

Citations: Kvon et al., 2016, Cell 167, 633–642 October 20, 2016. 2016 Elsevier Inc.
<http://dx.doi.org/10.1016/j.cell.2016.09.028>

The goal of this study was to determine the reason behind the loss of limbs in snakes. Within the Sonic hedgehog enhancer the ZRS limb enhancer is the consistent non-conserved sub-region that is not present or active in snakes. The authors determined this partly by doing multiple sequence alignment on the ZRS regions using 18 various species, which included various snakes. The authors then replaced the ZRS regions of mouse embryos with ZRS regions from various species to see if limb development would occur. They were also able to use this to measure the limb truncation based on evolutionary distance. The ancestral allele of the 17 base pair deleted region of snakes was used in a transgenic mouse enhancer reporter assay to determine if normal limb growth would occur. Finally, the authors wanted to identify the specific transcription factors that might have led to the loss of ZRS enhancer by removing them in mice.

Their final findings determined that the loss of limb development within snakes can be attributed to the loss of the ZRS limb enhancer being deactivated by either the E1 ETS transcription factor coupled with the loss of other ETS or homeodomain transcription factors. They determined that the snakes had a consistent 17 base pair deletion that was identified as the ZRS region. When a mouse's cut out ZRS region was replaced with ZRS regions of other vertebrates, such as fish or bats, there was limb development once more. This was traced phenotypically to determine the evolutionary distance between non-limb species and limbed species was large and beyond that between snakes. Knowing the region responsible, they next determined which transcription factor might have caused the deactivation of the ZRS region. They found that by removing just the E1 ETS motif in mice, it was not enough to cause limb truncation. However when the E1 ETS transcription factor was coupled with other ETS TFs or the homeodomain TF factor, there was loss of function of the limb enhancer. All this leads to conclude that the ZRS enhancer region is responsible for the loss of limb development in snakes.

The article's first part of the section of *In Vivo Resurrection of a Distant-Acting Snake Limb Enhancer* section corresponds to our analysis done in-class. This corresponded to identifying which sequences were actually responsible for limb development which was part 8 after we determined the ZRS region through gene expression analysis. Their gene expression analysis was different in the form of observing mouse embryos while ours was done using kallisto and sleuth software. We both ended our analysis by aligning the sequences using MAFFT.

Our results from MAFFT yielded the multiple sequence alignment of 8 species, including 4 snake species. Their results yielded the alignment of 18 species. Our results were similar but not as precise. Their alignment had a single nucleotide deletion that was part of the ZRS region that our alignment did not show. This means that their alignment yielded 17 base pairs while our yielded 16 base pairs, that were conserved in vertebrates but not snakes. The difference is likely to come from the fact that our alignment used significantly less species, so it did not account for the nucleotide of lizards that indicates another deleted nucleotide of the ZRS region. Overall the results were similar enough to conclude this was the enhancer region resulting in the loss of limb expression in snakes.