AP-1/J un Is Required for Early Xenopus Development and Mediates Mesoderm Induction by Fibroblast Growth Factor but Not by Activin*

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In Xenopus, normal mesoderm formation depends on signaling through the fibroblast growth factor (FGF) tyrosine kinase receptor. An important signaling pathway from receptor tyrosine kinases involves Ras/Raf/MAP kinase. However, the downstream pathway that occurs in the nucleus to finally trigger gene expression for mesoderm formation remains unknown. We report here that a high level of activator protein-1 (AP-1)-dependent transcripational activity is detected during the early development of Xenopus embryos. Injection of a dominant negative mutant jun (DNM-jun or TAM67) RNA into the two-cell stage embryos inhibited endogenous AP-1 activity and blocked normal embryonic development with severe posterior truncation in tadpoles. The inhibition of AP-1 activity and the phenotypic change induced by TAM67 was rescued by co-injection of wild-type c-jun RNA, but not by the control β-galactosidase RNA. The FGF-stimulated mesoderm induction was markedly inhibited in animal cap explants from the embryos injected with TAM67. Activin induction of mesoderm, on the other hand, was normal in the embryos injected with TAM67 RNA. These findings suggest that AP-1 mediates FGF, but not activin, receptor signaling during mesoderm induction and the AP-1/J un is a key signaling molecule in the development of posterior structure.

Using stage 8 Xenopus embryo animal pole explants, it has been shown that mesoderm induction occurs at an early stage of vertebrate embryogenesis and is stimulated by growth factors, e.g., bFGF† and members of the FGF family (1). Experiments with a dominant-negative mutant of FGF receptor indicated that FGF signaling is required in the early embryo for the formation of posterior and lateral mesoderm (2). Similar experimental results were obtained for activin receptor signaling (3–5). Disruption of activin signaling blocks mesoderm formation (3, 4). Although the detailed molecular mechanism is not clear, it appears that both Ras and Raf are involved in FGF signaling, while only Ras is involved in the activin-stimulated signaling pathway (6, 7). More recently, it has been reported that MAP kinase is required for FGF-induced mesoderm formation (8, 9). AP-1 activity has been reported to be modulated through the Ras/Raf and MAP kinase signaling pathways in many cell lines (10). Stimulators of AP-1 include the protein kinase C activator phorbol 12-myristate 13-acetate (TPA), growth factors such as platelet-derived growth factor, epidermal growth factor, FGF, and interleukins and oncogene products (11–17). The AP-1 complex consists of dimers of jun and fos multigene families and is a sequence-specific DNA binding transcription factor that is part of a pathway by which intracellular signals are converted into changes in gene activity (10).

Although AP-1 is downstream of the signal transduction pathway of Ras/Raf in many biological systems and Ras/Raf and MAP kinase are involved in FGF-induced mesoderm induction (7, 10), these experiments do not reveal whether Ras/Raf and MAP kinase act through AP-1 to induce mesoderm or whether pathways involving other transcription factors are implicated. In the present study, we investigated the role of AP-1 activity in the Xenopus development in response to FGF.

EXPERIMENTAL PROCEDURES

DNA and RNA Preparation—The dominant negative c-jun (DNM-jun or TAM67) and c-jun were subcloned into SP64TEN (18, 19). DNM-ras, and DNM-rf were inserted into Bluescript II SK+. These vectors contained SP6, T7, or T3 promoter which is required for in vitro RNA transcription. Each of these constructs was linearized and used for in vitro transcription by using an in vitro transcription kit (Ambion, Austin, TX), and capped mRNAs were prepared as described by Moan and Christian (20).

mRNA Injection and Explant Culture of Embryonic Tissues—Xenopus laevis embryos were obtained by artificial insemination after induction of females with 500 units of human chorionic gonadotropin. Developmental stages were designated according to Nieuwkoop and Faber (21). The jelly layer was removed with 2.5% thioglycolic acid (pH 8.1). The two-cell stage embryos were injected with fixed amount of synthetic capped mRNAs. The injected embryos were allowed to develop up to 45 stages.

Analysis of AP-1 Activity—We have used an AP-1 luciferase reporter (AP-1)-Luc in this study. (AP-1)-Luc containing four AP-1 binding sequences (TGAC/GTCA) was inserted into a luciferase construct with the minimal promoter sequences from the albumin gene. (AP-1)-Luc plasmid DNA was injected at 50 pg/embryo alone or together with various mRNAs into two blastomeres of the two-cell stage embryos (22). After injection, five embryos per group were pooled at various developmental stages and homogenized to prepare the cell extract. AP-1-dependent luciferase activity in the extract was determined using the...
luciferase assay reagent from Promega and a luminometer from Analytical Luminescence Laboratory (Monolight 2010) for 10 s after mixing the extract and assay reagent (22).

Detection of Molecular Markers—Total RNA was isolated from animal caps and oligonucleotide primers for the reverse transcription-polymerase chain reaction (RT-PCR) were as described previously (18, 19). Molecular markers for development including muscle actin, elongation factor-1α (EF-1α), Xbra, and goosecoid were analyzed by RT-PCR as described previously (18, 19).

RESULTS AND DISCUSSION

In order to study the role of AP-1 activity in the development of Xenopus, the two-cell stage Xenopus embryos were injected with (AP-1)4-Luc, a construct that contains four tandem AP-1 binding sites (TPA-responsive cis-enhancer elements, TRE) linked to a luciferase reporter gene (22, 23). The embryos were collected at various developmental stages, and AP-1-dependent luciferase activity of the embryo lysates was measured. As shown in Fig. 1, AP-1 activity was detectable at stage 10 and increased with increasing developmental stage. The corresponding vector Al-Luc without AP-1 binding sites was used as a control, and the luciferase activity was found to remain at the background level (Table I).

AP-1 activity was detectable at stage 10 and increased with increasing developmental stage. The corresponding vector Al-Luc without AP-1 binding sites was used as a control, and the luciferase activity was found to remain at the background level (Table I). The high levels of AP-1 activity in the early embryos suggest that AP-1 might play an important role in early development. It has been reported that fos was expressed at a low level at the mid-blastula, late neurula, and tadpole stage (24). By using a specific antibody (Ab-1, Oncogene Sciences), we detected a c-Jun protein band that can compete with a c-Jun peptide at stages 10 and 33 (data not shown) (22, 23).

If AP-1 is a mediator of FGF/Ras/Raf signaling for the induc-
Defects produced in embryos injected with dominant negative jun (TAM67) RNA

| RNA injected          | No. of experiments | No. of surviving embryos | Normal | Tail truncation | Bent axis | Nonspecific abnormality |
|-----------------------|--------------------|----------------------------|--------|-----------------|-----------|------------------------|
| c-jun, 1 ng           | 4                  | 72                         | 54 (77)* | 0               | 6 (8)     | 12 (17)                |
| TAM67, 1 ng           | 8                  | 286                        | 99 (35) | 113 (39)        | 60 (21)   | 14 (5)                 |
| c-jun, 2 ng + TAM67, 1 ng | 4              | 72                         | 20 (69) | 4 (6)           | 5 (7)     | 13 (18)                |
| β-gal, 3 ng           | 2                  | 76                         | 70 (92) | 0               | 4 (5)     | 2 (3)                  |
| Water                 | 8                  | 249                        | 224 (90) | 0               | 13 (5)    | 12 (5)                 |
| Uninjected            | 8                  | 261                        | 256 (98) | 0               | 5 (2)     | 0                      |

* Numbers in parentheses are percentages.

Rescue of TAM67-inhibited AP-1 activity by wild-type c-jun

Fifty pg of (AP-1)Luc plasmid DNA with 1–3 ng of mRNA were injected into two blastomers of two-cell stage embryos, and AP-1 activity was measured at different developmental stages. Results are mean ± standard error for three independent experiments. p values are determined by Student’s t test.

| Developmental stage | AP-1 activity | p value |
|---------------------|---------------|---------|
|                     | β-gal (3 ng)  | TAM67 (1 ng) | c-jun (2 ng) + TAM67 (1 ng) | β-gal versus c-jun + TAM67 |
| 8                   | 14 ± 2        | 15 ± 0.6   | 13 ± 3.3               | >0.1                      |
| 12                  | 2545 ± 402    | 1500 ± 151 | 2814 ± 519            | >0.1                      |
| 16                  | 21,499 ± 1917 | 10,230 ± 670 | 18,907 ± 1196          | >0.1                      |
| 18                  | 20,143 ± 2396 | 15,013 ± 714 | 25,953 ± 1443          | >0.05                     |
| 20                  | 21,167 ± 3836 | 10,365 ± 468 | 25,415 ± 4919          | >0.05                     |
| 22                  | 28,111 ± 1700 | 16,247 ± 862 | 25,283 ± 2876          | >0.01                     |
| 33                  | 59,499 ± 1425 | 29,164 ± 5213 | 55,534 ± 5436          | >0.05                     |

* Relative light units.
truncated tails (39%) and bent anterior-posterior axis (21%), similar to the lithium chloride-induced phenotype. A small fraction (5%) of the water-injected embryos displayed bent axis as a result of mechanical damage during injection. The incidence of abnormality of TAM67-injected embryos was significantly higher than that of the water-control group. Co-injection of wild-type c-jun with TAM67 at 2:1 ratio of RNA clearly rescued the TAM67 defects. The bent axis embryos were also reversed to the normal background level (7%). As a control, overexpression of a wild-type c-jun alone resulted in an essentially normal phenotype (Table II). Moreover, co-injecting TAM67 with wild-type c-jun RNA also rescued TAM67-inhibited AP-1 activity (Table III). At stages 16, 18, 20, and 22, AP-1 activity was significantly inhibited by TAM67 \((p < 0.05, \text{or } p < 0.01)\). After co-injection of 2 ng of c-jun RNA with TAM67, AP-1 activity in these stages was not significantly different from the corresponding β-gal controls (Table III). These experiments show that the effects of TAM67 on embryonic development were specifically due to the inhibition of AP-1 and/or other J un-containing transcription factor activity. There are several mesoderm-inducing factors including Vg1, FGF, activin, and bone morphogenetic proteins (31, 32). Their functions differ in the induction of different parts of mesoderm. For example, FGF signaling is required for formation of posterior and lateral mesoderm, while activin induces anterior dorsal mesodermal tissues (5, 27, 28, 32–35). Bone morphogenetic protein-4, on the other hand, induces ventral mesodermal tissues and antagonizes dorsal and neural inducing signals (18, 19, 32, 36–38). As discussed above, DNM-jun (TAM67)-induced posterior truncations were similar to those observed when a dominant negative FGF receptor or a DNM-raf mRNA was introduced into Xenopus embryos (5, 11). Therefore, AP-1/jun appears to be involved in the FGF-ras/raf signaling. This model was further tested by an animal pole explant experiment. As shown in Fig. 4, after injection of TAM67 mRNA into the two-cell stage embryos, bFGF-induced elongation of animal caps was inhibited by TAM67 mRNA. Moreover, the bFGF-induced muscle formation was completely blocked in TAM67 explants. By contrast, the explants derived from TAM67-explants responded well to activin, a dorsal-type mesoderm inducer. By RT-PCR analysis, a muscle-specific actin signal was completely blocked in TAM67 explants after induction by bFGF, but only slightly decreased after induction by activin (Fig. 4). After normalization with internal control EF-1α, expression of the activin-induced early dorsal marker goosecoid was not affected by TAM67 (Fig. 4).

In summary, this report provides evidence for a role of AP-
1/jun in Xenopus development and suggests that AP-1 mediates mesoderm induction by FGF. By contrast, AP-1 appears not to mediate activin-stimulated mesoderm induction, implying that the AP-1 pathway is relatively specific for a particular group of growth factors, typified by FGF receptors. These findings demonstrate that AP-1/jun is a key signaling molecule, possibly downstream of FGF-Ras/Raf in the development of Xenopus posterior structure.

REFERENCES
1. Jessell, T. M., and Melton, D. A. (1992) Cell 86, 257–270
2. Amaya, E., Musci, T. J., and Kirschner, M. W. (1991) Cell 66, 257–270
3. Hemmati-Brivanlou, A., and Melton, D. A. (1992) Nature 359, 609–614
4. Hemmati-Brivanlou, A., and Melton, D. A. (1994) Cell 77, 273–281
5. Green, J. B. A., New, H. V., and Smith, J. C. (1992) Cell 71, 731–739
6. Whitman, M., and Melton, D. A. (1992) Nature 357, 252–254
7. MacNicol, A. M., Muslin, A. J., and Williams, L. T. (1993) Cell 73, 571–583
8. LaBonne, C., Burke, B., and Whitman, M. (1995) Development 121, 1475–1486
9. Umbhauer, M., Marshall, C. J., Mason, C. S., Old, R. W., and Smith, J. C. (1995) Nature 376, 58–62
10. Angel, P., and Karin, M. (1991) Biochim. Biophys. Acta 1072, 129–157
11. Curran, T., and Franzoa, B. R. (1988) Cell 55, 395–397
12. Kerr, L. D., Hidt, L. T., and Matrisian, L. M. (1988) Science 242, 1424–1427
13. Vogt, P. K. (1992) Cancer 69, 2610–2614
14. Wasylyk, C., Imler, J. L., Perez-Mutul, L., and Wasylyk, B. (1987) Cell 40, 525–534
15. Lee, W., Mitchell, P., and Tjian, R. (1987) Cell 49, 741–752
16. Muller, R., Bravo, R., Burckardt, J., and Curran, T. (1984) Nature 312, 716–720
17. Quantin, B., and Breathnach, R. (1988) Nature 334, 538–539
18. Xu, R.-H., Dong, Z., Maeno, M., Kim, J., Ueno, N., Sredni, D., Colburn, N. H., and Kung, H.-f. (1995) Proc. Natl. Acad. Sci. U. S. A., in press
19. Xu, R.-H., Kim, J., Taira, M., Zhan, S., Sredni, D., and Kung, H.-f. (1995) Biochem. Biophys. Res. Commun. 212, 212–219
20. Moon, R. T., and Christian, J. C. L. (1989) Technique 1, 76–89
21. Nieuwkoop, P. D., and Faber, J. (1967) Normal Table of Xenopus laevisi, Daudine, North-Holland, Amsterdam
22. Dong, Z., Lavrovsky, V., and Colburn, N. H. (1995) Carcinogenesis 16, 749–756
23. Dong, Z., Birrer, M. J., Watts, R. G., Matrisian, L. M., and Colburn, N. H. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 609–613
24. Kindy, M. S., and Verma, M. (1989) Cell Growth Differ. 31, 27–37
25. Lazarus, P., and Calcagnotto, A. (1994) Cancer Lett. 82, 201–208
26. Alani R., Brown, P., Binetruy, B., Dosaka, H., Rosenberg, R. K., Angel, P., Karin, M., and Birrer, M. J. (1991) Mol. Cel. Biol. 11, 6286–6295
27. Smith, J. C., Price, B. M., VanNimmen, K., and Huylebroeck, D. (1990) Nature 345, 729–731
28. Ueno, H., Gunn, M., Dell, K., Tseng, A., and Williams, L. T. (1992) J. Biol. Chem. 267, 1470–1476
29. Brown, P. H., Sanders, D. A., Alani, R., Birrer, M. J. (1992) Oncogene 8, 877–886
30. Brown, P. H., Chen, T. K., and Birrer, M. J. (1994) Oncogene 9, 791–799
31. Domann, F. E., Levy, L. P., Birrer, M. J., and Bowden, G. T. (1994) Cell Growth Differ. 5, 9–16
32. Thomsen, G., Woff, T., Whitman, M., Sokol, S., Vaughan, J., Vale, W. W., and Melton, D. A. (1990) Cell 63, 485–493
33. Harland, R. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10243–10246
34. Asashima, M., Nakano, H., Shimada, K., Kinoshita, K., Ishi, K., Shibai, H., and Ueno, N. (1990) Roux's Arch. Dev. Biol. 199, 330–335
35. Mathews, L. S., and Vale, W. M. (1991) Cell 65, 973–982
36. Kessler, D. S., and Melton, D. A. (1994) Science 266, 596–604
37. Suzuki, A., Thies, R. S., Yamaji, N., Song, J. J., Watanabe, J. M., Murakam, K., and Ueno, N. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10255–10259
38. Maeno, M., Ong, R. C., Suzuki, A., Ueno, N., and Kung, H.-f. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10260–10264
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