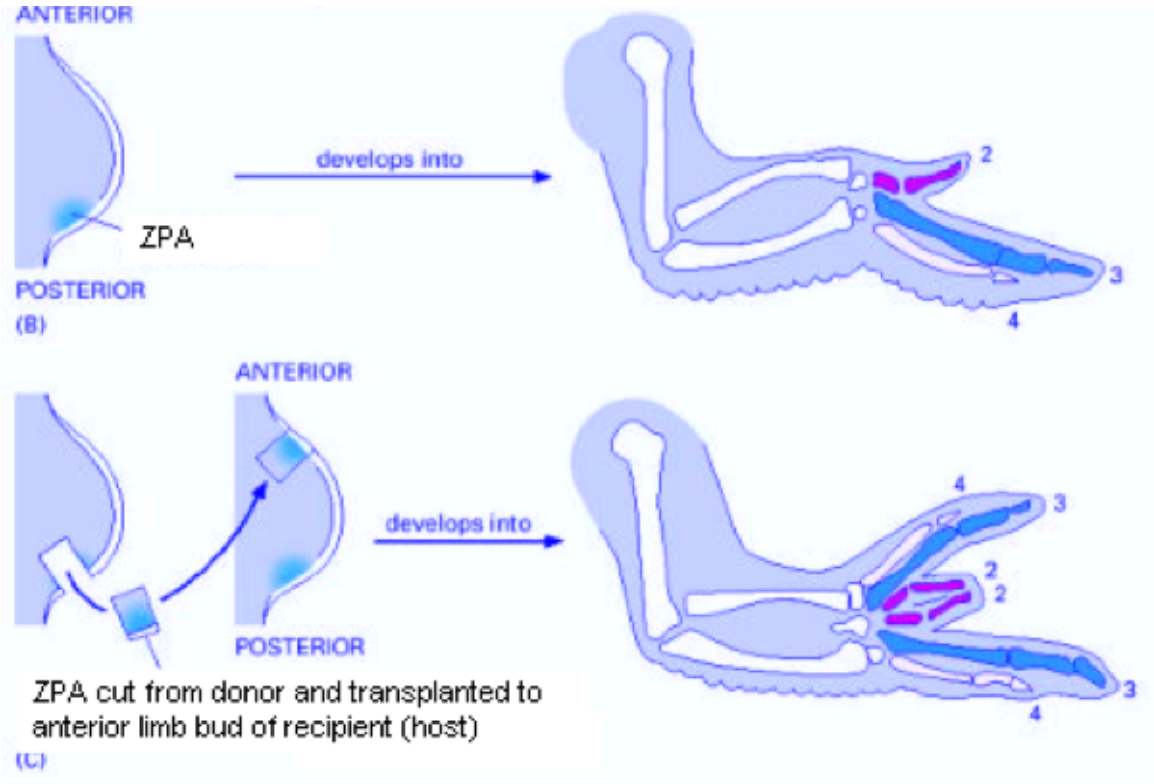
Signaling in Limb Development_KEY
BIOL125PS November 6, 2017

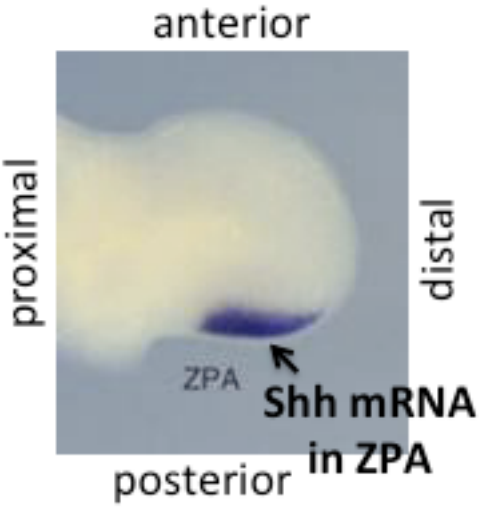
We have learned that FGF signaling between the limb mesenchyme and the apical ectodermal ridge (AER) is important in directing the outgrowth of the limb bud in chicken embryos. Another important signaling center, the ZPA (we'll learn what the initials stand for later), is located at the posterior edge of the limb bud. In a classic experiment, the ZPA from a donor limb bud was transplanted to the anterior edge of a recipient limb bud in a chick embryo, as diagrammed below. The limb that resulted is pictured, below a normal limb for comparison. (By convention, the chick's digits (2, 3, and 4) are numbered according to their corresponding homologs in the human hand.)



1. Based on the outcome of this experiment, propose two functions for the ZPA in limb development.

ZPA triggers growth of digits
ZPA secretes signals that determine the order of the digits – PATTERNING

2. The picture below shows an *in situ* hybridization* of a chick limb bud showing expression of the mRNA for the sonic hedgehog gene (Shh) in the ZPA. (Yes, that's really the name of the gene.) The sonic hedgehog gene encodes a secreted growth factor protein. (**in situ* hybridization is a method in which a labeled antisense nucleic acid probe is used to specifically stain mRNA for a single gene.)



   a. Would you expect the sonic hedgehog protein to be found in the same location as the sonic hedgehog mRNA? Why or why not?
   The protein would probably be found in a broader region than the mRNA because it is secreted and can diffuse away from the cell in which it is transcribed and translated. I would expect to see it form a concentration gradient that is high in the posterior limb bud and low in the anterior limb bud.

   b. Based on the experiment shown in (1), can you predict in what regions of the limb bud that receptors for Shh would be found?

   Because the transplanted region has effects on the limb mesenchyme (the part that forms most of the limb bones and other tissue), I would expect to find Shh receptors on the mesenchyme cells. The receptors could also be expressed in other regions as well (even in every cell of the embryo)

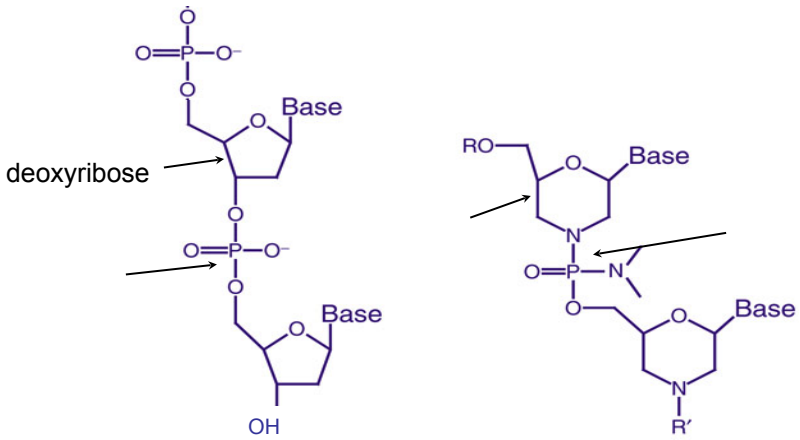
3. Imagine that you are a researcher studying induction during limb development. You would like to know if Shh is sufficient to perform the functions of the ZPA. Outline an experiment to test this, and diagram the result that you would expect to see if Shh is sufficient to perform the functions of the ZPA. What controls would you include in your experiment?

1. Put an Shh-soaked bead on the anterior of the limb bud early in limb development. If Shh is sufficient to perform all of the ZPAs functions, the results should be the same as the ZPA transplant experiment described in question 1 (a limb that is duplicated on the anterior/posterior axis). Control: bead without Shh (would expect that you don't get a duplicated limb)

OR

2. Remove the ZPA and replace it with an Shh-soaked bead. If Shh is sufficient to perform all of the functions of the ZPA, the limb should develop normally. Control: bead without Shh (would expect that you no longer get digit development, because you've taken away the ZPA, so there is no Shh)

4. You are also interested in knowing whether Shh is necessary for the ZPA to perform its functions and decide to use a morpholino antisense oligonucleotide technique to test this. Morpholino antisense oligonucleotides ("morpholinos") are chains of nucleotides in which the sugar-phosphate backbone has been replaced by a very stable, uncharged morpholine ring backbone. Because morpholinos contain nucleic acid bases, they can bind to complementary RNA molecules. Once they bind, morpholinos form stable double-stranded duplexes with RNA molecules.



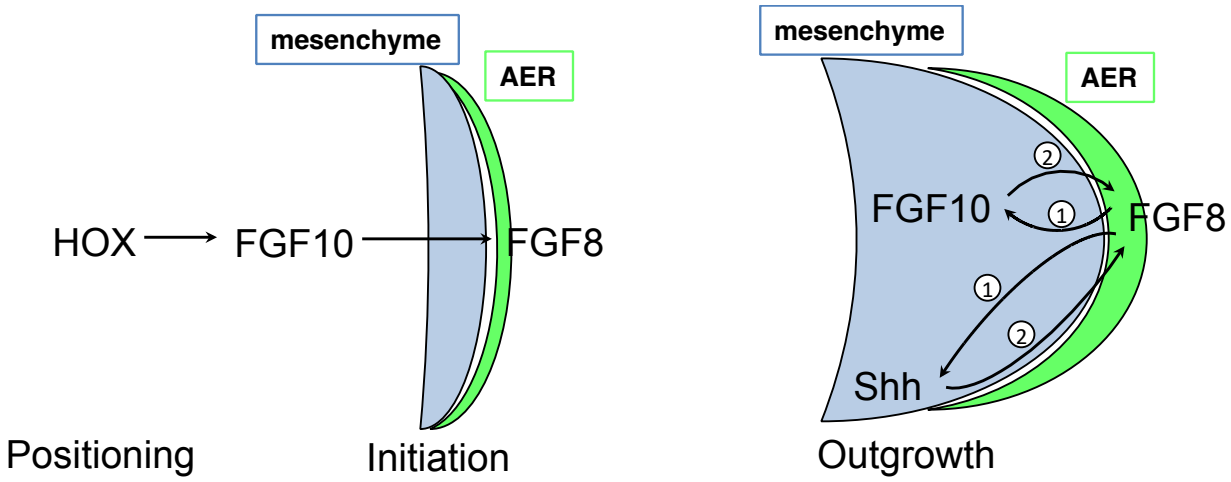
a. You inject your **DNA** antisense chick limb bud oligonucleotide **Morpholino** with a morpholino that is complementary to the translational start site on the Shh mRNA molecules. What do you predict will happen to the mRNA molecules that base pair with the morpholino antisense oligonucleotide?

   The morpholino will block translation of these mRNAs, and no Shh protein will be made.

b. How do you predict eliminating Shh in the limb bud will affect limb development in your embryos? Draw a diagram of your hypothesized outcome.
   Many possibilities. One is that the limb would develop with fewer digits, and perhaps the anterior-posterior patterning would be abnormal with digit 2 replacing digit 4.

c. When you look closely at the embryos that you have treated with the Shh morpholinos, you notice that the limb bud has stopped growing, and the AER has disappeared. What are some possible explanations for this surprising result?
The Shh in the ZPA is required to maintain the AER so that it produces FGF8. Without the AER, the limb bud stops growing. Alternatively, Shh in the ZPA could be required for the mesenchyme to continue to produce FGF10. Without FGF10, the AER would deteriorate and stop producing FGF8.

5. Based on this finding and on the other experiments we have talked about, diagram a regulatory network showing how the ZPA (and Shh) might influence other limb signaling centers and their growth factors. Use your regulatory network to make predictions about what would happen to FGF expression if Shh or the ZPA were removed. Can you predict what might happen to Shh expression if the AER were removed?



Expression of both FGF10 and 8 should be reduced or absent if the ZPA is removed. The experiments described here don't really tell you what will happen to Shh in the absence of the AER. However, other experiments have shown that Shh is also maintained by FGF8 expression from the AER. When the AER is removed, Shh expression becomes reduced.