Biography of Jan-Åke Gustafsson

Nuclear receptors, proteins that regulate transcription through binding hormones or other molecules in a cell's cytoplasm or nucleus, are involved in essentially all aspects of physiology and disease. Until the past 30 years, however, little was known about the receptors' structure or mechanism of action. In the early 1970s, Swedish researcher Jan-Åke Gustafsson began making his first pivotal contributions to this field. He has since published more than 1,000 articles, with findings ranging from the elucidation of the receptors' DNA-binding mechanism (1) to the more recent discovery of a second estrogen receptor (2).

His prolific works have earned him numerous awards, including a 1997 election to the Swedish Academy of Sciences and the 2000 European Medal from the British Society for Endocrinology. In 2002, Gustafsson was elected a foreign associate of the National Academy of Sciences. His Inaugural Article, published in this issue of PNAS, identifies inherent differences in signaling between the two estrogen receptors (3). These findings clarify the roles that each receptor plays in mammary cell proliferation and point toward possible new therapeutics for breast cancer.

Jan-Åke Gustafsson (center bottom) and his laboratory members.

Making a Difficult Choice

Growing up in Stockholm, Sweden, Gustafsson initially had his eye on a career as a clergyman in the Protestant church. However, his father, a narcotics police officer, strongly encouraged Gustafsson to enter an academic profession. "In a sense, he was disappointed about his own profession because he felt intellectually malnourished," Gustafsson said.

Seeking to honor his father's wishes, Gustafsson eventually decided to pursue a career in medical research. After entering Stockholm's Karolinska Institute in 1962, he completed a bachelor's degree in medicine in 1964 and continued on to pursue a doctoral degree, a necessary step to perform medical research in Sweden. For his graduate work, he chose Karolinska's chemistry department, headed by Sune Bergström, an M.D. and a biochemist who later won the 1982 Nobel Prize in Physiology or Medicine. Working under the leadership of mentors Jan Sjövall, a former pupil of Bergström, and Bengt Gustafsson (no relation to Jan-Åke), head of Sweden's medical research council, Gustafsson compared steroid metabolism between normal rats and "germ-free" animals specially bred to live without intestinal microflora. He and his colleagues identified and described several new steroid metabolites in the animals, such as 15β-hydroxy-*allo*-tetrahydrocorticosterone 21-sulfate, the predominant corticosterone metabolite in rats (4). The researchers also discovered that some steroids follow an enterohepatic circulation pattern, interacting with intestinal microflora while cycling between the liver and the gut (5). Gustafsson published these findings for his thesis and graduated with a doctorate in chemistry in 1968.

Gustafsson continued his medical coursework and completed an M.D. degree at Karolinska in 1971. After graduation, he was faced with a difficult choice: Gustafsson not only enjoyed working with patients but also wanted to continue laboratory research, goals that could be difficult to combine. Seeking an expert opinion, he conferred with a clinical endocrinologist he had met during his medical studies. With a discouraging tone, the endocrinologist steered Gustafsson away from clinical medicine, urging him to choose research instead. Gustafsson thus continued on at Karolinska as an associate professors in chemistry. "It took me about a year before I had gotten rid of those feelings that I wanted to go back to patients. It was difficult, but gradually I got accustomed to full-time basic research," he said.

An Equal-Level Collaborator

After setting up his laboratory at Karolinska, Gustafsson focused on studying the interaction between steroid hormones and their corresponding receptors. As he became more excited with his research, he also realized that he possessed a particularly useful gift. "I noticed that I had a certain capacity in building up a group and stimulating young medical students to do science," he said. Gustafsson's enthusiasm helped him attract a talented group of 15 students and technicians. Gradually, the group's focus shifted to studying the mechanism of glucocorticoids, hormones that affect carbohydrate metabolism and have antiinflammatory properties. A particular goal of their work was to describe the glucocorticoid receptor's basic structure, then a long-held goal for hormone research.

Gustafsson's team tagged receptors in rat cells with radioactively labeled corticosterone, a glucocorticoid ligand. After running crushed cells through a gel-filtration column, Gustafsson's group found three different particle sizes for what they previously had supposed was a uniformly sized receptor. "We were puzzled by that, as were other people," he said. Further investigation, however, showed that proteases digested the receptors after the cells were crushed. By combining the use of proteases and protease inhibitors, Gustafsson and his colleagues found that the receptor was composed of three domains: one that bound hormones, another that bound DNA, and a third that bound antibodies. His laboratory published their findings in three articles in 1977, 1978, and 1982 (6–8).

This is a Biography of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 3739.

Gustafsson's team then turned its attention to elucidating how the glucocorticoid receptor binds and interacts with DNA. After purifying the receptor (9), a challenging feat that had never before been accomplished, Gustafsson began a collaboration with Keith Yamamoto, a biochemist at University of California, San Francisco. The two researchers worked together to understand how the receptor identifies and attaches to specific binding sites on DNA in mouse mammary tumor virus (MMTV). "The idea was a true, equal-level collaboration where Yamamoto supplied the responsive MMTV gene, which he could produce in sufficient quantities, and we supplied the purified protein," Gustafsson said.

Starting in 1979, Gustafsson's team sent samples of purified human glucocorticoid receptor to Yamamoto on a weekly basis. Three years later, the researchers were confident they had identified the receptor's DNA-binding mechanism. "The receptor sort of glides along the strand with a low binding affinity, searching for a high-affinity site. Then, it gets stuck at that site because it's found the right parking place," said Gustafsson. Importantly, Gustafsson's and Yamamoto's teams also discovered that the receptors could confer hormone responsiveness onto downstream genes that previously had been unresponsive. Gustafsson and his collaborators published their findings on these "hormone response elements" in 1983 (10).

The Yin and Yang of Receptors

Gustafsson continued to work with Yamamoto to clone the glucocorticoid receptor gene, a subject of hot competition among several laboratories. Although the two researchers managed to clone a portion of the receptor in 1984 (11), publishing their first results in *Nature*, they were beaten at cloning the receptor's full length in 1985 by Ronald Evans, a biochemist at the Salk Institute (12). Seeking a new focus, Gustafsson's team turned to elucidating the receptor's 3D structure. Collaborating with Robert Kaptein, an NMR specialist at Utrecht University, Holland, Gustafsson and his colleagues solved the structure of the glucocorticoid receptor's DNA-binding domain in 1990 (13). Their work revealed an α-helix that associates with DNA, a structure later found to be typical for nuclear receptor DNA-binding domains.

During the 1990s, Gustafsson also became interested in orphan receptors, so called because their corresponding ligands are unknown. Focusing on a particular orphan receptor known as peroxisome proliferator-activated receptor, or PPAR, he and his laboratory showed that its ligands were fatty acids (14). The finding was a surprise to many nutrition scientists, who had previously assumed that fat supplied mainly calories and had no hormonal action on the body. "It meant a more complex understanding of what happens when we eat," said Gustafsson. The group's discovery, published in 1992, founded the new discipline of molecular nutrition, a field that continues to grow today.

Gustafsson's most recent work centers on his fortuitous 1996 discovery of a second estrogen receptor (ER), which he named ERβ (2). Gustafsson's article reporting this discovery was communicated to PNAS by Elwood Jensen, a biochemist at the Institute for Hormone and Fertility Research in Hamburg, Germany, and the discoverer of the first estrogen receptor, now called ERα. Gustafsson identified and cloned ERβ receptors in prostate tissue. "Nobody in their right mind would look for estrogen receptors in the prostate. We were actually looking for new orphan receptors, and we came up with this," said Gustafsson.

Gustafsson's team discovered that the β receptor had similarities to the original "α" ER. Further investigation, however, showed that the two receptors exert some opposing effects (15). "They have an intricate interplay, like a yin–yang principle," Gustafsson said. Activation of either α or β receptors can stem osteoporosis, depression, urinary incontinence, and heart disease, menopausal symptoms that many women seek to avoid with hormone replacement therapy. However, whereas ERα can cause a host of unwanted effects, such as an increased incidence of endometrial and breast cancer, ERβ does not cause proliferation in the breast or endometrium. Because ERβ confers such favorable effects, some pharmaceutical companies currently are working to develop ligands that avoid ERα in favor of binding to ERβ (16).

Gustafsson's Inaugural Article, published on page 3739, investigates the role that ERβ plays in cell proliferation. Although researchers have long known that stimulating ERα receptors encourages cell proliferation, these receptors are conspicuously missing from dividing cells. Thus, some investigators have theorized that estrogen exerts its proliferative effects indirectly through an unknown mechanism. Here, Gustafsson and his colleagues show that ERβ is not lost during cell proliferation, but ERα receptors are rapidly degraded as soon as a cell has committed itself to proliferation. The researchers suggest that ERβ receptors may be necessary for restoring ERα receptors to the nucleus after a cell has divided.

Practical Medicine

Encouraged by his successes working with the two estrogen receptors, Gustafsson sees his future work headed in different directions. Although he plans to continue studying basic science "for science's sake," he also wants to direct his research toward more practical problems in collaboration with the drug industry. To that end, Gustafsson and fellow National Academy of Sciences member John Baxter, an M.D. and biochemist at the University of California, San Francisco, cofounded a biotechnology company called KaroBio in 1987. The company, based on Karolinska University's South Campus, seeks to develop nuclear receptor ligands into marketable drugs.

Gustafsson said that this commercial mission finally combines his original goal of doing both research and clinical medicine. By focusing his skills on creating drugs to treat diseases, Gustafsson hopes to see his work help patients during his lifetime. "For a medical scientist, it is extremely fulfilling to see what you are doing is really of medical value," he said. "My youthful joy is there again. Every day, you go the lab and find new stuff. It's wonderful."

Christen Brownlee, *Science Writer*

1. Payvar, F., Wrange, Ö., Carlstedt-Duke, J., Okret, S., Gustafsson, J.-Å. & Yamamoto, K. R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6628–6632.
2. Kuiper, G. G. J. M., Enmark, E., Pelto-Huikko, M., Nilsson, S. & Gustafsson, J.-Å. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 5925–5930.
3. Cheng, G., Weihua, Z., Warner, M. & Gustafsson, J.-Å. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 3739–3746.
4. Gustafsson, J.-Å. & Sjövall, J. (1968) *Eur. J. Biochem.* **6,** 236–247.
5. Eriksson, H., Gustafsson, J.-Å. & Sjövall, J. (1968) *Eur. J. Biochem.* **6,** 219–226.
6. Carlstedt-Duke, J., Gustafsson, J.-Å. & Wrange, Ö. (1977) *Biochim. Biophys. Acta* **497,** 507–524.
7. Wrange, Ö. & Gustafsson, J.-Å. (1978) *J. Biol. Chem.* **253,** 856–865.
8. Carlstedt-Duke, J., Okret, S., Wrange, Ö. & Gustafsson, J.-Å. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4260–4264.
9. Wrange, Ö., Carlstedt-Duke, J. & Gustafsson, J.-Å. (1979) *J. Biol. Chem.* **254,** 9284–9290.
10. Yamamoto, K. R., Payvar, F., Firestone, G. L., Maler, B. A., Wrange, Ö., Carlstedt-Duke, J., Gustafsson, J.-Å. & Chandler, V. L. (1983) *Cold Spring Harbor Symposia on Quantitative Biology* (Cold Spring Harbor Lab. Press, Plainview, NY), Vol. XLVII, pp. 977–984.
11. Miesfeld, R., Okret, S., Wikström, A.-C., Wrange, Ö., Gustafsson, J.-Å. & Yamamoto, K. (1984) *Nature* **312,** 779–781.
12. Hollenberg, S. M., Weinberger, C., Ong, E. S., Cerelli, G., Oro, A., Lebo, R., Thompson, E. B., Rosenfeld, M.G. & Evans, R. M. (1985) *Nature* **318,** 635–641.
13. Härd, T., Kellenbach, E., Boelens, R., Maler, B. A., Dahlman, K., Freedman, L. P., Carlstedt-Duke, J., Yamamoto, K. R., Gustafsson, J.-Å. & Kaptein, R. (1990) *Science* **249,** 157–160.
14. Göttlicher, M., Widmark, E., Li, Q. & Gustafsson, J.-Å. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4653–4657.
15. Weihua, Z., Saji, S., Mäkinen, S., Cheng, G., Jensen, E. V., Warner, M. & Gustafsson, J.-Å. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 5936–5941.
16. Gustafsson, J.-Å. (2003) *Trends Pharmacol. Sci.* **24,** 479–485.