phenicol acetyl-transferase (CAT) reporter system have shown that 10 nM E2 could further enhance AR transcriptional activity in the presence of ARA70.[30] We were interested in determining if this ARA70-mediated E2-AR new pathway could also occur with other AR coactivators. We found that E2 (1 nM to 10 nM) could not enhance significantly the AR transcriptional activity in the absence of AR coactivators. Addition of ARA70 could then enhance the AR transcriptional activity 2–4 fold at 1 nM E2 and above 20 fold at 10 nM E2. Among the other AR coactivators we tested, most have only slight induction (2–3 fold) at 10 nM E2 treatment with the exception of SRC-1, which was able to enhance E2-mediated AR transcriptional activity 7–8 fold. The enhancement effect of E2-mediated AR transcriptional activity by various AR coactivators is therefore, quite different from the enhancement effect of DHT-mediated AR transcriptional activity by various AR coactivators. Together, these contrasting data indicating the E2-mediated induction of AR transcriptional activity in the presence of ARA70 may suggest that ARA70 represents a unique and important cofactor among all AR coactivators.

*HF-mediated AR transcriptional activity in the presence of different AR coactivators*

During the androgen ablation therapy, the partial agonist activity of antiandrogen hydroxyflutamide (HF), has been proposed as one of the possible reasons to explain why most prostate cancers will progress into an androgen independent stage. The detailed molecular mechanism of this phenomenon, the so-called flutamide withdrawal syndrome, remains unclear. As ARA70 can confer the androgenic activity to E2, we were also interested in knowing whether AR coactivators could enhance the agonist activity of HF. Our results indicated that ARA70 and ARA55 could enhance the HF-mediated wtAR transcriptional activity 3–6 fold at 1 to 5 μM.[19,33,34] Other coactivators, such as ARA54, RAC3/ACTR/AIB1, SRC-1, and Rb, showed only marginally activity on wtAR. Together, these data indicate that some selective AR coactivators can promote the agonist activity of HF at pharmacological concentrations, which may help us to explain why HF can switch to an agonist activity during androgen ablation therapy of prostate cancer.

*Can AR, ARA70, ARA55, and ARA54 interact to the proteins with histone acetyltransferase activity?*

It has been speculated that specific sets of proteins were recruited by the steroid receptors as coactivators that may function as bridge factors between the receptors and general transcription factors in the preinitiation complex.[35–37]

Identifying and understanding the function of individual components of these complexes are part of the key to answer how nuclear receptors regulate their target genes. More significantly, recent progress in the study of coactivators further linked the transcriptional activation of steroid receptors to chromatin acetylation. Some of these coactivators, such as CBP/p300,[5] SRC-1,[38] and RAC3/ACTR,[9] have been found to either have intrinsic histone acetyltransferase (HAT) activity or have the capacity to recruit the p300/CBP-associated factor (PCAF) that has HAT activity.[39] However, the physiological significance of these cofactors and their involvement in development, differentiation, and reproductive disease remain to be further studied.

Whereas the histone acetylase PCAF has been suggested to be part of a transcriptional complex, the physical interactions between PCAF and AR, or between PCAF and ARAs have remained unclear. Currently, we have applied the mammalian two-hybrid system and immunoprecipitation to test the chemical and functional association of p300/CBP or PCAF to the ARA70, ARA55, and ARA54.

Our results suggest that the essential step of chromatin acetylation during the transcriptional activation of AR could be achieved not only through the CBP-AR interaction, but also is possible through the association of PCAF with AR or AR coactivators, ARA70, ARA54, and ARA55 (Yeh *et al.*, unpublished observations, 1999).

## Discussion

Transcriptional activation or repression by nuclear hormone receptors can be augmented by transcriptional coactivators and corepressors, which can serve as a bridge between the nuclear receptor and the basal transcriptional machinery. In an effort to understand transcriptional regulation by the AR LBD, we have identified ARA70, ARA55, and ARA54 as ligand dependent coactivators.

While SRC-1 has been reported as an effective steroid receptor coactivator,[6] our results from DU145 cells indicated SRC-1 is a relatively weaker AR coactivator. Previous reports indicated SRC-1 had only 2 fold coactivator effect in CV-1 cells.[11] Similar contrasting results also occurred with another coactivator, TIF2, with 20 fold coactivator effect in COS-7 cells vs only 4 fold coactivator effect in HeLa cells.[32] These contrasting results suggest the relative effects by various coactivators may depend heavily on the cell environment. Different cell lines, cell growth conditions, or transfection methods using various vectors and varying ratios of AR to AR coactivators can all contribute to the coactivator effects. Indeed, we also found that ARA70