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Chemistry comes to the cell: ASCB 2005

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Chemical biology continues to find its way into biomedical research in new and exciting ways. The recent American Society of Cell Biology meeting showed how this discipline is making an impact in areas such as cell biology.

The American Society of Cell Biology held its 45th Annual Meeting from 10 December to 14 December, 2005 at the Moscone Center in San Francisco, California. From its modest beginnings involving a few hundred scientists in 1961, this meeting has grown into a major scientific conference with nearly ten thousand attendees from around the world. Like many other areas of modern biomedical research, cell biology increasingly uses the approaches and methodologies of chemical biology. The view of cells as black boxes has given way to an understanding of cellular processes as molecular processes occurring in a chemical context. Here we review some of the chemical biology highlights from the meeting.

Quantitative biology of signaling networks

The meeting got off to an excellent start with a symposium about new quantitative studies of cellular signaling networks, chaired by Marc Kirschner (Harvard University). The talks in this session focused on the quantitative analysis of cellular signaling using a variety of existing and newly developed techniques. One exciting development in cell biology is the degree to which signaling pathways can now be modeled as chemical engineering problems. This is a striking departure from the traditional manner of studying these pathways as a series of binary interactions between individual components. By using techniques such as Bayesian network analysis, it is possible to detect emergent properties of these signaling networks that are otherwise obscured by the torrent of data.

Kirschner presented an insightful talk about the role of ubiquitination and protein degradation in cell-cycle progression, including an interesting comparison of processive versus distributive regulation of signaling networks[1,2]. To ensure that unidirectional progression through the cell cycle occurs with high fidelity, the E3 anaphase-promoting complex (APC) degrades specific cell-cycle regulators with a remarkable degree of precision. Although this process is known to be tightly regulated, how APC recognizes its substrates in a specific order has been unclear. Kirschner showed that the interaction of APC with substrates in a processive manner yields full multiubiquitin chains in a single step, whereas distributive interaction with other substrates requires multiple rounds of binding and competes with the deubiquitination reaction. Subtle changes in the substrate-APC interaction can lead to changes in substrate ordering and have large effects on cell-cycle progression. It will be interesting to see whether this principle is applied in other signaling processes such as protein phosphorylation-dephosphorylation cascades.

Gary Nolan (Stanford University) described his laboratory's work on the use of multiparameter fluorescence-activated cell sorting (FACS) to analyze kinase signaling pathways in the immune system[3,4]. He described how, by monitoring the activation status of up to 11 kinases in one experiment using state-specific antibodies, the signaling pathways involved in inflammation and oncogenic transformation can be studied at the single-cell level with unprecedented detail. Layered on top of this wealth of detail about intracellular signaling pathways, Nolan described how cell surface markers could be used to discriminate subtypes of immune cells in mixed populations from patient blood samples. This ability may allow the response of patients to particular drug regimens to be precisely monitored and related to clinical outcome. One of the goals of modern molecular medicine has been tailoring therapy to suit the individual patient, and Nolan's work should be a significant step toward meeting this goal.

The 45th Annual Meeting of the American Society for Cell Biology was held at the Moscone Center in downtown San Francisco.

Peter Sorger (Massachusetts Institute of Technology) described his group's work on the quantitative signaling of cell death and survival[5,6]. In collaboration with Doug Lauffenburger and others involved in MIT's systems biology initiative, Sorger has tackled the problem of how cancer cells interpret the signals that induce cell death or promote survival. One key to understanding these

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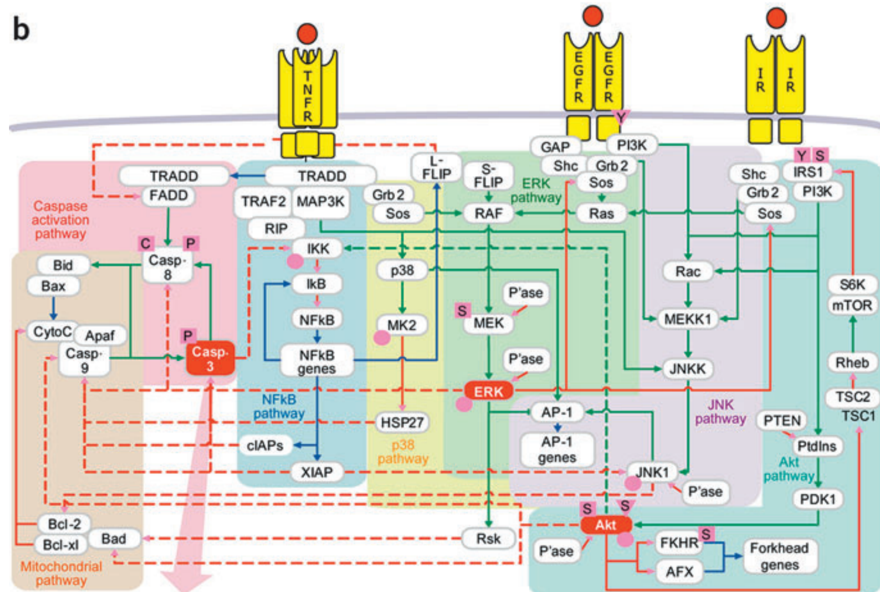

Gaudet *et al*./Mol Cell Proteomics 2005

Figure 1 The cell death and survival signaling cascades. (**a**) An oil refinery as a conceptual analog to the chemical process engineering of cell signaling. Courtesy of Emerson Process Management. (**b**) The TNFR, EGFR and insulin receptor signaling cascades. Reproduced from ref. 5 with permission.

pathways, according to Sorger, is to adopt the paradigm of reaction engineering from process chemistry (**Fig. 1**). With this idea, individual reactions can be modeled as either continuously variable, as in the case of reversible protein phosphorylation, or as sharp 'snap action' steps, as in the case of irreversible protein cleavage. The logic of this becomes clear when one considers cell death, in which a half-dead state would be highly undesirable. Emerging from these studies are several feedback loops in these signaling pathways that had previously been unknown.

Modulating the cytoskeleton with small-molecule inhibitors

Small-molecule inhibitors have been used extensively for cell biology experiments for decades. However, the development of highly specific inhibitors has been elusive and remains the focus of much of the pharmaceutical industry today. One company that has worked to develop such drugs that target cytoskeleton proteins is Cytokinetics, Inc. A group within the company has focused on finding molecules that affect the polymerization of F-actin. Through the use of high-throughput pyrene actin polymerization screens, Cytokinetics has identified two small molecules that inhibit the function of the Arp2/3 complex. Arp2/3 is an important and highly conserved protein complex that is required for the branching of actin filaments and F-actin polymerization *in vivo*. The work presented at the meeting by N. Tomasevic and colleagues showed that two compounds, CK-0944636 and CK-0993548, both inhibited actin polymerization induced by Arp2/3 activation. In this screen, either the bacterial protein ActA from *Listeria monocytogenes* or the VCA domain from N-WASP, a known Arp2/3 activator, were used to activate the Arp2/3 complex and therefore induce actin polymerization. In either case, both CK-0944636 and CK-0993548 prevented actin polymerization. Interestingly, the compounds have no effect on spontaneous F-actin polymerization or on polymerization induced by other actin nucleators such as the formin protein Cdc12. To demonstrate an *in vivo* use for these small molecules, the compounds were shown to prevent *L. monocytogenes* comet tail formation. *L. monocytogenes* motility requires the Arp2/3 complex for comet tail formation, implying that the compounds could be efficacious *in vivo* against the bacteria. The authors used total internal reflection microscopy to visualize the effects of the small molecules on F-actin filaments. Interestingly, they observed distinct differences between the two compounds in the ways that they inhibited actin polymerization. CK-0993548 inhibited any branching of actin filaments, indicating that it may prevent Arp2/3 association with the mother filament. By contrast, CK-0944636 allowed branching to occur, but prevented extension of either the mother or the daughter filament. These drugs will likely be extremely useful tools for studying actin dynamics and the Arp2/3 complex.

Inhibitors that target more than one protein can potentially affect multiple pathways and this can lead to serious misinterpretation of experiments. One widely accepted inhibitor, Y-27632, is used to inhibit ROCK2, a kinase downstream of Rho GTPases that is important for altering actin-myosin contractility of the cytoskeleton[7]. Work presented by members of the Mandato laboratory (McGill University) used genetic approaches to reveal some of the potential problems with this compound. Through RNAi techniques, they were able to show distinct morphoplogical differences in cells that lacked ROCK2 expression as compared to cells treated with Y-27632. Loss of ROCK2 decreased the abundance of some but not all internal actin structures, whereas Y-27632 decreased stress fibers and induced thin protrusions on the

periphery of the cell. This result indicates that other proteins beside ROCK2 may be affected by this compound. Although inhibitors are invaluable for determining the role of signaling events within a cell, careful interpretation of results is always required. The use of RNAi and gene knockouts will be particularly important in target validation.

Arginylation of β-actin

Post-translational modifications, such as ubiquitination, acetylation, methylation and phosphorylation, have been shown to affect protein-protein interactions and protein localization, function and degradation. Proteins can actually be post-translationally modified in a variety of exotic ways, but the significance of many types of modifications is poorly understood. One such modification is arginylation. One of the more interesting talks at the ASCB meeting, given by Anna Kashina (University of Pennsylvania), focused on the importance of the N-terminal arginylation of cellular proteins. Arginine-tRNA protein transferase (ATE1) is an enzyme that adds arginyl groups to the N-terminal ends of proteins[8]. More specifically, ATE1 has been shown to arginylate aspartic acid, glutamic acid and cysteine residues located on the N terminus of proteins, and requires the deamination of methionine residues from the N terminus. Until recently, arginylation was poorly understood and was not believed to be important owing to the fact that deletion of *ATE1* in yeast was not lethal. Intriguingly, deletion of the *Ate1* gene in mice leads to embryonic death, indicating that this gene has a significant role in mammals[9]. Previous work had indicated that the loss of ATE1 caused defects in embryogenesis and cardiac development. By comparing changes of electromobility of proteins from wild-type versus *Ate1*-knockout embryonic fibroblasts, Kashina's group determined that numerous cellular proteins seem to be arginylated *in vivo*.

One of the surprising proteins to be identified as an important substrate of ATE1 was β-actin. Both biochemical and *in vivo* studies indicate that arginylation affects the stability of F-actin structures in cells. To determine the importance of arginylation for proper actin function, pyrene-actin was added to extracts from wild-type and *Ate1*-null cells. Actin polymerization assays indicated that initial polymerization in the $Ate1^{-/-}$ cell extract was normal, but pyrene fluorescence dropped sharply at later timepoints, suggesting that actin aggregation occurs in the absence of arginylation. This postulated aggregation was confirmed by visual microscopy. In support of the *in vitro* evidence, $Ate1^{-/-}$ fibroblasts also seemed to have aggregated F-actin at the leading edge of migrating cells. In a key control experiment, the researchers found that this defect in proper actin organization could be rescued by reintroducing β-actin containing the N-terminal arginyl group into arginylation-free cells. Interestingly, other F-actin structures, such as stress fibers, appeared to be normal in these cells. This may be due to the fact that most of the β-actin is found at the leading edge and not integrated into the stress fiber network. Together, these data suggest that arginylation of β-actin provides a charged side group that keeps filaments from collapsing into clumps in cells (**Fig. 2**). As up to 50% of all β-actin (20–25% of total actin) in cells seems to be arginylated, this may be an important regulatory pathway for controlling actin dynamics *in vivo*. Determining whether other important proteins are also arginylated will also be of interest. Previous studies have shown that actin is acetylated, but the importance of this modification has remained elusive. Because acetylation also occurs at the N terminus, it is possible to believe that these two modifications may work in concert for proper regulation of β-actin.

New fluorophores for live-cell imaging

One of the most important interfaces between chemistry and cell biology is the development of biophotonic tools (dyes, fluorescent proteins and so on) to interrogate dynamic processes within cells. A number of interesting advances in this area were presented at this year's meeting. Gerald Marriott (University of Wisconsin–Madison) described his group's work on a new class of optical switches using divalent metal-ion chelates[10]. These thiol-reactive fluorophores have two states: a colorless spiro state and a fluorescent merocyanine state. Illumination with 365-nm light converts the spiro state into the merocyanine state, whereas 543-nm light excitation will return the fluorescent complex to the nonfluorescent spiro state. These optical transitions are rapid and fully reversible and will allow highly sophisticated fluorescent highlighting and quenching experiments. As proof of concept, cells were microinjected with actin conjugated with one of these chelates, BIPS. The researchers showed that these actin molecules could be optically switched between spiro and merocyanine states over 20 excitation cycles with little evidence of bleaching. This reagent will allow the use of light to modulate the fluorescence state of fluorophores reversibly and may provide extremely powerful tools for monitoring molecular dynamics in cells.

Numerous other interesting innovations in biophotonic chemistry were also presented. Wen-Hong Li's group (University of Texas Southwestern Medical Center) demonstrated a new photoactivatable fluorophore called NPE-HCCC2/AM in their work on gap junction communication[11]. These coumarin fluorophore cages show more than 200-fold fluorescence enhancement after 330–380-nm UV photolysis. NPE-HCCC2/AM is a neutral, lipophilic molecule that can easily penetrate into cells. After intracellular esterases hydrolyze the acetoxymethyl esters, NPE-HCCC2 can be uncaged by UV in a dose-dependent manner to release the quencher group 1-(2-nitrophenyl)ethyl, and 7-hydroxy-6-chloro-coumarin 3-carboxamide shows high fluorescence in aqueous environment. Because this fluorophore (after uncaging) possesses very high multiphoton excitation at 700 nm, it will be ideal for studies involving molecular tracking using multiphoton imaging *in vivo*.

Brent Martin from Roger Tsien's group (University of California, San Diego) demonstrated the group's work on optimization of tetracysteine sequences for biarsenical dyes[12]. A mammalian cell-based screen using retrovirus and FACS picked up an optimized sequence, "FLNCCPGCCMEP," that was shown to have higher fluorescence quantum yield and markedly improved dithiol resistance. This work continues to advance the use of FlAsh and ReAsh technology in cellular protein detection.

Rapidly expanding use of quantum dots in cell biology

Many cell biologists have begun using a new class of fluorophores called quantum dots, which contain a nanocrystal core, inorganic

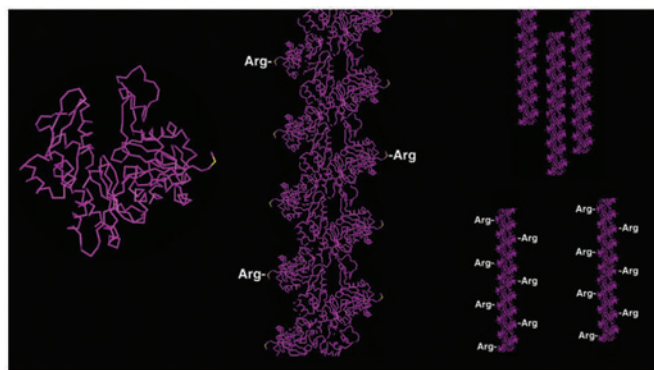

Figure 2 A model for the role of arginylation in β-actin function. In this model, actin filaments are prevented from collapsing into aggregates by the addition of charged arginyl modification to a subset of actin monomers.

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shell, organic coating and biomolecular surface. Quantum dots are highly fluorescent and essentially unbleachable, but surface chemistry has been somewhat limited until recently, and blinking behavior remains a problem for some applications. Work from Mark Ellisman's group (National Institute for Microscopy and Imaging Research, University of California, San Diego) demonstrated a way to correlate light and electron microscopy using quantum dots based on the fact that different quantum dots give different light microscopy patterns (differing emission wavelength) and are distinguishable at the electron microscopy level by size. Along these lines, Paul Matsudaira's group (Massachusetts Institute of Technology) demonstrated the visualization of the actin cytoskeleton using quantum dots and wet electron microscopy. When they compared different stains (uranyl acetate, colloidal gold particles and quantum dots), quantum dots produced the best results. Wei Chen in Da-hong Zhang's lab (Oregon State University) microinjected trace amounts of Alexa 488 phalloidin and Qdot 655 phalloidin into cells to study contractile ring assembly. She found that quantum dots intermittently decorate actin filaments and form speckles along the Alexa dye–labeled actin filaments. Taking advantage of the fact that quantum dots are highly resistant to photobleaching, the group was able to use the quantum dot speckles as precise reference points for the analysis of actin filament dynamics in the contractile ring of dividing cells. Kinneret Keren from Julie Theriot's group (Stanford University) gave an elegant talk on "Intracellular Fluid Dynamics in Motile Fish Keratocytes," in which she described using polyethylene glycol–coated quantum dots as inert tracers in living cells. In this work, she showed that the fluid flow does not contribute appreciably to transport of essential components of the actin machinery in the lamellipodia of migrating cells.

Perspectives

As cell biology moves forward into the twenty-first century, chemical biology has become an increasingly important intellectual force in this field. The recognition of chemical aspects of cell biological processes will continue to inform experiments in many areas, from signaling to membrane trafficking to motility. In addition, the collaboration between chemists and cell biologists continues to produce more and more precise tools to interrogate the cell.

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