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Meeting report – Arf and Rab family G proteins

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A FASEB Summer Research Conference entitled 'Arf and Rab family G proteins' was held in July 2013 at Snowmass Village, Snowmass, Colorado. Arfs and Rabs are two families of GTPases that control membrane trafficking in eukaryotic cells, and increasing evidence indicates that their functions are tightly coordinated. Because many workers in this field have focused on only one family, this meeting was designed to integrate our understanding of the two families. The conference was organized by Elizabeth Sztul (University of Alabama, Birmingham, USA) and Jim Casanova (University of Virginia, Charlottesville, USA), and provided an opportunity for ~90 scientists to communicate their work and discuss future directions for the field. The talks highlighted the structural, functional and regulatory properties of Arf and Rab GTPases and the need to develop coordinated approaches to investigate them. Here, we present the major themes that emerged from the meeting.

Coincidence detection provides spatial and temporal regulation to GTPase activity

The activation and inactivation of GTPases requires guanine-nucleotide-exchange factors (GEFs) and GTPase-activating proteins (GAPs), respectively. Accumulating evidence indicates that most, if not all, GEFs and GAPs are autoinhibited in the cytosol and are activated upon recruitment to a membrane. One of the best-understood autoinhibitory mechanisms is the masking of the catalytic domain by an adjacent pleckstrin homology (PH) domain. Interaction of the PH domain with membrane components (phospholipids and/or proteins) relieves this inhibition, resulting in local site-specific activation. David Lambright (University of Massachusetts, Worcester, MA, USA) described the activation of the cytohesin/ARNO family of Arf GEFs through simultaneous recognition of two membrane components: an activated Arf and a phosphoinositide. Structural studies showed that simultaneous binding causes a major conformational shift that displaces the PH domain and frees the catalytic Sec7 domain for interaction with its substrate, Arf-GDP. His data support a positive-feedback model in which the product of cytohesin activation (Arf-GTP) further stimulates cytohesin activity.

In contrast, structural data presented by Jacqueline Cherfils (CNRS, Gif-sur-Yvette, France) showed that the PH domain of a different Arf GEF, BRAG2, is not autoinhibitory; instead it is part of the catalytic mechanism, potentiating nucleotide exchange in the presence of negatively charged lipids. Thus, although they act through distinct mechanisms, the PH domains of cytohesins and BRAG2 control Arf activation through recognition of specific membrane microenvironments.

Many Arf GAPs are also regulated by their PH domains, and Paul Randazzo (NIH, Bethesda, USA) presented a new regulatory mechanism for ASAP1. In this case, autoinhibition is mediated by an N-terminal BAR domain, and is relieved upon binding to an appropriate membrane. ASAP1 also contains a PH domain, and analogous to the role of the PH domain in the GEF BRAG2 (see above), binding of PtIns(4,5)P_2 results in a conformational change that allows the PH domain to present the substrate (ARF1-GTP) to the ASAP1 GAP domain.

A different form of autoinhibition is found in a large family of Rab GEFs, the DENN domain family, as presented by Peter McPherson (McGill University, Montreal, Canada). Here, the autoinhibition of connecdenn/DENN1A (a GEF for Rab35) is relieved through the phosphorylation of serine residues within its C-terminal region, indicating that kinases can also function as components of the coincidence detection network.

The large, Golgi-associated Arf GEFs of the GBF1/BIG1/BIG2 subfamily do not contain PH domains, and are regulated through other mechanisms. Paul Melancon (University of Alberta, Edmonton, Canada) showed that recruitment of mammalian GBF1 to membranes is stimulated by inactive GDP-bound Arf1, but the underlying mechanism remains to be determined. Another layer of regulation was discussed by Rick Kahn (Emory University, Atlanta, USA), who proposed that the recruitment and activation of large Arf GEFs is linked to the presence of cargo, with other membrane components (e.g. phosphoinositides) providing additional specificity. An important concept that emerged from his talk is that different cargoes recruit different modules of GEFs, Arfs and coat components, implying that cargo imparts specificity to GEF output.

A key aim in the field is to identify all the components of the coincidence detection mechanisms through which GEFs and GAPs are positioned and activated at specific membrane sites. Apart from the obvious factors, such as phosphoinositides and cargo, additional binding partners of GEFs and GAPs (scaffolds, other GTPases and kinases) are likely to participate in coincidence detection and should be investigated in depth.

Many Arfs and Rabs exist in regulatory GTPase cascades

Directionality of membrane traffic can be achieved by ordering of regulatory events such that events that occur later in a transport pathway cannot occur without the prior early events that set them up. The Rab cascades that regulate the secretory pathway were discussed in several talks. Nava Segev (University of Illinois at Chicago, USA) discussed how Golgi entry and exit is coordinated by Ypts (yeast Rabs) and their GEFs. The idea is that Ypt1, which is involved in ER-to-Golgi trafficking, is activated by the multisubunit GEF TRAPPI; Ypt1 then recruits TRAPPII, a GEF for the late-Golgi Rabs Ypt31 and Ypt32. Such a

cascade ensures vectorial traffic of cargo from the ER through the Golgi to the plasma membrane. Alternatively, TRAPPI can also convert into TRAPPIII to stimulate the function of Ypt1 in the autophagic pathway, thereby coordinating the function of a single Rab in shuttling cargo to two different destinations. Peter Novick (University of California San Diego, San Diego, USA) described a Rab cascade that acts in the formation of yeast secretory vesicles, in which the Golgi-associated Rab Ypt32 binds and recruits Sec2 (a GEF for the vesicle-associated Rab Sec4) to nascent transport vesicles. Sec2 also binds Sec15p (a component of the exocyst tethering complex) and PtdIns(4)*P*. Importantly, Sec2 binding to Ypt32 and Sec15 is inversely regulated by PtdIns(4)*P*; as PtdIns(4)*P* levels decrease, Sec2 dissociates from Ypt32 and its binding to Sec15 is enhanced. This 'maturation cascade' results in secretory vesicles that are primed for vesicle fusion at the plasma membrane. A third example of a Rab cascade was provided by Suzanne Pfeffer (Stanford University, Stanford, USA), who described cross-talk between Golgi Rabs Rab33B and Rab6. She showed that the medial Golgi-localized Rab33B recruits a heterodimeric GEF comprised of Ric1 and Rgp1, that subsequently activates the trans-Golgi network (TGN)-localized Rab6. Finally, Anne Spang (University of Basel, Basel, Switzerland) discussed a Rab cascade in the endocytic pathway, in which the transition from early endosomes (EEs) to late endosomes (LEs) is facilitated by the loss of Rab5 and acquisition of Rab7. She showed that SAND-1/Mon1 plays a key role in this process by displacing rabex5 (a GEF for Rab5) from EEs, thus causing the dissociation of Rab5 from membranes, and simultaneously acting as a GEF for Rab7, promoting maturation to LEs. Thus, cascades involving Rabs, their GAPs and GEFs promote maturation of transport intermediates within the secretory and endocytic pathways.

Rabs also participate in mixed cascades with Arfs to regulate each other's activity, as showcased by Peter McPherson (McGill University, Montreal, Canada) who described a cascade involving Rab35 and Arf6. In this pathway, Rab35 recruits the Arf6 GAP ACAP2 to regulate Arf6 activation. The balance between Rab35 and Arf6 activities affects cell migration by modulating the recycling of cadherins and integrins, and reduced Rab35 levels that lead to enhanced Arf6 activity correlate with enhanced metastatic potential of cancer cells. Julie Donaldson (NIH, Bethesda, USA) focused on the Rab35–Arf6 cascade in the context of clathrin-independent endosomal trafficking. She presented evidence that blockade of clathrin-mediated endocytosis caused retention of Rab35 at the plasma membrane. Although this led to a slight elevation in Arf6-GTP, it actually increased degradation (rather than recycling) of clathrin-independent cargo. The molecular basis for this result remains to be determined. Another example of a mixed Arf–Rab regulatory cascade in retinal photoreceptor cells was presented by Duska Deretic (University of New Mexico, Albuquerque, NM). In these cells, activation of Arf4 at the TGN triggers the recruitment of the Arf GAP ASAP1, which in turn binds to Rab11. The resulting complex serves as a scaffold for the assembly of a Rab11–Rabin8–Rab8 complex that is necessary for delivery of rhodopsin to the light-sensing organelles of these cells.

Arf GEFs also participate in signaling cascades, as illustrated in the talk by Elizabeth Sztul (UAB, Birmingham, AL), who presented insights into the GBF1–Arf–BIG1/BIG2–Arf cascade at the TGN. At the top of the cascade, GBF1 activates its substrates (Arf1, Arf4 and Arf5), which then bind the N-terminal domains of BIG1 and BIG2 and recruit them to membranes to facilitate the activation of their substrates (Arf1 and Arf3). GBF1 mediates the Arf-dependent recruitment of COPI at the ER–Golgi interface, whereas BIG1 and BIG2 recruit Arf-dependent TGN coats. This suggests that GBF1, by affecting the activity of BIG1 and BIG2, also (indirectly) regulates the recruitment of TGN coats and thus might coordinate trafficking of cargo through the entire secretory pathway.

The identification of BIG1 and BIG2 as effectors of Arf4 and Arf5 raises the key question of what constitutes an Arf-effector interaction domain? Julie Menetrey (CNRS, Gif-sur-Yvette, France) showed that although effectors bind to the same switch regions on active Arfs, the Arf-binding domains in different effectors vary greatly. Indeed, such domains can be either β-sheets (as in the Arf6 effector MKLP1), α-helices (as in GGA proteins) or contain mixed folds (as in ARHGAP21). Her findings were greeted with uniform disappointment because they preclude the prediction of possible effector domains based on structural information alone.

Taken together, an extensive functional crosstalk among Arfs and Rabs, as well as their regulators and effectors highlights the complex and multi-dimensional networks that must be unraveled to provide a detailed understanding of how these proteins function in vesicular transport.

Many Rabs and Arfs are 'hijacked' by pathogens

Many infectious agents reorganize intracellular membranes to optimize replication and/or inhibit the host secretory pathway to prevent the release of proinflammatory signaling molecules. Because Rabs and Arfs control compartment identity and regulate trafficking pathways, many pathogens evolved mechanisms to subvert their function. For example, Craig Roy (Yale University, New Haven, CT) discussed how the intracellular bacterial pathogen *Legionella pneumophila* diverts the activity of Rab1, resulting in the conversion of a plasma-membrane-derived phagosome into an ER-derived vacuole that supports bacterial replication. In this example, the bacterial protein DrrA recruits Rab1 to the *Legionella*-containing vacuole and activates it in a process requiring distinct DrrA domains: a C-terminal PtdIns(4)*P*-binding domain that anchors DrrA at the membrane, a central GEF domain that activates Rab1, and an N-terminal nucleotidyl transferase activity that attaches an AMP moiety onto the switch II region of Rab1. This covalent modification inhibits the ability of host cell GAPs to inactivate Rab1, resulting in its permanent activation.

The theme of Rab1 exploitation by pathogens was extended by Neal Alto (UT Southwestern, Dallas, USA) who showed that the *Escherichia coli* protein EspG and the *Shigella flexneri* protein VirA contain a related domain with Rab1 GAP activity that inactivates Rab1 to disrupt host protein secretion. Additionally, EspG contains a domain that interacts with Arf6, suggesting that EspG can rewire both Arf6 and Rab1 signaling. Furthermore, the *Shigella* protein IpaJ also targets Arfs, but by a distinct mechanism: IpaJ is a protease that cleaves the N-terminal glycine residue of Arfs, which is required for myristoylation. This irreversible modification renders

Arfs (and possibly other myristoylated proteins) non-functional, and blocks host protein secretion.

Whereas *Shigella* inactivates Arf, poliovirus might need Arf function to generate viral 'replication factories' in host cells. poliovirus causes the amplification of membranous structures that are likely to be derived from the ER–Golgi intermediate compartment (ERGIC) and/or the Golgi, on which viral replication and virion assembly occurs. George Belov (University of Maryland, College Park, MD) showed that viral replication itself but not the membrane remodeling requires the Arf GEF GBF1, and that the N-terminal region rather than the catalytic GEF domain is essential for viral replication. This implies that inhibitors that target the N-terminal region in GBF1, but do not inhibit Arf activation might be used to combat viral infection.

Pathogens also subvert cellular degradative pathways, presumably to prevent their own clearance. Kim Orth (UT Southwestern, Dallas, TX) described a mechanism by which *Vibrio parahaemolyticus* manipulates autophagic flux in host cells. The bacterial protein VopQ binds to the V_o domain of the vacuolar-type H^+-ATPase and forms a gated channel in the lysosome membrane that mediates the outward flux of ions, thus decreasing intralysosomal pH. Disrupting lysosomal homeostasis alters autophagic flux in the cell, and disrupts the ability of the cell to destroy the bacteria.

Defects in Rabs or their regulators and effectors cause disease

The absolute requirement for a tightly regulated Rab activation is underscored by human diseases that are caused by mutations in Rabs, their regulators or effectors. Angela Wandinger-Ness (University of New Mexico, Albuquerque, NM) discussed mutants of Rab7 that cause Charcot–Marie–Tooth Disease type 2B. These mutations can alter Rab7–effector interactions, or GEF- and GAP-dependent regulation of Rab7, and perturb endocytic trafficking. In neurons, this interrupts nuclear transmission of growth factor receptor signals that are important to neuronal cell differentiation and survival, causing axonal loss and peripheral neuropathies.

Diabetes is another disease affected by mutations in Rabs, owing to their roles in regulating trafficking of the glucose transporter type 4 (GLUT4). David James (Garvan Institute, Sydney, Australia) presented insights into the role of Rab10 and the Rab GAP TBC1D4/AS160 in GLUT4 trafficking. He also described a new Rab10 GEF that, like TBC1D4/AS160, is phosphorylated in response to insulin and might also modulate GLUT4 trafficking. Therefore, there might be multiple inputs into the activity of Rab10 to regulate GLUT4 trafficking.

The importance of Rab interactions with motor proteins was discussed by Jim Goldenring (Vanderbilt University, Nashville, USA) who described an inherited mutation in myosin Vb that causes microvillus inclusion disease (MVID), a fatal condition in which intestinal epithelial cells fail to develop brush borders. He showed that the interaction of myosin Vb with Rab8a is necessary for brush border assembly in cultured epithelial cells and that the inability of the disease-causing myosin Vb mutant to bind to Rab8a contributes to MVID.

Arfs and Rabs have multiple roles in vesicle formation, lipid dynamics, autophagy and cytokinesis

One of the most intensively studied roles of Arfs is in vesicle formation. It has been proposed that activated Arfs directly recruit coat complexes to the donor membrane to form a vesicle, whereas subsequent recruitment of Arf GAPs leads to GTP hydrolysis and drives vesicle uncoating. However, Victor Hsu (Harvard University, Boston, MA) reported that ArfGAP1 is present in Golgi-derived vesicles prior to uncoating and might act as an Arf effector that links cargo proteins to Arfs and COPI coats during vesicle formation. This view is supported by the observation that ACAP1 has a similar role in clathrin coat assembly on recycling endosomes.

On the other hand, Frank Adolf and Felix Wieland (Heidelberg University Biochemistry Centre, Heidelberg, Germany) presented evidence that ArfGAPs do not represent a component of COPI-coated vesicles and instead catalyze uncoating of COPI vesicles. This group also revisited the requirement of GTP hydrolysis for vesicle scission and uncoating, and showed that Arf catalyzes scission independently of GTP hydrolysis.

An important Arf function in vesicle formation is through modifying the local lipid environment. In budding yeast, transport between the TGN and endosomes requires the phospholipid flippase Drs2, which increases membrane curvature during vesicle formation. Todd Graham (Vanderbilt University, Nashville, TN) showed that Drs2 activity is enhanced by binding to the Arf GEF Gea2 (a yeast GBF1 ortholog); this promotes the local accumulation of phosphatidylserine, which in turn stimulates the recruitment of the Arf1GAP Gcs1. Thus, Gea2 and Drs2 act in concert to recruit coat protein complexes and Arf effectors, and bend the lipid bilayer.

A role of Arf in the trafficking of glucosylceramide (GlcCer) was discussed by Antonella DeMatteis (Telethon Institute of Genetics and Medicine, Naples, Italy). GlcCer is the precursor of all glycosphingolipids and is transported from the cis-Golgi (where it is synthesized) to the late Golgi (where it undergoes additional glycosylation) through both vesicular and non-vesicular pathways. The non-vesicular traffic is mediated by Arf effectors, including the four-phosphate-adaptor protein2 (FAPP2) and Antonella DeMatteis described the molecular mechanism through which FAPP2 mediates directional transport of GlcCer. She showed that without bound GlcCer, FAPP2 targets to early Golgi membranes, and, upon binding GlcCer, undergoes a conformation shift that increases its affinity for PtdIns(4)*P* and promotes its targeting to the TGN where PtdIns(4)*P* is enriched. There, FAPP2 releases GlcCer for its further glycosylation.

A novel role for Arf GEFs in lipid metabolism was described by Cathy Jackson (CNRS, Gif-sur-Yvette, France) who showed that GBF1 localizes to lipid droplets (in addition to the ERGIC and Golgi) and is required for localization of two proteins involved in lipid metabolism, adipose triglyceride lipase (ATGL) and perilipin, to the droplet surface.

A number of talks discussed the roles of Rabs in autophagy. Juan Wang (UCSD, San Diego, CA) reported that TRAPPIII, a specific form of the yeast Ypt1 GEF TRAPP, is required for early stages of autophagy in yeast; it is recruited to the preautophagosomal structure (PAS) by Atg17, a scaffolding protein that coordinates the formation of the

autophagy initiator complex. Activated Ypt1, in turn, recruits the kinase Atg1, which is required for phagophore formation, and that also interacts with Atg17. Thus, the local activation of Ypt1 by TRAPPIII ensures that Atg1 is recruited selectively to the PAS, rather than to other compartments where Ypt1 is also activated. These findings also showcase the ability of Ypt1 to regulate both the secretory and autophagic pathways. Mark McNiven (Mayo Clinic, Rochester, MN) showed that the large GTPase dynamin (Dyn2) is required for lipid droplet metabolism by mediating the formation of nascent lysosome budding from autophagolysosomes. Under starvation conditions, lipid-loaded hepatocytes in which Dyn2 function has been perturbed display long tubular LAMP1- or LC3-positive compartments than cannot contribute to autophagic-based lipophagy. These findings imply that there is a new cellular location and function for this large mechano-GTPase.

Sharon Tooze (LRI, London, UK) described results from a screen for RabGAPs that function in autophagy, in which 11 of the 38 known GAPs inhibited some aspect of autophagy. One of these, TBC1D14, interacts with Ulk1 (the mammalian ortholog of yeast Atg1) and binds to Rab11. Thus, Rab11, which normally regulates endosomal recycling, is required for phagophore formation, further supporting the notion of single Rabs regulating multiple pathways.

The concept of Rab11 'multi-tasking' was further developed by Rytis Prekeris (University of Colorado, Denver, CO) and Kazuhisa Nakayama (Kyoto University, Kyoto, Japan) who discussed the FIP family of Rab11 effectors. Both showed that Rab11 and FIP3 deliver recycling endosomes to the intercellular bridge during cytokinesis, presumably to provide components needed for cleavage furrow formation and subsequent abscission. Rytis Prekeris also showed that another Rab11 effector, FIP5, is involved in the development of an apical lumen in polarizing epithelia. Thus, Rab11 appears to control multiple functions including recycling traffic, autophagy, cytokinesis and cell polarity.

Arfs and Rabs are crucial modulators of signaling pathways

Signaling can be modified by changes in receptor localization that are dictated by their trafficking itinerary, which itself can be modified by signaling inputs. The role of Arfs in trafficking of signaling receptors was discussed by Morag Park (McGill University, Montreal, Canada) who presented evidence for a direct role of the adaptor protein GGA3 and Arf6 in the recycling of the HGF receptor Met. She showed that GGA3/Arf6-mediated recycling of Met maintains the high levels of ERK activation and mitogenic signaling and supports cell migration, whereas in the absence of GGA3, Met is degraded in lysosomes, attenuating ERK activity and inhibiting HGF-mediated cell migration.

A role for Arf6 in the recycling of Met in the context of tumor development and metastasis was discussed by Crislyn D'Souza-Schorey (University of Notre Dame, Notre Dame, IN). She showed that in 3D cell models of epithelial cysts, Arf6 hyperactivation downstream of extracellular agonists, such as canonical Wnts, prevents Met recycling, and keeps it within 'signaling' endosomes. This causes sustained ERK signaling and results in the formation of cysts with multiple lumens instead of normal cysts with a single lumen.

Arf GEFs also influence signaling as discussed by Jim Casanova (University of Virginia, Charlottesville, VA) who described a subfamily of Arf GEFs, BRAG1–BRAG3, that localize to postsynaptic densities in the brain where they modulate the trafficking of neurotransmitter receptors to regulate signaling at the synapse. Mutations in the catalytic domain of BRAG1 or its calmodulin-binding IQ-like domain have been implicated in familial X-linked mental disability, and corresponding mutations were shown to either inhibit AMPA receptor trafficking or uncouple it from Ca^{2+}-calmodulin signaling in hippocampal neurons.

The role of Rabs in regulating signaling was also discussed by Jenny Stow (University of Queensland, Brisbane, Australia). She showed that knockdown of Rab8 or of phosphoinositide 3-kinase (PI3K) in macrophages impairs the activation of Akt and affects internalization of the Toll-like receptor TLR4 after stimulation by bacterial lipopolysaccharides. Such altered signaling enhances the production of pro-inflammatory cytokines (e.g. TNFα) and reduces anti-inflammatory cytokines (IL-10). The emergence of Rabs as regulators of inflammation elicited considerable discussion.

Conclusions and perspectives

The conference concluded with an open discussion on the outstanding questions in the field; the key issues for future exploration were coincidence detection specificity, regulation of the regulators (e.g. GEFs and GAPs), and the interplay between lipid and protein signaling. Among the practical issues was the need for close collaboration between laboratories to integrate individually generated information into a cohesive network of understanding. Additionally, there is a strong need to share reagents (plasmids, antibodies, cell lines, expression patterns, etc.) to optimize resources and speed of discovery. Much remains to be done in exploring the role of Rabs and Arfs in cell function, and future discussions are planned to define best means of organizing this community to ensure rapid progress.

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