Activation of Apoptosis Signal–Regulating Kinase 1 (ASK1) by the Adapter Protein Daxx

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The Fas death receptor can activate the Jun NH_2-terminal kinase (JNK) pathway through the receptor-associated protein Daxx. Daxx was found to activate the JNK kinase kinase ASK1, and overexpression of a kinase-deficient ASK1 mutant inhibited Fas- and Daxx-induced apoptosis and JNK activation. Fas activation induced Daxx to interact with ASK1, which consequently relieved an inhibitory intramolecular interaction between the amino- and carboxyl-termini of ASK1, activating its kinase activity. The Daxx-ASK1 connection completes a signaling pathway from a cell surface death receptor to kinase cascades that modulate nuclear transcription factors.

Fas is a cell surface receptor that induces apoptosis upon oligomerization (*1*). Fas belongs to a family of related death receptors, including the receptors for tumor necrosis factor–α (TNF-α) and the cytotoxic ligand TRAIL (*1*, *2*). Fas-induced apoptosis has a critical role in maintaining peripheral immune tolerance (*1*). Fas can activate two independent signaling pathways. One well-characterized pathway involves the adapter protein FADD, which recruits procaspase-8 and activates a protease cascade leading to apoptosis (*1*, *3*). The second pathway is mediated by Daxx, which can enhance Fas-induced apoptosis by activating the JNK kinase cascade, culminating in the phosphorylation and activation of transcription factors such as c-Jun (*4*, *5*).

Because Daxx might activate JNK through a mitogen-activated protein (MAP) kinase kinase kinase (MAP3K) (*6*–*8*), we focused on ASK1. It is a MAP3K that can activate apoptosis, is activated by TNF-α, and the dominant negative form of which can block TNF-α–induced death (*8*). Using an immunoprecipitation (IP)-kinase assay after expression in human embryonic kidney 293 cells (*8*, *9*), we found that ASK1 activity was potentiated by coexpression with Daxx (Fig. 1A). Another MAP3K that can activate the same kinase cascade, TAK1 (*6*), was not activated by Daxx. The Daxx domain that encodes its JNK activation and apoptotic activities (amino acids 501 to 625) and fragments incorporating it (*4*), but not other parts of Daxx, also increased ASK1 activity (Fig. 1B). These data implicate ASK1 as a downstream target of Daxx. Consistent with this notion, endogenous ASK1 activity was activated rapidly by Fas cross-linking in a dose-dependent manner in Jurkat cells; low but detectable ASK1 activation was evident 5 min after Fas cross-linking (Fig. 1C).

To determine the functional role of ASK1 in Daxx and Fas signaling, we tested the effect of altering ASK1 activity on the apoptotic activities of Daxx and Fas (Fig. 2). An activated deletion mutant of Daxx, DaxxC501, can induce cell death in a Fas-independent manner in 293 cells but not in HeLa cells (*4*). However, coexpression of ASK1 and DaxxC501 in HeLa cells synergistically induced apoptosis (Fig. 2A). A conservative point mutation in the ATP binding loop of ASK1 (K709R) completely abrogated cell killing (Fig. 2A). ASK1(K709M), which has less residual kinase activity than ASK1(K709R), inhibited apoptosis by Fas and DaxxC501 in a dose-dependent manner (Fig. 2, B and C). ASK1(K709M) also inhibited the ability of DaxxC501 and Fas to activate JNK, whereas the caspase inhibitor crmA did not (Fig. 2D). Collectively, these results imply a critical role for the ASK1 kinase in JNK activation and apoptosis induced by Fas binding of Daxx.

Because MAP3Ks such as Raf directly interact with upstream signaling proteins (*5*), we assayed physical interaction between Daxx and ASK1 by coimmunoprecipitation from transfected 293T cells. Full-length human Daxx specifically coimmunoprecipitated with ASK1 (Fig. 3A), indicating that these two proteins physically interact in mammali-

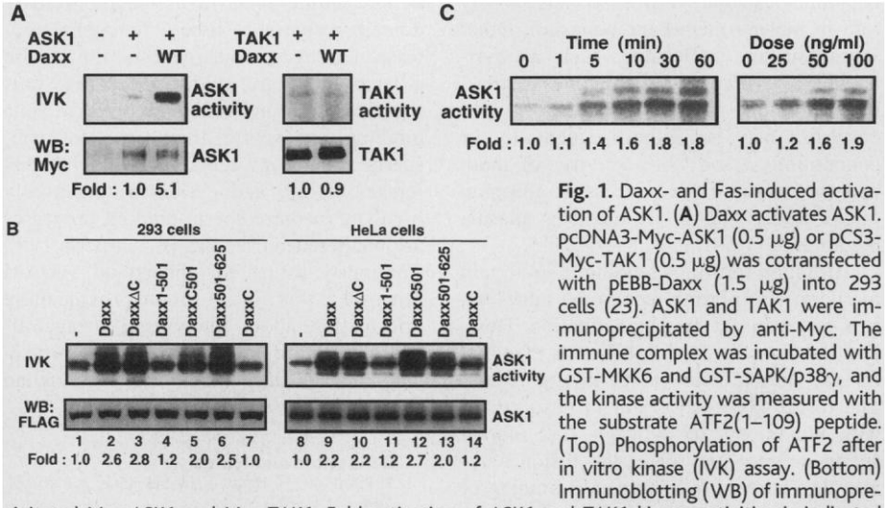

Fig. 1. Daxx- and Fas-induced activation of ASK1. (**A**) Daxx activates ASK1. pcDNA3-Myc-ASK1 (0.5 μg) or pCS3-Myc-TAK1 (0.5 μg) was cotransfected with pEBB-Daxx (1.5 μg) into 293 cells (*23*). ASK1 and TAK1 were immunoprecipitated by anti-Myc. The immune complex was incubated with GST-MKK6 and GST-SAPK/p38γ, and the kinase activity was measured with the substrate ATF2(1–109) peptide. (Top) Phosphorylation of ATF2 after in vitro kinase (IVK) assay. (Bottom) Immunoblotting (WB) of immunoprecipitated Myc-ASK1 and Myc-TAK1. Fold activation of ASK1 and TAK1 kinase activities is indicated below. Kinase activities relative to the amount of ASK1 or TAK1 proteins were calculated, and the activities are shown as fold activation relative to the activities of ASK1 or TAK1 from Daxx-negative cells. (**B**) ASK1 activation by Daxx deletion mutants. pcDNA3-FLAG-ASK1 (0.5 μg) and each Daxx mutant (1.5 μg) were cotransfected into 293 cells (left) or HeLa cells (right), and ASK1 was immunoprecipitated with anti-FLAG. The immune complex was incubated with GST-MKK6, and then the kinase activity was measured with the substrate GST-SAPK/p38γ(KN). The sequences incorporated in each Daxx construct are as follows: Daxx [amino acids (aa) 1 to 739], DaxxΔC (aa 1 to 625), Daxx1–501 (aa 1 to 501), DaxxC501 (aa 501 to 739), Daxx501–625 (aa 501 to 625), DaxxC (aa 626 to 739). (Top) Phosphorylation of GST-SAPK3/p38γ(KN). (Bottom) Expression of FLAG-ASK1. Fold activation of ASK1 kinase activities is indicated below. (**C**) Fas-induced activation of ASK1. Jurkat cells (5×10^6) were treated with CH-11 anti-human Fas (MBL, Nagoya, Japan) (100 ng/ml) for the indicated times (left) or with the indicated concentrations for 30 min (right). The endogenous ASK1 was immunoprecipitated with anti-ASK1 (DAV) (*24*), and the ASK1 kinase activity was measured as described in (B).

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an cells. FLAG-tagged FADD was not coprecipitated by ASK1 under the same condition. To evaluate the observed Daxx-ASK1 interaction under more physiological conditions, we examined the association of endogenous Daxx and ASK1 by coimmunoprecipitation in L/Fas cells, a mouse fibroblast cell line expressing murine Fas (*4*). Daxx became associated with ASK1 after Fas ligation by an

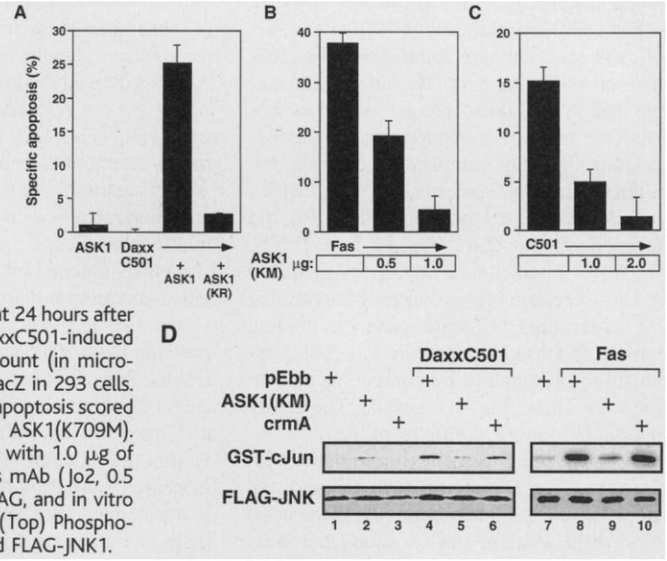

Fig. 2. Role of ASK1 in Daxx- and Fas-induced apoptosis and signaling. (**A**) Synthetic lethality of ASK1 with DaxxC501. HeLa cells were transfected with 0.5 μg of pcDNA3-ASK1 or pcDNA3-ASK1(K709R) (*23*) and 1.0 μg of pEBB-DaxxC501 along with 0.5 μg of pCMV-lacZ reporter by calcium phosphate precipitation. Total amount of transfected DNA was made constant by adding vector DNA. Twenty-four hours after transfection, the cells were stained with X-Gal and scored for apoptotic morphology (*4*). Specific apoptosis was calculated as the percentage of apoptotic blue cells in each experimental condition minus the percentage of apoptotic blue cells (~5%) in parallel vector-transfected cells. The data shown are the mean ± SD of two to four independent experiments. (**B**) Inhibition of Fas-induced apoptosis by ASK1(K709M). HeLa cells were transfected with 0.5 μg of pEBB-Fas and pCMV-lacZ and the indicated amount (in micrograms) of ASK1(K709M). Jo2 antibody (12.5 ng/ml) was added 16 hours later. X-Gal staining was done at 24 hours after transfection. Specific apoptosis was calculated as in (A). (**C**) Inhibition of DaxxC501-induced apoptosis by ASK1(K709M). pEBB-DaxxC501 (2.0 μg) and the indicated amount (in micrograms) of pcDNA3-ASK1(K709M) were cotransfected with 0.5 μg of pCMV-lacZ in 293 cells. Twenty hours after transfection the cells were stained with X-Gal and specific apoptosis scored as in (A). (**D**) Inhibition of DaxxC501- and Fas-induced JNK activation by ASK1(K709M). Expression constructs for each indicated protein (1.0 μg) were cotransfected with 1.0 μg of pCMV-FLAG-JNK1 in 293 cells. Cells in lanes 7 to 10 were treated with Fas mAb (Jo2, 0.5 μg/ml) for 30 min before assay. JNK1 was immunoprecipitated with anti-FLAG, and in vitro kinase assay with 1 μg of GST-cJun(1–79) was performed as described (*4*). (Top) Phosphorylation of GST-cJun(1–79). (Bottom) Immunoblotting of immunoprecipitated FLAG-JNK1.

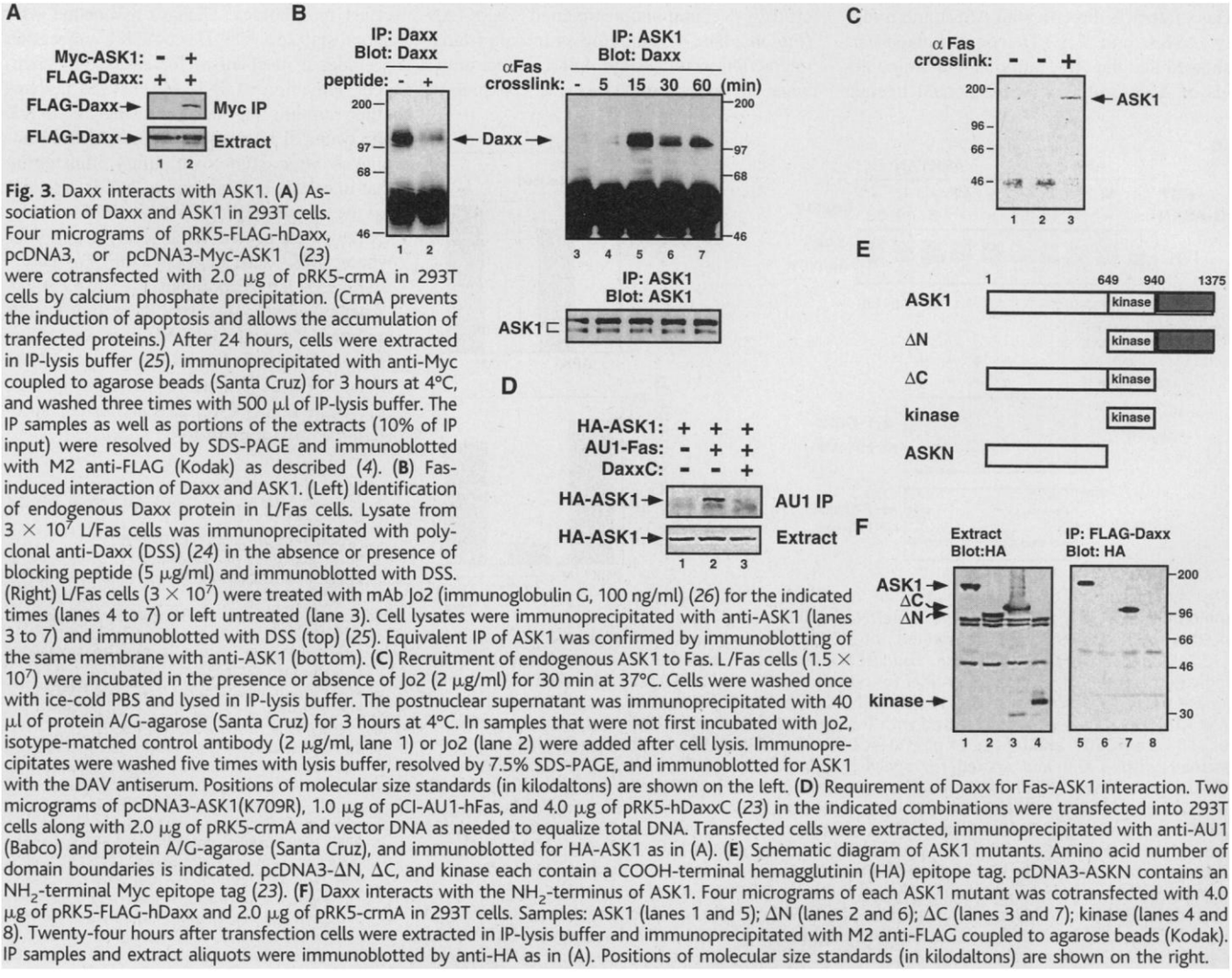

Fig. 3. Daxx interacts with ASK1. (**A**) Association of Daxx and ASK1 in 293T cells. Four micrograms of pRK5-FLAG-hDaxx, pcDNA3, or pcDNA3-Myc-ASK1 (*23*) were cotransfected with 2.0 μg of pRK5-crmA in 293T cells by calcium phosphate precipitation. (CrmA prevents the induction of apoptosis and allows the accumulation of tranfected proteins.) After 24 hours, cells were extracted in IP-lysis buffer (*25*), immunoprecipitated with anti-Myc coupled to agarose beads (Santa Cruz) for 3 hours at 4°C, and washed three times with 500 μl of IP-lysis buffer. The IP samples as well as portions of the extracts (10% of IP input) were resolved by SDS-PAGE and immunoblotted with M2 anti-FLAG (Kodak) as described (*4*). (**B**) Fas-induced interaction of Daxx and ASK1. (Left) Identification of endogenous Daxx protein in L/Fas cells. Lysate from 3×10^7 L/Fas cells was immunoprecipitated with polyclonal anti-Daxx (DSS) (*24*) in the absence or presence of blocking peptide (5 μg/ml) and immunoblotted with DSS. (Right) L/Fas cells (3×10^7) were treated with mAb Jo2 (immunoglobulin G, 100 ng/ml) (*26*) for the indicated times (lanes 4 to 7) or left untreated (lane 3). Cell lysates were immunoprecipitated with anti-ASK1 (lanes 3 to 7) and immunoblotted with DSS (top) (*25*). Equivalent IP of ASK1 was confirmed by immunoblotting of the same membrane with anti-ASK1 (bottom). (**C**) Recruitment of endogenous ASK1 to Fas. L/Fas cells (1.5×10^7) were incubated in the presence or absence of Jo2 (2 μg/ml) for 30 min at 37°C. Cells were washed once with ice-cold PBS and lysed in IP-lysis buffer. The postnuclear supernatant was immunoprecipitated with 40 μl of protein A/G-agarose (Santa Cruz) for 3 hours at 4°C. In samples that were not first incubated with Jo2, isotype-matched control antibody (2 μg/ml, lane 1) or Jo2 (lane 2) were added after cell lysis. Immunoprecipitates were washed five times with lysis buffer, resolved by 7.5% SDS-PAGE, and immunoblotted for ASK1 with the DAV antiserum. Positions of molecular size standards (in kilodaltons) are shown on the left. (**D**) Requirement of Daxx for Fas-ASK1 interaction. Two micrograms of pcDNA3-ASK1(K709R), 1.0 μg of pCI-AU1-hFas, and 4.0 μg of pRK5-hDaxxC (*23*) in the indicated combinations were transfected into 293T cells along with 2.0 μg of pRK5-crmA and vector DNA as needed to equalize total DNA. Transfected cells were extracted, immunoprecipitated with anti-AU1 (Babco) and protein A/G-agarose (Santa Cruz), and immunoblotted for HA-ASK1 as in (A). (**E**) Schematic diagram of ASK1 mutants. Amino acid number of domain boundaries is indicated. pcDNA3-ΔN, ΔC, and kinase each contain a COOH-terminal hemagglutinin (HA) epitope tag. pcDNA3-ASKN contains an NH_2-terminal Myc epitope tag (*23*). (**F**) Daxx interacts with the NH_2-terminus of ASK1. Four micrograms of each ASK1 mutant was cotransfected with 4.0 μg of pRK5-FLAG-hDaxx and 2.0 μg of pRK5-crmA in 293T cells. Samples: ASK1 (lanes 1 and 5); ΔN (lanes 2 and 6); ΔC (lanes 3 and 7); kinase (lanes 4 and 8). Twenty-four hours after transfection cells were extracted in IP-lysis buffer and immunoprecipitated with M2 anti-FLAG coupled to agarose beads (Kodak). IP samples and extract aliquots were immunoblotted by anti-HA as in (A). Positions of molecular size standards (in kilodaltons) are shown on the right.

agonistic monoclonal antibody (mAb); this interaction peaked after 15 min and decreased thereafter (Fig. 3B). The Daxx-ASK1 interaction raised the possibility that ASK1 may interact indirectly with Fas through Daxx. In L/Fas cells, the endogenous ASK1 was specifically coimmunoprecipitated with Fas after mAb cross-linking (Fig. 3C, lane 3), indicating that ASK1 does interact with Fas and therefore may be a component of the Fas receptor signaling complex. In contrast, addition of mAb to Fas after cell lysis, which immunoprecipitates monomeric Fas (*10*), did not coprecipitate ASK1 (Fig. 3C, lane 2). The Fas-ASK1 interaction is apparently mediated by Daxx because coexpression of DaxxC, the COOH-terminal 112 amino acid Fas-binding domain of Daxx, blocked the Fas-ASK1 interaction, presumably by competing out endogenous Daxx (Fig. 3D, lane 3). The ability of DaxxC to block ASK1 recruitment to Fas can explain the documented dominant negative effects of DaxxC on both Fas-induced apoptosis and JNK activation (*4*). In the yeast two-hybrid system, ASK1 interacted with Daxx but not with Fas (*11*), suggesting that Daxx interacts directly with ASK1 and bridges ASK1 and Fas. Deletion mutagenesis showed that the NH_2-terminal 648 amino acids of ASK1, termed ASKN, could interact with Daxx (Fig. 3E and F, lane 7), whereas other parts of ASK1 could not interact.

Deletion of the NH_2-terminal 648 amino acids of ASK1, forming ASK1ΔN, caused the constitutive activation of kinase activity (*12*) as it does in other MAP3Ks (*6*). Purified recombinant glutathione S-transferase (GST)–ASKN inhibited the in vitro kinase activity of ASK1 but not ASK1ΔN immunoprecipitated from cells (Fig. 4A), suggesting that one or more interacting cellular factors regulate ASKN autoinhibition. ASK1ΔN exhibited constitutive cell death activity in HeLa cells in the absence of added Daxx (Fig. 4B). Apoptosis induced by ASK1ΔN was quantitatively similar to that induced by ASK1 plus DaxxC501 and was not enhanced by coexpression with DaxxC501 (Fig. 4B). These results indicate that an activated allele of ASK1 functions as a genetic bypass of Daxx and suggests that with regard to ASK1 activation, the function of Daxx is to relieve the inhibition caused by the NH_2-terminal regulatory domain. We tested this model directly by in vivo interaction assays. ASKN interacted with Daxx (Fig. 4C, lane 2). It also specifically coimmunoprecipitated ASK1ΔN (Fig. 4C, lane 4), implying an intramolecular interaction in full-length ASK1. Importantly, when an excess of Daxx was coexpressed with ASKN and ASK1ΔN, ASKN associated with Daxx but not ASK1ΔN (Fig. 4C, lane 6). This supports a model whereby Daxx activates ASK1 activity by displacing an inhibitory intramolecular interaction between the NH_2- and COOH-termini of the kinase and "opening up" the kinase into an active conformation. In support of this model, ASKN can inhibit the constitutive apoptotic activity of ASK1ΔN in trans, and this inhibition is fully reversed by the coexpression of Daxx (Fig. 4D).

The present results suggest a Fas-Daxx-ASK1 axis in activating JNK and p38 MAP kinase cascades. The mechanism by which ASK1 is activated by Daxx is similar to that described for the activation of Byr2, a MAP3K in the *Schizosaccharomyces pombe* mating pheromone pathway, by its activators Ste4 and Shk1 (*13*). Fas activation has been reported to activate JNK by caspase-dependent (*14*) and -independent pathways (*4*, *15*). During apoptosis, caspases can cleave and activate PAK2 and MEKK (*16*, *17*), two kinases that can activate the JNK pathway; JNK activation in this context is believed to effect morphologic changes associated with apoptosis (*16*). The Daxx-ASK1 connection provides a mechanism for caspase-independent activation of JNK by Fas and perhaps other stimuli. In mice deficient for JNK3, hippocampal neurons are protected from apoptosis after excitotoxic injury, illustrating that in certain circumstances JNK is essential for the apoptotic program (*18*). In this study, we have used several tumor-derived cell lines where JNK activation by the Fas-Daxx-ASK1 axis led to apoptosis. Because *FADD*-deficient embryonic fibroblasts and T cells are blocked for Fas-induced apoptosis (*19*), at least in these cells Daxx does not provide an independent death pathway. The physiologic role of the Daxx-ASK1 axis and its cell specificity in vivo remain to be addressed.

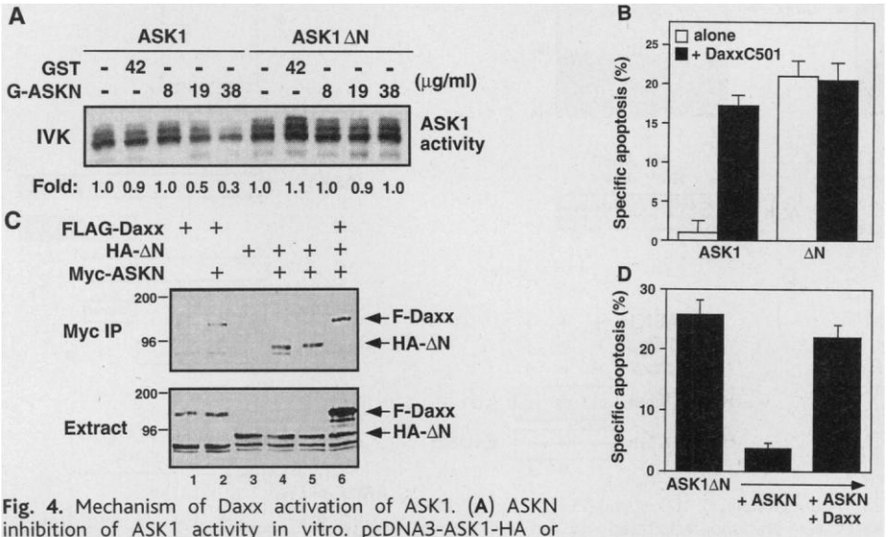

Fig. 4. Mechanism of Daxx activation of ASK1. (**A**) ASKN inhibition of ASK1 activity in vitro. pcDNA3-ASK1-HA or pcDNA3-ASK1ΔN-HA were transfected into 293 cells and immunoprecipitated with anti-HA (12CA5) and protein A–Sepharose. Equalized input kinase activities were incubated with the indicated amount of GST or GST-ASKN for 60 min at 4°C, and subjected to the immune complex kinase assay as described in Fig. 1B. G-ASKN, GST-ASKN. (**B**) Constitutive apoptotic activity of ASK1ΔN. HeLa cells were transfected with 0.5 μg of each indicated ASK1 mutant, 1.0 μg of pEBB or pEBB-DaxxC501, and 0.5 μg of pCMV-lacZ reporter. Twenty-four after transfection, cells were stained with X-Gal and scored for specific apoptosis as in Fig. 2A. (**C**) Daxx releases the COOH-terminus of ASK1 from the NH_2-terminus of ASK1. 293T cells were transfected with pcDNA3-Myc-ASKN, pcDNA3-ASK1ΔN, and pRK5-FLAG-hDaxx as indicated along with 2.0 μg of pRK5-crmA and vector DNA as needed. Two micrograms of each indicated DNA was transfected in lanes 1 to 4; in lanes 5 and 6, 0.5 μg of ASKN, 2.0 μg of ASK1ΔN, and 4.0 μg of Daxx were transfected. Transfected cells were extracted, immunoprecipitated with anti-Myc coupled to agarose beads, and immunoblotted with anti-HA and anti-FLAG as in Fig. 3A. (**D**) One microgram each of pcDNA3-ASK1ΔN, pcDNA3-ASKN, and pRK5-FLAG-hDaxx was cotransfected as indicated with 0.5 μg of pCMV-lacZ and vector DNA as needed in HeLa cells. Twenty-four hours after transfection cells were stained with X-Gal and scored for specific apoptosis as in Fig. 2A.

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9. 293 and HeLa cells were maintained in Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS), glucose (4.5 μg/ml), and penicillin (100 U/ml) and transfected with Tfx-50 (Promega). Jurkat cells were cultured in RPMI 1640 medium containing 10% FBS and antibiotics in an atmosphere of 5% CO_2 at 37°C. SAPK3/p38γ and ATF2 peptide (1–109) were provided by M. Goedert and Z. Yao, respectively. Cells were extracted and immunoprecipitated with Myc mAb (Ab-1, Calbiochem), FLAG mAb (M2, Kodak), or antiserum to ASK1 (DAV) (*19*) with protein G (for

Ab-1 or M2)–or protein A (for DAV)–Sepharose. Immune complex assays were performed essentially as described (*12*). Phosphorylation of ATF2 peptide or GST-SAPK3/p38γ was analyzed by a Fuji BAS2000 image analyzer. ASK1 or TAK1 protein was detected by immunoblotting and enhanced chemiluminescence (ECL), which in exposures less than 10 min did not detect ^{32}P radioactivity from kinase autophosphorylation. Protein levels from immunoblot were quantified by densitometry (Quantity One program, pdi).

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11. EGY48 yeast strain was tranformed with EG202-ASK1(K709R), pJG4-5 vector, pJG4-5-mFas(192–295), or pJG4-5-mDaxx, and JK101 reporter plasmids, and quantitative liquid β-galactosidase (β-Gal) assay was performed (*4*). Relative β-Gal units ± SD for ASK1(KR) alone, 2.4 ± 0.2; ASK1(KR) plus Daxx, 119 ± 8.8; ASK1(KR) plus Fas, 1.8 ± 0.4.
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22. 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.
23. pEBB-Daxx (*4*), Daxx mutants (*4*), pEBB-Fas (*4*), pcDNA3-ASK1(*8*), pcDNA3-ASK1(K709R) (*8*), Myc-TAK1(*6*), pCMV-FLAG-JNK1(*4*), pRK5-crmA (*20*), EG202-ASK1(K709R) (*12*), and pJG4-5-mDaxx (*4*) were as described. ASK1ΔN, ΔC, kinase, FLAG-ASK1, Myc-ASK1, ASK1 (K709M)-HA, and Myc-ASKN were constructed in pcDNA3 (Invitrogen) by polymerase chain reaction (PCR). FLAG-tagged human Daxx and hDaxxC were derived from EST clone AA085057 and constructed in pRK5 (*20*) by PCR. pCI-AU1-hFas was constructed by J. Wang and M. J. Lenardo. The plasmids of GST–human MKK6, GST SAPK3/p38γ(KN), and GST-ASKN for bacterial fusion protein were constructed in pGEX-4T-1 (Pharmacia Biotech) by PCR.
24. Antiserum to ASK1 (DAV) was raised to the peptide sequence DAVATSGVSTLSSTVSHDSQ, amino acids 1217 to 1236 in human ASK1, as described (*21*). Rabbit polyclonal antibody to mouse Daxx (DSS) was raised against the peptide sequence DSSTRVDSP-SHELVTSSLC (amino acids 680 to 698) (*22*).
25. 293T cells (2×10^6) [grown in DMEM supplemented with 10% FBS, penicillin-streptomycin (100 U/ml), and glutamine (1 mM)] were plated onto a 60-mm dish the day before transfection. Twenty-four hours after transfection, cells were washed once in ice-cold phosphate-buffered saline (PBS) and lysed in 300 μl of IP-lysis buffer [50 mM Hepes (pH 7.4), 1% NP-40, 150 mM NaCl, 10% glycerol, 1 mM EDTA, 2 mM dithiothreitol] supplemented with 1 mM phenylmethylsulfonyl fluoride and 1% aprotinin. Extract (50 μl) was diluted in IP-lysis buffer (500 μl) and immunoprecipitated with antibody reagents as described in the figure legends. In Fig. 3B, L/Fas cells were lysed in 1 ml of lysis buffer. Cell lysates were immunoprecipitated with antiserum to ASK1 through use of protein A–Sepharose. The beads were washed twice with the washing buffer, separated by SDS—polyacrylamide gel electrophoresis (PAGE), and immunoblotted with anti-Daxx (DSS).
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Promotion of Dendritic Growth by CPG15, an Activity-Induced Signaling Molecule

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Activity-independent and activity-dependent mechanisms work in concert to regulate neuronal growth, ensuring the formation of accurate synaptic connections. CPG15, a protein regulated by synaptic activity, functions as a cell-surface growth-promoting molecule in vivo. In *Xenopus laevis*, CPG15 enhanced dendritic arbor growth in projection neurons, with no effect on interneurons. CPG15 controlled growth of neighboring neurons through an intercellular signaling mechanism that requires its glycosylphosphatidylinositol link. CPG15 may represent a new class of activity-regulated, membrane-bound, growth-promoting proteins that permit exquisite spatial and temporal control of neuronal structure.

The *cpg15* gene was identified in a forward genetic approach designed to isolate activity-regulated genes that mediate synaptic plasticity (*1*). In the adult rat, *cpg15* is induced in the brain by kainate (KA) and in visual cortex by light (*2*). During development, *cpg15* expression is correlated with times of afferent ingrowth, dendritic elaboration, and synaptogenesis (*2*). Sequence analysis predicts a small, secreted protein (*2*) that is membrane-bound by a glycosylphosphatidylinositol (GPI) linkage (*3*).

Antiserum generated against bacterially expressed rat CPG15 recognizes a protein from rat brain dentate gyrus extracts (Fig. 1A) (*4*) of the size predicted by sequence analysis. A protein of similar size is induced in *Xenopus laevis* after KA injections into the brain ventricle (Fig. 1A) (*5*). In situ hybridizations using a partial clone of *Xenopus cpg15* indicate that the CPG15 mRNA is expressed in retinal ganglion cells and in differentiated neurons throughout the central nervous system (CNS) of stage-47 tadpoles (*6*). *Xenopus* CPG15 protein is present in neurons and axons throughout the CNS (*7, 8*). In the optic tectum, differentiated neurons label in a honeycomb pattern similar to N-CAM (neural cell adhesion molecule) and other cell-surface antigens, while cells in the proliferative zone have undetectable levels of CPG15 (Fig. 1C).

To investigate the cellular function of CPG15, we used a recombinant vaccinia virus (VV) to express CPG15 in optic tectal cells of albino *Xenopus* tadpoles (*9, 10*). Tadpoles were infected by ventricular injection with VV carrying rat *cpg15* and β-galactosidase (β-gal) cDNAs in a dual promoter vector, or with a control virus containing only the β-gal cDNA (*11*). Two days after viral infection and approximately 24 hours after the beginning of expression of foreign protein (*9*), single tectal cells were labeled with DiI (*10, 12*). Confocal images through the entire structure of each neuron were collected at 24-hour intervals over a period of 3 days, and three-dimensional (3D) images were reconstructed from this (*13*).

The most prominent effect of CPG15 on the morphology of tectal projection neurons was that the dendritic arbors of neurons from CPG15VV-infected animals increased their total dendritic branch length (TDBL) and became more complex than arbors of neurons from β-gal–infected or uninfected animals (Fig. 2) (*14*). This effect was quantified as an increase in averaged TDBL (Fig. 3A) and by Sholl analysis (Fig. 3B).

We measured the distribution of dendritic arbor sizes, expressed as TDBL, within the population of neurons from CPG15VV-infected animals and from control animals (Fig. 3C). All three populations of neurons showed a gradual shift toward larger TDBLs as their dendritic arbors grow. The shift toward larger TDBLs was greatest in neurons from CPG15VV-infected animals. This analysis also demonstrates the presence of a subpopulation

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