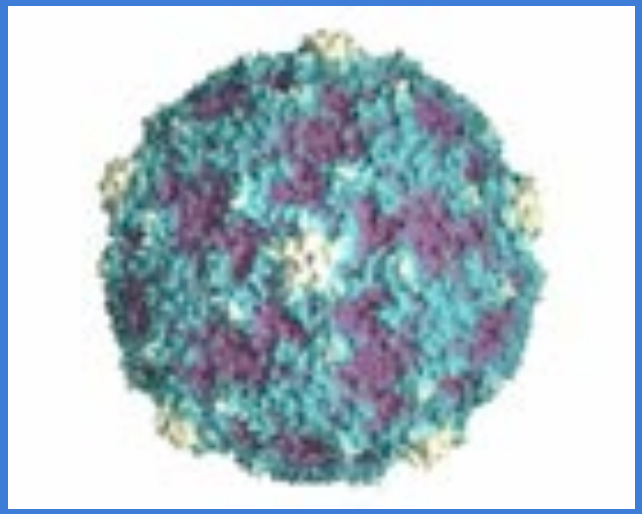
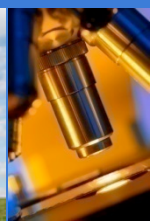
Replication Defective Enterovirus Infections: Implications for Type I Diabetes

N. M. Chapman
Department of Pathology & Microbiology
University of Nebraska Medical Center

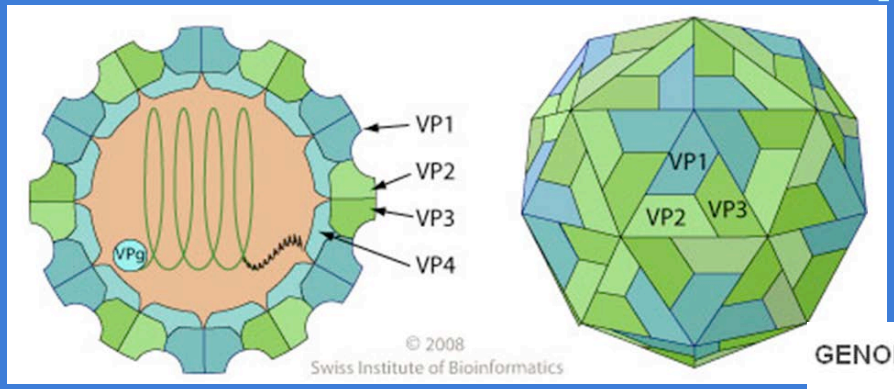


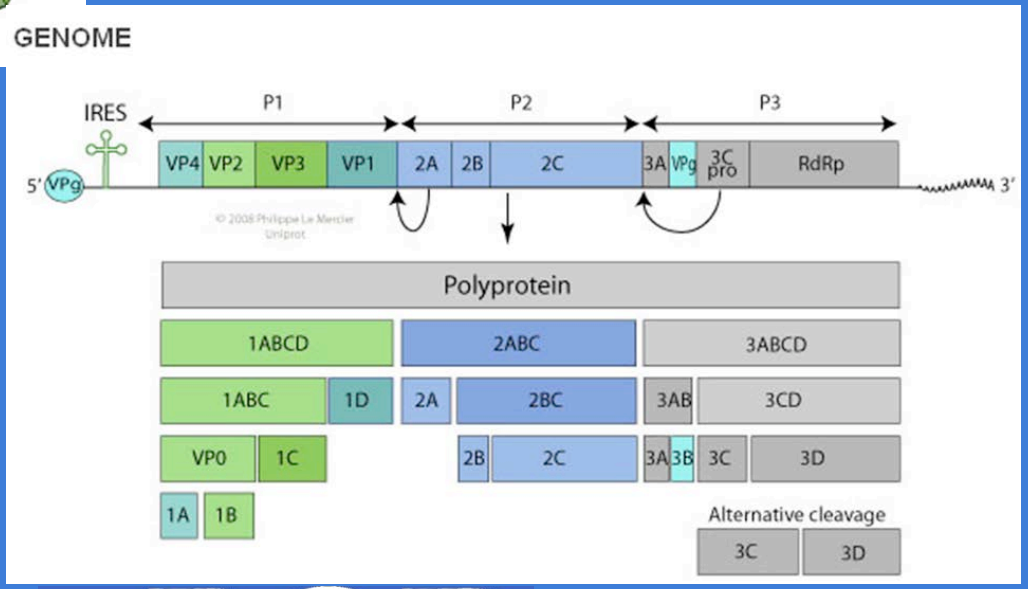


nPOD
Network for Pancreatic Organ Donors with Diabetes

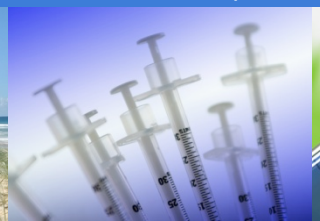



Enterovirus Genome and Capsid

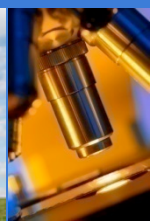




(Source: *ViralZone:www.expasy.org/viralzone, Swiss Institute of Bioinformatics*)



nPOD
Network for Pancreatic Organ Donors with Diabetes

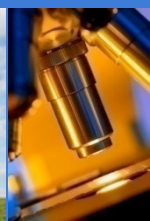


Coxsackie B viruses (CVB) are typical human enteroviruses (HEV)

- 4 Human enteroviruses (HEV) species
- More than 100 characterized (acknowledged or identified) HEV serotypes
  - this number goes up much higher when including the human rhinoviruses
- HEV circulate worldwide
  - spread most commonly by a fecal-oral route of transmission
  - aerosol less commonly (although for human rhinoviruses, aerosol is the primary route)
- With increasing hygienic standards, HEV infections are not as frequent as they once were from birth onwards




nPOD
Network for Pancreatic Organ Donors with Diabetes

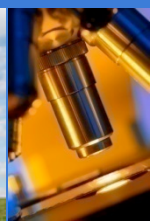


CVB infections cause…

- myocarditis (in up to 1% of hearts in autopsy studies)
- cardiomyopathy (5.5 cases/100,000 population/year)
- pericarditis
- endocarditis
- pancreatitis
- aseptic meningitis
- fulminant/often deadly neonatal/pediatric infections
- polymyositis
- a trigger of type 1 diabetes



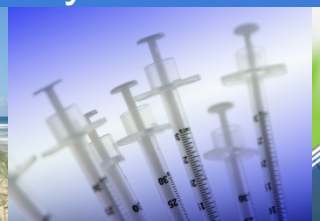

nPOD
Network for Pancreatic Organ Donors with Diabetes



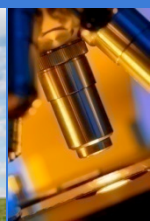
T1D and the HEV connection

- Does such a connection exist?
  - The link is not as firm as in other HEV diseases
    - poliovirus and poliomyelitis
    - CVB and myocarditis or aseptic meningitis
    - rhinoviruses and the common cold

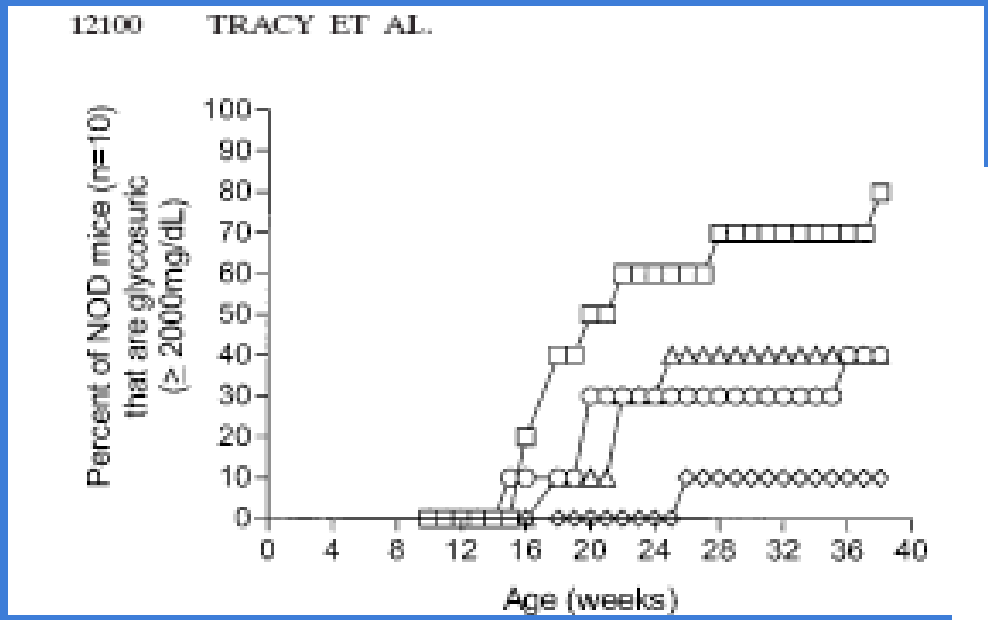
- Nonetheless, data support an HEV etiology in at least some, if not many, cases of human T1D
  - Some cases in which CVB have been isolated at or shortly after T1D onset
  - Other non-CVB HEV (*all HEV-B*, echovirus types 1,3,4,6,9,30) have been isolated in connection with T1D cases
  - Immunohistochemical evidence of HEV protein in islets
  - Experimental rodent models of virus-induced T1D
  - Ability to infect isolated human or murine islets in culture with HEV

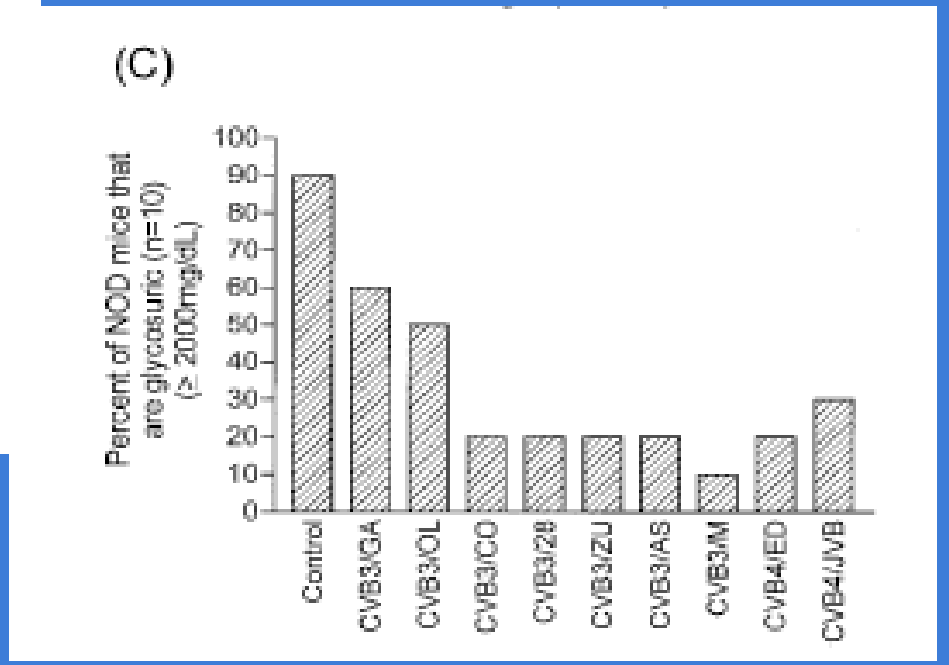



nPOD
Network for Pancreatic Organ Donors with Diabetes



CVB infection of **young** NOD mice without insulitis reduces T1D incidence relative to mock-infected control mice





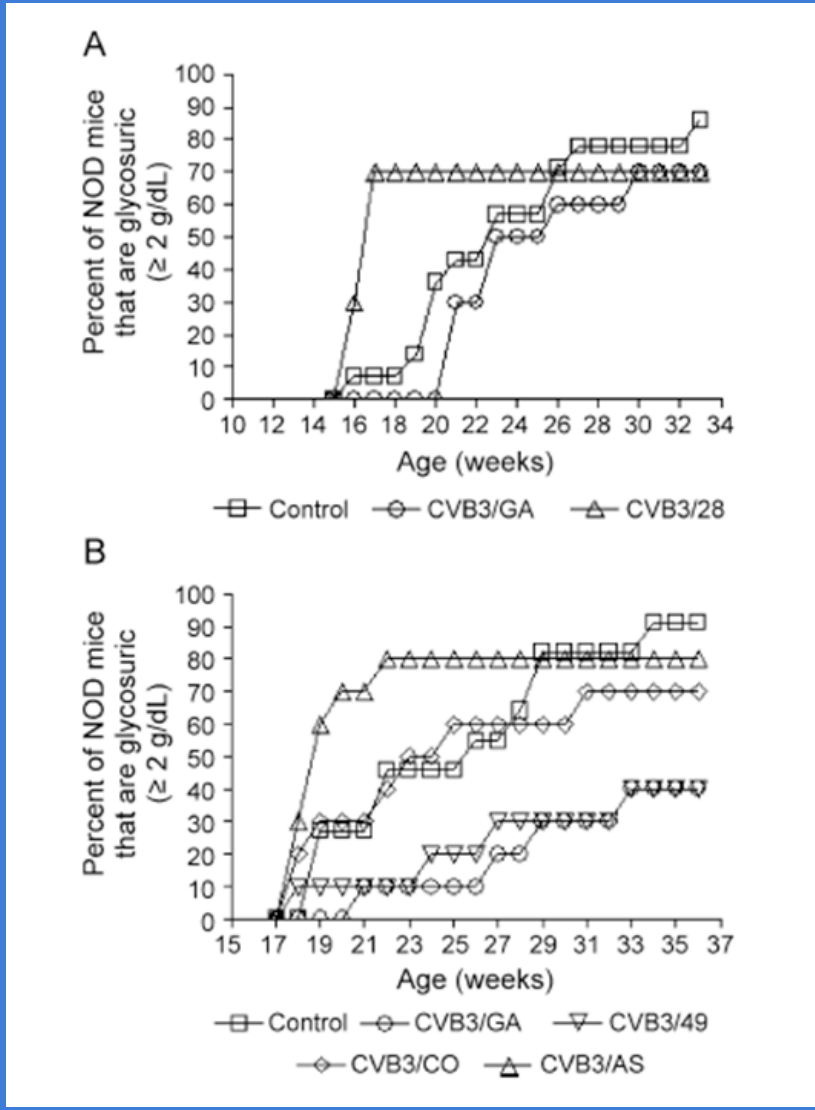
Tracy et al., 2002 J. Virol 76:12097

nPOD
Network for Pancreatic Organ Donors with Diabetes

In **older** mice with existing and
increasing insulitis,
three basic outcomes can be observed:

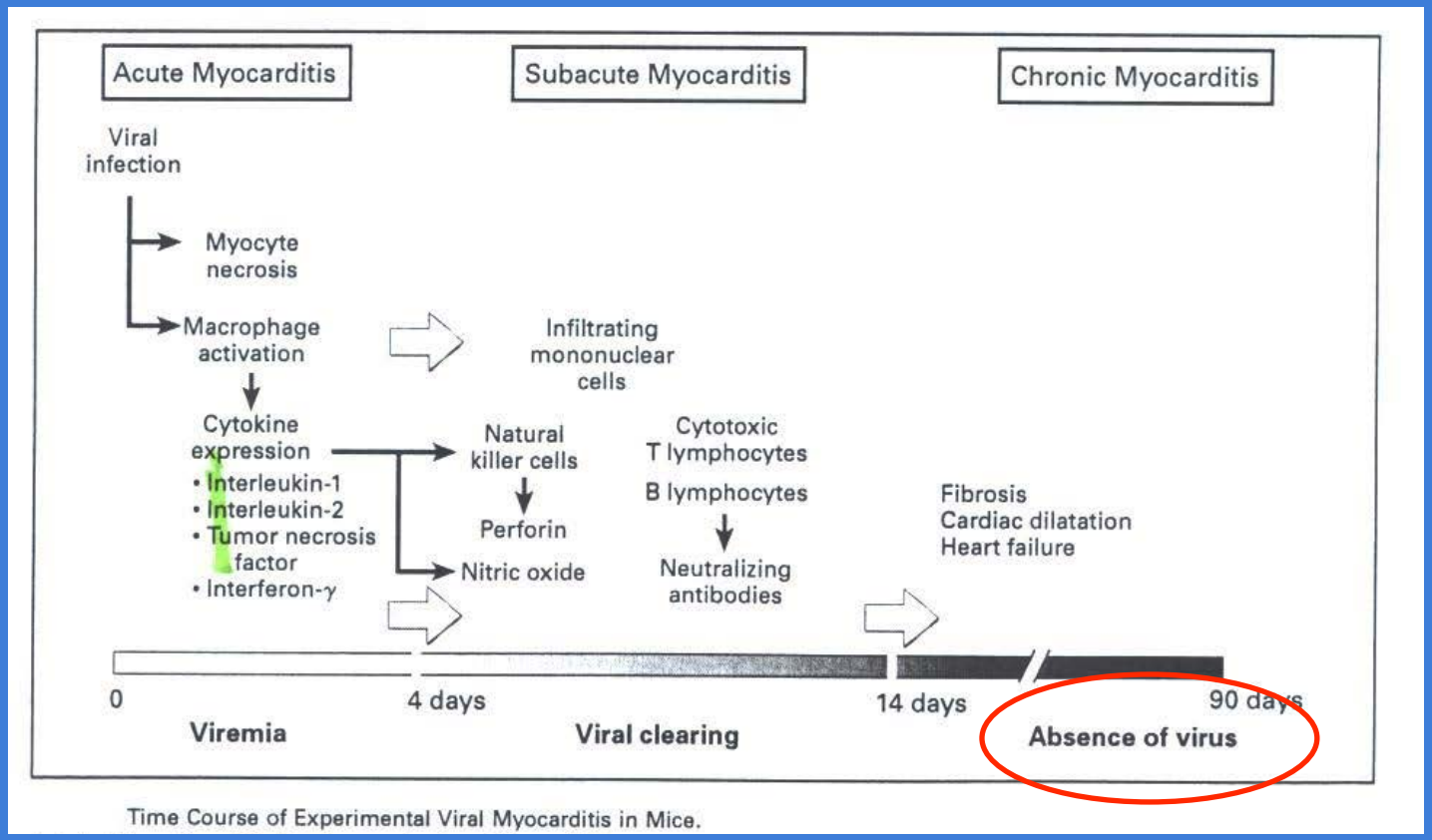
accelerated T1D onset
-or-
no change from uninfected
-or-
slowed onset

From Drescher 2004 Virology 329: 384



nPOD
Network for Pancreatic Organ Donors with Diabetes

Enteroviral myocarditis provides a model of how enterovirus persistence plays a role in human disease

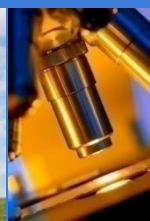


Time Course of Experimental Viral Myocarditis in Mice.

From Kawai C. Circulation. 1999 99:1091-100.



nPOD
Network for Pancreatic Organ Donors with Diabetes

Enterovirus RNA can persist in human heart in the apparent absence of cytopathic or infectious virus

- Enterovirus RNA is detected in about 20-25% of cases of human myocarditis and dilated cardiomyopathy

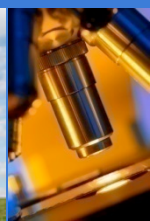
- It is extremely rare to isolate infectious virus from adult hearts (defined as the ability to lyse cells in culture)

  Rey et. al. 2001 J Med Virol. 64:133-40.

  ... Of the 55 biopsy specimens aseptically collected from the explanted hearts of 55 patients, 21 (38.2%) were positive by RT-PCR microplate assay whereas only 19 (34.5%) were positive by nested RT-PCR assay and none were positive by classical cell culture assays. No enterovirus was detectable by RT-PCR or classical cell culture assays in any of the 55 heart biopsy specimens taken from organ donors without any known heart disease. …




nPOD
Network for Pancreatic Organ Donors with Diabetes

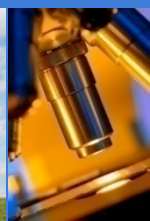


Expression of coxsackievirus proteins alters cardiomyocyte function, but how can function of the cardiomyocyte be altered by a lytic virus infection?

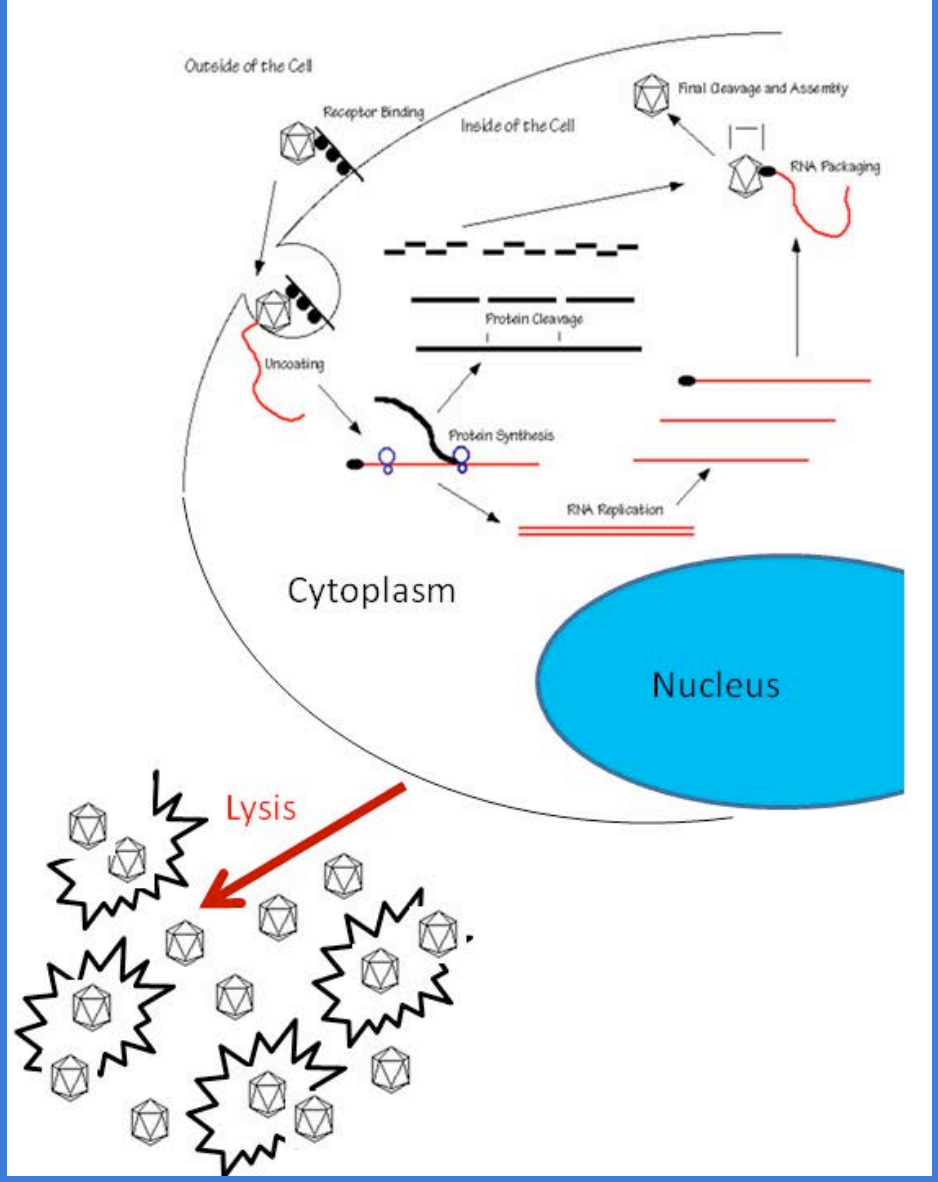
- Infection of myocytes with coxsackievirus B3 causes dystrophin cleavage: Badorff C, et al. Nat Med. 1999 5:320-6.
- Expression of coxsackievirus proteins or just the coxsackievirus 2A protease in the heart of transgenic mice results in cardiomyopathy: Wessely R, et al. J Clin Invest. 1998 102:1444-53; Xiong D, et al. Circulation. 2007 115:94-102.
- Enteroviruses are “hit and run” viruses which lyse the cells they infect (within 8-10 hours in cell culture).
- Normally enterovirus infections are cleared by the generation of enterovirus-specific antibodies.
- How can a cardiomyocyte infected with an enterovirus survive long enough to have effects upon the cytoskeleton?



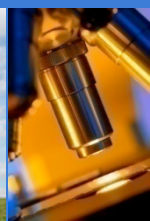

nPOD
Network for Pancreatic Organ Donors with Diabetes



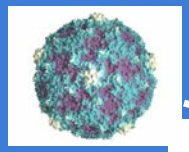
Enterovirus Replication



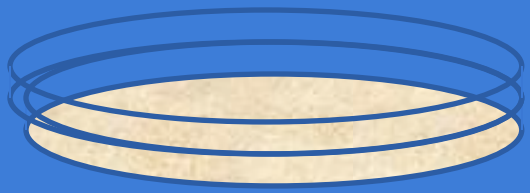
nPOD
Network for Pancreatic Organ Donors with Diabetes



Coxsackievirus B3 (CVB3) persists in myocarditis-susceptible mice without cytopathic virus



Homogenize heart, freeze-thaw, clear debris, filter, inoculate cell culture

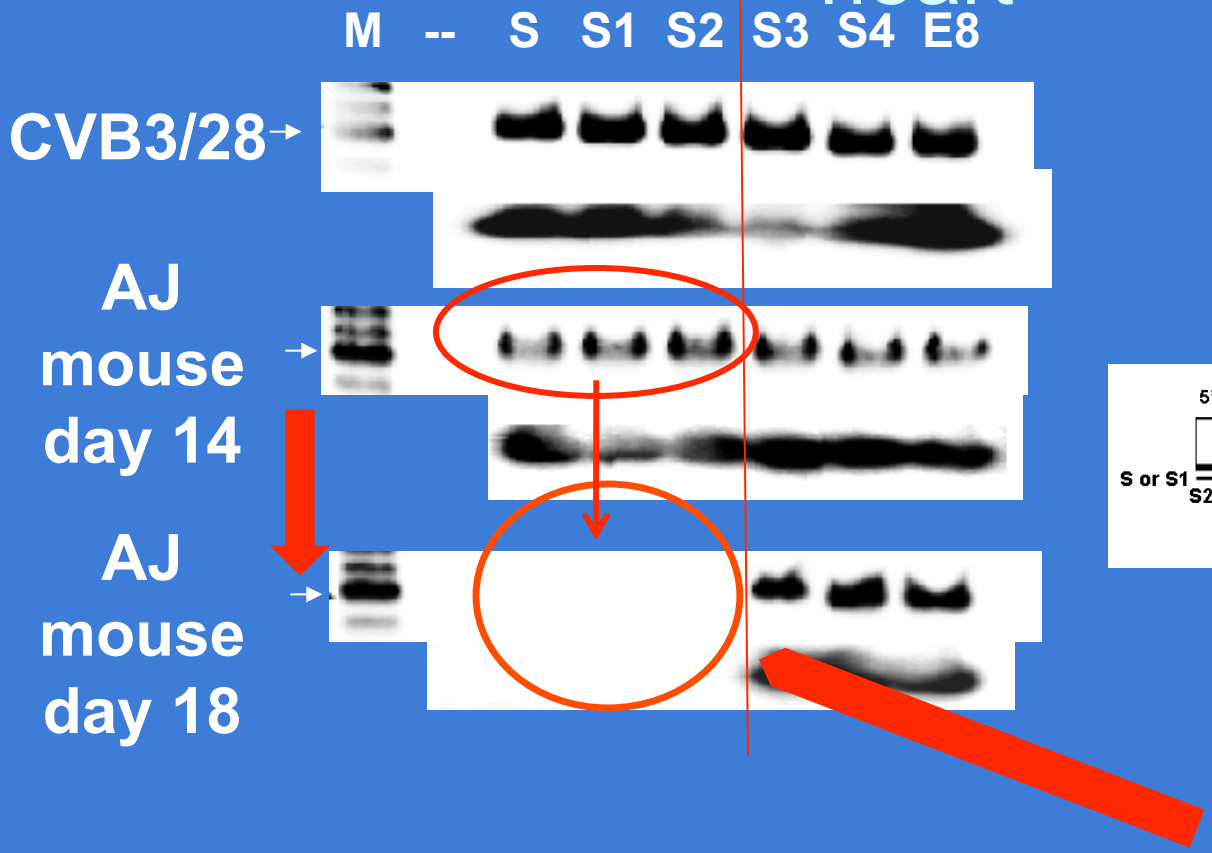


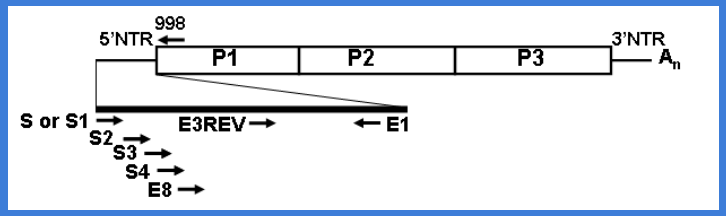
Day p.i.	4	8	14	21	28	53
CPE	3/3	2/2	2/5	1/3	0/5	0/9
RT-PCR	3/3	2/2	5/5	3/3	5/5	1/9

From Kim 2005
J Virol 79: 7024

nPOD
Network for Pancreatic Organ Donors with Diabetes

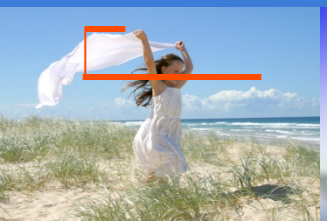
A cardiovirulent CVB3 population evolves to a 5' *deleted* virus population in the mouse heart



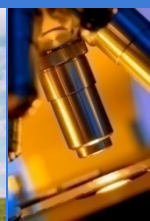


Within just a few days, a viral population could develop that appears to completely lack the 5' end

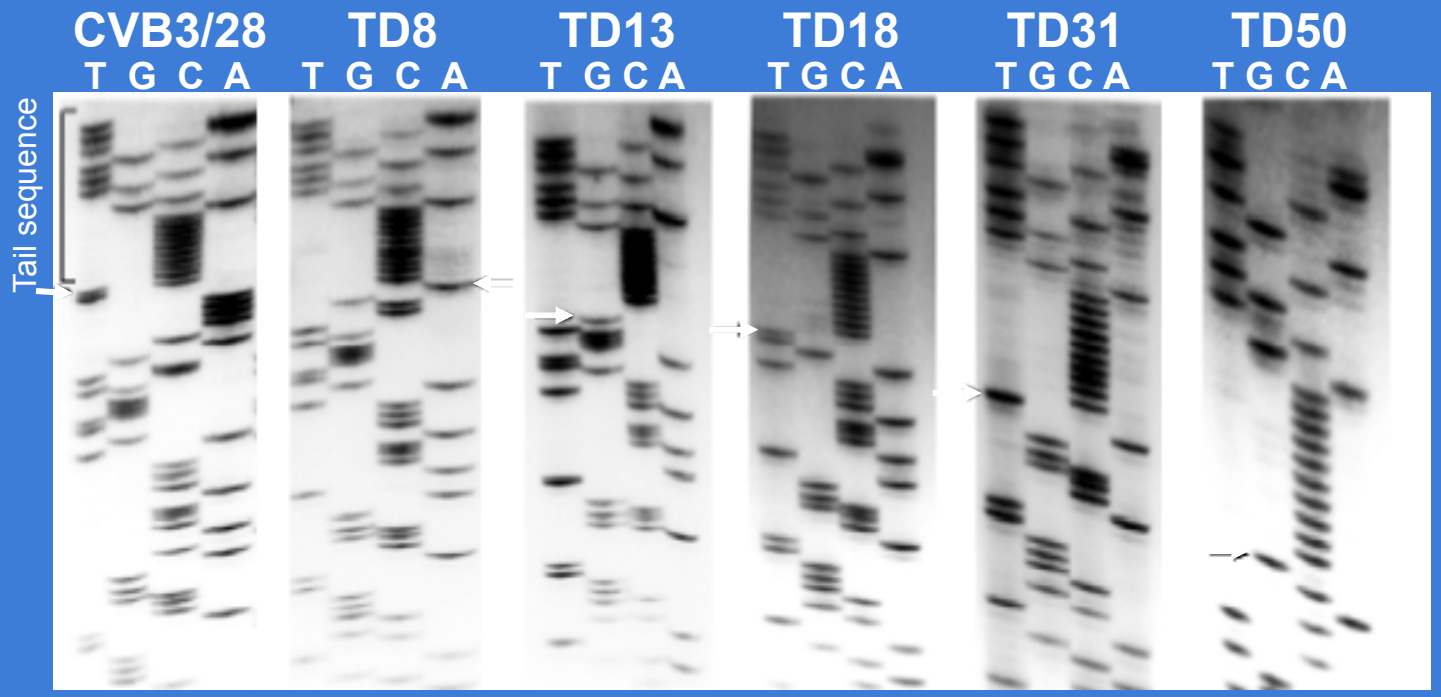
Kim 2005 J Virol 79: 7024



nPOD
Network for Pancreatic Organ Donors with Diabetes

Sequence analysis of cloned ends demonstrates 5' terminal deletions

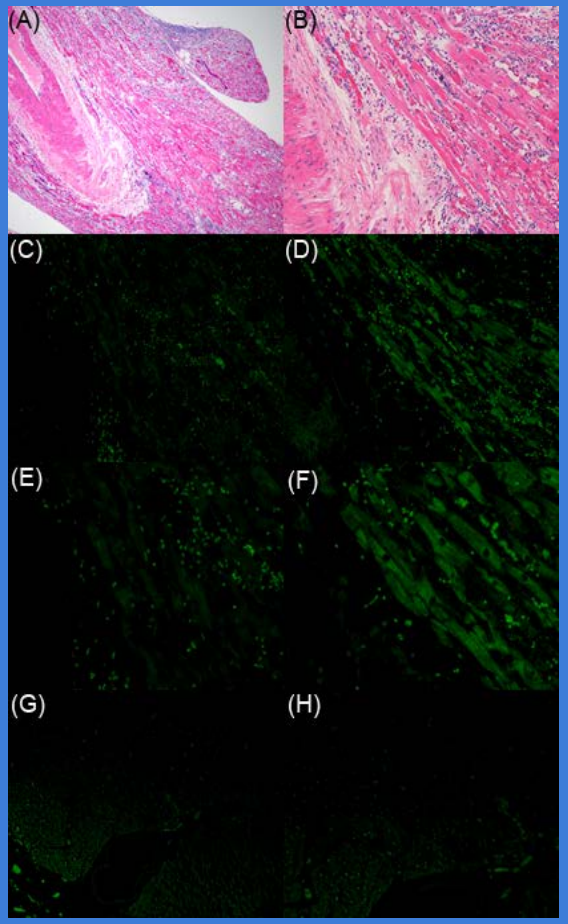



```
CVB3/28  UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGGCCCAUUGGGCGCUAGCACUCU-
TD8            AGCCUGUGGGUUGAUCCCACCCAUAGGGCCCAUUGGGCGCUAGCACUCU-
TD13                GUGGGUUGAUCCCACCCAUAGGGCCCAUUGGGCGCUAGCACUCU-
TD18                     UUGAUCCCACCCAUAGGGCCCAUUGGGCGCUAGCACUCU-
TD31                               UAGGGCCCAUUGGGCGCUAGCACUCU-
TD50                                                  GCACUCU-
```

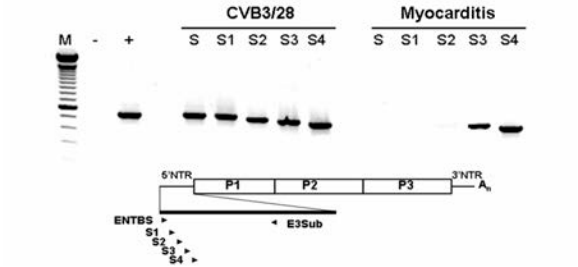


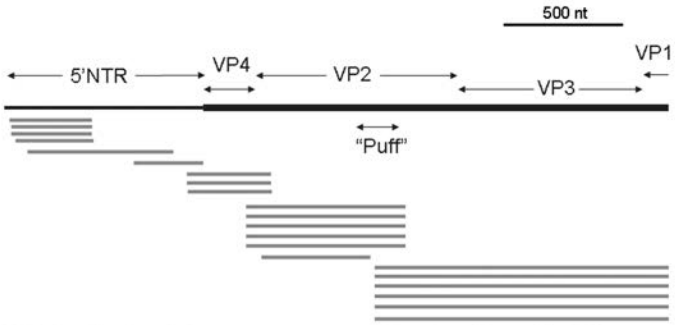
nPOD
Network for Pancreatic Organ Donors with Diabetes


Coxsackievirus B2 from human myocarditic heart has deleted 5’ terminus



A heart from a fatal case of adult fulminant myocarditis in Japan shows not only in an HEV present but that it lacks the 5’ terminal sequences, as seen in cell culture and in mouse myocarditis models.

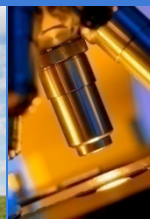




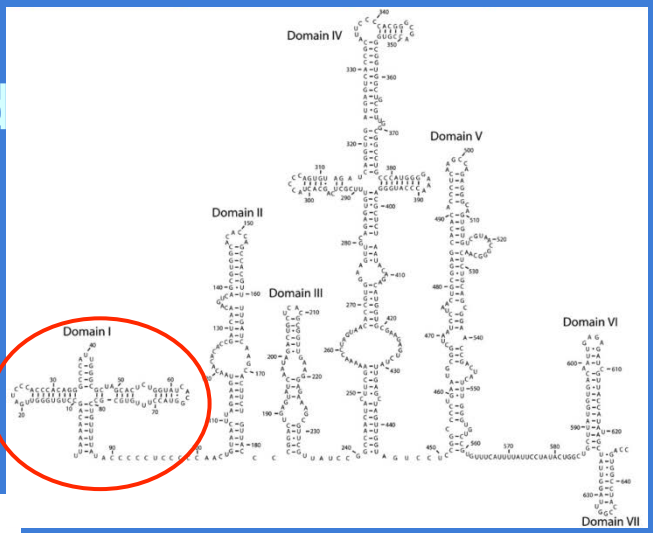
2,563 nt of the heart virus sequence were determined from 21 cDNAs generated from RNA purified from formalin-fixed tissue. Primers were designed to amplify conserved CVB or HEV-B sequences; to proceed into the genome, primers were then designed based upon sequence from the heart virus genome.

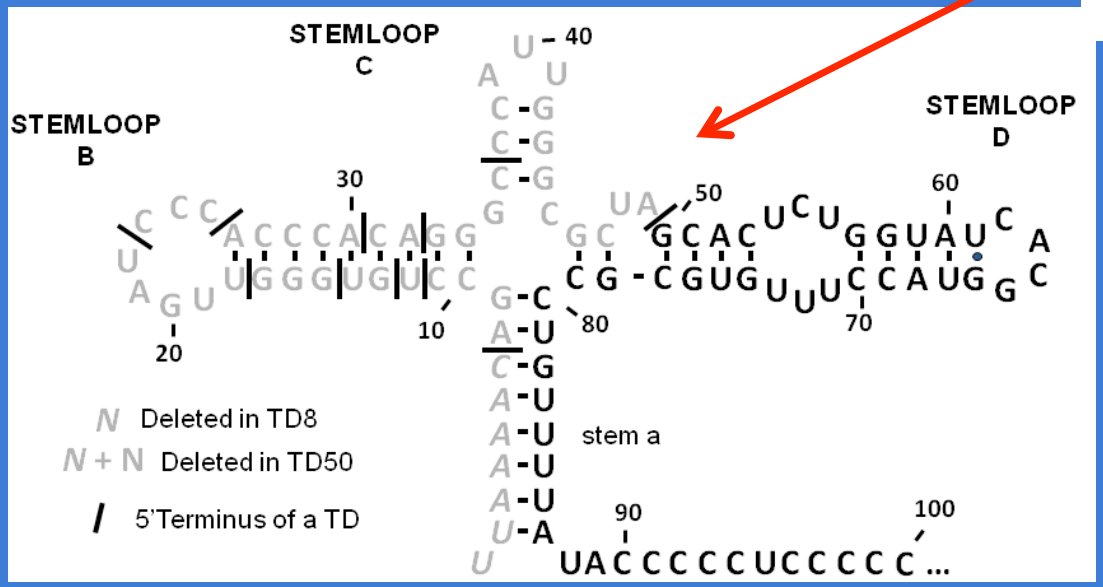
Chapman 2008 Virol 375: 480

nPOD
Network for Pancreatic Organ Donors with Diabetes



The CVB3 domain I (cloverleaf) in the 5' NTR. CVB3-TD genomes from heart have been characterized with deletions from 7 to 49 nt (lines show 5' ends of characterized TD genomes).

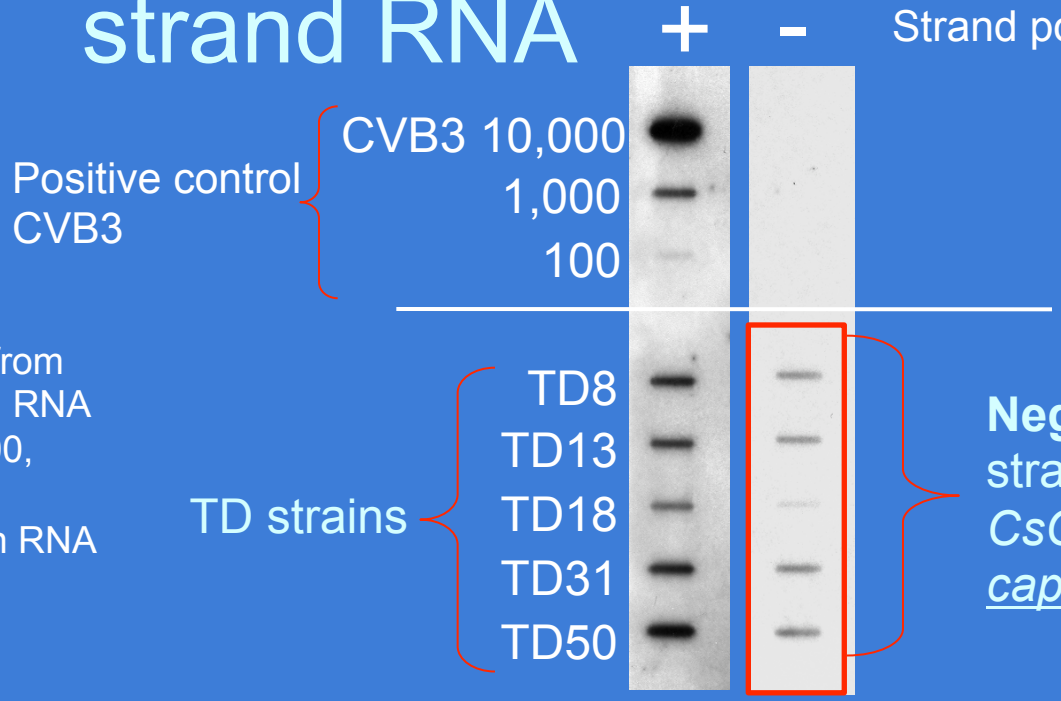




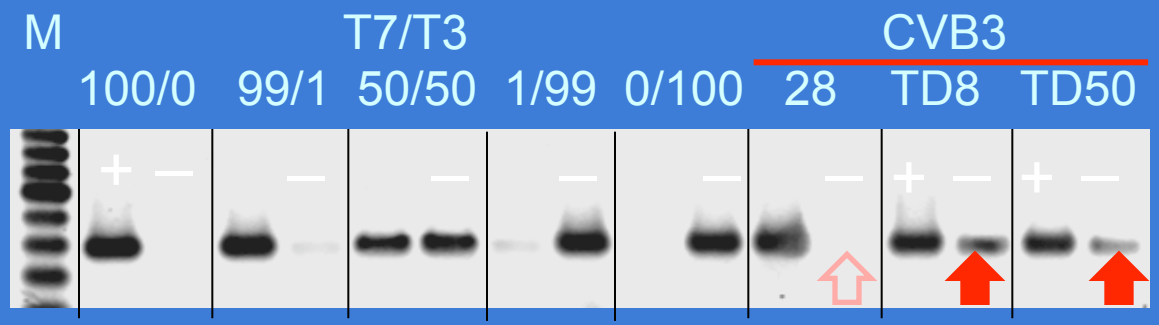


nPOD
Network for Pancreatic Organ Donors with Diabetes


CVB3/TD strains encapsidate negative strand RNA



RT-PCR using strand-selected RNA from CsCl purified virions. Negative strand RNA is not detectable at about 1 part in 100, *nor* in wildtype virus, *but* it *is* readily detectable in TD virion RNA

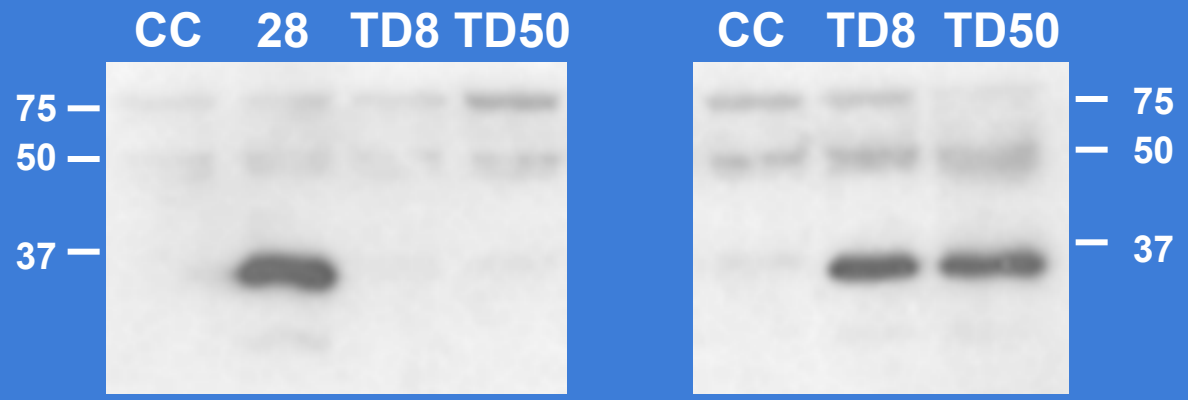


From Kim 2005 J Virol 79: 7024



nPOD
Network for Pancreatic Organ Donors with Diabetes


CVB3/TD strains produce viral proteins in cell culture replication



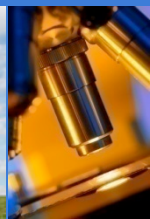
6 Hours

24 Hours

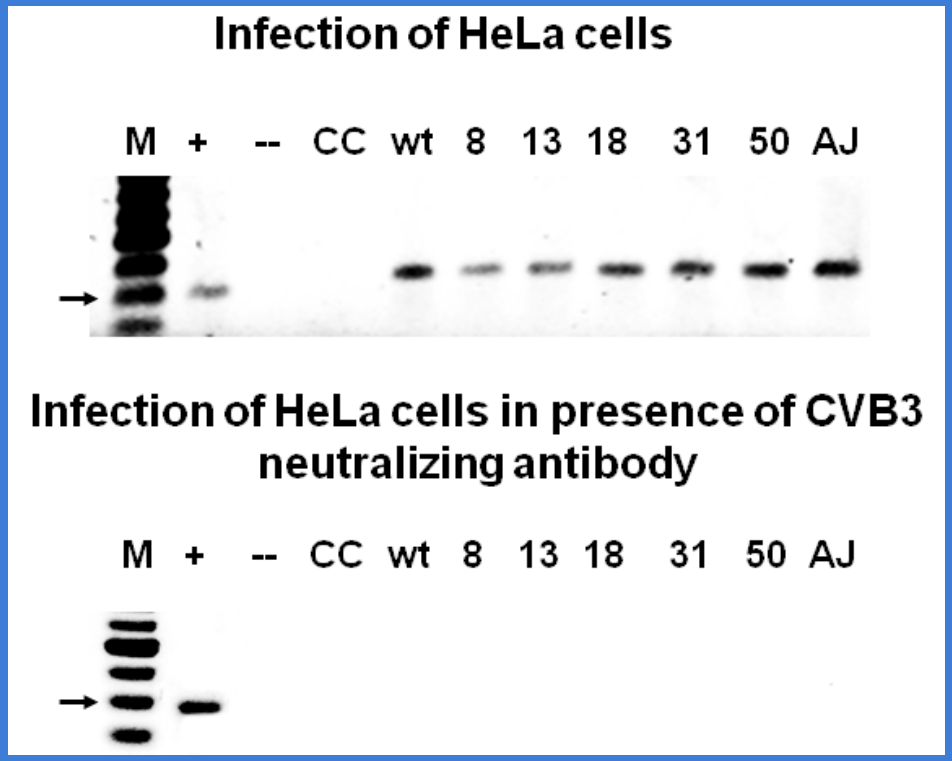
Expression of CVB3 proteins in heart cells alters function and causes heart remodeling: persisting enteroviruses express all the viral proteins.

From Kim 2005 J Virol 79: 7024

nPOD
Network for Pancreatic Organ Donors with Diabetes



CVB3/TD produce encapsidated virus: neutralized by anti-CVB3 polyclonal serum

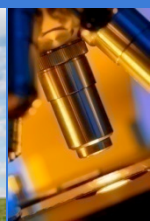


As TD virus makes infectious virus particles further infection of cells can continue

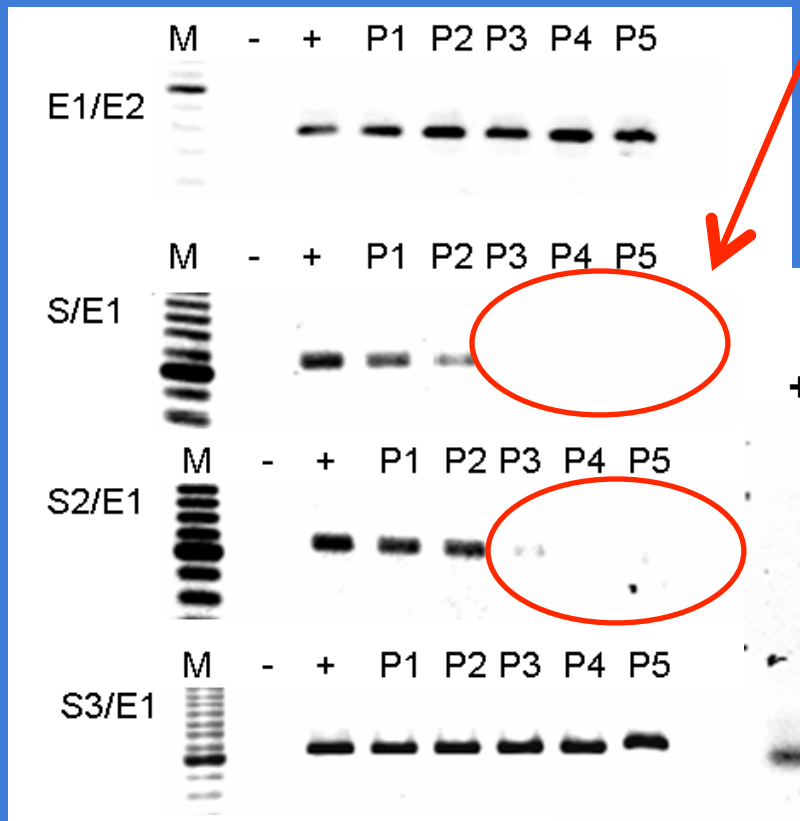
From Kim 2005 J Virol 79: 7024

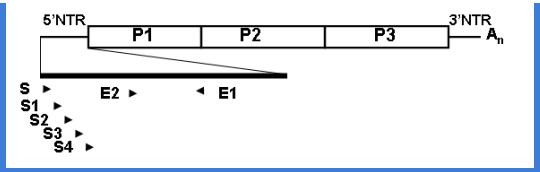



nPOD
Network for Pancreatic Organ Donors with Diabetes



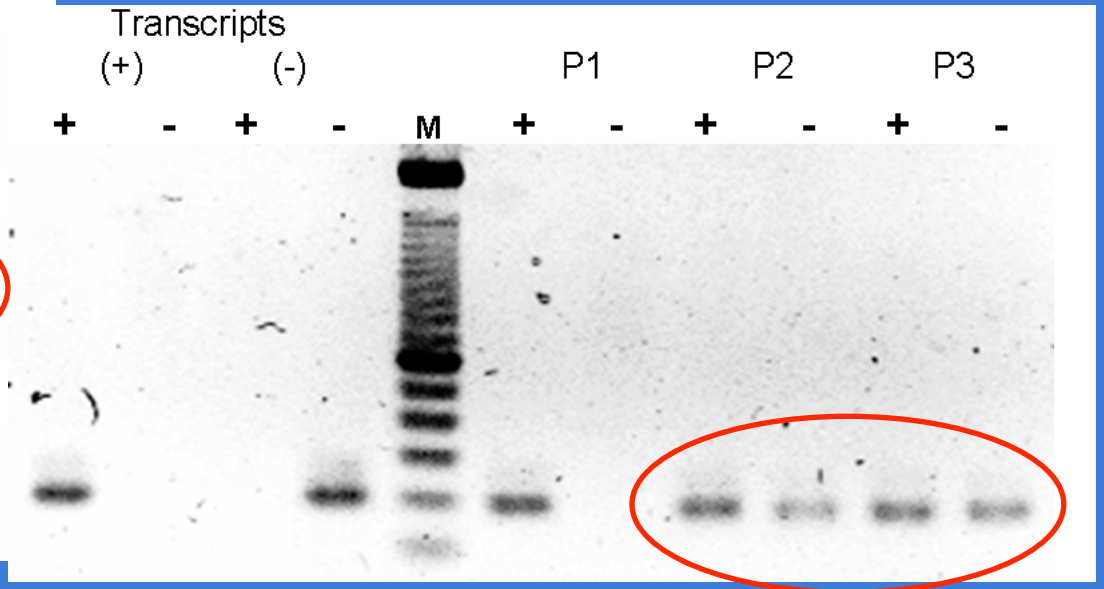
TD are selected in primary pancreatic cell passaged CVB3-28





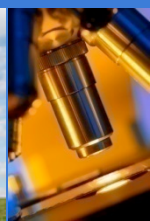
By pass 3, TDs have replaced wt

RT-PCR of strand selected RNA from CsCl purified virus



By pass 2, negative strand RNA is detectable in purified virions

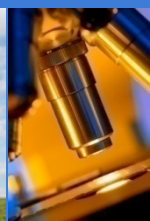
nPOD
Network for Pancreatic Organ Donors with Diabetes



How terminal deletions occur…the short version

- Virus infects a quiescent cell which lacks a host cell factor important for positive strand replication in the cytoplasm
- Positive strand priming sites within domain I are apparently random in this situation
- If priming occurs between the terminus and stemloop d, the RNA is a TD and it is viable
- Multiple passages in quiescent cell culture are required to generate a purely TD population, suggesting that a mixed population of TD and wt is generated in these cells.



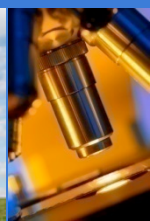



Summary

- After an acute infection with enteroviruses, some infected cells have selection of a defective virus
- These viruses replicate at a level too low for detection by cytopathic effect but can be detected by sensitive RT-PCR and immunohistochemistry
- These viruses produce nearly equal levels of positive strand and negative strand RNA
- These viruses persist at a stage at which levels of neutralizing antibody should clear enterovirus infection, presumably due to slow replication producing long term intracellular states.
- In the mouse model of CVB3 infection, CVB3 RNA persists in the pancreas at this stage.




nPOD
Network for Pancreatic Organ Donors with Diabetes

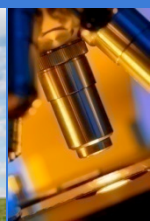


Implications

- Defective enterovirus from past infection of the pancreas may persist well after the adaptive immune response to the virus.
- The viral RNA and protein from that persisting virus may be at reduced levels PER CELL compared to levels found with wild type virus.
- Virus may persist in this form at sites other than the pancreas.




nPOD
Network for Pancreatic Organ Donors with Diabetes

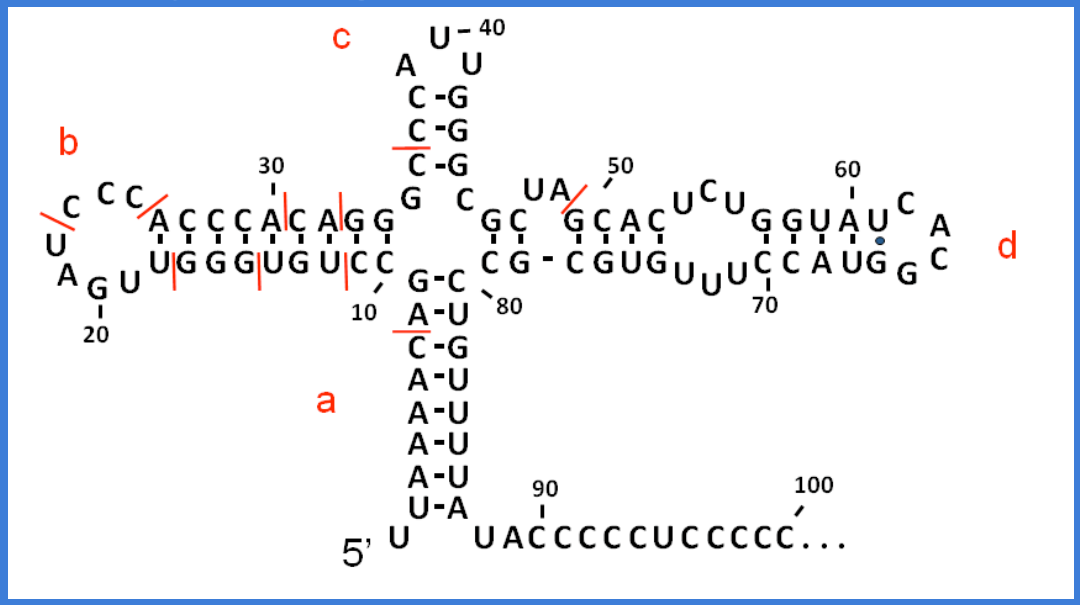


Collaborators in this work

Steven Tracy, Ph.D.

Kristen Drescher, Ph.D

Kyung-Soo Kim, Ph.D.



nPOD

Network for Pancreatic Organ Donors with Diabetes