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Coactivator Function Defines the Active Estrogen Receptor Alpha Cistrome▽†

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Proper activation of transcriptional networks in complex organisms is central to the response to stimuli. We demonstrate that the selective activation of a subset of the estrogen receptor alpha (ERα) cistrome in MCF7 breast cancer cells provides specificity to the estradiol (E2) response. ERα-specific enhancers that are subject to E2-induced coactivator-associated arginine methyltransferase 1 (CARM1) action are critical to E2-stimulated gene expression. This is true for both FoxA1-dependent and independent enhancers. In contrast, a subset of E2-suppressed genes are controlled by FoxA1-independent ERα binding sites. Nonetheless, these are sites of E2-induced CARM1 activity. In addition, the MCF7 RNA polymerase II cistrome reveals preferential occupancy of E2-regulated promoters prior to stimulation. Interestingly, E2-suppressed genes tend to lie in otherwise silent genomic regions. Together, our results suggest that the transcriptional response to E2 in breast cancer cells is dependent on the interplay between polymerase II pre-occupied promoters and the subset of the ERα cistrome associated with coactivation.

The transcriptional response to estrogen in numerous tissues, including mammary gland, bone, and uterine tissues, and in diseases such as breast cancer is dependent on estrogen receptor alpha (ERα). Genome-wide positional analyses defining the set of *cis*-regulatory elements recruiting ERα, known as its cistrome, in breast cancer cells have revealed its predominant recruitment to enhancers as opposed to promoter regions (6, 7, 37, 39). As for many other transcription factors, genomic recruitment of ERα is restricted to a small proportion of its putative binding sites (<4.4%) offering a primary means of defining the response to estradiol (E2) (5, 7, 37). Similarly, the promoter predominant Pol II recruitment in breast cancer cells is restricted to a subset of promoters upon E2 stimulation (7, 32, 33, 35). Epigenetic modifications are central to the lineage-specific recruitment at enhancers and promoter regions. Indeed, promoters of activated genes harbor trimethylated histone H3 lysine 4 (H3K4me3) favoring the recruitment of chromatin remodeling enzymes and histone acetylases (1, 18, 42, 52, 56, 58). In contrast, promoters associated with transcriptional repression harbor trimethylated H3K27 (H3K27me3) (1, 3, 36, 42). Similarly, functional enhancers are associated with mono- and dimethylation of H3K4 (H3K4me1, me2) restricting the recruitment and the chromatin remodeling activity of the pioneer factor FoxA1, required for ERα binding, in a lineage-dependent manner, while levels of H3K9me2 are elevated on nonfunctional enhancers (15, 25, 40).

Despite these epigenetic constraints, RNA polymerase II (Pol II) and ERα together are recruited to more than 9,000 independent high-confidence (false discovery rate [FDR], 1%) sites across the genome of breast cancer cells upon E2 stimulation (7). Studies limited to a small number of ERα target sites have implicated coactivators, such as the coactivator associated arginine methyltransferase 1 (CARM1), in the E2 response (22). As they are recruited to ERα binding sites, coactivators allow for a series of posttranslational modifications on histones and other coactivator proteins in order to facilitate chromatin remodeling and cycling of the transcriptional unit essential for the E2 response (41, 57). In the case of CARM1, this involves dimethylation of arginine residues on histone H3 as well as on the coactivator AIB1 (8, 48). In addition, recent studies in *Drosophila* have revealed the dominant presence of poised Pol II at promoters of genes involved in the response to stimuli and developmental signals (47, 69). In the present study, we investigated the impact of CARM1 coactivator's activity on ERα binding sites and of Pol II at promoters in the transcriptional response to E2 through genome-wide positional analyses in human breast cancer cells.

MATERIALS AND METHODS

ChIP-microarray preparation. Cells were hormone deprived for 3 days in phenol red-free medium (Invitrogen) supplemented with 10% charcoal dextran-treated fetal bovine serum. Cells were stimulated with the estrogen 17β-estradiol (10^{-8} M) for 45 min and cross-linked by using 1% formaldehyde. Samples were sonicated (Fisher Sonic Desmembrator, model 500) and immunoprecipitated, as previously described (40), using an antibody against histone H3 arginine 17

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