Signalling in space and time: systems dynamics of intracellular communication

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The second edition of the EMBO Spatial meeting took place in May 2011 in Engelberg, Switzerland. Although the work presented at the meeting covered a challengingly broad range of topics, it accomplished the rare goal of promoting interactions between cell biologists, neuroscientists, systems biologists and mathematicians, all united by a common interest in understanding how cells integrate signals in space and time.

The second meeting in the EMBO Conference Series on Systems Dynamics of Intracellular Communication (SPATIAL 2011), organized by Philippe Bastiaens (MPI of Molecular Physiology, Dortmund, Germany), Joerg Stelling (ETH Zurich, Switzerland) and Jeff Twiss (Drexel U., USA), was held in the beautiful mountain resort of Engelberg in central Switzerland in May 2011. The stunning alpine views and fresh air provided the perfect backdrop to a very stimulating meeting.

Location, location, location

A fundamental feature of signalling pathways is that the spatial organization and subcellular localization of proteins dictates specificity. John Scott (Howard Hughes Medical Institute, U. Washington, USA) described a family of A-kinase anchoring proteins (AKAPs) that tether signalling enzymes and locally activate kinases and phosphatases. Targeting determinants within the anchoring protein dictate the subcellular localization of PKA–AKAP complexes. AKAP220 creates a signalling platform by recruiting PKA, IQGAP2 and effectors such as Rac GTPases to a specified cellular location. While PKA is constitutively bound to AKAP220, IQGAP2 binds to AKAP220 in a calcium-dependent manner to form the ternary complex IQGAP2–AKAP220–PKA (Logue *et al.*, 2011). A simultaneous rise in cAMP concentration activates AKAP220-anchored PKA allowing the kinase to

phosphorylate IQGAP2, which in turn enhances activated-Rac-GTPase binding to the complex. Downregulation of either IQGAP or AKAP220 alters microtubule dynamics and reduces cell motility.

Glycosylphosphatidylinositol (GPI)-anchored proteins are believed to associate with lipid rafts—membrane microdomains enriched in cholesterol and sphingolipids—which are hypothesized to act as signal-transduction platforms. Satyajit (Jitu) Mayor (National Centre for Biological Sciences, Bangalore, India) reproduced the organization of GPI-anchored protein nanoclusters by a model transmembrane molecule bearing a cytosolic actin-binding protein domain, strengthening the idea of a connection with a sub-membranous cortical actin. Mayor also showed that GPI-anchored proteins exhibit non-brownian density fluctuations and presented a new theoretical

framework, derived in collaboration with soft-matter physicist Madan Rao (Raman Research Institute, Bangalore, India), on the basis of the active dynamics of actin and myosin. This provided a consistent explanation for both the dynamic properties of GPI-anchored proteins and their spatial organization template by actin.

How can we get information about the spatial distribution of proteins in the cell? To answer this question, Ruedi Aebersold (ETH Zurich, Switzerland) used isotope-tagged crosslinking reagents and mass spectrometry to generate distance constraints for a computational model that enabled complex stoichiometric identification and computation of complex topologies by homology modelling and docking (Bohn *et al.*, 2010). To get both spatial and temporal resolution of freely diffusing or bound proteins in a cell, Johan Elf (Uppsala U., Sweden) followed the location of RelA-Dendra2 in living bacteria using high-resolution stroboscopic single-molecule imaging. The kinetics of RelA binding to the bacterial ribosome was characterized by taking advantage of the different diffusive properties of the free and ribosome-bound isoforms of RelA (English *et al.*, 2011). Ribosome-bound RelA is highly sub-diffusive and an order of magnitude slower than the rapidly diffusing free RelA. The ribosome-binding kinetics obtained using this method, provided insight into the mechanism of the

intracellular catalytic cycle of RelA. RelA dissociates from the ribosome on starvation and seems to spend extended periods of time off before rebinding. This suggests a modified model in which most of the catalytic function of RelA happens in its free state, after it is activated by the ribosome.

Breaking symmetry

Previous studies have identified the yeast protein Kar9 as a crucial component in the establishment of spindle pole polarity. Yves Barral (ETH Zurich, Switzerland) reported that Kar9 asymmetry is a highly dynamic process that is controlled by a complex network of phosphorylation and feedback loops. Barral presented new evidence suggesting an interesting ‘tug-of-war’ between Kar9 and the cell-cycle kinase Cdk1 at each spindle pole, which sets up spindle pole asymmetry. Cell fusion is a fundamental event in many cell types, and an intriguing question is how are organelles and macromolecules redistributed between the two fusing cells? Alan Tartakoff (Case Western Reserve U., Cleveland, USA) described an organelle checkpoint that is likely to contribute to uniparental inheritance of the mitochondrial genome when two yeast cells fuse and budding subsequently occurs at the terminus of the zygote. Cell fusion is followed by the rapid flux of soluble proteins between the parental cells, followed by nuclear fusion and, after a further delay, mitochondrial fusion. The delay in mitochondrial fusion seems to be due to a barrier including septin proteins, which transiently form a partition at the midpoint of the zygote. Delay could ensure that mitochondrial fusion primarily occurs when one parental mitochondrial genome has already associated with the nascent bud. This barrier provides a prototype for investigation of the spatial segregation of cytoplasmic constituents in other contexts.

In theoretical modelling, a combination of three elements—stochastic fluctuations, a positive feedback loop and the presence of a limiting factor—can lead to symmetry breaking. Daniel Lew (Duke U., USA) studied the singularity of budding in yeast cells. The molecular mechanism for the positive-feedback loop in this case consists of the PAK–Bem1–GEF complex. GTP–Cdc42 can bind to PAK and thereby recruit cytoplasmic PAK–Bem1–GEF complexes, which can then activate neighbouring Cdc42 molecules, growing an activated Cdc42 cluster. The number of cytoplasmic complexes in the

cell is limiting, and thus the different clusters would compete for complexes. Rapid filming enabled the detection of early stages of polarity establishment, and provided direct evidence for competition between clusters of Cdc42, with the largest one ‘winning’ to become a bud. Initial cluster formation was unexpectedly oscillatory, indicating that an additional negative-feedback loop is built into the yeast polarity circuit. With negative feedback, it is possible to find conditions in which, instead of a competing, two clusters would equalize, resulting in the formation of two buds. This was observed in some cells overexpressing Bem1.

Leah Edelstein-Keshet (U. British Columbia, Canada) used theoretical modelling to study mechanical–biochemical coupling in cell polarization. Polarity can be obtained by using a simplified biochemical circuit consisting of a single protein that, similarly to most GTPases, cycles between an active, membrane-bound, slowly diffusing form and an inactive cytosolic rapidly diffusing form. Two important elements are a positive-feedback loop for the active form and a constant amount of protein. Such a regulatory network leads to polarization through a wave-pinning mechanism, by which a high concentration of activated protein propagates as a front until it comes to a halt midway through the cell. The mechanical properties of the system have their effect in the model through the elastic perimeter of the cell, which represents the actin cortex. A direct link between the local concentration of the activated protein form and the force generated by the network, couples the mechanical and biochemical networks. The coupling of the biochemical system to the mechanical system makes the cell shape less sensitive to parameters such as membrane stiffness and actin-generated protrusive forces.

Cytoskeletal dynamics

Jennifer Lippincott-Schwartz (National Institutes of Health, USA) reported on the coordination between two actin-based modules that regulates the migratory behaviour of epithelial cells. Previous studies have reported that cell migration depends on two actin networks: the lamellipodia at the leading edge and the lamella that forms just behind it. However, whether these modules formed a continuous network or were structurally distinct entities remained unclear. With the elegant use of fluorescence speckle microscopy and photoconvertible reporters,

Lippincott-Schwartz and colleagues were able to gain the spatial and temporal resolution needed to visualize the highly dynamic behaviour of these discrete actin pools (Burnette *et al*, 2011). She reported that during edge retraction, but not protrusion, the lamellipodial actin converts to arc-shaped actin bundles that then transition into the underlying lamella. This behaviour is driven by the motor myosin II, which localizes to the cell edge and compresses the lamellipodial actin, resulting in the formation of actin arcs during the retraction phase of cell crawling. By identifying actin arcs as a structural element connecting the two modules during retraction, but not protrusion, Lippincott-Schwartz and colleagues were able to resolve some of the outstanding questions in the field regarding the precise interactions between the two actin networks during migratory behaviour.

Francois Nedelec (European Molecular Biology Laboratory, Heidelberg, Germany) and co-workers used computer simulations to study the intricate balance between microtubule assembly, sliding and disassembly that leads to the formation of the spindle structure (Loughlin, 2010). Two active processes control the steady-state spindle structure, the anti-parallel flux driven by kinesin5 and the minus-end depolymerization of microtubules induced by kinesin13. These processes balance each other when the spindle has its normal length. The equilibrium length is stable because the kinesin13-dependent depolymerization is faster for a longer spindle. This is because as the pole extends further, the number of microtubules arriving at the pole decreases due to plus-end catastrophes. Having fewer microtubules to work on, kinesin13 depolymerizes them more quickly. Consequently, a spindle that is longer than the steady-state length would be shortened, whereas a shorter one would be lengthened. Such feedback is essential to stabilize the spindle around its equilibrium length.

Signalling in a noisy environment

Biological systems exhibit stochastic fluctuations. These fluctuations are necessary to accommodate changing environmental conditions, but create a challenge in discriminating between information and noise. Martin Howard (John Innes Centre, UK) studied the generation of a pom1 concentration gradient in fission yeast, which is important for length-sensing and site

of cytokinesis positioning. The positional information has to be precisely obtained inside each cell, despite variable pom1 expression levels and intrinsic fluctuations arising from mechanisms such as diffusion that generate the membrane-associated gradient. A mathematical model based on fluorescence imaging and tracking measurements suggests that pom1 exists on the membrane in two forms: a fast-diffusing, potentially monomeric form and a slow-diffusing, clustered form. This two-component model reduces the sensitivity of the concentration profile to the pom1 expression level. Furthermore, time-averaging of the fluctuating pom1 profile by downstream elements such as cdr2, which have comparatively long membrane-residence times, reduces intrinsic fluctuations. Together, these mechanisms ensure that the pom1 profile can supply precise positional information. Boris Kholodenko (Conway Institute, U. College Dublin, Ireland) described the Raf-MEK-ERK biological circuit (Sturm *et al.*, 2010). This network topology is analogous to a negative feedback amplifier (NFA), a design which leads to a decrease in ERK phosphorylation output sensitivity relative to changes in reaction rates and the level of proteins within the NFA. These results suggest that for inhibitory drugs to be effective, targets outside the negative-feedback loop should be chosen.

Benjamin Kaupp (Research Center Caesar, Germany) described the ability of sea-urchin sperm to respond to single molecules of chemoattractant. Crucial signalling events include the binding of a chemoattractant molecule to a receptor and the synthesis of the intracellular messenger cGMP, followed by membrane hyperpolarization due to the opening of potassium-selective cyclic-nucleotide-gated (CNGK) ion channels. CNGK is uncooperative, operates in the nanomolar regime, and binding of a single ligand molecule is sufficient to open the gate (Bonigk *et al.*, 2009). The CNGK polypeptide consists of four repeat sequences with only the third cyclic-nucleotide-binding domain being functional. To avoid high levels of noise as a result of fluctuations in cGMP concentration, hyperpolarization beyond a threshold must be achieved in the sperm before a calcium signal is initiated.

Intracellular transport

In all eukaryotic cells, the intracellular transport of membrane-bound organelles,

messenger RNAs (mRNAs) and protein complexes is a fundamental process. A high-resolution imaging approach integrating the ultra-structural details offered by immuno-electron microscopy coupled with live imaging of fluorescent proteins (correlative-light electron microscopy) allowed Judith Klumperman and colleagues (U. Medical Center, Utrecht, Netherlands) to identify a new route for the delivery of newly synthesized membrane proteins to lysosomes. Klumperman reported that lysosome-associated membrane proteins (LAMPs) could be transported from the *trans*-Golgi network (TGN) to late endosomes and lysosomes by a set of carriers that are not clathrin-associated. Loss-of-function analyses showed that components of the homotypic fusion and protein sorting (HOPS) tethering complex are crucial for the maturation of endo-lysosomal compartments, as well as the delivery of the LAMP carriers to late endosomes, whereas transport of mannose-6 phosphate receptors (M6PRs) remained unaffected. Thus, it seems that there is more than one way to get from the TGN to endo-lysosomes, and that each pathway is regulated differently and mediates the transport of a distinct set of cargo.

Majid Hafezparast (U. Sussex, UK) suggested that mutations in the microtubule-based motor dynein could contribute to neurodegenerative diseases by perturbing growth factor signalling. Hafezparast reported aberrant mitogen-activated protein kinase (MAPK) signalling and deficits in endosomal transport in motor neurons isolated from *Loa* (legs at odd angles)-mutant mice that carry a spontaneous mutation in the dynein heavy chain subunit. Valeria Cavalli (Washington U., USA) identified a new regulatory mechanism for the anterograde microtubule motor kinesin 1. She presented new evidence that syd (sunday driver, also known as JIP3) is not merely an adaptor for kinesin motors, as was previously believed, but is a positive regulator of kinesin 1 activity and motility in neurons. These findings suggest more complex functions for adaptor proteins in 'driving' microtubule motility, rather than their conventional structural roles of linking motors with their cargo. Juan Carlos Arevalo (U. Salamanca, Spain) reported that depletion of an E3 ubiquitin ligase, Nedd4-2, results in enhanced accumulation of the neurotrophin receptor TrkA in early endosomes and at the plasma

membrane, through increased recycling from intracellular compartments. Protection of TrkA from Nedd4-2-mediated degradation results in enhanced neurotrophin signalling and neuronal survival. These results suggest that ubiquitination of the TrkA receptor is an important post-translational determinant of its transport and signalling.

One mechanism to spatially control signalling in cells is to locally synthesize proteins at their site of action. In neurons, local protein synthesis in axons allows the axons to autonomously respond to growth-promoting stimuli during development and to injury in later life. The localization of mRNAs into axons is mediated by RNA-binding proteins that typically bind to *cis*-acting elements in the 3'-untranslated (3'UTR) region. Dianna Willis (Cornell U., USA) reported that overexpressing a reporter construct harbouring the 3'UTR region of an axonally transported mRNA (β -actin) in transgenic mice results in the depletion of endogenous mRNAs in axons and reduces neurite outgrowth after injury. These deficits are probably due to the reporter construct sequestering endogenous levels of a crucial RNA-binding protein, zipcode-binding protein 1 (ZBP1), as ZBP1 overexpression rescued RNA transport and axon growth deficits. These results suggest that ZBP1 is a common and limiting element in the transport of growth-promoting mRNAs to axons, and provides evidence for the importance of axonally synthesized proteins in regeneration.

ACKNOWLEDGEMENTS

We apologize to the authors whose work we could not include because of space constraints.

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Published online 19 August 2011

EMBO reports (2011) **12**, 877–879.
doi:10.1038/embor.2011.169