Vesicle Trafficking: A Rab Family Profile


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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 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.

References

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,
Rabs are inactive when bound to GDP and are activated by exchanging GDP for GTP. GTP can then be cleaved by the endogenous GDP- to GTP-bound states provides GTPases [1–3]. Switching of Rabs from members of the Rab family of small fusion — are regulated by specific tethering, vesicle formation and vesicle hydrolysis by the GTPase, and by regulating (GDIs) interact with Rabs by inhibiting GTP factors (GEFs), which promote the exchange Guanine nucleotide dissociation inhibitors (GDIs) interact with Rabs by inhibiting GDP hydrolysis by the GT Pase, and by regulating the membrane association of the Rab.

Figure 1. Rab GTPases act as molecular switches.

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 (Rab27Gal4-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 Rab27Gal4-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...
**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. Collett¹ and Matthew Collett²**

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 orientation roughly along a North–South axis. As the instructions will have taken many generations to evolve, the

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### References


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