Gap Junction Expression and Function in Xenopus Laevis Embryos

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Connexins are intermembrane proteins that form protein complexes called connexons. These complexes bind together to form intercellular pores, called gap junctions, by binding to connexons of adjacent cells. These pores allow for passive transport of small molecules and ions involved in intercellular communication. Because gap junctions are required to transport of these ions, it is hypothesized that gap junctions are involved in developmental processes such as convergent extension during the processes of gastrulation and neurulation. In order to determine the role these genes play in development we must first determine the stages where they are expressed. To do this, cDNA (DNA reverse transcribed from RNA to only contain expressed genes) was made from developmental stages of Xenopus laevis embryos. The stages used were stage 6 (prior to activation of embryonic transcription), stage 8 (blastula), stages 10 and 12 (gastrula), stages 14, 16, 18 (neurula) and stage 20 (tailbud). This cDNA was then standardized by PCR using primers specific to ef1 alpha. This series of cDNA was then used as a template for PCR reactions of Xenopus connexin genes. The PCR reactions were then analyzed via gel electrophoresis in order to determine at what stages of development each gene is expressed. Gap junction gene alpha 3 was found to be expressed throughout neurulation and Gap junction gene alpha 7 was found to be expressed at all stages that were tested. Gap junction gene beta 1 and beta 2 were found to be expressed throughout gastrulation and neurulation. Gap junction gene alpha 2 was found to be maternally expressed as well as expressed in early gastrula, while Gap junction gene alpha 5 and alpha 4 are only expressed though early gastrulation. The next step of this project will be to use In Situ Hybridization to detect localized gene expression in embryotic tissue.