RESEARCH ARTICLE SUMMARY

AVIAN GENOMICS

Core and region-enriched networks of behaviorally regulated genes and the singing genome

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INTRODUCTION: Brain activity drives both behavior and regulated gene expression in neurons. Although past studies have identified activity-induced signaling and gene regulation cascades in cultured neurons, much less is known about how activity-dependent transcriptional networks are affected by the variations in cell-type composition, network interconnections, and firing patterns that comprise behaviorally active brain circuits in vivo.

RATIONALE: We tested the hypothesis that behaviorally regulated gene expression is anatomically and temporally diverse and that the key determinants of this diversity are networks of transcription factors, their genomic binding sites, and epigenetic chromatin states. We analyzed genome-wide, singing-regulated gene expression across time in the four major forebrain regions of the song control system in songbirds, a model of speech production in humans. We then performed a transcription factor motif analysis to identify gene regulatory networks enriched in

each song nucleus and measured acetylation of histone 3 at lysine 27 (H3K27ac) to identify chromatin regions that were transcriptionally active in the genomes of song nuclei before and after singing.

RESULTS: We found that singing was associated with differential regulation of about 10% of all genes in the avian genome that came in several waves across time. Less than 1% of these genes were comparably regulated in all song nuclei tested, and these comprised a core set dominated by immediate-early gene (IEG) transcription factors. By contrast, the vast majority of singing-regulated genes were regulated in only one or a subset of song nuclei, such that each song nucleus had its own dominant subset of genes regulated with defined temporal profiles, controlling a variety of functions. The promoters of many of the singing-regulated genes contained binding motifs for known early-activated transcription factors (EATFs) that become active in response to neural firing, some of which were expressed differentially between

song nuclei at baseline. One EATF, calcium-response factor (*CaRF*), was tested with RNA interference knockdown in cultured neurons and found to regulate the predicted genes in response to neural activity, but was also found to modulate their expression even at baseline. More strikingly, we found with H3K27ac analysis that many song nucleus–specific singing-regulated genes did not show increased chromatin regulatory element activity after singing but rather already had primed region-specific regulatory activity before singing began.

CONCLUSIONS: We propose a dual mechanism for the diversity of behaviorally regulated genes across different brain regions in vivo (see the figure). First, the neural activity associated with singing activates EATFs, and some TFs differentially expressed in brain regions at baseline, to trigger region-specific expression of their target genes. Second, brain region–specific enhancers near activity-regulated genes are waiting in an epigenetically primed state, ready to modulate transcription of general and song nucleus–specific genes at a moment's notice when the neurons fire. The combination of these two mechanisms underlies a great diversity of behaviorally regulated gene expression, permitting each nucleus to perform its particular function in this complex behavior. ■

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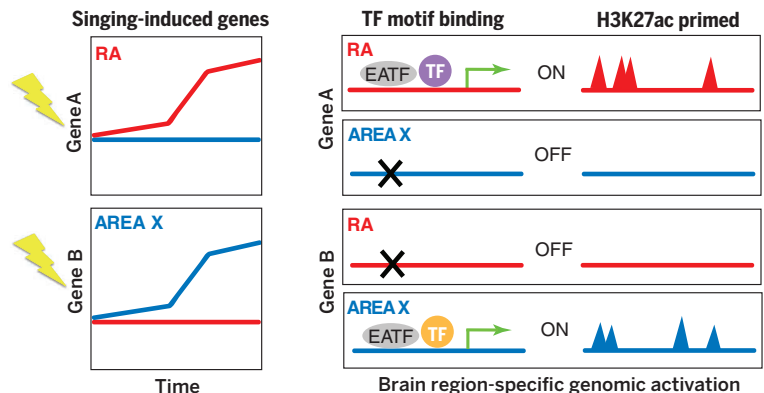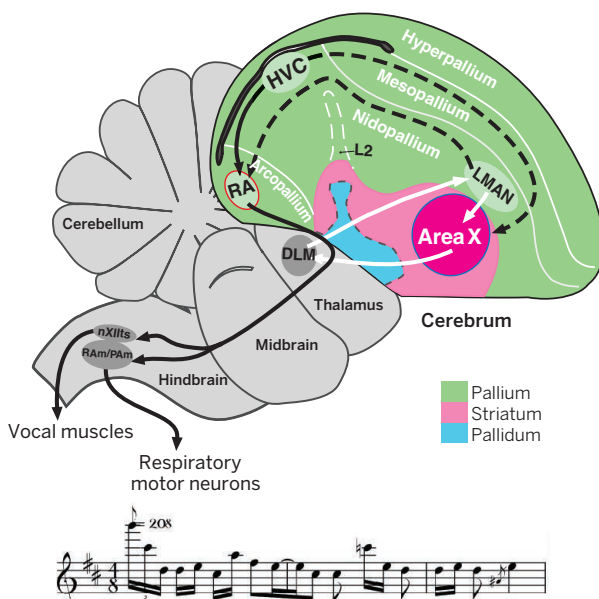
Dual mechanism model for behaviorally regulated gene expression diversity. (**Left**) Song brain circuit and zebra finch song motif (transcribed using https://my.scorecloud.com). (**Middle**) Song nucleus–specific (RA, red; Area X, blue) singing-regulated genes (gene A and gene B) in response to neural firing (yellow). (**Right**) Region-general EATF and region-specific TF only bind to genomic DNA (lines) with region-specific acetylated histone 3 (H3K27ac peaks) and then transcribe their mRNAs (green arrow).