Trends in Genetics

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Review

Transcription and epigenetic factor dynamics in neuronal activity-dependent gene regulation

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Neuronal activity, including sensory-evoked and spontaneous firing, regulates the expression of a subset of genes known as activity-dependent genes. A key issue in this process is the activation and accumulation of transcription factors (TFs), which bind to cis-elements at specific enhancers and promoters, ultimately driving RNA synthesis through transcription machinery. Epigenetic factors such as histone modifiers also play a crucial role in facilitating the specific binding of TFs. Recent evidence from epigenome analyses and imaging studies have revealed intriguing mechanisms: the default chromatin structure at activity-dependent genes is formed independently of neuronal activity, while neuronal activity modulates spatiotemporal dynamics of TFs and their interactions with epigenetic factors (EFs). In this article we review new insights into activity-dependent gene regulation that affects brain development and plasticity.

Highlights

Genetically determined cis-elements, promoters, and enhancers, are the first requirement of activity-dependent gene expression.

Chromatin states are pre-prepared at the activity-dependent gene sites before the elevation of neuronal activity but are further modified by neuronal activity.

Neuronal activity promotes transcription factor (TF) binding to cis-elements by increasing the binding time and/or the repetition of binding and dissociation.

TF activation and increased TF concentrations are likely necessary and sufficient for activity-dependent gene regulation.

Neuronal activity induces the expression of a subset of genes

Neuronal activity, including sensory-evoked and spontaneous firing, plays a pivotal role in shaping neuronal circuits [1–3]. Even after establishing neuronal circuits, neuronal activity continues to affect synaptic transmission and synapse formation. In these processes, a subset of genes, known as activity-dependent genes, is regulated by neuronal activity [4–6]. The disruption in this regulation leads to various neuropathological diseases [7].

To date, the fundamental molecular mechanisms of activity-dependent gene expression have been demonstrated: activation of TFs occurs in the nucleus through calcium ion influx and calcium-dependent signaling pathways [8]. These TFs bind to specific DNA sequences known as cis-elements within enhancers and promoters of the genome and trigger the downstream gene expression. Based on their transcriptional responses, activity-dependent genes are classified as immediate early genes (IEGs) and late response genes (LRGs) [9]. IEGs show burst-like transcription in rapid response to neuronal activity and often encode TFs, which in turn regulate the expression downstream LRGs encoding neuronal effector molecules [8]. Concurrently, EFs involved in open or closed chromatin structures also affect TF binding and recruitment of the preinitiation complex, including RNA polymerase (RNAP) [10].

An intriguing problem is how activity-dependent gene expression is regulated spatiotemporally in the nucleus of neurons. More specifically, how does neuronal activity selectively regulate a subset of genes? How does neuronal activity affect the dynamics of TFs and EFs and their interactions in order to induce appropriate gene expression? These questions are being clarified by recent studies using imaging techniques, including single-molecule imaging (SMI) (Box 1) as well as epigenome analyses.

How are activity-dependent gene loci selected in the nucleus?

While activity-dependent gene loci are present in all cells in our body, transcriptional responses to neuronal activity are uniquely induced in neuronal cells. This specificity raises the question: how

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Box 1. Single-molecule imaging

To perform SMI, proteins of interest (POIs) are tagged with genetically encoded fluorescent proteins or with self-labeling proteins such as HaloTag and SNAP-tag, which can be covalently coupled to cell-permeable organic fluorophores. The latter labeling method is preferred in terms of photostability and brightness. Fluorescently labeled molecules inside the nucleus, such as TFs, are visualized as fluorescent spots by highly inclined and laminated optical sheet (HILO) microscopy, which produces high signal-to-noise ratio images by generating thin-layer illumination and reducing out-of-focus background signals. Each labeled spot obtained in this way is thought to represent a single molecule, as each spot suddenly disappears by prolonged excitation light. Thus, the dynamic aspect of TFs can be observed at a single-molecule level with a temporal resolution of 10–100 ms. An important analysis is to measure the dwell times of fluorescently labeled spots. The residence time of each spot varies, ranging from short to long. The residence time distribution could tell TF dynamic aspects by combining with experiments using mutant proteins. In the case of CREB, the appearance of spots with a long residence time is markedly decreased in mutant CREB, which lacks a DNA-binding site, indicating that the long-residence components represent the DNA-binding of CREB. Another interesting point in SMI allows us to observe the localization of POIs with super spatial resolution. Indeed, the precise location of a single molecule can be obtained by fitting a 2D image of each spot into a 2D Gaussian function. This spatial analysis efficiently estimates colocalization with other fluorescent-labeled molecules or nuclear organelles. Moreover, dual SMI of two different molecules can show more accurate colocalization of two nuclear proteins.

are the specific populations of genes selected and activated as activity-dependent genes among thousands?

Sequence-specific mechanisms

Activity-dependent genes and their response characteristics are genetically determined by the genomic sequence of the regulatory regions, which include enhancers and promoters. IEG transcription involves calcium-responsive TFs such as cAMP response element binding protein (CREB), serum response factor (SRF), and myocyte-specific enhancer factor 2 (MEF2), which are already present in the nucleus prior to neuronal activity and are ready to bind to specific cis-elements in the genome [11–13]. Correspondingly, the promoters and enhancers of typical IEGs contain cis-elements for these TFs, including the CREB binding cAMP response element (CRE), the SRF binding CArG box, and the MEF2 binding MEF2 response element (MRE) [11–13]. The well-characterized IEGs, Fos and Arc, contain these cis-elements in their regulatory regions [14,15]. On the other hand, the regulatory regions of the LRGs contain cis-elements to which IEG-encoded TFs bind, including AP-1 (a heterodimer of FOS family proteins and JUN family proteins), EGR1, and NPAS4 [16–18]. Thus, the composition of cis-elements makes a large contribution to determining the structure of activity-dependent genes and their transcriptional responsiveness (Figure 1A).

The sequences of cis-elements are genetically defined and commonly shared among many activity-dependent genes [19,20]. However, variations and combinations of TFs binding to cis-elements regulate the timing and level of transcription in each gene [21,22]. Small differences in the cis-element sequence within individual genes can also influence the binding affinity of TFs, thereby affecting subsequent transcription processes [23]. Moreover, the number and position of enhancers play a crucial role in establishing the basal structure of genes, leading to unique transcription patterns by regulating chromatin structures, including 3D genome folding [20,24]. While cis-element sequences are conserved across species, evolutionary changes in these sequences can lead to the formation of novel cis-elements and give rise to species-specific activity-dependent genes [25–28]. For example, primate neurons exhibit activity-dependent Osteocrin expression, which depends on MEF2-binding in their enhancer regions. By contrast, mouse neurons lack these sites due to several sequence differences and do not show such expression [25].

Chromatin state formation at activity-dependent gene loci

The chromatin state is crucial for the function of enhancers and promoters, particularly regarding TFs binding at cis-elements. During neuronal development, chromatin modifiers act to set up

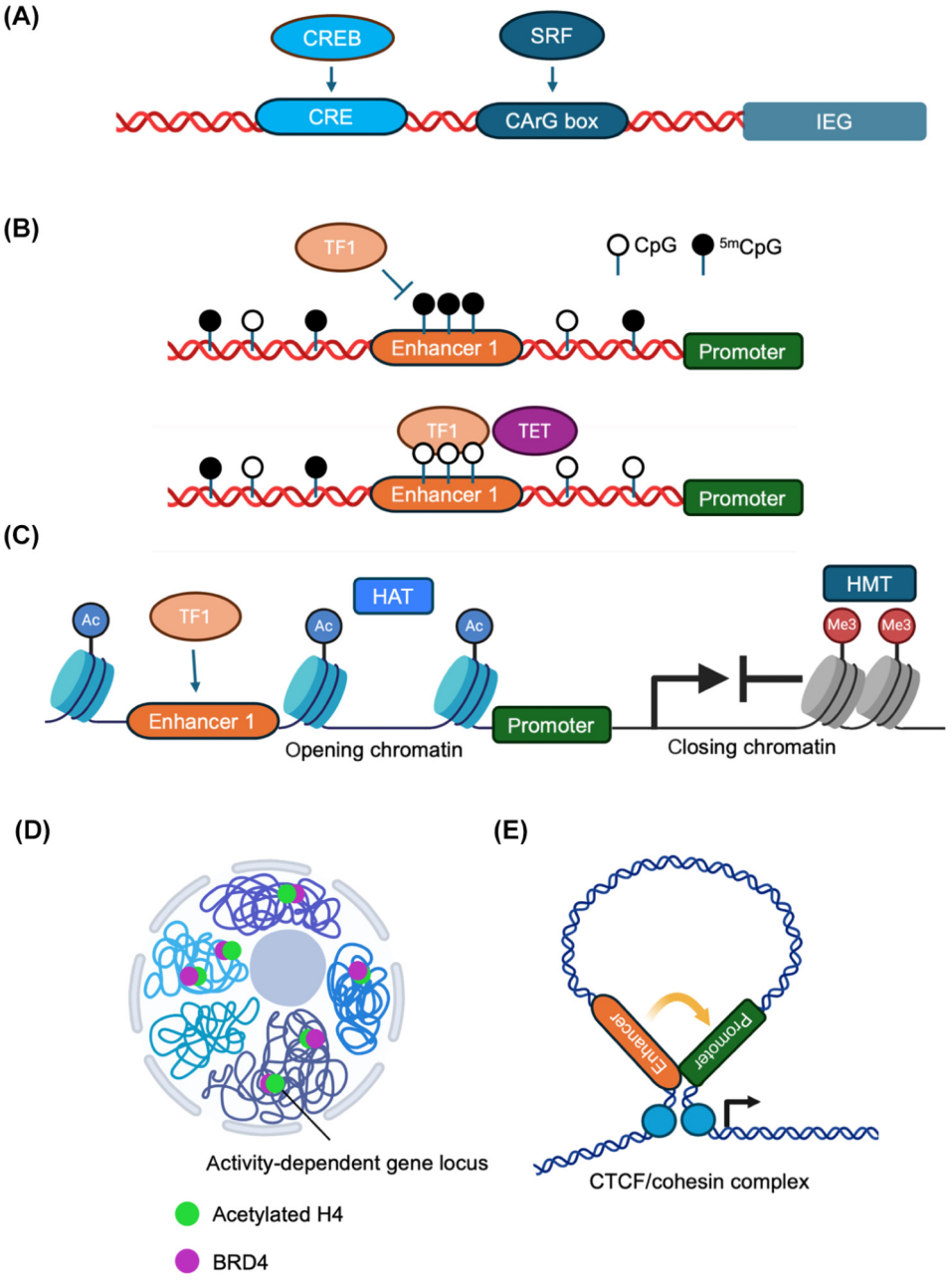


Figure 1. Structure of activity-dependent gene loci in developing neurons. (A) The composition of cis-elements in promoters and enhancers genetically determines the hallmark of activity-dependent genes. (B) DNA methylation at CpG sites of cis-elements inhibits transcription factor (TF) bindings. In immature neurons, hypo-DNA methylation in enhancers, caused by active DNA demethylation, is likely the first signature in forming chromatin structure in activity-dependent genes. (C) During prenatal development, the chromatin state of immediate early gene (IEG) loci, which contain both activating and repressive features due to histone modifications, provides a standby state for activity-dependent expression. Histone acetyltransferases (HATs) promote histone acetylation of H3K27 in transcriptional regulatory regions, facilitating TF binding. By contrast, polycomb complexes, including histone methyltransferases (HMTs), maintain histone methylation marked by H3K27me3 and a closed chromatin state in the gene body. (D) Imaging studies reveal that activity-dependent gene loci are localized in distinct chromatin

(Figure legend continued at the bottom of the next page.)

activity-dependent gene loci. DNA methylation patterns likely serve as the initial signatures that shape chromatin structures in enhancers and promoters in developing neurons [29]. DNA methylation at CpG sites directly inhibits the binding of many TFs [30]. For instance, CREB is unable to bind to its cis-element if it contains a methylated CpG [31]. However, the initial methylation pattern is changed by DNA demethylation processes during neuronal development. In cortical neurons, just after the final mitosis, hypomethylated enhancers are enriched in neuronal genes [29,32]. This DNA demethylation may represent the first step to set up default chromatin states at activity-dependent gene loci independently of neuronal activity (Figure 1B) [32–34]. Selective DNA demethylation requires heterochromatin- and DNA-methylation-insensitive TFs, known as pioneer TFs, such as NeuroD1, NeuroD2, and Ngn2 [35–38]. Pioneer TFs bind to cis-elements and recruit the active demethylation enzyme Ten-eleven translocation (TET) family proteins, which lack sequence-specific binging ability [35]. In accordance with this, mice lacking Tet1 show hypermethylation in the regulatory region of IEGs, followed by decreased expression levels [39].

In addition to DNA methylation patterns, histone modifications at IEG loci provide a 'standby' state for rapid transcriptional induction [15,40,41]. During mouse prenatal development, IEGs display a unique bipartite chromatin signature [40]. In this state, histone H3K27ac, a marker of active chromatin, is enriched at enhancers and promoters. These acetylated nucleosomes facilitate the efficient binding of TFs to specific cis-elements, which subsequently recruit transcription regulatory factors. Simultaneously, the repressive chromatin mark, histone H3K27me3, is formed by the polycomb suppressor histone methyltransferase EZH2 at the gene body and inhibits transcription. These activating and repressive histone modifications create a standby state for IEG expression (Figure 1C). Moreover, in the nucleus of developing human cortical neurons, the IEG loci overlap with immunocytochemically visualized spots marked by active chromatin signatures, such as histone H4 acetylation (H4ac) and its associated Brd4 (Figure 1D) [42]. This histone acetylation may have been set up at activity-dependent gene loci before neuronal activity becomes active [43–45].

What causes these high histone acetylation sites in the nucleus? At least in part, the histone acetyltransferase (HAT) activity is responsible for their formation [42]. Transfection of a dominant negative mutant CREB binding protein (CBP), which lacks the HAT domain, into developing neurons inhibits the formation of H4ac and BRD4 spots and suppresses CREB binding to cis-elements. Therefore, CBP and its paralog p300 likely contribute to histone acetylation at activity-dependent gene loci [46].

An essential question is how CBP/p300 selectively acetylates specific nucleosomes of activity-dependent genes, given that these proteins lack sequence-specific DNA-binding ability [47]. It is possible that a sequence-specific factor that interacts with CBP/p300, independently of neuronal activity, initially targets these specific nucleosomes [48]. Although this factor remains unidentified, one possibility is that pioneer TFs could initiate both histone acetylation and DNA demethylation, as nucleosomes around their binding sites are frequently acetylated [37,38,49]. Additionally, developing spontaneous neuronal activity may promote acetylation by CBP/p300 which binds to TFs [15,16,40,41].

Pre-formed spatial interactions between enhancers and promoters are also likely required for setting up activity-dependent gene induction [24]. CTCF/cohesin complexes regulate the proximity

modification sites marked by acetylated histone H4 and BRD4 in the nucleus. (E) 3D chromatin construction mediated by CTCF/cohesin complex facilitates the formation of proximity between enhancers and promoters in activity-dependent genes.

between enhancers and promoters, facilitating transcription by constructing 3D chromatin structures such as chromatin loops and topologically associating domains (TADs) [50]. Super-resolution imaging – which visualizes specific genome positions using repetitive sequences and fluorescent proteins that bind to those sequences in living cells – has revealed spatiotemporal 3D genome folding involved in these interactions before transcription [51–54]. These 3D chromatin structures support cell-type-specific neuronal gene expression during differentiation [55–59]. For activity-dependent genes, particularly those with distal enhancers, transcription regulation likely occurs within these structures (Figure 1E). It is known that the AT-rich DNA-binding protein SATB2, expressed in the developing cortex, is also involved in this 3D genome organization [59].

Neuronal activity promotes TF binding to cis-elements and modification of the chromatin structure

Spatiotemporal dynamics of TFs regulated by neuronal activity

Once chromatin modifiers create the standby state structure of chromatin, neuronal activity activates TFs and promotes the interaction with promoters and enhancers at activity-dependent gene loci [15]. How does neuronal activity facilitate interactions of activated TF with the cis-element? A notable aspect is the temporal profile in the TF dynamic interactions. Basically, two temporal patterns are possible. One possibility is that neuronal stimulation increases the binding time of TF to the cis-element, which enhances RNAP recruitment and subsequent RNA synthesis. Another possibility is that TF binding frequency is increased by neuronal stimulation.

To address this issue, SMI is a powerful technique that allows us to observe the spatiotemporal dynamics of TFs (Box 1). ChIP-Seq analysis is used to investigate the binding of TFs to specific sites in the genome, but is not necessarily useful to study temporal aspects of binding and dissociation or spatial localization in the nucleus. To date, studies using SMI techniques have revealed the dynamic behavior of TFs in the nucleus of various cell types [60–77]. In these investigations, individual TF molecules are detected as fluorescent spots representing single molecules (Figure 2). Although different theoretical models have been adopted to analyze the behavior of these spots [78], a common feature is the existence of fluorescent spots with short and long residence times. The short residence time component (<1 s) is considered to represent non-specific or weak binding of TFs to the genomic DNA, while the long residence time component (ranging from seconds to tens of seconds) is considered to represent specific binding of TFs to cis-elements. Most results indicate that stimuli such as hormone application extend the duration of the long residence time component in many cell types, indicating that TF binding time is the primary factor driving target gene expression [61–63,66,70–73,75]. In line with this, simultaneous imaging of TF binding and RNA synthesis has shown that the frequency and duration of transcription bursts increase with TF residence time at specific nuclear locations [64,65,74,76].

In neuronal cells, SMI of SRF has also demonstrated that stimulation with brain-derived neurotrophic factor (BDNF) increases the long residence time of SRF binding (Figure 2) [67,69]. This finding supports the view that the length of the residence time is crucial for transcription, consistent with observations in other cell types. By contrast, the SMI of CREB reveals different activity-dependent TF dynamics: neuronal activation with KCl-induced depolarization or optogenetic stimulation leads to repetitive binding of CREB to specific nuclear locations without altering its residence time distribution (Figure 2) [42,68]. Furthermore, simultaneous imaging of active RNAPII with CREB SMI has demonstrated that RNAPII accumulation is increased proportionally with repetitive CREB appearance at particular nuclear locations [42,79], highlighting the importance of repetitive TF binding in gene expression. This repeated binding and dissociation behavior has also been observed in TFs which are critical for maintaining pluripotency in ESCs [73]. Such

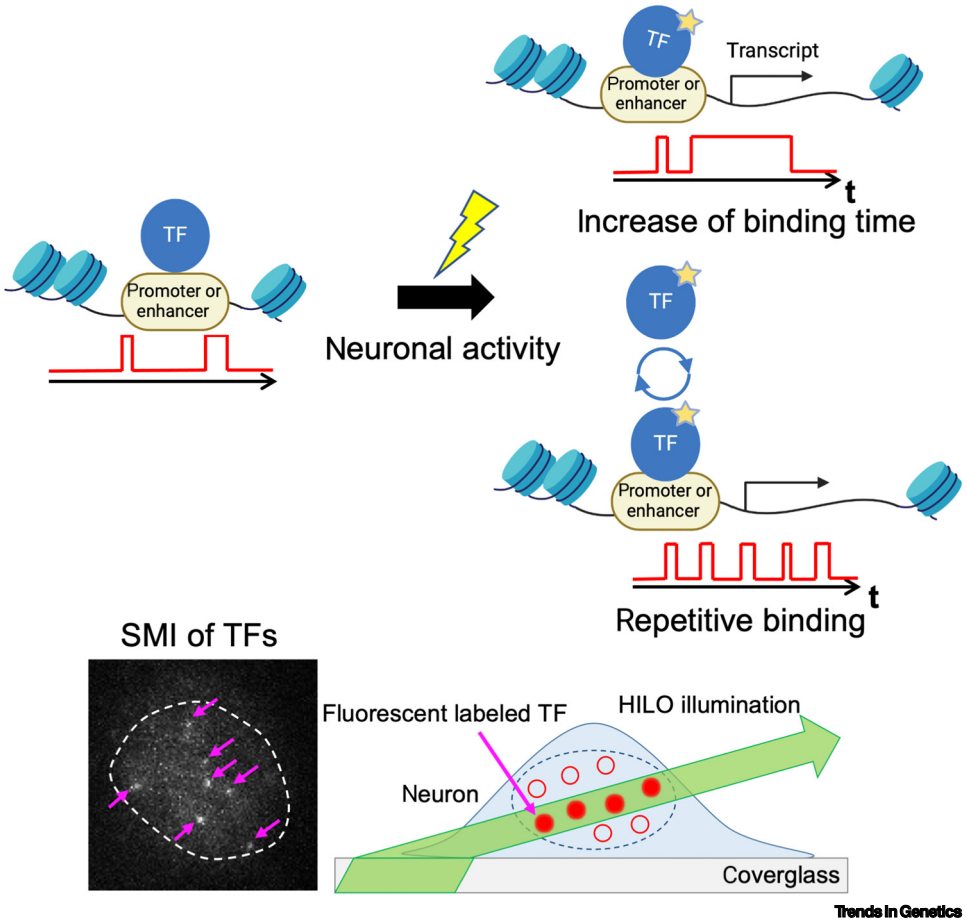


Figure 2. Dynamics of transcription factor (TF) binding to cis-elements. The highly inclined and laminated optical sheet (HILO) microscope shows fluorescent-labeled TF spots (single molecules, indicated by magenta arrows) in the nucleus of cultured neurons. Quantitative analysis, such as dwell time and position of TF spots, demonstrates that neuronal stimulation increases the binding time of the TF or induces its repetitive binding to cis-elements in enhancers and promoters.

repetition may be more efficient for driving transcription bursts than sustained long-term binding [72,76].

Roles of TF activation state and concentrations

What drives the increase in residence time or repetitive binding of TFs? In the case of CREB, phosphorylation via calcium influx is essential. Indeed, an increase in repetitive binding of CREB, coinciding with the recruitment of RNAPII, has been found in cortical neurons through the overexpression of a constitutively phosphorylated CREB mutant, even in the absence of neuronal activity [42]. Nuclear receptors acting as TFs also often require phosphorylation for their activation [80]. However, analysis of the residence time distribution using SMI has shown the presence of other activation modes: SRF is activated via interactions with other TFs [67], and the activation of TP53 requires acetylation of C-terminal domain [70]. Thus, regardless of the pathway, the activation state of TFs is critical for promoting TF actions.

In addition to activation, the concentrations of TFs are likely involved in TF dynamics [74,81]. An extreme example of this mechanism is shown in developing zebrafish embryos by using the SMI

technique: as nuclear size is gradually decreased during development, TF concentrations are increased, leading to a rise in overall transcription [75]. In neuronal cells, the nuclear envelope has been shown to be folded by elevation of neuronal activity [82]. Such a morphological change might contribute to not only a skewed distribution of TFs in the nucleus but also to the increased local TF concentrations. However, recent imaging studies have revealed that TFs can rapidly accumulate at target gene sites without nuclear structural changes in response to stimulation, followed by an increase in transcription burst [54,65]. This suggests that specific chemical reactions underlie the local accumulation of TFs. It has been shown that intrinsically disordered regions (IDRs) of TFs are involved in self-interactions and phase separation, which can enhance the local TF concentrations [83–85]. The IDR domain also appears to contribute to the formation of condensates comprising TFs and other transcription regulatory factors [86–88]. The role of neuronal activity in the condensation of TFs is an area of research that is expected to yield new insights.

Chromatin modification by neuronal activity

Chromatin modifications are induced by neuronal activity and are involved in TF binding/dissociation and the recruitment of transcriptional regulatory proteins. Histone acetylation and deacetylation, mediated by HATs and histone deacetylases (HDACs), are key contributors to this process [15,16,89]. Imaging analysis using fluorescent protein tags has revealed that nucleocytoplasmic translocation of class IIa HDACs (HDAC4, 5, and 9) depends on neuronal activity levels [90,91]. In immature cortical neurons, HDAC9 is initially localized in the nucleus but moves into the cytoplasm after depolarization. By contrast, when inhibiting neuronal firing in matured cortical neurons, HDAC9 is more distributed in the nucleus [91,92]. In the adult hippocampus, a subset of neurons exhibits transient nuclear localization of HDAC4 and HDAC5 during a process associated with memory acquisition, possibly due to a temporary decrease in neuronal activity within these circuits [93]. Despite the lack of the sequence-specific binding ability, nucleocytoplasmic translocation of these HDACs affects specific gene loci rather than inducing global changes. This is due to the specificity with which HDACs bind to certain TFs [94]. Nuclear HDACs may bind to MEF2 at enhancers and promoters, reducing acetylation levels of surrounding nucleosomes and thereby suppressing the transcription of activity-dependent genes (Figure 3) [91,95,96].

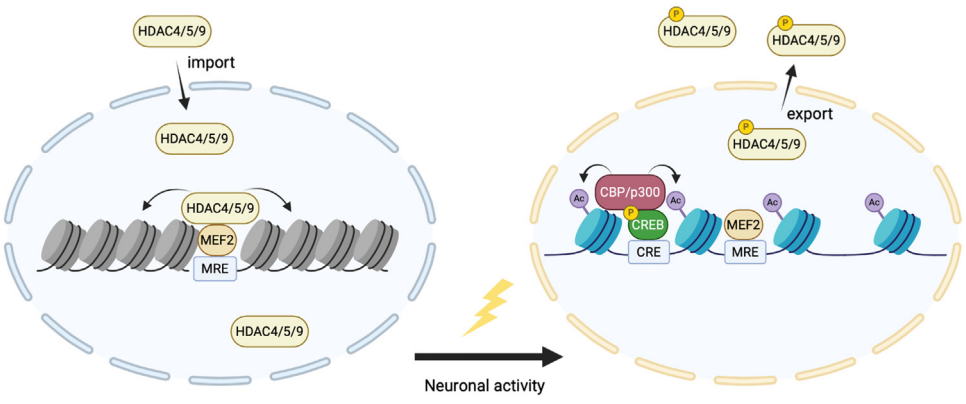


Figure 3. Neuronal activity-dependent modulation of nucleosome acetylation levels. Neuronal activity modulates nucleosome acetylation levels via histone acetyltransferases/histone deacetylases (HATs/HDACs), affecting activity-dependent gene expression. In the low-activity state (left), class IIa HDACs 4/5/9 are predominantly localized within the nucleus and bind to MEF2, reducing histone acetylation levels at the regulatory regions, thereby suppressing activity-dependent gene expression. In the high-activity state (right), calcium signaling-responsive protein kinases (e.g., CaMK) phosphorylate HDACs and cAMP response element binding protein (CREB). HDACs are dissociated from MEF2 and finally exported into the cytoplasm. Instead, phosphorylated CREB can bind to cAMP response element (CRE), and CREB binding protein (CBP) intensively acetylates nucleosomes around its binding sites. Abbreviation: MRE, MEF2 response element.

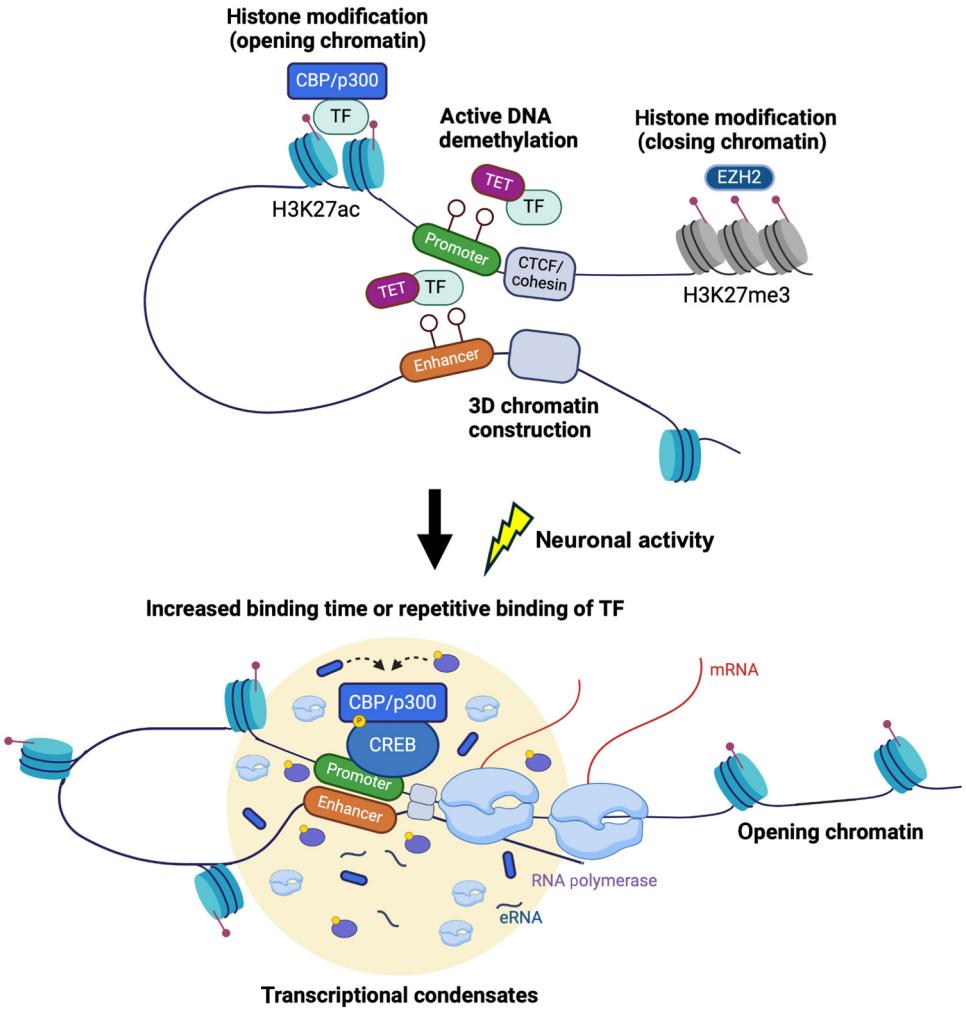
When HDAC translocation from the nucleus to the cytoplasm is promoted by neuronal activity, the relative effect of antagonistic HAT activity becomes more prominent. The HAT enzyme CBP/p300, which binds to phosphorylated CREB, contributes to the acceleration of nucleosome acetylation at enhancers and promoters of activity-dependent gene loci (Figure 3) [15,89]. Indeed, dual SMI has demonstrated that neuronal activity increases the colocalization frequency of CBP and CREB, indicating enhanced CBP recruitment [42]. This HAT-mediated, activity-dependent chromatin opening efficiently recruits transcriptional regulatory proteins, including RNAPII [89,97]. The repetitive binding of phosphorylated CREB (see earlier text) might rely on these highly acetylated nucleosomes.

Neuronal activity also promotes multiple chromatin modifications in parallel with histone acetylation. These modifications vary across activity-dependent genes, involving locus-specific mechanisms. First, during perinatal stages, neuronal activity, including spontaneous activity, rapidly relieves polycomb-mediated repression at the bipartite chromatin state of IEGs, which allows their expression from the standby state (see earlier text) (Figures 1C and 4) [40]. Second, active DNA demethylation reduces methylation levels at specific CpG sites [98,99]. In mouse dentate granule neurons, these unmodified CpG sites are enriched in brain-specific genes following neuronal activation [100]. Among these, EGR1, an IEG product, recruits TET1 to the enhancer and promoter regions of downstream genes [101]. Third, dephosphorylation of the chromatin chaperone protein DAXX by calcineurin leads to the incorporation of histone variant H3.3 into nucleosomes at the regulatory regions of Fos and Bdnf loci [102]. Fourth, activity-dependent topoisomerase IIβ-induced DNA double-strand breaks within promoters alter DNA topology in IEG loci such as Fos and Npas4 [103]. Fifth, neuronal activity induces CTCF/cohesin-mediated 3D chromatin reorganization, facilitating long-range loop formation due to the greater distance between the interacting enhancer and promoter, as seen at the Bdnf locus [104–107]. Finally, additional activity-dependent processes, including other histone modifiers and ATP-dependent chromatin remodelers, further influence chromatin structure [108–115]. These neuronal activity-induced chromatin structures at specific gene loci may result in unique dynamics of TFs and transcriptional regulatory proteins.

Patterns and history of neuronal activity influence gene expression

The effect of neuronal activity in gene expression is likely not an all-or-nothing process but rather occurs in a gradual manner as neuronal activity is composed of various firing patterns. In developing thalamocortical axons, the frequency of spontaneous neuronal firing regulates the expression of Robo1 which suppresses axon growth [116]. In olfactory circuit formation, phasic and tonic firing patterns differentially regulate the expression of different axon growth-promoting factors [117]. These findings strongly support the importance of firing patterns in activity-dependent gene regulation [41]. A live imaging study has further shown that Bdnf promoter activity is altered differently by various stimulation patterns that induce distinct intracellular calcium concentrations [118]. Given that phosphorylation of CREB is necessary for its repetitive binding (see earlier text) [42], the quantity of phosphorylated CREB regulated by intracellular calcium concentrations would be a key determinant. Thus, it is likely that neuronal activity patterns regulate the concentrations of activated TFs via intracellular calcium concentrations, which in turn determine TF binding properties and downstream gene expression.

Each neuron has a unique history of differentiation and neuronal activity, leading to its responsiveness to future stimuli, a phenomenon known as meta-plasticity [119]. Chromatin states are considered to play a pivotal role in this process. As described above, chromatin states at activity-dependent genes are likely set up at early developmental stages and further develop by increasing spontaneous activity and the subsequent sensory-evoked activity [15,40]. As a result, a chromatin state in each neuron is established, allowing the binding of TFs to cis-elements at the specific genome sites and inducing expression of genes that influence synaptic modifications,



Outstanding questions

What is the spatiotemporal relationship between activity-dependent gene loci, nuclear organelles, and multiple nuclear factors?

How does neuronal activity lead to the formation of TF condensates?

Are there evolutionary and human-specific aspects in the neuronal activity-dependent dynamics of TFs and EFs?

What is the relationship between TF and EF dynamics at activity-dependent gene loci and neuropathological diseases?

Figure 4. Spatiotemporal dynamics of multiple chromatin modifiers and transcription factors (TFs) in activity-dependent gene loci in developing and mature neurons. Activity-dependent genes in the genome are genetically determined by DNA sequences in promoters and enhancers, including cis-elements bound to calcium-signal responsive TFs. In an activity-independent manner, chromatin structures that are constructed by DNA demethylation, histone modification, and 3D structure are also prepared as standby states for expression from embryonic stages. Neuronal activity induces opening chromatin with multiple chromatin modifiers such as CBP/p300 and increases the binding time of TFs to the cis-elements or the number of repetitive binding of TFs by elevating the local concentrations of activated TFs and RNAPs, which rapidly leads to effective RNA synthesis. Abbreviations: CBP, CREB binding protein; CREB, cAMP response element binding protein; RNAP, RNA polymerase; TET, ten-eleven translocation protein.

such as pre- and post-synaptic protein expression and synaptic morphological changes [10,120,121]. However, the chromatin state is not permanently fixed. The different neuronal activity patterns induced by sensory or motor experience could form a new chromatin state [41,106,107] which may serve as the basis for future rounds of gene expression and play important roles in learning and memory [93,122,123].

Concluding remarks

Expression of a subset of genes is upregulated by neuronal activity. For this regulation, cis-elements, promoters, and enhancers, which are genetically determined as primary DNA

sequences, are inevitable. It is likely that pre-prepared histone modification is also required at the genome loci in an activity-independent fashion (Figure 4). In addition, a history of neuronal activity could affect the chromatin states. Neuronal activity increases the binding time of TFs to the cis-elements or the number of repetitive binding of TFs by elevating the local concentrations of activated TFs, which leads to effective RNA synthesis (Figure 4). These mechanisms of activity-dependent gene expression are important to understand how various sensory-evoked and spontaneous neuronal activities alter development and plasticity of the brain (see Outstanding questions).

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Declaration of interests

The authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT4 and DeepL exclusively for grammatical error correction and stylistic changes. Additionally, BioRender (www.biorender.com) was utilized for creating graphical illustrations. The authors thoroughly reviewed and edited the content following the use of these tools and take full responsibility for the final content of the publication.

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