

# People & Ideas

## Arnaud Echard: *Adieu, ma soeur*

Echard studies membrane trafficking and the cytoskeleton, with a focus on cytokinesis.

**D**uring cytokinesis, the two daughter cells begin separating from each other through constriction of an actomyosin ring. The cells then remain connected via a narrow cytoplasmic intercellular bridge for several hours while the membrane is remodeled at the fission site. Only then can they part ways.

Arnaud Echard wants to know how cytokinesis is achieved and regulated. By leveraging his background in membrane dynamics (1, 2) and cytokinesis (3), Echard has demonstrated that trafficking (4) and modification of membrane lipids (5) drive cytoskeletal reorganization at the cytoplasmic bridge. To learn more, we called him at his lab at France's Institut Pasteur.

### FIRST CONNECTION

#### *Do you recall your first encounter with biology?*

When I was 11 or 12 I received a little book for kids on protozoa. It had beautiful pictures of these wild-looking organisms, and it also described a protocol you could use to culture protozoa. My brother had a children's microscope, so I followed the protocol and looked at a homemade broth under the microscope. But back then I was also interested in chemistry and astronomy.

At that time it was easy to get chemicals for experiments, and for some reason my parents let me do this.

Maybe they didn't realize the danger. [Laughs] I actually recently came across a little lab notebook that I kept when I was 16, describing the chemistry experiments I conducted in the garden shed behind our house. It wasn't until I was in the Ecole Normale Supérieure in Lyon that I was first exposed to the field of cell biology.

#### *How did you first come to work on Rab proteins?*

It was when I was a graduate student in Bruno Goud's lab at the Institut Curie in

Paris. His lab has a lot of expertise on the Golgi apparatus, and I remember that when I came to the lab I had the choice to characterize several different partial cDNAs encoding potential interactors of a Golgi-localized Rab protein. Just by chance I picked one that turned out to be a kinesin. At the time the genome was not yet sequenced, so I spent about six months creating a library of phages to use in cloning this kinesin-encoding gene—something that today takes only two days. It is now so simple to click and order a full-length clone from a company. [Laughs]

Anyway, this kinesin was one of the first kinesins to be associated to the Golgi, and we also found that the Rab6 GTPase interacts with it. At that time, I would've loved to have RNAi to study the function of these proteins, but that hadn't been discovered yet, so for my doctorate we instead used dominant-negative constructs to show that they are important for trafficking from the Golgi.

### STRONG TIES

#### *Why did you switch fields for your postdoc?*

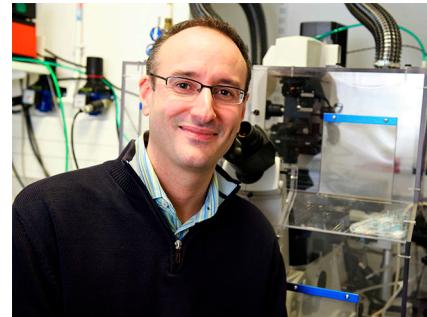
Towards the end of my PhD, we realized that the expression of our Golgi-associated kinesin is strongly up-regulated during mitosis and that it is essential for cytokinesis. But no one in our lab worked on cytokinesis, so I decided I would study cell division for my postdoc. I was interested in Patrick O'Farrell's lab at the University of California, San Francisco because his lab had published some beautiful papers characterizing cyclin A, cyclin B,

and Cdc25 in *Drosophila*. When I got to his lab, I felt quite at home because San Francisco has a very European feel to it.

**"It was the perfect project for me to bring back to Paris."**

#### *What were you working on with O'Farrell?*

During cytokinesis the cell contracts at the equator. But the cell must wait for the



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chromosomes to be fully segregated before it starts contracting. So the question I wanted to ask was: How is the timing of cytokinesis determined? We knew that cytokinesis onset is linked to the decrease of CDK activity, but at that time we didn't have the molecular details for how this worked. We could at least establish that cyclin-CDK inhibits the function of a cytokinesis-specific Rho guanine nucleotide exchange factor essential for furrow contraction. It was a pretty exciting time.

Then I got lucky because, while I was at UCSF, the first papers were published showing that RNAi works in *Drosophila*. Soon, the Vale, Davis, and O'Farrell labs produced a fantastic library of RNAi clones covering the fly genome. So I did one of the first RNAi screens for new genes involved in *Drosophila* cytokinesis. Most of the hits were related to actin dynamics, but we did find some new genes. Surprisingly, many of these were genes previously known to be involved in membrane trafficking. I had come from the membrane trafficking field, and by chance we found out that membrane trafficking was very important for cytokinesis. So it was the perfect project for me to bring back to Paris.

### CLEARING THE WAY TO CUT

#### *Why did you return to France?*

I left UCSF when I did because I was hired for a permanent position at the CNRS, the National Center for Scientific Research in France. I had to return quickly to France or lose the position.

At that time, it was quite common for French researchers to go abroad for their postdoc. They'd then return to their graduate advisor's lab to work as a junior researcher for a time before becoming completely independent. So that is what I did. It was then that we discovered Rab35-regulated pathways involved in cytokinesis—a project that I'm still working on now that I have my own lab.

When we first started working on Rab35, nothing was known about its function or localization; we started completely from scratch. We found that Rab35 is important for recycling lipids and proteins from the endosomes back to the plasma membrane. Later, we showed that the function of Rab35 is to locally remove the lipid PI(4,5)P<sub>2</sub>, which in turn is important for limiting the local accumulation of actin at the intercellular bridge that joins two daughter cells. It does this by recruiting a protein called OCRL, a lipid phosphatase that hydrolyzes PI(4,5)P<sub>2</sub> into PI(4)P. We found that cells lacking OCRL have too much PI(4,5)P<sub>2</sub> and therefore too much actin at the bridge, so the bridge cannot be cut by the abscission machinery.

***So you need actin to begin cytokinesis, but you need to remove it to finish...***

Exactly! And it turns out that OCRL is mutated in an extremely rare genetic disease called Lowe syndrome. In patients with this disease, which is currently in-

curable, the proximal tubular renal cells are unable to recapture proteins from the primary urine. Through collaboration with physicians at the nearby Necker hospital, we have obtained cells from some of the French patients affected with this disease.

We've found that, although these patients don't have binucleated kidney cells, their cells do take a very long time to complete cytokinesis. Our earlier studies had suggested this delay might be due to having too much PI(4,5)P<sub>2</sub> and therefore too much actin, so we asked if we could correct this defect by treating the cells with latrunculin, a drug that depolymerizes actin. And indeed we found that we could completely restore normal cytokinesis timing by treating patient cells with tiny amounts of latrunculin, which had no effect on normal cells. We now suspect the problems with proximal cells in the kidneys could be due to deficiency in endocytic recycling, caused by having too much actin on specific endosomal compartments. We're now setting up a novel animal model for this disease to test whether low doses of latrunculin could alleviate disease symptoms.

So that is one major project in the lab, but we're also still interested in cytokinesis. We've recently begun looking at the midbody, which is a structure that is formed at the center of the cytokinetic bridge and is inherited by one of the two daughter cells after cytokinesis. We're also studying how the actin cortex affects spindle orientation, using micro-patterning technology developed by my colleague Manuel Théry to orient the spindle in a predictable manner. This technology is so much fun; Manuel has also used micropatterning to create some neat images to advertise a meeting I am helping organize for next September. It's a French Society for Cell Biology international meeting called "Building the Cell."

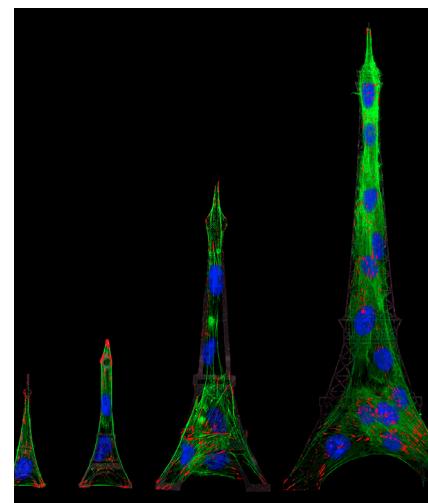


IMAGE COURTESY OF MANUEL THÉRY, CEA

**HeLa cells cultured on fibronectin micro-patterns advertise the French Society for Cell Biology by emulating an iconic shape.**

***Organizing a meeting must take a lot of time...***

Yes. [Laughs] I am Vice President of the Society so I have a lot to do, but there are many other great people working on it. I still have enough time to do other things, including teaching. Researchers at CNRS have no teaching responsibilities, but I love spreading excitement about science, so I teach at a grande école, the École Polytechnique. If I have any time left over from that, I like to spend it with my kids or playing the harp, which I've been doing for 33 years now. It's my main hobby; I love transcribing baroque era music from keyboard or organ and playing it on the harp.

1. Echard, A., et al. 1998. *Science*. 279:580–585.
2. Miserey-Lenkei, S., et al. 2010. *Nat. Cell Biol.* 12:645–654.
3. Echard, A., et al. 2004. *Curr. Biol.* 14:1685–1693.
4. Chesneau, L., et al. 2012. *Curr. Biol.* 22:147–153.
5. Dambourne, D., et al. 2011. *Nat. Cell Biol.* 13:981–988.

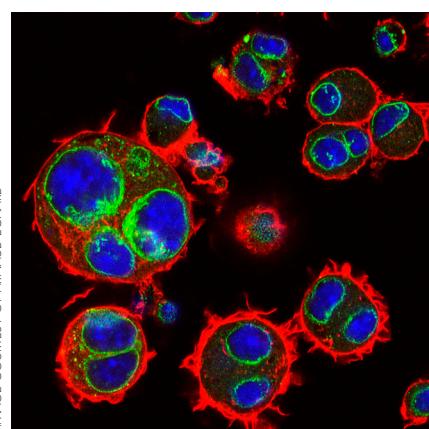


IMAGE COURTESY OF ARNAUD ECHARD

**Multinucleated cells appear in a screen for genes affecting cytokinesis.**