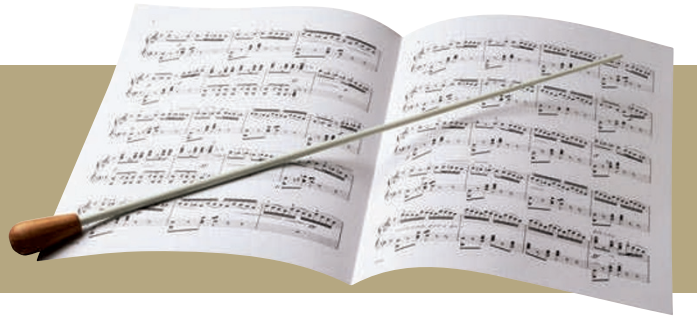
NEURONAL ACTIVATION

The symphony of transcription



Part of the neuronal response to stimulation involves temporally and spatially controlled changes in gene expression. Most studies have elucidated the regulation of transcription through the activation of promoters, but mechanisms by which enhancers contribute to gene expression are poorly characterized. Greenberg and colleagues have now identified thousands of enhancers that recruit the transcriptional co-activator CBP and RNA polymerase II (RNAPII) in an activity-dependent manner and drive the synthesis of a novel class of enhancer RNAs (eRNAs).

Transcription factors regulate gene expression by binding to DNA regulatory elements including enhancers and promoters. Enhancers are localized far from transcription start sites and are defined as sites that bind CREB-binding protein (CBP; also known as CREBBP) at active chromatin regions.

The authors stimulated primary neurons *in vitro* by increasing the K^+ concentration in the medium, and identified 12,000 enhancer sites that bound CBP in an activity-dependent manner. By using a luciferase reporter assay, they showed that these enhancers could induce activity-regulated gene expression, but only when the promoter was intact.

Next, the authors investigated whether transcription factors that are known to mediate activity-regulated gene expression, such as cyclic AMP-responsive element-binding protein (CREB), serum response factor (SRF) and neuronal PAS domain-containing protein 4 (NPAS4), bind to enhancers in an activity-regulated manner. They found constitutive binding of CREB and SRF to most enhancers, whereas NPAS4 was recruited to thousands of promoter and enhancer sites only upon neuronal activation. All three transcription factors bound nearby CBP-binding sites, suggesting that these may cooperate to regulate transcription.

At promoters, CBP is known to recruit components of the transcription machinery, including RNA polymerase II (RNAPII). Indeed, the binding of RNAPII to 3,000 activity-regulated enhancers increased about twofold upon stimulation, and this binding might be mediated by CBP. The authors investigated the role of enhancer-bound RNAPII and found that it drove the bi-directional synthesis of a new class of short RNAs (eRNAs) from CBP–RNAPII-occupied enhancer binding sites.

Next, the authors addressed whether eRNAs might specifically mark enhancers that are actively engaged in promoter activation. Neuronal activity-induced changes in eRNA expression levels correlated with changes in mRNA expression of nearby genes, suggesting that eRNA synthesis depends on an interaction between enhancer and promoter. In neurons in which the gene encoding activity-regulated cytoskeleton-associated protein (*Arc*) and its promoter were deleted, the binding of SRF and RNAPII to the *Arc* enhancer was not affected, but eRNA synthesis was abolished. This indicates that the occupation of enhancer sites is not sufficient to drive eRNA synthesis, and suggests that eRNA synthesis requires the interaction of enhancer and promoter.

The finding that RNAPII binds to thousands of enhancers and triggers the synthesis of eRNAs upon neuronal activation suggests a general mechanism in activity-dependent transcriptional control. The identification of a novel class of RNAs also poses questions about their specific biological function. This study might have wider implications for neuroscience and other disciplines, especially in processes that are driven by transcriptional networks, such as the regulation of cell differentiation.

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> “RNAPII binds to thousands of enhancers and triggers the synthesis of eRNAs upon neuronal activation”

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