FEDERAL INSTITUTE
FOR RISK ASSESSMENT

BfR
Bundesinstitut für Risikobewertung

Non-mammalian animal models in developmental toxicology

Dr. Michael Oelgeschläger

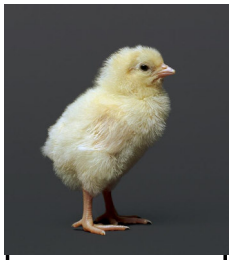
Emergence of animal models in experimental developmental biology

Nick Hobgood, wikipedia

H. Driesch
Sea urchin

André Karwath, wikipedia

T.H. Morgan
D. melanogaster



Hamburger
Hamilton
Gallus gallus

D. melanogaster
Screen

G. Streisinger
Brachydanio rerio

1900

1950

2000

W. Roux
Rana esculenta

Grand-Duc, Wikipedia
http://de.wikipedia.org/wiki/
Benutzer:Grand-Duc

E. Browne
Hydra

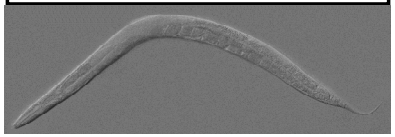
Frank Fox
www.mikro-foto.de

H. Spemann
Triturus vulgaris

Christian Fischer, wikipedia

Nieuwkoop
Faber
Xenopus laevis

S. Brenner
Caenorhabditis elegans



Zeynep F. Altun, www.wormatlas.org

Boston/ Tübingen
Screen (D. rerio)

Advantages of the various model systems

	C. elegans	D. melanogaster	Xenopus	Zebrafish	Chicken	Mouse
Number of Eggs	± 300	± 100	> 1000	± 150	1	5-10
Embryo accessibility	++	++	++	++	+	+/ -
Generation time	Very short	Very short	X.l. Long X.t. Medium	Medium	Medium	Mediu m
Genome known	Yes	Yes	Yes	Yes	Yes	Yes
Genetics Gain-of function Loss-of-function	+ + +	+++ +++ +++	(+/-) + (+/-)	++ ++ ++	- - -	+++ +++ +++
Micromanipulation	+ /-	+/-	++	+	++	+/-
ES cells available	No	No	No	No	Yes	Yes
HTS	++	++	++	++	-	-
Costs	Low	Low	Low	Low	Medium	High
Evolutionary distance	High	High	Medium	Medium	Medium	Close

Comparison of animal models reveal a high degree of evolutionary conservation

1. Transcription regulation of cell fate determination and positional information

- **Highly conserved Hox gene clusters with highly similar genomic organisation and biological functions have been identified in species throughout the animal kingdom**

- **Mutation of Pax6 causes aniridia in humans and an "*eyeless*" phenotype in mouse or fly. Transgenic mouse Pax6 can induce the formation of ectopic compound eyes in fly**

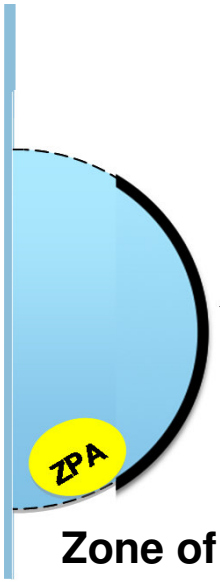
2. Control of early embryonic patterning by conserved signalling pathways

- **Role of various key signalling pathways first identified in fly were found to regulate comparable processes in mammals**
  - **BMP: inhibition of neural cell fate**
  - **WNT: inhibits foforebrain formation**

Comparison of animal models reveal a high degree of evolutionary conservation

3. Limb development

Highly conserved in vertebrates:



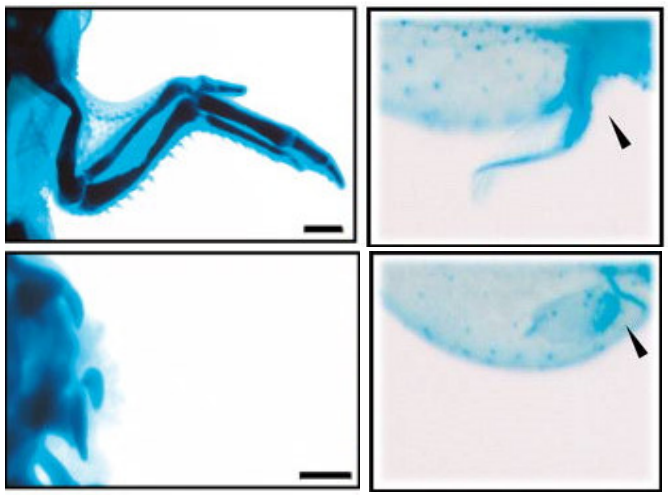
Apical Ectodermal Ridge expressing Fgf (outgrowth)

AER

Zone of Polarising Activity expressing Shh (AP axis)

e.g. Thalidomid

- **Inhibits limb /fin outgrowth in chicken / zebrafish**
- **Target (CRBN) identified using chicken and zebrafish embryos as functional readout**
- **Relevance of the regulation of CRBN by Thalidomid verified in human cells (regulation of Ikarus in myelomas)**



co

Thal

Ito et al. 2010 Science 327: 1345-1350

1. Drosophila melanogaster

- Genetics (various mutant and transgenic strains available)
- High similarity between genes regulating embryogenesis in fly and vertebrates
- Identification of genes related to human disease (drug discovery)

High Evolutionary Distance

Sex-linked Recessive Lethal (SLRL) genotoxicity test: TG 477
Somatic Mutation And Recombination Test (SMART)
Teratogenicity testing performed applying various protocols

Problems:	false negatives
	evolutionary distance
HTS capacity:	automated embryo sorting followed by imaging

2. Caenorhabditis elegans

- Highly defined cell lineages
- Conserved molecular mechanisms and signalling pathways
- Basic mechanism of apoptosis discovered in C. elegans

- genetic screens
- gain- or loss-of-function studies
  (transgenic approaches, interfering RNA)
- various mutant and transgenic lines available

High Evolutionary Distance

Common use .
- neurobiology (Alzheimer, Parkinson)
- ageing
- drug screening

Proposed for studies on developmental / neurotoxicity

3. Chicken

- **Highly conserved molecular mechanisms and signaling pathways**

  - **microsurgical procedures possible**
  - **local exposure using beads**
  - **gain- / loss-of-function studies (viral transduction, in ovo electroporation)**

Medium Evolutionary Distance

Avian Reproduction Test: OECD TG 206

Chick Embryotoxicity Screening Test (CHEST)
Problems : false positive rate, route of exposure, maternal <=> embryonal toxicity

4. Xenopus laevis

- Highly conserved molecular mechanisms and signalling pathways (BMP, Wnt)
- > 1000 eggs /female / day

  - microinjection of RNA, DNA, Morpholinos or Protein in single blastomere
  - detailed fate map available
  - micromanipulations, incl. explant culture (animal caps) possible

Medium Evolutionary Distance

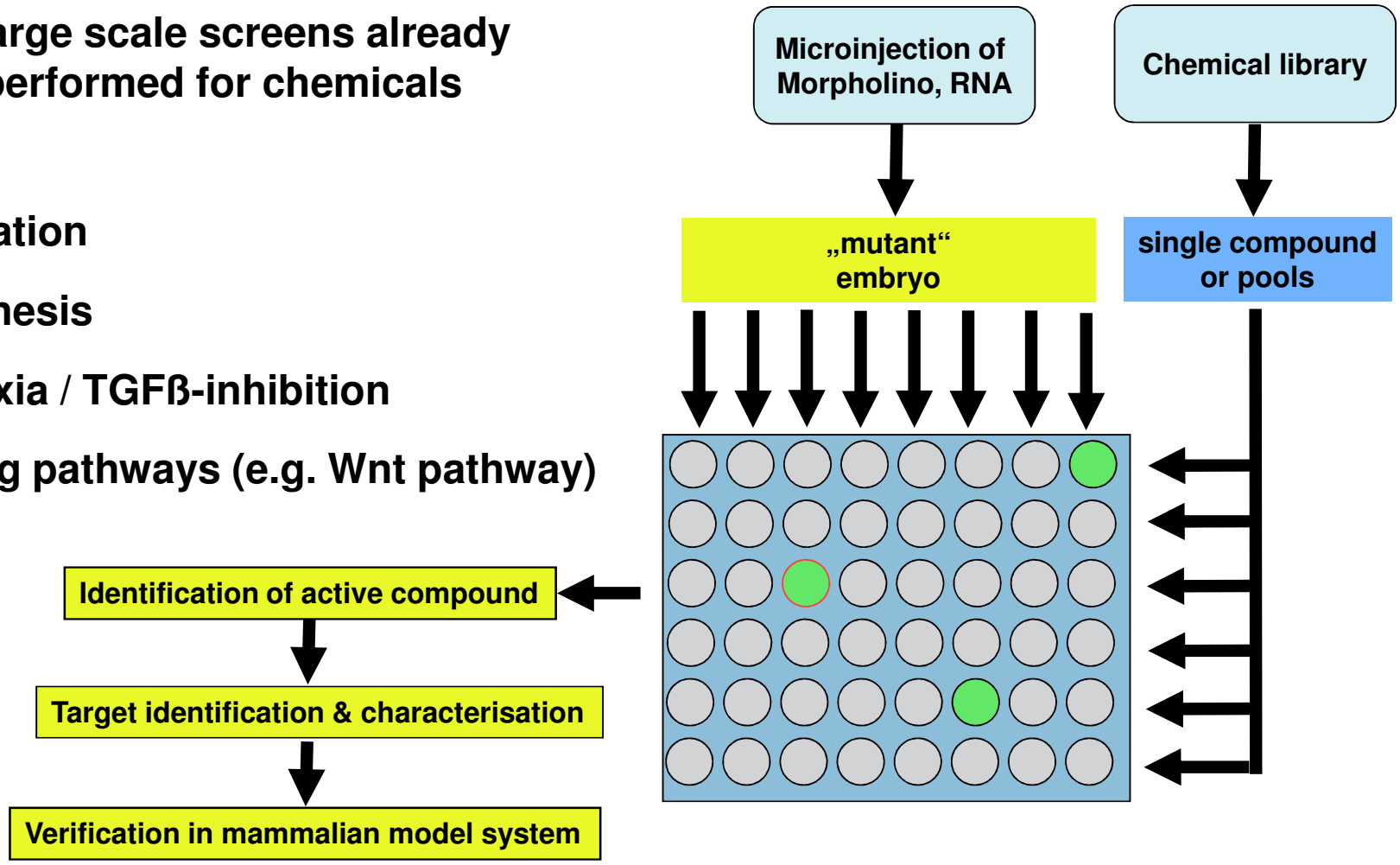
Amphibian metamorphosis assay (AMA): TG 231
Frog Embryo Larval Amphibian Growth and Development Assay (LAGDA)
Transgenic approaches to determine effects on thyroid hormone activity

Frog Embryo Teratogenesis Assay (FETAX)
Problems: equivocal results
embryotoxicity <=> maternal toxicity

4. Xenopus laevis

A number of large scale screens already successfully performed for chemicals affecting:

- **pigmentation**
- **angiogenesis**
- **heterotaxia / TGFß-inhibition**
- **signalling pathways (e.g. Wnt pathway)**



5. Zebrafish

- Embryonic patterning (Boston/ Tübingen screen)
- Highly conserved molecular mechanisms and signalling pathways
- Especially suited for:
  - (genetic) screens
  - gain- or loss-of-function studies (microinjection RNA/ Morpholinos)
  - various mutant and transgenic lines available

Medium Evolutionary Distance

Fish Embryo Acute Toxicity (FET) Test: OECD TG 236
Fish Sexual Development Test (FSDT): OECD TG 234
Fish, 21 Day Assay (FA): OECD TG 230
Fish, Short Term Reproduction Assay (FSTRA): OECD TG 229
Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages: OECD TG 212
Fish, Early-life Stage Toxicity Test: OECD TG 210

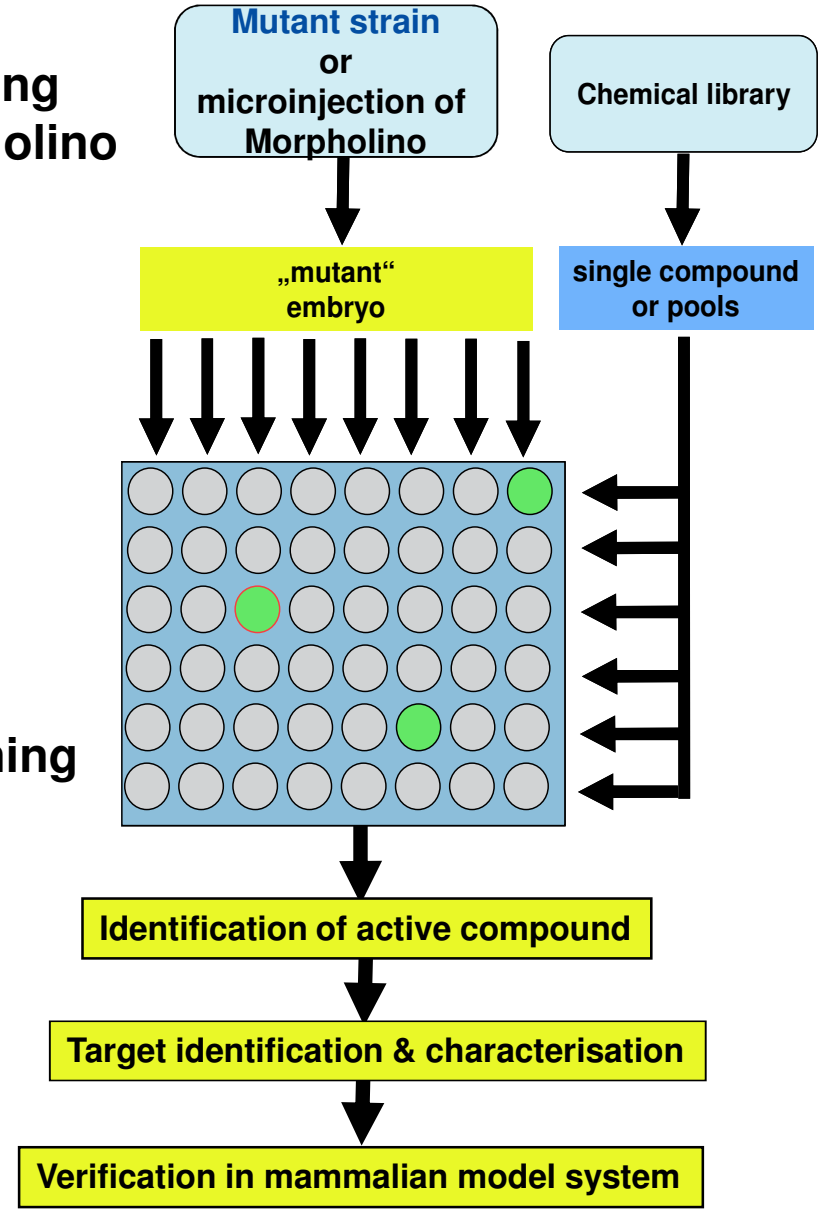
5. Zebrafish

Various HT screens successfully performed, using wild-type, mutant and transgenic lines or morpholino knockdown:

- Cancer
- Cardiovascular disease
- Neurodegenerative diseases
- Drug-induced toxicity

Reporter lines for developmental toxicity screening

- Early patterning (dharma, Wnt8)
- Neurogenesis (ngn)
- Angiogenesis (fli-1, flk-1)
- Myogenesis (mhc)



5. Zebrafish

Developmental toxicity assays

Padilla et al (2012)

Toxicology 22:174–87

309 ToxCast chemicals

62 % toxicity (191)

Concentration: 80mM to 1nM

Exposure: 6-120 hpf

6 endpoints

Truong et al. (2012)

Toxicological Sciences 137:212-33

1.060 ToxCast chemicals

46% toxicity (487)

Concentration: 640 μM to 0.064 μM

Exposure: 6 to 120 hpf

18 endpoints

Gustafson et al (2012)

Reproductive Toxicology 33: 155-164

20 chemicals

blind study (4 Labs)

Concentration: 1000 μM to 1μM

Exposure: 5-120 hpf

10 endpoints

Selderslaghs et al (2012)

Reproductive Toxicology 33:142– 154

27 chemicals

Concentration ranges determined in preliminary experiments for each compound (1μM – 162 mM)

Exposure: 2-144 hpf

11 endpoints

Potential problems using non-mammalian models

- (inter-)laboratory reproducibility (dep. on the complexity of the assay)
- Species differences
  - Phenotype ?
  - Molecular mechanism ?
    - Transcriptomics
    - Proteomics
    - Metabolomics
- Exposure
  - Toxicokinetics / Toxicodynamics
  - Relevance of test substance concentration
- Applicability domain
  - Number of substances tested that can be compared with reliable mammalian / human data

Use of non-mammalian models for the identification of conserved (specific) toxicity pathways

- Screening for phenotypic effects of compounds or mixtures
  - transgenic reporter lines
  - sensitized mutants
- HT- HC screening to identify the key pathways involved in mediating toxicity
  - use of the various distinct experimental advantages, including iRNA, morpholino knock down, micromanipulations, mutant and transgenic lines
  - use of mutant or transgenic strains or knock-down technologies to verify the relevance of potential (specific) mediators of toxicity identifies in “omics” studies
- Identification of conserved toxicity and adverse outcome pathways
- Establishment of novel (non - mammalian) assays
- Identification of new predictive endpoints for mammalian testing

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Thank you for your attention

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