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Sex Steroid Hormones : Action and Dynamism

(Special Lecture)

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Sex Steroid Hormones and Their Receptors

Sex steroid hormones are small lipid-soluble molecules that differ from one another in both chemical structure and function. Nonetheless, they all act using a similar mechanism. Hydrophilic signaling molecules such as peptides or proteins are unable to cross the plasma membrane, and bind to cell-surfaced receptors which in turn generate complex intracellular signal transduction. Hydrophobic signaling molecules, in contrast, enter cells by penetrating directly across the plasma membrane of the target cells and bind to intracellular receptor proteins. Liganded receptors translocate either intracellularly or intranuclearly and form large protein complexes with cofactors to induce or repress gene transcription. Therefore steroid hormone receptors are ligand-dependent transcription factors.

Visualization of Sex Steroid Hormone Receptors

With the advent of green fluorescent protein (GFP) and its color variants, the subcellular distribution of many steroid hormone receptors has been found to be much more dynamic than previously thought, with some of the receptors shuttling between the cytoplasm and nucleus in the culture system. Steroid hormone receptors can be divided into three categories based on their unliganded distribution: those that are primarily in the nucleus, those in the cytoplasm, and those with mixed cytoplasmic and nuclear distribution. However in all cases, addition of ligand leads almost complete nuclear translocation of the receptors. Hormonal stimulation induces intranuclear receptor distribution from a homogeneous pattern to heterogeneous dot-like image. Ligand binding to steroid hormone receptors leads to the recruitment of many proteins including cofactors to provoke the redistribution of receptor complexes in the nucleus. This focal organization could involve more complex events than simple DNA binding sites for transcription.

Estrogen Receptor Dynamism in the Nucleus

Upon estradiol treatment ERa and b in the same cell were relocalized to show discrete pattern, and they were localized at the same discrete cluster, suggesting that both subtypes of ERs were bound to the same nuclear sites. In fact, FRET (fluorescence resonance energy transfer) clearly showed the interaction of ERa and ERb. In the presence of the estradiol, however, the discrete staining pattern of ERa and b were mostly overlapped with Brg-1, indicating that most of the ERs clusters are involved in the chromatin remodeling machinery. FRAP (fluorescence recovery after photobleaching) analysis showed that nuclear ERa and b are most dynamic and mobile in the absence of the ligand, but its mobility was slightly decreased after the ligand treatment. When ATP was deleted from the culture medium, even liganded ERa significantly lost its free mobility in the nucleus, indicating that ERa dynamism is ATP-dependent. Nuclear matrix which is scaffolding sites within the nucleus is composed of actin and actin-related peptides. Treatment by detergent caused complete loss of ERa in the unliganded condition, but liganded ERa sticked to the nuclear matrix. Nuclear matrix is an important factor to determine the dynamism of unliganded and liganded ERa.

Interaction of ERa and AR

AR (androgen receptor) and ERa showed punctate colocalization in the nucleus with estrogen, but not androgen. N-terminus AR deletion mutant did not form a nuclear punctate pattern with either

androgen or estrogen. In the presence of AR, but not ERa, formed a punctate nuclear pattern with androgen. AR had different mobility depending on the ligand and the presence of ER a. On the other hand, AR had little effect on the stability of ER a. ER a mutant that does not bind coactivators did not alter the mobility of AR.

ERa-GFP transgenic mouse

Transgenic (Tg) mice in which GFP was expressed under the ERa promoter activity were generated. In the ovary of several lines of these Tg, granulosa cells of ovarian follicle showed GFP fluorescence. In the brain many GFP cells were distributed and its distribution was in general similar to the previous data from ERa immunohistochemistry. Comparison of the GFP cells and ERa immunoreactive cells were made at cell-by-cell basis by using confocal microscopy. Ovariectomy caused significant reduction of cell bodies of GFP neurons containing ERa in the medial preoptic area, but not in the ventromedial nucleus. These results suggest that estrogen affects ERa containing cells at the region-specific manner.