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Spotlight

Epimetronomics: m6A Marks the Tempo of Corticogenesis

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Yoon et al. (2017) uncover a key role for the m6A RNA mark in regulating the timing of cerebral cortex development in mouse and human. This discovery opens new avenues of exploration into how the epitranscriptome helps orchestrate central nervous system formation.

The cerebral cortex plays a role in all higher brain functions, including social interactions, decision-making, behavioral output, and other complex behaviors. The development of the cerebral cortex is a symphony of precisely timed dynamic processes, including cell proliferation, differentiation, migration, process outgrowth, and connectivity. The principal progenitor cells in the cortex, the radial glia cells (RGCs), generate consecutive waves of neurons designated for different cortical layers, with the later-born neurons migrating past previously born neurons to eventually produce six distinct cortical laminae. The timing of this process is exquisitely regulated, and mistakes in the timing can have egregious consequences, resulting in mal-patterned cortex and contributing to disease (Marín, 2016). The molecular machinery governing the orchestrated timing of this complex process remains one of the major questions in neurodevelopment.

Prior work has shown that each cell stage in the corticogenesis process expresses a set of distinct transcripts, and that specific networks of transcription factors (TFs) drive production of each cortical layer. Pax6+ Sox2+ RGCs give rise to Tbr2+ intermediate progenitor cells (IPCs), which in turn produce neuronal progeny with distinct TF expression. For instance, *Bcl11b*, also known as CTIP2, promotes production of deep-layer subcortical projection neurons and *Satb2* represses *Bcl11b* to promote production of callosal neurons that predominantly arise later, being destined for more superficial layers. Now, new work by Yoon et al. has implicated a role for the epitranscriptome, marks on RNA, in regulating the timing of corticogenesis: they identify the N6-methyladenosine (m6A) RNA modification as the metronome that sets the pace of corticogenesis (Yoon et al., 2017).

RNA modifications have been demonstrated to engage in the gamut of cellular processes, ranging from roles in immunity and protein diversity to regulating protein synthesis and cell-cycle progression. The m6A mark, the most abundant internal modification of mRNA, was discovered more than 40 years ago. A renewed interest in the role of RNA modifications in regulating biological systems and the discovery of an m6A demethylase in 2011 have led to a resurgence in studies of m6A (Cao et al., 2016). Since 2011, multiple enzymes responsible for placing or removing the m6A mark have been revealed, increasing our understanding of m6A functions. The best-characterized role for m6A is to increase the turnover of mRNA by inducing instability of targeted mRNAs, but it has also been shown to affect translation and RNA splicing (Cao et al., 2016).

An intriguing role of the m6A modification is that it can control the timing of a biological process. For instance, m6A methylation of multiple mRNAs in the circadian clock pathway sets the pace of circadian rhythms. Loss of the methylase responsible for depositing these m6A marks greatly elongates the circadian period and misregulates the circadian clock (Fustin et al., 2013). In a developmental context, m6A has been linked to coordination of differentiation. Knockout in germ cells of *Mettl3*, an RNA methylase that creates the m6A modification, leads to stalling of meiosis, resulting in defective spermatogenesis (Xu et al., 2017). Pluripotent stem cell (PSC) studies have produced apparently conflicting results regarding the role for the m6A mark in both driving differentiation and maintaining pluripotency (Cao et al., 2016). These apparently paradoxical results were resolved by examining the transcriptomes of two distinct mouse PSC states: naive, which is skewed toward self-renewal, and primed, which is skewed toward lineage commitment (reviewed in Weinberger et al., 2016). In both cell states, the m6A mark was associated with genes driving differentiation and genes driving self-renewal, and when the mark was lost, the prevalent class of transcripts dominated the fate of the cell. So after loss of m6A marks, naive PSCs manifest a state of hyper-self-renewal, while primed PSCs lose self-renewal potential and become lineage committed (Cao et al., 2016). In both situations, the m6A mark regulates the rate of mRNA decay and balances self-renewal and differentiation appropriate to the PSC state. Here, in the subject of this spotlight, Yoon et al. bolster our understanding of m6A as a regulator of biological timing. They examined the impact of loss of *Mettl14*, which produces m6A, and discovered that this greatly extends the timing of cerebral cortex formation.

Yoon and colleagues began their study with a simple question: does the epitranscriptome, and in particular the m6A modification, play a role in corticogenesis? They first analyzed published single cortical progenitor cell RNA sequencing data for expression of genes regulating m6A deposition. They found that only *Mettl14* showed differential expression across the identified cell types, with RGCs having the highest expression. After creating a conditional knockout (cKO) of *Mettl14* in the developing cortex, they discovered a severe defect in the timing of corticogenesis. Notably, while RGCs

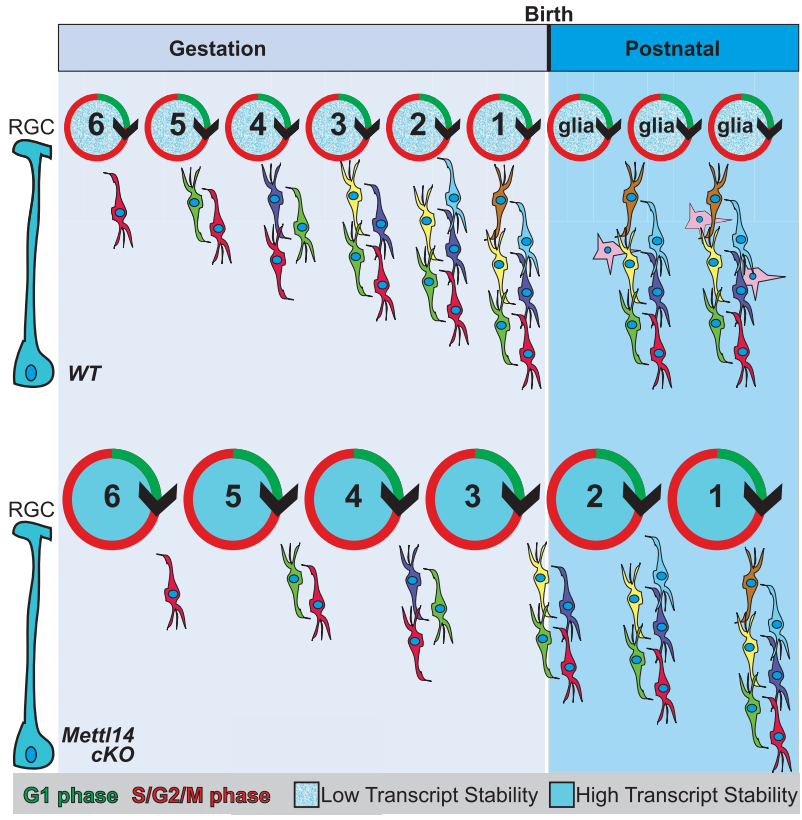

Figure 1. Loss of m6A Has Dual Effects that Delay Cerebral Cortex Development
Loss of m6A lengthens S/G2/M, which promotes progenitor proliferation and delays differentiation. Loss of m6A also decreases decay of RGC transcripts associated with stem cell maintenance, neurogenesis, and differentiation. Together, these lead to prolonged progenitor cell maintenance and delayed differentiation. Accumulation of pro-differentiation transcripts due to the loss of mRNA decay eventually drives RGCs to differentiate, albeit at a slower pace than in wild-type cells.

and neuron generation are normally extinguished by birth, in the *Mettle14* cKO, proliferating RGCs and neuron-producing IPCs still remain active postnatally.

A persistent neurogenic cortical germinal zone in the postnatal mouse could mean either that the progenitors had a differentiation defect or that there was a delay in corticogenesis. The postnatal *Mettl14*cKO mouse had similar numbers of deep-layer neurons, but a deficit of upper-layer neurons, suggesting a potential defect in differentiation. However, the embryonic *Mettl14*cKO cortex also showed a deficit in deep-layer neurons and the postnatal mice showed a decrease in glia, which are normally born after neurons, suggesting a potential delay. The m6A mark was previously shown to affect the proliferation of neural progenitors in the adult brain (Li et al., 2017) and the cell cycling of germ cells (Xu et al., 2017). Yoon et al. demonstrated that the cell cycle of *Mettl14*-deficient RGCs had lengthened, consistent with a delay in the progression of corticogenesis being the primary culprit rather than a differentiation block.

To better grasp how m6A was controlling the pace of corticogenesis, Yoon et al. used m6A sequencing to uncover the targets of m6A methylation. While they did find targets among genes controlling the cell cycle, a more provocative discovery was that many of the previously identified TFs regulating later cortical development were also targets of the m6A machinery. Increased levels of these transcripts were seen in early cortical progenitors in *Mettl14* cKO mice. Blocking mRNA decay produced similar results to losing *Mettl14*, indicating that the increased transcript levels were due to lack of mRNA decay and not to increased transcription. These results imply that lineage commitment genes are already expressed in RGCs, effectively pre-patterning their fate, but are normally subject to rapid decay via m6A modification. As with PSCs, the prevalent class of extant transcripts within RGCs, the sum of production and degradation, pro-proliferation and pro-differentiation, appears to determine their fate.

Collectively, these experiments illustrate a role for the m6A mark in the temporal regulation of corticogenesis in the mouse. Recent insights into human brain development have highlighted the differences between human and mouse (Bae et al., 2015), prompting Yoon and colleagues to examine the role of m6A in a human model system. Utilizing organoids derived from human induced PSCs, they examined the effect of knocking down *METTL14* and found that loss of m6A decreased the proliferation of forebrain progenitors, consistent with their observations in the mouse. Then, using m6A sequencing, they examined the targets of m6A modification in the forebrain organoids and in fetal tissue and compared these to the mouse targets. The human samples had many more transcripts marked by m6A than the mouse. Nevertheless, there was a significant overlap of targeted genes involved in neurogenesis and cortical development in mouse and human, supporting a similar role for m6A in regulating the timing of corticogenesis in both species. Intriguingly, the m6A targets specific to the human samples were enriched for genes linked to many nervous system diseases including mental disorders, implicating a potential role for epitranscriptomic misregulation in disease etiology. This experiment exemplifies the insight into brain development and disease development that can be gained using human organoid model systems in conjunction with mouse experimental systems.

Putting the work of Yoon and colleagues into context, a complex picture of the temporal regulation of corticogenesis emerges (Figure 1). The m6A modification governs the tempo of corticogenesis by two separate but potentially interconnected mechanisms: increased cell-cycle length and decreased mRNA decay. Prior work has shown that the period of time in a given phase of the cell cycle is a key determinant

of neural progenitor cell fate, establishing the "cell-cycle length hypothesis" (Hardwick et al., 2015). A G1 phase longer than a certain threshold is required to elicit differentiation, thought to be due to the extended period for neurogenic factor accumulation. RGCs in the *Mettl14* cKO mice had an elongated S/G2/M phase, which is expected to increase proliferation rather than differentiation (Hardwick et al., 2015), which could contribute to the extended germinal period. Given that, as a proportion of the entire cell cycle, G1 is effectively shortened, how is the threshold for differentiation eventually reached? A plausible answer is that concomitant reduction in mRNA decay through reduced m6A marks serves this purpose, leading to a gradual buildup of pro-differentiation factors that eventually tips the balance. The *Mettl14* cKO mice do have increased numbers of RGCs with detectable levels of pro-neuronal protein expression, supporting this explanation.

In this study, Yoon and colleagues have helped shed light on a central mystery of cerebral cortical development by identifying m6A modification as the metronome setting the pace of corticogenesis. Like all good answers, it leads to even more questions. How does the cellular machinery target m6A to the correct transcripts? Which of the m6A targets are key to regulating the tempo of corticogenesis? And could delving into this mechanism explain the dramatically different gestational periods of human and mouse corticogenesis? Answering these questions will be essential next steps in understanding brain development more completely and the role that the epitranscriptome plays to orchestrate development and instigate disease processes.

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