137 to 141 (Cys-Arg-His-Lys-Leu) with Val-Met sequence. The HBXRsr mutant has been described previously.

To obtain a structure-function correlation of this interaction, the mutants were also tested for their transactivation activities. Since HBX has been shown to activate transcription through AP-1, AP-2, AP-3, NF-κB and SP-1 factors (Seto et al., supra; Maguire et al., supra; Kekule et al., Nature 361:742-745, 1993), the effects of these mutants were tested on five reporter constructs, each of which contains a cis-acting sequence responsive to each of the five factors. Rous Sarcoma Virus (RSV0 LTR, which has been shown to be transactivated by HBX, was also tested. The results are shown in Fig. 5.

In Fig. 5, reporter constructs directed by AP-1, AP-2, Ap-3, NF-kB, SP-1 and RSV were co-transfected with HBX mutant expression constructs into HepG2 cells. The reporter activity of each mutant was shown as percentage of activity of wild-type HBX (indicated as transactivation index in the figure). Interactions of HBX mutants and XAPC7 are shown below the transactivation activity of each mutant. The light grey color of the MT5, suggestive of diminished binding, was consistently observed in various experiments.

HBX mutants were generated by PCR and confirmed by sequencing. Wild-type and mutant HBX genes were cloned into the pCD.1 expression vector (Invitrogen) for transactivation studies. HBX mutants were also constructed into pEG202 for interaction studies in yeast. CAT reporter constructs containing either 4 AP-1, 6 AP-2, 5 AP-3, or 3 NF-kB sites in front of a minimal human metallothionein IIA promoter (MT-IIA) were described previously (Maguire et al., supra). Another reporter construct contains 2 SP-1 sites (5' GGGCGGGGCAGGG 3') cloned into the pTK81Luc plasmid which consists of a minimal HSV TK promoter in front of the luciferase gene (Nordeen, S.K., Biotechniques 6:454-457, 1988). The reporter construct for RSV is RSV-Luc. The minimal MT-IIA and TK promoter-driven reporter constructs had low activities in transfected cells and were not transactivated by co-transfection with HBX expression construct (not shown). Calcium phosphate co-transfection of HBX expression constructs and reporter plasmids were performed at a ratio of 1 to 5 with a total DNA of 0.3 μg per well in 6-well plate. Reporter activities were assayed two days later. Data presented are the mean values of transfections done in triplicate and the results are representative of three separate experiments.

The mutations tested here appeared to affect the transactivation of all the reporters equally, suggesting that HBX exerts its transactivation function through a single functional pathway. This observation was also corroborated by transfection studies in CV-1 cells (data not shown). The glycine (MT7) and histidine (MT10) mutations in the second domain eliminated both the transactivation and binding properties. The cystein mutation (MT8) did not affect the binding or transactivation. MT11 with a partial deletion of the second domain also had no activities in both transactivation and binding. Other conserved residues in the second domain, such as phenylalanine (MT6) and arginine (MT9), appeared not to be essential for XAPC7 binding or function of HBX. Mutagenesis analysis in the second domain of HBX shown in Fig. 4 demonstrated a close association between the ability of the mutants to interact with XAPC7 and the transactivation activity of the mutants.

Mutagenesis studies of the first domain of HBX (shown in Fig. 5) demonstrated that this domain is not important for interaction with XAPC7 but is critical for HBX transactivation function. The glycine (MT2) and two cysteine (MT1 and 4) mutations in the first domain appeared to abrogate the transactivation function of HBX without affecting the binding to XAPC7. The mutant with proline to alanine substitution (MT3) in the first domain seemed to retain 50-60% of the transactivation activities of wild-type HBX but bound to XAPC7 as WT HBX did. These data are consistent with the phenotype of the HBXRsr and HBXEn mutants described previously. This observation suggests that HBX may interact with another cellular factor through the first domain and this interaction is equally critical for the function of HBX. The two cysteine residues in the first domain might be important in the formation of disulfide bond. Finally, because of the highly conserved tryptophan residue at aa #120, an additional mutation was introduced at this position (MT5, Trp to Arg). Transactivation and interaction analyses showed that this HBX mutant does not transactivate but retains part of the binding activity to XAPC7. This conserved tryptophan residue, therefore, may contribute to the binding of the second domain of HBX to XAPC7.

EXAMPLE V

The invention also encompasses a method of screening for compounds that interfere with binding of HBX protein to the XAPC7 proteasome subunit. Thus, a candidate polypeptide according to the invention may be tested for ability to interfere with interaction between HBX and XAPC7, as follows.

An *in vitro* screening method for candidate polypeptides that interact with HBX or XAPC7 is as follows. This system is described above with respect to demonstrating HBX/XAPC7 interaction.

Two glutathione-S transferase (GST)-fusion expression plasmids are generated as described above, one encoding a GST-HBX fusion protein, the other encoding a GST-XAPC7 fusion protein. These proteins are generated in a rabbit reticulocytelysate system using RNAs transcribed *in vitro* from the genes, or are generated in bacteria as fusion proteins which are purified with glutathione beads, as described above, Binding reactions include an ^{35}S-Met labeled GST-fusion protein and a candidate polypeptide fusion protein in NETN buffer (see above). Binding reactions with GST only are performed as controls in each binding assay. The beads are then washed and bound proteins are analyzed on SDS-PAGE. The labeled GST-fusion protein is used above to determine the control migration position. Binding of a candidate polypeptide is determined by comparison of migration of the candidate polypeptide with respect to the unbound labeled protein. That is, if a larger protein complex is apparent on the gel in the appropriate sample lane, then this indicates binding of the candidate polypeptide to the labeled fusion protein. Failure of the candidate polypeptide to bind is indicated by migration of the sample protein mixture to the control label position.

An *in vivo* method of screening candidate compounds according to the invention for interaction with the HBX or XAPC7 proteins is as follows. Fig. 6 schematically illustrates the yeast two hybrid system. This system is described above for identification of the XAPC7 protein as a cellular factor that interacts with HBX. The system is also described above for identification of HBX mutants that fail to transactivate the reporter gene when combined in the system with XAPC7. As shown above, some of these mutants may, however, retain the ability to bind to XAPC7.

Other compounds, e.g., polypeptides, may be tested for transactivation and binding using this system, which is briefly described below.

With reference to Fig. 6, in brief, two different DNA constructs capable of replicating in yeast are prepared. The first construct includes a gene coding for a protein or polypeptide to be tested fused to a gene encoding a known DNA-binding polypeptide that does not activate transcription by itself, for example, the DNA binding domain of GAL4 (amino acids 1-147) or LexA (amino acids 1-202). The second construct includes a gene coding for an activation domain, i.e., a transcriptional activator, such as that of GAL4 (amino acids 768-881), fused to one of HBX or XAPC7 to provide a binding domain. Thus, if the candidate polypeptide comprises an amino acid sequence from the HBX protein, the first construct is a fusion protein of GAL4 (amino acids 1-147) and the candidate HBX polypeptide, and the second construct will encode a fusion protein of GAL4(768-881) and XAPC7 (or a domain fragment thereof that binds to HBX). Alternatively, if the candidate polypeptide comprises an amino acid sequence from the XAPC7 protein, the first construct is a fusion protein of GAL4 (amino acids 1-147) and the candidate XAPC7 polypeptide, and the second construct will encode a fusion protein of GAL4(768-881) and HBX (or a domain thereof that binds to XAPC7). The second construct by itself does not activate transcription because it contains no DNA binding domain (that is, it contains only a binding domain specific for the HBX/XAPC7 interaction). The reporter construct contains the cognate DNA binding sequence of the DNA binding domain upstream of a gene that produces a protein with enzymatic activity, such as ß-galactosidase, or an auxotrophic marker, such as Leu-2, which is required for leucine synthesis. The interaction of two proteins will bring the DNA binding and activation domains, e.g., of GAL4, together to activate transcription of the reporter gene, thus enabling identification of a yeast clone containing the candidate polypeptide that interacts with the HBX or XAPC7 protein.

In addition, in another alternative assay similar to the two hybrid system described above, the first and second constructs may include HBX and XAPC7 fusion proteins, as described in the above Examples. A third construct may be provided to the cell which encodes and allows for expression of a candidate polypeptide. Thus, if the expressed candidate polypeptide interferes with interaction between the HBX and XAPC7 fusion proteins, the reporter gene will not be expressed. In contrast, absent an interfering candidate polypeptide, the HBX and XAPC7 interaction will occur and the reporter gene will be expressed.

Preparation of DNA constructs, yeast transformation, and identification of transactivating or non-transactivating clones are described in detail above for identification of the XAPC7 cellular proteasome subunit and in Fields et al., 1989, supra, and Gyuris et al., 1993, supra). Appropriate modifications of the system to accomplish substitution of DNA encoding a candidate polypeptide rather than a complete protein will be readily apparent to one of skill in the art.

Polypeptides of the invention may also be tested in a suitable animal model. Polypeptides that are able to interfere with the Hepatitis B virus X protein/XAPC7 interaction may be tested in the woodchuck system, as described in detail in Chen et al., Jour. Virol. 67;1218 (1993) and Zoulim et al., Jour. Virol. 68;2026 (1994), both of which are hereby incorporated by reference.

Polypeptides that are found to interfere with the HBX/XAPC7 interaction via any one of the *in vitro* or *in vivo* tests described above may be of further use in stimulating the immune response to HBV infection. That is, without being bound to any one theory, it is proposed that the binding of HBX to XAPC7 of the proteasome interferes with proteasome function, thus preventing viral antigen presentation in HBV-infected cells.

EXAMPLE VI

Proteins and polypeptides of the present invention will have sequences as described herein and may be produced by de novo synthesis, or by expression of a suitable nucleic acid in a suitable expression system *in vitro* or in transfected cells, or they may be obtained by extraction from natural sources in which case the proteins will be substantially pure and free from other protein and non-protein material with which they are naturally associated *in vivo*.

The polypeptides and proteins of the invention may be used for treatment of HBV infection in a mammal or for generating antibodies.

In a particular aspect, the invention relates to polypeptides having a sequence of at least 5 contiguous amino acid residues corresponding to a sequence of the same length as presented in Figs. 1 to 4 or alleles thereof. Polypeptides will preferably be 7 or even 10, 20, 50, 100 or 200 or more residues in length. Those longer than 5 residues may differ in one or more residues from the sequence presented in Figs. 1 to 4. Differences may be by substitution deletion or insertion. Polypeptides preferably have at least 75%, more preferably 85%, 90% or even 99% homology with the relevant portion of the sequence presented in Figs. 1 to 4 or alleles thereof.

In another aspect, the invention relates to proteins and polypeptides which contain epitopes corresponding to one or more epitopes of the XAPC7 or S4 proteins, or mimetopes thereof, or which are capable of interrupting association of HBX and XAPC7 or HBX and S4, or which are capable of interrupting association of HBX and XAPC7 or HBX and S4 so as to prevent function of the proteasome by specific reversible or irreversible binding. Such proteins and polypeptides may be identified by appropriate assay techniques, for instance as described herein.

Antibodies may be whole antibodies such as IgM or, preferably IgG; they may be polyclonal or monoclonal. The term "antibody" includes fragments of antibodies containing the antigen-recognition site such as F(ab) and $F(ab^1)_2$ fragments, single domain antibodies (DABs), complementarity-determining regions (CDRs) and minimal recognition units (MRUs). The term "antibody" further includes anti-idiotypic antibodies and anti-idiotype-2 antibodies (i.e. antibodies against anti-idiotype-2 antibodies) and fragments,DABs, CDRs, and MRUs thereof. Anti-idiotypic antibodies are those which recognize the antigen-recognition site of an antibody. Anti-idiotypic antibodies therefore mimic the antigen whereas anti-idiotype-2 antibodies mimic the antibody.

Antibodies may be obtained by conventional immunization techniques and extracted from the body fluids of immunized animals. Alternatively, they may be obtained by culturing antibody secreting cells obtained from such immunized animals, preferably after immortalization by fusion with myeloma cells and other well known techniques. In another alternative, antibodies may be obtained by expressing genetic material, obtained from such antibody secreting cells, in transfected cells.

Antibodies specific for polypeptides or proteins of the present invention are useful in detection of HBX or XAPC7 or S4 proteins used in the screening methods described herein, or for preventing association of HBX and XAPC7 or HBX and S4, and thus for interfering with the HBV life-cycle or for preventing proteolysis by the proteasome.

Cells which secrete antibodies may be obtained by conventional techniques and include antibody-secreting cells obtained from animals immunized against an appropriate antigen, antibody-secreting cells obtained from immunized animals and immortalized by fusion with myeloma cells to form hybridomas, by use of Epstein Barr virus and other known immortalizing techniques and cells capable of expressing and, preferably, processing antibodies on the basis of exogenous nucleic acid inserts.

Antibodies specific for polypeptides of the invention have a variety of uses, including interfering with HBX/XAPC7 or HBX/S4 interaction, identification of the cognate epitope (protein) in the cell, or for identification of the cognate antigen or the HBX/XAPC7 or HBX/S4 complex via polyacrylamide gel electrophoresis or Western blotting (see Maniatis et al., 1990, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, CSH, NY, hereby incorporated as reference).

EXAMPLE VII

Polypeptides corresponding to portions of the XAPC7 carboxy terminus, i.e., the region encompassing amino acids 137-248, were tested for the ability to interfere with the HBX/XAPC7 interaction or to bind to HBX, as follows.

XAPC7 polypeptide fragments were tested for binding to HBX, as follows. Deletion constructs of XAPC7 polypeptide-encoding DNA were generated and tested for binding to HBX in yeast cells. Deletion mutants were generated using convenient restriction sites. These mutants are DM.4, DM.5, DM.6, DM.7 and DM.8. The mutants were generated using restriction sites within the XAPC7 cDNA followed by T4 DNA polymerase treatment and ligation: DM.4 contains an in-frame deletion of aa #143-163 (Avr II to Ban 1); DM.5 deletion of aa #143-198 (Avr II to Xmn I); DM.6 deletion of 143-220 (Avr II to Dra I); DM.7 deletion of aa #188-198 (BstXI to Smn I); DM.8 deletion of aa #188-220 (BstX I to Dra I). DM.1, DM.2, DM.3, DM.9 and DM.10 were generated by PCR and contain: DM.1 (containing amino acids 137-190; DM.2 (containing amino acids 137-207); DM.3 (containing amino acids 137-228); DM.9 (containing amino acids 171-248); DM.10 (containing amino acids 194-248).

The deletion mutants were cloned into JG4-5 and analyzed for interaction with pEG202HBX in yeast, as described above. The results, shown in Fig. 7, reveal that aa 137-248 of XAPC7 contains the HBX-interacting domain. The data demonstrates that the interaction domain includes two regions: the middle and the very C-terminal region of C7. The C-terminal 20 amino acids appears to be essential for interaction between C7 and HBX. Deletion of 143-163 (DM.4) interacted positively with HBX, whereas deletion of 137-170 (DM.9) was weakly reactive. A small deletion of aa 188-198 (DM.7) appeared to eliminate interaction with HBX completely. These data suggest that, in addition to the C-terminal 20 amino acids of C7, amino acid sequences in the middle of C7 (163-198) are also important for interaction with HBX.

In order to better define the contact points within C7 for interaction between C7 and HBX, amino acid residues 178-200 and 239-246 will be subject, individually or together, to mutational analysis, as follows: Asp-178 will be replaced with an asparagine, Glu-182 with glutamine, Asp-185 with asparagine, Lys-189 with glutamine, Lys-193 with glutamine, Leu-196 with threonine, Glu-197 with gln, Val-198 with thr, Val-199 with thr, Gln-200 with glu, Asn-239 with lys, Glu-240 with gln, Lys-241 with gln, Lys-242 with gln, Lys-243 with gln, Gln-244 with gly, Lys-245 with gln, and Lys-246 with gln. Single amino acid mutants will be made by oligonucleotide-directed mutagenesis, and tested as described hereinabove for deletion mutants. Where a single or several amino acid mutation(s) results in a failure of C7 to bind to HBX, that amino acid(s) will thus be indicated as important for binding of C7 to HBX and as a residue that is preferred in a polypeptide of the invention.

EXAMPLE VIII

Except as otherwise required, nucleic acids of the invention may be DNA or RNA. The nucleic acids may be single stranded or double stranded. Double stranded nucleic acids may be blunt ended or have 5′ or 3′ extensions at one or both ends; they may contain one or more restriction endonuclease recognition and/or cutting sites and one or both ends may be cut ends obtained by restriction endonuclease cutting or designed for ligation. Single stranded DNA may be a template strand or a complementary (non-template) strand. DNA may be cDNA, genomic DNA or synthetic DNA. RNA may be mRNA, sense or antisense RNA.

Nucleic acids of the invention may be produced by *de novo* synthesis and, if necessary, by assembly of fragments to create longer sequences, or obtained from natural sources such as from human cells or by cloning or amplification of natural or synthetic nucleic acids including by transcription or reverse transcription *in vitro* or in host cells.

Nucleic acids of the invention are useful in diagnosis for instance as hybridization probes for following alleles of HBX or XAPC7 or S4 genes through family trees to identify at risk individuals where particular alleles are associated with particular diseases, or to identify mutated or damaged genes in an individual. These nucleic acids are also useful as probes for HBV productive infection, or as primers for amplification of a selected region of HBX or XAPC7 or S4 DNA.

In a particular aspect, the invention relates to nucleic acids having a sequence of at least 17 contiguous nucleotide bases or base pairs corresponding to a sequence of the same length within the sequence set out in Figs. 1 and 4 or alleles thereof. Preferably the nucleic acid contains at least 20, more preferably 50, 100 or even 200 or more bases or base pairs in a sequence corresponding to that of Figs. 1 and 4. Nucleic acid of a total of 17 bases or base pairs will have a sequence identical to the relevant portion of the sequences of Figs. 1 and 4 or alleles thereof or will be exactly complementary thereto. Nucleic acids longer than 17 bases or base pairs may have a sequence exactly the same as or differing from the relevant portion of the sequence of Figs. 1 and 4 or alleles thereof in one or more bases or base pairs or complementary to such a sequence. Differences may be by substitution, deletion or insertion but should not preserve the reading frame where the nucleic acid is intended to be expressed. Preferably such nucleic acids have at least 75%, more preferably 85%, 90% or even 99% homology with the relevant portion of the sequence of Figs. 1 and 4 or alleles thereof.

In another aspect, the invention relates to nucleic acids encoding proteins or polypeptides of the invention by use of alternative codons to those used in the normal HBX and XAPC7 genes.

Cloning vectors, expression vectors, viral genomes (and virus particles containing such genomes), transfected prokaryotic and eukaryotic cells and transgenic animals of the invention all contain exogenous nucleic acids of the invention and may be produced by conventional techniques.

These vectors and expression systems may variously be used for amplification of nucleic acids of the invention, as diagnostic reagents or therapeutic agents and as sources of materials such as nucleic acids, proteins or polypeptides and antibodies which are themselves products of the invention.

For *in vitro* and *in vivo* procedures and for other reasons well known to those skilled in the art, it is often convenient to provide products such as the nucleic acids, proteins, peptides and antibodies of the invention with detectable labels. Such labels, for instance radio isotopes, fluorescent chromophores, enzymes, metal particles and polyester beads may be bonded to the products of the invention, used and detected by conventional techniques. Labeled products form a particular aspect of the invention.

For therapeutic applications and *in vivo* diagnostic procedures and for other reasons well known in the art, it is often useful to target HBX to a particular site using the recognition properties of a targeting entity such as an antibody.

Diagnostic and screening methods of the invention include all conventional techniques practiced *in vitro* including nucleic acid hybridizations and immunoassays based on the use of antibodies or proteins or polypeptides of the invention including direct or competitive protein/protein binding assays, ELISA, RIA and fluorescent assays.

USE

The invention provides compositions and methods used for treatment of Hepatitis B viral infection in mammals when administered as described herein. The compound is preferably administered via oral, intravenous or parenteral modes. A therapeutically effective amount of a polypeptide composition of the invention will be in the range of about 0.1 mg - 50mg/kg body weight/day; preferably in the range of 5-20 mg/kg/day. The compound may be administered with a pharmaceutically acceptable carrier substance, e.g., magnesium carbonate, lactose, or a phospholipid with which the compound can forma micelle, together can form a therapeutic composition, e.g., a pill, tablet, capsule or liquid for oral administration to the mammal. Other forms of compositions are also envisioned, e.g., a liquid capable of being administered nasally as drops or spray, or a liquid capable of intravenous, parenteral, subcutaneous, or intraperitoneal administration. The compound may be in the form of a biodegradable sustained release formulation for intramuscular administration. For maximum efficacy, zero order release is desirable, e.g., using an implantable or external pump, e.g., an Infusaid™ pump (Infusaid Corp, MA).

OTHER EMBODIMENTS

The invention may be embodied in other specific forms without departing from the spirit and scope thereof. Accordingly, other embodiments are within the following claims.

CLAIMS

1. An isolated polypeptide comprising a polypeptide that prevents interaction of Hepatitis B virus X protein and XAPC7 protein.

2. An isolated polypeptide comprising a polypeptide that prevents interaction of Hepatitis B virus X protein and S4 protein.

3. The isolated polypeptide of claim 1 or 2 selected from the group consisting of:

an amino acid sequence encompassed by an amino acid sequence of Hepatitis B virus X protein that interacts with XAPC7, amino acid sequence encompassed by an amino acid sequence of XAPC7 protein that interacts with Hepatitis B virus X protein, and an amino acid sequence encompassed by an amino acid sequence of S4 protein that interacts with Hepatitis B virus X protein.

4. The isolated polypeptide of claim 1 comprising an amino acid sequence encompassed by amino acids 220 - 248 of XAPC7 presented in Fig. 1.

5. The polypeptide of claim 1, comprising an amino acid sequence encompassed by amino acids 198 - 248 of XAPC7 presented in Fig. 1.

6. The polypeptide of claim 1, comprising an amino acid sequence encompassed by amino acids 163 - 198 of XAPC7 presented in Fig. 1.

7. The polypeptide of claim 1, comprising an amino acid sequence encompassed by the XAPC7 carboxy terminal amino acids 171-248 presented in Fig. 1.

8. The polypeptide of claim 1, comprising an amino acid sequence encompassed by the XAPC7 carboxy terminal amino acids 188 - 198 presented in Fig. 1.

9. The polypeptide of claim 2, comprising an amino acid sequence encompassed by residues 184 - 320 of S4 presented in Fig. 8A.

10. An isolated polypeptide comprising an amino acid sequence encompassed by amino acids 132 - 145 of Hepatitis B virus X protein presented in Fig. 4.

11. The polypeptide of claim 10, comprising an amino acid sequence encompassed by the amino acid sequence of Hepatitis B virus X protein presented in Fig. 4.

12. An isolated polypeptide selected from the group consisting of:

amino acids 198 - 248 presented in Fig. 1;
amino acids 155 - 248 presented in Fig. 1;
amino acids 220 - 248 presented in Fig. 1;
amino acids 171 - 248 presented in Fig. 1;
amino acids 163 - 198 presented in Fig. 1;
amino acids 188 - 198 presented in Fig. 1;
amino acids 184 - 320 of presented in Fig. 8A;
amino acids 132 - 145 presented in Fig. 4; and
the amino acid sequence presented in Fig. 4.

13. A recombinant nucleic acid comprising a nucleotide sequence encoding an isolated polypeptide as claimed in claim 1 or 2.

14. The recombinant nucleic acid of claim 13 comprising the nucleotide sequence shown in Fig. 1.

15. A host cell containing the recombinant nucleic acid of claim 13.

16. A method of interfering with Hepatitis B virus infection in a mammal, comprising

administering to a mammal suspected of harboring Hepatitis B virus a therapeutically effective amount of a polypeptide according to claim 1 or 2.

17. A method of screening for a polypeptide that interferes with interaction of Hepatitis B virus X protein and a proteasome, comprising

providing Hepatitis B virus X protein, proteasome XAPC7 or S4 protein, and a candidate polypeptide suspected of inhibiting interaction of said X protein and said XAPC7 or said S4 protein; and

combining said proteins under conditions sufficient to allow for interaction of said X protein and said XAPC7 or S4 protein to form a complex, wherein the failure of said X and said XAPC7 or said S4 proteins to form a complex in the presence of said candidate polypeptide is indicative of inhibition of said interaction by said candidate polypeptide.

18. The method of claim 17 wherein said candidate polypeptide comprises an amino acid sequence encompassed by the XAPC7 carboxy terminal amino acids 198 - 248 presented in Fig. 1.

19. The method of claim 17 wherein said candidate polypeptide comprises an amino acid sequence encompassed by the XAPC7 carboxy terminal amino acids 155 - 248 presented in Fig. 1.

20. The method of claim 19 wherein said candidate polypeptide comprises an amino acid sequence encompassed by amino acids 220 - 248 presented in Fig. 1.

21. The method of claim 17 wherein said candidate polypeptide comprises an amino acid sequence encompassed by amino acids 220 - 248 presented in Fig. 1.

22. The method of claim 17 wherein said candidate polypeptide comprises an amino acid sequence encompassed by amino acids 171 - 248 presented in Fig. 1.

23. The method of claim 17 wherein said candidate polypeptide comprises an amino acid sequence encompassed by amino acids 163 - 198 presented in Fig. 1.

24. The method of claim 17 wherein said candidate polypeptide comprises an amino acid sequence encompassed by amino acids 188 - 198 presented in Fig. 1.

25. The method of claim 17 wherein said candidate polypeptide comprises an amino acid sequence encompassed by amino acids 184 - 320 of presented in Fig. 8A.

26. The method of claim 17 wherein said candidate polypeptide comprises an amino acid sequence encompassed by the Hepatitis B virus X protein amino acids 132-145 presented in Fig. 4.

27. The method of claim 26 wherein said candidate polypeptide comprises an amino acid sequence encompassed by the Hepatitis B virus X protein amino acid sequence presented in Fig. 4.

28. A kit for interfering with Hepatitis B virus infection, the kit comprising an isolated polypeptide as claimed in claim 1 or 2, and container means therefore.

29. The kit of claim 28, further comprising written instructions comprising: the polypeptide is to be administered to a mammal suspected of or known to harbor the Hepatitis B virus.

30. A kit for screening candidate compounds that may interfere with interaction between HBX and a proteasome

subunit, the kit comprising the XAPC7 or S4 protein and container means therefore.

31. The kit of claim 30, further comprising the HBX protein.

32. The kit of claim 31, wherein one or both of said proteins are labeled.

```
                                                   ggagcccggccgcccgccggc
  1  atgagctacgaccgcgccatcaccgtcttctcgcccgacggccacctcttccaagtggag   60
     MetSerTyrAspArgAlaIleThrValPheSerProAspGlyHisLeuPheGlnValGlu

 61  tacgcgcaggaggccgtcaagaagggctcgaccgcggttggtgttcgaggaagagacatt  120
     TyrAlaGlnGluAlaValLysLysGlySerThrAlaValGlyValArgGlyArgAspIle

121  gttgttcttggtgtggagaagaagtcagtggccaaactgcaggatgaaagaacagtgcgg  180
     ValValLeuGlyValGluLysLysSerValAlaLysLeuGlnAspGluArgThrValArg

181  aagatctgtgctttggatgacaacgtctgcatggcctttgcaggcctcaccgccgatgca  240
     LysIleCysAlaLeuAspAspAsnValCysMetAlaPheAlaGlyLeuThrAlaAspAla

241  aggatagtcatcaacagggcccgggtggagtgccagagccaccggctgactgtagaggac  300
     ArgIleValIleAsnArgAlaArgValGluCysGlnSerHisArgLeuThrValGluAsp

301  ccggtcactgtggagtacatcacccgctacatcgccagtctgaagcagcgttatacgcag  360
     ProValThrValGluTyrIleThrArgTyrIleAlaSerLeuLysGlnArgTyrThrGln

361  agcaatgggcgcaggccgtttggcatctctgccctcatcgtgggtttcgactttgatggc  420
     SerAsnGlyArgArgProPheGlyIleSerAlaLeuIleValGlyPheAspPheAspGly

421  actcctaggctctatcagactgacccctcgggcacataccatgcctggaaggccaatgcc  480
     ThrProArgLeuTyrGlnThrAspProSerGlyThrTyrHisAlaTrpLysAlaAsnAla

481  ataggccggggtgccaagtcagtgcgcgagttcctggagaagaactatactgacgaagcc  540
     IleGlyArgGlyAlaLysSerValArgGluPheLeuGluLysAsnTyrThrAspGluAla

541  attgaaacagatgatctgaccattaagctggtgatcaaggcactcctggaagtggttcag  600
     IleGluThrAspAspLeuThrIleLysLeuValIleLysAlaLeuLeuGluValValGln

601  tcaggtggcaaaaacattgaacttgctgtcatgaggcgagatcaatccctcaagatttta  660
     SerGlyGlyLysAsnIleGluLeuAlaValMetArgArgAspGlnSerLeuLysIleLeu

661  aatcctgaagaaattgagaagtatgttgctgaaattgaaaaagaaaaagaagaaaacgaa  720
     AsnProGluGluIleGluLysTyrValAlaGluIleGluLysGluLysGluGluAsnGlu

721  aagaagaaacaaaagaaagcatcatgatgaataaaatgtctttgcttgtaatttttaaatt 780
     LysLysLysGlnLysLysAlaSerEndEnd

781  catatcaatcatggatgagtctcgatgtgtaggcctttccattccatttattcacactgag 840

841  tgtcctacaataaacttccgtattttt (poly a)
```

FIG. 1

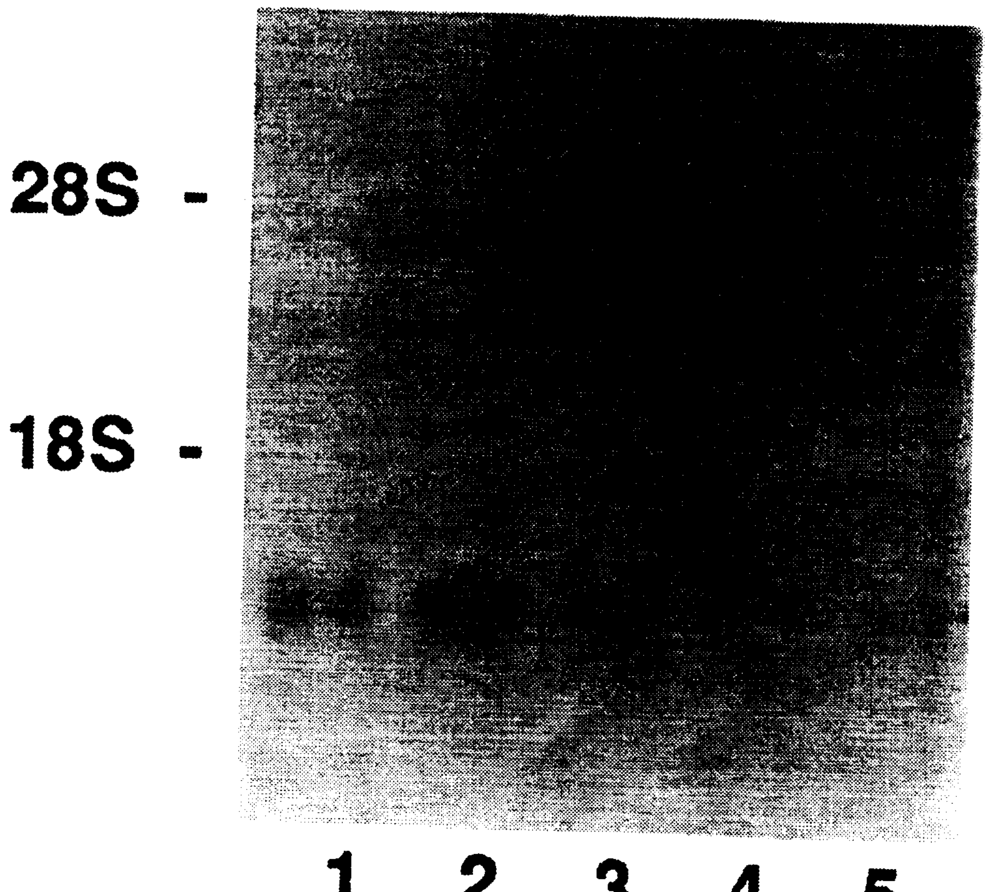


FIG. 2

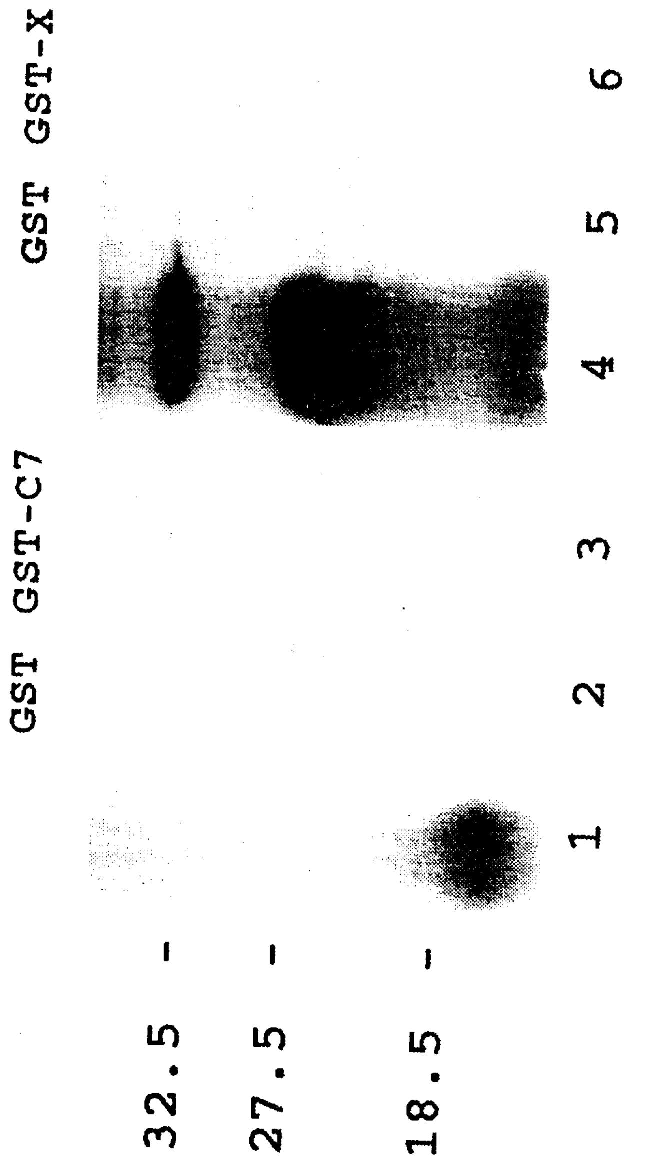


FIG. 3

```
aa #          60         70
              |          |
HBV   ...GLPVCAFSSAGPCALRFTSA.....................
WHV   ...RLPACLASGSGPCCLVFTCA.....................
              S     AAS

aa #               120        130        140
                    |          |          |
HBV   ...YFKDCLFKDWEELGEEIRLKVFVLGGCRHKLVCSPAP...
WHV   ...YIKDQLLTKWEEGSIDPRLSIFLLGGCRHKCMRLL*
                    R           Y   VSQD
```

FIG. 4

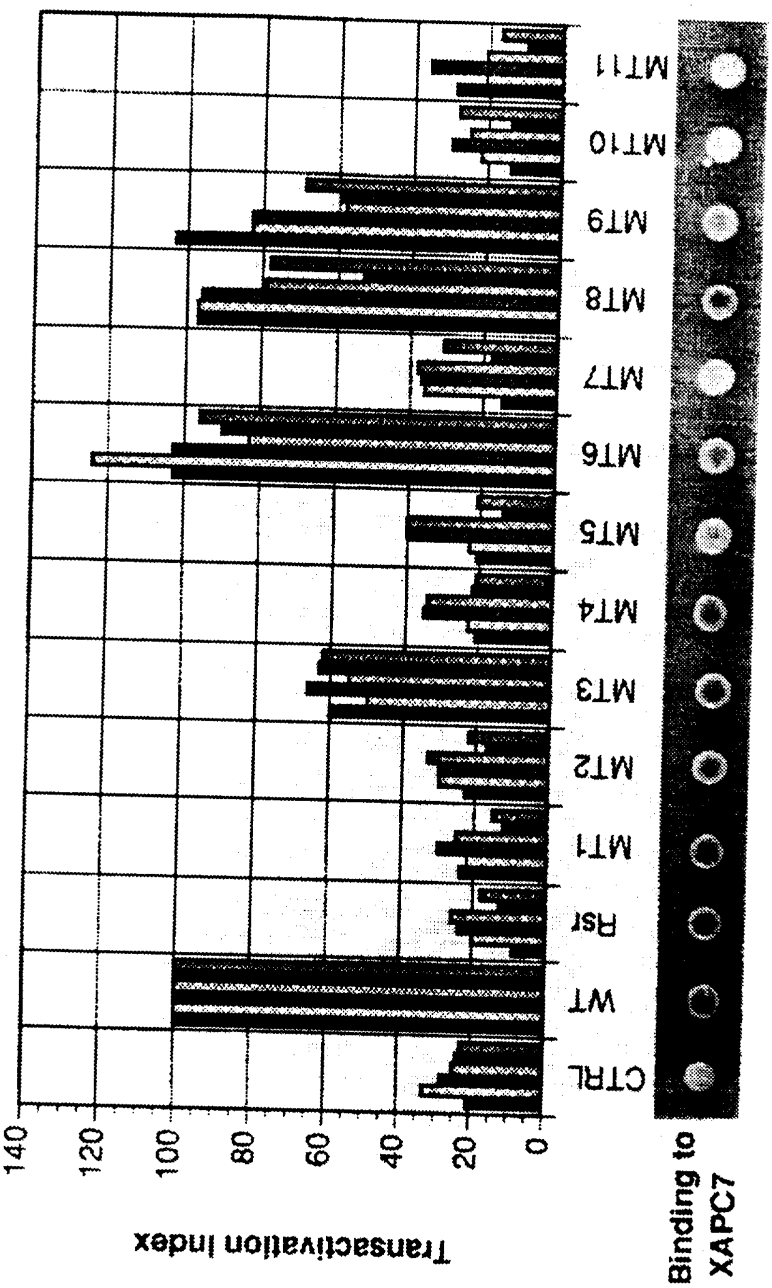

Transactivation Index
140
120
100
80
60
40
20
0
CTRL
WT
Rsr
MT1
MT2
MT3
MT4
MT5
MT6
MT7
MT8
MT9
MT10
MT11
Binding to XAPC7

FIG. 5

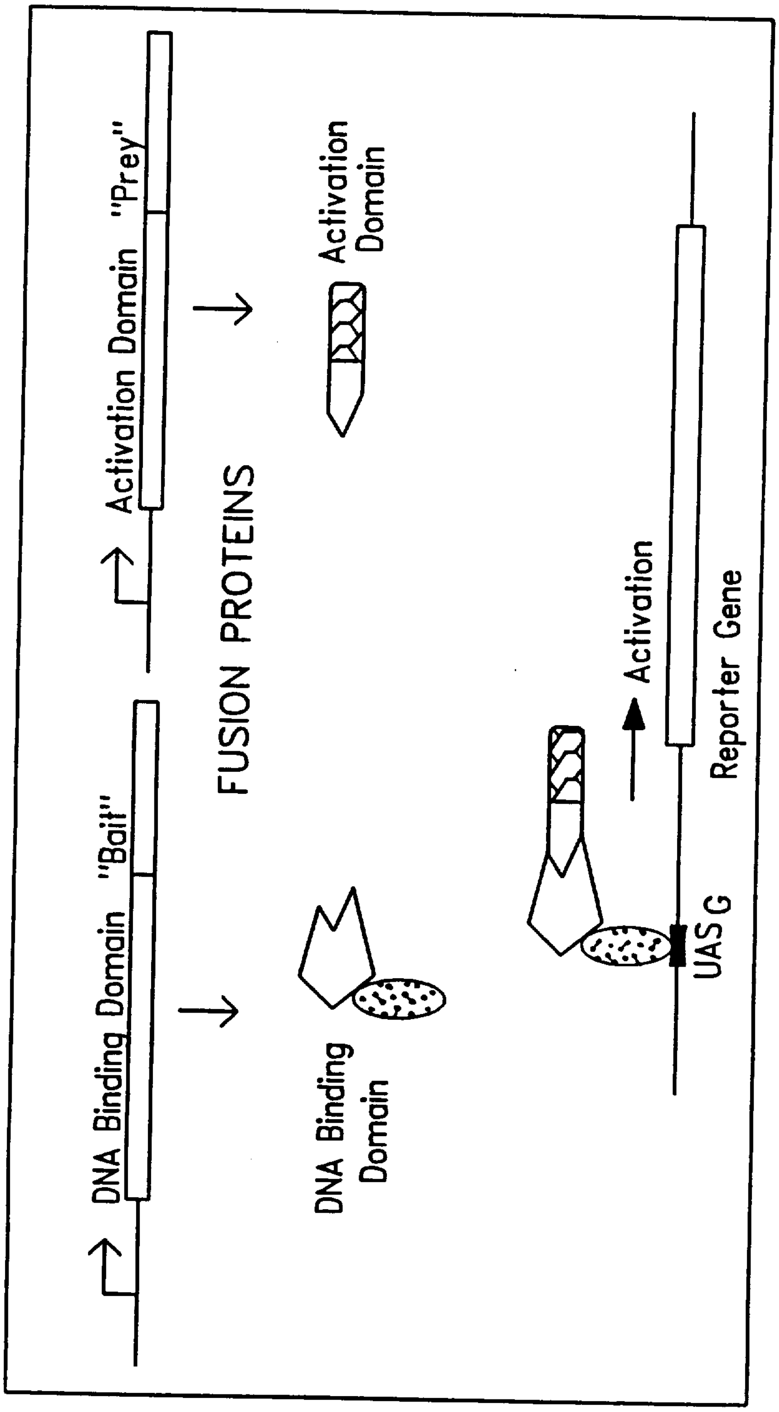

DNA Binding Domain "Bait"
Activation Domain "Prey"
FUSION PROTEINS
DNA Binding Domain
Activation Domain
Activation
UAS G
Reporter Gene

FIG. 6

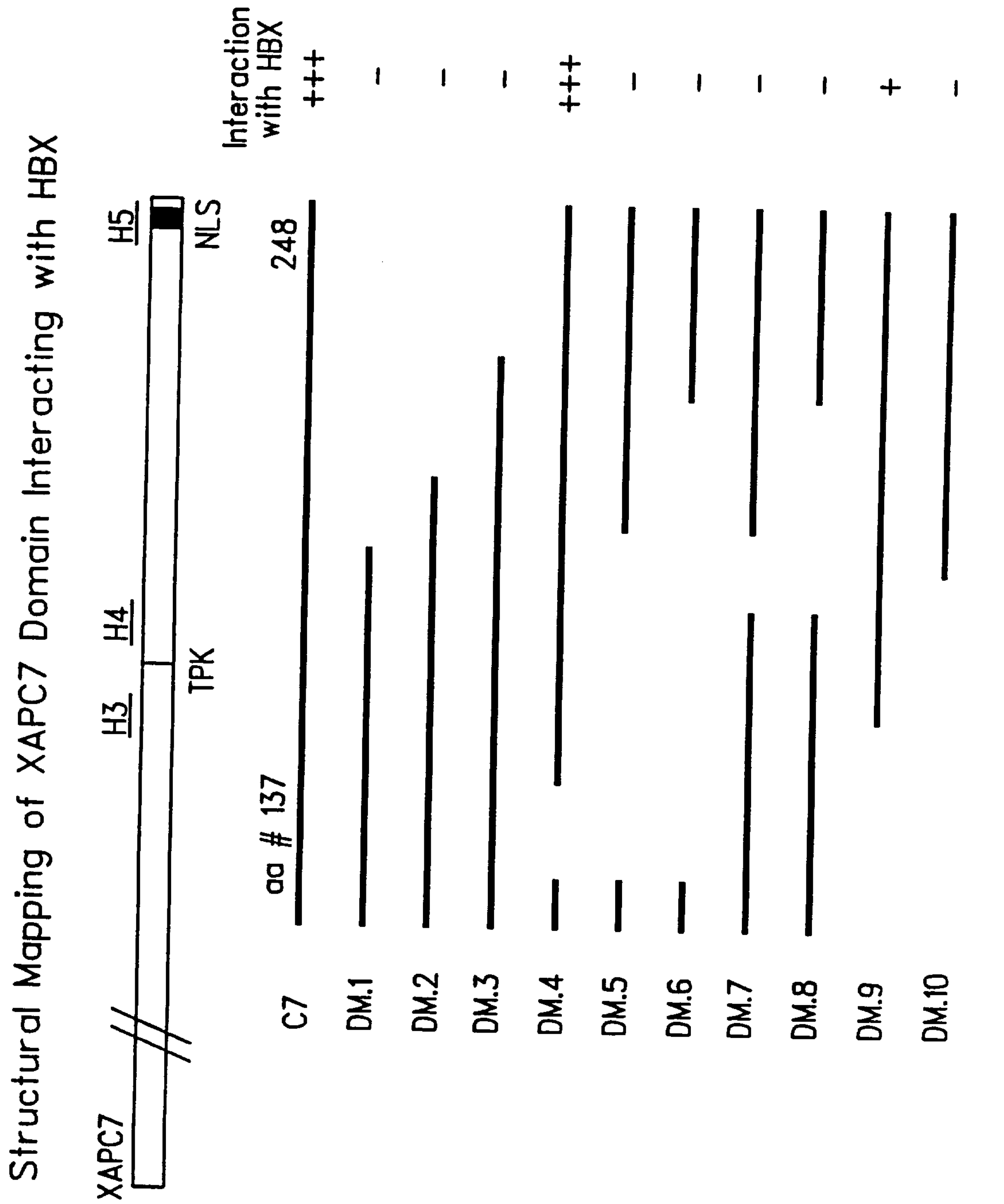

Structural Mapping of XAPC7 Domain Interacting with HBX
XAPC7
H3
H4
H5
TPK
NLS
aa # 137
248
Interaction with HBX
C7
DM.1
DM.2
DM.3
DM.4
DM.5
DM.6
DM.7
DM.8
DM.9
DM.10
+++
-
-
-
+++
-
-
-
-
+
-

FIG. 7

"MGQSQSGGHGPGGGKKDDEDKKKKYEPPVPTRVGKKKKKTKGPD
AASKLPLVTPHTQCRLKLLKLERIKDYLLMEEEFIRNQEQMKPLEEKQEEERSKVDDL
RGTPMSVGTLEEIIDDNHAIVSTSVGSEHYVSILSFVDKDLLEPGCSVLLNHKVHAVI
GVLMDDTDPLVTVMKVEKAPQETYADIGGLDNQIQEIKESVELPLTHPEYYEEMGIKP
PKGVILYGPPGTGKTLLAKAVANQTSATFLRVVGSELIQKYLGDGPKLVRELFRVAEE
HAPSIVFIDEIDAIGTKRYDSNSGGEREIQRTMLELLNQLDGFDSRGDVKVIMATNRI
ETLDPALIRPGRIDRKIEFPLPDEKTKKRIFQIHTSRMTLADDVTLDDLIMAKDDLSG
ADIKAICTEAGLMALRERRMKVTNEDFKKSKENVLYKKQEGTPEGLYL"

FIG. 8A

```
  1 AATTCCGGCG GAAGTGGTGG AGGAACTTCC GGCAGCGGCA GCTCAAGTGG
 51 CCAAGGCAAG ATGGGTCAAA GTCAGAGTGG TGGTCATGGT CCTGGAGGTG
101 GCAAGAAGGA TGACGAGGAC AAGAAAAAGA AATATGAACC TCCTGTACCA
151 ACTAGAGTGG GGAAAAAGAA GAAGAAAACA AAGGGACCAG ATGCTGCCAG
201 CAAACTGCCA CTGGTGACAC CTCACACTCA GTGCCGGTTA AAATTACTGA
251 AGTTAGAGAG AATTAAAGAC TATCTTCTCA TGGAGGAAGA ATTCATTAGA
301 AATCAGGAAC AAATGAAACC ATTAGAAGAA AAGCAAGAGG AGGAAAGATC
351 AAAAGTGGAT GATCTGAGGG GGACCCCGAT GTCAGTAGGA ACCTTGGAAG
401 AGATTATTGA TGACAATCAT GCCATCGTGT CTACATCTGT GGGCTCAGAA
451 CACTACGTCA GCATTCTTTC ATTTGTAGAC AAGGATCTGC TGGAACCTGG
501 CTGCTCGGTC CTGCTCAACC ACAAGGTGCA TGCCGTGATA GGGGTGCTGA
551 TGGATGACAC GGATCCCCTG GTCACAGTGA TGAAGGTAGA AAAGGCCCCC
601 CAGGAGACCT ATGCAGATAT TGGGGGGTTG GACAACCAAA TTCAGGAAAT
651 TAAGGAATCT GTGGAGCTTC CTCTCACCCA TCCTGAATAT TATGAAGAGA
701 TGGGTATAAA GCCTCCTAAG GGGGTCATTC TCTATGGTCC ACCTGGCACA
```

FIG. 8B

```
751 GGTAAAACCT TGTTAGCCAA AGCAGTAGCA AACCAAACCT CAGCCACTTT
801 CTTGAGAGTG GTTGGCTCTG AACTTATTCA GAAGTACCTA GGTGATGGGC
851 CCAAACTCGT ACGGGAATTG TTCCGAGTTG CTGAAGAACA TGCACCGTCC
901 ATCGTGTTTA TTGATGAAAT TGACGCCATT GGGACAAAAA GATATGACTC
951 CAATTCTGGT GGTGAGAGAG AAATTCAGCG AACAATGTTG GAACTGCTGA
1001 ACCAGTTGGA TGGATTTGAT TCTAGGGGAG ATGTGAAAGT TATCATGGCC
1051 ACAAACCGAA TAGAAACTTT GGATCCAGCA CTTATCAGAC CAGGCCGCAT
1101 TGACAGGAAG ATTGAGTTCC CCCTGCCTGA TGAAAAGACG AAGAAGCGCA
1151 TCTTTCAGAT TCACACAAGC AGGATGACGC TGGCTGATGA TGTAACCCTG
1201 GACGACCTGA TCATGGCTAA AGATGACCTC TCTGGTGCTG ACATCAAGGC
1251 AATCTGTACA GAAGCTGGTC TGATGGCCTT AAGAGAACGT AGAATGAAAG
1301 TAACAAATGA AGACTTCAAA AAATCTAAAG AAAATGTTCT TTATAAGAAA
1351 CAGGAAGGCA CCCCTGAGGG GCTGTATCTC TAATGAACCA TGGCTGTCAT
1401 CAGGAAAATG GTTGGGAGAT TTCTCAATCC CTGAAAGGGA TGAGGTTGGG
1451 GGAGTTGCCC AGAGGAATCC CTGTTCCCAC TGATTTTTAT TAGCAAAACA
1501 TCCTGTGTCT TTTGGAGTAC GATGTGTAAG TGCCCATTGG GTGGCCTGTT
1551 GGTCACTGTG CAGCAGTCTG CTTCCCAATA AAGCGTGCTC TTTCACAAG
```

FIG. 8C

```
XAPC7  141 TPRLYQTDPSGTYHAWKANAIG...RGAKSVREFLEKNYTDEAIETDDLT 187
           | |:   ||. . .:: .. |:   .:.|. : ::: : |.  . .||:|
S4     331 TNRIETLDPALIRPGRIDRKIEFPLPDEKTKKRIFQIH.TSRMTLADDVT

XAPC7  188 IKLVIKALLEVVQSGGKNI..ELAVMR.RDQSLKILNPEEIEKYVAEIEK 234
           :. :|.|  :: ..: |.|  | ::|  |:..:|: |        .::.|
S4     380 LDDLIMAKDDLSGADIKAICTEAGLMALRERRMKVTN........EDFKK 421

XAPC7  235 EKEENEKKKQKKAS*      250
           .||:   |||. ..
S4     422 SKENVLYKKQEGTPEGLYL* 441
```

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13426

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL : 435/5, 6, 69.1, 70.1, 71.1, 71.2, 320.1; 436/501; 530/350, 412
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/5, 6, 69.1, 70.1, 71.1, 71.2, 320.1; 436/501; 530/350, 412

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG
Hepatitis B, X antigen, Proteasome, XAPC7, S4, XAPP13

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,777,240 (MORIARTY ET AL.) 11 October 1988, column 6, lines 20-35, column 7, lines 4-57, column 13, line 38-column 16, line 26, column 16, lines 37-46, column 17, lines 2-40.	1, 2, 13, 15, 16, 28-32
Y	US, A, 5,204,446 (KUMAZAWA ET AL.) 20 April 1993, column 8, claims 1 and 4	1, 2, 13, 15, 16, 28-32
Y	PCT, A, WO 92/11289 (TROWSDALE ET AL.) 09 July 1992, page 10, line 5-page 12, line 14.	1, 2, 4-9, 12-16, 28-32
Y, E	JP, A, 7,255,476, 09 October 1995 (Abstract; Derwent WPI Acc. No. 95-378535/49)	1, 2, 4-9, 12-16, 28-32

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 JANUARY 1996	13 FEB 1996

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Deborah Freese for HENRY E. AUER
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13426

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP, A, 4,077,497, 11 March 1992 (Abstract, Derwent Acc. No. 92-136766/17)	1, 2, 4-9, 12-16, 28-32
A, P	US, A, 4, 425,942 (TANAKA) 20 June 1995, Entire document	1, 2, 4-9, 12-16, 28-32
A	Cell, Volume 78, issued 09 September 1994, K. L. Rock et al, "Inhibitors of the Proteasome Block the Degradation of Most Cell Proteins and the Generation of Peptides Presented on MHC Class I Molecules", pages 761-771.	1, 2, 4-9, 12-16, 28-32
P, Y	Virus Genes, Volume 10, No. 1, issued February 1995, M. Fischer et al, "HBx Protein of Hepatitis B Virus Interacts with the C-Terminal Portion of a Novel Human Proteasome Alpha-Subunit", pages 99-102, see entire document.	1, 2, 4-9, 12-16, 28-32
P, X	Hepatology, Volume 20, No. 4 part 2, issued 1994, 45th Annual Meeting of the American Association for the Study of Liver Diseases, November 11-15, 1994, J. K. Huang et al., "Identification and characterization of proteasome as the cellular target of hepatitis B viral X protein", p. 305A, Abstract No. 833, see entire abstract.	1, 2, 4-9, 12-16, 28-32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13426

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched bv this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
   1, 2, 4-9, 12-16, and 28-32.

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07K 1/00,14/00, 17/00; C12N 15/00, 15/09, 15/63, 15/70, 15/74; C12P 21/04, 21/06; C12Q 1/68, 1/70; G01N 33/566

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I. Claim 1, 2nd member of the group of claim 3, claims 4-8, first six members of the group of claim 12, polypeptides directed to XAPC7 in claims 16 and 28-32, and nucleic acids coding fro XAPC7 peptides of claims 13-15; drawn to polypeptides related to XAPC7 and nucleic acids coding therefor, and first method of use.

Group II. Claim 2, 3rd member of the group of claim 3, claim 9, 7th member of the group of claim 12, polypeptides directed to S4 in claims 16 and 28-32, and nucleic acids coding for S4 peptides of claims 13 and 15; drawn to polypeptides related to S4, nucleic acids coding therefor, and first method of use.

Group III. Claims 10, 11, last two members of the group of claim 12, and polypeptides directed to HBX of claim 16; drawn to polypeptides related to HBX and first method of use.

Group IV. Claims 17-27; drawn to a second method of use of the polypeptides of Groups I-III.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I-III relate to three distinct substances, and a first method of use for each. Group IV relates to a second method of use for each of the substances of Groups I-III. Rule 13.2 does not provide for multiple products and methods.