Amphibian Limb Regeneration and Cell Cycle Regulation

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Partial or full regeneration of limbs is an exclusive capability owned by a few amphibians, and this occurrence is often a potent factor to the survival of a species or its ability to thrive in an environment. A restricted ability, a greater understanding would have a profound impact and clear applications in the treating of human diseases, ailments, and injuries. Further grasping the mechanisms behind natural regeneration and its occurrence at different stages and under different stimuli may allow for mimicry in the influence of tissues for human benefit. In an antecedent study, it was observed that ecotropic viral integrative factor 5 (EVI5) was perpetuating itself at a greater rate in sample tissues of the regeneration-competent axolotl salamander (*Ambystoma mexicanum)* as opposed to control tissue where this increase was null. EVI5 arrests the cells after Synthesis phase and G2 phase before the cells enter into Mitosis until prospective cells dedifferentiate and there is a an established blastema-the instrument by which regeneration continues. This study wished to show what proteins are expressed in the blastema tissue at these stages and to ascertain these previous findings. Samples of the axolotl were taken at 1, 4, and 7 days post-amputation where the amputation was made either midway or two-thirds towards the distal end of the tibia-fibula pairing. Samples were embedded and cut into sections with a cryostat at approximately -20 degrees Celsius. Immunofluorescent staining was utilized with EVI5 as primary antibody for the target site and anti-goat as the second antibody; in addition, H&E (hematoxylin and eosin) staining was employed to more definitively identify the nuclear structures of the cut and stained sections. Slides were observed under microscope to decipher protein expression and compare results. Positive identification of EVI5 reinforces its importance in delaying Mitosis so that a blastema can form and regeneration can occur.

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