Assignment 1

Hedgehog transcription during Xenopus Development

Hedgehog (Hh) was originally identified as an extra cellular signaling molecule. Hh proteins in Xenopus display overlapping expression but their MRNA levels peak during neural induction and early organogenesis. Hh proteins also mediate epithelial-mesenchymal interactions that are required for the development of aerodigestive tract. Hh receptors include Patched (PTC) which function as a negative regulator of the Hh signal smoothed (SMO). The active mutant smoM2 can deregulate the Hh signaling pathway in cells. In this experiment the effects of expressing smoM2 in Xenopus development was tested. This expression of smoM2 yield developmental consequences such as eye disruptions, absence of muscle development in the coelomic body wall, absence of the pancreas and arrested cytodifferentiation in the midgut epithelium. Through this mutation with smoM2 it can be concluded that the undisrupted Hh pathway is transcribed in the eye, coelomic body wall, and the gut during Xenopus development.

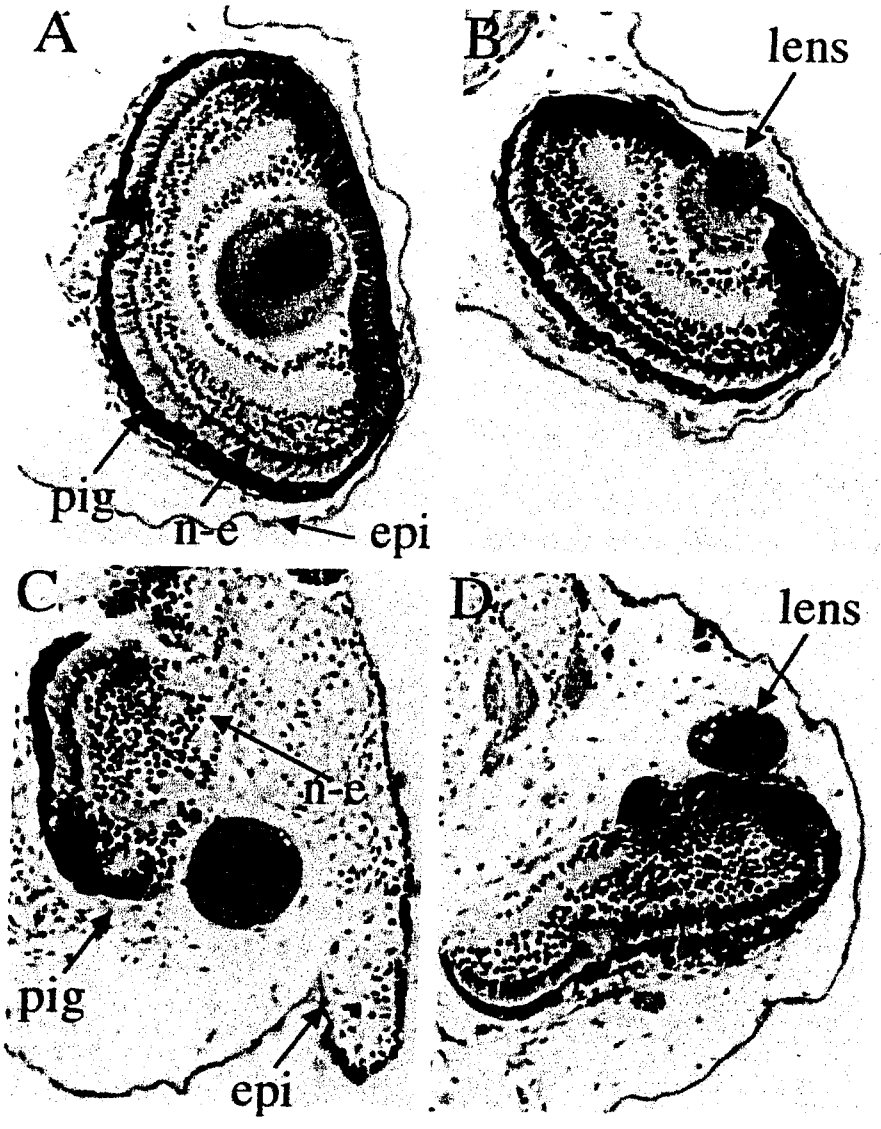

FIG. 2. Histology of eye defects seen with overexpression of SmoM2. In comparison to the normal organization of epithelia in control tadpoles injected with wild-type Smo (A, B), the eye fields of SmoM2-injected tadpoles (C, D) show a normal-appearing pigmented epithelium (pig) but a highly disorganized neuroepithelium (n-e), displaced but developed lens, and considerable distance from the epidermal surface of the skin (epi).

To determine effects of direct Hh stimulation, *Xenopus* tadpoles were harvested at Nieuwkoop–Faber Stage 41 and maintained over the next 16–18 h at 21°C in 0.1× MMR in the presence of 0, 1, 5, or 10 μg/ml recombinant amino-terminal Shh (N-Shh). Hexahistidine-tagged recombinant N-Shh was expressed in bacteria and purified by nickel chromatography. In parallel experiments, the intestines were isolated from about 20 tadpoles per group by dissection under the microscope and cultured for the next 16–18 h at 21°C in 0.1× MMR supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 0, 1, 5, or 10 μg/ml recombinant N-Shh. Tadpoles and organ explants were subsequently fixed and processed for histologic examination as outlined below.

cDNA Library and Screening

Xenopus Smo and *Ptc* cDNA clones were isolated by screening a stage 20 cDNA library and a testis cDNA library with human *Smo* and rat *Ptc* cDNA probes, respectively, at low stringency (30% formamide, 5× SSC, 1% SDS, 1× Denhardt's reagent, 50 mM

(2)

Assignment 2

10/28/04
Developmental Biology

Introduction

Embryonic development is an extremely vital process in the context of an organism's life. It is not until relatively recently that the mechanisms driving development have been studied. Through the study of the model organisms such as sea urchins and drosophila we can understand aspects of development that may aid in the understanding of our own complex development.

Within a developing embryo there are two major cell types or arrangements; epithelial cells and mesenchymal cells. Epithelial cells are organized into sheets while mesenchymal cells operate as independent units (Gilbert, 2003). Mesenchymal cells are extremely important during the archenteron invagination during embryonic gastrulation and thus play a vital role in embryonic development (Gilbert, 2003). Mesoderm structures in the sea urchin embryo have been found to originate from two types of mesenchyme cells; primary(PMC) and secondary(SMC) mesenchyme cells. Both these types of mesenchyme cells are involved in the archenteron formation (Fernandez, 2004). It has also been shown that the ingression of PMC'S and SMC'S are dependant on the activation of the ERK/MEK/RAF signaling pathway. Inhibition of this signaling pathway prevents PMC ingression and the appropriate functioning of SMC'S (Rottinger, 2004). It has also been shown that compound UO126 will act as an inhibitor of the MEK signaling pathway (Kumano, 2001).

In this experiment UO126 was added to fertilized sea urchin eggs to disrupt the movement of mesenchymal cells. Through the use of immunocytochemistry the effect of UO126 on mesenchymal cells during the blastula stage was tested. It is hypothesized that the embryos treated with UO126 will express disrupted development specifically in relation to the progress of mesenchymal cells compared to those embryos without the presence of UO126.

Assignment #3 (3)

11/18/04
Developmental Biology

Discussion

After the staining and incubation of the collected Sea urchin embryos, epifluorescence microscopy was utilized to observe the results. In summary, the results obtained were inconclusive. Both the control and all three variables (1um, .5um, .05um) of diluted U0126 concentrations exhibited normal mesenchymal development and ingression. More importantly, the expected primary mesenchymal cell staining was non-existent. The staining observed was non-specific and prominent background staining was observed.

If the inhibition of primary mesenchymal cell ingression was achieved by U0126 then the results would have been different than observed. Effects of successful mesenchymal ingression can be seen in fig 3 and in table 1(Fernandez, 2004). With the treatment of U0126 primary mesenchymal cell ingression is greatly altered and disturbed. Control blastulae show normal primary mesenchymal cell (PMC) ingression, while treated blastulae lack PMC's. Control gastrulae show normal gastrulation, while treated gastrulae lack most mesenchyme cells and gut invagination (Fernandez, 2004).

The lack of PMC inhibition by U0126 may be explained by the protocol used. Perhaps the number of washes with seawater was not sufficient enough to complete the staining process. The amount of time between washes may have also been too long and thus may have affected the results. It is also suggested that the U0126 was added too early during development.

For future experiments one could add the inhibitor U0126 to different urchin developmental stages and observe the various results. Perhaps the addition of U0126 to later developmental stages yields the inhibition of other cells associated with Sea urchin development. One could also use the calcium ionophore A23187 as a PMC inhibitor in place of U0126. In addition, U0126 could be used on other model organisms such as zebra fish or chicks, to test if their mesenchyme development would be altered as well.

References

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Assignment #5 (5)

Drosophila melanogaster genes *amnesiac* and *neuralized* are expressional regulated by ethanol

12/17/04
Biology 302

Title Page ?

Introduction

Many factors can affect genes within an organism during development. An example of one of these factors is ethanol. Ethanol can affect the physical processes of an organism, on direct exposure; multicellular organisms show hypersensitivity, uncoordination, and sedation. (Kwon et al, 2004). Influences such as ethanol can also affect the genes or receptors within an organism upon exposure. It has been found that G-protein receptors, such as dopamine, opioid, and adenosine receptors are up- or down-regulated in response to the exposure of ethanol (Diamond and A.S. Gordon, 1997). To understand and analyze the precise effects of ethanol on genes, model organisms have been used. Examples of frequently used model organisms are mice and the *Drosophila* fly species. Previous research has shown that transgenic mice overexpressing TGF-*B* show an increased sensitivity to the effects of ethanol (L. Hilakivi-Clarke and R. Goldberg, 1995). It has also been found that mice lacking the protein kinase Cγ are less sensitive to the hypnotic and hypothermic effects of ethanol (R.A. Harris et al, 1995).

neuralized in *Drosophila melanogaster* can facilitate further studies on additional ethanol responsive genes not only in Drosophila but in other model organisms as well.

Materials and Methods

Isolation of RNA

Embryos of *Drosophila melanogaster* were collected at approximately the twelve-hour stage of development. Embryos were washed with water and then introduced to varying concentrations of ethanol (0%, 0.5%, 1.0%, 2.0%). Embryos were decorianated through incubation in a 50 % bleach solution for three minutes. They were then washed with water to remove all traces of bleach and weighed to ensure that an appropriate quantity of embryos was collected. Embryos were then incubated in *RNA Later.* RNA was isolated using the protocol for animal tissues in the Qiagen RNeasy (or Not-so-easy) Mini Handbook; 3rd edition, June 2001. The RNA isolation protocol was started at step 4a. (Note: Optional step 10a centrifugation was omitted). Isolated RNA was then incubated for five days at 4°C. Presence of RNA in samples was quantified using a spectrophotometer. Presence of RNA was probed using an absorbance wavelength of 260nm. The presence of protein was probed using an absorbance wavelength of 280nm (Table 1). An additional Wild Type sample was collected based on low RNA volumes calculated from A_{260} for the initial Wild Type sample.

Reverse Transcription

products were then incubated for an additional 10 min at 72°C. PCR products were stored at 4°C for four days.

Preparation and Running of Agarose Gels

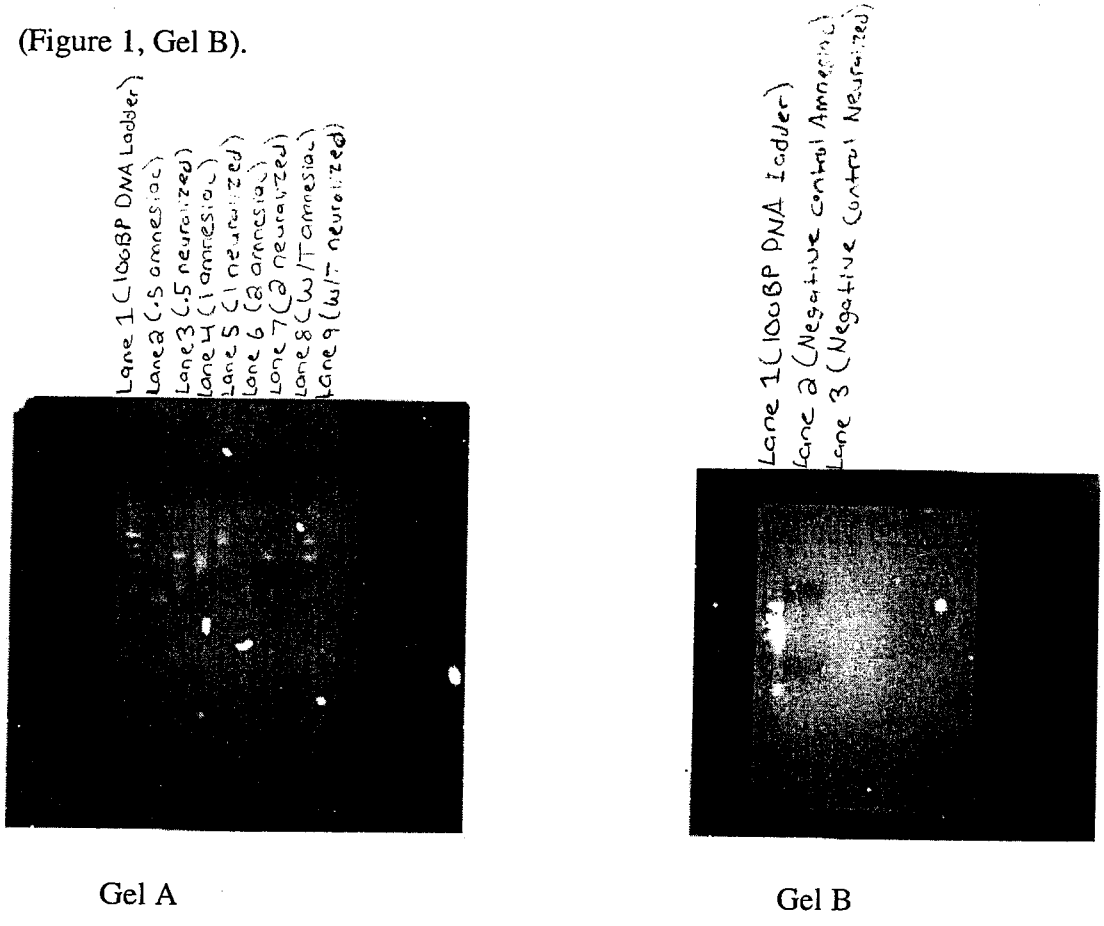
An agarose gel was prepared at a 1.8% weight per volume agarose concentration in a base of 0.5X TBE. The PCR products were then run on the agarose gel using "Preparation and Running of Agarose Gel" protocol (Dr. Deborah Eastman: Laboratory Handout, Developmental Biology, 2004). The gel was run for ninety minutes at 60Volts and 10 minutes at 75 Volts. The gels were then examined and photographed under ultraviolet light on a transilluminator (Fig 1).

Results

Quantification of isolated RNA

The amount of RNA isolated from the embryos collected was determined through the use of a spectrophotometer. The 260nm absorbance was used to determine the amount of RNA present while the 280nm absorbance was used to determine the amount of protein present. Results show that the initial wild type (W/T) samples contained less RNA present than that of the blank (dH_2O). The samples with the most RNA were 1.0 A and 1.0 B with 1.60 μg/μl and 1.82 μg/μl of RNA. Sample 0.5 A and 0.5 B had 0.04 μg/μl and

amnesiac and *neuralized* genes were run on a separate gel (gel B) . These negative control samples lacked any cDNA. Lane 1 contained the 100 BP DNA Ladder for comparative reference. The negative control for the *amnesiac* gene was run in Lane 2 and the negative control for *neuralized* was run in Lane 3. Neither lanes show any banding (Figure 1, Gel B).



Gel A

Gel B

Figure 1: RT-PCR products for wild type (wt), and ethanol exposed embryonic RNA. 100 BP DNA Ladder and Sample RT-PCR Products (Gel A). 100 BP Ladder and Negative control for *amnesiac* and *neuralized* genes of *Drosophila melanogaster* (Gel B).

Drosophila neuralized gene exhibit hypersensitivity to ethanol (Ruan et al, 2001). Recent research has also found that the mutant *cheapdate*, an allele of *amnesiac*, shows an increased sensitivity to the exposure of ethanol (Moore et al, 1998). Both of these past reports indicate that *amnesiac* and *neuralized* are ethanol sensitive and thus it seems likely that their regulation would indeed be affected by ethanol exposure.

Our results show that *amnesiac and neuralized* are ethanol sensitive. Specifically, *amnesiac* seems to be up-regulated at 1% ethanol while *neuralized* seems to be down-regulated at 1% ethanol. Further research may investigate the effect of other concentrations of ethanol upon these genes. Other ethanol responsive genes may be analyzed as well. The amnesiac gene is involved with cAMP pathway and thus it would be interesting to analyze its role with cAMP when it is exposed to ethanol. It would also be interesting to analyze the effects of ethanol on these genes during various periods of *Drosophila* development.

Acknowledgments

References

1) I. Diamond and A.S. Gordon, Cellular and molecular neuroscience of alcoholism. *Physiol. Rev.* **77** (1997), pp. 1–20.

2) J.Y. Kwon, M. Hong, M.S. Choi, S. Kang, K. Duke and S. Kim *et al.*, Ethanol-response genes and their regulation analyzed by a microarray and comparative genomic approach in the nematode *Caenorhabditis elegans*, *Genomics* **83** (2004), pp. 600–614.

(b)

12/14/04

Regulation of FG10 Expression

The researchers in this experiment looked at Lung Bud formation during chicken embryonic development. It has been previously shown that Tbx4, a member of the T-box transcription factor gene family is expressed in the mesoderm of the lung primordium. During this research the function of Tbx4 was analyzed. This was accomplished through the ectopic expression of Tbx4 in the visceral mesoderm of the foregut. It is important to note that the respiratory primordium is positioned in the foregut. It was found that ectopic Tbx4 induced ectopic bud formation in the esophagus by activating the expression of Fgf10. It was also found that the interference of Tbx4 caused the repression of Fgf10 expression.

Fig 3 shows that ectopic Tbx4 induces ectopic Fgf10 expression. Fgf10 expression was analyzed in Tbx4 misexpressed embryos. In the Tbx4 misexpressed embryos, ectopic Fgf10 expression was found in the visceral mesoderm at the same location of exogenous Tbx4 expression (Fg 3A,B blue arrowheads). This suggests that Tbx4 is a transcription factor of the downstream Fgf10 signalling molecule.

Fig 5 shows the further analysis of the regulation of Tbx4 on Fgf10. This was performed by the designed misexpression of variant forms of Tbx4. Tbx4-VP16 was used as an active form. Tbx4-Enr was used as a dominant negative form. Tbx4 misexpression in the presumptive esophagus-respiratory region results in Fgf10 expression (F A-B). With Tbx4-VP16 misexpression, Fgf10 expression could also be seen through the Tbx4-VP16 mesoderm (5 C-D). However, Tbx4-Enr misexpression resulted in the reduction of Fgf10 expression (5 E-F).

Thus it can be seen that Tbx4 is one of the players involved with the regulation of Fgf10 expression.

* = ectopic expression was accomplished through electroporation

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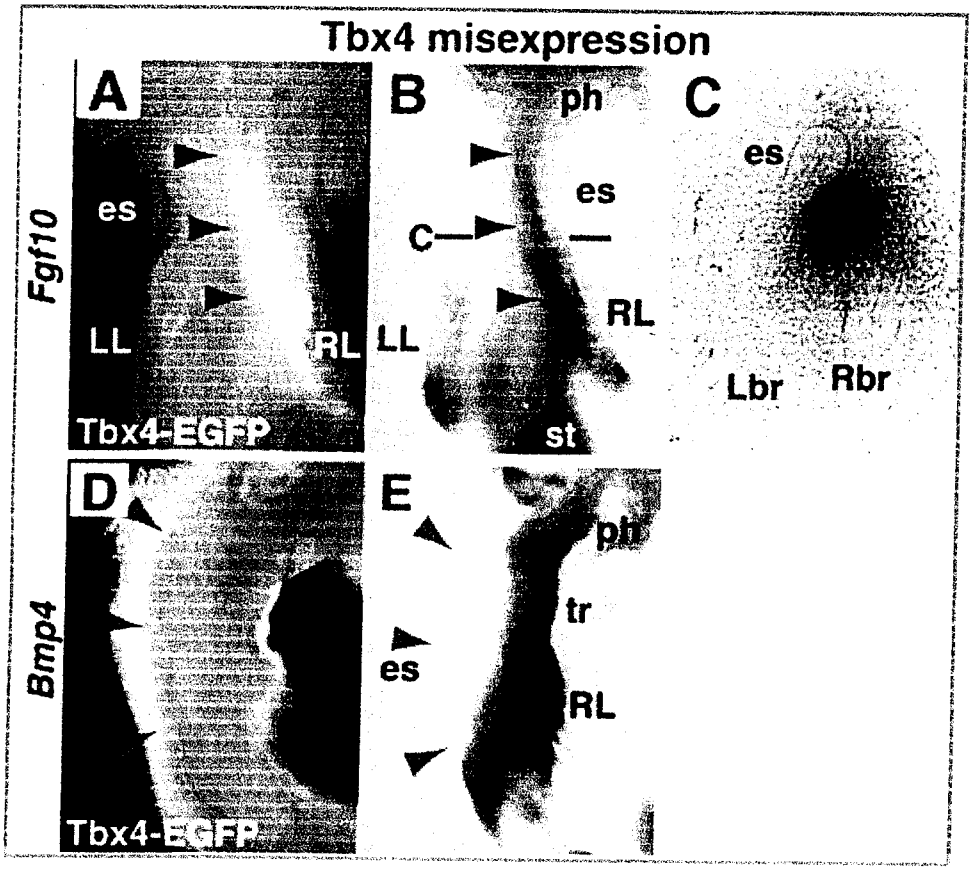

Fig. 3. *Fgf10* and *Bmp4* expression in the Tbx4 misexpressing foregut. (A-C) Induction of ectopic *Fgf10* expression in the Tbx4-misexpressing mesoderm. (A) The Tbx4-transfected mesoderm was monitored by GFP fluorescence from the dorsal view 2 days after electroporation. (B) *Fgf10* expression in the same specimen from the same viewpoint as in A. Blue arrowheads indicate the esophagus mesoderm misexpressing exogenous Tbx4. (C) Cross-section as indicated in B. (D,E) Tbx4 misexpression does not affect *Bmp4* expression. (D) The Tbx4-transfected region was monitored by GFP fluorescence 2 days after electroporation. (E) *Bmp4* expression in the same specimen from the same view point as in D. Red arrowheads in D,E indicate the esophagus mesoderm misexpressing exogenous Tbx4. es, esophagus; ph, pharynx; st, stomach; tr, trachea; LL, left lung; RL, right lung; Lbr, left main bronchus; Rbr, right main bronchus.