I am grateful to Peniel Dimberu (Yale University) for corrections to this page

VIROLOGY CHAPTER EIGHTEEN

HEPATITIS VIRUSES

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Several diseases of the liver, collectively known as hepatitis, are caused by viruses. The viruses involved, five of which have been reasonably well characterized, come from a wide range of virus families.

- Hepatitis A virus is a picornavirus, a small single strand RNA virus
- Hepatitis B virus belongs to the hepadnavirus family of double stranded DNA viruses (see below)
- Hepatitis C virus is a flavivirus, a single stand RNA virus
- Hepatitis D which is also known as Delta agent is a circular RNA that is more similar to a plant viroid than a complete virus
- Hepatitis E, also an RNA virus, is similar to a calicivirus
- Hepatitis G virus is a flavivirus. Its involvement in human disease, if any, is obscure

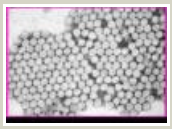


Figure 1A
Hepatitis A virus
CDC

Flavivirus
Calicivirus
Why is there no Hepatitis F Virus?

For a summary of the hepatitis viruses, see Table1.

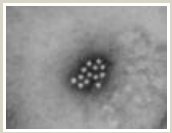


Figure 1B
An electron micrograph of the Hepatitis A virus (HAV)
CDC - Betty Partin

HEPATITIS A VIRUS

This picornavirus (figure 1) is the causative agent of infectious hepatitis. Picornaviruses have a single strand, 3'-polyadenylated, positive sense RNA genome surrounded by a naked (unenveloped) icosahedral capsid that is around 28 nm in diameter (figure 2). At the 5' end of the RNA strand is a viral protein called VPg. There is only one serotype of HAV.

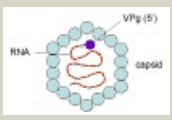


Figure 2
Hepatitis A virus - a picornavirus

Replication

The virus binds to a receptor that is found on the surface of hepatocytes and a few other cells. HAV cellular receptor 1 (havcr-1) has an ectodomain that contains an N-terminal cysteine-rich immunoglobulin-like region, followed by a mucin-like region that extends the immunoglobulin-like region well above the cell surface. The immunoglobulin-like region is required for binding of HAV. The virus spends its entire life in the cytoplasm where it replicates using a virus-encoded RNA-dependent RNA polymerase. For further information on picornavirus replication see Virology Section Chapter Four.

HEPATITIS B VIRUS

Figure 3A
Transmission electron micrograph of hepatitis B virions, also known as Dane particles
CDC/Dr. Erskine Palmer

Figure 3B
Hepatitis B virus © Dr Linda Stannard, University of Cape Town, South Africa. Used with permission

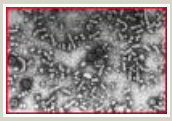


Figure 3C
Hepatitis B virus
CDC

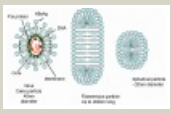


Figure 3D Hepatitis B virus. Dane particle and incomplete particles that are found in patient's serum

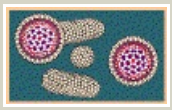
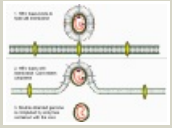


Figure 3E
Hepatitis B virus structure © Dr Linda Stannard, University of Cape Town, South Africa. Used with permission



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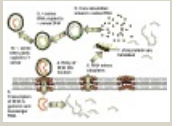

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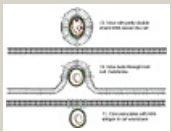

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Figure 4A
Hepatitis B replication

Human hepatitis B virus (figure 3) is the prototype virus of the hepadnavirus family and causes serum hepatitis. HBV has a diameter of about 40nm. It infects humans and chimpanzees but there are closely related members of this family that infect other mammals and birds. HBV is a DNA virus and is enveloped. The DNA is only partly double stranded and forms a circle of around 3,200 bases. Although surrounded by a host cell-derived envelope, HBV is remarkably stable to organic solvents. It is also heat- and pH-resistant. The genome is associated with the P (polymerase) protein and this complex is, in turn, surrounded by the core antigens (HBcAg and HBeAg). These two proteins have most of their sequence in common and most of the HBeAg is secreted since it is processed differently from the HBcAg and thus not assembled into progeny virus. Embedded in the surface lipid bilayer is the surface antigen (HBsAg). The HBsAg (Australia antigen) is made up of three glycoproteins that are encoded by the same gene. The proteins are translated in the same reading frame but start at a different AUG start codon; thus, all have the same C-terminus. The largest protein is the L protein (42kd) and contained within this is the M glycoprotein. The S glycoprotein (27kD) is contained within the M protein. The HBsAg protein is also secreted into the patient's serum where it can be seen as spherical (mostly self-associated S protein) or filamentous particles (also mostly S protein but with some L and M). The former are smaller than the true virus but the filaments can be quite large (several hundred nanometers). This large amount of free HBsAg accounts for the inability to detect antibodies against the protein early during infection (the so-called "window" between the presence HBsAg (indicative of the presence of virus) and the presence of anti-HBsAg).

The glycoproteins on the virus surface contain antigenic determinants that are group specific and type specific. Using these determinants, epidemiologists identify eight subtypes of HBV. HBV virions are also known as Dane particles.

Replication

HBV has a very curious way of replicating itself since (figure 4A), although it is a DNA virus, it uses a RNA proviral intermediate that has to be copied back to DNA. The copying of RNA to DNA is not a normal function of an uninfected cell but is found in retroviruses that also have an RNA genome and a DNA intermediate that gets integrated into host cell chromosomes. For the purpose of copying RNA to DNA, retroviruses and HBV have a virally-encoded DNA polymerase (P) called reverse transcriptase.

After the HBV has attached to the cell surface receptor (which has yet to be identified but may be a member of the ovalbumin family of serine protease inhibitors), the viral membrane fuses with the cell membrane releasing the core into the cytoplasm. The core proteins dissociate from the partially double stranded DNA. DNA polymerase now completes the DNA so that it is completely double stranded. This is done by the virally-encoded polymerase in the cytoplasm that is one of the core proteins (whereas the cell's DNA polymerase is in the nucleus). The double stranded DNA enters the nucleus and the ends are ligated by host enzymes so that the virus is in the form of a circular episome. The viral DNA associates with host nuclear histones and is transcribed by cellular RNA polymerase II into mRNAs. In contrast to the situation with retroviruses, however, the DNA form of HBV is usually not integrated into cellular DNA; rather it is found as an independent episome. This is because, unlike retroviruses, hepadnaviruses have no integrase activity. However, integrated parts of the HBV genome are found in the chromosomes of many hepatocellular carcinoma patients.

Four mRNAs are made from the HBV genome. The host cell RNA polymerase interacts with four promoters but transcription always ceases at the same polyadenylation site so that the overlapping mRNAs have a common 3' terminus. One of these mRNAs is slightly longer than the DNA sequence because of the polyadenylation at one end and a repeated region. This is the full length c-RNA that will be the template for the genome. The full length messenger RNA codes for the polymerase and core HBcAg and HBeAg proteins. The latter are very similar because they are translated in the same reading frame from two different start codons. Two smaller mRNAs (2.4 and 2.1 bases) which overlap code for the surface glycoproteins. There is also a small mRNA of 700 bases that codes for a protein that is a protein kinase and is a transactivator of transcription.

In the cytoplasm, the full-length (3,500 base) positive strand c-RNA is encapsidated by core proteins. Inside the core, the RNA is transcribed to minus strand DNA by the same DNA polymerase (reverse transcriptase) that completed the double stranded DNA and, at the same time, the RNA is degraded by a

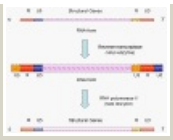
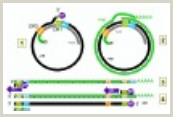


Figure 4B
Genome replication in retroviruses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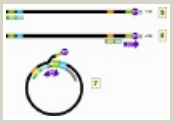
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Figure 4C
Genome replication in hepadnaviruses

ribonuclease H that is also part of the reverse transcriptase. Unlike the reverse transcriptase of the retroviruses, the HBV reverse transcription reaction does not require a tRNA primer. Rather, the polymerase itself acts as a primer and remains covalently attached to the 5' end of the negative strand DNA. A host cell chaperone protein, heat shock protein 90, is also necessary. The chaperone associates with the reverse transcriptase allowing it to fold into an active conformation.

The virus now buds through the endoplasmic reticulum and/or Golgi Body membranes (or perhaps a novel pre-Golgi compartment) of the host cell from which it acquires HBsAg. At this stage or later, the minus stand of DNA is partly transcribed into a plus strand. When the viral DNA polymerase is used to transcribe RNA to DNA, it is acting as a reverse transcriptase similar to that found in retroviruses; in fact, HBV DNA polymerase and retroviral reverse transcriptase are very similar, and may have evolved from a common ancestor.

Virus particles that contain RNA or DNA at various stages of replication can be found in the bloodstream suggesting that nucleic acid replication is not tightly controlled with the passage out of the cell. In addition, empty envelopes containing the envelope proteins embedded in a lipid bilayer are continuously being shed.

RNA polymerase problem

There is a distinct problem posed by using host cell RNA polymerase II to transcribe a DNA viral genome to an RNA form (See section on retroviruses). The normal function of RNA polymerase II is to transcribe a gene into messenger RNA for subsequent translation into protein. In the mRNA, all that is required is the information to make the protein. In the DNA gene, additional information is present that is needed to make the RNA. This extra information (that is not transcribed into RNA) includes the promoter (the site at which the RNA polymerase binds), the enhancers that are up- and down- stream of the region transcribed to mRNA and the polyadenylation site. Thus, a messenger RNA is smaller than the DNA gene, even if there are no introns.

Retroviruses overcome the loss of promoter/enhancer information as a result of using RNA polymerase II transcription by carrying internal copies of the promoter and enhance regions (these are the U3 and U5 sequences respectively). They duplicate their internal U3 promoter sequence and transpose it to the opposite end when the DNA is transcribed from RNA. Similarly, the enhancers and other 3' information are stored internally (as U5) and transposed to the other end. These events give rise to the long terminal repeats (LTRs) that are only found in the DNA form of the virus. When the RNA polymerase recognizes the promoter in the U3 region, it finds the transcription initiation site at the border between the U3 and R and starts transcribing at the beginning of the R region. This leads to a faithful copy of the original RNA as the terminal U3 and U5 are lost (figure 4B).

The same problem occurs in hepadnaviruses which also have a DNA form of their genome that is copied to RNA by host cell RNA polymerase II before copying the RNA back to DNA using reverse transcriptase. However, the mechanism is different; in this case, the DNA form of the virus is smaller than the RNA form, quite the opposite of what occurs in the retroviruses.

The hepadnaviruses are small DNA viruses and, in contrast to the retroviruses, it is the DNA that is packaged into the viral particle. This DNA is copied to RNA in the infected cell by RNA polymerase II and the resulting RNA is copied back to DNA by reverse transcriptase in the maturing virus particle.

In the viral particle, the DNA is only partially double stranded. The negative strand is complete, though not ligated into a circle. There are free 5' (with an attached reverse transcriptase protein molecule) and 3' ends. The DNA is in the form of a relaxed circle because it is hybridized to a partial copy of the positive strand. The DNA contains two direct repeats (DR1 and DR2). DR1 is close to the 5' end of the negative strand and DR2 is close to the 5' end of the partial positive strand.

On entering the nucleus, the negative strand is ligated to form a covalently closed circle. This is then copied by host RNA polymerase II. The polymerase starts about 6 bases to the left (in figure 4Ci-2) of the DR1 and proceeds (clockwise in figure 4Ci-2) around the circle past both the initiation site and the DR1 and stops at the termination/poly A site (light blue) that is a little further downstream. The

RNA becomes polyadenylated. The RNA copy is therefore larger than the covalently closed circular DNA (compare the situation in retroviruses) because the DR1 region has been duplicated and poly A has been added.

This RNA moves to the cytoplasm where encapsidation by viral proteins occurs. There is an encapsidation signal at the 5' end of the RNA and thus only one RNA molecule is found in each virion (compare the situation in retroviruses). Now, in the virus particle itself, the RNA is copied to DNA using reverse transcriptase. All DNA polymerases need a primer and in the case of the retroviruses this is a host cell tRNA that is packed in the virion. In the hepadnaviruses, the polymerase is packaged in the virion as it is in the retroviruses, though there are fewer polymerase proteins per virus particle in the hepadnaviruses. The reverse transcriptase is itself the primer for the synthesis of the negative DNA strand and it remains attached to the 5' end of the DNA via a tyrosine residue.

The DNA initiates on a hydroxyl group of the tyrosine using, as a template, a region near the 5' end of the RNA (fig 4Ci-3). The polymerase copies through the DR1 near the 5' end of the RNA and terminates at the end of the RNA molecule. Next, a template exchange occurs in which the nascent negative strand DNA moves to the DR1 near the 3' end (fig 4Ci-4). Why this is necessary is obscure since the initiation could have occurred near the 3' DR1. From the 3' DR1, the DNA is extended accompanied by RNase H digestion of the template RNA strand. Synthesis stops when the 5' end of the RNA is reached (figure 4Ci-4). The negative strand is now terminally redundant. The RNA is not completely destroyed and the last 15 or so nucleotides remain (figure 4Cii-5) to serve as a primer for the second (positive) DNA strand synthesis. This is translocated to the DR2 at the 5' end of the first DNA stand (figure 4Cii-6). Extension continues to the 5' end of the first DNA strand. There now occurs a switch of template in which the DR1 at the 5' end of the negative strand is replaced by the DR1 at the 3' end so circularizing the template (figure 4Cii-7). The reverse transcriptase now copies around the circle for a variable distance to form the DNA that is found in mature virus particles.

Carcinogenesis

It is clear that individuals who are HBsAg positive are at a much higher risk of hepatocellular carcinoma than those who are negative. In patients with chronic hepatitis, there is destruction of hepatocytes as a result of the immune response to the virus. This results in regeneration (by cell division) of liver cells that may ultimately cause the cancer. Although the virus does not integrate during the course of normal replication, parts of the HBV genome are found integrated into the DNA of hepatocellular carcinoma patients. This may result in the activation of a cellular proto-oncogene in much the same way as occurs in some retrovirus-caused cancers; in fact, in most cases of woodchuck hepatocellular carcinoma (a widely used model system), viral DNA is found close to the myc or a similar proto-oncogene. Hepatocellular carcinoma takes many years to develop and this may reflect the rarity of integration in the absence of an integrase enzyme. The tumor that does develop is thus likely to be clone of a single cell where this process has occurred.

An HBV protein called protein X is known to activate the src kinase and this may also underlie HBV carcinogenesis. This protein may also interact with p53, one of the cell's tumor suppressor genes.

HEPATITIS C VIRUS

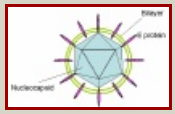
Figure 5 Hepatitis C structure

Hepatitis C is a flavivirus (of which yellow fever is the prototype) that causes non-A, non-B hepatitis. Flaviviruses (figure 5) are icosahedral, positive strand RNA viruses and gain an envelope from their host cell. The virus particle is about 30 to 60nm across. The genome of 9,600 bases codes for ten proteins. In many ways, the flaviviruses are similar to picornaviruses with the prominent exception that they are enveloped. The viral RNA does not have a 5' cap or 3' poly A tract. Translation of the viral RNA is mediated by the internal ribosome entry site (IRES).

There is one protein product from one open reading frame. The hepatitis C virus polyprotein is cleaved by both a virally-encoded protease activity and a cellular protease. The nascent protein contains a signal sequence that results in the translating ribosome attaching to the cytoplasmic surface of the endoplasmic reticulum. The envelope protein (E) thus crosses and embeds in the membrane and the signal sequence is removed by a cellular signal protease. This results in the remainder of the protein, the core protein, becoming cytoplasmic. It is cut by two viral proteases. The C-terminal domain of NS2 is a cysteine

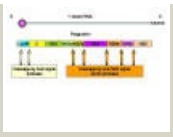
Figure 6
Flavivirus polyprotein processing

protease and cleaves at the NS2/NS3 junction. Another protease (NS3/4A serine protease) cleaves the remaining junctions.

Thus, the core protein is cut into NS1, NS2, NS3 and NS4 proteins. NS2 and NS4 are then cut again (to give NS2a, NS2b, NS4a and NS4b)

HCV binds to either the CD81 antigen or low density lipoprotein (LDL) receptor on hepatocytes via its E2 glycoprotein. There is also some evidence that it may bind to glycosaminoglycans.

HEPATITIS DELTA AGENT


Figure 7
Hepatitis Delta agent
CDC

Hepatitis D (figure 7) is a highly defective virus since it cannot produce infective virions without the help of a co-infecting helper virus. This helper virus is hepatitis B virus that supplies the HBsAg surface protein. In budding out of the cell, HDV acquires a membrane containing HBsAg. HDV is similar to a plant viroid in that it has a small circular RNA genome (1,700 bases) but unlike the plant viroids, the RNA encodes a protein called the delta antigen. This complexes with the RNA. The RNA is single stranded negative sense and is a covalently closed circle. Because of a large amount of base pairing, the RNA takes on a rod-like structure (figure 8).

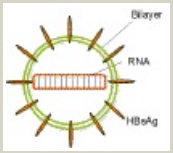

Figure 8
Hepatitis Delta agent - structure

HDV can only form an infectious particle if the cell in which it replicates is co-infected with HBV since the latter provides the surface HBsAg which is required for reinfection of another cell. The HBsAg of HDV binds to the same surface receptor as HBV and the virus fuses with the cell membrane. The tropism of HDV is therefore the same as HBV. The RNA genome is coated with delta antigen, the only protein encoded by the RNA. The delta antigen, which is exposed when the envelope is lost, has a nuclear localization signal that targets the genome to the nucleus. Here the genome is copied by host cell RNA polymerase II, the enzyme that normally makes mRNA. RNA polymerase II is used by some other viruses to copy their genomes, for example, the retroviruses, but in that case the polymerase copies DNA to RNA (which is the normal function of the enzyme in the uninfected cell). In HDV replication, the polymerase is copying RNA to RNA. The negative sense genomic RNA is copied to a positive strand that is also circular. The genomic RNA can also be transcribed into a linear 5' capped and 3' polyadenylated mRNA which is smaller than the genomic RNA and contains the small open reading frame from which the delta antigen is translated; or it can be generated from the circular positive sense genomic-sized RNA by an autocatalytic process that cleaves the RNA. Thus, the RNA is acting as a ribozyme, that is a catalytic RNA (figure 9).

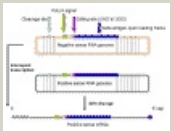
Figure 9
Hepatitis delta agent. Three RNA forms.
Adapted from Wagner and Hewitt.: Basic Virology. Blackwell Publishing

Delta antigen, translated from the mRNA has two forms that differ in size by 19 amino acids (195 compared to 214 residues). The formation of the large delta antigen happens by a rather strange mechanism in which a host cell enzyme called double stranded RNA-activated adenosine deaminase converts a UAG (stop) codon into a UGG that allows translation to proceed to the next stop codon. The small delta antigen is involved in the replication of the genome but the larger form suppresses replication. This leads to the promotion of viral particle assembly.

HEPATITIS E VIRUS

This virus (figure 10), which causes enteric non-A, non-B hepatitis, seems to be related to the Caliciviruses but its classification is undecided since the genome organization is not the same as that of the Caliciviridae. In sequence, HEV is more similar to rubella which is a Togavirus than to any Calicivirus. HEV is a small (approximately 34nm), round, icosahedral, positive strand RNA virus that does not have an envelope. It has a rather smooth surface but not as smooth a HAV. The genome has a poly A tract and is capped at the 5' end. There are three open reading frames that overlap; each is in a different coding frame. Based on sequence motifs, open reading frame 1 (ORF1) appears to have several enzymic activities. These may be involved in RNA capping, proteolysis and an RNA-dependent RNA polymerase activity. ORF2 is the structural protein and may be glycosylated. It appears to have a signal sequence suggesting that its encoded protein may enter the endoplasmic reticulum. The third ORF codes for a phosphoprotein of unknown function that interacts with the host cell's cytoskeleton. Not much is known about HEV replication but it is likely that the positive strand RNA is copied to a negative strand intermediate by a viral polymerase


Figure 10
Hepatitis E virus CDC

HEPATITIS G VIRUS

Hepatitis G virus is a flavivirus, like HCV to which it is closely related. It is associated with some cases of acute or chronic non-A, non-B, non-C, non-D, non-E hepatitis. Although it seems common in human blood, it may not he a significant cause of hepatitis in humans.

Table 1

	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis Delta	Hepatitis E
Virus family	Picornavirus	Hepadnavirus	Flavivirus	Circular RNA similar to plant viroid	Similar to Calicivirus
Nucleic acid	RNA (+ sense)	DNA (partially double strand)	RNA (+ sense)	RNA (- sense)	RNA (+ sense)
Disease caused	Infectious hepatitis	Serum hepatitis	Non-A, non-B hepatitis		Enteric non-A, non-B hepatitis
Size	~ 28nm	~40nm	30 - 60nm	~ 40nm	30 - 35 nm
Envelope	No	Yes	Yes	Yes	No

 Return to the Virology section of Microbiology and Immunology On-line

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