Supplementary data

YFP PCA analysis and imaging

The fluorescence of reconstituted YFP was evaluated with a fluorescence microscope (Olympus 1×70). The relative amount of YFP intensity was determined by transferring cells to 96-well plates and using fluorometric analysis (Wallac 1420 Multilabel Counter, PE) at excitation and emission wavelengths of 485 and 535 nm, respectively. The intensity of fluorescence was normalized to the total protein concentration in the cell lysate. The background fluorescence intensity corresponding to non-transfected cells was subtracted from the fluorescence intensities of all of the samples. Values are mean ± SD of three independent determinations. For confocal imaging, HEK293 cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton X-100 for 8 minutes, directly mounted with Vectashield medium (Vector Laboratories), and imaged with a Leica TCS SP2 microscope. Acquisition of images was performed using LCS software.

Gel-shift and ChIP Assays

Nuclear extracts of LNCaP cells were prepared (Liu et al., 2003), and then subjected to Gel shift analysis as described previously (Wu et al., 2003; Li et al., 2006). The [α-32P]dCTP-labeled probe of the consensus for ARE is as follows:

AAGTCTGTACAGGTGTTCTTTTTCG. For the ChIP assay, LNCaP cells were stimulated using 10nM DHT for the specified time and treated with formaldehyde. Cells were lysed and subjected to sonication. AR and Smad1 were immunoprecipitated as previously described (Wang et al., 2006). 1% cell lysate taken for the
immunoprecipitation serve as input control. DNA extracted from immunocomplexes was amplified with primer sets as follows: PSA promoter containing AREII: (forward): GTGGAGCTGGATTCTGGG; (reverse): TGGGTACGATCCCCGATT), and PSA enhancer containing AREIII: (forward): CCTCCCAGGTTC AAGTGATT; (reverse): GCCTGTAATCCCAAGCCTTT.

References


