Drawing of Hepatitis C virus structure removed due to copyright restrictions.

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Tech Spec Review
Hepatitis C Treatment

Introduction

- What is hepatitis C?
- Why we chose it?

How it works?

Chimp study- why nucleosides

- Study tested in vivo effects of nucleoside in chimpanzees
- Injecting 0.2-2 mg of nucleoside/ kg of body weight for 12 days
- Found that there was a >5log10(original IU concentration) decrease to the point where they could no longer detect the presence of IU
- Improvement through cell uptake

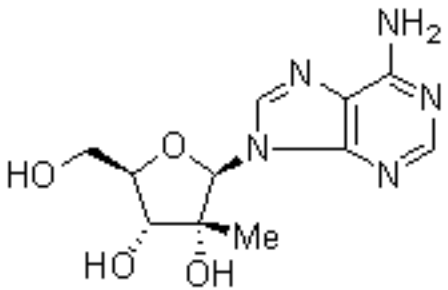
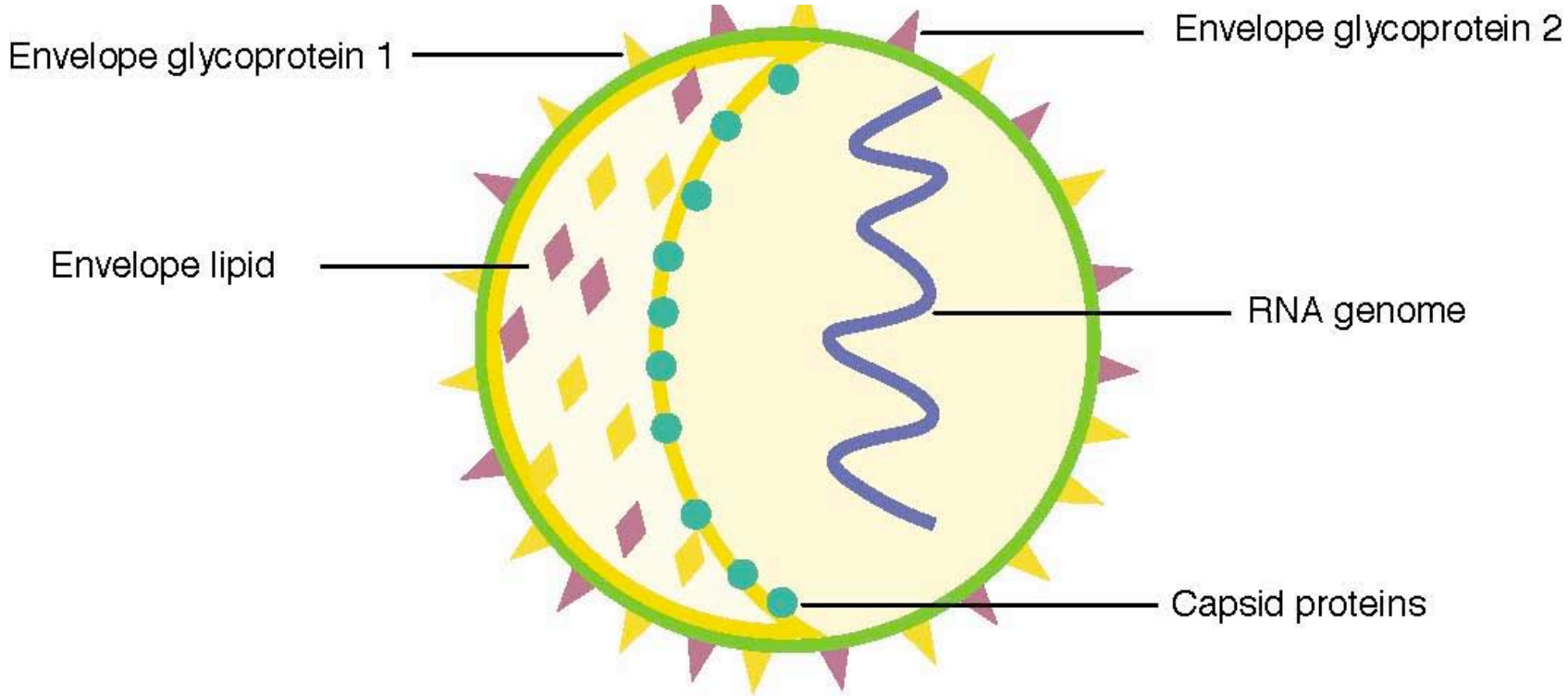


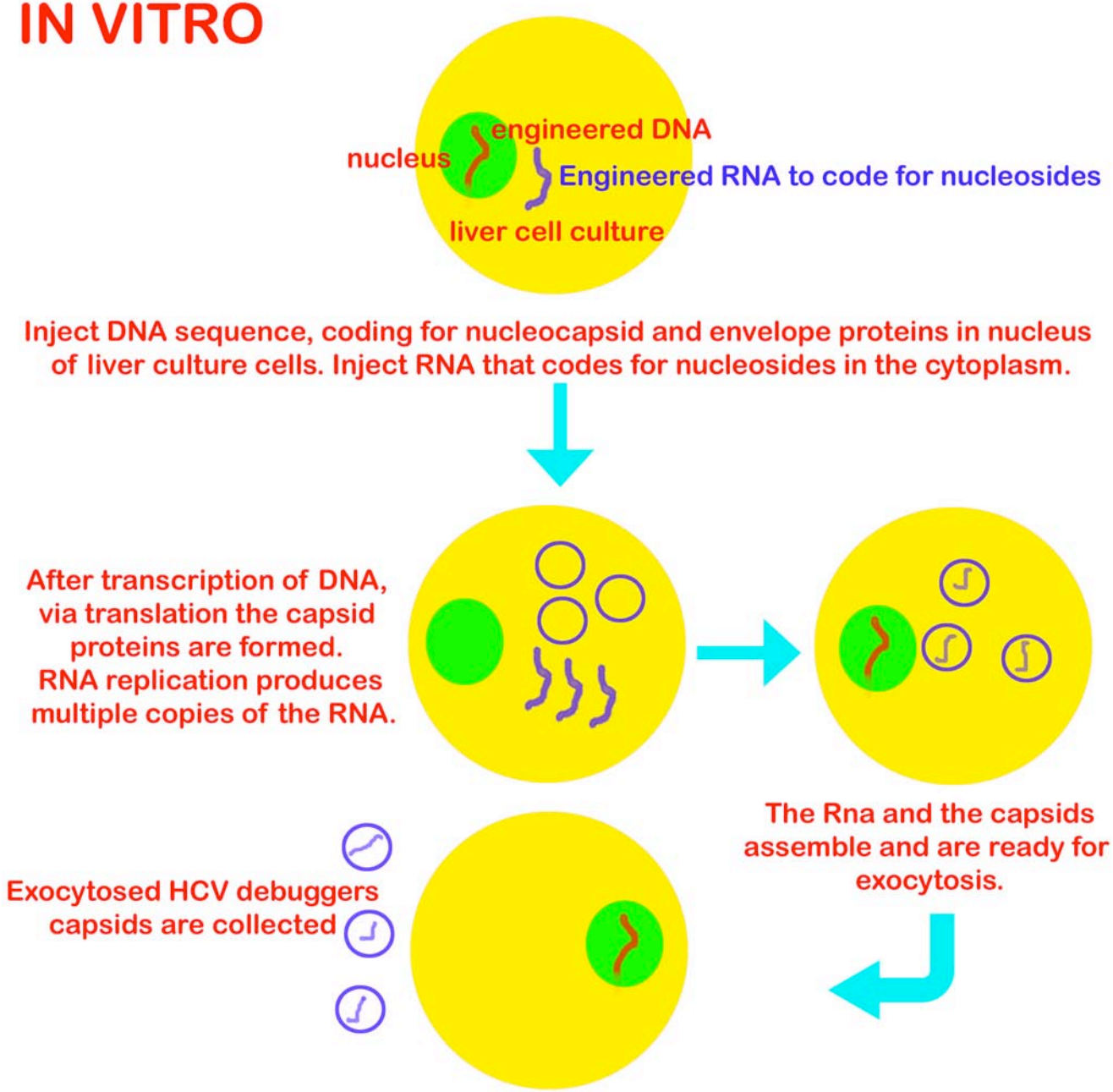
Image courtesy of Ori2uru on Flickr.

Model Structure of HCV



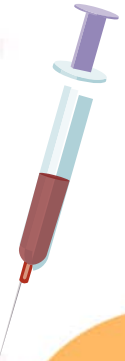
System level diagram

IN VITRO

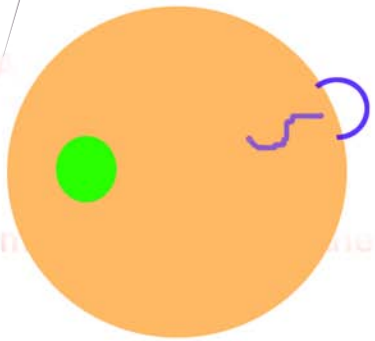


IN VIVO

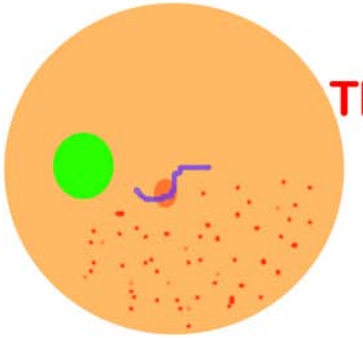
Collect HCV debuggers from liver cell culture



Inject them into blood stream of human infected with HCV



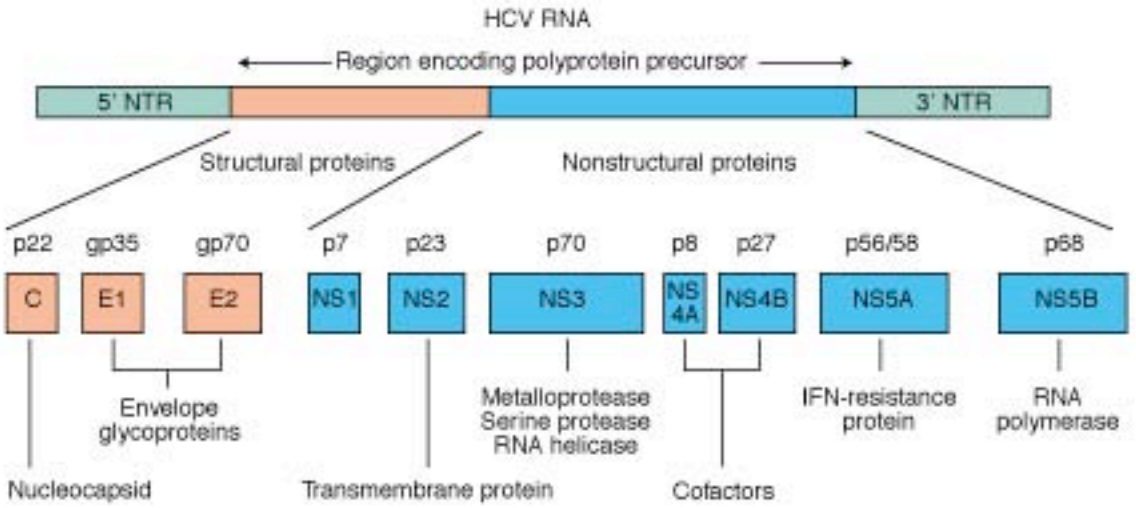
The HCV debuggers are directed to the liver.The endocytose into the liver cell, injecting the engineered RNA.

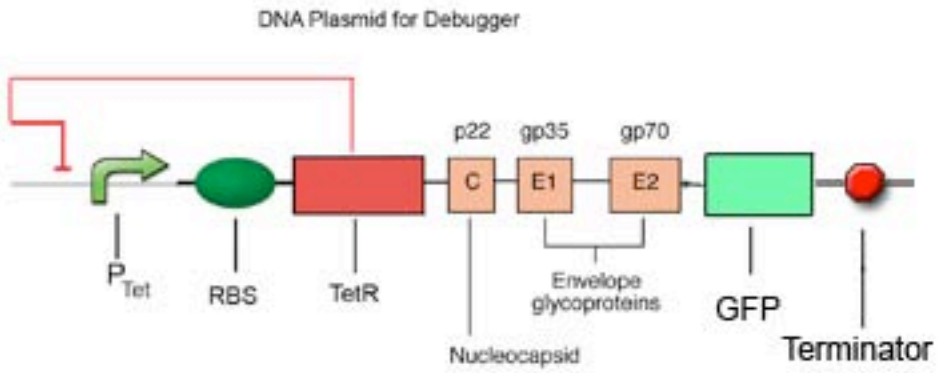


The RNA is translated to make the specialized nucleosides, which should prevent viral replication.

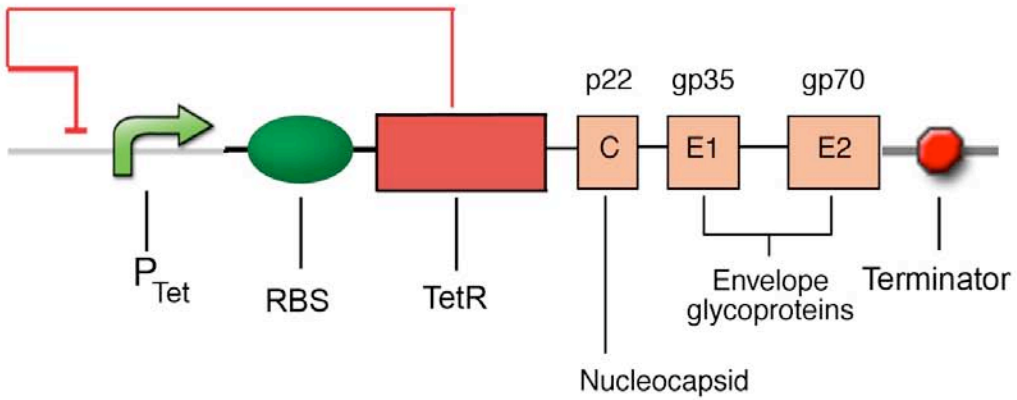
Figures by MIT OpenCourseW

Engineering the capsid producing DNA



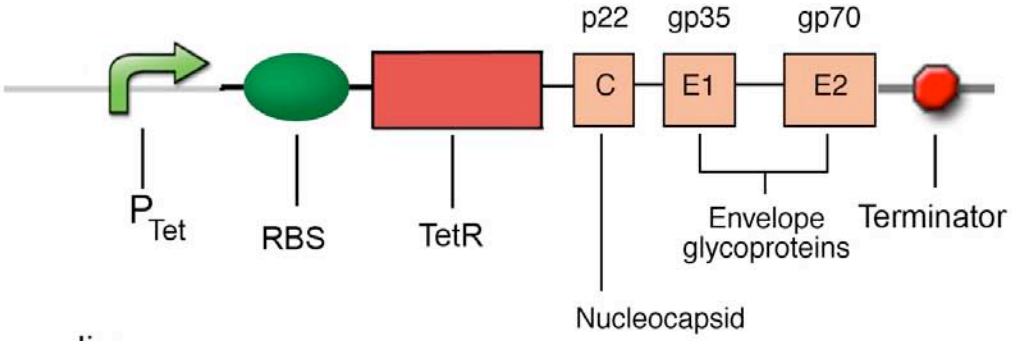


DNA Plasmid for Debugger



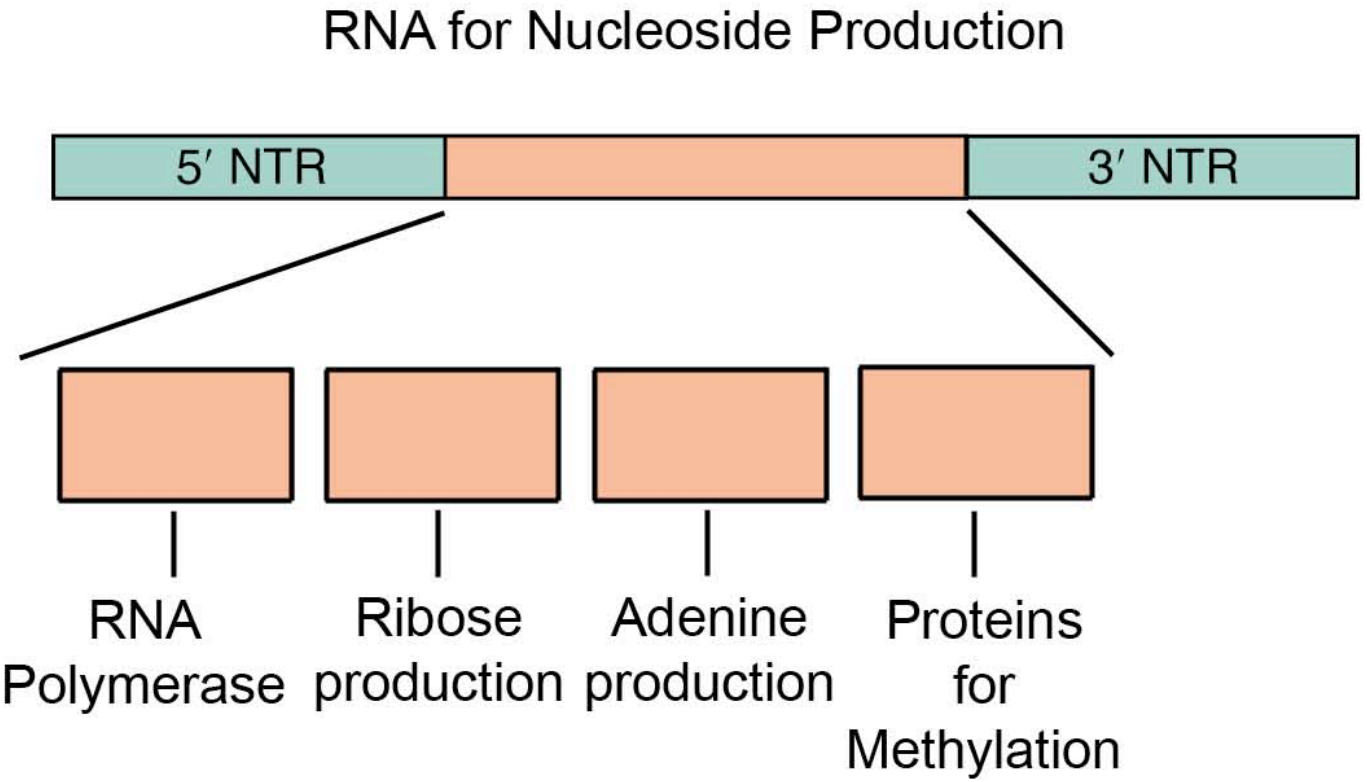
-Doxycycline

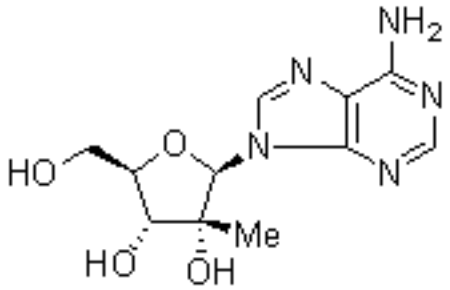
DNA Plasmid for Debugger



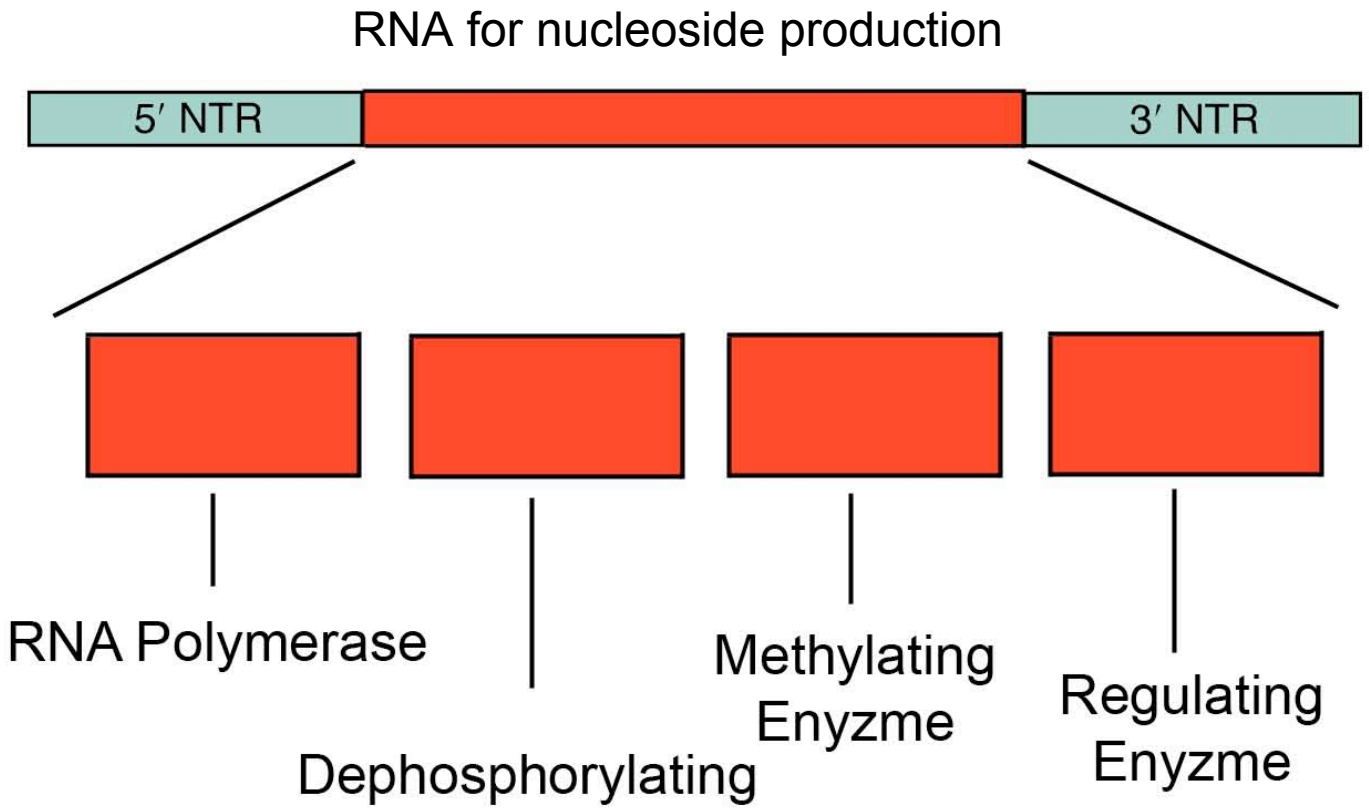
+Doxycycline

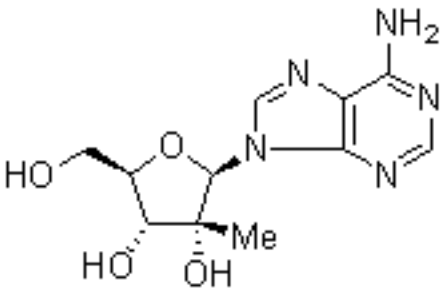
Engineering nucleoside RNA - 1



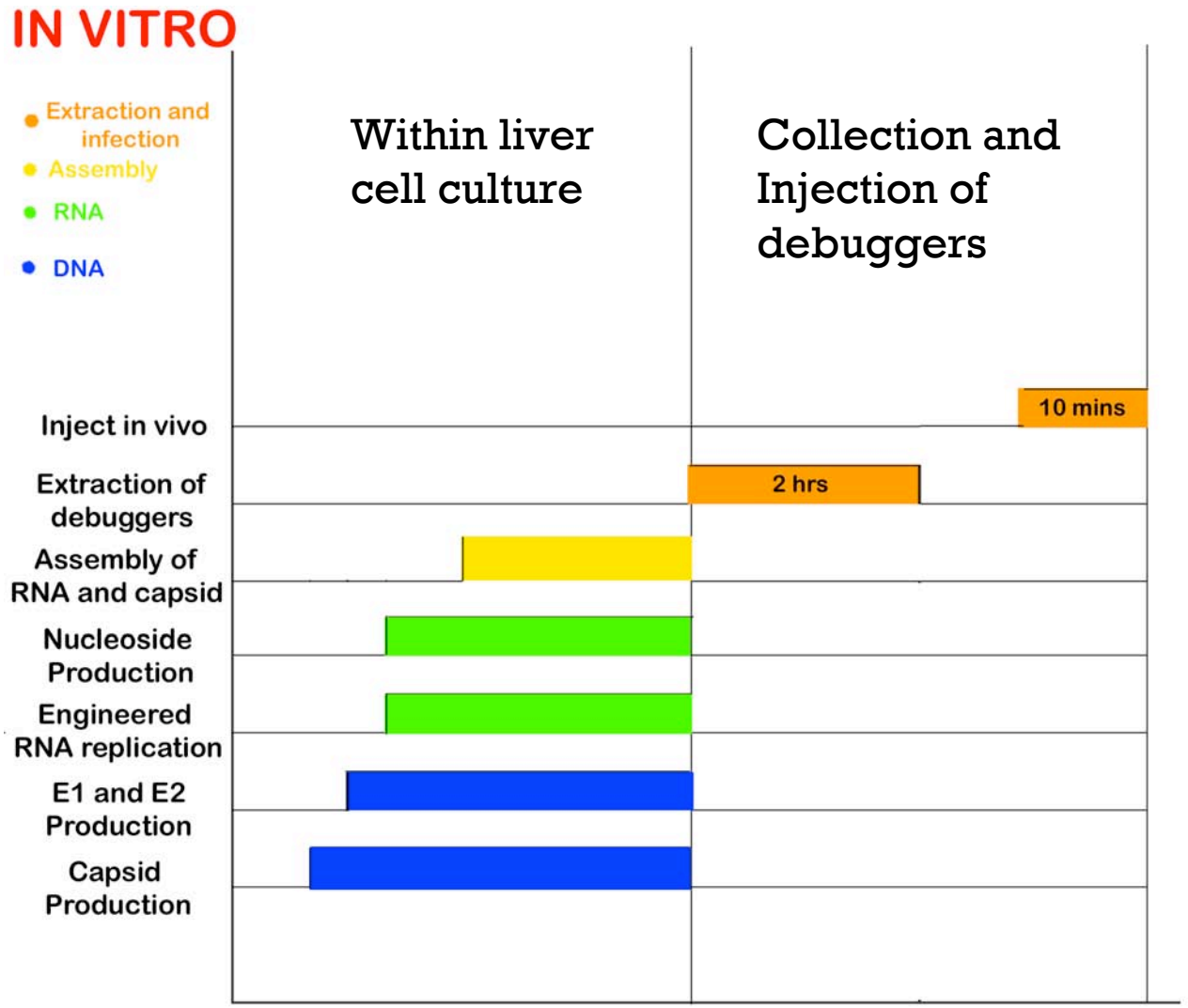


Engineering nucleoside RNA - 2





Time Diagram



After injecting debuggers in vivo, we would test for a reduction in viral load in 48 hours

Parts list – In Vitro

Parts	Sourcing	Description	Annotation
Liver Cells	PromoCell	Human Hepatocyte	Will produce HCV capsids
DNA (Capsid, E1, E2, GFP, terminator)	Integrated DNA Technologies	Deoxyribonucleic acid	Codes for capsid and proteins
Tet repressor	sequence from Reg. Standard Biological Parts	Controls expression of our DNA	When Tet is present, gene will not be expressed
Doxycycline	Next Tag	Antibiotic	Allows DNA to be expressed
Capsid, E1, E2	Sequence from National Center for Biotechnology Information	The capsid and glycoproteins from HCV	Capsid will form around our synthetic RNA and target it to liver cells
RNA	Integrated DNA Technologies	Ribonucleic acid	codes for mechanism that will make our nucleosides
Plasmid Backbone	Sequence from Registry of Standard Biological Parts	Circular DNA	Shuttle for DNA

Parts List – for RNA

Parts	Description	Annotation
RNA	Ribonucleic Acid	Codes for mechanism that will make our nucleosides
Sequence for RNA Polymerase	Will code for RNA Polymerase	Copy itself, don't have to inject every day
Adenine Production Enzyme	Codes for Enzyme that constructs Adenine	Basic structure of nucleoside
Ribose Sugar Enzyme	Adds Ribose Sugar to Adenine	Adenine becomes basic, common nucleoside: Adenosine
Proteins for Methylation	Proteins methylate adenosine	Methylation makes the nucleoside specific to viral RNAP
Sequence for GFP	Codes for Green Fluorescent Protein	Will assist in debugging and validation

Cost

Part	Source	Price
Doxycycline	Next Tag	$ 3.50
Liver Cells	Promocells	$ 794 / 3-6* 10^6 cells
DNA	Integrated DNA Technologies	$ 1 / base
RNA	Integrated DNA Technologies	$ 1 / base

$$$

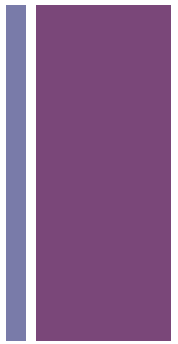
Plan for debugging and validation

- In vitro:
- GFP – to detect capsid formation
- Validation of nucleosides – infect liver culture cells, and then use debuggers to see if they stop viral replication.
- In vivo:
- Taqman assay on infected human and chimp

Impact of solution

- In the chimps experiment, decrease in Infectious Units per milliliter by 1.0 log10(original IU/ml) when Chimps injected with 0.2 mg/kg of body weight per day.
- If we succeed…by enhancing the uptake of nucleoside by the liver cells, we should see a greater decrease in IU/ml of viral load !

Buildable



- Everything exists !
- Black boxes need to be filled in !

Safety

- HCV debuggers are non-infectious.
- The methylated nucleosides are not toxic in the known dosages.
- In vitro work should be carried out in a biosafety lab 3 environment, with necessary precautions.

Security

- Design should not be available to the general public, due to potential for remodeling HCV into another infectious virus !
- Do not want to disclose of the hook that helps RNA assemble with capsid !

Unknowns

- Detailed mechanism for nucleoside production remains unknown.
- Exact mechanism for methylation of nucleosides due to commercialization of product.
- How nucleoside production would affect the energy of the liver cell.

???

Open issues and ethics

- Human and chimp test subjects will be needed for final validation of design …
- We are using the outer proteins of HCV … but just as a vaccine…
- Nucleoside production vs. Liver cell energetics

Go/No Go decision

Photo adapted by MIT OpenCourseWare, original courtesy of Laverrue on Flickr.

References

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20.020 Introduction to Biological Engineering Design

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