New Clue Found to Growth Factor Action

Discovery of the "profilin connection" may help explain epidermal growth factor's effects on the cell skeleton

REMARKABLE THINGS HAPPEN WHEN growth factors encounter their target cells. Take epidermal growth factor (EGF). It triggers a whole series of responses in its targets: cells change shape and send out needle-like projections from their surfaces; they may migrate toward the growth factor; they may divide; in some cases, EGF may even set the cells on a course toward cancerous growth. Which raises a long-standing scientific conundrum of considerable import: How, cell biologists want to know, can a growth signal, acting outside a cell, cause such profound changes inside?

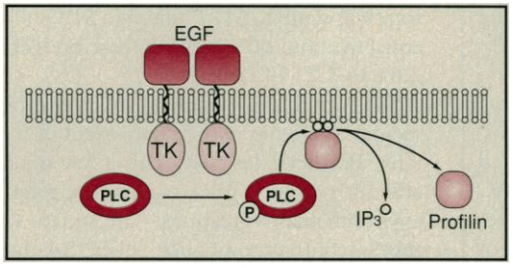
Despite a great deal of work, and some recent progress, the answer to that question is largely unknown. But now researchers from Johns Hopkins University School of Medicine and the National Heart, Lung, and Blood Institute have provided an important new piece in the EGF puzzle, filling in a major gap in the pathway that conveys the growth factor's signals to the cell interior (also see p. 1231).

Researchers have known for some time that EGF and other chemical signals activate a cascade of biochemical events at the cell membrane. They also know that many of EGF's cellular responses, especially the changes it induces in cell shape and motility, are mediated through a remodeling of the cell skeleton, an internal meshwork of tiny filaments made of the protein actin.

What was missing was the link between the membrane events and the cytoskeletal remodeling, and that's what the current work, performed by Johns Hopkins cell biologists Pascal Goldschmidt-Clermont and Thomas Pollard and their colleagues, may have provided. The discovery, says Uno Lindberg of Stockholm University, whose own work focuses on signal transmission pathways, "is a crucial observation. We had few clues previously about how the microfilament system was regulated."

The first inkling that there might be such a connection came about 6 years ago. Ingrid Lassing, a student in Lindberg's Stockholm lab, found that a protein called profilin binds tightly to a membrane phospholipid called PIP_2 (for phosphatidylinositol-4,5-bisphosphate). PIP_2 is an important participant in several membrane signalling pathways, including EGFs.

Indeed, one of the first things that happens after EGF binds to its receptor on the cell membrane is the splitting of PIP_2 into two components, diacylglycerol and inositol trisphosphate, which serve as "second messengers" for bringing about many of the growth factor's cellular effects. Lassing's discovery that PIP_2 binds profilin caught people's attention because profilin also binds to actin and was thought to regulate its polymerization to form the cytoskeletal filaments. The result suggested then that profilin might be a bridge between the early activity induced by EGF at the cell membrane and the cytoskeleton. But no one understood the significance of profilin binding to PIP_2 or how that might be related to EGF's cytoskeletal effects.



Membrane events. *When EGF binds to its receptor, the kinase segment (TK) attaches a phosphate to phospholipase Cγ1, which then splits PIP_2, releasing profilin and inositol trisphosphate (IP_3).*

Enter the Johns Hopkins group. Their work not only sheds light on those issues, but also helps clear up another mystery that had plagued EGF work for some time. The splitting of PIP_2 in response to the growth factor is carried out by an enzyme called phospholipase Cγ1, which is phosphorylated by the activated EGF receptor. Although cell biologists always assumed that phosphorylation was needed to turn on the phospholipase activity, they had a hard time proving it. In fact, in test tube studies, the unphosphorylated enzyme appeared just as adept at breaking down PIP_2 as the phosphorylated version. "No one could understand why it had to be phosphorylated if the activity was not increased," Goldschmidt-Clermont says.

The Johns Hopkins researchers made their first key discovery last spring when they found that profilin binding to PIP_2 prevents the phospholipid from being broken down by unphosphorylated phospholipase Cγ1. But while that suggested some role for profilin in regulating PIP_2 breakdown in response to EGF, in itself the finding had little meaning. "It was kind of a dead end," says Goldschmidt-Clermont. "To have any physiological relevance, we had to show that the effect was reversible." And that's what the researchers have now done.

Goldschmidt-Clermont and his colleagues in Pollard's lab and in that of Sue Goo Rhea at the National Heart, Lung, and Blood Institute have shown that phosphorylated phospholipase Cγ1 *can* break down PIP_2 that has bound profilin. Taken together, the results indicate that profilin prevents the phospholipase from acting on PIP_2—until the enzyme is phosphorylated by the activated EGF receptor kinase. "The inhibition of phospholipase C caused by profilin binding to PIP_2 per se could have been trivial," Lindberg says. "However, together with the facts added now it bears a great deal of significance."

And the earlier experiments apparently failed to demonstrate much difference in activity between phosphorylated and unphosphorylated phospholipase Cγ1 because they were done without profilin. In the absence of profilin, Goldschmidt-Clermont says, both forms of the enzyme have about the same level of activity.

Finally, Goldschmidt-Clermont suggests a possible way in which PIP_2 breakdown in response to EGF may lead to such cellular responses as changes in shape and increased motility. As mentioned previously, these responses may be mediated by alterations the growth factor induces in the cell skeleton, but how the biochemical events EGF causes at the cell membrane get translated into cytoskeletal changes is unclear. But Goldschmidt-Clermont says profilin may indeed be the link as Lassing's work suggested. "When PIP_2 is broken down," he explains, "the profilin is eventually released." That means it's free to interact with actin and influence its polymerization to form the cytoskeletal filaments.

There's still a great deal of uncertainty about the way that profilin might alter actin polymerization. As Lindberg notes, "The unravelling of the functional relationships between transmembrane signalling and microfilament-based motility has only begun." Nevertheless, profilin may well be an important piece of the signalling puzzle.

■ MICHELLE HOFFMAN