Overcoming Plant Defense Mechanisms: Evolutionary Arms Race Between Plants and Viruses

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How do we, mammals, battle diseases?

Immune System

~ Kingdom *Plantae* ~

Lack immune system, yet thrive!

Overview of Plant Toxic Proteins

Family	Source	Structural Features	Biological Activity	References
Lectins	Ubiquitous in plants	One or more CRDs	Carbohydrate-binding activity	Van Damme et al. (2008), Van Damme (2014)
Ribosome inactivating proteins (RIPs)	Widely distributed	*N*-glycosidase domain	*N*-glycosidase activity	Peumans et al. (2001), Shang et al. (2014)
Protease inhibitors / α-Amylase inhibitors	Widely distributed, rich in storage tissues	N/A	Inhibition of protease / α-amylase	Leung et al. (2000), Murdock & Shade (2002), Svensson et al. (2004)
Urease and canatoxin-like proteins	Mainly in legumes	A 10 kDa region, with a β-hairpin motif	Ureolytic activity Pore-forming activity	Follmer et al. (2001), Barros et al. (2009)
Arcelins	Seeds of *Phaseolus* sp.	Legume lectin fold	N/A	Acosta-Gallegos et al. (1998), Zaugg et al. (2013)
Thionins	A number of monocot & dicot plants	~ 5 kDa cysteine containing proteins	Increase of cell membrane permeability	Stec (2006)
Cyclotides	Widely distributed	Cyclic cysteine knot	Pore-forming activity	Craik et al. (2012)
Pore-forming toxins	Some plants, e.g., *Enterolobium contortisiliquum*, wheat	Membrane-spanning region (β-barrel / α-helical)	Pore-forming activity	Bittencourt et al. (2003), Puthoff et al. (2005)

Dang, L. and Van Damme, E.J. (2015) *Phytochemistry* **117**, 51-64.

Various Plants Produce Ribosome Inactivating Proteins (RIPs)

Castor bean plant
(*Ricinus communis* L.)

Jequirity bean plant
(*Abrus precatorius* L.)

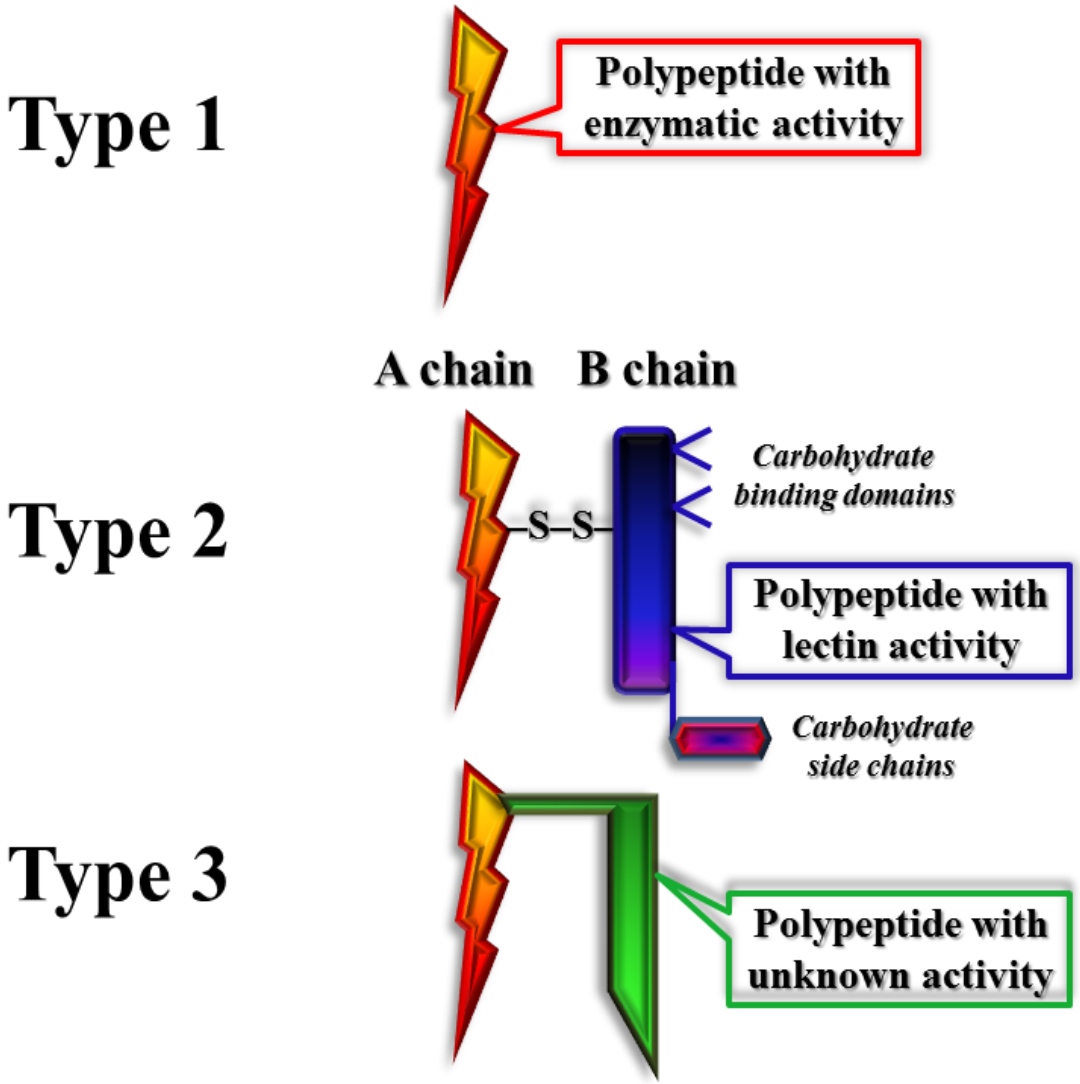
Common pokeweed
(*Phytolacca americana* L.)

Common soapwart
(*Saponaria officinalis* L.)

Corn
(*Zea mays* L.)

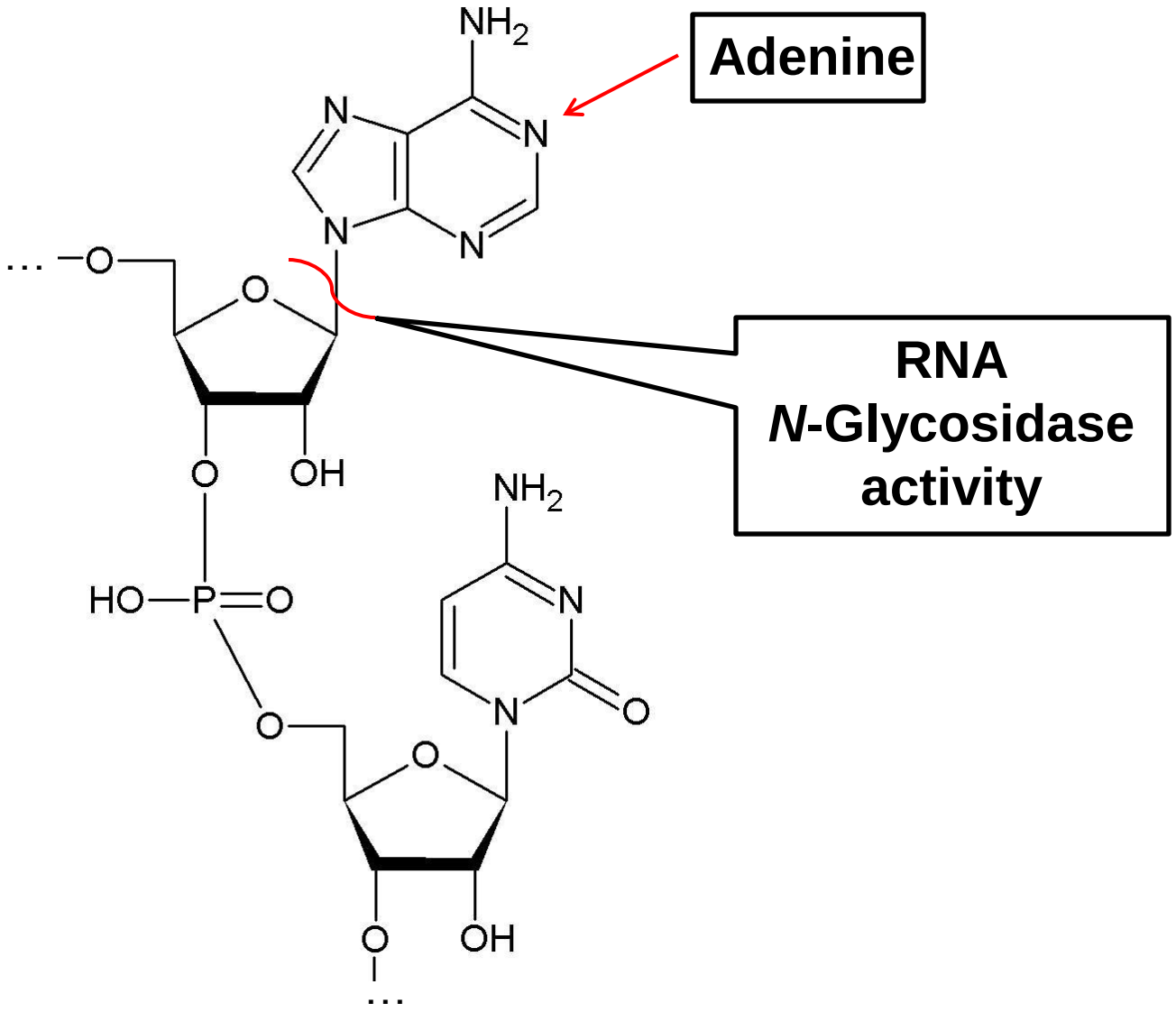
Barley
(*Hordeum vulgare* L.)

Structure of ribosome inactivating proteins (RIPs)

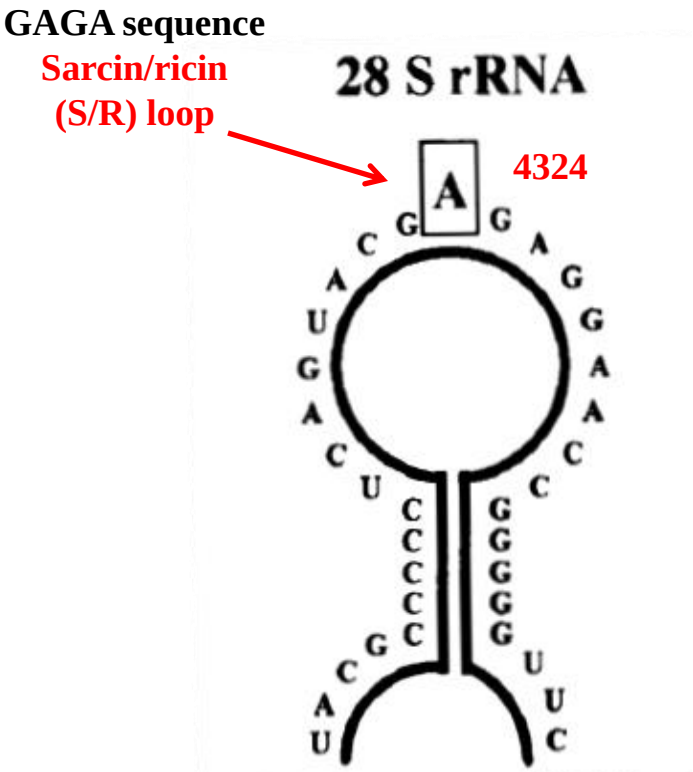


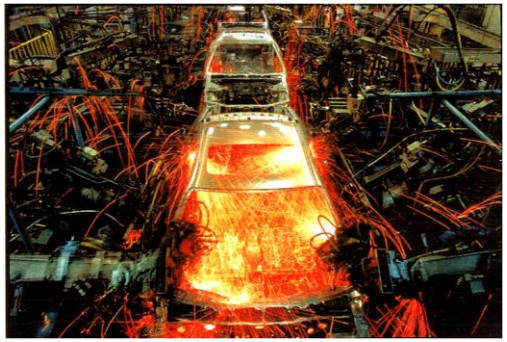
Domashevskiy, A.V. and Goss, D.J. (2015) *Toxins* **7**, 274-298.

Schematic representation of the action site for RNA *N*-glycosidase activity of RIPs

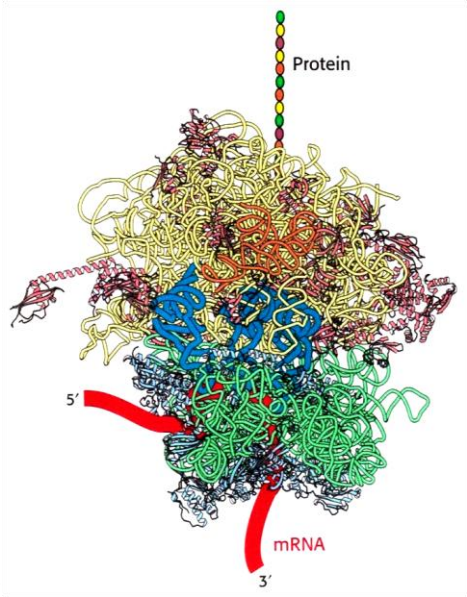


Structure of rRNA substrates for *N*-glycosidase activity of RIPs



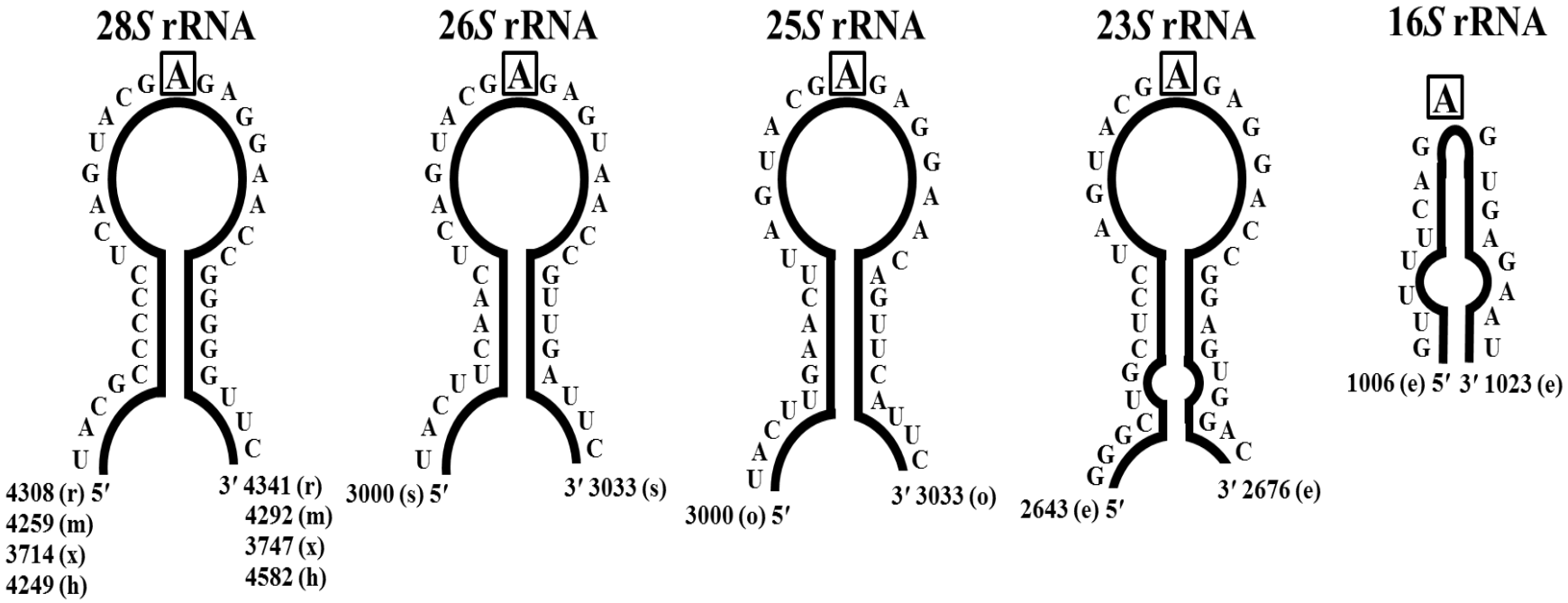


Protein Synthesis



Gutell, R. R., and Fox, G. E. (1988) *Nucleic Acids Res.* **16 Suppl.,** r175-269.

Structure of rRNA substrates for *N*-glycosidase activity of RIPs: (e) *E. coli*, (h) *Homo sapiens*, (m) *Mus musculus*, (o) *Oryza sativa*, (r) *Rattus rattus*, (s) *Saccharomyces cerevisiae*, (x) *Xenopus laevis*.



Domashevskiy, A.V. and Goss, D.J. (2015) *Toxins* **7**, 274-298.

Selective toxicity of RIPs has been explored

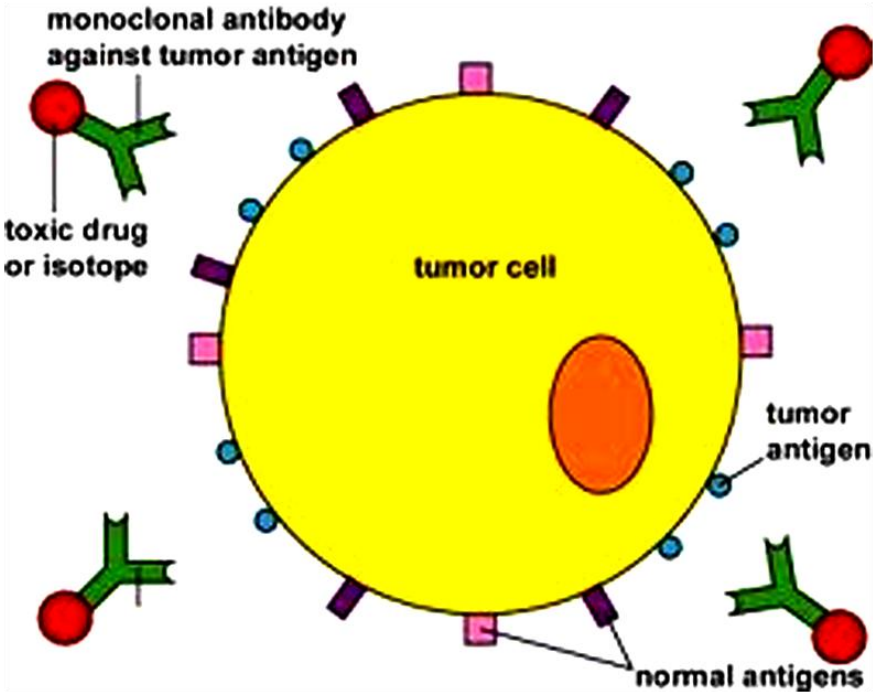
By biologists to create transgenic plants resistant to viral & fungal infections

***Nicotiana tabacum* infected with Tobacco mosaic virus (TMV)**

Transgenic *Nicotiana tabacum* plants

Tumer, N. E., Hwang, D., Bonness, M. (1997) *Proc. Natl. Acad. Sci. USA.* **94**, 3866-3871.

Cancer researchers to investigate immunoconjugate therapeutics



Frankel, A.E., *et al.* (1996) *Cancer Research* **56**, 926-932.
Kreitman, R.J. (1999) *Curr. Opin. Immunol.* **11**, 570-578
Pastan, I. and FitzGerald, D. (1991) *Science* **254**, 1173-1177

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Flashback: Dissident's poisoning

Georgi Markov: Journalist killed by poisoned umbrella

The poison ricin, which has been found by the British police at an address in London, was famously used to murder Bulgarian dissident Georgi Markov in 1978.

Markov, a BBC World Service journalist and a strong critic of the communist regime, was killed in London when he was injected with ricin while he waited at a bus stop.

Nobody has ever been charged with the murder, but it is widely believed that the Bulgarian secret service and the KGB were behind it.

Bulgarian prosecutors said their investigation produced inconclusive results, and the case remains open.

Accounts of the incident differ. Some say a ricin-laced pellet was either fired or injected from an umbrella tip as

"The clever thing about ricin is it appears in hospital investigations as natural disease"

See also:

- 07 Jan 03 | Health
  Q&A: What is ricin?
- 12 Sep 02 | Health
  Vaccine hope for lethal toxin
- 07 Sep 98 | Europe
  Markov murder 'Bulgaria's darkest hour'

Internet links:

- Ricin guidelines (pdf file)
- Public Health Laboratory Service
- Ricin
- Metropolitan Police

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Top UK stories now:

- No rush to war, says Blair
- 10,000 chased for congestion fine
- Omagh detectives make arrest
- Beckham forgives Ferguson
- Waiter jailed for underage sex
- Britons 'baffled over euro rate'
- Sleepy drivers who kill face jail
- Man charged after boy stabbed

Links to more UK stories are at the foot of the page.

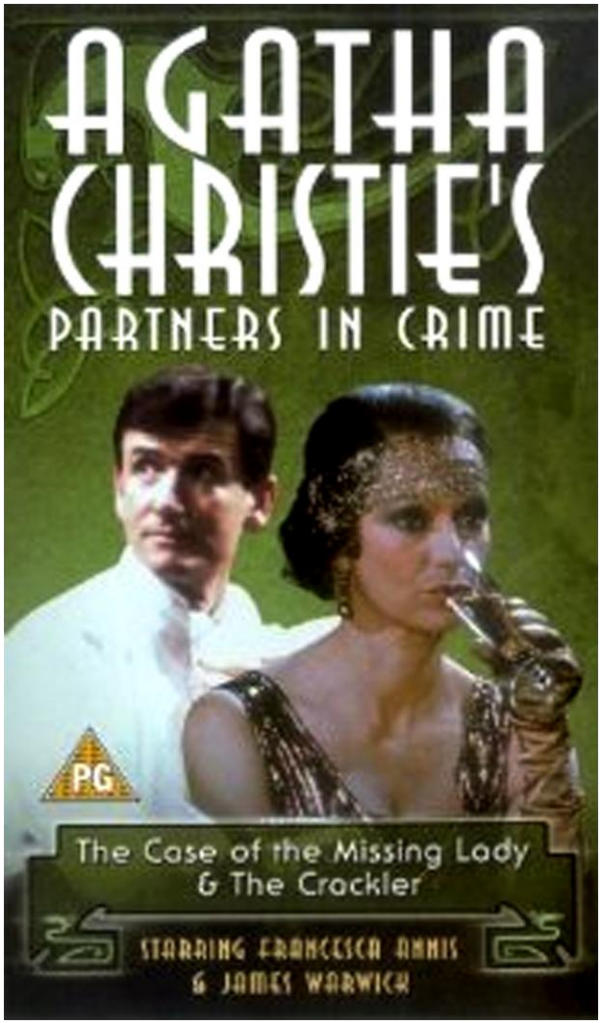
Political & military groups to create biological weaponry

Ricin & abrin are homicidal poisons

Ricin = Compound W (1952)

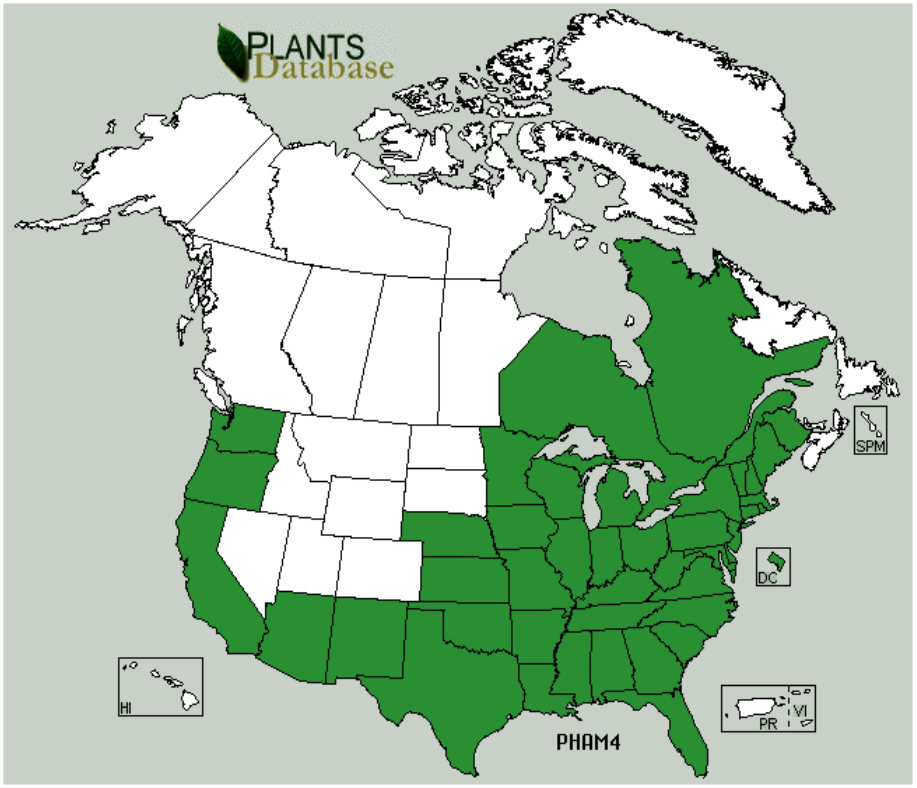
Assassination of Georgi Markov (1978) & Vladimir Kostov

By mystery writers to engage their readers



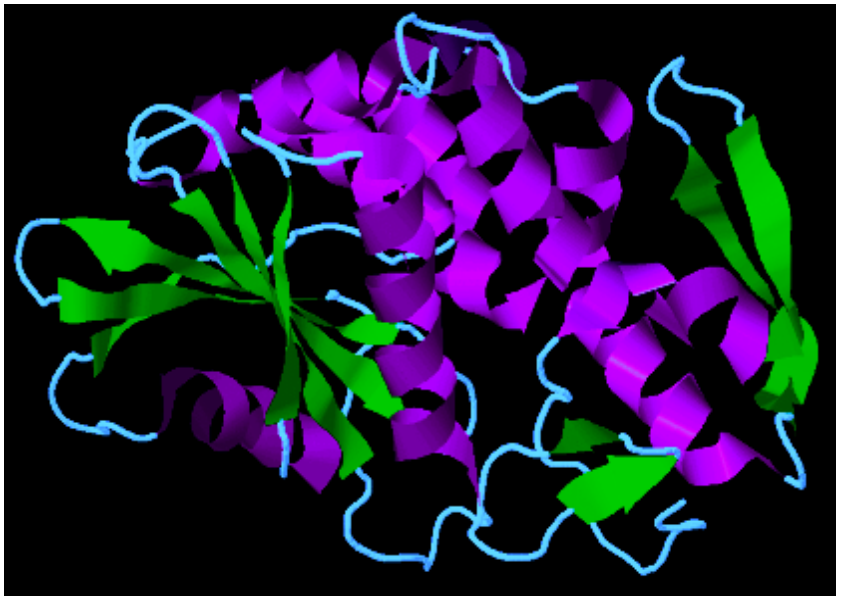
Christie, A. (1929) House of lurking death., In *Partners in Crime.*, Dodd, Mead and Company, New York.

Common pokeweed (*P. americana,* common pokeberry) produces pokeweed antiviral protein (PAP)

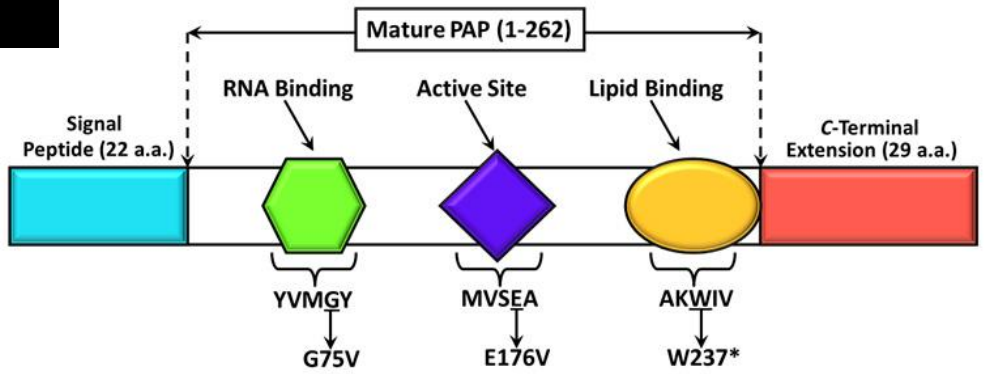


McMillian, J. (2011) Phytolacca americana L., USDA-NRCS PLANTS Database.

Pokeweed Antiviral Protein (PAP)



PDB ID 1QCI.

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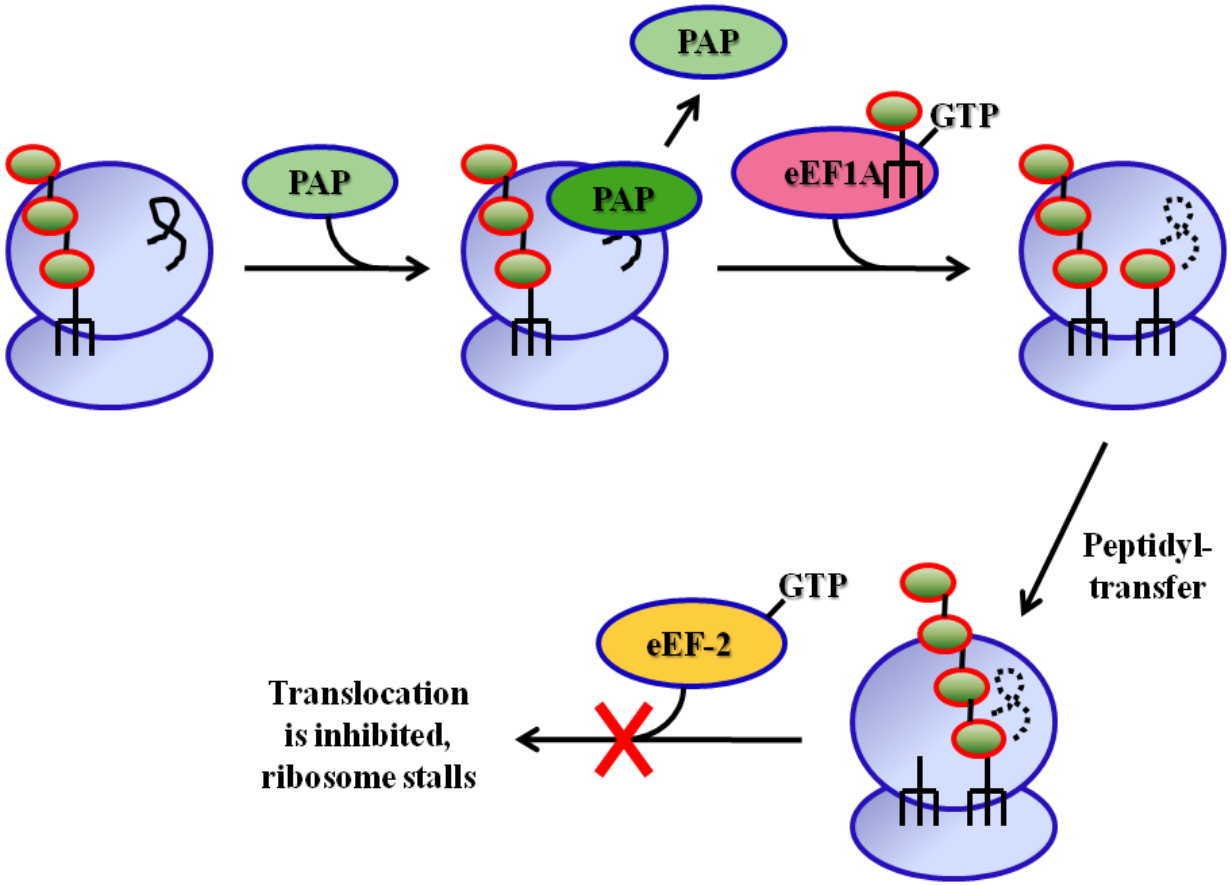
MKSMLVVTIS  IWLILAPTST  WAVNTIIYNV  GSTTISKYAT  FLNDLRNEAK  DPSLKCYGIP   60
MLPNTNTNPK  YVLVELQGSN  KKTITLMLRR  NNLYVMGYSD  PFETNKCRYH  IFNDISGTER  120
QDVETTLCPN  ANSRVSKNIN  FDSRYPTLES  KAGVKSRSQV  QLGIQILDSN  IGKISGVMSF  180
TEKTEAEFLL  VAIQMVSEAA  RFKYIENQVK  TNFNRAFNPN  PKVLNLQETW  GKISTAIHDA  240
KNGVLPKPKE  LVDASGAKWI  VLRVDEIKPD  VALLNYVGGS  CQTTYNQNAM  FPQLIMSTYY  300
NYMVNLGDLF  EGF                                                       313
```

Domashevskiy, A.V. & Cheng, S.Y. (2015) *J. Nat. Sci.* ***1***, e82.

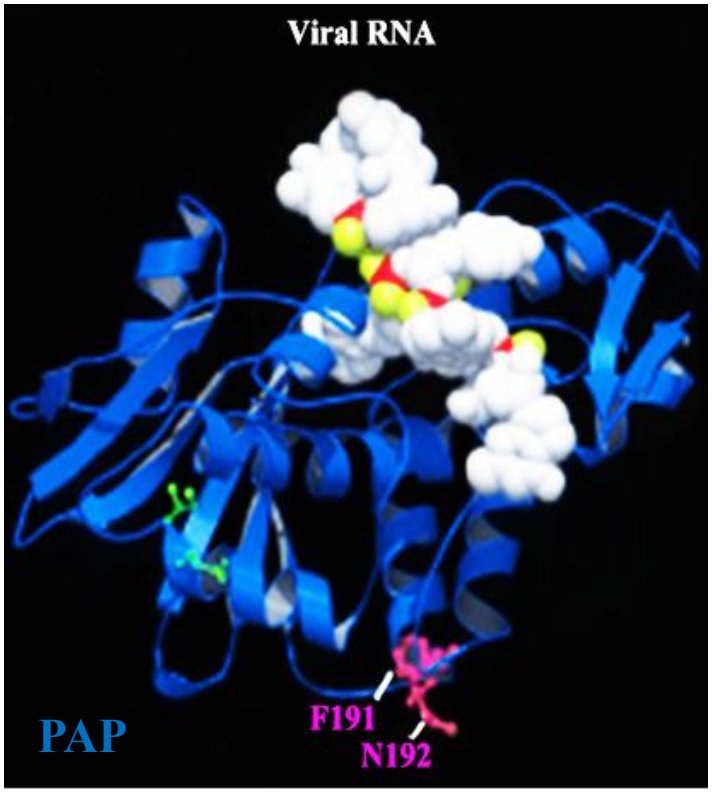
Model illustrating the step of the elongation cycle at which PAP depurinates the ribosome.

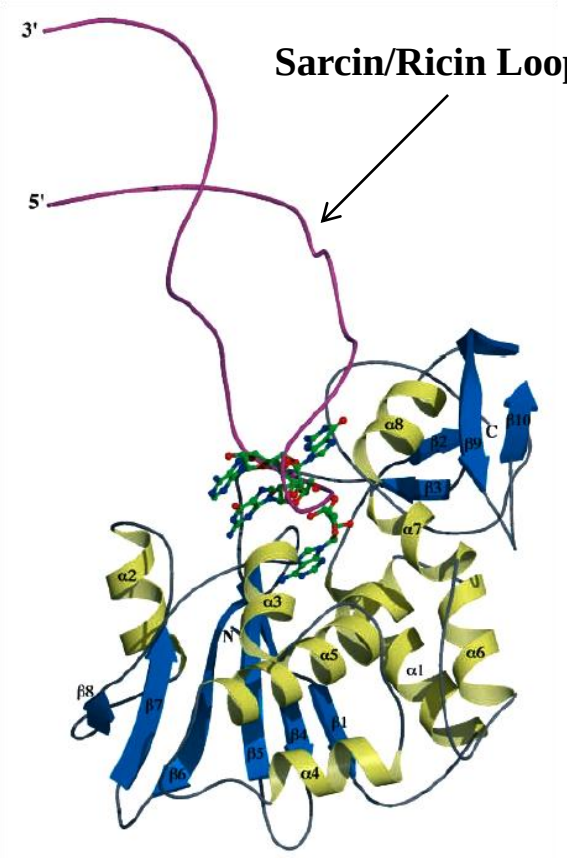


Mansouri, S., Nourollahzadeh, E., and Hudak, K. A. (2006) **RNA** *12*, 1683-1692.

Molecular Model of PAP-RNA Interactions.

Although rRNA of native ribosomes is ideal substrate for RIPs,
PAP depurinates naked rRNA (multiple sites); DNA; tRNA; mRNA;
viral RNA!!! (Both capped & uncapped)
Removal of G residues.





Uckun, F. M., *et al.* (2003) **Antimicrob. Agents Chemother.** *47*, 1052-1061.
Rajamohan, F., *et al.* (2000) **J. Biol. Chem.** *275*, 3382-3390.

Model for PAP's antiviral mechanism:

when virus infects a cell, PAP also gains entrance,
disrupts cellular protein synthesis,
thus killing virus-infected cells
& thereby preventing viral replication.

• Ready, M. P., Brown, D. T., and Robertus, J. D. (1986) **Proc. Natl. Acad. Sci. U S A** *83*, 5053-5056.

Viral genome-linked protein (VPg) from turnip mosaic virus (TuMV)

Uninfected cabbage of Cruciferous family.

Cabbage infected with Turnip mosaic virus.

Genus *Potyvirus*, Plant family *Potyviridae*

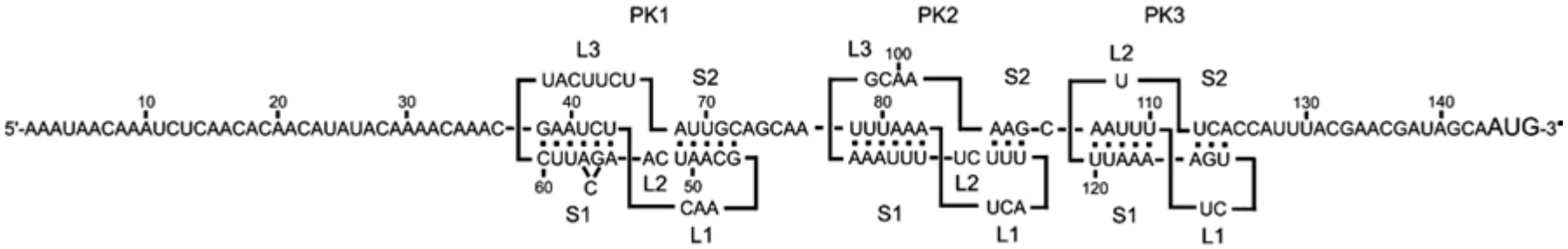
- Viral infection cycle
- Replication
- Cell-to-cell movement
- Overcoming plant defense mechanisms

H_2N – akgkrqrqkl kfrnardnkm grevygdddt iehffgdayt kkgkskgrtr gighknrkfi nmygfdpedf savrfvdplt gatlddnplt ditlvqehfg nirmdllged eldsneirvn ktiqayymnn ktgkalkvdl tphiplkvcd lhatiagfpe renelrqtgk aqpinidevp rannelvpvd he hhhhhh - COOH

Tobacco etch virus (TEV)

Uninfected pepper plant of Piperaceae family.

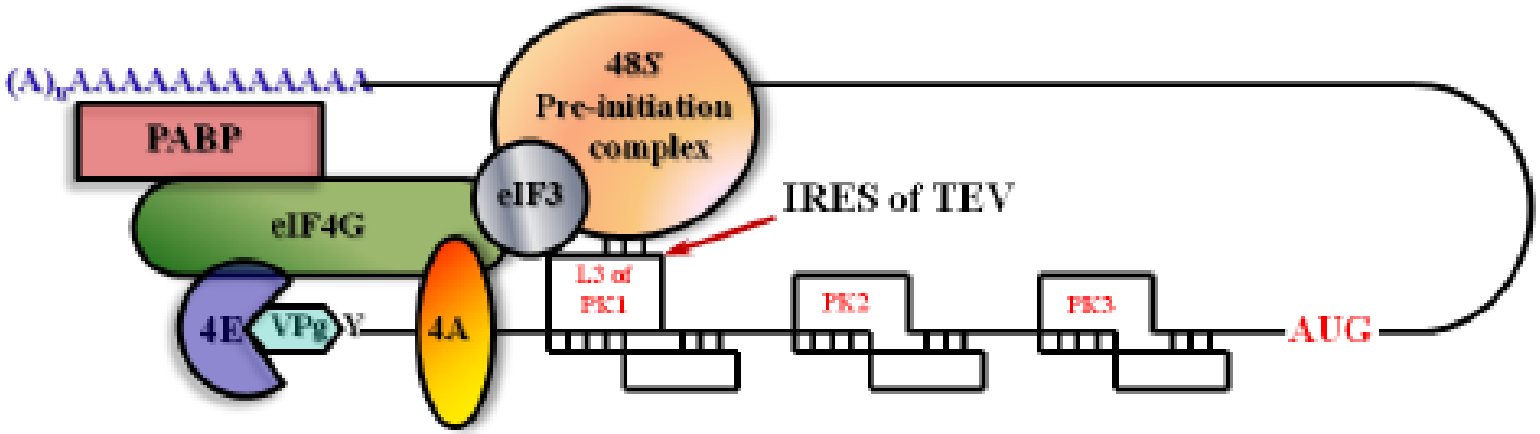
Pepper plant infected with TEV.



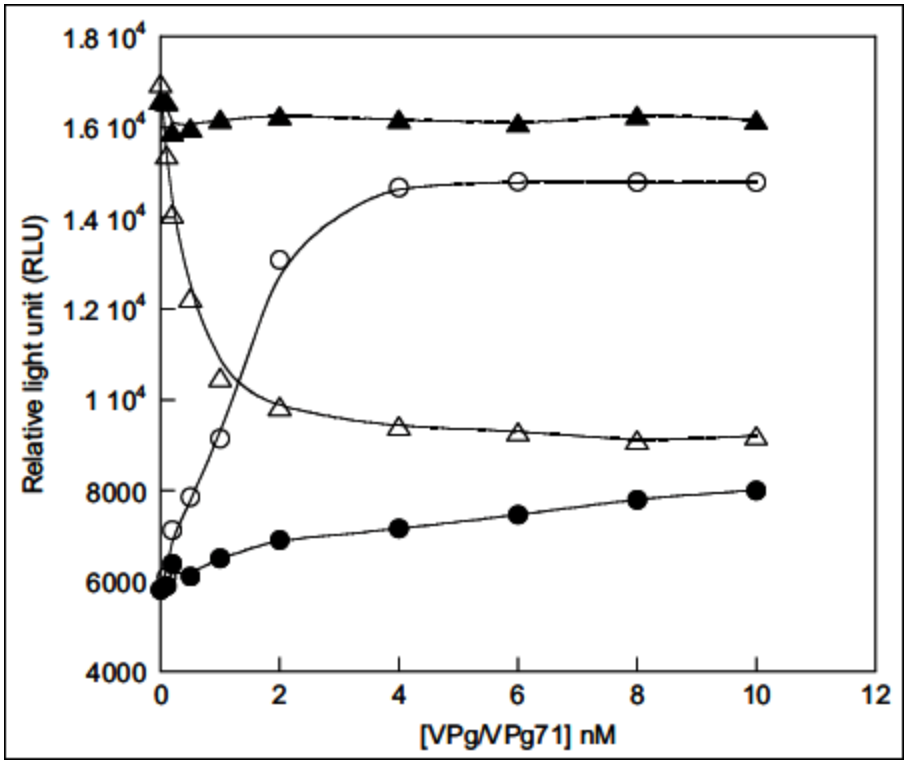
The predicted structure of the TEV 5’-leader sequence

Zeenko, V. and Gallier, D.R. (2005) *J. Biol. Chem.* **280**, 26813-26824.

Depiction of VPg functioning as an alternative cap-structure in initiation in translation of uncapped tobacco etch virus (TEV) RNA that has a poly(A) tail and a 5' VPg covalently linked via a tyrosine residue (Y).



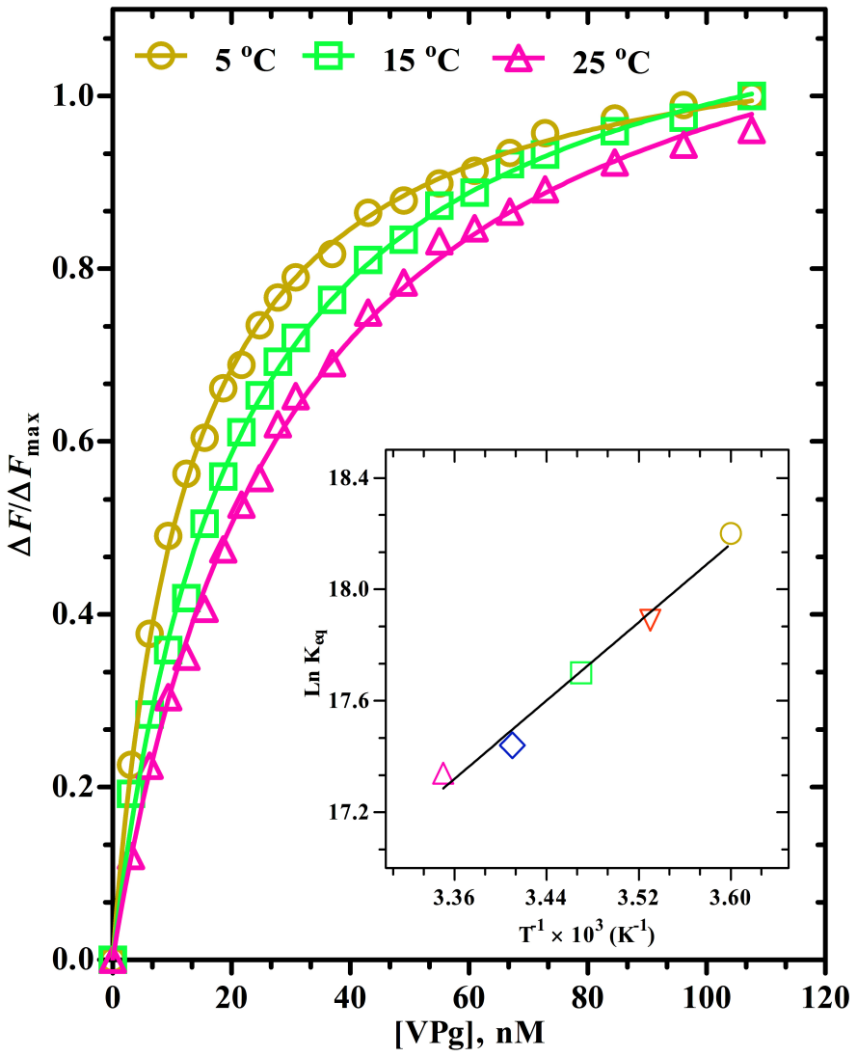
Translation of luciferase reporter TEV RNA constructs in wheat germ extracts.



Capped TEV1-143-*luc*-A50 RNA + wt VPg (Δ), Capped TEV1-143-*luc*-A50 RNA + VPg71 (▲), Uncapped TEV1-143-*luc*- A50 RNA + wt VPg (○), & TEV1-143-*luc*-A50 RNA + VPg71 (●).

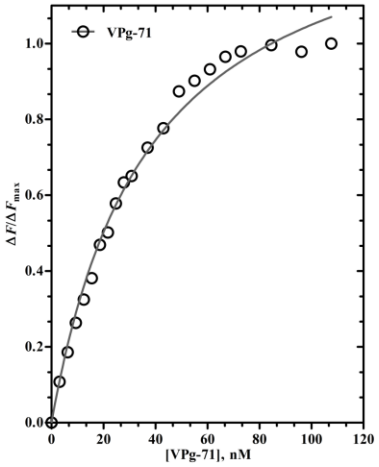
Khan, M.A., Miyoshi, H., Gallie, D.R., Goss, D.J. (2008) *J. Biol. Chem.* **283**, 1340-1349.

Temperature dependence of PAP-VPg interactions.



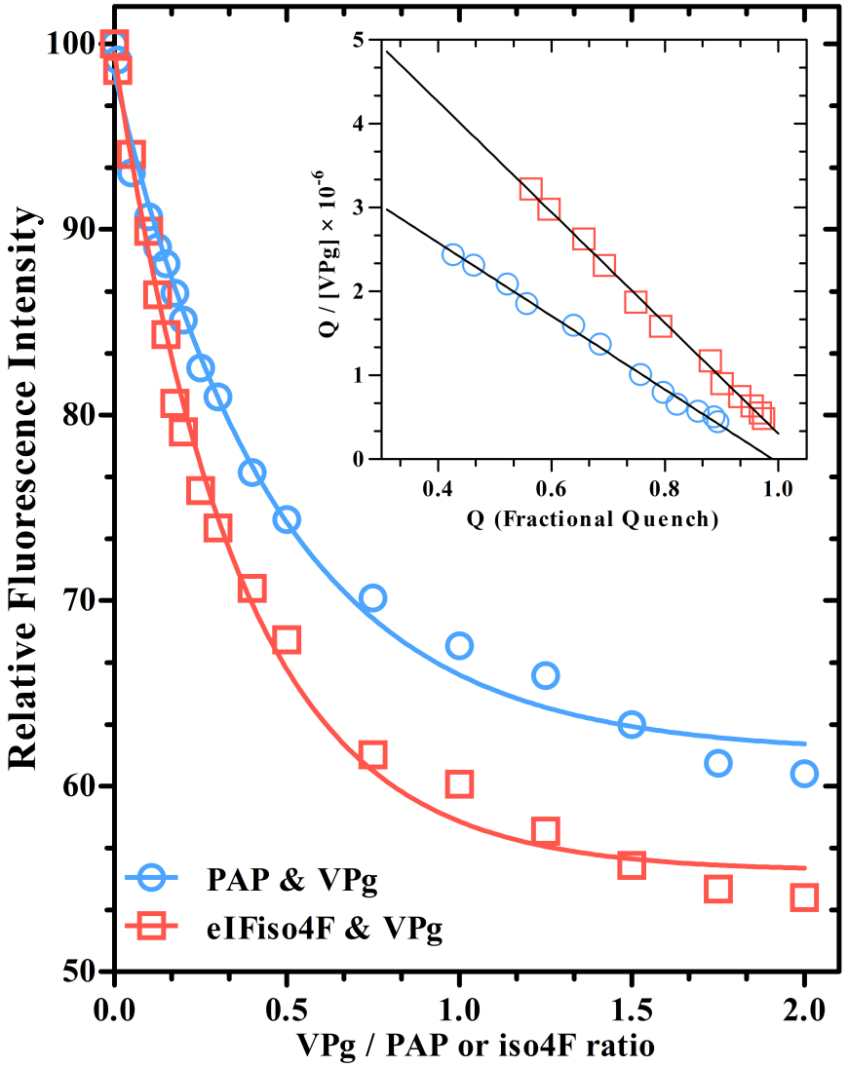
Complex	Equilibrium Dissociation Constant, K_d, [nM]				
	5 °C	10 °C	15 °C	20 °C	25 °C
PAP – VPg	12.5 ± 0.6	17.0 ± 0.7	20.9 ± 1.2	26.7 ± 1.3	29.5 ± 1.8
PAP – VPg-71	ND	ND	ND	ND	37.4 ± 3.0

Complex	Enthalpy, ΔH^o	Entropy, ΔS^o	Gibbs Free Energy, ΔG^o
	kJ·mol^{-1}	J·K^{-1}·mol^{-1}	kJ·mol^{-1}
PAP – VPg	– 29.2 ± 0.9	+ 46.0 ± 3.0	– 43.0 ± 1.8



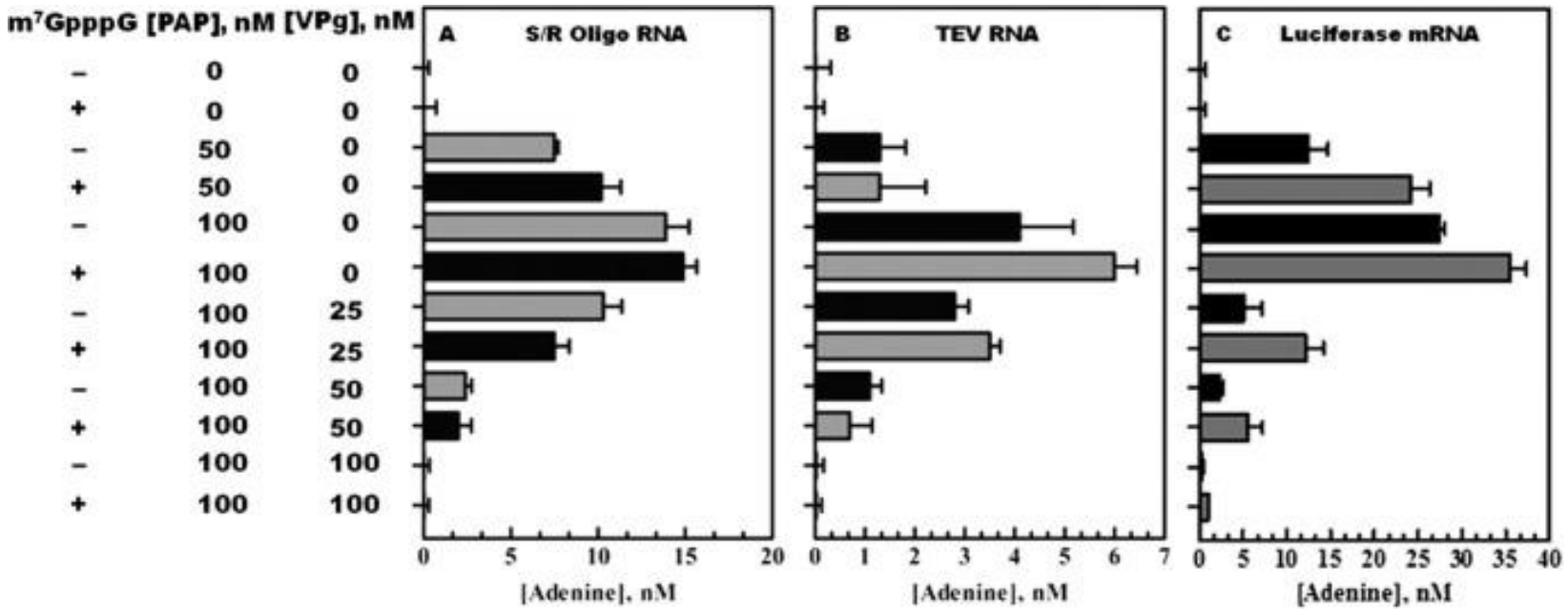
Domashevskiy, A.V., Miyoshi, H, Goss, D.J (2012) *J. Biol. Chem.* **287**, 29729-29738.

Competition of eIF*iso4F (complex of eIFiso4E-eIFiso4G) (—□—) and PAP (—○—) binding with VPg.*



Domashevskiy, A.V., Miyoshi, H, Goss, D.J (2012) *J. Biol. Chem.* **287**, 29729-29738.

VPg inhibits PAP depurination of S/R oligo, TEV RNA, & luciferase mRNA.



Domashevskiy, A.V., Miyoshi, H, Goss, D.J (2012) *J. Biol. Chem.* **287**, 29729-29738.

PAP reduces the infectivity of capped plant viruses

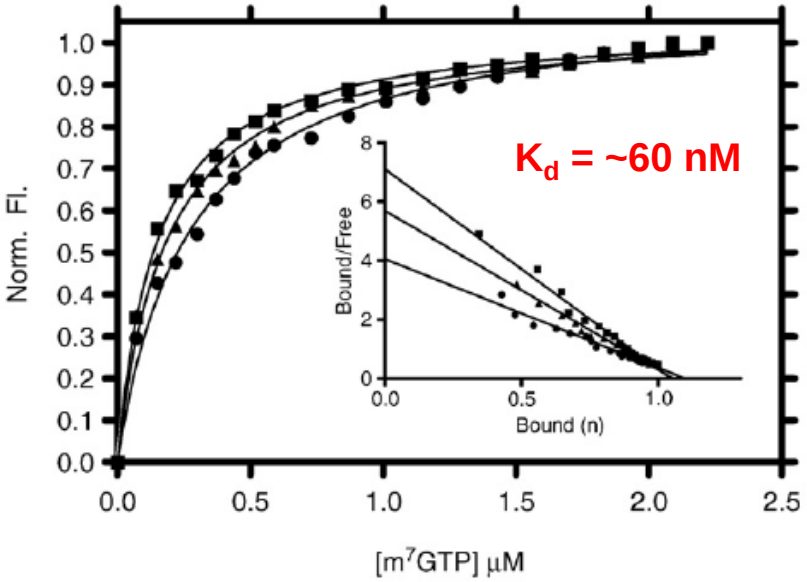
- **tobacco mosaic virus** (Grasso and Shepherd, 1978; Stevens *et al.,*1981)**,**
- **brome mosaic virus** (Hudak *et al.,* 2002)**,**
- **cucumber mosaic virus** (Tomlinson *et al.,* 1974)**,**

  & a variety of others (Chen *et al.,* 1991)**.**

PAP is a cap binding protein (Hudak *et al.,* 2002; Baldwin *et al.*, 2009)

Model how PAP selects capped RNA as its target:

PAP binds to the cap structure at the 5'-end of viral RNA and, thus accesses it for depurination, reducing infectivity of plant and animal viruses



PAP is selectively toxic to cells infected with uncapped viruses

¿¿¿ Uncapped Viruses ???

- **poliovirus** (Ussery *et al.,* 1977),
- **influenza virus** (Tomlinson *et al.,* 1974),
- **herpes simplex virus** (Aron and Irvin, 1980; Teltow *et al.,* 1983),
- **human immunodeficiency virus (HIV-1)** (Zarling *et al.,* 1990).

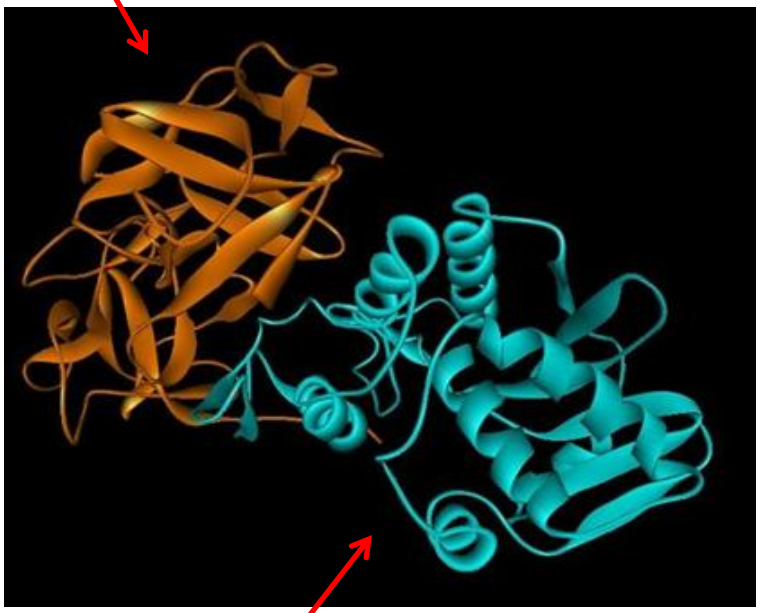
Vivanco *et al.*: PAP does not depurinate every capped RNA & it can inhibit translation of uncapped viral RNAs *in vivo*!

The cap structure is not the only determinant for inhibition of translation by PAP, & thus the overall mechanism of PAP antiviral activity remains to be elucidated.

• Vivanco, J. M. and Tumer, N. E. (2003) **Phytopathology** *93,* 588-595.

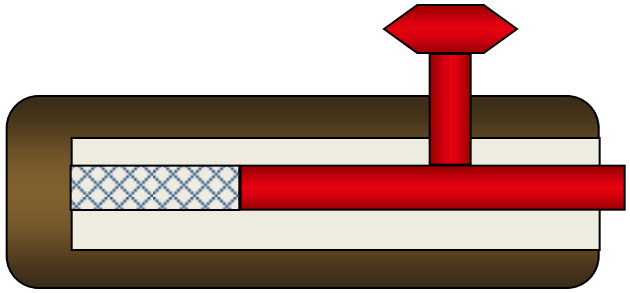
Castor Plant (*Ricinus communis*)

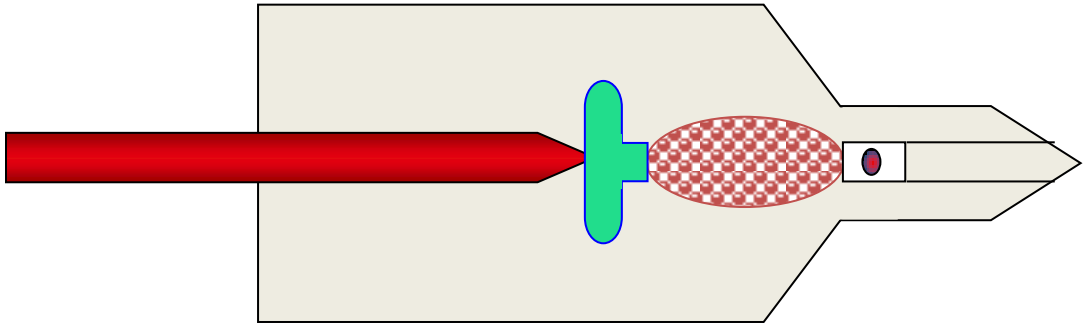
***N*-Glycosidase Domain**

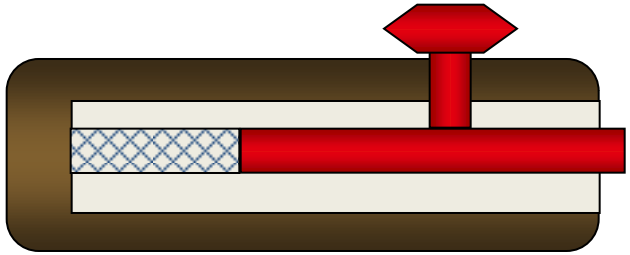


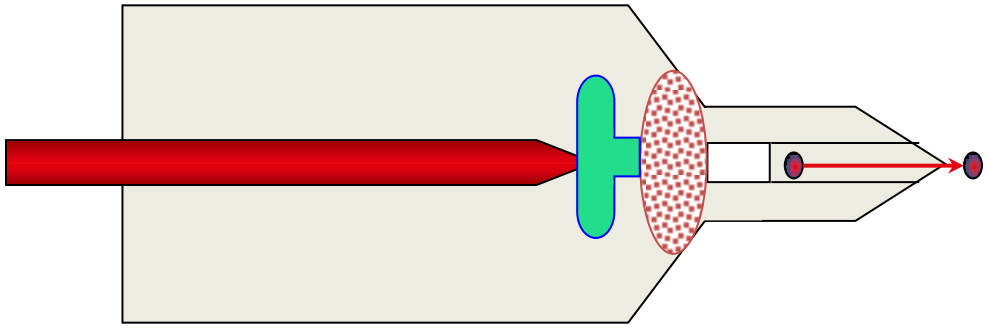
Lectin-binding Domain

Proposed Umbrella Gun Design







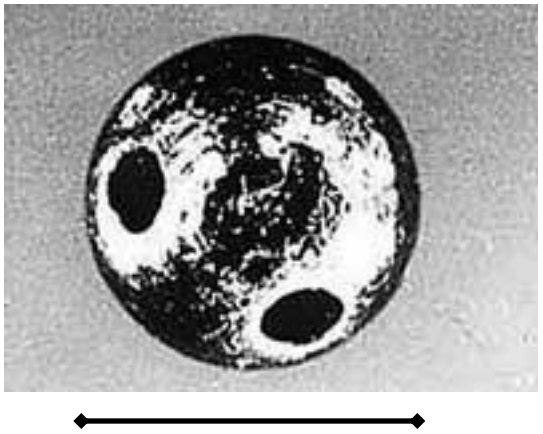


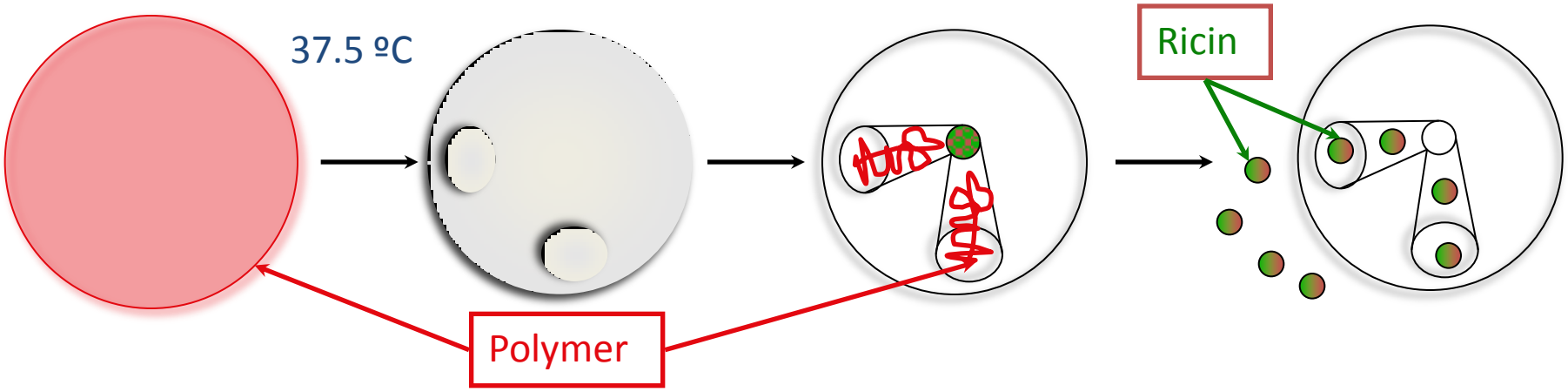
Umbrella handle trigger

Gas injected pellet

Poison Delivery System

- Polymer coated “bullet” containing ricin
- Bullet = 90% Platinum & 10% Iridium
  - Immunologically Inert
- Polymer degrades at body temperature



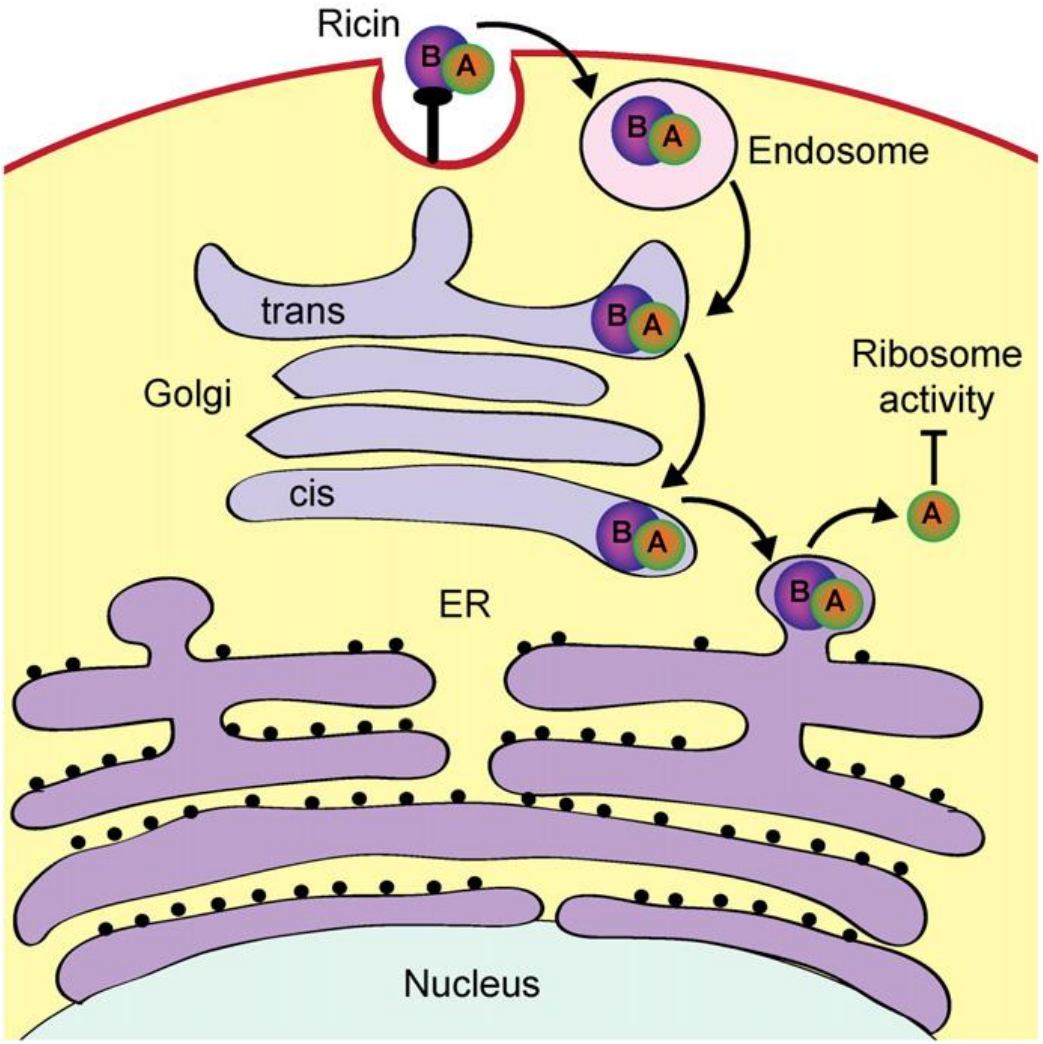


Ricin from Castor Beans

- 5% of mass contains toxin
- 8 beans are considered dangerous
- Subcutaneous Toxicity
  - 24 µg / 100 hrs
- Markov likely injected with 500 µg subcutaneous

Intracellular uptake of ricin and ribosome inactivation.

Clathrin-dependent endocytosis

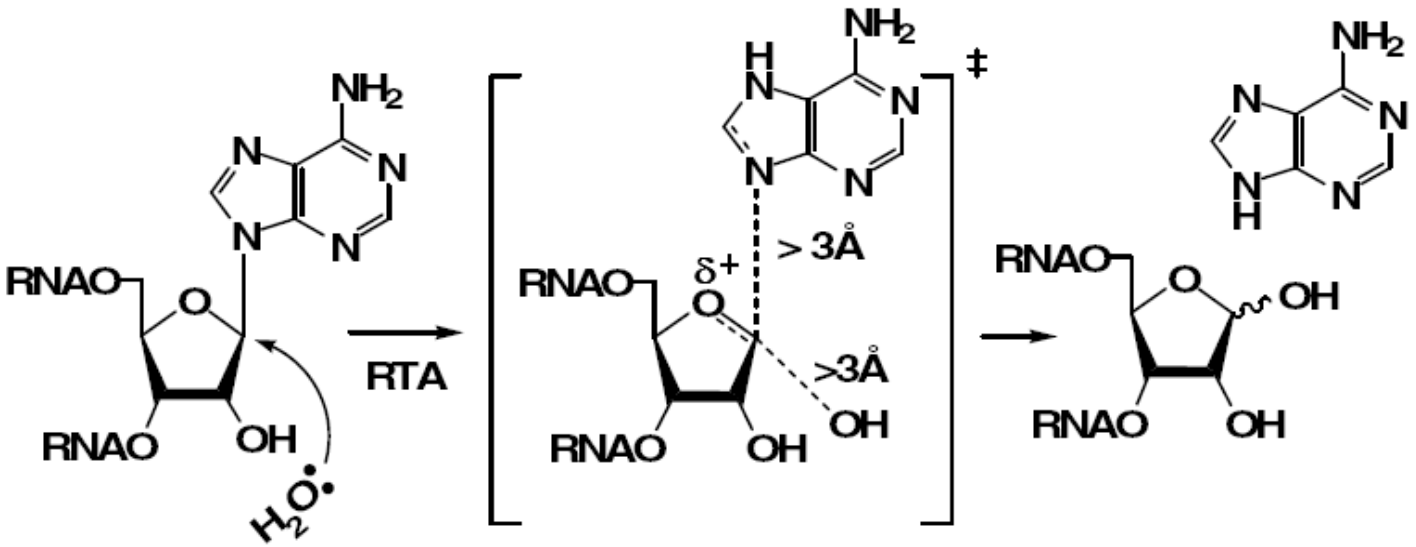


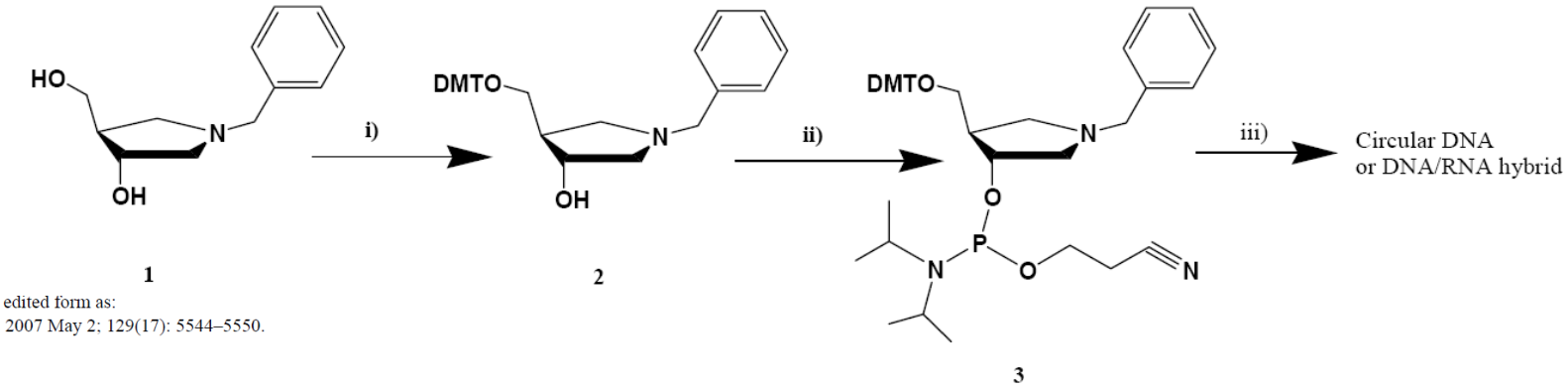
Clathrin-independent endocytosis

Retrograde traffiking

Bozza, W.P. *et al.* (2015) *Biotechnology Advances* **33**, 117-123.

A plausible mechanism for ricin and the RIP family





Published in final edited form as:
J Am Chem Soc. 2007 May 2; 129(17): 5544–5550.

Circular DNA and DNA/RNA Hybrid Molecules as Scaffolds for Ricin Inhibitor Design

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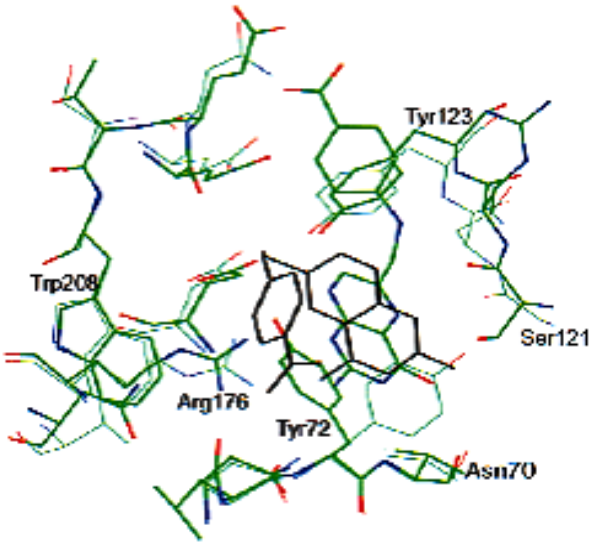
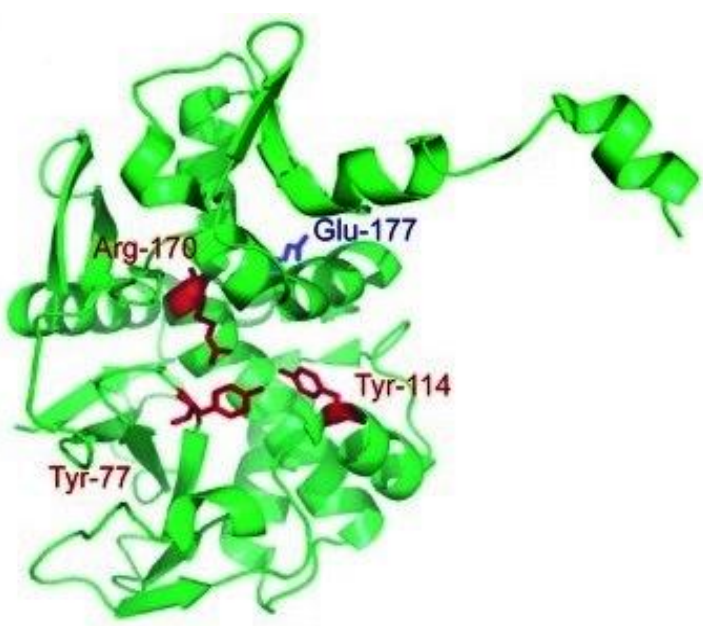
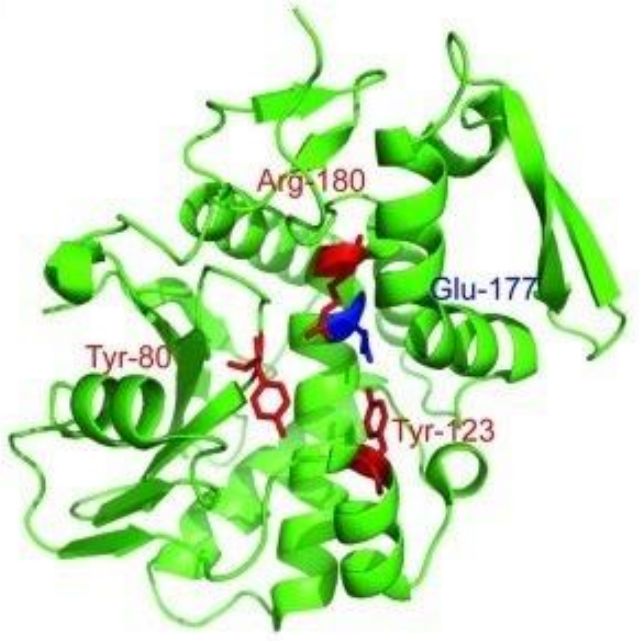


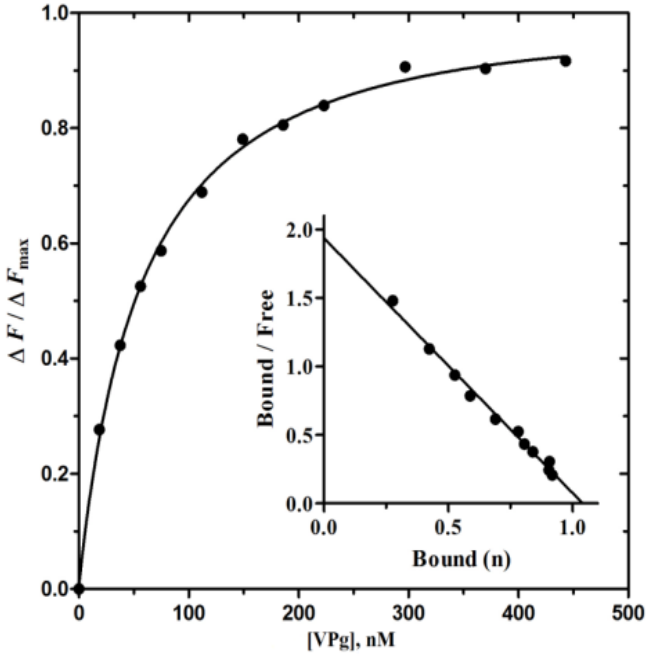
Superimposition of PAP and RTA.

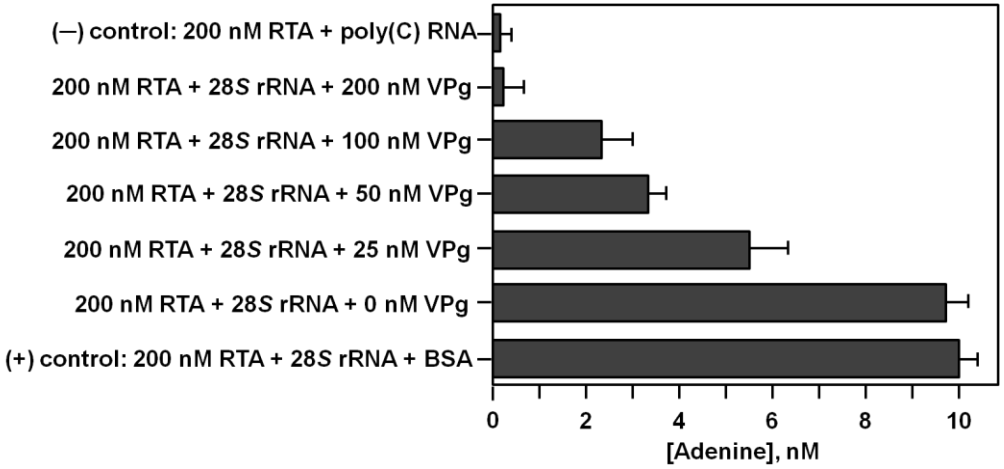




Structures of ricin A-chain and Shiga toxin Stx2.

Fluorescence binding curve for a binary complex of RTA with VPg from TuMV at 25 °C. Increasing concentrations of VPg inhibit RTA depurination of 28*S* rRNA *in vitro.*





Depurination inhibition by VPg may confer an advantage for viral replication, AND suggests the possible use of this protein against cytotoxic activity of RIPs and inhibition of their biological potency!!!

Thank you