

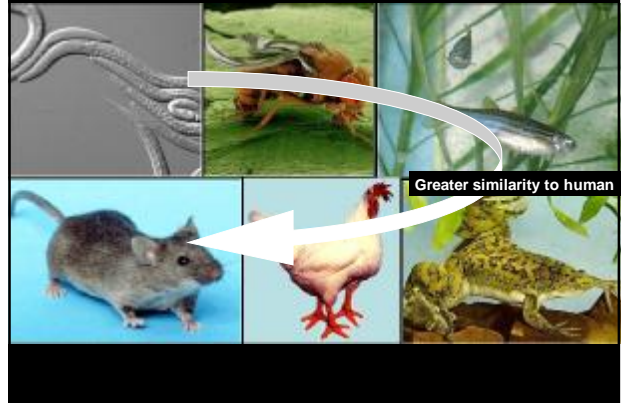
# Techniques & Strategies in Molecular Medicine

Dec. 10<sup>th</sup> 2007

## MODEL ORGANISMS

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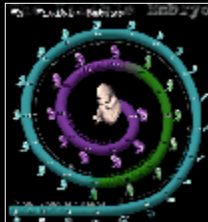


## Why Use Model Organisms ?

✓ Gain understanding at an *in vivo* level, integrative biology.

✓ *In vitro* (test-tube) data may not translate into equivalent results *in vivo* (e.g. drug screens)

✓ some biological processes need to be studied in whole animal (e.g. memory formation, vision, behaviour.....)



*"Model organisms act as surrogates that enable experiments to be carried out under a more favourable environment than would be available in the original system"*

### -advantages common to model organisms

- 1) rapid development with short life cycles.
  - 2) small adult size.
  - 3) ready availability.
  - 4) fewer ethical constraints.
  - 5) tractability (easily manipulated).
  - 6) well characterised
  - 7) generate research data
- } *Reduced maintenance costs*

## C. Elegans (worm)

The diagram illustrates the life cycle of *C. elegans*. It begins with an 'Adult' worm, which produces a 'Fertilized Egg'. This egg undergoes 'Cleavage' and 'Embryogenesis' (indicated by a series of small circles). The resulting embryo hatches into a 'Hatching' stage, which then develops through larval stages labeled 'L1', 'L2', and 'L3'. The cycle completes by returning to the 'Adult' stage. A central circular clock indicates 'Hours after fertilization at 20°C', with markers at 0, 12, 24, 36, 48, and 60 hours.

- ü small size (1.5 mM)
- ü Short life cycle (3 days)
- ü hermaphrodites (self-fertilisation)
- ü External & rapid development
- ü Transparent
- ü Transgenesis
- ü Mutagenesis
- ü Used to study apoptosis, aging
- ✗ invertebrate

Wolpert: Principles of Development

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## Drosophila melanogaster (fly)

- small size
- Short life cycle (2 weeks)
- External & rapid development
- Transgenesis
- Transposition
- Mutagenesis
- Used to study development
- invertebrate

The diagram illustrates the life cycle of Drosophila melanogaster. It begins with a 'Fertilized egg', which develops through 'Diapause' and 'Sexual Maturation' (stages 1-4) into a 'Neuroblastoma'. This stage leads to 'Embryonic' development (stage 13), which then undergoes 'Metamorphosis' to become an 'Adult fly'. The adult fly is shown with a segmented body and wings. A separate branch from the adult fly shows 'Germ cells' leading to 'Meiosis and fertilization', which then leads to 'Stage 1' of the embryo. The embryo is shown in various stages of development, including 'Stage 1', 'Stage 2', 'Stage 3', and 'Stage 4'. A 'Dorsal view' and 'Ventral view' of the embryo are also shown. A microscopic image of a fly embryo is included in the bottom left corner.

- ✓ small size
- ✓ Short life cycle (2 weeks)
- ✓ External & rapid development
- ✓ Transgenesis
- ✓ Transposition
- ✓ Mutagenesis
- ✓ Used to study development
- ✗ invertebrate

## Danio rerio (zebrafish)

The diagram illustrates the life cycle of *Danio rerio* (zebrafish). It begins with an **Adult** zebrafish, which produces an **Oocyte** and a **Sperm**. These fuse to form a **Zygote**, which undergoes **Cleavage** to form a **2-cell stage**, then a **4-cell stage**, and finally a **16-cell stage**. The **16-cell stage** is labeled as **30 minutes after fertilization**. The **16-cell stage** develops into a **Gastrulation and epiboly** stage, which is labeled as **1 hour after fertilization**. This stage is characterized by **75% epiboly (velvet)**. The **Gastrulation and epiboly** stage leads to the **Organogenesis** stage, which is labeled as **10-12 hours stage**. The **Organogenesis** stage leads to the **Body plan established** stage, which is labeled as **Major organs visible**. The **Body plan established** stage leads to the **Hatching** stage, which is labeled as **First swimming**. The **Hatching** stage leads to the **Adult** stage, which is labeled as **30 minutes after fertilization**. The **Adult** stage is also labeled as **30 minutes after fertilization**.

Key features of *Danio rerio* (zebrafish) include:

- Medium size
- Short life cycle (9 weeks)
- External & rapid development
- Transgenesis
- Transposition
- Used to study development
- vertebrates
- Mutagenesis

Wolpert: Principles of Development

- *medium size*
- *Short life cycle (9 weeks)*
- *External & rapid development*
- *Transgenesis*
- *Transposition*
- *Used to study development*
- *vertebrates*
- *Mutagenesis*

## Mus musculus (mouse)

The diagram illustrates the life cycle of a mouse and the timeline for various experimental procedures. The life cycle starts with an adult mouse, which produces a fertilized egg. This egg undergoes cleavage to become a blastocyst, which is then implanted into the uterus. After implantation, the embryo develops through gastrulation and turning, leading to organogenesis. The resulting fetus undergoes fetal growth and development, eventually giving birth to a new adult mouse. The experimental timeline is shown as a circular clock starting from fertilization (0 hours). Key events include: Cleavage (0-12 hours), Blastocyst (12-16 hours), Implantation (16-24 hours), Gastrulation (24-48 hours), Turning (48-72 hours), Organogenesis (72-120 hours), Fetal growth and development (120-180 hours), and Birth (180-210 hours).

- Adult
- Fertilized egg
- Cleavage
- Blastocyst
- Implantation
- Gastrulation
- Turning
- Organogenesis
- Fetal growth and development
- Birth
- Days after fertilization

- medium size
- Short life cycle (9weeks)
- Transgenesis
- Knockout
- mammalian
- v. commonly used
- Internal development

Wojcik: Principles of Development

- ü medium size
- ü Short life cycle (9weeks)
- ü Transgenesis
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- ✗ Internal development

### Functional Genomics in Zebrafish

1. I identify Novel Gene ... .. What does it do...FUNCTION ?
2. Biological Entity of Interest ... .. What genes regulate it ?

### WHAT'S THE STORY ?

Whole Genome (25 chromosomes)  $2 \times 10^9$  base pairs .....The letters

Single Gene .....The words

mRNA/protein

*What does each gene do ?* .....The story  
(post-genome sequence)

Gene Function

- 1) expression pattern
- 2) "gain-of-function" analysis (transgenics)
- 3) "loss-of-function" analysis ( 1. random mutagenesis  
2. targeted knockout/knockdown)

### 1) Gene Expression

RT-PCR

wholemount in-situ hybridisation

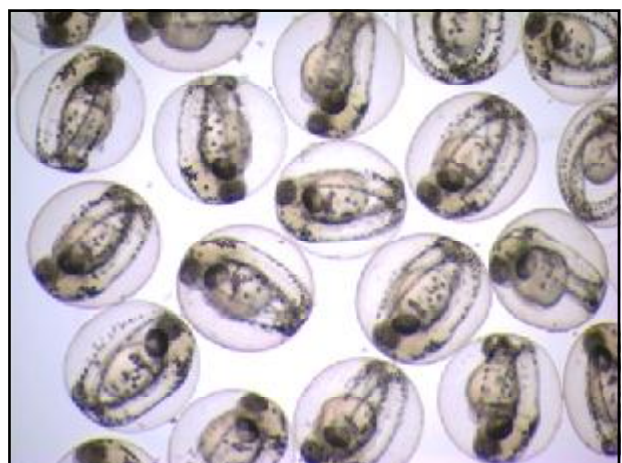
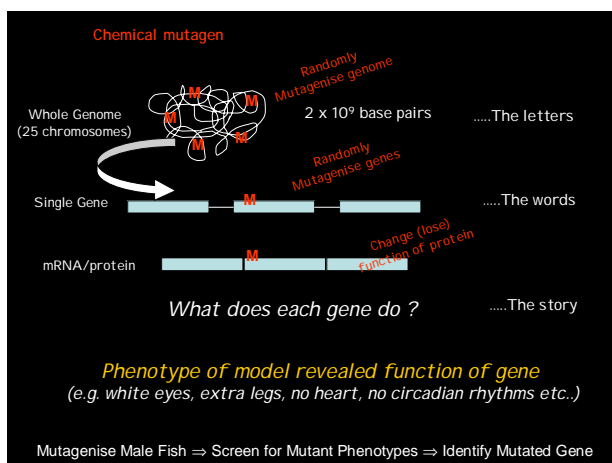
arrays

Gene	Expression Pattern	Expression Pattern
smc3	0.5h	1h
smc3	3h	6h
smc3	12h	24h
smc3	48h	72h

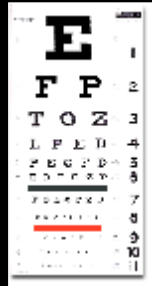
## 2) Functional Genomics

- Forward Genetics----Random Knockouts.  
MUTAGENESIS SCREENS = **Mutants**
- Transgenics ---- Transgene Expression  
..GFP lines  
.. "Disease Models"  
.. "Gene Therapy"
- Reverse Genetics---Targeted Knockdown.  
MORPHOLINOS = **Morphants**

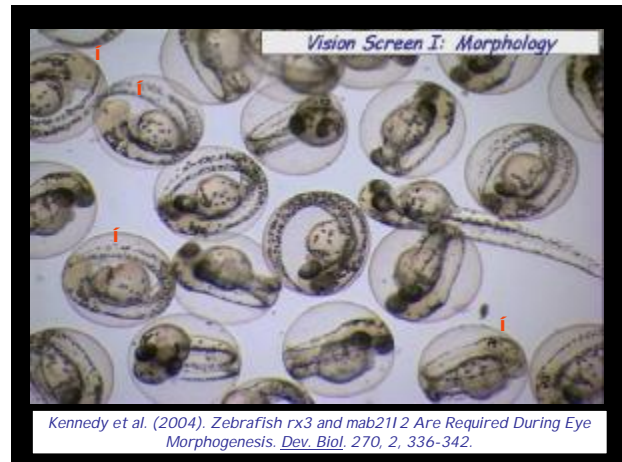
## Mutagenesis Screens



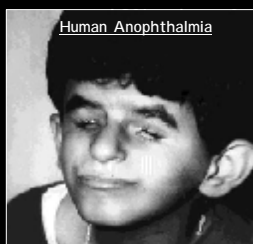
How can we "rapidly" tell if zebrafish are blind ?



1. Morphology (*eym* = *anophthalmia*)
2. Visual Behaviour Assay (*nof* = *achromotopsia*)
3. GFP Photoreceptor Screens.....

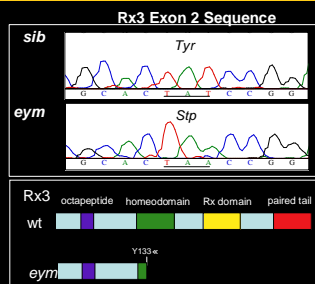


Premature Stop Codon In Rx3 is Responsible for Eyeless Phenotype

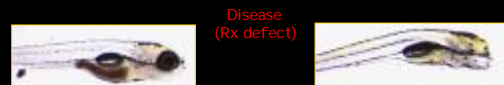


Mutations in human *Rx* linked with anophthalmia.  
Voronina et al. *Hum. Mol. Genet.* (2004).

Knockout of mouse *Rx* results in anophthalmia.  
Mathers et al. *Nature*, (1997).

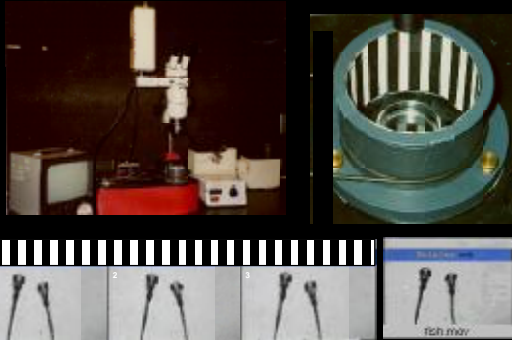


Can we cure anophthalmia in zebrafish ?



Yes ! When we re-introduce a non-defective version of the *rx* gene the fish have normal eyes...

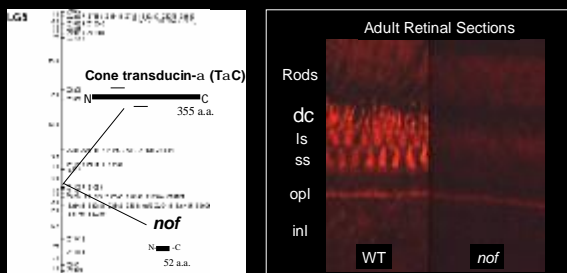
### Vision Screen II: Visual behaviour (OKR)



Brockerhoff et al. (1995) A behavioral screen for isolating zebrafish mutants with visual system defects. PNAS 92(23):10545-9.



### The Blind Mutant *nof* Results from Mutations in *TaC*

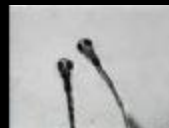


Brockerhoff et al. (2003). Light stimulates a transducin-independent increase of cytoplasmic  $Ca^{2+}$  and a suppression of current in cones from the zebrafish mutant *nof*. J. Neurosci. 23, 470-480.

Model of Human Achromatopsia.  
Mutations in human *TaC* also result in achromatopsia  
Aligianis et al. J. Med. Genet 0; Kohl et al. Am. J. Human Genetics (2002)

### Can we cure achromatopsia in zebrafish ?

2.



Yes !! When we re-introduce a wt cone transducin alpha gene regulated by cone-specific promoter, the fish regain OKR visual behaviour





