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Tension gets chromosomes oriented

During cell division pairs of chromosomes are pulled apart into the two newly forming cells. Before this can occur, kinetochores are repeatedly connected to and disconnected from microtubules until paired kinetochores are attached to opposite poles and the chromosomes are said to be bioriented. The first suggestion of how cells discard the wrong configurations, such as when both members of a chromosome pair are attached to the same pole, while selecting the correct ones, came from the work of R. Bruce Nicklas and his colleagues at Duke University (Nicklas and Koch, 1969).

By the late 1960s scientists knew that unipolar kinetochore-to-pole attachments are unstable and easily come undone. They also knew that by a somewhat random process, stable bioriented attachments are eventually established. “I had a sequence of pictures of dividing cells on the wall in my office,” says Nicklas. “The challenge was thinking that it would take years to make some sense of [the process by which chromosomes find the right orientation].”

But the answer came earlier than expected. A paper by Ronald Dietz first raised the possibility that tension between two kinetochores, generated only in the bioriented state, might discriminate between correct and incorrect attachments (Dietz, 1958). “I thought it was an easy idea to test and that we should do the experiment,” says Nicklas. As chance would have it, a recently designed micromanipulator (Ellis, 1962) made the experiment possible. “From a technical point of view, in 1969 this was a very easy experiment to do,” says Nicklas. “The challenge was thinking of a way to test the role of tension in chromosome orientation.”

Using grasshopper cells in meiosis, Nicklas’ group showed that the kinetochore-to-pole connections of chromosomes that were improperly attached could be stabilized by using a glass needle to pull on one of the chromosomes. As a result of the applied tension, the two chromosomes would remain in a unipolar orientation for many hours, and would not achieve the correct orientation. In contrast, in the absence of tension, the same two chromosomes would reorient into the correct configuration within several minutes.

“One problem with our paper was that the act of pulling made the chromosomes point straight to a spindle pole, so that position rather than tension could have been the decisive factor,” says Nicklas. To address this point, several years later Nicklas and Ward (1994) were able to repeat the same experiment applying tension in a way that would not affect the position of the chromosomes, thereby confirming that tension was responsible for stabilizing kinetochore-to-pole connections. More current work has shown that the correction of unipolar attachments requires the activity of the Aurora B protein kinase, an enzyme that is highly conserved in yeast and vertebrates (Tanaka et al., 2002; Hauf et al., 2003).

Nicklas’ pioneering experiments were conducted in cells undergoing meiosis, so it was not clear whether the same mechanisms would be at play during mitosis. However, recently Dewar et al. (2004) showed that the combination of tension and Aurora B activity is indeed sufficient to ensure that sister chromatids are appropriately aligned during mitosis. JCB

Dewar, H., et al. 2004. Nature. 428:93–97.
Dietz, R. 1958. Chromosoma. 9:359–440.
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Hauf, et al. 2003. J. Cell Biol. 161:281–294.
Nicklas, R.B., and C.A. Koch. 1969. J. Cell Biol. 43:40–50.
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Tanaka, T.U., et al. 2002. Cell. 108:317–329.

Not actin, not myosin, but intermediate

Early studies of skeletal muscle revealed the existence of two kinds of protein filaments: thick, ~150 Å–diameter myosin filaments and thin, ~60 Å–diameter actin filaments. These filaments combine to form myofilaments in developing and mature muscle cells. The year 1968 brought the discovery of another class of filaments ~100 Å in diameter ( Ishikawa et al., 1968). These intermediate filaments constitute the majority of free filaments in the cytoplasm of most eukaryotic cells.

“There were many reports of free, thin cytoplasmic filaments in many kinds of cells. At the time, these were generally thought to be actin,” says Howard Holtzer. “We thought that they might be a completely new kind of filament because of their failure to be decorated with heavy meromyosin. And, unlike actin, they were of indefinite lengths.”

Earlier experiments by Inoué (1952) and Tilney (1965) had shown that the mitotic inhibitor colchicine caused depolymerization of another set of protein fibers found in most cells, the microtubules. When Holtzer and colleagues rearred any of a wide variety of cells in colchicine, they noted that as the microtubules disappeared, the individual cytoplasmic filaments aggregated laterally into “immense, meandering, translucent cables,” says Holtzer. When the colchicine was washed...
Seeing peroxisomes

At about the same time that Christian de Duve and his colleagues were describing the biochemistry of lysosomes (see “Catching sight of lysosomes” JCB 168: 174), they biochemically identified (Baudhuin et al., 1965) and purified (Leighton et al., 1968) another enzyme-containing organelle. Initially the organelle was known as the microbody, and de Duve declined to give it a more specific name in 1965 because “too little is known of their enzyme complement and of their role in the physiology of the liver cells to substantiate a proposal at the present time” (Baudhuin et al., 1965). But in an abstract presented at the 1965 American Society for Cell Biology annual meeting and a year later in print (de Duve and Baudhuin, 1966), de Duve proposed that the new organelle be called a peroxisome, because it appeared to both generate and break down hydrogen peroxide.

A Swedish graduate student, J. Rhodin, had first described microbodies in his dissertation in 1954, after spotting their distinctive morphology. A year later, they were described in a paper that mistakenly suggested, based on appearance and location, that they were precursors to mitochondria (Rouiller and Bernhard, 1956). Subsequently other researchers observed similar structures by microscopy “but no one knew the function of these particles,” says de Duve. “There were all kinds of wild speculations about what they might do.”

de Duve’s group modified a cell fractionation method devised by Robert Wattiaux and colleagues for separating peroxisomes, lysosomes, and mitochondria, which required injecting animals with Triton WR-1339 (Wattiaux et al., 1963). “The compound accumulates in lysosomes and causes them to float in a sucrose gradient,” says de Duve. This technique led to a full identification of peroxisomes using microscopy and biochemistry (Baudhuin et al., 1965), when they were clearly shown not to be related to mitochondria.

In a landmark paper published in JCB in 1968 (Leighton et al., 1968), de Duve described the first large-scale preparation of peroxisomes—a feat that made possible more conclusive and precise characterization of their biochemical and morphological properties. “The same technique was to be used for many years to come in the study of the biogenesis of peroxisomes,” says de Duve.

The key to scaling up the separation technique was an automated rotor. “That machine was remarkable,” says deDuve. “Belgian scientist Henri Beaufay designed the rotor, and its construction was completed at the Rockefeller [University] instrument lab. It was a transatlantic collaboration.” The automated rotor had several advantages over the conventional swinging bucket rotor. It could accommodate larger sample volumes and allowed loading and unloading of samples while the rotor was running, thereby suppressing artifacts associated with starting and stopping the centrifuge.

With these advantages, de Duve and colleagues were able to use 100 g of liver from mice in a single experiment to obtain significantly more concentrated and cleaner preparations of peroxisomes, lysosomes, and mitochondria. “We were able for the first time to get a sufficiently thick preparation that you could see different colors of the peak fractions,” says de Duve. One of the figures in the paper shows the peroxisomes fraction as having a greenish tinge, presumably reflecting their richness in catalase.

These highly enriched and purified fractions lent themselves to further characterization, thus putting peroxisomes on a much firmer footing as distinct structures in the cell and allowing their identification despite differences in morphology in different cell types. The authors confirmed, for example, that peroxisomes contain essentially all the i-ox hydroxyacid oxidase, d-amino acid oxidase, and catalase in a liver cell. “The 1968 paper was an upgrading and scaling up of the work we had done before,” says de Duve. “But most importantly it laid the groundwork for subsequent studies on peroxisomes.” JCB

Baudhuin, P., H. Beaufay, and C. de Duve. 1965. J. Cell Biol. 26:219–243.

de Duve, C., and P. Baudhuin. 1966. Physiol. Rev. 46:323–357.

Leighton, F., et al. 1968. J. Cell Biol. 37:482–513.

Rouiller, C., and W. Bernhard. 1956. J. Biophys. Biochem. Cytol. 2:355–360.

Wattiaux, R., M. Wibo, and P. Baudhuin. 1963. In Ciba Foundation Symposium on Lysosomes. A.V.S. de Reuck and M.P. Cameron, editors. J. & A. Churchill Ltd., London.

Out, the cables disassembled into their constituent filaments. Colchicine, however, had no obvious effects on the maturing actin filaments of the myofibrils.

By measuring the diameter of individual filaments by electron microscopy, Holtzer and colleagues were able to determine that the free cytoplasmic filaments, and those in the coochicine-induced cables, had a diameter different from that of actin filaments. These filaments were named “intermediate filaments” because their size was between that of myosin and actin filaments in muscle cells.

The 1968 study was followed by an explosion of research that quickly lead to the identification of many intermediate filament isoforms, such as the nuclear lamins, vimentin-like filaments, keratins, and neurofilaments. Many of these give mechanical stability to cells (Janmey et al., 1991), but some, says Holtzer, “are almost certain to be involved in cell differentiation and cell maturation.” Changes in the state of their aggregation following stress, infection, or mutation are diagnostic of specific human diseases, and their varied expression profiles in different epithelia make them particularly useful in classifying the tissue of origin of many tumors. JCB

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Inoué, S. 1952. Exp. Cell Res. 2:305–314.

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Janmey, P.A., et al. 1991. J. Cell Biol. 113: 155–160.