The creation and destruction of a virus

Approximately 2 billion people worldwide have been infected with the hepatitis B virus (HBV), with 350 million of them becoming chronically infected. HBV infection causes a wide range of serious illnesses, including acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma: around 1 million fatalities each year are associated with HBV infection.

HBV is a member of the Hepadnaviridae family, which also includes related virus species such as duck hepatitis B virus. All hepadnaviruses contain a small, partially double stranded, relaxed circular DNA genome. The virus replicates this DNA genome via an RNA intermediate using a process called reverse transcription. The virus itself is an enveloped virus with the relaxed circular DNA genome housed within an inner protein shell, or nucleocapsid. The HBV capsid shell enclosing the DNA is composed of multiple copies of a single viral protein, HBV core protein (HBc).

Normally, there are 240 copies of this protein which make up the viral capsid. The primary cell type infected by HBV is liver cells, or hepatocytes. Once in the nucleus of the hepatocyte, the relaxed circular DNA needs to be converted to a different type of DNA – covalently closed circular DNA (CCC DNA) – in order for viral replication to be established and sustained.

A prerequisite for CCC DNA formation is the uncoating of the virus in order to expose their DNA. Only viruses with relaxed circular genomes are considered mature viruses as they have the ability to be secreted from infected cells and then internalised by the next host cells. Interestingly, empty capsids containing no viral DNA or RNA are also produced and secreted as enveloped empty virions.

There are two fates of the mature nucleocapsids containing relaxed circular DNA. They can either be enveloped by viral envelope proteins, followed by secretion out of the cell as a complete virus particle, or the nucleocapsid is disassembled to release relaxed circular DNA into the host cell nucleus to form CCC DNA. Immature nucleocapsids are unable to do either of these processes. Structural changes associated with nucleocapsid maturation are likely to play a part in the selective uncoating of mature capsids to allow CCC DNA formation and its preferential envelopment for virion secretion.

Lab approaches used to investigate HBV capsid assembly usually centre around over-expression of HBc in bacteria. Using this system, it is possible to interface with the conditions in which the virus must assemble. For example, Professor Hu’s group, as well as many others around the world, have shown that the first 140 amino acids of the HBc protein (the major component of the capsid), the ‘assembly domain’, are sufficient for virus assembly. Amino acids found at the opposite end of the protein in the C-terminal domain, and the linking region between the two domains, are dispensable. In contrast, Professor Hu’s group has recently demonstrated, using mutants which mimic physiological conditions in infected human cells, that the C-terminal domain is also required to facilitate capsid assembly. Indeed, Professor Hu’s lab has developed a mammalian-cell-free system in which HBc is expressed at physiologically low levels and which assembles into capsids under near physiological conditions. This is in contrast to the traditional systems which rely on non-physiological levels of protein and salts to induce HBc assembly into capsids. In addition, the phosphorylation state of the C-terminal domain which regulates capsid assembly and the packaging of RNA in the cell-free system, is the same as occurs in human cells.

In order for the viral genetic material (genome) inside the virus to do its job, it must be released from the virus particle in which it is packaged. Once the virus has undergone binding to its target cell in the host, the disassembly process can begin. The uncoating, i.e., disassembly of the nucleocapsid, process is subject to host regulation, which therefore influences HBV species tropism, i.e. which species the virus is able to infect and replicate within. The capsid disassembly process is poorly understood. However, the work of Professor Hu’s group has shown that this step may be regulated by the capsid itself, as well as the host.

Previous studies have been hampered by the lack of convenient laboratory or animal model systems. In particular, mouse hepatocytes are incapable of supporting CCC DNA formation, which has prevented the development of this convenient small animal model as a fully-permissive host for HBV infection study. Intriguingly, Professor Hu’s lab was able to show recently that an immortalised mouse liver cell line could support HBV CCC DNA formation, hence suggesting that mouse hepatocytes may be manipulated to produce HBV CCC DNA and thus, it may be possible to develop a mouse model that supports HBV CCC DNA formation.

Professor Hu and his group have shown using mutants of the HBc protein in human cells, as well as the mouse liver cell line, that efficient CCC DNA conversion is associated with enhanced nucleocapsid uncoating, i.e., release of the relaxed
As HBV can be recognised by the host immune system in some situations through exposed viral DNA, it may provide a situation which can be exploited therapeutically.

**Reference**


