Techniques & Strategies in Molecular Medicine

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MODEL ORGANISMS

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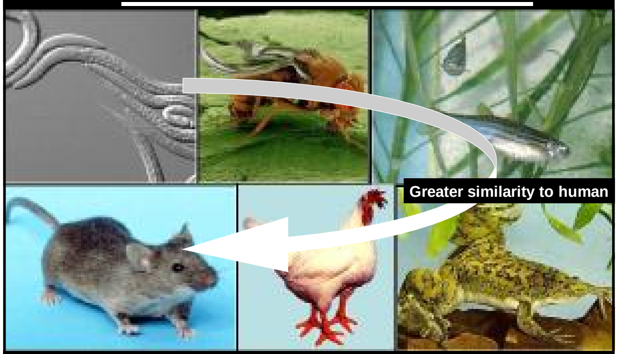


Why Use Model Organisms ?

- 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"

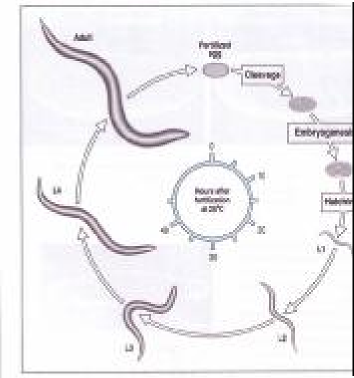
-advantages common to model organisms

1) rapid development with short life cycles.
2) small adult size.
3) ready availability.

} *Reduced maintenance costs*

4) fewer ethical constraints.
5) tractability (easily manipulated).
6) well characterised
7) generate research data

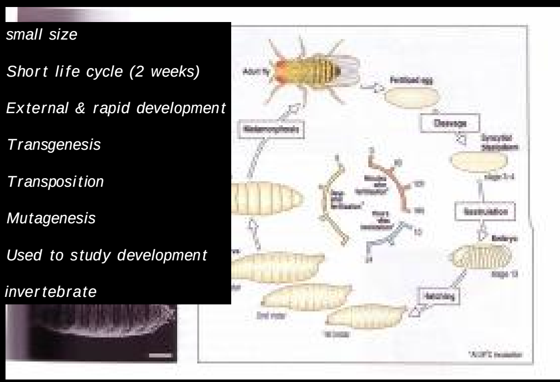
C. Elegans (worm)



- 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
- X invertebrate

Wolpert: Principles of Development

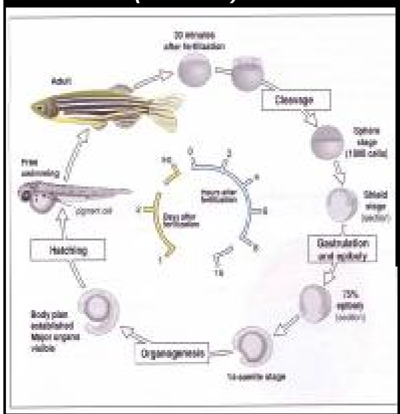
Drosophila melangoster (fly)



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

Wolpert: Principles of Development

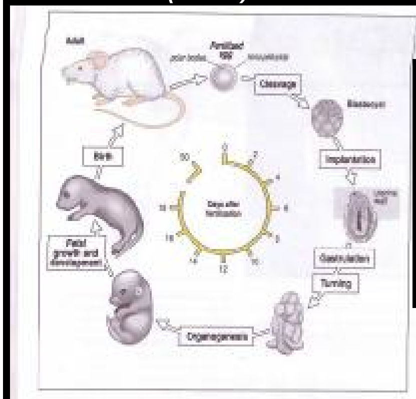
Danio rerio (zebrafish)



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

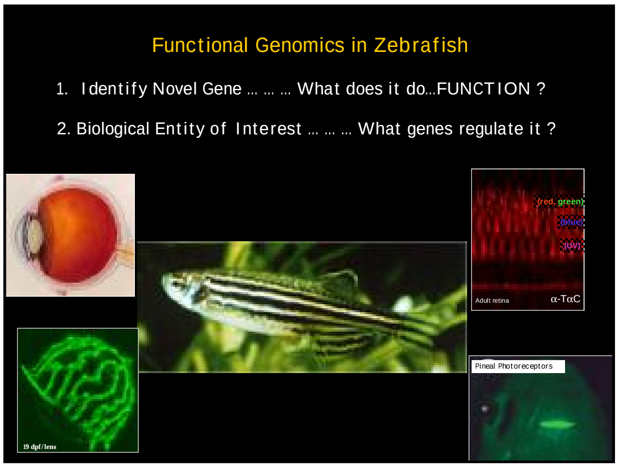
Wolpert: Principles of Development

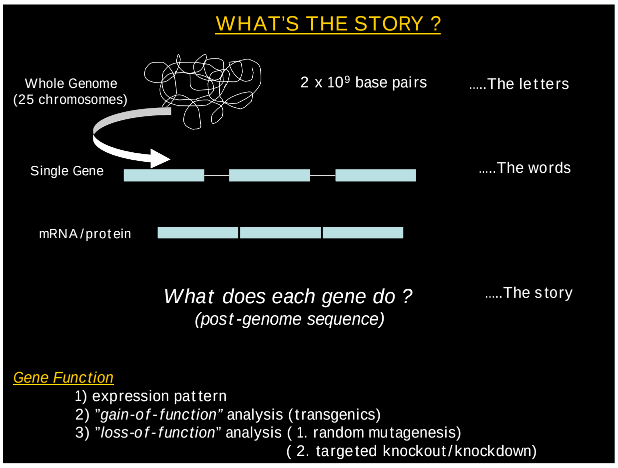
Mus musculus (mouse)

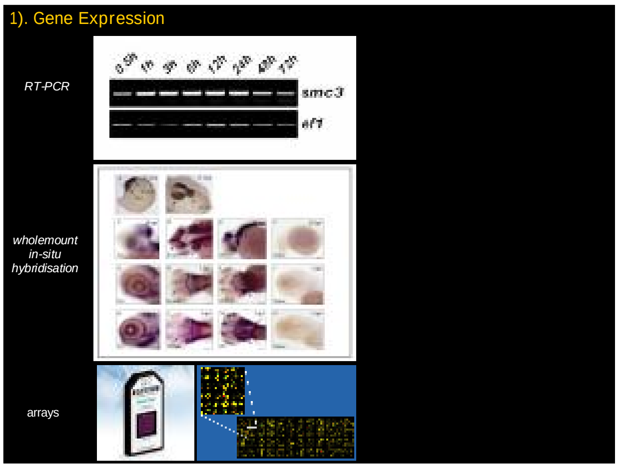


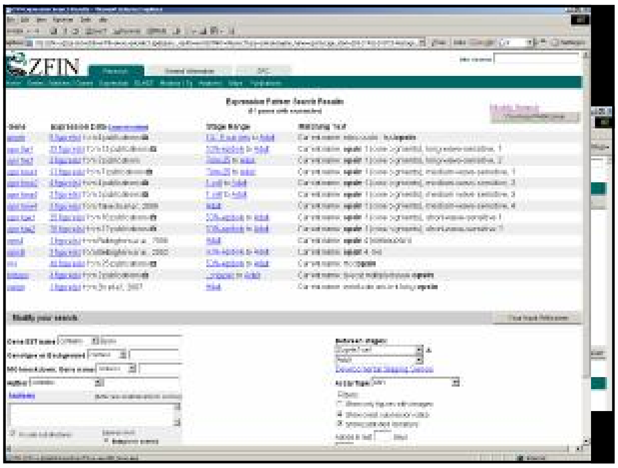
- ü medium size
- ü Short life cycle (9weeks)
- ü Transgenesis
- ü Knockout
- ü mammalian
- ü v. commonly used
- X Internal development

Wolpert: Principles of Development


Functional Genomics in Zebrafish
1. Identify Novel Gene … … … What does it do…FUNCTION ?
2. Biological Entity of Interest … … … What genes regulate it ?
(red, green)
(blue)
(UV)
Adult retina
α-TαC
Pineal Photoreceptors
19 dpf lens


WHAT'S THE STORY ?
Whole Genome
(25 chromosomes)
2 x 10⁹ base pairs
…..The letters
Single Gene
…..The words
mRNA/protein
What does each gene do ?
(post-genome sequence)
…..The story
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
smc3
ef1
wholemount
in-situ
hybridisation
arrays


ZFIN

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

Chemical mutagen

Whole Genome (25 chromosomes) — Randomly Mutagenise genome — 2 x 10^9 base pairs .....The letters

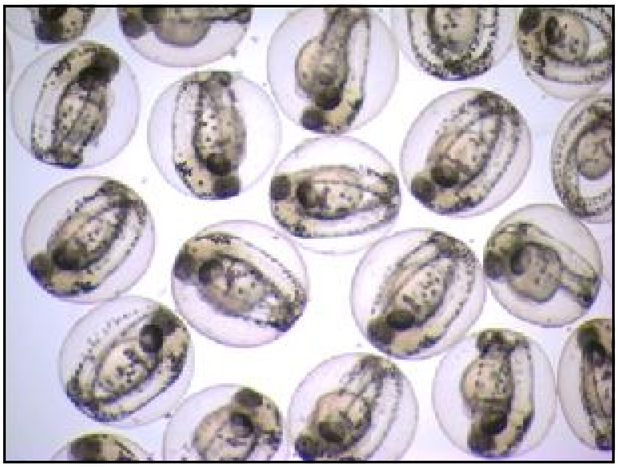
Single Gene — Randomly Mutagenise genes .....The words

mRNA/protein — Change (lose) function of protein

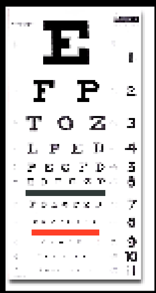
What does each gene do ? .....The story

Phenotype of model revealed function of gene
(e.g. white eyes, extra legs, no heart, no circadian rhythms etc..)

Mutagenise Male Fish ⇒ Screen for Mutant Phenotypes ⇒ Identify Mutated Gene



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

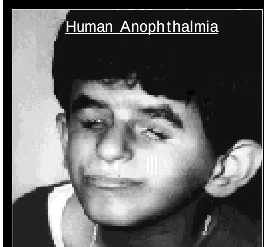


1. Morphology (*eym* = *anophthalmia*)
2. Visual Behaviour Assay (*nof* = *achromotopsia*)
3. GFP Photoreceptor Screens......

Kennedy et al. (2004). Zebrafish rx3 and mab21l2 Are Required During Eye Morphogenesis. Dev. Biol. 270, 2, 336-342.

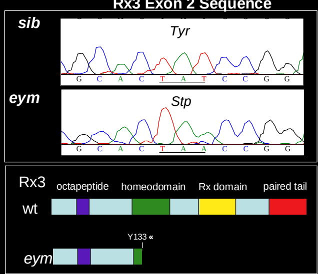
Premature Stop Codon In Rx3 is Responsible for Eyeless Phenotype

Human Anophthalmia

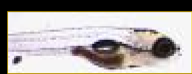


Mutations in human *Rx* linked with anophthalmia.
Voronina et al. Hum. Mol. Genet. (2004).

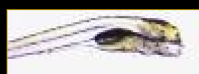
Knockout of mouse Rx results in anophthlamia.
Mathers et al. Nature, (1997).



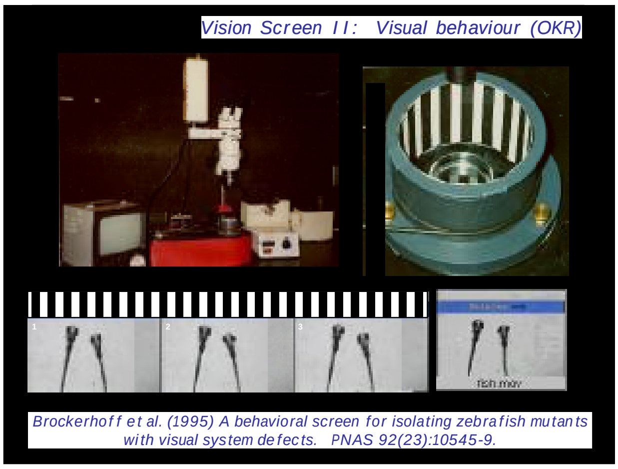
Can we cure anophthalmia in zebrafish ?

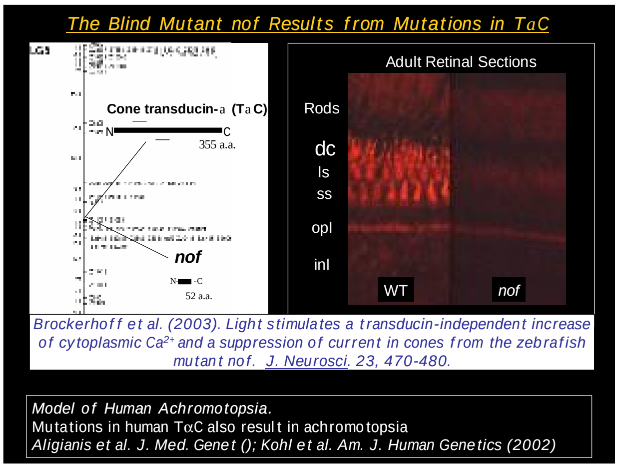


Disease
(Rx defect)

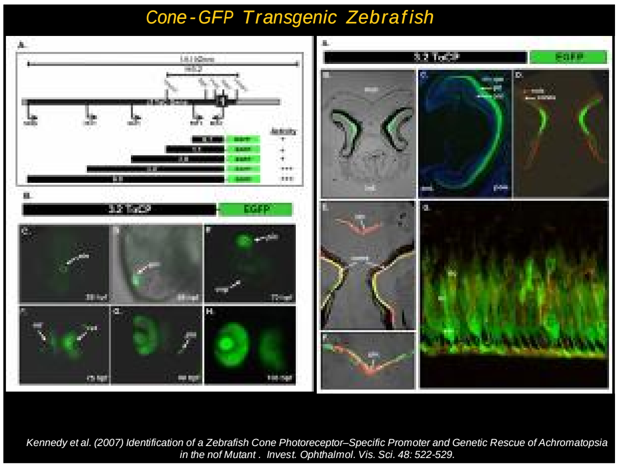


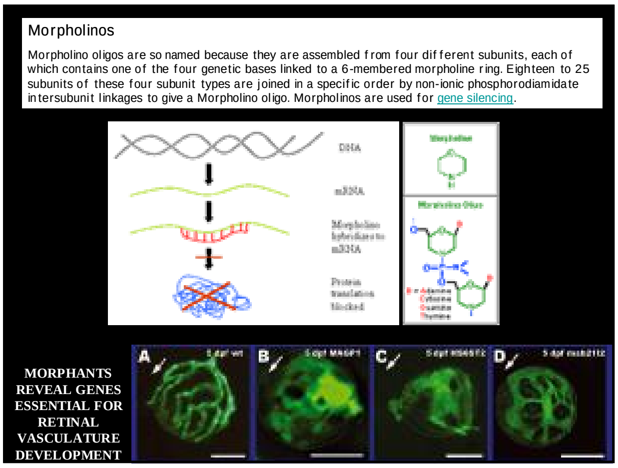
Yes ! When we re-introduce a non-defective version of the rx gene the fish have normal eyes...

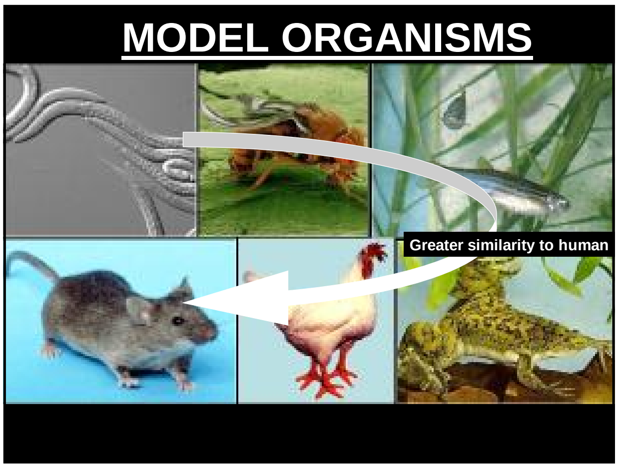

Vision Screen II: Visual behaviour (OKR)
fish.mov
Brockerhoff et al. (1995) A behavioral screen for isolating zebrafish mutants with visual system defects. PNAS 92(23):10545-9.


The Blind Mutant nof Results from Mutations in TαC
LG5
Cone transducin-α (TαC)
N
C
355 a.a.
nof
N
-C
52 a.a.
Adult Retinal Sections
Rods
dc
Is
ss
opl
inl
WT
nof
Brockerhoff et al. (2003). Light stimulates a transducin-independent increase of cytoplasmic Ca2+ and a suppression of current in cones from the zebrafish mutant nof. J. Neurosci. 23, 470-480.
Model of Human Achromotopsia.
Mutations in human TαC also result in achromotopsia
Aligianis et al. J. Med. Genet (); 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
3.2 TαCP
TαC


Cone-GFP Transgenic Zebrafish
3.2 TaCP
EGFP
Kennedy et al. (2007) Identification of a Zebrafish Cone Photoreceptor–Specific Promoter and Genetic Rescue of Achromatopsia in the nof Mutant . Invest. Ophthalmol. Vis. Sci. 48: 522-529.


Morpholinos
Morpholino oligos are so named because they are assembled from four different subunits, each of which contains one of the four genetic bases linked to a 6-membered morpholine ring. Eighteen to 25 subunits of these four subunit types are joined in a specific order by non-ionic phosphorodiamidate intersubunit linkages to give a Morpholino oligo. Morpholinos are used for gene silencing.
DNA
mRNA
Morpholino hybridizes to mRNA
Protein translation blocked
MORPHANTS REVEAL GENES ESSENTIAL FOR RETINAL VASCULATURE DEVELOPMENT
A
B
C
D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Greater similarity to human