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Trafficking of nuclear receptors in living cells

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Abstract

Most of the steroid receptor family, with the exception of the estrogen receptor, are classically viewed as 'translocating receptors'. That is, they move from an exclusively, or principally, cytoplasmic distribution in the absence of hormone to a predominately nuclear localization in hormone stimulated cells. The estrogen receptor and the nuclear receptor family are found exclusively in the nucleus, both in hormone stimulated and hormone free cells. This behavior has now been studied with GFP-fusions in living cells, and has in general been confirmed. However, there are important exceptions, and new findings, particularly with regard to sub-nuclear localization. We propose that the intracellular distribution of both receptor classes is dependent not only on subcellular localization signals directly encoded in the receptors, but also on the nature and composition of the large, macromolecular complexes formed by each receptor. Furthermore, we find that most members of the receptor superfamily form focal accumulations within the nucleus in response to ligand, and suggest that these structures may participate in the biological life cycle of the receptors. Finally, we propose that receptor movement in the nucleus is highly dynamic, with the receptors undergoing constant exchange between genomic regulatory elements, multi-protein complexes with other transcription factor partners, and subnuclear structures that are as yet poorly defined. Published by Elsevier Science Ltd.

Keywords: Steroid receptor; Estrogen receptor

1. Introduction

The distribution of steroid/nuclear receptors within the major subcellular compartments is an important component of their biological activity. For members of the steroid receptor family, the mechanisms governing the cytoplasmic/nuclear distribution have been modeled primarily in terms of the conditional interaction of nuclear localization signals with the import/export apparatus present in nuclear pores [1]. In the absence of hormone, these receptors are found in association with a complex set of chaperones in a large complex [2] interaction of the cognate ligand with these receptors induces a conformational rearrangement that results in dissociation of the complex and loss of many of the associated factors. This reorganization is thought in some cases to expose previously masked translocation signals, and the receptors are then recognized by the transport machinery.

In contrast, members of the 'nuclear' subgroup are found constitutively in the nucleus, and are believed not to interact with the heat shock protein/chaperon complex. Some of these receptors, the thyroid hormone receptor in particular, have been shown to interact with specific DNA regulatory elements in the absence of ligand, and also repress selective genes in the hormone-free cells [3,4]. These observations have led to the general hypothesis that the 'nuclear' receptors are nuclear localized because they are constantly present on chromatin. The dominant paradigm for subcellular localization of the receptors thus focuses on ligand-independent interaction with chaperone complexes for the steroid receptors, and ligand-independent binding to DNA elements for the nuclear receptors. Despite the wide-spread acceptance of this model, there are significant disparities in the literature. For example, the estrogen receptor clearly moves from a chaperone complex (8s) form to a dissociated form (4s) in a ligand dependent fashion, but it is constantly present in the nucleus.

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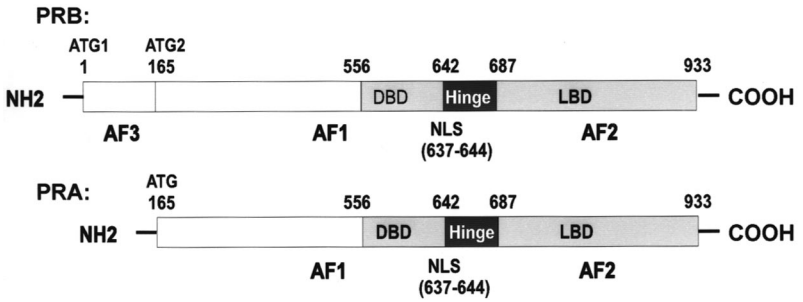

Fig. 1. Domain structure of the progesterone receptor A and B forms. AF1, 2, 3 correspond to activation functions 1, 2, or 3. DBD, Hinge, and LBD indicate the DNA binding domain, hinge region, and ligand (hormone) binding domain respectively. NLS indicates location of the nuclear localization signals, both constitutive and hormone-dependent.

Furthermore, with the exception of the nuclear matrix, which is a controversial structure, current models of receptor trafficking view the nucleus essentially as a soluble compartment with little internal structure.

With the advent of the green fluorescent protein (GFP) [5], and the exciting potential this development provides for real time monitoring of protein trafficking [6], we initiated a program to utilize this reagent to study intracellular distribution and movement for members of the steroid/nuclear receptor family. The reagent has proven to be quite useful. Receptors labeled with GFP in general retain their normal transcriptional activity and ligand dependence [7–16]. We now have approximately five years of experience using these chimeras, and several unexpected findings have emerged regarding subcellular trafficking for members of the receptor superfamily.

2. Progesterone receptor

The progesterone receptor (PR) is found in mammalian cells as two variants, the A and B forms. The A form is an expression variant, utilizing an alternate initiation signal, with the N-terminal 165 a.a. of the B form deleted (Fig. 1). These receptors are identical within the remaining 768 a.a. identical. PR-A can exert a dominant negative effect on PRB in some systems [17] and variation in dimerization efficiencies have been reported [18]. The formation of AA dimers appears more likely than AB dimers, with BB dimers being the least efficient. Since the nuclear localization signals [19], and chaperone protein binding regions [20] of the two forms are identical, a similar subcellular distribution for these molecules should be observed. However, these receptor forms traffick quite differently in living cells (Fig. 2). The A form is found predominantly in the nucleus in the absence of ligand, while significant numbers of cells have the B form primarily cytoplasmically localized in hormone free cells [14].

It thus appears that features other than the classic chaperone and translocation protein interacting domains are important for PR subcellular trafficking. Differences in localization are unlikely to result from size differences between the two forms of the receptor, as both molecules (94 kDa A form; 120 kDa B form) are well above the exclusion limit of 45 kDa for free diffusion across the nuclear pore complex (separating the nucleus from the cytoplasm) [21]. Also, differential localization of PR-A and PR-B is unlikely to be mediated by heterodimerization, because addition of an excess of PR-A has no effect on the localization of GFP–PR-B, and excess PR-B has no effect on the localization of GFP–PR-A [14].

Several hypotheses can be advanced to explain the differential subcellular distribution observed. PR-B may adopt a tertiary conformation that leads to a less effective nuclear localization signal (NLS). Alternatively, a difference in overall conformation of the A form of the receptor may allow for a more effective nuclear localization signal. Of course, there may be as yet unidentified nuclear export signals in the N-terminal region of the PR. If there are such translocation signals, altered kinetics between import into and export from the nucleus could explain the differential localization.

A more probable model would involve the differential interaction of PR-A and PR-B with any of the many coactivators, chaperones, and other complexes that characterize the intracellular organization of recep-

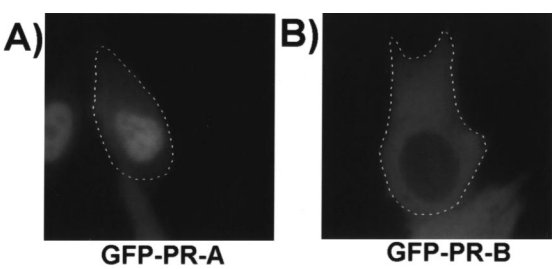

Fig. 2. Intracellular distribution of GFP-PR-A and -B in living cells. Distribution profiles representative for each of the receptor forms are shown for ligand free cells (1471.1 cells). The discontinuous white line indicates the cell outline. The image in panel A corresponds to PR-A and that in panel B PR-B (see [14] for details of expression).

tors and other transcripiton factors. A general concept that emerges from the past decade of research in the field of transcriptional regulation is that regulatory proteins have the potential to form many partner complexes in the living cell. Indeed, many of the characterized complexes are quite large. It seems likely, therefore, that the A and B forms of the receptor, because of the additional domains present in PR-B, enter into different multi-protein complexes, or enter into similar complexes with different efficiencies. Determining the exact status of these very high molecular weight structures, with many protein components, may be one of the major challenges of the next decade. The ability to label selective members of these complexes with various fluorescent markers in living cells could in principal contribute significantly to understanding these structures. Several sophisticated technologies that detect direct molecular interactions between fluorescently tagged proteins are under intense investigation. These include fluorescence energy transfer (FRET) [22,23]; proximity imaging (PRIM) [24]; fluorescence lifetime imaging microscopy (FLIM) [25], and other approaches. While these methodologies are not yet standard reserach tools, the emergence of one or more into general use would provide direct data concerning the interaction of macromolecular complexes in living cells, information that can only be obtained from these approaches.

3. Estrogen receptor-alpha

While the alpha form of the estrogen receptor (ER) is classically a member of the steroid receptor family, and was the first to be identified in a molecular chaperone complex, it is unique among this group in its strictly nuclear localization. We recently confirmed this distribution in living cells with GFP derivatives of the human estrogen receptor [12]. Human ER labeled at the N-terminus with GFP was found to be expressed as a stable protein, and retains transactivational properties very similar to those of the wild type receptor. GFP–ER was found localized completely in the nucleus of transfected cells, both in the absence and presence of ligand, in agreement with findings from biochemical cell fractionation studies, and indirect immunofluorescence on fixed cells [26].

High resolution microscopy of GFP–ER distribution in living cells clearly indicates, however, that the intranuclear location of the receptor is strongly affected by ligand. The receptor in hormone free cells is distributed rather uniformly throughout the nucleus [12] (Fig. 3). In ligand-treated cells, the receptor adopts a more focal distribution, with nuclei developing a markedly punctuate organization of the receptor. This effect of hormone is not unique to the estrogen receptor. In fact, most of the receptors we have examined, including the progesterone receptor [14], the glucocorticoid receptor (Fig. 4) [13], the thyroid hormone receptor (see below) [27], and the aryl hydrocarbon receptor (AhR) (Elbi et al., unpublished), all show a more focal nuclear organization in the presence of ligand. It seems clear, therefore, that ligand-dependent intranuclear reorganization of the steroid/nuclear receptors involves more complex events than simple recognition of specific chromosomal DNA binding sites. While some of the focal structures associated with the activated receptors may include specific DNA sequences, there is no evidence to date that the preponderance of these elements

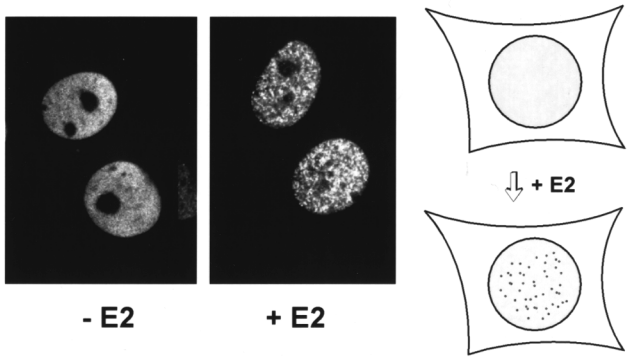

Fig. 3. Intracellular distribution of the green fluorescent protein labeled estrogen receptor (alpha form) in living cells (MCF-7 cells). The receptor is found exclusively in the nucleus, but as indicated in schematic (right panel), the intranuclear distribution is distinctly altered upon addition of ligand (see [12] for a quantitative description of the distribution).

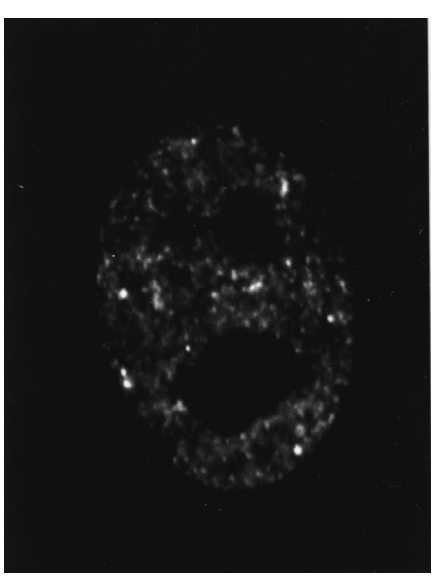

Fig. 4. Subcellular distribution of GFP–GR-C656G in living cells. Upon treatment with 10 nM dexamethasone, GFP–GR moves completely to the nucleus (1471.1 cells). The intranuclear disposition of the receptor is non-uniform, with a distinctly punctuate accumulation superimposed on an overall nuclear distribution. (see [13] and [33] for further examples of the non-uniform distribution).

are sites of specific receptor regulated gene activity. These structures remain at this point essentially a mystery. It clearly is important to establish whether the focal distribution associated with activated receptors represents an important functional structure in the biology of receptor action.

4. Thyroid hormone receptor

The thyroid hormone receptor (TR) has been described since its first identification as a nuclear receptor, that is, it is nuclear localized both in the absence and presence of ligand [28–30]. Several observations suggest that hormone-independent association with DNA is responsible for this localization. Hormone-free TR can bind to specific DNA elements in vitro [31], and is found in chromatin in association with a set of corepressors. Furthermore, unliganded TR can repress some promoters in a TRE-dependent fashion, suggesting strongly that the unactivated receptor does interact with specific genetic elements. In living cells, however, we discovered that a significant portion of TR can be cytoplasmically localized [16]. These findings suggest that intracellular trafficking of TR could be more complex than previously assumed. Indeed, in recent experiments [27], we find that mutants of TR that interfere with corepressor interactions can cause a major relocation to the cytoplasm, and these mutant receptors will move to the nucleus in a hormone-dependent fashion. This is a previously undescribed behavior for 'nuclear' receptors, and suggest that TR trafficking is not determined simply by its ligand-independent DNA affinity. We suggest, rather, that for TR, as for PR and probably GR and ER, the intracellular distribution of these receptors is determined by multiple mechanisms, including the action of hormone-dependent or -independent NLS signals in the receptors, but also the nature of the macromolecular complexes that are formed in hormone-free and ligand-stimulated cells, and the location in turn of these large multicomponent structures.

5. Dynamics of receptor movement

A classic view of receptor trafficking has often invoked a static location for a given receptor in a continuous ligand state. The steroid receptors are seen as continuously bound to a hormone response element in the constant presence of ligand, leaving the regulatory elements only when hormone is withdrawn. Conversely, a nuclear receptor, with TR as the prototype, is envisaged in a chromatin bound state in the absence of T3, in association with corepressors and chromatin silencing activities such as histone deacetylases [32]. Under these models, DNA binding, either hormone dependent for the steroid family, or hormone independent for the nuclear family, plays a major role in subcellular localization.

We recently reported the direct, real-time observation of glucocorticoid receptor binding to a set of regulatory elements in living cells [33]. GFP–GR was observed to bind directly to an artificial, amplified array of MMTV reporter elements on chromosome 4 in a mouse cell line. Two photobleaching approaches were used to study the real-time interaction of receptor with its regulatory sites in chromatin, FRAP (fluorescence recovery after photobleaching), and FLIP (fluorescence loss in photobleaching). With both protocols, the surprising finding was obtained that GR exchanges rapidly and continuously in the constant presence of ligand. This finding stands in contrast to the classical view that liganded steroid receptors bind statically to their response elements, and induce the formation of a stable transcriptional preinitiation complex.

6. Conclusions

These observations on receptor trafficking in living cells suggest a more complex and dynamic view of receptor movement than entertained in current models. We suggest that the distribution of nuclear/steroid receptors between the nucleoplasmic and cytoplasmic compartments is dependent not only on the intrinsic NLS signals present on the receptors, but equally subject to constraints imposed by the many partners found in the large macromolecular complexes with which the receptors associate (Fig. 5). Since focal concentration of receptor protein in a ligand-dependent fashion now appears to be a common feature of many steroid/nuclear receptors and interacting proteins, we further suggest that some fraction of the receptor population traffics to "accumulation centers" within the nuclear compartment. A functional role for these focal structures is completely unknown at this time, but we note that in several cases the movement to these centers requires the receptor to be occupied by agonist; focus formation with the relevant antagonist does not occur [see [9] for mineralocorticoid receptor and [13] for glucocorticoid receptor]. Information concerning the molecular composition of these focal structures will be an early goal in determining the role, if any, of these structures in receptor action.

Finally, we have shown [33] that the glucocorticoid receptor engages in constant and rapid exchange with regulatory elements, and we propose (Fig. 5) that this is a common feature of the steroid receptor family. Recent findings with a GFP derivative of the progesterone receptor (Mueller et al., unpublished observations, 2000) indicate that rapid exchange is observed also for PR. Indeed, we predict that the nuclear receptors may

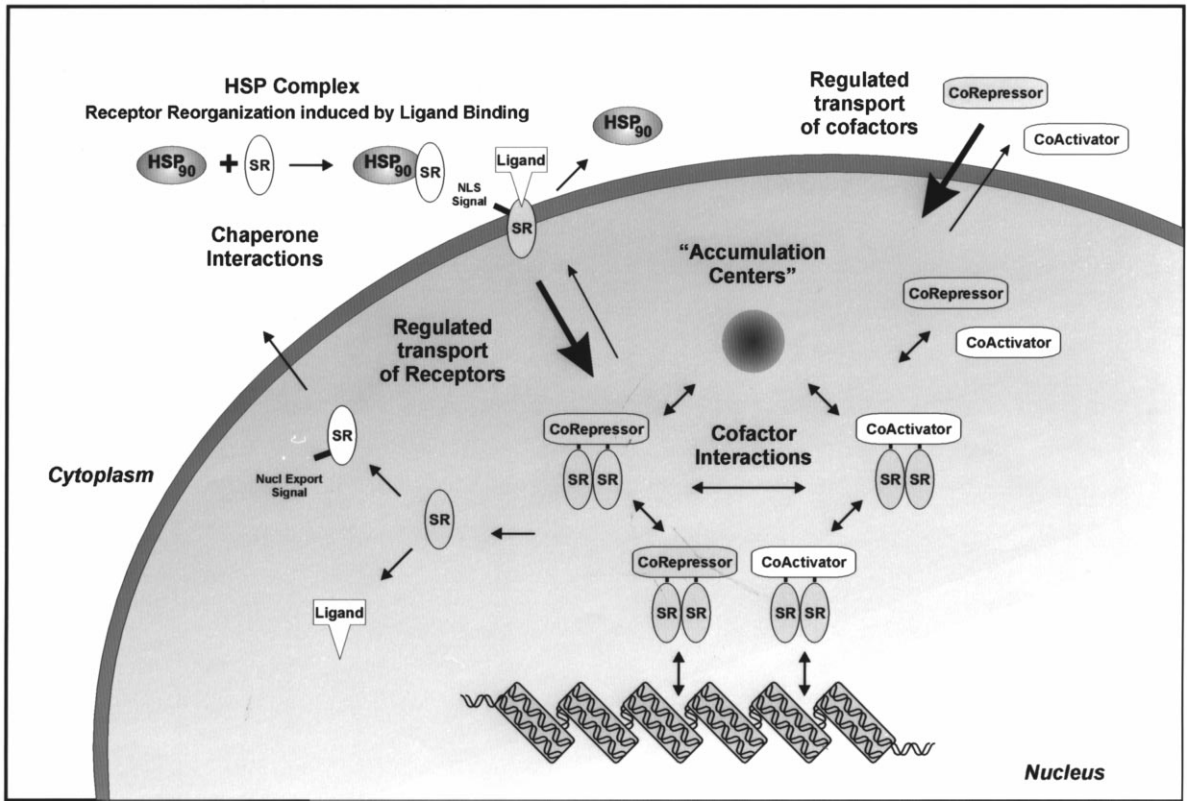

Fig. 5. Integrated model for the subcellular distribution of steroid receptors. Trafficking of the steroid/nuclear receptor superfamily (SR indicates members of the superfamily) is seen as dependent on a large number of separate mechanisms. Some of the receptors interact with chaperones, and are dissociated in a ligand-dependent fashion, leading to (a) a reorganization of receptor protein that may be involved in ligand assimilation into the hydrophobic ligand pocket, and/or (b) exposure of NLS signals that interact with the pore complex transport apparatus. Intranuclear receptors are associated with a number of large macromolecular complexes (corepressors and coactivators), and these interactions are regulated by ligand. Since the subcellular distribution of the other members of these complexes is also regulated, the status of receptor in these multi-protein complexes impacts receptor distribution. Both receptors and their cofactors can also be observed in large focal structures (referred to here as "accumulation centers"). The nature and function of these structures is not currently understood, but they have been observed for many different factors, and likely play some role in the receptor life cycle. Finally, receptors bind to chromatin, and these interactions also impact the subcellular distribution. In contrast to the classical view of static binding, however, we argue that the genomic interactions are highly dynamic, and influence trafficking by affecting the equilibrium distribution, not the static location.

also exchange with regulatory sites in the absence of ligand. Although the equilibrium for these receptors is clearly biased to the nucleus in hormone-free cells, there is no direct evidence that static binding to chromatin governs this equilibrium. The interaction of the nuclear receptor class with genomic targets may also be dynamic. It is clearly of substantial importance to measure directly the interaction of members this receptor group with response elements in living cells.

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