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Filling in the gap: cloning a connexin

David Paul, Daniel Goodenough, Nalin Kumar, and Norton Gilula clone the first connexin proteins.

How do you construct a channel between cells? The first step in answering this question was to isolate the components of one type of channel—the gap junction. Daniel Goodenough had been pursuing gap junction proteins since his days as a graduate student in the mid-1960s with Jean-Paul Revel, who helped discover the structures (Revel and Karnovsky, 1967; see "Defining gap junctions" *JCB* 169:379). In 1972, Goodenough demonstrated that gap junctions could be purified biochemically (Goodenough and Stoeckenius, 1972). By the mid-1970s, when Goodenough had his own lab at Harvard Medical School (Boston, MA), the field was struggling with spotty antibodies in its efforts to identify which proteins were forming the junctions.

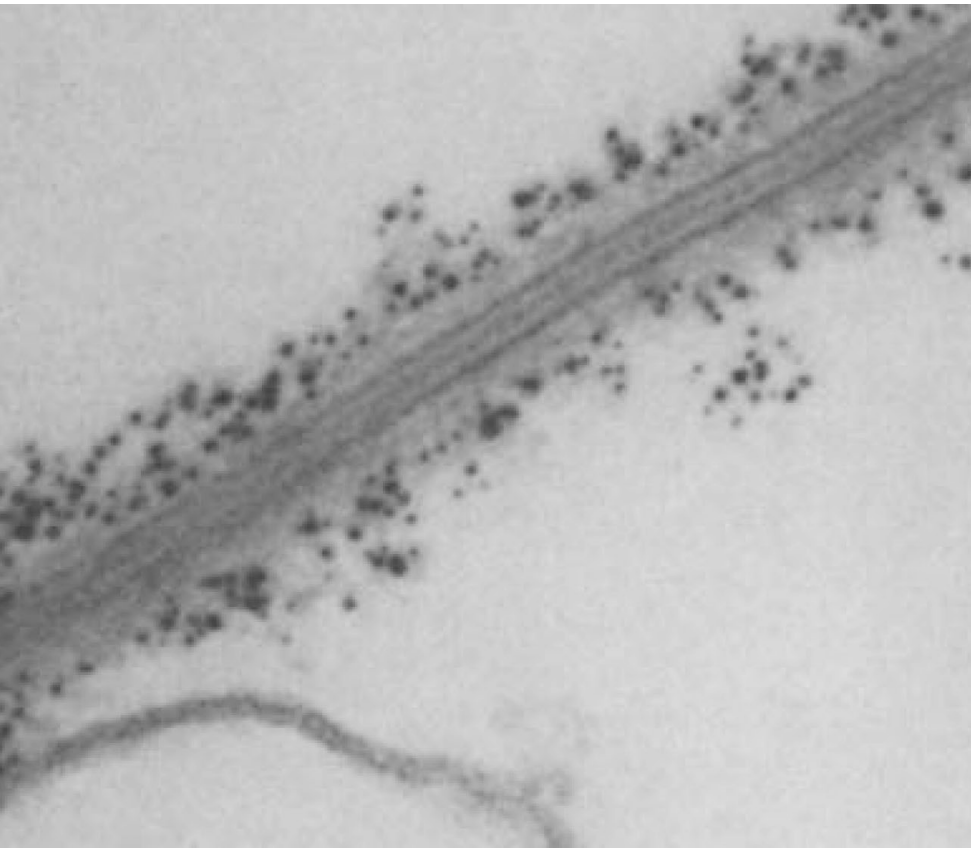
When then graduate student David Paul joined Goodenough, they strove to make a clean enough preparation of rat liver gap junctions to generate a better antibody—hopefully one that could be used for expression cloning. Paul says there was nothing "technically miraculous" about their approach, but it was the right time to clone the 1.5 Kb cDNA of a 32-kD gap junction protein (Paul, 1986).

Norton "Bernie" Gilula's lab cloned the same rat liver connexin, now known as connexin 32 (Cx32), the same year (Kumar and Gilula, 1986). This group used an oligonucleotide probe based on a partial protein sequence to pull out cDNAs for the human and then rat liver proteins.

Paul admits that being the first to work with DNA in Goodenough's lab was "scary," but his molecular leap paid off. Northern blots revealed three related mRNAs—one in liver, brain, kidney, and stomach tissues, and others in heart and lens. That prompted the lab to formally name the family of related proteins as connexins and to clone the genes encoding a heart gap junction protein, Cx43 (Beyer et al., 1987), and a lens gap junction protein, Cx46 (Paul et al., 1991). "Everyone was struggling with the antibodies," Goodenough recalls, but the cDNAs moved things forward.

The cDNAs also allowed expression experiments in cells that did not normally make gap junctions, thus demonstrating that the proteins could form channels that allowed communication (Dahl et al., 1987). Later studies turned up the sequence-unrelated innexins (invertebrate connexins), the mammalian pannexins (a group more ancient than mammalian connexins), and the insect virus vinnexins.

Connexins were also linked to disease. Cx26 mutations account for about half of all cases of genetic deafness, Cx46 and Cx50 mutations cause familial cataracts, and Cx32 mutations are a common cause of the peripheral nerve disorder Charcot-Marie-Tooth disease (Bergoffen et al., 1993). JCB



PAUL

An anticonnexin antibody stained only junctional plasma membranes.

Bergoffen, J., et al. 1993. *Science.* 262:2039–2042.

Beyer, E.C., et al. 1987. *J. Cell Biol.* 105:2621–2629.

Dahl, G., et al. 1987. *Science.* 236:1290–1293.

Goodenough, D.A., and W. Stoeckenius. 1972. *J. Cell Biol.* 54:646–656.

Kumar, N.M., and N.B. Gilula. 1986. *J. Cell Biol.* 103:767–776.

Paul, D.L. 1986. *J. Cell Biol.* 103:123–134.

Paul, D.L., et al. 1991. *J. Cell Biol.* 115:1077–1089.

Revel, J.P., and M.J. Karnovsky. 1967. *J. Cell Biol.* 33:C7–C12.

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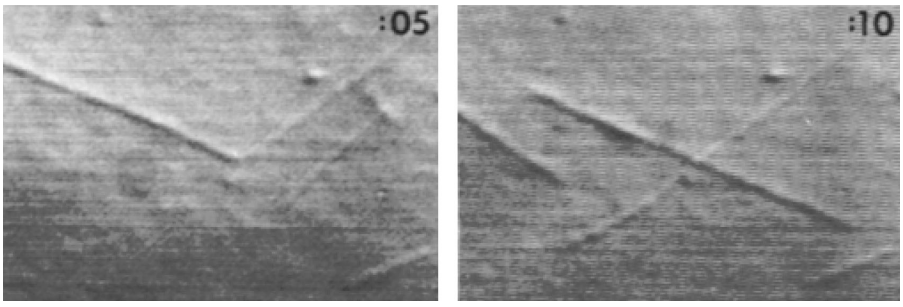
MAP 1C is a motor

When the protein dynein was discovered to provide the flagellar bending force to axonemal microtubules (MTs) (Gibbons, 1963), "people had jumped to the reasonable hypothesis that dynein was perhaps also involved in other microtubule movements," recalls Richard Vallee (Columbia University Medical Center, New York, NY). But by the 1980s, no such cytoplasmic motor proteins had been found.



MAP 1C, now known as dynein, translocates microtubules.

Kinesin and MAP 1C

In the summer of 1985, Vallee recalls seeing work on axonal transport in the squid axoplasm from the labs of Michael Sheetz and Ray Lasek at the Woods Hole Marine Biology Laboratory. "The thinking about axonal transport was all over the place before that," he says. Observations of fast axonal transport argued against a passive mechanism, but no one had found a mechanism to support theories like cytoplasmic streaming along the MTs. "But now," says Vallee, "there was good evidence that there might be specific molecules responsible for transport." The Sheetz lab identified and named the new molecule kinesin and showed it could move both MTs on glass and axonal organelles along MTs (Vale et al., 1985a).

At about this time, Bryce Paschal joined Vallee's lab at The Worcester Foundation for Experimental Biology (Shrewsbury, MA) as a graduate student. A few months into his Ph.D., Paschal had second thoughts about graduate school and took a leave of absence, but he continued working with Vallee as a technician. He began working on a project to test whether kinesin had an MT-stimulated ATPase activity. He purified kinesin, tested column fractions for ATPase activity, and noticed that he got two peaks of activity—one that tracked to kinesin, but another in fractions containing MT-associated protein 1C (MAP 1C), previously identified in the lab. Another lab reported that kinesin's activity was dependent on MTs (Kuznetsov and Gelfand, 1986), so Paschal turned his attention to MAP 1C.

If it looks like a motor...

Trace MAP 1C had always been found in the lab preparations of calf brain MTs. Preliminary characterization had shown that MAP 1C was insensitive to proteolysis, unlike other MAPs (Bloom et al., 1984). In 1982 Vallee had developed a new protocol for MT preparations using taxol, which was such a potent promoter of MT assembly that preps could be made in the absence of ATP or GTP nucleotide (Vallee, 1982).

In this situation, MAP 1C became much more abundant. Nucleotide-sensitive MT association "was very characteristic of a motor protein," says Vallee. In previous preps using nucleotide, he realized, "all of us had been throwing milligrams and milligrams of motor proteins down the drain. It explained why these proteins had not popped up before."

Thinking they were on the trail of the elusive cytoplasmic dynein, Vallee wanted more definitive proof. The best test was to do scanning transmission EM on the protein itself and compare its structure directly to that of flagellar dynein. The first images, produced early in the project, were conclusive. "There was no question that this thing was dynein," says Vallee. Paschal set to work to show that MAP 1C acted as an MT motor. He took on the tricky MTs-on-glass motility assay and demonstrated that, in a kinesin-free prep, MAP 1C could translocate MTs in a unidirectional manner (Paschal et al., 1987a).

Anterograde transport by kinesin had been demonstrated (Vale et al., 1985b). "There was no indication that kinesin could mediate bidirectional transport, but decades of neurobiology had established the retrograde movement of proteins," says Paschal, now at the University of Virginia (Charlottesville, VA). Using *Chlamydomonas reinhardtii* flagella that have a defined polarity, he showed that MAP 1C and kinesin moved the axonemes in opposite directions (Paschal and Vallee, 1987), and that MAP 1C was the retrograde motor.

The clincher was the publication of EM pictures showing that MAP 1C was in fact a two-headed cytoplasmic dynein (Vallee et al., 1988). Paschal went on to show that flagellar dynein isolated from sea urchin sperm behaved similarly in his MT motility assays (Paschal et al., 1987b). In all, it was a banner year for him, with four major publications that largely solved the vexing question of how cells moved things along MTs in two distinct directions. It was definitely worth the 5 a.m. drives to Cambridge, MA to pick up calf brains from a slaughterhouse and, "needless to say," says Paschal, "I decided to go back to graduate school." JCB

Bloom, G.S., et al. 1984. *J. Cell Biol.* 98:320–330.
Gibbons, I.R. 1963. *Proc. Natl. Acad. Sci. USA.* 50:1002–1010.
Kuznetsov, S.A., and V.I. Gelfand. 1986. *Proc. Natl. Acad. Sci. USA.* 83:8530–8534.
Paschal, B.M., et al. 1987a. *J. Cell Biol.* 105: 1273–1282.
Paschal, B.M., et al. 1987b. *Nature.* 330:672–674.
Paschal, B.M., and R.B. Vallee. 1987. *Nature.* 330:181–183.
Vale, R.D., et al. 1985a. *Cell.* 42:39–50.
Vale, R.D., et al. 1985b. *Cell.* 43:623–632.
Vallee, R.B. 1982. *J. Cell Biol.* 92:435–442.
Vallee, R.B., et al. 1988. *Nature.* 332:561–563.