TheScientist
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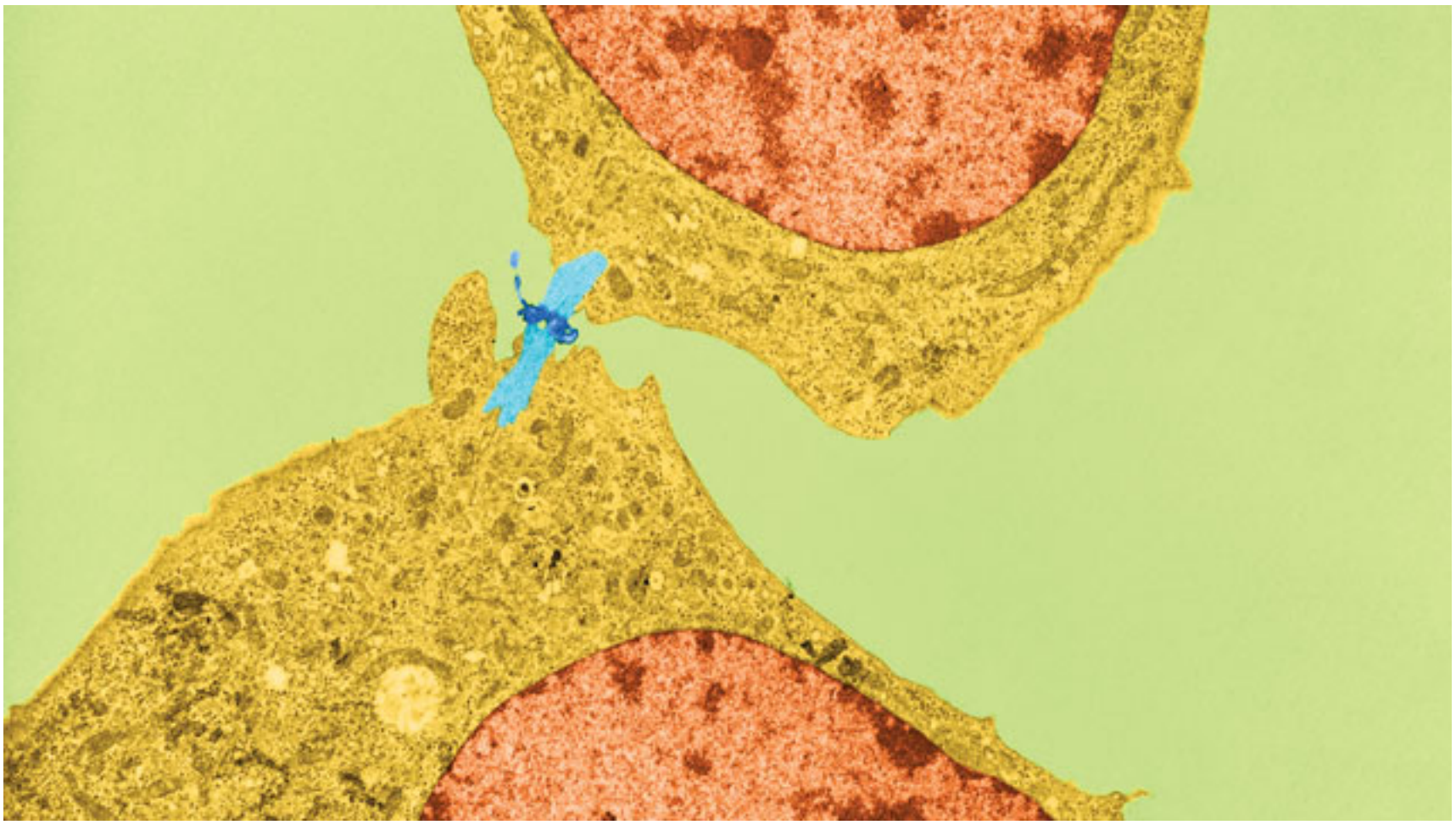
One Man's Trash...

Scientists who dared to waste their time looking at the midbody, a remnant of cell division, have catapulted the organelle to new prominence.

By Kerry Grens | December 1, 2013



SISTER CELLS: This color-enhanced transmission electron micrograph shows the midbody (blue) linking two epithelial cells that have just divided.

During her search for a postdoctoral advisor in 1997, cell biologist Ahna Skop grew accustomed to getting turned down. Again and again, she rang the bell at the labs of faculty members only to have the door shut in her face. Her problem was that she was dead set on investigating what was, to many, an uninteresting vestige of cell division: the midbody.

The existing literature painted the midbody as "a garbage dump," Skop recalls. Studies of mitosis had led researchers to believe that the small cluster of microtubules and proteins, found at the exact point where

two daughter cells are last connected, was "stuff that just gets thrown away after cell division," she says. But Skop thought the midbody was something more. Although the organelle had been identified more than a century earlier, its function was still unclear, and the primary mystery that Skop wanted to solve—what exactly the midbody was made of—was wide open.

In 1998, proteomics and mass spectrometry were just revealing themselves to be useful tools for studying the specific proteins associated with mitotic structures. Skop wanted to apply these techniques to investigate the midbody. Eventually, her appeal fell upon the sympathetic ears of two faculty members at the University of California, Berkeley. For four months, Skop worked in the lab of cell biologist Rebecca Heald to isolate the organelle from Chinese hamster ovary cells, using a protocol she had developed with the help of midbody pioneers of decades prior. She then brought her midbodies down to Scripps Research Institute in La Jolla for protein identification, which yielded a list of hundreds of proteins that constituted the mammalian midbody. Many, such as the actin- or microtubule-associated proteins involved in cell division, were expected to be there, but there were others that didn't seem appropriate for a cellular junkyard.

Back at UC Berkeley, in the lab of geneticist Barbara Meyer, Skop knocked down the homologous genes, one by one, in *Caenorhabditis elegans* to see what would happen. Loss of the first randomly chosen protein caused a cell-division defect. "I went and did the next one, and that one had a cell-division defect. And then the next one, and the next one," says Skop. "Then I thought, 'This is getting boring.' It was working."

> The midbody was called a remnant, a scar, and other nasty names.
>
> —Stephen Doxsey, University of Massachusetts School of Medicine

A handful of researchers had predicted in the 1960s and '70s that the midbody was important for cell division. Skop had now demonstrated it. In the summer of 2004, she published her results in *Science*, where midbodies splashed across the journal's cover.[1] Even before the paper came out, Skop had been offered—and had accepted—a faculty position at the University of Wisconsin–Madison. Midbodies, with Skop as their ambassador, were finally getting due recognition. In the decade since, a surge of interest in the midbody's role during and after abscission—the very final cut between two daughter cells—have thrust this erstwhile bit of cellular detritus, now considered a true organelle, into the limelight.

History and foresight

In the late 1800s, a German cell biologist named Walther Flemming drew beautiful pictures of a newly identified structure that conjoined two daughter cells just before they made a complete separation. The structure came to be known as the Flemming body and later, the midbody. At the end of mitosis, the mother cell develops a waist that cinches progressively tighter as it forms two daughter cells. In this cleavage furrow is a bundle of microtubules, vesicles, and a variety of associated proteins. As the waist gets narrower and the daughter cells form distinct boundaries, this collection of material persists in connecting the two daughters, flanked on either side by a thin intercellular canal of cytoplasm and microtubules.

"The midbody was called a remnant, a scar, and other nasty names," says Stephen Doxsey, a cell biologist at the University of Massachusetts School of Medicine. Cell biologist Dick McIntosh of the University of Colorado, Boulder, concurs: "I don't know who said it first—I know I said it—that the midbody is a garbage can." Those who weren't dismissing the midbody paid it no mind at all. But in the early 1960s, as electron microscopy (EM) first offered scientists a way to view the fine structure of cells, it became impossible to ignore. Appearing as a conspicuous black blob, full of electron-dense material and positioned between daughter cells, it caught the eye of Breck Byers, a graduate student in the Harvard laboratory of renowned cell biologist Keith Porter, an EM pioneer.

Byers was investigating the microtubule-generated forces that cause cells to elongate. He was aware of papers dating back to the 1800s that mentioned the midbody, but the organelle had rarely been studied

since, so he prepared a set of time-lapse microscopy images to track the dark structure as daughter cells divided.

Byers observed that the midbody tethered daughter cells for as long as three hours or more. "It wasn't that the cell cycle didn't end at telophase, but this took a lot longer," he says. During this waiting period, the intercellular bridge rippled in a wave-like motion, then snapped on just one side of the black nubbin. Whereas some researchers would later report the midbody matrix—the dark, dense knob—spit out into the extracellular space, Byers observed that it was inherited by one of the daughter cells.[2]

"What I'd imagined was, somehow, the daughter cells 'care' who gets it. That was my thought at the time, and I thought about that for years and years," says Byers, now at the University of Washington. Four decades later, other researchers would generate evidence to support his hunch that the asymmetric inheritance of the midbody may actually mean something. But during those decades, a handful of other midbody enthusiasts began to address more fundamental questions: What is this elusive organelle made of, and what does it do?

On form and function

Michael Mullins was a graduate student at the University of Texas at Austin in the early 1970s when he came across Byers's descriptions of the midbody. "I was fascinated, because I never realized there was a structure post-furrow," says Mullins, now a professor at Catholic University of America in Washington, DC. Like Byers, Mullins turned to cutting-edge imaging technologies and observed the waves of motion along the intercellular bridge before it broke. He also reported that the bridge constricted down to a small diameter prior to snapping, a process now considered to be an important step in abscission. (See illustration.)

Mullins discovered another peculiar aspect of the midbody. Normally, he observed, the midbody stayed intact until the bridge broke, then tagged along as a tail on one of daughter cells. In one case, however, Mullins saw the midbody break apart before the bridge split. Shockingly, rather than separate as daughter cells normally would, those cells merged back together. In a 1973 *Tissue and Cell* paper, Mullins and his colleague cautiously posited that the midbody plays some role in cell division. "While no general conclusions can be drawn from a single observation, the possibility certainly exists that the midbody provides a structural means by which one daughter cell is effectively distinguished from the other and by which the activities leading to separation are organized," they wrote in the paper.[3]

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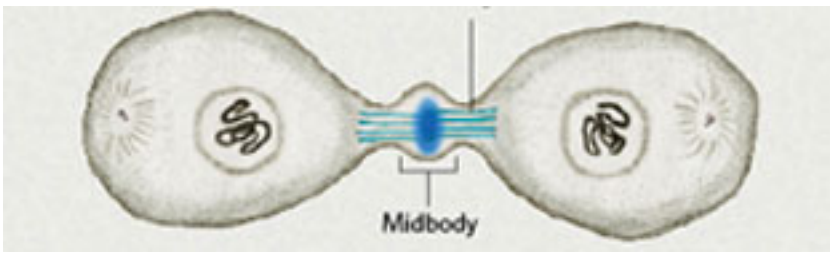
> —Breck Byers, University of Washington

It would be many years before the process of abscission would be interrogated at a molecular level and the midbody's functional role in cell division solidified, but Mullins's work over the next decade did yield evidence that the midbody is essential for the separation of daughter cells. As a postdoc in McIntosh's lab in the late 1970s, Mullins had developed a protocol to isolate midbodies for the first time,[4] and started to tease out one of the proteins associated with the midbody. "It was fascinating, fundamental science," says Mullins, "and it was just satisfying."

But just as Mullins was making headway on developing probes to uncover the identity of midbody proteins, he got scooped. Ryoko Kuriyama, a cell biologist at the University of Minnesota, developed a monoclonal antibody to a midbody-associated protein she called CHO1 antigen. This allowed her to track the protein during cell division as it appeared in short lines along the spindle axis, then clustered at the midbody as division progressed.[5] And because CHO1 was present before anaphase—the stage when sister chromatids of DNA begin to separate—the result lent the midbody a new functional facet, further distinguishing it from cellular junk. Maybe, Kuriyama thought, the midbody could be playing an active role early in the cell-division process.

Two years later, Kuriyama teamed up with McIntosh's group to figure out what CHO1 was up to. Just as Skop would later see with her C. elegans mutants, the researchers found that messing with the mammalian midbody arrested mitosis, suggesting the organelle was indeed an important player in the cell cycle before abscission occurred.[6] McIntosh's group went on to show in vitro that CHO1, later known as MKLP-1 for mitotic kinesin-like protein, is a motor protein that cross-bridges microtubules oriented in opposite directions, causing them to slide, similar to the microtubule movements in spindle elongation during mitosis.[7]



FORMATION AND FATE OF THE MIDBODY: How the midbody forms during cell division, and its role in mediating the process

See full infographic: JPG | PDF

Over the next decade, midbody research began to accelerate as interested scientists identified more and more associated proteins, including passenger proteins, cross-linkers, kinases, and others. In the mid-2000s, Doxsey's group reported on midbody elements such as secretory vesicles, recycling endosomes, and membrane-tethering complexes that are important for the division of the cytoplasm into two daughter cells, a process called cytokinesis. One gene, encoding a protein called centriolin, turned out to be essential for abscission: without it, cells would never fully separate. They would remain joined at the hip, with each daughter cell progressing into the next cell cycle.

These experiments evoked a change of thinking among some cell biologists, who had once thought of abscission as a simple snapping of cell membranes. "I think people ignored abscission for a long time," says Tim Mitchison of Harvard Medical School. "I think it's now recognized as a very important piece of cytokinesis, and, at least under some circumstances, it's a regulated, orchestrated event."

One midbody component that researchers are currently striving to understand is the endosomal sorting complex required for transport (ESCRT) pathway, a set of protein complexes involved in moving molecules to endosomes. Mullins didn't know 40 years ago that he was observing the handiwork of the ESCRT machinery when he noticed the series of ripples in the plasma membrane of the intercellular bridge. Those ripples, it turns out, are caused by ESCRT-III, one of the subcomplexes of the ESCRT-driven pathway. Daniel Gerlich, now at the Institute of Molecular Biotechnology in Vienna, found in 2011 that wiping out ESCRT-III eliminated the ripples at the constriction site.[8]

The discovery of ESCRT-III's role offered a new model for abscission, says Gerlich. Vesicles were not completing the closure of the plasma membrane from the inside out with new material at the site of abscission. Rather, the membrane of the intercellular bridge was being constricted to pinch down and cleave the connection. "The ESCRT-III complex is predisposed to perform that function, because it can deform the membrane and catalyze membrane fission events," says Gerlich. It's an attractive possibility, but has yet to be definitively proven.

Many other questions remain. One that continues to intrigue Gerlich is why dividing cells wait so long before undergoing abscission—often an hour or more after the chromosomes have segregated. Gerlich speculates that the abscission delay may be necessary for midbody assembly. The midbody is, after all, a highly complex organelle. Another lingering question—one that has haunted Breck Byers since the 1960s—is what happens to the midbody after abscission. What is its ultimate fate?

Midbody inheritance

Part of the reason that the midbody was once considered junk was that researchers found it was often jettisoned out into the extracellular space following daughter cell separation. "It pretty much had been thought of, until very recently, mainly [as] just a scaffold for proteins to complete abscission," says John Schiel, a postdoc in Doxsey's lab. After abscission, researchers believed, it got chewed up or spit out.

But not always, it turns out. Doxsey and a few others began confirming earlier observations, such as

Byers's, that in some cases one of the daughter cells actually retains the midbody. In 2009, a German group showed that certain cells would accumulate midbodies, and then dispose of them through autophagy, the digestion of cellular parts and the recycling of the material. At the time, "it was heresy," says Doxsey. Now, it's accepted that autophagy is one midbody destiny.

Another possible midbody fate is hoarding. In a striking set of experiments, Doxsey's group showed that stem cells in mouse and human tissues were accumulating midbodies, but the differentiating cells surrounding them were not.[9] "That was one of my eureka moments, when we saw where the midbodies were in the mouse tissue," he says. "That blew us away."

Doxsey's group also found that blocking autophagy increased midbody retention and led to greater tumor growth. The findings offered yet another role for the organelle—perhaps midbody accumulation has something to do with the "stemness" of stem cells or the aggression of cancer cells. Could midbody absence somehow signal a cell to differentiate? While these questions remain unanswered, Doxsey's group did confirm that midbody inheritance was deliberate: the daughter cell with the older centrosome, the original centrosome from the mother, got the midbody.

Doxsey's results conflicted with those from other groups, however. Just a couple of years earlier, Wieland Huttner at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden demonstrated "without any doubt" that the midbody was released following the division of neural progenitor cells, Huttner says. And shortly after Doxsey's 2011 paper, Huttner's group again provided evidence of midbodies being discarded into the extracellular space.[10]

Doxsey and Huttner now agree that midbody retention must be cell type-specific, though each scientist has his own hypothesis about what purpose the midbody serves. In brain cells, Huttner proposes, midbodies sent off into extracellular space could serve as a signal to surrounding cells that a cell has divided, perhaps as a means of regulating tissue growth.

Doxsey also suspects that midbodies are signaling centers, though he focuses on the information they can share with the cell that retains them. As massive organelles—twice the size of centrosomes—midbodies carry a wealth of information. "My model is that they allow themselves to be bathed in cytoplasm so they can sense signal-transduction pathways, [then] give off signals that would diffuse" within the cell, Doxsey says. Schiel points out that many of the midbody components are involved in cell fate, polarity, and behavior. Potentially, "having more of these [retained proteins could] go on to help control future divisions," he says.

In October of this year, however, a bit of cold water was splashed on this hypothesis. Guangshuo Ou, working in Pierre Gönzcy's lab at the Swiss Federal Institute of Technology in Lausanne, found that during early *C. elegans* development midbodies follow a pattern of being spit out extracellularly following the first two cell divisions. They are then internalized most times by the same cell type in the embryo. The finding doesn't necessarily conflict with the idea that midbody retention is important, but Ou found that the mechanism regulating how the midbody is internalized is not essential for normal development. Mutants of the proteins involved in the midbody-internalization process had no obvious defects—confusing the picture about whether the midbody plays a role in embryonic development.[11]

"The midbody, at least from the first cell division, . . . may not contribute to cell fate," says Ou, now a professor at the Institute of Biophysics at the Chinese Academy of Science in Beijing. Of course, he adds, it could well be that this result in a worm won't apply to higher organisms. "It's quite possible that in humans or mice the midbody has additional functions, which *C. elegans* doesn't have," he says.

Regardless of its role in development, there seems to be agreement now among cell biologists that the midbody is important for the cell. Additionally, midbody retention delegitimizes an assumption to which most biologists ascribed for decades: that daughter cells are essentially the same, receiving equal contributions from the parent cell. "When you say that cells are identical when they're divided, it's not true," says Doxsey: the cell that internalizes the midbody carries hundreds of proteins that its sister does not.

What exactly this means to the cell, however, remains to be seen. Midbodies "could be completely irrelevant," Mitchison says, "or they could be doing something interesting."

The Midbody: A Time Line

1891
Walther Flemming discovers the midbody.

1962
Robert Buck and James Tisdale use electron microscopy to show that the midbody forms as two cells begin to divide, but before the cleavage process begins.

1968
Breck Byers and David Abramson observe asymmetric inheritance of the midbody; some daughter cells reabsorb the protein cluster.

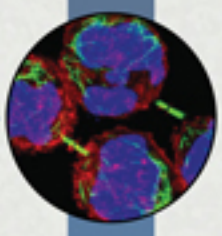
1973
Michael Mullins and John Biesele capture images of constriction on one side of the midbody bulge in the intercellular bridge that precedes abscission, the final cut between the two cells. "It seems likely that the midbody has some functional significance for cell activities, most likely those of cytokinesis," the duo wrote in *Tissue and Cell*.

1977
Mullins and Biesele observe the midbody breaking apart before abscission. This leads to incomplete cytokinesis and the merging of the two daughter cells.

1980
Ursula Euteneuer and Dick McIntosh reveal the polarity of microtubules passing through the midbody.

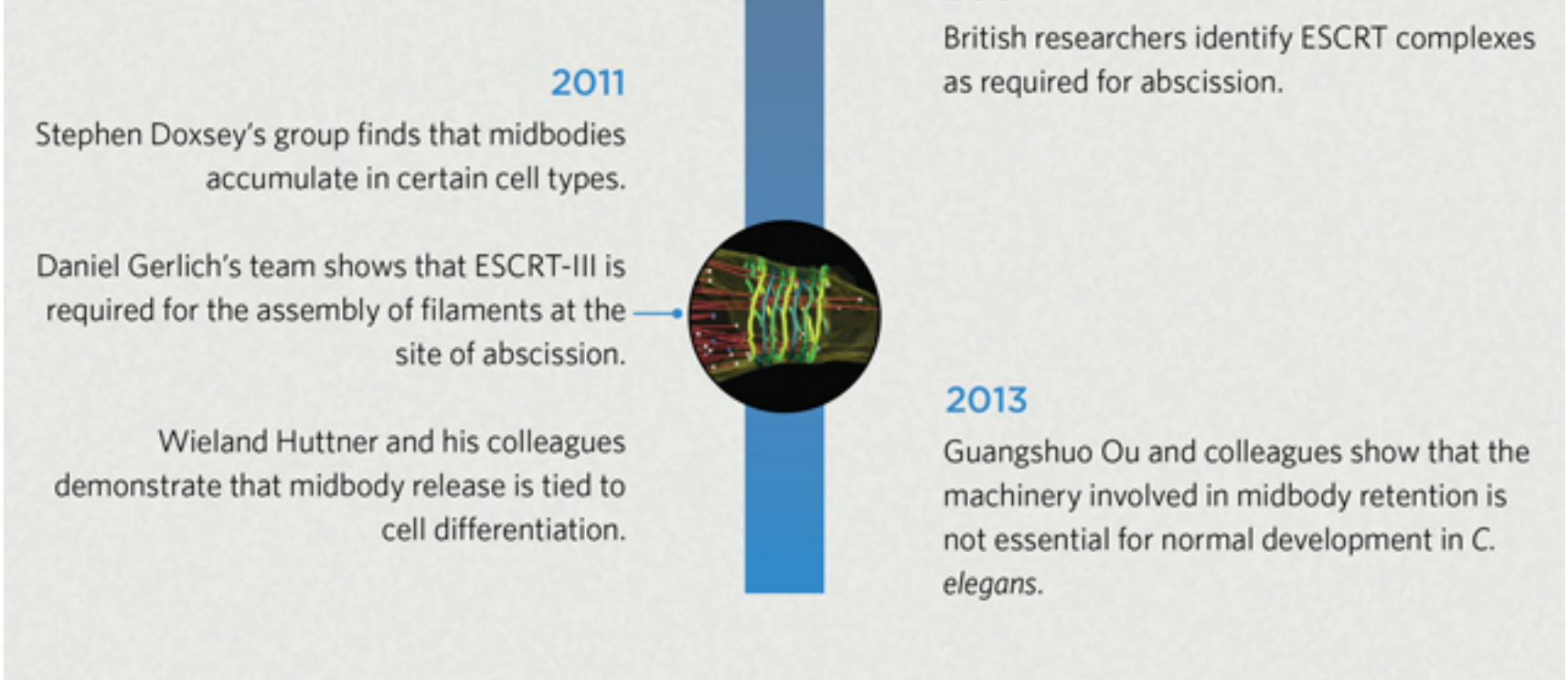
1982
Mullins isolates the midbody for the first time.

1990
Ryoko Kuriyama, McIntosh, and others show that interrupting a midbody-associated protein blocks mitosis before the onset of anaphase.



2004
Ahna Skop publishes the proteomics of the midbody.

2007



1891: wikimedia commons; 1973: *Tissue & Cell* 1973 5(1) 47-61; 1977: © 1977 Rockefeller University Press. Originally published in *Journal of Cell Biology*. 73:672-684; 1982: © 1982 Rockefeller University Press. Originally published in *Journal of Cell Biology*. 94:654-661; 2004: *Science* 305:61-66, 2004. Reprinted with permission from AAAS. 2011: *Science* 331:1616-1620, 2011. Reprinted with permission from AAAS.

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