Joint Graduate Seminar Dec 2014
Department of Microbiology
Faculty of Medicine
The Chinese University of Hong Kong

Mutualistic Viruses with Different Species

M.Phil Candidate : Leon Lai Tsz On
Supervisor: Prof. Paul Chan
Date : 15 / 12 / 2014

Introduction

Intercellular obligate parasites?
Disease-causing agents?
Pathogens?

What if

Viruses ≠ Pathogens
Mutualistic Viruses

Virus and Reproduction

Polydnaviruses

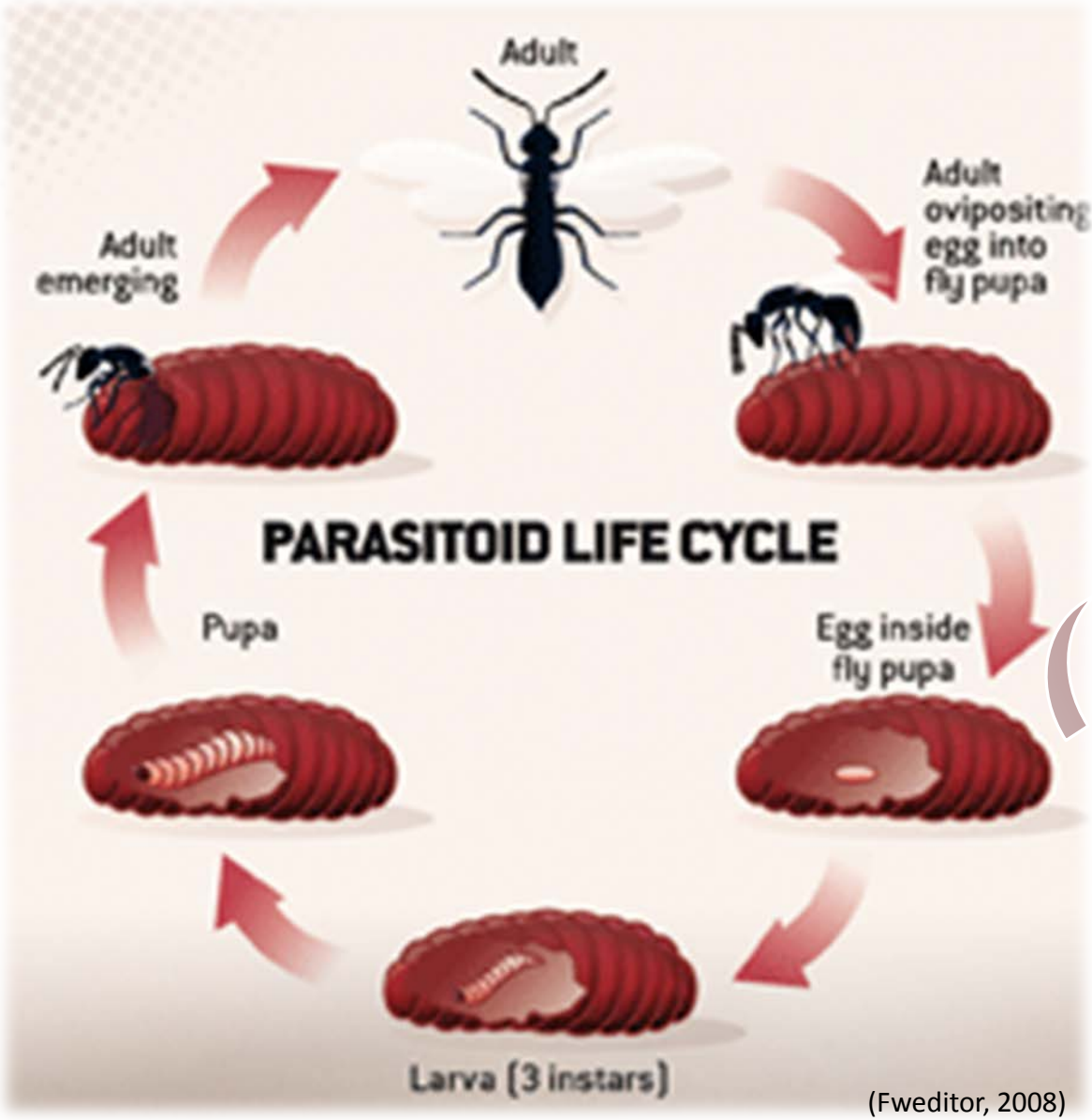
- dsDNA virus
- Over thousands types

Campoletis sonorensis virus (CsV)
for *C. sonorensis* wasps

Endoparasitoid wasps

- Insect
- Over 30,000 types

Cotesia congregata bracovirus (CsBV)
for *C. congregata* wasps



(Fweditor, 2008)

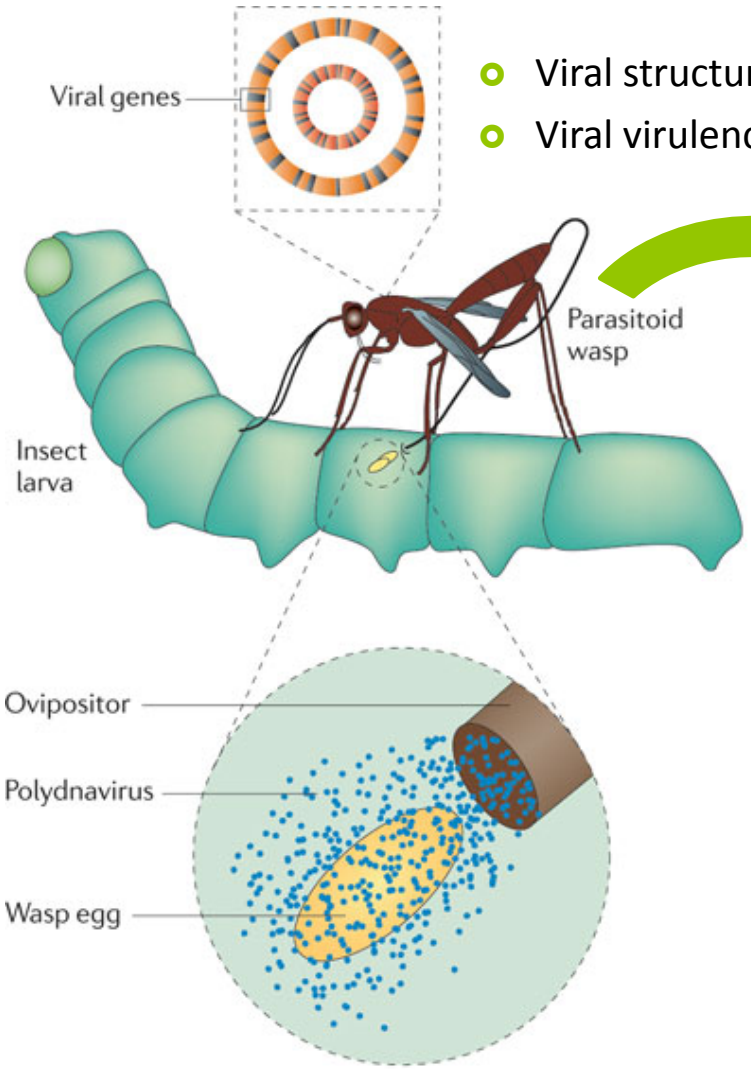
Host Innate Immune Response

- Encapsulation
- Melanization

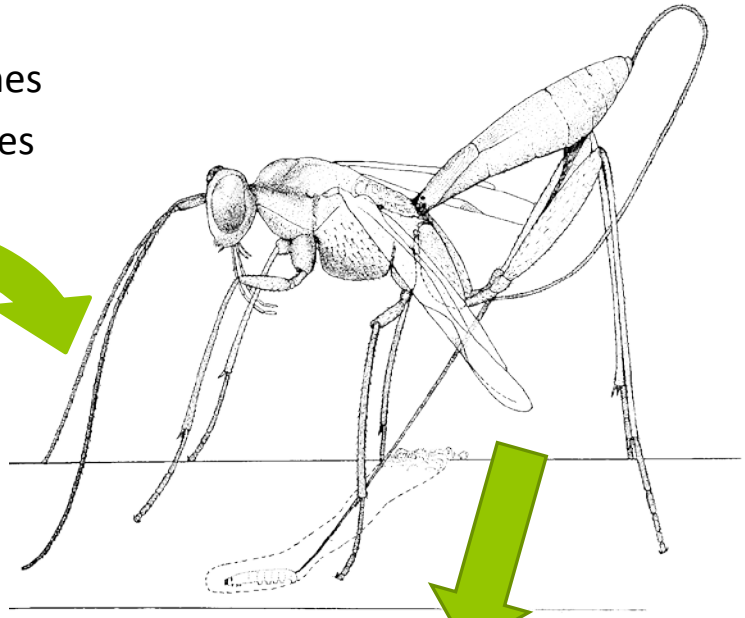
Melanin

Parasitoid larvae growth stopped

- Viral structural genes
- Viral virulence genes



Nature Reviews | Microbiology

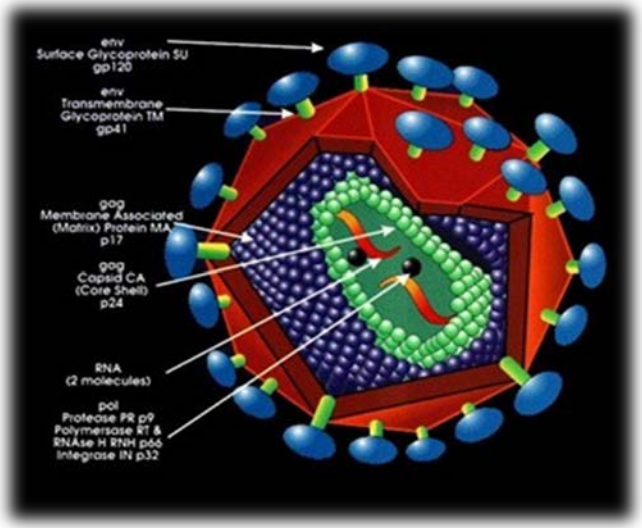


Wasps Calyx

Virus and Development

Endogenous Retrovirus (ERV)

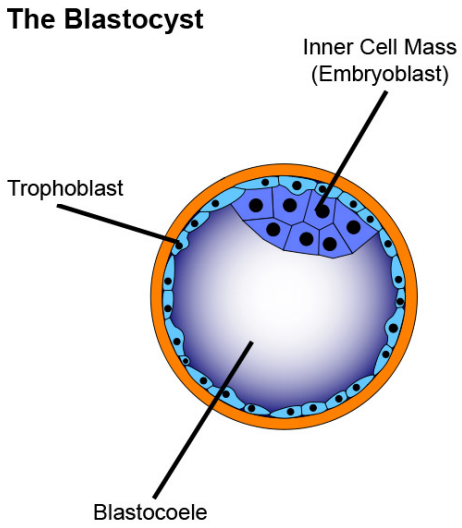
- About 8% of human genome
- Germline-integrated
- Human and mammalian placenta



(Evidence for Evolutionary Model, 2012)

Retroviral envelope *env* proteins

- Placental syncytium formation
- Localized maternal immunosuppression
- Initial trophoblastic attachment + invasion
- Overall placental survival



(Rowland T., 2009)

Endogenous retroviruses regulate periimplantation placental growth and differentiation

Kathrin A. Dunlap*, Massimo Palmarini†, Mariana Varela†, Robert C. Burghardt‡, Kanako Hayashi*, Jennifer L. Farmer*, and Thomas E. Spencer*§

*Center for Animal Biotechnology and Genomics, Department of Animal Science, and ‡Image Analysis Laboratory, Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843; and †Institute of Comparative Medicine, University of Glasgow Veterinary School, Glasgow G61 1QH, United Kingdom

Edited by George E. Seidel, Jr., Colorado State University, Fort Collins, CO, and approved August 8, 2006 (received for review May 10, 2006)

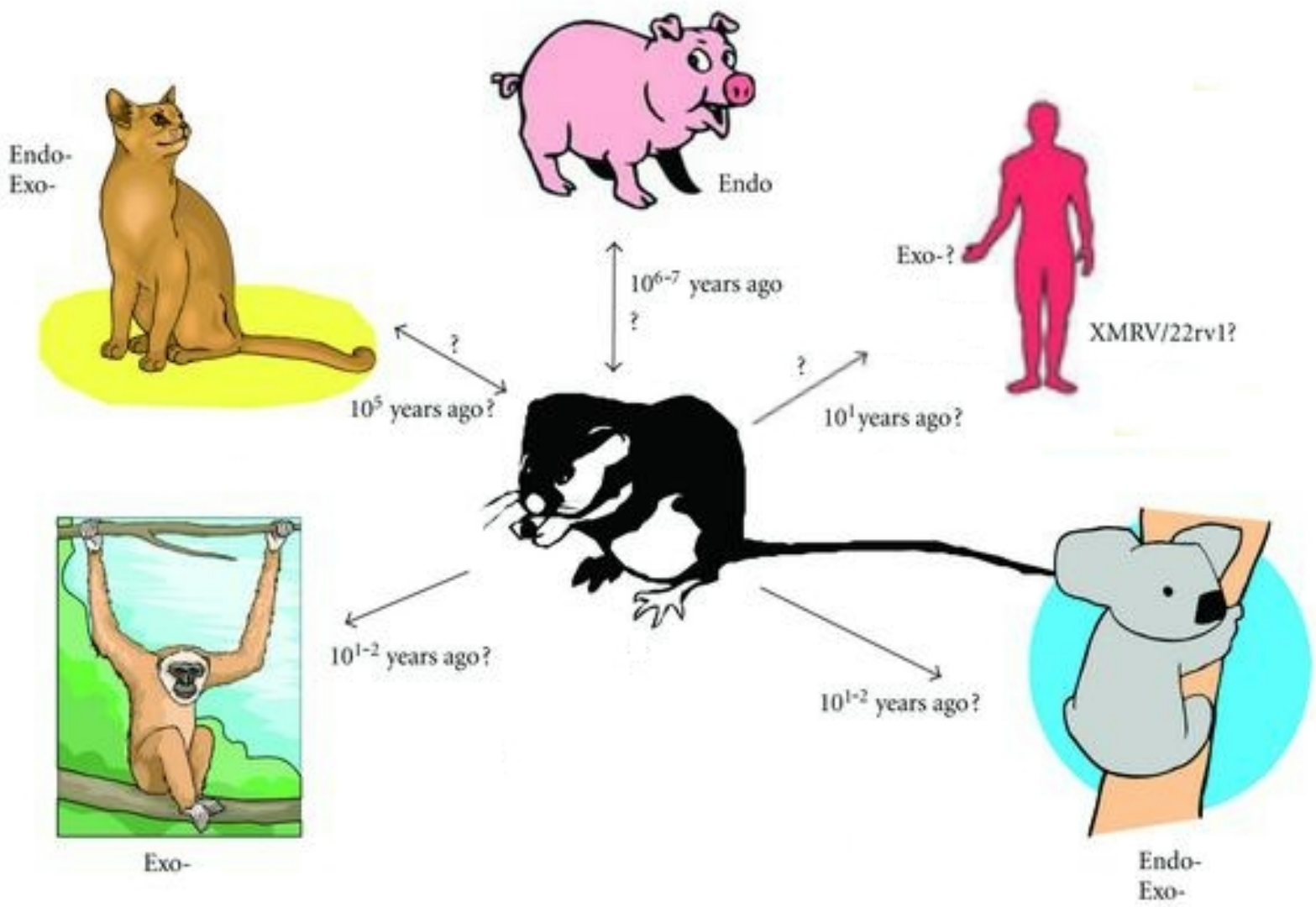
Endogenous retroviruses (ERVs) are fixed and abundant in the genomes of vertebrates. Circumstantial evidence suggests that ERVs play a role in mammalian reproduction, particularly placental morphogenesis, because intact ERV envelope genes were found to be expressed in the syncytiotrophoblasts of human and mouse placenta and to elicit fusion of cells *in vitro*. We report here *in vivo* and *in vitro* experiments finding that the envelope of a particular class of ERVs of sheep, endogenous Jaagsiekte sheep retroviruses (enJSRVs), regulates trophectoderm growth and differentiation in the periimplantation conceptus (embryo/fetus and associated extraembryonic membranes). The enJSRV envelope gene is expressed in the trophectoderm of the elongating ovine conceptus after day 12 of pregnancy. Loss-of-function experiments were conducted *in utero* by injecting morpholino antisense oligonucleotides on day 8 of pregnancy that blocked enJSRV envelope protein production in the conceptus trophectoderm. This approach retarded trophectoderm outgrowth during conceptus elongation and inhibited trophoblast giant binucleate cell differentiation as observed on day 16. Pregnancy loss was observed by day 20 in sheep receiving morpholino antisense oligonucleotides. *In vitro* inhibition of the enJSRV envelope reduced the proliferation of mononuclear trophectoderm cells isolated from day 15 conceptuses. Consequently, these results demonstrate that the enJSRV envelope regulates trophectoderm growth and differentiation in the periimplantation ovine conceptus. This work supports the hypothesis that ERVs play fundamental roles in placental morphogenesis and mammalian reproduction.

development | placenta | sheep | trophectoderm

Hyaluronidase 2 (HYAL2) is a glycosylphosphatidylinositol-anchored cell-surface protein that can serve as a cellular receptor for exogenous JSRV Env as well as for retroviral vectors pseudotyped by enJSRV Env (13, 14). By RT-PCR analyses, *HYAL2* mRNA is first detected in the conceptus on day 16, which is associated with the onset of BNC differentiation (5). Throughout pregnancy, *HYAL2* mRNA can be detected in the BNCs and multinucleated syncytia of sheep placentomes but not in the mononuclear trophectoderm cells of the conceptus or any cells of the endometrium.

Of great interest for comparative physiology is that enJSRV *env* expression in the developing ovine placenta is strikingly similar to that observed for syncytin 1 and 2, products of human ERV *env* in humans and primates (15–19) and possibly of two related *env* genes (*syncytin A* and *syncytin B*) in mice (20). Syncytins encode highly fusogenic retroviral envelope proteins that are expressed in the syncytiotrophoblast layer generated by mononuclear cytotrophoblast cell fusion at the maternal–fetal interface. Syncytins are fusogenic when expressed *in vitro*, thereby advancing the hypothesis that they are involved in placental morphogenesis (15–19). Thus, circumstantial evidence gleaned from studies of primates, sheep, and rodents supports the concept that independently acquired ERVs have been positively selected for a convergent physiological role in placental morphogenesis (21, 22).

In these studies we tested the hypothesis that enJSRV Env has a biological role in periimplantation ovine conceptus development and placental morphogenesis by using an *in vivo* morpholino loss-of-function approach (23) to block enJSRV Env production *in utero*.



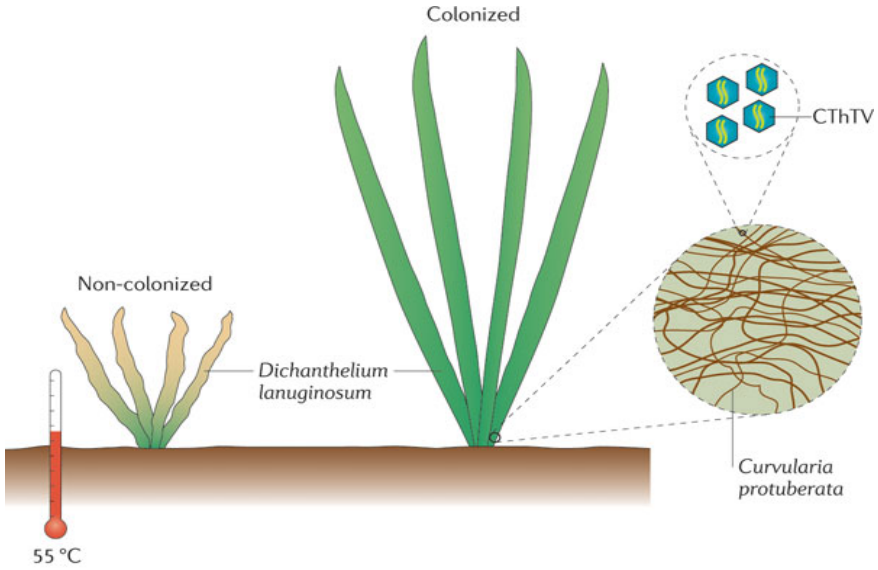
Modified from (Hindawi, 2011)

Virus and Tolerance

Thermotolerance

- *Dichanthelium lanuginosum*
- Geothermal soils
- Three-way mutualistic symbiosis:
  Grass *Dichanthelium lanuginosum* +
  Fungus *Curvularia protuberata* +
  Virus Curvularia protuberata thermal tolerance virus (CThTV)

Dichanthelium lanuginosum



A Virus in a Fungus in a Plant: Three-Way Symbiosis Required for Thermal Tolerance

Luis M. Márquez,[1] Regina S. Redman,[2,3] Russell J. Rodriguez,[2,4] Marilyn J. Roossinck[1]*

A mutualistic association between a fungal endophyte and a tropical panic grass allows both organisms to grow at high soil temperatures. We characterized a virus from this fungus that is involved in the mutualistic interaction. Fungal isolates cured of the virus are unable to confer heat tolerance, but heat tolerance is restored after the virus is reintroduced. The virus-infected fungus confers heat tolerance not only to its native monocot host but also to a eudicot host, which suggests that the underlying mechanism involves pathways conserved between these two groups of plants.

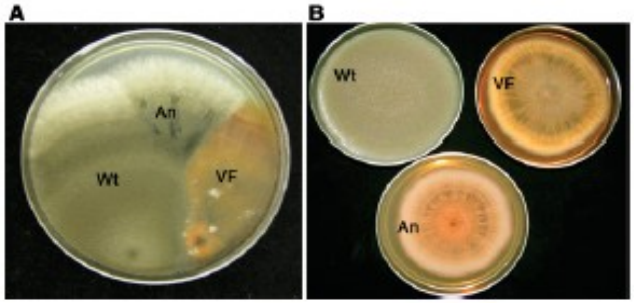


Fig. 3. (**A**) Anastomosis of the wild-type virus-infected isolate of *C. protuberata* (Wt) and the virus-free hygromycin-resistant isolate (VF) to produce a virus-infected hygromycin-resistant isolate (An). (**B**) After single-spore isolation to produce pure cultures, the isolate newly infected with the virus (An) retained the hygromycin-resistance and the morphology of the VF isolate.

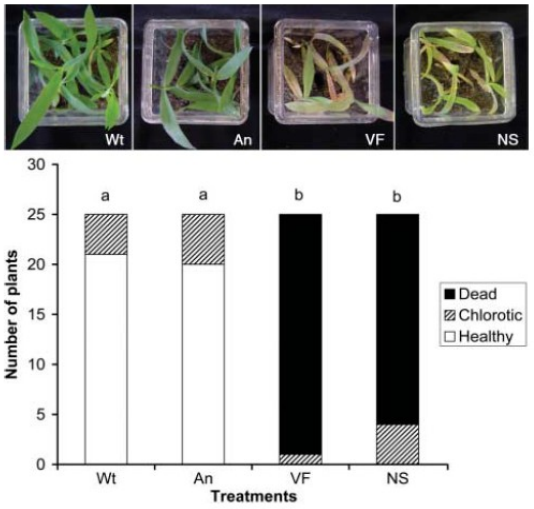
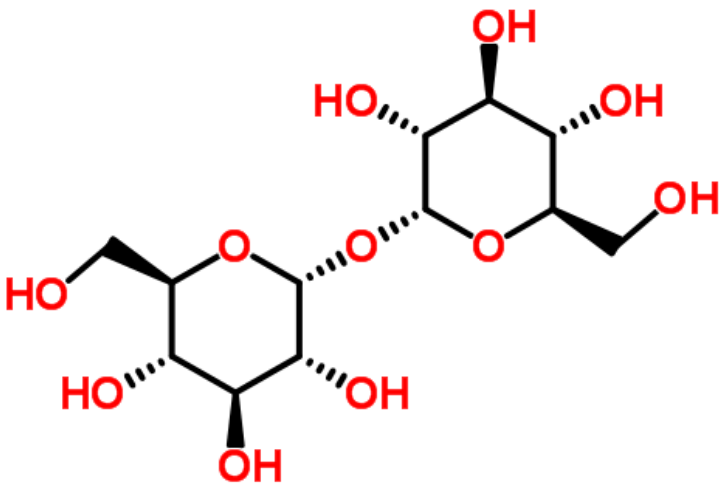


Fig. 2. (**Top**) Representative *D. lanuginosum* plants after the heat-stress experiment with thermal soil simulators. Rhizosphere temperature was maintained at 65°C for 10 hours and 37°C for 14 hours/day for 14 days under greenhouse conditions. Plants were nonsymbiotic (NS) and symbiotic with the wild-type virus-infected isolate of *C. protuberata* (Wt), the hygromycin-resistant isolate newly infected with the virus through hyphal anastomosis (An), or the virus-free hygromycin-resistant isolate (VF). (**Bottom**) The histogram presents the number of plants chlorotic, dead, and alive at the end of the experiment. The small letters on top of the bars indicate statistical differences or similarities (chi-square test, $P < 0.01$).

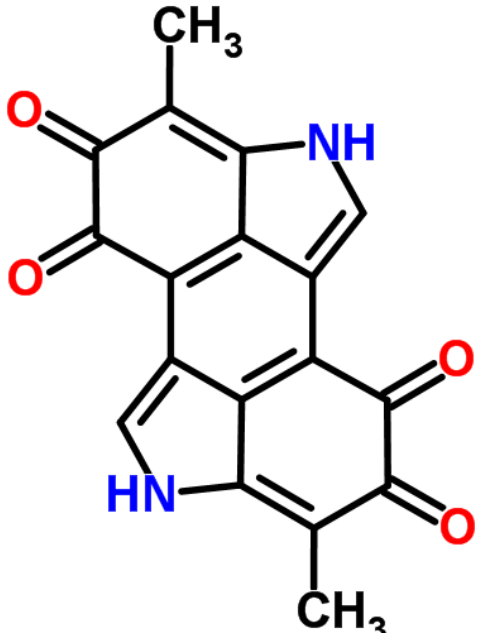
Trehalose

- Sugar related to drought and heat tolerance



Melanin

- Pigment associated with fungi abiotic-stress tolerance



Freezing and Drought Tolerance

- *Nicotiana benthamiana*
- Tobacco mosaic virus (TMV)
  Cucumber mosaic virus (CMV)
  Brome mosaic virus (BMV)
  Tobacco rattle virus (TRV)
- Resistance for water-withdrawn and freezing condition

New Phytologist

Research

Virus infection improves drought tolerance

Ping Xu, Fang Chen, Jonathan P. Mannas, Tracy Feldman, Lloyd W. Sumner and Marilyn J. Roossinck

The S. R. Noble Foundation, Ardmore, OK 73401, USA

Author for correspondence:
Marilyn J. Roossinck
Tel: +1 580 224 6630
Fax: +1 580 224 6692
Email: mroossinck@noble.org

Received: *14 July 2008*
Accepted: *10 August 2008*

Summary

- Viruses are obligate intracellular symbionts. Plant viruses are often discovered and studied as pathogenic parasites that cause diseases in agricultural plants. However, here it is shown that viruses can extend survival of their hosts under conditions of abiotic stress that could benefit hosts if they subsequently recover and reproduce.
- Various plant species were inoculated with four different RNA viruses, *Brome mosaic virus* (BMV), *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* and *Tobacco rattle virus*. The inoculated plants were stressed by withholding water. The onset of drought symptoms in virus-infected plants was compared with that in the plants that were inoculated with buffer (mock-inoculated plants). Metabolite profiling analysis was conducted and compared between mock-inoculated and virus-infected plants before and after being subjected to drought stress.

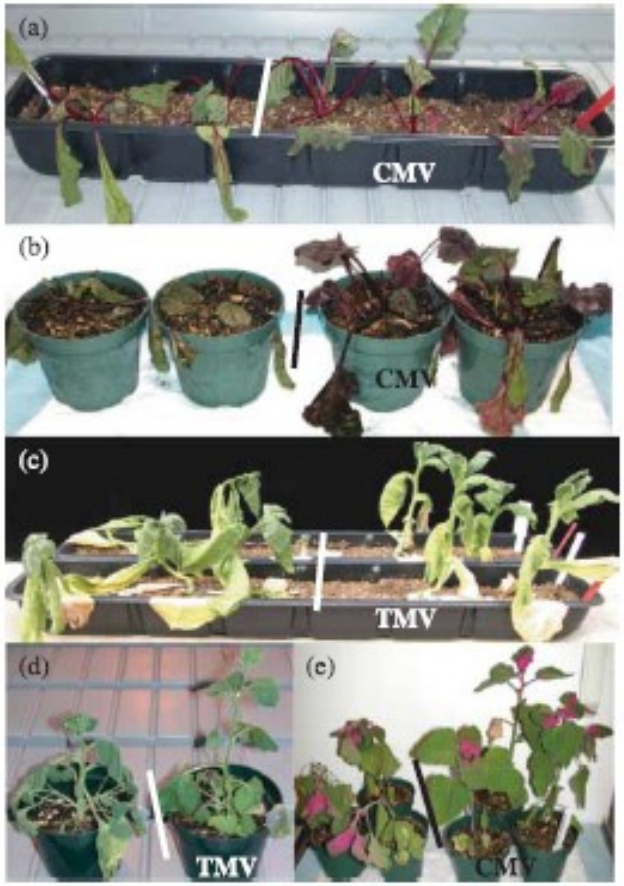
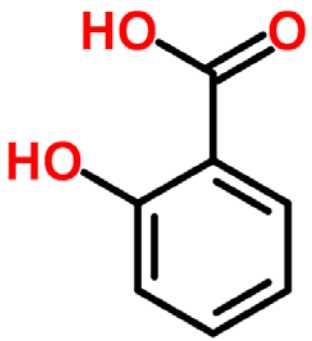
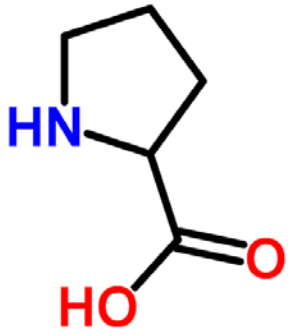


Fig. 1 Comparison of the symptoms in mock-inoculated and virus-infected plants caused by drought or frost stress. (a) Beet (*Beta vulgaris*) plants at 4 d after withholding water (daww). (b) Beet plants placed at 15°C for 16 h in the daytime and −2°C for 8 h at night for 2 d followed by another 15°C for 16 h and −4°C for 8 h. (c) Tobacco (*Nicotiana tabacum*) plants at 12 daww. (d) *Nicotiana benthamiana* plants at 8 daww. (e) *Chenopodium amaranthicolor* plants at 4 daww. Plants on the left side of the black or white lines are mock-inoculated plants and on the right are virus-infected plants.

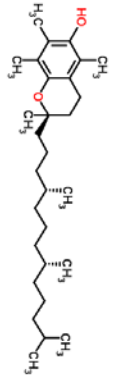
Salicyclic Acid



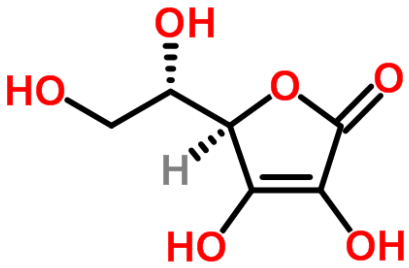
Proline



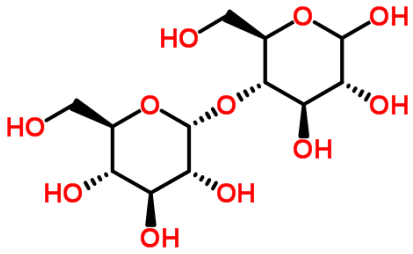
Tocopherol



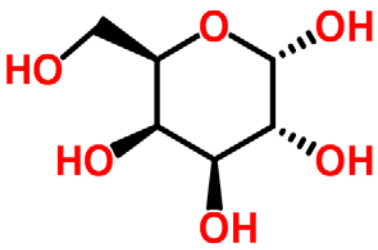
Ascorbic Acid



Maltose



Galactose

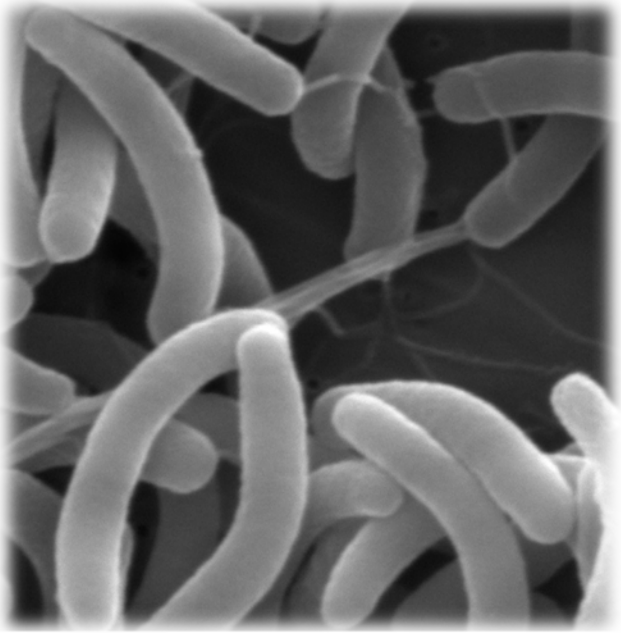


Plant Osmoprotectants and Anti-oxidants

Virus and Virulence

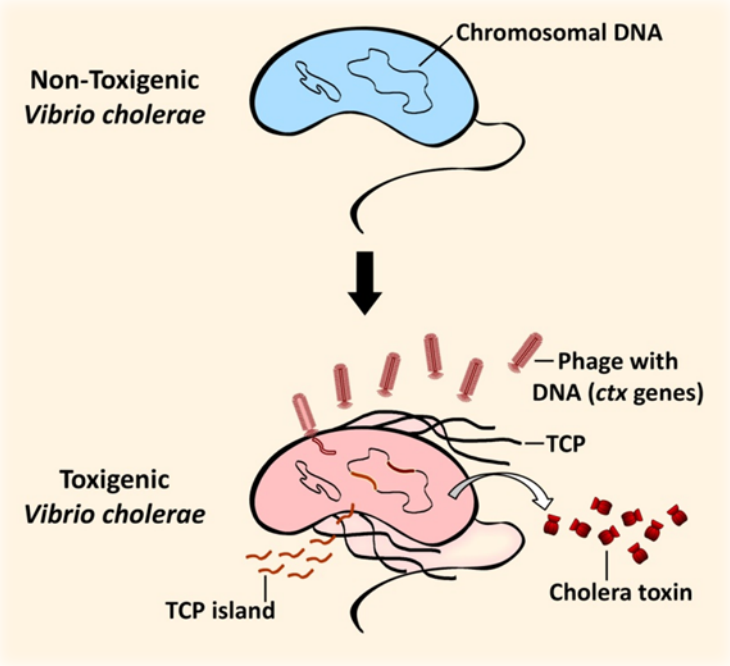
Vibrio cholerae

- Gram-negative, facultative anaerobic bacterium
- Cholera-causing agent



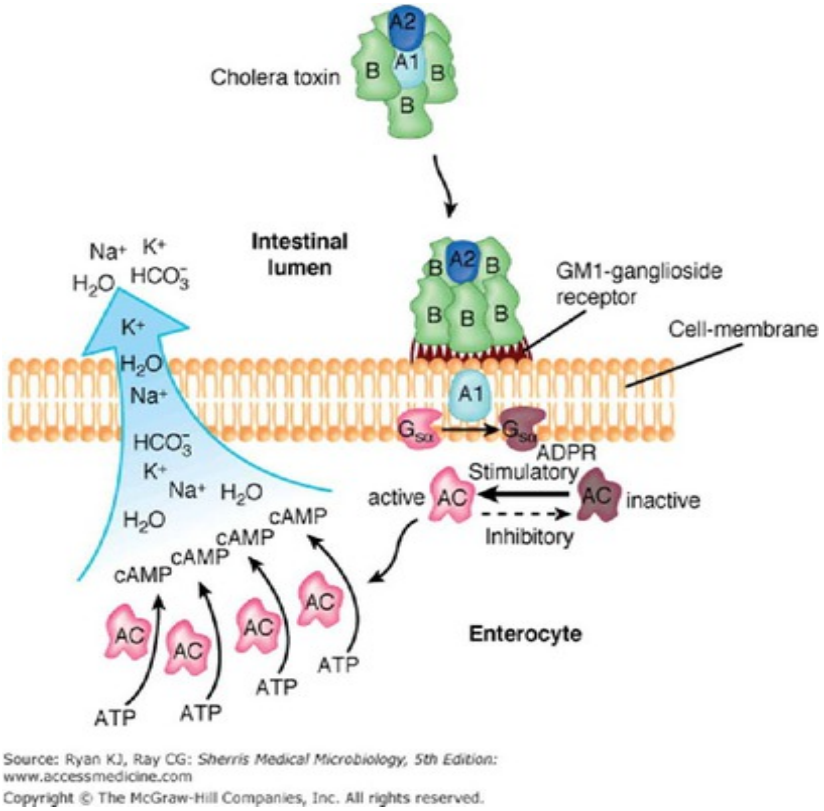
Vibrio cholerae

- Gram-negative, facultative anaerobic bacterium
- Cholera-causing agent



CTXϕ

- Filamentous bacteriophage
- Origin of cholera toxin gene
- 6.9 kb viral genome



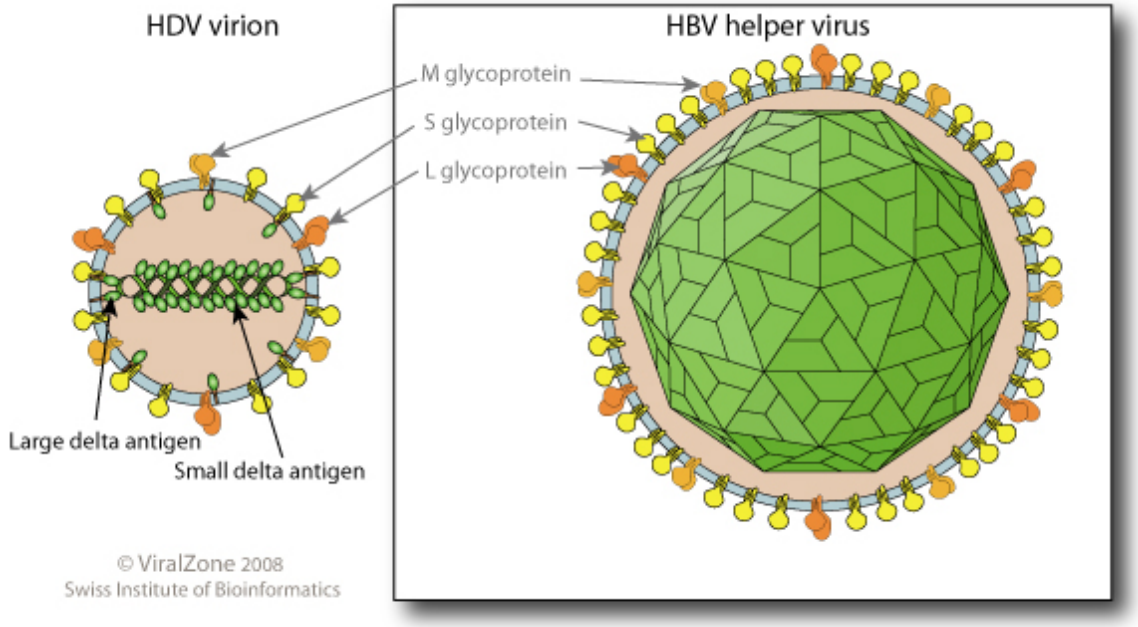
(Boucher Y., et al, 2014)

Hepatitis D Virus (HDV)

- Defective virus
- 1.7 kb RNA genome

Hepatitis B Virus (HBV)

- Helper virus
- 3 kb DNA genome



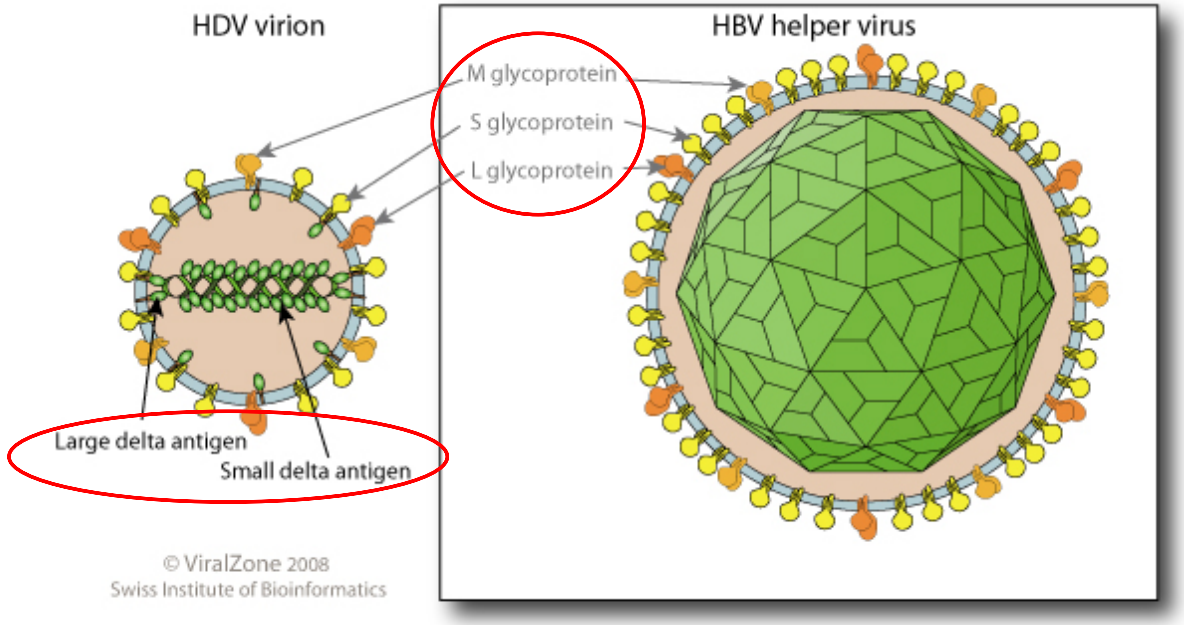
HDV Coinfection - occurs simultaneously when first infected with the hepatitis B virus
HDV Superinfection - occurs in persons with an existing chronic hepatitis B infection

Hepatitis D Virus (HDV)

- Defective virus
- 1.7 kb RNA genome

Hepatitis B Virus (HBV)

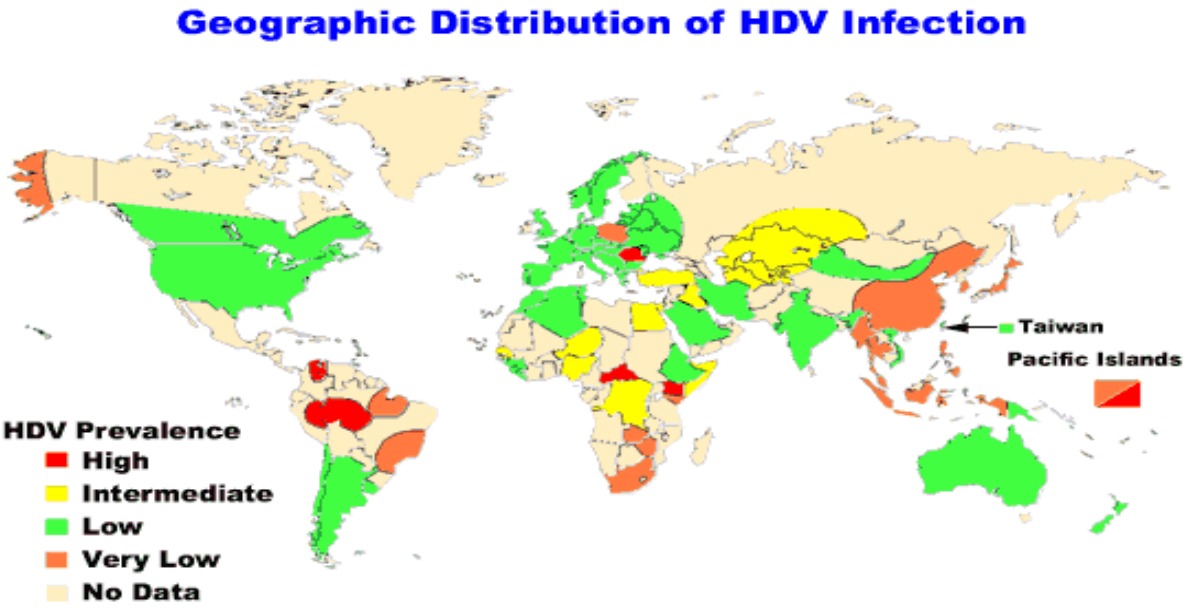
- Helper virus
- 3 kb DNA genome



Small delta antigen (SDAg) - used for HDV replication
Large delta antigen (LDAg) - required for HDV RNA packaging and new HDV virion assembly with the association of HBV surface antigens (HBsAgs) and envelope proteins.

HDV – HBV Infection

- Exaggerated disease state
- About 350 million HBV carriers worldwide
- More than 15-20 million coinfected by HDV
- More severe acute viral hepatitis
- Higher liver failure chances
- Rapider liver cirrhosis progression
- Liver cancer

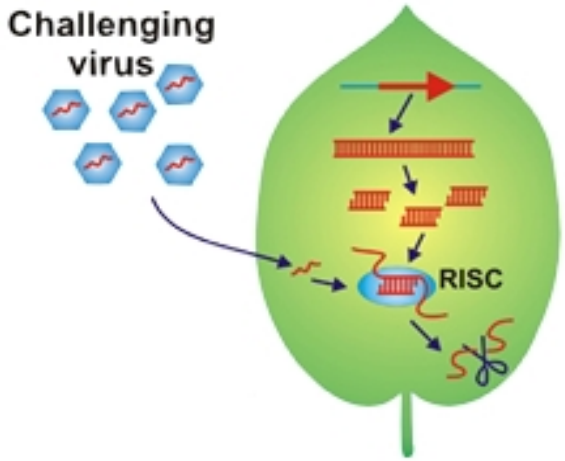


(CDC, 2014)

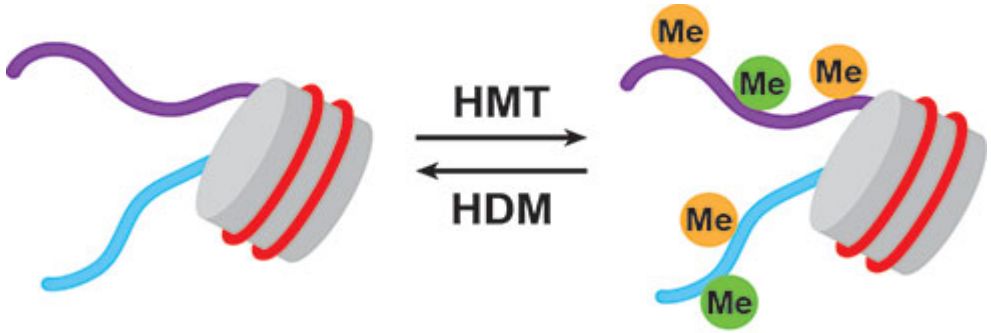
Virus and Disease Prevention

Plants with Pararetrovirus

- Tobacco, rice, banana and potato
- Against viral infections



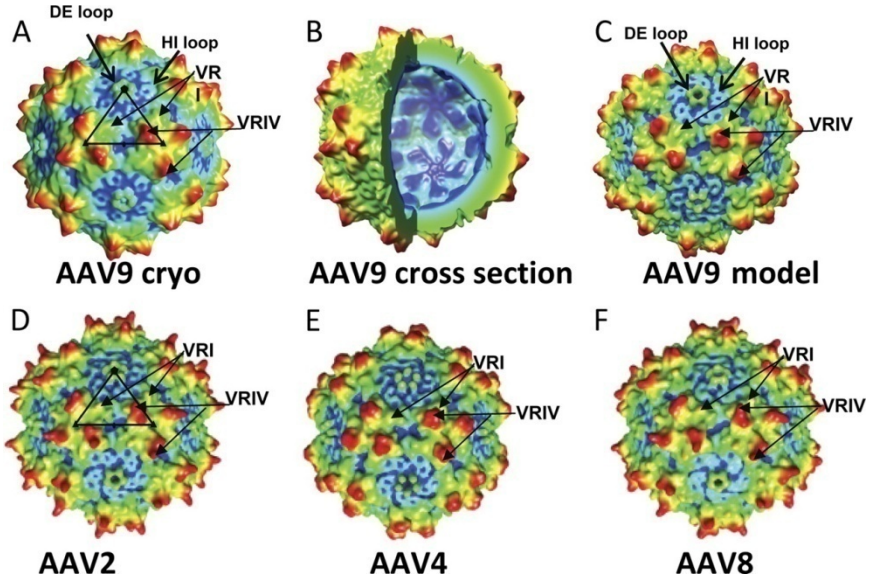
Post-transcriptional gene silencing

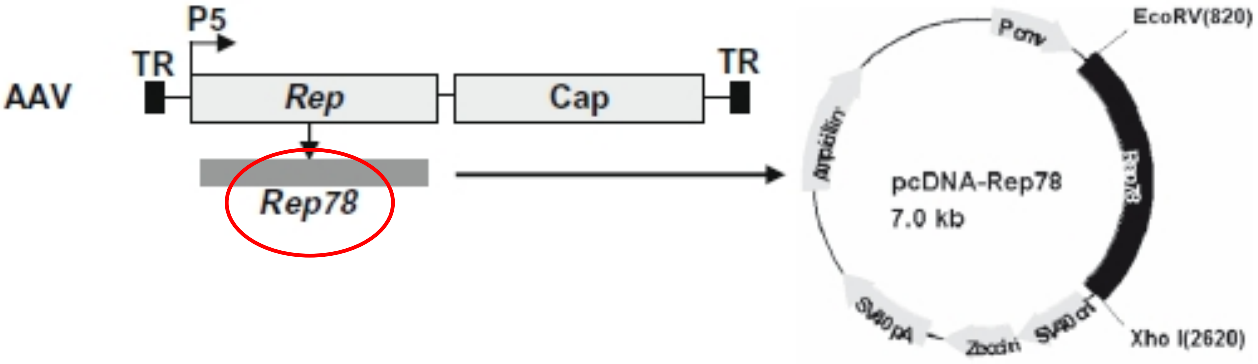


RNA-mediated epigenetic modifications (RNA-directed DNA or histone methylation)

Humans with Adeno-associated Virus (AAV)

- Parvoviridae family
- Not known to cause any human diseases
- 4.7 kb ssDNA genome





AAV Rep78 Protein vs HBV

- Inhibition of promoter activities of proto-oncogenes (such as *ras* and c-*myc*)
- Interference with the transcription and expression of Hepatitis B virus core promoters

Adeno-associated virus Rep78 protein inhibits Hepatitis B virus replication through regulation of the HBV core promoter

Tianhui Liu[a], Min Cong[a], Ping Wang[a], Jidong Jia[a], Yong Liu[b], Paul L. Hermonat[b], Hong You[a,*]

[a] Liver Research Center, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, PR China
[b] Gene Therapy Center, Department of Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

ARTICLE INFO

Article history:
Received 9 April 2009
Available online 3 May 2009

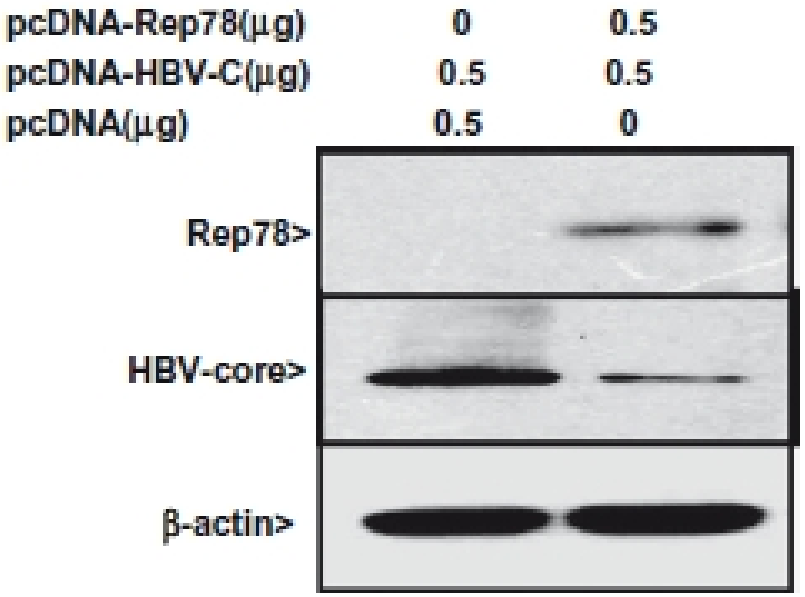
Keywords:
Hepatitis B virus
Core promoter
Rep78
Replication
Transcription

ABSTRACT

Rep78, the *rep* gene product of adeno-associated virus (AAV), has been shown to inhibit the replication of several DNA viruses. This study investigated the effects of Rep78 on replication of Hepatitis B virus (HBV) and possible mechanisms of inhibition. We have shown that HBV DNA replication and secretion of HBsAg and HBeAg in HepG2 2.2.15 cells were inhibited by Rep78. We have also demonstrated, using *in vitro* transcription and luciferase assay, that Rep78 binds to the HBV core promoter (HBV CP) and inhibits HBV CP activity. Furthermore, after Rep78 and HBV core protein expression plasmids were co-transfected into HepG2 cells, the expression of HBV core protein was inhibited significantly. These results suggest that Rep78 can inhibit the replication of HBV, correlating strongly with suppression of HBV CP activity.

AAV Rep78 Protein vs HBV

- Inhibition of promoter activities of proto-oncogenes (such as *ras* and c-*myc*)
- Interference with the transcription and expression of Hepatitis B virus core promoters



AAV Rep78 Protein vs HPV

- AAV infection negatively correlated with HPV-associated cervical carcinoma
- Apoptosis of HPV-infected keratinocytes
- Inhibition of transcription initiation of HPV-16 and HPV-18 LCR

Adeno-Associated Virus Type 2 Induces Apoptosis in Human Papillomavirus-Infected Cell Lines but Not in Normal Keratinocytes[▽]

Samina Alam and Craig Meyers*

Department of Microbiology and Immunology, Pennsylvania State University College of Medicine, 500 University Drive, Hershey, Pennsylvania 17033

Received 16 February 2009/Accepted 13 July 2009

The results of seroepidemiological studies suggest that infection with adeno-associated virus type 2 (AAV2) is negatively correlated with the incidence of human papillomavirus (HPV)-associated cervical cancer. We studied the potential of AAV2 oncosuppression of HPV and showed that HPV/AAV2 coinfection of cells culminated in apoptotic death, as determined by DNA laddering and caspase-3 cleavage. The induction of apoptosis coincided with AAV2 Rep protein expression; increased S-phase progression; upregulated pRb displaying both hyper- and hypophosphorylated forms; increased levels of $p21^{WAF1}$, $p16^{INK4}$, and $p27^{KIP1}$ proteins; and diminished levels of E7 oncoprotein. In contrast, normal keratinocytes that were infected with AAV2 or transfected with the cloned full-length AAV2 genome failed to express Rep proteins or undergo apoptosis. The failure of AAV2 to productively infect normal keratinocytes could be clinically advantageous. The delineation of the molecular mechanisms underlying the HPV/AAV2 interaction could be harnessed for developing novel AAV2-derived therapeutics for cervical cancer.

AAV Rep78 Protein vs HPV

- AAV infection negatively correlated with HPV-associated cervical carcinoma
- Apoptosis of HPV-infected keratinocytes
- Inhibition of transcription initiation of HPV-16 and HPV-18 LCR

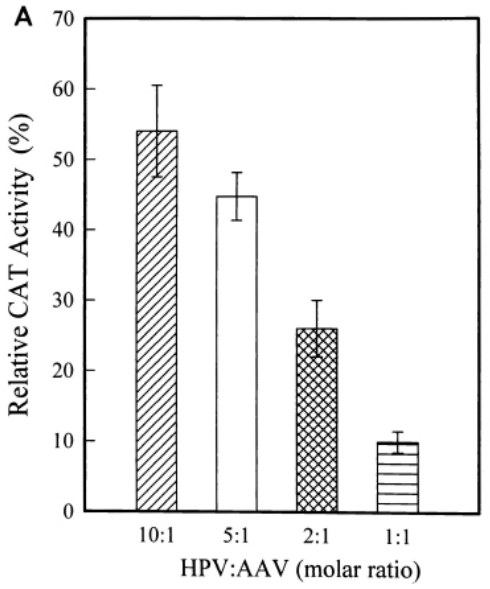
Adeno-Associated Virus Major Rep78 Protein Disrupts Binding of TATA-Binding Protein to the p97 Promoter of Human Papillomavirus Type 16†

PEI-FEN SU,[1,2] SHU-YUAN CHIANG,[1] CHENG-WEN WU,[1]* AND FELICIA Y.-H. WU[1]

Division of Cancer Research, Institute of Biomedical Sciences, Academia Sinica,[1] and Institute of Life Sciences, National Defense Medical Center,[2] Taipei, Taiwan, Republic of China

Received 24 June 1999/Accepted 7 December 1999

Adeno-associated virus type 2 (AAV) is known to inhibit the promoter activities of several oncogenes and viral genes, including the human papillomavirus type 16 (HPV-16) E6 and E7 transforming genes. However, the target elements of AAV on the long control region (LCR) upstream of E6 and E7 oncogenes are elusive. A chloramphenicol acetyltransferase assay was performed to study the effect of AAV on the transcription activity of the HPV-16 LCR in SiHa (HPV-positive) and C-33A (HPV-negative) cells. The results reveal that (i) AAV inhibited HPV-16 LCR activity in a dose-dependent manner, (ii) AAV-mediated inhibition did not require the HPV gene products, and (iii) the AAV replication gene product Rep78 was involved in the inhibition. Deletion mutation analyses of the HPV-16 LCR showed that regulatory elements outside the core promoter region of the LCR may not be direct targets of AAV-mediated inhibition. Further study with the electrophoretic mobility shift assay demonstrated that Rep78 interfered with the binding of TATA-binding protein (TBP) to the TATA box of the p97 core promoter more significantly than it disrupted the preformed TBP-TATA complex. These data thus suggest that Rep78 may inhibit transcription initiation of the HPV-16 LCR by disrupting the interaction between TBP and the TATA box of the p97 core promoter.



Conclusion

- Extraordinary diversity in replication strategies
- Mutualism with their hosts

Reproduction	Successful reproduction of parasitoid wasp larvae
Development	Establishment of placental mammals
Tolerance	Enhancement of tolerance to adverse conditions
Virulence	Greater virulence for invasion
Disease Prevention	Protection of hosts from other infections

Reference

- Alam S. and Meyers C. (2009). *Adeno-Associated Virus Type 2 Induces Apoptosis in Human Papillomavirus-Infected Cell Lines but Not in Normal Keratinocytes*. Journal of Virology, Oct. 2009, p. 10286–10292 Vol. 83, No. 19
- Alibrandi A., Cacciola I., Calvi C., Colucci G., Gaeta G.B., Iannello G., Levrero M., Raffa G., Raimond G., Santantonio T., Squadrito G. and Pollicino T. (2011*). Replicative and Transcriptional Activities of Hepatitis B Virus in Patients Coinfected with Hepatitis B and Hepatitis Delta Viruses*. Journal of Virology, Jan. 2011, p. 432–439 Vol. 85, No. 1
- Alves C., Branco C. and Cunha C. (2013). *Hepatitis Delta Virus: A Peculiar Virus*. Advances in Virology, Volume 2013, Article ID 560105, 11 pages
- Andersson A., Eriksson L., Larsson E., Scherer J., To¨njes R.R. and Venables P.J.W. (2002). *Developmental Expression of HERV-R (ERV3) and HERV-K in Human Tissue*. Virology 297, 220–225
- Asgari S. (2004). *SoLS News*. The University of Queensland. Website: http://sci-s03.bacs.uq.edu.au/sib/sib-news/march04/johannes.htm
- Aslanidi G., Baker T.S., Bennett A., Byrne B.J., DiMattia M.A., Gurda B.L., McKenn M.A., McKenna R., Mitchell M., Muzyczk N., Nam H.J., Olson N.H., Potter M., Sinkovits R.S., Vliet K.V. and Zolotukhin S. (2012) *Structural Insight into the Unique Properties of Adeno-Associated Virus Serotype 9. Journal of Virology*, Volume 86, Number 12, p. 6947–6958.
- Bao X.D. and Roossinck M.J. (2013) *A life history view of mutualistic viral symbioses: quantity or quality for cooperation?* Current Opinion in Microbiology 2013, 16:514–518
- Be´zier A., Drezen J., Herniou E.A., Huguet E., Periquet G. and The´ze´ J. *When parasitic wasps hijacked viruses: genomic and functional evolution of polydnaviruses*. (2013). Phil Trans R Soc B 368: 20130051.
- BioNews. (2014) *Koala holds clues about origins of the human genome*. BioScholar. Website: http://news.bioscholar.com/2014/11/koala-holds-clues-origins-human-genome.html
- Bloom D.C., Camerini D., Hewlett M.J. and Wagner E.K. (2008). Basic virology, third edition, P.9. Blackwell publishing.
- Borroto-Fernández E.G., Gregor W., Machado M.L.C., Matzke M., Mette M.F., Schwarzacher T., Staginnus C. and Teo C.H. (2007). *Endogenous pararetroviral sequences in tomato (Solanum lycopersicum) and related species*. BMC Plant Biology 2007, 7:24 doi:10.1186/1471-2229-7-24
- Boucher Y., Keim P.S. and Orata F.D. (2014) *The 2010 Cholera Outbreak in Haiti: How Science Solved a Controversy.* Website: http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1003967
- Burghardt R.C., Dunlap K.A., Farmer J.L., Hayashi K., Palmarini M., Spencer T.E. and Varela M. (2006). *Endogenous retroviruses regulate periimplantation placental growth and differentiation*. PNAS , vol. 103, no. 39.
- Carrapiço F. and Rodrigues T. (2005). *Symbiogenesis and the Early Evolution of Life*. Proc. of SPIE Vol. 5906, 59060R.
- CDC (2014) *Hepatitis D.* Website: http://www.cdc.gov/hepatitis/HDV/index.htm#
- Chen F., Feldman T., Mannas J.P., Roossinck M.J., Sumner L.W. and Xu P. (2008). *Virus infection improves drought tolerance*. New Phytologist, doi: 10.1111/j.1469-8137.2008.02627.x
- Chiang S.Y., Su P.F., Wu C.W. and Wu F.Y.H. (2000). *Adeno-Associated Virus Major Rep78 Protein Disrupts Binding of TATA-Binding Protein to the p97 Promoter of Human Papillomavirus Type 16*. Journal of Virology, Mar. 2000, p. 2459–2465
- Cong M., Hermonat P.L., Jia I.D., Liu T.H., Liu Y., Wang P. and You H. (2009). *Adeno-associated virus Rep78 protein inhibits Hepatitis B virus replication through regulation of the HBV core promoter*. Biochemical and Biophysical Research Communications 385 (2009) 106–111
- Davis B.M. and Waldor M.K. (2003). *Filamentous phages linked to virulence of Vibrio cholerae*. Current Opinion in Microbiology 2003, 6:35-42
- Denner J. and Young P.R. (2013). *Koala retroviruses: characterization and impact on the life of koalas*. Retrovirology 2013, 10:108.

- Eiden M.V., Farrell K.B. and Oliveira N.M. (2006). *In vitro characterization of a koala retrovirus. J Virol*. 2006 Mar;80(6):3104-7.
- Evidence for Evolutionary Model (2012) *Three Layers of Endogenous Retroviral Evidence for the Evolutionary Model*. Website: http://www.evolutionarymodel.com/ervs.htm
- Faruque S.M. and Mekalanos J.J. (2012). Phage-bacterial interactions in the evolution of toxigenic Vibrio cholerae. Virulence 3:7, 1–10.
- Fleming J.G. and Summers M.D. (1991). *Polydnavirus DNA is integrated in the DNA of its parasitoid wasp host*, Vol. 88, pp. 9770-9774. Proc. Nati. Acad. Sci. USA
- Fweditor (2008) *Parasitic wasps can help control flies on poultry units*. Website: http://www.fwi.co.uk/poultry/parasitic-wasps-can-help-control-flies-on-poultry-units.htm
- Greig D., McBride R. and Travisano M. (2008). *Fungal Viral Mutualism Moderated by Ploidy*. Evolution 62-9: 2372–2380
- Harris J.R. (1998). *Placental endogenous retrovirus (ERV): structural, functional, and evolutionary significance*. Bio-Essays 20:307-316, 1998.
- Hindawi (2011) *MLLVs.* Website: http://www.hindawi.com/journals/av/2011/341294/fig5/
- Lo S.C. and Huang C.R. (2014). *Hepatitis D virus infection, replication and cross-talk with the hepatitis B virus*. World J Gastroenterol 2014 October 28; 20(40): 14589-14597
- Manns M.P. and Wedemeyer H. (2010). *Epidemiology, pathogenesis and management of hepatitis D: update and challenges ahead*. Nature Reviews, Gastroenterology & Hepatology volume 7
- Márquez L.M., Redman R.S., Rodriguez R.J. and Roossinck MJ. (2007). *A virus in a fungus in a plant – three way symbiosis required for thermal tolerance*. Science 315: 513–515.
- Molecular Cytogenetics Research Group. (2014). *Paradigm*. University of Leicester, Department of Biology. Website: http://www.le.ac.uk/bl/phh4/prv.htm
- Roossinck M.J. (2011). *Changes in Population Dynamics in Mutualistic versus Pathogenic Viruses*. Viruses 2011, 3, 12-19.
- Roossinck M.J. (2005). *Symbiosis Versus Competition in Plant Virus Evolution*. Nature Review, Microbiology, volume 3, Devember 2005, 917-924
- Roossinck M.J. (2011). *The good viruses: viral mutualistic symbioses*. Nature Reviews , Microbiology Volume 9.
- Rowland T. (2009) *Showtime for Stem Cells*. Website: http://www.independent.com/news/2009/nov/21/showtime-stem-cells/
- SIB Swiss Institute of Bioinformatics. (2014). *Deltavirus*. SIB Swiss Institute of Bioinformatics. Website: http://viralzone.expasy.org/all_by_species/175.html
- Spadafora C. (2008). *A Reverse Transcriptase-Dependent Mechanism Plays Central Roles in Fundamental Biological Processes*. Systems Biology in Reproductive Medicine, 54:11–21.
- The Hepatitis Blog. (2011) *What is Hepatitis D*? The Hepatitis Blog. Website: http://thehepatitisblog.com/?p=788
- Vibrio-cholerae.org. (2013) *Vibrio cholerae morphology and culture*. Vibrio-cholerae.org. Website: http://www.vibrio-cholerae.org/

Thank You

Q & A