Vesicle Trafficking: A Rab Family Profile

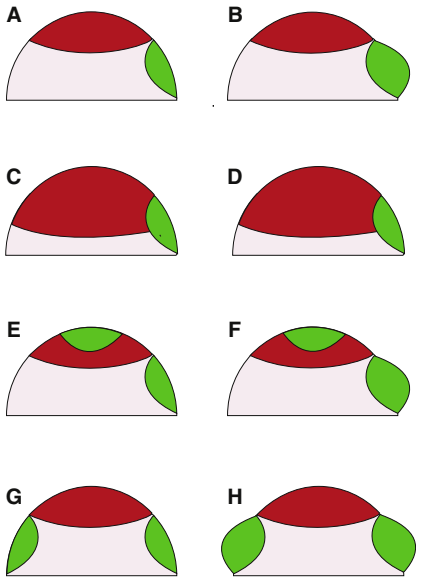

Figure 1. Schematic diagram of the role of tissue mechanics in organ initiation

(A) The meristem is partitioned into a central zone characterised by a relatively stiff extracellular matrix (red), surrounded by a peripheral zone of relatively pliant material (beige). Regions within the peripheral zone are demarcated for organ initiation (green) by an auxin-based patterning system. As a consequence of cell wall loosening in this region, morphogenesis occurs (B). If the central region of tissue stiffness extends into the flanks of the meristem (C), then, despite the presence of the endogenous signals for leaf initiation, morphogenesis does not occur since the downstream cell wall effectors cannot overcome the preset local tissue mechanics (D). Similarly, if auxin signaling is ectopically induced in the central zone (E), the local tissue stiffness blocks morphogenesis (F). In contrast, ectopic signaling in the peripheral zone (G) leads to cell wall loosening and ectopic leaf initiation (H).

extracellular matrix, rather than the epidermis, are important for the system to function, whereas other work in this area has suggested the opposite [10,11]. The mechanical interactions of cell layers are liable to be complex and trying to define linear cause and effect may be too simplistic an approach, with the meristem being set up as a truly integrated system. The further application of tools such as atomic force microscopy will hopefully provide more data to provide a deeper insight into this issue. A second surprise is that the stiffness response of the tissue to altered pectin methylation status was the opposite of that expected from extant models; decreased pectin methylation is expected to make the extracellular matrix stiffer, not more pliant. Although we have extensive data on the composition of the plant cell wall, our understanding of how these components fit together and how they influence the mechanical properties of the matrix is largely based on models that still need to be stringently tested [12].

Finally, much of developmental biology has viewed the process of morphogenesis as a one-way process (gene transcription leading to form), but there are a number of lines of evidence indicating that feedback loops must occur so that the transcriptional apparatus is itself sensitive to and modulated by the physical stresses and strains that underpin morphogenesis [13,14]. These ideas are most advanced in animal development and differentiation [15,16], but the field is now opening up for plant biologists working at the interface of developmental mechanics to explore this area and to close the loop of genetic regulation and morphogenesis.

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Vesicle Trafficking: A Rab Family Profile

A new tool-kit has been developed for profiling expression and function of Rab GTPases on a genome-wide scale. Use of this tool-kit has revealed unexpectedly that at least half of *Drosophila* Rabs have neuronal-specific expression patterns and localize to synapses.

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Vesicle trafficking between compartments is essential for cellular function and intercellular communication. Many distinct steps during trafficking — including cargo sorting, vesicle transport, targeting,

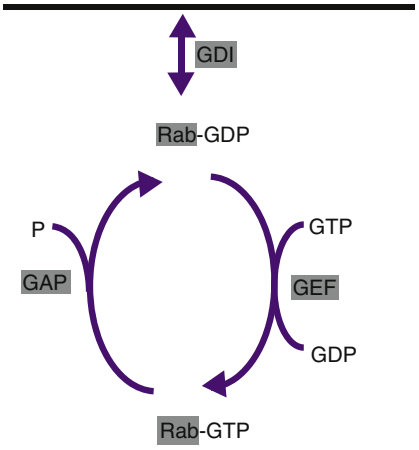

Figure 1. Rab GTPases act as molecular switches.

Rabs are inactive when bound to GDP and are activated by exchanging GDP for GTP. GTP can then be cleaved by the endogenous GTPase activity of the Rab to produce the GDP-bound form. Changes in activation state are catalyzed by guanine nucleotide exchange factors (GEFs), which promote the exchange to GTP, and GTPase-activating proteins (GAPs), which promote GTP cleavage. Guanine nucleotide dissociation inhibitors (GDIs) interact with Rabs by inhibiting GTP hydrolysis by the GTPase, and by regulating the membrane association of the Rab.

tethering, vesicle formation and vesicle fusion — are regulated by specific members of the Rab family of small GTPases [1–3]. Switching of Rabs from GDP- to GTP-bound states provides on/off switches that regulate multiple steps in the life cycle of a vesicle (Figure 1). In neurons, where neurotransmitter release places increased demands on the membrane trafficking system, Rabs are thought to be of particular importance [1,4,5].

The genome of the fruitfly *Drosophila* includes 31 *rab* or *rab*-like genes, the majority of which have clear orthologs amongst the >75 vertebrate Rabs [6]. Studies characterizing *Drosophila* Rab proteins support a tight conservation from flies to mammals with respect to Rab function and localization [6–10]; however, only a handful of Rab family members have been characterized *in vivo*.

As they report in this issue of *Current Biology*, Chan *et al.* [11] have generated a tool-kit for characterizing the expression pattern and function of the Rab family in *Drosophila*. The authors cloned a large genomic region surrounding each *rab* gene, with the aim of capturing all regulatory elements within the genomic fragment. The authors then replaced the open reading frame of the *rab* with that for yeast transcription factor Gal4, creating a reporter cassette that would express Gal4 under the control of that *rab* gene's regulatory elements. For some Rabs, only the start site and first exon were replaced, if removing the entire open reading frame was deemed likely to remove regulatory sequences. These Gal4 knock-in cassettes were inserted in a landing site in the *Drosophila* genome, creating 'driver' lines that can direct expression of constructs downstream of a UAS promoter. The authors were able to create Gal4 knock-ins for 25 *rab* loci, allowing for a detailed comparison of expression patterns across the Rab family.

Strikingly, they found that about half of the Rabs are expressed either exclusively or predominantly in neurons. The other Rabs appear to be more ubiquitous, being expressed in a variety of neuronal and non-neuronal cell types, and not surprisingly these include common endosomal compartment markers such as Rab5, Rab7 and Rab11. These findings support a key role of trafficking regulation by Rabs in neuronal function. Furthermore, the neuronally-enriched Rabs are expressed in distinct subsets of neurons, suggesting the existence of diverse mechanisms of trafficking regulation amongst neuronal cell types [11].

To characterize the subcellular distribution of Rab proteins, the authors overexpressed YFP-tagged Rabs [6] under the control of their own regulatory elements. This analysis revealed that Rabs that are specifically expressed or strongly enriched in neurons typically localize to synapses [11]. In contrast, ubiquitously expressed Rabs typically localize to both the cell body and synapse, or just the cell body. The synaptic Rabs colocalize with a variety of compartment markers (Figure 2), including the early endosomal marker Rab5, the late endosomal marker Rab7, and the synaptic vesicle marker cysteine string protein (CSP).

Interestingly, most synaptically-enriched Rabs colocalize with the recycling endosome marker Rab11, often causing an enlargement of this compartment [11]. These findings provide a glimpse at the diverse functionality of Rabs at the synapse. Coupled with the varied expression patterns of Rabs across neuronal subtypes, it will be fascinating to dissect the intersecting functionalities and redundancies of the Rab family of proteins in *Drosophila* neurons. For example, each of the seven Rabs that colocalize with Rab11 has a distinct neuronal expression profile. This may reflect a deep redundancy, or perhaps, specialized mechanisms in different cell types.

What makes this Rab tool-kit particularly appealing is the inclusion of a gene-targeting cassette [11]. The authors developed a recombineering vector (P[acman]) [12] that contains ends-out homologous recombination sequences [13]. This allows for the Gal4 knock-ins to be mobilized *in vivo* and incorporated into the endogenous locus, replacing the Rab in question with Gal4. The ability to systematically produce knock-outs for 25 of the *Drosophila rab* genes will prove an invaluable tool for fully characterizing the function of the Rab family *in vivo*. Furthermore, such knock-out lines will contain Gal4 within the genomic locus, allowing other constructs to be expressed in the knock-out under that gene's regulatory elements.

As a proof of principle, the authors produced and characterized a knock-out of Rab27 ($Rab27^{Gal4\text{-}KO}$). Rab27 localizes to synaptic vesicles and is found specifically in mushroom bodies [11], a region of the brain implicated in learning, memory and sleep in *Drosophila* [14–17]. Behavioural testing reveals that $Rab27^{Gal4\text{-}KO}$ flies exhibit a specific sleep phenotype, where they have a reduction in sleep-bout length during daytime. These findings demonstrate a remarkably specific function for Rab27 in the brain that is supported by its cell-specific expression pattern.

The diversity of cell-specific expression patterns exhibited by neuronal Rabs leads to several fascinating questions. To what extent is each Rab versatile or redundant? Is each Rab's role essential or modulatory? One can argue that most Rabs appear to be modulatory given that expression of dominant negative Rabs rarely results in a loss of neuronal viability [11]. Ideally, the eventual production and analysis of knock-outs of each *rab* will provide even stronger evidence of this. But insight into such questions might also come from an analysis with an inverted focus — that is, to begin with a cell type and define its Rab profile. Increasingly, cross-talk

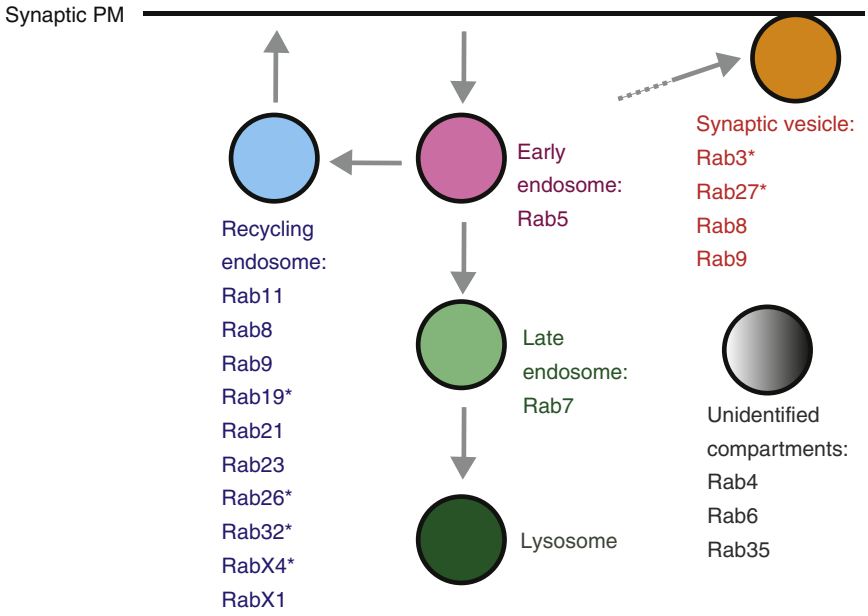

Figure 2. Synaptic localization of Rabs.

At synapses, Rab family proteins decorate several vesicular compartments, including synaptic vesicles, early endosomes, late endosomes and recycling endosomes. A majority of synaptic Rabs label Rab11-positive recycling endosomes. Some Rabs do not colocalize with any of the markers used in this study. Asterisks indicate neuronal-specific Rabs. PM, plasma membrane.

between Rabs is thought to be an important part of their regulation, for example through sharing of effector molecules [18]. Thus, understanding the complement of Rabs expressed in a given cell, and then having the tools to knock out or misexpress each or all of them in that cell, will help to clarify how Rabs cooperate to modulate the trafficking machinery in a specific biological context.

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Animal Navigation: Following Signposts in the Sea

The directional responses of turtles to simulated magnetic coordinates of positions in the sea have given insight into the turtles' route-like and map-like behaviour.

Thomas S. Collett[1] and Matthew Collett[2]

Many young animals embark on long migratory journeys with only inherited instructions to guide them. Such migrations are often seasonal and oriented roughly along a North–South axis. As the instructions will have taken many generations to evolve, the guidance cues that the instructions exploit must be long-lasting and, of course, must operate over long distances. The known cues are either astronomical or geophysical. For example, Monarch butterflies born in late summer migrate southwards from North America to over-wintering sites in Mexico [1]. Their direction is guided at least in part by a time-compensated sun compass [2,3]. Indigo buntings, migrating southwards at night, set their direction of flight by constellations around the North Star [4]. Such