differences, which will be discussed in the following sections. The precise role of these three cofactors may affect the different physiological influences on prostate cells. Together, it will be of great interest to further characterize the functions of these three AR coactivators.

The SRC-1 and RAC3/ACTR/AIB1 also function as AR cofactors

To investigate whether two other steroid receptor coactivators, SRC-1 and RAC3/ACTR/AIB1,[6,9,10,11] can function as AR coactivators, we have co-transfected these two cofactors with AR. Our data indicated that these coactivators could enhance AR transcriptional activity at 1 nM 5 α-dihydrotestosterone (DHT).[30] Although it has been speculated that SRC-1 and RAC3/ACTR are the coactivators for many steroid hormone receptors, our results provided the first evidence showing that SRC-1 and RAC3/ACTR do functionally enhance the transactivation activity of AR.

DHT-mediated AR transactivation activity in the presence of different AR coactivators

Among the cell lines we tested (CHO, DU145, LNCaP, PC-3, HeLa, and MCF7), the human prostate cancer cell line, DU145, was used due to its low background of AR transcriptional activity in the absence of exogenous AR. To compare the relative enhancement of DHT-mediated AR transcriptional activity with different AR coactivators, human AR and all available AR coactivators (ARA70, ARA55, ARA54, SRC-1, and RAC3) were inserted into the pSG5 expression vector for the same transfection efficiency. When human AR and/or individual AR coactivators were transiently expressed in DU145 cells without adding DHT, there was no AR transcriptional activity. However, AR transcriptional activity could be induced to 5–7 fold when AR was expressed in the presence of 1 nM DHT. The addition of various AR coactivators, at a 1:3 AR:AR coactivator ratio, could further enhance the AR transcriptional activity to 22–45 fold in the following order: ARA55 > ARA70 > ARA54 > RAC3 > SRC-1. Together, these data suggest that ARA55 and ARA70 are the two most effective AR coactivators in prostate DU145 cells.

Coexpression of different cofactors, ARA70, ARA54, or SRC-1 could additively enhance AR transcriptional activity

It has been demonstrated that coexpression of SRC-1 and CBP can stimulate estrogen receptor (ER) and progesterone receptor (PR) transcriptional activity in a synergistic manner.[31] In addition, both ARA70 and SRC-1 could act as coactivators for AR transcriptional activation.[17,30] Therefore, we were interested to know if the coexpression of ARA54 with ARA70 or SRC-1 can enhance synergistically the AR-mediated transcriptional activity. While ARA70, ARA54 and SRC-1 could individually induce AR transcriptional activity in DU145 cells, our data further suggested that when two cofactors were expressed simutaneously, the increase in AR-mediated transactivation was additive but not synergistic relative to that observed in the presence of each of them alone.[20] These results indicate that these cofactors may contribute individually to the proper or maximal AR-mediated transcriptional activity.

Interactions between AR and ARA70, ARA55, ARA54 are androgen-dependent

To test whether these three ARAs, identified by our group, can interact with AR in an androgen-dependent manner, we first applied a yeast two-hybrid assay. We found DHT and testosterone could promote the specific interaction of ARAs and wtAR or mtAR at concentrations of greater than 1 nM.

Next, we applied a mammalian two-hybrid assay to confirm this DHT-dependent interaction between AR and ARAs *in vivo*. DU145 cells were co-transfected with a plasmid encoding the LBD of wtAR fused to the GAL4 DNA binding domain (GAL0AR) and a plasmid encoding ARA55, ARA54, or ARA70 fused to the activation domain of VP16. Interaction was estimated by determining the level of luciferase activity from the reporter plasmid, and SV40 large T-antigen was used as a negative control. In the absence of androgen, the combination of GAL0AR and individual ARA showed a negligible amount of activity. A significant level of luciferase activity was induced by the co-transfection of GAL0AR with VP16-ARA70 or VP16-ARA54, only in the presence of 1 nM DHT. The induction of VP16-ARA55 was not as high as ARA70 and ARA54, but still above 3 fold. Together, results from yeast two-hybrid assay and mammalian two-hybrid assay indicate that the specific interaction between ARAs and AR is an androgen-dependent process. However, using glutathione S-transferase (GST) pull-down assay, Alen *et al.*[32] reported that AR could also interact with ARA70 in the absence of androgen. While the answer to this discrepancy is unclear, different assays using different cell environments may contribute to these differences.

17 β-estradiol (E2)-mediated AR transcriptional activity in the presence of different AR coactivators

Previous data using the mouse mammary tumor virus (MMTV)-androgen response element (ARE)-chloram-