The RNA Coregulator SRA, its Binding Proteins and Nuclear Receptor Signalling Activity

Shane Colley and Peter Leedman*
Centre for Medical Research, Western Australian Institute for Medical Research and School of Medicine and Pharmacology, University of Western Australia, Perth, WA 6000
*Corresponding author: peterl@waimr.uwa.edu.au

Nuclear receptor (NR) coregulators are key modulators of hormone signalling. The discovery of Steroid receptor RNA Activator (SRA), a coregulator that is active as an RNA, transformed thinking in the field of hormone action. The subsequent identification of SRA-binding coregulator proteins, including p72, SHARP and more recently SLIRP, has provided important insight into SRA’s mechanism of action, and potentially offers new opportunities to target NR signalling pathways for therapeutic gain. This review outlines advances in the field of NR coregulator biology, with an emphasis on recent progress in understanding SRA-protein interactions.

The Nuclear Receptor Superfamily and Coregulators

As described in the article by Myers et al., NRs are ligand-inducible transcription factors that regulate the expression of target genes involved in metabolism, development and reproduction (1,2). The discovery of NR coregulators that are recruited to receptor complexes and modulate their activity has dramatically changed our understanding of hormone action. The past decade has revealed a host of such molecules that are recruited to cognate promoters, communicate with the general transcriptional machinery and RNA polymerase II (1), and modulate NR-directed transcription. Some coregulators possess enzymatic activity that can alter chromatin structure (e.g. histone acetylase) or methylation status (e.g. methyl transferase). Generally, coactivators facilitate transactivation, and corepressors reduce the transcriptional rate of target genes. Many coregulators exist in precomplexes that associate in promoter-specific configurations. For example, SRC-1 is recruited to the LBD of multiple NRs facilitating transactivation (1).

SRA – a Remarkable RNA Coactivator

Until 1999, all NR coregulators identified were proteins. However, with the isolation of SRA (3), a unique RNA coactivator molecule was uncovered (Fig. 1). Several laboratories have demonstrated SRA’s ability to coactivate a range of NRs, including ERα and β, AR, GR, RARα, PPARδ and γ, TR and VDR (3-6). Some studies, however, suggest that SRA can also corepress NR activity (7). Extensive studies demonstrated that SRA-mediated NR coactivation did not require the expression of a SRA protein (3).

The SRA gene is well conserved across species and is composed of five exons in human, rat and mouse genomes. There are several alternative splice products of SRA. Initially, three SRA isoforms were described, with unique 5’ and 3’ ends but a shared common ‘core’ domain (3). Protein initiation and polyadenylation sequences were identified in the SRA isoforms, however, a reading frame of no greater than 162 amino acids was
predicted. Some newly discovered human SRA isoforms contain larger open reading frames (8). For example, SRA1 codes for a 236 amino acid protein, referred to as SRAP, the carboxy-terminal 162 residues of which are identical to that theoretically coded for by the SRA sequence but with an additional 73 amino-terminal residues. Consistent with the presence of two initiating methionines in the SRA1 transcript, two proteins of 31/32 kDa have been detected, with SRAP present in both the nucleus and cytoplasm. As discussed below, differential splicing of the SRA transcript may generate a non-coding 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 (9).

SRA – an Emerging Role in Human Tumourigenesis?

Multiple lines of evidence implicate SRA in human tumourigenesis. Elevated and/or aberrant SRA expression has been reported in cell lines and human breast, ovary and uterine tumours (10,11). In studies of breast cancer samples, expression of an exon 3 deletion mutant of SRA (SRA-Del), but not the wild type molecule, correlated with a higher tumour grade (10,12). Notably, mutation of SRA stem-loop SDM10, encompassed by the SRA-Del deletion, results in a 50% reduction in transactivation compared with wild type SRA in transient transfection assays (13). There is mounting evidence that SRAP may also play a role in breast cancer tumour growth. Recent data suggest that patients with SRAP-positive tumours have lower recurrence rates and improved outcomes (14). Consistent with this observation are results showing that overexpression of SRAP reduces estrogen signalling activity in the ER-positive MCF-7 breast cancer cell line. Interestingly, transgenic mice overexpressing SRAP demonstrate increased mitosis and elevated cell death of mammary epithelium but no apparent increase in tumour incidence (11). When the SRA transgenic mice were crossed with MMTV-ras mice, the frequency of mammary tumour development was surprisingly reduced compared with MMTV-ras single transgenic animals. Taken together, these data suggest that SRA is an important regulator of mammary epithelial cell growth, but that the roles of SRA and SRAP in tumourigenesis are yet to be fully elucidated.

Mechanism of SRA Action as an RNA Coactivator

Secondary structure predictions suggest the existence of multiple stem-loops within SRA. Extensive mutational analysis has shown that some of these stem-loops are critical for SRA’s coactivation activity (13). In fact, it appears multiple RNA substructures work together to effect SRA’s overall coactivator function. Initial studies indicated that SRA coactivates a range of NR activities, including ER α and β, PR and GR in a ligand-dependent manner. SRA could also augment ERα activity in a ligand-independent manner through its AF-1 domain involving MAPK but this was not the case for ERβ (4). Overexpression of SRA1 in ER-positive MCF-7 cells generated contrasting results (14). In stable 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 (14). 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 coding and functional non-coding RNAs and the overall effect of SRA gene expression (9).

SRA-Binding Proteins Involved in NR Signalling

The identification of RNA-binding domains within multiple coregulators, including SHARP, PGC-1, CoAA, CoAM, CAPER α and β, and p72 (1), has generated much interest in understanding RNA-protein interactions in NR signalling (Fig. 1). It also has raised the possibility that some coregulators could target specific components of the transcriptional machinery as a critical part of their function. A number of coregulators with RNA recognition motif (RRM) RNA-binding domains play roles in splicing (e.g. CoAA, CAPER α and β), whilst others appear to target SRA and more directly impact on NR transactivation. For example, SHARP (SMRT/HDAC1 associated repressor protein) is a NR corepressor that interacts with SRA in vitro and contains an RNA-binding domain comprised of three RRRMs (15). These RRRMs are required by SHARP to repress SRA-augmented estrogen-induced transactivation (15). Another ER coregulator which binds SRA in vitro and copurifies with SRA from cell extracts is p72 (16). However, to date the specific details of how each of these proteins interacts with SRA, and which stem-loop is targeted, remain to be determined.

Adding a further layer of complexity to the regulation of NR activity, pseudouridinyl synthase (PUS) has been reported to bind and pseudouridylate SRA transcripts (5). In this manner, PUS may stabilise base stacking and hydrogen bonding within RNA molecules and between binding partners, as demonstrated for other non-coding RNAs, thereby augmenting NR activity. The existence of this posttranscriptional mechanism of SRA regulation further highlights the importance of this RNA activator in the regulation of NR activity.

SLIRP – a Novel SRA-Binding Protein Corepressor

In order to identify novel SRA-binding proteins and better understand detailed SRA-protein interactions, we screened a human breast cancer library with a SRA probe containing a key stem-loop implicated in SRA activity and identified SLIRP (SRA Stem-Loop Interacting RNA- Binding Protein) (6). SLIRP contains a highly conserved RRM domain that is required to repress signalling of a range of NRs. SLIRP augments SHARP’s corepressor activity and the effects of tamoxifen on estrogen signalling. Notably, these repressive effects occur with both agonist-induced and basal NR activity, consistent with SLIRP binding SRA and compromising its ability to augment AF-1 domain dependent, non-ligand effects of NRs. Chromatin immunoprecipitation studies confirmed
SLIRP’s presence in the nucleus associated with NR target genes and that its depletion by siRNA impacted on the recruitment of NRs and other coregulators, such as ER and NCoR (6).

**SLIRP – a Predominantly Mitochondrial Coregulator**

SLIRP is widely expressed in normal human tissues but elevated in high energy demand organs such as liver, skeletal and cardiac muscle. In addition, SLIRP is expressed in a range of human cancer cell lines and readily detected in human breast cancer tissue. We were surprised, however, to observe that SLIRP is predominantly a mitochondrial protein. Although unexpected, the presence of SLIRP in the mitochondria adds to the mounting evidence describing NRs such as ER (17), GR (18) and TR (19) in this organelle. For the GR, mitochondrial translocation correlates with susceptibility to glucocorticoid-induced apoptosis (18). In addition, the mitochondrial DNA contains putative hormone-response elements that can be bound by NRs in DNA gel-shift studies (19). The presence of NRs in both the nucleus and mitochondria raises the possibility that they could coordinate signalling pathways between the nucleus and other organelles.

To our knowledge, SLIRP is the only NR coregulator that influences transcription in the nucleus but resides predominantly in the mitochondrion. Interestingly, although not located in the mitochondrion, there are parallels between SLIRP and another coregulator, RIP140. The latter is also preferentially expressed in tissues similar to SLIRP where it can suppress oxidative metabolism and mitochondrial biogenesis (20). Furthermore, RIP140 is a powerful negative regulator of insulin-responsive hexose uptake and oxidative metabolism in mouse adipocytes. Depletion of RIP140 from these cells upregulates clusters 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 (6), 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 body metabolism and mitochondrial biogenesis.

**Summary**

The discovery of the RNA coactivator SRA represented a paradigm shift in our understanding of coregulator regulation of NR signalling. 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 normal energy homeostasis and metabolic disease, but how they may coordinate signalling pathways between the nucleus and other organelles.

**References**