Mechanisms of androgen receptor signalling via steroid receptor coactivator-1 in prostate

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Abstract

The androgen receptor (AR) is a member of the nuclear receptor superfamily. These ligand-activated transcription factors usually contain two activation functions, a ligand-independent activation function 1 (AF1) in the divergent N-terminal domain and a ligand-dependent AF2 in the more conserved C-terminal ligand-binding domain. To promote transcription from target promoters, DNA-bound nuclear receptors recruit coactivator proteins that promote transcription by modifying histones within nucleosomes, resulting in altered topology of chromatin to allow access of the basal transcriptional machinery, or stabilising the pre-initiation complex. It is well known that most coactivators interact with AF2 of many nuclear receptors via conserved, helical LxxLL motifs (where L is leucine and x is any amino acid). The AF2 of the AR is very weak, but we were able to demonstrate that its intrinsic ligand-dependent activity is potentiated by steroid receptor coactivator-1 (SRC1) and that this region interacts with coactivators via LxxLL motifs. However, a mutant SRC1 coactivator with no functional LxxLL motifs was still able to potentiate AR activity. We found that SRC1 can also be recruited to (and increase activity of) AF1 of the AR via a conserved, glutamine-rich region. Point mutations within this region abolish SRC1 interaction with AF1 and also abolish or severely impair its ability to potentiate AR activity on all promoters tested. Thus the AR interacts with SRC1 via two different regions and the AF1 interaction is functionally the more important, although the contribution of the two interactions varies in a promoter-dependent fashion. SRC1 then potentiates receptor activity via recruitment of CBP/p300, a histone acetyltransf erase.

This is important in the context of prostate cancer as SRC1 and other coactivators including CBP are coexpressed with AR in the luminal epithelial cells of the prostate, where over 90% of prostate tumours arise. There is a need for effective second-line prostate cancer therapy aimed at blocking the AR pathway when anti-androgen therapy has failed. Since there is growing evidence that nuclear receptor cofactors may be implicated in the progression of hormone-dependent tumours to hormone-independent states, novel targets could include the interaction of AR with coactivator proteins. We suggest that the N-terminal interaction would be a more specific and effective target in the case of prostate cancer than the LxxLL/AF2 interaction.

Introduction

Prostate cancer is the most commonly diagnosed male cancer in the UK and the second leading cause of male cancer death (Cancer Research UK 2003a,b). The prostate is an androgen-dependent organ, requiring androgens for growth during foetal and pubertal development. After puberty, prostate growth ceases in spite of the high circulating levels of androgen, which are required to maintain its secretory function. However, in cases of
benign prostatic hyperplasia and malignant prostatic carcinoma, androgen-dependent growth resumes. In the case of prostate cancer this is treated by removing circulating androgens by chemical castration and/or opposing their effects using anti-androgens (combined this is termed total androgen blockade). This treatment works well in the majority of patients, preventing further growth and even leading to regression of the tumour, but after a median of 13 months therapy fails and tumour growth resumes. This growth is often termed ‘hormone independent’ as it occurs despite the absence of androgens and the presence of anti-androgens. This term is misleading since the vast majority of tumours continue to express androgen receptor (AR) and appear to remain dependent on AR signalling.

The AR is a ligand-activated transcription factor that mediates signalling by all androgens including dihydrotestosterone (DHT), the major androgen involved in prostate development. In up to 30% of advanced prostate tumours, overexpression or amplification of the AR has been observed, which could lead to an amplified response to low doses of residual androgens, or weak adrenal androgens (Visakorpi et al. 1995, Koivisto et al. 1997, Linja et al. 2001). Mutation of the AR has also been reported at similar frequencies and some of the identified mutations cause the receptor to become activated by alternative ligands, such as oestrogens or anti-androgens (Culig et al. 1993, Veldscholte et al. 1994, Peterziel et al. 1995, Taplin et al. 1995, Gottlieb et al. 1999). As such alternative ligands are present in patients, this aberrant activation could contribute to ‘hormone-independent’ tumour growth. However, in many cases the cause of growth is not clear and the explanation could lie in other alterations in the AR signalling pathway. It is essential that we know more about the ways in which AR promotes activation of target genes, in order that we can understand how this process goes awry in prostate cancer and to suggest potential new targets for therapy to block this pathway once anti-androgen therapy has failed.

When activated by androgen the AR binds to response elements in target gene promoters, including those encoding proteins involved in mitosis, differentiation and apoptosis in the prostate (Nelson et al. 2002, Verrijdt et al. 2003). Research on the mechanism of activation of target genes by nuclear receptors has identified many ‘coactivator’ proteins: proteins that bind to an activated receptor in a ligand-dependent manner and enhance its ability to promote activation of a target gene (Bevan & Parker 1999). DNA transcription involves chromatin remodelling prior to recruitment of the basal transcriptional machinery, the assembly of the pre-initiation complex on the promoter, and the movement of the RNA polymerase along the gene (Kadanaga 1998). Coactivators can enhance transcription by promoting one or more of these steps. The best characterised coactivators are the p160 family, consisting of three highly homologous 160kDa proteins called (i) steroid receptor coactivator-1 (SRC1) (Oñate et al. 1995), (ii) transcriptional intermediary factor-2 (TIFF2) (Voegel et al. 1996) (the human homologue of mouse glucocorticoid receptor interacting protein 1 (GRIP1) (Hong et al. 1996)) and (iii) amplified in breast cancer 1 (AIB1) (Anzick et al. 1997) (also known as ACTR (Chen et al. 1997), RAC3 (Li et al. 1997) and TRAM1 (Takeshita et al. 1997), the human homologue of mouse pCIP (Torchio et al. 1997)). These recruit the general coactivators CREB binding protein (CBP) and p300. p160 coactivators and CBP contain histone acetyltransferase (HAT) activity, allowing them to acetylate lysine residues in the tails of histone proteins associated with the DNA in chromatin (Bannister & Kouzarides 1996, Ogryzko et al. 1996, Chen et al. 1997, Spencer et al. 1997). This appears to promote the recruitment of other complexes containing ATP-dependent chromatin remodelling activity, such as the SWI/SNF and ISWI complexes (Becker & Horz 2002). The modifications these induce loosen the DNA–protein association and ‘relax’ the chromatin, allowing easier access to the basal transcriptional machinery and other transcription factors. ATP-dependent chromatin remodelling complexes can interact directly with nuclear receptors (Fryer & Archer 1998, Belandia et al. 2002) but may also be recruited via p160/CBP (Huang et al. 2003). Another class of coactivators acts by stabilising the interactions of receptors with the basal transcriptional machinery. The thyroid hormone receptor-associated protein (TRAP) complex appears to have such a role. This large multiprotein complex was first identified as binding to the thyroid hormone receptor (Yuan et al. 1998), while parallel work led to the characterisation of related complexes called SRB- and MED-containing complex (SMCC) (Ito et al. 1999) and vitamin D receptor interacting proteins (DRIP) (Rachez et al. 1999). Recruitment of the TRAP complex to nuclear receptors appears to be via a single protein termed TRAP220. Recently a similar complex has been shown to interact with steroid receptors (Hittelmann et al. 1999, Burakov et al. 2000). It has emerged that these complexes, containing at least 12 polypeptides, are almost identical to one another and about half the peptides are similar to those found in the yeast mediator complex. The mediator complex may serve several functions but importantly it recruits RNA polymerase II to target genes by its association with the C-terminal repeat domain of the large subunit. Although due to the plethora of coactivator proteins, most of which are ubiquitously expressed, there is likely to be a degree of redundancy between them such that one coactivator may compensate when another is removed.
(Xu et al. 1998), it is likely that nuclear receptors absolutely require the recruitment of coactivators in order to promote transcription.

Materials and methods

Immunohistochemistry

Sections (4 μm) of formalin-fixed, paraffin-embedded benign prostate tissue were dewaxed in xylene, rehydrated in decreasing concentrations of ethanol, and endogenous peroxidase activity was blocked using 2% hydrogen peroxide. Antigen retrieval was carried out by microwaving at 750 W in 0.01M trisodium citrate, pH 6, three times for 5 min. Sections were blocked with rabbit or goat serum (1:10 dilution) before incubation overnight at 4°C with the primary antibody (1:150 dilution): a rabbit polyclonal antibody to AR (AR C-19; Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse monoclonal antibody to SRC1 (SRC1-1135, H-4; Genetex, San Antonio, Texas, USA) or a mouse monoclonal antibody to CBP (CBP C-1; Santa Cruz Biotechnology). After washing, sections were incubated with biotinylated rabbit anti-mouse or goat anti-rabbit immunoglobulin (1:200, 45 min; DakoCytomation, Ely, UK) followed by peroxidase conjugated with streptavidin (1:100, 30 min; DakoCytomation). Sections were then washed and enzyme activity developed in 1mg/ml 3,3′-diaminobenzidine tetrahydrochloride (DakoCytomation) and counterstained with haemotoxylin (Vector Laboratories, Burlingame, CA, USA).

Yeast culture, transformation and reporter assays

The yeast strain W303-1B transfected with an oestrogen receptor (ER)-responsive lac Z reporter, and plasmids expressing AR ligand-binding domain (LBD) or SRC 1240-1441 fused to the heterologous ER DNA-binding domain (DBD) or the VP16 activation domain, or full-length SRC1e, have been previously described (Bevan et al. 1999). Yeast was transformed by electroporation and cultured overnight in the presence of 7 M mibolerone. -Galactosidase activity was measured and normalised against protein content. All details of experimental procedures were as described by Bevan et al. (1999). Results shown are the mean ± S.E. of results from three independent transformants.

Mammalian cell culture, transfection and reporter assays

The expression vectors for full-length AR and AR lacking the LBD were generous gifts from Dr A O Brinkmann and the reporter plasmid TAT-GRE-E1BTAT-luc was a kind gift from Dr G Jenster. Other reporter plasmids used are described by Verrijdt et al. (2003). The VP16-activation function 1(AF1) plasmid has been described previously (Christiaens et al. 2002). Gal4 DBD-SRC1 fragments and the Gal4-luc reporter vector were kind gifts from Professor M G Parker. Mutant Gal4 DBD-SRC1 plasmids and SRC1e wild-type (WT) and mutant expression vectors have been described previously (Bevan et al. 1999, Christiaens et al. 2002).

COS cells were maintained and transfected as described previously (Bevan et al. 1999, Christiaens et al. 2002). After transfection, cells were treated with medium containing vehicle (ethanol) or a saturating concentration of a synthetic DHT analogue, either 10⁻⁸ M mibolerone or 10⁻⁹ M R1881 (both Perkin Elmer and Analytical Life Sciences Boston, MA, USA). Luciferase activity was assayed and normalised against β-galactosidase activity to correct for transfection efficiency. Unless otherwise stated, experiments were carried out in duplicate or triplicate and results shown are the mean ± S.E. of three or more independent experiments.

Results

AR coactivators in prostate

We used immunohistochemistry to study the expression of the p160 coactivator SRC1 and the general coactivator CBP in formalin-fixed prostate sections. We found that both SRC1 and CBP were expressed in the prostate in the same cell types as the AR: the luminal epithelial cells and a subpopulation of the stromal cells (Fig. 1). This staining was predominantly nuclear. Only in the basal cell population was the pattern of expression different for the three proteins as these cells were uniformly AR negative while all were CBP positive (not shown) and the majority were SRC1 positive. This, coupled with the fact that SRC1 is a strong coactivator of AR activity in transient transfection assays (see Fig. 3), makes SRC1 a potentially important regulator of AR activity in prostate, as is also suggested by the impairment of prostate development in mice lacking the SRC1 gene (Xu et al. 1998). This paper explores the ways in which SRC1 interacts with the AR to promote signalling.

The LBD of AR contains a ligand-dependent activation function and interacts with SRC1 via LxxLL motifs

The AR is a member of the nuclear receptor superfamily and, like other nuclear receptors, has a conserved functional domain arrangement consisting of a central DBD, a C-terminal LBD and an N-terminal region.
It is well documented that other steroid and nuclear receptors contain a second, ligand-dependent activation function in the LBD, termed AF2, which in many receptors (including the ER) is the major activation function and shows separable, ligand-dependent activity in receptors lacking the N-terminal domain. AF2 comprises a coactivator-binding interface, consisting of residues from several of the conserved α-helices that form the structure of the LBD (Wurtz et al. 1996, Darimont et al. 1998), notably the most C-terminal helix 12. These critical residues are well conserved in the AR but a separable AF2 was difficult to observe since a construct with deletion of the whole N-terminal region was completely inactive in mammalian cells. However, we and others have found that the isolated LBD, fused to a heterologous DBD, showed dose-dependent activation of a reporter gene in yeast, demonstrating the presence of an intrinsic, ligand-dependent activity in this region (Moiänen et al. 1998, Bevan et al. 1999). Further, we also demonstrated that coexpression of SRC1e in yeast was able to increase the activity of AF2 sevenfold (Fig. 2B). Thus we concluded that AR has an AF2 function, which is much weaker than the AF1 function, and that AF2 activity is increased by p160 coactivators.

It has been well documented that the major receptor interaction domains in many coactivators are LxxLL motifs, where L is leucine and x is any amino acid (Heery et al. 1997, Torchia et al. 1997). The region of ER involved in this interaction is AF2, and mutations of conserved hydrophobic residues in helix 12 of the ER LBD abolish it (Heery et al. 1997). We used a yeast two-hybrid assay to investigate this interaction for the AR and found that the AR LBD showed a strong ligand-dependent interaction with a fragment of SRC1 containing an LxxLL motif. This interaction was abolished by mutation of the LxxLL motif in SRC1 (Fig. 2D) and by mutation of conserved residues in helix 12 of the AR (Fig. 2C). Thus we concluded that, as for other nuclear receptors, the AR AF2 function interacts with SRC1 via LxxLL motifs.

SRC1 exists in two isoforms, termed a and e. These differ only at the C terminus, which is longer and contains an LxxLL motif in SRC1a but not SRC1e (Kalkhoven et al. 1998). Both isoforms contain three LxxLL motifs in a central receptor-interacting region and mutation of two or all three of these to LxxAA (A = alanine) in full-length SRC1e is sufficient to abolish its potentiation of many receptors including ER, due to an inability to interact with the receptor (Heery et al. 1997, Ding et al. 1998, Kalkhoven et al. 1998). We cotransfected increasing amounts of SRC1e mutated in all three LxxLL motifs (SRC1e-LxxAA) with AR and found that, surprisingly, it was able to increase AR activity to a similar extent as wild-type (WT) SRC1e, while in parallel experiments it
was unable to potentiate ER or GR activity (Bevan et al. 1999). Subsequent experiments using different reporter constructs showed that in fact the ability of SRC1e-LxxAA to potentiate AR was dependent on the promoter context, with significant impairment evident on two of the seven reporters tested (Fig. 3). We have concluded that the AF2 interaction with LxxLL motifs is not essential for recruitment of SRC1 to AR in vivo, although on some promoters (notably mouse mammary tumour virus promoter fragment (MMTV)) it is required for maximal interaction, and that another interaction is taking place between the two proteins, directly or indirectly.

AF1 of AR interacts with a glutamine-rich region of SRC1

Several lines of evidence led us to the hypothesis that a second interaction with SRC1 was occurring via AF1 of the AR. Firstly, in a yeast two-hybrid experiment using full-length AR as ‘bait’ to interact with fragments of SRC1 fused to VP16, we found a ligand-independent interaction with the fragment SRC1 989-1240 (Bevan et al. 1999). The fact that this was ligand independent, coupled with the fact that AF1 is the most important activation domain in AR and is also ligand independent, made this the obvious candidate for the interacting region.
of AR. Secondly, the ligand-independent activity of isolated AF1 can be strongly enhanced by full-length SRC1 in reporter gene assays (Fig. 4A). We confirmed and extended the finding from the yeast two-hybrid assay in a mammalian two-hybrid assay using SRC1 fragments as bait and measuring interaction with AF1 fused to VP16 – the strongest (ligand-independent) interaction was seen with the same 989–1240 fragment of SRC1 (Fig. 4B).

Further investigation of this region showed that it contains a high percentage of glutamine residues and shows conservation between the three p160 family members, with three highly conserved motifs we have termed the A-box, B-box and C-box (Fig. 5A and B).

We used fragments of the glutamine-rich region of SRC1 to determine the minimal interacting region with AF1 in two-hybrid assays. None of the three motifs alone was able to mediate the interaction and the whole region (1050–1185) was required for maximum interaction. However, a minimal interacting region that contains motifs A and B (1050–1120) was defined and this was further strengthened by the inclusion of more C-terminal sequence (1050–1145) (Fig. 5C). This minimal interacting

Figure 3 Interaction of SRC1 with the N terminus but not the LBD is essential for potentiation of AR activity. COS cells were transfected with expression vectors for AR and SRC1 with luciferase reporter vectors under the control of varying androgen-responsive elements (AREs). Activity in the presence of a saturating concentration of ligand is shown. LxxAA = SRC1e with all three LxxLL motifs mutated to LxxAA; Abox = SRC1 with the A box mutation described in the text. Promoters include: TAT-GRE = response element from the rat tyrosine aminotransferase gene; MMTV; PSA = prostate-specific antigen promoter fragment; PB-promoter = promoter fragment from rat probasin gene; C3-ARE2 = two copies of ARE from the first intron of the rat C3 gene; SC-ARE2 = two copies of ARE from human secretory component promoter; PB-ARE2 = two copies of ARE from probasin promoter.
The region is required for SRC1 recruitment \textit{in vivo}, as shown in a reporter assay where an internal deletion of 1053–1123 in full-length SRC1 abolished its ability to potentiate AR activity (Bevan et al. 1999).

To determine whether one or both motifs are required for the AF1 interaction, we introduced point mutations into the minimal interacting fragments and assayed their interaction in the two-hybrid assays. Mutation of only two or three amino acids in either of the motifs (in motif A, R1055V/Q1057V; in motif B, A1106P/Q1107V/M1108V) was sufficient to severely impair the interaction while mutation of both motifs abolished it (Fig. 5C). This has been confirmed using an \textit{in vitro} interaction assay (Christiaens et al. 2002). Thus, both motifs appear to be required for maximal interaction although neither is sufficient alone.

To investigate whether the A-box is required \textit{in vivo} for the interaction with AR, we tested the ability of full-length SRC1 carrying the A-box mutation to potentiate activity of full-length AR on several different promoters (Fig. 3). We found that the A-box mutant was impaired in its ability to potentiate ligand-dependent AR activity on all of the promoters tested, in contrast to the LxxAA mutant. This implies that recruitment of SRC1 to AF1 of AR requires an intact A-box and is essential for SRC1 activity on many promoters, whereas recruitment to the LBD via LxxLL motifs is not usually required for functional interaction of SRC1 and AR. However, the differing extent of the impairment of the two mutants on the different promoters we have now tested confirms our original theory that the relative involvement of AF1 and AF2 in AR transcriptional activity differs on different promoters.

\textbf{SRC1 potentiates AR activity via recruitment of histone acetyltransferases, but not methyltransferases}

SRC1 contains two intrinsic activation domains, AD1 and AD2. AD1 maps to residues 900–950 and interacts with the HAT coactivator CBP/p300 (Kalkhoven et al. 1998, Korzus et al. 1998, McInerney et al. 1998, Sheppard et al. 2001). AD2 maps to residues 1241–1385 and recruits methyltransferases such as coactivator-associated arginine methyltransferase (CARM1) and protein arginine methyltransferase 1 (PRMT1), which may increase transcriptional activation by altering methylation of histones (Chen et al. 1999, Koh et al. 2001). We investigated the relative importance of AD1 and AD2 on the potentiation of AR activity. Deletion of AD1 abolished the activity of SRC1 on all of the reporters tested (Fig. 6) and similar results were seen for the ER (Sheppard et al. 2001). Deletion of AD2, however, appears to increase SRC1 activity on most of the promoters tested. From this we concluded that (i) histone acetyltransferases such as CBP/p300 are required for SRC1 to potentiate AR activity and (ii) methyltransferases are not only not required but may inhibit AR activity. Since in these assays the target
promoter is on a transiently transfected expression vector, it is unlikely to be methylated fully, which could explain the redundancy of AD2. Recently in \textit{in vivo} studies in LNCaP cells in which an engineered GRIP1 promotes the G1 to S transition, deletion of AD1 abolished this ability while deletion of AD2 had little effect (Shang et al. 2002). Methyltransferases may therefore not be required for AR activity at all promoters even \textit{in vivo}. The observation that the AD2-containing region is apparently inhibiting SRC1 activity may be explained by a weak interaction we observed in yeast between the glutamine-rich 989–1240 region and the C-terminal 1241–1385 regions of SRC1 (data not shown). Such an interaction could compete with the AF1/SRC1 interaction if it occurs \textit{in vivo}, and so reduce recruitment of SRC1 to AR. Interestingly, the ΔAD2 mutant did not show such a strong increase in

\textbf{Figure 5} (A) Schematic of the major functional domains of SRC1. (B) Alignment of the amino acid sequence of the three p160 coactivators in the most highly conserved regions of the glutamine-rich fragment of SRC1 that interacts with AF1. (C) The minimal interacting region contains motifs A and B and mutation of either impairs interaction. (Data previously shown in Christiaens et al. 2002.) In a mammalian two-hybrid assay, COS cells were transfected with AF1 fused to VP16 activation domain (or VP16 alone), fragments of SRC1 fused to Gal4 DBD, and a Gal4-responsive reporter vector whose activity was proportional to the interaction between the two fusion proteins. bMLH, basic helix-loop-helix domain; PAS, Per-Arnt-Sim domain.
activity on ER (Sheppard et al. 2001 and data not shown), consistent with the observation that ER AF1 does not interact strongly with the SRC1 glutamine-rich region (Christiaens et al. 2002).

Discussion

The AR, although ostensibly very similar to the other steroid and nuclear receptors, has long been known to have key differences. Chief among these was the seeming lack of AF2 activity. We and others have now shown that it is possible to observe a separable, ligand-dependent AF2 activity in the isolated LBD. This was first seen in yeast cells, where it is strongly enhanced by p160 coactivators. It has also been observed in mammalian cells in the presence of overexpressed coactivator proteins (Alen et al. 1999). The AF2 of AR acts like that of other nuclear receptors in that it interacts with coactivators via LxxLL motifs, in a strictly ligand-dependent manner. However, AR is very different from other steroid and nuclear receptors in that a mutant p160 coactivator with no functional LxxLL motifs can still potentiate its activity. We have found this to be due to ligand-independent interaction of SRC1 with the AF1 region, via a conserved glutamine-rich fragment of SRC1 that previously had no known function. Thus SRC1 can interact with both termini of the AR, in common with several other cofactors including CBP (Frønsdal et al. 1998). The N and C termini of AR have long been known to interact (Zhou et al. 1995, Doesburg et al. 1997, Ikonen et al. 1997, Berrevoets et al. 1998, Langley et al. 1998, Scheller et al. 1998, He et al. 2002) and this interaction is believed to be necessary for maximal AR activity on certain promoters, as demonstrated by mutational analysis of the AR on selective versus non-selective androgen response elements (ARE) (Callewaert et al. 2003). One hypothesis is that SRC1 or other coactivators may act as bridging factors between the termini or stabilise the N/C-terminal interaction, but this has not been possible to prove directly. We believe that SRC1 is able to potentiate the AF1 and AF2 functions independently by means of the two interactions, and the relative importance of the two interactions is dependent on the promoter. On some promoters, AF1 contributes all of the activity and on these the LxxLL motifs are not required at all. On other promoters, AF2 contributes to the overall activity of the receptor and, on these, mutation of the LxxLL motifs reduces the effect of SRC1. However, on all the promoters tested, mutation in the A-box either abolished or severely reduced SRC1 activity, so we have concluded that AF1 is always required for AR activity.

ER, in contrast to AR, has a short N-terminal region that does not appear to be essential for maximum activity of the receptor in many contexts (Lees et al. 1989). Further, deletion of the LBD results in an inactive receptor, so AF1 contributes little to the overall ligand-induced activity of ER (although it may contribute more to activation by partial agonists such as tamoxifen). Mutation of the LxxLL motifs completely abolished the ability of SRC1 to potentiate ER. We have concluded that the most important interaction between SRC1 and the ER, in common with most nuclear receptors, is that between the LBD and the LxxLL motifs. However, although the interaction we observed between ER AF1 and SRC1 was vanishingly weak compared with the AR interaction (Christiaens et al. 2002), an interaction has been observed between the ER and GRIP1 in the glutamine-rich region (Webb et al. 1998). It appears that, rather than really being activated by SRC1 in a fundamentally different way, ER and AR are at opposite ends of a continuous spectrum. On the one hand, some receptors and/or some promoters require a strong AF1 interaction with p160, while other receptors or promoters only require interaction with AF2. Others may require both or either.

Several laboratories have reported that p160 coactivators have HAT activity, but it is very weak in comparison with that of CBP/p300 (Chen et al. 1997, Spencer et al. 1997). Also, the direct interaction of CBP/
p300 with receptors is weak in comparison with the interaction of p160 coactivators with either receptor or SRC1 (Heery et al. 2001, Sheppard et al. 2001), and the ability of p300 to potentiate receptor activity was strongly reduced by deletion of the p160-interacting region but not of the receptor-interacting region (Kraus et al. 1999, Li et al. 2000). Thus the major function of the p160 coactivators appears to be to act as a platform for the recruitment of other cofactor proteins, such as CBP/p300 and CARM1, which modify the chromatin to promote recruitment of the basal transcriptional machinery. CBP in turn recruits another HAT coactivator, p300/CBP associated factor (pCAF) (Yang et al. 1996), so a complex of HAT coactivators may build on a DNA-bound receptor (Sheppard et al. 2001, Shang et al. 2002). In Fig. 7, we have summarized the interactions of SRC1 with the AR and with other cofactors. There are multiple interactions since CBP is recruited by SRC1 but has also been shown to interact with both the LBD and AF1 of AR (Frønsdal et al. 1998) while pCAF is recruited by CBP but may also interact directly with the AR and acetylate the DBD (Fu et al. 2000). Whether all of these interactions occur in vivo or simultaneously is not known. Further, Huang et al. (2003) have recently shown that CBP also recruits the Mediator complex to the AR, so rather than sequential recruitment of different coactivator complexes, the HAT coactivators and Mediator may be recruited simultaneously.

The role of AF1 has been comparatively little studied to date. However, it is now becoming clear that this is a major functional domain for steroid receptors and perhaps especially the AR. As well as coactivators that interact with both N and C termini, several specifically AF1-interacting coactivators have been identified, such as p68 and AR27 (Endoh et al. 1999, Markus et al. 2002).

Further, AF1 of AR has recently been shown to be a target for corepressors, proteins that may be required in order for anti-androgens to have their repressive effects (Dotzlaw et al. 2002). Thus AF1 could be a key region in determining the effectiveness of anti-androgen action, and further studies of this region may be important to understand exactly how anti-androgens work and why they eventually fail.

Understanding the androgen signalling pathway is key to understanding prostate cancer progression. Current therapies (such as anti-androgens or chemical castration) aimed at inhibiting this pathway at one or another stage inevitably fail, and so-called hormone-independent growth resumes. This growth is unlikely to be truly hormone independent, since AR expression is retained in the majority of tumours. In cases where AR levels and coding sequence remain normal, another mechanism by which the AR signalling pathway could function aberrantly is by an alteration in the amounts of, or interactions with, coactivator proteins. We have found that several different coactivators are coexpressed with AR in the luminal epithelial cells of the prostate, where the majority of cancers arise. Although studies measuring expression of individual coactivators in prostate cancer have shown few convincing changes in levels correlating with progression to hormone independence, this could be a consequence of small subsets of cofactors being investigated (Fujimoto et al. 2001, Gnanapragasam et al. 2001, Li et al. 2002, Mestayer et al. 2003). Over 200 potential cofactors have been identified so far, many of which appear to function as part of large, multiprotein complexes, so if alterations in coactivators do contribute to hormone resistance, it may well be subtle changes in ratios between various types of cofactor. Global approaches such as microarray analysis of expression, or

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**Figure 7** Model of the interactions between the HAT complex that builds on DNA-bound AR. The major domains of AR are shown in grey, the major domains of SRC1 are shown as solid boxes. The Q-rich region of SRC1 (Qr) interacts with the AR N terminus, probably via both the Tau1 and Tau5 subdomains. The LxxLL motifs interact with the AR LBD. The AR LBD and AF1 also interact, probably via a FQNLF motif at the extreme N terminus (He et al. 2000, Callewaert et al. 2003). SRC1 then recruits CBP/p300 via AD1, which further recruits pCAF. CBP and pCAF may also both directly interact with AR. These coactivators then acetylate histones and each other/the AR (curved arrows). AD2 of SRC1 may recruit methyltransferases that modify histone tails (straight arrow).
mutation, may allow the identification of signature cofactor changes associated with disease progression, for diagnostic and prognostic purposes. Breakthroughs in understanding the mechanisms of nuclear receptor activation have also identified novel therapeutic targets for hormone-dependent cancers. For instance, a peptide containing an LxL motif could act as a competitive inhibitor to prevent coactivators binding to the AF2 of hormone receptors and would have application in any hormone-driven pathway (Hall et al. 2000). However, since AF2–coactivator interactions occur for all nuclear receptors tested, and involve highly conserved receptor domains, the problem would lie in refining this approach to prevent global inhibition of hormone pathways, either by targeting or by increasing our knowledge of receptor specificity for different motifs. The AF1–coactivator interaction described here might be a better target for inhibition since it involves a more diverse region of the receptor and appears to be more important for AR signalling, so its inhibition could inhibit AR signalling in the prostate without severely impairing other steroid or signalling, so its inhibition could inhibit AR signalling in the prostate without severely impairing other steroid or nuclear receptor pathways. Currently there is a lack of effective second-line therapies for prostate cancer for use after anti-androgen therapy has failed, to prolong the progression, for diagnostic and prognostic purposes. Breakthroughs in understanding the mechanisms of nuclear receptor pathways. Currently there is a lack of effective second-line therapies for prostate cancer for use after anti-androgen therapy has failed, to prolong the future, therapies could be aimed at such novel targets as the interactions described here.

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