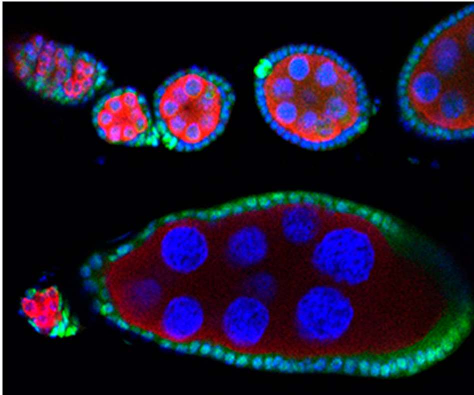
Research Roundup

Dividing via miRNA


RUOHOLA-BAKER/MACMILLAN

Fewer oocytes are produced in flies lacking Dicer-1 activity (bottom).

Stem cells use the miRNA pathway to drive mitosis forward through a stage when other cells would stall, according to results from Steven Hatfield, Halyna Shcherbata, Hannele Ruohola-Baker (University of Washington, Seattle, WA), and colleagues.

The remarkable ability of stem cells to divide relentlessly while surrounding cells are quiescent is key to their function. Stems cells have been noted to express specific miRNAs, suggesting that translational inhibition by these small RNAs might be involved. Ruohola-Baker's group now shows that Dicer-1, the enzyme that makes miRNAs, is required for the strong proliferative capability of germline stem cells (GSCs) in flies.

Compared with the wild type, mutant GSCs lacking Dicer-1 were blocked at the G1-to-S transition. Cyclin E, which normally drives cells into S phase, was high in the mutant GSCs. But Dacapo, a cyclin E inhibitor, was also elevated. As a result of the reduced GSC proliferation, female mutants made only 20% of the normal number of eggs. Egg production could be partially rescued in the Dicer-1 mutants by reducing Dacapo levels.

As Dacapo has several putative miRNA-binding sites in its 3′ UTR, the authors suspect that these miRNAs normally down-regulate Dacapo in GSCs, when other cell types become quiescent. The group is now testing whether the relevant miRNAs inhibit the translation of a reporter linked to the Dacapo 3′ UTR. Other stem cells, and possibly even cancer cells, might use a similar strategy to proliferate beyond the norm. **JCB**

Reference: Hatfield, S.D., et al. 2005. *Nature*. doi:10.1038/nature03816.

Infection in trans

The twist of a peptide bond in a viral protein starts a timer for infection, according to Barbara Eckert, Franz Schmid, and colleagues (Universität Bayreuth, Germany). To be successful, the virus must find its host's receptor before this timer goes off.

Bacterial infection by the phage fd is initiated by its gene-3-protein (G3P), which is folded into a protected conformation that is stable, robust, and fairly resistant to degradation, but not infective. Before infection, G3P must be opened via an initial contact with the bacterial F pilus to expose the binding site for its ultimate receptor, TolA. This opening converts a glutamine–proline bond in the hinge between the two binding domains from the cis to the trans conformation. This (nonnative) trans proline locks G3P in the open, infectious form.

The trans proline bond takes several minutes to isomerize spontaneously back to cis. "The back reaction is so slow because of the peculiar local sequence [glutamine–proline–proline] around the proline," says Schmid. Now his group shows that this sluggish isomerization gives phage fd enough time to infect the cell.

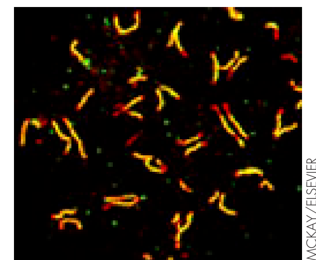
Mutations at and around the hinge proline that hastened the return to cis reduced viral infectivity, presumably because mutant G3P refolded and thus closed its domains before it found TolA. Indeed, trans-to-cis isomerization abolished G3P binding to TolA. Enzymes that accelerated isomerization also interfered with infection. As open G3P is more protease sensitive, keeping it closed probably protects the virus. It might also improve viral specificity by requiring two host interactions. **JCB**

Reference: Eckert, B., et al. 2005. *Nat. Struct. Mol. Biol.* doi:10.1038/nsmb946.

Rec'd recombination

The mammalian recombination machinery is determined to act, according to results from Huiling Xu, Michael McKay (Peter MacCallum Cancer Centre, Melbourne, Australia), and colleagues. If meiotic homologues are not properly positioned, the machinery sets up on sister chromatids instead.

Proper homologue positioning required Rec8, McKay's group showed. In yeast, Rec8 is known to be essential for recombination and for the formation of the synaptonemal complex (SC), which first links the homologues. In mice lacking Rec8, however, the SC was still laid down—but between sister chromatids rather than homologues. Rec8 thus somehow prevents SC formation between sisters normally.


MCKAY/ELSEVIER

Formation of the SC (yellow) reveals the synapsis of sister chromatid pairs in Rec8 mutants.

Chromosome pairing appeared normal in the mutant germ cells, but the pairs might not be presented correctly. "Early on in premeiotic prophase," says McKay, "Rec8 may have a structural role in creating a competent core. With Rec8, you get two homologues presenting a nice unified core to the SC machinery. If you don't have it, maybe the homologues are never close enough together. The only thing left is the sisters."

Sisters even underwent early recombination events, including DNA double-strand breaks. Later events were not seen, possibly because the unusual SC formation caused apoptosis before crossovers could be resolved. Germ cell death left the mice sterile, as expected. Less expected were the somatic effects, including growth defects, which could not be predicted based on the phenotype of yeast Rec8 mutants. **JCB**

Reference: Xu, H., et al. 2005. *Dev. Cell.* 8:949–961.

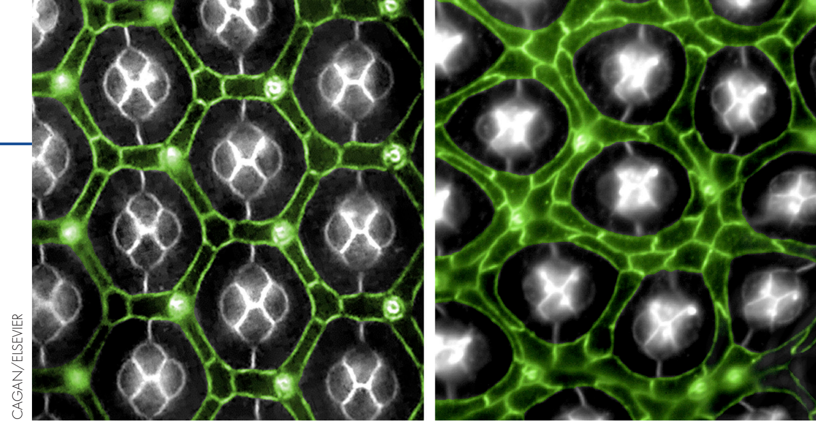
Downloaded from http://rupress.org/jcb/article-pdf/170/1/10/1884034/jcb170110.pdf by guest on 29 November 2023

Text by Nicole LeBrasseur

lebrasn@rockefeller.edu

Patterned by adhesion

Elaborate tissue patterns are formed by two cell types with complementary adhesion molecules, as revealed by Sujin Bao and Ross Cagan (Washington University, St. Louis, MO). Favored heterophilic attractions between the two adhesion molecules, they show, pattern the fly eye.



CAGAN/ELSEVIER

IPC (green) movements and patterning are disturbed in a Roughest mutant (right).

Patterning has often taken a back seat to fate determination in cell biology studies. "We've spent buckets of time," says Cagan, "finding all sorts of signaling molecules that explain cell fate, but not patterning. We can make kidney cells [in vitro], but how do you sort them into a glomerulus? What we really want to know is not what makes a lung cell, but what makes a lung."

A hint came when others found that mutation of the Ig family member Roughest caused disorganized ommatidia in flies but did not affect cell fate. Differential adhesion had been shown previously to control patterning by minimizing surface free energy—highly adherent cells congregate in the center, excluding cells with lesser adhesion strengths. But the fly eye is much more elaborate; each ommatidium is surrounded and separated by a single layer of cells derived from a pool of interommatidial precursor cells (IPCs) that rearrange to form a hexagon-shaped lattice.

For this lattice, complementary cell types expressing different adhesion proteins were needed. IPCs expressed Roughest, while the ommatidia expressed its homologue, Hibris. Roughest bound more strongly to Hibris than to itself, thus creating competition between IPCs for contact with ommatidia and minimizing interactions between IPCs. The result is that "the IPCs stretch out between the ommatidia," says Cagan. "The lowest free energy shape they can take is a hexagon."

Heterophilic interactions led to the formation of lasting junctions between the two cell types. IPCs that lost out in the competition for contact with ommatidia died by apoptosis; Roughest expression level was a determining factor in survival versus death. An IPC that had extra Roughest often inhabited a niche normally occupied by two IPCs. Deletion of Roughest from an IPC, by contrast, increased that cell's chance of dying.

As mammalian homologues of Roughest are strongly expressed in the kidneys, Cagan suspects that they are also a driving force behind glomerulus formation. "The way you can get a complex refined structure," he says, "is to use a two-piece system and then control the expression of those two pieces." JCB

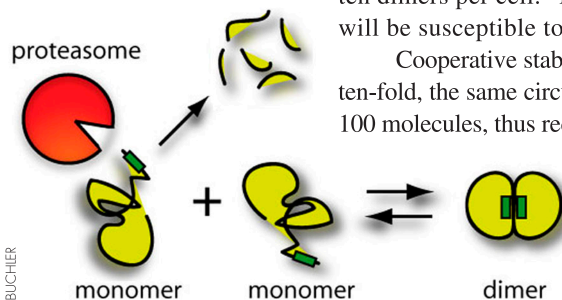
Reference: Bao, S., and R. Cagan. 2005. *Dev. Cell.* 8:925–935.

Stability in numbers

Two subunits are better than one when the goal is a bistable or oscillating circuit. The findings, based on a mathematical model by Nicolas Buchler (The Rockefeller University, New York, NY) and colleagues, suggest another reason why homodimers are common in biology. "When dimers are more stable than monomers," says Buchler, "it can have a big impact on genetic circuits."

Experimental evidence has shown that dimerization can hide a proteolytic tag or stabilize unfolded monomers. For such proteins, the degradation rate will decrease as the concentration of the protein (and thus of its dimer) increases. Buchler et al. modeled the effect of this cooperative stability in a simple, bistable bacterial genetic circuit in which a transcription factor dimer activates its own gene expression.

This bistable circuit can settle in either of two states at a high or low protein level, respectively. For typical transcription factors at physiological conditions, the bacterial cell was shown to have only two to ten dimers per cell. "For the high state, that's not many molecules," says Buchler. "The circuit will be susceptible to stochastic noise."



BUCHLER

By blocking degradation, dimerization may extend the range at which a bistable circuit operates.

Cooperative stability relieved this problem. By elevating the degradation rate of the monomers ten-fold, the same circuit could now be bistable at higher concentrations. The high state increased to 100 molecules, thus reducing the susceptibility of the bistable circuit to stochastic fluctuations.

Since many other proteins (e.g., enzymes, kinases) can form dimers, cooperative stability might also influence metabolic or signaling networks. "We still need experiments to know how common this is," says Buchler. "But we hope our results highlight the potential consequences of dimerization and degradation of regulatory proteins". JCB

Reference: Buchler, N., et al. 2005. *Proc. Natl. Acad. Sci. USA.* doi:10.1073/pnas.0409553102.

Downloaded from http://rupress.org/jcb/article-pdf/170/1/10/1884034/jcb170110.pdf by guest on 29 November 2023