pharmacological inhibitors of transcription and translation during constant light [10]. However, circadian rhythm in PRX sulphonylation in constant light is blocked by inhibition of the proteasome, establishing the necessity of proteasomal function to rhythmicity [4]. In the dark, however, transcription ceases. Under these conditions, proteasomal inhibition failed to block rhythmic PRX sulphonylation, indicating that proteasomal degradation was necessary for rhythmicity only under conditions in which protein synthesis persisted. However, inhibitors of other post-translational modifications had similar effects on the period of PRX sulphonylation as they did in the light [9]. This argues that the transcription/translation feedback loop (TTFL) and the post-translational feedback loop (PTFL) are normally tightly coupled under physiologically relevant conditions. However, in the abnormal and stressful condition of extended dark, encountered perhaps when *O. tauri* cells are carried deeply into the water column, the transcription/translation feedback loop is absent due to the cessation in transcription. The cessation of transcription is presumably a survival mechanism to endure a period of energy starvation. Nonetheless, the persistence of the post-translational rhythm in PRX sulphonylation suggests that there is still a survival value associated with rhythmicity, presumably in coordinating metabolism in these near-dormant conditions [4].

PRX proteins are widely distributed among taxa. Apparently rhythms in PRX sulphonylation are similarly widespread, because PRX proteins exhibit a robust circadian rhythm in PRX sulphonylation in human red blood cells [12]. This is a striking result, because human red blood cells lack nuclei and so are incapable of transcription. Of course, the demonstration that the cyanobacterial KaiA, KaiB, and KaiC proteins together with ATP are sufficient to reconstitute a robust temperature-compensated *in vitro* rhythm in KaiC phosphorylation had already established that rhythmicity was possible without transcription and translation [13], but now this has been extended to two eukaryotes of quite distinct lineages. Interestingly, 50 years ago it was observed that circadian rhythms in photosynthesis persist in enucleated Acetabularia major and *A. crenulata* [14] and almost 40 years ago a rhythm in respiration was reported in dormant onion seeds [15]. Obviously, these multiple observations of circadian rhythmicity without de novo transcription in cyanobacteria, *Acetabularia, O. tauri*, onions, and humans fully refute the general necessity of transcription for circadian clock function.

Are there two circadian clocks present in most cells, one based on transcription/translation feedback loops and one based on transcription/translation-independent mechanisms (Figure 1)? Certainly there are multiple examples of circadian rhythmicity in genotypes in which the known transcription/translation feedback loop mechanism is disrupted [16]. Although it seems premature to claim the ubiquity of these two types of clocks, it nonetheless seems likely that the exploration of the interaction between these two clock mechanisms is likely to offer important insights. The implications of these two types of clocks for the evolution of circadian rhythmicity are profound.

### References


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### Synaptic Growth: Dancing with Adducin

Manipulations of the actin-capping protein adducin in *Drosophila* and mammalian neurons provide new insights into the mechanisms linking structural changes to synaptic plasticity and learning. Adducin regulates synaptic remodeling, providing a molecular switch that controls synaptic growth versus disassembly during plasticity.

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Developing neural circuits are often highly plastic and not only form new synaptic contacts, but also eliminate unnecessary or redundant synapses. Once the brain has matured, extensive remodeling of circuits is rare, but connections between neurons can be modified in an activity-dependent fashion as well as in response to injury or disease [1]. Alterations of synaptic connections are hypothesized to underlie learning and memory and can occur through several mechanisms. The strength of a synapse can be increased or decreased by changing the properties of presynaptic release or
by altering the postsynaptic response to neurotransmitters. The addition or removal of contacts between neurons can also modify the strength of a connection or the wiring of a circuit. Remodeling of the underlying actin cytoskeleton plays a role in altering structural features of connectivity, including synapse formation and retraction. In a recent issue of Neuron, two papers by Pielage et al. [2] and Bednarek and Caroni [3] examined how adducin, a regulator of the actin cytoskeleton, controls synaptic stability and improves memory upon environmental enrichment.

Actin has the ability to alter synaptic function and plasticity through a variety of mechanisms. Presynaptically, actin plays a role in controlling the recruitment of synaptic vesicles from the reserve pool to the recycling pool [4]. Actin also modulates the recovery of synaptic vesicles via endocytosis after neurotransmitter release [5].

Postsynaptically, actin can affect the insertion or removal of AMPA receptors during long-term potentiation (LTP) or long-term depression (LTD) [6,7].

Dendritic spine formation and retraction are also dependent on structural rearrangements of the actin cytoskeleton. Therefore, regulation of actin polymerization, as well as the interactions between actin and other cytoskeletal and structural proteins, is important for a variety of processes that potentially contribute to learning and memory. Adducin controls actin polymerization by capping the fast-growing ends of actin filaments and promoting the interaction of actin with the cytoskeletal protein spectrin [8,9]. As such, adducin is capable of integrating actin fibers into other cellular structures, such as the spectrin network, by recruiting spectrin to the ends of actin filaments. Loss of other actin-capping proteins has been shown to promote the growth of actin-rich filopodia in culture [10,11], while overexpression of the actin-binding domain of β-spectrin in dendritic spines can stabilize actin filaments by inhibiting depolymerization, reducing the morphological plasticity of spine heads [12]. Regulation of adducin activity could therefore alter both the stability and the morphology of synapses.

Mammalian genomes encode three closely related adducin proteins termed α-, β- and γ-adducin. These proteins form tetramers of either α/β or α/γ heterodimers. The α- and γ-adducins are ubiquitously expressed, while β-adducin is enriched in erythrocytes and the brain, where it is present in dendritic spines and growth cones [13]. Drosophila has a single adducin homolog named Hu-li tai shao (Hts). The hts locus encodes four potential isoforms, one of which, Hts-M, is expressed in the larval brain [2]. Adducins contain a highly conserved carboxy-terminal region similar to the myristoylated alanine-rich C kinase substrate (MARCKS protein). The MARCKS-related domain is required but not sufficient for the actin-capping and spectrin-recruiting activities of adducins [13]. This region appears to be a key regulatory domain, with binding sites for protein kinase C (PKC), protein kinase A (PKA) and calcium–calmodulin [13], all of which play critical roles in synaptic plasticity (Figure 1A).

Figure 1. Organization and regulation of adducin function.

(A) Schematic of an adducin monomer, which contains a globular head region, a neck region required for oligomerization, and a tail region containing the MARCKS-related domain. Several key phosphorylation sites are indicated by arrows. (Modified from [13,20].) (B) Adducin caps the fast-growing end of filamentous F-actin and prevents the addition of monomeric G-actin. Environmental enrichment and long-term facilitation promote the phosphorylation of adducin by PKC, causing the dissociation of the actin–spectrin complex and inhibiting actin capping, thereby destabilizing the filament. (Modified from [13,20].)
Adducin deletion
Wild-type NMJ
Type Ib bouton
Type II/III bouton
Retracting type Ib bouton
Actin-rich protrusion
Presynaptic adducin overexpression

Figure 2. Adducin modulates synaptic growth at the Drosophila larval NMJ. Deletion of Hts-M/adducin results in an overgrowth of large-diameter type Ib boutons, as well as an increase in synaptic retractions and the appearance of actin-rich protrusions at the Drosophila larval NMJ. Presynaptic overexpression of Hts-M/adducin inhibits the formation of small-diameter type II and type III boutons.

The decreased stability of synapses in mouse and fly adducin mutants implies that synaptic plasticity and learning may also be affected. Indeed, the β-adducin knockout mouse has defects in hippocampal LTP and LTD, as well as deficits in several learning assays [15,16]. Furthermore, the loss of β-adducin eliminates many of the benefits of environmental enrichment, during which animals are exposed to additional sensory, social and motor stimuli relative to standard housing conditions. The β-adducin knockout mice raised under enriched conditions show the expected increase in dendritic spine numbers in the CA1 region of the hippocampus, but lack a concomitant increase in the number of functional synapses [3]. Bednarek and Caroni [3] found that β-adducin knockout mice raised in standard housing conditions had no defects in contextual fear conditioning or novel object recognition. When raised under enriched conditions, however, the mutant mice had levels of learning below those of mice raised under standard conditions. These deficits may not be merely a result of altered LTP, as mice lacking Rab3A, a synaptic protein required for LTP in hippocampal mossy fibers, show improved learning under enrichment conditions [17].

To understand how adducin can affect synaptic stability it is important to determine how adducin activity itself is regulated. PKC phosphorylation of the MARCKS-related domain inhibits the actin-capping and spectrin-recruiting activities of adducin in both mammals and invertebrates [13] (Figure 1B). In Aplysia, phosphorylation of adducin by PKC occurs during serotonin-induced long-term facilitation between sensory and motor neurons — a process that is associated with structural changes at synapses [18]. At the Drosophila larval NMJ, Hts-M is phosphorylated in the more dynamic type II and type III boutons, but is primarily dephosphorylated in larger type Ib boutons. This observation may explain the differences in dynamics seen between small- and large-caliber boutons at the NMJ in the study by Pielage et al. [2]. Phosphomimetic mutations in the MARCKS-related domain in Drosophila Hts-M result in increased synaptic accumulation of the protein, suggesting additional control at the level of adducin trafficking. In mice, adducin phosphorylation by PKC occurs upon environmental trafficking and is required for synapse disassembly [3]. Together, these results imply that phosphorylation of adducin by PKC leads to a loss of rigidity in the cytoskeleton, allowing synapses either to assemble or to disassemble. These structural changes are critical for modifications associated with learning and memory. Adducin activity can also be modified by PKA, Rho kinase, Fyn kinase, as well as calcium–calmodulin, all of which have been implicated in learning and memory [13,19].

These two recent studies add to the growing body of evidence that adducin is a key regulator of synaptic stability. Through PKC phosphorylation, adducin can act as a switch to control critical steps of synapse disassembly and reassembly that occur during the rearrangement of neural connections. In the future, it will be interesting to determine to what extent adducin is involved in the initial establishment of neural circuits, as well as the relative contributions of adducin activity pre- and postsynaptically.

References


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