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PROSPECT

Proteins Interacting with the Mammalian Estrogen Receptor: Proposal for an Integrated Model for Estrogen Receptor Mediated Regulation of Transcription

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Abstract Two forms of estrogen receptor (ER) that exist in the mammalian uterus have been examined in this review. (1) ERα, or the classical estrogen receptor that is considered to influence the transcriptional process; (2) the non-activated estrogen receptor (naER), an alternative form of ER with no DNA binding function, localized in the plasma membrane. An integrated model is being proposed to highlight the functional roles of both receptors in transcriptional regulation. The proteins with which ER interacts during various stages of its existence are being examined. These stages include: (1) transport from the cytoplasm to the nucleus; (2) interaction with the nuclear transcription machinery; (3) involvement in post-transcriptional control mechanisms; and (4) degradation through ubiquitination. The proteins with which naER interacts during its plasma membrane-to-nucleus movement have also been identified; the results have not yet been published. Within the nucleus it dimerizes with a DNA-binding protein, the estrogen receptor activation factor (E-RAF). It is being proposed that the purpose behind the dimerization between naER and E-RAF is to transport E-RAF to the transcription initiation site as the naER in the heterodimer is a RNA-polymerase binding protein. Deglycosylated naER fails to dimerize with the E-RAF. Deglycosylation of the naER therefore dissociates the heterodimer and this transformed naER is now identified as nuclear estrogen receptor II (nER II). The dissociated E-RAF is free either to destabilize (E-RAF II) or stabilize (E-RAF I) the DNA while the naER remains bound to the RNA polymerase II. nER II phosphorylates certain subunits in RNA polymerase; the functional significance of this phosphorylation remains to be known. J. Cell. Biochem. 80:571–579, 2001.

Key words: estrogen receptor; non-activated estrogen receptor; estrogen receptor activation factor

The historical 'two-step mechanism in estrogen action' proposed by Jensen and co-workers during the late 1960s and early 1970s suggested that the 4S estrogen receptor, in its non-activated form existed as a 8S complex [Jensen et al., 1968]. Hormone binding was thought to dissociate the 4S ER from the other proteins, thereby facilitating its nuclear entry and the eventual conversion to the activated 5S species. This 5S receptor, as we know today is a homodimer of 4S ER. The concept of ER 'activation' fascinated the imagination of many investigators during the 1970s. It was proposed that the 4S ER associated with a non-hormone binding protein with DNA binding ability during its transformation to the 5S ER. Yamamoto [1974] identified this protein as the 'x' subunit. The first experimental observation that demonstrated the presence of this 'x' subunit was that of Thampan and Clark [1981] who observed that in the rat uterus existed a DNA binding protein which did not bind estradiol but dimerized with the 4S ER, giving rise to a 5S (4.8S) complex. Thus, a working model of 'activated' ER was developed in which the activated ER existed as a heterodimer where one subunit bound the hormone while the other bound to the DNA. This 'x' subunit was identified as the estrogen receptor activation factor (E-RAF). The discovery of the primary structure of the human estrogen receptor (hER) was achieved during the mid-1980s [Kumar et al., 1986]. The observation that one

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and the same protein carried both the DNA binding and the hormone binding domains generated a shadow of doubt on the very existence of the E-RAF. For the investigators who believed in the E-RAF concept this was virtually an uncertain period until it came to the light that the ER that dimerized with the E-RAF was not the regular or the classical ER but an alternative form. Purification of this alternative form of ER from the goat uterus and its subsequent functional characterization generated some unforeseen results [Karthikeyan and Thampan, 1996]. This receptor, identified as the non-activated ER (naER), is a glycoprotein, a tyrosine kinase and is localized in the plasma membrane. Additionally, it was observed that a non-glycosylated form of naER existed in the uterine nucleus and that this transformed naER (identified here as nuclear estrogen receptor II or nER II) lost its capacity to dimerize with the E-RAF [Karthikeyan and Thampan, 1995]. The transformation was also found to result in a loss in the naER affinity for estradiol at the same time gaining additional binding sites for the hormone. A major nuclear target for nER II binding appeared to be the nuclear RNA polymerase II.

There has been a renewed interest in identifying the factors involved in ER-mediated transcription during the past 4 years, a period which also witnessed the discovery of ERβ. Several co-factors (co-activators, co-integrators and co-repressors) which interact with the basal transcription machinery and the ERα have been identified [reviewed by Freedman, 1999; Horwitz et al., 1994; Xu et al., 1999]. The basic aim of the current review is to integrate these findings on the ERα with those of the naER–E-RAF system to evolve a working model which could be subjected to experimental scrutiny.

Structure of the Mammalian ER

The ER belongs to the nuclear receptor superfamily that mediates ligand dependent transcription. They bind selectively, as a homodimer, to the estrogen responsive element (ERE) in the target gene. The hER is the most extensively characterized estrogen receptor [Kumar et al., 1986]. The 595 amino acids long protein molecule (Fig. 1) is divided into A/B, C, D, E and F regions of which C and E represent the DNA binding domain (DBD) and the ligand binding domain (LBD), respectively.

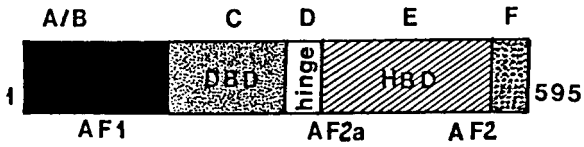

Fig. 1. Functional domains on the human estrogen receptor (hER) [Based on he information reviewed by Freedman, 1999]. Four major regions have been identified in the 595 amino acids long protein A/B, C, D, E and F. Of these 'C' represents the DNA binding domain, 'E' is the hormone (ligand) binding domain and 'D' is the hinge region. Three main domains involved in transactivation function (AF) have been recognized: AF1 which represent the A/B region, AF2 localized at the C-terminal of the ligand binding domain and a minor AF2a at the intersection between regions 'D' and 'E'.

The region D is also referred to as the 'hinge'. The N-terminal A/B domain and the C-terminal region of the E-domain are found to be involved in activation functions (AF) that have been identified as AF1 and AF2, respectively. Additionally, a narrow AF2a lies in the intersection between the hinge and the LBD.

Cytosol-to-Nucleus Movement of the ER

The ER is believed to be transported into the nucleus immediately after its synthesis on the cytoplasmic ribosomes eventhough we do not have the kinetic data to clearly measure the time taken for the ER to enter the nucleus after its synthesis. The nucleus has been shown to be the primary site of localization of the ER [King and Greene et al., 1984; Welshons et al., 1984]. Transport of proteins to the nucleus has been a major target of investigation by cell biologists eversince the identification of nuclear localization signals (NLS) [De Rebertis et al., 1978]. The first identification of NLS in hER was made by Chambon and co-workers [Ylikomi et al., 1992]. The NLS in the hER was observed to exist in a 'split' condition as three separate stretches of amino acids with several non-NLS amino acids in between. These were identified as the proto nuclear localization signals (pNLSs), the pNLS-1, pNLS-2 and pNLS-3. The pNLS-1 (amino acids 299–303) was found to exist in the hormone binding domain, the pNLS-3 (amino acids 256–260) in the DNA binding domain and the pNLS-2 (amino acids 265–273) in the hinge region.

The very first report on the NLS binding protein which mediates the nuclear entry of goat uterine estrogen receptor (gER) was made by Nirmala and Thampan [1995a]. It was ob-

served that, similar to several other classical systems of protein transport into nuclei, the nuclear transport of ER also underwent two distinct mechanisms: an energy independent phase during which the receptor was transported to the nuclear pore complex (NPC) and an energy-dependent phase during which the nuclear entry of the ER through the nuclear pore complex took place. It was observed that the energy independent phase involved the transport of ER to the nuclear pore complex (NPC) by a 55 kDa, NLS binding protein (p55). The 'docking' of this complex at the nuclear pore complex was found to be mediated by a 12 kDa protein. Details associated with the energy-independent phase have been investigated recently [Sai Padma and Thampan, 2000; Sai Padma et al., 2000]. These studies showed that under hormone-free conditions, two additional proteins remain associated with the goat uterine ER (gER). These are: (1) a 28 kDa protein (p28) that binds to the NLS on the gER and thereby prevents the p55 from binding to the NLS; (2) a 73 kDa protein, p73 that remains bound to the hormone binding domain (LBD) of the gER. It appears that in a complex formed between p28 and p55 the NLS binding domain of p55 remains overshadowed by p28. Consequently, p28 gains access to the NLS on the gER. Since p28 is incapable of interaction with p12 the 'docking' of the complex at NPC fails to materialize. Moreover, p55 is an actin binding protein, a function that is not associated with p28, underlining a possibility that cytoskeletal elements are actively involved in the nuclear entry of gER. This association of proteins has been illustrated in Figure 2. When estradiol binds to the LBD, it appears that the resultant change in LBD conformation precipitates an interaction between p73 and p28. The p73–p28 complex dissociates from the NLS site on the gER, paving the way for the binding by p55. The p55–gEr complex interacts with a smaller protein, p12 which in turn gets docked at the NPC. It has been observed that both p55 and p12 are Ca^{++}/Mg^{++} activated ATPases. Inhibition of these activities by the flavonoid, quercetin, resulted in the prevention of the nuclear entry of the gER.

The transport proteins which facilitate nuclear entry of nuclear proteins have been described as belonging, in general, to two categories, importin α and importin β. Importin α recognizes the NLS in the protein that requires

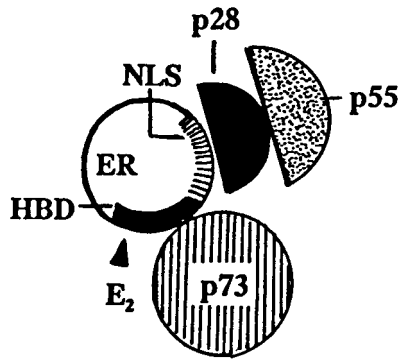

Fig. 2. Proteins associated with the goat uterine estrogen receptor (gER) under estrogen-free conditions [based on the data reported by Sai Padma et al., 2000]. HBD represents the hormone binding domain on the gER and the NLS indicates nuclear localization signals. Both p28 and p55 are NLS binding proteins while p73 binds to the hormone binding domain. p28 in spite of its capacity to recognize NLS is incapable of transporting gER to the nucleus. It lacks the properties found in p55 which include the actin-binding function and the capacity to interact with p12, the protein that 'docks' at the nuclear pore complex. p73 interacts with p28, but not with p55. p73–p28 interaction leads to the dissociation of p28 from the NLS, facilitating the NLS recognition by p55. It appears that in the complex formed between p28 and p55 and NLS binding domain of p55 gets masked due to which NLS binding of p55 does not take place. Therefore, it is a necessity that p25 should dissociate from the complex to effect the nuclear entry of gER.

to be transported while importin β recognizes the importin α on the one hand and the nucleoporins of the nuclear pore complex on the other [Gorlich and Mattaj, 1996]. While the p55 can easily be identified as representing the importin α, due to the smaller size of p12, the protein fails to be categorized as importin β in spite of its display of its capacity to interact with the NLS binding p55 and also the NPC-'docking' function.

ERβ

A new form of ER, ERβ, was discovered 4 years ago following which the classical ER came to be identified as ERα. cDNA of clone 29 isolated from a rat prostate cDNA library encoded a protein of 495 amino acids with a calculated molecular mass of 54.2 kDa [Enmark et al., 1996]. In contrast to ERα, ERβ showed conservations of 96 and 58%, respectively, for the DBD and LBD while the A/B, hinge and the F regions displayed practically no conservation at all. Transactivation properties of the two proteins, complexed with estradiol, at the ERE and AP-1 sites showed opposite results [Paech et al., 1997]. While ERα activated transcrip-

tion, ERβ inhibited it. On the other hand, ERβ complexed with the antiestrogen tamoxifen, raloxifene or ICI 164384 activated transcription at an AP-I site. Recent studies on the ER distribution in rodent mammary glands showed that 8S ER formation in low salt sucrose gradients was achieved with ERα and not with ERβ [Saji et al., 2000].

Transcription Regulation by Estrogen Receptor

The exact mechanism by which ER brings about transcriptional regulation still remains shrouded in mystery. The ER has been implicated in the formation of pre-initiation complexes. Transcription initiation by RNA polymerase II involves a step-wise assembly of general transcription factors or basal factors designated TF II A to TF II J on the promoter forming the pre-initiation complexes. Of the basal factors TF II D is the TATA element binding protein which by itself is a complex made of TATA binding protein (TBP) and up to 13 TBP-associated factors (TAFs). TF II B serves a bridging function between TBP and RNA polymerase II [reviewed by Horwitz et al., 1996].

Transcription stimulation by ER is mediated by two activation domains: AF1 located in the N-terminal A/B domain and AF2 located in the C-terminal ligand binding domain (LBD) or E region of the estrogen receptro, whose activity varies depending on the responsive promoter and cell type [Tora et al., 1989]. A third activation function, AF2a has been identified which is located at the boundary between D domain (Hinge region) and domain E (LBD) [Pierrat et al., 1994]. Both AF1 and AF2a appears to function in ligand independent manner whereas AF2 which forms a short α-helical motif functions in a ligand dependent manner.

Direct interaction of ER with some of the basal factors like TF II B, TBP and TAF II 30 has been reported [reviewed by Horwitz et al., 1996]. The site of interaction of TF II 30 has been traced to the AF2a on the ER [Pierrat et al., 1994]. The proteins that have been shown to interact with ER have been categorized into three groups: co-activators; co-integrators; and co-repressors. Majority of the co-activators identified so far have been shown to interact with AF2 domain of ER. A distinct structural characteristic of these proteins is the presence of LXXLL signature motifs (also called LXDs, NR boxes or NIDS) which represents the feature necessary for interaction with the LBD. The LXXLL ('L' represents leucine while 'X' can represent any other amino acid) forms the core of a short amphipathic α-helix. This, is turn, is recognised by a highly complimentary hydrophobic groove formed by residues from helices 3, 4, 5 and 12 and the turn between the helices 3 and 4 on the surface of the receptor [reviewed by Xu et al., 1990]. Since many co-activators do interact with other steroid receptors, it has been suggested that the selectivity between the receptor concerned and the LXXLL motif on the co-activator is determined by amino acid residues located at both amino terminal and carboxy terminal regions of the LXXLL motif. In a complex formed between tamoxifen and ER, the helix 12 has been shown to occlude the co-activator recognition groove [reviewed by Freedman, 1999].

One mechanism that has been postulated by which co-activators influence transcription is by remodelling chromatin and thereby facilitating access of general transcription factors to the transcription initiation site. Some co-activators possess intrinsic histone acetyl transferase activity. Histone acetylation reduces the DNA binding affinity of the protein and this is believed to result in enchanced transcriptional activity [reviewed by Xu et al., 1999]. Whether the dissociation of histones from the DNA also leads to DNA helix destabilization is not clear. Some co-activators have been found to interact with memebers of the basal transcription machinery as well as ligand activated ER, indicating a possibility that they may be acting as molecular bridges between the ER and the transcription apparatus. Thus, co-activators are thought to act as adapters of the general transcription machinery and modulators of chromatin structure. Candidate proteins that have been recognized to function as to co-activators are as follows:

(1)	SRC/NCoA-1	Steroid receptor co-activator-1/nuclear receptor co-activator 1
(2)	TIF2/GRIP-1/ NCoA2	Transcription intermediary factor 2/ glucocorticoid receptor interacting protein 1/nuclear receptor co-activator-2

(3)	pCIP/ACTR/RAC-3/AIB-1	p300/CBP cointegrator associated protein/ activator of thyroid and retinoic acid receptor/receptor associated co-activator-3/activated in breast cancer-1

These proteins have an approximate molecular mass of 160 kDa and similar overall domain structure. They possess two functional domains: CBP interaction domain (CID) and nuclear receptor interaction domain (NID), both of which employ multiple LXXLL motifs [reviewed by Xu et al., 1999]. Interaction of p160 proteins with ER is mediated by LXXLL motifs, three of which are conserved both in space and sequence in all the free family units mentioned above. Among the three conserved motifs, mofif II is the preferred site for interaction with ER. The transcriptional activation domains of SRC-1/ NCoA-1 and GRIP-1/TIF-2/ NCoA-2 have been mapped to two activation domains: AD1 and AD2 [Kalkhoven et al., 1998]. Since AD1 co-localizes with CBP binding domain, it is suggested that the AD1 function may be mediated through CBP-binding. In contrast, AD2 domain is CBP-independent. p160 members have also been shown to interact with two proteins (CBP and p/CAF) possessing histone acetyl transferase (HAT) activity [reviewed by Freedman et al., 1995]. Other members of the p160 family like SRC-1/NCoA-1 and pCIP/ACTR/RAC-3/ AIB-1 have been shown to display intrinsic yet week, histone acetyl transferase activity. Structurally, however, these proteins have not been shown to contain domains homologous to the CBP/p300 or p/CAF domains displaying HAT activity [reviewed by Xu et al., 1999].

An example of co-integrator is the CBP/p300. Cyclic AMP responsive element binding protein (CREB)-binding proteins (CBP) and its close homologue p300 was found to associate with ligand-bound nuclear receptors including steroid receptor family. The glutamine-rich carboxy terminus of CBP interacted with the amino terminus of SCR-1. The AF2 domain of the ER interacts with CBP in a ligand-dependent manner. p/CAF, a protein that possesses HAT activity has also been shown to interact with CBP [reviewed by Freedmann, 1999]. CBP/p300 is required for transcriptional activation by different classes of transcription factors including CREB and AP-1. Therefore, the function of CBP is considered to be that of a nuclear integrator of multiple signal transduction pathways. For this reason, CBP/p300 was designated as a co-integrator [reviewed by Xu et al., 1999]. p/CAF is p300/CBP associated factor that possess HAT activity that has been found to interact with ER, CBP/p300 and p160 family of co-activators.

Little is known about the co-activators that are specific for AF1 of ER-α. Members of SRC-1/ TIF-2 protein have been reported to stimulate AF1 activity synergestically with AF2, showing only partial support for the AF1 function [reviewed by Xu et al., 1999]. The N-terminal activation function of AF1 is ligand-independent [Tora et al., 1989]. Phosphorylation of ser118 in human ERα by mitogen activated protein kinase (MAPK) has been shown to activate AF1 and AF2 functions [Kato et al., 1995]. Phosphorylation of ser106 and ser124 of ERβ by MAPK stimulates the recruitment of SRC-1 to AF1 [Freedman, 1999].

p68 RNA helicase of MCF-7 cells has been shown to interact with the A/B domain but not the LBD of hER-α [Endoh et al., 1999]. It enhances the function of AF1 function but not that of AF2. It is possible that p68 serves as an adaptor protein to be associated with AF2 co-activator since it has been shown to interact with CBP. The p68–ER-α interaction is regulated by MAPK-induced phosphorylation of ser118 in ERα [Endoh et al., 1999].

Co-repressors identified for nuclear receptors, have mostly been shown to display histone deacetylase (HDAC) activity. This activity stabilizes the ordered chromatin structure and thereby prevents access of basal transcription factors to the ER. Co-repressors like NCoR and SMRT interact with ER only in the presence of estrogen antagonists. This interaction has been found to be essential for the full expression of antiestrogen function [reviewed by Xu et al., 1999]. REA (repressor of estrogen receptor activity) is a 37 kDa protein which is the single most ER-specific co-repressor ever identified [Montano et al., 1999]. REA has been shown to potentiate two inhibitors of ER action; antiestrogens and dominant negative ER. It has also been observed that REA and SRC-1 compete for binding sites on AF2, suggesting that the

proteins compete for modulating ER mediated signal transduction.

Estrogen Receptor and Post-Transcriptional Control of Gene Expression

Investigations which have approached this subject have been extremely limited. Biochemical [Thampan, 1985, 1988] and ultra structural [Vazquez-Nin et al., 1991] studies carried out on the rat uterus showed that withdrawal of estrogen from the system, through ovariectomy, resulted in the retention within the nucleus of ER-associated messenger ribonucleoprotein (mRNP). Exposure of these uteri either in vivo or in vitro to estradiol resulted in the immediate transport of the mRNP from the nucleus to the cytoplasm [Thampan, 1985]. This transport appeared to be preceded by the activation of a nuclear Ca^{++}/Mg^{++} dependent ATPase and a cGMP-dependent protein kinase [Thampan, 1988]. Inhibition of the ATPase activity prevented the estrogen-mediated movement of the ER–mRNP complex.

Recent studies [T. Sebastian and R.V. Thampan, manuscript communicated] have added a new dimention to the findings mentioned above. The ER has been localized in a class of small nuclear ribonucleoproteins (snRNPs) particularly those containing U1 and U2 snRNAs. It is generally accepted that export of RNA from the nuclei, in the form of RNP, is mediated by specific transport factors [Izaurralde and Mattaj, 1995]. The nuclear export is specified by nuclear export signals (NES) present in the protein moiety of RNPs and characterized by a short stretch of hydrophobic amino acids primarily leucine. HIV-1 Rev protein is perhaps the most extensively studied transport protein containing a leucine rich NES [Pollard and Malim, 1998]. The estrogen-dependent RNP transport from the uterine nucleus to the cytoplasm appears to be mediated by a RNP associated protein that apparently recognizes the NES on the ER. One significant information generated from these studies will be the nature of the estrogen receptor that associated with the RNP, whether the ER is the regular ERα/ERβ or the alternative form (nER II) that does not bind to the DNA.

Degradation of Estrogen Receptor Through Ubiquitination

Ciechanover et al. [1980] demonstrated that several molecules of ubiquitin were conjugated to the proteins to be degraded in an ATP-dependent manner. This involved isopeptide linkages, linking ubiquitin to the ε-amino lysine of the protein substrate. Since protein-ubiquitin conjugates undergo rapid degradation resulting in the release of free and reusable ubiquitin, a model was proposed according to which protein conjugation with ubiquitin is an obligatory event in protein breakdown. The C-terminus of ubiquitin is linked by an isopeptide bond to specific internal lysine residues of the target protein in a multistep process. Nirmala and Thampan [1995b] showed that the rat uterine ER is degraded by the ubiquitin pathway and also that the ubiquitination of ER is enhanced by estradiol. The observations were extended further by Nawaz et al. [1999] who demonstrated that treatment of mammalian cells with proteasome inhibitor MG 132 blocked ER degradation, suggesting that the ER is turned over through the ubiquitin-proteasome pathway. The enzymes responsible for the linking of ER to ubiquitin have not yet been isolated and characterized.

The Alternative Estrogen Receptor Systems

The mammalian uterus contains an alternative system of estrogen receptor in which the receptor, naER with no DNA-binding function to perform, dimerizes with a DNA-binding protein, the E-RAF. naER is a glycoprotein localized in the plasma membrane and is a tyrosine kinase. In vitro studies have shown that exposure of isolated, goat uterine plasma membrane to estradiol results in the release of naER to the medium and also that the process is inhibited by tamoxifen [Karthikeyan and Thampan, 1996]. The estrogen-binding affinity and specificity of the naER are indistinguishable from the corresponding features of the regular ER. The naER–E-RAF dimerization takes place within the nuclei since prior dimerization by the proteins prevents the nuclear entry of the complex [Thampan et al., 2000]. The same negative effect is produced by cholesterol binding to E-RAF indicating that cholesterol binding altered E-RAF conformation and also that the E-RAF of altered conformation failed to interact with the docking protein at the NPC. What has been highlighted by the studies on the NLS binding proteins is the observation that the proteins display actin-binding property indicating the possible involvement of

cytoskeletal proteins in the nuclear entry of naER and E-RAF. The naER which enters the nucleus undergoes deglycosylation and gets transformed to an ER of altered conformation. This molecule is now identified as the nuclear estrogen receptor II (nER II) [Karthikeyan and Thampan, 1995]. Recent experiments carried out in our laboratory have succeeded in isolating and purifying a 62-kDa transforming factor that converts naER to nER II [Jaya and Thampan, 2000].

E-RAF is a 66 kDa protein existing in two molecular forms, E-RAF I and E-RAF II [Thampan, 1989]. While E-RAF II destabilizes DNA double helix and enhances transcription in vitro, E-RAF I does the opposite function. Synthesis of E-RAF appears to be regulated by both estradiol and progesterone. As mentioned before there is a distinct possibility that E-RAF is a transcription factor, involved either in enhancing (E-RAF II) or inhibiting (E-RAF I) gene expression. The site on the gene where E-RAF binds is yet to be identified. An endogenous calcium activated neutral protease (CANP) of the goat uterus was employed recently as a tool in the structural characterization of E-RAF. The 66 kDa E-RAF gets cleaved, as a result of CANP action, into a 32 and 30 kDa fragment, termed α and β, respectively. While α retains the DNA-binding and dimerization domains, β carries with it the ATPase function and the ability to interact with the 'docking protein' associated with the NPC. Cholesterol binding function appeared to be shared by both α and β [Kumar et al., 1999]. Recent studies carried out in our laboratory [A.P. Govind and R.V. Thampan, manuscript in preparation] have shown that, favoured by high intracellular concentration of estradiol, E-RAF remains anchored to an endoplasmic reticulum protein. Studies on this anchor protein are in progress. Lowering of estradiol concentration or an increase in intracellular progesterone concentration causes the dissociation of E-RAF from the anchor protein. The E-RAF enters the nucleus unaided by an NLS binding protein. We are not in a position to preclude the involvement of a NPC-'docking' protein that mediates nuclear entry of E-RAF, at this stage.

The integrated model (Fig. 3A) takes into consideration the basal transcription machinery of an estrogen responsive gene where the ERα homodimer remains bound to the ERE. The co-activators serve to bridge ER with the basal transcription factors while the co-integrators evolve an additional level of influence by interacting with CREB proteins bound to cyclic AMP responsive element. naER, in view of its affinity for binding to RNA polymerase II is shown to interact with RNA polymerase II on the one hand and E-RAF on the other. This complex formation brings the E-RAF to the transcription initiation site. Deglycosylation of naER by the transforming factor (Fig. 3B) serves to dissociate E-RAF from the naER (nER II). E-RAF II destabilizes the DNA, providing the template required for RNA polymerase action. nER II continues its association with the RNA polymerase through phosphorylation of the enzyme subunits. The role of subunit phosphorylation in RNA polymerase action is not clear at this stage.

Undoubtedly, research work accomplished during the past one decade in laboratories dealing with the molecular biology of estrogen action has contributed substantially towards our understanding of the protein–protein interactions in which ERα is involved prior to the activation of transcription. The number of proteins that are being identified as co-activators or co-integrators is enormous. Nevertheless, a careful review of these data still underscores the reality that we are far from identifying the fundamental mechanisms that lead to the activation of transcription by the ER. There is an urgent need to integrate the available data on the classical and alternate forms of ER and the associated proteins. In the process, it might come to the light the possibility of the nER II/E-RAF system forming contracts with the RNA-polymerase associated transcription factors which, in turn, interact with ERα through co-activators. naER has been shown to phosphorylate a 40 kDa subunit in RNA polymerase II and following its transformation to nER II, the same protein phosphorylates the 20 and 91 kDa subunits of the enzyme [Karthikeyan and Thampan, 1995]. Dissociation of E-RAF from the endoplasmic retiluclum anchor protein is dependent on the intracellular concentration of estradiol/progesterone and the dissociated E-RAF enters the nucleus for its subsequent dimerization with the naER. These features on their own qualify naER/nER II and E-RAF as targets for further studies probing deeper into the estrogen receptor mediated regulation of transcription.

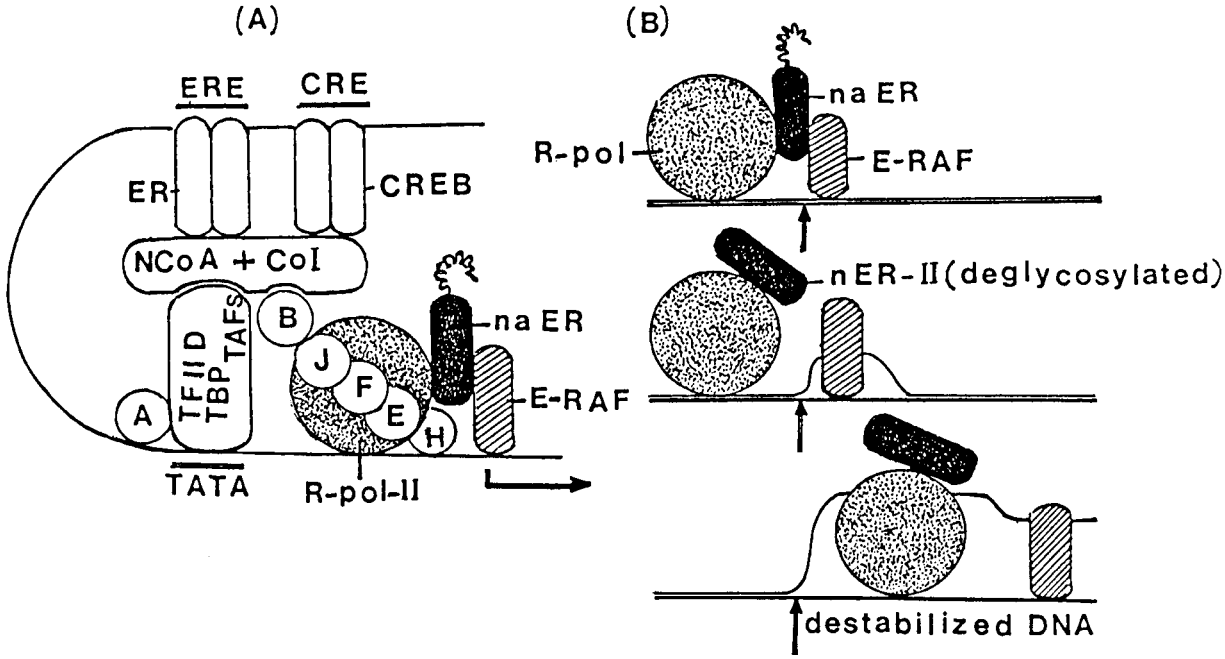

Fig. 3. An integrated model that takes into consideration functional roles of both ERα and naER (nER II) in transcription of an estrogen-regulated gene. (A) This displays the interaction of basal transcription factors, estrogen receptor homodimer (ER), cyclic AMP response element (CRE), binding protein (CREB) and RNA polymerase II with an estrogen responsive gene. ERE is the estrogen response element. Factors A to F are transcription factors. NCoA represents coactivators and CoI represents cointegrators. naER–E-RAF heterodimer binds to RNA polymerase II, being influenced by the RNA polymerase binding property of the naER. This positioning of the heterodimer in relation to RNA polymerase II is shown to bring E-RAF to the transcription initiation site. (B) The upper panel represents naER as a glycoprotein, dimerized with the E-RAF. The E-RAF binds to the transcription initiation site. The middle and lower panels show helix destabilization by E-RAF (E-RAF II). Deglycosylation of naER leads to its transformation to nuclear estrogen receptor II (nER II) which displays a capacity for a closer interaction with RNA polymerase than that of naER. nER II is incapable of dimerization with E-RAF. The dissociated E-RAF is now free to destablize the DNA and facilitate the transcription process by RNA polymerase II.

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