Molecular BioSystems

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Cite this: *Mol. BioSyst.*, 2011, **7**, 667–676

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PAPER

Comparative analysis of nuclear estrogen receptor alpha and beta interactomes in breast cancer cells†

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Received 5th August 2010, Accepted 18th November 2010
DOI: 10.1039/c0mb00145g

Estrogen Receptor alpha and beta (ER-α and -β) are members of the nuclear receptor family of transcriptional regulators with distinct roles in mediating estrogen dependent breast cancer cell growth and differentiation. Following activation by the hormone, these proteins undergo conformation changes and accumulate in the nucleus, where they bind to chromatin at regulatory sites as homo- and/or heterodimers and assemble in large multiprotein complexes. Although the two ERs share a conserved structure, they exert specific and distinct functional roles in normal and transformed mammary epithelial cells and other cell types. To investigate the molecular bases of such differences, we performed a comparative computational analysis of the nuclear interactomes of the two ER subtypes, exploiting two datasets of receptor interacting proteins identified in breast cancer cell nuclei by Tandem Affinity Purification for their ability to associate *in vivo* with ligand-activated ER-α and/or ER-β. These datasets comprise 498 proteins, of which only 70 are common to both ERs, suggesting that differences in the nature of the two ER interactomes are likely to sustain the distinct roles of the two receptor subtypes. Functional characterization of the two interactomes and their topological analysis, considering node degree and closeness of the networks, confirmed this possibility. Indeed, clustering and network dissection highlighted the presence of distinct and ER subtype-specific subnetworks endowed with defined functions. Altogether, these data provide new insights on the protein–protein interaction networks controlled by ER-α and -β that mediate their ability to transduce estrogen signaling in breast cancer cells.

Introduction

Estrogens are involved in promotion of cell growth and differentiation in the mammary gland. Their physiological and pathological effects depend on the presence of Estrogen Receptors (ERs) that directly regulate transcription of target genes such as those involved in cell cycle control, including proto-oncogenes or cyclin genes.[1–3] The control of cellular activities by estrogens is mediated by binding to two nuclear receptors, ERα and -β subtypes, acting through genomic and non-genomic signal transduction pathways. In the first case, hormone binding to ERs induces a conformational change of these transcription factors, driving their dimerization, DNA binding to cognate estrogen response elements (ERE),[4] recruitment of co-activators and co-repressors and, finally, regulation of chromatin organization and gene transcription.[5,6] In the second case, estrogens induce rapid effects on extranuclear signaling pathways, including activation of protein kinases and phosphatases or enhancement of ion exchanges across membranes.[7] Since integration of these pathways mediates the mitogenic actions of estrogens, and clinical and experimental data point to these hormones as pathogenic factors in breast and uterine cancers, drugs interfering with their activity are extensively used in the treatment of these diseases.

Although the two ERs show a conserved structure, sequence homology with each other and share similar mechanisms of action, they substantially differ in their ability to control gene

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† Electronic supplementary information (ESI) available: Figures S1 and S2. See DOI: 10.1039/c0mb00145g
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transcription, including unique behaviour of the ER-β subtype.[8,9] When compared, the amino acid sequences of the two ERs show a significant conservation in the DNA-binding domain (DBD; central) and ligand-binding domain (LBD; C-terminus), but marked divergence in the AF-1 (N-terminus) and in the D-domain (hinge region between DBD and LBD). In particular, the AF-1 region of ERβ exhibits a weak transcriptional activity, with respect to ERα, and contains a repressor domain causing reduction of the overall transcriptional activity of this receptor,[10,11] contributing to the ability of ERβ to repress ERα-mediated transactivation, when they are co-expressed in the cell.[12] Moreover, ERα and -β differ in the affinities for various ligands and in the transcriptional response these elicit.[13] For example, tamoxifen is a cell- and tissue-specific mixed agonist–antagonist for ERα and a pure antagonist for ERβ.[14] They can recruit the same co-activators or co-repressors, but for example SRC-3 up-regulates the transcriptional activity of ERα more than that of ERβ.[8] These proteins may interact with different regions of each ER and/or differentially recruit other factors. Both ERs induce the transcription of a reporter plasmid containing an Estrogen Response Element (ERE), but ERβ is a weaker activator than -α.[9] The ERE sequence and the cell context are important factors that determine the differences in the transcriptional activity of the two ERs,[15] which are often co-expressed in cells and tissues, although ERα is expressed mainly in uterus, vagina and mammary glands, while ERβ can be found in several tissues.[6] When activated, in the cells where both are expressed, they can form either homodimers (ERα/α and ERβ/β) or heterodimers (ERα/β). It has been described that heterodimers are less active than ERα homodimers, while ERβ homodimers oppose ERα mediated transcription, probably through a competition for ERE binding.[16] For this reason, the ratio of ERα : ERβ represents the key determinant for the cell specific response to estrogen. In breast this ratio is higher in cancer than in normal tissues; this is due to up-regulation of ERα and down-regulation of ERβ expression in cancers,[17,18] where loss of ERβ mRNA levels in breast cancer cells can occur consequent to promoter methylation.[19] Overexpression of ERβ in ERα positive breast cancer cells inhibits cell proliferation in response to E2 by increasing the expression of anti-proliferative genes and/or decreasing the expression of proliferative and anti-apoptotic genes.[20–22] This has suggested an anti-proliferative and a positive prognostic value of this receptor subtype.[22] As such, ERα and -β are both key mediators of the estrogen signal transduction cascade, with different and/or antagonistic biological roles. These observations suggest that the pattern of genes regulated by the two receptors is only partially overlapping. Indeed, genes differentially regulated by ERα and -β, such as cyclin D1[23] and Fibulin-1C,[24] have been identified. Inactive ERα interacts with Hsp90, immunophilin and p23.[5] Experiments, performed in human breast cancers, have shown the presence of two distinct peaks of estradiol binding detectable in low-salt extracts upon sucrose gradient centrifugation: the 8S peak containing ERα and the 4S containing exclusively ERβ. Differences between several breast samples in the amount of estradiol bound and the ratio between the two peaks have been verified,[13,25] suggesting that ERα is involved in larger protein complex than ERβ. Moreover, ERβ specific interaction with the MAP kinase interacting kinase Mnk2[26] and specific phosphorylation and activation only of ERα by Rsk2 have been demonstrated.[27] These results suggest that different transduction pathways are involved in ER subtype-specific regulation of cellular responses (gene expression or cell proliferation), dependent upon the receptor form present or predominantly expressed within the cell. Despite these evidences, understanding the nature of the functional partners of ERs in different cell types and compartments, and how these molecules contribute to the hormone-dependent cancer phenotype, remains an open issue in breast cancer biology and pharmacology. Indeed, it is well known that most effects of estrogens are cell type specific and this is achieved by differentiated expression not only of ERs but also of functional partners of these receptors. These are believed to include transcriptional co-regulators, signaling effectors, molecular adapters and other intracellular molecules, which participate in estrogen signal transduction by physically interacting with ERs and, thereby, constituting modular multi-protein complexes with different biological activities, depending upon their absolute composition, stoichiometry and conformation of their components. Tandem Affinity Purification (TAP)[28] has been extensively used for the identification of different protein interactomes in several systems. In our previous studies we applied TAP coupled to MS analysis in order to identify multi-protein complexes co-purifying with ERα or ERβ in human breast cancer cell lines stably expressing either TAP-ERα or TAP-ERβ together with endogenous ERβ.[29,30] By this approach we could show the molecular basis of ERα involvement in regulation of the transcriptional activity by nuclear actin network.[29] Here we present a comprehensive bioinformatics analysis aimed at the identification of the molecular cascades that mediate the specific activities of each receptor subtype. The analyses have been performed on two datasets, represented by proteins isolated in complexes with ERα or -β in a breast cancer cell line expressing tagged ERs.[30,31] We performed an in depth functional network dissection to search for specific and primary interacting partners of the receptors. Results provide a new perspective on the functional diversities between these two proteins, based upon the differences in their interactomes, as summarized in Fig. S1 (ESI†).

Experimental

In silico functional analyses

To identify statistically over-represented "Biological Process" (BP) and "Molecular Function" (MF) Gene Ontology terms among sets of ERs interacting proteins identified by MS analyses, we used the Database for Annotation, Visualization and Integrated Discovery (DAVID; http://david.abcc.ncifcrf.gov/) and relative functional annotation tools.[32,33] To this aim, lists of detected genes, derived from gene expression profiling data obtained in the same cell lines under comparable experimental conditions used for this study, were used as background.

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Table 1 Main topological parameters of ERα and ERβ networks

Parameter	Symbol	ERα	ERβ
Number of nodes	$\langle N \rangle$	187	149
Number of edges	$\langle E \rangle$	1495	612
Clustering coefficient	$\langle cc \rangle$	0.388	0.326
Network diameter	$\langle d \rangle$	12	12
Average node degree	$\langle avk \rangle$	15 989	8188

The Table summarizes the following parameters: *Degree* (k): for undirected graphs, the degree of a node is the number of its adjacents, so in this work it represents the number of interactors of a node. *Average degree* ($\langle k \rangle$): represents the average of the degree of all nodes. *Clustering coefficient* ($\langle cc \rangle$): is the average of all the clustering coefficients of nodes, representing the fraction of the number of edges between the nodes within the i-neighborhood divided by the number of edges that could possibly exist between them. *Mean shortest path length* (mspl): is the average of the steps (number of links) needed to connect every pair of nodes through their shortest path. *Diameter* (d): is the longest among all shortest paths, *i.e.* the minimum number of links that separate the two most distant nodes in a network. *Closeness centrality* (ccl): Closeness centrality is a measure of how fast information spreads from a given node to other reachable nodes in the network. The closeness centrality [P9] of a node ccl(n) is calculated as the reciprocal of the average shortest path length and is computed as follows: $cc(n) = 1/avg(L(n,m))$, where m indicates all the reachable nodes from n and $L(n,m)$ the length of the shortest path between two nodes n and m. The closeness centrality of each node is a number between 0 and 1 and that of isolated nodes is equal to 0.

Topological analyses of protein–protein interaction networks

The protein datasets obtained from the TAP experiments were used to extract from UniHI[34] information concerning known, validated protein–protein interactions. Retrieved information were integrated to build an interaction network with Cytoscape.[35] In this way we obtained two networks, one for each ER subtype. Representation as spring embedded (or circular layout) was applied to enhance the readability of such networks. Then both networks were analyzed in order to extract topological parameters, centrality measures and key subnetworks. In particular, we measured the following parameters: number of nodes ($\langle N \rangle$) and edges ($\langle E \rangle$), average clustering coefficient ($\langle cc \rangle$), degree ($\langle k \rangle$) and its distribution among nodes, average node degree $\langle avk \rangle$, diameter ($\langle d \rangle$) and closeness centrality of each node ($\langle ccl \rangle$).[36] Table 1 lists the main topological parameters obtained for each network. The first and second steps were performed using the Network Analyzer plugin for Cytoscape.[37] Subnetworks were extracted by using MCODE[38] and Nemo algorithms.[39]

Results and discussion

Functional analyses reveal both specific and common pathways for ERα and ERβ

Assuming as background the known differences between ERα and -β, also supported by our previous research that allowed the identification of proteins copurifying with ERs, the main focus of this study was to investigate how the multiprotein complexes formed by each of the two receptors can provide useful information concerning the mechanism of action of these two proteins. To this aim, we performed an in depth computational analysis and dissection of ERα and ERβ interactomes, focusing on the main nodes of each network driving key functional differences relative to the signal transduction pathway.

Several ERα interacting proteins have been identified in the past years by genetic and molecular cloning approaches, including transcriptional coregulators associated to the DNA-bound receptor.[29,40,41] More recently, by applying TAP method coupled to glycerol gradient sedimentation and nano LC-MS/MS analysis we were able to identify 264 proteins associated with ligand activated ERα and 304 bound to ERβ in MCF-7 cells nuclei.[30,31,42,43] These experimental results were exploited to perform a comparative analysis of the two interactomes, in order to highlight and functionally characterize common and/or unique key interacting partners and complexes involving the two ER subtypes in breast cancer cells.

As shown in the Venn graph in Fig. 1A, the overlap between the ERα and ERβ interactomes indicates that, among all the identified proteins, only 70 proteins are in common between both ERs. Consequently, a large number of proteins (194 for ERα and 234 for ERβ) that are co-purified in complexes only with one or the other receptor subtype. These data indicate that these datasets represent a useful starting point to investigate the molecular basis of the functional differences among the two ERs in the same cellular background. For this reason, we performed a functional analysis on the three lists of interactors (ERα-specific, ERβ-specific and common) to highlight informative differences, reported schematically in Fig. S1 (ESI†). The GO terms relative to MFs and BPs significantly enriched in each list are reported in Fig. 1B. These results show different functional pathways converging on either ERα (ERα only), ERβ (ERβ only) or both receptors (ERα + ERβ), highlighting significant differences between the two receptors. In particular, considering the MF terms found enriched, ERα interactors result involved in RNA binding, ATP dependent RNA–DNA helicase activity and RNA–DNA dependent ATPase activity. In addition, terms associated with transcription regulator activity and transcription factor binding are found significantly represented among ERβ interactors. On the other hand, ERα and -β interactomes share some functional annotations, such as actin binding, ribosome organization and biogenesis and the generic category represented by macromolecule metabolic and biosynthetic processes.

Topological investigation of ERα and ERβ networks

To clarify the observed functional differences we performed several targeted computational analyses of the ERs interaction networks. Based on database searching, two interaction networks, for ERα, containing 187 nodes (proteins) and 1495 edges (interactions), and ERβ (149 nodes and 612 edges) were built. For both we measured as centrality topological parameters[36] the following, listed in Table 1: number of nodes (the number of proteins), average degree (average number of interactions for each protein), clustering coefficient (*i.e.* how many interactions has a protein with respect to the maximum number of possible interactions), network diameter (maximum distance between two proteins in terms of number of intermediate proteins), node degree

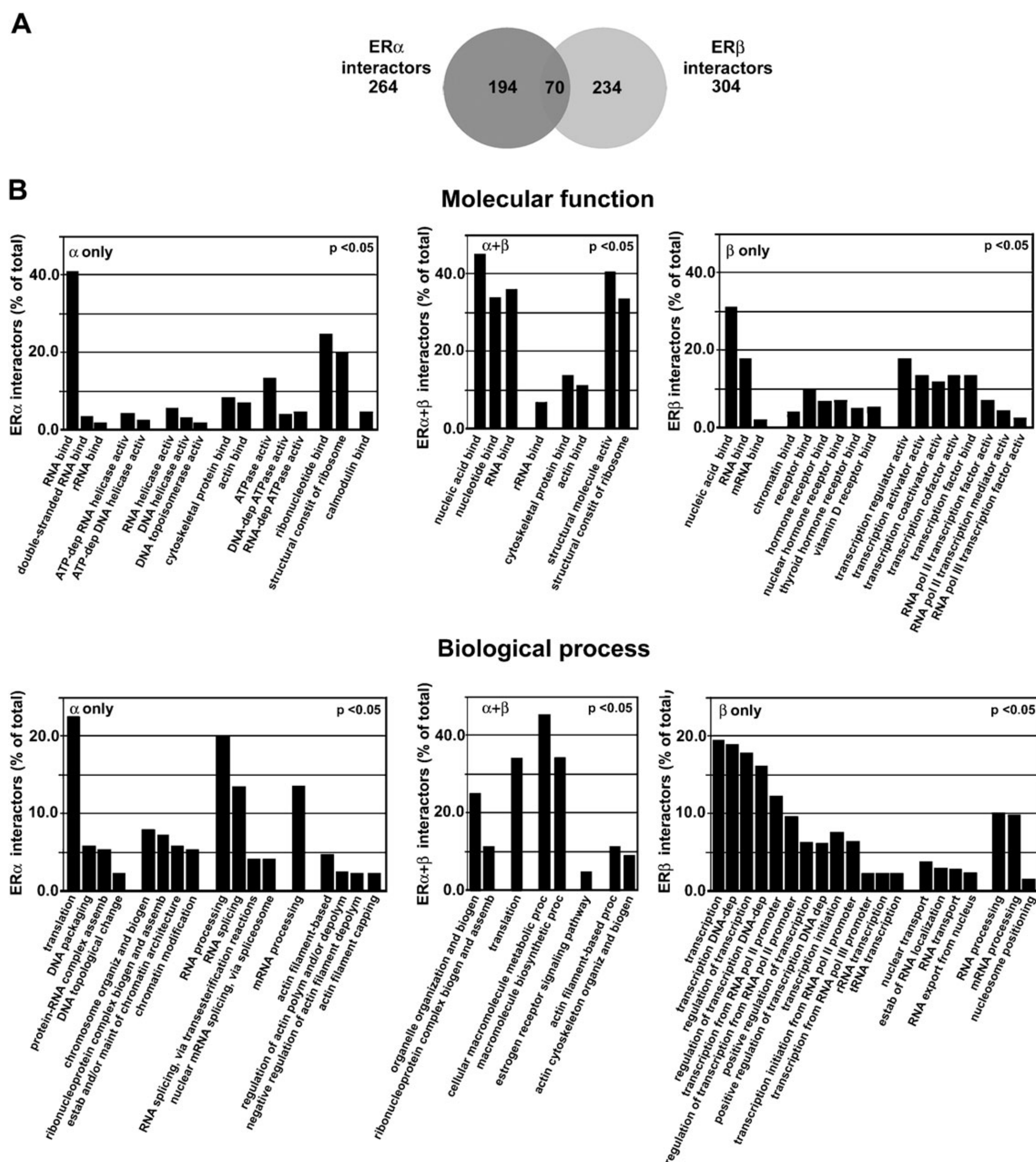

Fig. 1 (A) Venn diagram of total ERα and -β specific interacting proteins identified. (B) Functional annotation tool according to Gene Ontology analysis of total ERα specific, ERβspecific and ERα-βcommoninteracting proteins.

distribution (*i.e.* how many interactions has each node) and closeness (a measure of the average number of nodes connecting a protein to all other proteins). All these information are integrated in the network representations shown in Fig. 2 and 3. We found that ERα (the diamond-shaped node labelled ESR1 in Fig. 2) presents a lower average degree with respect to the average degree of the network (4 compared to 15 989, $p < 0.05$). Conversely, in the ERβ network shown in Fig. 3A and B, both receptors (indicated as the diamond-shaped nodes labelled ESR1 and ESR2) present a degree value comparable with respect to the average degree of the networks (respectively 7 for ESR1 and 8 for ESR2 and 8188 for the network). This result suggests that ERα participates in a lower number of biological functions in its networks compared to ERβ. In other words, a lower number of interactions suggests a lower number of functions. The average node degree (*i.e.* the average number of interactors for each proteins) is appreciably different between the two interactomes (15 989 for ERα *vs.* 8188 for ERβ) suggesting that the proteins of the first interactome could exert more biological functions. In contrast, both networks present a comparable value of the clustering coefficient (0.388 and 0.326, respectively) and the same network diameter (12). This similarity is not surprising, as both networks were discovered with the same experimental platform (TAP coupled to LC-MS/MS) and may demonstrate a lack of high level of modularity (*i.e.* a high number of subnetworks/protein complexes) in both networks. Subsequently, we performed a connectivity analysis, aimed at investigating the distribution of interactors. Fig. 2 and 3 report the behaviour of node degree (number of interactors) and closeness (*i.e.* how a protein is close to all the others) for the two networks. This analysis has two main objectives: (i) to evidentiate a subset of

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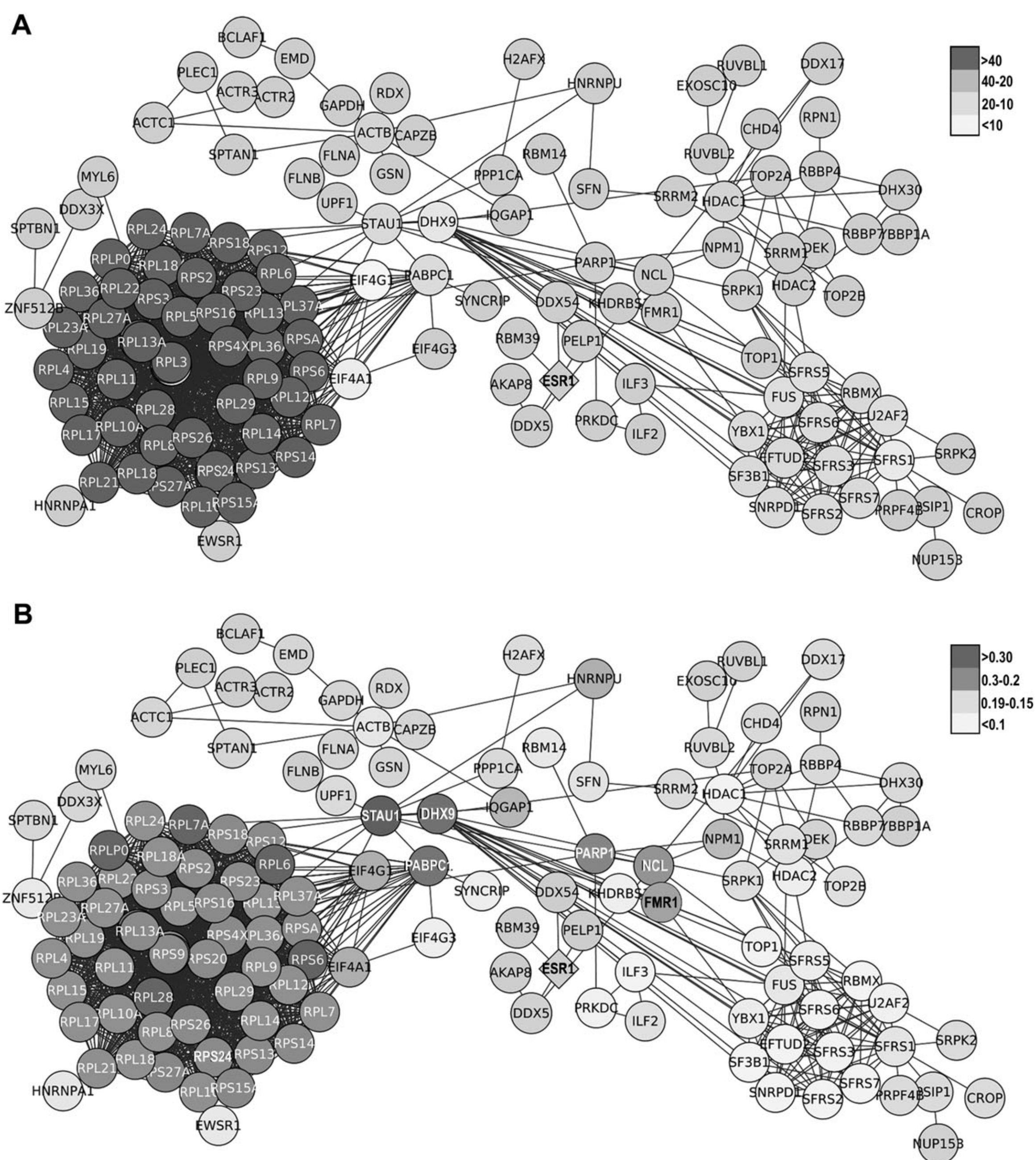
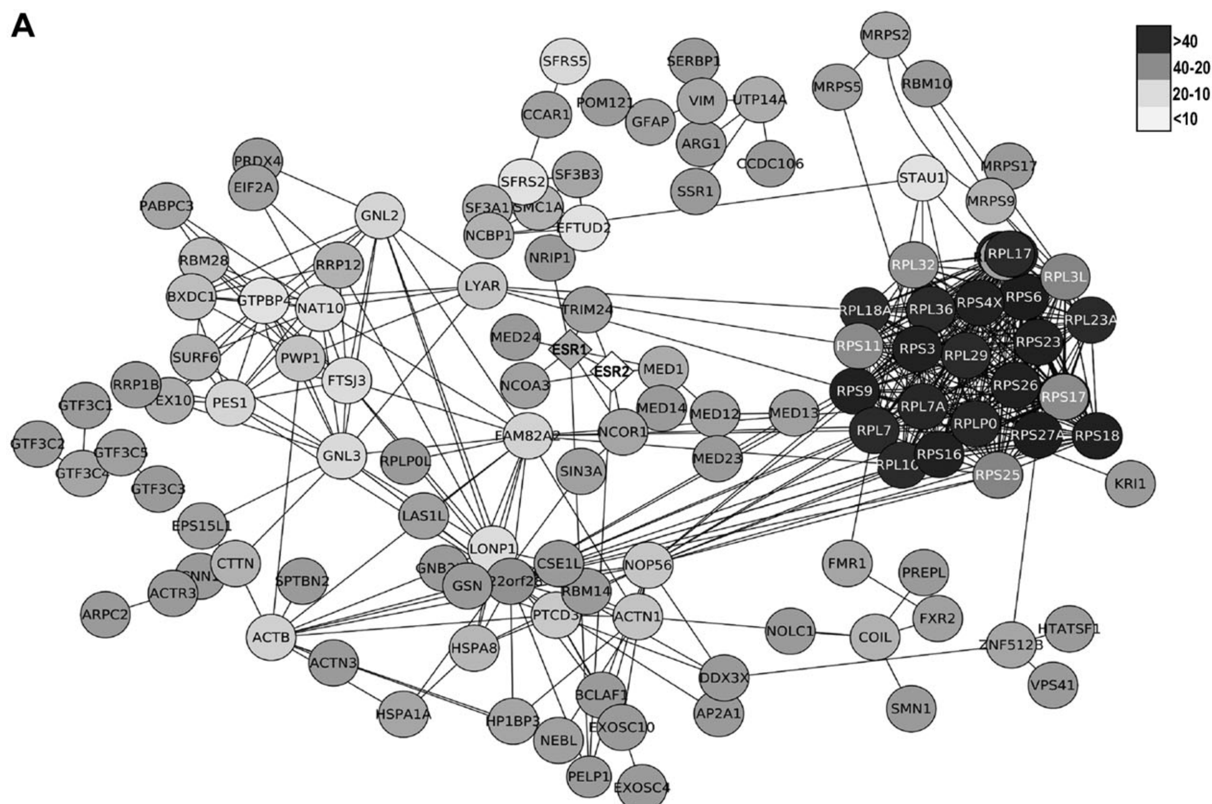


Fig. 2 Distribution of node degree (panel (A)) and closeness centrality (panel (B)) in ERα network, determined using Cytoscape software. Lighter grey shades indicate nodes with a low value of node degree and closeness centrality, respectively. Proteins are depicted using round shaped nodes except for ESR1 represented by a diamond shaped node.

relevant proteins, *i.e.* a subset of proteins with a high number of interactions and possibly participating in a high number of functions; (ii) to identify differences between two interactomes or two receptors. In particular, Fig. 2A and B depict the node degree and closeness distribution for ERα network,while Fig. 3A and B show the same parameters for ERβ. For better readability, the lighter grey shades represent low values of node degree (interactors) and closeness centrality. As expected, a positive correlation of the two parameters is evident for a considerable subset of proteins, such as ribosomal proteins in ERα and ERβ networks. In this way a considerable number of proteins have a high value of both parameters. ERs, on the other hand, exhibit a slightly different behaviour. Considering, in fact, ESR1 in ERα interactome, we found a low value of closeness centrality with respect to the network corresponding to the low value of neighbours. This suggests that ESR1 needs a considerable number of intermediate proteins to play its role. On the other hand, for ESR1 and ESR2 in ERβ networkwe found a positive correlation between the average degree and the closeness centrality, suggesting a key role for both proteins in the interactomes. Concerning connectivity analysis,[44,45] the combined use of degree values and closeness centrality reveals a remarkable relevance of a subset of proteins in the



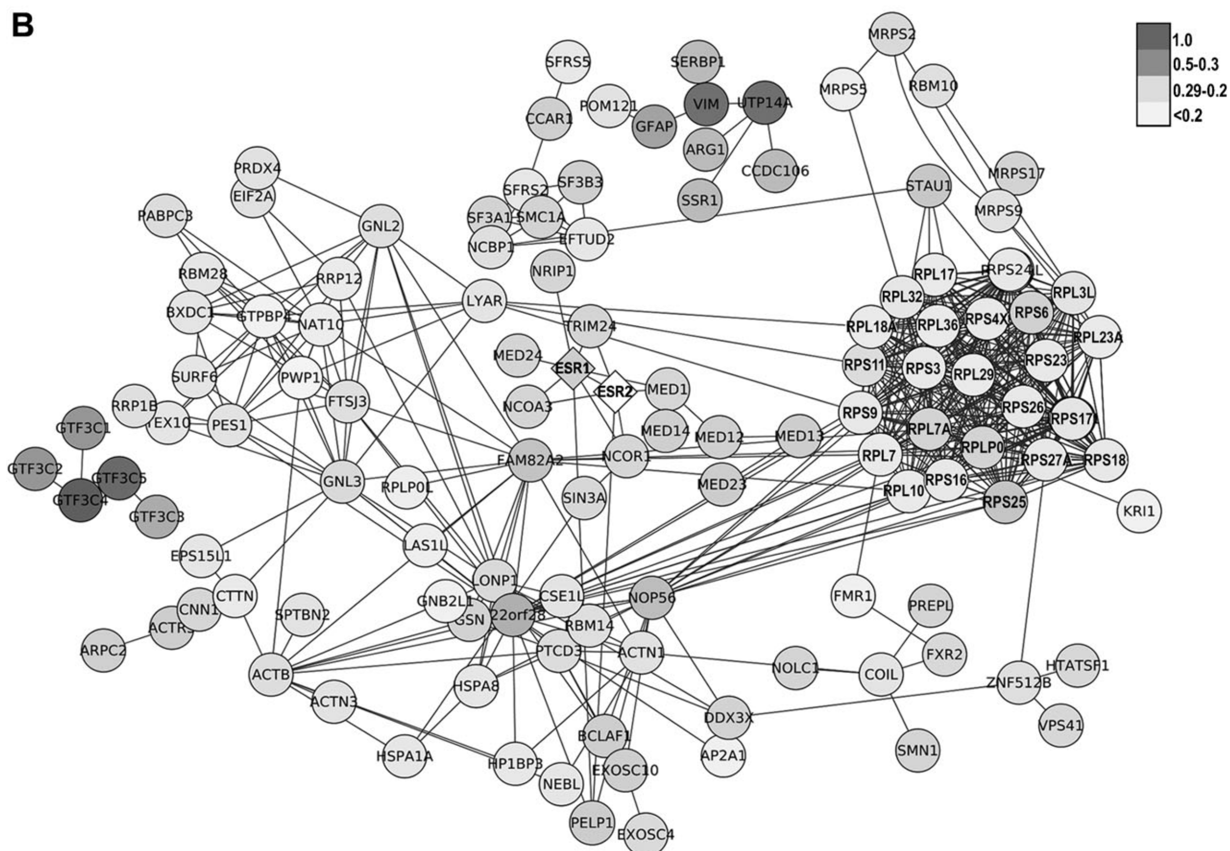


Fig. 3 Distribution of node degree (panel (A)) and closeness centrality (panel (B)) in ERβ network, determined using Cytoscape software. Lighter grey shades indicate nodes with a low value of node degree and closeness centrality, respectively. Proteins are depicted using round shaped nodes, except for ESR1 and ESR2 that are represented by diamond shaped nodes.

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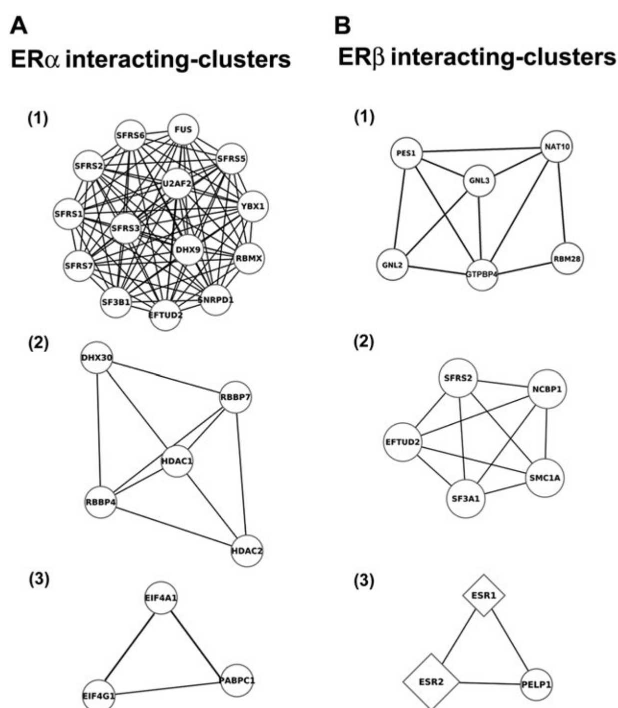

Fig. 4 Representative, non-redundant clusters from either network. In particular ERα subnetworks are drawn in panel (A) and those of ER > in panel (B).

two networks. In particular, considering ERα our study suggests a preeminent role for ACTB, DHX9, PABPC1, STAU1, EIF4A1, and EIF4G, while for ERβ the analysis reveals a relevant role for C22orf28, NOP56, FAM82A2, GNL2, GNL3, and ACTB proteins. All these proteins are characterized by a high value of degree and closeness centrality. This result suggests that all these proteins participate in many biological functions in the interactome. After the global topological analysis, we performed an exhaustive search for biologically relevant and statistically significant subnetworks, summarized in Fig. S2 (ESI†). This analysis revealed 3 non-redundant protein complexes for both ERα (Fig. 4A) and ERβ (Fig. 4B). These results, when combined, indicate that the differences observed in the global functional analysis shown in Fig. 1 were confirmed by bioinformatic dissection of the two networks.

Functional differences between ERα and ERβ revealed by sub-network analysis of their interactomes

The results described above provide interesting information concerning the mechanisms sustaining the different actions of the two receptors in the control of key biological processes in breast cancer cells. This is based on the assumption that the biological activity of any regulatory protein depends upon its ability to associate with specific partners in the cell. The numerical gap between the common interactors and those specific for each receptor was functionally confirmed by Gene Ontology analysis, in which both common and specific BP and MF were outlined. The next step, concerning the study of the topological features within ERα and -β complexes, again underlined a peculiar behavior for each ER-driven interaction network. The differences between the two ER subtypes are clearly highlighted also from the network dissection analysis. This analysis points to a relevant role in the network of several proteins among the interacting partners of each receptor.

In particular, concerning ERα, network dissection revealed 3 primary clusters (Fig. 4A). Cluster 1 indicates the presence of a sub-network whose components are involved in both transcription and splicing. These fundamental biological processes involve, in addition to DNA-protein and RNA-protein, also protein–protein interactions. They are coordinated and, sometimes (*e.g.* in alternative splicing), contextual within the cell, depending upon the relative abundance of the different SR proteins and the degree of promoter occupancy by different transcription factors.[46] Besides the previously described role of splicing factors such as SR proteins, U2AF2, FUS and RBMX in coupling these events,[30] we observed their clusterization with EFTUD2 and SNRPD1, also important for spliceosome assembly,[47] and YBX1. This last ERα partner is particularly interesting, since it is a transcription factor that, on one side regulates alternative splicing site selection through the interaction with SR proteins,[48] and on the other it promotes cell proliferation in breast cancer cells by transcriptional activation of cell cycle genes such as cyclins, whose expression is induced in response to mitogenic signals.[49] This is in line with the well known ERα effects on breast cancer cell proliferation. Indeed, On the other hand, ERα-copurifying Cluster 2 represents a chromatin remodelling complex also called 'core histone deacetylase complex', involved in regulation of ER functions in breast cancer. Histone modifications regulate a dynamic transition between transcriptionally active and silent chromatin. HDAC1 and -2 are known to interact with ERα both *in vitro* and *in vivo* and participate in the regulation of cell proliferation, apoptosis and gene expression. Their role in breast cancer is still poorly understood, but it has been reported that HDAC inhibitors are able to repress cell growth and proliferation.[50,51] HDACs are known to act in concert with retinoblastoma associated proteins 46 and 48 (RBBP4 and -7), also present in this cluster, in modulating gene expression.[52] DHX30 also belongs to the HDAC co-repressor complex, that is believed to be strongly associated with ERα in the absence of hormone. Finally, Cluster 3 reveals ERα interaction with a key complex involved in translational regulation. The proteins belonging to this cluster are predominantly found associated to the translational initiation complex form that plays a major role in the binding of the 3′ Poly (A) tail of mRNA (PABPC1) and in 5′ cap recognition (EIF4A1 and EIF4G1).[53] It is known that high expression of these proteins is frequently observed in cancer, where it is associated with malignant progression, since they regulate translation of proteins correlated with cell growth, such as cyclin D1.[54] Among the other components of this sub-network, EIF4G1 can enhance cap-independent translation of mRNAs containing internal ribosome entry site[55] and, moreover, it has been found over-expressed and correlated with angiogenesis in inflammatory breast cancer.[56] Indeed, these proteins have also been demonstrated to shuttle between the cytoplasm and nucleus, where they are mainly localized into the speckles, subnuclear structures rich in pre-messenger RNA splicing factors. Here they are supposed

to be involved in trafficking of RNA-binding proteins and pre-messenger RNA processing.[57,58]

On the other hand, ERβ network Cluster 1 (Fig. 4B) is characterized by the presence of Nucleolar GTP-binding protein 1 (GTPBP4), Guanine nucleotide-binding protein-like 2 (GNL2), Guanine nucleotide-binding protein-like 3 (GNL3) and Pescadillo homolog (PES1) play an important role in regulation of cell growth and proliferation. Nucleolar proteins, in particular GNL3, have been shown to regulate cell growth by controlling ribosome biogenesis,[59] whose deregulation contributes to tumorigenesis. The components of this cluster are able to modulate these processes through their direct physical interaction with p53 and/or a role in modulating its activity.[60] Moreover, GTPBP4 is involved in 60S ribosome biogenesis and is directly involved in breast cancer cell survival through reciprocal regulation with p53. Indeed, overexpression of this protein is associated with reduced survival and p53 expression in these cells.[61] PES1 also exhibits a similar behaviour, since it is involved in ribosomal biogenesis and its downregulation inhibits proliferation and tumorigenesis of the same cell type.[62] Since both GTPBP4 and PES1 are upregulated in breast cancer compared to normal mammary epithelial cells,[61,62] their physical association to ERβ could influence their activity, thereby interfering with their ability to promote p53 downregulation and/or cyclin D1 upregulation, with significant effects on cell survival and proliferation. This evidence supports the suggested role of ERβ in tumor suppression and antiproliferative activity. The ERβ interacting proteins of Cluster 2, on the other hand, are involved in multiple key cellular mechanisms. Together with the RNA splicing factors SFRS2 and EFTUD2, in common with ERα (see Fig. 4A), other components of this cluster have been described to have interesting properties. In particular, structural maintenance of chromosomes protein 1A (SMC1A) is involved in chromosome cohesion during cell cycle and in DNA repair,[63] plays a role in spindle pole assembly during mitosis and participates in DNA repair *via* its interaction with BRCA1.[64] BRCA1 is a known tumor suppressor that plays a role also in the inhibition of breast cancer cell proliferation.[65] These data are in agreement with the known ability of ERβ to regulate cell cycle in breast cancer cells. Proline glutamic acid and leucine-rich protein 1 (PELP1), present together with both ERα and -β in the third sub-network identified (Cluster 3 in Fig. 4B), provide a link with both genomic and non-genomic actions of these receptors. This protein, first identified as an ERα transcriptional co-regulator and recently shown to act as a reader of H3 histone methylation marks playing a crucial role in modulating the histone code at estrogen target genes,[66] is also one of the components of the non-genomic ER signalosome that control breast cancer cell migration and metastatic potential.[67]

Finally, among the proteins in common between the two networks[30,31] it is worth mentioning β-actin (ACTB) for its known role in estrogen signaling.[29,67,68] ERα and β-actin are involved in several nuclear pathways, including regulation of target gene activity, chromatin remodelling and ribosome biogenesis, as well as reorganization of nuclear territories enhancing interchromosomal interactions among specific ERα-bound transcription units.[67] We observed significant differences in β-actin interaction with ERβ with respect to ERα (R. Tarallo, G. Nassa and A. Weisz, unpublished results), suggesting a differential role of the two ER subtypes in control of the nuclear actin network by estrogens.

Conclusions

Our study demonstrates that combination of experimental and computational analyses represents a useful approach to investigate large sets of proteins, discriminating peculiar activities involving specific protein–protein interactions creating differential functional clusters. The data obtained provide new clues on specific functions of each ER in breast cancer cells. Global analysis of the interactomes and specific subnetworks associated with either ERα or ERβ suggests the frequent involvement of the two receptors in the same process, that results however in divergent effects based upon recruitment of different key proteins.

Acknowledgements

Work supported by the European Union (CRESCENDO I.P., contract n.er LSHM-CT2005-018652), the Italian Association for Cancer Research (Grant IG-8586), the Region Campania (grant Legge 5/2007), the Italian Ministry for Education, University and Research (grant PRIN 2008CJ4SYW_004) and Fondazione per il Sud (grant 2009-PdP-22) to AW.

This work was presented at the 5th Annual National Conference of the Italian Proteomics Association held in Florence 9–12th June 2010.

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