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VIRAL INFECTION

Changing sides to get in

> this slow maturation process prevents particle binding to HSPGs on cells other than hepatocytes to promote effective infection

The human hepatitis B virus (HBV) causes acute and chronic infections that have been linked to liver cirrhosis and hepatocellular carcinoma. The virus replicates exclusively in the liver, and it was shown that virions attach to hepatocytes through binding of the PreS domain in the large (L) surface glycoprotein to heparan sulfate proteoglycans (HSPGs) presented on the host cell surface. How the virus achieves such a specific tissue tropism while binding to ubiquitous HSPGs remained elusive. In this study, Seitz *et al.* report that virions acquire receptor-binding competence by a slow maturation process that involves the translocation the PreS domain across the membranous viral envelope to avoid non-productive attachment to non-hepatocytes.

Previous studies have shown that the L protein exhibits a dual transmembrane topology: during co-translational membrane insertion, the PreS domain of the L protein is located on the cytosolic surface of the endoplasmic reticulum (where it has been implicated in capsid binding during envelopment), whereas this domain is exposed on the particle surface as a result of post-translational passage through the membranous viral envelope to facilitate entry into hepatocytes.

Using affinity chromatography, the authors of this study identified two HBV subpopulations: heparin non-binders, which they denoted as N-type particles, and a population that binds to heparin, which they termed B-type particles. Interestingly, B-type particles were highly infectious when added to cells, and these binding-competent particles presented the PreS domains of most of the L protein molecules in their envelopes on the outside. By contrast, non-binding N-type particles were inactive and the PreS domain was mostly located on the inside of the particle. These findings suggest that a structural change of the L protein facilitates a conversion from non-infectious into infectious particles. Indeed, incubation at 37 °C spontaneously converted immature N-type particles into binding-competent virions with a half-time conversion rate of 4.7 hours, and these converted particles could infect primary human hepatocytes. In addition, the L protein of those converted particles was trypsin-sensitive, which suggests that the PreS domain of this protein is translocated to the external side of the particle to increase infectivity.

Kinetic analyses of virion secretion and conversion revealed that most virions are released as non-infectious N-type particles; over time, the numbers of non-binders decreased to a plateau while the numbers of binding-competent particles increased inversely, which correlated with an increase in infectivity. The authors suggest that this slow maturation process prevents particle binding to HSPGs on cells other than hepatocytes to promote effective infection in target cells.

Consistent with this, mice that had been transplanted with human hepatocytes were inoculated with labelled B-type virions. The authors did not observe a preferential accumulation of the virions in the liver and instead observed the injected virions in all organs, which suggests that the circulating B-type virions non-selectively attach to available HSPGs on various cell types. Importantly, infection of mice with high doses of either N-type particles or *in vitro* matured B-type particles led to viraemia, whereas under conditions of low-dose infection, mice became viraemic only upon inoculation with N-type but not B-type particles.

In summary, these findings suggest that under low-dose transmission conditions, a slow maturation process not only enables immature N-type particles to evade the PreS-specific humoral immune response but also to travel from the entry site to the liver during circulation without binding to cell surface receptors present on non-hepatic tissues to establish an infection.

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ORIGINAL ARTICLE Seitz, S. *et al.* A slow maturation process renders hepatitis B virus infectious. *Cell Host Microbe* http://dx.doi.org/10.1016/j.chom.2016.05.013 (2016)