OF MICRORNAs AND FISH

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17th Annual Workshop of the National Reference Laboratories for Fish Diseases
European Union Reference Laboratory for Fish Diseases
National Veterinary Institute, Technical University of Denmark
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DTU
Technical University of Denmark
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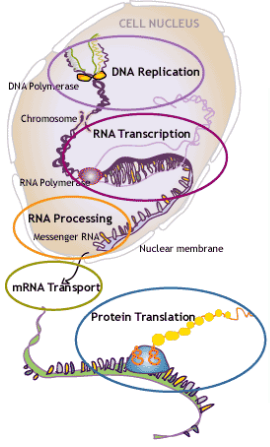
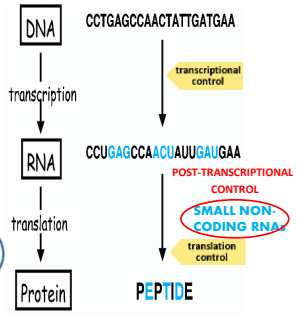
Outline

- Overview of gene expression and its control
- MicroRNAs and Post-transcriptional control by RNA interference
- Studies on microRNAs in teleost fish
- VHSV-induced microRNAs in rainbow trout
- Summary/Take-home messages



Control of gene expression



microRNAs (miRNAs)

- 18-22 nucleotides
- Endogenous, non-coding
- Bind to 3'-UTR of target mRNAs

```
miRNA:  3'CGUUGAAUCAUUACACGUUAU5'   hsa-miR-32
            |||:|||       |||||||
mRNA:   5'GCAGCUU-------UGCAAUA3'   PFV-1 ORF2
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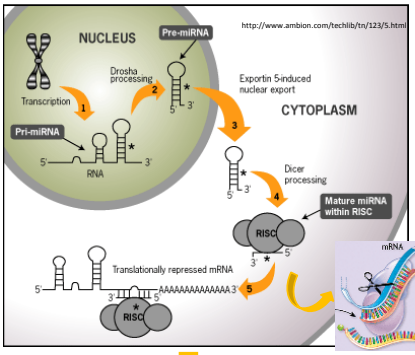
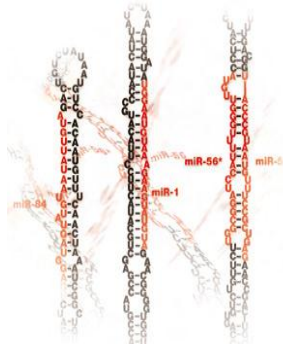


RNA Interference (RNAi)

ALTERED PHENOTYPE

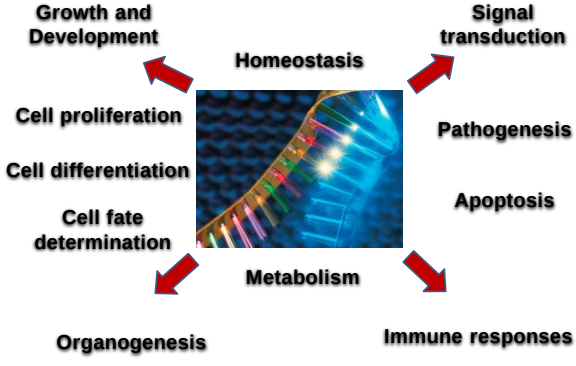
microRNAs (miRNAs)



- > 20, 000 miRNAs identified in 193 species
- Highly conserved (e.g. dre-miR-155, hsa-miR-155)

(http://www.mirbase.org/)

Why are miRNAs important?



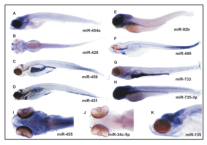
Why are miRNAs important?

- miRNAs are associated with disease

Age-related diseases
Cancer
Neurological disorders
Immune function disorders

- Development of miRNA-based therapeutics
- miRNA profiling for disease diagnosis

How about miRNAs in fish?



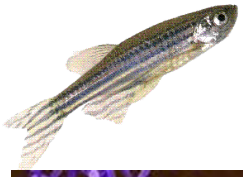
- miRNA expression, functions, and mechanisms during embryonic development

How about miRNAs in fish?

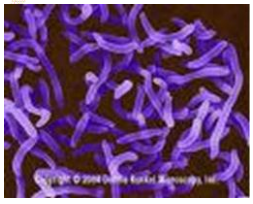
- miRNA transcriptomic analysis of rainbow trout eggs

→ molecular markers for egg quality and embryonic development potential (Ma et al., 2012)

How about miRNAs in fish?

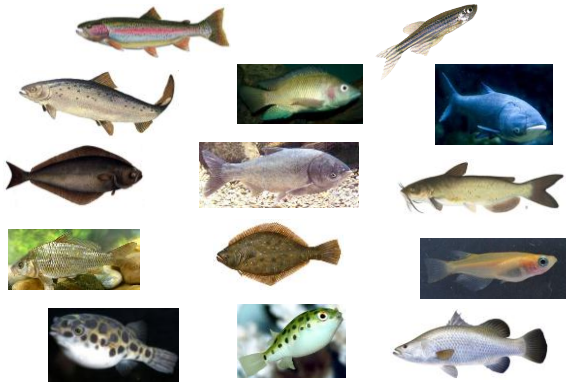


- miRNA responses to bacterial infection in zebrafish



→ potential roles of miRNAs in regulating immune response genes (Wu et al., 2012)

Teleost fish miRNAs



Teleost fish miRNAs

Fish species	Common name	Sequences available in miRBase	Reference
Oryzias latipes	Medaka	168 precursors, 147 mature	Li et al., 2010; Tani et al., 2010
Takifugu rubripes	Fugu	129 precursors, 108 mature	Ref? in miRBase Mihaela Zavolan
Tetraodon nigroviridis	Pufferfish	132 precursors, 109 mature	Ref? in miRBase Mihaela Zavolan
Danio rerio	Zebrafish	134 precursors, 146 mature	Kloosterman et al., 2006 + Many others
Lates calcarifer	Asian seabass	Not yet available	Xia et al., 2011
Oncorhynchus mykiss	Rainbow trout	Not yet available	Salem et al., 2010
Cyprinus carpio	Common carp	134 precursors, 146 mature	Zhu et al., 2012; Yan et al., 2012
Paralichthys olivaceus	Japanese flounder	20 precursors, 38 mature	Fu et al., 2011
Oreochromis niloticus	Nile tilapia	Not yet available	Huang et al., 2012
Hypophthalmichthys nobilis	Bighead carp	Not yet available	Chi et al., 2011
H. molitrix	Silver carp	Not yet available	Chi et al., 2011
Hippoglossus hippoglossus	Atlantic halibut	1 precursors, 1 mature	Bizuayehu et al., 2012a,b
Ictalurus punctatus	Channel catfish	Not yet available	Xu et al., 2013
Salmo salar	Atlantic salmon	Not yet available	Reyes et al., 2012

Why study miRNAs in fish

- >30,000 species living in diverse environments
- Fish are important models for studying aspects of vertebrate biology
- Fish are important commercial and food species
- miRNAs might have enormous impact in regulating fish biological processes

→understand miRNA evolution and functions

→Provide resource for further studies on post-transcriptional gene regulation

→Knowledge of gene regulation important for developing new techniques for disease control

Our research

www.env.gov.bc.ca

http://dnr.wi.gov/fish/health/vhs.html

- Identification of virus-induced miRNAs in rainbow trout

http://www.dfw.state.or.us/fish/diseases/docs/vhsv_fact_sheet.pdf

Our research

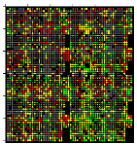
- Identify miRNA biomarkers

→ allow understanding of web of interactions in underlying immune responses to infection

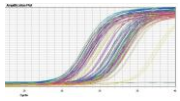
→miRNA signatures of immune responses may be used as selection criteria for identifying disease-resistant fish

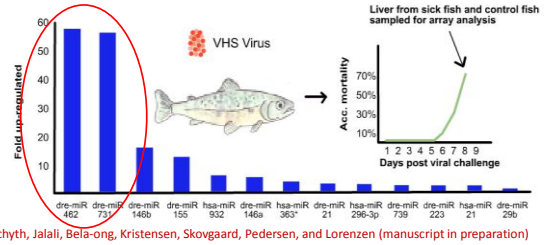
- Determining the potential role of miRNAs in host-pathogen interactions

Transcription profiling of liver samples in VHSV-infected fish



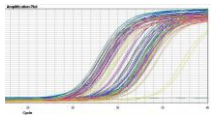
- **miR-462** and **miR-731**

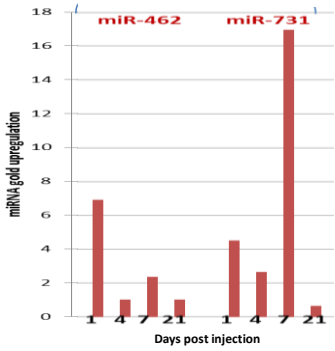




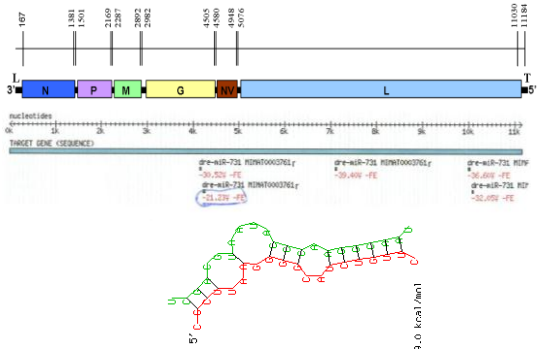
Schyth, Jalali, Bela-ong, Kristensen, Skovgaard, Pedersen, and Lorenzen (manuscript in preparation)

Expression of miR-462 and miR-731 elicited by Type I IFN at injection site





In silico analysis of putative targets in the VHSV genome

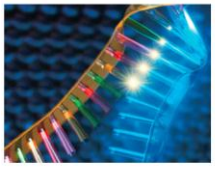


Evaluation of potential function(s) of miRNAs

- Treat cells and fish with **antagomiRs/anti-microRNAs/anti-miRs**
  - short oligonucleotides
  - anti-sense microRNA
- Infect/Challenge with VHSV
- Monitor cytopathic effect/disease development

Take-home message

- microRNAs are novel regulators of gene expression impacting a wide range of biological proceses



Cell differentiation

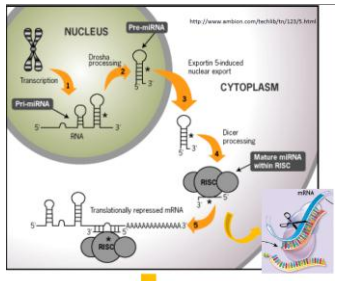
Development

Homeostasis

Immune responses

……and many more

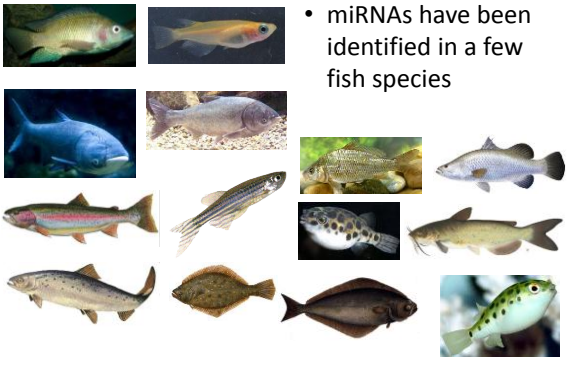
Take-home message



ALTERED PHENOTYPE

- microRNAs regulate gene expression via the RNAi pathway, regulating mRNA translation

Take-home message



- miRNAs have been identified in a few fish species

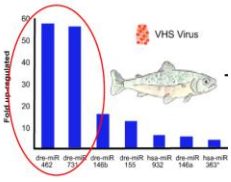
Take-home message

- Studies on teleost fish miRNAs include:

  - analysis of expression, functions, and mechanisms of miRNAs during embryonic development, immune responses to infection

Take home message

- In rainbow trout, two fish-specific microRNAs were highly upregulated by VHSV infection



miR-462

miR-731

- Testing the potential roles of these miRNAs in fish interaction with VHSV on-going

http://www.naturalfayre.com/2009/08/01/salmon-steaks-cooked-in-foil/

Acknowledgment

- Danish Technical Research Council Grant 247-08-0530 (Co-evolution)
- EEC Sixth Framework Programme (Food Quality and Safety) IMAQUANIM Contract No. 007103
- EU Network of Excellence, EPIZONE Contract No. FOOD-CT-2006-016236

THANK YOU FOR YOUR ATTENTION

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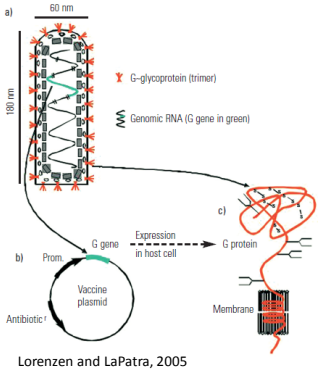
Fish rhabdovirus DNA vaccine

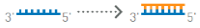


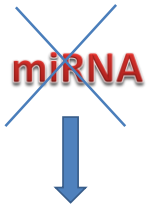
Lorenzen and LaPatra, 2005

- glycoprotein G: protective antigen
- Provides strong protection
- Early onset of protection related to anti-viral interferon response

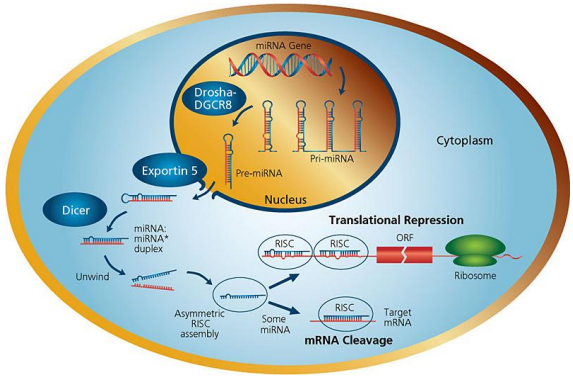
Summary

- We plan to test whether these two VHSV-induced miRNAs possess pro-viral or anti-viral activity





Loss of function



http://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/mirna/learning-center/mirna-introduction.html

- ANIMATION

http://www.nature.com/nrg/multimedia/rnai/animation/index.html

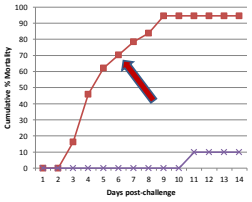
General questions

- Which miRNAs are involved in fish responses to VHSV infection and DNA vaccination?
- What is/are the function(s) of miRNAs in fish?
- Do microRNAs in fish play a role in host interaction with viral pathogens?

What do we expect to see?

If miR-462 and miR-731 possess anti-viral activity:

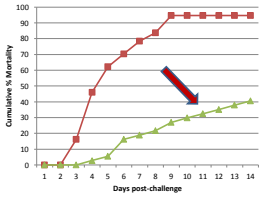
silencing the miRs will:



levels of infection, disease progression

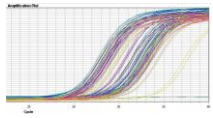
If miR-462 and miR-731 promotes viral replication:

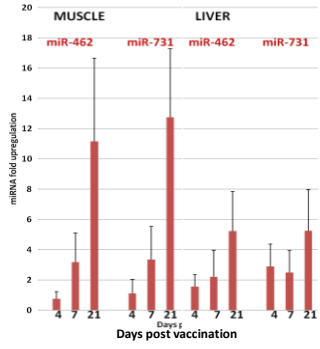
silencing the miRs will:



levels of infection, disease progression

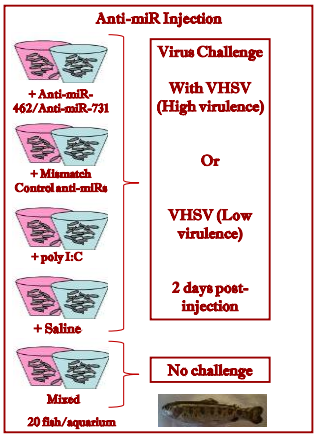
miR-462 and miR-731 were upregulated in the muscle (vaccination site) and liver





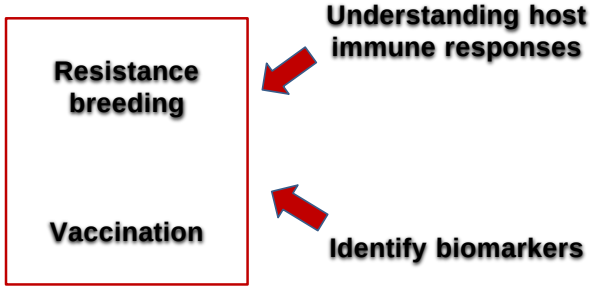
Are miR-462 and miR-731 involved in the interaction of fish with VHSV?

- Do miR-462 and miR-731 have anti-viral activity?
- Do miR-462 and miR-731 promote VHSV infection?



- Fish size: ca. 1 g
- Injection route: Intraperitoneal
- Dose: 1µg/g in 25 µL saline per fish

Mitigating Potential Outbreaks



Identifying Biomarkers

- gene expression profiling identified molecular markers of immunological relevance
- analysis of mRNA expression and regulatory factors key to understanding cellular processes
- newly discovered small regulatory RNAs (miRNAs) add another layer of the molecular diversity of cellular processes

Significance

- miRNAs might potentially have an enormous impact in the regulation of immune responses
- identification of miRNAs and their target genes important toward understanding
  - regulatory networks
  - gene silencing mechanisms
  - practical use for gene manipulations

Future Work

- identify mRNA and miRNA signatures of immunological relevance upon vaccination
- Identify specific cells that express these miRNAs
- Identify genes they regulate
- Combine profiles with in vitro work in cell culture to describe target relationships between miRNAs and mRNAs and the effect of this targeting in fish

Perspective

- Use of small RNAs in antiviral therapy
- Targets and functions of miRNAs using knowledge on their pathway interactions in the choice of targets for developing new antiviral treatments in aquaculture
- These regulated microRNAs could potentially be used as biomarkers of immune responses and as suitable selection markers to identify VHSV-resistant fish.