FMDV as a model for lethal mutagenesis

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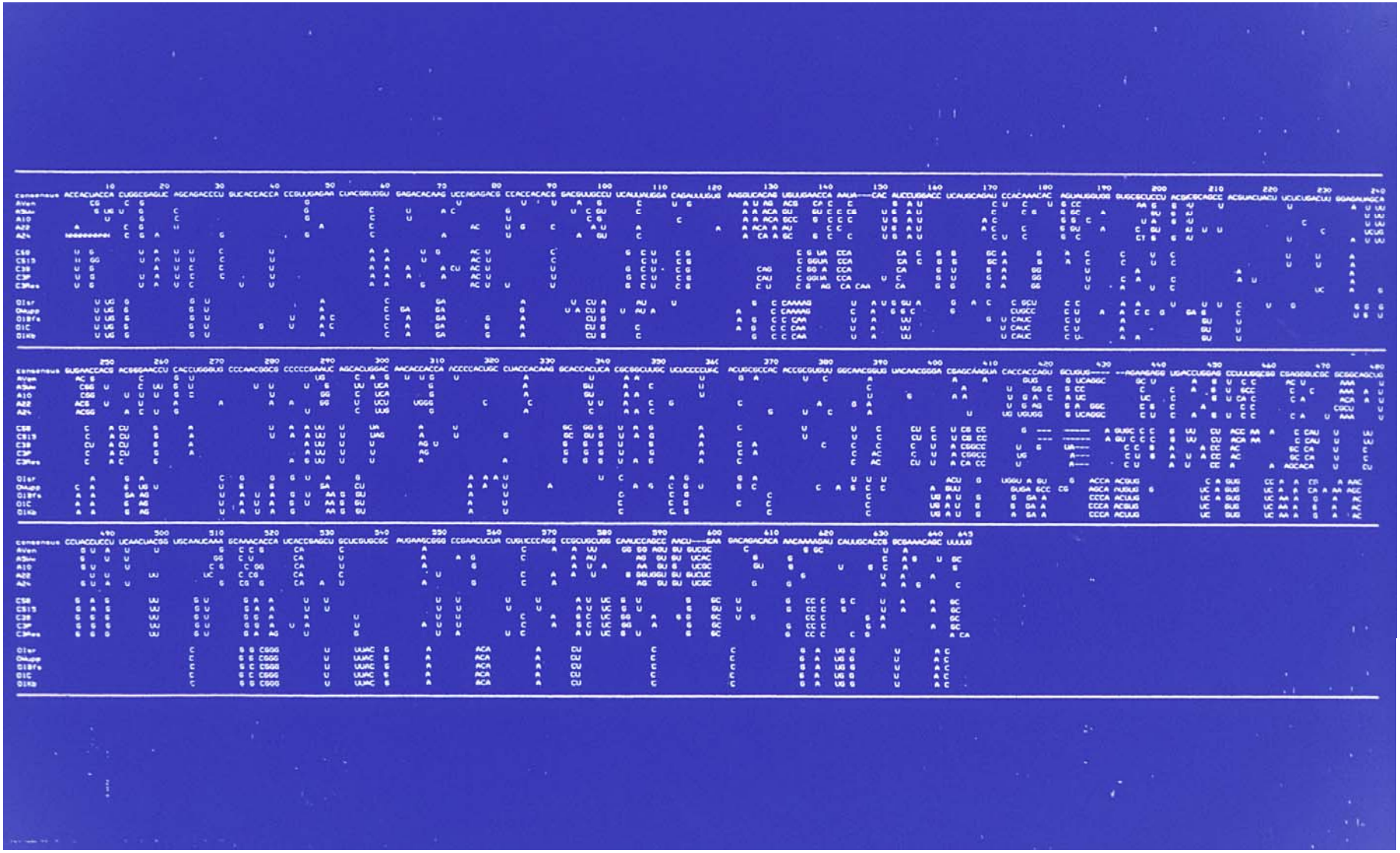
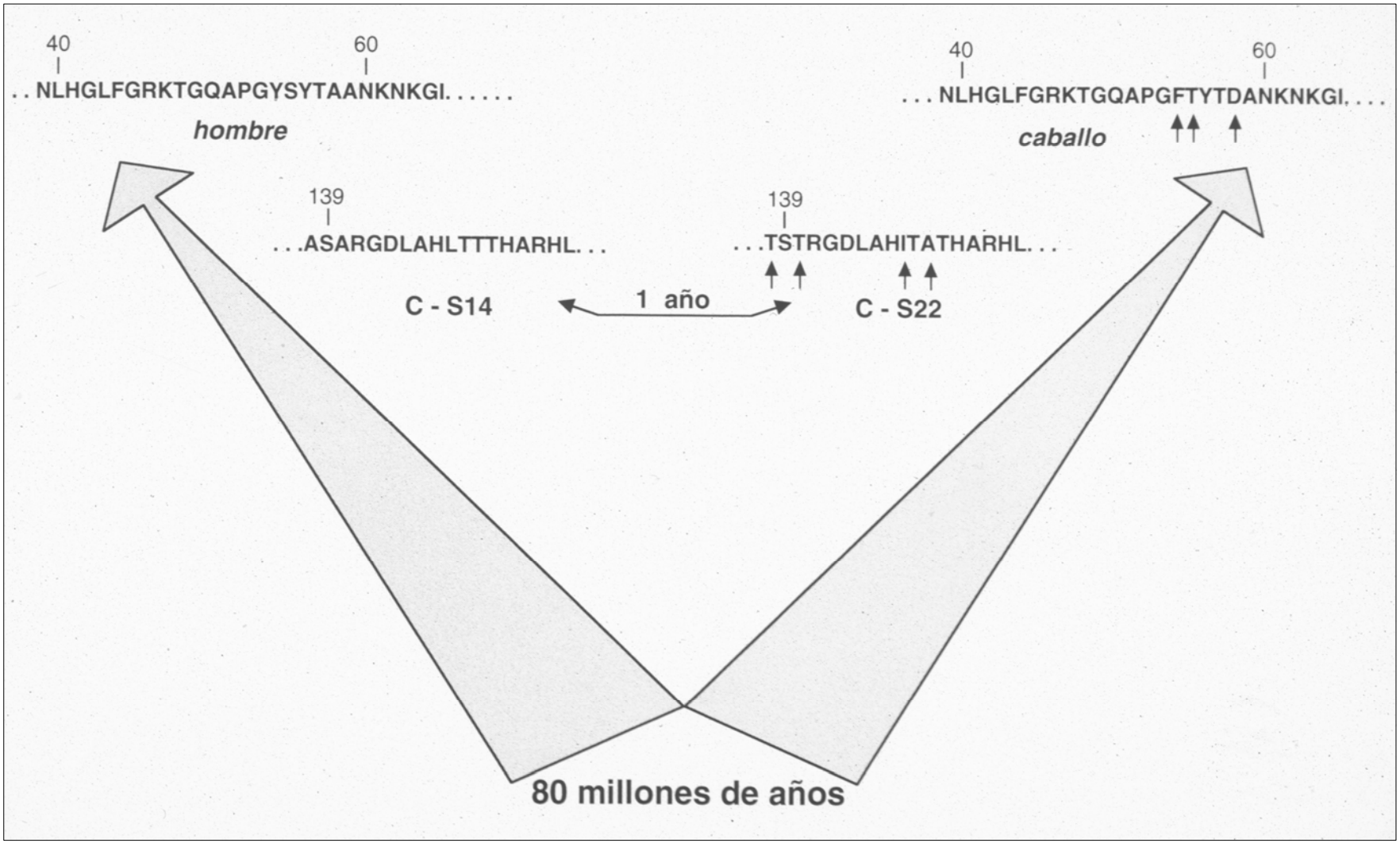
1. *Some historical slides, seen 28 years later*

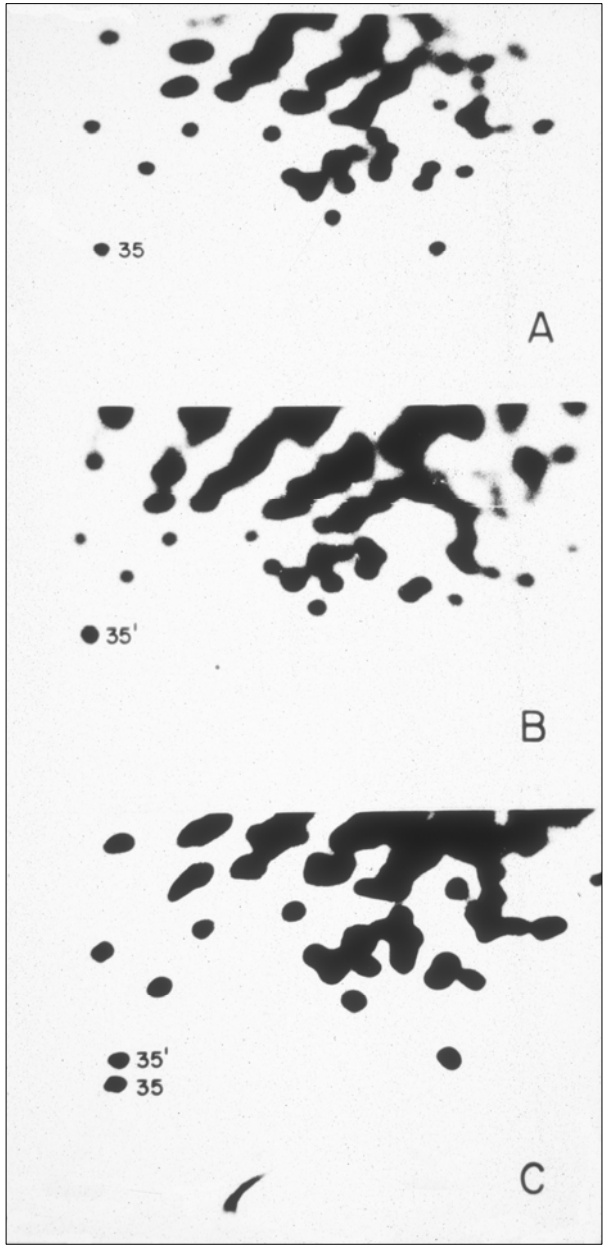
2. *High error rates and virus population dynamics demand new antiviral approaches*

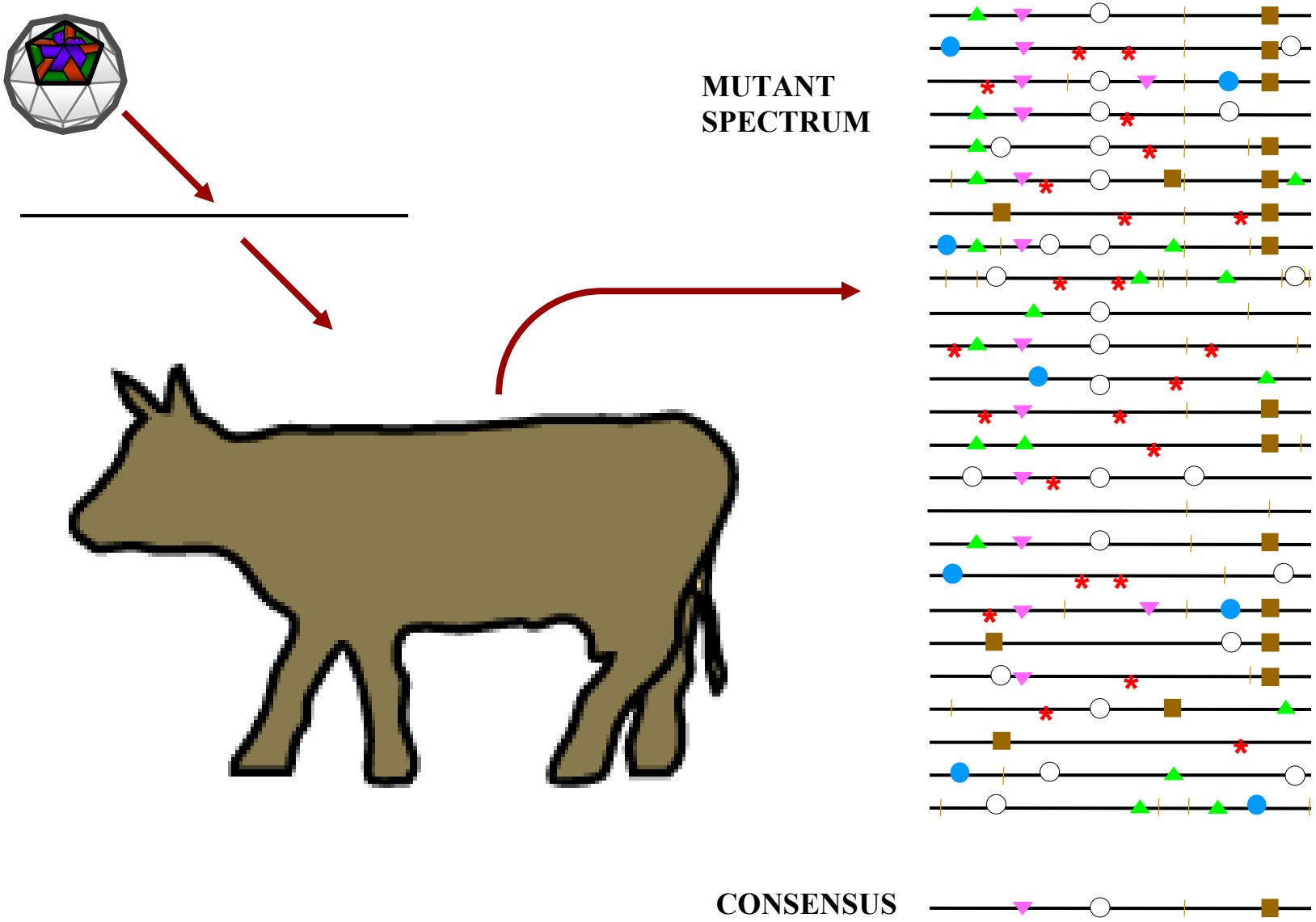
3. *How FMDV has helped in the current development of lethal mutagenesis*

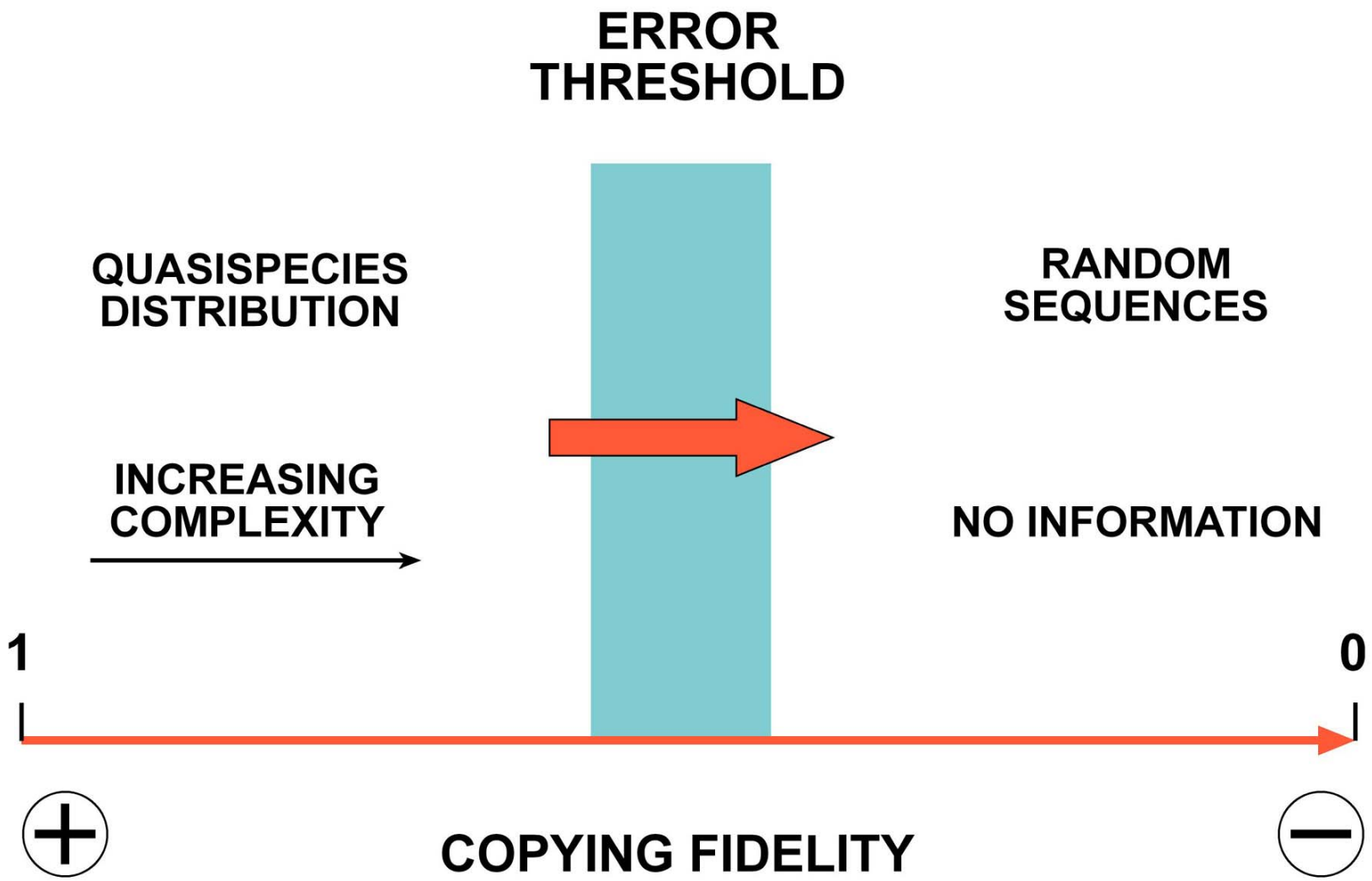
4. *Prospects. Links with anti-cancer therapy*



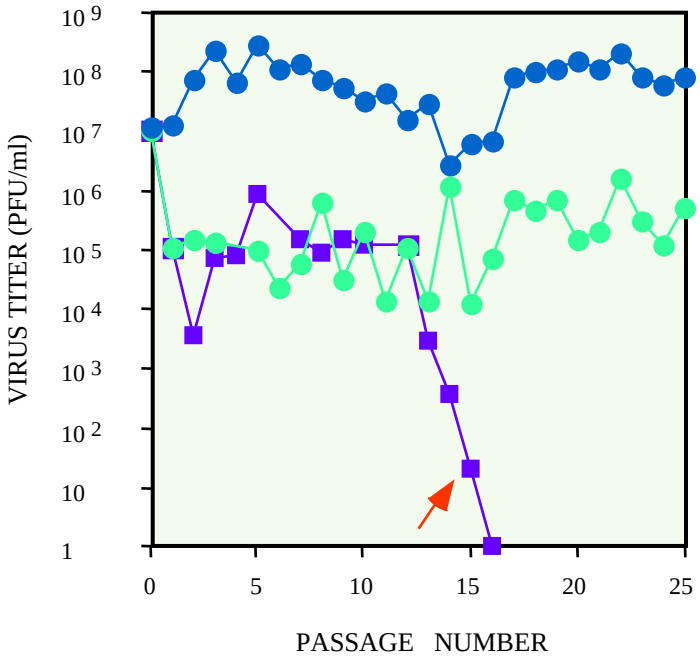

40
60
. . NLHGLFGRKTGQAPGYSYTAANKNKGI. . . . . .
hombre
40
60
. . . NLHGLFGRKTGQAPGFTYTDANKNKGI. . . .
caballo
139
. . .ASARGDLAHLTTTHARHL. . .
139
. . .TSTRGDLAHITATHARHL. . .
C - S14
1 año
C - S22
80 millones de años


35
A
35'
B
35'
35
C

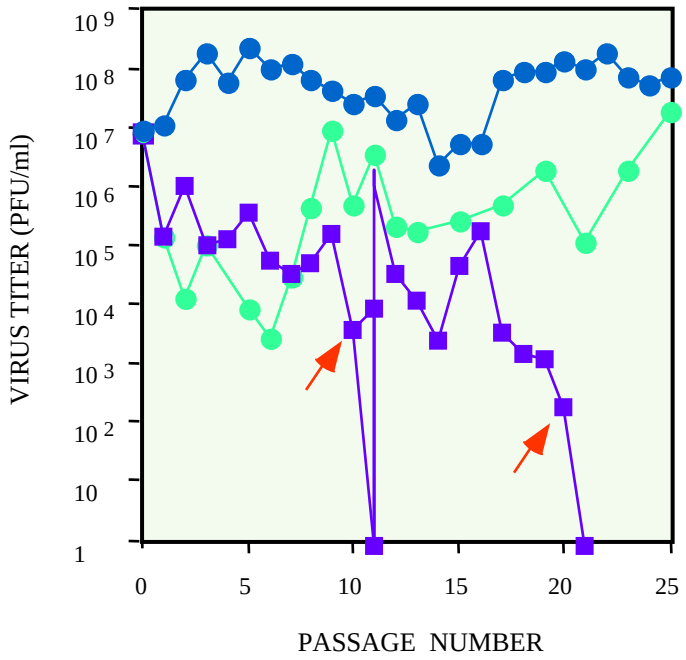

MUTANT
SPECTRUM
CONSENSUS

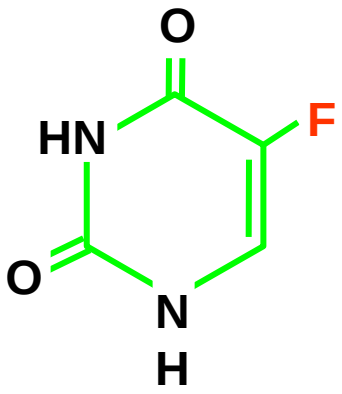

ERROR
THRESHOLD
QUASISPECIES
DISTRIBUTION
RANDOM
SEQUENCES
INCREASING
COMPLEXITY
NO INFORMATION
1
0
COPYING FIDELITY

FU

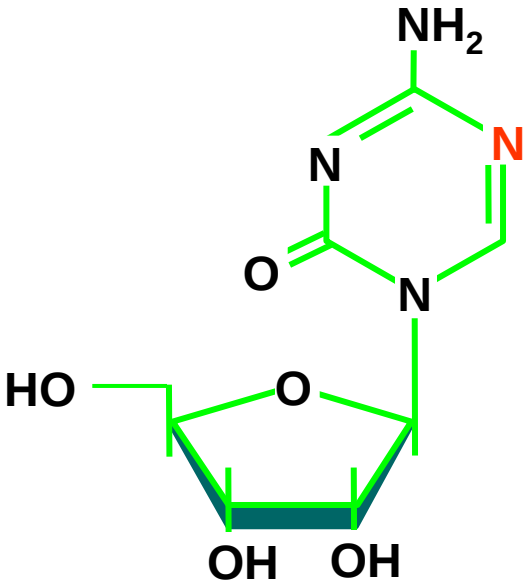

VIRUS TITER (PFU/ml)
10 9
10 8
10 7
10 6
10 5
10 4
10 3
10 2
10
1
0
5
10
15
20
25
PASSAGE NUMBER

AZC

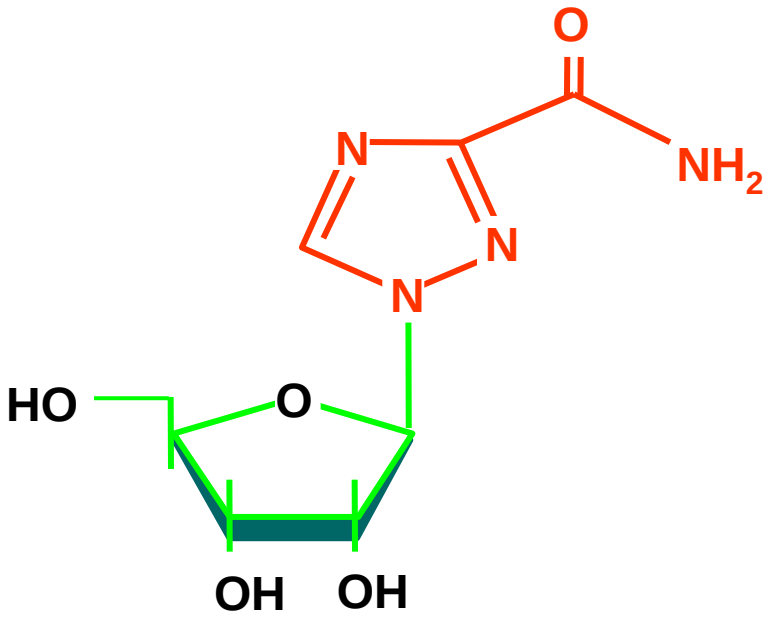

VIRUS TITER (PFU/ml)
10 9
10 8
10 7
10 6
10 5
10 4
10 3
10 2
10
1
0
5
10
15
20
25
PASSAGE NUMBER



5 - Fluorouracil

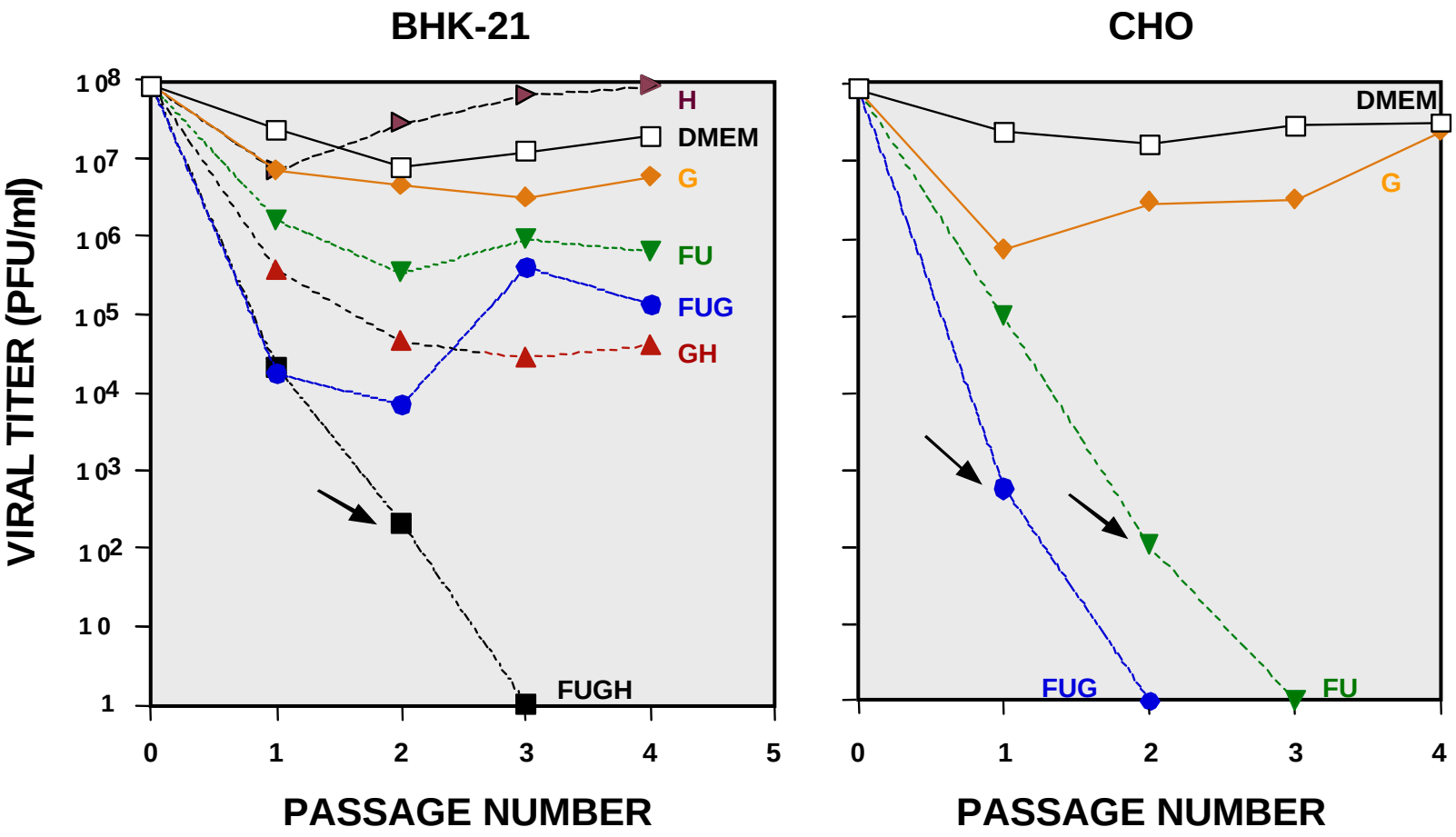


5 - Azacytidine



Ribavirin

FMDV MARLS (RELATIVE FITNESS 130 IN BHK-21 CELLS)



- ***Preextinction FMDV RNA interferes with infectious FMDV RNA (despite low relative fitness of preextinction FMDV: ≤ 0,183 relative to parental virus, fitness 1)***

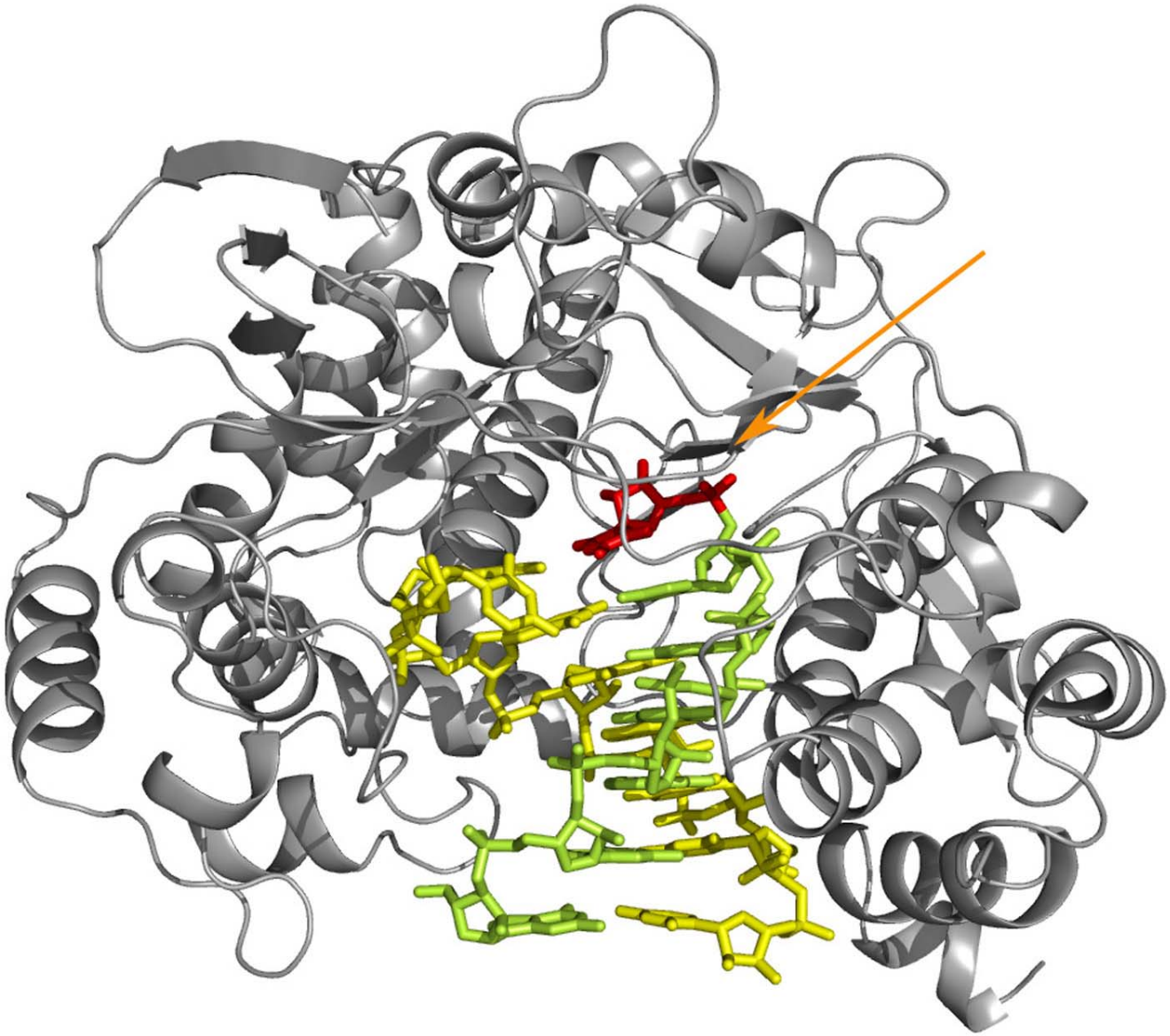
- ***The transition of FMDV towards extinction is accompanied of:***

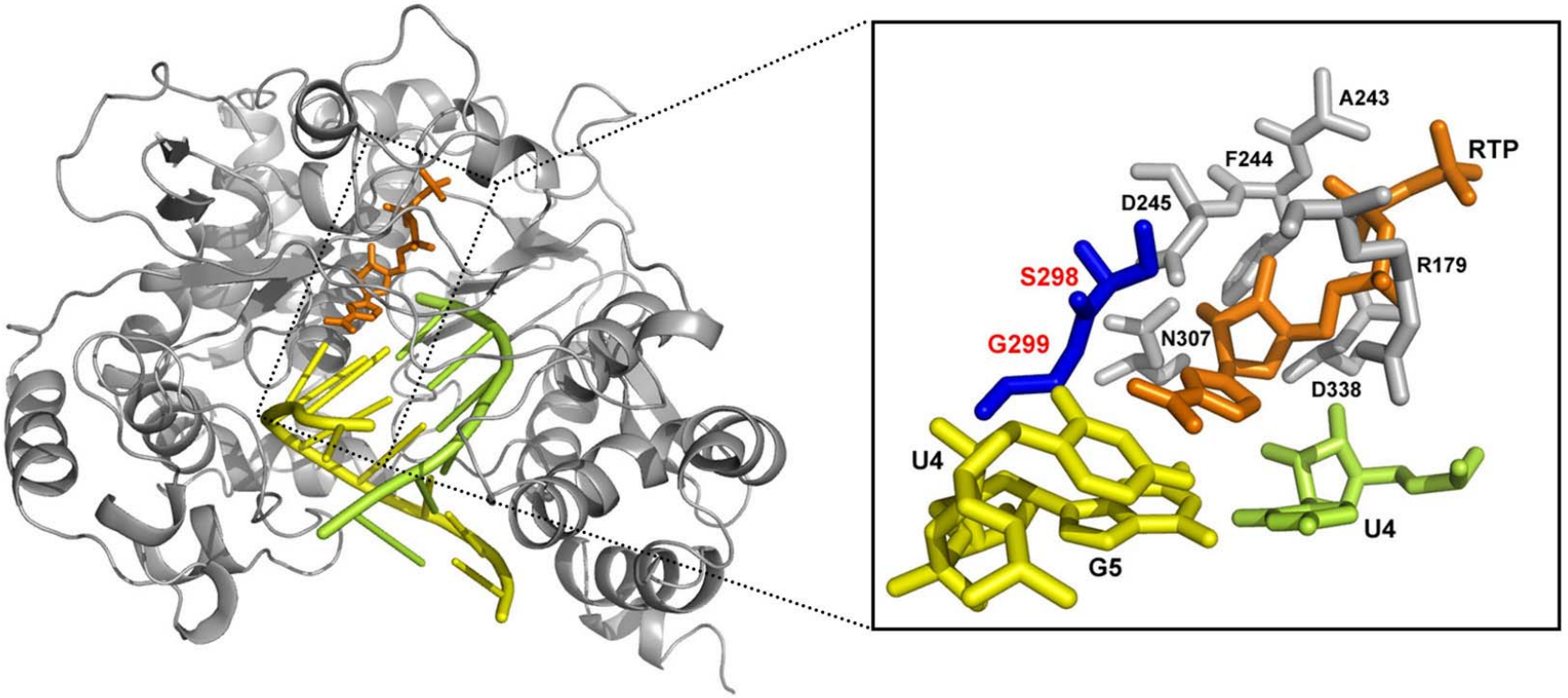
  - Increases of 2.0- to 11.1- fold in mutation frequency (measured with components of mutant spectra)
  - Decreases of about 10^3-fold in specific infectivity (infectious RNA/total RNA ~$2.5x10^{-7}$)
  - Invariant consensus nucleotide sequence

- ***The same initial inhibitory activity but without a mutagenic agent does not lead to extinction***

Molecular basis of mutagenesis

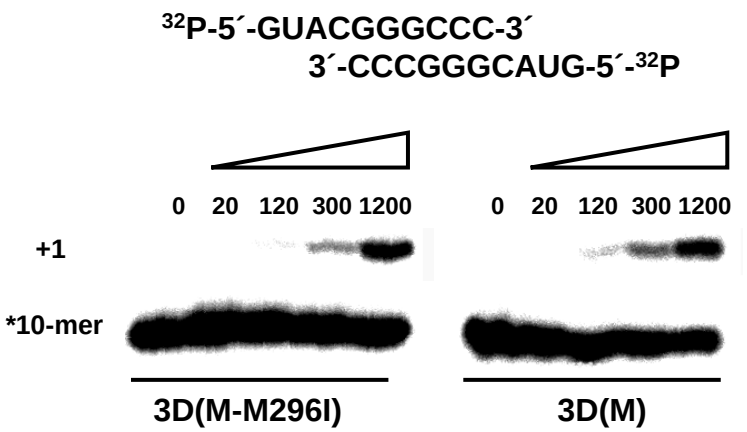
- ***Base and nucleoside analogues are converted to their nucleotides which can be active in mutagenesis by at least two mechanisms:***
  - ***The nucleoside-triphosphates can be incorporated by the viral polymerases and induce transition mutations***
  - ***Nucleotide analogues may inhibit enzymes of nucleotide metabolism thereby altering intracellular nucleotide pools***

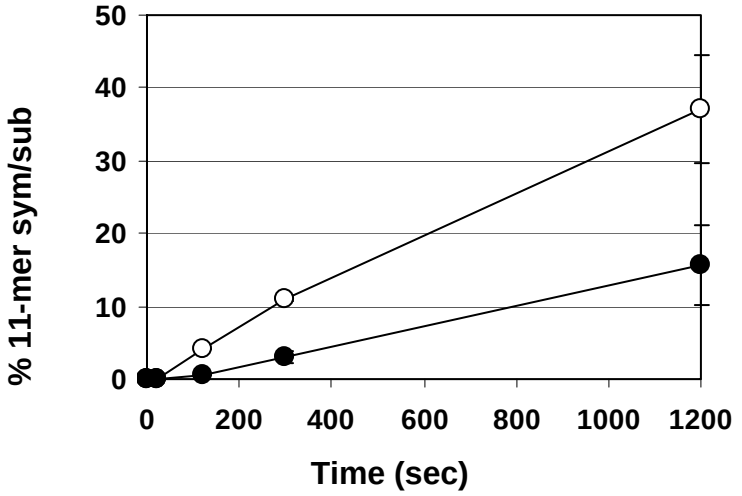


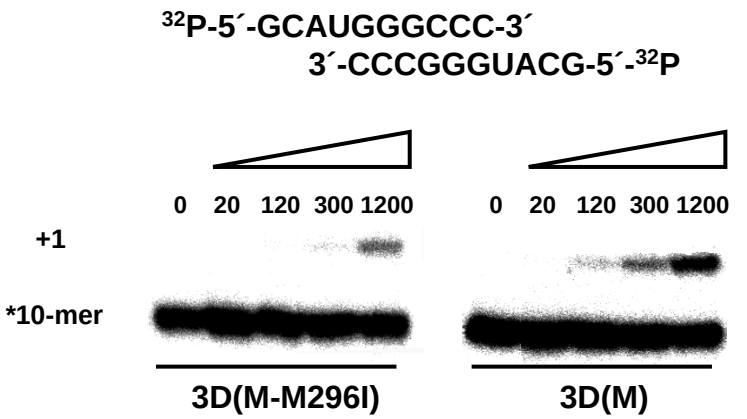

A243
F244
RTP
D245
R179
S298
N307
G299
D338
U4
U4
G5

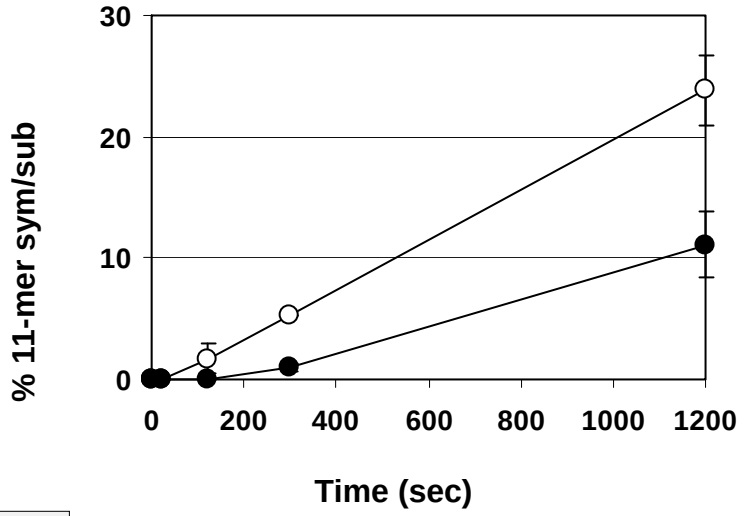
FMDV 3D mutant M296I selected by ribavirin

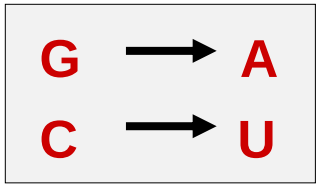
- ***The substitution increases viral fitness in the presence of ribavirin, but not in the absence of ribavirin***
- ***The mutant 3D shows decreased capacity to incorporate ribavirin monophosphate in the place of GTP or ATP***
- ***In other biochemical reactions (standard polymerization assays, VPg uridylylation, RNA binding) the mutant 3D behaves as the wild type 3D***


32P-5´-GUACGGGCCC-3´
3´-CCCGGGCAUG-5´-32P
0 20 120 300 1200
0 20 120 300 1200
+1
*10-mer
3D(M-M296I)
3D(M)

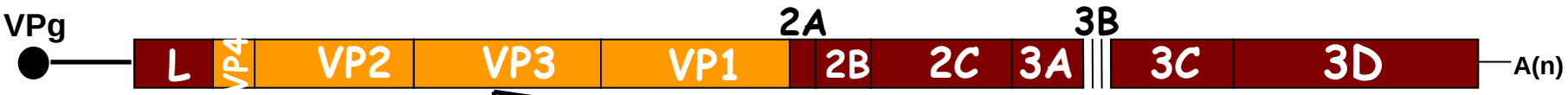

% 11-mer sym/sub
50
40
30
20
10
0
0 200 400 600 800 1000 1200
Time (sec)


32P-5´-GCAUGGGCCC-3´
3´-CCCGGGUACG-5´-32P
0 20 120 300 1200
0 20 120 300 1200
+1
*10-mer
3D(M-M296I)
3D(M)


% 11-mer sym/sub
30
20
10
0
0 200 400 600 800 1000 1200
Time (sec)


G → A
C → U

FMDV CAPSID MUTANTS



Cápsid

Q2027A

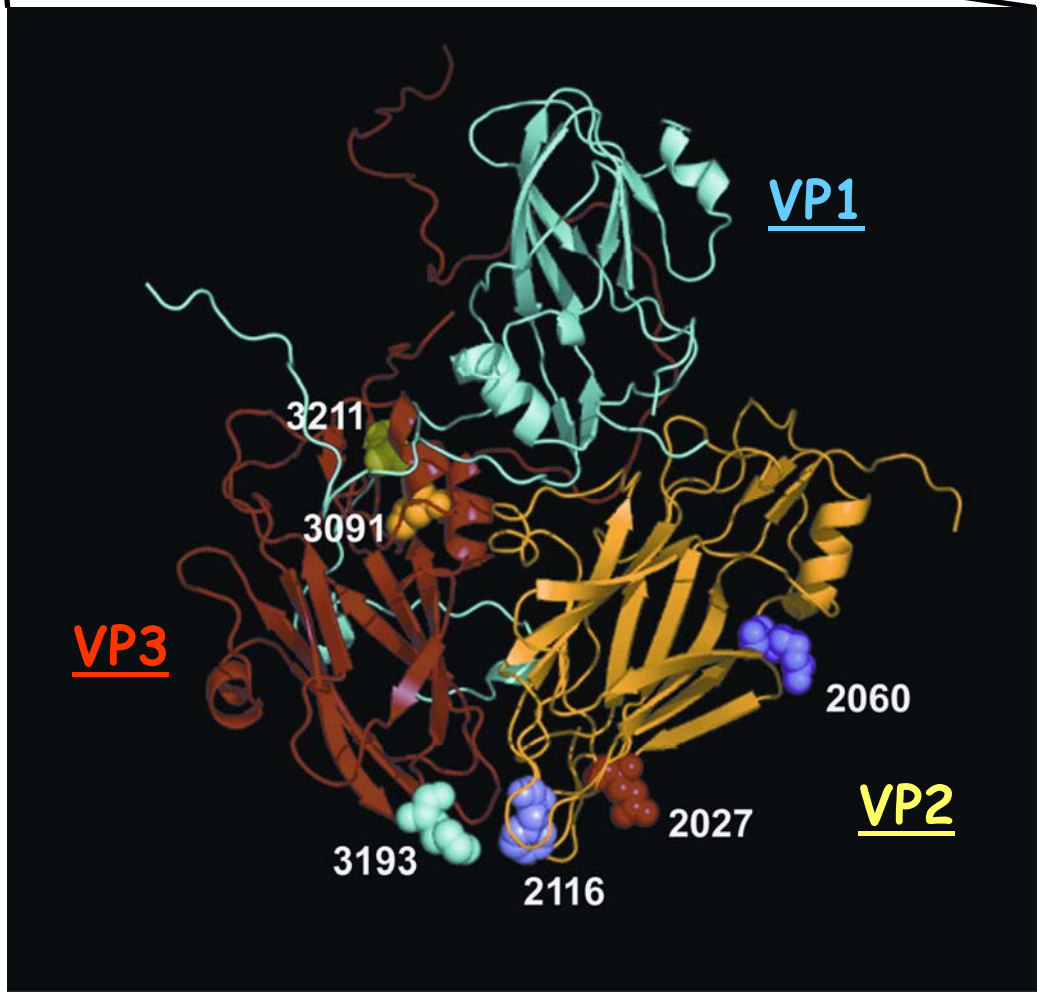
R2060A

F2116A

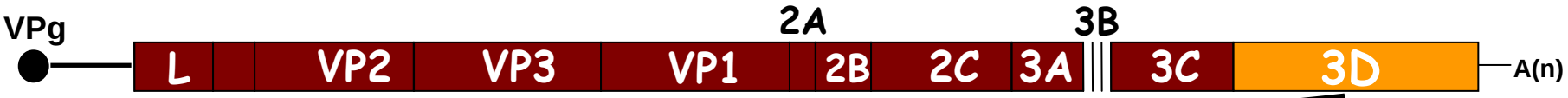
K3193A

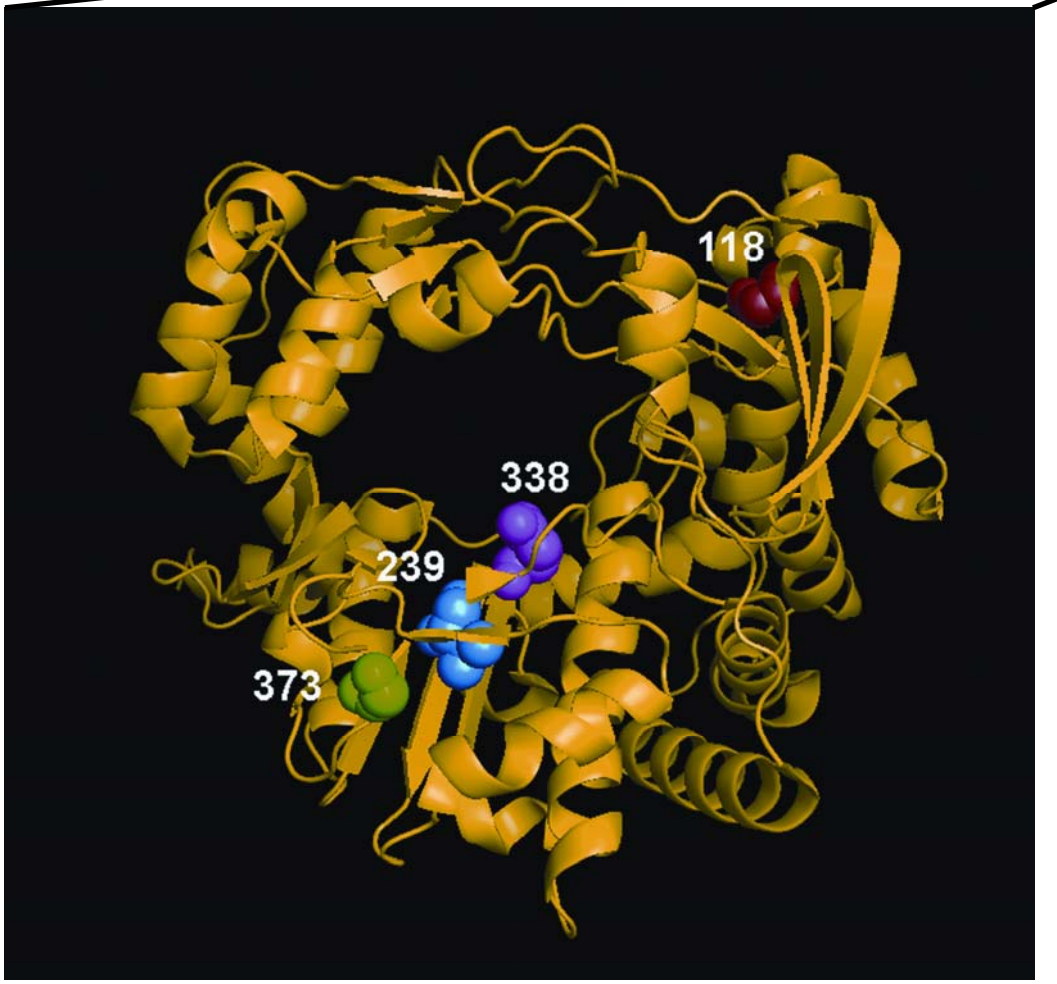
L3091R

L3211P



FMDV POLYMERASE MUTANTS

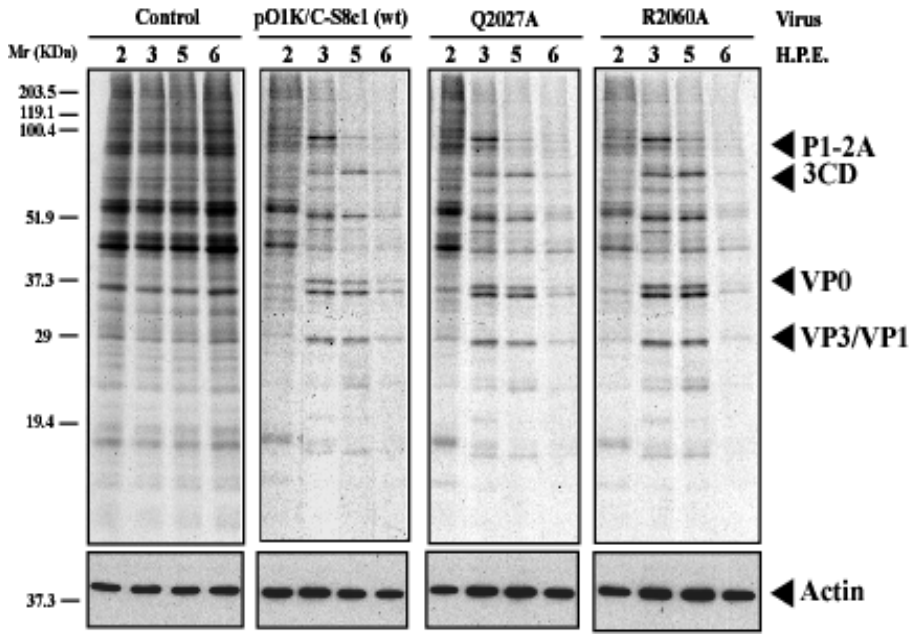


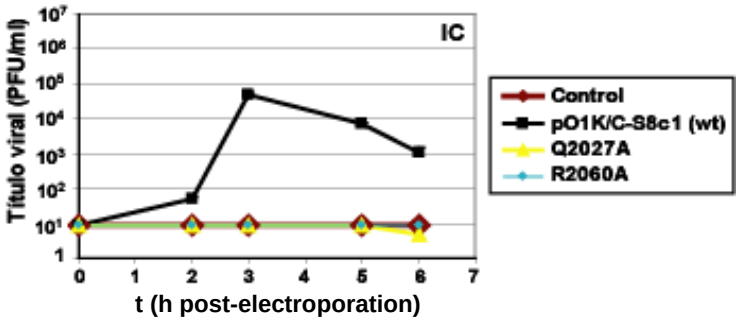


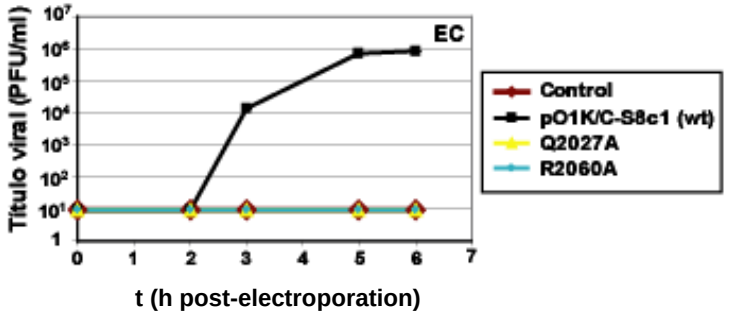
Polymerase

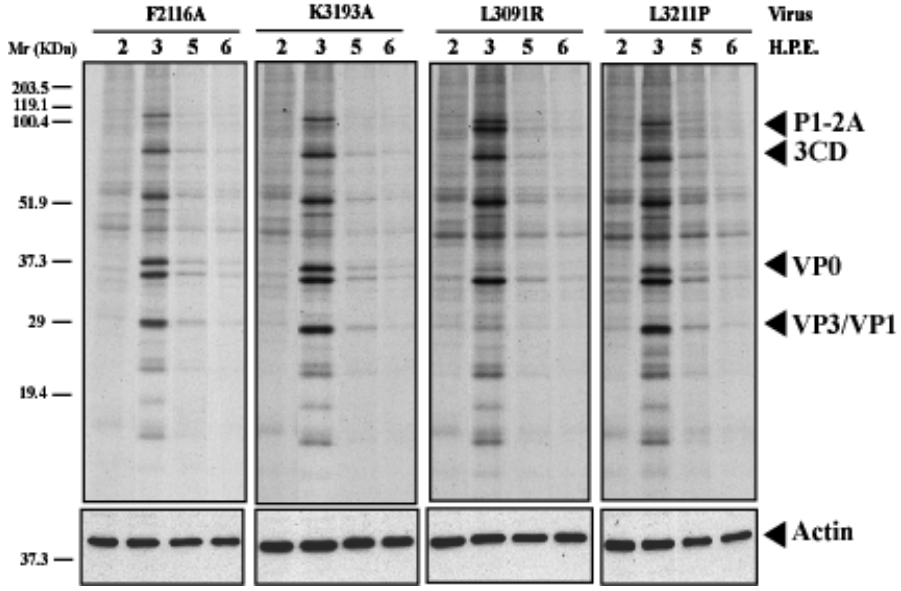
DMD	G118D V239M G373D
D3	D338A
MD	V239M G373D

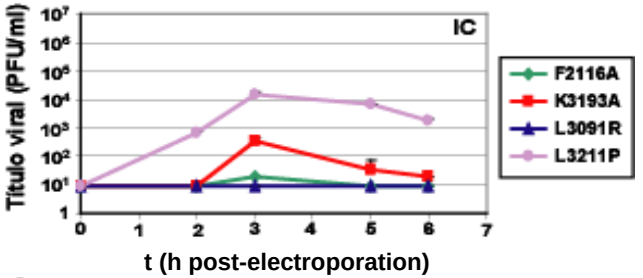
CAPSID MUTANTS OF FMDV

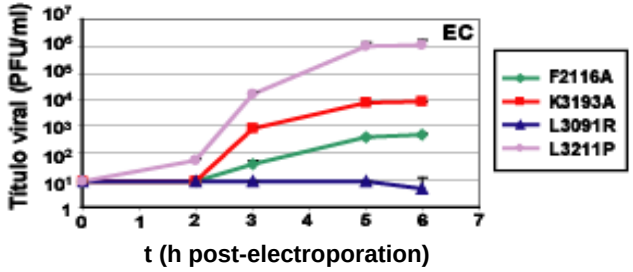




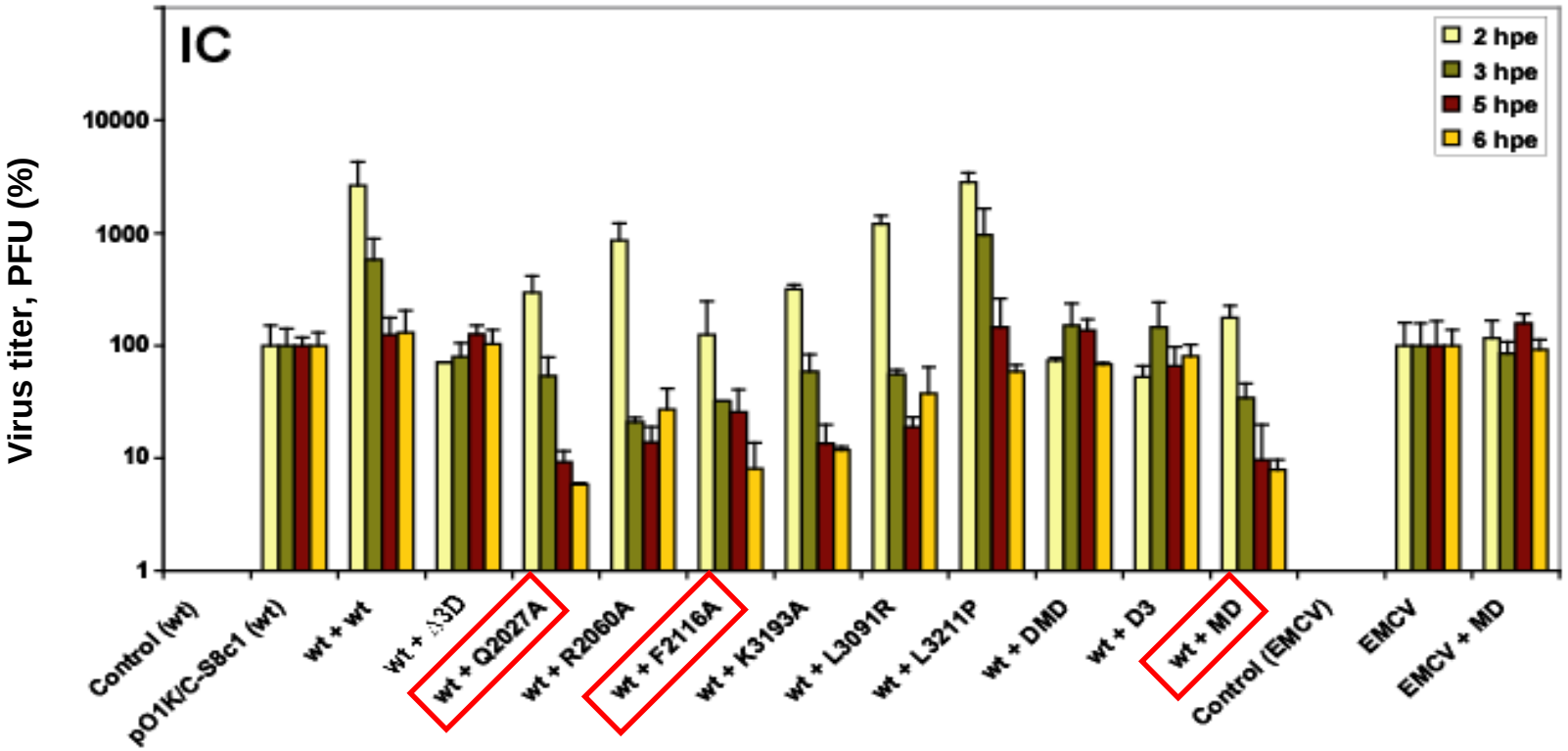








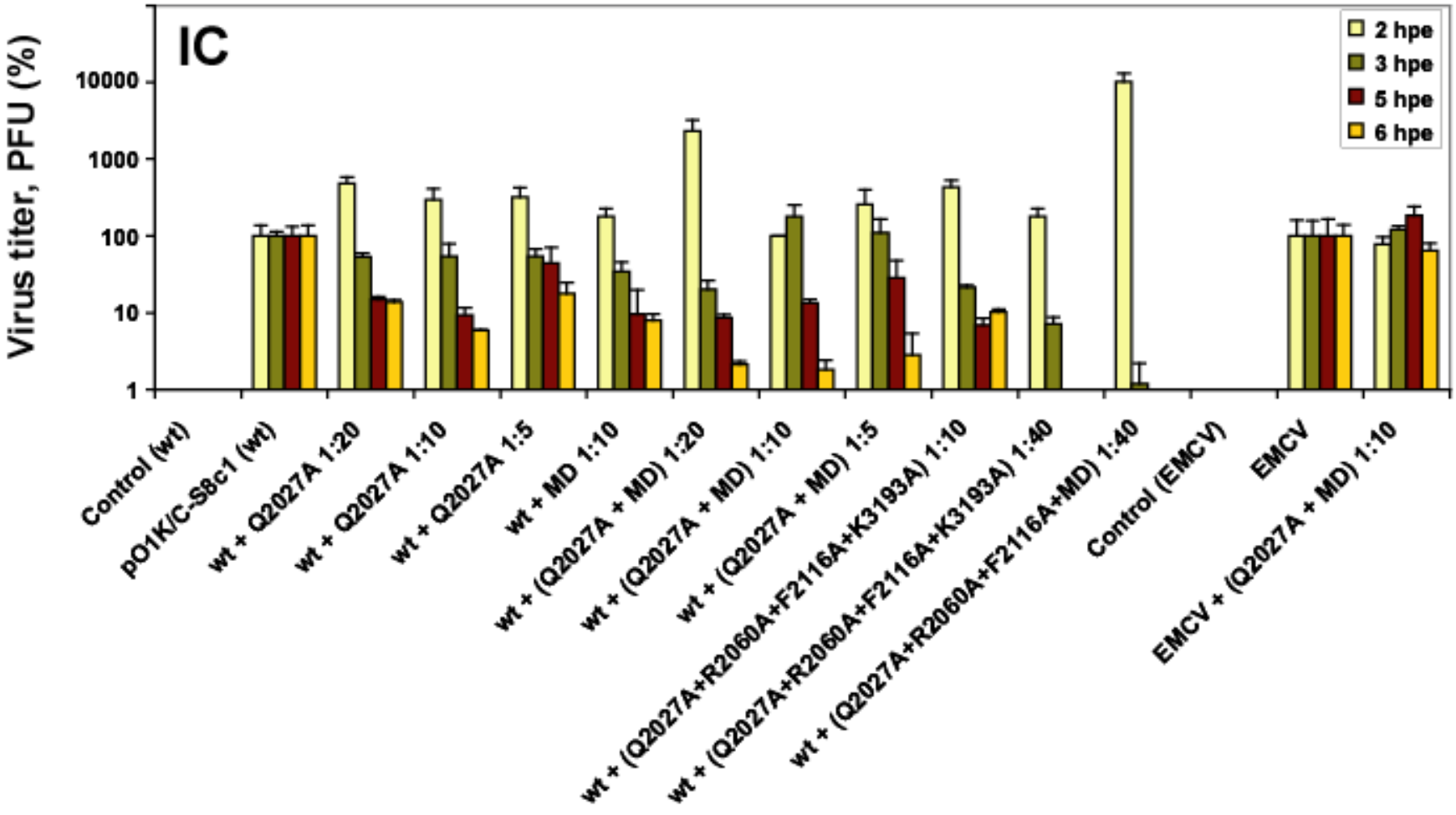
INTERFERENCE BY INDIVIDUAL FMDV MUTANTS



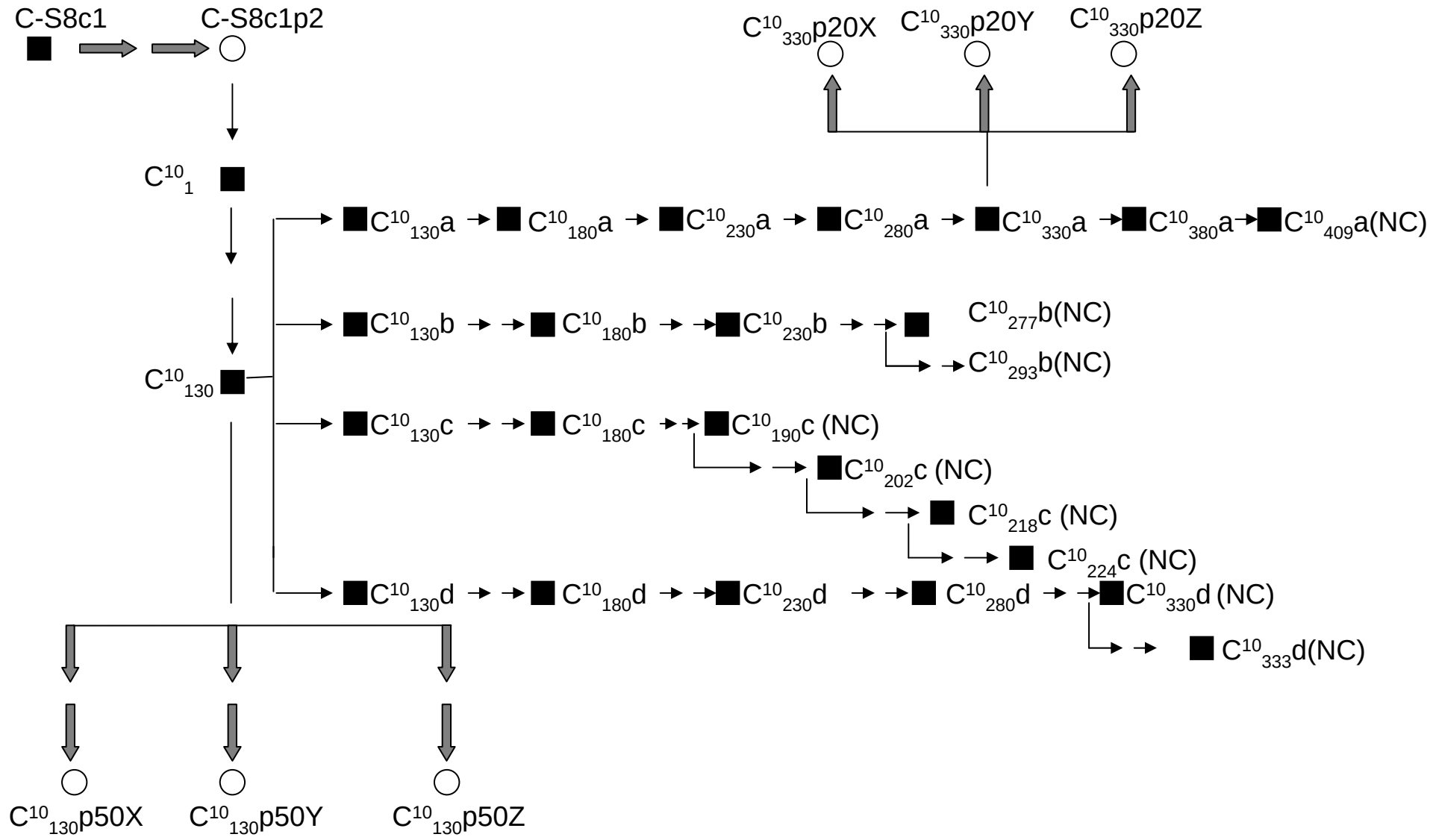
SUMMARY OF REPLICATIVE AND INTERFERENCE PROPERTIES OF FMDV MUTANTS

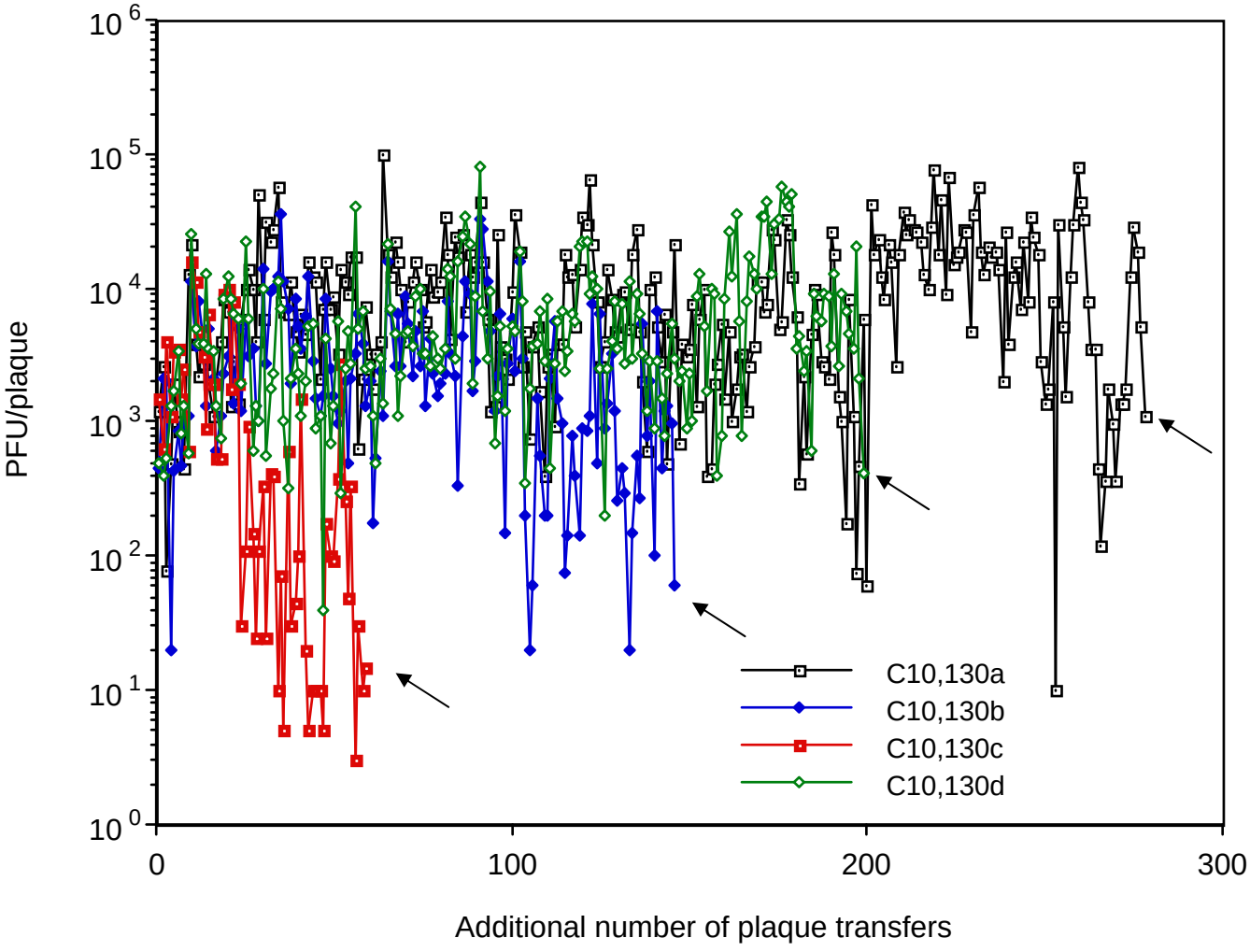
Virus	Amino acid substitution	Protein	Intracellular replication	Infectious progeny		Interference
				Intracellular	Extracellular	
pO_1K/C-S8c1 or pMT28	–	–	+ +	+ +	+ +	–
Q2027A	Q27A	VP2	+ +	–	–	+ +
R2060A	R60A	VP2	+ +	–	–	+
F2116A	F116A	VP2	+ +	–	–	+ +
K3193A	K193A	VP3	+ +	+/-	+/-	+
L3091R	L91R	VP3	+ +	–	–	+
L3211P	L211P	VP3	+ +	+ +	+ +	–
DMD	G118D V239M G373D	3D	–	–	–	–
D3	D338A	3D	–	–	–	–
MD	V239M G373D	3D	+ +	+	+	+ +

INTERFERENCE BY MIXTURES OF FMDV MUTANTS



- ***Specific mutants can interfere with FMDV replication***
- ***Some lethal mutants did not interfere, while some replication-competent mutants (notaby polymerase mutant MD) exerted a strong interference***
- ***Interference was stronger with combinations of capsid and polymerase mutants***

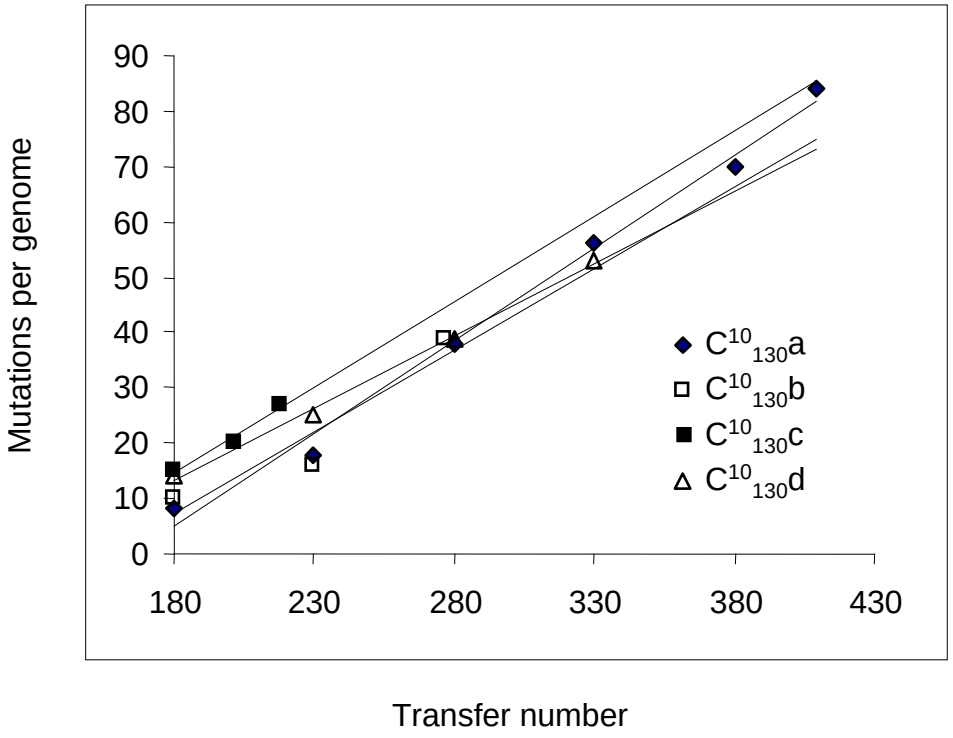

C-S8c1
C-S8c1p2
C^{10}_{330}p20X
C^{10}_{330}p20Y
C^{10}_{330}p20Z
C^{10}_{1}
C^{10}_{130}a
C^{10}_{180}a
C^{10}_{230}a
C^{10}_{280}a
C^{10}_{330}a
C^{10}_{380}a
C^{10}_{409}a(NC)
C^{10}_{130}
C^{10}_{130}b
C^{10}_{180}b
C^{10}_{230}b
C^{10}_{277}b(NC)
C^{10}_{293}b(NC)
C^{10}_{130}c
C^{10}_{180}c
C^{10}_{190}c (NC)
C^{10}_{202}c (NC)
C^{10}_{218}c (NC)
C^{10}_{224}c (NC)
C^{10}_{130}d
C^{10}_{180}d
C^{10}_{230}d
C^{10}_{280}d
C^{10}_{330}d (NC)
C^{10}_{333}d(NC)
C^{10}_{130}p50X
C^{10}_{130}p50Y
C^{10}_{130}p50Z


PFU/plaque
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
100
200
300
Additional number of plaque transfers
C10,130a
C10,130b
C10,130c
C10,130d

Specific infectivity

FMDV	RNA molecules in plaque	PFU in plaque	Specific infectivity PFU / RNA
$C^{10}1$	4.5×10^{8}	1.1×10^{5}	2.4×10^{-4}
NC clones (218-409 transfers)	3.0×10^{6} - 3.8×10^{6}	< 5	$< 1.7 \times 10^{-6}$ - $< 1.3 \times 10^{-6}$

- *NC clones establish a persistent infection in BHK-21 cells, without a phase of cell killing*


Mutations per genome
90
80
70
60
50
40
30
20
10
0
180
230
280
330
380
430
Transfer number
C10130a
C10130b
C10130c
C10130d

Plaque – to- plaque transfers

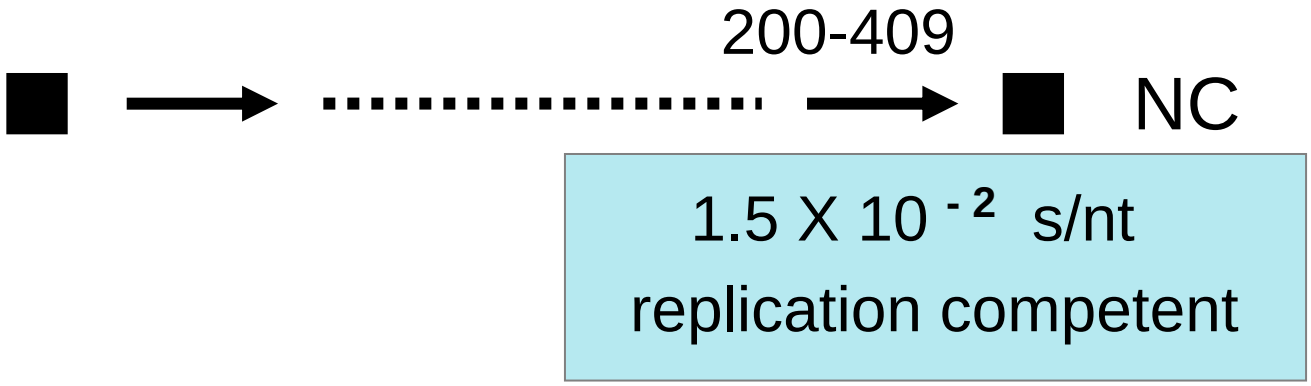
0.26 – 0.34 mutations per genome per transfer

dn / ds = 0.13 – 0.16

Large population passages

0.10 – 0.25 mutations per genome per passage

dn / ds = 0.50 – 1.20


200-409
NC
1.5 X 10 - 2 s/nt
replication competent
Mut 1-20
4 X 10 - 4 - 3 X 10 - 3 s/nt
no RNA, no infectivity

Our current view of intra-quasispecies interactions and lethal mutagenesis

- ***Positive interactions (complementation) and negative interactions (interference) occur wihin the viral quasispecies. In well adapted mutant distributions, complementation dominates***

- ***With enhanced mutagenesis, negative interactions (interference) operate, and contribute to a decrease of infectivity. Infectivity loss precedes replication collapse, probably due to the involvement of more functions in infectivity than in genome replication***

- ***As mutagenesis progresses, the proportion of genomes with interfering mutations and lethal mutations increases, leading eventually to the complete replicative collapse and virus extinction***

- ***The transition into error catastrophe does not occur through "evaporation" into the entire sequence space. This is physically impossible. A "phenotypic" or "extinction" threshold intervenes prior to the classical genotypic "error" threshold. This is an obvious extension of "error catastrophe" theory to real viruses***

Prospects of lethal mutagenesis as an antiviral therapy

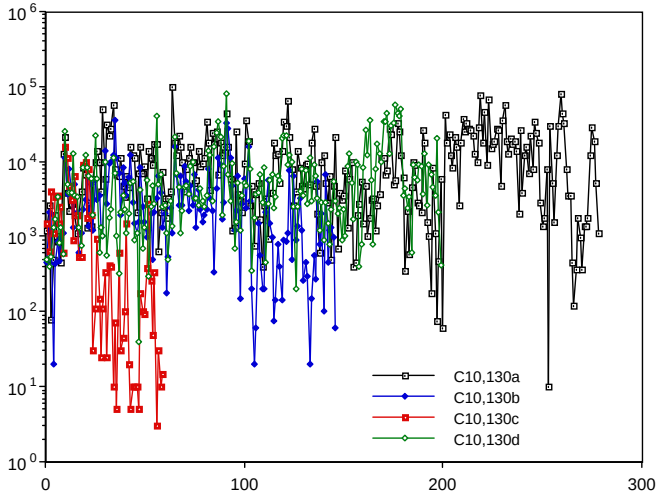
- ***An increasing number of specific mutagenic nucleoside analogues are under study. Links with anti-cancer chemotherapy***

- ***A clinical trial (phase 1b) was initiated in 2005 with about 40 HIV-1-infected patients who failed HAART. Phase 2 scheduled for 2007. It involves administration of a new nucleoside analogue KP-1461 (Koronis Pharmaceuticals Inc., Redmond, WA, USA)***

References

- ***Sierra et al. (2000) J. Virol 74: 8316-8323***
- ***Ferrer-Orta et al. (2007) Proc. Natl. Acad. Sci. 104: 9463-9468***
- ***Perales et al. (2007) J. Mol. Biol. 369: 985-1000***
- ***Escarmís et al. (2008) J. Mol. Biol. 367: 367-379***

CBMSO



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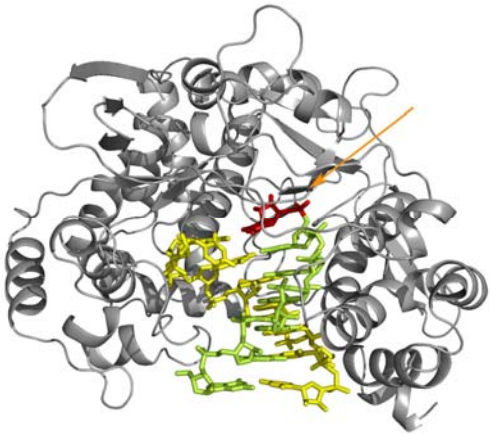

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