sion, could well account for the differences in skeletal phenotypes between isthmus and FGF8 bead grafts. Therefore, the variability in the duplications observed by Noden may also be explained by the variability of local FGF8 concentration present in grafted tissue. In contrast to the isthmic grafts, the duplicated first-arch structures observed in the *Hoxa2* mutants exhibit a mirror image polarity. This implies that other factors must be involved in patterning different axes of polarity in these duplications. The transposition of a signaling center might disrupt the mechanisms that influence polarity or axis patterning.

These experiments underscore the important role played by *Hoxa2* in branchial arch identity. Recently, functional inroads have been made into understanding the precise mechanisms by which *Hoxa2* influences the morphogenesis of second-arch elements (*30*). *Hoxa2* is widely expressed in the second-arch mesenchyme but is excluded from the chondrogenic domains and acts very early in the chondrogenic pathway upstream of *Sox9*, *Col2a1*, and *Cbaf11* to repress their expression. During normal development, both endochondral and dermal (intramembranous) ossification occurs in first-arch morphogenesis; however, endochondral ossification primarily occurs in second-arch morphogenesis. Therefore, one of the roles of *Hoxa2* in the second branchial arch may be the prevention of dermal bone formation. Overexpression studies of *Hoxa2* in chick and zebrafish embryos have now confirmed its role as a true selector gene (*28*, *29*). Therefore, *Hoxa2* not only inhibits development of the lower jaw skeleton but is also primarily responsible for specifying second branchial arch fate.

The presence of the isthmus as a mechanistic basis for first-arch duplications also helps resolve two puzzling aspects of Noden's work (*2*). First, there was considerable variability in the frequency of duplications observed, presumably arising through variable inclusion of the isthmus and, consequently, the local concentration of FGF8. Second, it explains why both first-arch and frontonasal neural crest develop similar duplicated skeletal structures when transposed posteriorly, even though they are derived from different axial levels.

Therefore, rather than providing evidence for the prepatterning of neural crest cells, Noden's experiments (*2*) highlight the importance and effects of local signaling centers, such as the isthmus, in A-P patterning and regulation of *Hox* gene expression (*26*, *27*). This study, together with recent evidence from mouse, chick, and zebrafish transplantation studies, argues as a general principle that cranial neural crest cells are not prespecified or irreversibly committed before their emigration from the neural tube (*5*, *11*, *16*–*18*). Rather, neural crest patterning is based on plasticity and the ability of neural crest cells to respond to environmental influences in the branchial arches, and future attention will be focused on the nature of these signals.

References and Notes

1. N. Le Douarin, *The Neural Crest* (Cambridge Univ. Press, Cambridge, 1983).
2. D. Noden, *Dev. Biol.* **96**, 144 (1983).
3. ———, *Development* **103**, 121 (1988).
4. A. Lumsden, R. Krumlauf, *Science* **274**, 1109 (1996).
5. P. Trainor, R. Krumlauf, *Nature Rev. Neurosci.* **1**, 116 (2000).
6. A. Lumsden, N. Sprawson, A. Graham, *Development* **113**, 1281 (1991).
7. G. Serbedzija, S. Fraser, M. Bronner-Fraser, *Development* **116**, 297 (1992).
8. J. Sechrist, G. N. Serbedzija, T. Scherson, S. E. Fraser, M. Bronner-Fraser, *Development* **118**, 691 (1993).
9. N. Osumi-Yamashita, Y. Ninomiya, H. Doi, K. Eto, *Dev. Biol.* **164**, 409 (1994).
10. T. F. Schilling, C. B. Kimmel, *Development* **120**, 483 (1994).
11. P. M. Kulesa, S. E. Fraser, *Development* **127**, 1161 (2000).
12. P. Kulesa, M. Bronner-Fraser, S. Fraser, *Development* **127**, 2843 (2000).
13. F. M. Rijli *et al.*, *Cell* **75**, 1333 (1993).
14. M. Gendron-Maguire, M. Mallo, M. Zhang, T. Gridley, *Cell* **75**, 1317 (1993).
15. O. Chisaka, M. Capecchi, *Nature* **350**, 473 (1991).
16. T. Schilling, *Dev. Biol.* **231**, 201 (2001).
17. P. Trainor, R. Krumlauf, *Nature Cell Biol.* **2**, 96 (2000).
18. J. Saldivar, C. Krull, R. Krumlauf, L. Ariza-McNaughton, M. Bronner-Fraser, *Development* **122**, 895 (1996).
19. J. R. Saldivar, J. W. Sechrist, C. E. Krull, S. Ruffin, M. Bronner-Fraser, *Development* **124**, 2729 (1997).
20. P. Hunt, J. D. W. Clarke, P. Buxton, P. Ferretti, P. Thorogood, *Dev. Biol.* **198**, 82 (1998).
21. G. F. Couly, A. Grapin-Bottom, P. Coltey, N. M. Le Douarin, *Development* **122**, 3393 (1996).
22. N. Itasaki, J. Sharpe, A. Morrison, R. Krumlauf, *Neuron* **16**, 487 (1996).
23. A. Grapin-Botton, M.-A. Bonnin, L. Ariza-McNaughton, R. Krumlauf, N. M. LeDouarin, *Development* **121**, 2707 (1995).
24. A. L. Joyner, *Trends Genet* **12**, 15 (1996).
25. G. Couly, A. Grapin-Botton, P. Coltey, B. Ruhin, N. M. Le Douarin, *Development* **128**, 3445 (1998).
26. P. Crossley, G. Minowada, C. MacArthur, G. Martin, *Cell* **84**, 127 (1996).
27. C. Irving, I. Mason, *Development* **127**, 177 (2000).
28. M. Pasqualetti, M. Ori, I. Nardi, F. M. Rijli, *Development* **127**, 5367 (2000).
29. G. A. Grammatopoulos, E. Bell, L. Toole, A. Lumsden, A. S. Tucker, *Development* **127**, 5355 (2000).
30. B. Kanzler, S. J. Kuschert, Y.-H. Liu, M. Mallo, *Development* **125**, 2587 (1998).

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MAPKK-Independent Activation of p38α Mediated by TAB1-Dependent Autophosphorylation of p38α

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Phosphorylation of mitogen-activated protein kinases (MAPKs) on specific tyrosine and threonine sites by MAP kinase kinases (MAPKKs) is thought to be the sole activation mechanism. Here, we report an unexpected activation mechanism for p38α MAPK that does not involve the prototypic kinase cascade. Rather it depends on interaction of p38α with TAB1 [transforming growth factor-β–activated protein kinase 1 (TAK1)–binding protein 1] leading to autophosphorylation and activation of p38α. We detected formation of a TRAF6-TAB1-p38α complex and showed stimulus-specific TAB1-dependent and TAB1-independent p38α activation. These findings suggest that alternative activation pathways contribute to the biological responses of p38α to various stimuli.

Mitogen-activated protein kinases (MAPK) have crucial roles in cellular responses to various extracellular signals (*1*). The prototypical module of MAP kinase activation is a cascade of three kinases, consisting of MAP3K (MAP kinase kinase kinase), MAPKK, and MAPK (*2*). p38α is a MAPK activated by MAPKKs MKK3 and MKK6 (*2*–*7*). Although the protein kinase cascade is unquestionably a mechanism controlling p38α activation (*2*–*7*), we have identified an alternative p38α activation mechanism that has not previously been addressed.

We used the yeast two-hybrid system with a library constructed from human gastrointestinal tract tissue to search for proteins that

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interact with p38α. By screening 1.5×10^7 transformants, we isolated six clones encoding TAB1 (*8*). Recombinant p38α also bound to glutathione *S*-transferase (GST) fusion TAB1 isolated with glutathione-agarose (Fig. 1A). When TAB1 and flag-tagged p38α were expressed in HEK 293 cells, TAB1 was associated with flag-p38α immunoprecipitated from cell lysates (Fig. 1B). TAB1 was not detected in the immunoprecipitates prepared from cells expressing other p38 isoforms or members of the JNK and ERK families of MAP kinases (*8*). Interaction between endogenous TAB1 and p38α was detected in HEK 293 cells treated with tumor necrosis factor-α (TNF) by immunoprecipitation (Fig. 1C). Coexpression of TAB1 with p38α increased phosphorylation of p38α as detected by an antibody against phospho-p38 (Fig. 2A). The amount of p38α phosphorylation mediated by TAB1 was comparable to that noted with coexpression of dominant active MKK6 [MKK6(E)]. p38α activation was confirmed by kinase activity in immunoprecipitates obtained from the same cells. TAB1 also binds to TAK1 and activates its kinase activity (*9*, *10*). However, it is unlikely that TAB1-mediated phosphorylation of p38α is through a kinase cascade of TAK1 and MAPKKs such as MKK3 and MKK6. Expression of dominant negative MKK3 [MKK3(A)], MKK6 [MKK6(A)], MKK4 [MKK4(A)] (*11*), or TAK1 [TAK1(K63W)] failed to prevent TAB1-mediated p38α activation (Fig. 2B).

SB203580, an inhibitor of p38α but not of MKK3 or MKK6 (*11*, *12*), prevents phosphorylation of p38α in many experimental systems (*13*–*16*). This may occur if the intrinsic kinase activity of p38α accounts for phosphorylation and activation of the kinase. Treatment of cells expressing TAB1 and flag-p38α with SB203580 prevented TAB1-induced phosphorylation of p38α (Fig. 2C). Thus, TAB1-mediated phosphorylation of p38α appears to require the intrinsic kinase activity of p38α. We further examined the effect of TAB1 on mutated forms of p38α where the TGY dual phosphorylation site is changed to AGF [p38α(AF)], the adenosine triphosphate (ATP)–binding site is modified [K^{53} to M mutant, termed p38α(M)], or where p38α is inactivated by mutation in which D^{168} is replaced by A [p38α(DA)] (*17*, *18*). p38α(AF), p38α(M) and p38α(DA) were coprecipitated with TAB1 (*11*), indicating that kinase activity is not required for TAB1 binding. No phosphorylation of p38α(AF), p38α(M), or p38α(DA) was detected when these proteins were expressed with TAB1 (Fig. 2C). However, p38α(M) and p38α(DA) were efficiently phosphorylated by coexpressed MKK6(E) (Fig. 2C). Thus, TAB1 binds to p38α and causes autophosphorylation and consequent activation of the kinase.

Recombinant TAB1 and p38α were expressed in Sf9 cells or bacteria then purified as histidine-tagged (His) or GST fusion proteins. GST-p38α (0.5 μg) was incubated with various amounts of His-TAB1 in a kinase reaction buffer containing nonradioactive ATP. The extent of phosphorylation of p38α, detected by Western blotting with an antibody against phospho-p38, was dependent on the amount of added TAB1 (Fig. 3A). The phosphorylation was time-dependent (*8*) and sensitive to SB203580 inhibition (Fig. 3B). TAB1 did not stimulate phosphorylation of catalytically inactive p38α mutants in vitro (Fig. 3B). Incubation of p38α with various amounts of GST-TAB1 increased its kinase activity toward myelin basic protein (MBP) and GST-ATF2(1-109) (Fig. 3C). In contrast, GST had no effect on p38α activity. TAB1-mediated p38α phosphorylation is most likely an intramolecular reaction, because

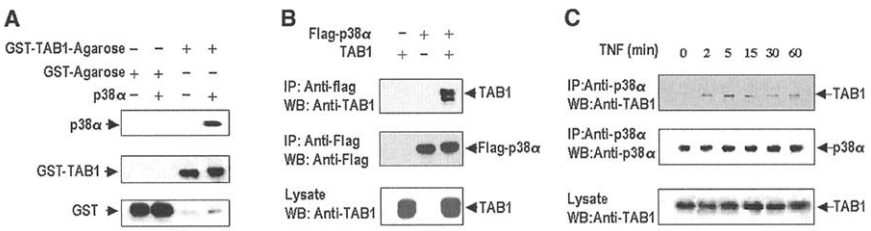

Fig. 1. Interaction of p38α with TAB1. (**A**) Binding of p38α to TAB1 in vitro. p38α was incubated with GST-TAB1 or GST bound to glutathione-agarose beads (*24*). p38α was detected in Western blotting with p38α-specific antibody; GST-TAB1 and GST were detected with GST-specific antibody. (**B**) Binding of p38α to TAB1 in cells. Flag-p38α and TAB1 were expressed together in HEK 293 cells (*8*). Proteins immunoprecipitated with flag-specific antibody or cell lysates were subject to immunoblotting with antibodies against TAB1 or flag as indicated (*8*). (**C**) Association of endogenous p38α and TAB1 in HEK 293 cells treated with TNF (100 ng/ml). p38α was immunoprecipitated with p38α-specific antibody, and the precipitates were subjected to immunoblotting with antibodies against TAB1 or p38α.

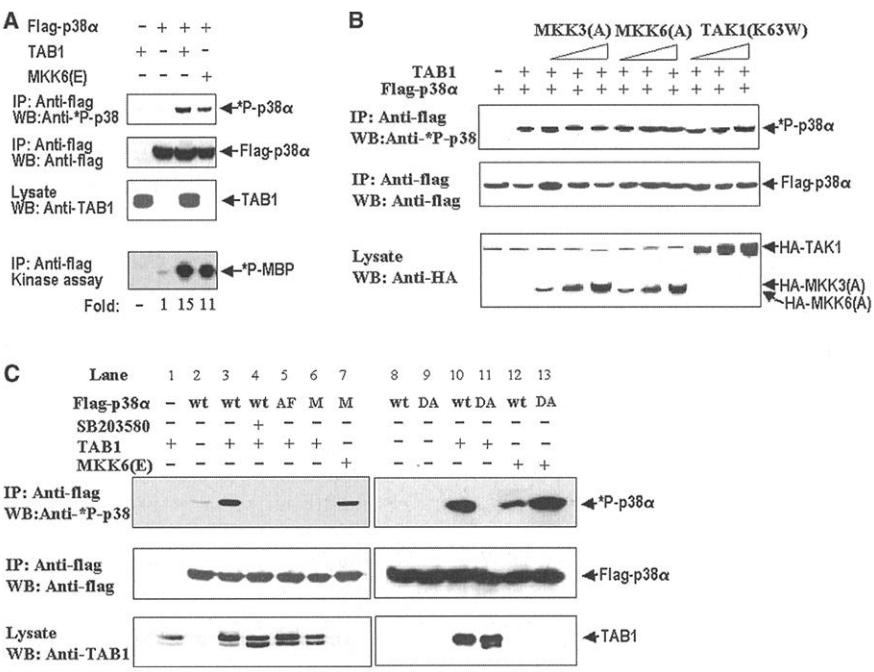

Fig. 2. TAB1-mediated autophosphorylation of p38α. (**A**) Phosphorylation and activation of p38α expressed with TAB1. Flag-p38α was expressed with or without TAB1 or MKK6(E) in HEK 293 cells. Proteins immunoprecipitated with flag-specific antibody were subjected to immunoblotting with antibodies against phospho-p38 or flag. The cell lysates were analyzed by immunoblotting with TAB1-specific antibody. Immunoprecipitates with flag-specific antibody were subjected to kinase reaction using MBP (2 μg) as substrate (*24*). Quantification of MBP phosphorylation was done with a scintillation counter. (**B**) Effect of dominant-negative MKK3 [MKK3(A)], MKK6 [MKK6(A)] or TAK1 [TAK1(K63W)] on p38α phosphorylation. HEK 293 cells were transfected with expression vectors of flag-p38α and TAB1 and increasing amounts of plasmid DNA of MKK3(A), MKK6(A), and TAK1(K63W) (0.1, 0.2, and 0.4 μg). Immunoprecipitation and Western blotting were done as in (A). (**C**) Requirement of intrinsic p38α activity for TAB1-mediated p38α phosphorylation. Coexpression of flag-p38α, flag-p38α(AF), flag-p38α(M), or flag-p38α(DA) with TAB1 or MKK6(E) was done as in (A). SB203580 (5 μM) was added into cell culture medium 4 hours after transfection in the sample indicated. Immunoprecipitation and Western blotting were done as in (A). Data shown are representative of two to three independent experiments.

p38α(M) cannot be phosphorylated when incubated with wild-type p38α in the presence of TAB1 (*8*). Collectively, these data support our model of TAB1-mediated p38α phosphorylation occurring by an autocatalytic mechanism.

To identify the exact phosphorylation sites in p38α, we incubated recombinant p38α or its mutants, T^{180} to A [p38α(A)], Y^{182} to F [p38α(F)], or T^{180} and Y^{182} to A and F [p38α(AF)], with TAB1 or MKK6(E) in kinase buffer containing [^{32}P]ATP. TAB1-induced phosphorylation of p38α only occurred in wild-type p38α, whereas wild-type p38α and single phosphorylation site mutants, p38α(A) and p38α(F), were all phosphorylated by MKK6(E) (Fig. 3D). Phosphopeptide mapping revealed two major phosphopeptides of phosphorylated p38α that resulted from the incubation with either TAB1 or MKK6(E). Peptide 1 contained phosphotyrosine and phosphothreonine (Fig. 3D), whereas peptide 2 was phosphorylated only on tyrosine (*11*). Thus, the dual phosphorylation of p38α can be mediated by either upstream kinase MKK6 or TAB1-mediated autophosphorylation.

Deletion of the COOH-terminal 86 amino acids of TAB1 impairs its interaction with TAK1 (*9*). To map the p38α interaction region in TAB1, we made progressive deletions of TAB1. Their interaction with p38α and effect on p38α phosphorylation was analyzed. Deletion of the COOH-terminal 86 amino acids in TAB1 [TAB1(1-418)] increased its binding affinity for p38α and enhanced p38α phosphorylation (Fig. 4). Recombinant TAB1(1-418) also efficiently activated p38α in vitro (*11*). These data further support our contention that the effects of TAB1 on p38α are independent of its effects on TAK1. TAB1(1-373), containing a deletion of all amino acids following amino acid 373, failed to bind to and activate p38α (Fig. 4). Thus, amino acid sequences between 373 and 418 of TAB1 are required for p38α interaction.

TAB1-induced p38α phosphorylation is sensitive to SB203580 in vitro and in vivo (Figs. 2C and 3B). We therefore tested whether TAB1-dependent p38α phosphorylation induced by extracellular stimuli was also sensitive to SB203580. SB203580 inhibited p38α phosphorylation induced by TNF or peroxynitrite in HEK 293 cells (Fig. 5A). The effect of SB203580 on anisomycin-induced p38α phosphorylation was less pronounced, and SB23580 had almost no effect on hyperosmolarity (sorbitol)-induced phosphorylation of p38α. RPMI 8226 cells, from a human B cell line, respond to bacterial components such as CpG oligonucleotides, lipopolysaccharides (LPSs), and bacterial outer member lipoproteins via various toll-like receptors (TLRs) (*19*). SB203580 treatment inhibited CpG- and LPS-induced phosphorylation of p38α, but had no effect on phosphorylation induced by bacterial lipoprotein (Fig. 5B). In the experiments on the same cell line, it is unlikely that SB203580 functioned differently in the cells treated with different stimuli. Thus, the differential sensitivity to SB203580 is most likely a reflection of differing p38α activation mechanisms.

To further examine whether the particular TLRs couple to different signaling pathways, we studied p38α phosphorylation in TLR2- or TLR4-expressing cells. When TLR2 or TLR4 was expressed in HEK 293 cells, increased basal phosphorylation of p38α was detected (Fig. 5C). Expression of TLR2 conferred phosphorylation of p38α in response to bacterial lipoprotein (Fig. 5C) (*19*). That was insensitive to SB203580 or expression of TAB1(Δ313-418), a mutant that lacks the p38α binding domain and can act as a dominant negative mutant to inhibit TAB1-dependent activation of p38α (*8*). However, LPS-induced phosphorylation of p38α mediated by TLR4 was sensitive to inhibition by SB203580 or TAB1(Δ313-418). In addition, LPS-induced phosphorylation of catalytically inactive p38α mutants was reduced, whereas the inactive mutants were phosphorylated to the same extent as wild-type p38α in lipoprotein-treated cells. Thus, p38α activation can be mediated by distinct mechanisms, and both TAB1-dependent and TAB1-independent p38α activation pathways are likely to mediate the effects of physiologically important extracellular stimuli.

The TNF receptor-associated factor 6 (TRAF6) forms a complex with TAB1 and TAK1 (*20*). Thus, we examined whether TRAF6 interacts with TAB1 and p38α. Flag-TAB1 was expressed in TLR4-293 cells together with Myc-TRAF6 or HA-p38α or both (Fig. 5D). When p38α was expressed alone

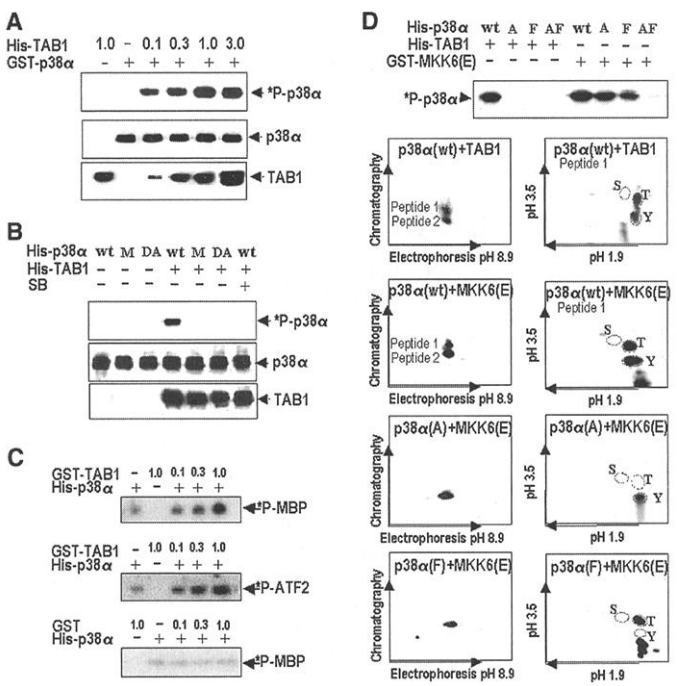

Fig. 3. Interaction of p38α and TAB1 in vitro. **(A)** TAB1 is sufficient to cause p38α phosphorylation. Recombinant GST-p38α (0.5 μg) was incubated with the indicated amounts of His-TAB1 (in μg) for 30 min in kinase buffer. The p38α phosphorylation was determined by immunoblotting with antibody against phospho-p38. The proteins used in the in vitro reaction are shown in the lower panels. **(B)** Requirement of intrinsic p38α kinase activity for TAB1-mediated p38α phosphorylation in vitro. His-p38α, His-p38α(M), or His-p38α(DA) was incubated with His-TAB1 as in (A). SB203580 (1 μM) was included in the reaction as indicated. The p38α phosphorylation was determined by immunoblotting with antibody against phospho-p38. The proteins used in the in vitro reaction were shown in the lower panels. **(C)** Enhancement of p38α activity by TAB1 in vitro. Recombinant p38α (0.5μg) was incubated with or without different amounts (0.2, 0.4, and 0.8 μg) of recombinant GST-TAB1 or GST for 15 min in kinase buffer, and p38α substrate MBP or GST-ATF2(1-109) (2 μg) was added. The kinase reactions were continued for another 30 min and analyzed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. **(D)** Tryptic phosphopeptide mapping and phosphoamino acid analysis of p38α phosphorylated by TAB1-mediated autophosphorylation or by MKK6(E) in vitro. His-p38α, His-p38α(A), His-p38α(F), or His-p38α(AF) (2 μg) was incubated with His-TAB1 (2 μg) or GST-MKK6(E) (2 μg) for 30 min in kinase buffer containing [^{32}P]ATP. Phosphorylated p38α and its mutants were resolved on SDS-PAGE (top panel). Two-dimensional tryptic peptide maps were obtained from ^{32}P-labeled p38α and its mutants (left panels). The phosphopeptides were subjected to phosphoamino acid analysis (right panels) (*24*).

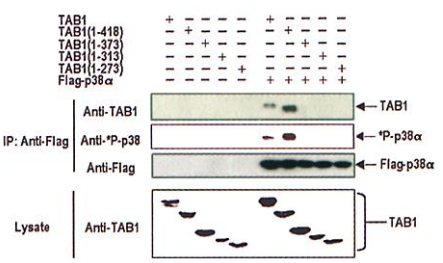

Fig. 4. Binding of p38α to deletion mutants of TAB1. Flag-p38α was expressed with deletion mutants of TAB1 in HEK 293 cells. Immunoprecipitation and immunoblotting were done as in Fig. 2A. Data shown are representative of two independent experiments.

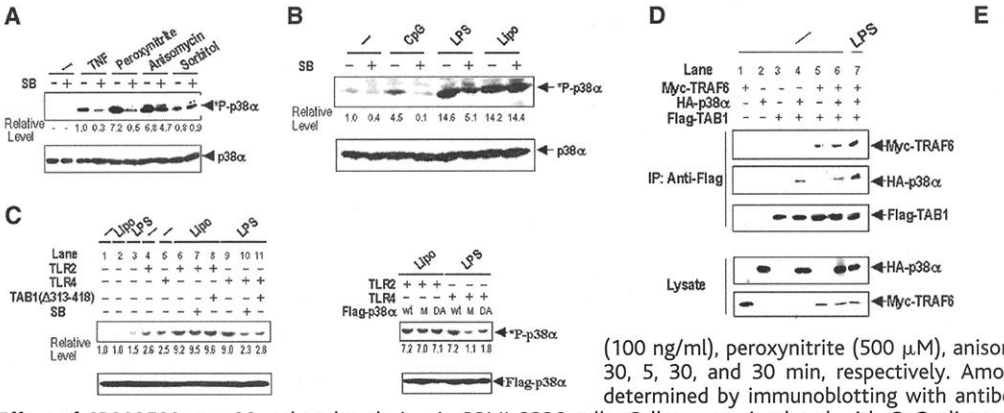

Fig. 5. Selective activation of TAB1-dependent and TAB1-independent p38α activation pathways. (**A**) Requirement of intrinsic p38α activity for p38α phosphorylation induced by extracellular stimuli. The HEK 293 cells, pretreated with or without SB203580 (5 μM) for 30 min, were stimulated with TNF (100 ng/ml), peroxynitrite (500 μM), anisomycin (50 ng/ml), or sorbitol (0.4 M) for 30, 5, 30, and 30 min, respectively. Amounts of p38α and phospho-p38α were determined by immunoblotting with antibodies against flag and phospho-p38. (**B**) Effect of SB203580 on p38α phosphorylation in RPMI 8226 cells. Cells were stimulated with CpG oligonucleotide (5 μg/ml), LPS (100 ng/ml), or lipoprotein (lipo) (200 ng/ml) for 30 min. Amounts of phospho-p38α and p38α were determined by immunoblotting. (**C**) Distinct signaling to p38α by TLR2 and TLR4. Flag-p38α, flag-p38α(M), or flag-p38α(DA) was expressed in HEK 293 cells with or without TLR2 or TLR4, and with or without TAB1(Δ313-418) as indicated. The cells were treated with SB203580 and stimulated with lipoprotein or LPS as indicated. Amounts of p38α and phospho-p38α were determined by immunoblotting. (**D**) Interaction of TRAF6 with TAB1-p38α. TLR4-293 cells were transfected with expression plasmids encoding Myc-TRAF6, HA-p38α, and flag-TAB1 in various combinations and exposed to LPS as indicated. Immunoprecipitates with flag-specific antibody and cell lysates were analyzed by immunoblotting with antibodies against Myc, HA, and flag. (**E**) Proposed signaling pathway upstream of TAB1-mediated p38α activation. Data shown in (A to D) are representative of two to three independent experiments.

or together with TRAF6, the complexes were coimmunoprecipitated with TAB1. Stimulation of cells with LPS increased the amount of coprecipitated-TRAF6 and -p38α (Fig. 5D), suggesting enhanced formation of a TRAF6-TAB1-p38 complex. Thus, the TAB1-p38α pathway may be directly linked with TRAF6 (Fig. 5E).

Signal transduction is controlled not only by enzymes, but also by nonenzymatic adapters, scaffolds and other "inert" proteins. Much like these adapters, TAB1 binds various kinases such as TAK1 and p38α. However, a difference between TAB1 and the other nonenzymatic modulators of the MAP kinase pathway is that binding with TAB1 results in kinase activation. Direct mediation of p38α activation by TAB1 represents a new mechanism of activation distinct from the well-known activation by MAPKK (*1*–*7*). Although autophosphorylation of MAP kinase has been observed in vitro, it occurred at such a low level that it was not considered a primary activation mechanism (*21*–*23*). The autoactivation of p38α MAP kinases facilitated by interaction with regulatory molecule(s) could be an important alternative activation pathway operating in parallel with kinase cascades in regulating intracellular signaling.

References and Notes

1. M. J. Robinson, M. H. Cobb, *Curr. Opin. Cell Biol.* **9**, 180 (1997).
2. K. J. Blumer, G. L. Johnson, *Trends Biochem. Sci.* **19**, 263 (1994).
3. B. Derijard *et al.*, *Science* **267**, 682 (1995).
4. J. Han *et al.*, *J. Biol. Chem.* **271**, 2886 (1996).
5. T. Morguchi *et al.*, *J. Biol. Chem.* **271**, 26981 (1996).
6. J. Raingeaud *et al.*, *Mol. Cell Biol.* **16**, 1247 (1996).
7. A. Cuenda *et al.*, *EMBO J.* **15**, 4156 (1996).
8. Supplementary materials are available on *Science* Online at www.sciencemag.org/cgi/content/full/295/5558/1291/DC1
9. H. Shibuya *et al.*, *Science* **272**, 1179 (1996).
10. K. Kishimoto et al., *J. Biol. Chem.* **275**, 7359 (2000).
11. B. Ge, H. Gram, F. Di Padova, J. Han, data not shown.
12. S. Kumar. *et al.*, *Biochem. Biophys. Res. Commun.* **263**, 825 (1999).
13. B. Frantz et al., *Biochemistry* **37**, 13846 (1998).
14. A. Galan et al., *J. Biol. Chem.* **275**, 11418 (2000).
15. S. Zhuang *et al.*, *J. Biol. Chem.* **275**, 25939 (2000).
16. T. Matsuguchi *et al.*, *J. Immunol.* **165**, 5767 (2000).
17. J.-H. Her *et al.*, *Biochem. J.* **296**, 25 (1993).
18. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
19. J. Imler, J. A. Hoffmann, *Trends Cell Biol.* **11**, 304 (2001).
20. J. Ninomiya-Tsuji *et al.*, *Nature* **398**, 252 (1999).
21. J. Wu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 9508 (1991).
22. D. J. Robbins *et al.*, *J. Biol. Chem.* **268**, 5097 (1993).
23. A. J. Rossomando *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5779 (1992).
24. J. Han *et al.*, *Nature* **386**, 296 (1997).

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Cytoskeletal Regulation by the Nedd8 Ubiquitin-Like Protein Modification Pathway

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The Nedd8 ubiquitin-like protein modification pathway regulates cell-cycle progression. Our analysis of Nedd8 requirements during *Caenorhabditis elegans* embryogenesis indicates that the cytoskeleton is another target. Nedd8 conjugation negatively regulated contractility of the microfilament-rich cell cortex during pronuclear migration and again during cytokinesis. The Nedd8 pathway also was required after meiosis to negatively regulate katanin, a microtubule-severing complex, permitting the assembly of a large mitotic spindle. We propose that Nedd8-modified cullin, as part of an E3 ubiquitin ligase complex, targets katanin for degradation during the transition from meiosis to mitosis.

Ubiquitin (UBQ) and ubiquitin-like proteins (UBLs) are a family of small, conserved polypeptides that become covalently attached to other proteins. UBQ usually targets proteins for degradation by the proteasome (*1*, *2*), whereas modification by UBLs can affect instead the subcellular localization of target proteins, their conformation, and their association with other proteins (*3*). Related pathways mediate the attachment of UBQ and UBLs to targeted proteins (*4*). In reactions that require ATP, UBQ and UBLs are first covalently attached to cognate E1-activating enzymes and then transferred to E2-conjugating enzymes that, directly