

Meeting Report

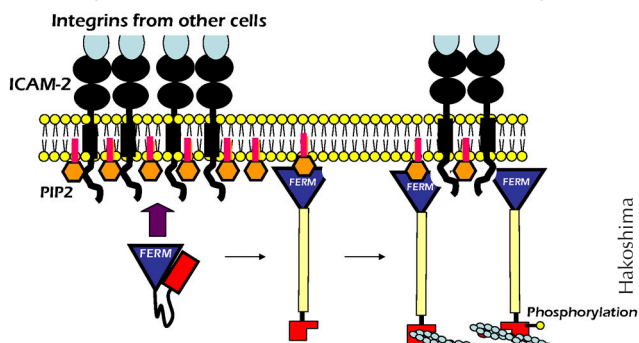
In May, the Japan Society for Cell Biology held their annual meeting in Osaka. For some of us, this year's meeting was a special treat; presentations were given in English for the first time in the meeting's 57-year history. The conference organizers, including President Yoshimi Takai (Osaka University), did a superb job of attracting top cell biologists from not only Japan, but also far overseas. Each symposium was an especially cohesive collection of presentations, and topics included microtubule targeting and function, tissue remodeling, membrane trafficking, ubiquitylation in DNA repair, noncoding RNAs, structural analysis of transmembrane signaling, and advances in live-cell imaging. Poster sessions included discussions in Japanese when appropriate, but English was also heard throughout. Attendees and speakers included both established and young, up-and-coming scientists who enjoyed top-rate science in an international atmosphere.

PIP₂ unlocks linkers

ERM proteins link adhesion molecules to the cytoskeleton, but this linkage must be restricted to the necessary place and time. Structural analyses by Toshio Hakoshima (Nara Institute of Science and Technology, Nara, Japan) suggest that phospholipids take care of this regulation.

Inactive ERMs, such as radixin, are folded to mask both their actin- and adhesion protein-binding (FERM) domains. This fold is lost in vitro if either the actin-binding domain is phosphorylated or the FERM domain binds to PIP₂. Both modifications occur in vivo, so Hakoshima examined which was critical for radixin activation.

The analysis revealed that the phospholipid binding activates ERMs. In the radixin crystal structure, the PIP₂-binding site was accessible in the folded form, whereas the phosphorylation site was buried within the interface. Phospholipid binding to FERM opened the structure, thus exposing both actin- and adhesion molecule-binding sites.



PIP₂ binds to and activates the closed form of ERMs.

PIP₂-mediated activation restricts ERM opening to the membrane. Adhesion molecules can then lock the ERMs at adhesion sites.

The adhesion molecules may also recruit ERMs by pooling PIP₂. Hakoshima showed that adhesion proteins such as ICAM-2 contain positively charged regions just inside the cytoplasmic side of the membrane that remain accessible when ERMs bind. ICAM2 clustering may therefore concentrate negatively charged PIP₂ at adhesion sites, bringing in more ERMs.

PIP₂ production is enhanced by Rho, which also activates a candidate ERM kinase. ERM phosphorylation stabilized the open structure, suggesting that high Rho activity may strengthen adhesions via multiple routes. ■

References: Hamada, K., et al. 2000. *EMBO J.* 19:4449–4462.
Hamada, K., et al. 2003. *EMBO J.* 22:502–514.

Kinesins deliver stabilizing goods

Some kinesins destroy microtubules by chewing them up. But others have stabilizing effects that have not been explained. David Pellman (Harvard Medical School, Boston, MA) presented evidence that, in yeast, these motors can be stabilizing because they deliver plus-end binding proteins to microtubule tips.

In budding yeast, astral microtubules are more stable during mitosis than during interphase, which allows them to grow toward the bud site for correct spindle positioning. Pellman finds that these changes in microtubule stability correlate with fluctuations in the levels of the Kip2 kinesin.

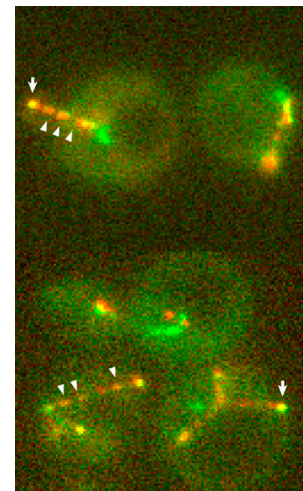
Pellman was able to track this motor and its cargo on individual microtubules. Kip2's cargo included Bik1, a plus-end binding protein known to stabilize

microtubules. During mitosis, Kip2 levels were highest and so Bik1 was carried out to the plus ends.

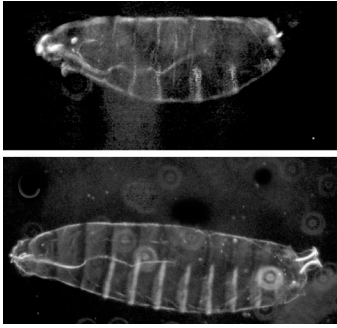
Kip2 was also needed to bring dynein, a minus-end-directed motor needed to pull the spindle into the bud at anaphase, to microtubule plus ends. From there, dynein was transferred to the cortex. A receptor on the cortex, possibly Num1, seems to activate dynein. "When [dynein] hits the cortex, we see showers of [dynein] speckles tracking toward the minus ends," says Pellman.

Kip2 levels are low until mitosis, so the plus ends of interphase microtubules have less dynein and Bik1. Their absence seems to favor other plus-end binding proteins, such as Kar9, which works with a myosin in G₁ to guide spindle microtubules to the bud site. ■

Reference: Carvalho, P., et al. 2004. *Dev. Cell.* 6:815–829.



Kip2 (red) takes Bik1 (green) to the plus ends of microtubules.



Siomi/CSTHL

RNAi inhibition of *ftz* (top) does not happen if AGO2 is missing (bottom).

miRNA precursor, and with both unprocessed and mature forms of the miRNA. In the AGO1 mutant, there is much less mature miRNA, but its precursor did not accumulate in its place, so Siomi supposes that AGO1 stabilizes the mature miRNA after cleavage.

The separate AGO functions suggest that perhaps one RISC does not fit all. “I believe that the [RISCs] are distinguishable in terms of the protein components,” says Siomi, “because we do not detect AGO2 in the AGO1 complex, and vice versa.”

Making the RNAs is probably restricted to the complex with the appropriate AGO. The two complexes may talk to each other during later stages of silencing, however. “Perhaps at the second round or later . . . , the small RNAs are somehow exchangeable between RISCs, since we can clearly detect mature miRNAs both in the AGO1- and AGO2-containing complexes,” says Siomi. “But this is merely our speculation.” The biological significance of any such interchange remains to be determined. ■

Reference: Okamura, K., et al. 2004. *Genes Dev.* doi:10.1101/gad.1210204.

Silencing specificity

RNA-mediated silencing begins with two types of RNAs. Those completely complementary to their target (siRNAs) lead to message degradation, whereas those less perfectly matched (miRNAs) block translation of their target. Both RNAs are produced by and carry out their very different functions within an RNAi-induced silencing complex (RISC). Until recently, all RISCs were considered equal. But evidence presented by Mikiko Siomi (University of Tokushima, Japan) suggests that RISCs are customized for the two RNAs with an appropriate Argonaute (AGO) family member.

Flies have multiple AGOs, but Siomi shows that at least AGO1 and AGO2 are not interchangeable. AGO2 mutants were blocked in single-stranded siRNA production but could make miRNAs. The siRNA duplex is normally made by Dicer-2 cleavage of a long dsRNA, and AGO2 was needed to unwind this siRNA duplex, although no helicase domains have been identified in AGO2.

AGO1, in contrast, was needed for miRNA accumulation and formation of the miRNA-containing RISC, but was dispensible for siRNA function. AGO1 associated with Dicer-1, which cleaves the

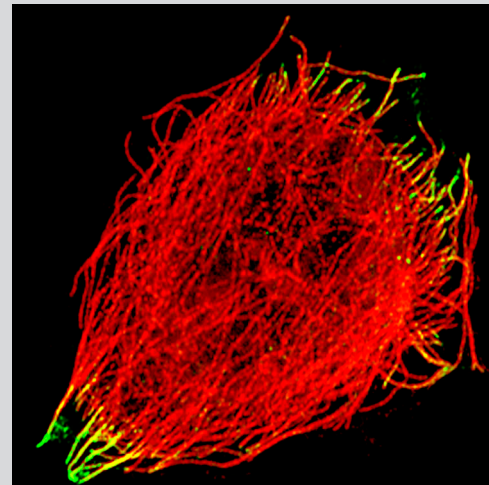
Microtubules: on the plus side

Microtubule plus ends hold the keys to their dynamics and, thus, their targeting, as was presented by Yuko Mimori-Kiyosue (KAN Research Institute, Kyoto, Japan).

Those keys are plus-end binding proteins such as CLASP and APC. Because the plus end is the primary site of growth and shortening, proteins that bind there are good candidates for regulating directional growth. Mimori-Kiyosue has found that this is the case for at least two classes of plus-end binding proteins, CLASPs and adenomatous polyposis coli (APC).

Based on their localizations, CLASP1 and CLASP2 are poised to act as linkers, as they were found to bind to plus ends and to the cortex independently. CLASP2 also bound to EB1, which sits specifically on the plus ends of growing microtubule. CLASP knock-down experiments reduced the number of microtubules at the very edge of the cell, suggesting that CLASPs keep growing, EB1-bound microtubules near the periphery. Microtubules in cells with reduced CLASPs also had shorter pause time and more depolymerization events. In the presence of CLASP, peripheral microtubules were stable yet dynamic, with more frequent transitions from growth to shortening and from shortening to growth than in the knock-down cells.

Mimori-Kiyosue's experiments were done in nonmotile HeLa cells, in which CLASPs were found at the edges of lamellipodia all around the cell. In migrating cells, CLASPs localize to the leading edge, as has been shown in neuronal growth cones and fibroblasts, and may therefore favor microtubule growth in the direction of migration.



Mimori-Kiyosue

Plus-end binding proteins such as APC (green) stabilize and orient microtubules (red).

Migrating epithelial cells also receive directional instruction from APC during wound migration. APC is transported to plus ends by the KIF kinesin. Mimori-Kiyosue showed that only when APC also binds to PDZ proteins, such as the DLG scaffolding protein, does directional migration result. DLG localizes to the basolateral plasma membrane and may thus help point stabilized microtubules in this direction. Wounds that slice through a tissue will therefore be repaired efficiently by sideways migration of cells. ■

Reference: Mimori-Kiyosue, Y., and S. Tsukita. 2003. *J. Biochem.* 134:321–326.