Meeting Report

This year, in addition to coverage of the ASCB Meeting from our science writers, the JCB invited session chairs from minisymposia and special interest subgroups to contribute summaries. Excerpts from some of these articles are printed here. Additional summaries and daily coverage from the meeting are available on our biowrites blog (http://jcb-biowrites.rupress.org), and a special ASCB edition of our biobytes podcast is also available (http://jcb.rupress.org/biobytes).

Mitosis and meiosis: Dividing cells are a win-win combination

Two of the speakers in this Minisymposium were recipients of ASCB awards. Arshad Desai (Ludwig Institute for Cancer Research, University of California, San Diego) received the prestigious “Early Career Life Scientist Award.” His C. elegans embryo work has identified the core microtubule-binding machinery of kinetochores. At the meeting, Desai presented recent work from his laboratory that shows the dynein/dynactin microtubule motor complex is recruited to kinetochores by a conserved coil–coil protein called spindly. Spindly also seems to mediate lateral interactions between unattached kinetochores and microtubules, important for chromosome positioning and segregation during mitosis.

The second ASCB award winner was Ekaterina Grishchuk (University of Colorado at Boulder), lead author on the Molecular Biology of the Cell “Paper of the Year” (Grishchuk et al., 2007). Grishchuk detailed her studies of microtubule coupling dynamics and chromosome movement. Based on biophysical experiments, structural reconstructions, and computer simulations Grishchuk and colleagues have shown that microtubule depolymerization produces a strong minus end–directed pulling force. Specialized kinetochore protein complexes transmit this force to chromosomes, while ensuring their stable attachment to the shortening microtubules during anaphase (Grishchuk et al., 2008; McIntosh et al., 2008).

Sadie Wignell’s talk (Stanford University, CA) also deserves mention. She demonstrated that chromosomes can align properly at the spindle equator in the absence of classical “end-on” kinetochore attachments during meiosis in C. elegans oocytes. Klp19-mediated sliding of kinetochores alongside microtubule bundles appears sufficient for chromosome congression. In contrast, end-on attachments are essential during anaphase.

A. Khodjakov (Wadsworth Center, Albany, NY) Grishchuk, E.L., et al. 2007. Mol. Biol. Cell. 18:2216–2225.
Grishchuk, E.L., et al. 2008. Proc. Natl. Acad. Sci. 105:15423–15428.
McIntosh, J.R., et al. 2008. Cell. 135:322–323.

Cell polarity and epithelial morphogenesis

It’s hard to find a biological process not linked in some way to cell polarity. Defects in polarization underlie many disorders, including cancer. This Minisymposium highlighted the extraordinary range of processes in which cell polarity is manifested and gave a fascinating overview of the wide-ranging functions of cell polarization in animal development.

Two speakers discussed the use of genetic screens to identify genes involved in specialized types of polarized cell migration. Joshua Ziel (Duke University, Durham, NC) uses vulval development in C. elegans as a powerful in vivo model to study invasion of cells through basement membranes. He showed that Netrin, a factor implicated in axon guidance, promotes invasion of the anchor cell through the basement membrane by polarizing the netrin receptor to the basal side of the anchor cell. The receptor initiates signal transduction cascades for localized secretion of basement membrane proteases (Ziel et al., 2008). Todd Nystul (Carnegie Institute/HHMI, Baltimore, MD) uses follicle stem cell maintenance within the Drosophila germarium as a model for polarized cell migration. He showed that stem cell loss on one side of the ovariole initiates division and migration of a replacement from the other side. A genetic screen identified a cell adhesion molecule, CRELD1, as an important regulator of this process.

Cells within epithelial sheets adopt planar cell polarity (PCP). Padmashree Rida (Emory University, Atlanta, GA) described how a protein called TBC1d19, which interacts with a PCP protein called Vangl2, may regulate cell movement during gastrulation and organization of hair cells in the inner ear—coupling the PCP pathway to microtubules and cilia.

Covering mechanisms of apical/basal polarity, Eli Knust (Max-Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany) discussed the role of Crumbs, an apical polarity factor, in photoreceptor development in flies (Bulgakova et al., 2008). Crumbs forms a complex with the polarity proteins Stardust and Patj at the apical junction of epithelial cells. Defects in these genes result in light-induced retinal degeneration. Luke McCaffrey (University of Virginia, Charlottesville) described a new model for studying gene function, in which murine mammary stem cells are transduced with lentivirus to knockdown gene expression, and then transplanted back into a host mouse to permit mammary gland regeneration. Silencing the expression of the polarity protein Par3 in this system blocks normal progenitor cell differentiation and duct formation. I. Macara (University of Virginia, Charlottesville) Ziel, J., et al. 2008. Nat. Cell Biol. (Dec 21 Epub ahead of print)
Bulgakova, N.A., et al. 2008. J. Cell Sci. 121:2018–2026.
Bridging engineering and life sciences: Cool next generation tools for cell biology

This Special Interest Subgroup hosted by the National Cancer Institute united biologists, physicists, chemists, and engineers to showcase multidisciplinary approaches for studying and controlling complex cellular processes.

“Micro” technologies provide new tools to examine cell activities such as cell adhesion and migration. In one approach, adhesive proteins in linear patterns generated by laser ablation of nonadhesive materials were shown to guide cell migration in one dimension. High-resolution microscopic analyses of these migrating cells provide information about cell shapes, membrane projections, and intracellular distributions of cellular organelles during cell movement. A second strategy called “microetched migration channels with hooks,” provides a unique substrate to separate motile cells by geometry. Cells that extend long thin protrusions at the leading edge grab onto hook tips bent in the direction of migration, whereas cells with wide lamelli migrate toward hook tips allowing small numbers of cells to be sorted by their migratory behaviors.

How micro technologies can extend into the diagnostic realm was illustrated with the integrated blood barcode chip. This protein detection system is built by decorating single-stranded DNA barcode arrays with DNA-tagged antibodies. This array is assembled into a microfluidic device to generate an antibody barcode chip that can capture target proteins from solutions flowing by. As proof of the chip’s capacity to analyze complex samples, small amounts of proteins in blood taken from a finger prick were identified and measured (Fan et al., 2008).

These highlights cover only a smattering of the techniques and interdisciplinary tools discussed in this subgroup. However, they illustrate how innovative applications of emerging technologies are providing new insights into biological problems and new potential for translation to the clinic.

J. Schwarzbauer (Princeton University, NJ)

Fan, R., et al. 2008. Nat. Biotechnol. 26:1373–1378.

Wikipedia needs cell biologists

For the first time this year, ASCB offered a crash course in Wikipedia for cell biologists. As an open access source of information, Wikipedia has immense potential for outreach by the scientific community. Scientific articles such as “The immune system” draw nearly 2,600 viewers a day (one million hits per year). The Wikipedia audience is truly global, and encompasses all aspects of the general public, including policy makers, voters, and students. Becoming a wikipedian is an excellent way to draw attention to your field of study, contribute to the dissemination of accurate scientific information, and provide a lasting, valuable electronic resource for the general public.

Wikipedia is sponsored by a non-profit educational charity, the Wikimedia Foundation, and its content is created entirely by volunteers. The ASCB Wikipedia workshop gave an overview of the Wikipedia site and had WikiStaff on-site to assist attendees with creating a Wiki account. Participants then learned how to edit existing articles and were given guidelines for the desired structure of Wiki articles when creating new ones.

WikiProjects are domains within Wikipedia that coordinate article coverage for a given field. The WikiProject on Molecular and Cell Biology, headed by Tim Vickers, currently has more than 200 members and aims to increase the quality of scientific articles. While there are nearly 2 million entries for molecular and cell biology-related topics, many of these articles lack sufficient amounts of useful information. Collaborative projects, such as ProteinBoxBot, an automated software program which creates Wikipedia “skeletons” for identified human genes (Huss, 2008), have generated many truncated articles, or “stubs,” that require annotation by scientists working in related fields of study.

Wikipedia is in need of experts on scientific topics, especially cell biologists, to improve the quality of these articles and to ensure that the information is accurate, correctly cited, and well-presented. The MCB WikiProject has created a prioritized “action” list in order to focus the group’s efforts on important, contemporary topics. The MCB WikiProject lists many ways you can help, such as collaborating to improve the “article of the month,” or “adopting a stub.”

If you are interested in becoming a Wikipedian, the presentation from the ASCB meeting and tutorials are available through the 2008 ASCB Meeting website. Alternatively, you can set up a Wikipedia account and contact the MCB WikiProject. AC

Huss, J.W. 3rd, et al. 2008. PLoS Biology. 6:e175.
Why models are attractive

How cellular structures assemble is a fundamental question that cell biologists try to address in a variety of ways. But, according to Wallace Marshall (University of California, San Francisco), one approach that will prove vital to answering such questions is computer modeling.

As Marshall explained at the beginning of a session called “Building the Cell,” researchers can directly study things at both the molecular and cellular levels, but observing processes in between these levels is much more problematic. It’s here that computer modeling comes into its own. In an attempt to engage the community in thinking about these issues, Marshall invited speakers from a range of disciplines—from cell biologists to physicists to computational biologists. In turn, the speakers presented their efforts to understand the assembly of a range of cellular structures.

Several talks dealt with how membrane-bound organelles achieve their characteristic size and shape. Some speakers presented their more traditional laboratory-based approaches to this question, such as Gia Voeltz (University of Colorado at Boulder), who studies a family of proteins called reticulons that bend parts of the ER membrane into its characteristic tubular form. Voeltz’s laboratory has recently shown that the oligomerization of reticulons into an immobile scaffold is important for the specific localization of the proteins and their function in the ER.

Jian Liu (University of California, Berkeley) also looks at membrane curvature, but takes a modeling approach. Many of the molecular players involved in endocytosis are well known, but Liu generates mathematical simulations of endocytosis to gain insights into how they interact and regulate one another to form vesicles. For example, the BAR domain proteins that deform the lipid bilayer assemble and disassemble on real endocytic vesicles with rapid kinetics. The model can only replicate this by incorporating feedback loops where curvature of the membrane promotes BAR protein assembly, which in turn causes increased curvature. Liu’s models also show that the GTPase dynamin, thought to drive the pinching-off of vesicles from the invaginating membrane, might not act so directly. The tension generated by differing concentrations of the phospholipid PIP(4,5)P2 at the neck of the vesicle, according to Liu, is sufficient to drive scission. This difference in PIP(4,5)P2 is governed by the dynamic localization of dynamin and a lipid phosphatase, whose localization is also dependent on membrane curvature. Modeling all this allows Liu to recreate endocytosis in silico, with impressive accuracy to the real thing.

Other talks in the session focused on the assembly and function of the cytoskeleton. Physicist Jennifer Ross (University of Massachusetts, Amherst), showed how microtubule architecture regulates microtubule-based motors. For example, if microtubules are overlayed with one another in vitro, dynein/dynactin motors carrying cargo may switch tracks, reverse, or fall off upon reaching a crossover point. But if the density of dynein/dynactin molecules is increased, the motors and their cargo will simply pause at the crossover. This is just one way in which cytoskeletal architecture regulates motor behavior: microtubule-associated proteins, post-translational modifications, and actin–microtubule interactions all have their effects too.

Then Alex Mogilner (University of California, Davis) showed how his computer simulations suggest an unexpected twist to mitotic spindle assembly. In mammalian cells, microtubules use a “search and capture” mechanism to assemble the spindle, whereby microtubules are stabilized when they successfully attach to a kinetochore. The process takes just 20 minutes. Simulating this in silico supports recent biological observations suggesting that growth of microtubules from the chromosomes as well as from spindle poles might be a critical factor for such rapid assembly. In Mogilner’s early simulations, the kinetochores were often incorrectly attached, with sister chromatids commonly attached to the same spindle pole. To get correct attachment, Mogilner had to model a scenario in which the attachment of one chromatid results in the chromosome rotating so that the sister chromatid faces the opposite pole.

It remains to be seen whether such a chromosome twist happens in vivo, but Mogilner’s talk showed how computational models can provide fresh insights to cell biological questions. BS

Rafelski, S.M., and W.F. Marshall. 2008. Nat. Rev. Mol. Cell Biol. 9:593-602.
Cilia keep moving in the right direction

Long neglected by biologists in favor of other, sexier organelles, the primary cilium is now sending all the right signals. Sören T. Christensen (University of Copenhagen, Denmark) is particularly attracted by a signal that helps cells navigate during wound healing.

Found in almost all mammalian cells, the primary cilium protrudes from the cell surface like an antenna. Interest in the organelle has risen with the realization that components of many signaling pathways localize there. Christensen and colleagues have now found that a receptor for platelet-derived growth factor (PDGFR-α) that localizes to the cilium is essential for cells to coordinate their migration.

In response to PDGF-AA, fibroblasts oriented their cilia toward the leading edge and PDGFR-α signaling in the cilium rearranged the cytoskeleton to allow the cells to move toward the source of the ligand. Fibroblasts with disrupted primary cilia, the team showed, could not coordinate themselves to move in the right direction.

In vivo, wound healing is initiated when blood cells release PDGF-AA to stimulate fibroblast migration and proliferation. Significantly, mice lacking primary cilia have defects in repairing wounds. The primary cilium functions like a cellular GPS, says Christensen, receiving and transmitting signals to help the cell navigate correctly in damaged tissue. BS

Schneider, L., et al. 2005. Curr. Biol. 15:1861–1866.

Fibronectin feels the tension

The extracellular matrix (ECM) component fibronectin is not simply a passive player in adhesion-based signaling, according to data presented by David Boettiger (University of Pennsylvania, Philadelphia).

After binding to the ECM and clustering, integrin cell–adhesion receptors can activate nonreceptor tyrosine kinases such as FAK, promoting cell migration, proliferation, and survival. It was generally thought that fibronectin only acted as a simple substratum for the integrin α5β1, but Boettiger suggested that a tension-driven switch in fibronectin’s conformation signals through α5β1 to activate FAK and its downstream pathways.

Using both chemical cross-linking and force detachment assays, Boettiger could distinguish two types of fibronectin–integrin bonds. The “relaxed” bond represents the initial interaction between them, and when force is applied, the bond becomes “tensioned,” stimulating FAK signaling. Inhibiting myosin II activity or mutating a domain of fibronectin called the synergy site could prevent this process.

Generating the tension depends on matrix stiffness as well as myosin II activity. When cells were plated on a softer surface, fewer tensioned bonds formed and less FAK was activated; this may explain how cells can sense and respond to changes in ECM stiffness. The findings also have implications for understanding cell migration, since myosin II can drive the initial protrusions of the cell’s leading edge and then strengthen cell adhesions as they form and pull the cell forward. It’s a wholly different way of looking at how integrins function, says Boettiger. BS

Boettiger, D. 2007. Methods Enzymol. 426:1–25.

iPS cells as tools and treatments

Induced pluripotent stem (iPS) cells were a major topic of discussion at the working group on the “Impacts of Stem Cell Research on Cell Biology,” with many participants eager to discuss their potential for research tools and therapies.

iPS cells are cells that have been taken from adult tissue and reverted to an embryonic stem cell-like state by the introduction of just a few key genes. A major concern over their potential for treating human disease is that chimeric mice derived from iPS cells tend to develop tumors. Using plasmids rather than retroviruses to generate iPS cells (so that the key genes are not integrated into the host genome) reduces this risk, which is one way that the originator of the technique, Shinya Yamanaka (Kyoto University, Japan) is attempting to make the technology safer. But even if the mice don’t form tumors, they still show higher mortality rates than wild-type mice. It’s unclear why this is so, but Yamanaka speculated that it could be due to incomplete reprogramming of the cells.

The session moderator, Larry Goldstein (University of California, San Diego) cautioned the community against settling on iPS techniques as the only way to make pluripotent cells. Other approaches, such as cell fusion and nuclear transfer, should continue to be explored in case they prove to be safer and more efficient techniques. Nevertheless, his group is generating iPS cells from the dermal fibroblasts of Alzheimer’s disease (AD) patients that can then be differentiated into neurons. They think these cells will be a better model system for studying sporadic AD than current animal models that rely on a single genetic mutation and only replicate a subset of AD symptoms. BS

Okita, K., et al. 2008. Science. 322:949–953.

Schneider, L., et al. 2005. Curr. Biol. 15:1861–1866.
Cancer cells bound for the brain

Joan Massagué (Memorial Sloan-Kettering Cancer Center, New York, NY) and colleagues have identified a protein that grants metastatic breast cancer cells access to the brain.

The tissues that metastatic cancer cells infiltrate and colonize are determined by the original tumor type and by the particular proteins the cells express. Massagué’s team has done extensive work in mice and with human cancer samples to characterize the specific proteins expressed by breast cancer cells bound for the lung. Now the team has done the same for brain-bound cells.

The lung work revealed 54 genes whose expression indicated a lung fate, 18 of which can be expressed in the primary tumor even before cells have set out on their journey. These early expressers tend to code for proteins that come in handy for exiting the blood vessels of the lung. The brain-bound cells share a number of these handy proteins, Massagué said, but they also have one protein that appears to be crucial for brain access—perhaps necessary because the blood–brain barrier is particularly recalcitrant to infiltrators.

The protein is an undisclosed member of the sialyltransferase family—enzymes that attach sialic acid onto sugar moieties of glycoproteins. Massagué explained that sialyltransferases have been reported to regulate cell–cell recognition and adhesiveness, but exactly how the identified sialyltransferase works during breast-to-brain metastasis is not yet known. Whatever the underlying mechanism, when the team knocked down the sialyltransferase in the cancer cells, brain entry was reduced—an important finding because brain tumors are among the most difficult to treat. RW

Nguyen, D.X., and J. Massagué. 2007. Nat. Rev. Genet. 8:341–352.

Could bone save brain?

Bone marrow transplants might delay a devastating neurodegenerative disease called mucolipidosis, according to a presentation by Craig Montell (Johns Hopkins University School of Medicine, Baltimore, MD).

Mucolipidosis type IV (MLIV) is a lysosome storage disease (LSD) and like most other LSDs causes severe childhood neurodegeneration. The cause of MLIV—mutation of a gene called trpml1—was discovered in 2000, but since then there has been little progress in understanding MLIV’s cellular pathology, or in designing an effective treatment.

To learn more about MLIV pathology, Montell’s team mutated the single trpml1 homologue in flies (trpml). The mutant flies had impaired motor skills, and showed progressive neurodegeneration due to increased cell death. A closer look at the flies’ cells revealed that their lysosomes contained a buildup of undegraded proteins and lipids. This lysosomal failure was apparently causing a failure in autophagy—a process for disposing of damaged cellular components.

Autophagy is a general housekeeping mechanism in cells, and the TRPML protein is widely expressed but the defects in the flies (as in humans) appeared to be neuron specific. Indeed, expression of TRPML in the mutant flies’ neurons eradicated the disease symptoms. Amazingly however, expression of TRPML in hemocytes or glial cells also greatly improved symptoms. This seems to be due to increased clearance of dying neurons from the flies’ brains and retinas—both glia and hemocytes dispose of dying cells and TRPML boosts this disposal by promoting lysosome function.

The results suggest that while neuronal death is the primary cause of MLIV, the disease is worsened when the dying cells are not cleared—probably because neighboring cells are damaged or killed by toxic cell debris. The team now plans to test whether bone marrow transplants improve disease symptoms in a mouse model of MLIV. RW

Venkatachalam, K., et al. 2008. Cell. 135:838–851.
How do flies smell?

Awful! It might sound like the set-up for a bad joke, but in fact it’s a question that drives Leslie Vosshall’s research. The answer is: they use ion channels. And Vosshall’s team has now identified in the fly a new breed of odor-responsive ion channels that are similar to glutamate receptors of the brain.

Earlier this year, Vosshall (The Rockefeller University, New York, NY), together with colleagues from the University of Tokyo, showed that unlike vertebrates—whose olfactory receptors (ORs) are G protein–coupled receptors that trigger signal transduction pathways—flies’ olfactory receptors are ion channels that trigger cation influx.

The fly ORs are expressed in olfactory neurons in the antennae and maxillary palps, but as Vosshall explained, there are certain olfactory neurons that don’t express ORs. Her team has now shown that IRs (ionotropic receptors) do the odor sensing in these neurons. Like ORs, IRs are ion channels, but the two are structurally unrelated. Instead, IRs are evolutionarily linked to glutamate receptors that sit at neuronal synapses. Interestingly, a family of glutamate receptor-like proteins has been identified in plants. Although nothing is yet known about the physiological function of these plant receptors, chances are they are involved in chemosensing rather than in nerve transmission. RW

A view to a kill(er)

Bojana Gligorijevic (Albert Einstein College of Medicine, New York, NY) and colleagues have made it possible to view the metastatic behavior of tumor cells in a living mouse—by installing a window.

They call it a Mammary Imaging Window (MIW) to be precise. The 8-mm diameter glass porthole is mounted in two plastic rings that are sutured into the skin. The tumors are easy to spot because they glow green (mice are injected with fluorescent cancer cells that home to the mammary gland and develop into tumors).

To watch activity within the tumor, the team photoswitches individual cells (or small groups of cells) from green to red by targeting them with a blue laser. Using this approach, they have looked at how different microenvironments of the tumor affect cell behavior. In regions without detectable blood vessels, there was limited cell migration and the number of photoswitched cells increased, suggesting proliferation. In environments surrounding blood vessels, however, cells underwent considerable migration but reduced proliferation. These cells tended to line up next to the vessels before gradually disappearing, suggesting exit into the bloodstream. Indeed, red fluorescent cells turned up in the lungs.

The MIW can remain in the mouse for a number of weeks, and though photoswitched cells remain red for a few divisions only (before becoming too dim to see), Gligorijevic says that it might be possible to photoswitch individual cells a second time and thus follow their path over a longer period. RW

And finally…

Two stories from the meeting that can only be described as weird and wonderful.

First, the discovery by Mimi Shirasu-Hiza (Stanford University, CA) that disease progression after infection depends not only on the pathogen, but on the time of day that infection occurs—at least if you’re a fruit fly. The underlying cause seems to be a change in the activity of phagocytes, which Shirasu-Hiza showed eat less of certain pathogens during the day than at night. She and her colleagues are now determining the molecular links between circadian rhythm and phagocyte activity to try and uncover why these cells get the midnight munchies for certain bugs.

A second helping of peculiar pie came from Michael Overholtzer (Memorial Sloan-Kettering Cancer Center, New York, NY). He reported that breast cancer cells, in vitro and in vivo, can invade other breast cancer cells and continue living inside them. Though the living arrangement will usually result in death for the inner cell, this is not always the case. Both inner and outer cells can divide, and can also give up their relationship and go back to living separate lives. The story gets weirder. Once a cell has invaded another, these two can invade a third. This might be described as the cellular equivalent to Russian dolls except that big cells can monstrosely squeeze themselves into smaller neighbors!

Since most internalized cells die, this could be a novel suicide mechanism for tumor cells. But Overholtzer’s latest work suggests that cell-in-cell arrangements might also lead to the formation of multinucleated cells, thus prompting tumor progression. RW

Kedrin, D., et al. 2008. Nat. Methods. 5:1019–1021.

Shirasu-Hiza, M.M., et al. 2007. Curr. Biol. 17:R353–R355.

Overholtzer, M., and J.S. Brugge. 2008. Nat. Rev. Mol. Cell Biol. 9:796–809.

Benton, R., et al. 2009. Cell. 136:1–14.