CHANGES IN TRANSCRIPTIONAL ENHANCER ACTIVITY OF THE SECOND-FASTEST-EVOLVING HUMAN GENOMIC REGION

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The recently discovered Human Accelerated Regions (HARs) are human genomic regions highly conserved in mammals but with extensive substitutions since our last common ancestor with the chimpanzee[1,2]. The functions of these elements largely await experimental validation, but are likely to provide clues towards the specifics of uniquely human evolution. We have focused our studies on the second-highest ranking HAR, HAR2, which is a 119 base-pair non-coding element containing 12 human-specific substitutions. We hypothesize HAR2 functions as a distal transcriptional regulatory element, and further that the human-specific substitutions lead to an altered regulatory capacity in human relative to other mammals. We show using a LacZ reporter assay in transgenic mice that HAR2 functions as a neural-specific transcriptional enhancer. To further investigate this finding we have developed an assay using a luciferase reporter to test the ability of HAR2 to regulate transcription throughout the differentiation of mouse embryonic stem cells (mESCs) to neurons. From preliminary experiments we demonstrate HAR2 is capable of driving expression in undifferentiated mESCs. We show in addition that the human and chimpanzee versions of HAR2 have differing activity. To assess which of the human-specific substitutions contribute to this altered activity, we analyzed the human and chimpanzee sequences for transcription factor binding sites. Results from two independent methods indicate several novel binding sites are created and two are ablated in human HAR2 as a result of the human-specific substitutions. Based on this analysis, we attempt to reconstitute human-like activity in chimpanzee HAR2 using site-directed mutagenesis; current progress with this work will be presented. Our comparative study of human and chimpanzee HAR2 activity may shed light on regulatory changes that led to human-specific features of the human brain.

1. Pollard et al., PLoS Genetics. 2006, 2(10):e168.
2. Pollard et al., Nature. 2006, 443(7108):167–172.