Viral infections and metabolism: a clue to antiviral therapy?

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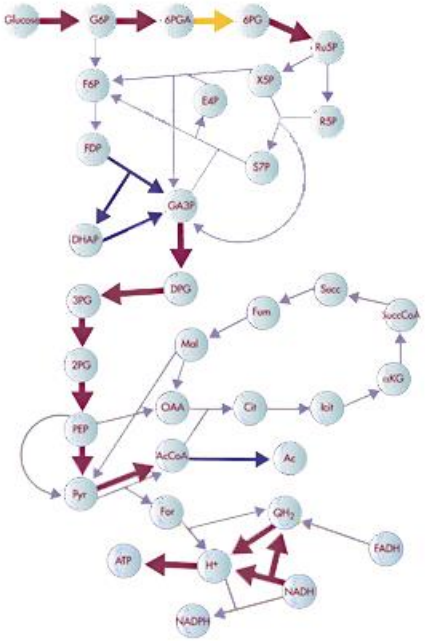
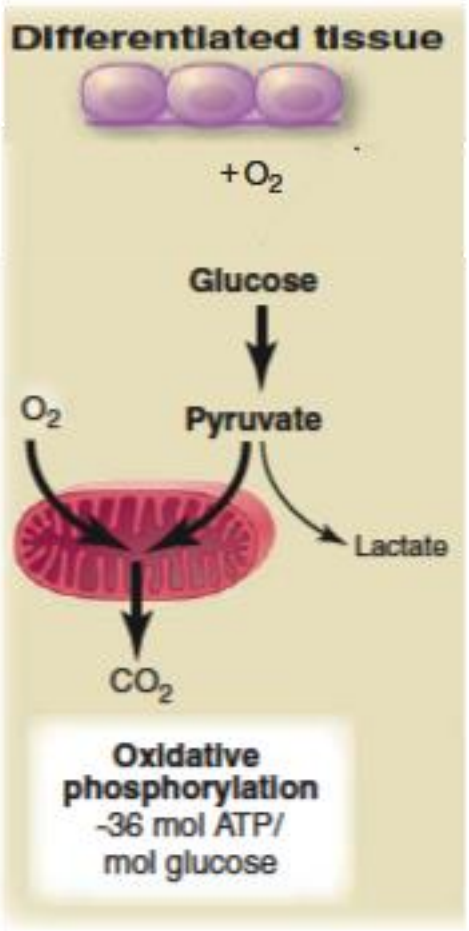
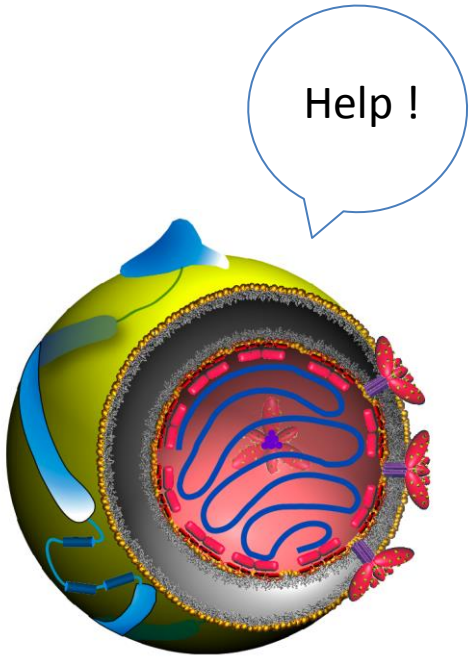
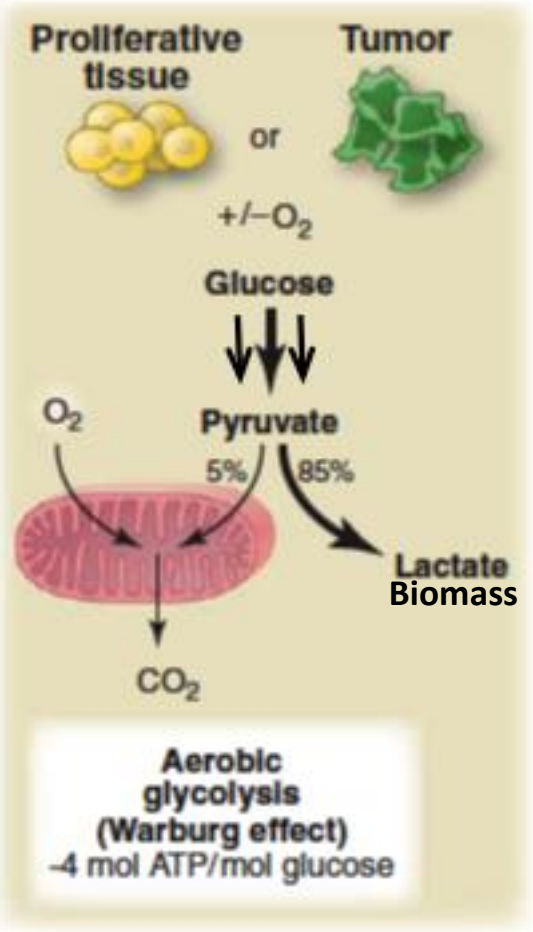
What are the general rules which define the Topology of neuronal connections ?



How is regulated the flux of metabolites in metabolic pathways ? How stable is a steady state equilibrium ?


Differentiated tissue
+O2
Glucose
O2
Pyruvate
Lactate
CO2
Oxidative phosphorylation
-36 mol ATP/ mol glucose


Help !

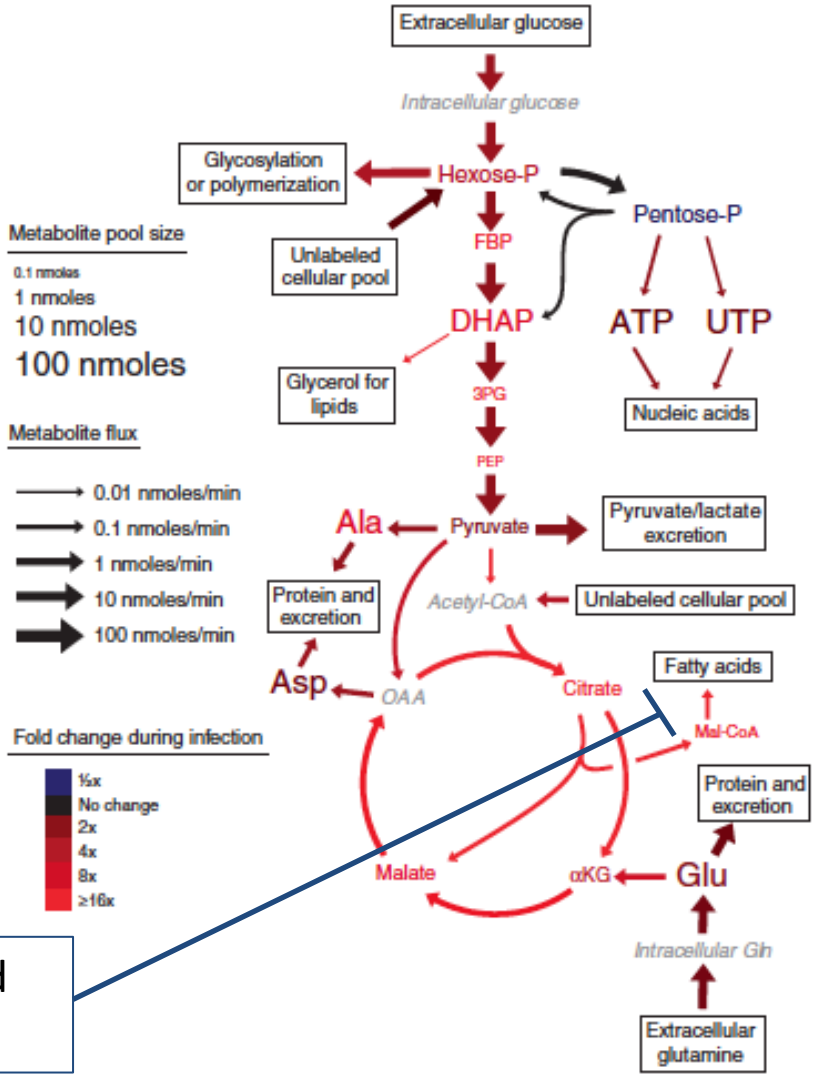

Proliferative tissue
or
Tumor
+/–O2
Glucose
O2
Pyruvate
5%
85%
Lactate
Biomass
CO2
Aerobic glycolysis (Warburg effect)
-4 mol ATP/mol glucose

Questions

- Do viruses replicate only in cells which have the appropriate metabolism or do they modify the cell metabolism ?

Metabolo- and fluxomic of fibroblasts infected by human cytomegalovirus

- Infection of primary human fibroblast by human cytomegalovirus
- Increased glycolysis with increased lipid, aminoacid and nucleotide synthesis
- Increased TCA feed by Glu

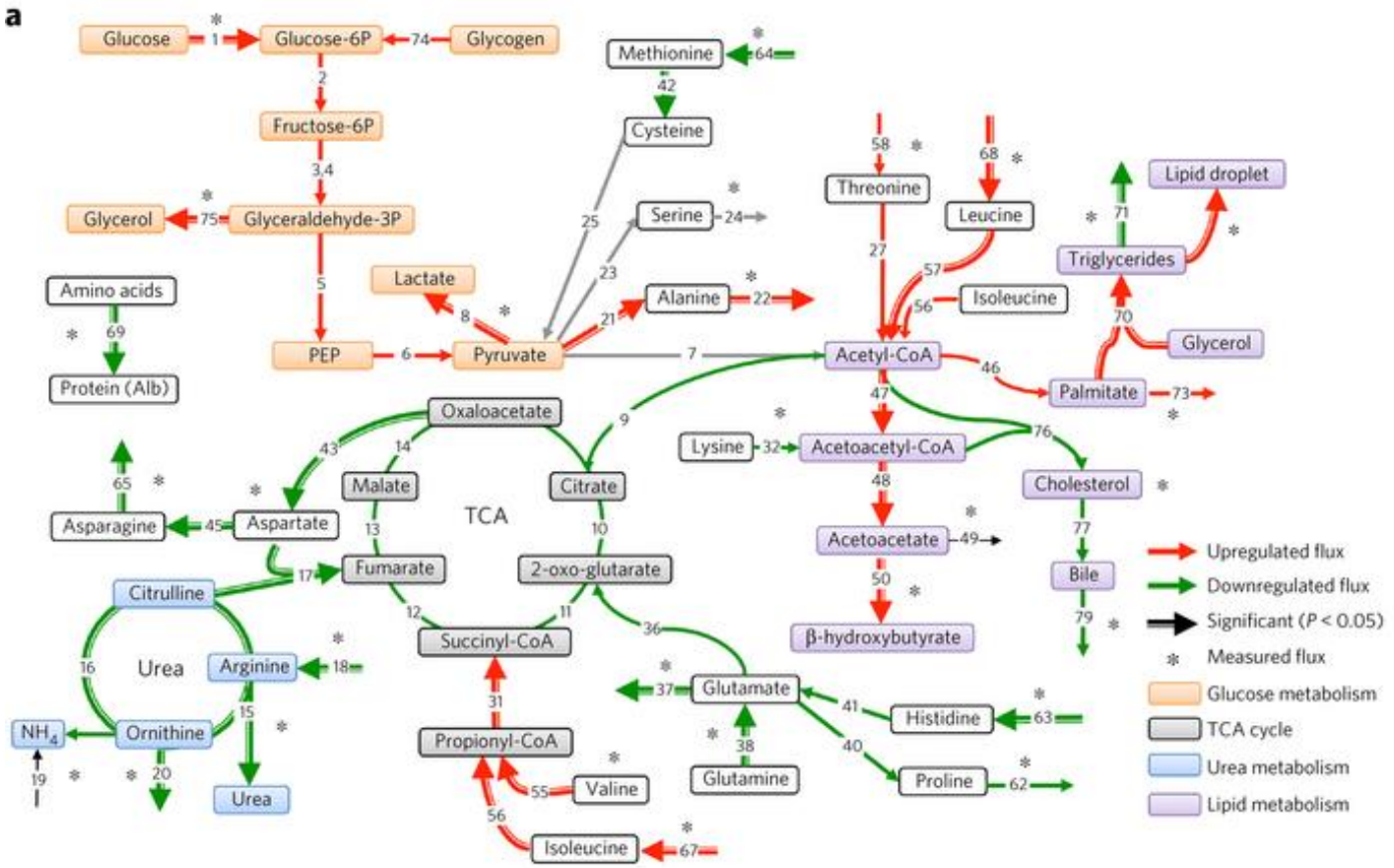


Fatty acids synthesis inhibitors (ACC and FAS inhibitors) repress viral replication

Munger, Nature, 2008

All-omic studies of hepatitis C virus infection

Studies in primary human hepatocytes, at 10 days post-infection

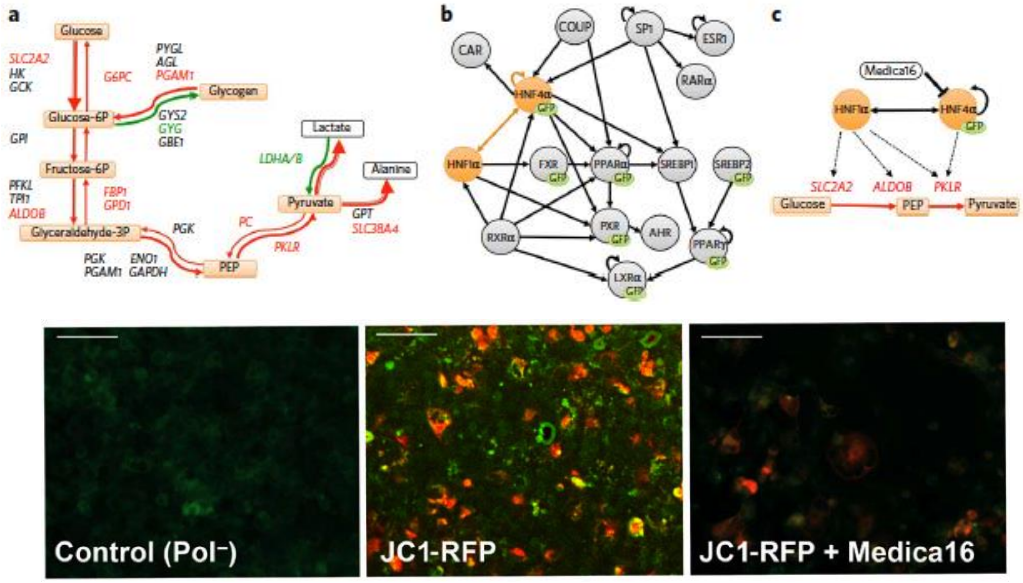


Levy, Nat Chem Biol, 2016

All-omic studies of hepatitis C virus infection

- Transcriptional regulatory analysis of glycolysis showed enrichment of HNF4α targets

- HNF4α inhibitor Medica 16 represses HCV RNA replication

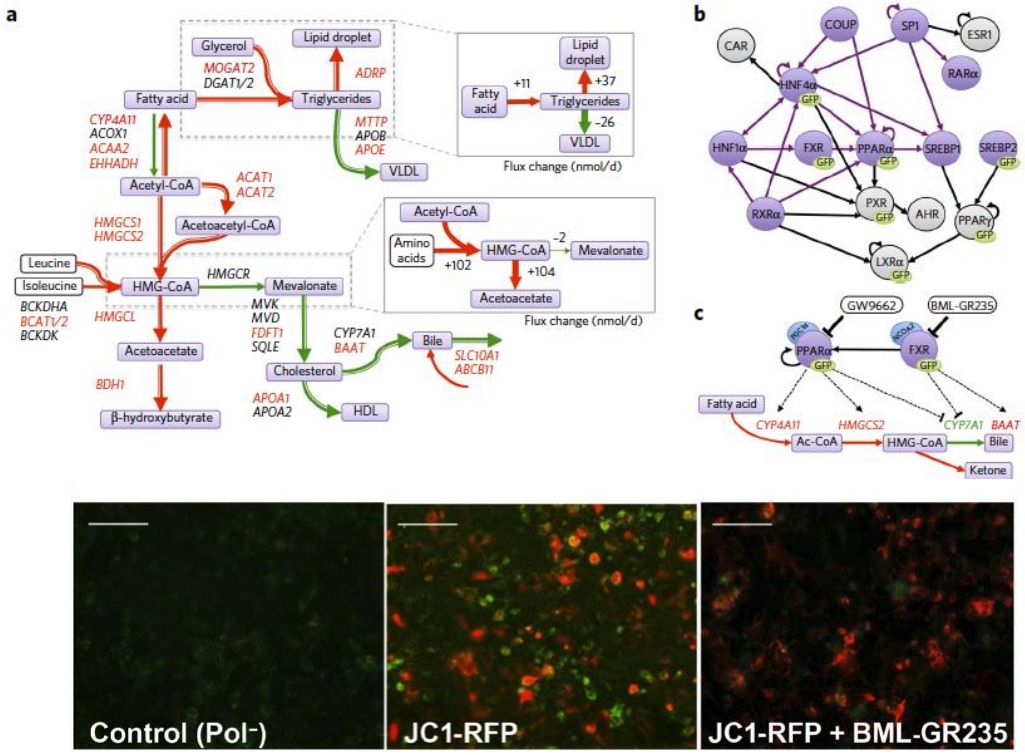


➡ Infection-induced metabolism changes **favor** viral replication

Levy, Nat Chem Biol, 2016

All-omic studies of hepatitis C virus infection

- Increased lipid oxidation and decreased bile salts synthesis
- Transcriptional regulatory analysis of fatty acid oxidation showed significant enrichment of FXR targets
- FXR antagonists favor HCV RNA replication



➡ Infection-induced metabolism changes **limit** viral replication

Levy, Nat Chem Biol, 2016

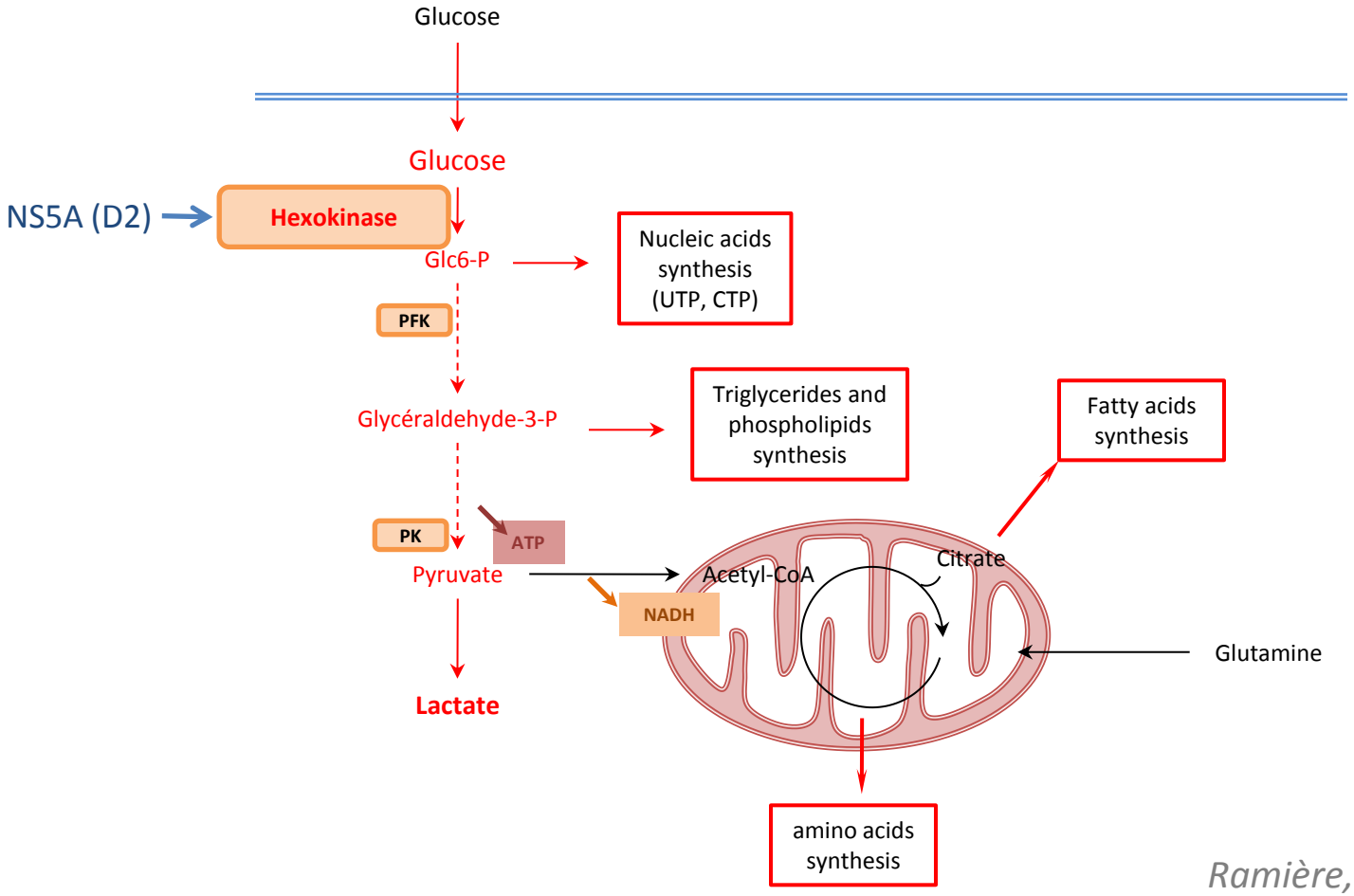
Answers and new questions

- Viral infections alter cell metabolism
- Some metabolic modifications favor and others limit virus replication (defining a new equilibrium supporting both viral replication and cell survival ?)
- Limiting the virus-induced metabolic modifications by chemicals targeting specific metabolic pathways alter viral replication

- Do viruses directly modify metabolic factor ?
- Does metabolites consumption by viral replication oblige cell metabolism to respond and reach a new equilibrium ?

Investigating the mechanisms underlying the metabolism changes

Pairwise screen of interaction between HCV proteins and glycolysis enzymes by protein complementation assay in mammalian cells

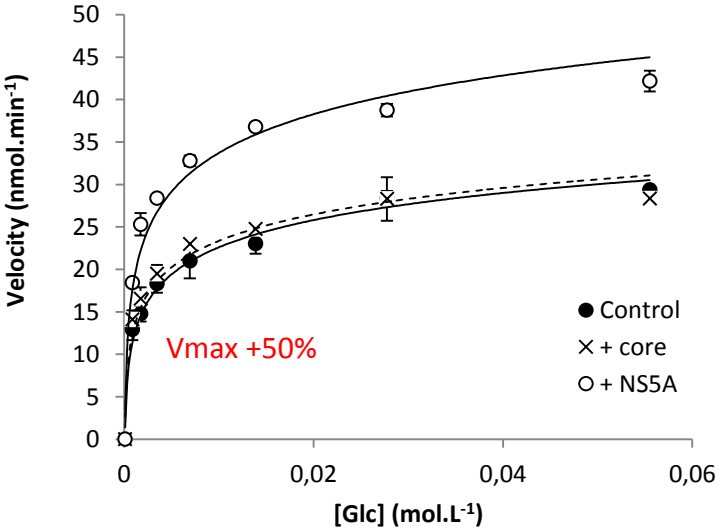


Ramière, J Virol, 2014

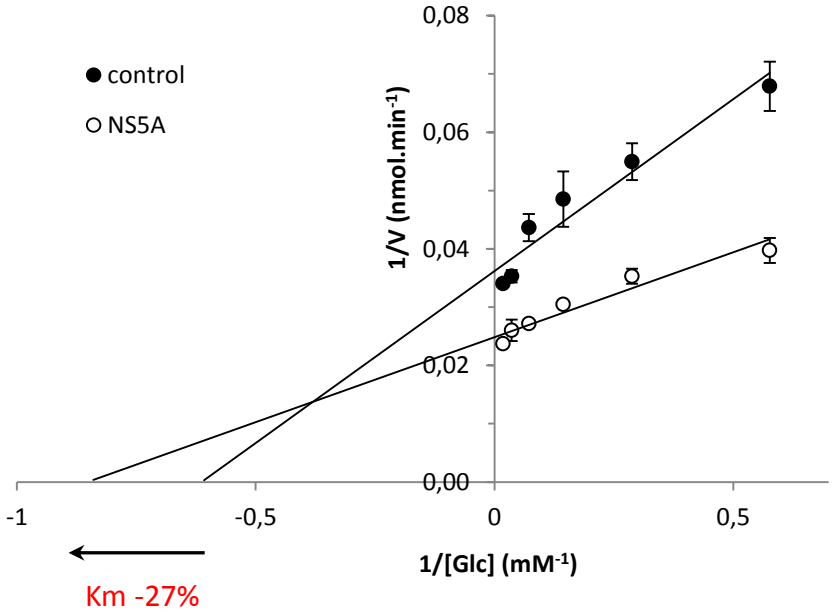
Functional impact of NS5A and HK interaction on HK activity

Purified recombinant HK2 protein, specific activity=0.1UI/mg
Purified NS5A full length and Core proteins produced with wheat germ cell-free expression system (Cell-Free Science, Japan) provided by F. Penin.

Michaelis and Menten curves



Lineweaver and Burk projection

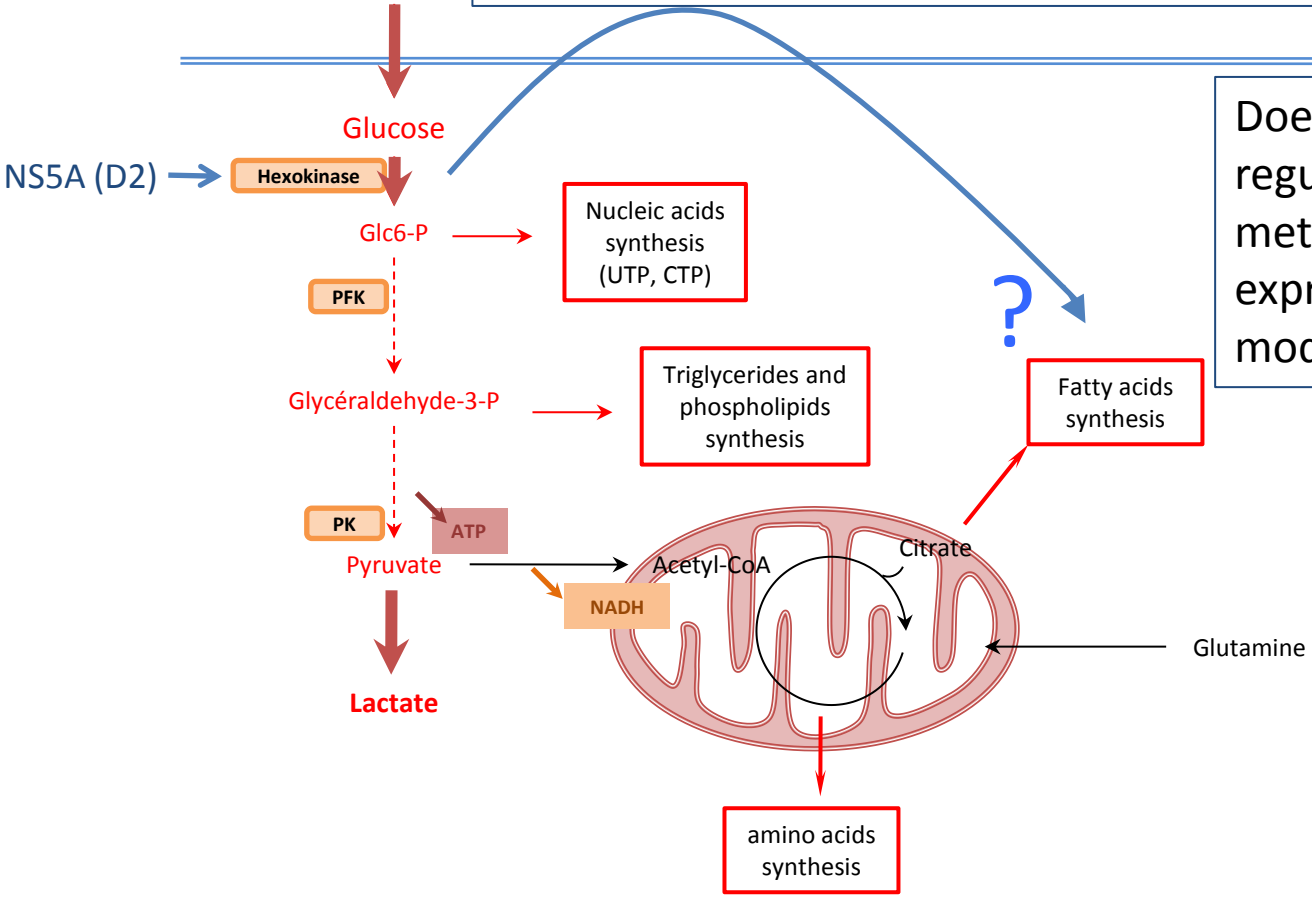


- NS5A = allosteric activator of hexokinases
- Cell expression of NS5A sufficient to increase the uptake of glucose and the excretion of lactate

Ramière et al, J Virol, 2014

On-going work and perspectives

Can virus-alteration of HK activity sufficient to induce remote metabolome modifications (e.g. fatty acids synthesis…) ?



Does NS5A modifies other regulatory loops within the metabolome (e.g. NR expression, enzyme activity modulation…) ?

On-going work and perspectives

- Comparative metabolomic and fluxomic analysis of Huh-7 cells infected or non-infected with HCV; expressing or not NS5A; expressing HK4 or 2 that have different catalytic activities
- Could modeling predict the role of HK and other factors in the observed metabolic changes
- What algorithms can be used, Recon2.2, topological, differential…

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