Cholesterol: fa(s)t-food for enterovirus genome replication

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Hijacking and remodeling of host membranes is an obligatory step in the replicative cycle of (+)RNA viruses, including enteroviruses. Ilnytska et al. unveiled in Cell Host & Microbe that enteroviruses usurp clathrin-mediated endocytosis to shuttle cholesterol to sites of genome replication and that cholesterol is needed for efficient replication.

Positive-strand RNA [(+)RNA] viruses are a large and diverse group of viruses that include many important human pathogens, such as enteroviruses (including poliovirus, coxsackievirus, rhinovirus, enterovirus-71), hepatitis C virus (HCV), dengue virus, chikungunya virus, West Nile virus, norovirus, and SARS- and MERS-coronavirus. All (+)RNA viruses remodel host membranes into replication organelles (ROs), which support viral RNA synthesis, but the origin of the membranes varies between viruses [1]. Enteroviruses hijack membranes at the endoplasmic reticulum (ER)–Golgi interface to generate a complex tubulovesicular network [2,3]. Rather than using this cellular compartment as a whole, these viruses seem highly selective in recruiting specific host factors to build new organelles with a unique protein and lipid composition [1]. For example, enteroviruses, as well as HCV, specifically recruit the lipid-modifying kinase phosphatidylinositol-4-kinase IIIβ (PI4KIIIβ) to ROs for the production of PI4-phosphate (PI4P), an essential lipid for viral RNA replication [4]. Furthermore, poliovirus was recently shown to increase the uptake of fatty acids to use them for the highly upregulated synthesis of phosphatidylincholines, essential building blocks of membranes [5].

In the September issue of Cell Host & Microbe, the group of Nihal Altan-Bonnet unveiled that enteroviruses induce internalization of the plasma membrane and extracellular cholesterol pools and channel it towards the ROs [6]. Cholesterol is of vital importance for cellular membranes as it influences membrane fluidity and permeability as well as the formation of nanodomains called lipid rafts. Cells normally acquire cholesterol via de novo synthesis or through uptake of extracellular cholesterol. The plasma membrane is the largest source of free cholesterol (i.e., non-esterified) in most eukaryotic cells; and extracellular cholesterol is present in low-density lipoprotein (LDL) complexes that bind to the LDL receptor and are internalized via clathrin-mediated endocytosis (CME). Free cholesterol is subsequently redistributed from the endosomal compartments to various organelles, while excess cholesterol is esterified with a fatty acid into cholesterylester and stored in lipid droplets (Figure 1).

Although many viruses, including enteroviruses, require cholesterol for cell entry [7], the Altan-Bonnet laboratory now reports that modulation of the cellular cholesterol content affected replication of enteroviral RNA [6]. Viral RNA replication was reduced when the authors lowered free cholesterol levels by various treatments (e.g., depletion using methyl-β-cyclodextrin, or blocking uptake by pharmacological inhibition or knockdown of CME components). By contrast, replication was enhanced when cellular free cholesterol was elevated (e.g., by knockdown of the non-CME component caveolin 1 or in cells from Niemann-Pick Type C disease patients). In addition, they show that enteroviruses actively increased CME to stimulate the uptake of cholesterol from the medium (Figure 1). Concomitantly, the amount of cholesterylster, which reflects the amount of cholesterol stored in lipid droplets, decreased during infection. It remains to be determined whether enteroviruses merely inhibited the deposition of new cholesterylsters in lipid droplets or that they mobilized stored cholesterol from lipid droplets as a source of free cholesterol in addition to the increased uptake of cholesterol. Collectively, these data convincingly demonstrate that uptake of cholesterol and delivery to ROs is important for enterovirus genome replication.

The authors then wondered how enteroviruses achieved the enhanced endocytosis of cholesterol. The most likely candidates for this effect are viral proteins 2B and/or 2BC, which were previously shown to increase uptake of cell surface proteins [8]. The Altan-Bonnet laboratory demonstrated that ectopic expression of 2BC alone was sufficient to increase uptake of the endocytic marker AM4-65 and to raise cellular free cholesterol levels, pointing to an important role of 2B (which was not tested) and/or 2BC in the enhanced uptake of cholesterol in infected cells. But how is the endocytosed cholesterol subsequently channeled to ROs? Normally, recycling endosomes (REs) transport a portion of the internalized cholesterol back to the plasma membrane. The authors show that in infected cells REs are re-routed to the replication sites, making them a likely supplier of cholesterol. The viral protein 3A plays an important role in this re-routing by recruiting PI4KIIIβ [4], which directly binds the RE protein Rab11 (Figure 1). It remains to be established whether cholesterol is delivered

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stepwise cleaved by viral proteases (i.e., 2A\textsuperscript{pro}, 3C\textsuperscript{pro}, 3D\textsuperscript{pol}) to generate the individual viral proteins. One of the cleavage intermediates is 3CD\textsuperscript{pro}, which functions in RO formation, viral RNA synthesis, and cleavage of the viral capsid precursor protein. Upon autocleavage, 3CD\textsuperscript{pro} is cleaved into 3C\textsuperscript{pro} and 3D\textsuperscript{pol}, the RNA-dependent RNA polymerase that replicates the viral genome. The Altan-Bonnet group observed that depletion of cholesterol enhanced cleavage of 3CD\textsuperscript{pro}, but not of intermediates 2BC and 3AB [6]. How cholesterol influences the efficiency of 3CD\textsuperscript{pro} cleavage at the RO and whether the altered cleavage efficiency of 3CD\textsuperscript{pro} is indeed critical for optimal viral replication are topics for future investigation. Furthermore, it remains to be established whether there are any additional roles for cholesterol at ROs. Given its profound effects on membrane fluidity, permeability, and lipid raft formation, it will be interesting to study whether the cholesterol content is important for the generation and/or architecture of the tubulovesicular RO network.

In conclusion, Altan-Bonnet and colleagues elegantly show that enteroviruses remodel the host cell cholesterol landscape for building ROs and efficient viral RNA replication.

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