Meeting Report

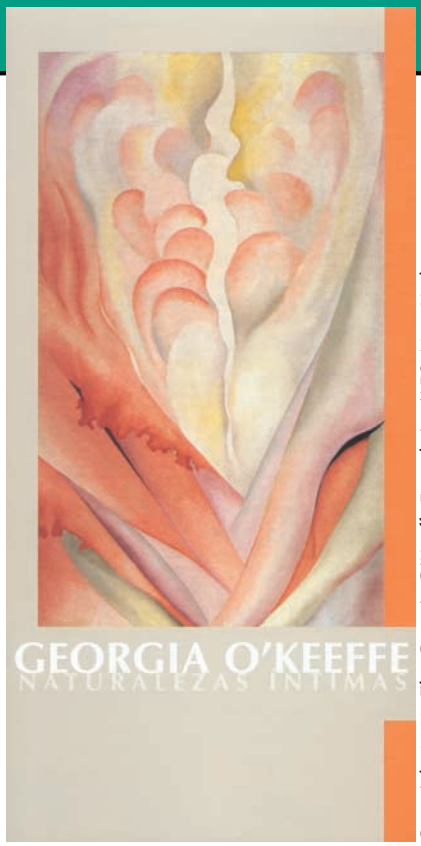
The Fundación Juan March is one of the most active and important cultural institutions in Spain. Since 1955, it has promoted excellence in music and art, specializing in the support of young Spanish artists and musicians. In 1986, the Fundación's mission was extended to the promotion of biological sciences with the organization of the Instituto Juan March de Estudios e Investigaciones. Since its inception, the Institute has hosted a remarkable series of lectures and meetings, each held in the elegant Madrid headquarters of the Fundación.

The most recent Juan March workshop, which was organized by Francisco Sánchez-Madrid and Rick Horwitz and held from June 3–5, 2002, focused on a problem of central importance in cell biology: the mechanisms and functions of cell polarity. Until fairly recently, the study of cell polarity comprised a fairly restricted range of problems, mostly involving the analysis of membrane and cytoskeletal protein distribution in epithelial cells, neurons, and yeast. However, with the growing appreciation that the principles governing asymmetry at the cellular level are among the most fundamental and therefore conserved in all of biology, the field of cell polarity has grown to involve an increasing array of problems, ranging from asymmetric cell division and cell fate decisions in bacteria to the morphogenesis of vertebrate embryos to the directed of migration of metastatic cancer cells. Although the small three-day meeting did not attempt to cover this entire range of problems, it did include an appropriately eclectic mix of topics. It also provided an exceptional forum that allowed diverse and often noninteracting segments of the field to come together to discuss common problems and exciting new results in quite detail.

The meeting began with a unique opportunity for attendees to view an impressive show of paintings of American artist Georgia O'Keefe. Held in the first floor gallery of the Fundación, the exhibition combined some of O'Keefe's best known works with others seen less often in public. More scientific meetings should start on such a high note. Perhaps it was the inspiration of O'Keefe's uninhibited vision that stimulated so many of the meeting's participants to freely present and discuss their unpublished and most recent data—a feature becoming all too infrequent at many such gatherings.

The meeting's first session, "Establishment and maintenance of cell polarity," chaired by Kai Simons (Dresden, Germany), began with a consideration of polarity in the budding yeast, *Saccharomyces cerevesiae*. The systematic genetic analysis of how yeast polarize their secretory pathway during mating or budding has over the past decade allowed the problem to be defined in precise molecular terms. We know now that yeast buds form in response to the deposition of a membrane-associated spatial cue that orients the actin cytoskeleton, and thus the traffic of secretory vesicles, to the forming bud tip, in part due to the concerted action of a variety of GTPases, nucleotide exchange factors, actin binding proteins, and regulatory kinases.

David Drubin (Berkeley, CA), whose group has contributed extensively to our understanding of the gene products involved in orienting actin filaments toward the bud tip, reviewed this work and raised a new issue in yeast budding. When budding is complete, the newly formed daughter cell must be released from the mother. This process involves secretion of the cell wall–digesting enzyme chitinase at the mother–daughter junction, a function uniquely performed by the new daughter cell. Thus, although the budding process is carefully contrived to allow both mother and daughter to partition virtually all of a yeast's organelles between the two cells, budding also involves an asymmetric cell fate decision. Drubin focused on the highly conserved Cbk1 kinase and its associated protein Mob2p, which act at the daughter cell cortex to promote polarized growth. Mob2 and Cbk1 were also found to enter the daughter cell nucleus in response to a cell cycle signal, ultimately turning on transcription of chitinase and other genes involved in cell separation. These findings establish a link between



control of events at the cell cortex with a mechanism for executing a cell fate decision critical to completing polarized bud growth.

Tony Bretscher (Ithaca, NY) next reviewed in detail the regulation and organization of the actin cytoskeleton during yeast budding, demonstrating a role for tropomyosin in maintaining the actin cables extending from the bud into the mother cell. The Type V myosin, Myo2p, uses these cables to translocate new secretory vesicles into the bud (Schott et al., 2002). The system works for a variety of organelles including, with some modification, the nucleus. Bretscher has now described in great detail the role of formins (e.g., Bni1p) in ensuring the initial orientation of the actin cables from the bud tip (Evangelista et al., 2002). Formins are activated by Rho GTPases in the bud tip, and they bind to profilin, nucleate actin assembly, and remain bound to the growing barbed end of actin filaments. The filament association promotes polarized actin cable growth and does so in a manner apparently independent of the Arp2/3 complex, which instead causes the branched growth of actin required for cortical patch formation (Pruyne et al., 2002).

Regulation and Functional Insights in Cellular Polarity
Madrid, Spain June 3–5, 2002

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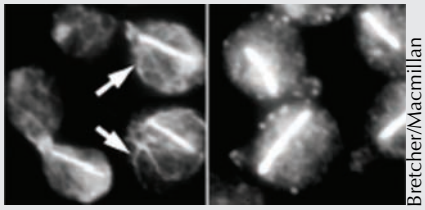
In the case of fission yeast (*S. pombe*), quite a different mechanism was later described by Phong Tran (Philadelphia, PA). Working in the laboratory of Frad Chang (New York, NY), Tran showed that the nucleus of interphase cells must be accurately positioned at the cell center before cell division. This occurs by attaching the nucleus to multiple bundles of antiparallel microtubules, allowing the nucleus to sense its distance from either pole of the growing cell (Tran et al., 2001).

The next talk, by Ira Mellman (Yale University, New Haven, CT), introduced the problem of polarity in epithelial cells, specifically the issue of how polarized cells provide for the asymmetric distribution of their membrane proteins and lipids at distinct plasma membrane domains. He updated his group's work showing how transport of membrane proteins to the basolateral domain of epithelial cells is controlled by the interaction of the epithelial cell–specific clathrin adaptor complex AP-1B with discrete cytoplasmic domain targeting signals (Fölsch et al., 2001). Regulation of these interactions may now explain how some proteins undergo transcytosis from the basolateral to the apical surface—AP-1B is recognized on the secretory pathway, but not in endosomes following internalization at the basolateral surface. AP-1B also helped recruit other downstream components (members of the mammalian exocyst complex, Rab GTPases) to secretory vesicles in the Golgi region, thus ensuring their targeting to docking sites at the basolateral plasma membrane. This paradigm is somewhat different from what had been predicted from the initial yeast work. However, it is one which received independent support later in the meeting from Philippe Chavrier (Paris, France), who found that at least one exocyst component that interacted with a plasma membrane Arf GTPase was also recruited to vesicles in the Golgi region of AP-1B–expressing MDCK cells.


Bretcher/Macmillan

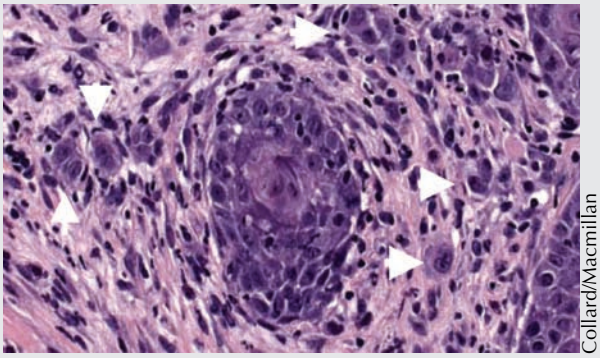
Actin cables fall apart without formins (right), but do not need Arp2/3 to form (left).

Targeting to the apical surface of epithelial cells was next discussed by Miguel Alonso (Madrid, Spain) and by Simons. Here, evidence for the involvement of lipid rafts was discussed, although Alonso also stressed the importance of the apical tetra span raft protein MAL. In MDCK cells, reduction of MAL expression had previously been shown to interfere with apical targeting, and this concept was extended further. Hepatocytes and Caco-2 intestinal epithelial cells, initially found not to express MAL, were found indeed to express a second very closely related gene designated MAL-2. Again using the antisense approach, Alonso's group suggested that MAL-2 provides an analogous function in these cell types (Martin-Belmonte et al., 2000). Conceivably, the MAL proteins are important for providing the stable association of nascent apical proteins in raft domains. This issue was also taken up by Chiara Zurzolo (Naples, Italy), whose work has strongly suggested a relationship between protein oligomerization and stable raft assembly.

Simons concentrated more on his group's efforts to characterize lipid raft formation in budding yeast (Bagnat et al., 2000). A variety of proteins found at the tip of mating projections, such as Fus1p and the GPI-anchored protein GFP, were found to be raft associated. Ergosterol mutants, which reduce sterol levels, abolished this polarity with a concomitant decrease in mating efficiency; the projections themselves, however, were still present, indicating that rafts were not required for this aspect of cell polarity. Furthermore, not all raft-associated proteins in yeast were even found at mating projections in wild-type cells. This finding emphasized, as was reiterated throughout the meeting, that rafts do not comprise a homogeneous species whose multiple members cannot be easily distinguished by the relatively crude techniques available.

Migrating eukaryotic cells are polarized, with a front (or leading edge) rich in polymerizing actin filaments, and a back/rear containing a contractile actomyosin filament network. Several speakers discussed how the distribution of proteins and membranes becomes polarized in migrating cells. Fred Maxfield (New York, NY) described how membranes show different properties at the back and front of neutrophils, consistent with the back being raft like, or more detergent resistant (Seveau et al., 2001). Carlos Martinez-A (Madrid, Spain) reported that polarized T cells, in contrast to fibroblast-like cells, had two raft-like structures, one at the front containing the chemokine receptors, and one at the rear (uropod) bearing transmembrane proteins such as CD44. These structures also have distinct lipid compositions; whereas the uropod is enriched in GM1, the leading edge is enriched in GM3 (Gómez-Moutón et al., 2001). Francisco Sánchez-Madrid (Madrid, Spain) emphasized the role of ezrin/radixin/moesin proteins in T cell polarization, where moesin is needed for localization of a number of transmembrane receptors to the uropod (Serrador et al., 2002).

Rick Horwitz (Charlottesville, VA) and Vic Small (Salzburg, Austria) discussed mechanisms that regulate adhesive formation and turnover in polarized migrating cells. The studies by Small indicate that microtubule dynamics are involved in adhesive turnover. Although the relaxing agent is not known, kinesin-1 is involved (Krylyshkina et al., 2002). The polarity of the delivery appears to be determined by a mechano-sensing system guided by the actin cytoskeleton (Kaverina et al., 2002). Horwitz presented complementary studies that addressed the mechanisms by which adhesions form and dissipate at the cell front and rear, respectively, in the context of a sequential mechanism. He also showed, using video microscopy, the polarized trafficking of integrins in vesicles and of GIT1, an ARF-GAP–containing signaling adaptor, in large macromolecular complexes that move from adhesions



Collard/Macmillan

Skin tumors are highly invasive in Tiam1$^{-/-}$ mice.

as they release and into adhesions as they form (Laukaitis, et al., 2001; Manabe et al., 2002).

How cells initially become polarized to migrate is still a mystery, although those working in Dictyostelium are probably closest to identifying the first polarizing signals. Rick Firtel (San Diego, CA) described how the generation of PIP3 [PI(3,4,5)P_3] is polarized to the front of cells stimulated with chemoattractant. He then reported that this polarization is achieved through the localization of PI 3-kinases (which generated PIP3) to the front of cells, together with the localization of PTEN (which converts PIP3 to PIP2) to the sides and back of cells. Together, this ensures that PIP3 accumulates selectively at the front, but what is still not known is how PI 3-kinases and PTEN become localized (Funamoto et al., 2002).

Downstream of PIP3 lies the Rac GTPase, which together with its close relative Rho is important in cell migration, both through regulating the actin cytoskeleton and microtubules. Several talks provided novel insight into how these proteins function. Shuh Narumiya (Kyoto, Japan) described how two downstream targets of Rho, ROCK and mDia1, act antagonistically to regulate Rac activity. Inhibiting ROCK enhanced Rac activity and cell extension, whereas dominant–negative forms of mDia1 inhibited neurite extension and cell migration (Tsuji et al., 2002). Miguel Vicente-Manzanares (Madrid, Spain) reported that Rho and Rac both act through their effects on actin and microtubules and found that inhibition of Rho reduced microtubule stability, consistent with previous reports showing that Rho induces increased microtubule stability. Isabel Olazabal (Birmingham, UK) described a novel role for ROCK in phagocytosis, where it appears that myosin IIA is localized to phagosomes induced by complement-coated particles, and contributes in some unknown way to recruitment of the Arp2/3 complex. The role of a different GTPase, ARF6, in polarized delivery of membrane to phagosomes was reported by Chavrier. He described a novel interaction between ARF6 and a component of the exocyst complex, previously shown to be required for polarized delivery of vesicles in budding yeast.

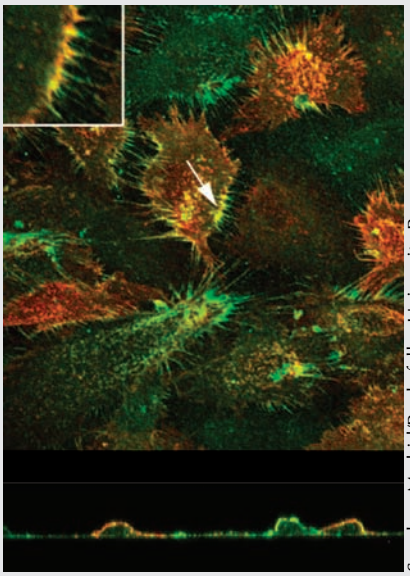
John Collard (Amsterdam, Netherlands) moved into in vivo studies, and described the phenotype of mice lacking Tiam1, an activator of Rac. *Tiam1* was isolated as a gene inducing T lymphoma invasion. In mice, it is highly expressed in keratinocytes in the skin. Although mice lacking Tiam1 are less susceptible to skin cancer, the cancers they do develop are more invasive. The results are consistent with a requirement for Tiam1/Rac in cell survival and proliferation, but suggest that if cells are able to bypass this requirement, they then become more motile if they lack Tiam1 (Malliri et al., 2002).

Another type of polarized cell migration is the transmigration of leukocytes across endothelial cells. Anne Ridley (London, UK) discussed the important contribution of endothelial cells to this response, and in particular how Rho is activated following engagement of leukocyte-binding receptors on the apical surface of endothelial cells (Thompson et al., 2002). Francisco Sanchez-Madrid looked at the interaction zone between endothelial cells and T cells and found that adhesion molecules VCAM-1 and ICAM-1 and a number of cytoskeletally associated proteins in endothelial cells localized to this zone (Barreiro et al., 2002).

Another zone of polarized cell–cell interaction that has been extensively studied is the immunological synapse between T cells and antigen-presenting cells. This synapse forms when T cell receptors (TCRs) recognize peptide bound to major histocompatibility complex (MHC) proteins. T cells then segregate surface proteins and intracellular signaling proteins into supramolecular activation clusters (SMACs), with the TCR in the center and integrins such as LFA-1 at the periphery (Bromley et al., 2001).

Mark Davis (Stanford, CA) stressed the remarkable sensitivity of the TCR. Using finely tuned, quantitative fluorescence microscopy, the most recent data from his group now indicate that only a single TCR–peptide-MHC complex is sufficient to induce signaling and thus activation in the T cell. Since such monomeric interactions would not by themselves induce the TCR cross-linking long thought to be required for signaling, he has proposed from recent structural data that dimer formation can be induced by "trans" interactions involving the TCR-associated CD4 molecule: i.e., that the CD4 associated with a given TCR may interact with an MHC class II molecule other than the one recognized by the TCR itself.

Michael Dustin's laboratory (New York, NY) has developed an in vitro system for analyzing formation of the immunological synapse, where the antigen-presenting cell is replaced with



Sanchez-Madrid/Rockefeller University Press

Moesin (green) and VCAM (red) interaction helps leukocytes stick to endothelial cells.

a lipid bilayer containing MHC/peptide and ICAM-1, the receptor for the T cell integrin LFA-1. Remarkably, these two signals are enough to induce T cell polarization and activation (Grakoui et al., 1999). Investigating the timing of signaling events during T cell–APC interaction, he reported that the peak tyrosine phosphorylation of ZAP70, a key molecule transducing signals from the TCR, occurs before formation of the immunological synapse (Lee et al., 2002). This begs the question of what signals are being produced by the immunological synapse. One possibility is that protein kinase Cθ is active: as Abraham Kupfer (Denver, CO) described, PKCθ is the only PKC localized to the immunological synapse and is required for signaling by the TCR (Monks et al., 1997, 1998; Sun et al., 2000).

Although in vitro immunological synapses to B cells can be very stable, in vivo, or at least when dendritic cells are involved as antigen-presenting cells, they may be far more dynamic. Using dendritic cells stabilized within a collagen matrix to simulate the structure of a lymphoid organ, Peter Friedl (Wurzburg, Germany) has found that individual T cells rarely form long-term complexes with any one antigen presenting cell (Friedl and Gunzer, 2001). This concept has emerged from an extensive series of quantitative live cell imaging studies in which T cells were seen to continuously scan the surface of dendritic cells, crawling on and off the cells at great rates occasionally delayed by an interaction between the presenting cell and the T cell's trailing uropod. In these conditions, a basic synapse structure might well still form, but be far more dynamic than originally thought. All of this may somehow be related to why the dendritic cell is so much more effective at T cell stimulation than other antigen presenting cell types. In any event, such studies emphasize the importance of developing appropriate techniques for live cell imaging in tissues and three-dimensional systems, to complement the work done in vitro. For example, Rick Horwitz (Charlottesville, VA) showed beautiful movies of fluorescently labelled cells migrating in situ in slices of tissues from chick and mice. Cells in these systems extend a single, unusually long process forward that seems to explore the environment and determine direction of migration (Murase and Horwitz, 2002). By introducing genes into the cells, this work is corroborating that done on cell migration in vitro, for example showing that regional Rac activation is critical for local process extension and directed cell translocation (Knight et al., 2000).

The overall message from the different polarized cell systems described at this meeting was that we have now identified many molecules (proteins and lipids) that show a polarized distribution, we are beginning to understand some of the mechanisms responsible for generating and maintaining these asymmetric distributions, but we as yet know very little about the initial signals that establish cell polarity in the first place. Whether the stimulus is a pheromone, a chemoattractant, or an antigen, we have yet to establish how proteins and lipids become localized in one part of the cell, and how the cytoskeleton becomes reoriented to maintain the polarity. New and more sensitive imaging techniques and reagents are being developed to allow detection of signals within seconds of cell stimulation, and it is clear that single cell analysis is essential for us to dissect the process of cell polarization. ■

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