Connexin expression and function in Xenopus Laevis embryos

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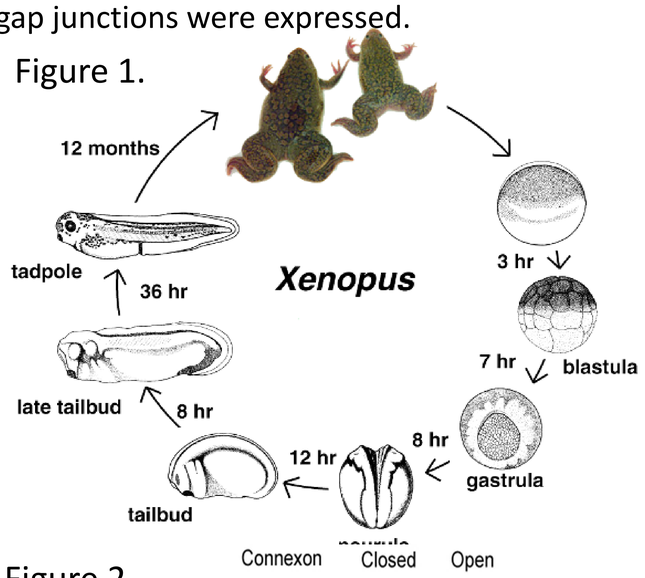
Introduction

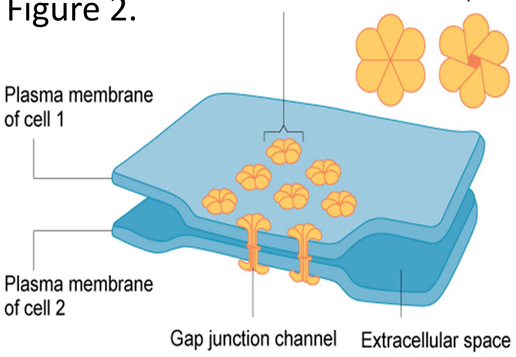
Connexins are proteins that form selective intercellular ion channels. Due to the involvement of ions in intercellular signaling it is hypothesized that connexins would be involved in the developmental processes of gastrulation and neurulation. Connexins are known to be involved in intercellular communication in Xenopus embryos (Landesman et al. 2002). The goal of this project was to use PCR and electrophoresis analysis to determine what stages particular gap junctions were expressed.

Figure 1.



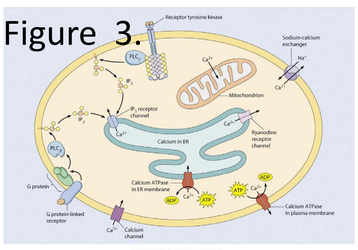
The embryos studied were between stage 7 and 20 primarily undergoing gastrulation or neurulation figure 1 shows the approximate amount of time these stages occur after fertilization.

Figure 2.



Connexins bind to other connexins of the same type and form a gap junction that allows for ion specific passive transport.

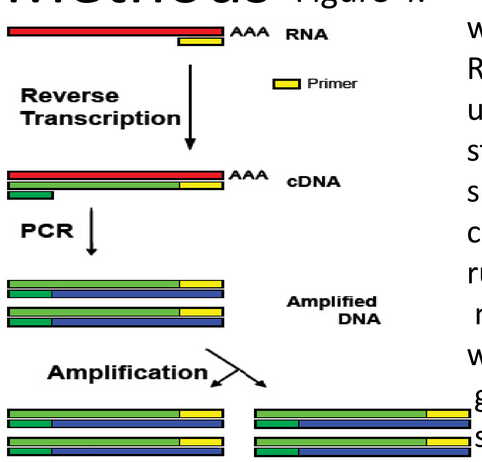
Figure 3.



Calcium is one of the ions gap junctions are permeable to. This ion is also heavily involved in cellular communication and gene expression regulation, through pathways like those in figure 3 (Clapham D. 1995). Calcium's ability to regulate cell function is central to our hypothesis that gap junctions regulating the intercellular flow of calcium will be involved in the coordination, initiation and termination of developmental processes.

Methods

Figure 4.



Rt (reverse transcriptase) PCR was used to reverse transcribed RNA into cDNA which was then used as a template to run standard PCR with primers specific to connexin genes. By comparing the results of PCR run on genomic DNA and cDNA made form RNA at each stage it was possible to determine if genes were expressed at each stage of development.

In order to reverse transcribe RNA into DNA the genomic DNA of the embryos must be degraded. This is done using the enzyme reverse transcriptase. This reaction doesn't eliminate 100% of DNA. Figure 4 shows the product of a DNase reaction at top and the product of that reaction used as a template for PCR. This demonstrates that the presence of a PCR product alone doesn't indicate gene expression as the re will inevitably be genomic DNA contamination.

Figure 5.

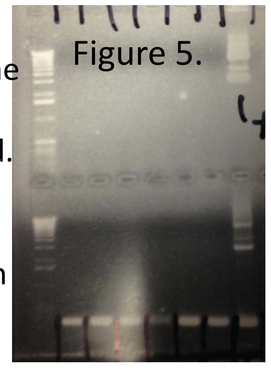
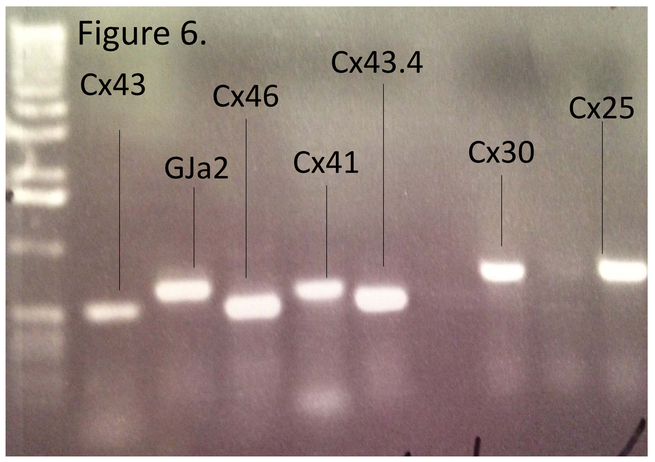
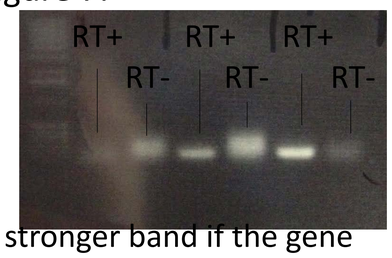


Figure 6.



In order to use PCR primers as a diagnostic tool their functionality had to first be established using PCR of genomic DNA. Figure 6. shows the results of that PCR as shown on .8% agarose gel.

Figure 7.



In order to determine if a gene is expressed the PCR product of the cDNA is compared to the control genomic DNA of the same stage the cDNA will produce a stronger band if the gene is expressed. Figure 7 demonstrates this with a control gene EF1alpha that is known to be expressed at all stages. The narrow bands labeled RT+ represent cDNA while the unfocused bands are genomic DNA.

Figure 8.

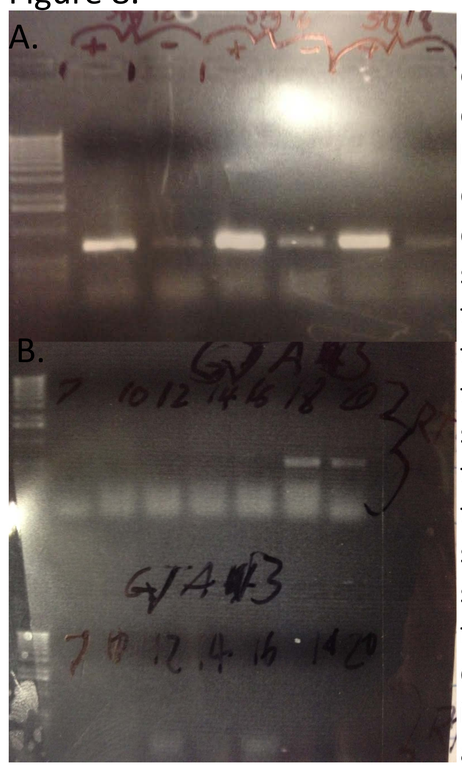


Figure 8 A. shows the expression of Cx 30 by comparing the RT+ and RT- PCR products at different stages. The difference in band strength demonstrates the presence of mRNA of the desired gene, and therefor expression is shown. Figure 8 B. shows the same for Cx 46. The top row is RT+ PCR at stages 7-20. The bottom shows the PCR product from RT- template. This data indicates that Cx 46 is expressed at stages 18, and 20.

Results

Table 1.

Gene name	Landesman et al 2002	experimental expresion	landesman expresion
GJalpha1/CX 43	Cx 43	none	maternal
GJalpha2	none	none	not tested
Gjalpha3/Cx 46	none	stg18,stg20	not tested
GJalpha 4/Cx 41	Cx 41	none	maternal
Gjalpha7/Cx43.4	Cx 43.4	stg18,stg20	maternaly, stg 15, stg 30
Gjbeta1/Cx30&32	Cx 30	stg12-stg20	stg15,stg30
GJbeta3/cx31	Cx 31	not tested	maternaly, stg 15, stg 30
Gjbeta7/cx25	none	none	not tested
Cx38	Cx 38	not tested	maternal
Gjb2/cx 26	none	not tested	not tested

The results of this project are summarized in table 1. The experimental data is also compared with data from (Landesman et al. 2002) that conducted similar experiments on several connexins. The only inconsistency between the two experiments was the lack of expression of Cx 43.4 at stage 15.

Future Work

The next step on this project is to determine if other gap junctions are expressed in these stages of development. This will be done with the same process used here. Once all connexins have been identified in situ hybridization will be done to determine where these connexins are expressed. Based on known information about developmental processes it will be possible to determine what specific processes each gene is involved in. Once the location of expression is it will be possible to use morpholinos to eliminate gene translation and determine what the exact function of each gene is.

Refernces

Landesman Y, Postma F, Goodenough D and Paul D. 2003. Multiple connexins contribute to intercellular communication in the *Xenopus* embryo. *Journal of Cell Science* 116: 29-38

Clapham D. 1995. Calcium signaling.*Cell* 80: 259-268

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