factor between the receptor and the basal transcriptional factor complex to promote the chromatin structure change.[5]

Because of the homology of the receptor interaction domain (RID) and similar functional domains, some of the cofactors have been grouped as the steroid receptor coactivator (SRC)/p160 family containing SRC-1/NCoA-1,[6] GRIP1/TIF2/NCoA-2,[7,8] and RAC-3/ACTR/P/CIP/NCoA-3.[9–11] The RIDs of these factors are highly conserved and contain three repeated motifs of the consensus sequence LXXLL (where X is any amino acid).[12] In addition, other receptor cofactors such as transcriptional intermediate factor 1 (TIF1), receptor interacting protein 140 (RIP140), TATA-binding protein-associated factor II30 (TAFII30), and PPAR (peroxisomal proliferator-activated receptor) gamma coactivator-1 (PGC-1) also have been identified.[13–16] Some of these coactivators have been shown to bind multiple receptors, but the others might possess some specificity.[16]

The coactivators identified in our lab, such as AR associated protein (ARA)70, ARA55, ARA54, and retinoblastoma protein (Rb)[17–20] all have different RIDs, transactivation domains, and signature functional domains, as compared to the SRC/p160 family.[17–20] It will be interesting to systematically study the differential effects and characteristics among these unique cofactors and SRC-1. Currently, some other cofactors have been identified to associate with AR DNA binding domain or AR N-terminal domain, such as ARA160, ARA24, CREB binding protein (CBP), a small nuclear ring finger protein (SNURF), a novel nuclear binding protein (ANPK), *etc* (Hsiao *et al.*, unpublished observations, 1999).[21–23] We will mainly focus on the cofactors which associate with the LBD of AR in this review.

Identification of AR ligand binding domain-associated cofactors

To identify the cofactors by protein-protein interaction, the strategies include yeast two-hybrid system, modified yeast one-hybrid system, Far-Western screening of phage expression library, and microsequencing the protein purified by immunoprecipitation.

The hypothesis that the mutant AR (mtAR) may change the antiandrogen specificity and contribute to the progress of prostate cancer from an androgen-dependent to an androgen-independent stage has been widely accepted.[24] Therefore, we were interested in investigating if cofactors are required for wild type AR (wtAR) or mtARs to exert this distinct function. The LBD of wtAR or mtAR t877s (codon 877 was changed from threonine to serine) was used as a bait to screen the potential positive clones from a human prostate cDNA library by the yeast two-hybrid system. As a result, we have obtained more than 20 candidates including SRC-1. After characterization, three of the positive cDNA clones, ARA70, ARA55, and ARA54, were further studied.

The functional domain of different AR cofactors

The unique sequence of ARA70, ARA55, and ARA54 places these three AR coactivators outside the family of the common SR coactivators p160 family that include SRC-1, TIF2/GRIP1, and RAC3/ACTR/AIB1.[6–11] For example, these three AR coactivators lack some common motifs [such as basic helix-loop-helix (bHLH) domain, Per-AhR-Sim (PAS) domain and LXXLL motifs] that are shared by p160/SR coactivators. Furthermore, it has been well documented that LXXLL is the signature motif for the member of p160 cofactor family to interact with SR.[12] Although there is a LXXLL motif in the N-terminal domain of ARA70 and ARA55, this motif is not located in the RID of ARA55 and ARA70 (Yeh *et al.*, unpublished observations, 1999).[19] Furthermore, ARA55 does have three LIM (lin-11 isl-1 mec-3) motifs in the interaction domain of the C-terminal region. The LIM motif is a cysteine-rich motif that is found in several proteins (including Trip 6), with diverse functions and subcellular distributions. The biochemical properties and the function of the LIM motifs have not been fully defined and it has been suggested that their main function is in developmental regulation.[25,26] However, Schmeichel and Beckerle reported that LIM motifs might be involved in protein-protein interaction.[27] Therefore, the LIM motifs in the C-terminal region of ARA55 may contribute to its interaction with AR.

ARA54 is another AR coactivator with a novel sequence. Interestingly, ARA54 contains a conserved RING finger motif and a B-box-like structure. Proteins in the RING finger family are ubiquitously expressed in species ranging from human to virus, participate in diverse cellular processes, and may be involved in some aspects of transcriptional regulation and protein-protein interaction.[28] In addition, it has been reported that mutant promyelocytic leukemia (PML) proteins without the RING finger motif could become the potential dominant-negative inhibitors of the wild type PML.[29] Although the significance of the RING finger domain in ARA54 remains unclear, it is possible that ARA54 might use this domain to interact with other key factors in the activated nuclear receptor-mediated signaling complex and function as a bridge factor between AR and general transcription machinery.

There is also no homology among ARA55, ARA54, and the first identified AR coactivator, ARA70.[17,19,20] Although these AR coactivators enhance AR transcriptional activity in DU145 cells, they show distinct