Thursday, 23 Sept '21

Finish HOX and start 10. Learning from (clawed) frogs

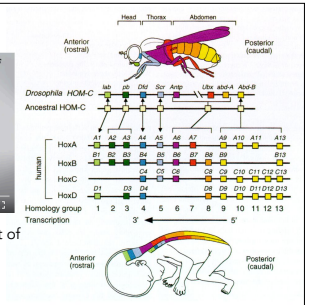
Remember to turn on zoom

Put in your iPods

confusions / clarifications / questions?

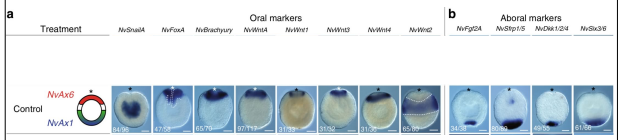
What makes a good model system?
Is it just relevance to humans?

Homeotic (HOX) genes



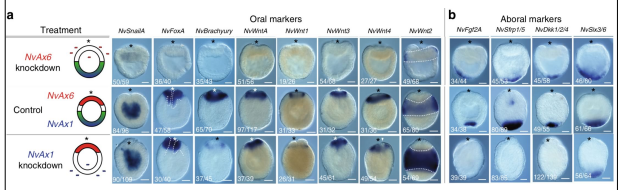
Q: What does this imply as to the effect of Abd-A on the more anterior Hox gene?

Q: What does presence of HOX cluster in fruit flies and mouse lead you to conclude? How might you test it?



Hox and Wnt pattern the primary body axis of an anthozoan cnidarian before gastrulation

Timothy Q. DuBuc[1], Thomas B. Stephenson[2], Amber Q. Rock[2] & Mark Q. Martindale[2]



Q: How might you determine which genes are direct and which indirect targets of the oral and aboral HOX genes Ax6 and Ax1?
(**Q**: what is a direct target?)

Hox and Wnt pattern the primary body axis of an anthozoan cnidarian before gastrulation

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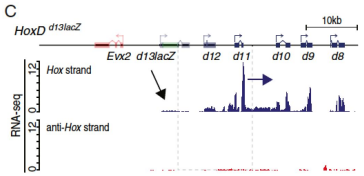
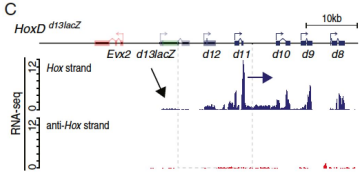
Q: How does the HOX cluster organization impact the expression of specific HOX genes?



- Strand-specific RNA profiles from homozygous HoxDd13lacZ **E13.5 metanephros**.
- Hoxd13lacZ was not transcribed
- Hoxd12 showed a low expression.
- RNAs leaking from Hoxd11 to Hoxd10 are shown

Q: How does the HOX cluster organization impact the expression of specific HOX genes? How would you study it?



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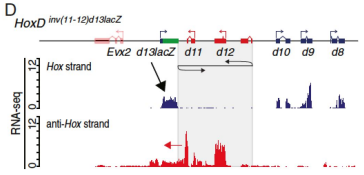


- A region of the HOX cluster was inverted
- Strand-specific RNAs from homozygous metanephros.
- Hoxd11 transcripts now leak over Hoxd13lacZ (red arrow)
- Together with ectopic transcription of Hoxd13lacZ

HOX introduced:

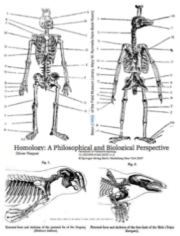
The term homologous (or homolog) is often used in biology to describe genes, structures, and processes.

Is it possible (↓) for two biological objects to be partially homologous.?

- yes
- no
- no idea

At the molecular, behavioral, or the anatomical levels (→) how might you distinguish whether two genes, structures, processes, or behaviors are homologous or analogous ? (↓)

what does the term homolgous imply?

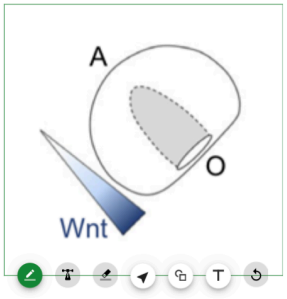


A common feature of metazoans is the presence of an anterior-posterior (oral-aboral) Wnt signaling gradient. Depending upon the organism, a number of factors can influence where such a gradient forms.

Would you expect to find such a Wnt gradient in the ancestor of bilaterians?

justify your answer

- yes
- no
- no idea



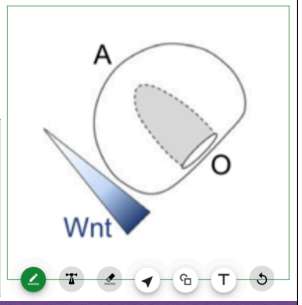
In this figure the strength of Wnt signaling is indicated. Generate a model, using the expression of Wnt ligands, receptors, and antagonists, that produces a stable signaling gradient (→) and describe your thinking (↓)

mechanistically

If high levels of Wnt signaling induce the expression of the BMP signaling antagonist chordin, what might the strength of BMP signaling look like, assuming that BMP and its receptor(s) are expressed ubiquitously in the early embryo (↓).

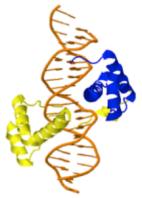
Assume you know nothing about how chordin works to inhibit BMP signaling. How would the level of BMP signaling change if chordin were cytoplasmic compare to if it were a secreted protein. (→) describe your reasoning (↓)

justify your answer mechanistically



Based on this image of two homeobox domains interacting with (and binding to DNA in a sequence specific manner), do you think the homeobox domain interacts with the minor or the major groove of the DNA?

- major
- minor
- no idea



What secondary structural features are primarily involved in this interaction?

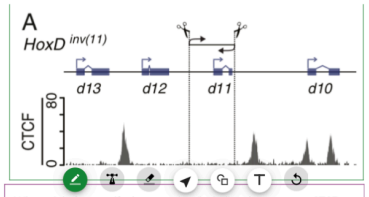
- α-helices
- β-sheets
- unstructured regions
- hydrophobic stretches
- no idea

What features of a homeodomain protein could enable it to act as a transcriptional repressor at one DNA binding site and a transcriptional activator at another (in the same genome)?

how might the DNA sequences bound influence the protein's behavior?

Here is a CHIP SEQ map for the DNA-binding protein CTCF in a region of the HoxD gene cluster. CTCF acts as a "insulator protein". Regions of chromatin flanked by CTCF binding sites are relatively independent of regulatory interactions with enhancers outside of those boundaries.

Predict how the gene flip indicated (→) would influence the expression of d10 (↓).



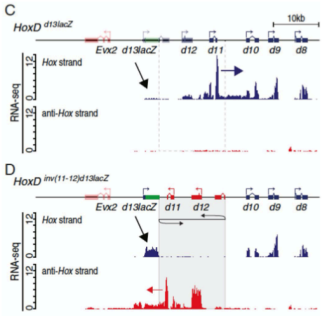
be as mechanistic as possible

What might happen if a larger region (including the upstream CTCF binding site was included in the flip? Indicate the location of CTCF binding sites after flipping (↑) and predict the impact on d13 gene expression (↓).

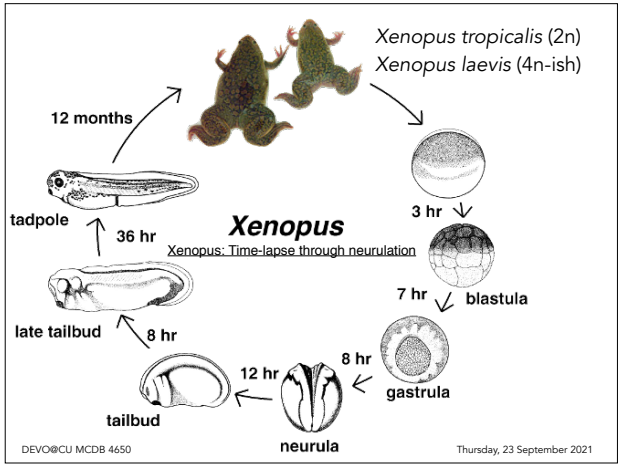
be as mechanistic as possible

Here is a map (→) of the number of RNA transcripts detected in the wild type hox cluster (top) and after the orientation of d11 and d12 gene region has been flipped (bottom).

Describe the observed effects of flipping on the expression of d11, d12, and the neighboring d13lacZ and d10 genes and suggest a model that produces such effects.



what does flipping change?



Xenopus laevis (tropicalis)

- **fertilization**: external
- **development**: external
- **early embryonic asymmetries**: built in
- **time to sexual maturity**: slow (faster in tropicalis)
- **lab history & adaptations**: hardy breeding of lab stocks
- **No growth early:** just mitosis/cytokinesis

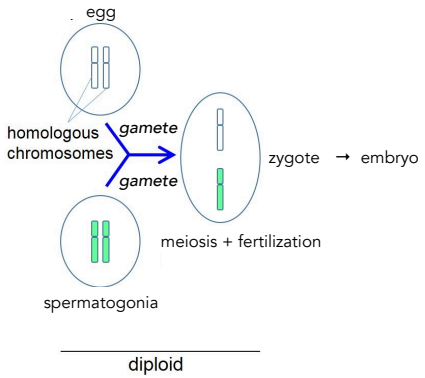
- **Timing of fertility:** gonadotropin inducible

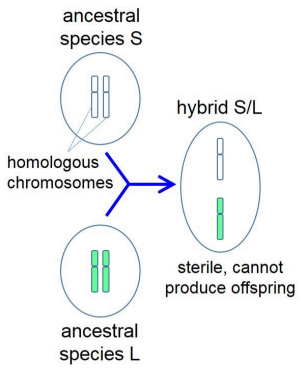
The Hogben Pregancy Test!

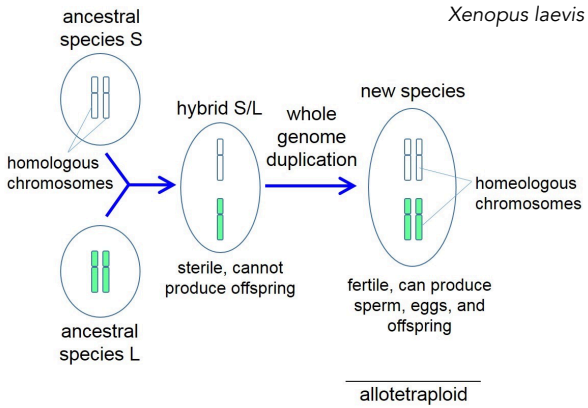
Xenopus females laid eggs in response to human chorionic gondadotropin.

en.wikipedia.org
Lancelot Hogben

Xenopus tropicalis



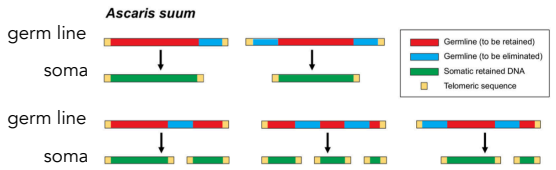




What has and can be learned from *Xenopus*

- Are genes lost during cell differentiation? (nuclear transplant)
- How sperm entry point combines with oocyte asymmetry to generate axes in Xenopus
  - role of β-catenin-Wnt signaling system
- cellular mechanisms driving morphogenesis

Somatic differentiation & genome diminution



Q: how does dna elimination insure irreversible differentiation?

Q: what about mitosis? (How would centromeres behave?)

Q: what would expect to find DNA elimination in the to occur in the germ line?

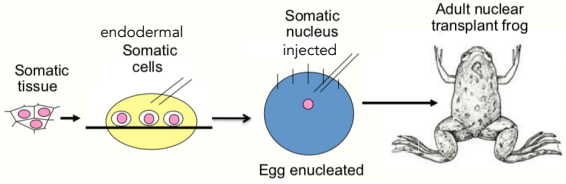
Wang et al., 2017 Comparative genome analysis of programmed DNA elimination in nematodes

Organisms with programmed DNA elimination.

Organism	First discovered (year; organism)	Common name
Nematodes	1887; *Parascaris univalens*	Roundworm
Insects	1914; *Phragmatobia fuliginosa*	Moth
Arachnids	1939; *Pediculopis graminum*	Grass mite
Crustaceans	1959; *Cyclops strenuus*	Copepod
Ciliates	1965; *Stylonychia mytilus*	Ciliate
Mammals	1965; *Isoodon macrourus*	Bandicoot
Chondrichthyans	1984; *Hydrolagus colliei*	Spotted ratfish
Hagfishes	1986; *Eptatretus burgeri*	Inshore hagfish
Birds	1998; *Taeniopygia guttata*	Zebra finch
Lampreys	2009; *Petromyzon marinus*	Sea lamprey

Programmed DNA elimination in multicellular organisms
Jianbin Wang and Richard E Davis

Is genome diminution occurring in *Rana*?



TRANSPLANTATION OF LIVING NUCLEI FROM BLASTULA CELLS INTO ENUCLEATED FROGS' EGG

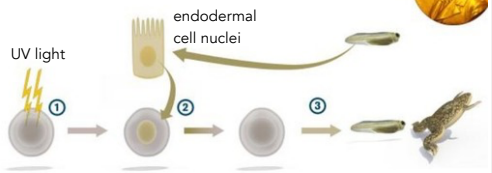
By Robert Briggs and Thomas J. King

Institute for Cancer Research and Lankenau Hospital Research Institute, Philadelphia, Pennsylvania

Communicated by C. W. Metz, March 15, 1952

The Nobel Prize in Physiology or Medicine 2012

John B. Gurdon



John B. Gurdon eliminated the nucleus of a frog egg cell (1) and replaced it with the nucleus from a specialised cell taken from a tadpole (2). The modified egg developed into a normal tadpole (3). Subsequent nuclear transfer experiments have generated cloned mammals (4).

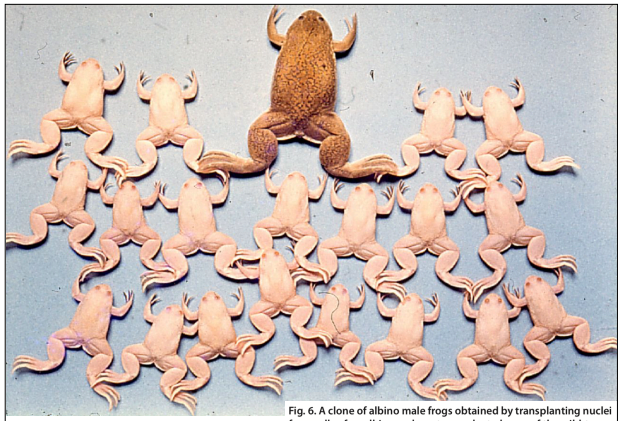
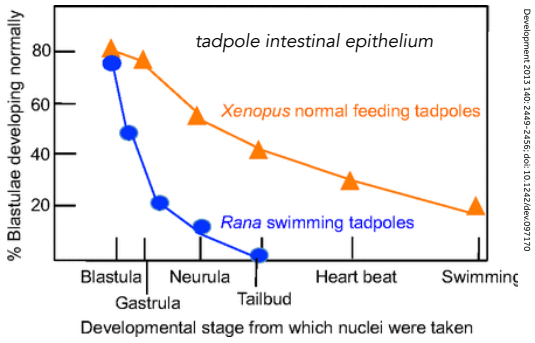
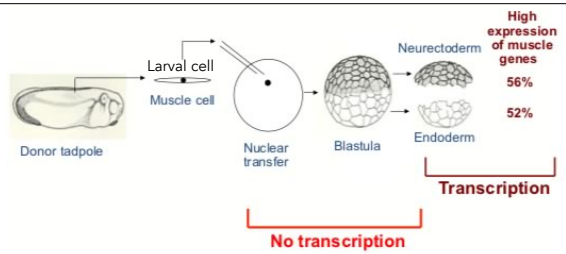


Fig. 6. A clone of albino male frogs obtained by transplanting nuclei from cells of an albino embryo to enucleated eggs of the wild-type female shown. The albino frogs are genetically identical and will accept skin grafts from each other.



The egg and the nucleus: a battle for supremacy
J.B. Gurdon
Development 2013 140: 2449-2456; doi: 10.1242/dev.097170

Fig. 2. Survival of nuclear transplant embryos in *Rana pipiens* and *Xenopus laevis*. Even advanced donor cells from the endoderm of *Xenopus* tadpoles have nuclei that can sometimes yield normal individuals after nuclear transfer [data taken from Briggs and King (Briggs and King, 1957) for *Rana* and from Gurdon (Gurdon, 1962) for *Xenopus*].

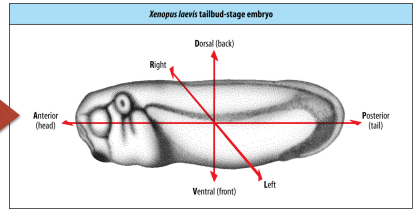


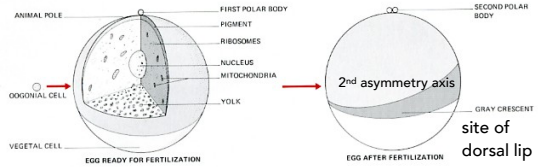
Q: What does this result imply about the stability of gene expression (during development)?
Q: How might the behavior change if the transplanted nuclei as from an adult muscle cell?

asymmetry axes

animal

vegetal

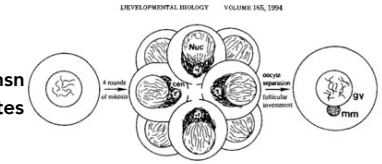




site of dorsal lip

Where does animal-vegetal asymmetry come from?

mitotic divisionsn giving rise to oocytes



Inherent asymmetry: