Science amongst the vines

Meeting on Signalling Systems

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The third Barossa Meeting on Signalling Systems took place between 14 and 17 November 2007, in the Barossa Valley, Australia, and was organized by a committee chaired by A. Lopez.

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these pathways are often regulated not only at the post-translational level, but are also subject to transcriptional regulation, as well as post-transcriptional control by microRNAs. The widespread involvement of several positive and negative feedback loops in these networks adds yet further complexity.

The third Barossa 'Science Amongst the Vines'TM meeting brought together leaders in diverse research areas to discuss 'Signalling Systems' and to gain an understanding of the global rules that control cell signalling, and their implications for fundamental biological processes and therapeutic strategies. This conference expanded on the 2005 'Signalling Networks' meeting of this series (Guthridge *et al*, 2006), and focused on cellular regulation beyond isolated signalling pathways towards a more integrated approach.

Signal timing, location and strength

How growth factors and cytokines are able to mediate specific, and often unique, cellular responses has remained one of the crucial unresolved questions in cell biology and was a recurrent theme of the meeting. For example, how is it that more than 200 type I growth factor and cytokine receptors are able to regulate pleiotropic biological responses through the regulation of what seems to be a limited repertoire of signalling pathways (Guthridge & Lopez, 2007)? One explanation has been that growth factors and cytokines differentially regulate generic signalling pathways in quantitatively different manners to promote distinct cellular responses. Many investigators have examined the mechanisms by which extracellular-signal-regulated kinase 1/2 (ERK1/2) can be activated either in a sustained or transient manner (Marshall, 1995), whereas J. Blenis (Boston, MA, USA) took a different approach and examined how sustained versus transient signalling can lead to specific cellular responses. He reasoned that the 'molecular sensors' for sustained signalling—that is, the targets of sustained ERK1/2 activation—would only be induced following prolonged growth factor stimulation. He also hypothesized that despite the observations that growth factors triggering transient or sustained ERK1/2 activation induce similar patterns of immediate-early gene (IEG) expression (Fambrough *et al*, 1999), these IEGs are still obvious candidates for such 'molecular sensors'. In support of this, Blenis showed that both transient and sustained ERK1/2 activation induced transcription of the IEG c-Fos, but that only sustained ERK1/2 activation promoted subsequent c-Fos

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Introduction

Classically, cell signalling has been viewed in terms of linear pathways that lead from external or internal stimuli to cellular effectors. But this simple view developed more as a means to visualize and to understand the individual components of these complex systems, rather than to reflect reality. It is now clear that, in most cases, cell signalling is much more complex and involves the integration of many pathways in terms of timing, amplitude and cellular localization. Furthermore,

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Fig 1 | The discovery of phosphotyrosine in proteins: 'X' marks the spot. Acid hydrolysis of polyomavirus middle T antigen followed by cellulose thin layer electrophoresis at pH 1.7 allowed visualization of phosphotyrosine ('X') for the first time in 1979. Image reprinted from Hunter T, Eckhart W (2004) The discovery of tyrosine phosphorylation: it's all in the buffer! *Cell* 116 (Suppl 2): S35–S39, © (2004), with permission from Elsevier.

phosphorylation and stabilization. This resulted in increased and sustained activator protein 1 (AP1) transcriptional activity and the regulation of a gene programme important for cell proliferation (Murphy & Blenis, 2006). Thus, the revealing insight presented by Blenis was not only the identification of a crucial 'molecular sensor' (c-Fos), but also the mechanism by which sustained ERK1/2 activation leads to a distinct biological response.

The work of Blenis clearly emphasized the importance of timing and amplitude of intracellular signalling, but it was also clear from the work of J. Scott (Portland, OR, USA) that spatial compartmentalization is also a pivotal factor. Scott detailed how A-kinase anchoring proteins (AKAPs) are crucial to the coordination of cAMP-responsive events through diverse mechanisms. For some time, AKAPs have been recognized to differentially localize the cAMP-dependent protein kinase A (PKA) to specific subcellular sites, thereby differentially directing its activity to different substrates. More recently, Scott has shown that AKAPs regulate the differential localization of enzymes involved in cAMP metabolism such as adenylyl cyclases and phosphodiesterases to establish localized intracellular gradients of cAMP, and to regulate the spatio-temporal dynamics of the activity of PKA and other cAMP-effectors (Bauman *et al*, 2006). Scott also detailed additional emerging roles of AKAPs in differentially integrating cAMP signalling with other pathways such as the one involving cyclin-dependent kinase 5 (Cdk5; Beene & Scott, 2007).

T. Hunter (San Diego, CA, USA) continued this theme of molecular interactions mediating exquisite regulation of cellular signalling and discussed the mechanisms of activation of the ataxia telangiectasia mutated (ATM) protein kinase by DNA double-strand breaks. Remarkably, total activation of ATM in a cell is rapidly induced by as few as 18 double-strand breaks (You *et al*, 2007). However, the molecular mechanisms responsible for this impressive signal amplification are not fully understood. Hunter provided some answers by describing the role for ATM interaction with the Mre1–Rad50–Nbs1 protein complex at the site of the DNA double-strand break in inducing ATM autophosphorylation and activation (You *et al*, 2005). Further studies outlined by Hunter now also implicate the association of ATM with DNA regions flanking the site of DNA breakage as important in ATM activation (You *et al*, 2007). Therefore, it seems that two different molecular interactions of ATM—one involving specific proteins, and the other involving DNA/chromatin—can lead to the same induction of autophosphorylation.

Systems biology and cell signalling

As the true picture of the complexity of cellular signalling emerges, the approaches of systems biology to understanding these regulatory networks become increasingly appealing. A. Aderem (Seattle, WA, USA) described such an approach to understanding host–pathogen interactions in the innate immune system. Macrophages use an array of pattern recognition receptors to detect diverse microbial-specific structures with the set of receptors activated directing tightly controlled, but extremely complex, pathogen-specific immune responses. By using a systems biology approach incorporating transcriptomics, Aderem deduced that the activating transcription factor ATF3 is a negative regulator of the innate immune response, a hypothesis that was validated using ATF3-null mice (Gilchrist *et al*, 2006). This elegant study shows the power of systems biology to reveal new regulatory mechanisms and possible new drug targets.

The importance of coalescence of signalling complexes was a recurrent theme throughout the meeting. W. Lim (San Francisco, CA, USA) described a systems biology approach to examining the functional outputs of manipulating scaffolding proteins in the mitogen-activated protein (MAP) kinase cascade. Scaffolding proteins are often viewed as passive assembly platforms, but Lim showed that the yeast MAP kinase pathway scaffold protein Ste5 has an active role in allosterically inducing autophosphorylation and activation of the yeast MAP kinase Fus3. Notably, activated Fus3 has a negative feedback effect, attenuating the functional output from the Ste5–MAP kinase pathway complex, while leaving other non-Ste5-dependent MAP kinase effects unaltered (Bhattacharyya *et al*, 2006).

Cellular signalling by lipids

Lipid signalling pathways continue to be widely recognized as having crucial roles in regulating diverse biological processes. B. Vanhaesebroeck (London, UK) presented data examining the roles of specific isoforms of the type I family of phosphoinositide 3-kinases (PI3Ks). Early studies in which knockouts of specific catalytic (p110 α , β , γ and δ) and regulatory (p85 α , p85 β , p55 α and p50 α) subunits of PI3K were generated in mice were sometimes difficult to interpret as the deletion of one subunit often had marked effects on the levels of other catalytic and regulatory subunits and/or the ratios of various catalytic:regulatory heterodimers within the cell (Vanhaesebroeck *et al*, 2005). To avoid such effects, Vanhaesebroeck

Fig 2 | Total internal reflection fluorescence imaging of live platelet spreading at the surface of immobilized fibrinogen. Platelet membranes were stained before settling and spreading. Red pseudocolour regions represent membrane within 25 nm of the coverslip surface. Note the dynamic reorganization of the adhesion footprint over time. Image courtesy of W. Nesbitt (Melbourne, Australia).

highlighted the need to generate knock-in mutants that abolish PI3K activity without affecting expression levels. So far, Vanhaesebroeck has reported knock-in mutant mice for the p110 α (Foukas *et al*, 2006) and p110 δ (Okkenhaug *et al*, 2002; Ali *et al*, 2004) PI3K isoforms. This approach has revealed an essential role for p110 δ in immune signalling, and for p110 α in metabolic regulation and glucose stasis in mice. Interestingly, p110 α is the only catalytic subunit identified so far that is mutated in cancer. As p110 α is directly coupled to growth factor signalling pathways and has essential roles in glucose metabolism, Vanhaesebroeck conjectured that p110 α represents a preferential oncogenic target co-opted by cancer cells for deregulated cell growth and survival. R. Pearson (Melbourne, Australia) developed this theme further by presenting data on the crucial downstream target of PI3K, Akt. He showed that Akt is essential and sufficient to drive ribosome biogenesis and cell growth through its ability to regulate both the synthesis and processing of mature ribosomal RNA (Hannan *et al*, 2003).

Tyrosine kinase signalling and therapeutics

One of the highlights of this series of biennial meetings is the awarding of the Clifford Prize for Cancer Research. The 2007 recipient was Hunter for his original discovery of tyrosine phosphorylation and his subsequent substantial body of work that has contributed to the current understanding of the importance of tyrosine phosphorylation and tyrosine kinases in cancer. He gave a historical perspective of aspects of this work, including a description of the initial discovery in 1979 of tyrosine phosphorylation (Fig 1). The significance of this discovery cannot be overstated, with the link between cancer and deregulated tyrosine kinase signalling now representing a central paradigm in biology and forming the cornerstone for most approaches for the treatment of cancer, with several tyrosine kinase inhibitors (TKIs) approved for clinical use.

A. Ullrich (Martinsreid, Germany) presented data that might shift one of the central dogmas that has guided the development of TKIs for the treatment of cancer. The traditional pathway charted for the development of anti-cancer therapies has been first to identify the main oncogenic tyrosine kinase in a cancer, and second to target this enzyme using a specific TKI. Originally, strong emphasis was placed on developing highly specific TKIs so as to limit off-target effects. However, as Ullrich pointed out, cancer cells are genetically unstable and capable of accelerated evolution that allows positive selection in the presence of specific drugs, leading to the emergence of drug-resistant clones and

disease relapse. In essence, cancer is a 'moving target' in which highly specific monotherapies are almost certain to fail. Ullrich illustrated this concept by presenting some recent outcomes of clinical trials using SU11248 (SUTENT/Sunitinib). This TKI was originally intended to target specifically the vascular endothelial growth factor (VEGF) receptor and to block tumour angiogenesis. SU11248 has shown clinically significant responses in patients with Gleevec-resistant gastrointestinal stromal tumors and renal cell carcinomas, and was approved for clinical use in 2006. However, Ullrich highlighted that the success of SU11248 might not lie in its specificity, but rather in its ability to inhibit a broad spectrum of tyrosine kinases, each of which might constitute part of the 'moving target' within a transformed cancer cell.

Regeneration

N. Rosenthal (Monterotondo, Italy; Melbourne, Australia) posed the question: Why is it that mammalian species have such a restricted capacity for tissue regeneration, whereas Amphibia such as the salamander are able to regenerate whole organs and tissues? One possibility suggested by Rosenthal was that perhaps the trade-off for less regeneration was less cancer. Recent work in the laboratories of Rosenthal and others offers some support for such an intriguing hypothesis. Rosenthal showed that knocking out I κ B kinase 2 (IKK2), a crucial regulator of the transcription factor NF- κ B, promoted muscle hypertrophy and decreased denervation-induced muscle atrophy (Mourkioti *et al*, 2006). Therefore, IKK inhibitors might be useful in the treatment of degenerative muscle diseases. However, as M. Karin (San Diego, CA, USA) discussed at the previous Barossa 2005 'Signalling Networks' meeting (Guthridge *et al*, 2006), knocking out IKK subunits can lead to an increased incidence of cancer in mouse models (Maeda *et al*, 2005). Therefore, improved regenerative capacity and an increased incidence of cancer are both regulated by IKK subunits, and might represent two inextricably linked biological outcomes for which the evolutionary solution in mammals has been to compromise on regenerative capacity.

Platelet biology

The analysis of signalling in platelets has long proved difficult, but S. Jackson (Melbourne, Australia) showed how the application of new imaging technologies can allow the spatio-temporal analysis of intracellular signalling events in single platelets both *ex vivo* and *in vivo*. By using total internal reflection fluorescence (TIRF)

microscopy, Jackson showed how the various phases of platelet activation proceed in a sequential manner and how Ca^{2+} fluxes are regulated following the activation of mechanotransduction pathways (Fig 2). B. Kile (Melbourne, Australia) then presented a series of elegant studies that describe a new biological clock that determines the lifespan of platelets. This biological clock is composed of an antagonistic balance between the relative abundance of the pro-survival protein, Bcl- x_L , and the pro-apoptosis protein, Bak. Kile suggested that the Bcl- x_L :Bak ratio provides a molecular link to the apoptotic machinery and allows old platelets to execute programmed cell death and be removed from the circulation (Mason *et al*, 2007).

MicroRNAs in cellular regulation

One of the more recently discovered signalling systems that controls complex information flows to regulate diverse cellular responses is that of microRNAs. The intersection of microRNAs with transcription factor pathways was featured in several talks throughout the meeting. E. Hornstein (Rehovot, Israel) discussed how microRNAs provide a robustness to genetic programmes, by using elegant expression, knockout and microRNA sensor methods in chick and mouse embryos. Sonic hedgehog (Shh) sets up the posterior–anterior axis in limb and digit development. In the forelimb, retinoic acid induces Hoxb8, which mediates the induction of Shh expression, but in the hindlimb the induction of Hoxb8 by retinoic acid is muted by the presence of the miR-196 microRNA, which is expressed from a gene embedded in the Hox gene cluster and that directs the cleavage of the Hoxb8 mRNA.

Concluding remarks

Although it is clear from this conference that considerable progress has been made in understanding the complexities of cellular signalling, it is also clear that much still remains unknown. Many important signalling pathways have been defined, and some of the mechanisms controlling their regulation and cross-talk between other pathways are now being elucidated. The future challenges remain to develop better quantitative assessments of these pathways and to integrate all of this information to gain a clearer global picture of cellular regulation. Such an objective is likely to require systems biology approaches, which, although showing tremendous potential, so far have only been applied to understanding a limited number of cellular responses. As knowledge on the regulation of the individual components of these pathways grows and new theoretical methods are developed to characterize network topology, systems biology approaches will become broadly viable. We look forward to hearing more on the progress in these areas at the next ‘Science Amongst the Vines’ meeting to be held again in the picturesque Barossa Valley, one of Australia’s premier wine regions, in November 2009.

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