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REVIEW ARTICLE

SRA and its binding partners: an expanding role for RNA-binding coregulators in nuclear receptor-mediated gene regulation

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Abstract

The discovery that SRA RNA can function as a nuclear receptor (NR) coactivator resulted in a fundamental change in the perception of how NRs and their coregulators may regulate hormone signaling pathways. The subsequent identification of molecules capable of binding SRA, including SHARP, p68, and more recently SLIRP, which also function as coregulators, has further broadened our understanding of NR-dependent gene regulation. The integral role that NRs play in directing developmental, metabolic and pathological programs of transcription has defined them as paramount targets for treating a broad range of human diseases. Thus with a greater understanding of SRA and its interactions with its binding partners, novel RNA–protein interactions may be identified and exploited for therapeutic gain. Here we discuss the isolation of SRA, its impact on NR activity and interactions with known binding partners.

Keywords: *SRA; RNA-binding proteins; SLIRP; coregulators; nuclear receptor*

The nuclear receptor superfamily and coregulators

The nuclear receptor (NR) superfamily of ligand-inducible transcription factors are key regulators of pathways directing metabolism, development and reproduction (McKenna and O'Malley, 2002). Ligands activating these receptors, and ultimately the transcription of hormone-responsive genes, include sex steroids (progestins, estrogens, and androgens), glucocorticoids, mineralocorticoids, vitamin D, thyroid and retinoid hormones, as well as a variety of metabolic ligands. Membership of the NR family is based on structural characteristics with each containing an amino-terminal activation function (AF-1) domain, DNA-binding domain, a hinge region, and a carboxy-terminal ligand-binding region containing a second AF-2 domain (Westin et al., 2000). The molecular events associated with ligand binding, receptor activation and nuclear translocation, dimerization and binding of cognate DNA response elements have been well characterized for many of the individual NRs (Robyr et al., 2000; McKenna and O'Malley, 2002; Nettles and Greene, 2005).

The discovery that NR coregulators are recruited to receptor conjugates modulating their activity has dramatically changed our understanding of hormone action. The past decade has revealed a host of such molecules that, in response to ligand, are either recruited or released from complexes with NRs, modify binding to hormone response elements, interact with elements of the cellular transcriptional and translational machinery and ultimately modulate NR activity (McKenna and O'Malley, 2002; Roeder, 2005). Specific coregulators possess enzymatic activity that can alter chromatin structure (histone acetylase) or methylation status (methyl transferase), act as kinases, ubiquitin ligases or have ATPase activity. In general, coactivators augment transactivation while corepressors reduce NR target gene transcription. Significantly, the ratio of coactivator to corepressor molecules has been shown to alter the

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responsiveness of individual tissues to both NR agonists and antagonists (Smith and O'Malley, 2004).

The biological significance of coregulator activities has been highlighted in knockout studies where the majority are embryonically lethal (Lonard et al., 2007). Viable knockouts and transgenic animal investigations have exposed what may be considered subsets of NR action. For example, female mice lacking SRC-3 have reduced fertility and attenuated mammary gland development (Xu et al., 2000) while mammary gland hyperplasia and accelerated differentiation with high incidence of mammary tumour development characterize mice over-expressing SRC-3 (Torres-Arzayus et al., 2004). Differential expression of coregulators can have significant clinical relevance in humans. Again using SRC-3 as an example, its over-expression is associated with increased tamoxifen resistance in breast cancer patients and a poorer clinical outcome (Osborne et al., 2003). Together these studies demonstrate that changes to NR coregulator expression and activity may have biological consequences comparable to those caused by alterations to receptors they modulate.

SRA – a remarkable RNA coactivator

Until 1999, each of the NR coregulators identified were proteins. However, with the identification of Steroid Receptor RNA Activator (SRA) it was found that noncoding transcripts (SRA) as well as the protein products (SRAP) of this gene were capable of acting as NR coregulators (Lanz et al., 1999) (Figure 1). As detailed below, the SRA clones originally identified contained the hallmarks associated with coding sequences; however, initially no protein product was detected by antisera raised against peptides coded for by the cDNAs isolated and their *in vitro* translation failed to yield detectable peptides. This led to an extensive study of the activities of the SRA RNA which was found to coactivate as a transcript (Lanz et al., 1999; 2002). This characteristic was confirmed by mutational studies demonstrating no change in coactivation as a result of altering putative protein initiation sites or inducing frame shift mutations in the transcript. Further, discrete loops of the RNA were required for the full coactivator activity of SRA (Lanz et al., 2002).

Following its isolation in conjugates with progesterone receptor (PR), SRA has been demonstrated to coactivate a range of NRs, including estrogen (ERα and β), androgen (AR), glucocorticoid (GR,) retinoic acid (RARα), peroxisome proliferator activated receptors (PPARδ and γ), thyroid (TR) and vitamin D receptors (VDR) (Lanz et al., 1999; Deblois and Giguere, 2003; Kawashima et al., 2003; Zhao et al., 2004; Hatchell et al., 2006). In addition to NRs, SRA has been shown to coactivate MyoD activity (Caretti et al., 2006); however, its failure to potentiate the activities of GAL4, Sp1, E2F, E47, and CREB suggests it is not a general coactivator of all transcription factors (Lanz et al., 1999; Zhao et al., 2007).

The SRA gene is well conserved across species and is composed of five exons in human, rat and mouse genomes (Figure 1). Conventional Northern analysis indicated that SRA expression is ubiquitous, but with elevated transcript levels being present in skeletal muscle, heart and liver, while very little is present in brain (Lanz et al., 1999). Several alternative SRA transcripts have been identified (Leygue, 2007). Initially three SRA isoforms were described, with unique 5′ and 3′ ends but a shared common 'core' domain (Lanz et al., 1999). Protein initiation and polyadenylation sequences were identified in these SRA isoforms; however, a reading frame of no greater than 162 amino acids (aas) was predicted, and as discussed above, no evidence of a translated product was detected. Subsequently, human SRA isoforms containing larger open reading frames have been found (Emberley et al., 2003). For example, SRA1 codes for a 236 aa protein, referred to as SRAP, the carboxy-terminal 162 aas of which are identical to those predicted by the SRA sequence originally described by Lanz and co-workers

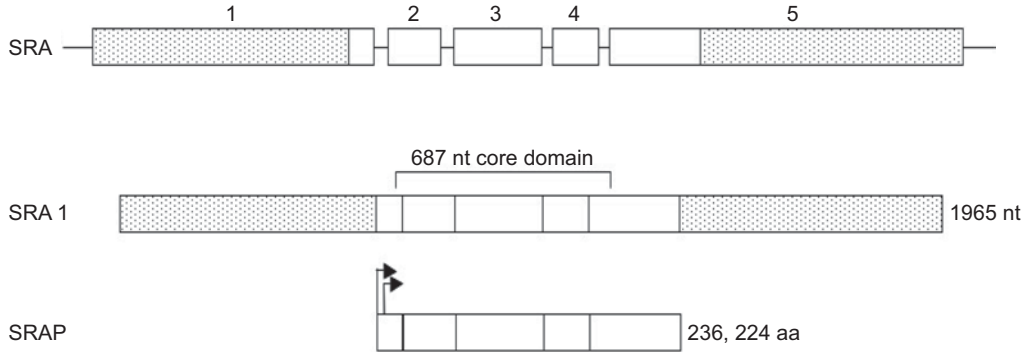

Figure 1. SRA and its products. The human SRA gene comprises five exons over a region of approximately 6.5 kb at 5q31.3 (Lanz et al. 2002). Multiple SRA transcripts containing a 687 nt core domain have been isolated and found to coactivate nuclear receptor activity (Leygue, 2007). The presence of two initiation codons in exon 1 leads to the translation of two SRAP proteins of 236 or 224 aa from the SRA1 isoform transcript (Emberly et al. 2003).

but with an additional 73 amino-terminal aas. Sequence database analyses predict that over 20 chordata species express a SRAP protein (Leygue, 2007) and anti-human SRAP antisera was able to detect appropriately sized proteins in cow, rabbit, chicken, pig, sheep and avian species (Chooniedass-Kothari et al., 2004). Consistent with the presence of two initiating methionines separated by 36 nucleotides in the SRA1 transcript, two SRAP proteins of 31 and 32 kDa have been detected (Figure 1), which are present in both the nucleus and cytoplasm. As discussed below, differential splicing of the SRA transcript may generate a noncoding RNA that is the target for a range of RNA-binding coregulators and a translated product whose expression may be important in human breast cancer (BCa) (Hube et al., 2006).

SRA and tumorigenesis

Multiple lines of evidence implicate SRA in human tumorigenesis of various tissues. Elevated and/or aberrant SRA expression has been observed in human breast, ovary and uterine tumors and a range of cell lines (Leygue et al., 1999; Lanz et al., 2003; Hussein-Fikret and Fuller, 2005). In studies of BCa samples, expression of an exon 3 deletion mutant of SRA (SRA-Del) but not the wild type molecule correlated with a higher tumor grade (Leygue et al., 1999; Murphy *et al.*, 2000). Notably, mutation of SRA stem-loop SDR10, encompassed by the SRA-Del deletion, results in a 50% reduction in transactivation compared with wild type SRA in transient transfection assays (Lanz et al., 2002). Protein products of the SRA gene may also play a role in BCa tumor growth, as recent data suggests that patients with SRAP-positive tumors have lower recurrence rates and improved outcomes (Chooniedass-Kothari et al., 2006). Consistent with this observation, over-expression of SRAP reduced ER-luc reporter activity in the MCF-7 BCa cell line (Chooniedass-Kothari et al., 2006). However, contrasting data resulted from antisense oligonucleotide directed SRA depletion in the MCF-7 line, where expression of the endogenous ER target gene pS2 was elevated in response to the loss of SRA and a mild increase in cell proliferation was observed (Cavarretta et al., 2002). Interestingly, MMTV-SRA transgenic mice over-expressing the core region of SRA but not encoding the full length SRAP protein in breast tissue demonstrate increased mitosis and elevated mammary epithelium cell death but no significant increase in tumor incidence (Lanz et al., 2003). When these MMTV-SRA transgenic mice were crossed with MMTV-ras mice, surprisingly, there was reduced frequency of mammary tumor development in the resultant progeny compared with MMTV-ras single transgenic animals. Together these data suggest that SRA gene products influence mammary epithelial cell growth, but the precise roles of SRA and SRAP in tumorigenesis remain to be determined.

Mechanism of SRA action as an RNA coactivator

Secondary structure predictions suggest the existence of multiple stem-loops within SRA RNA (Lanz et al., 2002). Extensive mutational analysis showed that discrete stem-loops are critical for SRA's coactivation activity, although deletion of individual structures only partially reduced the ability of mutant molecules to coactivate relative to wild type transcripts (Lanz et al., 2002). This led to the conclusion that multiple RNA substructures work together to effect SRA's overall coactivator function. Initial studies indicated that SRA coactivates a range of NRs, including ERα and β, PR, and GR in a ligand-dependent manner. Subsequently it has been reported that SRA can also augment ERα activity in a ligand-independent manner through its AF-1 domain involving MAPK and phosphorylation of S118 of the receptor (Watanabe et al., 2001; Deblois and Giguere, 2003). Further supporting this AF-1 dependent activity was the lack of coactivation of both ERβ and S118 mutated ERα in the absence of ligand. In contrast, Coleman and co-workers recorded only a minor loss of coactivation when the activity of an S118A ERα AF-1 GAL4 DBD chimeric protein was compared with that of the wild type representative in a mammalian transactivation assay (Coleman et al., 2004). This group also demonstrated that SRA enhances 4-hydroxytamoxifen agonist activity, but not that of the pure antagonist ICI 182,780 suggesting that the ability of SRA to coactivate ER activity is not only NR subtype but ligand and potentially model specific.

In studies more closely examining the activity of SRAP, over-expression of SRA1 in MCF-7 cells generated conflicting results (Chooniedass-Kothari et al., 2006). In clones constitutively expressing SRAP, ER reporter gene activity was lower than in control cells; however, when expression of the endogenous ER target PR was assessed, its levels were elevated (Chooniedass-Kothari et al., 2006). Given the existence of both coding and non-coding SRA RNAs, and their opposing activities, it has been proposed that differential splicing of its transcripts may regulate the balance between each product and the overall effect of SRA gene expression (Hube et al., 2006).

SRA, p68 and p72 coactivation of MyoD

SRA is present in conjugates with the skeletal muscle differentiation factor MyoD and p68/p72 (Caretti et al., 2006). Over-expression of SRA augments MyoD activity, while its siRNA mediated reduction results in

reduced myoblast differentiation and muscle specific gene expression (Caretti et al., 2006). Coactivation of MyoD by SRA is potentiated by both p68 and p72, while depletion of p68 expression inhibited both adipocyte differentiation and lineage specific gene expression (Kitamura et al., 2001) along with myoblast differentiation (Caretti et al., 2006). Complementing these data, chromatin immunoprecipitation (ChIP) assays showed that in response to decreased p68/72, reduced TATA and pol II recruitment to the myosin heavy chain IIb promoter occurred. Notably, not all genes activated by MyoD are modulated by p68/72 or SRA (Caretti et al., 2006). p68 is recruited to a subset of regulatory regions directing muscle gene expression in differentiated skeletal muscle cells but is absent from the same sites in myoblasts (Caretti et al., 2007). Curiously, p68 mutants lacking the ability to bind ATP still coactivated MyoD, indicating that the helicase activity of these DEAD box proteins is not required for augmenting SRA's activity. It has been proposed that p68 and p72 may act as RNA chaperones regulating the assembly/disassembly of RNA–protein complexes by facilitating the formation of optimal RNA secondary structures resulting in NR coactivation (Watanabe et al., 2001; Caretti et al., 2007).

SRA and binding proteins involved in NR signaling

Rare amongst members of the NR super family is the ability to directly bind RNA. To date, this characteristic has been demonstrated for DAX-1, an orphan receptor involved in steroidogenesis, the mutation of which is associated with adrenal hypoplasia congenita and associated hypogonadotrophic hypogonadism (see review Lalli and Sassone-Corsi, 2003). This group also demonstrated RNA homopolymer binding for RARα and γ (Lalli et al., 2000).

At present the only NRs demonstrated to directly bind SRA are TRα1 and 2 and TRβ1 (Table 1). TRα and β are transcribed from separate genes and produce several proteins via alternate splicing and promoter usage (Brent, 1994). TRα1 and 2 are identical for their first 370 aas but the carboxy-terminal 40 aas of TRα1 are replaced by a unique 122 aa carboxy-terminus in TRα2. Unlike TRα1 and β, TRα2 does not bind triiodothyronine (T3) and transactivate TR target gene expression. However, all three forms can bind SRA, via a 41 aa region between their second zinc finger and ligand-binding domains (Xu and Koenig, 2004; 2005). Investigations of TRα1 and 2 have shown that phosphorylation of TRα2 at CK2 kinase sites results in its loss of SRA binding and a change in its localization from predominantly nuclear to mainly cytoplasmic. In contrast, alterations in the phosphorylation state of TRα1 have little impact on its ability to bind SRA or alter its nuclear localization. These data support a model whereby SRA is bound by the ligand insensitive and transcriptionally silent TRα2 in the nucleus when phosphorylated, acting as a repressor by sequestering SRA in inactive NR complexes (Figure 2). However, following phosphorylation,

Table 1. Proteins demonstrated to bind directly to SRA or be present with it in complexes.

Protein	Direct interaction	Complex formation	Method of detecting interaction	RNA-binding domain	References
SHARP	✓		*In vitro* binding assays, IP-RT-PCR, REMSA	RRM (x3)	Shi *et al.*, 2001; Hatchell *et al.*, 2006
p68	✓		IP-RT-PCR	DEAD-Box	Watanabe *et al.*, 2001; Caretti *et al.*, 2006
p72	✓		REMSA, IP-RT-PCR	DEAD-Box	Watanabe *et al.*, 2001
PUS1	✓		Pseudouridylation, *in vitro* pull down-RT-PCR	None	Zhao *et al.*, 2004
PUS3	✓		Pseudouridylation	None	Zhao *et al.*, 2007
TRα&β	✓		*In vitro* binding assays, IP-RT-PCR	Unique, ssRNA affinity	Lanz *et al.*, 1999, Xu & Koenig 2004
SLIRP	✓		Y3H, UVXL, REMSA, IP-RT-PCR	RRM	Hatchell *et al.*, 2006
SRC-1		✓	Co-purification, IP-RT-PCR	None	Lanz *et al.*, 1999, Hatchell *et al.*, 2006
AR		✓	Co-purification, IP-RT-PCR	None	Lanz *et al.*, 1999
PR		✓	Co-purification	None	Lanz *et al.*, 1999
SRC-2/TIF2		✓	IP-RT-PCR	None	Watanabe *et al.*, 2001
ERα		✓	IP-RT-PCR	None	Watanabe *et al.*, 2001
RAR		✓	IP-RT-PCR	None	Zhao *et al.*, 2004
Myo D		✓	IP-RT-PCR	None	Caretti *et al.*, 2006

IP-RT-PCR = immunoprecipitation RT-PCR assay; REMSA = RNA gel shift assay; Y3H = yeast three-hybrid screening.

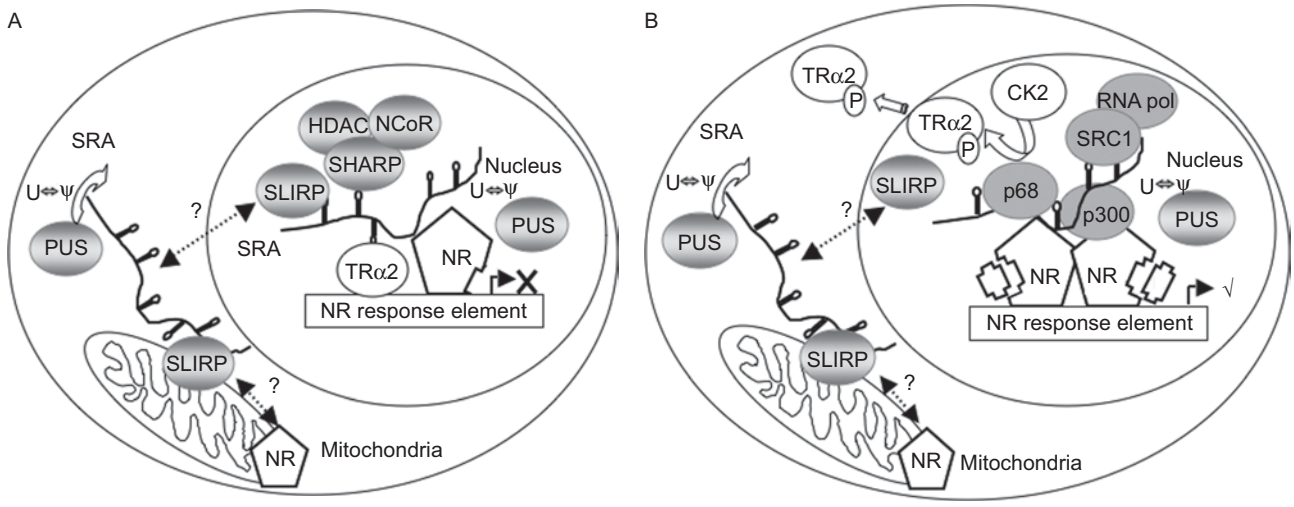

Figure 2. SRA in nuclear receptor complexes and signaling. Coactivation of NR activity by SRA may be regulated at multiple points within corepressed and coactivator settings. (A) Typically in the absence of ligand (L), nuclear receptors (NRs) present at target response elements form conjugates with corepressor molecules such as SHARP, NCoR and SLIRP. In the absence of ligand, some NRs (eg TRα2) may be able to bind and sequester SRA reducing its coactivator capacity. In addition to recruiting enzymes including HDACs that repress transcription through interactions with the chromatin, corepressors may compete for SRA binding with coactivator molecules such as SRC-1 and p68. Also, in a repressed state, PUS3 may act on discrete uridines (U) within the SRA transcript, converting them to pseudouridine (Ψ) to induce secondary structures that are conducive to repressor not coactivator recruitment. (B) Following exposure to ligand, corepressors are released from NR complexes in favor of coactivators which interact with elements of the cells transcriptional machinery including RNA polymerase II (RNA pol). In addition, ligand may promote phosphorylation of NRs, which in the case of TRα2 could result in relocation from the nucleus to the cytoplasm, potentially releasing SRA for coactivation function. Recruitment of coactivators (eg SRC-1) may then be facilitated by SRA acting as a scaffold, the secondary structure of which is modified by PUS and p68 to alter coregulator binding affinities. The mitochondrial and nuclear localization of individual coregulators and NRs themselves suggest organelle-specific activities for these molecules and that communication between separate compartments is required to coordinately regulate NR function.

TRα2 would lose its ability to bind SRA and exit the nucleus, thereby releasing SRA for participation in coactivator complexes. Further validation of this model is required, as over-expression of TRα2 only weakly inhibits TRα1 and β activity but such a system does offer a mechanism where down regulation of NR activity is the default as CK2 activity is constitutively active (Meggio and Pinna, 2003).

Adding a further layer of complexity to the regulation of NR activity, two pseudouridine synthases (PUS1p and 3p) have been reported to pseudouridylate SRA transcripts and potentiate SRA coactivation of NR activity (Zhao et al., 2004; 2007). PUS 1 has been shown to coactivate a range of NRs including RARγ, TR, ER, GR, AR and PR-B. In contrast, although PUS3 can coactivate VDR, TR and GR, it does not have a similar effect on the sex steroid receptors ER, PR and AR. In investigating this reduced range of activity, the Spanjaard laboratory found that PUS3 immunoprecipitated with RARγ but not ER, inferring that NR association is required for SRA-dependent coactivation by the PUS enzymes. While both these enzymes have common targets within SRA, they have been shown to modify different residues within the transcript as well. Further, the order in which SRA is processed by the individual PUS enzymes can alter patterns of pseudouridlyation, potentially influencing the binding of other coactivators and repressors. Significantly, mutation of U206 to adenine results in SRA becoming hyperpseudouridylated and a transcriptional repressor (Zhao et al., 2007). This has led to speculation that the activities of PUS1 and 3 may control a coactivator/corepressor switch in SRA. Alterations to PUS activity have clinical significance as mutation of human PUS1p causes mitochondrial myopathy and sideroblastic anemia (Bykhovskaya et al., 2004), suggesting a role for defective SRA-NR signaling in this disease (Patton et al., 2005). The existence of this posttranscriptional mechanism of SRA regulation further highlights its importance in regulating NR activity.

SLIRP, a small SRA binding protein, is a nuclear receptor corepressor

The identification of RNA-binding domains in multiple NR coregulators such as SHARP (Shi et al., 2001), PPARγ coactivator-1 (PGC-1) (Monsalve et al., 2000), coactivator activator (CoAA), coactivator modulator (CoAM) (Iwasaki et al., 2001), PIMT (Zhu et al., 2001), p68 (Katze et al., 1991), p72 (Watanabe et al., 2001), CAPERα and β (Dowhan et al., 2005) and a limited number of NRs,

including TRα1 & 2, TRβ (Xu and Koenig, 2004; 2005) and DAX-1 (Lalli et al., 2000) has generated much interest in the role of RNA–protein interactions in NR signaling (Table 1). Interestingly, the RNA recognition motif (RRM) RNA-binding domain containing proteins such as CoAA, CAPERα and β have been demonstrated to influence transcriptional and splicing events associated with NR target genes (Auboeuf et al., 2004; Dowhan et al., 2005). In the case of SMRT/HDAC1 associated repressor protein (SHARP), its repression of SRA-augmented transactivation is via the recruitment of histone deacetylase to receptor complexes (Shi et al., 2001).

The unique role of SRA as an RNA NR coregulator prompted further investigations to identify molecules that bound to it and potentially influence NR activity. To this end, our laboratory performed yeast three-hybrid analysis utilizing discrete loops of SRA shown to be required for maximal coactivation by SRA (Lanz et al., 2002). Using STR7, a key stem-loop implicated in SRA's transactivation capacity, to screen a human breast cancer library, we isolated a number of SRA binding proteins including a previously unidentified molecule SRA Stem-Loop Interacting RNA binding Protein (SLIRP) (Hatchell et al., 2006). The SLIRP gene was found to code for a small, 109 aa protein with a putative mitochondrial localization signal at its amino-terminal and a central RRM RNA-binding domain required to bind SRA. Overexpression of SLIRP was found to repress a broad range of NRs including ER, GR, AR, PPAR, TR and VDR, while its depletion by siRNA resulted in both increased NR reporter activity and endogenous target gene expression. Associated with these changes in transcriptional activity, chromatin immunoprecipitation (ChIP) studies showed that in response to reducing SLIRP levels in the absence of ligand, ER recruitment to target promoters was increased to levels approximating that observed following estrogen treatment of SLIRP replete controls. Complementing this increase in ER recruitment, markedly reduced NR corepressor-1 (NCoR) repressor binding was detected at the estrogen-responsive pS2 gene. It was further observed that, in the absence of SLIRP, there was a marked increase in SRA binding by the SRC-1 coactivator. Taken together, these data indicate that SLIRP acts as a repressor of NR activity by both influencing NR conjugate formation at promoter sites within the nucleus and coregulator interactions.

To investigate the interaction between SLIRP and SRA further, a series of experiments was performed using mutant forms of both molecules. The presence of an intact RRM was found to be essential for SLIRP's ability to bind SRA and repress transcription (Hatchell et al., 2006). Consistent with the observations of the O'Malley group (Lanz et al., 2002), over-expression of SRA with mutations to STR7 (i.e. the loop bound by SLIRP) resulted in reduced reporter gene coactivation compared with wild type transcript. Significantly, overexpression of SLIRP did not repress coactivation resulting from cotransfection with STR7 mutated SRA. From these and earlier yeast three-hybrid and REMSA data it was concluded that SLIRP specifically associates with, and mediates its activity via, its interactions with the STR7 loop of SRA. We were also able to demonstrate that SHARP also binds to SRA STR7 via its RRM domain (Hatchell et al., 2006). As a number of SRA-binding proteins have now been identified, detailed studies of their interactions with sequences within SRA are required to more fully elucidate the mechanisms of SRA mediated, NR coactivation.

SLIRP – a predominantly mitochondrial coregulator

SLIRP is widely expressed in normal human tissues but elevated in high energy demand organs such as liver, cardiac and skeletal muscle. Given SLIRP's NR repressive activities and demonstrated presence in the nucleus via ChIP assays, we were surprised to observe that it is predominantly a mitochondrial protein. However, this is consistent with the presence of a predicted mitochondrial localization signal at its amino terminus and that alterations to the amino terminus of SLIRP altered its cellular distribution (Hatchell et al., 2006). A similar pattern of expression has been reported for SRA (Lanz et al., 1999). Also, *in situ* hybridization studies by different groups have shown that SRA is predominantly found in the cytoplasm (Lanz et al., 2003; Zhao et al., 2007). Although experiments resolving SRA localization to the organelle level have not been presented, these data confirm that SLIRP and SRA are present in both nuclear and cytoplasmic compartments and may therefore be expected to perform tasks in both locations.

Although unexpected, the presence of SLIRP in the mitochondria adds to the mounting evidence describing NRs such as ER (Pedram et al., 2006), GR (Sionov et al., 2006) and TR (Morrish et al., 2006) in this organelle. For GR, mitochondrial translocation correlates with susceptibility to glucocorticoid-induced apoptosis (Sionov et al., 2006). In addition, mitochondrial DNA contains putative hormone-response elements that can be bound by NRs in DNA gel-shift studies (Morrish et al., 2006). The presence of NRs in both the nucleus and mitochondria raises the possibility that they could coordinately regulate gene expression in both locations directing key processes in metabolism and cell growth. The localization of PUS3 with its specific activities toward SRA in the cytoplasm and suspected role in the mitochondrion also raises the possibility of organelle specific regulation and or activity of SRA and its binding partners (Zhao et al., 2007) (Figure 2).

Although not located in the mitochondrion, there are parallels between SLIRP and another NR coregulator, RIP140. The latter is also preferentially expressed in tissues similar to SLIRP where it can suppress oxidative metabolism and mitochondrial biogenesis (Powelka et al., 2006). Furthermore, RIP140 is a powerful negative regulator of insulin-responsive hexose uptake and oxidative metabolism in mouse adipocytes and skeletal muscle (Seth et al., 2007). Depletion of RIP140 promotes the expression of genes involved in glucose uptake, glycolysis, the TCA cycle, fatty acid oxidation, mitochondrial biogenesis and oxidative phosphorylation, together with increased mitochondrial oxygen consumption. As predicted, RIP140 null mice are resistant to diet-induced obesity. Given that SLIRP corepresses PPARδ (Hatchell et al., 2006), is predominantly mitochondrial, and is expressed in high energy demand tissues, it will be of great interest to determine what genes are regulated by SLIRP and whether it plays a role in regulating energy homeostasis and mitochondrial biogenesis.

Targeting SRA–protein interactions for therapeutic benefit

The pivotal roles played by NRs such as the ER in breast and PPARs in energy metabolism define them and the pathways they direct as critical chemotherapeutic targets for the treatment of human disease. By defining critical interactions down to the residue level of resolution, molecules that can inhibit pro-proliferative interactions between SRA and its binding partners may be useful in the treatment of cancer, while augmentation of such interactions in bone or metabolic processes may be exploited for treating osteoporosis and type 2 diabetes. Significant lessons have been learned in the examination of other RNA coregulator interactions, such as that between Trans-activator of Transcription (TAT) and Trans Activation Responsive region RNA (TAR), resulting in the development of inhibitors for the treatment of HIV (for reviews see Stevens et al., 2006; Yang, 2005). Similar studies of SRA and its binding partners may provide the basis for manipulating these interactions for clinical benefit.

Summary

The identification of SRA as an RNA coactivator represented a paradigm shift in our understanding of coregulator regulation of NR signaling. The subsequent isolation of SRA-binding proteins such as SLIRP has opened up new avenues of investigation that will lead to a better understanding of the mechanism of SRA action and may yield novel targets for the treatment of diseases ranging from breast cancer to diabetes. In addition, the discovery of NRs and coregulators in mitochondria not only provokes challenging questions regarding the role of these molecules in controlling energy homeostasis and metabolism, but how they may coordinate signaling pathways between the nucleus and other organelles.

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