EMBO reports

meeting point

Inositides—confusion and clarification in Shangri-La

Robin Irvine

The meeting 'Inositide Signalling in Health and Disease', sponsored and organized by the National Centre for Biological Research, Bangalore, and the Indian Academy of Sciences, was held in late November 2012 in the luxurious destination of Orange County Resort, Coorg, India.

For the most memorable meetings there are fine venues, then outstanding venues, and finally those that transcend any previous experience. Somewhere, perhaps above all of those, is the veritable Shangri-La of the Orange County Resort, Coorg, located 4,000 feet above sea level in the forests of the Western Ghats in India. About 60 scientists gathered there for four days to address some of the major issues that compromise our understanding of how inositides (inositol lipids and phosphates) contribute to cell function and disease. The schedule was relaxed and, as there was no escape (who would want to?), there was a wealth of informal discussion and exchange. This mostly took place by the pool between senior scientists from all continents and a large handful of bright and enthusiastic Indian students, while plum-headed parakeets and scarlet minivets tumbled in the canopy overhead.

Inositide signalling is at a stage where we thought we knew almost exactly what is going on in some aspects, and were getting nowhere with others. I have selected some talks at the meeting that showed how wrong we were on both counts!

PI(3)K signalling

This is an area in which we think we know most of the essentials. For example, it is generally accepted that the regulation of the production of phosphatidylinositol 3-,4- and 5-trisphosphate (PIP_3) in neutrophils by G-protein-coupled receptors—for example the f-Met-Leu-Phe (fMLP) receptor—is by βγ-subunits that bind to the p101 regulatory subunit of PI(3)Kγ1, and that the PIP_3 thus produced by the activated PI(3)Kγ acts on a Rac-GEF called P-Rex to activate the monomeric G-protein Rac [2]. Phill Hawkins (Babraham Institute, UK) showed that if you start to look at real cells (mouse neutrophils) and physiological agonists, instead of transfected cells activated by phamacological agonist doses, this picture is simplistic to the point of being wrong. Elegant use of knockout and knock-in mice revealed that, for example, there are 'flavours' of PIP_3 in the neutrophill—when βγs activate PI(3)Kγ that forms a complex with the 'other' regulatory subunit p84 rather than with p101, the PIP_3 produced regulates a different set of effector pathways. Moreover, the activation of Rac by P-Rex is not entirely direct, but is mostly though another small G-protein called RhoG, which activates another Rac-GEF, that is, a Rac activator called ELMO/DOCK2. Even worse—for those who prefer simplicity—the βγ-activation of PI(3)Kγ occurs not only through direct binding to the enzyme itself, but also proceeds through βγ-subunit activation of phosphoinositide-phospholipases C (PI-PLCs) β2 and β3 with the resulting diacylglycerol (DAG) activating the Ras-GEF RasGRP4, and the subsequent GTP-Ras activating PI(3)Kγ through its Ras-binding domain [3].

…it was truly exciting to hear about this satisfying resolution of the 'plasma membrane PI4P synthesis problem'…

Mercifully, not all of the PI(3)K science was telling us that we needed to go back to square one. Roger Williams (LMB, Cambridge, UK) presented structural data on PI(3)K activation, gained largely through hydrogen and deuterium exchange mass spectroscopy (HDX-MS), which he explained in exquisite detail to show how βγ-subunits directly activate PI(3)Kβ. This isoform was considered to be solely tyrosine-kinase-regulated, but increasing evidence has implicated G-protein-coupled receptors—through βγ-subunits—in their regulation. Elegant use of HDX-MS enabled the Williams group to show unambiguously that the βγ-subunit is interacting with the C2-helical domain linker domain of the PI(3)Kβ catalytic subunit to cause a change in the enzyme structure that leads to an increase in membrane affinity of the PI(3)Kβ. Williams also presented data from his lab taken from a paper published in *Proceedings of the National Academies of Science USA* [4] that examines the key set of mutations that occur naturally in PI(3)Kα in many human cancers. Again by using HDX-MS, the data show with breathtaking clarity how those single amino-acid mutations change the structure of the enzyme to activate it in a variety of ways and, thus, contribute to the oncogenic potency of this enzyme. This is a remarkable molecular explanation of a disease state.

Inositol lipid synthesis

One part of inositide metabolism that still baffles us is how and where the main polyphosphoinositol lipids PI4P and $PI(4,5)P_2$ are synthesized. Not least, PI4P is made rapidly in the plasma membrane to act as a synthetic precursor for $PI(4,5)P_2$, among other things. Yet, all four of the mammalian PI 4-kinases that are responsible for making PI4P have so far resolutely refused to be detected in the plasma membrane in mammalian cells. This contradiction has been particularly puzzling for PI4KIIIα, which earlier work strongly suggested is the major synthesizer of the plasma membrane PI4P.

Pietro DeCamilli (Yale U., USA) solved this conundrum by showing that we have hitherto all been working with an amino-terminally truncated version of PI4KIIIα, and that the full-length protein, as with its yeast counterpart Stt4, interacts with a plasma membrane-targeting protein EfrA/B. This protein, aided by another associated protein TTC7A/B, associate PI4KIIIα with the plasma membrane to generate the crucial pool of PI4P. Even the association of transfected full-length PI4KIIIα is transient unless its two partners are co-transfected and are present at similar levels.

Removing PI4KIIIα in mouse embryonic fibroblasts—by a tamoxifen-inducible knockout—leads to a severe depletion of the plasma membrane PI4P, and consequent loss of many inositol lipid-dependent plasma membrane functions. It was noteworthy that after PI4KIIIα loss, cells upregulate their PI4P 5-kinases in what seems a desperate attempt to maintain their $PI(4,5)P_2$ levels. Indeed, an interesting by-product of this 'desperation' are abnormal levels of $PI(4,5)P_2$ in intracellular membranes. This phenomenon also suggests a loss of the plasma membrane 'identity' if PIP is depleted there. Subsequently, there has been discussion of the data from the Irvine lab, published in *Science* [5], which showed—by a different approach—that the plasma membrane $PI(4,5)P_2$ levels are maintained even if PI4P drops. Their evidence also showed that PI4P is important in contributing to plasma membrane charge and thus identity, as well as being a $PI(4,5)P_2$ precursor. Such a concept has only served to heighten our previous frustrating ignorance of how PI4P is synthesized in the plasma membrane, and it was truly exciting to hear about this satisfying resolution of the 'plasma membrane PI4P synthesis problem' from De Camilli.

Another aspect of inositol lipid synthesis and function that is being systematically and slowly—it is mind-bogglingly complex—teased out is PI3P and intracellular transport. Pete Cullen (Bristol) gave us a fascinating example by showing how a PI3P-binding protein called SNX27 is involved in regulating endosome-to-plasma membrane recycling. This regulates the plasma membrane location—and thus function—of a large handful of proteins identified by a RNA interference/SILAC mass spectrometry screen. GLUT-1, a crucial regulator of glucose uptake, was just one example that Cullen showed is regulated in this way. There was also a lively and absorbing discussion of how cargo recognition and targeting is regulated by cell transport, which at times seems to resemble the organized chaos of the traffic we saw in downtown Bangalore before travelling to Coorg.

PI5P 4-kinases

The principal organizer of the meeting was Raghu Padinjat of the National Centre for Biological Research (Bangalore, India), and it was appropriate that there was a significant focus reflecting his interest in the family of PI5P 4-kinases (also known as type II PIP kinases or PIP4Ks). These enzymes, which generally serve to regulate the levels of their substrate PI5P, are usually perceived to fall into the 'we seem to know nothing' category described at the start, but data revealed at the meeting showed that we really are getting somewhere (I should add that in all the topics discussed, a huge amount of unpublished data was revealed and exchanged freely at this 'open' meeting—I have been careful here to seek permission from all speakers whose work I mention).

Two completely independent lines of enquiry pointed to some PI5P4K connections with the TOR pathway. Raghu showed that knocking out the only *Drosophila* PI5P4K gene led to larval growth retardation and reduced cell size that could be mimicked by rapamycin treatment, which also augmented and exaggerated the *dPIP4K* phenotype. Changes in S6 kinase and Akt phosphorylation in the mutant flies, plus rescuing of the phenotype by S6 kinase, also implicate the dTOR signalling pathway in the actions of dPIP4K.

…if you start to look at real cells (mouse neutrophils) and physiological agonists […] this picture is simplistic to the point of being wrong

Independent evidence came from Lucia Rameh (Boston Biomedical Research Institute, USA), who has built up a compelling connection between the γ-isoform of PI5P4K and mTOR. By using several cell lines, and in particular a *TSC2*$^{-/-}$ line in which mTOR is constitutively activated, she showed that PI5P4Kγ is probably phosphorylated on two serine residues by mTOR1. The functional consequences of this are being teased out, and might involve a phosphorylation-driven recruitment of PI5P4Kγ to an endomembrane compartment, and/or some link with 4E-BP—one of the targets of mTOR—but where exactly PI5P4Kγ fits into the grand scheme of things remains far from clear.

The challenge to understand the function of PI5P4Kγ in particular is made harder by my own estimates, presented at the meeting as part of a detailed molecular dissection of PI5P4K family enzymatic activities. PI5P4Kγ's PI5P 4-kinase activity is so low—unless we are missing something really central to how it works—that it is unlikely to have an impact on cellular PI5P levels; it might serve only to target the more active PI5P4Kα to the right location or simply to be a protein kinase. It is relevant to add here that Raghu reported that the PI5P 4-kinase activity of the *Drosophila* enzyme dPIP4K is high—comparable with mammalian PI5P4Kα.

…this is the first time […] that a detailed molecular pathway involving PI5P and a PI5P4K […] has been shown to regulate a physiological function of major significance

We have a much clearer picture of how the other two mammalian isoforms PI5P4Kα and PI5P4Kβ might function from two pathways described at the meeting by Nullin Divecha (Paterson Institute for Cancer Research, Manchester, UK). These enzymes are involved in cellular stress responses, for example, in response to ultraviolet light or H_2O_2. PI5P4Kβ is primarily a nuclear protein, and can heterodimerize with PI5P4Kα to target it to the nucleus [6,7], and it is in the nucleus that the clearest PI5P stories are emerging. Divecha's latest work shows that PI5P4Kβ interacts directly with PIN-1, a prolyl isomerase that targets proteins phosphorylated by proline-directed kinases, such as p38 mitogen-activated protein kinase—a stress kinase already implicated in PI5P4Kβ control by Divecha's group [8]. The evidence suggests that PIN-1 activates PI5P4Kβ, and thus feeds into a PI5P-regulation of stress genes that are downstream from a transcription factor known to be an important contributor to cell stress responses.

A second function for nuclear PI5P regulated by PI5P4Kβ comes from muscle, the tissue that expresses by far the highest levels of PI5P4Kβ relative to the other PI5P4K isoforms [9]. Divecha described the discovery and molecular manipulation of a PHD-finger protein, implicated in myogenesis, as a PI5P-regulated protein. He presented a convincing story that nuclear PI5P, regulated by PI5P4Kβ, thus has a key role in myogenesis. Although, in the past, hints and ideas have pointed to some molecular mechanisms of PI5P4K action, this is the first time to my knowledge that a detailed molecular pathway involving PI5P and a PI5P4K—specifically the β-isoform—has been shown to regulate a physiological function of major significance.

There were other revelations leading to extensive discussions in other parts of inositide signalling for which space does not allow a full description—for example, I have not mentioned the exciting progress in higher inositol polyphosphate function reported by John York (Vanderbilt U. School of Medicine, USA), Adolfo Saiardi (Medical Research Council Laboratory for Molecular Cell Biology, UK) and Rashna Bhandari (Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India); maybe we'll just have to go back to Coorg another year and check out those parakeets and minivets.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

REFERENCES

1. Stephens LR *et al* (1997) *Cell* **89:** 105–114
2. Welch HC *et al* (2002) *Cell* **108:** 809–821
3. Suire S *et al* (2012) *EMBO J* **31:** 3118–3129
4. Burke JE *et al* (2012) *Proc Natl Acad Sci USA* **109:** 15259–15264
5. Hammond GR *et al* (2012) *Science* **337:** 727–730
6. Wang M *et al* (2010) *Biochem J* **430:** 215–221
7. Bultsma Y *et al* (2010) *Biochem J* **430:** 223–235
8. Jones DR *et al* (2006) *Mol Cell* **23:** 685–695
9. Clarke JH *et al* (2008) *Am J Physiol Renal Physiol* **295:** F1422–F1430

Robin Irvine is a Royal Society research professor in the Pharmacology Department of the University of Cambridge, Cambridge, UK. E-mail: rfi20@cam.ac.uk

EMBO *reports* (2013) **14,** 216–218; published online 8 February 2013; doi:10.1038/embor.2013.7

