

## Weakness of innate immunity also contributes to susceptibility of *Xenopus* tadpoles to FV<sub>3</sub> infection




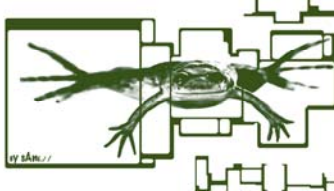
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## FV<sub>3</sub>

- Large dsDNA icosahedral virus (130nm, enveloped ~200nm)
- Genome of 105kb (98-100 ORF), Highly methylated (CC\*GG)
- Replicates both in the nucleus and the cytoplasm
- Can multiply ≤ 32°C even in mammalian cells but inactive at 37°C
- Can remain infectious in water and sediment for few days
- Genomes sequenced. Low variation among different sequences suggests that most virus isolates are related
- Increased prevalence of RV infections in wild and farmed amphibian populations worldwide
- In the wild, late-stage larvae and metamorphs more affected
- ✧ Pathogenesis and immune responses of amphibian hosts to RV infections, remain largely unknown


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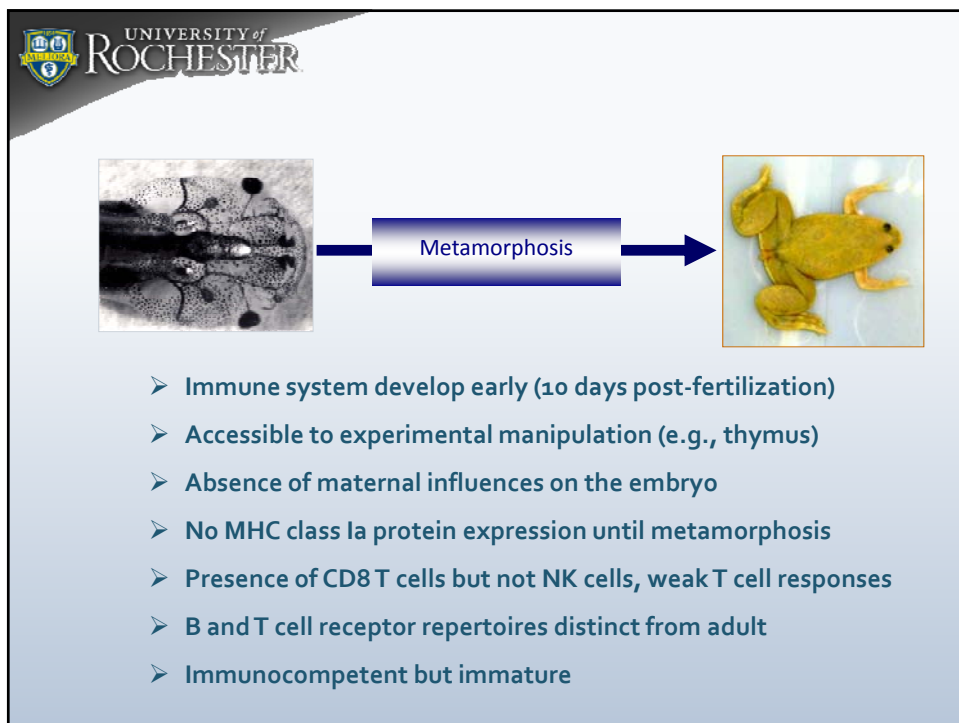
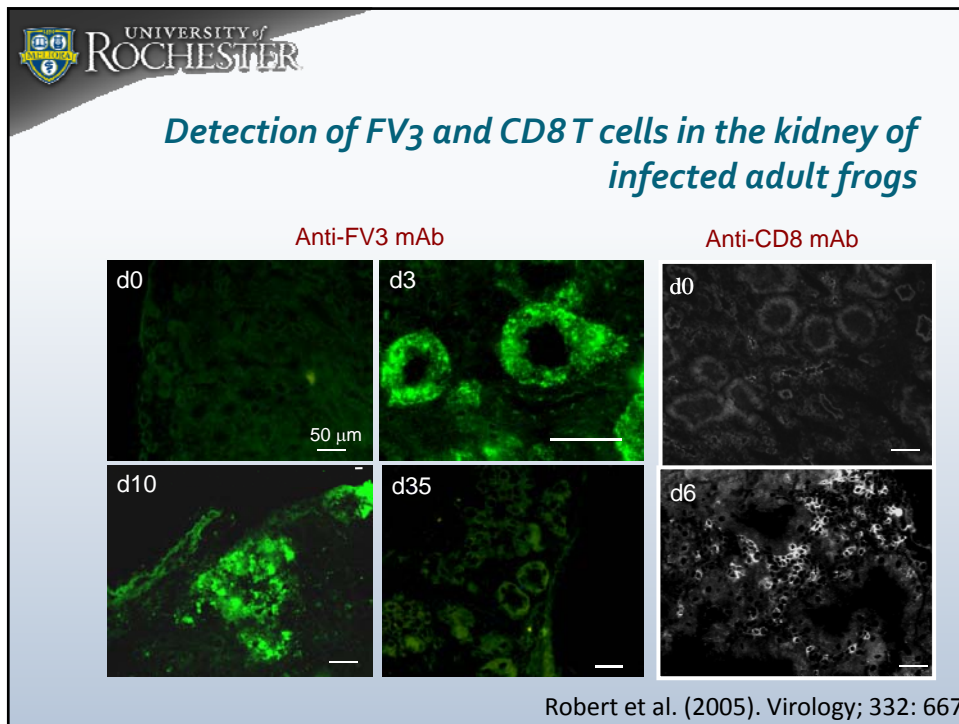

**Xenopus laevis**  
 Resource for Immunobiology

<http://www.urmc.rochester.edu/smd/mbi/xenopus>

- Inbred MHC-defined strains, and isogenetic clones (gynogenesis)
- Adoptive cell transfer, tissue transplantation
- Transplantable thymic tumor cell lines
- T cell deficient thymectomized animals
- Transgenesis ( $\Phi$ C31 Integrase, Transposase, meganuclease)
- cDNA libraries of leukocyte subsets ( $> 10^7$  ESTs)
- Panel of mAbs, molecular probes (B, T, NK cells)
- *Xenopus tropicalis* fully annotated genome sequence (*X. laevis* ongoing)
- Mutant strains by genome wide mutagenesis

## *Xenopus model*

- ❖ *Xenopus* instrumental laboratory model to study immunity and pathogenesis of RVs such as FV3
- ❖ In adults, the critical involvement of CD8 T cells and antibodies is now established
- ❖ Immunological memory: faster recovery, viral clearance upon secondary infection, protective anti-FV3 Ab, faster CD8 T cells proliferation in the spleen and infiltration in kidneys
- ❖ Class Ia-deficient larvae with a more immature immune system are more susceptible to FV3 infection (ip injection or waterborne)





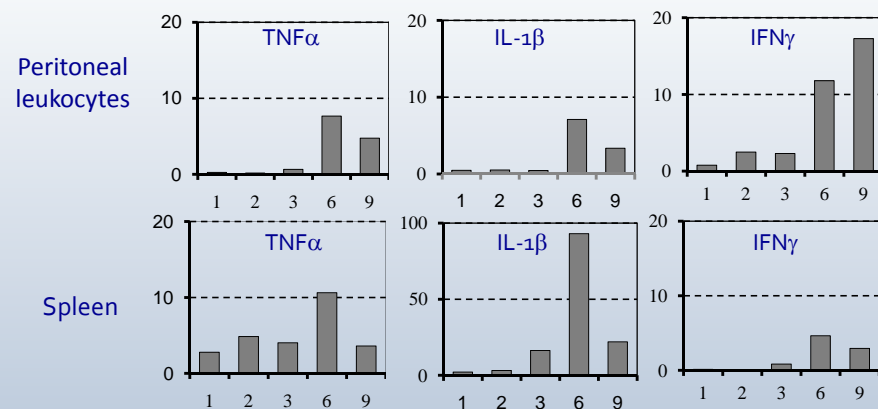
## Tadpoles' susceptibility to FV<sub>3</sub>

- Tadpoles more susceptible to FV<sub>3</sub> infection than adult (>90% death within 1 months)
- No CD8 T cell depletion and no effect on susceptibility by anti-CD8 mAb treatment
- Up-regulation of AID and IgY in larval spleen and kidneys from 3 to 10 dpi
- But attempts to detect anti-FV<sub>3</sub> Abs inconclusive
- Vaccination with heat-killed FV<sub>3</sub> with either Alum or IFA failed to provide protective immunity

✧ Can larvae develop an innate immune response to FV<sub>3</sub> ?



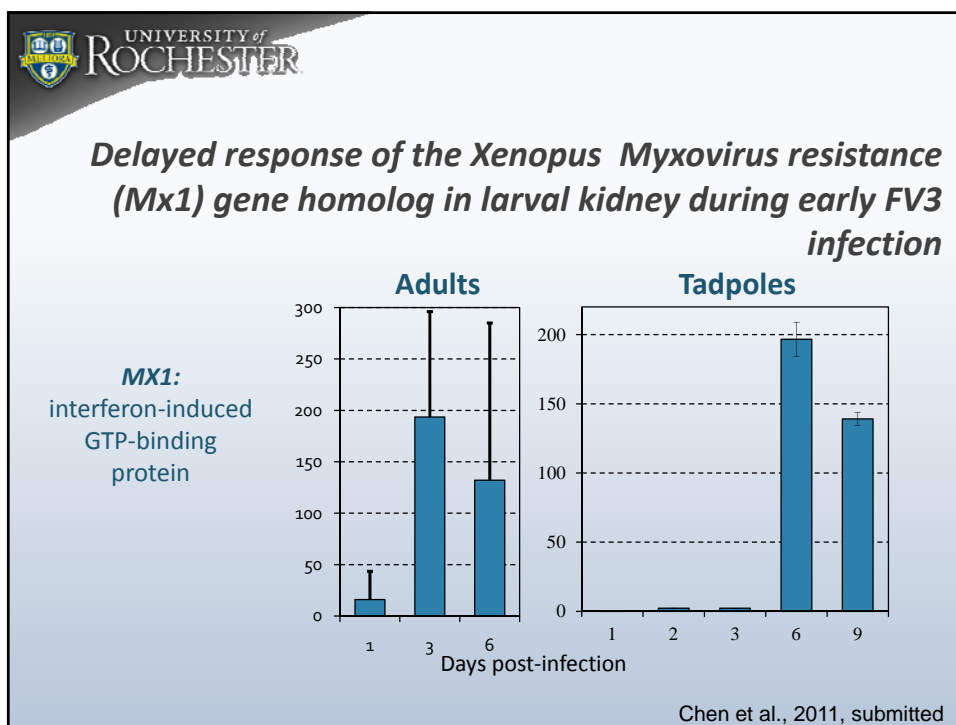
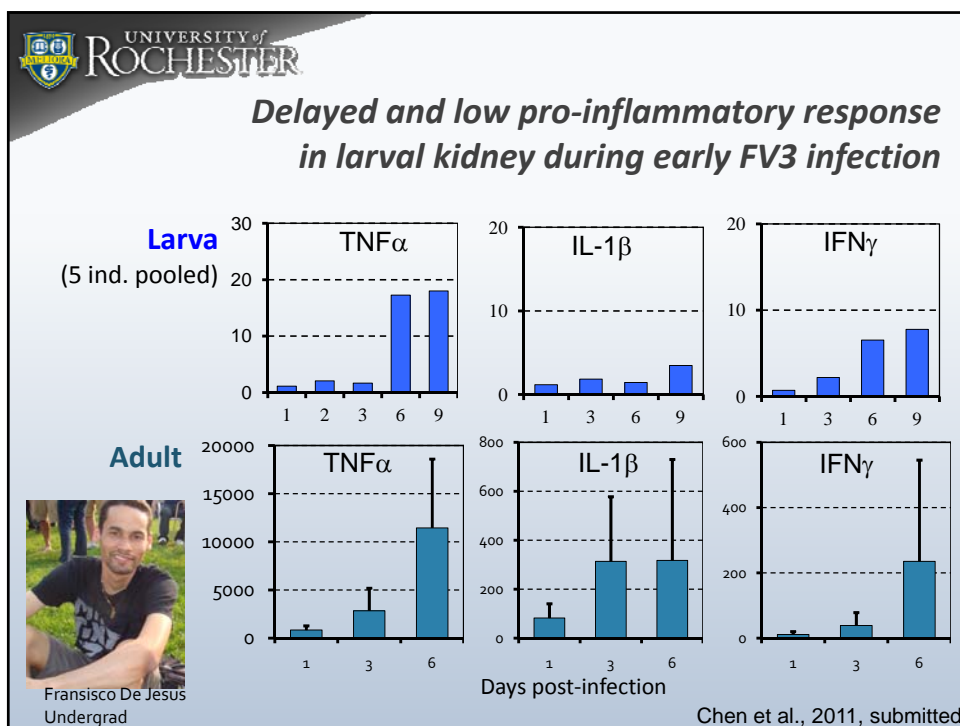
### Delayed and low pro-inflammatory response by larval leukocytes during early FV<sub>3</sub> infection

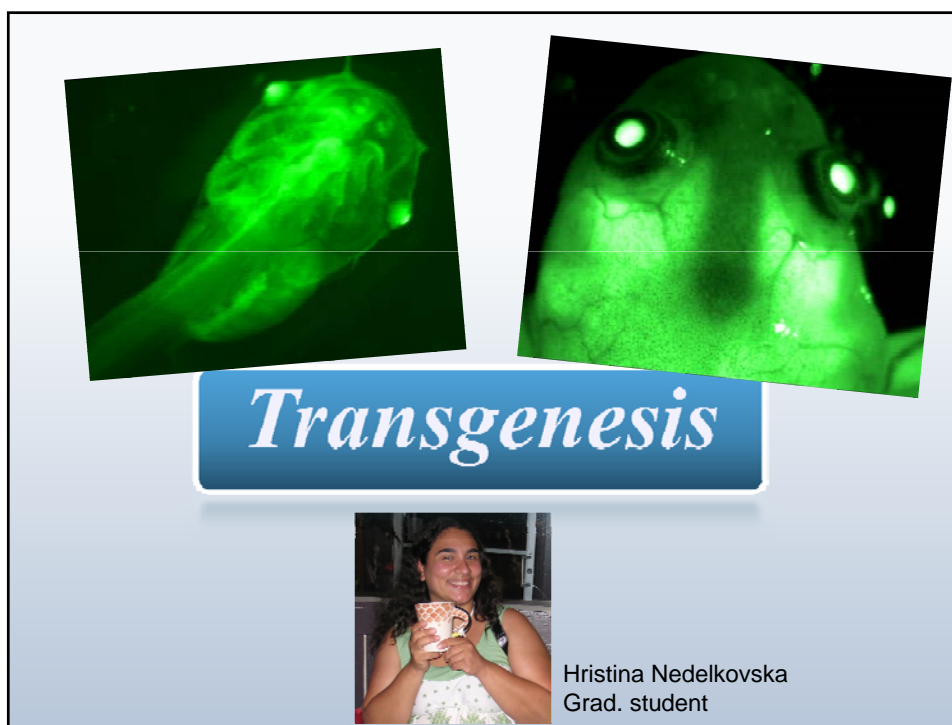
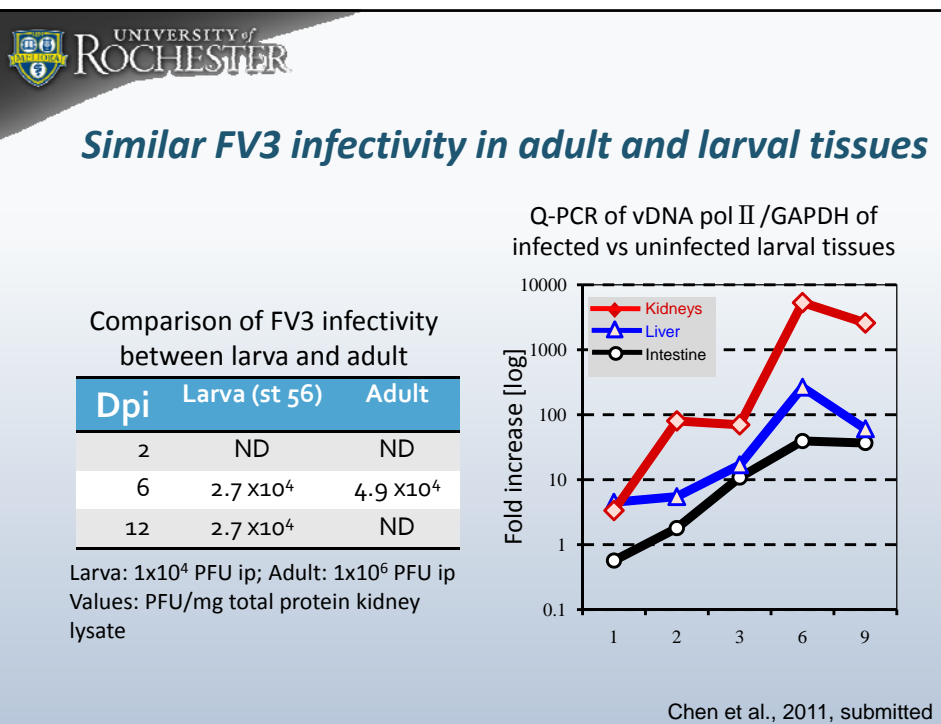


(from 5 pooled ind.)

Days post-infection

Chen et al., 2011, submitted

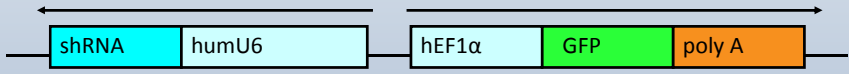




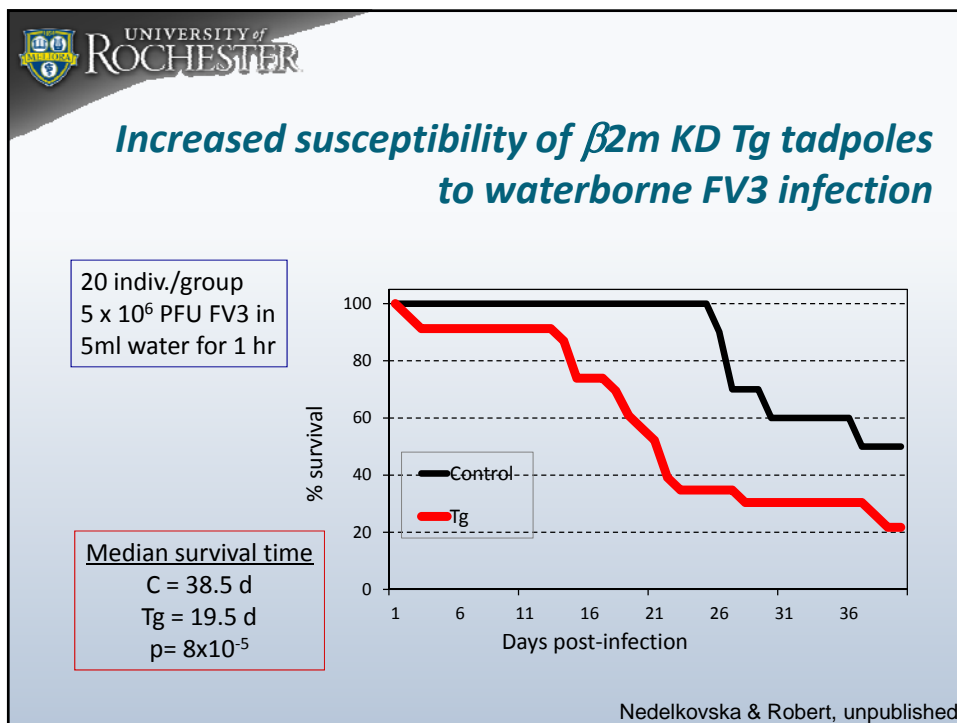
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### *In vivo $\beta$ 2-m silencing by transgenesis with the I-SceI Meganuclease technique*

- Requires a plasmid that carries transgene flanked by 18bp I-SceI recognition sequences
- Estimated 1 to 8 insertions in 1-2 different sites
- Transgenesis efficiency is 14% (33-50%) and germline transmission has been demonstrated
  - 2-12% non-mosaic expression (Ogino et al., 2006, Pan et al., 2006)



Robert et al., (2009). Immunol. Res. 45:114







## Summary - Tadpoles

- ✧ Besides weaker anti-FV<sub>3</sub> B and T cell responses, larvae appear to have a more delayed and weaker innate immune response compared to adult frog.
- ✧ FV<sub>3</sub> target mainly kidneys in larvae as in adult
- ✧ b2m knockdown by transgenesis (that impairs both MHC class Ia and class Ib function) increases susceptibility to FV<sub>3</sub> infection at early larval stage
- ✧ This suggests a critical role for class Ib molecules in viral immunity during early development when class Ia expression is suboptimal

## THE JUMPING FROG LAB

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<http://www.urmc.rochester.edu/smd/mbi/xenopus/>