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Calmodulin, a regulatory partner of the estrogen receptor alpha in breast cancer cells

Running title: Calmodulin regulates ERα

Dominique Gallo[a], Yves Jacquot[b], Guy Laurent[c] and Guy Leclercq[a, *]

[a] Laboratoire J.-C. Heuson de Cancérologie Mammaire, Université Libre de Bruxelles (U.L.B.), Institut Jules Bordet, 1 rue Héger-Bordet, B-1000 Brussels, Belgium.
[b] Université Pierre et Marie Curie-Paris6, CNRS-UMR 7613, "Synthèse, Structure et Fonction de Molécules Bioactives", FR 2769, Case courrier 45, 4, place Jussieu, 75005 Paris, France.
[c] Service d'Histologie et de Cytologie Expérimentale, Faculté de Médecine et de Pharmacie, Université de Mons-Hainaut, 6 avenue du Champ de Mars, B-7000 Mons, Belgium.

*** Corresponding author :**
Prof. Guy Leclercq
Laboratoire J.-C. Heuson de Cancérologie Mammaire
Institut Jules Bordet
1 rue Héger-Bordet
B-1000 Brussels
Belgium
Phone: +3225413744, Fax: +3225413498
E-mail: **lcanmamm@ulb.ac.be**

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Summary

Although calmodulin (CaM) interaction with estrogen receptor alpha (ERα) has been known for more than two decades, it is only recently that the molecular mechanism of CaM-mediated regulation of ERα is beginning to emerge. Others and we have identified a putative calmodulin binding site (P_{295}LMIKRSKKNSLALSTADQMVS$_{317}$) in ERα, at the boundary between the hinge and the ligand binding domain. ERα mutations affecting its association with CaM have been reported to generate high basal, estrogen-independent transactivation activity, indicating that the P_{295}-T_{317} sequence has an inhibitory function. Moreover, we found that a synthetic peptide (ERα17p : P_{295}-T_{311}) containing residues crucial for CaM binding exerts estrogenic effects on breast carcinoma cells. Finally, computer-aided conformational studies revealed that the CaM binding site might associate with a region located downstream in ERα (the ß turn/H4 region), this association likely resulting in an autoinhibitory folding of the receptor. Thus, we propose as a hypothesis that CaM acts as a positive regulator by relieving this ERα autoinhibition.

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1. Historical background

First indications concerning a possible contribution of calmodulin (CaM) to the mechanism of action of estrogen receptor alpha (ERα) appear in studies of Auricchio's group published in the early '80s, where it was reported that a cytosolic CaM-dependent kinase could restore the estradiol (E_2) binding capacity of a crude ERα preparation inactivated beforehand by the action of a nuclear phosphatase (Auricchio et al., 1981a; Auricchio et al., 1981b; Migliaccio et al., 1984). Subsequent investigations performed by these authors suggested that this kinase, which was found to target the receptor on Tyr-537 (Castoria et al., 1993), might also confer E_2 binding ability to ERα synthesized *in vitro* (Castoria et al., 1993; Migliaccio et al., 1989; Migliaccio et al., 1991). Finally, they proposed that the loss of such a CaM-dependent phosphorylation of ERα in breast cancer may explain the decrease of hormone dependence that occurs during tumor progression (Castoria et al., 1996; Migliaccio et al., 1992). Because of its clinical implications, this hypothesis aroused in our team a considerable interest for Auricchio's studies. While we failed to reproduce in serially transplanted mouse mammary tumors these observations on estrogen binding (Piccart et al., 1998), we confirmed that CaM can associate with ERα in a Ca^{++} dependent manner (Bouhoute, Leclercq, 1992; Bouhoute, Leclercq, 1995; Bouhoute, Leclercq, 1996), a property also described by these authors (Castoria et al., 1988). Nowadays, the involvement of a CaM-dependent kinase in ERα phosphorylation on Tyr-537, resulting in enhanced hormone binding capacity, appears at least questionable (Carlson et al., 1997; Yudt et al., 1999). In fact, some reported observations might result from the combined influence of various factors, such as a CaM-induced protection of ERα against proteolysis (Li et al., 2001), an

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increase of Tyr-537 phosphorylation and a more stable association with chaperones (i.e. heat shock proteins, Hsp_s), these effects contributing altogether to increase ligand binding capacity.

While the implication of CaM in ERα phosphorylation is not at all substantiated, there is no doubt that CaM contributes to E_2-induced, receptor-mediated transcription. Early studies from our laboratory revealed that CaM increases the ability of the receptor to associate with estrogen response elements (EREs) (Bouhoute, Leclercq, 1995). Subsequent independent investigations (Biswas et al., 1998) indicated that it operates as a co-regulator of major importance for the formation of stable ERα~ERE complexes. The additional observations of these authors that a CaM antagonist (W7) abrogates the transactivation of an E_2-responsive promoter led them to conclude that CaM participates to ERα-mediated transcription. Extensive studies conducted by Sacks (Li et al., 2005; Li et al., 2001) and Ramos (Garcia Pedrero et al., 2002) definitely established this concept.

The mechanism by which CaM enhances ERα-mediated transcription is still a matter of debate. The present paper, based on most recently reported data including ours, proposes a model aimed at explaining CaM involvement in this process. According to this model, CaM antagonizes the inhibitory activity of a receptor motif, the action of which is under the control of ligands as well as a panel of co-regulators. The potential implication of CaM in the regulation of ERα turnover rate, and thus in the control of ERα transactivation ability (Leclercq et al., 2006), is also evoked.

2. The regulatory function of CaM concerns the nuclear form of ERα

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Intracellular visualization of ERα has revealed that its localization is mainly nuclear. This finding does not mean that ERα is sequestered within the nucleus since it has been shown to undergo a dynamic intracellular shuttling (Maruvada et al., 2003; Nonclercq et al., 2007; Stenoien et al., 2000) which seems to determine the type of response to estrogen signaling (Leclercq et al., 2006). Thus, estrogens elicit quasi-immediate responses *via* membrane associated form(s) of ERα that can interact with signaling cascades and thereby modulate *in fine* the transcription of target genes (Membrane-Initiated Steroid Signaling, MISS) (Acconcia, Kumar, 2006; Pedram et al., 2006; Song, 2007). In contrast, nuclear form(s) of ERα directly regulate gene expression (Nuclear Initiated Steroid Signaling, NISS), the receptor functioning in this case as an estrogen-dependent transcription factor (or as a co-regulator of other transcription factors).

Inasmuch as CaM regulates intracellular processes in both the cytoplasm and the nucleus, one may wonder whether it interacts with one or several forms of ERα. In order to explore this issue, Sacks and coll. (Li et al., 2003) have transfected cells with expression plasmids coding for peptides able to selectively inhibit the cytoplasmic or the nuclear form of CaM. Results of their study demonstrated that CaM exerts its action on nuclear ERα, since E_2-stimulated gene transactivation was abrogated when the CaM-inhibitory peptide accumulated within the nucleus, while it was only slightly attenuated when the peptide was targeted to the membrane and the cytoplasm. This study supported the concept that CaM influences the action of ERα acting as a transcription factor at promoter level.

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3. CaM regulates ERα transactivation when complexed with Ca^{++}

Although Ca^{++} binding appears essential for the regulatory function of CaM in many physiological processes, it is not always necessary. Indeed, Ca^{++}-free CaM (apo-calmodulin) modulates the activity of several proteins essential for cell function (Jurado et al., 1999). With regard to ERα, studies have demonstrated the requirement of Ca^{++} not only for the formation of the CaM / ERα complex (Bouhoute, Leclercq, 1992) but also for its ability to regulate the transactivation ability of the receptor (Li et al., 2005). Thus, BAPTA-AM (1,2-bis(*o*-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid tetra(acetoxymethyl)ester), a cell-permeable Ca^{++} chelator, was shown to abrogate the enhancing effect of E_2 on ERE-driven gene transcription in ERα-positive breast cancer cells (MCF-7 cells). In these cells, ectopic expression (obtained by transfection) of a CaM mutant unable to bind Ca^{++} produced a similar effect and, moreover, largely decreased the basal transcriptional activity of the receptor, unambiguously establishing the implication of the Ca^{++}~CaM complex in ERα-mediated transcription. Hence, Ca^{++} oscillations that govern CaM activity may, as a metronome, regulate basal ERα-induced transactivation, contributing to its cyclic character (Métivier et al., 2003; Reid et al., 2003).

Because of what is said above, it should be noted that the abbreviation CaM used hereunder actually refers to Ca^{++}-complexed calmodulin.

4. A CaM binding domain has been localized in ERα

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Studies conducted in Sacks's laboratory as well as in ours localized the binding motif of CaM in a region of ERα extending from Pro-295 to Ser-317 (P_{295}LMIKRSKKNSLALSTADQMVS_{317}), thus at the boundary between the hinge and the ligand binding domain (Gallo et al., 2007a; Li et al., 2005). Although the contribution of most of these aminoacid residues in terms of interaction with CaM is unknown, the importance of Ile-298, and Lys-299, 302 and 303 must, however, be stressed. Deletion or substitution of these residues not only abrogates the association of ERα with CaM (Garcia Pedrero et al., 2002; Li et al., 2005) but may also suppress the ability of E_2 to enhance the transcription of an ERE-driven reporter gene, as shown for I_{298}Q and K_{299}D mutated receptors (Li et al., 2005). Hence, the amino-terminal half of the P_{295}-S_{317} sequence seems to contain the major determinants for ERα-CaM complexation.

To explore further ERα interaction with CaM, we synthesized a peptide containing these major determinants (ERα17p : P_{295}-T_{311}), as well as two control peptides in which Lys-302 and 303 were substituted by alanines (ERα17pAA) or glycines (ERα17pGG) (Gallo et al., 2007a). As expected, while ERα17p bound to CaM-Sepharose and inhibited the binding of ERα to immobilized CaM, the control peptides ERα17pAA and ERα17pGG both failed to show similar properties, confirming unambiguously the implication of the P_{295}-T_{311} sequence - and in particular of residues Lys-302 and 303 - in CaM binding. Incidentally, ERα17p behaved as a weak CaM antagonist as compared to a reference peptide derived from the CaM kinase II, suggesting that it may antagonize the ERα-CaM interaction without affecting main CaM-mediated processes. Toxicity tests confirmed this assumption. Indeed, MTT assays performed on MCF-7 cells exposed to ERα17p at a dose

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impeding the complexation of ERα with CaM (10 μM) failed to disclose impairment of metabolic activity, while conventional CaM inhibitors displayed a detectable inhibitory effect at the same or even lower concentration. Hence, ERα17p as well as ERα17pAA and ERα17pGG appeared as valuable tools to decrypt the function of ERα-CaM association (see # 8).

5. The CaM binding domain of ERα is located in a regulatory platform

Interestingly, Lys-299, 302 and 303 of ERα, the importance of which in CaM binding has been stressed above (# 4), are included in a nuclear localization signal (K_{299}RSKK_{303}), suggesting that binding of CaM to ERα may contribute to the intracellular shuttling of the latter (Sweitzer, Hanover, 1996). CaM may also protect the receptor against partial proteolysis, in view of the high sensitivity of the K_{299}-K_{303} motif to trypsin-catalyzed cleavage (Seielstad et al., 1995). The fact that Lys-302 and 303 as well as Ser-305 are subjected to post-translational modifications that have been reported to play a critical role in ERα-mediated transactivation (K_{302} and K_{303} : acetylation (Wang et al., 2001) and mono-ubiquitination (Eakin et al., 2007; Heine, Parvin, 2007); S_{305} : phosphorylation by PAK-1and/or PKA (Wang et al., 2002; Zwart et al., 2007)) suggests a complementary regulatory control of CaM at this level. Finally, as described hereunder, CaM has also been shown to decrease the ability of ERα to bind E_2, a property most likely related to the proximity of the CaM binding motif to the ligand binding pocket. Hence, CaM appears as a crucial modulator of a regulatory platform playing an important role in the mechanism of action of ERα.

6. CaM modifies ERα binding properties

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ERα exists under two forms that can be distinguished on the basis of their binding affinity for E_2 (Kd : ~0.06 nM and 0.8 nM respectively); a third form is totally unable to bind the hormone (Dayani et al., 1988). A phosphorylation/ dephosphorylation process involving several enzymes favors the conversion of one form into another. Interestingly, the addition of CaM to an ERα preparation with high binding affinity promotes its conversion into the form with low binding affinity (increase of Kd value without decrease of binding capacity); in contrast, when the receptor preparation is characterized by a high Kd value, CaM only induces a weak loss of binding capacity that can be reversed by the CaM kinase inhibitor KN62 (Bouhoute, Leclercq, 1996).

The changes in estrogen binding properties outlined here were found to be associated with an increase of ERα interaction with EREs (Bouhoute, Leclercq, 1995), enlightening the concept that CaM may confer to the ligand binding domain a conformation appropriate for co-activator recruitment, a step required for a stable association of the receptor with DNA. The potential dissociation of chaperones, which are known to maintain ERα in a high binding affinity, transcriptionally inactive form is an alternative interpretation. In fact, both possibilities are not mutually exclusive, co-regulator recruitment being often associated with the release of chaperones.

7. Tamoxifen modulates the complexation of CaM with ERα

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Unexpectedly, the triphenylethylenic antiestrogen tamoxifen, which antagonizes ERE-dependent transcription, has been reported to increase the amount of ERα bound to CaM in MCF-7 cells (Li et al., 2001). The effect of the compound culminated at 0.25 to 0.5 μM and was suppressed by E_2. Whether this observation is related to a stabilization of the ERα / CaM complex due to the interaction of the antiestrogen with the receptor ligand binding pocket or to a tamoxifen-induced protection of ERα against proteasomal degradation (Laïos et al., 2003; Leclercq et al., 1992; Wijayaratne, McDonnell, 2001) remains a matter of debate. Indeed, we have observed that the addition of 1 μM tamoxifen (as well as of its steroidal analogues RU 39411 and RU 45144) to cytosolic ERα preparations perturbs the binding of the receptor to CaM-Sepharose (Bouhoute, Leclercq, 1992; Bouhoute, Leclercq, 1994). Exposure of MCF-7 cells to a triphenylethylenic antiestrogen like GW7604 (Wijayaratne, McDonnell, 2001), unable to induce receptor accumulation, may be an adequate approach to clarify this important issue.

Tamoxifen at a concentration of 1 μM and higher displays a strong antitumor activity largely derived from its ability to interact with an array of targets implicated in the regulation of cell proliferation, including CaM (de Medina et al., 2004). Hence, the potential antagonism that tamoxifen exerts at this concentration against ERα-CaM association is not necessarily related to its capacity to fit within the ligand binding pocket of ERα. The study of a series of analogues and derivatives of tamoxifen with potent antitumor activity (Rowlands et al., 1997) confirmed this view. It revealed, indeed, that the inhibition of CaM / ERα complex formation is neither related to the affinity of these compounds for the receptor nor to their intrinsic anti-CaM activity (Fanidi et al., 1994; Hardcastle et al., 1995; Lopes et al., 1990). In fact,

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the major determinant of the negative action of tamoxifen on CaM-ERα complex formation was found to be its phenylethoxyaminodialkyl side chain, which confers to this compound its pharmacological properties (de Medina et al., 2004).

Since the binding of CaM to ERα stabilizes the association of the latter with EREs, one may reasonably assume that tamoxifen at 1 μM - which also interferes with CaM function at this concentration - would favor an ERE-independent anchorage of the receptor within the cell nucleus. This property of tamoxifen, along with the ability of this antiestrogen to induce the accumulation of a functionally inactive form of ERα in the nucleus (Laïos et al., 2003; Leclercq et al., 1992; Wijayaratne, McDonnell, 2001), may contribute to its antiproliferative activity.

8. The CaM binding domain inhibits ERα-mediated gene transactivation

Mutations (deletions/substitutions) of ERα affecting CaM binding have been reported to generate high basal, E_2-independent transcription of reporter genes (Gallo et al., 2007a; Li et al., 2005). From this finding one may surmise that the D/E border region to which CaM binds stabilizes the receptor in an inactive status. The fact that CaM contributes to E_2-induced gene transcription suggests further that its binding to this region may interfere with an auto inhibitory mechanism involving intramolecular interactions within ERα. Studies conducted with ERα17p and its AA/GG derivatives unable to bind CaM (# 4) enabled us to confirm this hypothesis, as well as to decrypt the mechanism by which the CaM binding site operates.

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Exposure of MCF-7 cells to these three synthetic peptides was found to induce responses normally observed under estrogenic stimulation (i.e. ERα down regulation, induction of pS2 and progesterone receptor, growth stimulation…) (Gallo et al., 2007a; Gallo et al., 2007b; Gallo et al., 2008a). Assessment of a panel of breast cancer cell lines revealed that the trophic effect of these peptides was restricted to ERα-positive cell lines, establishing the implication of the receptor in their biological effect. With regard to the underlying molecular mechanism, we opted for a peptide-induced disruption of putative inhibitory interactions between the P_{295}-T_{311} sequence and a cognate motif localized elsewhere in the receptor (or in another protein). Disruption of these inhibitory interactions would unblock the transactivation capacity of the receptor. A computer-based modeling approach described hereunder, and the finding that ERα17p associates with human recombinant ERα (Gallo et al., 2007b) as well as with the chaperone Hsp-70 (Gallo et al., 2008a) provided theoretical and experimental arguments in favor of this concept.

Our modeling approach identified indeed within the ligand binding domain of ERα a motif theoretically able to establish intramolecular interactions with the P_{295}-T_{311} sequence (Jacquot et al., 2007). This putative motif includes the ßturn/H4 subregion of the D_{351}-Q_{414} sequence, which contains the H3-H5/ßhairpin domain involved in the recruitment of co-regulators harboring an LxxLL consensus motif (Fig. 1). Thus, we postulate that the estrogenic response induced by ERα17p stems from the fact that this peptide competitively suppresses the inhibitory interaction between the P_{295}-T_{311} sequence and the ß turn/H4 region. Conformational changes associated with this relaxation process, along with the release of Hsp-70 (Gallo et al., 2008a), would favor the recruitment of LxxLL co-regulators. In this context, it should

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be stressed that an ELISA-type assay of ERα binding to an LxxLL co-regulator motif revealed that ERα17p modulates the association of a highly purified recombinant receptor to this motif, in a same manner as a control LxxLL peptide (Gallo et al., 2007b).

Pursuing this line of reasoning, one may logically postulate that co-regulators recruited at the D/E border region of ERα may, like ERα17p, abrogate the auto-inhibition resulting from the mutual interactions between the P_{295}-T_{311} sequence and the ß turn/H4 region. One may anticipate that CaM would be a privileged actor of this regulatory mechanism, which modulates *in fine* ERα-mediated gene transcription, even if ERα17p and its AA/GG derivatives peptides exert their stimulating effect in a CaM-independent fashion.

9. CaM contributes to the to regulation of ERα turnover rate

The rate at which ERα is synthesized and degraded (turnover) is a major factor regulating the cell responsiveness to various ligands. Studies conducted in various laboratories have demonstrated the implication of the ubiquitin-proteasome system (UPS) in the elimination of the mature receptor which has achieved transactivation and has become functionally obsolete (Alarid et al., 1999; El Khissiin, Leclercq, 1999; Laïos et al., 2005; Nawaz et al., 1999a; Wijayaratne, McDonnell, 2001). Natural and pharmacological ligands either accelerate (estrogens, pure antiestrogens) or reduce (partial antiestrogens) the proteasomal degradation of ERα (Laïos et al., 2005; Read et al., 1989; Wijayaratne, McDonnell, 2001) by selecting UPS with high or low degradation rate.

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In the UPS, target proteins committed to degradation are first tagged by ubiquitination (Deshaies, 1999). The first step of this process is the formation of a thioester bond between ubiquitin and a Cys residue of an ubiquitin-activating enzyme (E1). The second step consists in the transfer of activated ubiquitin from E1 to a Cys residue of a member of the ubiquitin-conjugating enzymes (E2). This is followed by a third step where ubiquitin is transferred from the E2 enzyme to a Lys residue of the target protein, thanks to the activity of a specific ubiquitin ligase (E3). This mechanism proceeds cyclically, grafting additional ubiquitin moieties to those already present in the target protein. E6AP has been identified as an E3 ubiquitin ligase implicated in ligand-induced elimination of steroid hormone receptors, (Khan et al., 2006; Nawaz et al., 1999b). Remarkably, CaM has been shown to attenuate the E6AP-mediated ubiquitination of ERα, as well as to decrease the capacity of E6AP to associate with the receptor, suggesting that it may protect the latter against proteasomal degradation (Li et al., 2006). The finding that the proteasome inhibitors MG-132 and lactacystin prevent a permeable CaM antagonist from reducing ERα level in MCF-7 cells is consistent with this assumption. This protective effect of CaM may be a key factor by which it may modulate ERα turnover rate and influence thereby events depending on ERα level.

Based on these considerations, we propose that the proteasomal degradation of ERα resulting from the release of bound CaM generates ERα17p-like peptides that, like CaM, might relieve ERα auto-inhibition (Gallo et al., 2008b). ERα proteasomal degradation occurring during the successive steps of this process would facilitate the access to EREs of newly synthesized receptors, after release of bound

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Hsp-70 and association with CaM, to initiate further transactivation cycles. Successive occurrences of these events would guarantee high gene expression. Of course, other actors may be implicated in this scheme, in view of the fact that the CaM binding site of ERα is located in a platform for various posttranslational changes.

10. Inappropriate CaM - ERα complexation may be involved in breast pathology

Events affecting interaction between ERα and CaM may be expected to have pathological consequences. According to the concept outlined in section 8 and 9, one may anticipate that high amounts of CaM would favor extensive relaxation of ERα, with a concomitant loss of the inhibitory effect exerted by the CaM binding site on the recruitment of LxxLL motif-containing co-regulators. Remarkably, high CaM levels have been reported to frequently occur in breast cancers (Singer et al., 1976), especially those classified as ERα-positive (Krishnaraju et al., 1991), suggesting an implication of CaM in breast cancer development and / or progression. This would explain the synergistic effect of anti-CaM drugs on the antagonistic activity of antiestrogens (Frankfurt et al., 1995; Strobl, Peterson, 1992).

The BRCA-1 protein plays a critical role in the control of ERα function. When bound to BARD-1, BRCA-1 indeed provokes the mono-ubiquitination of Lys-302 and 303 in ERα (Eakin et al., 2007). Inasmuch as these residues are implicated in the association of ERα with CaM (# 4), this posttranslational modification of the receptor may decrease its association with EREs with a concomitant arrest of transcription

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initiation (Heine, Parvin, 2007). Hence, loss of function mutations of BRCA-1, known to enhance the risk of breast cancer, could result in a disruption of this negative regulation of ERα-mediated transactivation. Alternatively, alterations within the P_{295}-T_{311} sequence may also contribute to breast cancer emergence. Proteolytic cleavage of the $K_{299}RSKK_{303}$ motif (Seielstad et al., 1995), frequently recorded in breast cancer samples (Maaroufi et al., 2000), is likely to withdraw ERα from the action of repressors like BRCA-1, the oligomeric nature of ERα maintaining such cleaved receptors in a compact structure still able to mediate transcription (Kraus et al., 1995). Finally, missense mutation causing substitution of Lys-303 by an arginine, which is known to confer a high cell sensitivity to E_2 (Fuqua et al., 2000), most probably facilitates receptor activation, with a concomitant enhancement of cell proliferation. Interestingly, this $K_{303}R$ mutation has been frequently reported in pre-neoplastic and neoplastic mammary tissues (Fuqua et al., 2000) as well as in high-grade breast tumors (Conway et al., 2005).

11. Perspectives and conclusions

The concepts outlined in the previous sections primarily concern ERα-mediated gene transactivation in breast cancer cells. These concepts are most probably valid for ERα-positive cells where the receptor acts as a transcription factor in the nucleus. Whether they are also relevant to cells with a different intracellular ERα distribution remains a matter of speculation. Potential implication of CaM in the nuclear translocation of the receptor (Sweitzer, Hanover, 1996) as well as in its intracellular trafficking (Maruvada et al., 2003; Stenoien et al., 2000) suggests that it might not be the case. However, the polyproline II / α-helix mixed secondary

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structure of the CaM binding site (P_{295}-T_{311} sequence), extensively discussed in previous publications of our group (Gallo et al., 2007b; Jacquot et al., 2007), suggests that this motif might interact with a number of regulatory proteins containing polyproline-binding modules implicated in both non genomic and genomic estrogenic responses. Co-activators PELP1/MNAR (Nair, Vadlamudi, 2007) and PNRCs (Zhou et al., 2006) involved in gene transactivation, and the adaptor protein Grb2 belonging to the MAPK pathway are candidates for such potential regulatory processes (Yang et al., 2004). Therefore, interference of CaM with ERα functions other than these directly associated with gene transactivation might be of importance.

On the other hand, the inhibition exerted by the P_{295}-T_{311} sequence on ERα-mediated transcription could be exploited for the design of drugs directed against AF-2-dependent transactivation. The recent identification in the androgen receptor (AR) of a surface cleft adjacent to this activation function and designated as BF3 has led to the development of drugs that modulate the association of this receptor with its co-regulators (Estebanez-Perpina et al., 2007). Interestingly, superimposition of AR on the ERα backbone revealed that the ERα-corresponding BF3 domain is composed of residues located in the ß turn/H4 subregion, implicated in the inhibitory interaction exerted by the P_{295}-T_{311} sequence on the recruitment of co-activators at AF-2 (Y. Jacquot, unpublished data, see #8, Fig. 1). Structure activity relationships established for AR may therefore orient the design of drugs for the treatment of breast cancer, especially those which fail to respond to antiestrogens.

Our review being devoted to ERα in the context of estrogen dependence of breast cancer, we did not discuss the function of the association of CaM with AR

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(Cifuentes et al., 2004), nor its interaction with the orphan estrogen-related receptor γ (ERRγ) (Hentschke et al., 2003). The latter receptor, unable to bind E_2, is nevertheless of peculiar interest because it has been proposed as a new target for the endocrine therapy of breast cancer (Ariazi, Jordan, 2006). Of note, sequence alignment of ERα with ERRγ disclosed in the latter a homology with the N-terminal part of the P_{295}-T_{311} motif, crucial for CaM binding (# 4). Consequently, one may anticipate that the mechanisms discussed here for ERα may to some extent apply to ERRγ.

Further studies are warranted to lend experimental support to the concepts outlined in the current review. Indeed, some of our proposals still remain quite speculative. Nevertheless, we consider that they may open new perspectives toward the understanding of clinical disorders associated with nuclear receptor dysfunctions and pave the way to the development of new therapeutical approaches. This should be an incentive for further research on other aspects of CaM-nuclear receptor interaction.

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References

Acconcia, F., Kumar, R., 2006. Signaling regulation of genomic and nongenomic functions of estrogen receptors. Cancer Lett 238, 1-14.

Alarid, E.T., Bakopoulos, N., Solodin, N., 1999. Proteasome-mediated proteolysis of estrogen receptor: a novel component in autologous down-regulation. Mol. Endocrinol. 13, 1522-1534.

Ariazi, E.A., Jordan, V.C., 2006. Estrogen-related receptors as emerging targets in cancer and metabolic disorders. Curr. Top. Med. Chem. 6, 203-215.

Auricchio, F., Migliaccio, A., Castoria, G., Lastoria, S., Schiavone, E., 1981a. ATP-dependent enzyme activating hormone binding of estradiol receptor. Biochem. Biophys. Res. Commun. 101, 1171-1178.

Auricchio, F., Migliaccio, A., Rotondi, A., 1981b. Inactivation of oestrogen receptor in vitro by nuclear dephosphorylation. Biochem. J. 194, 569-574.

Biswas, D.K., Reddy, P.V., Pickard, M., Makkad, B., Pettit, N., Pardee, A.B., 1998. Calmodulin is essential for estrogen receptor interaction with its motif and activation of responsive promoter. J. Biol. Chem. 273, 33817-33824.

Bouhoute, A., Leclercq, G., 1992. Antagonistic effect of triphenylethylenic antiestrogens on the association of estrogen receptor to calmodulin. Biochem. Biophys. Res. Commun. 184, 1432-1440.

Bouhoute, A., Leclercq, G., 1994. Estradiol derivatives bearing the side-chain of tamoxifen antagonize the association between the estrogen receptor and calmodulin. Biochem. Pharmacol. 47, 748-751.

Bouhoute, A., Leclercq, G., 1995. Modulation of estradiol and DNA binding to estrogen receptor upon association with calmodulin. Biochem. Biophys. Res. Commun. 208, 748-755.

Bouhoute, A., Leclercq, G., 1996. Calmodulin decreases the estrogen binding capacity of the estrogen receptor. Biochem. Biophys. Res. Commun. 227, 651-657.

Carlson, K.E., Choi, I., Gee, A., Katzenellenbogen, B.S., Katzenellenbogen, J.A., 1997. Altered ligand binding properties and enhanced stability of a constitutively

ACCEPTED MANUSCRIPT

active estrogen receptor: evidence that an open pocket conformation is required for ligand interaction. Biochemistry 36, 14897-14905.

Castoria, G., Migliaccio, A., Bilancio, A., Pagano, M., Abbondanza, C., Auricchio, F., 1996. A 67 kDa non-hormone binding estradiol receptor is present in human mammary cancers. Int. J. Cancer 65, 574-583.

Castoria, G., Migliaccio, A., Green, S., Di Domenico, M., Chambon, P., Auricchio, F., 1993. Properties of a purified estradiol-dependent calf uterus tyrosine kinase. Biochemistry 32, 1740-1750.

Castoria, G., Migliaccio, A., Nola, E., Auricchio, F., 1988. In vitro interaction of estradiol receptor with Ca2+-calmodulin. Mol. Endocrinol. 2, 167-174.

Cifuentes, E., Mataraza, J.M., Yoshida, B.A., Menon, M., Sacks, D.B., Barrack, E.R., Reddy, G.P., 2004. Physical and functional interaction of androgen receptor with calmodulin in prostate cancer cells. Proc. Natl. Acad. Sci. U. S. A 101, 464-469.

Conway, K., Parrish, E., Edmiston, S.N., Tolbert, D., Tse, C.K., Geradts, J., Livasy, C.A., Singh, H., Newman, B., Millikan, R.C., 2005. The estrogen receptor-alpha A908G (K303R) mutation occurs at a low frequency in invasive breast tumors: results from a population-based study. Breast Cancer Res. 7, R871-R880.

Dayani, N., McNaught, R.W., Smith, R.G., 1988. ATP mediated receptor interconversion as a model of estrogen action: isolation of the factor which converts the non-estrogen binding form of the receptor to the lower affinity binding form. J. Steroid Biochem. 30, 219-224.

de Medina, P., Favre, G., Poirot, M., 2004. Multiple targeting by the antitumor drug tamoxifen: a structure-activity study. Curr. Med. Chem. Anticancer Agents 4, 491-508.

Deshaies, R.J., 1999. SCF and Cullin/Ring H2-based ubiquitin ligases. Annu. Rev. Cell Dev. Biol. 15, 435-467.

Eakin, C.M., Maccoss, M.J., Finney, G.L., Klevit, R.E., 2007. Estrogen receptor alpha is a putative substrate for the BRCA1 ubiquitin ligase. Proc. Natl. Acad. Sci. U. S. A 104, 5794-5799.

El Khissiin, A., Leclercq, G., 1999. Implication of proteasome in estrogen receptor degradation. FEBS Lett 448, 160-166.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Estebanez-Perpina, E., Arnold, L.A., Nguyen, P., Rodrigues, E.D., Mar, E., Bateman, R., Pallai, P., Shokat, K.M., Baxter, J.D., Guy, R.K., Webb, P., Fletterick, R.J., 2007. A surface on the androgen receptor that allosterically regulates coactivator binding. Proc. Natl. Acad. Sci. U. S. A 104, 16074-16079.

Fanidi, A., Guichard, Y., Fayard, J.M., Pageaux, J.F., Laugier, C., 1994. Fluorimetric studies of calmodulin interactions with antiestrogens. Cancer Detect. Prev. 18, 471-478.

Frankfurt, O.S., Sugarbaker, E.V., Robb, J.A., Villa, L., 1995. Synergistic induction of apoptosis in breast cancer cells by tamoxifen and calmodulin inhibitors. Cancer Lett 97, 149-154.

Fuqua, S.A., Wiltschke, C., Zhang, Q.X., Borg, A., Castles, C.G., Friedrichs, W.E., Hopp, T., Hilsenbeck, S., Mohsin, S., O'Connell, P., Allred, D.C., 2000. A hypersensitive estrogen receptor-alpha mutation in premalignant breast lesions. Cancer Res. 60, 4026-4029.

Gallo, D., Haddad, I., Duvillier, H., Jacquemotte, F., Laios, I., Laurent, G., Jacquot, Y., Vinh, J., Leclercq, G., 2008a. Trophic effect in MCF-7 cells of ERalpha17p, a peptide corresponding to a platform regulatory motif of the estrogen receptor alpha-Underlying mechanisms. J. Steroid Biochem. Mol. Biol. 109, 138-149.

Gallo, D., Haddad, I., Laurent, G., Vinh, J., Jacquemotte, F., Jarman, M., Leclercq, G., 2008b. Regulatory function of the P295-T311 motif of the Estrogen Receptor alpha - Does the degradation of the receptor induce emergence of peptides implicated in estrogenic responses? Nucl. Recept. Signal. In press.,

Gallo, D., Jacquemotte, F., Cleeren, A., Laïos, I., Hadiy, S., Rowlands, M.G., Caille, O., Nonclercq, D., Laurent, G., Jacquot, Y., Leclercq, G., 2007a. Calmodulin-independent, agonistic properties of a peptide containing the calmodulin binding site of estrogen receptor alpha. Mol. Cell Endocrinol. 268, 37-49.

Gallo, D., Jacquot, Y., Cleeren, A., Jacquemotte, F., Laurent, G., Leclercq, G., 2007b. Molecular basis of agonistic activity of ERa17p, a synthetic peptide corresponding to a sequence located at the N-terminal part of the estrogen receptor a ligand binding domain. Letters Drug Design & Discovery 4, 346-355.

Garcia Pedrero, J.M., Del Rio, B., Martinez-Campa, C., Muramatsu, M., Lazo, P.S., Ramos, S., 2002. Calmodulin is a selective modulator of estrogen receptors. Mol. Endocrinol. 16, 947-960.

Hardcastle, I.R., Rowlands, M.G., Houghton, J., Parr, I.B., Potter, G.A., Jarman, M., Edwards, K.J., Laughton, C.A., Trent, J.O., Neidle, S., 1995. Rationally designed

ACCEPTED MANUSCRIPT

analogues of tamoxifen with improved calmodulin antagonism. J. Med. Chem. 38, 241-248.

Heine, G.F., Parvin, J.D., 2007. BRCA1 control of steroid receptor ubiquitination. Sci. STKE. 2007, e34-

Hentschke, M., Schulze, C., Susens, U., Borgmeyer, U., 2003. Characterization of calmodulin binding to the orphan nuclear receptor ERRgamma. Biol. Chem. 384, 473-482.

Jacquot, Y., Gallo, D., Leclercq, G., 2007. Estrogen receptor alpha - identification by a modeling approach of a potential polyproline II recognizing domain within the AF-2 region of the receptor that would play a role of prime importance in its mechanism of action. J. Steroid Biochem. Mol. Biol. 104, 1-10.

Jurado, L.A., Chockalingam, P.S., Jarrett, H.W., 1999. Apocalmodulin. Physiol Rev. 79, 661-682.

Khan, O.Y., Fu, G., Ismail, A., Srinivasan, S., Cao, X., Tu, Y., Lu, S., Nawaz, Z., 2006. Multifunction steroid receptor coactivator, E6-associated protein, is involved in development of the prostate gland. Mol. Endocrinol. 20, 544-559.

Kong, E.H., Heldring, N., Gustafsson, J.A., Treuter, E., Hubbard, R.E., Pike, A.C., 2005. Delineation of a unique protein-protein interaction site on the surface of the estrogen receptor. Proc. Natl. Acad. Sci. U. S. A 102, 3593-3598.

Kraus, W.L., McInerney, E.M., Katzenellenbogen, B.S., 1995. Ligand-dependent, transcriptionally productive association of the amino- and carboxyl-terminal regions of a steroid hormone nuclear receptor. Proc. Natl. Acad. Sci. U. S. A 92, 12314-12318.

Krishnaraju, K., Murugesan, K., Vij, U., Kapur, B.M., Farooq, A., 1991. Calmodulin levels in oestrogen receptor positive and negative human breast tumours. Br. J. Cancer 63, 346-347.

Laïos, I., Journe, F., Laurent, G., Nonclercq, D., Toillon, R.A., Seo, H.S., Leclercq, G., 2003. Mechanisms governing the accumulation of estrogen receptor alpha in MCF-7 breast cancer cells treated with hydroxytamoxifen and related antiestrogens. J. Steroid Biochem. Mol. Biol. 87, 207-221.

Laïos, I., Journe, F., Nonclercq, D., Vidal, D.S., Toillon, R.A., Laurent, G., Leclercq, G., 2005. Role of the proteasome in the regulation of estrogen receptor alpha

ACCEPTED MANUSCRIPT

turnover and function in MCF-7 breast carcinoma cells. J. Steroid Biochem. Mol. Biol. 94, 347-359.

Leclercq, G., Lacroix, M., Laïos, I., Laurent, G., 2006. Estrogen receptor alpha: impact of ligands on intracellular shuttling and turnover rate in breast cancer cells. Curr. Cancer Drug Targets. 6, 39-64.

Leclercq, G., Legros, N., Piccart, M.J., 1992. Accumulation of a non-binding form of estrogen receptor in MCF-7 cells under hydroxytamoxifen treatment. J. Steroid Biochem. Mol. Biol. 41, 545-552.

Li, L., Li, Z., Howley, P.M., Sacks, D.B., 2006. E6AP and calmodulin reciprocally regulate estrogen receptor stability. J. Biol. Chem. 281, 1978-1985.

Li, L., Li, Z., Sacks, D.B., 2003. Calmodulin regulates the transcriptional activity of estrogen receptors. Selective inhibition of calmodulin function in subcellular compartments. J. Biol. Chem. 278, 1195-1200.

Li, L., Li, Z., Sacks, D.B., 2005. The transcriptional activity of estrogen receptor-alpha is dependent on Ca2+/calmodulin. J. Biol. Chem. 280, 13097-13104.

Li, Z., Joyal, J.L., Sacks, D.B., 2001. Calmodulin enhances the stability of the estrogen receptor. J. Biol. Chem. 276, 17354-17360.

Lopes, M.C., Vale, M.G., Carvalho, A.P., 1990. Ca2(+)-dependent binding of tamoxifen to calmodulin isolated from bovine brain. Cancer Res. 50, 2753-2758.

Maaroufi, Y., Lacroix, M., Lespagnard, L., Journe, F., Larsimont, D., Leclercq, G., 2000. Estrogen receptor of primary breast cancers: evidence for intracellular proteolysis. Breast Cancer Res. 2, 444-454.

Maruvada, P., Baumann, C.T., Hager, G.L., Yen, P.M., 2003. Dynamic shuttling and intranuclear mobility of nuclear hormone receptors. J. Biol. Chem. 278, 12425-12432.

Métivier, R., Penot, G., Hubner, M.R., Reid, G., Brand, H., Kos, M., Gannon, F., 2003. Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. Cell 115, 751-763.

Migliaccio, A., Castoria, G., de Falco, A., Di Domenico, M., Galdiero, M., Nola, E., Chambon, P., Auricchio, F., 1991. In vitro phosphorylation and hormone binding

Accepted Manuscript

ACCEPTED MANUSCRIPT

activation of the synthetic wild type human estradiol receptor. J. Steroid Biochem. Mol. Biol. 38, 407-413.

Migliaccio, A., Di Domenico, M., Green, S., de Falco, A., Kajtaniak, E.L., Blasi, F., Chambon, P., Auricchio, F., 1989. Phosphorylation on tyrosine of in vitro synthesized human estrogen receptor activates its hormone binding. Mol. Endocrinol. 3, 1061-1069.

Migliaccio, A., Pagano, M., De Goeij, C.C., Di Domenico, M., Castoria, G., Sluyser, M., Auricchio, F., 1992. Phosphorylation and estradiol binding of estrogen receptor in hormone-dependent and hormone-independent GR mouse mammary tumors. Int. J. Cancer 51, 733-739.

Migliaccio, A., Rotondi, A., Auricchio, F., 1984. Calmodulin-stimulated phosphorylation of 17 beta-estradiol receptor on tyrosine. Proc. Natl. Acad. Sci. U. S. A 81, 5921-5925.

Nair, S., Vadlamudi, R.K., 2007. Emerging significance of ER-coregulator PELP1/MNAR in cancer. Histol. Histopathol. 22, 91-96.

Nawaz, Z., Lonard, D.M., Dennis, A.P., Smith, C.L., O'Malley, B.W., 1999a. Proteasome-dependent degradation of the human estrogen receptor. Proc. Natl. Acad. Sci. U. S. A 96, 1858-1862.

Nawaz, Z., Lonard, D.M., Smith, C.L., Lev-Lehman, E., Tsai, S.Y., Tsai, M.J., O'Malley, B.W., 1999b. The Angelman syndrome-associated protein, E6-AP, is a coactivator for the nuclear hormone receptor superfamily. Mol. Cell Biol. 19, 1182-1189.

Nonclercq, D., Journe, F., Laios, I., Chaboteaux, C., Toillon, R.A., Leclercq, G., Laurent, G., 2007. Effect of nuclear export inhibition on estrogen receptor regulation in breast cancer cells. J. Mol. Endocrinol. 39, 105-118.

Pedram, A., Razandi, M., Levin, E.R., 2006. Nature of functional estrogen receptors at the plasma membrane. Mol. Endocrinol. 20, 1996-2009.

Piccart, M.J., Trivedi, S., Maaroufi, Y., Debbaudt, A., Veenstra, S., Leclercq, G., 1998. Evolution towards hormone independence of the MXT mouse mammary tumor is associated with a gradual change in its estrogen receptor molecular polymorphism. Cancer Biochem. Biophys. 16, 169-182.

Read, L.D., Greene, G.L., Katzenellenbogen, B.S., 1989. Regulation of estrogen receptor messenger ribonucleic acid and protein levels in human breast cancer

Accepted Manuscript

ACCEPTED MANUSCRIPT

cell lines by sex steroid hormones, their antagonists, and growth factors. Mol. Endocrinol. 3, 295-304.

Reid, G., Hubner, M.R., Metivier, R., Brand, H., Denger, S., Manu, D., Beaudouin, J., Ellenberg, J., Gannon, F., 2003. Cyclic, proteasome-mediated turnover of unliganded and liganded ERalpha on responsive promoters is an integral feature of estrogen signaling. Mol. Cell 11, 695-707.

Rowlands, M.G., Grimshaw, R., Jarman, M., Bouhoute, A., Leclercq, G., 1997. Antagonism of estrogen receptor and calmodulin association by antiestrogens is not dependent on an interaction with calmodulin. Biochem. Pharmacol. 53, 241-244.

Seielstad, D.A., Carlson, K.E., Kushner, P.J., Greene, G.L., Katzenellenbogen, J.A., 1995. Analysis of the structural core of the human estrogen receptor ligand binding domain by selective proteolysis/mass spectrometric analysis. Biochemistry 34, 12605-12615.

Singer, A.L., Sherwin, R.P., Dunn, A.S., Appleman, M.M., 1976. Cyclic nucleotide phosphodiesterases in neoplastic and nonneoplastic human mammary tissues. Cancer Res. 36, 60-66.

Song, R.X., 2007. Membrane-initiated steroid signaling action of estrogen and breast cancer. Semin. Reprod. Med. 25, 187-197.

Stenoien, D.L., Mancini, M.G., Patel, K., Allegretto, E.A., Smith, C.L., Mancini, M.A., 2000. Subnuclear trafficking of estrogen receptor-alpha and steroid receptor coactivator-1. Mol. Endocrinol. 14, 518-534.

Strobl, J.S., Peterson, V.A., 1992. Tamoxifen-resistant human breast cancer cell growth: inhibition by thioridazine, pimozide and the calmodulin antagonist, W-13. J. Pharmacol. Exp. Ther. 263, 186-193.

Sweitzer, T.D., Hanover, J.A., 1996. Calmodulin activates nuclear protein import: a link between signal transduction and nuclear transport. Proc. Natl. Acad. Sci. U. S. A 93, 14574-14579.

Wang, C., Fu, M., Angeletti, R.H., Siconolfi-Baez, L., Reutens, A.T., Albanese, C., Lisanti, M.P., Katzenellenbogen, B.S., Kato, S., Hopp, T., Fuqua, S.A., Lopez, G.N., Kushner, P.J., Pestell, R.G., 2001. Direct acetylation of the estrogen receptor alpha hinge region by p300 regulates transactivation and hormone sensitivity. J. Biol. Chem. 276, 18375-18383.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Wang, R.A., Mazumdar, A., Vadlamudi, R.K., Kumar, R., 2002. P21-activated kinase-1 phosphorylates and transactivates estrogen receptor-alpha and promotes hyperplasia in mammary epithelium. EMBO J. 21, 5437-5447.

Warnmark, A., Treuter, E., Gustafsson, J.A., Hubbard, R.E., Brzozowski, A.M., Pike, A.C., 2002. Interaction of transcriptional intermediary factor 2 nuclear receptor box peptides with the coactivator binding site of estrogen receptor alpha. J. Biol. Chem. 277, 21862-21868.

Wärnmark, A., Treuter, E., Gustafsson, J.A., Hubbard, R.E., Brzozowski, A.M., Pike, A.C., 2002. Interaction of transcriptional intermediary factor 2 nuclear receptor box peptides with the coactivator binding site of estrogen receptor alpha. J. Biol. Chem. 277, 21862-21868.

Wijayaratne, A.L., McDonnell, D.P., 2001. The human estrogen receptor-alpha is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. J. Biol. Chem. 276, 35684-35692.

Yang, Z., Barnes, C.J., Kumar, R., 2004. Human epidermal growth factor receptor 2 status modulates subcellular localization of and interaction with estrogen receptor alpha in breast cancer cells. Clin. Cancer Res. 10, 3621-3628.

Yudt, M.R., Vorojeikina, D., Zhong, L., Skafar, D.F., Sasson, S., Gasiewicz, T.A., Notides, A.C., 1999. Function of estrogen receptor tyrosine 537 in hormone binding, DNA binding, and transactivation. Biochemistry 38, 14146-14156.

Zhou, D., Ye, J.J., Li, Y., Lui, K., Chen, S., 2006. The molecular basis of the interaction between the proline-rich SH3-binding motif of PNRC and estrogen receptor alpha. Nucleic Acids Res. 34, 5974-5986.

Zwart, W., Griekspoor, A., Berno, V., Lakeman, K., Jalink, K., Mancini, M., Neefjes, J., Michalides, R., 2007. PKA-induced resistance to tamoxifen is associated with an altered orientation of ERalpha towards co-activator SRC-1. EMBO J. 26, 3534-3544.

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Legend to the figure

Putative mechanism whereby CaM and ERα17p induce estrogenic responses. The three-dimensional model represented here is based on X-ray crystallography analysis of the ligand binding domain of ERα complexed with the estrogenic core of raloxifene (Wärnmark et al., 2002). It also incorporates our own structural studies of intramolecular interactions between, on the one hand, a part of the CaM binding motif (P_{295}-T_{311}) adopting a helical polyproline II (PPII) conformation (the S_{305}-T_{311} sequence), and, on the other hand, the ß turn/H4 subregion of a huge polyproline recognizing domain (PRD) delineated by residues D_{351}-Q_{414} (Jacquot et al., 2007). The PRD, which contains the H3-H5 subregion as well as the ß hairpin structure (S1, S2 anti-parallel ß strands) regulates the conformation of AF-2 (Kong et al., 2005; Warnmark et al., 2002). Interactions between the CaM binding motif and the PRD are thought to confer to AF-2 a conformation inappropriate for the recruitment of LxxLL-containing co-regulators. CaM, by binding to the P_{295}-T_{311} sequence, relieves this autoinhibition. ERα17p, by binding to the ß turn/H4 subregion of the PRD, produces a similar effect.

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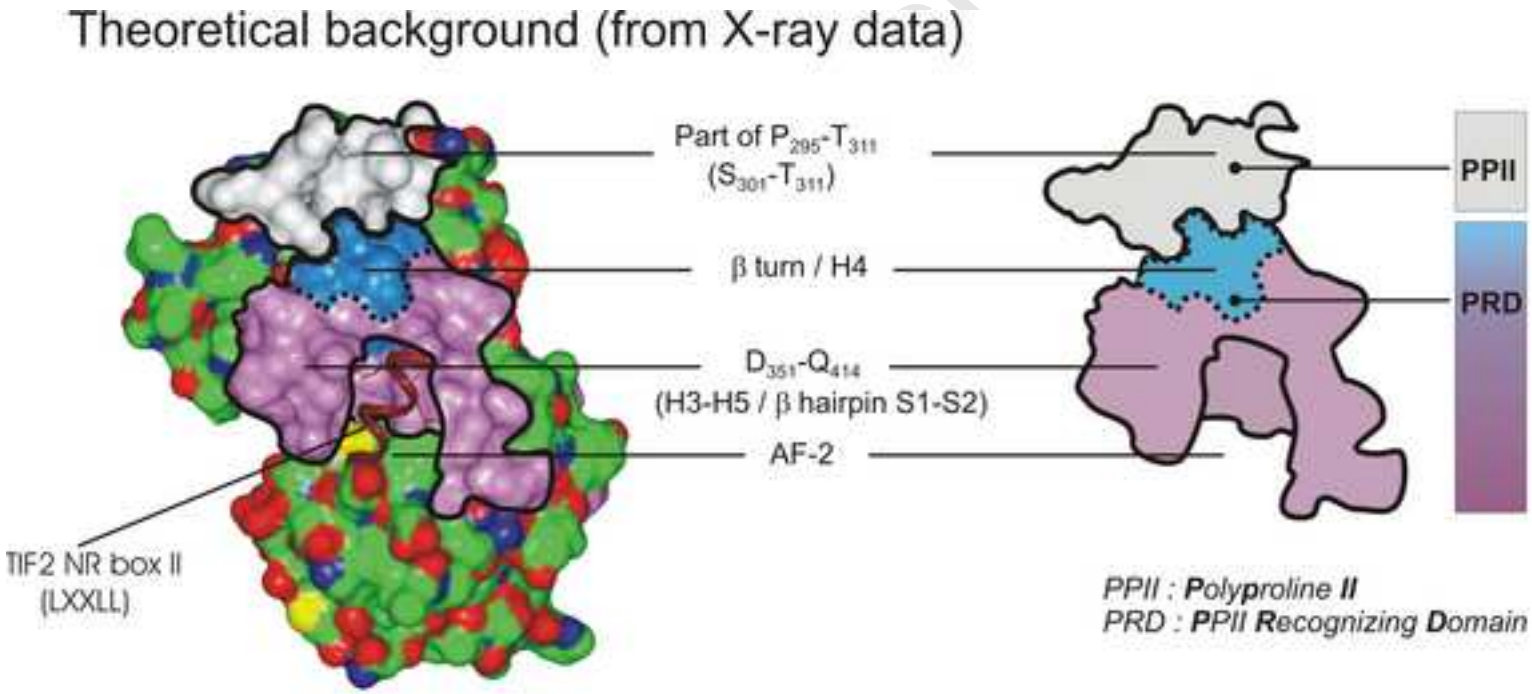
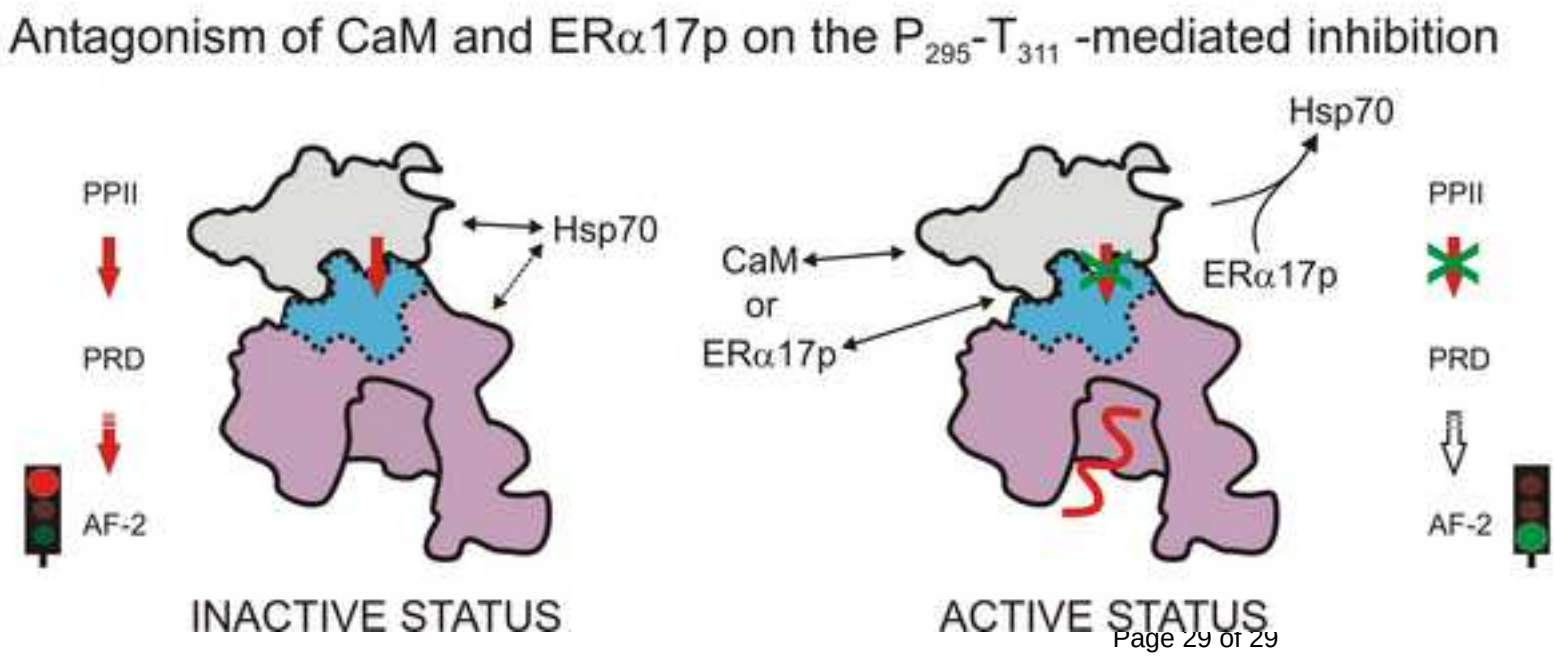
Figure


Theoretical background (from X-ray data)
Part of P_{295}-T_{311}
(S_{301}-T_{311})
β turn / H4
D_{351}-Q_{414}
(H3-H5 / β hairpin S1-S2)
AF-2
TIF2 NR box II
(LXXLL)
PPII
PRD
PPII : Polyproline II
PRD : PPII Recognizing Domain


Antagonism of CaM and ERα17p on the P_{295}-T_{311} -mediated inhibition
PPII
PRD
AF-2
Hsp70
INACTIVE STATUS
CaM
or
ERα17p
Hsp70
ERα17p
PPII
PRD
AF-2
ACTIVE STATUS