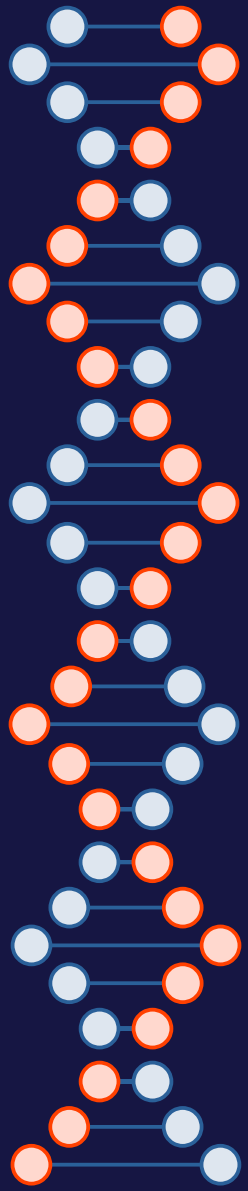
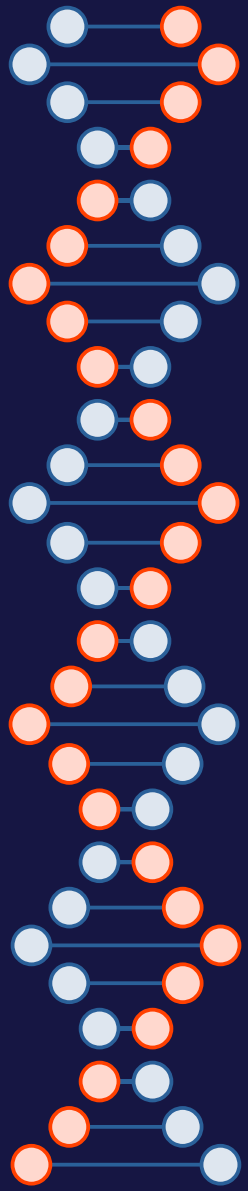


How To Kill Any Virus

(And Not The Patient)

by

Rűdiger Marcus Flaig

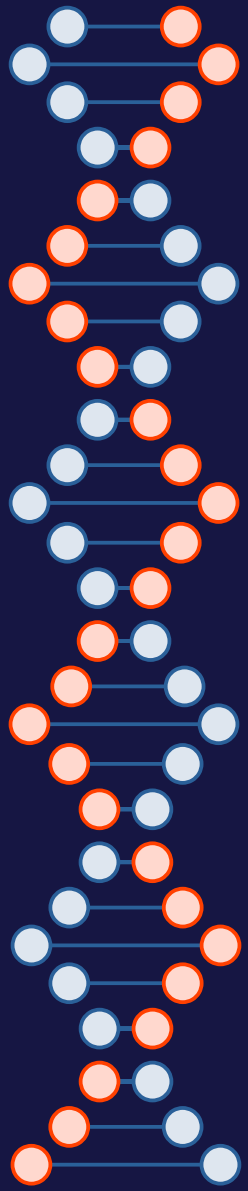


借刀杀人。

jiè	*dāo*	*shā*	*rén*
borrow	*blade*	*slay*	*person*

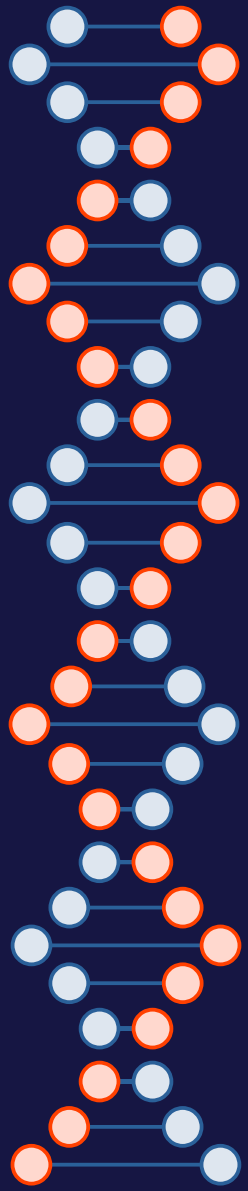
Strike with a borrowed knife.

- 3rd of the 36 Strategems of the Warring States



In other words:

Turn a virus's own components against it.

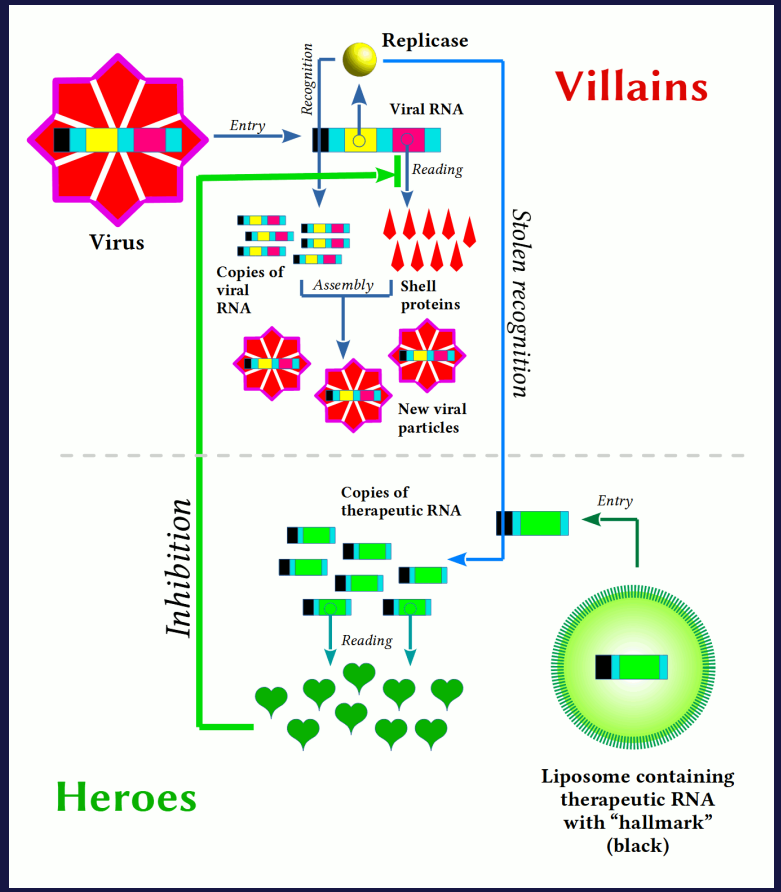


For the very curious:

Hijack the viral replication and packaging systems to produce antivirally active nucleic acid.

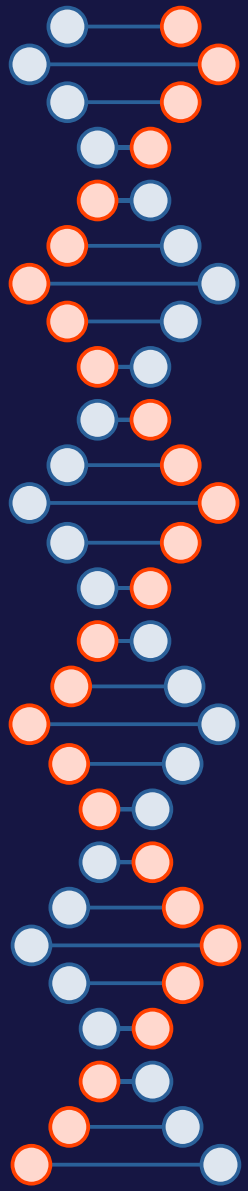
What does this mean?

Sneak preview:



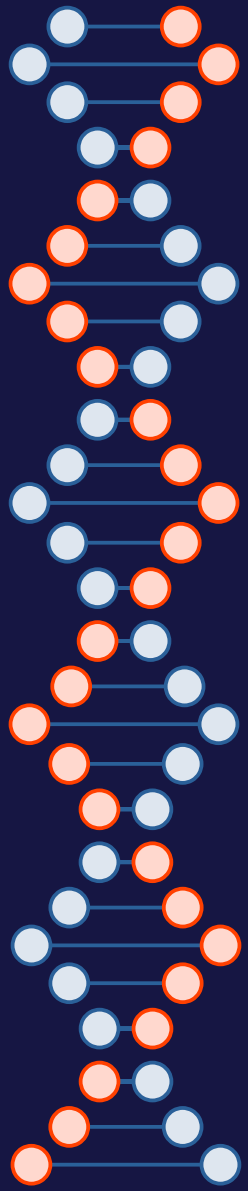
Do not worry if you do not understand this at first glance.

We will now discuss how this works.



Let us begin by looking at how viruses work.

Viruses are nucleic acid (genetic material) packaged into capsids (shells of protein and sometimes lipid).



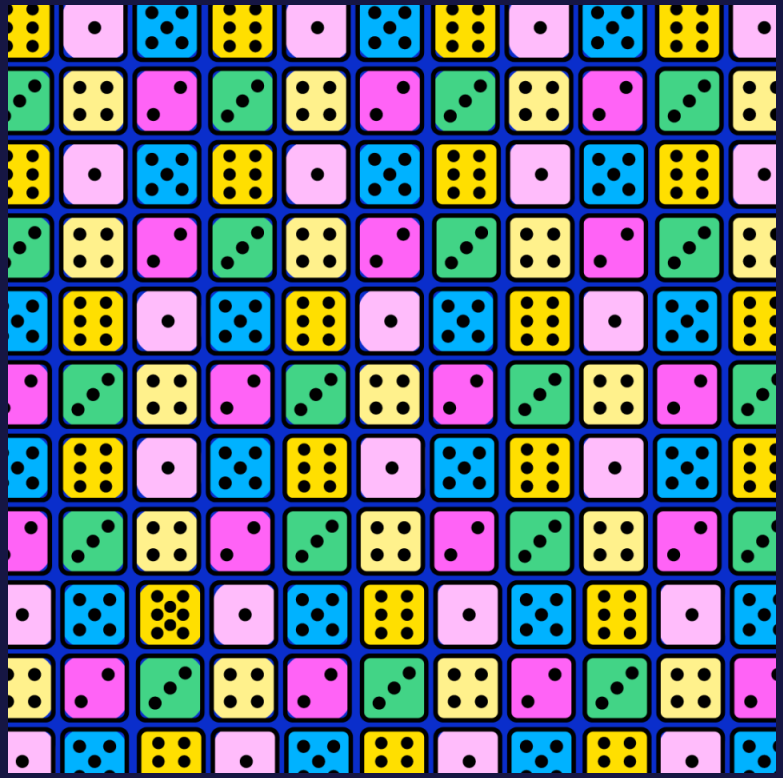
When a virus enters a cell…

- it makes copies of its nucleic acid (DNA or RNA),
- builds new shells, and
- packages the copies into the shells.

Thus, hundreds to hundreds of thousands of progeny are manufactured in one go before the cell dies.

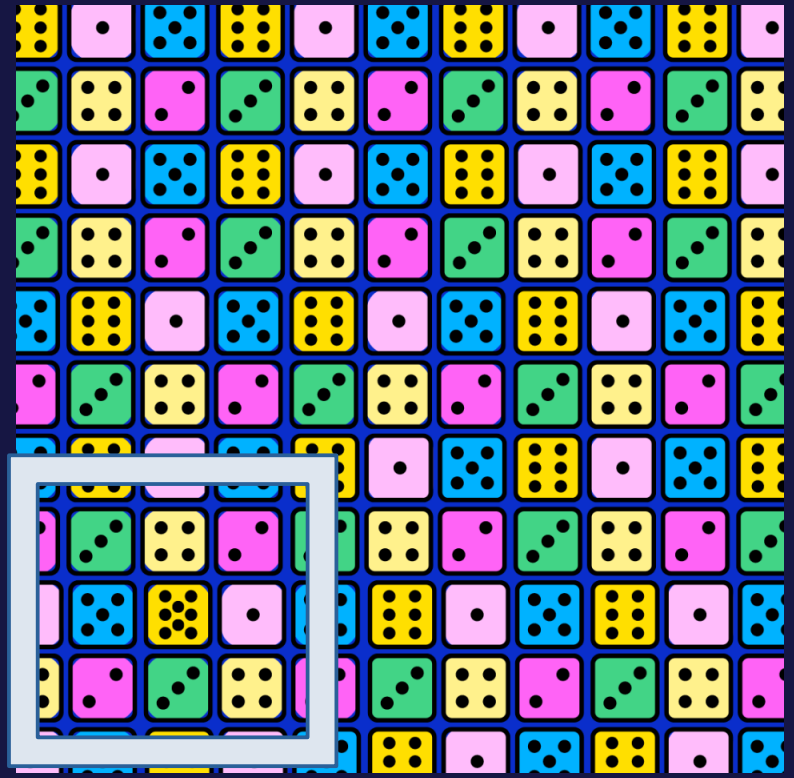
In this process, only the virus's own nucleic acid is to be copied and packaged, *not that of the host cell!*

The cell contains much more nucleic acid!



Being able to differentiate its own nucleic acid from that of the cell *(“needle in the haystack”)* is therefore pivotal to every virus.

The cell contains much more nucleic acid!



Being able to differentiate its own nucleic acid from that of the cell *(“needle in the haystack”)* is therefore pivotal to every virus.

Thus, every virus comprises…

(I) A gene for the **replication** mechanism.

(II) Gene(s) for the **shells**.

(III) A "**signal**" on its nucleic acid to show where replication starts, known as "ori".

(IV) Another "**signal**" that marks viral nucleic acid for packaging into the shells.

When a virus enters a cell...

(I) The **replication** mechanism creates copies of the viral genome.

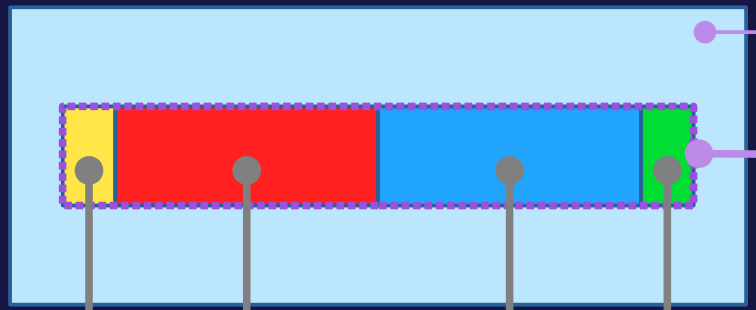
To this end, the replication mechanism must be able to identify the virus's nucleic acid.

(II) **Shells** are produced.

(III) The copies are packaged into the shells. Again, the copies must be identified.

GENERIC VIRUS

*The genetic information of a virus is stored on a **nucleic acid strand** encapsulated into a **protein shell** designed to infiltrate target cells.*



Shell

Nucleic acid

…like a CD in a casing!

Packaging identifier

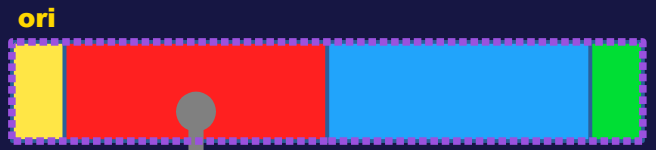
Gene for shell

Gene for copying mechanism (replicase)

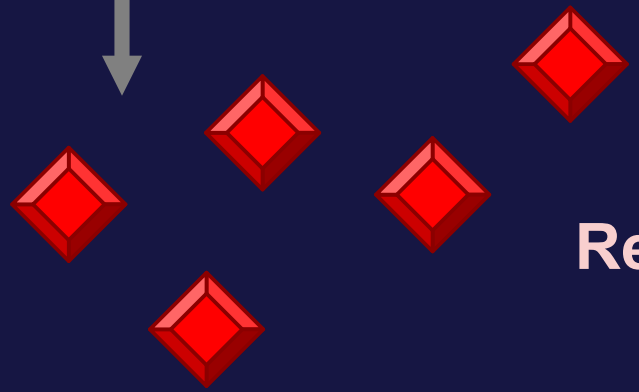
Copying identifier (“ori”)

Playlist

Step I: The virus abuses the cell to build its copying machine from the template on its nucleic acid



Reading of gene

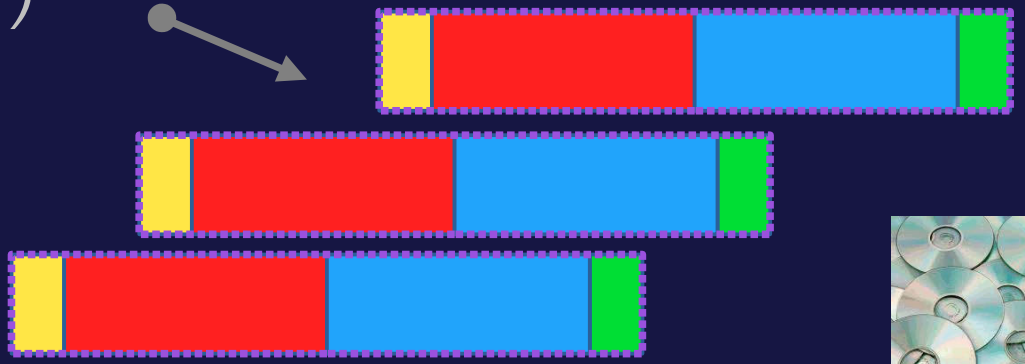


Replicase

Unpack and play.

Unlike a CD, this creates its own copying device.

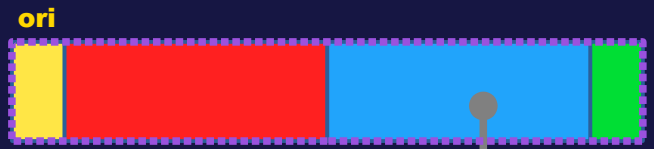
Step II: The copying machine copies the nucleic acid starting at the identification sequence



Copies of viral nucleic acid

Cellular nucleic acid is ignored due to lack of "ori" sequence.

Step III: The virus further abuses the cell to build new shells from the template on its nucleic acid



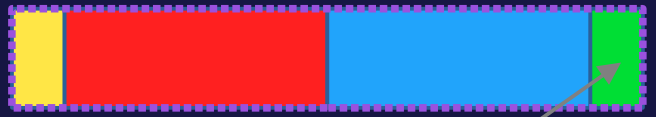
(By now, the viral nucleic acid has been copied – Step II –, so this reading is done on multiple templates in parallel, leading to large-scale production of shells.)

Reading of gene

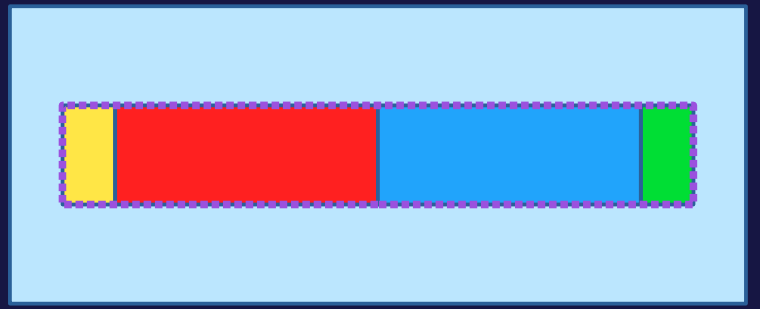
(Empty) shells

Step IV: The shells package the copies using the packaging identifiers

Copies from Step II

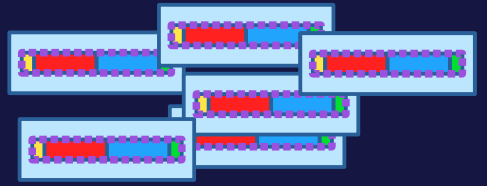


Detection



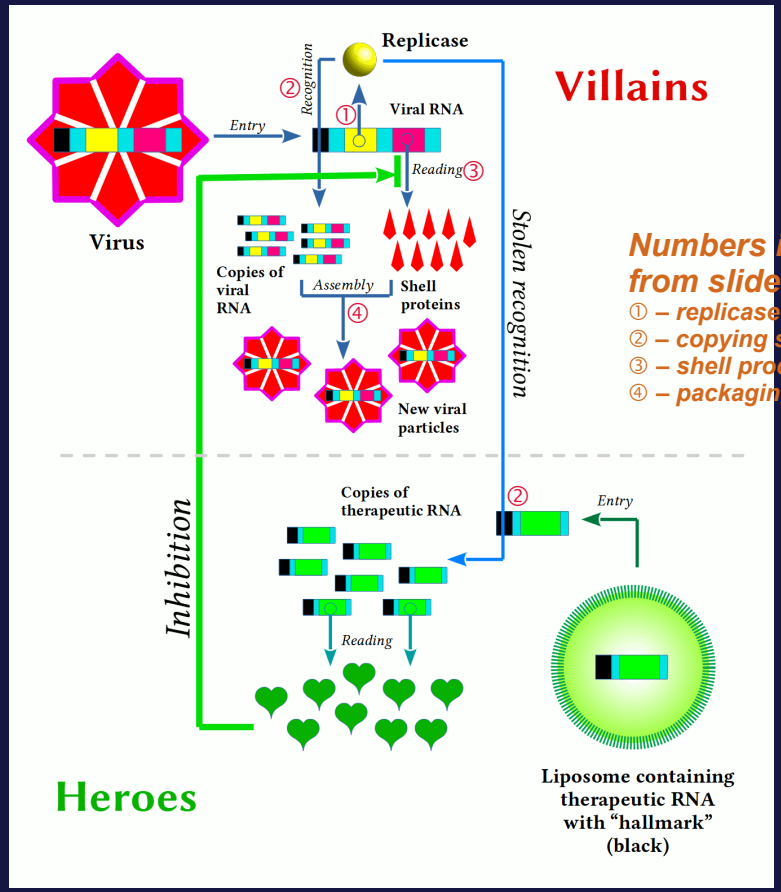
Infectious viral progeny

Shells from Step III



Cellular nucleic acid is ignored due to lack of packaging sequence.

This is the upper part ("Villains") of this diagram, coloured differently:

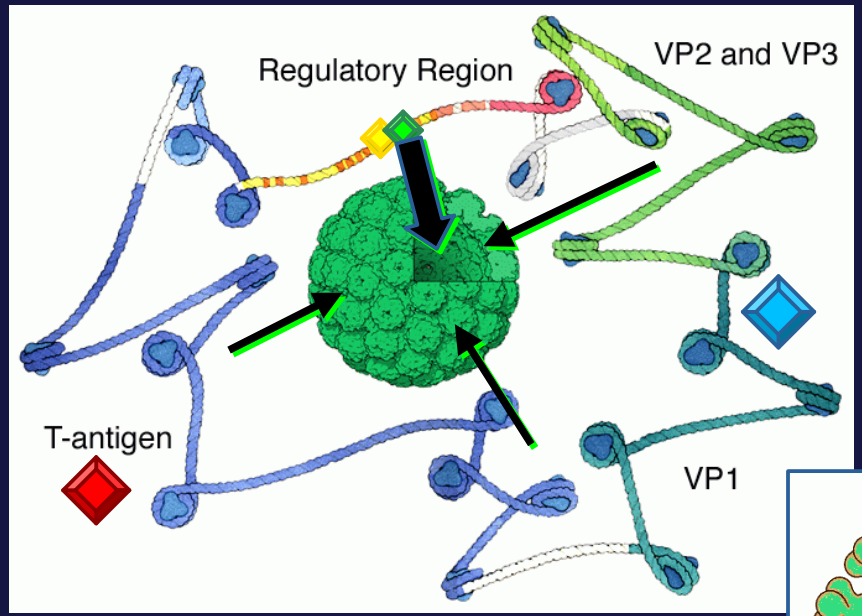


Numbers in circles are the steps from slides 13 – 16:

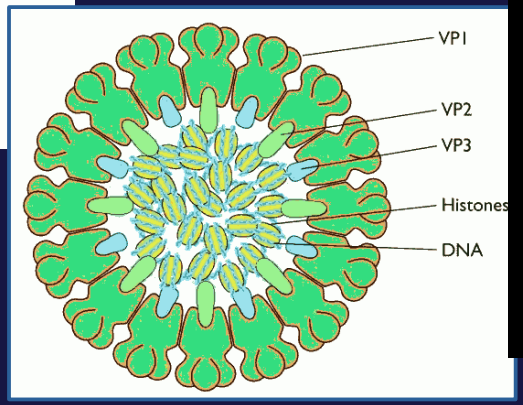
① – *replicase production*
② – *copying starting at ori*
③ – *shell production*
④ – *packaging*

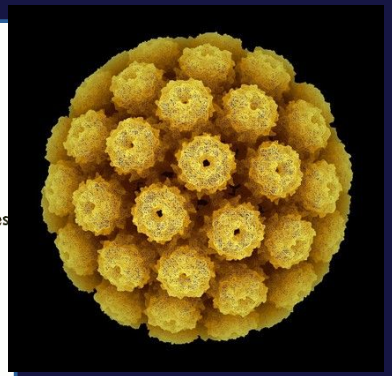
Now a few examples… *(or skip to page 28)*

Example 1 of 3: Papovavirus (DNA)



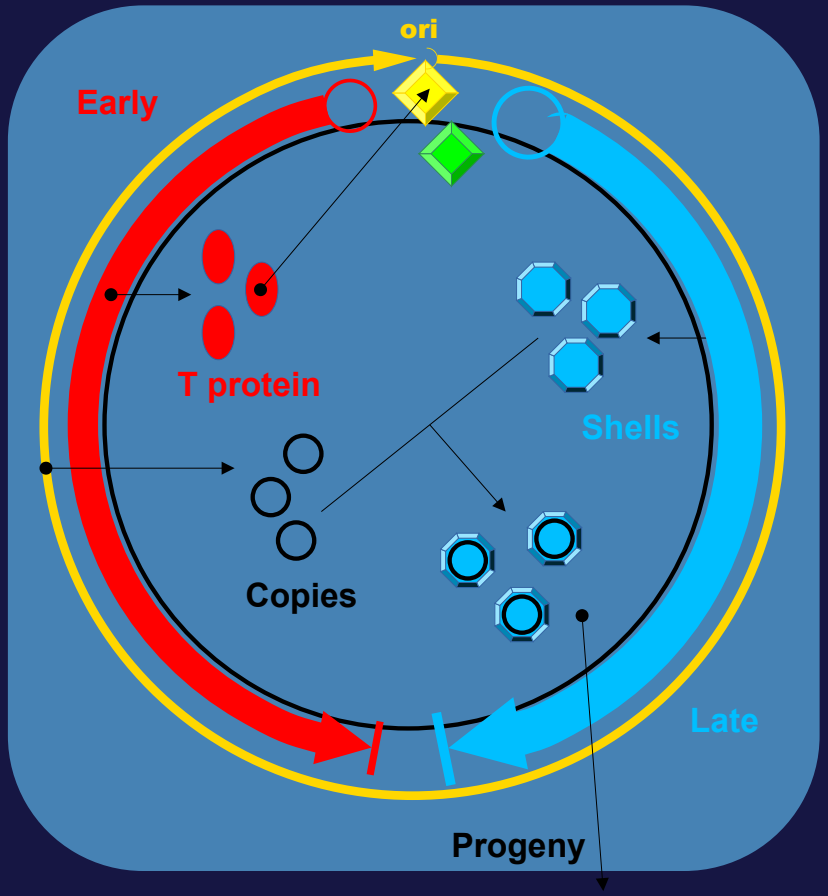
- **(I)** A gene for the replication mechanism.
- **(II)** Gene(s) for the shells.
- **(III)** A "signal" on its nucleic acid to show where replication starts, known as "ori".
- **(IV)** Another "signal" that marks viral nucleic acid for packaging into the shells.





Now remember this cutie...

Example 1 of 3: Papovavirus (DNA)



1. T protein is formed **("early")**.

2. T protein causes **replication**, starting at **"ori"**. To provide DNA material, the T protein switches the cell into growth mode… this may lead to warts or even tumours, hence "T".

3. Shell proteins are formed **("late")**. This is facilitated by presence of T protein.

4. Replicated DNA and shell proteins get together, using the **packaging signal**.

(A bit simplified… there are actually several T proteins and three shell proteins, but this is how it works.)

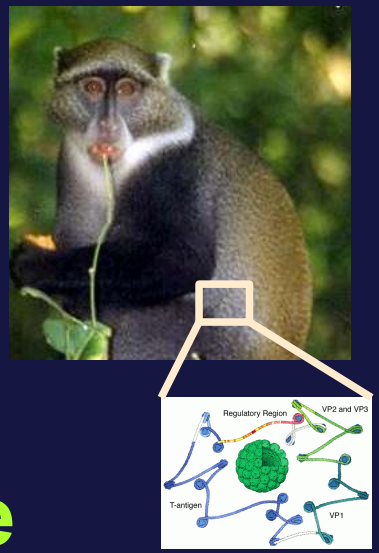
Total Eclipse Of The Mind

The **T proteins** are what causes tumours, but vaccination against papillomavirus is directed against the **shell proteins**.

*(Papillomaviruses are one group of papovaviruses – some scientists consider the name "papovavirus" obsolete: the **va**cuolating viruses are now ranked among the **po**lyoma viruses, leaving only these and the **pa**pilloma viruses)*

Yet Proven Effective

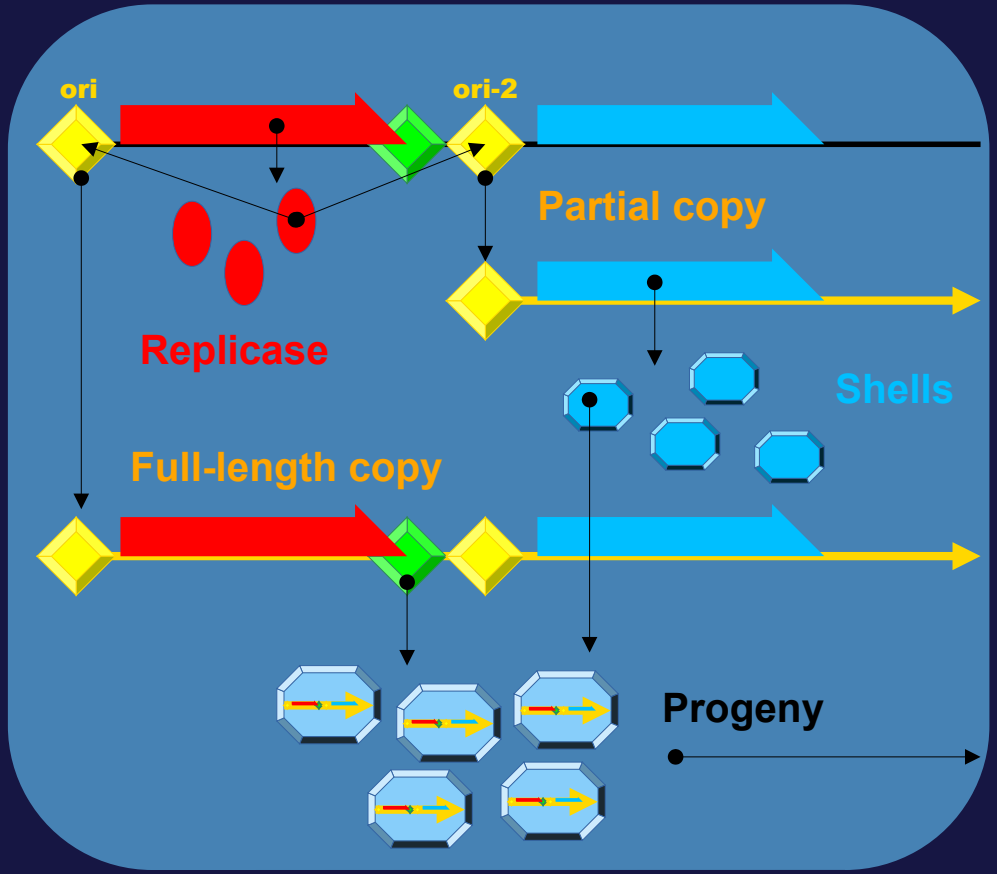
T proteins are constitutively expressed by **COS7** cells, where they are used as tools to transiently drive powerful **protein expression**.



Suppressing them is a **quantitative** problem.

Somebody *once did a lot of work on T proteins and ψSV40 shells…*

Example 2 of 3: Togavirus (RNA)



1. **Replicase** is formed.

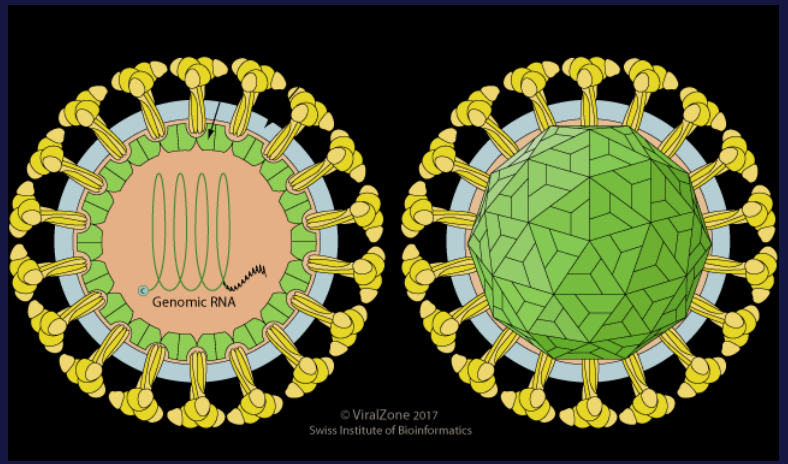
2. This causes **replication** of the viral RNA from each **ori**. (Full or partial, as only the first gene on an RNA is translated.)

3. **Shell proteins** are translated from the partial copies.

4. Replicated RNA and shell proteins get together (plus cellular components), using the **packaging signal**.

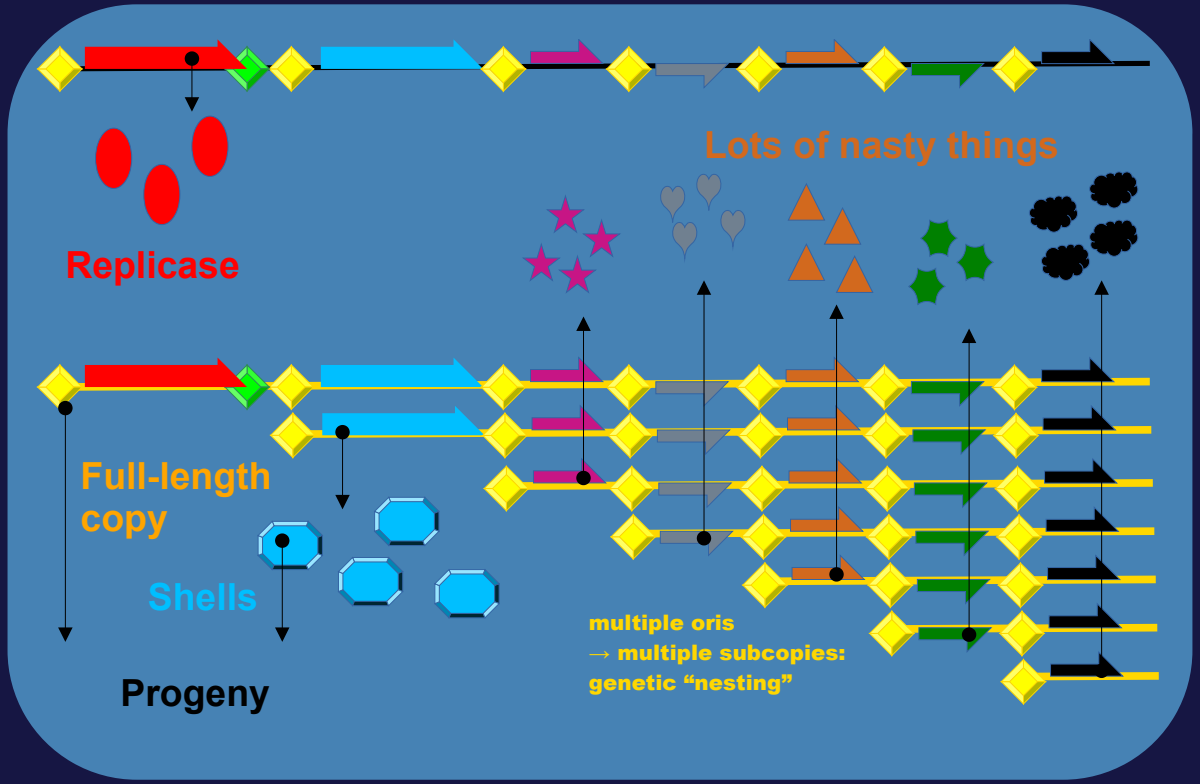
(A bit simplified, again… but this is how it works.)

Togavirus Structure (e.g. rubella virus)



Example 3 of 3: Coronavirus (RNA)

Coronaviruses are members of a larger group called nidoviruses (nested viruses).



For illustrative purposes only; does not reflect the actual genetic layout of any coronavirus.

Essentially just togavirus on steroids, with 7 levels...

1. **Replicase** is formed.

2. This causes **replication**. (Full or partial, as only the first gene on an RNA is translated.)

3. **Shell proteins** and **nasty things** are translated from the partial copies. *(More about nasty things on the next slides.)*

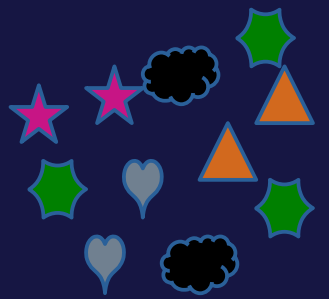
4. Replicated RNA and shell proteins get together (plus cellular components), using the **packaging signal**.

What Are Nasty Things?

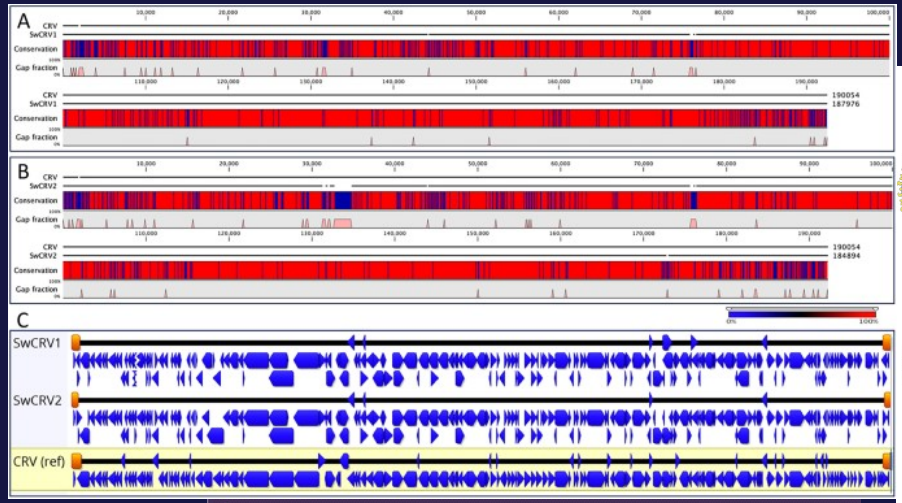
The immune system uses a complex network of sensors and signals to identify targets.

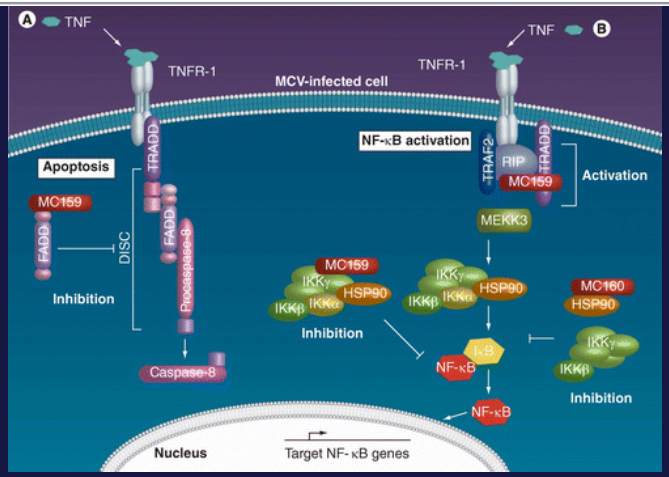
The larger a virus is, the more it relies on “hacking” this network to evade detection. Viruses use “nasty things” to:

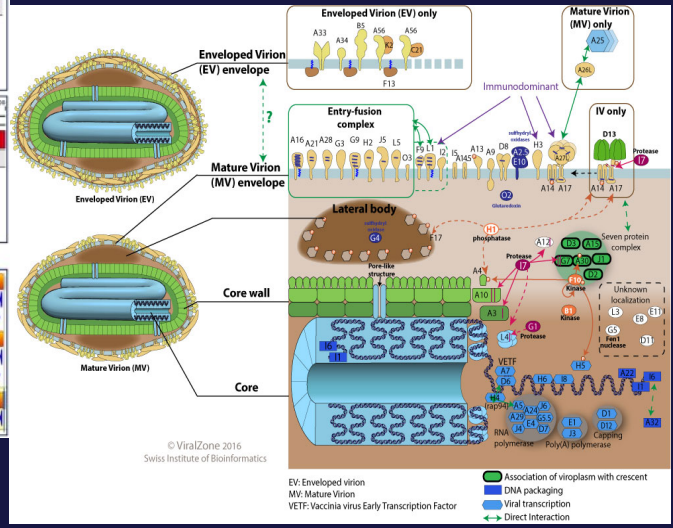
- generate false negative signals
  (i.e. broadcasting of “everything fine” messages)
- suppress true positive signals
  (i.e. blocking of alarm messages)
- interfere with signal processing
- induce an improper reaction
- cause autoimmunity



This Can Be Very Complex...

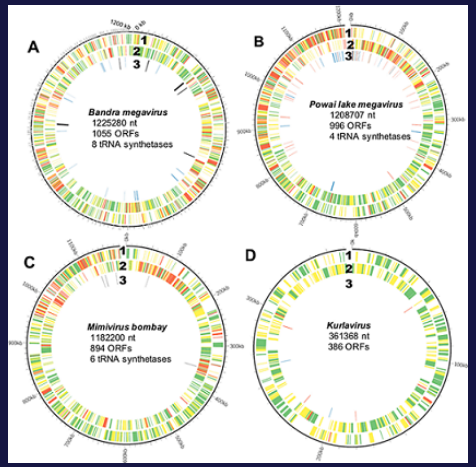


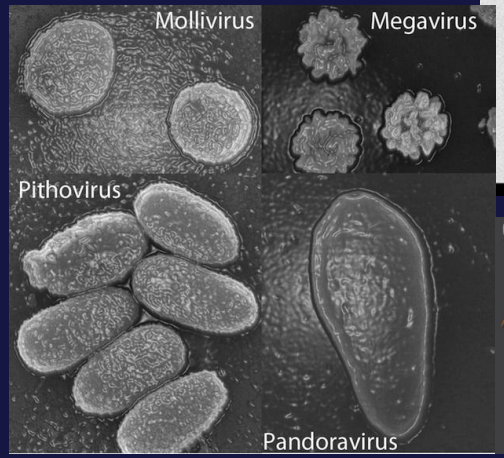


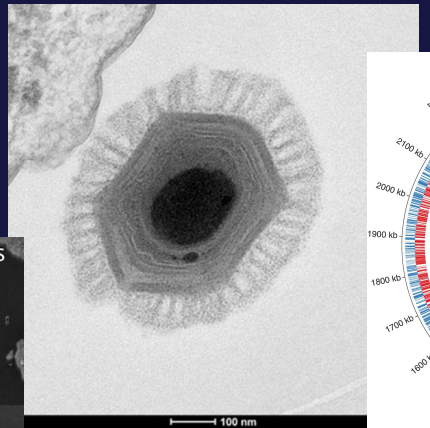


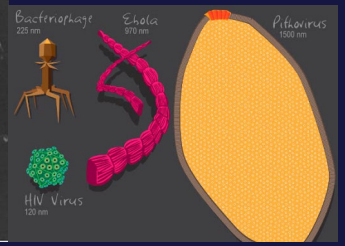
...as in the case of the sneaky MCV, a benign poxvirus with a vast array of defences.

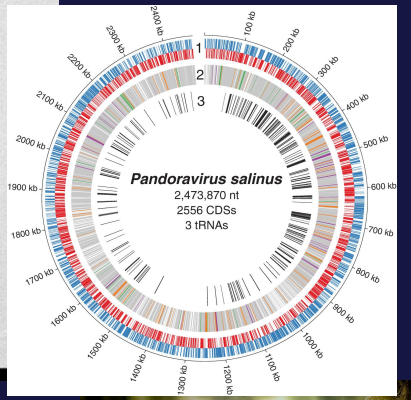
Just A Thought…

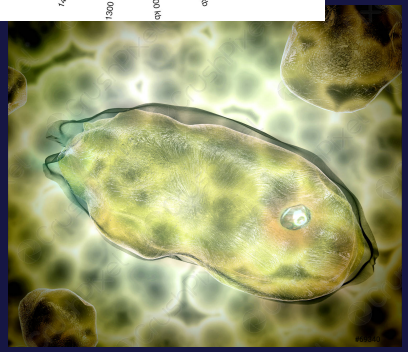












We are just beginning to appreciate the full complexity of really large viruses.
Pandoravirus has a genome about 12× as big as *Poxvirus*… and 500× as big as SV40.
Still, the two-stage design applies here, too.

Knowing Your Stuff (I)

RUPRECHT-KARLS-UNIVERSITÄT HEIDELBERG
FAKULTÄT FÜR BIOLOGIE

ZEUGNIS

über die Diplomprüfung im Studiengang Biologie

Rüdiger-Marcus Flaig

geboren am 28. April 1971 in Mannheim

hat am 07. Juli 1994

die Diplomprüfung
gemäß Prüfungsordnung vom 5.7.1985 - Nr. 812.110/5
bestanden.

Beurteilung der Einzelfächer:

Hauptfach	Genetik	sehr gut
Hauptfach	Botanik	sehr gut
Nebenfach	Zoologie	sehr gut
Nebenfach	Chemie	sehr gut - gut

Die Diplomarbeit mit dem Titel
Molekulargenetische und biochemische Untersuchungen zu
Ribonukleasen bei Bacillus und Escherichia

wurde mit der Note sehr gut - gut bewertet.

Gesamtnote: sehr gut (1,25)

Heidelberg, den 19. Oktober 1994

DER DEKAN
Prof. Dr. H.F. Moeller

DER VORSITZENDE
DES PRÜFUNGSAUSSCHUSSES
Prof. Dr. H.U. Schairer

Noten: sehr gut, gut, befriedigend, ausreichend (Zwischennoten sind zulässig)

QUOD BONUM FELIX FAUSTUMQUE SIT

NOS DECANUS CETERIQUE PROFESSORES
ORDINIS MEDICORUM IN LITERARUM

UNIVERSITATE RUPERTO-CAROLA

IN VIRUM ORNATISSIMUM

DIPL.-BIOL. RÜDIGER-MARCUS FLAIG

CUIUS PATRIA
MANNHEIM

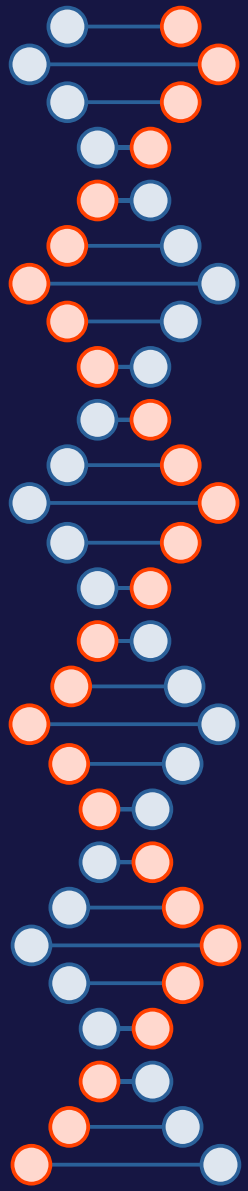
COMPROBATA DISSERTATIONE QUAE INSCRIBITUR

KONSTRUKTION PSEUDOVIRALER GENTRANSFERSYSTEME
ALS GRUNDLAGE FÜR DIE THERAPIE
ARTERIOSKLEROTISCHER ERKRANKUNGEN

ET EXAMINE RIGOROSO
IN MEDICINA INTERNA CUM LAUDE
SUPERATO
IURA ET PRIVILEGIA DOCTORIS SCIENTIARUM HUMANARUM
CONTULIMUS ET HOC DIPLOMATE SIGILLO
ORDINIS NOSTRI MUNITO TESTATI SUMUS

P.P. HEIDELBERGAE
D. XII. MENSIS DECEMBRIS
MCMXCVII

DECANUS
PROF.DR.DR.h.c. H.-G. SONNTAG



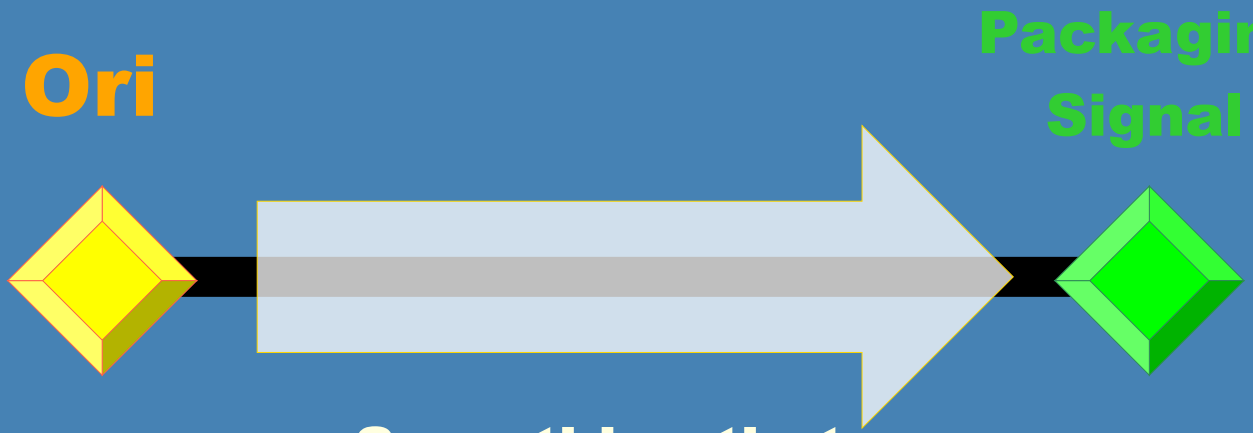
From the perspective of immunology…

Aimless Fire Makes The Situation Worse

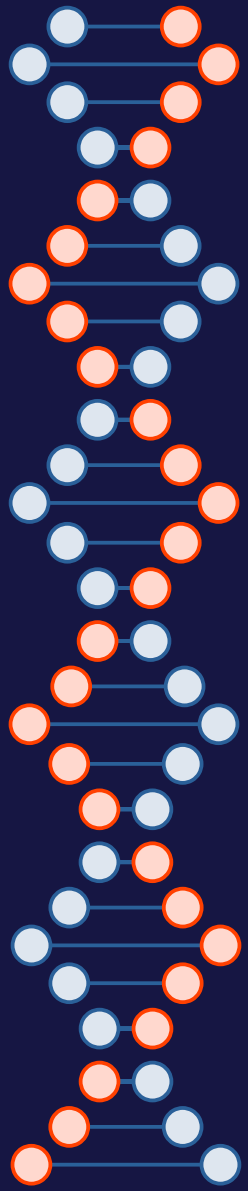
In Covid-19 and many other viral conditions, uncontrolled inflammation is the greatest problem.

So, Where's The Knife?

It's That Simple!



Something that the virus does not like at all

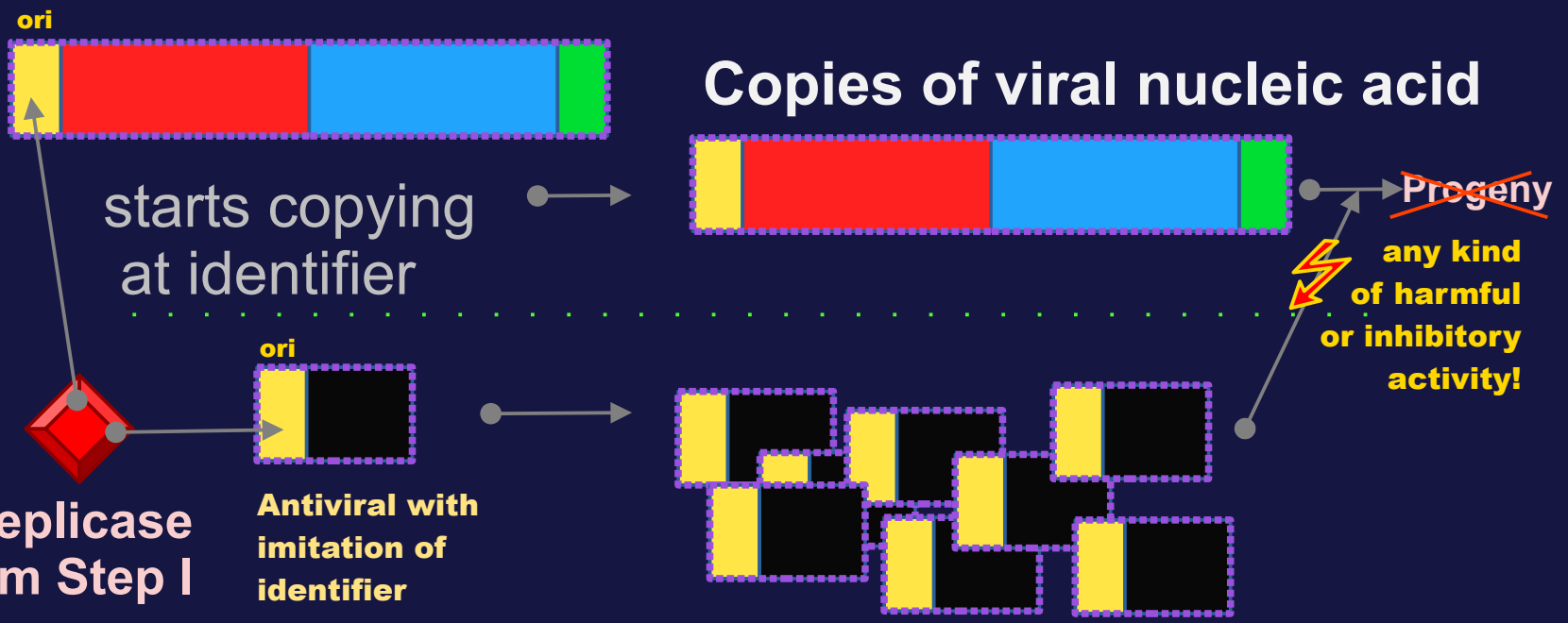


We attach the copying identifier *(ori)* to something the virus does not like.

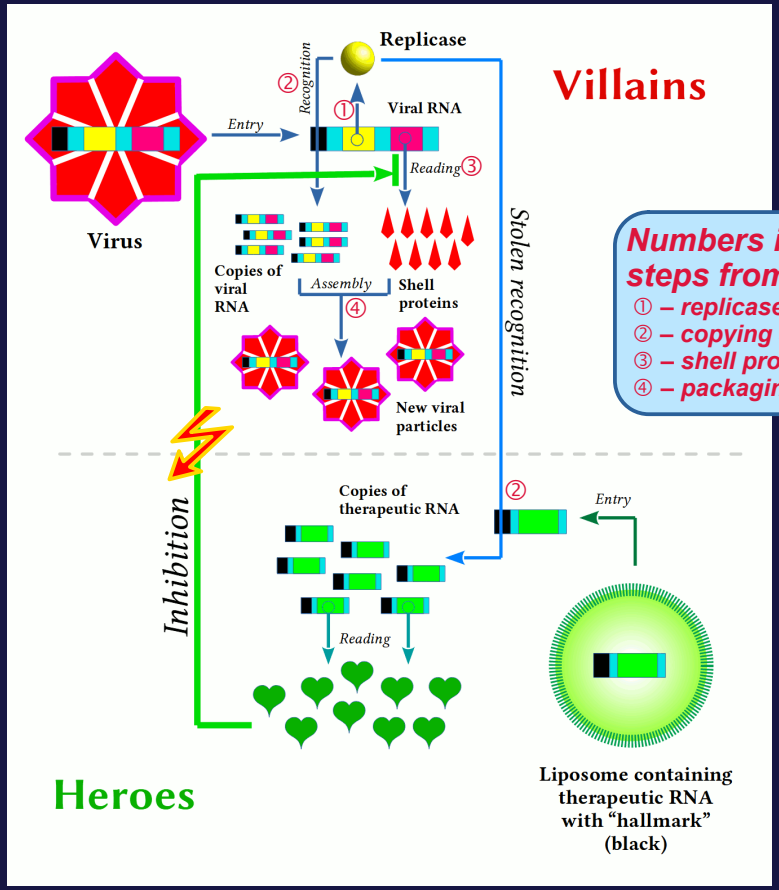
When the virus enters, it cannot help multiplying the "something" too.

The virus is fooled into copying something harmful to it.

Step IIb: The virus is fooled into copying the antiviral starting at the identification sequence



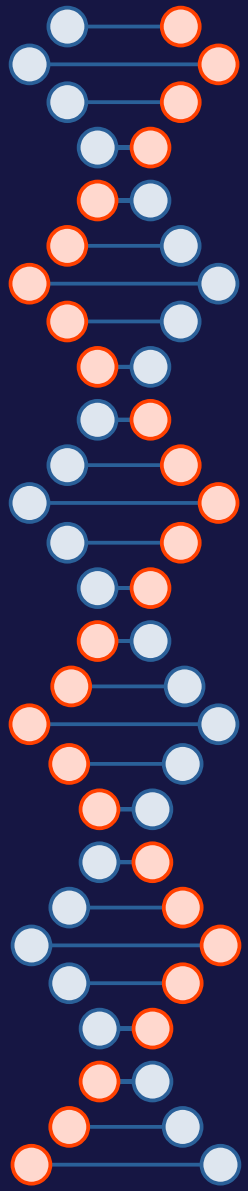
This is...



Numbers in circles are the steps from slides 13 – 16:

① – replicase production
② – copying starting at "ori"
③ – shell production
④ – packaging

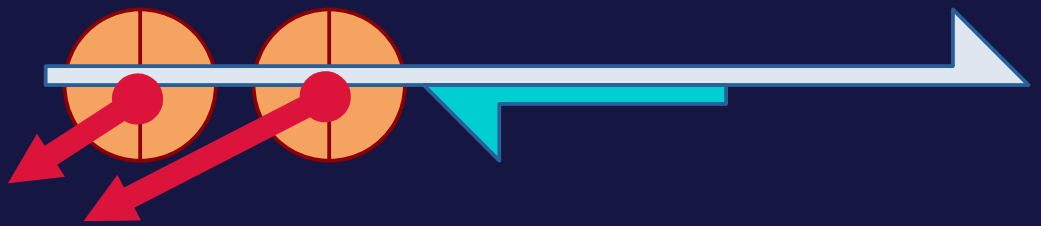
...what this is about.



What disturbs a virus?

Viruses Do Not Like Antisense

(Actually, Nobody Really Does)

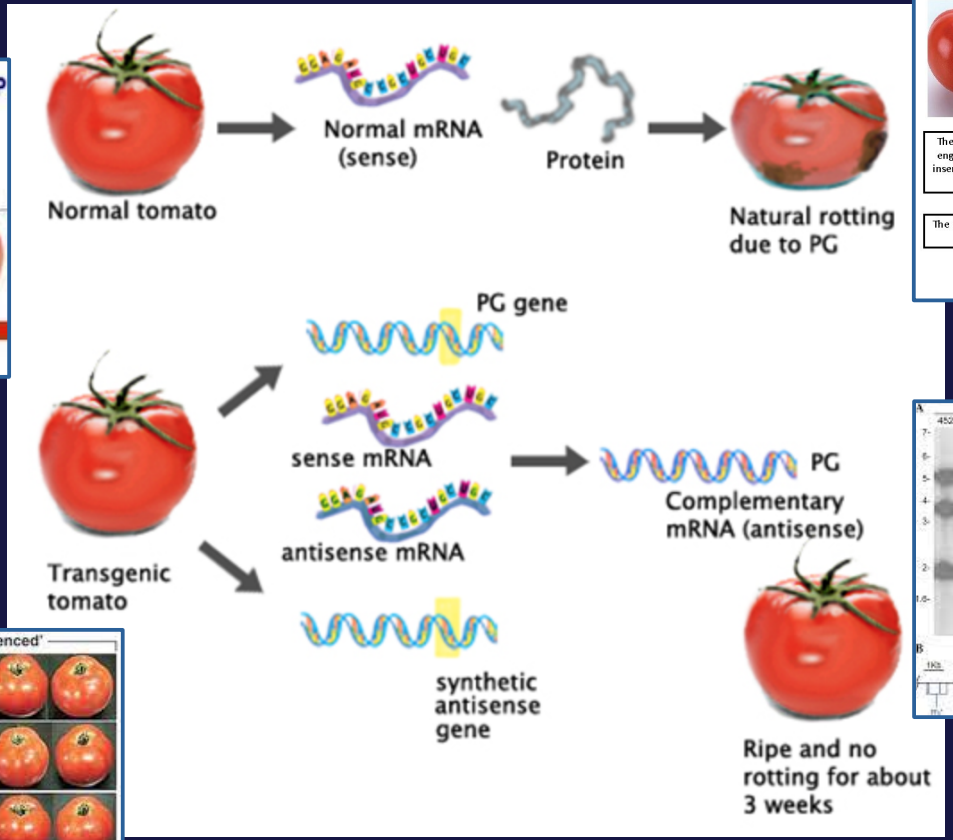


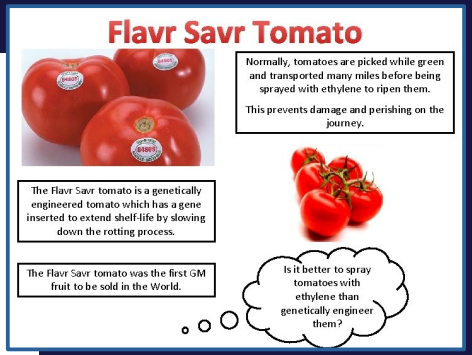
A complementary (A-T, G-C) RNA sequence can form an RNA double helix.

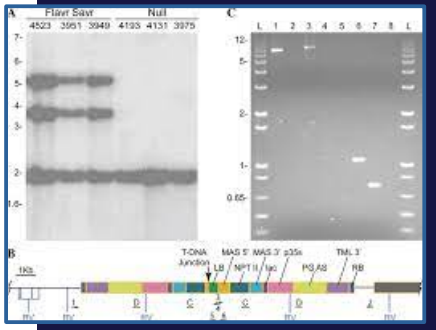
This hampers **translation** by knocking off the ribosomes and marks the RNA for destruction by a variety of mechanisms. In the latter case, innate antiviral mechanisms are triggered.

Antisense: Tried & Tested

(and causing hysteria since 1994)







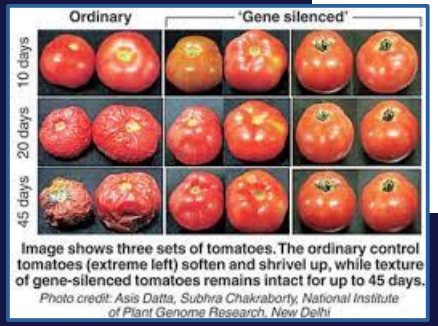
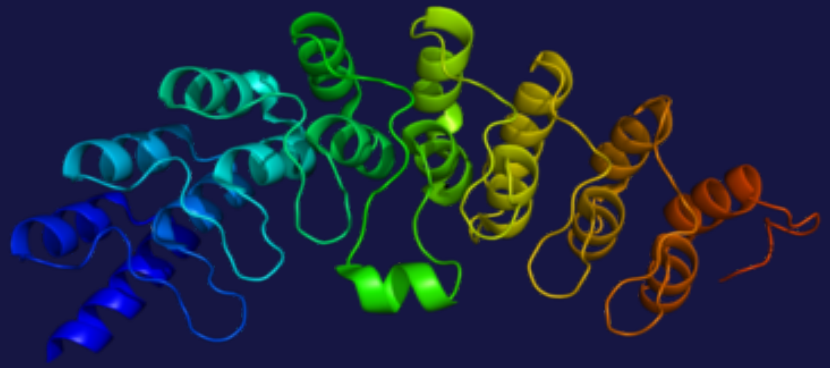


Image shows three sets of tomatoes. The ordinary control tomatoes (extreme left) soften and shrivel up, while texture of gene-silenced tomatoes remains intact for up to 45 days.

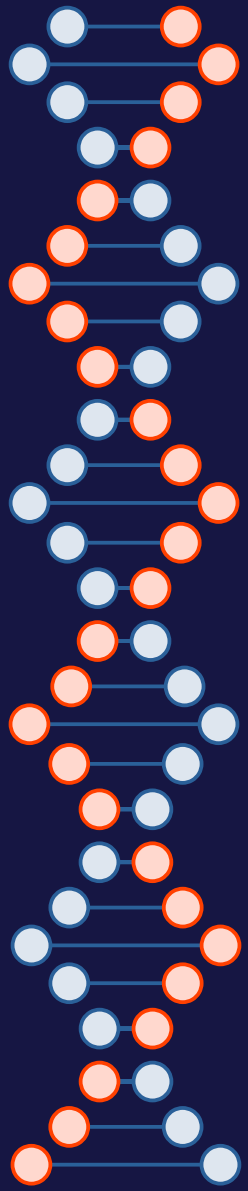
Photo credit: Asis Datta, Subhra Chakraborty, National Institute of Plant Genome Research, New Delhi

Community Action



Normally, cells react to RNA viruses by producing interferon, which induces **RNAse L**, which degrades all the RNA in the cell. The infected cell thus destroys itself ***and*** the virus, saving the others. Coronaviruses, however, are capable of disabling the interferon reaction.

RNAse L could thus also be used as a “blade”.



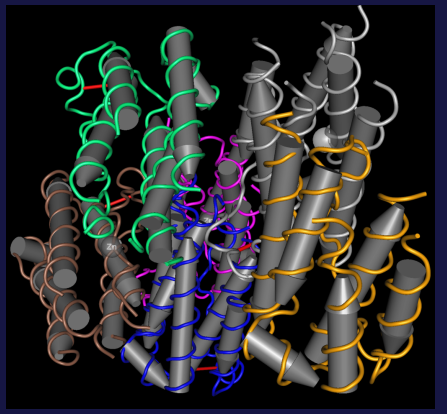
Viruses Downregulate Components Of The Immune System

When a virus destroys or “downregulates” components of the immune system, just supply lots of them to override the viral activity.

Or attack the viral elements responsible for the downregulation in turn.

Or modify viral components in such a way that they break the stealth mode, e.g. by producing “tagged” viral components.

Interferon



♦ In vertebrate cells, **interferons** are normally **induced by the presence of double-stranded RNA**, which is the hallmark of infection with an RNA virus.

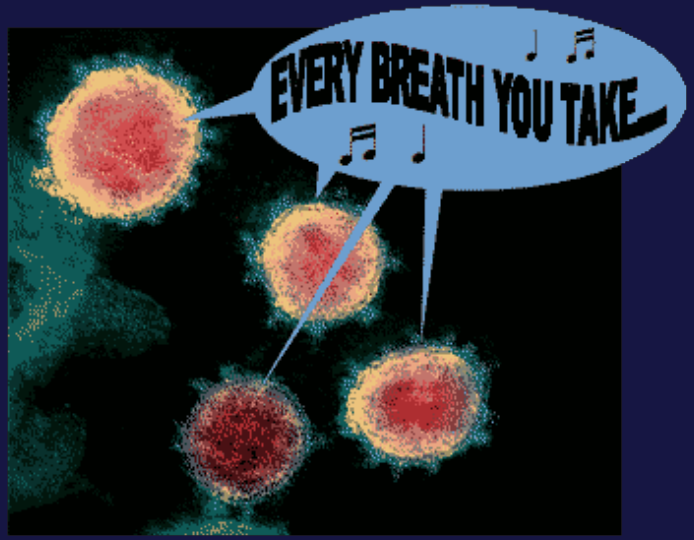
♦ They trigger the "**antiviral state**" with shutdown of protein synthesis and other antiviral measures.

♦ Hence, specific variants such as **siRNA** must be used in lieu of the vanilla antisense RNA in the "gene tomato".

♦ On the other hand, **RNA viruses generally disable interferon induction**; hence it might pay to use interferon as the "blade" gene, unless the point of interference is downstream.

→ **This is a complex but interesting issue.**

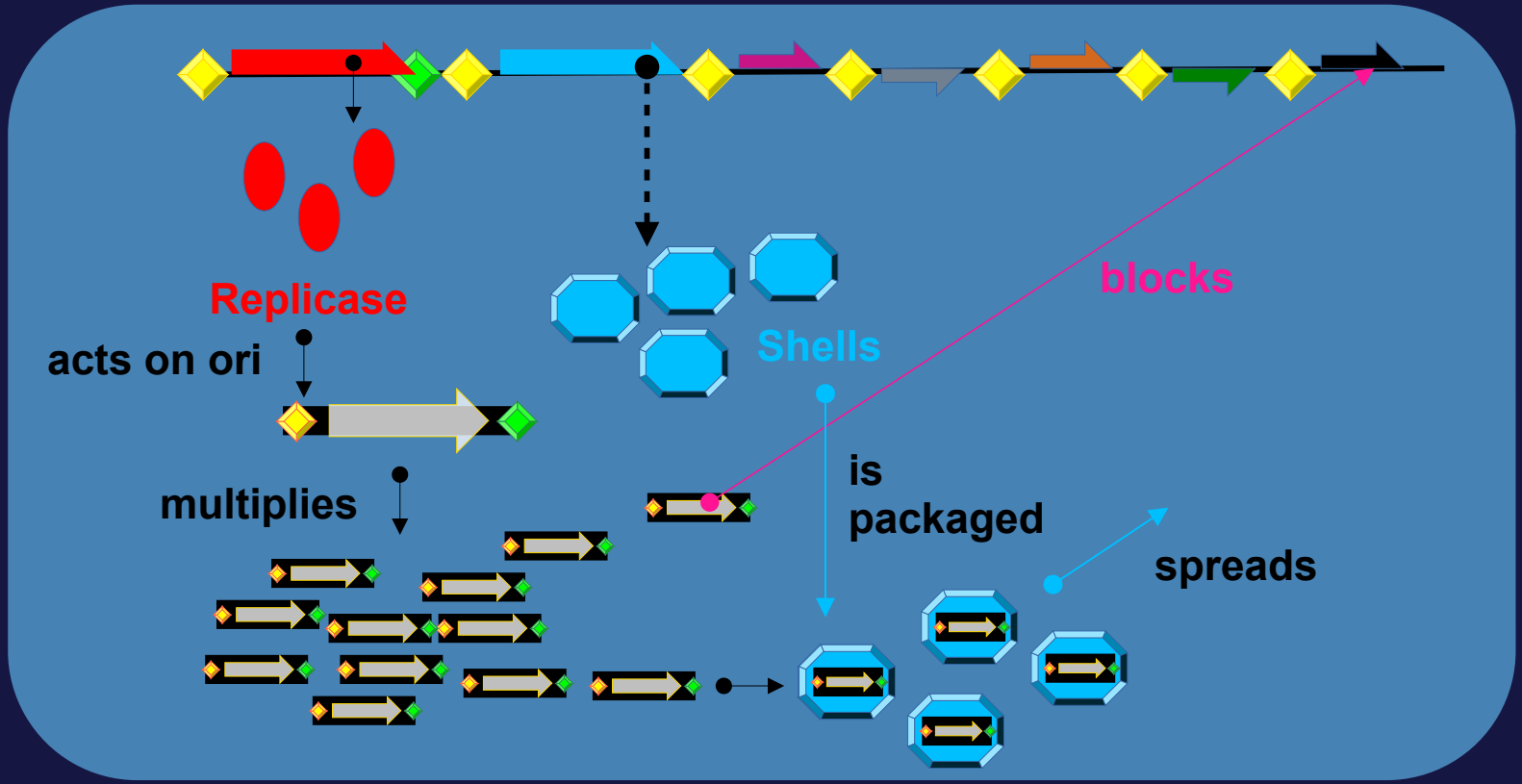
So, What Happens?



In the absence of the “knife” RNA, virally infected cells are cloaked from the immune system.

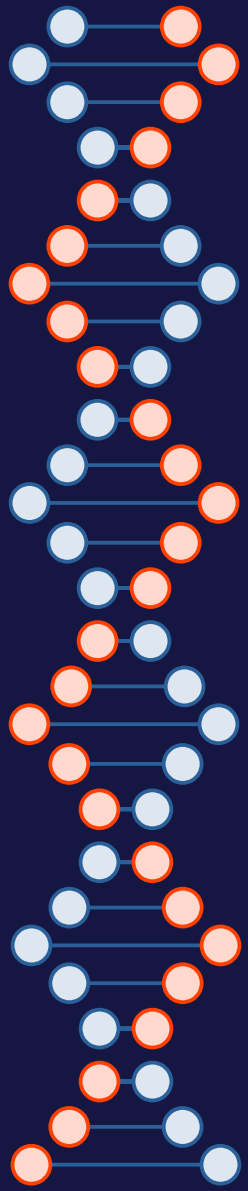
In a healthy cell, the “knife” RNA does not do anything.

But when the “knife” RNA meets the viral replicase...



...the "knife" RNA is multiplied by the virus.

It competes with viral RNA for replicase and packaging, blocks "nasty things" and is packaged into viral shells to spread the protection to other cells.



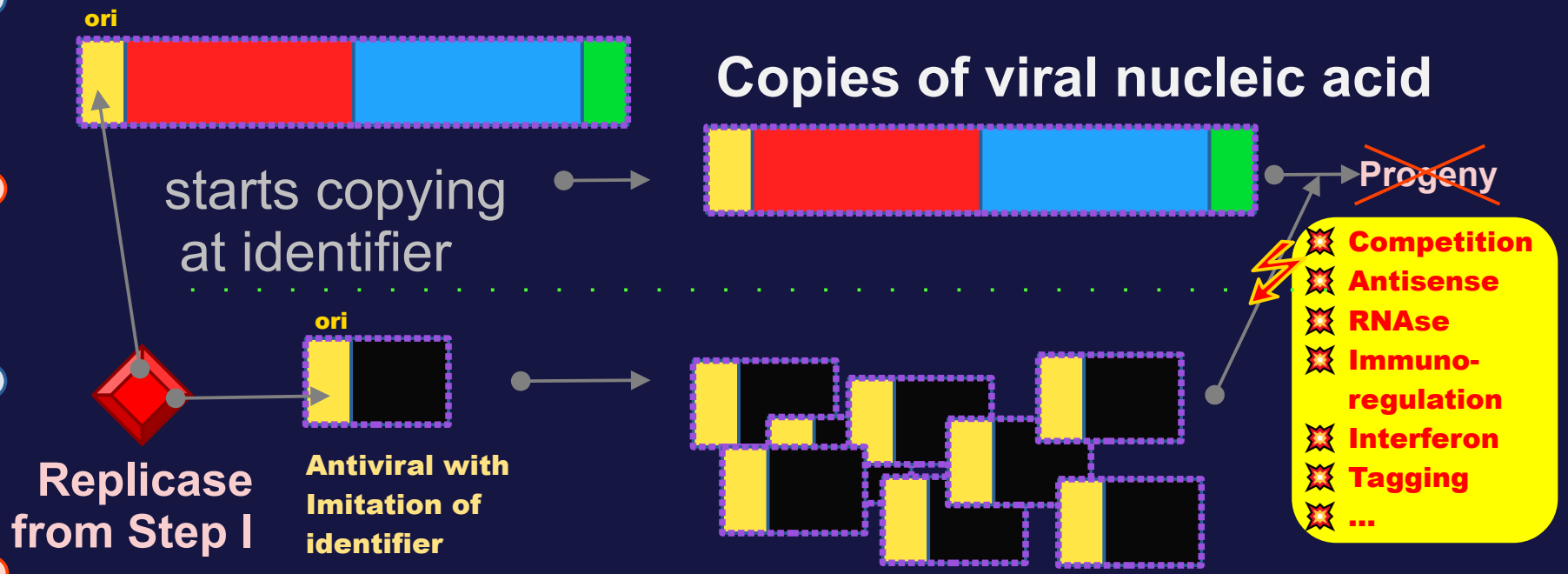
So the essence is:

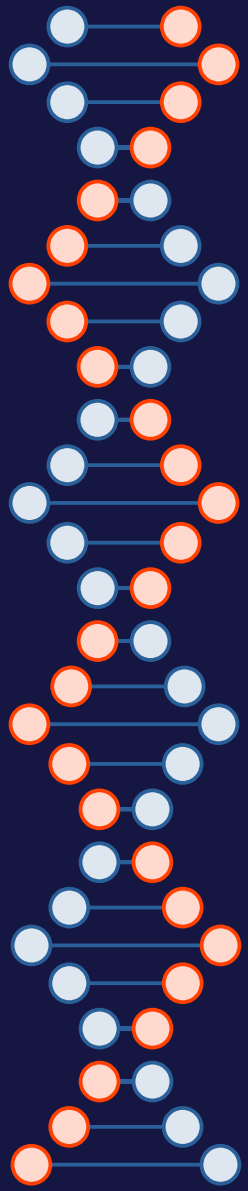
We hijack the viral replication system by imitating the recognition sequences ("ori") to produce antivirals.

And ideally we hijack also the viral packaging system to spread the antivirals through the body.

借刀杀人。

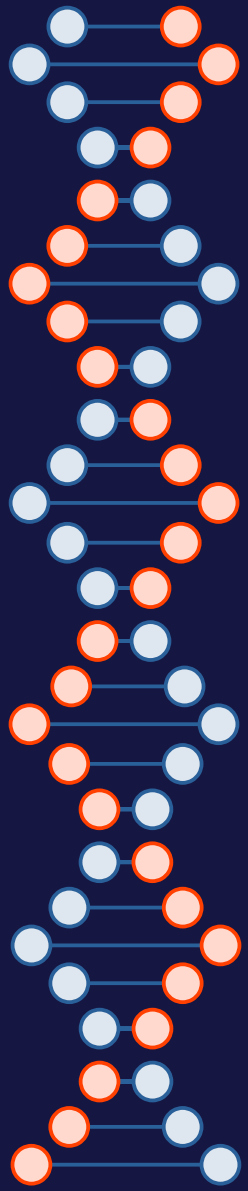
Step IIb: The virus is fooled into copying the antiviral starting at the identification sequence





Trickster tricked

The virus steals resources from the host cell – we steal replicase from the virus to hit back.

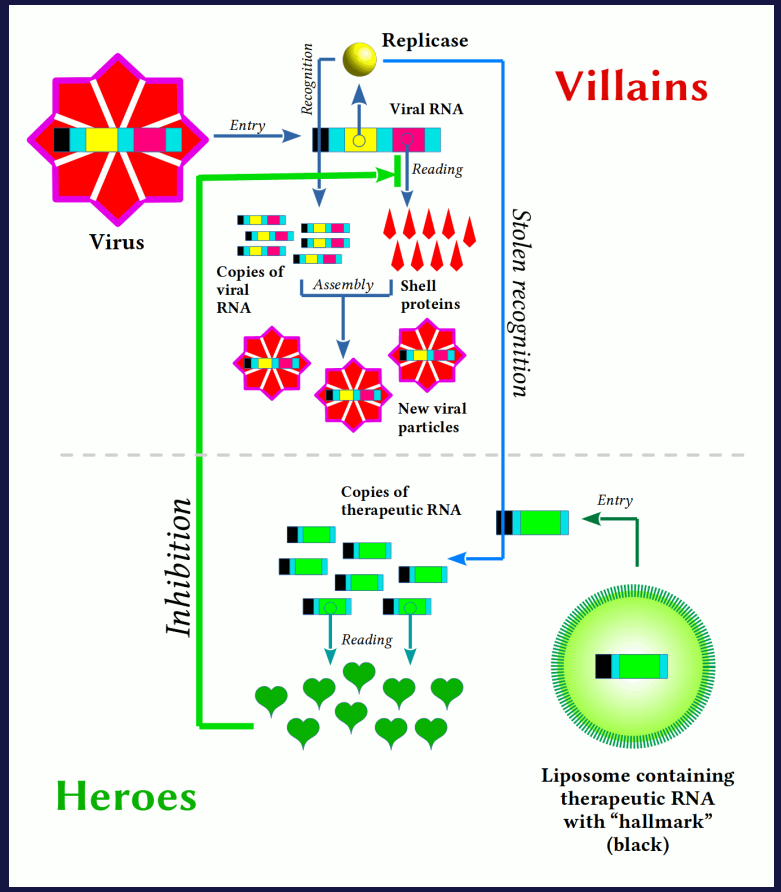


Trickster tricked

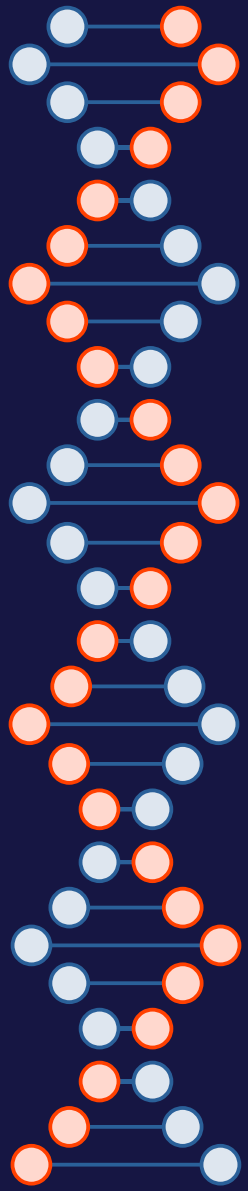
“There’s a fox among the chickens
And a killer in the hounds”

- IRON MAIDEN: “Back In The Village” (1984)

Remember this is...

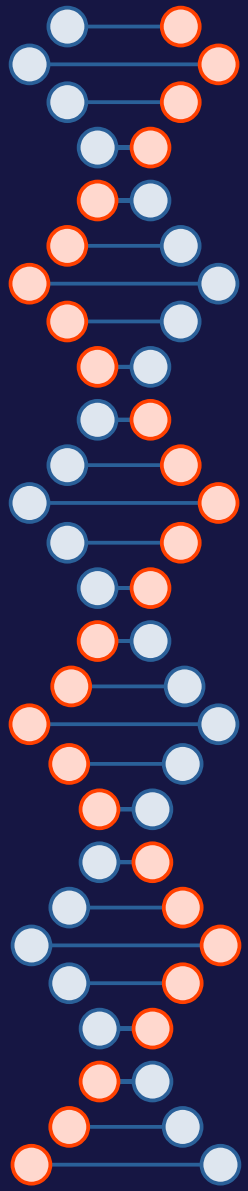


...what this is about.



Trickster tricked (II)

The virus steals resources to build new virus shells – by incorporating the packaging sequence, we also steal shells from the virus to expand the effect.



Will this work?

As early as 1992, Zhong, Dasgupta & Rueckert *made the following observation:*

into a 43-kDa (β) and a 4-kDa (γ) fragment after assembly into provirions (5, 14–16). RNA1 and RNA2 are required for viral infectivity (9). The mechanism by which RNA1 and RNA2, but not subgenomic RNA3, are selected for packaging into virions has been unclear.

FHV infects *Drosophila melanogaster* cells and normally causes lysis (17). However, about 1% of cells survive each infection cycle and become resistant to further infection (18). Such persistently infected cells synthesize, in addition to the genomic RNAs 1 and 2, defective RNAs, some of which are packaged into virions. Here we describe the use of one such molecularly cloned mutant, DI-634 with large deletions in RNA2, to identify a specific region of RNA required for packaging.

MATERIALS AND METHODS

Synthesis of Full-Length DNA Copy of DI-634, a Defective-

In other words: Sometimes this happens naturally.

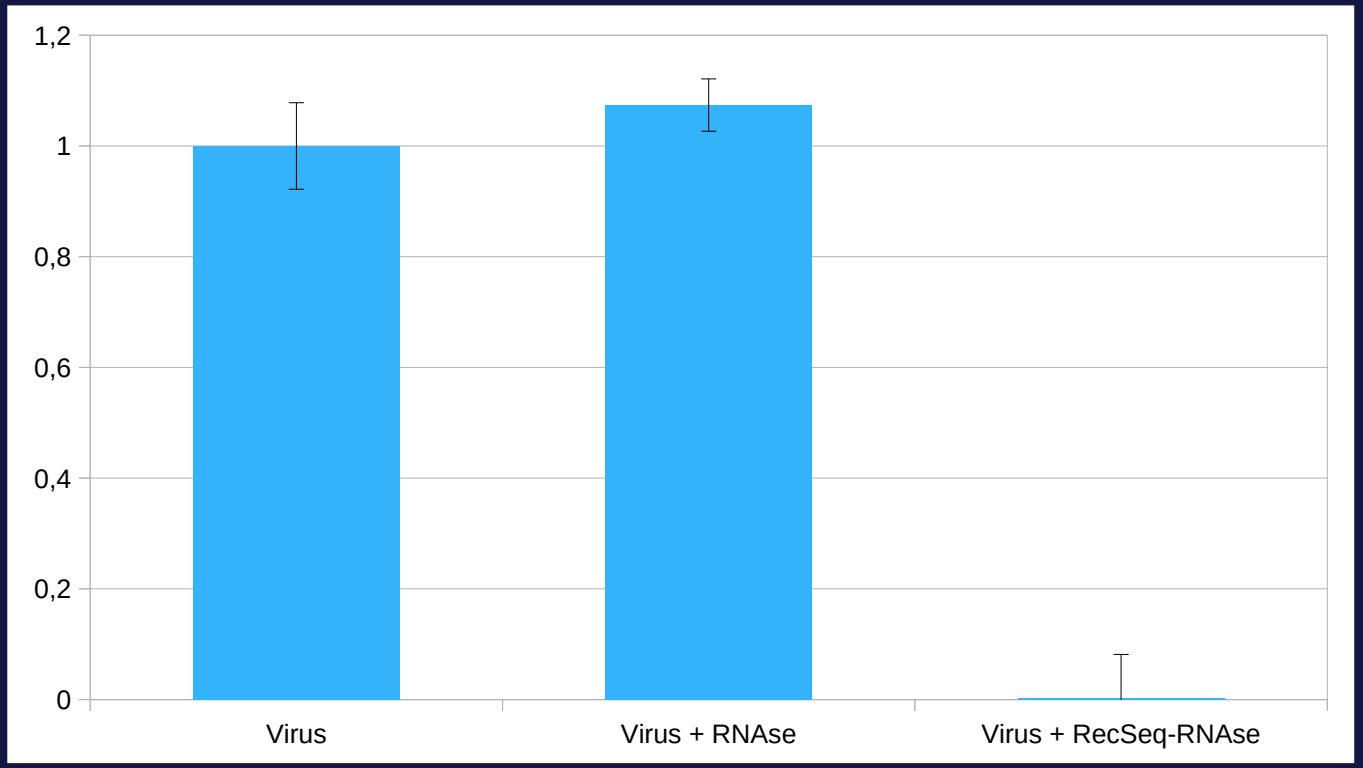
We also did a Monte Carlo simulation:

	Virus		Virus + RNAse		Virus + RecSeq-RNAse	
	Cyc+100	Progeny	Cyc+100	Progeny	Cyc+100	Progeny
	191	💀	240	💀	🏆	840
	203	💀	192	💀	🏆	852
	158	💀	183	💀	🏆	807
	193	💀	186	💀	🏆	758
	237	💀	187	💀	🏆	777
	200	💀	193	💀	🏆	752
	180	💀	177	💀	🏆	826
	198	💀	191	💀	🏆	740
	185	💀	180	💀	🏆	807
	179	💀	226	💀	🏆	748
	165	💀	213	💀	🏆	852
	292	💀	240	💀	🏆	744
	316	💀	206	💀	🏆	688
	182	💀	170	💀	🏆	937
	209	💀	180	💀	🏆	721
	169	💀	179	💀	🏆	794
	103,6	**10.000**	**96,4**	**10.000**	**2900**	**790**
Virions/cycle		96,6		103,7		0,272
Stdev		7,8 %		4,7 %		7,9 %
Propagation		100 %		107 %		0,28 %

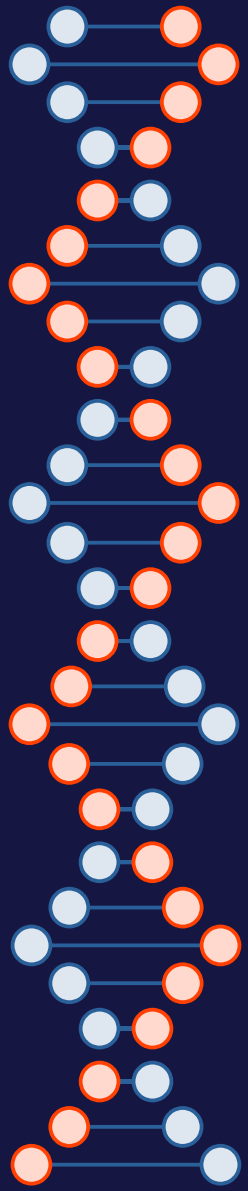
💀 Cell declared destroyed after production of 10,000 progeny virions
🏆 Equilibrium considered achieved after 2900 cycles post-infection

While presence of an unspecific RNAse alone actually increased virus production, the unspecific RNAse linked to a recognition sequence effectively suppressed the virus.

Some simulation results

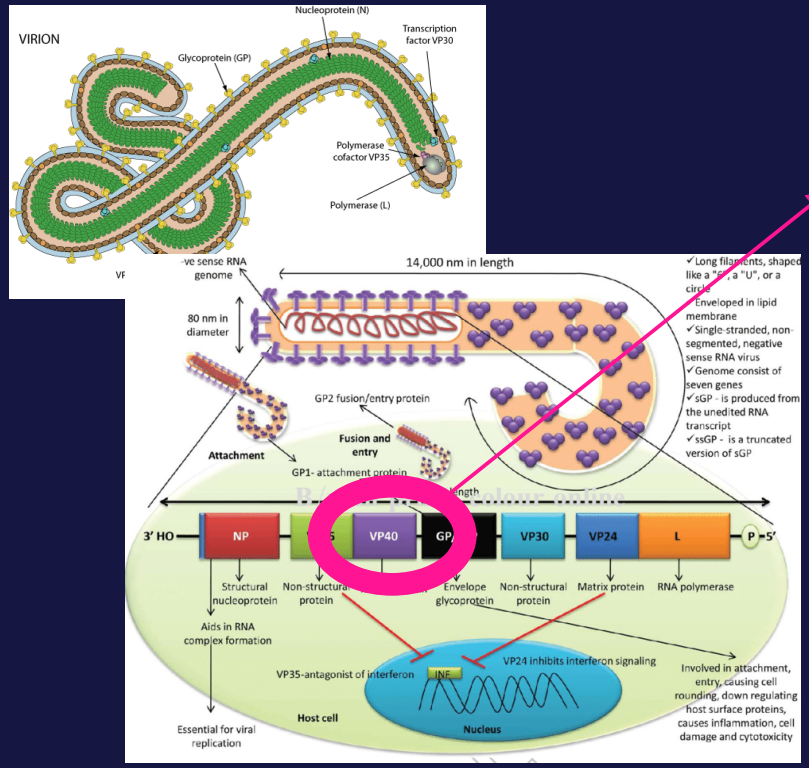


While presence of an unspecific RNAse alone actually increased virus production, the unspecific RNAse linked to a recognition sequence effectively suppressed the virus.



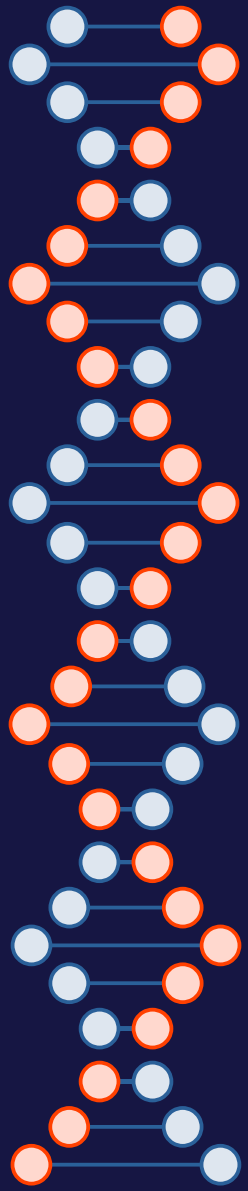
If it does, what next?

Tagging – Let's Play Dirty



Gene for surface protein...

...can be modified with a marker.



This requires some in-depth understanding of the immunological effects of the marker…

...in particular its interactions with the patient's MHCs.

Nevertheless, it opens the perspective of AI-based *personalised therapy*.

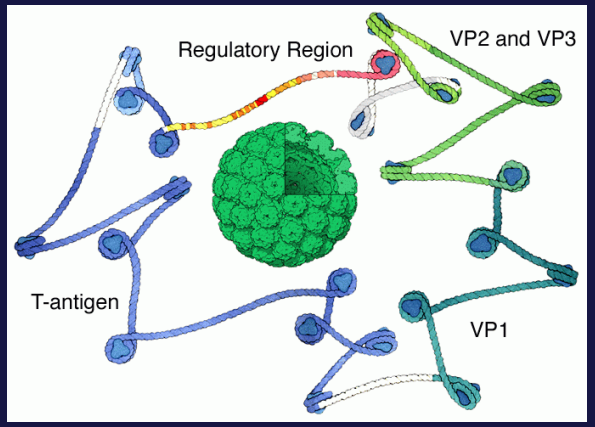
FORCE THEM TO UNCLOAK!

REMEMBER THIS:

Every virus has two basic functions

(I)
Replicate its nucleic acid (genetic material)

(II)
Build shells for the copies of its nucleic acid



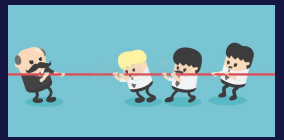
Component view of a papovavirus, with the shell open and the DNA outside.
(The bobbin-like structures are histone proteins stolen from the host cell.)

Everything else is just an add-on to this general two-stage design.

To Sum It Up

The anti-viral effect can be fivefold:

- **Competition**
  for replicase and shell proteins

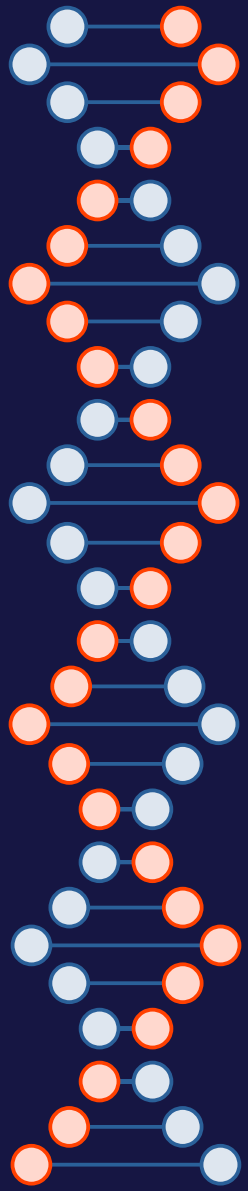


- **Blocking**
  of viral mechanisms by antisense RNAs

- **Overriding**
  of virally induced effects

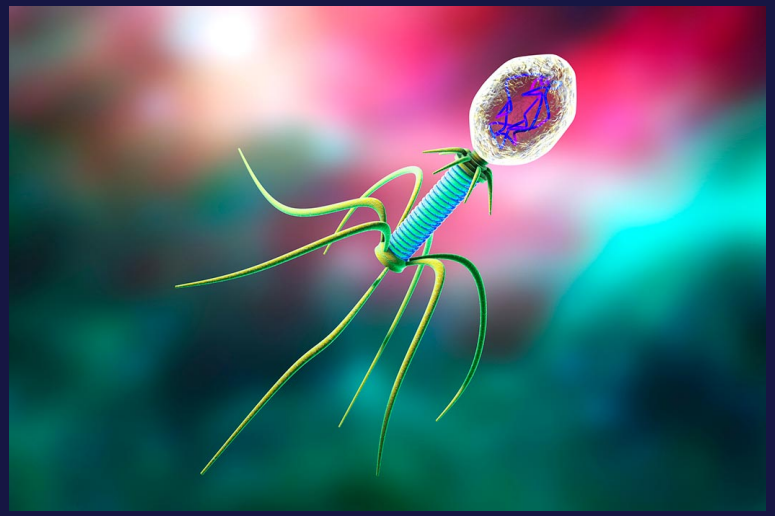
- **Hijacking**
  of nascent viral shells to spread the protection

- **Tagging**
  of viral structure elements to break the stealth



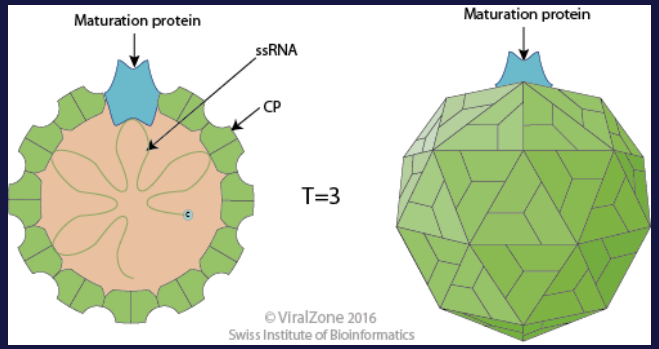
A direct application of the pattern abstraction approach, introduced originally into linguistics, by MA/Mag.Art. Lange Irén.

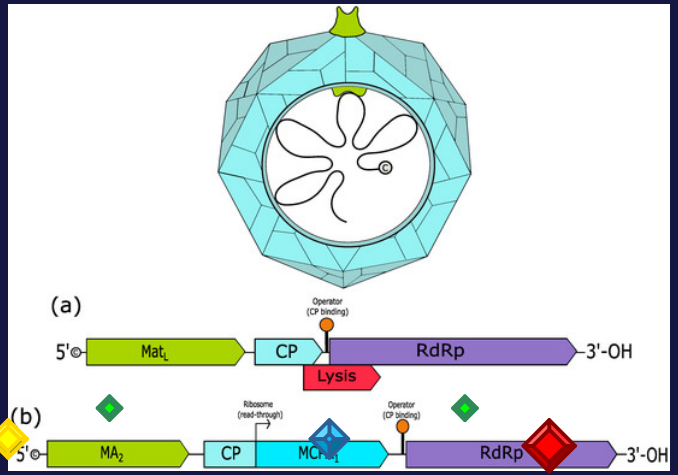
Proof Of Principle



...can be furnished
easily enough
with bacteriophages

...e.g. with Qβ





About $^1/_{10}$ the size of a coronavirus and featuring a straightforward genetic layout, the grenade-shaped Qβ is a lean and mean RNA-based bacteriophage that is exceedingly well characterised, including "Spiegelman's monster" – a genomic fragment capable of outcompeting the viral RNA for the attention of the replicase (RdRp).

An extremely handy model organism!

This Is Not a Vaccine...

This is a treatment for an acute or chronic disease.

However, it can be expected to lead to excellent immunity.

BIOTERRORISM

can design its agents to circumvent any existing drugs or vaccines…

…but this novel approach confers a distinct second-mover advantage:

Once released, any viral pathogen can be defeated by a tailored treatment!

"Chess is a game of complete information, and Black's information is always greater — by one move!"

- GM Mihai Suba

Historically, fortification has never thwarted a determined assault.

Emotionally, we are no longer capable of dealing with another pandemic, especially not by vaccination.

1% SAEs of vaccine → Disaster.
99% of moribund ICU patients saved → Triumph.

Can Viruses Develop Resistance to Our Nucleic Acids?

They can.

They will.

But we can adapt our countermeasures faster to their changes than they can to ours.

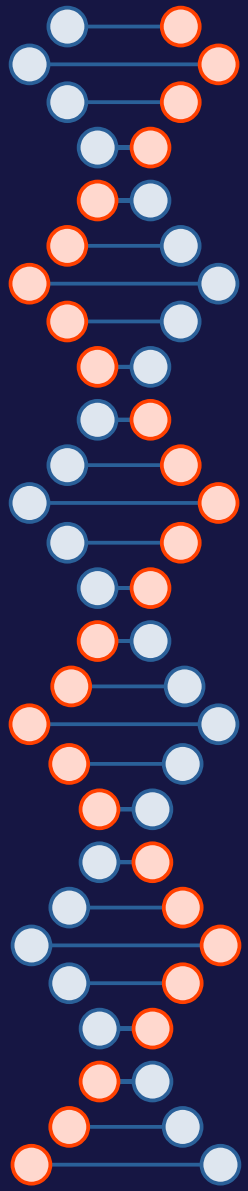
Mobility – today's paradigm

No “Escape Mutations”

This happens naturally:

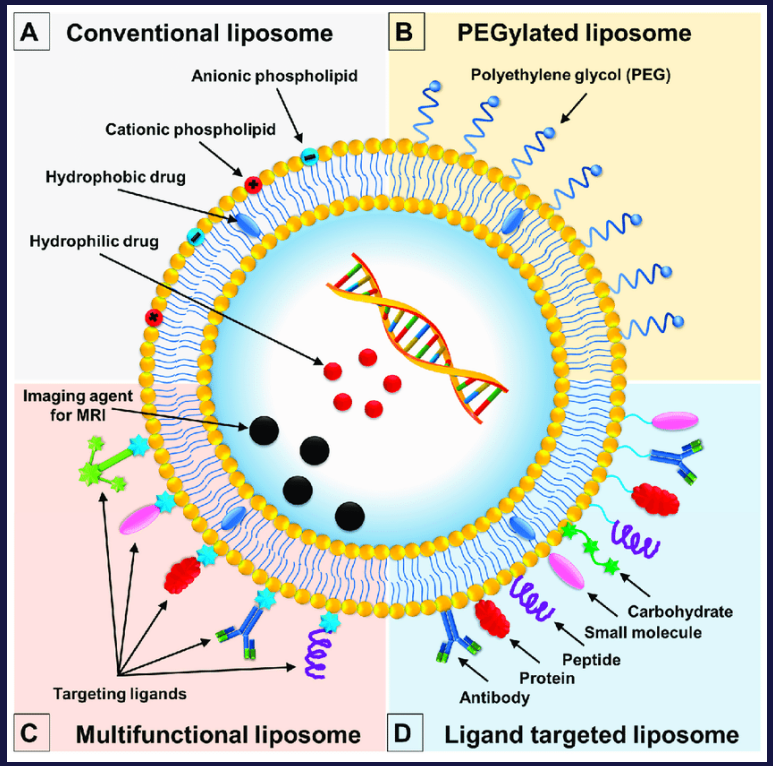
Viruses mutate to circumvent a vaccine. The more so, the vaster the pandemic and the more specific the vaccine are.

→ No problem for an acute-phase treatment for the individual virus – we can strike at the latest variant!



How Do We Get It Into The Cells?

How Do We Get It Into The Cells?



With liposomes. Or other molecular delivery systems. For Covid, this can be inhaled.

Knowing Your Stuff (II)

RUPRECHT-KARLS-UNIVERSITÄT HEIDELBERG

DIE NATURWISSENSCHAFTLICH-MATHEMATISCHE
GESAMTFAKULTÄT

verleiht

Herrn Diplom-Biologen Dr. sc. hum.
RÜDIGER-MARCUS FLAIG
geboren am 28. April 1971 in Mannheim

den Grad eines DOKTORS DER NATURWISSENSCHAFTEN
(DR. RER. NAT.)
nachdem er durch die Dissertation:

"Bdellosomen:
Ein neuartiges Arzneimitteltransportsystem auf der Basis
monomolekularer Polymerpartikel"

sowie durch die mündliche Prüfung seine wissenschaftliche Befähigung erwiesen
und dabei das Gesamturteil erhalten hat:

SUMMA CUM LAUDE

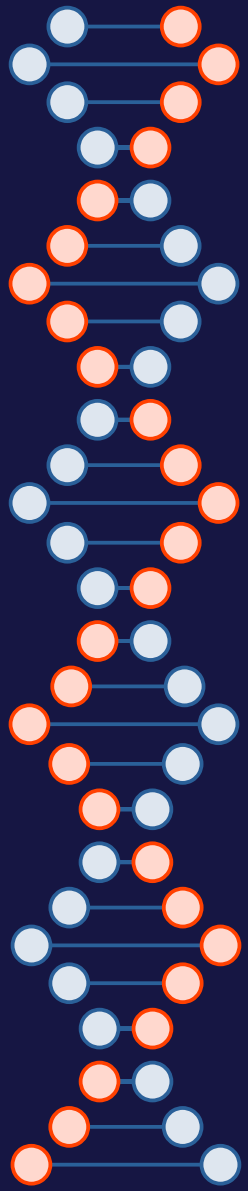
Heidelberg, 27. Juni 2001

DER REKTOR

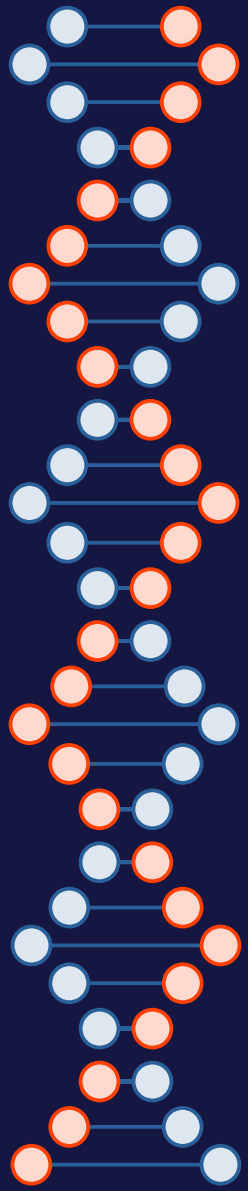
(Professor Dr. Jürgen Siebke)

DER DEKAN

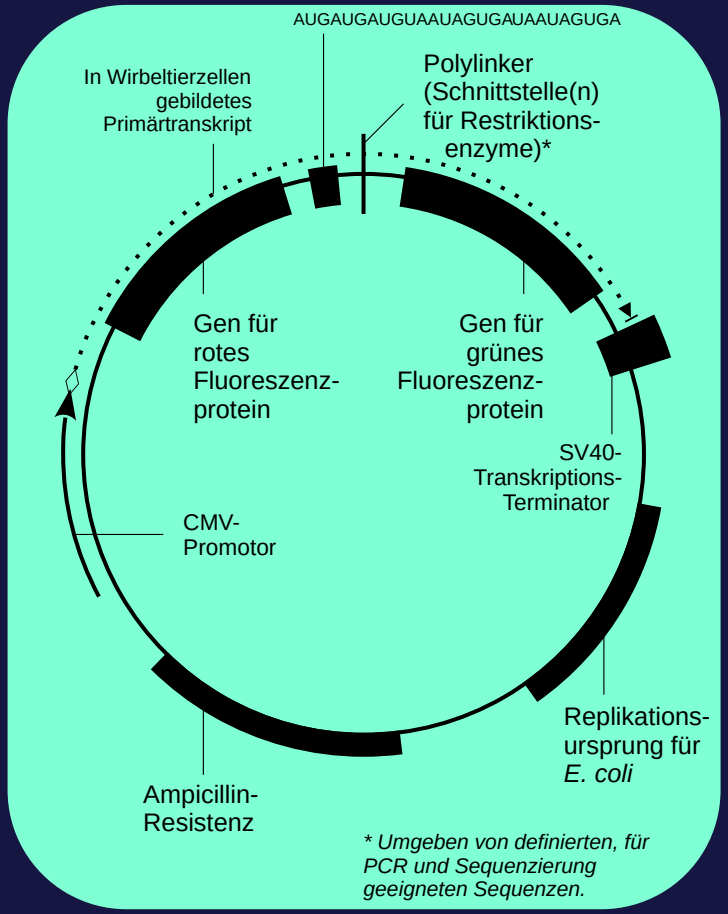
(Professor Dr. R. N. Lichtenthaler)

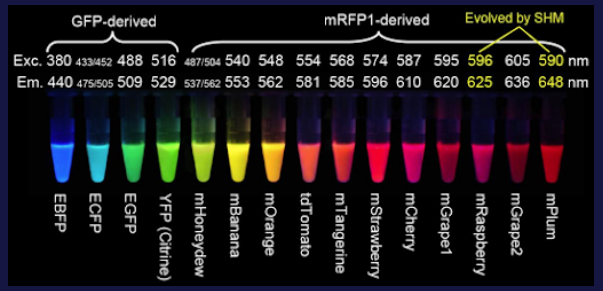


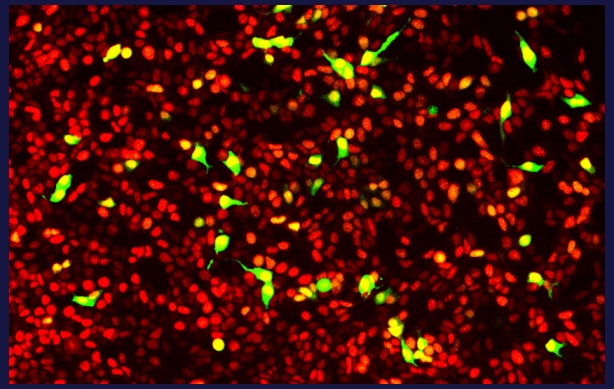
There are many other possibilities of putting this to good use against viruses, tumour cells and maybe other conditions.



Of course, there are lots of details not described here…

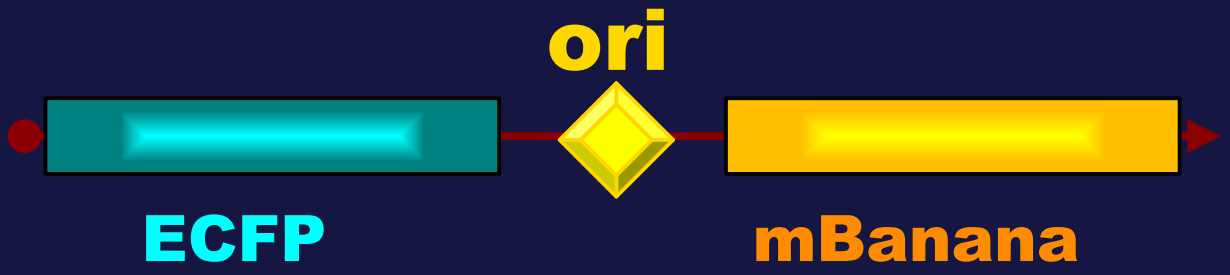






…such as a tool to scan an unknown RNA virus for its ori site.

Detection Kits

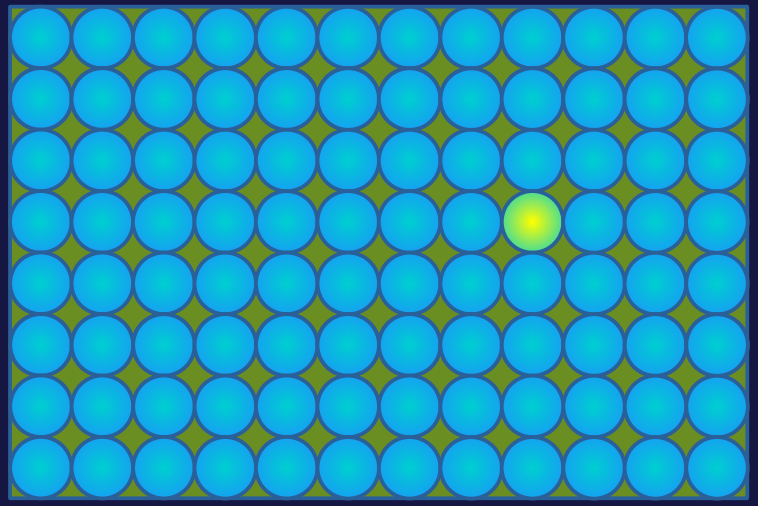


Place a nucleic acid like the one above in a commercially available *in vitro* eukaryotic expression system and contact it with the sample.

In the absence of replicase, there will be only moderate blue fluorescence.

In the presence of replicase, there will also be strong yellow fluorescence.

Detection Kits

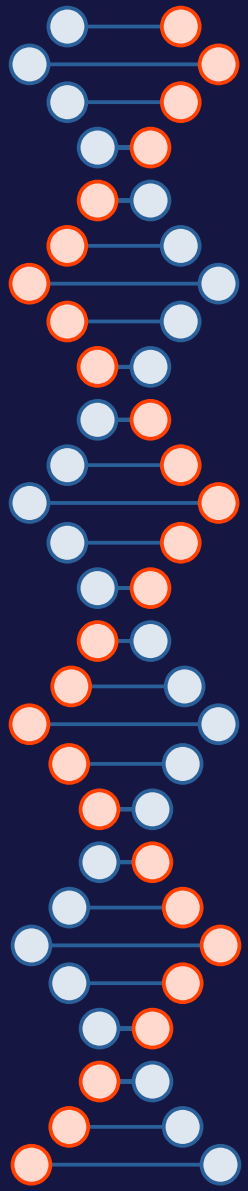


This can identify viral presence neither immunologically nor by PCR, but by its replicase activity.

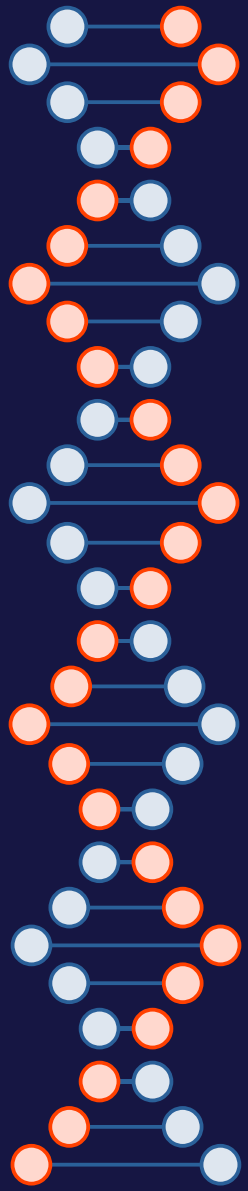
For mass screening in pandemics OR for virus classification in case of emergent diseases.

Vision

Package this into an emergency response kit and quench any emerging virus as soon as it rears its head.



Where to start?



Cost of a Novel Drug

from inception to marketing:

$ 100,000,000 – $ 1,000,000,000

Impossible to raise?

Maybe.

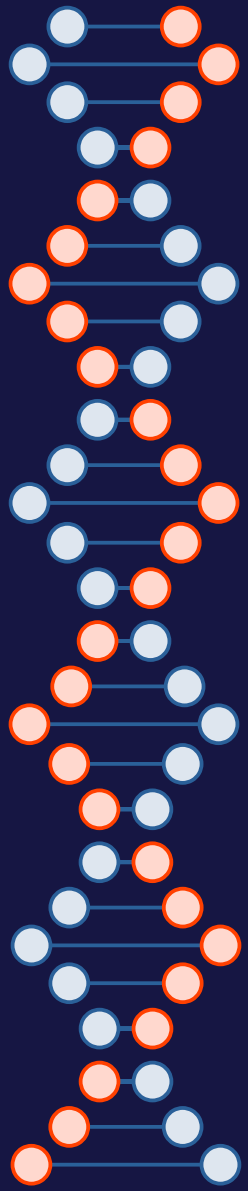
BUT…

A Persian Fairytale

A prince of Serendip was taken POW by a rival king and imprisoned in a high tower.

*Disguised as a servant, his wife came to the tower, bringing along a **stag beetle**, a **silk thread**, a **linen cord**, a **heavy rope** and some **honey**.*

She tied the silk string to the beetle, poured some honey on its antennae and placed it, head up, on the wall…



A Persian Fairytale (Ct'd.)

Following the honey, the beetle crawled up, dragging the thread behind it, until it had reached the window, and the prince took the thread and released the beetle.

*The lady **tied the linen cord to the thread**, and he pulled it up; next she **tied the heavy rope to the cord**, and he pulled that up too.*

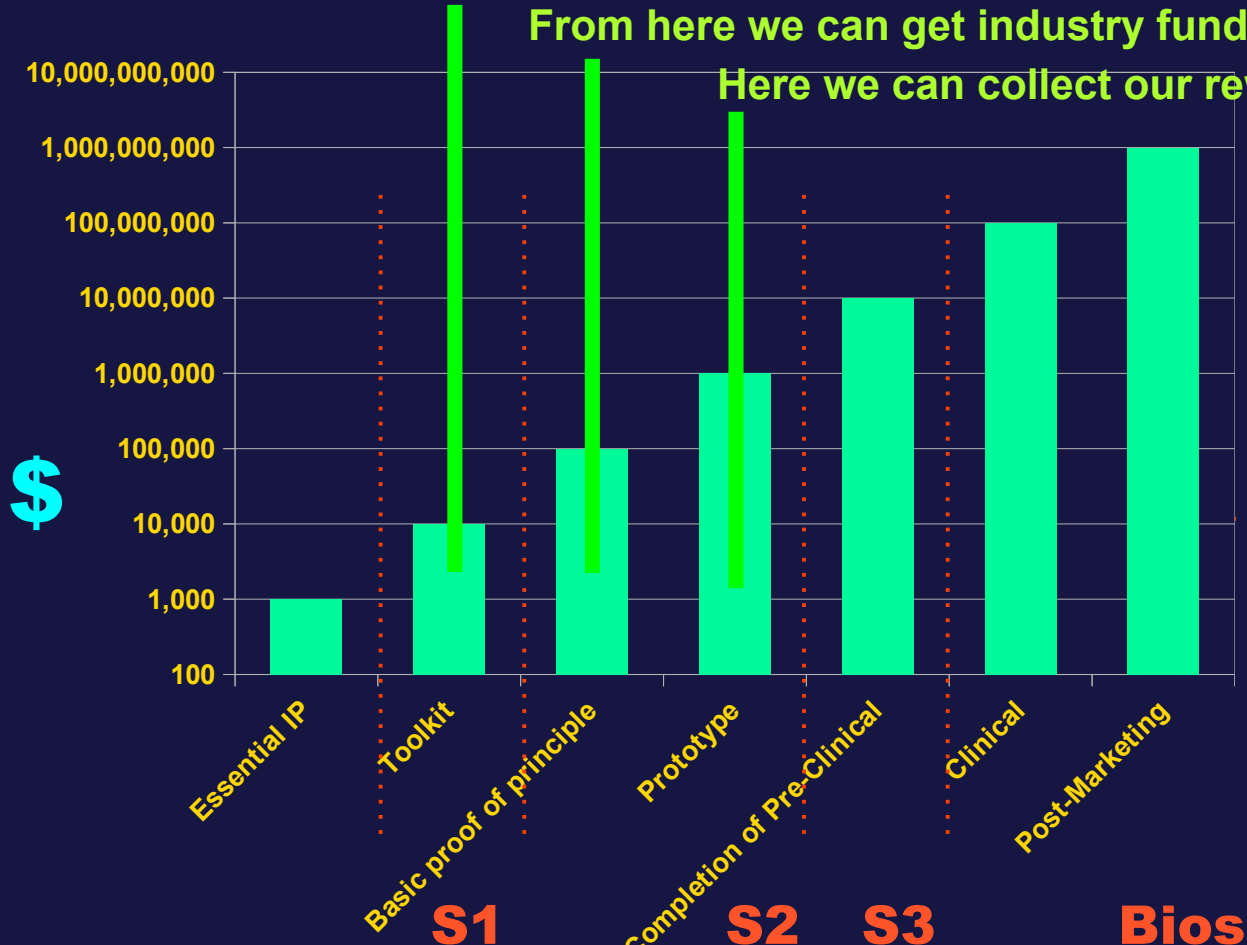
Then he tied the rope securely to his heavy bed, climbed out and down, and they went home and lived happily ever after.

How far to go?

From here we can get public funding. *(MSc thesis level)*

From here we can get industry funding. *(PhD thesis level)*

Here we can collect our rewards.



Phase 1: Triple-Beetle Approach

Beetle 1:

COS7 cells

A model for papovavirus-infected primate cells, yet S1!

Based on Flaig 1997

Beetle 2:

Qβ phage

A fully-fledged RNA bacteriophage suitable as a model, yet S1!

Well-characterised; mechanics comparable to coronavirus

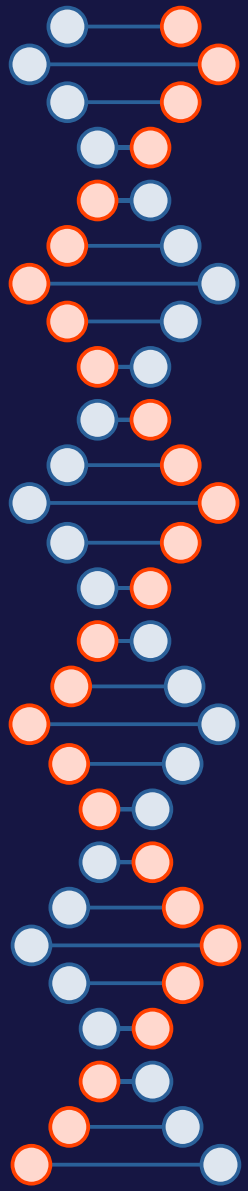
Beetle 3:

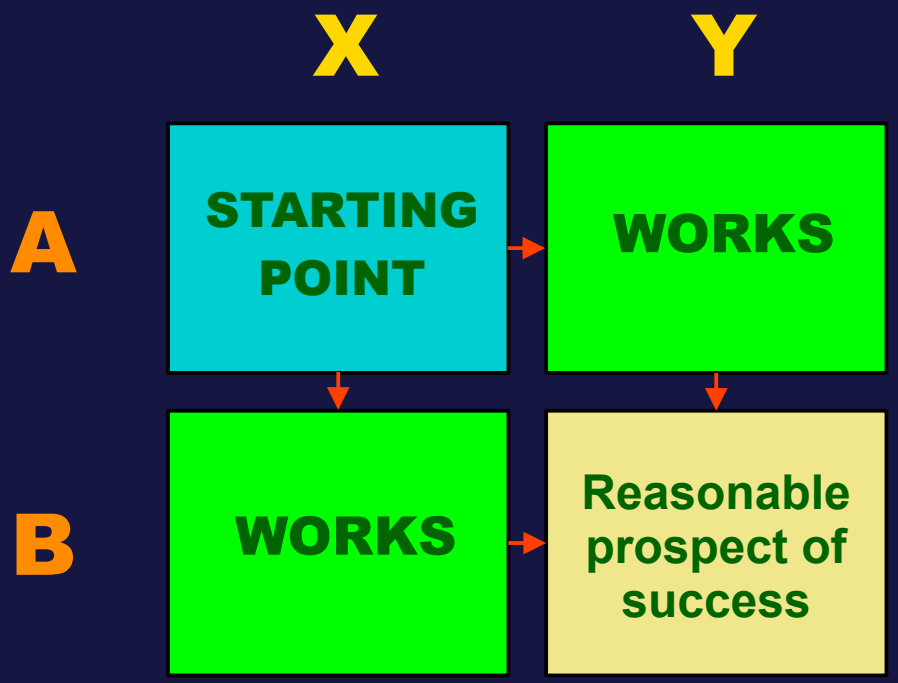
T7 phage

An autographic phage suitable as a model, yet S1!

Well-characterised; easy to handle; straight approach possible

Together, these can be used to establish a toolbox of components.





IF Beetle 1 provides all the individual tools for knocking down a eukaryotic virus,
and Beetle 2 or 3 shows that we can actually knock down a prokaryotic virus,
it is easy to assert that all that remains to be done is to put together the tools.

Phase 1b: Full Banzai

In case of availability of a BSL-3 lab, we could concomitantly go straight for the hub and directly test an expression construct against Covid-19. (E.g. with RNAse L.)

Cost: Very low (1 – 2 weeks)
Chances: Moderate (many error sources)
Result upon success: KO victory
Result upon failure: Pursue the beetle approach

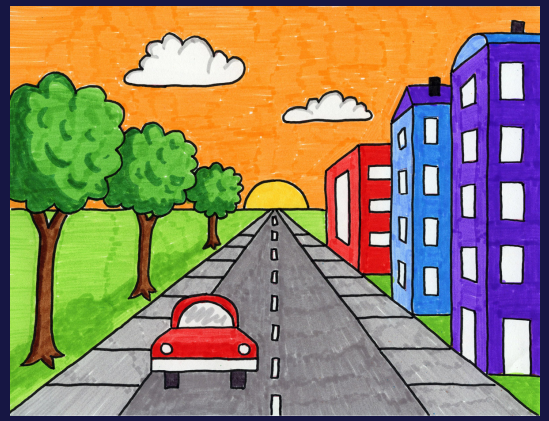
We have some further aces up our sleeve…

Just note that we can easily combine various "embodiments" by simply mixing them.

Perspectives

Once we succeed in “uncloaking” the virus, we can ultimately combine this with novel approached to unleashing an immune reaction: e.g. using peptides packaged into bdellosomes – Flaig 2001

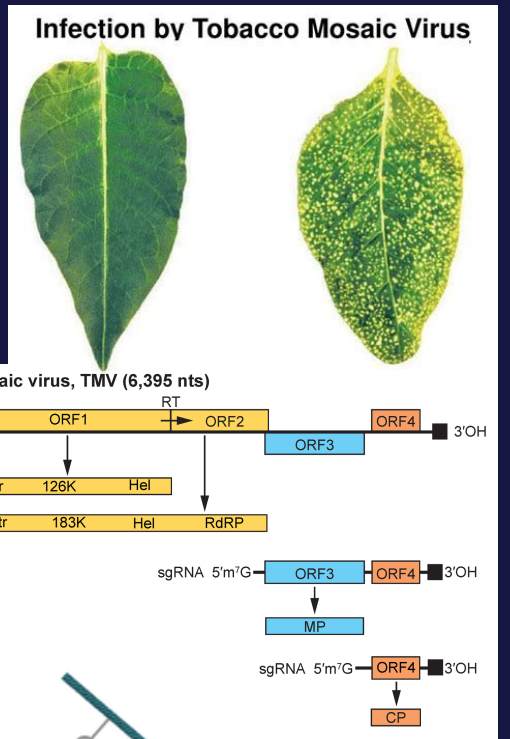
And we could look for interaction of viruses with other microorganisms: Facilitation of bacterial infections? Reactivation of resident viruses?...

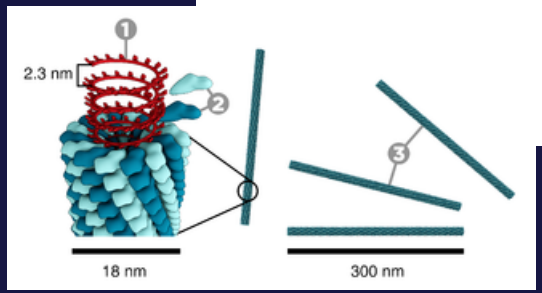


Once More Unto The Breach

Transgenic crops:

- **Model system (“Beetle 4”)**
- **Could be hardened against viral pathogens**
- **Might provide a cheap source of substances for the screening assay**





And Lest We Forget...

Based on Flaig 1997

and using the “knife” to knock down telomerase in cells expressing T proteins (and only there),

we could create a vaccine against T proteins rather than against shell proteins of papovaviruses without fearing the tumorigenic properties of T.

Suggested Reading

- German Patent Application 10 2021 001 841.9
- German Patent Application 10 2021 002 567.9
- German Patent Application 10 2021 003 360.4
- German Patent Application 10 2021 005 748.1
- Flaig 1997: “Konstruktion pseudoviraler Gentransfersysteme” (Dr. sci. hum. thesis)
- Flaig 2001: “Bdellosomen: Ein neuartiges Arzneimitteltransportsystem auf der Basis monomolekularer Polymerpartikel” (Dr. rer. nat. thesis)
- …

A Matter of Motivation

“Man kaufte mir das Kreuz,
Nun will ich’s mir verdienen”

(I was bought the cross —
Now I will merit it!)

Thank you.

Rűdiger Marcus Flaig

For questions: *rmf@sanctacaris.net*