Ranaviruses: Past, Present, and Future

1st Intl. Symposium of Ranaviruses, Minneapolis, MN

July 7, 2011

Outline

- Past: 100+ years of iridoviruses
- Present:
  - Molecular Virology: Elucidation of FV3 gene function
  - Ecology: Understanding the role of ranaviruses in die-offs and extinctions
- Future: Molecular, Genetic and Ecological studies.

Iridovirus/Ranavirus Timeline: 100+ years of Iridoviruses

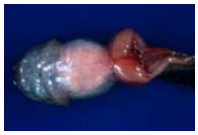
1900 | 1954 | 1965 | 1986 | 1997 | 2011

Lymphocystis disease identified

First Insect Iridovirus Identified

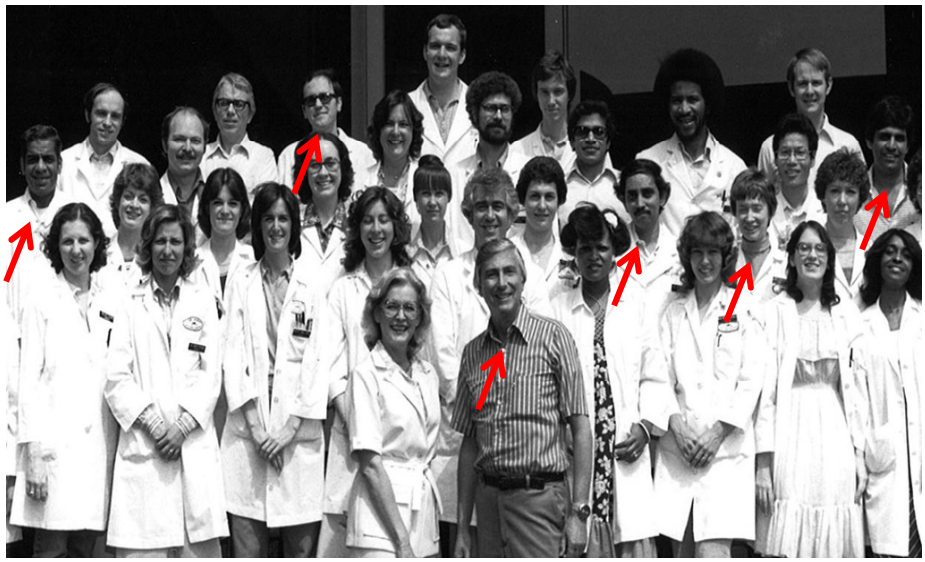
Xeros (1954)

FV3 identified



Granoff et al., 1965 (NY Acad. Sci.)

St. Jude Childrens Research Hospital, Division of Virology, circa 1980

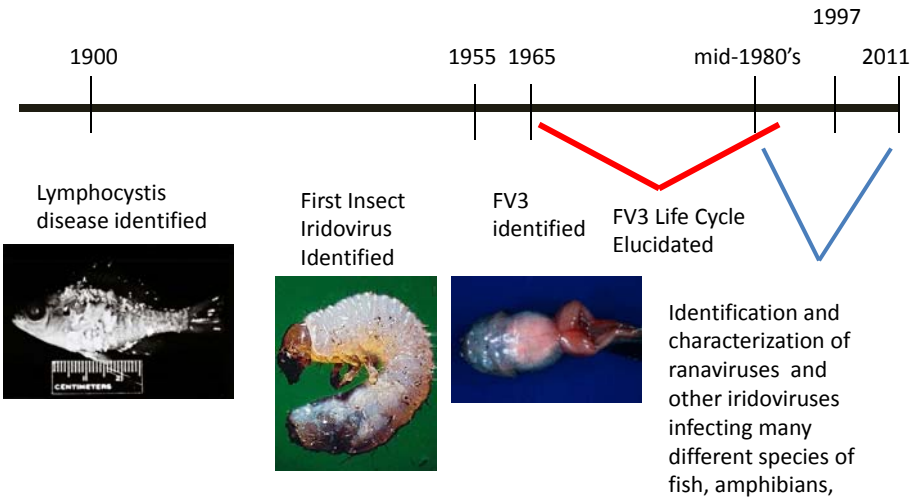


Allan Granoff, Rakesh Goorha, Dawn Willis, Raj Raghow, Gopal Murti, Greg Chinchar

Other early ranavirus workers

- Molecular Studies:
  - Aubertin, Drillien, Kirn (FR)
  - McAuslan (USA)
  - Elliot and Kelly (UK)
- Identification and Virus Characterization
  - Karzon, Clark
  - Wolf

Iridovirus/Ranavirus Timeline: 100+ years of Iridoviruses



A Ranavirus Renaissance

- Langdon and Humphrey (1987) Redfin perch [AU – EHNV]
- Ahne et al. (1989) Sheatfish [DE – ESV]
- Kanchanakhan (1989) *R. tigrina* [SE Asia]
- Pozet et al. (1992) *Ictalurus melas* [FR – ECV]
- Speare and Smith (1992) ornate burrowing frog [AU – BIV]
- Bloch and Larsen (1993) Turbot [Scandinavia]
- Chua et al. (1994) and Qin et al. (2001) brown spotted grouper [Singapore/Taiwan/PRC – SGIV/GIV]
- Cunningham et al. (1996) *R. tempora* [UK]
- Plumb et al., (1996) largemouth bass [USA/SC – LMBV]
- Mao et al., (1997) doctor fish [SE Asia –DFV]
- Jancovich et al. (1999) *Ambystoma tigrinum* [USA/AZ – ATV]
- Chen et al. (1999) soft shell turtle [PRC – STIV]
- Allender et al. (2006) box turtles [USA]

Family: *Iridoviridae*

- **Subfamily*: Invertiridovirinae***
  - Genus: ***Iridovirus***
  - Genus: ***Chloriridovirus***
- **Subfamily*: Chordiridovirinae***
  - Genus: ***Lymphocystivirus***…wart-like disease in freshwater and marine fish, disfigurement, but low mortality
  - Genus: ***Megalocytivirus***…life-threatening infections in >52 species of marine and freshwater fish in SE Asia
  - Genus: ***Ranavirus***…systemic disease in fish, reptiles, and amphibians accompanied by variable mortality.
    - **FV3, ATV, BIV, EHNV, ECV, SCRV**, *SGIV, RCV-Z*

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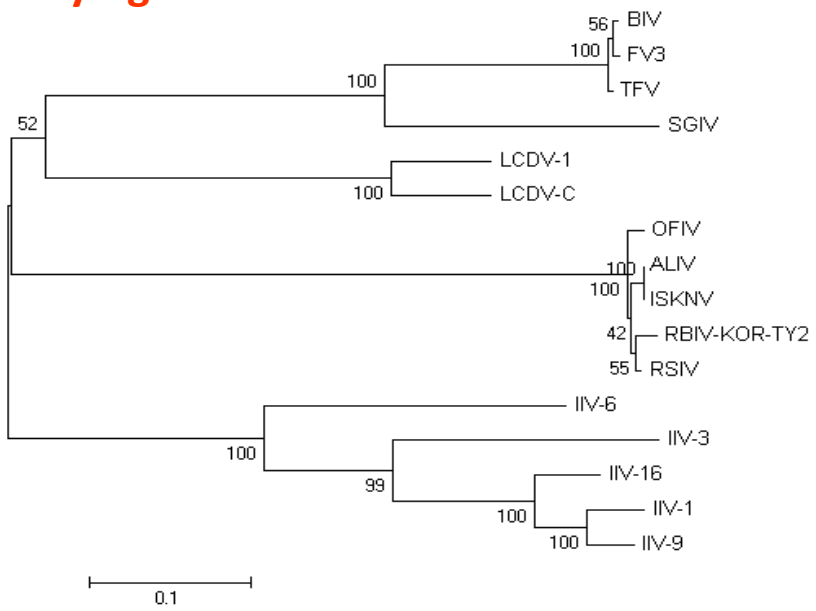
Phylogenetic Tree: MCP



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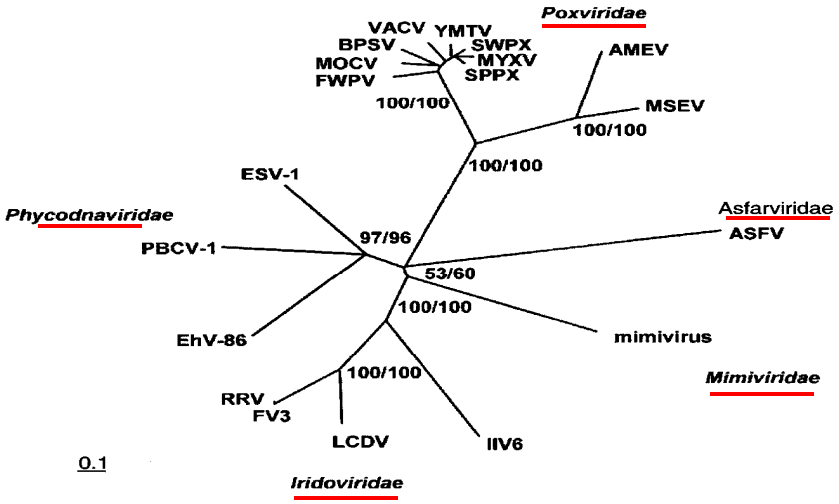
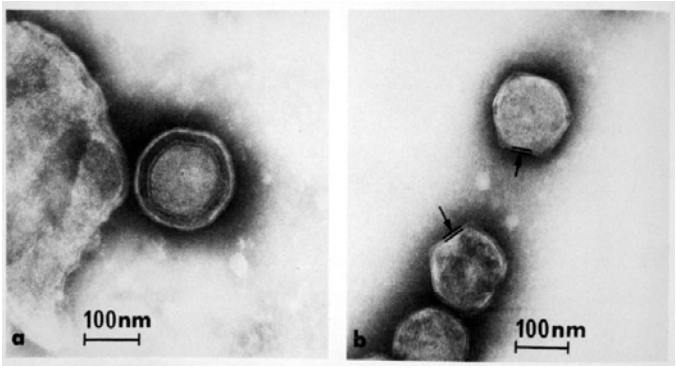


Allen et al., (2006) Virology J 3:15.

Nuclear Cytoplasmic Large DNA-containing Viruses (NCLDV)



Enveloped Virus

Non-enveloped FV3

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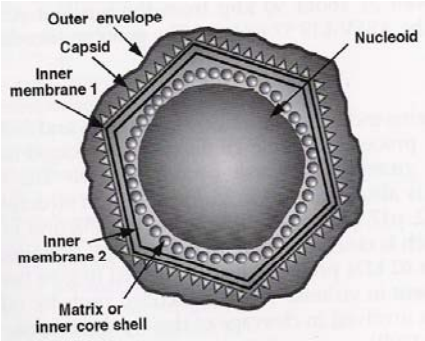
Ranavirus Virion Morphology



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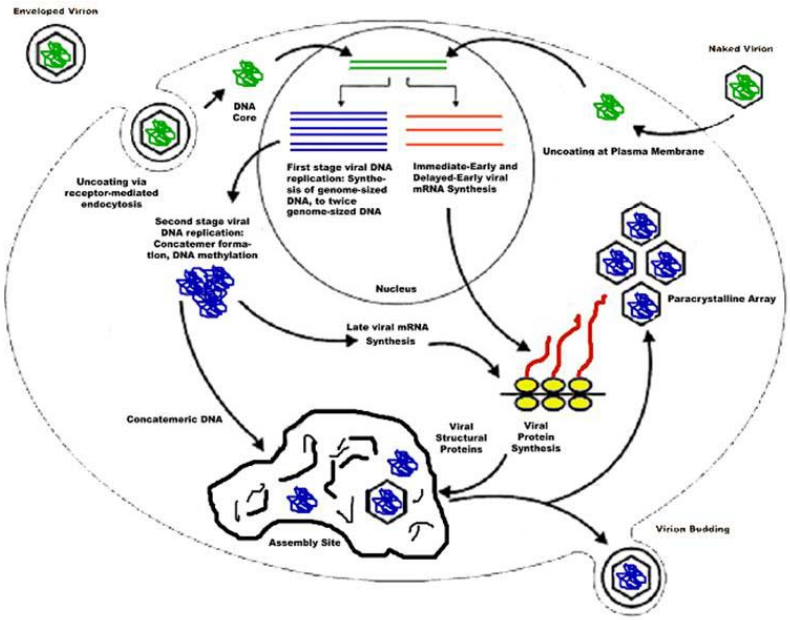
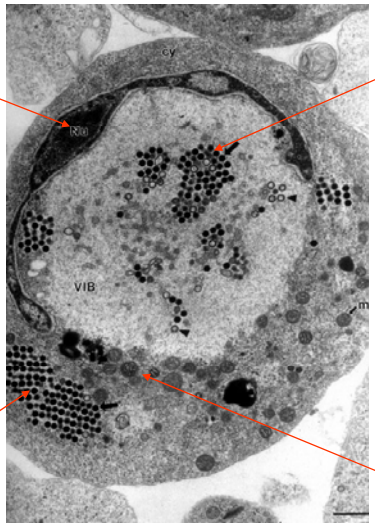


Nucleus showing chromatin condensation

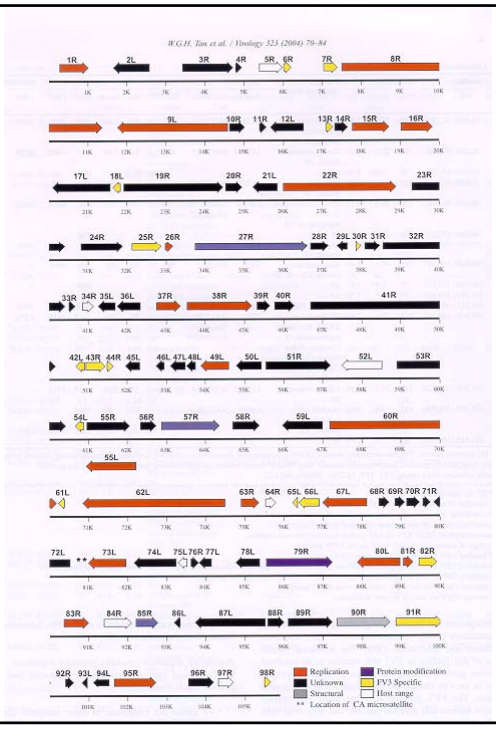
Virions within viral assembly sites



Virions within para-crystalline array

Mitochondria

Alex Hyatt (AAHL)



~100 ORFs:
19 RED (Replication)
5 Blue/Gray (structural)
51 Black (UNKNOWN)
15 Yellow (FV3-specific)

Tan et al., 2004

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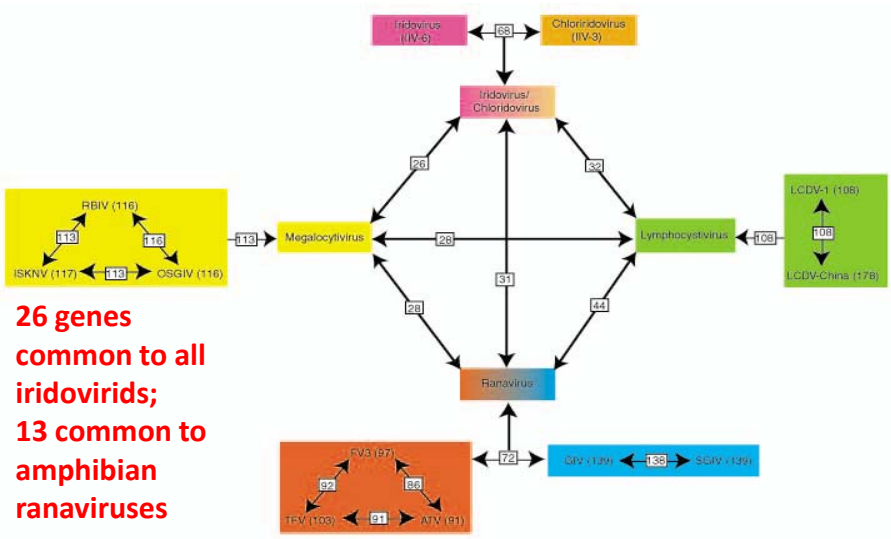

26 genes common to all iridovirids; 13 common to amphibian ranaviruses

Eaton et al., 2007 Virology J 4:11 and Jancovich et al., 2010.

FV3 Genes

- **Replicative Genes**
- DNA/RNA Pol
- Major capsid protein
- DNA repair
- Integrase
- Myristylated membrane protein

- **Immune evasion, HR, Virulence**
- vIF-2α
- Steroid synthesis (β-HSD)
- vCARD
- TNF receptor
- Bak-like, IAP-like
- dTTP synthesis: RR, TK, dUTPase
- **13 amphibian RV-specific genes; 27 RV-specific genes**

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FV3 *ts* mutants

- Naegele and Granoff, Virology 44: 286 – 295, 1971
- Purifoy et al., Virology 54: 525 – 535, 1973
- Chinchar and Granoff, J. Virology 58: 192 – 202, 1986.
- 28 mutants placed into 19 complementation groups and 4 classes
  - Class I: 12 CG, 16 mutants – E+ L+ DNA+ AS+ [Assembly]
  - Class II: 4 CG, 5 mutants – E+ L- DNA+ AS+/-
  - Class III: 1 CG, 1 mutant – E+ L- DNA+ AS-
  - Class IV: 2 CG, 5 mutants – E+ L- DNA- AS- [DNA]

Knock Down: Antisense Morpholino Oligonucleotides (asMOs)

asMOs are single-stranded 25-mers, which

If complementary to the region surrounding, or upstream of, the translational start site,

block protein synthesis by inhibiting ribosomal movement.

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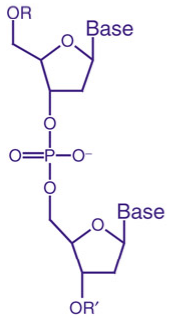
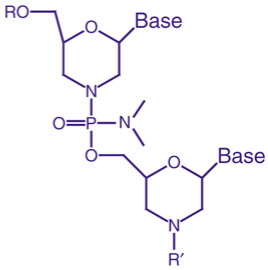
Structure of asMOs



Phosphodiester DNA



Morpholino

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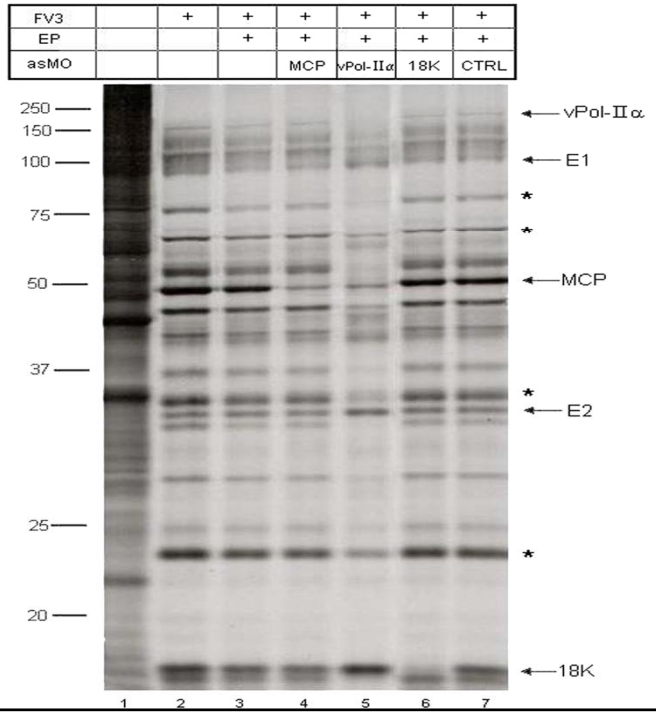
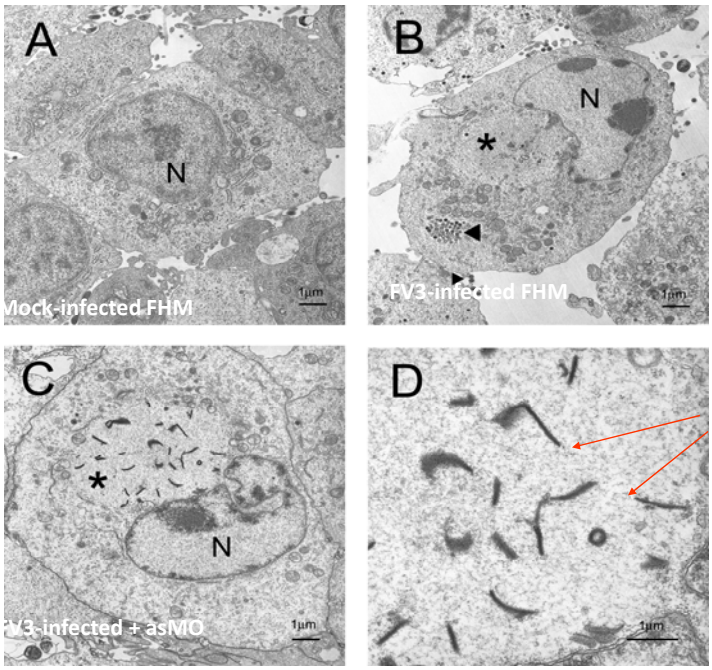

FV3 + + + + + +
EP + + + + +
asMO MCP vPol-IIα 18K CTRL
250
150
100
75
50
37
25
20
vPol-IIα
E1
MCP
E2
18K
1 2 3 4 5 6 7

21


A
N
Mock-infected FHM
1µm
B
N
FV3-infected FHM
1µm
C
N
FV3-infected + asMO
1µm
D
AE
1µm

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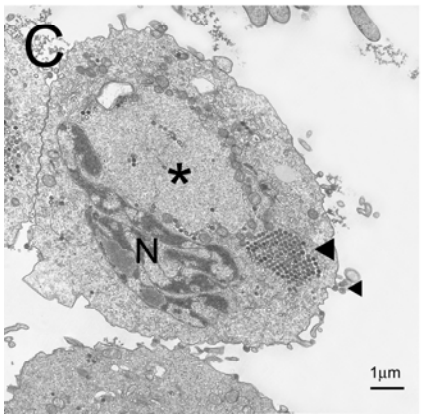
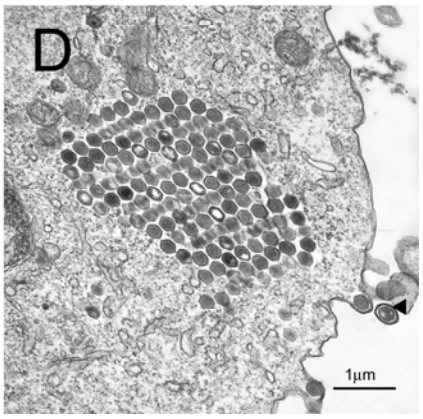
Knock down of 18K synthesis does not affect virion formation or viral infectivity



FV3 + anti-18K MO



FV3 + anti-18K MO

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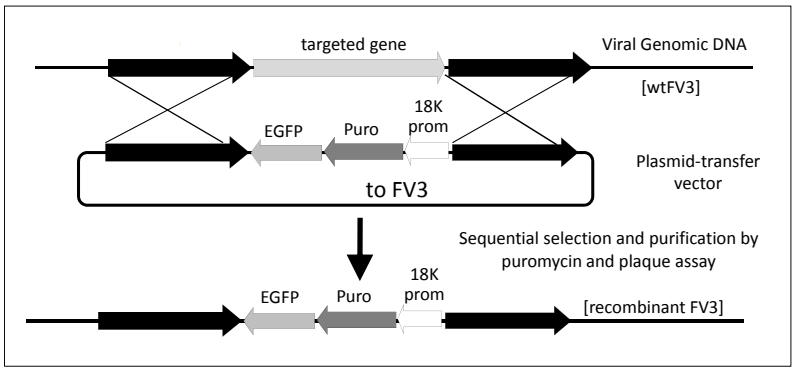
Knock Down Studies: Summary

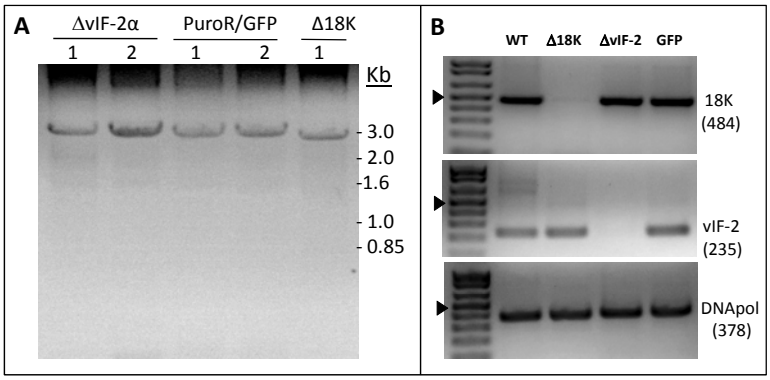
- KD of MCP, vPOL-II alpha, 53R, 46K, and 32R resulted in a marked drop in viral replication.
- KD distinguishes essential from non-essential genes.
- KD studies are limited by
  - inability to detect some viral proteins by SDS-PAGE,
  - sequence of target mRNA,
  - inability to function *in vivo*.

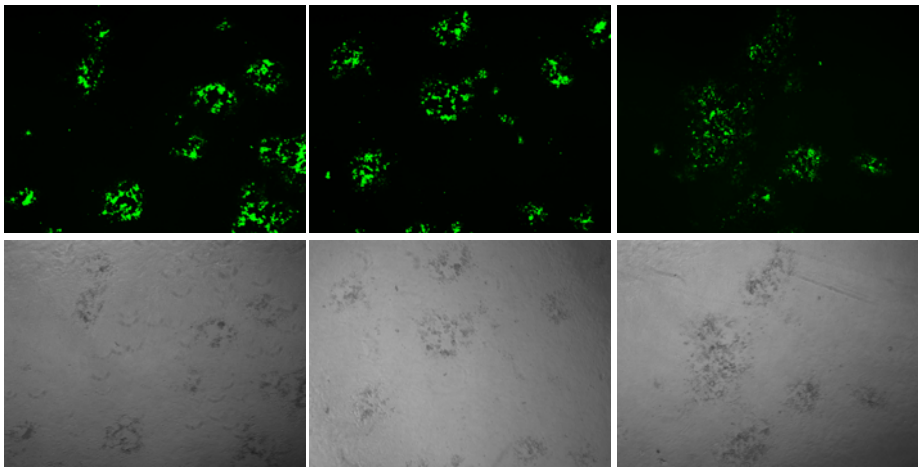
Knock Out



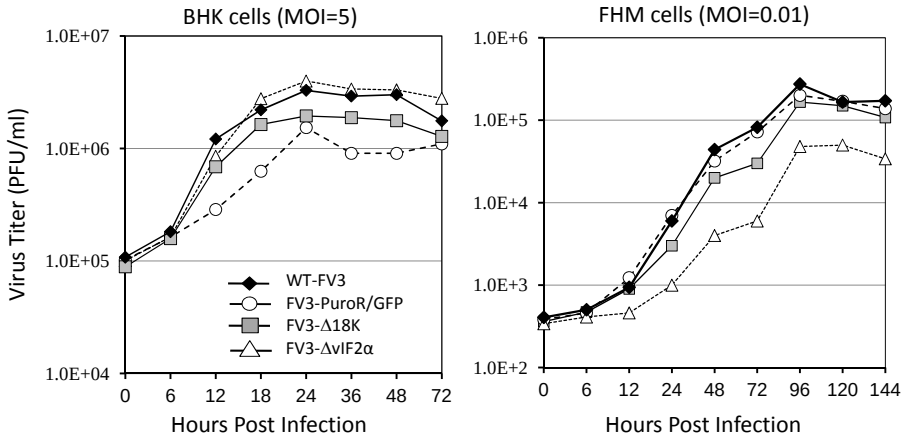
Chen and Robert (University of Rochester Medical Center)

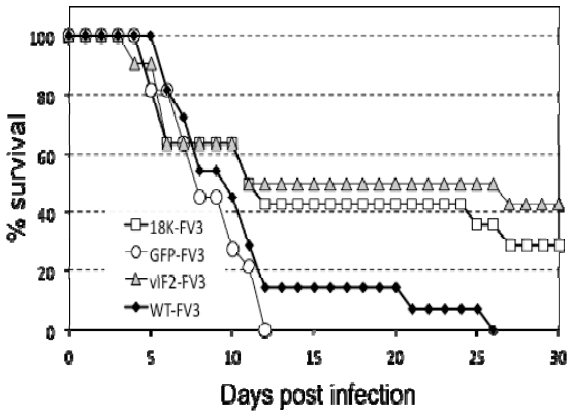
Confirmation of PuroR/GFP insertion and 18K and vIF-2 deletion



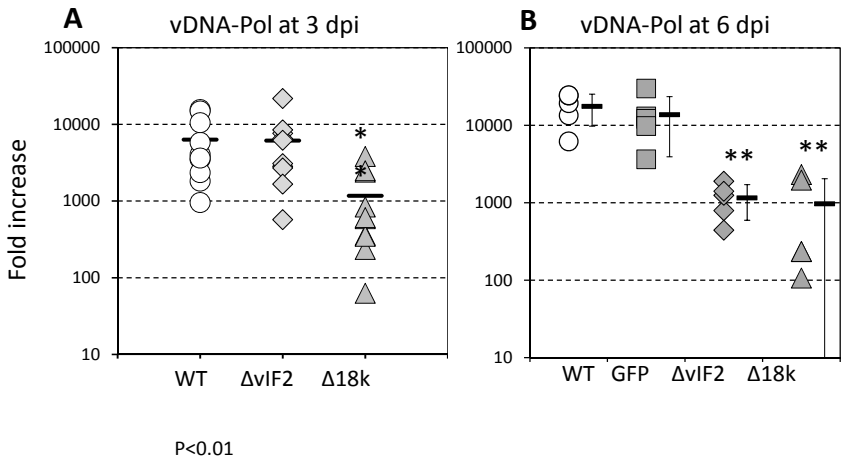


FV3-PuroR/GFP FV3-ΔvIF-2α FV3-Δ18K





Challenge of *Xenopus* tadpoles with control and KO mutants



KO mutants

- One KI and 5 KO mutants have been generated
  - FV3-PuroR/GFP (KI) – positive control
  - FV3Δ -18K, -vIF-2α, -βHSD, -dUTPase, -vCARD
- KOs can be used both *in vitro* and *in vivo,* and should be constant in their phenotype.
- Identify non-essential genes that may play key roles in virulence, host range, and immune evasion.

Ecological/Population Studies

- RVs and species declines (Gray, Storfer, et al.)
- Viral transmission and persistence (Brunner, Jensen, Picco)
- Host susceptibility and pathology (Hoverman, Green, Miller)
- Pesticides and RV infections (Kirby)
- Host shifts among RVs (Jancovich)
- Host-Pathogen co-evolution (Lesbarreres, Storfer)
- Host anti-viral immunity (Robert) and viral anti-host immunity (Robert, Chinchar)
- Identify viruses from various hosts and geographic regions (Balseiro, Schock, Duffus, Mazzoni, Une, Kanchankhan, Waltzek, Marschang, Allender, Ariel)

Ranaviruses: Past and Present

The old view

- RVs are relatively harmless viruses that provide insight into a poorly-characterized virus family and are useful molecular models for DNA methylation and its effect on transcription, host-shutoff, etc.

The new realization

- RVs are responsible for localized die-offs among ecologically and commercially important ectothermic animals .
- The "die-off trigger" is not known, but likely involves interplay between intrinsic viral functions and extrinsic factors (e.g., host immunity, stress, etc.)

The Future

- Understand the role of viral genes in virulence
  - Immune evasion, host range
- Identify host and reservoir species
  - Is FV3 really a "frog virus" ? Is LMBV an isolate of DFV ?
- Understand what host, viral, and environmental factors trigger disease/persistence/recrudescence
  - FV1 and FV2 were isolated from "healthy" frogs; what makes LMBV pathogenic?
- Determine if susceptible species can be protected by vaccination with KO mutants?
- Does the genus *Ranavirus* consist of 6+ unique species, or are there fewer species but multiple isolates displaying various host preferences and degrees of pathology?
  - A Regulatory/Taxonomic issue?