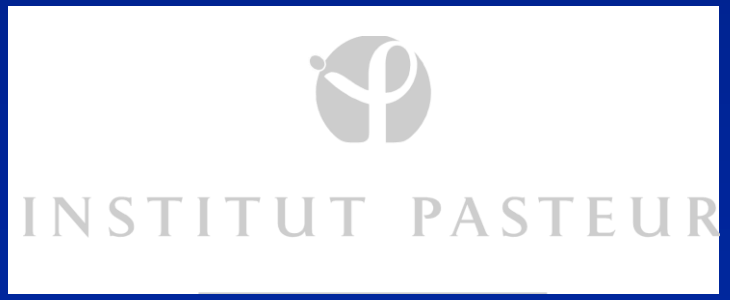
Perspectives in rabies therapies

Noël TORDO, Paris

Working together to stop the ongoing tragedy of rabies!

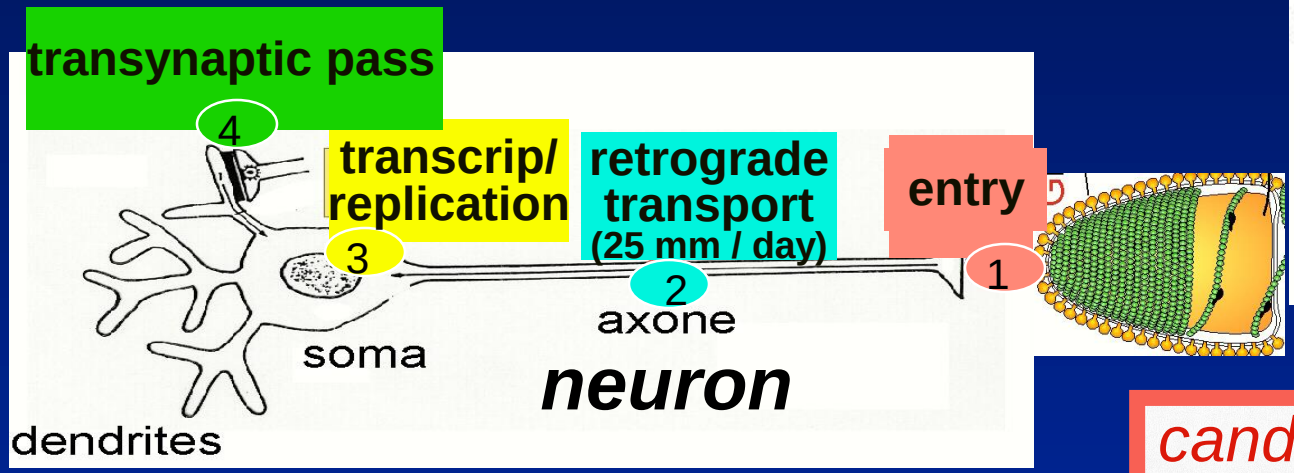
Make rabies history !

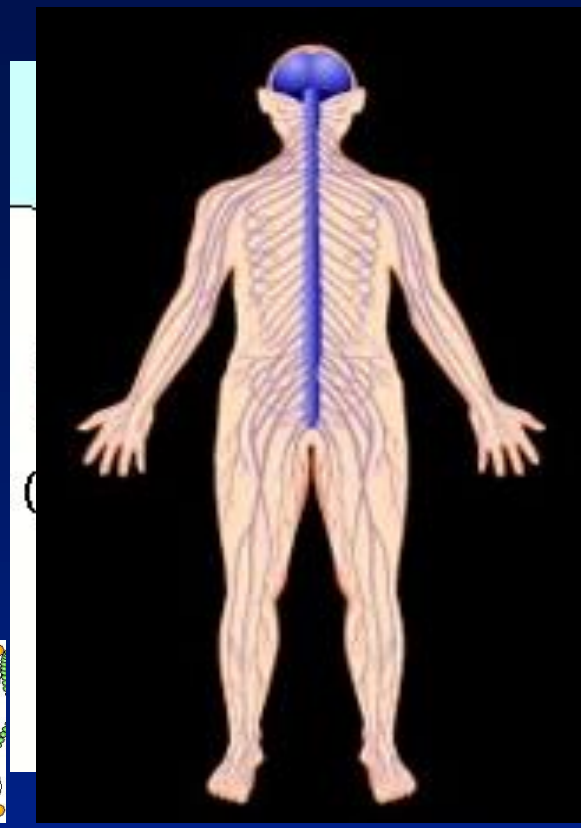


Rabies: a neurotropic virus

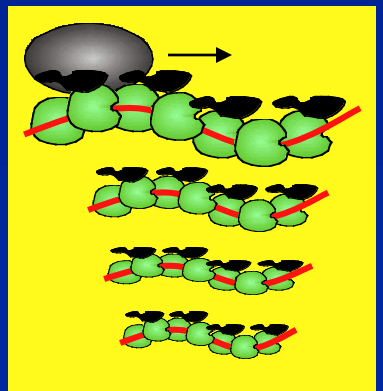
LONG INCUBATION PERIOD: 2 months (2 w / 7 y)

- peripheral inoculation (muscle, ...)
- nerve endings (neuro-muscular junction)
- from neurone to neurone up to CNS

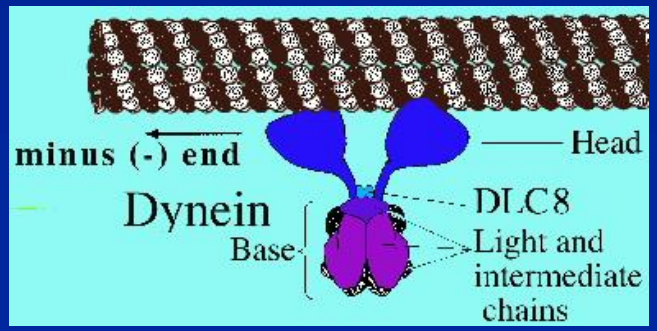




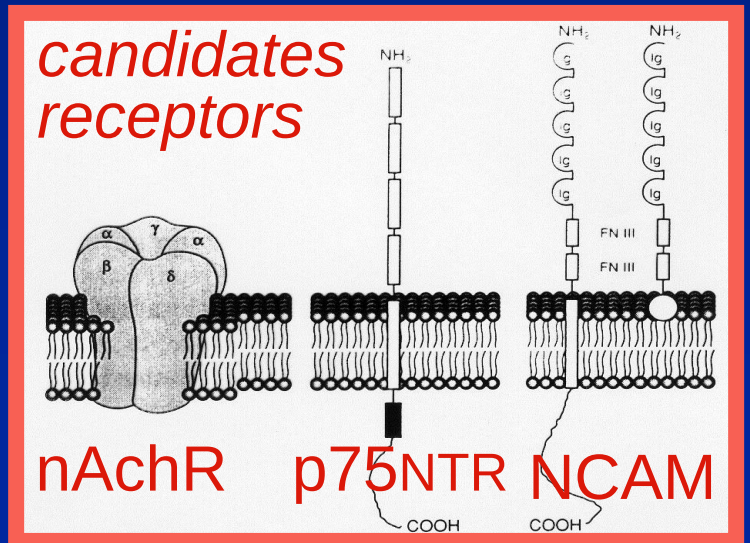
replication



microtubule based motors



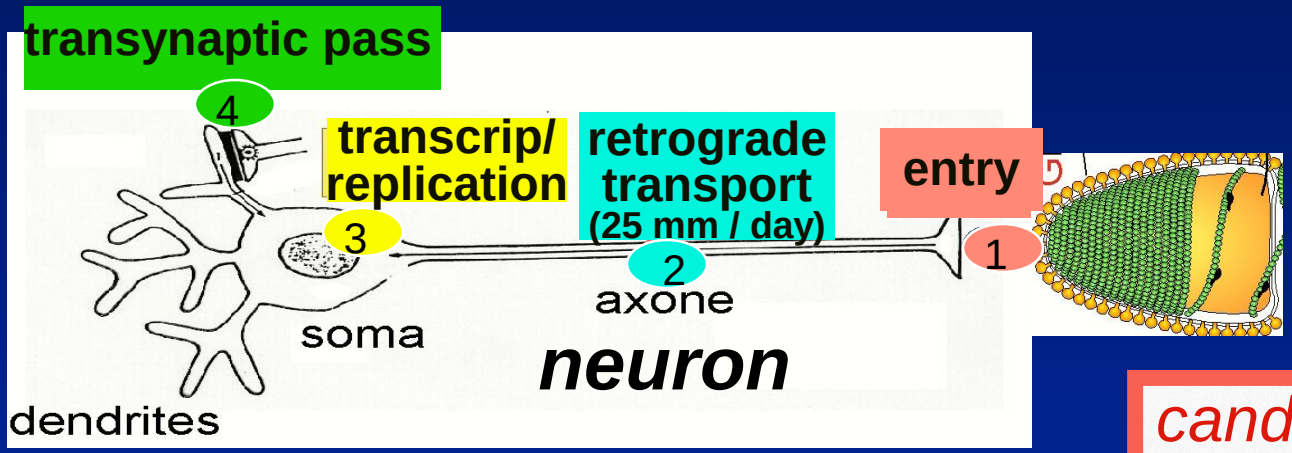
candidates receptors

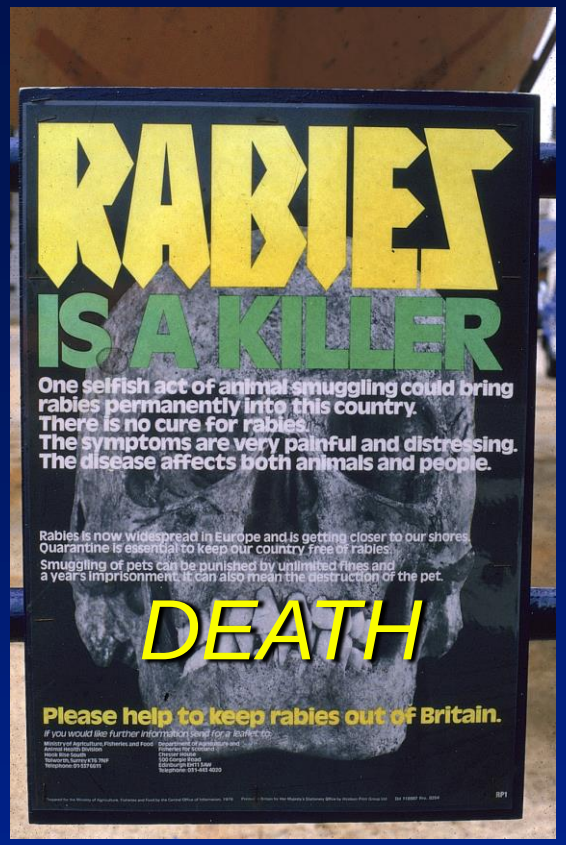


Rabies: a neurotropic virus

SHORT SYMPTOMATIC PERIOD (1 week)

- neuronal dysfonctions (neurotransmitters, ...)
- to non-neuronal tissues (salivary glands)
- transmission (bite, aerosol)





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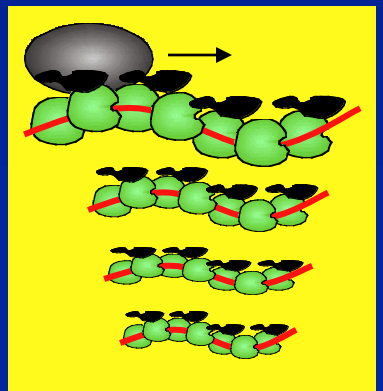

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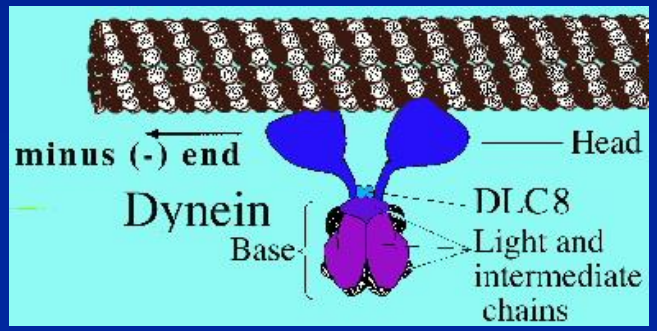
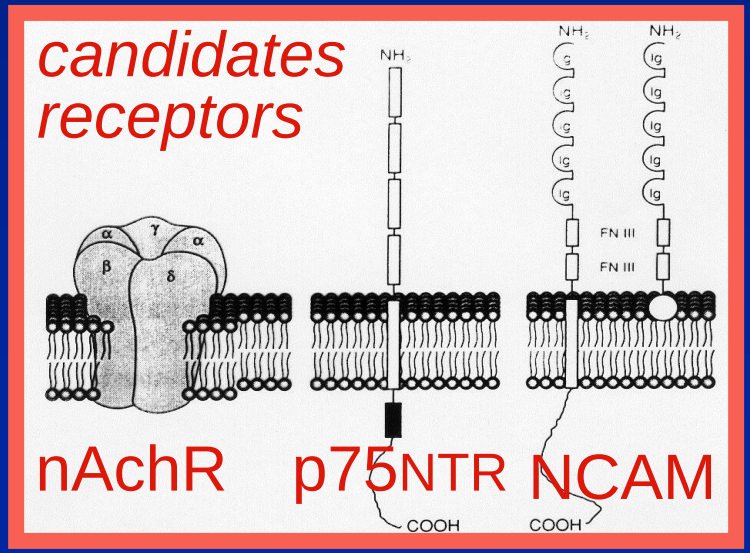




Tools for prevention/therapy

Pasteur's vaccine

rabid rabbit spinal cord
-> dessiccated

in 2015

human vaccines (prevention + therapy)

Not recommended by WHO

- cell culture: safe + efficient (expensive ?)

animal vaccines (prevention)

- nervous tissue (injection)
- cell culture (injection)
- attenuated/recombinant (oral, wildlife)

No efficient antiviral

Rabies and antivirals, an empirical arsenal

	in vitro	*animal*	*human*
• α-interferon: *Weinmann & al 1979 Infect Immun **24**, Merigan & al 1984 Ann Neuro **16*** *Warrell & al, 1989 Br Med J **299***		*monkey*	
• ribavirin (purine analogs, AraC): *Bussereau & al 1983 Ann Virol (I. Pasteur) **134**; 1988 Acta Virol **32*** *Warrel & al 1989 Br. Med. J. **299***		*mouse,fox*	
• interferon & vidarabine: *Dolman & Charlton 1987 Can J Neurol Sci **14***			
• ketamine (antagonist NMDA receptor): *Lockhart & al 1992 Antimic. Agents Chemother. **36*** *1991 Antiviral Chem Chemother **2**:9-15*		*rat stereotax*	
• heteropolyanions: *Pepin & Blancou 1985 Archiv. Virol **83***		*fox*	
• corticosteriods: *Enright et al 1970 Can J Microbiol **16***		*mouse*	
• amantadine, rifampicin, cinnabarin, chloroquin, neurotrophin, cholchicin, vinblastin, ascobic acid:			
• natural / semi-synthetic polymers, phenolic compounds plant extracts (red beans from South-America) :			
• antisens oligonucleotides, siRNA:			

Survival after treatment of rabies with induction of coma

(Willoughby et al. 2005, N. Eng. J Med 352:2508-14)

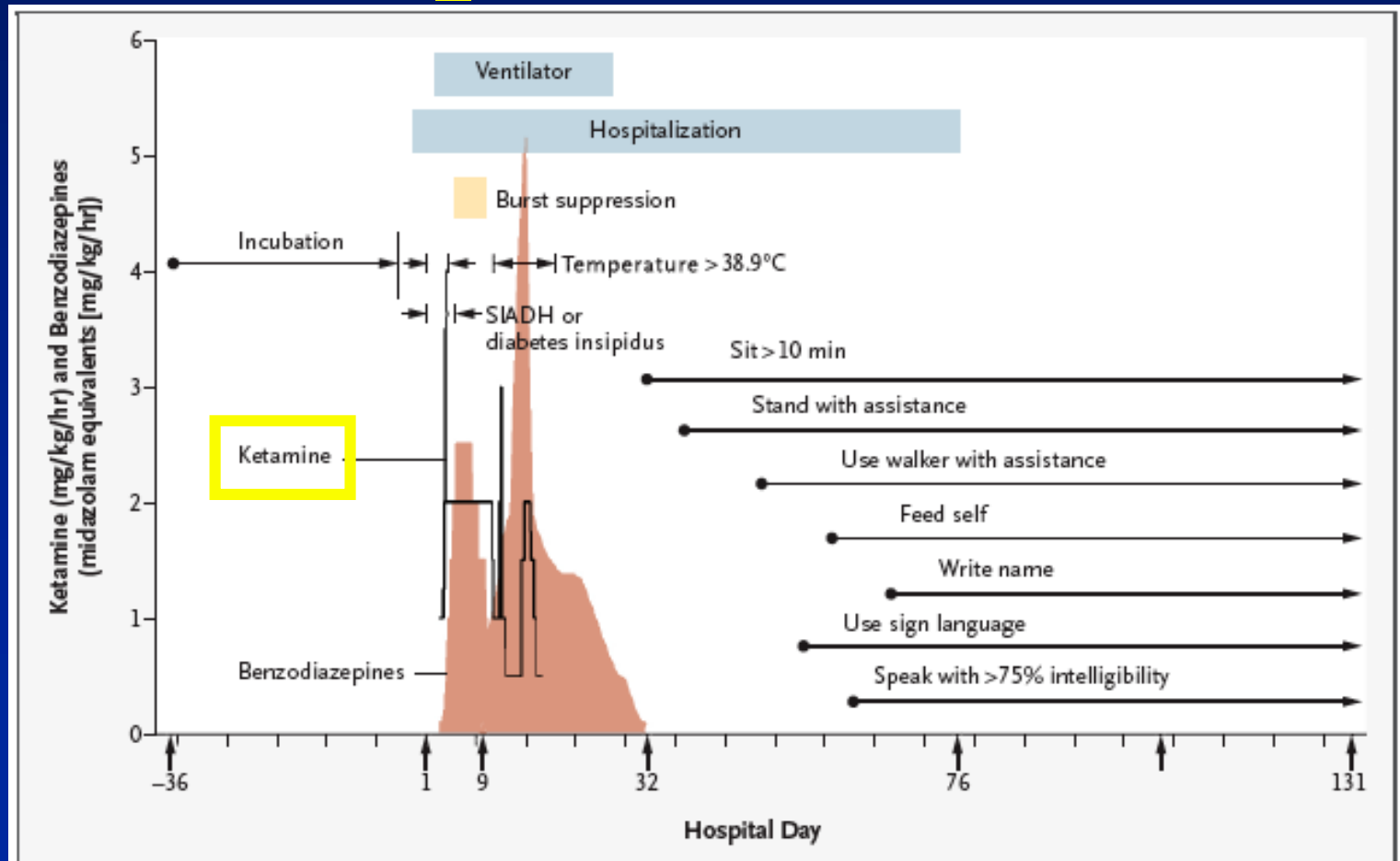
Scholar-athlete aged 15
Future veterinarian
Picked up downed bat
Laceration L index finger

No post-exposure prophylaxis

One month incubation

Amantadin

Ribavirin



Rabies and antivirals

Advantages

- No existing therapy (vaccine and RIG are « preventive »)
- long incubation period (2 weeks to 7 years: 2 months)
- increased interest (Millwaukee protocol)

Disadvantages

- tropism for the neurone (difficult to reach)
- neglected disease (poor countries)

Development of large spectrum antivirals targeting commom targets to negative strand RNA viruses

Two main strategies to find anti-rabies molecules

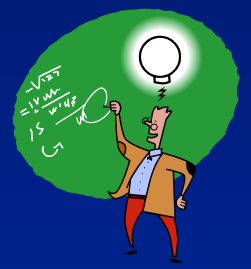
Combinatory approach

High-throuput screening assays on libraries of molecules

- *compounds*
- *siRNA*
- *peptides…*

Cognitive approach

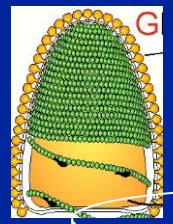
Design of molecules based on known functional interactions

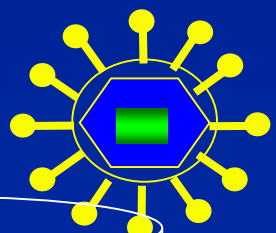


Lentivirus vector encoding GFP/Luc

Rabies G-protein

Rabies virus





Candidates molecules

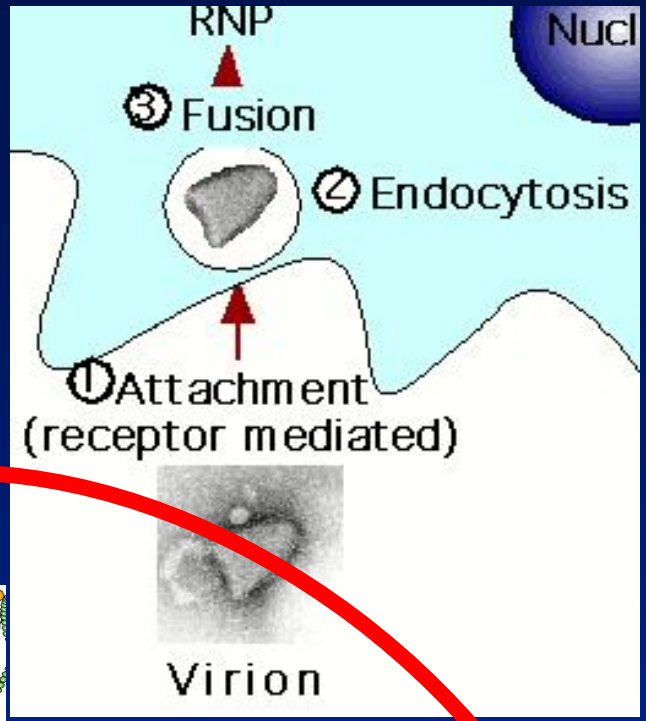
Validation by in vitro tests

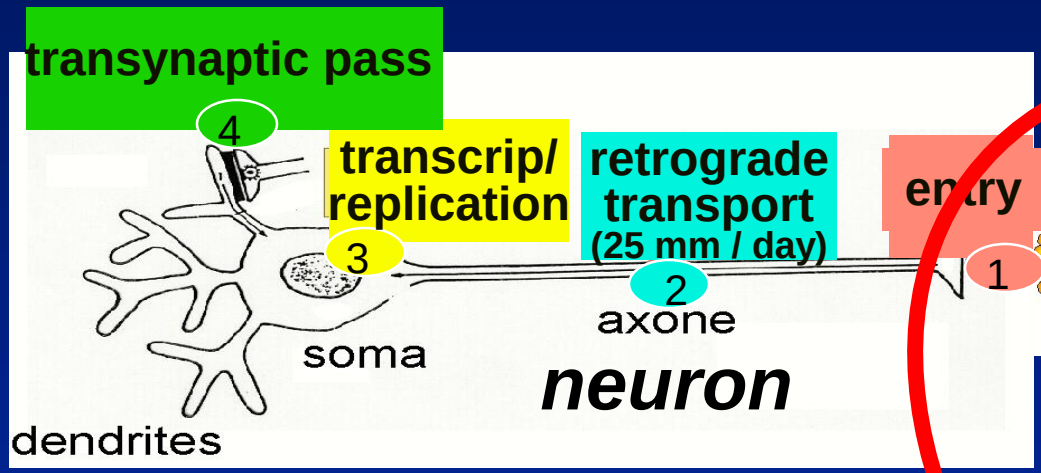
- Inhibition of rabies infection
- Inhibition of entry assay
- Inhibition of a minireplicon (replication)

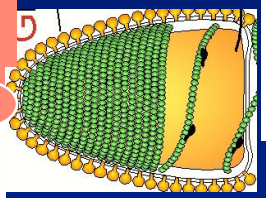
Inhibition of entry

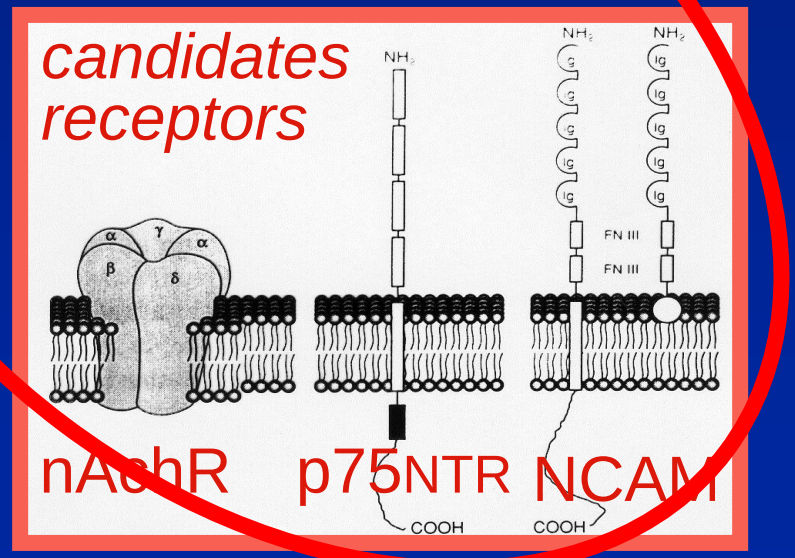
LONG INCUBATION PERIOD: 2 months (2 w / 7 y)

- peripheral inoculation (muscle, ...)
- nerve endings (neuro-muscular junction)
- from neurone to neurone up to CNS

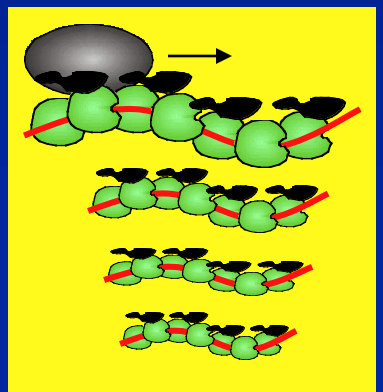




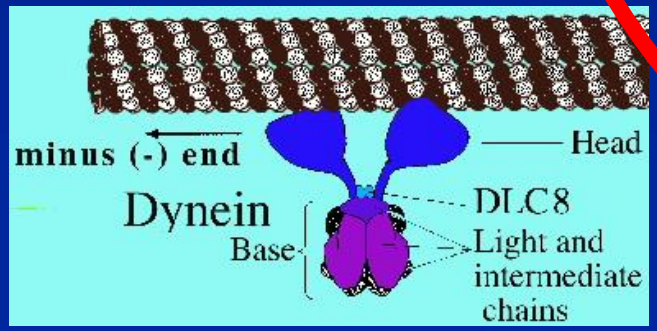




replication



microtubule based motors



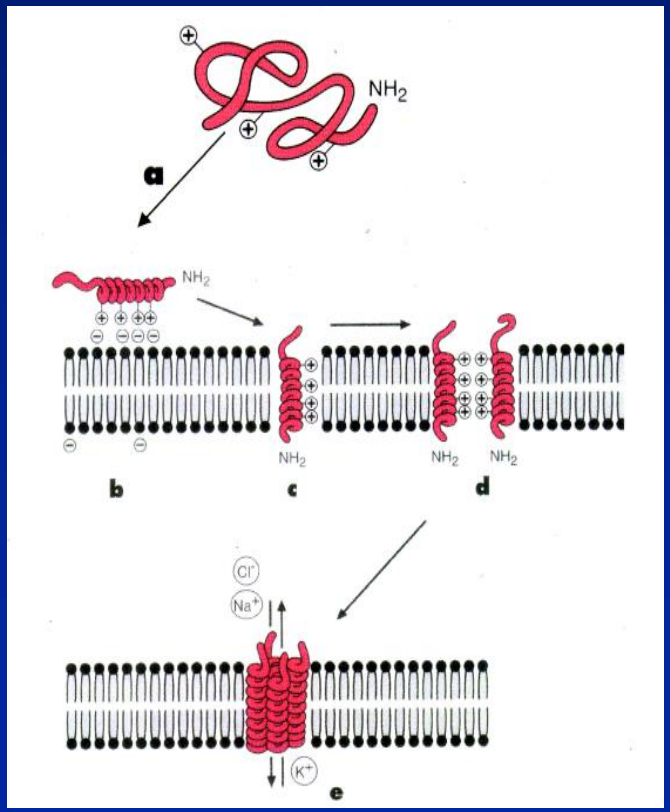
Rabies virus entry as a target : Dermaseptins

Dermaseptins

- *cationic peptides*
- *30 aa long, rich in lysine*
- *form amphiphilic α helix*
- *destabilize / disrupt membrane*

Antimicrobial potential

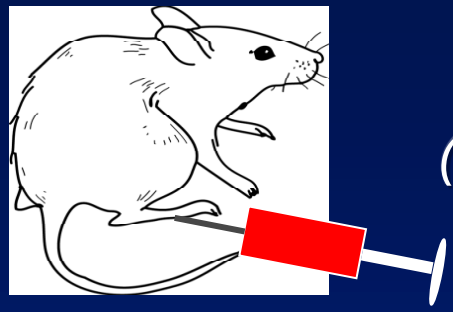
- **Bacteria** *(Navon-Venezia & al, 2002)*
- **yeast** *(Coote & al, 1998)*
- **protozoan** *(Brand & al, 2002)*
- **Enveloped Viruses**
  - **HSV** *(Belaid & al 2002)*
  - **HIV** *(Lorin & al 2005)*



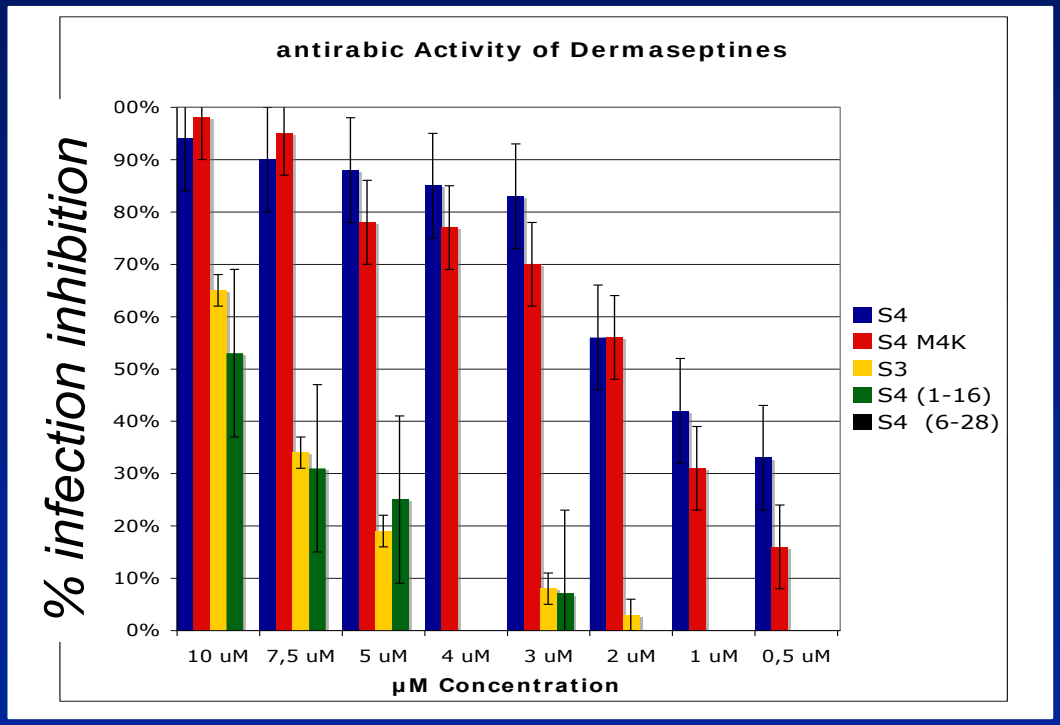
S1	A L W K T M L K K L G T M A L H A G K A A L G A A A D T I S Q G T Q
S1	A L W F T M L K K L G T M A L H A G K A A L G A A A N T I S Q G T Q
S3	A L W K N M L K G I G K L A G K A A L G A V K K L V G A E S
S4	A L W M T L L K K V L K A A A K A A L N A V L V G A N A
$S4_{M4K}$	A L W K T L L K K V L K A A A K A A L N A V L V G A N A
$S4_{1-16}$	A L W M T L L K K V L K A A A K - - - - - - - - - - - -
$S4_{6-28}$	- - - - - - L L K K V L K A A A K A A L N A V L V G A N A
S5	G L W S K I K T A G K S V A K A A A K A A V K A V T N A V

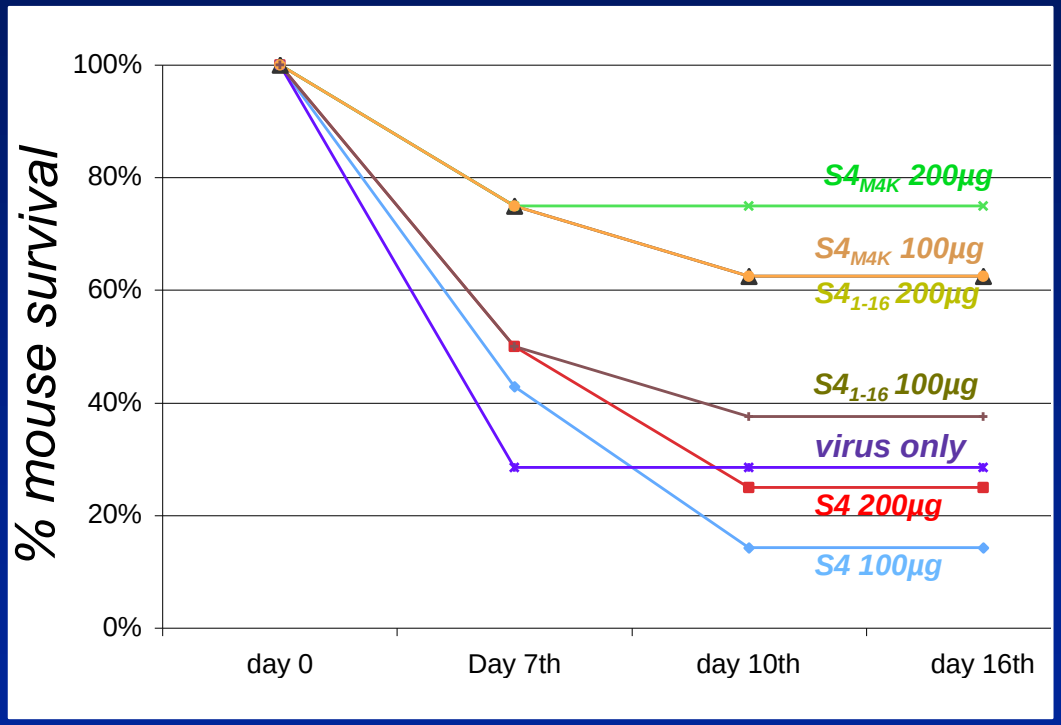
Anti-rabies activity of dermaseptins

in vitro
(co-infection)



in vivo
*(1h **post-infection** same site)*
10 mice/group





$S4 / S4_{M4K} > S3 / S4_{1-16} >> S4_{6-26}$

- *AntiRABV activity mostly in pos. 1-5*
- *Lysine in position 4 is important*

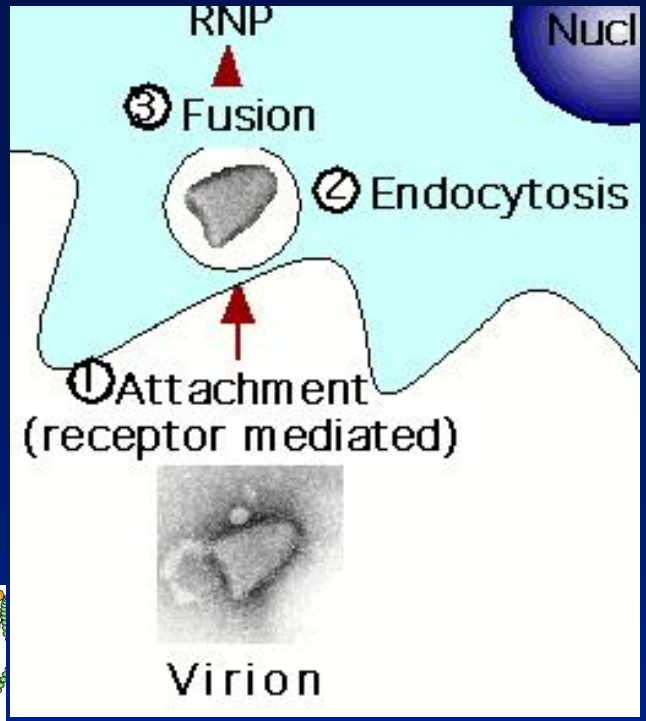
$S4_{M4K} > S4_{1-16}$

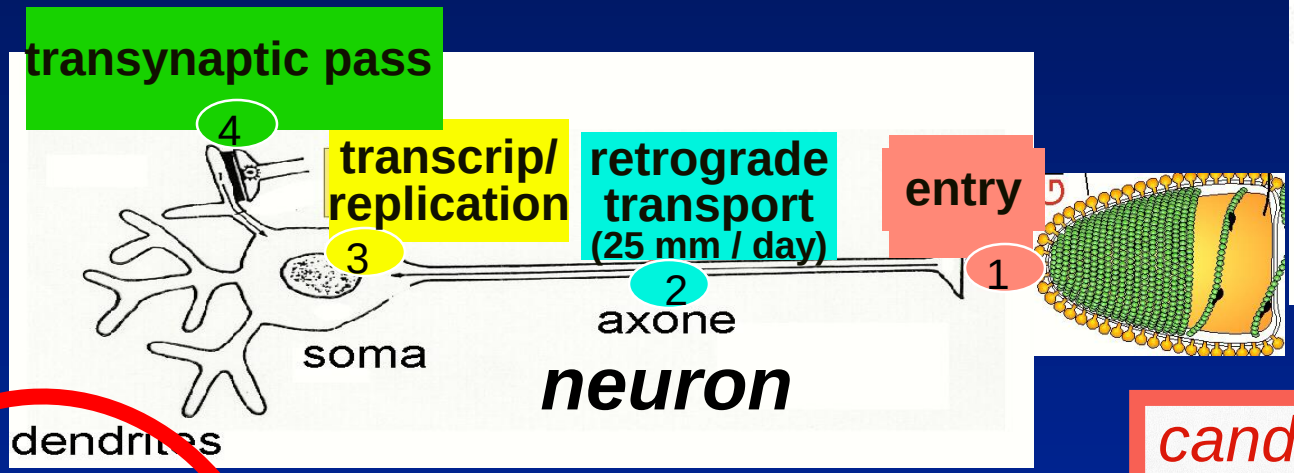
- *S4 not efficient post-exposure (size ? stability ? penetration ?)*

Inhibition of replication

LONG INCUBATION PERIOD: 2 months (2 w / 7 y)

- peripheral inoculation (muscle, ...)
- nerve endings (neuro-muscular junction)
- from neurone to neurone up to CNS





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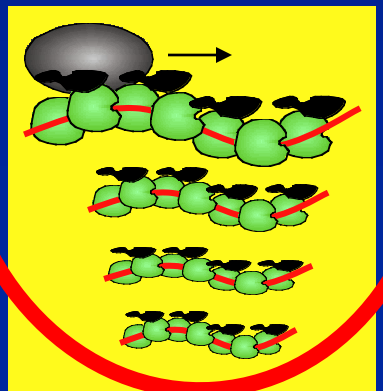
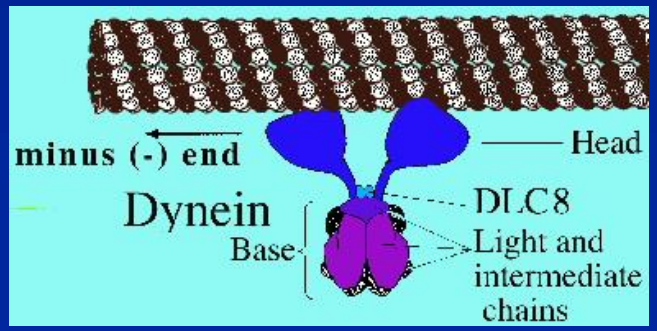
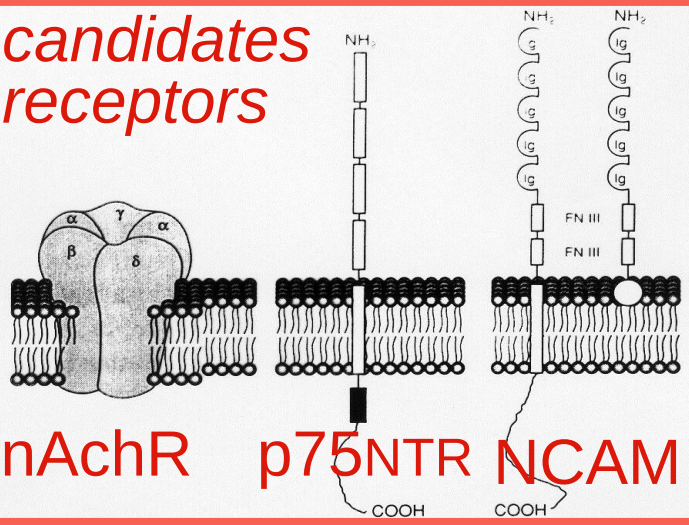


microtubule based motors



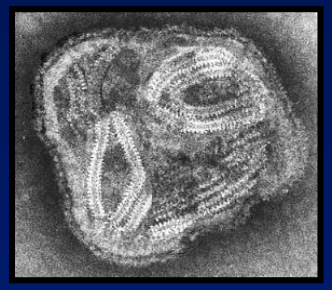
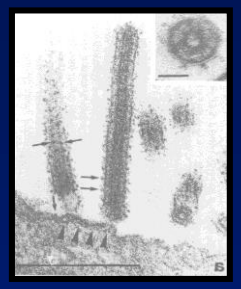
candidates receptors



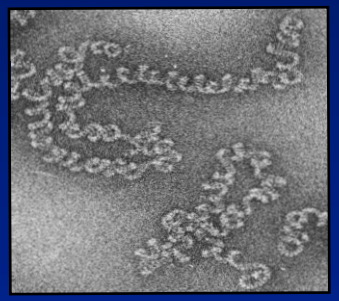
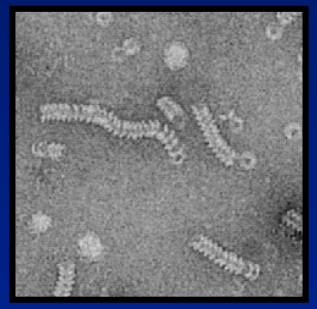
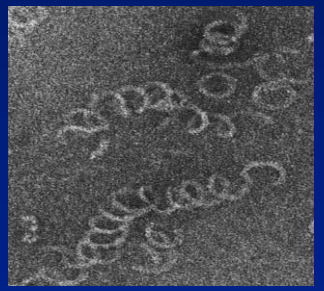
A similar transcription/replication complex (RNP)

Rabies v. *Measles v.* *Ebola v.*

virion

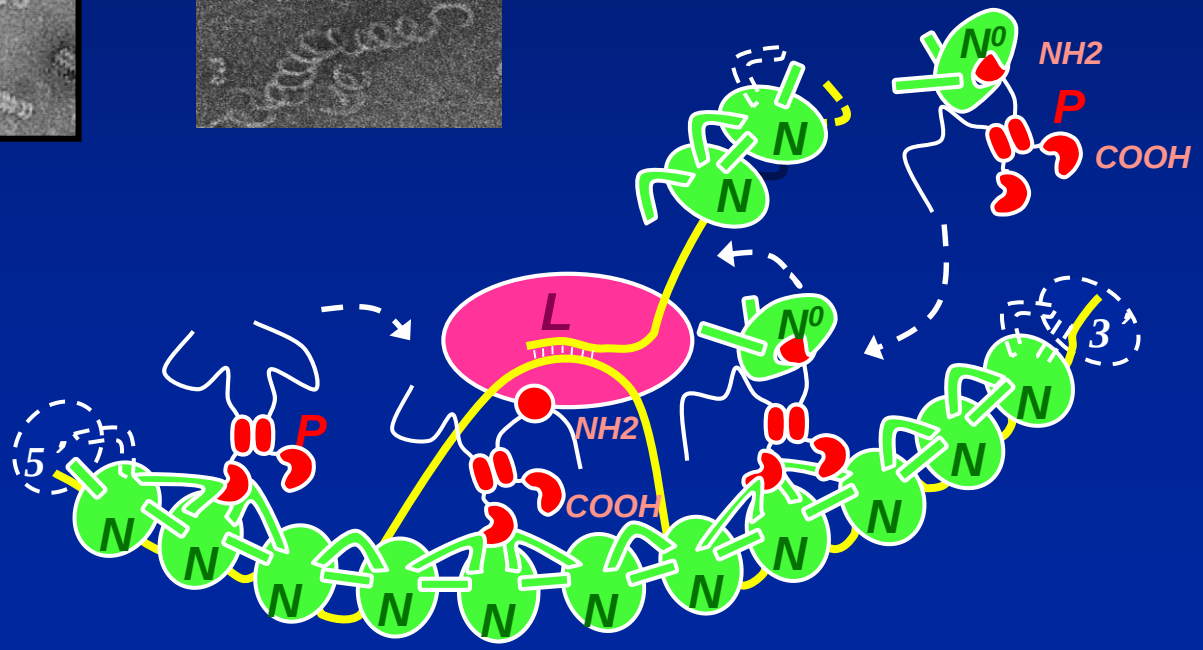




RNP

Rabies RNP

Leyrat et al. PLoS Pathog. 2011:e1002248



Template

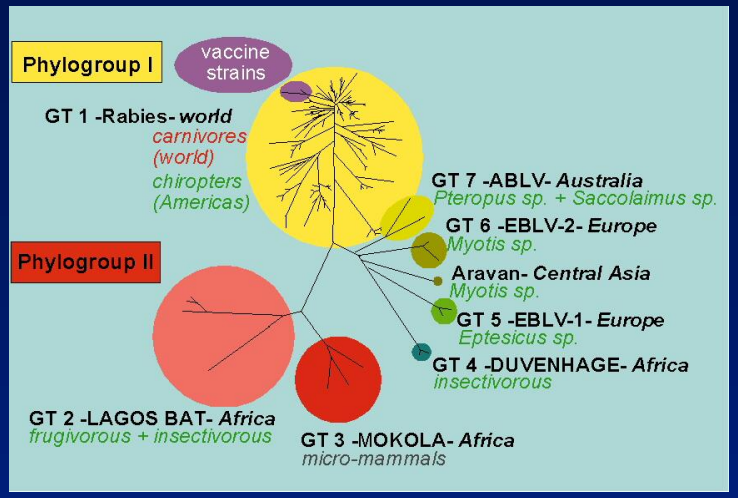
- RNA genome (-)
  + nucleoprotein N

Enzymes

- phosphoprotein P (cofactor)
- RNA polymerase L

Exemple 1: Peptides targeting the RNP complex

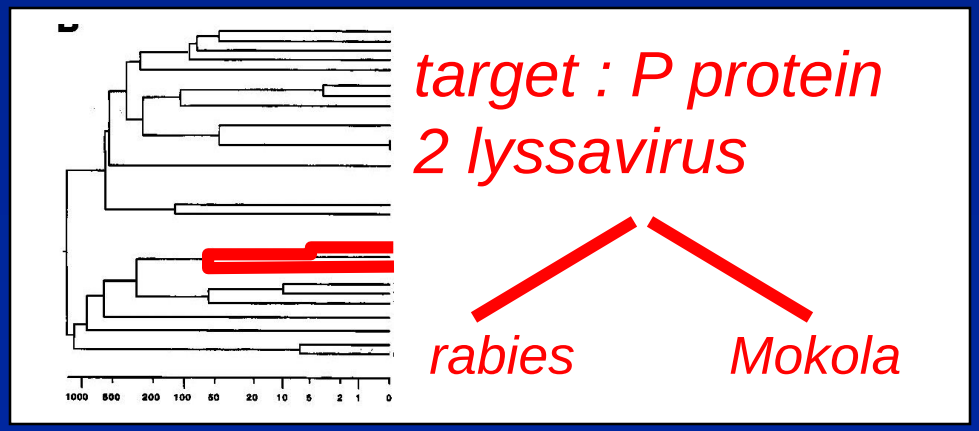
Combinatory approach



Screening of random peptide libraries for their affinity to the RNP

Candidate peptides

- 2-hybrid (Real et al. 2004)



Functional tests :

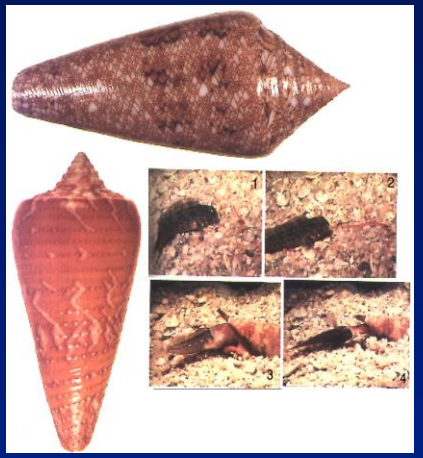
-Inhibition of a minireplicon (RNP)

-Inhibition of viral infection

combinatory library of auto-contraint peptides in *S. cerevisiae (2-hybrid)*

Toxins: conotoxins (mollusques); defensins (mammals)

constraint through disulfures bridges (cysteines)



Cys library (26 a.a) $1x10^7$ independent peptides

C-2x-C-5x-C-6x-C-5x-C-2x-C- Gal4 AD

Antimicrobial properties: lebocines, apidaecines (insects)

conformational constraint through prolines (turns)

Pro library (29 a.a) $3x10^7$ independent peptides

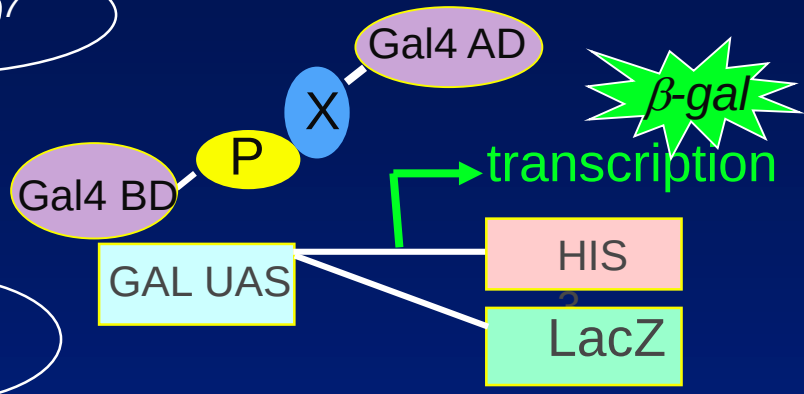
PP-5x-P-5x-PPP-5x-P-5x-PP - Gal4 AD

Selection steps *candidates*

peptide libraries — *8,55 10^7*

2-hybrid screening

P-Rab partners — *46.5% identity* — P-Mok partners — *755*

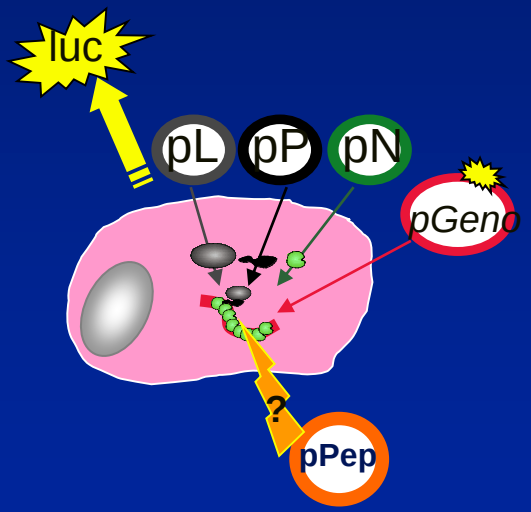


in silico analysis (phylogeny, groups) — *29*

inhibition of RNP transcription (minireplicon) — *11*

protein chip analysis (inhibition N/P interaction) — *8*

inhibition of cell infection — *4*



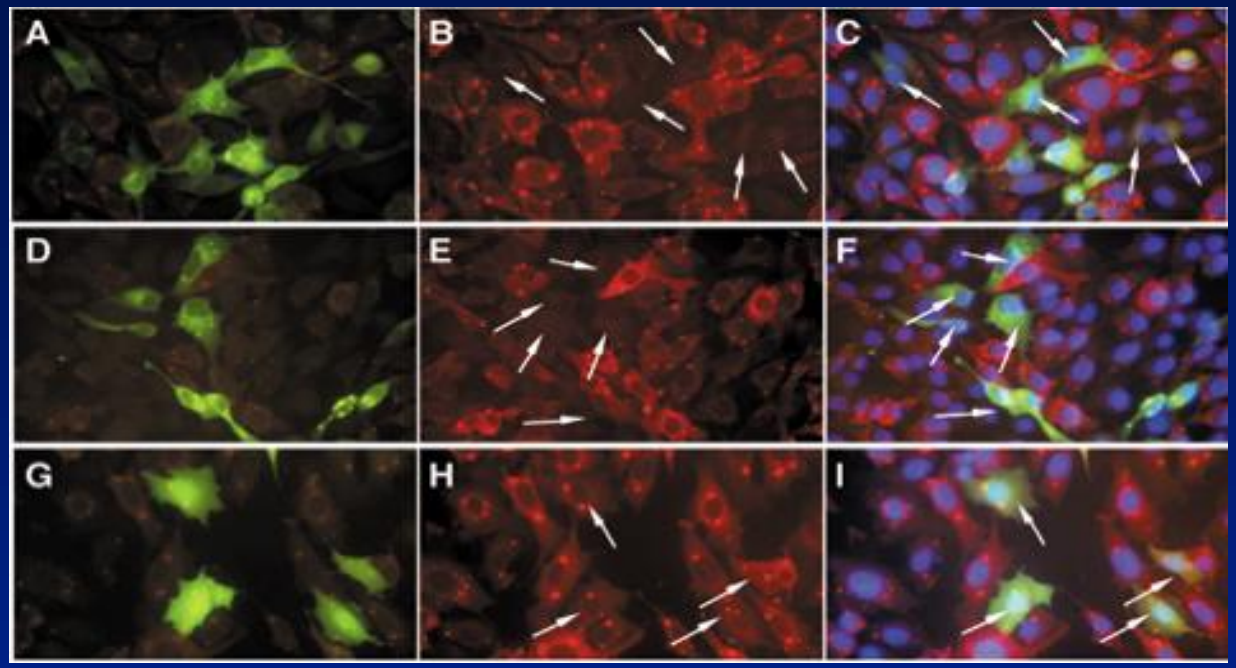
(Real et al., J. Virol 2004, 78: 7410-7)

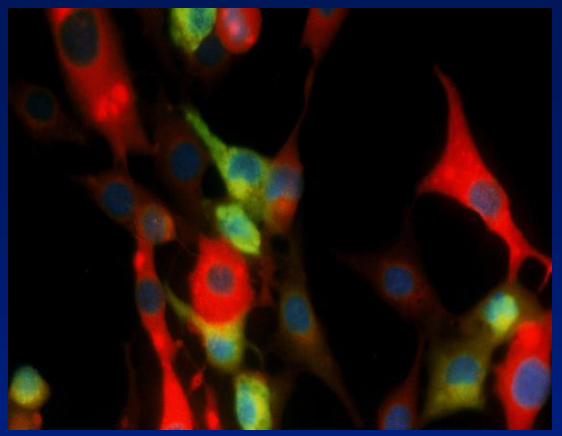
inhibition of Neuro-2A cell infection

C2-GFP

P16-GFP

GFP





Peptide	% inhibition infection	% inhibition replication (luciferase activity)
C2	**89 %**	**97.9 %**
C6	**83 %**	**97.2 %**
C8	**78 %**	**98.9 %**
P16	**71 %**	**96.3 %**

(Real et al., J. Virol 2004, 78: 7410-7)

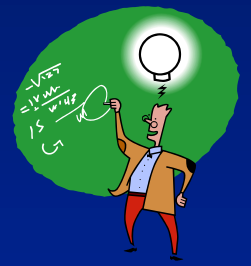
Exemple 3: Peptides targeting the RNP complex

Combinatory approach

Screening of random peptide libraries for their affinity to the RNP

Cognitive approach

Design of peptides based on known interactions in the RNP



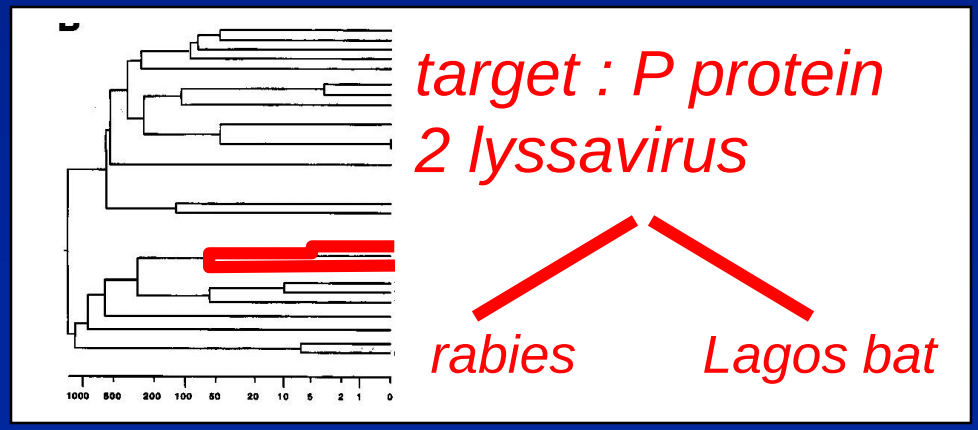
Candidate peptides

- *2-hybrid (Real et al. 2004)*
- *phage display (unpublished)*

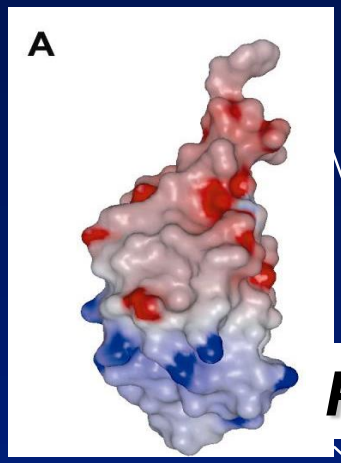
Functional tests :

-Inhibition of a minireplicon (RNP)

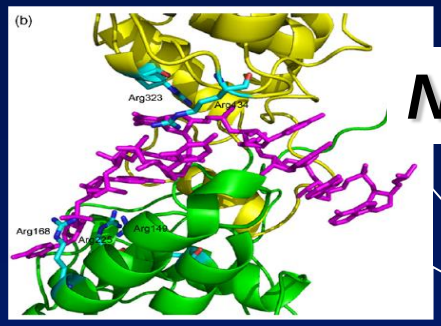
-Inhibition of viral infection



The rabies virus ribonucleocapsid as a target



P-N



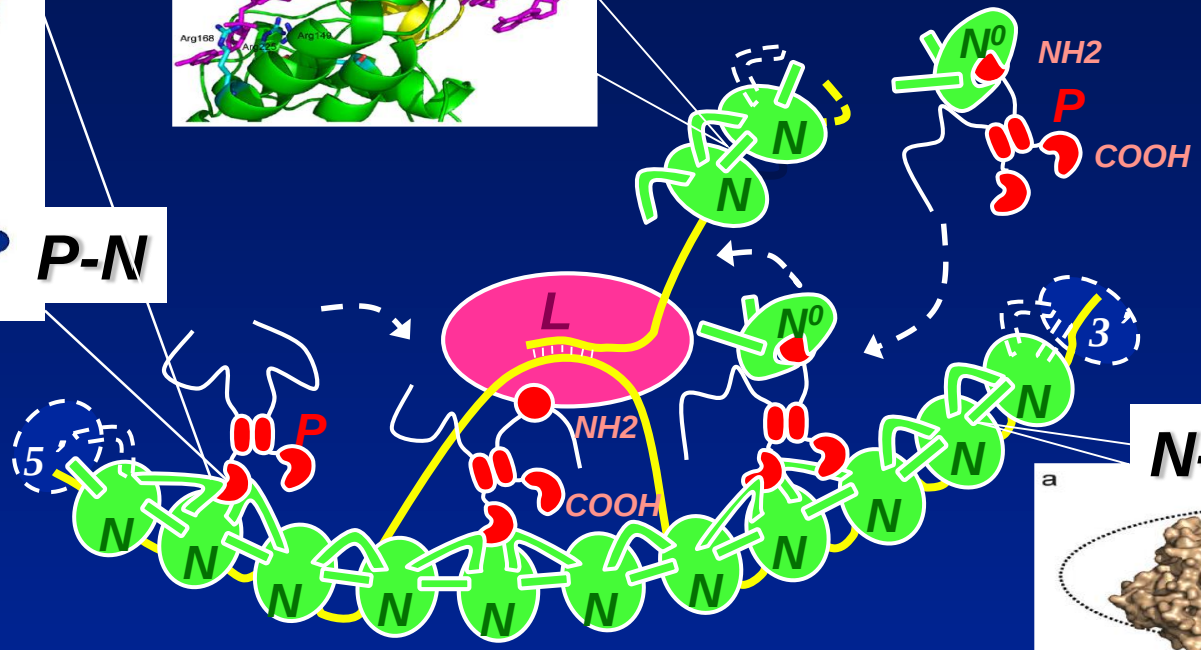
N-ARN

P - P dimers

P - N°

P - L

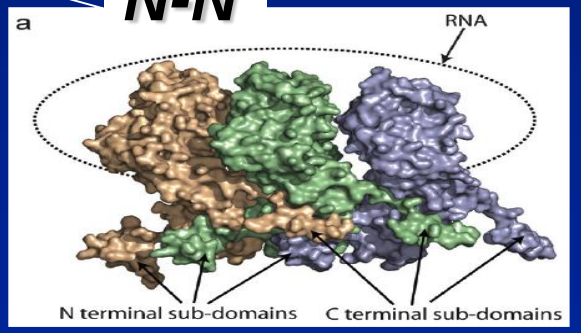
Leyrat et al.
PLoS Pathog.
2011:e1002248



Pos. 70-100

L

N-N



Pos. 4-42

N°

P60

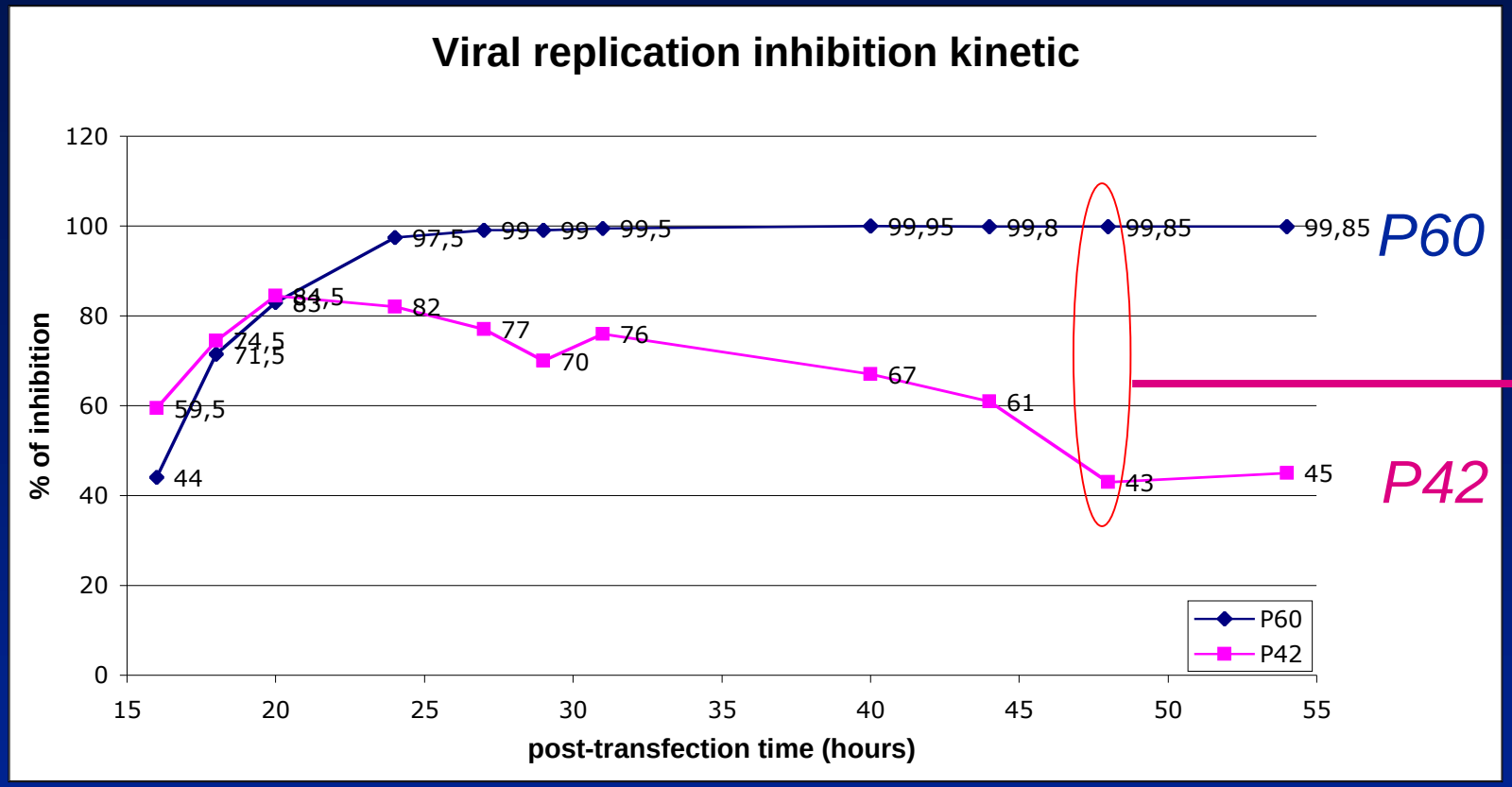
P42

Phosphoprotein

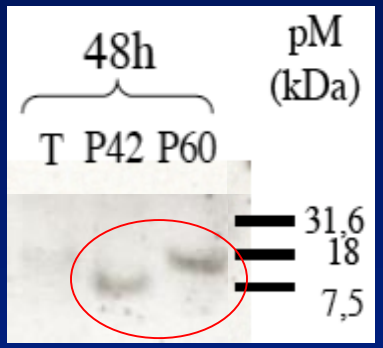
4 P-N° 40 70 P-L 100

1 297

Kinetic of inhibition of viral replication (minreplicon)



western blot



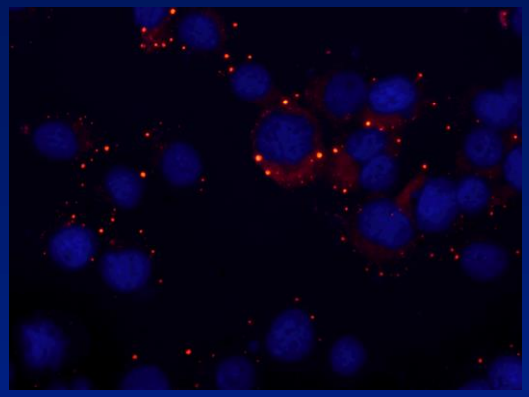
- P60 maintains its inhibitory effect up to 55h post-transfection
- the inhibitory effect of P42 progressively decreases after 24h

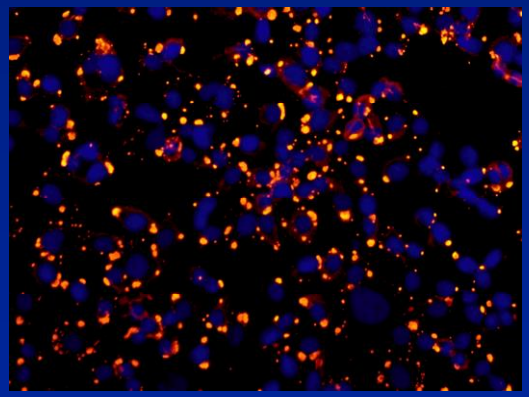
  → *not due to peptide degradation*

Castel et al, J Virol 2009, 83: 10808-20

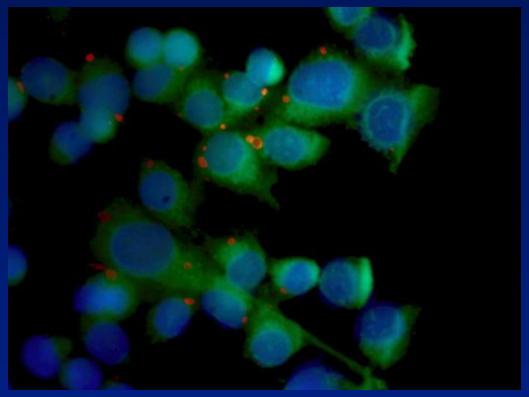
Inhibition of rabies virus infection by synthetic Tat-peptides

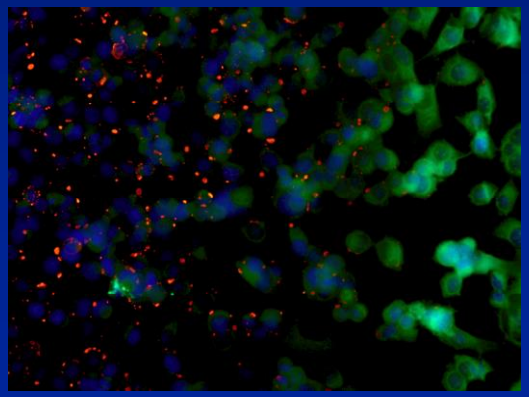
Control



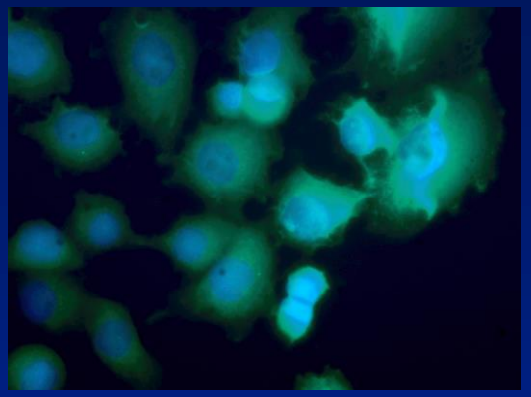


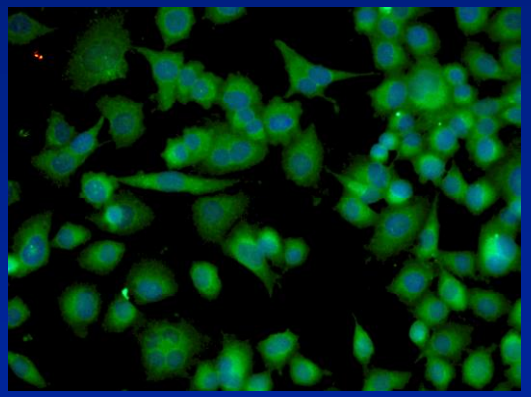
P42-Tat





P60-Tat





→ P42-Tat (≈60%) < P60-Tat (≈80%)

→ dose dependent effect

T=0 : Infection

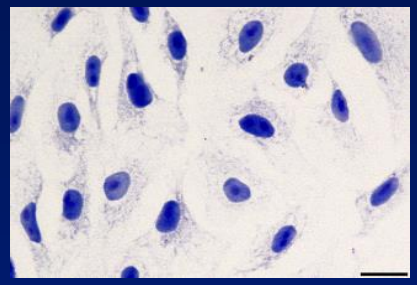
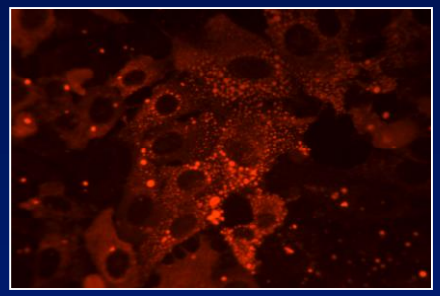
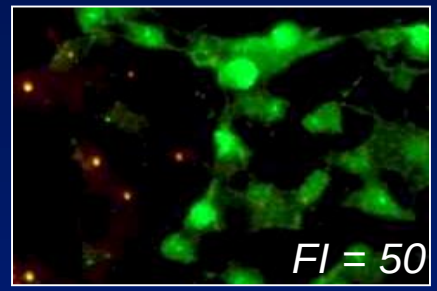
T=1h : 10μM peptide → T=14h

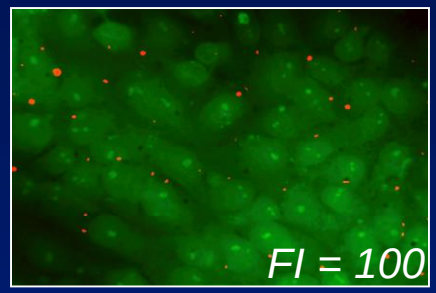
Castel et al, J Virol 2009, 83: 10808-20

Tat-peptides inhibit rabies virus infection in human neurons

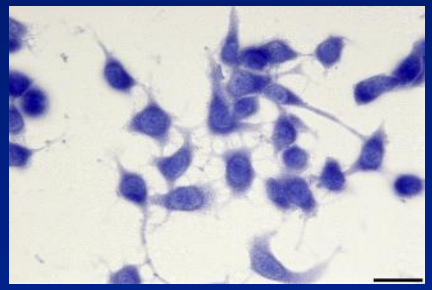
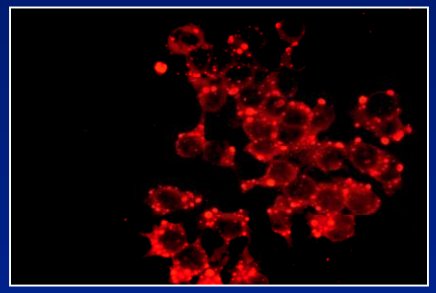
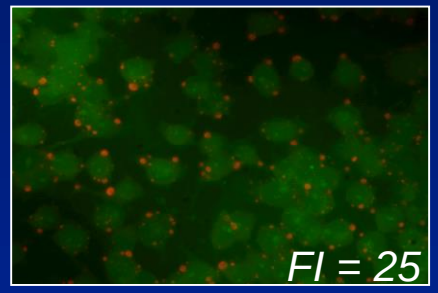
Infected cells | P42 | P1-20 + 20-40 + 40-60

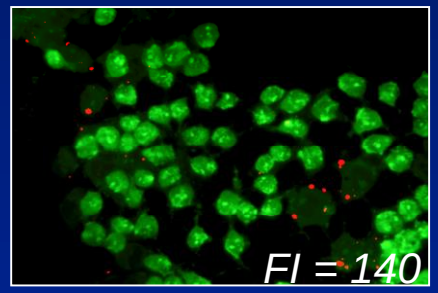
SK-N-SH neuroblastoma



SK-N-SH CP



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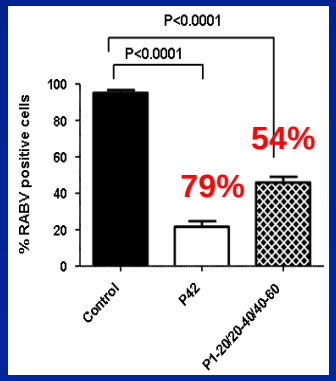

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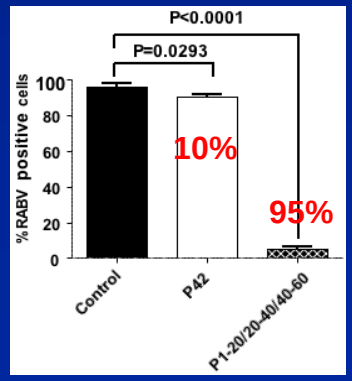
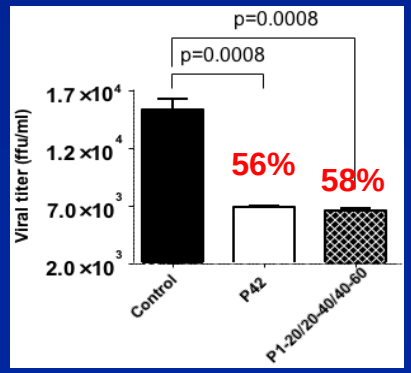


virus release

SK-N-SH



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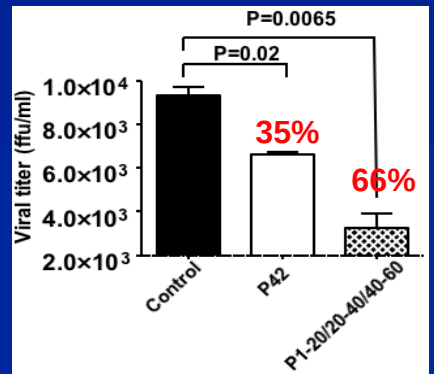
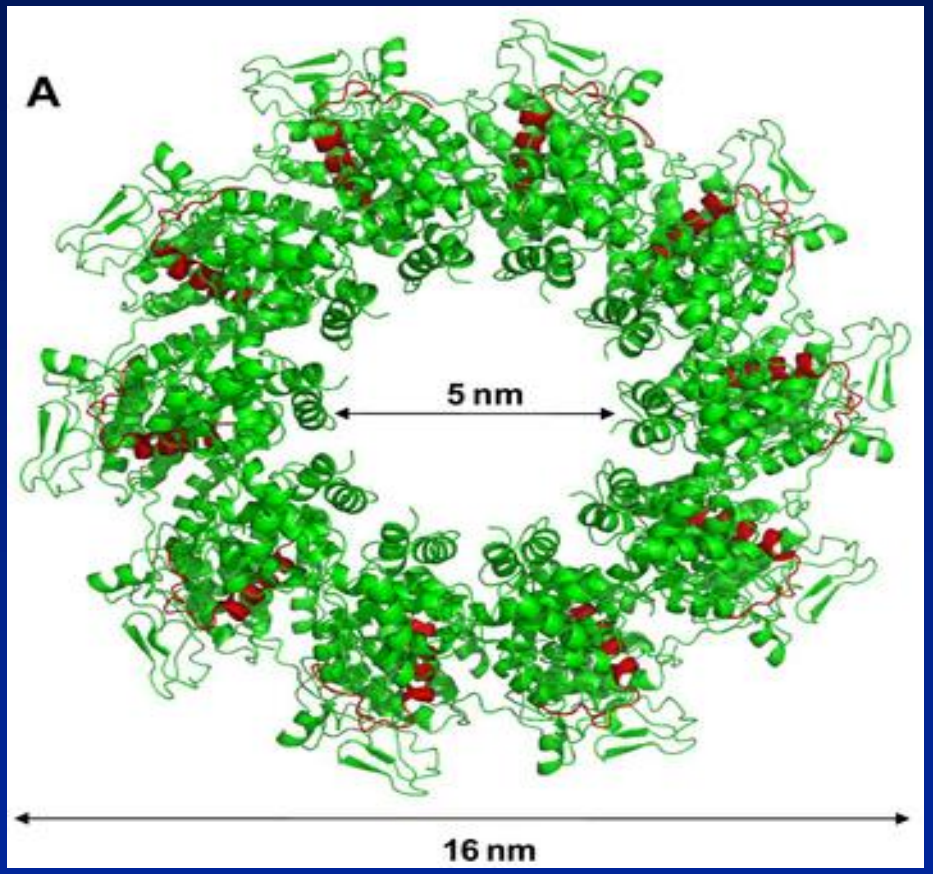
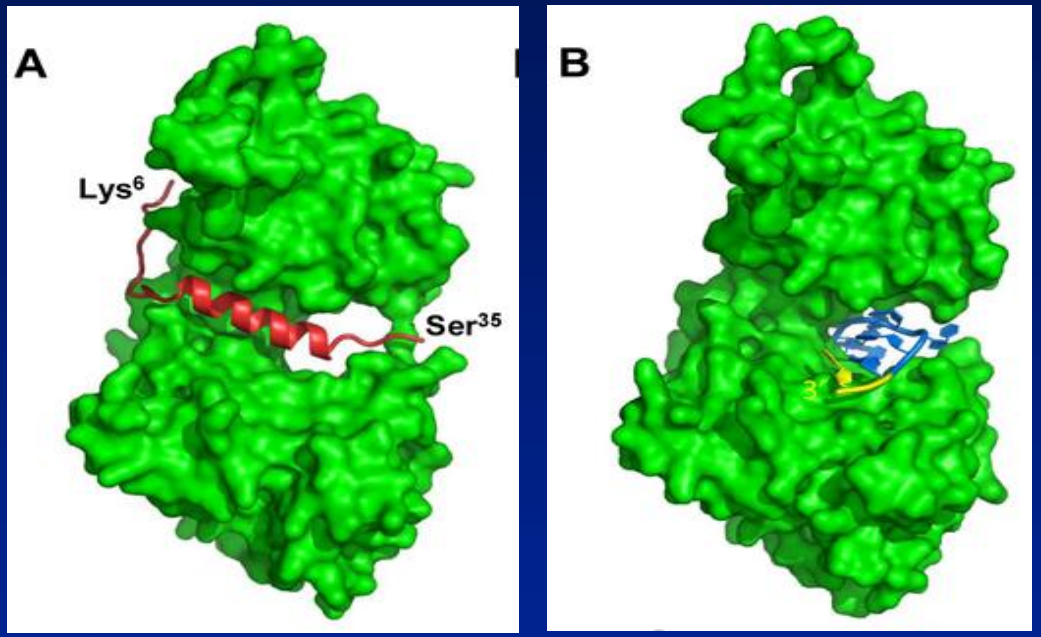


Castel et al, J Virol 2009, 83: 10808-20

Crystal structure of a decameric form of the NΔ210-P60 complex

N° -P60 N-RNA





P binding hinders RNA binding and self-assembly of soluble N°

Leyrat, et al. (2011). PLoS Pathog 7(9): e1002248

PLOS | PATHOGENS

Conclusions

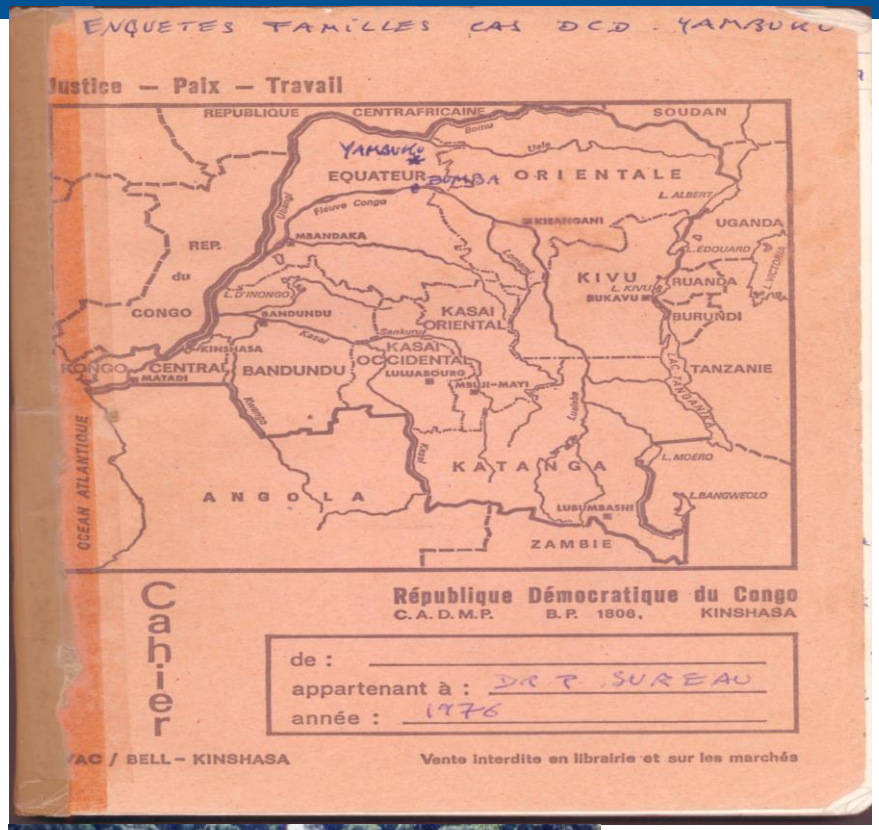
There is a need to develop rabies antivirals / therapies

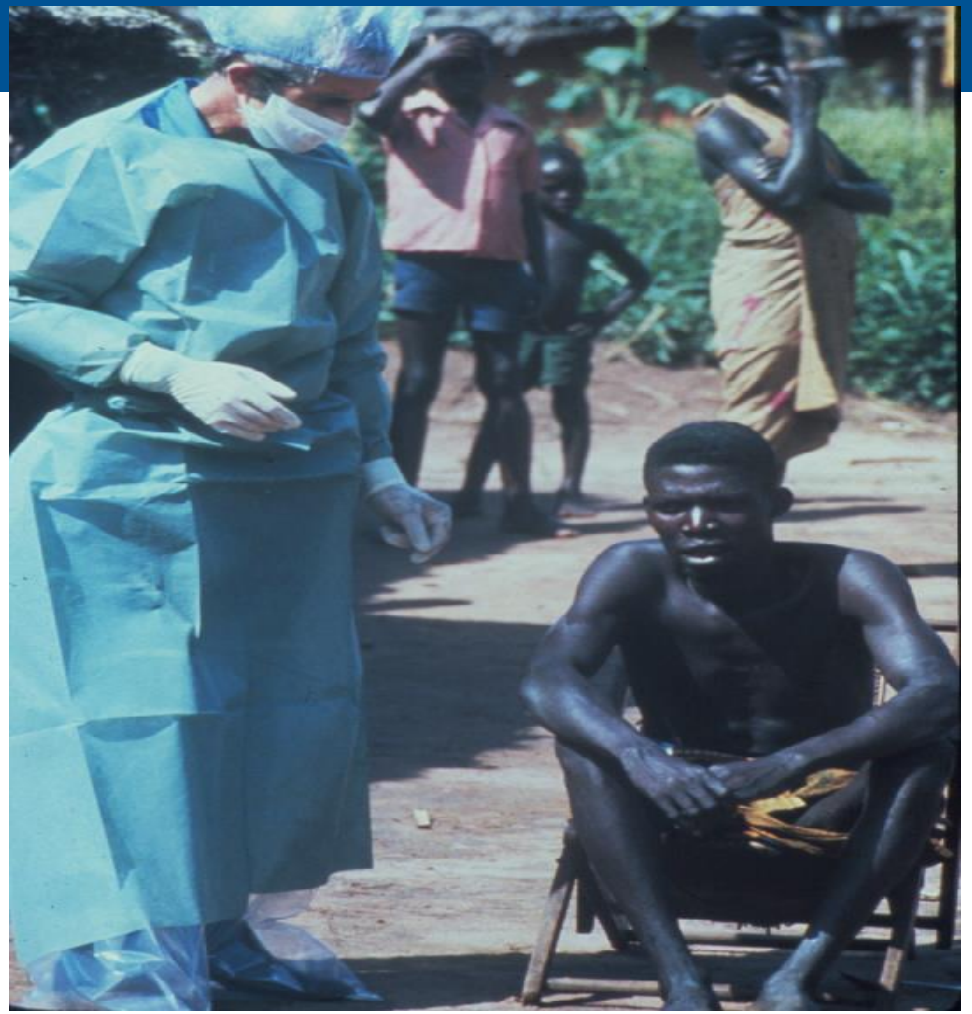
1. Post-exposure vaccination
   - → Shorten schedule (one week)
   - → Reduce vaccine volume required (ID rether than IM)

1. Replacing HRIG and ERIG (shortage, cost)
   - → Cocktail of human(ized) Mabs
   - → Dermaseptins

2. Developing large specturm (vaccines) antiviral strategies
   - → Random screening (molecules, peptides)
   - → Cognitive approach
     - ✓ Further dissection of interacting domains
     - ✓ Drug design : peptido-mimicry

Ebola Yambuku: 1976





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