

Evolution: Tinkering within Gene Regulatory Landscapes

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Recently evolved enhancers dominate mammalian gene regulatory landscapes. Mostly exapted from ancestral DNA sequences, many are linked to genes under positive selection. Just as RNA-seq some years ago, unbiased enhancer mapping is on the verge of changing evolutionary research.

“Their macromolecules are so alike that regulatory mutations may account for their biological differences.”

Mary-Claire King and Allan Charles Wilson, (1975)

This year marks the 40th anniversary of the landmark paper by Mary-Claire King and

Allan Wilson [1] that speculated about the importance of gene regulatory changes versus those in protein-coding sequences during evolution — in this particular case of humans and chimpanzees [1]. Two years later, François Jacob further expanded on some of these ideas in his influential essay ‘Evolution and Tinkering’ [2,3]. He recognized that evolution via

natural selection does not work like an engineer, but like a tinkerer. Instead of redesigning its components, it tinkers by fiddling with the existing bits. During the last decade, some fields, in particular evolutionary developmental biology (evo-devo), reinvigorated the discussion about the relative importance of protein versus regulatory changes

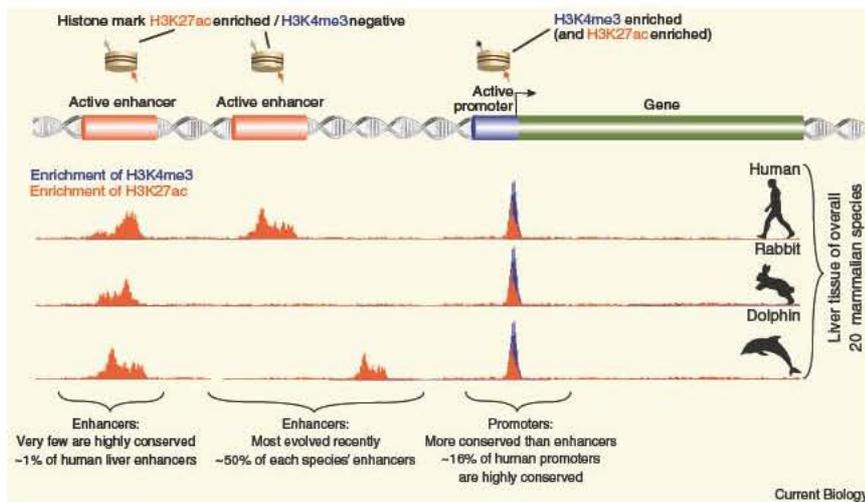


Figure 1. *In vivo* screen for regulatory activity in livers from 20 mammals. Functional genomic regions can be identified by enrichment of histone marks such as H3K27ac (identifying active enhancers) or H3K4me3 (identifying active promoters). Comparative analysis between 20 mammals showed that most enhancers evolved recently, and only a very small fraction are highly conserved.

during phenotypic evolution [4–6]. However, the discussion was – at least from the experimenters’ view – a most unequal fight. Due to technical hurdles and the complexity of regulatory landscapes, most research in evolutionary biology was forced to focus primarily on uncovering changes in the relatively small protein-coding fraction of genomes. There, the rules of change, synonymous vs. non-synonymous mutations, have been understood for decades. The regulatory parts of vertebrate genomes were, with a few exceptions [7–9], barely amenable to unbiased investigation outside of model organisms since the grammar of change still is largely unknown [10]. A recent paper by Diego Villar and colleagues [11] impressively demonstrates that these limitations might fade very soon and provides the most comprehensive overview of the evolutionary dynamics of vertebrate gene regulatory elements so far.

O Enhancer, Where Art Thou?

Villar and colleagues [11] take advantage of ChIP-seq, a method that allows for the experimental identification of active regulatory elements (enhancers and promoters). By performing a comprehensive set of ChIP-seq experiments in adult liver tissue of 20 different mammals they map the activity of both promoter and enhancer

elements in an unbiased genome-wide manner (Figure 1). For most, the liver might not be the first organ to think of as representative of mammalian diversity and evolution. However, its conserved function, distinctive morphology and broadly comparable gene expression profile [12,13] enable an understanding of the dynamics of the evolution of regulatory elements over larger evolutionary distances.

To investigate regulatory element evolution, the authors identified active promoters and enhancers for each species using histone modifications as a proxy. While active promoters are enriched for methylated histone 3 (H3K4me3), active enhancers can be identified by an enrichment of acetylated histone 3 (H3K27ac; Figure 1). The conservation of the underlying sequences was analysed by genome alignments. Hereby, the authors could dissociate alignable sequences that were found in the same, orthologous genomic region from non-alignable sequences that have been lost (or gained). Hence, by comparing between vertebrate species, they could distinguish between regulatory elements with conserved activity (histone-mark enrichment) and alignable sequence, elements that are conserved by sequence but not activity and sequences that simply could not be aligned.

Engineered Promoters – Tinkered Enhancers

Surprisingly, Villar and colleagues [11] found no clear differences in the frequency of loss (or gain) of enhancer and promoter sequences. It is almost equally likely to find the DNA sequence of a human liver promoter or a human enhancer in another mammal. However, the mere presence of the sequence does not seem to imply that the activity of the regulatory element is conserved as well (Figure 2A). Interestingly, the dynamics of enhancer and promoter evolution differ substantially. Despite sequence conservation far less enhancers retained their activity than promoters. This also implies that novel enhancers originate rather by ‘repurposing’ already existing, ancestral stretches of DNA (Figure 2B).

Conservation of both enhancer and promoter activity can be described as an exponential decay curve, if shown as a function of evolutionary distance. Activity of promoters is very conserved (around 83% retained their activity over a 100 million year divergence), while enhancer activity diverged much more quickly (only about 12% of enhancers retained their activity over a 100 million year divergence time). Of the approximately 41,000 active regulatory elements that were found in human liver tissue (roughly 2/3 of them are enhancers), around 2000 have conserved activity throughout the ten placental mammals with the highest-quality genomes. Of these conserved elements, 87% are promoters, confirming the apparently high constraints on promoters compared to enhancers. Every species uses approximately 10,000 enhancers (compared to only 1,000–2,000 promoters) that show species-specific activity, suggesting more rapid enhancer evolution and emphasizing their specific role during regulatory evolution. Roughly 2/3 of these evolutionarily young enhancers are composed of ancestral sequences (more than 100 MA old), while only a minority evolved *de novo* from repeat element expansions such as transposons. Surprisingly, for promoters the opposite was the case, novel promoters mainly evolved within younger DNA (Figure 2B).

Furthermore, Villar *et al.* [11] provide evidence that the genomic basis of lineage-specific adaptations might

reside in both coding sequences and non-coding regulatory sequences, possibly even as a result of a synergistic interplay between both. To test this hypothesis, they investigated the regulatory landscapes of genes under positive selection in the naked mole rat, (*Heterocephalus glaber*) and the common bottlenose dolphin (*Tursiops truncatus*). They found an over-representation of recently evolved enhancers around the thymopoietin gene (TMPO) in the naked mole rat and thyroid hormone receptor interactor 12 gene (TRIP12) in the dolphin. The preponderance of recently evolved enhancers surrounding genes showing positive selection provides compelling evidence for synergistic selection on both coding and regulatory mutations during adaptation.

Is Rapid Enhancer Evolution the Basis of Fast Diversification?

The study by Villar *et al.* [11] clearly demonstrates that enhancer evolution can be surprisingly fast and dynamic. However, the question remains: Are regulatory elements major “loci of evolution” despite or because of their frequent variation [3–5]? The high diversity of regulatory landscapes is likely to influence population divergence. For instance, single base pair substitutions (SNPs) can affect enhancer activity and even phenotypes such as hair coloration [14]. Already existing genetic variation in populations might thereby directly drive variation in enhancer activity (Figure 2C), resulting in ‘standing gene regulatory variation’ within populations that natural selection can act on. Mutations in non-conserved enhancers probably largely have small effects or are even without effect due to redundancies [15]. Due to that, most mutations resulting in a gain or loss of regulatory element activity will be nearly neutral. However, since enhancers are scattered over large genomic regions (an enhancer can be more than a million base pairs from the gene it controls), recombination might quickly lead to synergistic and beneficial effects on gene expression and possibly phenotypic variation.

Phenotypic diversification has been hypothesized to be effectively driven by developmental changes in the location (heterotopy), level (heterometry) or time of gene expression (heterochrony), as for

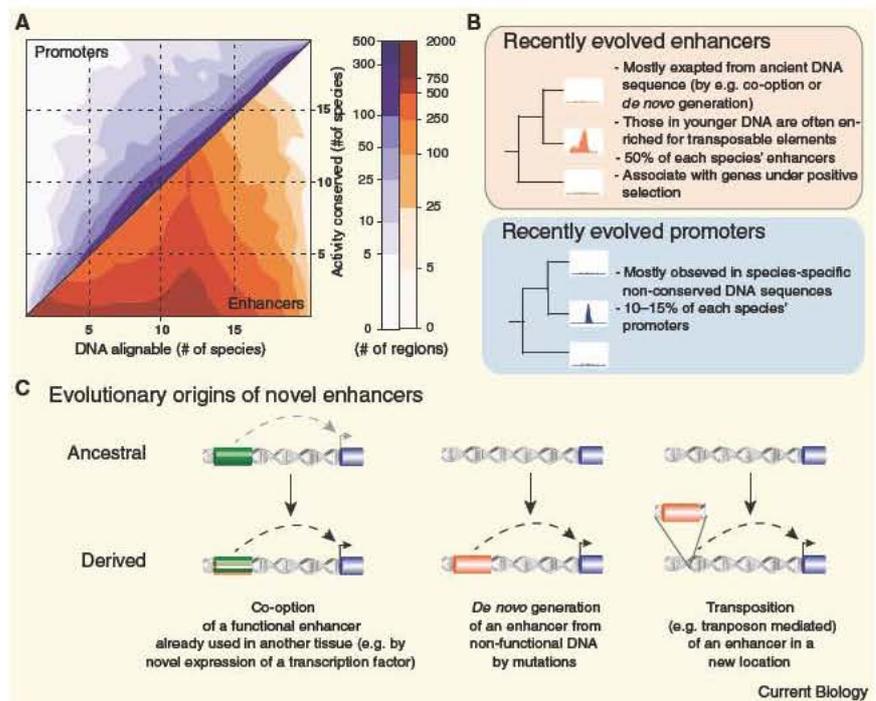


Figure 2. Rapid enhancer and slow promoter evolution.

(A) Comparison of DNA conservation (DNA alignable to other species) and activity conservation in comparison to a human data set. For promoters the conservation of the sequence strongly correlated with their activity, while for enhancers the presence of a similar, alignable sequence is a poor predictor for conserved activity. (B) Summary of the features of recently evolved enhancers and promoters. (C) Evolutionary origins of novel enhancers (modified from [19]). In the study by Villar *et al.* most enhancers evolved by exaptation from ancient sequences, probably either by co option of existing enhancers or by *de novo* generation. Origin by transposition occurs less commonly for liver enhancers (compared to e.g. [20]).

example shown in Darwin’s finches [16]. Similarly, roughly 4300 enhancers were found to be active during morphogenesis in developing embryonal face tissue in the mouse [17,18]. Surprisingly, knockouts of single enhancers from these 4300 enhancers resulted in subtle changes of the mice’ skull morphology. Taking these and other studies together, one might propose that the combinational power of putative small-effect enhancers might indeed be a powerful genetic mechanism to generate differences between individuals. Due to their redundancy and modularity that circumvents negative pleiotropic effects (different enhancers for different cell types), enhancers might thereby greatly contribute to phenotypic variation within populations that selection can act upon [3,5].

A better understanding of how regulatory landscapes change in a particular adult or developmental tissue during the course of evolutionary time will undoubtedly soon lead to a pronounced

increase of our understanding of the genomic basis for phenotypic diversification. Tools such as ChIP-seq allow us to finally uncover non-coding DNA stretches of regulatory importance outside of mice, cell-cultures, and zebrafish. Comprehensive studies across species such as this from Villar *et al.* [11] pave the way for a better understanding of how evolutionary tinkering with the regulatory machinery contributes to the molecular underpinnings of the diversification that Charles Darwin already talked about in the origin: “from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved” — often so quickly, one might add.

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