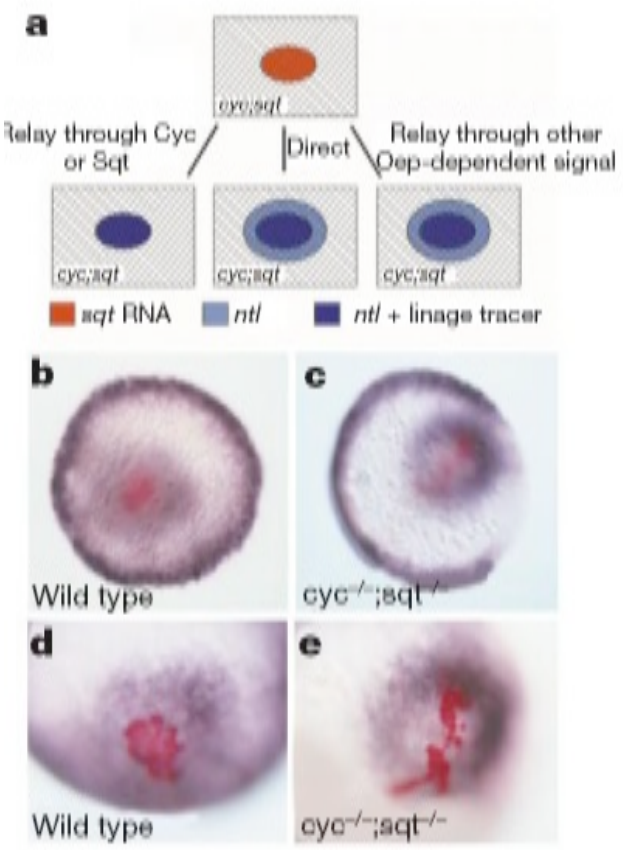


Figure 3 The long-range effect of Sqt does not depend on the induction of the endogenous cyc or sqt genes. a, Design and predictions for the experiments shown in b-e. b-e, Single-cell injection of 4 pg of sqt RNA plus lineage tracers biotin-dextran and rhodamine-dextran into wild-type (b, d, n = 18/18) and cyc;sqt double mutant embryos (c, e, n = 12/12) at the 128±256-cell stage. b, c, Focus is on the expression domain of ntl in marginal cells; cyc;sqt double mutants were distinguished from sibling embryos (b) by the lack of ntl expression on the dorsal side (c). d, e, High-magni®cation views of the embryos in b and c; the lineage tracer biotin-dextran was detected with the ABC kit from Vectastain (red).

The zebrafish Nodal signal Squint functions as a morphogen, by Yu Chen & Alexander F. Schier.

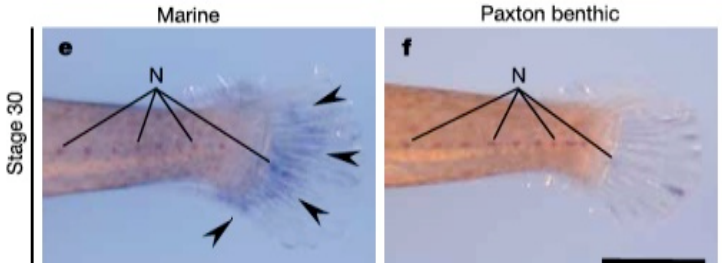
Figure 3, reproduced above, demonstrates that "The long-range effect of Sqt does not depend on the induction of the endogenous cyc or sqt genes." Discuss this experiment and how the authors can draw this conclusion.

Cells overexpressing squint were transplanted into host embryos. Even if the embryo was unable to itself make functional cyc or sqt mRNA, the cells expressing sqt from mRNA injection were able to induce a response (ntl expression) in cells at a distance from the injected cells. Thus, the sqt protein must have diffused through the tissue to trigger the response, there is no way that the secreted sqt from the injected cells could induce expression and secretion of functional cyc or sqt from the host cells. Thus, sqt must have diffused and acted directly on the neighboring cells.

Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks

Michael D. Shapiro1*, Melissa E. Marks1*, Catherine L. Peichel2*, Benjamin K. Blackman1, Kirsten S. Nereng1, Bjarni Jo´nsson3, Dolph Schluter4 & David M. Kingsley1

Hindlimb loss has evolved repeatedly in many different animals by means of molecular mechanisms that are still unknown. To determine the number and type of genetic changes underlying pelvic reduction in natural populations, we carried out genetic crosses between threespine stickleback fish with complete or missing pelvic structures. Genome-wide linkage mapping shows that pelvic reduction is controlled by one major and four minor chromosome regions. Pitx1 maps to the major chromosome region controlling most of the variation in pelvic size. Pelvic-reduced fish show the same left–right asymmetry seen in Pitx1 knockout mice, but do not show changes in Pitx1 protein sequence. Instead, pelvic-reduced sticklebacks show site-specific regulatory changes in Pitx1 expression, with reduced or absent expression in pelvic and caudal fin precursors. Regulatory mutations in major developmental control genes may provide a mechanism for generating rapid skeletal changes in natural populations, while preserving the essential roles of these genes in other processes.



The left figure from the Shapiro paper shows expression of *pitx1* in marine versus fresh-water sticklebacks. The paper was on pelvic reduction (abstract above). Propose a hypothesis to explain why there is more pitx1 expression in the caudal fin of the marine form.

Hypothesis 1: The promoter element that drives expression of pitx1 in the pelvic fins also is responsible for driving expression in the caudal fin. This promoter element is active in marine fish but inactivated in Paxton benthic.

Hypothesis 2: a new element has evolved in marine fish, such that there is now expression of pitx1 in the caudal fin in Marine fish.

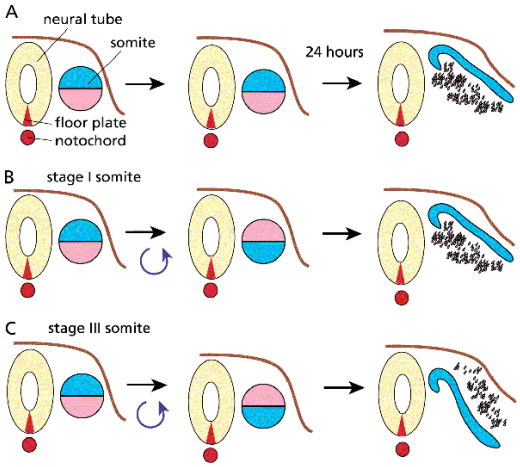
Note: There does not seem to be a huge difference in development of the caudal fin between populations. It may be that closer examination of the caudal fin will show that there is a slight difference between the species, or it may be that another gene can compensate for differences in pitx1 in the caudal fin (such a compensation doesn't exist in the pelvic fin).

When the AER is removed early, only proximal limb structures develop, while when the AER is removed later, more distal limb structures develop. How does the progress zone model explain this observation? *The cells that leave the progress zone early develop into proximal tissues.*

What is an alternative model to explain the stage-dependent effects on limb patterning of AER removal? *There is a constant, absolute number of cells that die after AER removal, and when this happens early (small limb bud), the cell death wipes out the proximal and distal elements (they're all in a small area). When the AER is removed later, the limb but is much bigger and thus the same amount of cell death leads to the loss of only distal structures.*

Explain the differences between *in situ* hybridization and immunohistochemistry. Be clear on what type of molecule is being detected, and what provides the specificity for each technique *In situ hybridization uses anti-sense RNA to bind specifically to a particular mRNA, while IHC uses an antibody specific to a particular protein to bind specifically to a particular protein.*

In many vertebrates, there is no dorsal-ventral gradient of BMP4 protein during gastrulation, however, there is a gradient of BMP4 <u>activity</u>. How might this be accomplished? *BMP4 inhibitors are not uniformly distributed, they are at higher concentrations on the dorsal side of the embryo.*



What do you conclude about somite patterning from the above experiments?

During normal somite development, the dorsal half of the somite develops into the dermomyotome. If the somite is rotated 180° early in development, it is the NEW dorsal half that develops into the dermomyotome. This means that dermomyotome fate is determined by the environment of the

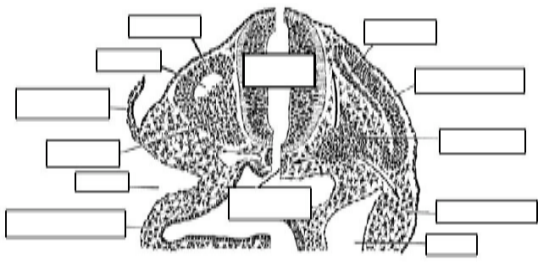
somite. The somite is not committed to form a specific tissue at this time, its neighboring tissues are responsible for patterning. When the somite is rotated later (stage III), the dermomyotome develops from the NEW ventral side (the old dorsal), we conclude therefore that the dorsal somite is already committed to form dermomyotome.

Pieces of the notochord, when grafted into the anterior of the limb bud, will cause digit duplications. Propose a hypothesis to explain this result. The notochord secretes the same signaling molecule that the zone of polarizing activity secretes (shh).

13. The following molecules play important roles in specifying cell fate. For each protein, write whether it is a secreted protein, a membrane protein, a cytoplasmic component of the signaling pathway, or a transcription factor (1 pt each).

Sonic Hedgehog Secreted Protein (Limb patterning, eye maintenance and taste bud development)
MyoD Transcription Factor (muscle specification and differentiation)
Ephrin Transmembrane Protein (Migration of Neural Crest cells and Sclerotome)
HoxC6 Transcription Factor (vertebral axial identity)
Chordin Secreted Protein (dorsal ventral polarity in xenopus gastrulation).

For ONE of the above proteins in any tissue or cell type, briefly describe how this molecule functions to specify cell fate (2 pts). I am more interested in the embryonic process that this molecule is involved in than the molecular basis for the molecule's action.
See Textbook for the processes listed adjacent to each protein.



Put the following terms in the appropriate boxes in this cross section of a chick embryo:

- Notochord (4pts) Bottom Center
- sclerotome (2 pts) Middle right

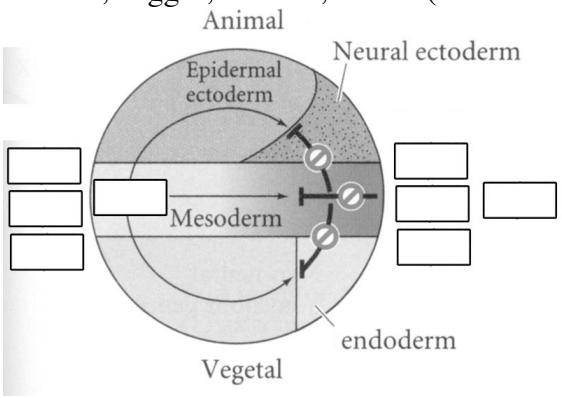
- dermomyotome (2 pts) Second from top on right
- spinal cord (2 pts) Top center

Fill in the blanks.

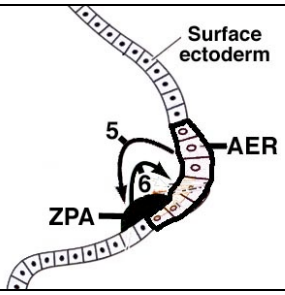
Fill in the blanks. All answers must come from the list of words and phrases; most of the above words will not be used, and some may be used more than once. Some questions may have more than one possible correct answer.
For questions 1-6, choose from the following secreted signals and transcription factors:

BMP4	chordin,	emx1	epidermal growth factor (EGF)
fibroblast growth factor (fgf)	goosecoid	noggin	pax2.1
siamois	sfrp	sonic hedgehog (shh)	tlc
vegT	wnt7a	hoxC6	ß-catenin

Fill in 4 boxes, putting gene products on the appropriate side of the *Xenopus* gastrula: chordin, noggin, siamois, BMP4 (not all boxes will be filled).



Dorsal is on the right, and would show expression of chordin, noggin, and siamois. Ventral is on the left, and would show expression of BMP4.

5. FGF 6. SHH	

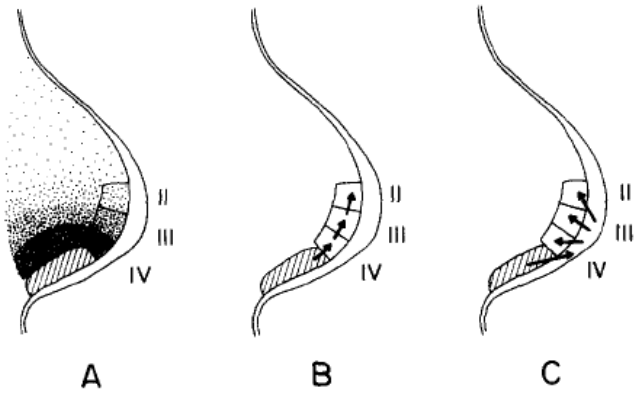


Figure 13. Possible Mechanisms by Which *Sonic hedgehog* May Act to Pattern the Mesenchyme

Sonic hedgehog may function in limb patterning as a diffusible morphogen (A) or it may function by initiating a series of cell–cell interactions (B). This instructive signal could directly affect limb mesenchyme or it could act through an AER intermediate (C). In each panel, the hatched regions along the posterior margin (the bottom of the limb bud) are ZPA cells expressing *Sonic hedgehog*.

(A) Here the intensity of the stippling in the limb bud is meant to suggest a graded distribution of the Sonic hedgehog protein. (B and C) Here the arrows are meant to suggest a potential signal cascade, initiated by *Sonic hedgehog*.

The last figure and figure legend in the Riddle paper is reproduced above. They raise three possibilities for the action of shh in patterning the anterior posterior identity of the limb. Based on the Chen paper (available to you in the exam), what kinds of experiments would you do to distinguish between these models. You have the corresponding technical ability and tools that Chen et al used in their paper.

1. Inject shh protein into the limb bud (or shh mRNA into a cell in the limb bud), ask if digit IV and III identity is established at a distance. This could be done in the posterior of a limb bud which does not have shh, or into the anterior of a limb bud. The latter experiment was essentially done in Riddle, and it worked.
2. Do the same treatment as above, but in a limb bud in which the progress zone and AER cannot respond to shh (a smoothened mutation, but you don't have to remember that). If there is no development of digit IV and III, then it demonstrates that cells unable to respond to shh are unable to make digit IV and III.
3. Do the same treatment as above, but in a situation where the cells being assayed can respond to shh, but there is a large group of cells which cannot respond, separating the assayed cells from the shh. If the effect of shh is

the same as in experiment 1, then a relay through the shh-unresponsive cells is not needed. This eliminates model B above.

4. Do the same treatment as above, but in a situation where the assayed cells can respond to shh, but the AER cells cannot respond. If the effect of shh is the same as in experiment 1, then a relay through the shh-unresponsive AER cells is not needed. This eliminates model C above.

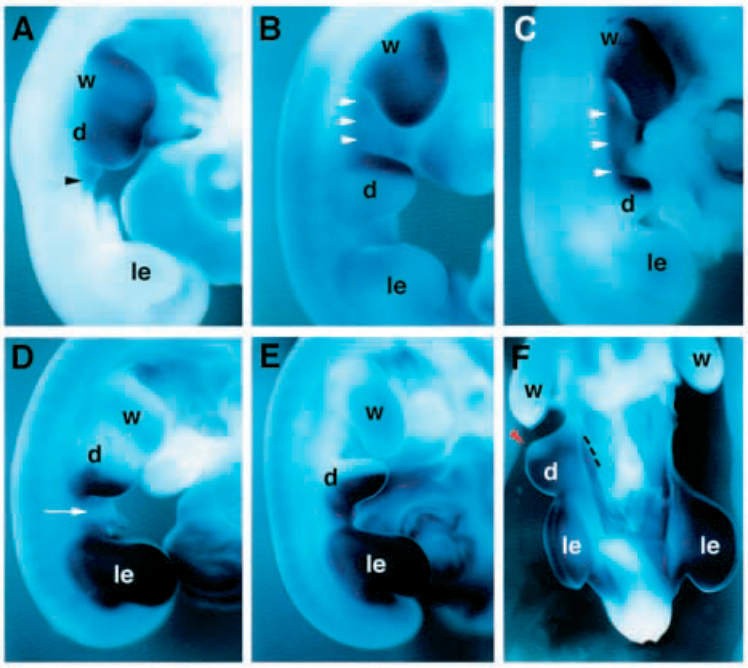
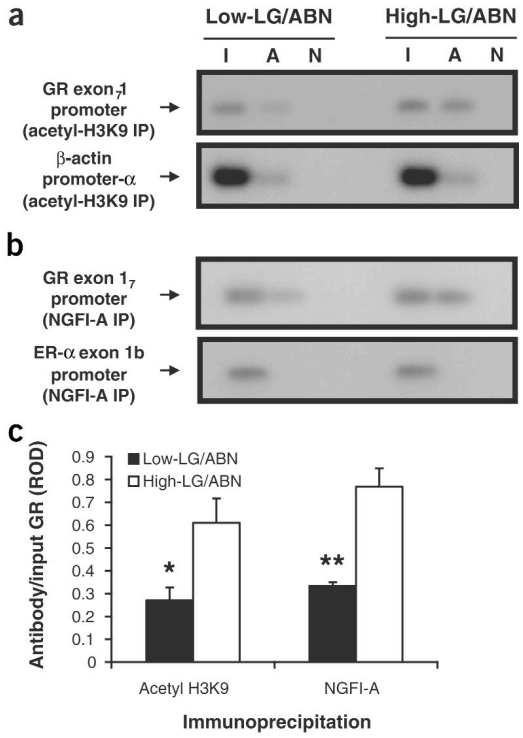


Fig. 5. Expression of Tbx5 (A-C) and Tbx4 (D-F) in three types of Dasokus (the extra limbs developed after experimental manipulation). As judged from their positions, (A,D) are wing-type, (B,E) are intermediate-type and (C,F) are leg-type. w, wing bud; d, Dasoku limb bud; le, leg bud. Anterior is to the top. (A) Tbx5 is expressed in the wing and Dasoku limb buds. The arrowhead indicates the posterior limit of Tbx5 expression in the flank. (B,C) Tbx5 is expressed in the cephalic region of the Dasoku limb buds. The arrowheads indicate Tbx5 expression in the flank. (D,E) Tbx4 is expressed in the leg bud and caudal region of the Dasoku limb buds. Tbx4 is not expressed in the flank between the Dasoku and the leg bud (arrow in D). (F) Ventral view of an embryo with a Dasoku on the right side. Tbx4 is expressed in the leg and Dasoku limb buds. Tbx4 expression appears weak in the cephalic margin of the Dasoku limb bud (arrowhead). The broken line indicates the expanded domain of Tbx4 expression on the right side, compared with the left.

How were the ectopic limbs induced? FGF expression in the surface ectoderm of the flank.

How does this experiment support the idea that tbx5 and tbx4 confer limb identity? There was a very good correlation between the type of limbs that were formed (anterior or posterior) and the version of tbx which was expressed.



Weaver et al. concluded from the figure on the left that maternal behavior (High versus Low) led to epigenetic effects.

What were the epigenetic effects demonstrated in this figures? Acetylation of Histone H3 on Lysine 9.
Do the dark bands in panel a represent protein, mRNA, or DNA? The bands represent DNA, because the immunoprecipitated acetyl-H3K9 was subjected to a pcr reaction to amplify the DNA in the particular promoter region indicated (GR rexon$_{7}$1 or β-actin).
How do the bands demonstrate that there were epigenetic effects? There is a difference in the amount of acetylated histone H3 between the Low and the High treatments.
Describe the importance of the B-actin bands? This indicates that the IP was equally effective in the High and the Low. It is a control for the actual immunoprecipitation and the PCR reaction. Thus, lower intensity in the GR rexon$_{7}$1 band in the Low is not a result of a failure to do a good IP in that sample.