Mice with targeted gene disruption of one of the estrogen receptor coactivators, p300/CBP-associated factor (PCAF), and its counterpart, PCAF-B, were used to investigate the possible involvement of PCAF and PCAF-B in estrogen receptor-mediated actions in vivo. Among ovariectomized mice that were treated with estrogen, PCAF and PCAF/PCAF-B knockouts showed abnormal growth of the uterus compared with the wild type. The level of c-fos gene expression in the uterus was not induced by estrogen in the knockouts. These observations suggest that PCAF and PCAF-B are required for estrogen-dependent normal growth of the uterus via estrogen receptor-mediated transcriptional regulations.

Key words: coactivator; histone acetyltransferase; p300/CBP-associated factor (PCAF); estrogen receptor; ovariectomy

Estrogens (E2) induce the differentiation and maintenance of reproductive tissues, including the uterus and ovaries, and control bone metabolism and remodeling. The physiological responses of the target organs to E2 are mediated by at least two nuclear receptors for E2 (ERs), ERα and ERβ. Current models of the action of ERs suggest that they modulate the rate of transcription initiation by interacting with the basal transcriptional machinery and by altering the status of chromatin organization at the promoter of target genes via the recruitment of a variety of coactivators, including p160 family members,1–3) p300/CBP,4) and p300/CBP-associated factor (PCAF).5)

The carboxy-terminal half of PCAF has sequence similarity to yeast GCN5 and has intrinsic histone acetyltransferase activity.6) Chromatin consists of nucleosomal core particles, which are histone octamers swapped with DNA. A histone octamer consists of a heterotetramer of the core histones H3 and H4 associated with two heterodimers of H2A and H2B. Each of the four core histones contains an amino terminal tail of amino acids that is rich in lysine residues, and many of these lysine residues can be acetylated in transcriptionally active chromatin. In in vitro assays on nucleosomal histones, recombinant PCAF primarily acetylated lysine 14 of H3 and lysine 8 of H4.6,7) PCAF acts as a transcriptional coactivator for several transcription factors, including ER, in vitro. However, the in vivo roles of endogenous ER-mediated physiological response of the PCAF remain unclear.

Due to the shared ability of PCAF and PCAF-B to bind p300/CBP and their extensive amino acid sequence identity, these two proteins cannot be distinguished by functional differences in vitro.7) To elucidate the in vivo roles of PCAF and PCAF-B, we generated a mouse line in which the PCAF and PCAF-B genes were disrupted.8) PCAF knockout mice are viable and have no overt phenotype; they develop normally throughout their life span and are fertile. In contrast, PCAF-B knockout mice die in utero.8,9) In the present study, we investigated the possible involvement of PCAF and PCAF-B in the ER-mediated actions of E2 in vivo.

PCAF knockout (PCAF−/−) mice were generated by crossing mice that were homozygous for the null allele of the PCAF gene. Since mice that are homozygous for the null allele of PCAF-B die in utero, we crossed mice that were homozygous for the null allele of PCAF with mice that were heterozygous for the null allele of PCAF-B to produce double mutant mice that were homozygous...
for the null allele of PCAF and heterozygous for the null allele of PCAF-B. The genotype of the offspring was determined by Southern blot analysis of genomic DNA. Representative data are shown in Fig. 1A. Both PCAF and PCAF-B protein levels were determined by Western blot analysis (Fig. 1B). The mice were housed under normal laboratory conditions in a 12-h light, 12-h dark cycle. The range of temperature was controlled, and the animals had free access to water and standard animal food (CE-2; Clea Japan, Tokyo, Japan). Adult (4–6 weeks old) females were ovariectomized (OVX) or underwent a sham (sham) operation on day 0, and were administered hypodermically with 17β-estradiol (Sigma-Aldrich Japan, Tokyo, Japan) (76 μg/kg/d) in vehicle (OVX+E2) or vehicle control (ethanol:DMSO:water = 1:4.5:4.5) from day 14 to day 21. The mice were sacrificed on day 21. Each bar represents the mean ± SD (n = 6).

Table 1. Effect of E2 Administration on the Wet Weight (mg) of the Uterus

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>PCAF−/−</th>
<th>PCAF−/−/PCAF-B+/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>sham</td>
<td>77.3 ± 24.9</td>
<td>89.0 ± 29.5</td>
<td>69.0 ± 18.9</td>
</tr>
<tr>
<td>OVX</td>
<td>22.0 ± 4.7</td>
<td>17.0 ± 5.0</td>
<td>16.4 ± 1.8</td>
</tr>
<tr>
<td>OVX+E2</td>
<td>260.0 ± 28.3</td>
<td>180.3 ± 0.6*</td>
<td>160.0 ± 17.9*</td>
</tr>
</tbody>
</table>

OVX or sham operation was performed on day 0. E2 was administered on day 14 to day 21, and the mice were sacrificed on day 21. Each bar represents the mean ± SD (n = 6).

*p < 0.001 vs. WT-OVX+E2.

Fig. 1. Genotyping and Protein Expression Profiles of Mice Used in This Study.
(A) Southern blot analysis. Restriction enzyme-digested genomic DNA isolated from offspring of mating mice was resolved by electrophoresis on agarose gel. For PCAF (upper), the probe hybridizes with 12.5- and 3.5-kb fragments in the wild type and targeted allele respectively. For PCAF-B (lower), the probe hybridizes with 13- and 10-kb fragments in the wild type and targeted allele respectively. See details in ref. 8). (B) Western blot analysis. Protein extract from the uterus of the animal was resolved by electrophoresis on SDS-polyacrylamide gel and immunoblotted with anti-human PCAF antibody (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) (upper), or anti-human PCAF-B antibody (Santa Cruz Biotechnology) (lower), respectively. The staining with Ponceau S of the transferred membrane shows that equivalent extracts were used (data not shown).

Fig. 2. Effect of E2 Administration on Endogenous c-fos Gene Expression in the Uterus.
Total RNA was extracted by TRIzol Reagent (Invitrogen, Carlsbad, CA, U.S.A.) from the uterus of WT and PCAF−/−/PCAF-B+/− mice with or without treatment of E2. Total RNA (10 μg) was separated on 1.1 M formaldehyde/1% agarose gel and then transferred to a nylon membrane (PerkinElmer Life Science, Boston, MA, U.S.A.). 32P-labelled full length rat c-fos cDNA was used as a probe. Mouse 36B4 cDNA was used as a probe for loading control.
induced growth, since they are all capable of amplifying the signal emanating from the interaction of E₂ and ER. Therefore, we propose that one of the possible explanations of PCAF⁻/⁻/PCAF-B⁻/⁻ uterus growth retardation in E₂ treatment is a defect in E₂-induced c-fos gene expression.

Blanco et al. found that PCAF binds directly to the DNA-binding domain of nuclear receptor family members. ERα and ERβ share identical amino acid sequences in their DNA-binding domain, and therefore might bind directly to PCAF (and also PCAF-B) in vitro and in vivo. Shang et al. found that ER and a number of coactivators rapidly associated with E₂ responsive gene promoters followed E₂ treatment in a cyclical fashion. In this model, p300, CBP, and PCAF are all involved in ERα-mediated gene transcription. These studies indicate that endogenous ER-mediated gene transcription is cooperatively regulated by multiple coactivator complexes. In agreement with these in vitro studies, our observations suggest that PCAF and PCAF-B are required for ER-mediated normal growth of the uterus in vivo.

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References


