Regulation of EVI5, VEGF and P53bp2 during Amphibian Limb Regeneration

Wiaam Y. Elkhatib. Department of Oral Biology, Indiana University School of Dentistry

Understanding limb regeneration on a molecular level could lead to new methods of healing for humans, therefore revolutionizing current medical treatments. The axolotl salamander possesses capabilities of limb regeneration that are lost in the *Xenopus laevis* froglet. The hypothesized reason is that elevated levels of EVI5 (ecotropic viral integration site 5) binding protein allow the axolotl to regenerate by delaying the mitosis of dedifferentiated cells until they have established a blastema. VEGF (vascular endothelial growth factor) and P53bp2 (tumor protein 53 binding protein 2) genes also take part in this process by stimulating blood vessel formation and regulating apoptosis and cell growth in regenerated tissue. The objective of this study is to clone EVI5, VEGF, and P53BP2 cDNA that can be used to detect their mRNA transcripts during limb regeneration in the axolotl and *Xenopus laevis*. To accomplish this, RNA is extracted from axolotl and *Xenopus laevis* limb tissue using an RNeasy kit. Total RNA concentration is then measured spectrophotometrically. RT-PCR (reverse transcription polymerase chain reaction) is used to clone the cDNAs, which are identified by Agarose gel electrophoresis and later sequenced for verification. It took half a year to get high enough RNA concentrations from both species' tissues and then clone the three genes. The EVI5 band size was determined to be about 200bps, VEGF about 370bps, and P53bp2 about 500bps using the Agarose gel electrophoresis, signifying successful gene cloning. The long-term goal is to determine the role these genes play in limb regeneration with the aim of applying that knowledge to new medical treatments.

Mentor(s): Fengyu Song, Department of Oral Biology, Indiana University School of Dentistry; Xiaoping Chen, Department of Biology, IUPUI