Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Virology

Flavivirus modulation of cellular metabolism

Tristan X Jordan and Glenn Randall

Over the last decade, we have begun to appreciate how flaviviruses manipulate cellular metabolism to establish an optimal environment for their replication. These metabolic changes include the stimulation of glycolysis, in addition to lipid anabolic and catabolic pathways. These processes are thought to promote an energetically favorable state, in addition to modifying membrane lipid composition for viral replication and virion envelopment. Importantly, many of these processes can be pharmacologically inhibited as successful antiviral strategies, at least in cell culture. In this review, we discuss the mechanisms by which flaviviruses alter cellular metabolism, remaining questions, and opportunities for therapeutic development.

Address
Department of Microbiology, The University of Chicago, Chicago, IL 60637, United States

Corresponding author: Randall, Glenn (grandall@bsd.uchicago.edu)

Current Opinion in Virology 2016, **19**:7–10

This review comes from a themed issue on **Viruses and metabolism**

Edited by **Richard E Lloyd** and **Mary K Estes**

For a complete overview see the Issue and the Editorial

Available online 7th June 2016

http://dx.doi.org/10.1016/j.coviro.2016.05.007

Introduction

Viral infections can modify many aspects of cellular physiology, including cell cycle, homeostatic pathways such as apoptosis and autophagy, and metabolic pathways. Viral strategies have evolved to either stimulate or inhibit the metabolic state of the cell depending on the desired outcome (productive or latent viral infection, or an altered cellular response to infection, reviewed in Ref. [9]). The flaviviruses are a closely related genus of (+) RNA viruses in the *Flaviviridae* family and include important human pathogens such as dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus, yellow fever virus, tick-borne encephalitis virus, and Zika virus, among others. Most flaviviruses are transmitted to humans via an arthropod vector (tick or mosquito) and produce an acute cytolytic infection. As such, flavivirus modulation of cellular metabolism does not result in prolonged cellular survival and may, in some cases, contribute to cytopathic effect.

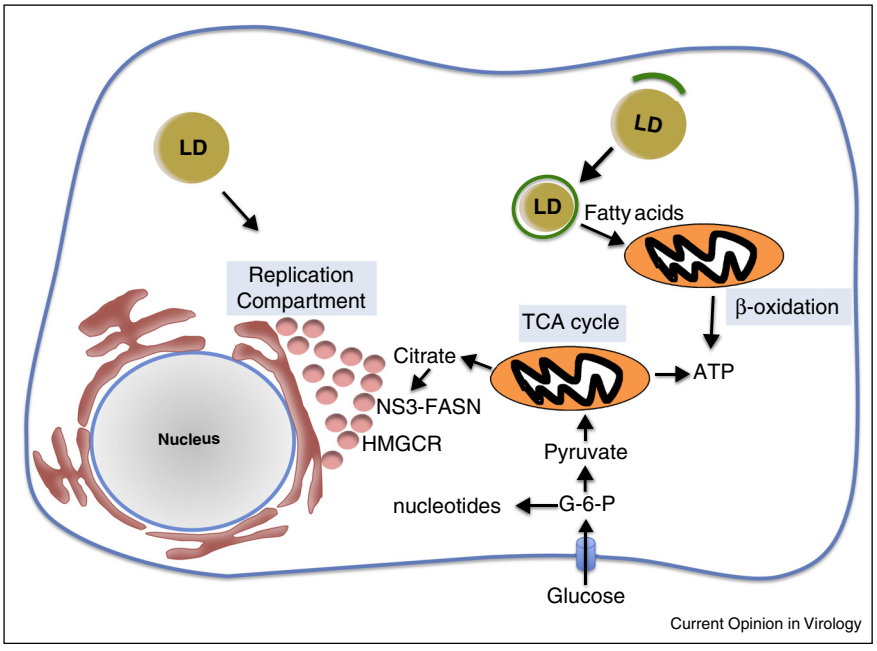
Our current understanding of the flaviviral benefits of modulating cellular metabolism includes the following conjectures. Flavivirus replication requires increased nucleotide pools and enzymatic cofactors, such as ATP for RNA helicase activity. As with all (+) RNA viruses, flaviviruses alter host membranes, in this case the endoplasmic reticulum (ER), to establish protected sites of replication. These replication compartments are thought to promote appropriate replicase scaffolding and concentration of replication substrates, in addition to shielding the RNA from cytosolic innate immune sensors and RNA degradation machinery [4,5]. Finally, as sites of replication and virion assembly are linked, modified ER lipid composition will be incorporated into virion envelopes, which may impact virion infectivity [33]. The following studies have investigated flavivirus modulation of cellular metabolism. One caveat is that these studies were performed in different cell lines, which may respond differently to flavivirus infection, and thus these metabolic changes may not be viewed holistically.

DENV and central carbon metabolism

DENV infection modulates and requires glycolysis for optimal virus replication (Figure 1) [2,7]. Because most cancer cell lines, at baseline, have an altered central carbon metabolism, Fontaine et al. performed a metabolomic analysis of mock and DENV infected primary human foreskin fibroblasts (HFFs). Infection of HFFs with DENV virus resulted in a significant change in all classes of metabolites (amino acids, carbohydrates, lipids, and nucleotides) as compared to uninfected cells over the course of infection [7]. DENV infection altered both glutamine and glucose utilization. Early glycolytic intermediates, such as glucose-6-phosphate and fructose-6-phosphate, increased in concentration over time, whereas late glycolytic intermediates decreased. While the authors were not able to determine whether these modulations were related to increased flux or decreased production of these intermediates, work from others has shown that the enzymatic activity of GAPDH is increased during DENV infection in an NS1-dependent manner [2]. This suggests that DENV actively drives flux through the glycolytic pathway.

Infection with DENV resulted in a modest increase in cellular glucose concentration, which likely resulted from an increase in the expression of glucose transporter 1 and hexokinase 2, the first enzyme of glycolysis [7]. Experimentally limiting glucose severely decreased viral replication, whereas limiting glutamine levels had only a modest effect on viral replication. Fontaine *et al.* further demonstrated that inhibition of glycolysis inhibited viral

Figure 1



Flavivirus modulation of metabolism. Early after infection, lipid droplets are reabsorbed into the ER. Later in infection, autophagosomes (green) liberate free fatty acids from lipid droplet triglycerides, which then undergo mitochondrial β-oxidation to produce ATP. In parallel, DENV stimulates glycolysis to produce ATP through the TCA cycle. This also generates citrate, which is a precursor for fatty acids biosynthesis. DENV NS3 recruits FASN to the ER in a Rab18-dependent fashion and stimulates fatty acid synthesis at RCs to modify the lipid composition. WNV recruits HMGCR to RCs to stimulate cholesterol synthesis. This establishes an optimal environment for flavivirus replication.

replication, thus showing that DENV replication requires active glycolysis for optimal virus replication. The authors also demonstrated the requirement for glycolysis in a second cell line of immortalized (but metabolically normal) endothelial cells, suggesting that the requirement for glycolysis is not cell-type specific. Glycolytic stimulation by DENV may enhance numerous processes, including glutamine production for increased ATP and nucleotide pools, in addition to citrate, which is a precursor for fatty acid synthesis, discussed later.

Modulation of lipids at the replication compartment (RC)

Flavivirus replication compartments (RCs) are convoluted invaginations of the endoplasmic reticulum (ER) membrane [8,33]. Numerous strategies exist to generate the membrane curvature of RCs, including modulation of membrane lipid content [5,6]. Insertion of polar lipids into membranes generates curvature, while some lipids, such as cholesterol, enhance membrane fluidity that facilitates the curvature of membranes by proteins [12]. WNV infection increases cholesterol synthesis and accumulation at RCs. The cholesterol biosynthetic enzyme 3-hydroxy-methyglutaryl-CoA reductase (HMGCR) is recruited to RCs. Inhibition of HMGCR inhibits WNV replication [18]. In addition to potential roles of cholesterol in RC formation, this redistribution of cholesterol to RCs perturbs lipid rafts that are important for antiviral interferon signaling [18].

DENV stimulates fatty acid biosynthesis to alter RC lipid composition (Figure 1). A targeted siRNA screen utilizing the DENV2 replicon identified acetyl-coA Carboxylase 1 (ACC1/ACACA) and fatty acid synthase (FASN) as important cofactors for DENV-replication, suggesting an important role for de novo fatty acid synthesis in DENV replication [10]. Pharmacological inhibition of FASN decreased DENV replication and infectious virus production. Infection with DENV caused a relocalization of FASN to RCs and increased de novo fatty acids synthesis in crude cellular biochemical fractions containing the DENV replicase. Yeast-two hybrid analysis identified DENV NS3 as binding FASN, and NS3 expression was sufficient to re-localize FASN to perinuclear regions associated with RC formation. Finally, the addition of purified NS3 was sufficient to increase FASN activity in cell lysates. Although NS3 can directly bind FASN, a role for the ras-related GTPase RAB18 in relocalization of Rab18 to ER-associated NS3 was uncovered. Both DENV replication and FASN relocalization required the active GTP-bound form of Rab18 [30].

Lipid synthetic enzymes further modify fatty acids to generate the vast majority of distinct membrane lipids. A