The synthesis of wall polymers, such as glucan, must also be ordered to couple bud growth with the cell cycle.

A small Ras-like guanosine nucleotide binding protein yeast Rho1p serves as a morphogenic coordinator. Such small guanosine triphosphatases (GTPases) act as molecular switches in many contexts, although most participate in signal transduction cascades that contain heterotrimeric GTP-binding proteins (G proteins) and protein kinases. Rho1p itself acts downstream of the G protein cascades for bud site selection (*3*) and it also activates a protein kinase, but now a long-anticipated downstream target involved in bud construction—glucan synthase—has been identified.

Prenylated Rho1p colocalizes with cortical actin to the inner surface of the plasma membrane at the growing bud tip and at the mother-daughter neck at cytokinesis (*4*). *RHO1* is an essential gene, and if it is absent, the cells stop growing when they have only tiny buds, which are bounded by cell walls that burst at their tips. One reason for this phenotype is that Rho1p is required to activate yeast protein kinase C, Pkc1p, which activates a mitogen-activated protein kinase signal transduction pathway required for cell wall integrity (*5*, *6*). But Rho1p has an additional function in cell wall biogenesis. The two new papers (*1*, *2*) show that activated GTP-bound Rho1p is a positive regulatory subunit of β(1→3)glucan synthase, the enzyme responsible for much of cell wall glucan synthesis. Although still incompletely characterized, β(1→3)glucan synthase contains two related, integral membrane protein subunits, Fks1p and Fks2p (*7*). Biochemical studies indicate that glucan synthase activity is stimulated by GTP, likely through a G protein (*8*). Rho1p is now identified as this activator, and Fks1p is shown to colocalize with Rho1p at the tip of growing buds and at the mother-daughter neck at cytokinesis. Thus, Rho1p regulates glucan synthesis so that it occurs at sites of wall growth and remodeling (see the figure).

Humans have a Rho1p homolog called RHOA; this GTPase is 72% identical to its yeast counterpart, and it is able to partially substitute for Rho1p in yeast (*9*). One role for RHOA in mammals is to regulate the formation of actin stress fibers, structures that emanate from small patches of the plasma membrane called focal adhesions, which allow the cytoskeleton to pull against the extracellular matrix and alter cell morphology. At these adhesions, integrin receptors bind to extracellular matrix proteins. These focal adhesions are formed in a RHOA-dependent manner in response to growth factors and phospholipids (*10*). How do stress fibers form? As shown in the figure, activated GTP-bound RHOA can bind to and activate protein kinases, including one called Rho-kinase, and may trigger a transduction cascade that ultimately results in stress fiber formation.

These provocative findings prompt an attempt to integrate the roles of Rho1p and RHOA in modulating polarized cell surface assemblages in yeast and mammals. Whether RHOA activates extracellular matrix polysaccharide biosynthesis in mammals is unknown. However, focal adhesion formation is triggered by extracellular signals, and human RHOA has partially retained the ability to replace Rho1p in yeast (*9*). Thus, RHOA may also promote ordering of the mammalian cell surface with the actin cytoskeleton at these focal contacts, possibly through the integrin or ERM-CD44 complexes (*11*). Pursuing this comparative theme, a bud could be viewed as a singular, specialized focal adhesion that consolidates its location by spinning an external glucan net.

There is still much to be learned; we do not fully know how Rho1p is localized or regulated, or how it acts as a multifunctional switch. The Rho subfamily of G proteins has five known members in yeast, some of which are required for bud growth (*12*). These may also activate other downstream targets effecting morphogenesis, such as localized cell wall chitin deposition. A process that can be recruited to form structures as apparently disparate as a yeast bud and a mammalian focal adhesion is likely to participate in creating an even wider range of cellular form. Rho1p and RHOA and their kind seem worth examining in other eukaryotes as regulators linking cellular polarity to morphogenesis.

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Arousal: Revisiting the Reticular Activating System

Mircea Steriade

Physiologists and clinical neurologists of the 18th and 19th centuries were aware that a structure located deep within the brain controls mental alertness. In the last few decades, neuroscience has defined this arousal system more clearly: It originates in the upper brainstem reticular core and projects through synaptic relays in the thalamus to the cerebral cortex, where it increases excitability (see figure) (*1*).

This arousal system was postulated to contain neurons with extensive dendritic and axonal domains that are driven by virtually all sensory modalities and have widespread projections to many cerebral structures (*2*). However, this concept fell into desuetude for more than three decades, because the connectivity and chemical codes of the hypothesized neural paths were unknown. In the past 10 to 15 years, the brainstem to cortex circuit, relayed through intralaminar and other thalamic nuclei (see figure), has been identified, and the main neurotransmitters (acetylcholine and glutamate) and their actions have been defined (*3*). Further, humans participating in a task requiring alertness and attention display increased regional blood flow (a measure of neuronal activity) in the midbrain reticular formation (MRF) and thalamic intralaminar nuclei (*4*); conversely, individuals with bilateral lesions of thalamic intralaminar nuclei are lethargic or somnolent (*5*). Now, two reports in this issue (*6*, *7*) describe how this arousal system changes the excitability of the cortex.

Ironically, this far-reaching concept of an ascending reticular system governing arousal was formulated on the basis of electroencephalographic (EEG) recordings in experiments on anesthetized animals in which the behavioral state did not change (*2*). In these initial experiments, MRF stimulation caused low-voltage or flattened EEG waves (*2*), similar to those of awake animals, suggesting an unexpected decrease in brain electrical activity (see figure, top). The epigones often used the term "desynchronization" to describe the EEG correlate of wakefulness [as well as that of rapid eye

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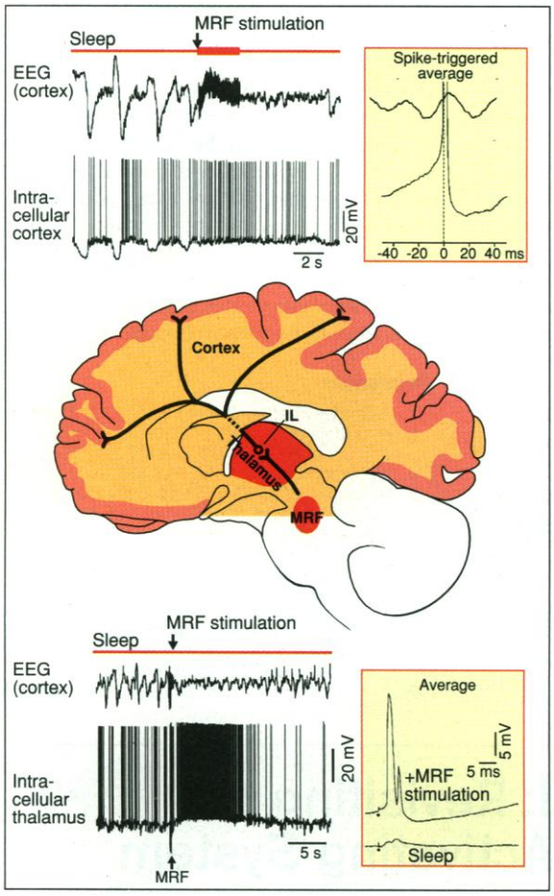

Arousal elicited by the brainstem-thalamic-cortical activating system. A parasagittal section of the human brain. Recordings are from in vivo experiments on cats. (**Top**) Simultaneous recording of cortical EEG and intracellular activity of a motor cortical neuron. The slow sleep oscillation (four cycles) was disrupted by high-frequency stimulation (horizontal bar) applied to brainstem cholinergic nuclei in the caudal part of MRF. At right, enlarged view of the intracellularly recorded action potentials and 30-Hz EEG waves during MRF-induced activation period (spike-triggered average). (**Bottom**) EEG and intracellular recordings, with test stimuli every 1 s, during sleep and MRF-induced (arrow) activation. At right, averaged intracellular responses show increased probability of responses, resulting in cell firing, during the activated period (top) and subthreshold responses during the sleep period (bottom); IL, intralaminar nuclei

movement (REM) sleep]. The desynchronization is now known to be more apparent than real: Electrophysiological recordings from cortex (and also thalamus) show that, although large slow waves disappear upon waking, smaller, fast spontaneous rhythms (30 to 40 Hz) that outlast MRF stimulation are actually enhanced and synchronized within intracortical and corticothalamic networks by MRF stimulation (*8*) (top recordings in figure). Thus spontaneous, fast oscillations in the membrane potential may underlie the coherent responses of cortical and thalamic neurons to messages from the outside world during waking or to internal messages during dreaming sleep (*8*). Indeed, in one of the new reports (*6*), Munk and his colleagues show that in lightly anesthetized animals, MRF stimulation facilitates synchronization among spatially separated pools of cortical neurons in response to visual stimuli. The occurrence of coherent discharges was not simply a result of increased firing rates. Interestingly, the MRF stimulation did not alter the specificity of cortical responses to different configurations of visual stimuli, suggesting that the increased synchronization of responses does not impair the selectivity for stimulus properties.

Even though MRF stimulation has such activating effects on coherent and stimulus-specific responses, it does not impair, and may even enhance, local inhibitory operations in the cerebral cortex (*9*, *10*).

Short-term plasticity, another aspect of cortical information processing, is also influenced by the arousal system. In the second report, Castro-Alamancos and Connors (*7*) show that a type of plasticity in the cortical neurons [a progressively larger response called the augmenting response, known since the 1940s (*11*)] elicited at 8 to 12 Hz is modulated by the level of vigilance and suggest that this state-dependent change in the responses may be related to short-term plasticity processes in the cortex. They propose that cortical augmenting responses are initiated in cortical pyramidal cells located in layer V when a second thalamic stimulus, delayed by 50 to 200 ms from a first stimulus, is delivered during the inhibitory postsynaptic potential produced by a first stimulus. In another example of modulation of plasticity by behavioral state, brief stimulation of the brainstem cholinergic nuclei not only enhances the coherence and probability of thalamic and cortical responses to an incoming message (see figure, bottom), but also potentiates the excitability of thalamic neuronal targets for up to 4 min, without disrupting the local inhibitory processes required for analytical processing (*12*).

The ascending brainstem reticular system potentiates thalamic and cortical responses not only during waking, but also during REM sleep, a state that electrophysiologically is virtually identical to waking (although thought is logical and can be stored in memory during wakefulness, whereas dreaming consciousness is characterized by bizarre imagery and illogical thoughts that are not retained in memory). Stimulation of the brainstem at the mesopontine junction, where cholinergic nuclei are located, gives rise to sharp waves that are similar to spontaneously occurring ponto-geniculo-occipital (PGO) potentials, stigmatic signs of REM sleep that may be the physiological correlate of tempestuous dreaming mentation. The brainstem-elicited PGO potentials are followed by synchronized fast oscillations of 30 to 40 Hz over a time window of about 600 ms (*8*). The widespread synchronization during dreaming sleep of fast (40 Hz) waves over the cerebral cortex (*13*) may be accounted for by the progressive synchronization of PGO potentials in REM sleep, giving rise to fast oscillations (*8*, *14*).

It is encouraging that the concept of brainstem activation of the cortical processes has been rescued from oblivion and substantiated (*3*, *4*, *6*–*8*). Future work should address the interactions occurring in arousal between the brainstem-thalamic-cortical system and extrathalamic activating systems and their neurotransmitters. These studies will need multiple-site, extra- and intracellular recordings in preparations with a rich behavioral repertoire, including attention-requiring tasks.

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