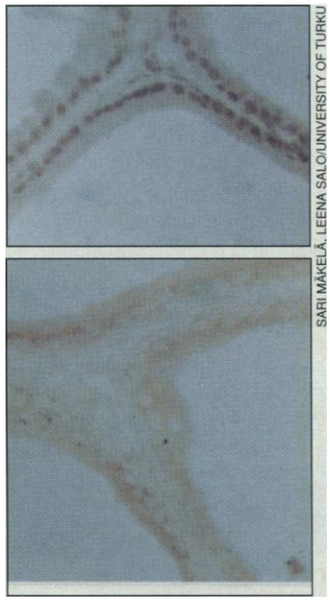
MOLECULAR ENDOCRINOLOGY

Differing Roles Found for Estrogen's Two Receptors

To the public, estrogen is one of the most familiar of all hormones. But to scientists, it is still among the most mysterious. Estrogen affects many tissues, including some, such as the ovaries and bladder, that until as recently as last year appeared to lack the receptors needed for estrogen responses. And estrogen-like anticancer drugs, which block the hormone's effects by binding to the same receptor molecules, can check tumor growth in some tissues while stimulating it in others. "There had been mysteries of estrogen action that had been intractable," says molecular biologist Peter Kushner of the University of California, San Francisco (UCSF).

On page 1508, chemist Thomas Scanlan, Kushner, and their UCSF colleagues, working with a group at the Karolinska Institute and the company Karo Bio in Huddinge, Sweden, now report a major step toward dispelling those mysteries. Their work follows up on a discovery made last year, when the Karolinska group, led by biochemist Jan-Åke Gustafsson, found a second estrogen receptor located on some tissues—including the ovaries and the prostate—that don't contain the first. Now the two teams have joined forces to show that although the new receptor, ERβ, looks like its older sibling, ERα, the two molecules can act quite differently, depending on the particular substance, or ligand, binding to them. For example, estrogen bound to ERα turns on certain genes, whereas in combination with ERβ, it has no effect.

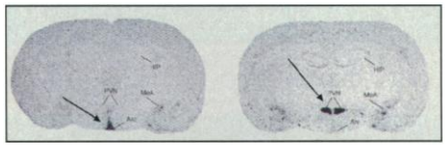


SARI MÄKELÄ, LEENA SALO/UNIVERSITY OF TURKU

Receptor pinpointed. Antibody stain (brown color, top) reveals ERβ in the nuclei of prostate cells. Unstained tissue is at bottom.

Until now, "it's been hard to find major differences between the receptors," comments Donald McDonnell, a pharmacologist at Duke University in Durham, North Carolina. "But it makes sense that at least in some circumstances, they'd have opposite effects." Such differences in activity, together with the different distributions of the two receptors in the body, may help explain estrogen's broad spectrum of activity and some of the paradoxical effects of estrogenlike drugs.

The results also suggest that it may be possible to develop "designer estrogens" that would have more specific effects than the drugs currently in use because they target one receptor and not the other. The goal would be, for example, an estrogen that could protect postmenopausal women against cardiovascular problems, Alzheimer's disease, and osteoporosis without raising their risk of developing breast or uterine cancers, as current supplements do, or a version of tamoxifen that can counter estrogen's cancer-promoting effects in the breast without increasing the risk of uterine cancer.



P. J. SHUGHRUE, I. MERCHENTHALER/WYETH-AYERST RESEARCH

Different distribution. ERα (left, arrow) and ERβ (right, arrow) occur in different brain regions.

To tease apart the differences between the two receptors, UCSF graduate student Kolja Paech first put the gene for either ERα or ERβ into cells growing in lab culture that have no estrogen receptors of their own. Then he and his colleagues added a so-called reporter gene linked to either of two different estrogen "response elements"—DNA sequences to which an estrogen-receptor complex has to bind to regulate the expression of other genes. If the reporter gene was activated by that binding, it then caused the cell to light up.

The researchers could detect no differences when they tested cells carrying one of the response elements. No matter which receptor they carried, they lit up in response to all the compounds used, including estrogen itself, the estrogenic compound DES, and three substances that are considered "antiestrogens" because they block some of the hormone's effects—tamoxifen, raloxifene, and one called Imperial Chemical Industries 164384. They got the same results with cells carrying the second element, called AP1, and ERα. But when the cells contained AP1 and ERβ, the researchers found that while the antiestrogens again increased the activity of the gene, estrogen and DES had no effect. "[The differences] were really startling," Kushner notes. "Up till now, it [had] looked like the ERα and ERβ worked the same."

In addition, the researchers picked up a hint of why tamoxifen sometimes ceases to be effective in stemming breast cancer growth and even seems to promote it. When they repeated the experiments using breast cancer and uterine cancer cell lines, they found that gene activation by the antiestrogens was particularly strong in one of the breast cancer cell lines containing ERβ. The UCSF team plans to look at whether tamoxifen might somehow induce breast cancer cells to make more of this receptor, and thus contribute to the drug's loss of effectiveness.

Although the genetically altered cells used for these experiments constitute a somewhat artificial experimental system, the researchers suspect that ERα and ERβ will behave similarly in the body, producing different effects depending on the type of cell where they are located and the identity of the ligand that binds to them. The presence of ERβ in the ovaries and urogenital tract, for example, may explain how these tissues are influenced by estrogen even though no ERα had been found there.

Sorting out the effects of the two receptors will not be easy, however. For one thing, there appears to be more than one form of ERβ. Sietse Mosselman, Rein Dijkema, and their colleagues at the Dutch-based drug company, N. V. Organon, have evidence that this receptor may also exist in a longer form than has been reported thus far. And while Gustafsson and his collaborators found that the receptor form they used is by far the most common in the tissues studied, they agree it will be necessary to determine the roles of these other forms.

In addition, just a few weeks ago, molecular biologist Paul Meltzer of the National Human Genome Research Institute in Bethesda, Maryland, and his colleagues described a gene called amplified in breast cancer–1 (AIB1) that makes a protein that works in the nucleus with other proteins to help receptors like ERα and ERβ turn on genes (*Science*, 15 August, p. 965). "It's a complicated story with multiple proteins vying for interactions with the receptor and with other transcription factors," says Meltzer.

All these complexities could make it harder to translate the new information into better synthetic estrogen compounds. Still, Gustafsson says, these are exciting times for estrogen researchers. After years of struggling with the hormone's mysteries, Kushner adds, "this [result] provides a potential explanation."

–Elizabeth Pennisi