It’s tough to get money to study something that most biologists doubt exists. Kristen Johansen of Iowa State University in Ames learned that firsthand in 1999 when the U.S. National Institutes of Health (NIH) in Bethesda, Maryland, rejected her grant application to investigate a hypothetical cellular structure called the spindle matrix. As one reviewer bluntly put it, recalls Johansen, “If it existed, it would have already been discovered.”

The spindle itself is well-known to cell biologists. This array of protein filaments separates a dividing cell’s two sets of chromosomes during mitosis, ensuring that each daughter cell has the right DNA. In the 1960s, a few scientists proposed that the spindle required a molecular underlayer—a spindle “matrix”—in order to work. But the idea never took hold, as cell biologists concentrated instead on the spindle filaments that they could observe and manipulate.

Fortunately for Kristen Johansen and Jørgen Johansen, her collaborator and husband, the grant reviewers at the National Science Foundation (NSF) were more open-minded than NIH’s about the spindle matrix. Since 2001, NSF has funded the pair as they try to breathe new life into this old idea. So far, the Johansens have pinpointed four matrix protein candidates. They are now working with mutant fruit flies to verify that these proteins are important to spindle function. Another researcher has identified at least one more matrix contender through her studies of spindles in a cell-free system. “I don’t think anyone rejects [the spindle matrix] out of hand anymore,” says Kristen Johansen.

However, because these proteins appear to be pulling double shifts—also having crucial roles in the nucleus of a nondividing cell—researchers have had trouble assessing just how important these proteins are to the spindle during cell division. And, says Jonathan Scholey of the University of California, Davis, until these “matrix” proteins are proven to have a role in cell division, he and many other cell biologists will remain skeptical. “It’s certainly an interesting topic that merits further research and discussion,” he says. “There has been progress, but the jury is still out.”

Unexplained forces
The spindle’s filaments, known as microtubules, are made of the protein tubulin. After a cell has duplicated its DNA and begun breaking down its nucleus in preparation for dividing, free tubulins polymerize into these filaments, arranging into an oval network. The cell relies on the spindle to segregate the two sets of chromosomes before it can pinch in at the middle to create two distinct cells.

Since the 1920s, cell biologists have produced stunning pictures of spindles. They really started to see spindles in action in the 1970s, when they observed live cells in which the protein tubulin and DNA were lit up with fluorescent markers. Once the spindle forms, chromosomes line up perpendicular to the spindle’s main filaments, at the midpoint between the spindle’s two ends, or poles. Sites on each chromosome called kinetochores attach to microtubules, which extend out to the poles. The chromosomes then start their march toward the spindle poles.

The spindle images that grace journal covers and textbook pages are so attractive that few cell biologists felt a need to look for something more to explain chromosome segregation, contends Yixian Zheng, a Howard Hughes Medical Institute investigator at the Carnegie Institution for Science in Baltimore, Maryland. “Nothing is as good looking as microtubules and kinetochores,” she says.

But what actually moves the chromosomes? Microtubules can lengthen or contract through...
the addition or removal of tubulin subunits. At first, researchers thought such changes might move the DNA. If a microtubule anchored at a spindle pole slowly shortened, it could reel in a chromosome. Alternatively, microtubules extending from the cell’s middle could push a chromosome out toward the pole as they added more tubulin subunits.

There are also motor proteins such as dynein attached to spindles, suggesting another way to move chromosomes. These molecules utilize a cell’s energy to change shape and inch along a substrate. They can transport tethered cargo and may push or pull chromosomes—or microtubules bearing DNA—toward the opposite ends of the cell.

Most mitosis researchers contend that microtubule growth and contraction, perhaps in combination with motor proteins, are all a dividing cell needs to get chromosomes to the right places. But a few cell biologists, chief among them Arthur Forer of York University in Toronto, Canada, and Jeremy Pickett-Heaps of the University of Melbourne, Australia, have long argued that microtubules and motor proteins don’t fully explain the movement of chromosomes along a spindle. As evidence, they point to what happens when they use a beam of ultraviolet light to sever spindle microtubules in dividing cells. “You would expect a chromosome to stop moving,” says Forer. Yet both the chromosome and the severed microtubules keep sliding toward the cell edge. “It looks like something is pushing them,” adds Forer.

For a motor protein to do the pushing, it “has to attach to something,” Forer points out. That something, he, Pickett-Heaps, and others argue, is the spindle matrix.

Reasonable doubts
Over the past decade, researchers have proposed various matrix ingredients, but none have yet convinced the skeptics. For example, Timothy J. Mitchison of Harvard Medical School in Boston and his colleagues reported in the 2 December 2004 issue of *Nature* that a polymer composed of sugars and nucleotides was needed to assemble the spindle. But his team has since found no evidence that the polymer is part of a spindle matrix; Mitchison calls himself “agnostic” on the matrix hypothesis.

The Johansens entered the matrix debate in 1996 when an antibody intended for a membrane protein instead bound to chromosomes in the nucleus. During the early stages of mitosis, these proteins left the chromosomes and, before any tubulin had started to form microtubules, created a suspiciously familiar filamentous oval. “It looked like a protein called titin appears in many of the same places within a dividing cell as skeleton, megalor, chromator, and microtubules. Given that titin’s distribution seems to mirror the spindle, Forer considers the protein an excellent candidate to provide musclcelike elasticity to a spindle matrix.

All this is provocative but far from proof that there is a spindle matrix composed of titin, skeleton, or anything else. One problem is that scientists have looked carefully for gene mutations that perturb spindle assembly, shape, or function, and thus far, none of the genes for the Johansens’ proteins has popped up. “What has been lacking up till now, and what people are clamoring for, is better evidence for a direct role of the matrix in spindle function,” acknowledges Kristen Johansen.

That proof isn’t easy to come by, she and other spindle-matrix fans stress. Because some of the key proteins have additional jobs in the nucleus—megator is part of the complex that regulates traffic across the nuclear membrane, for example—mutating them or depriving cells of the proteins may simply cause cells to die or have other problems that obscure any spindle defects.

Smoking gun?
Still, in work to be presented at the American Society for Cell Biology meeting in Washington, D.C., next week, the Johansens and their colleagues will describe strains of fruit flies with subtle mutations in chromator’s gene. These flies exhibit spindle defects—poorly organized microtubules or improper chromosome segregation, for example—as cells divide. The researchers further show that introducing the normal gene into cells corrects the problems. Finally, they provide evidence that chromator actually binds to microtubules and to a motor protein. “Hopefully, when these experiments are complete, we will be close to having a ‘smoking gun,’” says Kristen Johansen.

Zheng has taken another approach in an attempt to sway spindle-matrix skeptics. She adds a protein called RanGTPase to the extracts of frog eggs, which triggers the formation of spindles. With this simplified system, Zheng can concentrate on spindle function alone.

Last year, using this strategy, Zheng and her colleagues added a new name to the list of candidate spindle-matrix proteins: lamin B (*Science*, 31 March 2006, p. 1887). According to Zheng, this filamentous protein looks a bit like the putative matrix proteins studied by the Johansens. It’s likewise found in the nucleus, as part of the protein mesh that underlies the nuclear envelope.

Other researchers had shown that when this envelope breaks down at the beginning of mitosis, lamin B disperses and some of it ends up on spindle filaments. Using her egg-extract system, Zheng and her colleagues showed that lamin B is required for spindle creation and forms a matrix that harbors spindle assembly factors.

Zheng is now chasing down other possible spindle-matrix proteins using her system. And she proposes that as a nucleus dissolves for mitosis, many of its components likely become part of the spindle matrix. Still, she knows all too well how unfashionable the spindle matrix remains: Zheng recently lost her NIH funding and decided to downplay mention of her work on the matrix in a recent NIH grant application. “We’re not at the tipping point yet,” she says. —JOHN TRAVIS