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*Trans*forming our understanding of species-specific gene regulation

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Mechanisms underlying phenotypic divergence across species remain unresolved. In this issue of *Cell Genomics*, Hansen, Fong, et al.[1] systematically dissect human and rhesus macaque gene expression divergence by screening tens of thousands of orthologous elements for enhancer activity in lymphoblastoid cell lines, revealing a much greater role for *trans* divergence at levels equal to those of *cis* effects, counter to the prevailing consensus in the field.

Changes in noncoding DNA play a pivotal role in species divergence, significantly impacting gene regulation. Gene expression is influenced by two distinct mechanisms: (1) local *cis* effects driven by direct contacts of regulatory elements, such as promoter and enhancers, and (2) general *trans* effects driven by cellular environment, such as abundances of transcription factors (TFs) (Figure 1). An intriguing open question is the relative roles that *cis* and *trans* effects play in gene regulatory divergence across species.[2] Understanding these mechanisms will shed light on gene expression evolution. Previous research has employed innovative strategies to disentangle *cis* and *trans* effects. The use of hybrid cell lines[3] and organisms[4] has enabled the characterization of allele-specific expression, as a proxy for species' *cis* divergence, while controlling for *trans* differences. While these studies focus on *cis*-regulatory effects, estimates of overall *trans* divergence remain imprecise. Experiments employing massively parallel reporter assays (MPRAs) have directly tested *cis* and *trans* effects by comparing activities of orthologous putative regulatory elements across homologous cell lines from different species.[5] Generally, such studies have found that *cis*-acting changes account for a majority of gene expression divergence, with special cases of specific TFs driving a smaller proportion of *trans* differences. While MPRA studies are well controlled, they are often limited to a pre-selected set of DNA sequences, which may be insufficient in accounting for the complete gene regulatory landscape.

To mitigate some of the limitations of past studies, Hansen, Fong, et al.[1] used a modified MPRA approach, ATAC-STARR-seq, producing reporter libraries from DNA at open chromatin loci, effectively expanding the landscape of regulatory elements assayed by ~10-fold. Unlike standard synthetic MPRA methods, ATAC-STARR-seq also produces information on transposase-accessible open chromatin and DNA footprinting. Having previously demonstrated the efficacy of the approach using human lymphoblastoid cell line GM12878,[6] they apply it here to compare activity of 29,531 shared ATAC-accessible peaks between human and rhesus macaque. Specifically, using DNA derived from a single human (H) and rhesus macaque (M) lymphoblastoid cell line to generate species-specific ATAC-STARR-seq libraries, they transfected each back into its "native state" cell line (**H**H and **M**M) and compared activity of the top 10,000 most active regions. From this, they found 18% of elements exhibited conserved activity between species, while the remaining 82% of orthologs drove species-specific patterns of expression (equal proportion for human and macaque). To understand mechanisms, they next performed interspecies MPRAs, transfecting the human ATAC-STARR-seq library into the rhesus macaque cell line (**H**M) and vice versa (**M**H). In sharp contrast to previous work, they found that activity of elements were either retained (*cis* effects) or lost (*trans* effects) in the other species' cell lines at near equal proportions, with a surprising 60%–70% of the species-specific elements influenced by both *cis* and *trans* effects in both human and rhesus macaque. From this, they propose a model where *trans* changes, which alter the abundance of available TFs leading to immediate and impactful shifts in the cellular environment, likely precede *cis* changes that can subsequently serve to correct for possible longer-term deleterious effects.

Taking a closer look at the sequence content composing the assayed elements, *trans*-only features exhibited greater conservation at the nucleotide level, matching previous observations,[7] while *cis*-only features were enriched for variants associated with gene expression differences across modern humans. *Cis* and *trans* regions exhibited the most divergence, with enrichments in transposable elements (SINE/Alu elements). Further, nearly half of *trans* elements carry a footprint of TFs differentially expressed in human and rhesus lymphoblastoid cell lines, revealing only a handful of key immune regulators as driving a majority of *trans* species differences. Narrowing in on one putative *trans* driver (ETS1), they identified and experimentally validated *cis*-regulatory changes associated with increased expression of the TF in human cells. They also propose that a cascade of other *trans* changes may have contributed to its divergence, highlighting the efficacy of globally characterizing regulatory effects and the complexities of the system at large. Though an exciting first step, functional studies

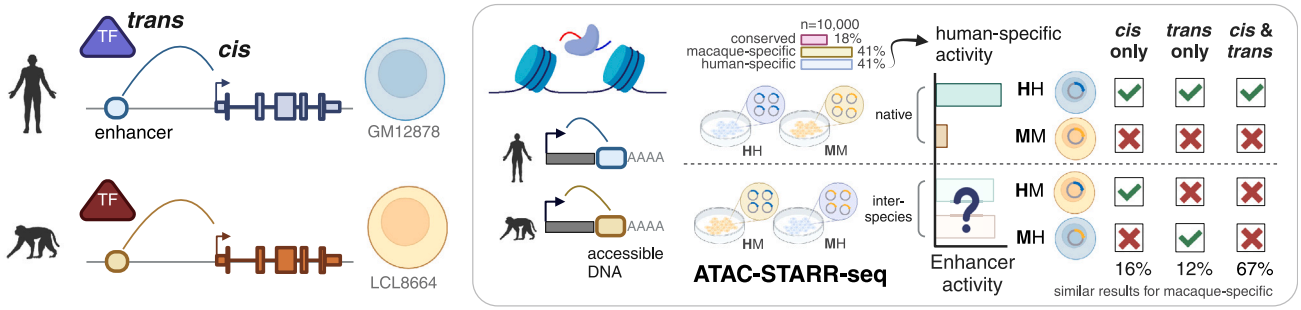

Figure 1. ATAC-STARR-seq to characterize regulatory effects on human and rhesus macaque expression differences
Created with BioRender.com.

assessing the subsequent impacts on predicted *cis* targets while precisely controlling expression of *trans*-driving TFs are necessary to ultimately verify their results.

With these new findings in hand, it begs the question: why did previous studies miss the significant *trans* effects observed here? Authors offer compelling arguments for their differing conclusions. Using the ATAC-STARR-seq approach allowed assessment of significantly more regulatory regions compared with similar studies using synthetic MPRAs, such as one directly comparing human/mouse elements that found 2× more *cis* versus *trans* differences but assayed 10× fewer orthologous elements.[8] While making gains in scale, their method also sacrifices the within-sample reproducibility provided by unique molecular tags of synthetic MPRAs that enable multiple measurements per element. This likely resulted in reduced sensitivity to detect regulatory activity, highlighted by their finding that, of the ~1,800 elements exhibiting species-conserved activity, 73% of human and 58% of rhesus sequences retained activity in the interspecies MPRA study. Though coordinated *cis* and *trans* changes could result in a similar finding, future work might test a subset of their 10,000 human-rhesus orthologous regions in a single synthetic MPRA library to strengthen confidence in results. Because ATAC-STARR-seq involves large-scale sequencing, this study analyzed just one human and one rhesus cell line to compare species expression differences. To avoid mistaking intraspecies variation for true differences and to bolster experimental results, replication across multiple individuals is crucial. While more research remains, the study's findings offer exciting insights into the mechanisms driving phenotypic divergence across species and open additional avenues of inquiry, including if *cis* and *trans* contributions vary across diverse cell types and at different evolutionary time scales.

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DECLARATION OF INTERESTS

The author declares no competing interests.

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