Organ-specific distribution of AP-1 in AP-1 luciferase transgenic mice during the maturation process

SHU PING ZHONG,1 WEI-YA MA,1 JAMES A. QUEALY,2 YIGUO ZHANG,1 AND ZIGANG DONG1
1The Hormel Institute, University of Minnesota, and 2Austin Medical Center, Austin, Minnesota 55912

Received 7 April 2000; accepted in final form 18 September 2000

Zhong, Shu Ping, Wei-Ya Ma, James A. Quealy, Yiguo Zhang, and Zigang Dong. Organ-specific distribution of AP-1 in AP-1 luciferase transgenic mice during the maturation process. Am J Physiol Regulatory Integrative Comp Physiol 280: R376–R381, 2001.—Activator protein-1 (AP-1), a dimeric complex consisting of proteins encoded by the jun and fos gene families, is a transcription factor induced by a variety of signals including those eliciting proliferation, differentiation, and neoplastic transformation. Although AP-1 has been widely studied in the last decade, physiological levels of AP-1 in different tissues are unclear. In the present study, we analyzed AP-1 activity in several organs (liver, kidney, brain, lung, spleen, heart, skin) of AP-1-luciferase transgenic mice of various ages. Results of these studies indicate that the level of AP-1 in young mice is much higher than that in older mice, and, second, that the skin contains considerably higher levels of AP-1 than other organs. The level of phosphorylated extracellular signal-regulated protein kinase (ERK) in skin was higher in 1- and 2-day-old mice than in mice of other ages. In addition, phosphorylated p38 kinase was high in 2-day-old and 1-wk-old mice, but phosphorylated c-Jun NH2-terminal kinase was not detected at any age. AP-1 activity and level of phosphorylated ERKs declined with maturation. These results imply that AP-1 activity mediated through an ERKs-dependent pathway may be involved in skin development.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Acknowledgments: We thank Dr. Marcelo C. Kato for the gift of fos reporter constructs. This study was supported by a grant from the Hormel Foundation and the University of Minnesota. This study was supported in part by U.S. Public Health Service Grants AI29984 and CA46013. SHPZ was supported in part by the National Science Foundation Graduate Research Fellowship and the University of Minnesota Postdoctoral Fellowship Program.

Address for reprint requests and other correspondence: Z. Dong, The Hormel Institute, Univ. of Minnesota, 801 16th Ave NE, Austin, MN 55912 (E-mail: zgdong@smig.net).
AP-1 DURING MATURATION

C57BL/6 male mouse carrying the 2× 12-O-tetradecanoylphorbol 13-acetate (TPA) response element (TRE)-luciferase transgene was crossed with DBA/2 females (SASCO, Omaha, NE) (20). The F1 offspring were screened by testing both the basal level and TPA-induced level of luciferase activity for the presence of the AP-1 luciferase reporter gene. Males and females were housed separately in solid-bottom polycarbonate cages on ventilated animal racks in temperature-, humidity-, and light-controlled conditions. Food and water were available ad libitum. The animal facility at the Hormel Institute is accredited by the American Association for Accreditation of Laboratory Animal Care. Different organs (liver, kidney, brain, lung, spleen, heart, and skin) were harvested from the transgenic mice of various ages.

Assay of AP-1 luciferase activity. Mice were killed by cervical dislocation. Samples from skin, liver, kidney, brain, spleen, and heart were harvested by a biops punch (1.5 mm, Acuderm, Ft. Lauderdale, FL) and immediately placed in 100 μl of lysis buffer [0.1 M potassium phosphate buffer at pH 7.8, 1% Triton X-100, 1 mM 1,4-dithiothreitol (DTT), 2 mM EDTA, and 4°C overnight]. Tissues were put on dry ice for immunobistochemical analysis. The AP-1-dependent luciferase activity of samples in the supernatant fraction was measured by luminometer (Monolight 2010, Analytical Luminescence Laboratory, San Diego, CA) for 10 s after mixing the extract and luciferase assay reagent as described (18). The luciferase assay reaction was verified to measure the linear range. The results are expressed as relative AP-1 activity.

Immunohistochemical analysis. For immunohistochemical analysis, the tissues from different organs were harvested and put on dry ice. Frozen sections were produced by using a freezing microtome. Mouse epidermal JB6 P+ 1–1 cells with 2× TRE-luciferase reporter and frozen slices of different tissues were first fixed with solution A (50% acetone and 50% methanol) and then blocked with 5% BSA. Primary antibodies of rabbit antiluciferase (Research Diagnostics) were incubated with the slices at 37°C for 60 min, and the slices were then washed three times (10 min/time) with PBS (50% acetone and 50% methanol) and then blocked with 5% BSA. Tissues were put on dry ice for immunobistochemical analysis. The AP-1-dependent luciferase activity of samples in the supernatant fraction was measured by luminometer (Monolight 2010, Analytical Luminescence Laboratory, San Diego, CA) for 10 s after mixing the extract and luciferase assay reagent as described (18). The immunohistochemical assay reaction was verified to measure the linear range. The results are expressed as relative AP-1 activity.

Mitogen-activating protein kinase analysis. Skin (equal weights) was harvested from mice of different ages and placed on dry ice. Samples were then cut into small pieces, placed on ice, and incubated in 500 μl of SDS lysis buffer (62.5 mM Tris-HCl pH 6.8, 2% (wt/vol) SDS, 10% glycerol, 50 mM DTT) for 60 min. The tissue lysates were sonicated for 20 s and centrifuged at 14,000 rpm at 4°C for 10 min. The supernate was saved and diluted with three volumes of acetone and left on ice for 10 min. The suspension was centrifuged in 14,000 rpm at 4°C for 10 min, and the pellets were subsequently resuspended in 800 μl acetone and centrifuged in 14,000 rpm at 4°C for 10 min. The pellets were then suspended in 200 μl of SDS lysis buffer. The protein concentration was measured using the Bradford method (Bio-Rad). An equal concentration of protein from each sample was resolved on a 10% SDS-PAGE after boiling for 5 min. The resolved proteins were then transferred to polyvinylidene difluoride (PVDF) membranes for Western blot analysis. PVDF membranes were blocked with 5% fat-free milk in PBS for 1 h at room temperature and incubated with the specific antibodies of rabbit anti-phospho-p42/44 mitogen-activated protein (MAP) kinase, phospho-p38 MAP kinase, and phospho-stress-activated protein kinase (SAPK) and/or c-Jun NH2-terminal ki-
These results show clearly that AP-1 expression in skin is markedly higher than that in other organs from different aged mice up to 3 mo old \((P < 0.0001)\).

**AP-1 activity in transgenic mice of different ages.** Although previous studies showed that aging is associated with decreased expression of c-fos/c-jun in different cell systems \((43)\), the way in which AP-1 transcriptional activity changes with maturation remains to be determined. In the present study, we analyzed AP-1 transcriptional activity during the maturation process in AP-1 luciferase transgenic mice. The level of AP-1 activity in different organs of transgenic mice decreased with maturation (Fig. 2). AP-1 activity in the various organs from 1-day-old mice was higher than AP-1 activity in organs of mice of all other age groups except the brain in 2-day- and 1-wk-old mice \((P < 0.05)\) (Fig. 2). On the other hand, AP-1 activity in six organs (liver, kidney, brain, lung, spleen, skin) decreased sharply (Fig. 2) from 1-day- to 3-mo-old mice. AP-1 activity detectable in the brain was maintained at a high level from 1-day- to 1-wk-old mice, and then the activity decreased between 1-wk- and 1-mo-old mice and remained at very low levels thereafter (Fig. 2). The marked reduction of AP-1 activity in the different organs with maturation suggests that AP-1 activity or expression may be inversely related to age.

**Tissue distribution of AP-1 luciferase activity in skin.** The above results clearly demonstrated that AP-1-dependent activity is highly expressed in skin compared with other organs. Immunohistochemistry staining of skin from 1-day-old mice revealed that the epidermis was stained by antiluciferase antibody conjugated with FITC and luciferase fluorescence and was located in cytoplasm (Fig. 3G), compared with nuclear staining in JB6 P+ 1–1 cells after UV-B irradiation (Fig. 3H). Photomicrograph images indicated that AP-1...
was expressed from the stratum basale (stratum germinativum) to the stratum corneum (Fig. 3A). AP-1 luciferase staining of skin from 1-day-old mice confirms that cell proliferation in the stratum basale is very actively occurring. The cells of the stratum basale were found to contain an abundance of AP-1 activity. AP-1-dependent luciferase activity in skin gradually diminished from 1-day- to 3-mo-old mice (Fig. 3, A-F). The mature 3-mo-old mice expressed only trace amounts of AP-1-dependent luciferase activity (Fig. 3F). In addition, we observed weaker staining of AP-1 luciferase in other organs (liver, kidney, brain, spleen, lung, and heart) from different age groups (data not shown), further confirming that, compared with skin, these organs contained considerably lower levels of AP-1 luciferase activity.

**Level of phosphorylated MAP kinases in the skin of different aged mice.** MAP kinases are proline-directed serine/threonine kinases that are activated by dual phosphorylation on threonine and tyrosine residues in response to a wide array of extracellular stimuli. Three distinct groups of MAP kinases have been identified in mammalian cells, including extracellular-regulated kinase (ERK), JNK, and p38 kinase. MAP kinases are mediators of signal transduction from the cell surface to the nucleus and AP-1 family proteins are target molecules of MAP kinases. Besides observing different levels of AP-1 activity in various organs from transgenic mice, we also analyzed organs for MAP kinase activity to determine the relationship between AP-1 and MAP kinases during maturation. In the skin, higher levels of phosphorylated ERKs were detected in 1-day- and 2-day-old mice (Fig. 4, A and D) and found to decrease gradually in 1-wk- to 3-mo-old mice. Higher levels of phosphorylated p38 kinase were detected in 2-day- and 1-wk-old mice (Fig. 4, B and E), whereas phosphorylated JNK was not detected in any of the various age groups compared with mouse epidermal JB6 cells after UV-B irradiation (Fig. 4C). These results indicate that phosphorylated ERK expression decreases as the mouse matures, corresponding to AP-1 activity in skin from 1-day- to 3-mo-old mice. On the other hand, phosphorylated p38 kinase did not display a similar pattern. These results indicate that AP-1 and ERKs might be involved in skin development.

**DISCUSSION**

In the present study, we provide experimental evidence that AP-1 activity in young mice is significantly higher than that found in older mice and that the skin of newborn mice contains considerably higher levels of AP-1 activity than found in other organs. The levels of phosphorylated ERKs in skin were higher in 1- and

---

**Fig. 4.** Level of phosphorylated mitogen-activated protein kinases from skin in different aged AP-1-luciferase transgenic mice. Skin proteins were extracted and resolved as described in MATERIAL AND METHODS. Phosphorylated (P) extracellular signal-related protein kinases (ERKs) and nonphosphorylated (NP) ERKs, P-p38 and NP-p38 kinases, and P c-Jun NH2-terminal kinase (JNKs) and NP-JNKs were detected with specific antibodies. A: P-ERKs and NP-ERKs in the skin. B: P-p38 and NP-p38 kinase in the skin. C: P-JNKs and NP-JNKs in the skin. D: the counts incorporated into P-ERKs in A were quantified using a Storm PhosphoImager 840 (Molecular Dynamics), and the results are displayed graphically. Values are represented as the means ± SE from skin in different aged transgenic mice with the AP-1 luciferase reporter gene. E: the counts incorporated in P-p38 the positions of P-ERK1/2 and NP-ERK1/2, P-p38 and NP-p38 kinase, and P-JNKs and NP-JNKs, respectively. Lane 1, 1 day; lane 2, 2 days; lane 3, 1 wk; lane 4, 1 mo; lane 5, 2 mo; lane 6, 3 mo; lane 7, JB6 C141 cells were harvested 30 min after 4 kJ/m² of UV-B irradiation.
of AP-1 declines with maturation, with AP-1 being almost undetectable in the mature transgenic mouse. AP-1 levels in each organ of 1-day-old mice were higher than all other age groups, but skin contained extraordinarily high levels of AP-1 activity. The tissue-specific expression of high levels of AP-1 in skin implies that AP-1 may play a key role in skin development.

MAP kinases, including ERKs, JNKs, and p38 kinases, are mediators in a protein kinase cascade for regulation of transcription factor AP-1 activity (17, 18, 29). c-Jun is phosphorylated and activated by JNK MAP kinase (8). In contrast, activating transcription factor-2 is phosphorylated and activated by both JNK and p38 MAP kinases (15, 31). Fos is phosphorylated by a kinase other than JNK MAP kinase (7). Our study shows that the levels of phosphorylated ERKs decline with the maturation process and AP-1 activity is associated with ERK phosphorylation during skin development in mice.

In summary, our experiments suggest that AP-1, MAP kinases, ERKs, and p38 may play an important role in skin development and regulation of AP-1 activity in the skin during the maturation process may be through an ERK-dependent pathway.

We thank Dr. Harald H. O. Schmid and Dr. Ann Bode for scientific discussion and editorial advice and Andria Hansen for secretarial assistance.

This research was supported by National Cancer Institute Grants CA-77646, CA-74916, and CA-81064 and by the Hormel Foundation.

REFERENCES


2-day-old mice than in older mice. In addition, levels of phosphorylated p38 kinase were high in 2-day- and 1-wk-old mice, but phosphorylated JNK was not detectable in any of the different age groups. AP-1 activity and the level of phosphorylated ERKs declined as the mice matured.

AP-1 is a transcription factor comprised principally of Jun and Fos protein family heterodimers that bind to a consensus cis element found on the transcriptional promoters of a number of genes whose expression is induced by tumor promoters (1, 10). AP-1/Jun is required for early Xenopus development and mediates fibroblast growth factor, but not activin receptor signaling during mesoderm induction and is a key signaling molecule in the development of the posterior structure (12). c-Jun-deficient mice die during mid- to late gestation and display morphological abnormalities in the liver and widespread edema (16, 24). Mice lacking c-Fos are viable but display defects in bone formation and in the hematopoietic system (23, 42).

Evidence for the critical importance of AP-1 activity in cellular transformation by tumor promoters and/or oncogenes has been extensively reported (2, 5, 6, 9, 10, 27). Transgenic mice overexpressing c-Fos developed osteosarcomas and chondrosarcomas, suggesting that aberrant expression of the c-fos gene can promote neoplastic transformation of bone tissue (14). Seez et al. (35) found that the c-fos gene is indispensable for malignant progression of skin tumors. UV-B irradiation highly induced the expression of AP-1 in JB6 cells and AP-1 luciferase transgenic mice (19). Inhibitors of AP-1 blocked tumor promotion in AP-1 luciferase transgenic mice (17). These data collectively suggest that skin carcinogenesis is associated closely with AP-1 activation.

Previous evidence indicates that AP-1 DNA binding activity might be related to the maturation and aging process. For example, Riabowol et al. (32) reported that serum-induced c-fos gene expression was impaired in aged human fibroblasts. Diminished concanavalin-A-induced AP-1 binding activity was observed in lymphocytes derived from the spleen of aged mice compared with younger mice (37). AP-1 transcription factor binding activity was reduced by 38% with age in unstimulated adrenal medulla (41). In contrast, AP-1 binding activity in nuclear extracts of whole brains was unchanged with age in unstimulated rats (3), and pentyleneetetrazole-induced AP-1 binding activity in the hippocampi of aged rats was unchanged compared with younger animals (25). Grassilli et al. (13) reported that fibroblasts from centenarians exhibited the same capacity to respond to different mitogenic stimuli as fibroblasts from young donors and that the well-preserved proliferative response is likely due to the fact that some pivotal regulators, c-fos, c-jun, and AP-1, are still fully inducible, despite a long process of in vivo senescence. Although the roles of replicative senescence and DNA binding activity of AP-1 have been studied (32, 40), whether AP-1 transcriptional activity changes during development remains unknown. In the present study, we provide evidence that the expression