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Review

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

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ABSTRACT

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 β turn/H4 region), this association likely resulting in an auto-inhibitory folding of the receptor. Thus, we propose as a hypothesis that CaM acts as a positive regulator by relieving this ERα auto-inhibition.

Contents

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,b; 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

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ERα synthesized *in vitro* (Castoria et al., 1993; Migliaccio et al., 1989, 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^{2+} dependent manner (Bouhoute and Leclercq, 1992, 1995, 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 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 and 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, 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

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 and 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 as a transcription factor at promoter level.

3. CaM regulates ERα transactivation when complexed with Ca^{2+}

Although Ca^{2+} binding appears essential for the regulatory function of CaM in many physiological processes, it is not always necessary. Indeed, Ca^{2+}-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^{2+} not only for the formation of the CaM-ERα complex (Bouhoute and 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^{2+} 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^{2+} produced a similar effect and, moreover, largely decreased the basal transcriptional activity of the receptor, unambiguously establishing the implication of the Ca^{2+}–CaM complex in ERα-mediated transcription. Hence, Ca^{2+} 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^{2+}-complexed calmodulin.

4. A CaM binding domain has been localized in ERα

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 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 Section 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 (Section 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 and 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 and 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

ERα exists under two forms that can be distinguished on the basis of their binding affinity for E_2 (Kd: ∼0.06 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 and Leclercq, 1996).

The changes in estrogen binding properties outlined here were found to be associated with an increase of ERα interaction with EREs (Bouhoute and 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

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 and 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 and Leclercq, 1992, 1994). Exposure of MCF-7 cells to a triphenylethylenic antiestrogen like GW7604 (Wijayaratne and 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, 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 and 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 (Section 4) enabled us to confirm this hypothesis, as well as to decrypt the mechanism by which the CaM binding site operates.

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 pro-

gesterone receptor, growth stimulation...) (Gallo et al., 2007a,b, 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 β turn/H4 subregion of the D_{351}–Q_{414} sequence, which contains the H3-H5/βhairpin 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 β 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 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 β 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 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 and Leclercq, 1999; Laïos et al., 2005; Nawaz et al., 1999a; Wijayaratne and 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 and McDonnell, 2001) by selecting UPS with high or low degradation rate.

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

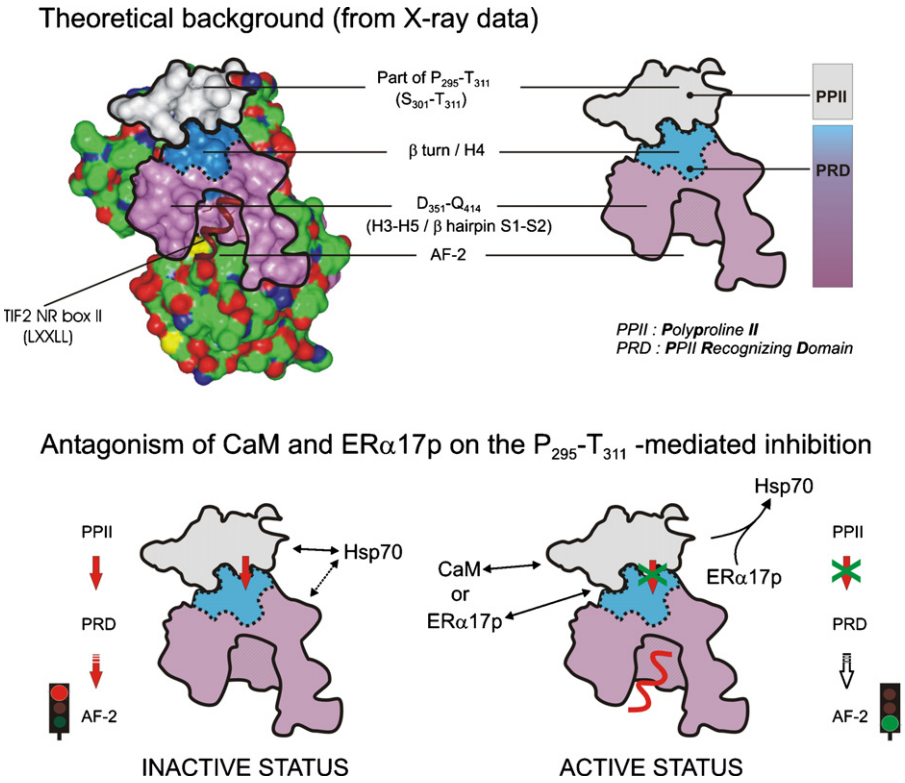

Fig. 1. Putative mechanism whereby CaM and ERα17p induce estrogenic responses. The three-dimensional model represented here is based on X-ray crystallography analysis of the ligand binding domain of ERα complexed with the estrogenic core of raloxifene (Wärnmark et al., 2002). It also incorporates our 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 β 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 β hairpin structure (S1, S2 anti-parallel β 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 auto-inhibition. ERα17p, by binding to the β turn/H4 subregion of the PRD, produces a similar effect.

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 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 post-translational 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 Sections 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 and 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 (Section 4), this post-translational modification of the receptor may decrease its association with EREs with a concomitant arrest of transcription initiation (Heine and 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 and 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 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 and 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 β 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 Section 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 (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 and 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 (Gallo et al., 2008b), crucial for CaM binding (Section 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 dysfunc-

tions 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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