THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 18, Issue of April 30, pp. 12605–12610, 1999
Printed in U.S.A.

MEK Kinase 1 (MEKK1) Transduces c-Jun NH_2-terminal Kinase Activation in Response to Changes in the Microtubule Cytoskeleton*

(Received for publication, January 25, 1999, and in revised form, February 22, 1999)

Toshiaki Yujiri‡, Gary R. Fanger‡, Timothy P. Garrington‡, Thomas K. Schlesinger‡, Spencer Gibson‡§, and Gary L. Johnson‡¶‖

From the ‡Program in Molecular Signal Transduction, Division of Basic Sciences, National Jewish Medical and Research Center, Denver, Colorado 80206 and the ¶Department of Pharmacology, University of Colorado Medical School, Denver, Colorado 80262

Cell shape change and the restructuring of the cytoskeleton are important regulatory responses that influence the growth, differentiation, and commitment to apoptosis of different cell types. MEK kinase 1 (MEKK1) activates the c-Jun NH_2-terminal kinase (JNK) pathway in response to exposure of cells to microtubule toxins, including taxol. MEKK1 expression is elevated 3-fold in mitosis and microtubule toxin-treated cells accumulated at G_2/M of the cell cycle. Targeted disruption of MEKK1 expression in embryonic stem cells resulted in the loss of JNK activation and increased apoptosis in response to taxol. Targeted disruption of the MEK kinase 2 gene had no effect on activation of the JNK pathway in response to microtubule toxins demonstrating a specific role of MEKK1 in this response. Cytochalasin D-mediated disruption of actin fibers activates JNK and stimulates apoptosis similarly in MEKK1$^{-/-}$ and wild type cells. The results show that MEKK1 is required for JNK activation in response to microtubule but not actin fiber toxins in embryonic stem cells. MEKK1 activation can protect cells from apoptosis in response to change in the integrity of the microtubule cytoskeleton.

Restructuring of the microtubule cytoskeleton occurs in response to a variety of events such as immune cell infiltration and invasion, interaction of cells with the extracellular matrix, and during the mitotic phase of the cell cycle. Cell shape change and the underlying restructuring of the cytoskeleton regulates gene expression and contributes to the commitment of cells to grow, undergo apoptosis, or differentiate. The importance of the cytoarchitecture and its regulation was realized when it was described that a reduction in cell spreading resulting in a spherical *versus* flat cell shape had an inhibitory effect on DNA synthesis (1, 2). More recently, it was demonstrated that cytoarchitecture determines whether a cell will grow or undergo apoptosis, as decreased cell spreading, which results in cell rounding using micropatterned substrates of various dimensions, induced an apoptotic response whereas cell spreading allowed survival and proliferation (3). The underlying restructuring of the cytoskeleton during these responses has been shown to influence gene expression. For example, microtubule disruption induces expression of the urokinase-type plasminogen activator and interleukin 1-β but inhibits tubulin synthesis (4–6). The promoter region for the B chain of platelet-derived growth factor was shown to contain a cis-acting response element that was regulated by shear stress induced by changes in cytoarchitecture (7). The collagenase-1 gene, which encodes a matrix metalloproteinase important for cell migration and invasion, is also regulated in response to cytochalasin D disruption of the actin cytoskeleton (8).

Reorganization of the cytoarchitecture regulates signaling pathways including the mobilization of intracellular calcium, activation of tyrosine kinases, Ras, extracellular signal-regulated kinase (ERK),[1] and c-Jun NH_2-terminal kinase (JNK) (9–12). Consistent with the activation of signal pathways, specific transcription factors are activated by cytoskeletal restructuring (8, 13). In this report, we show that targeted disruption of MEKK1 expression selectively inhibits JNK activation in response to microtubule toxins. Functionally, MEKK1 is the transducer for the specific regulation of the JNK pathway and promotes cell survival during changes in microtubule integrity.

EXPERIMENTAL PROCEDURES

Cell Culture—Cells were maintained in a humidified 7.0% CO_2 environment in Dulbecco's modified Eagle's medium supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin (Life Technologies, Inc.). Medium for T47D human breast adenocarcinoma cells was supplemented with 10% fetal bovine serum (Life Technologies, Inc.). Medium for MEKK1$^{-/-}$ and MEKK1$^{+/+}$ mouse embryonic stem (ES) cells was supplemented with 15% heat-inactivated fetal bovine serum (Summit Biotechnology, CO), 144 μM monothioglyerol (Sigma), and 1% leukemia inhibitory factor Chinese hamster ovary cell-conditioned medium.

Analysis of Kinase Activity—MEKK1 and JNK activies were measured as described previously (14), except 1 μg of recombinant purified kinase-inactive glutathione *S*-transferase-c-Jun NH_2-terminal kinase kinase (GST-$JNKK_{k-r}$) was used as a substrate to assay MEKK1 activity. To determine ERK and p38 activity, cell lysates were Western blotted with either an anti-phospho-ERK or anti-phospho-p38 antibody (New England Biolabs, MA) as a measure of kinase activity.

Apoptosis Assay—Apoptosis was measured using acridine orange/ethidium bromide staining. Apoptotic cell death was verified using DNA ladder formation assays.

Microscopy—Cells were plated onto uncoated glass coverslips 2 days before being fixed in a solution containing 3% paraformaldehyde and 3% sucrose in phosphate-buffered saline (pH 7.4). Cells were permeabi-

* This work was supported by National Institutes of Health Grants DK37871, DK48845, GM30324, and CA58157. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Leukemia Society Fellow.

‖ To whom correspondence should be addressed: Program in Molecular Signal Transduction, Division of Basic Sciences, National Jewish Medical and Research Center, 1400 Jackson St., Denver, CO 80206. Tel.: 303-398-1504; Fax: 303-398-1225; E-mail: johnsong@njc.org.

[1] The abbreviations used are: ERK, extracellular signal-regulated kinase; MEKK, MEK kinase; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; ES, embryonic stem; JNKK, JNK kinase; GST, glutathione *S*-transferase; $JNKK_{k-r}$, recombinant kinase-inactive JNKK; DAPI, 4′,6-diamidino-2-phenylindole.

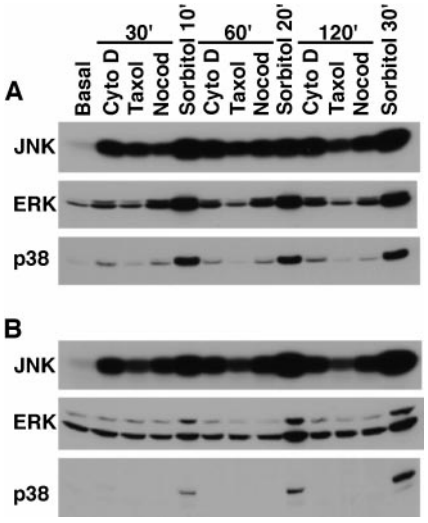


FIG. 1. **Regulation of JNK, ERK and p38 pathways by cytoskeletal toxins and hyperosmolarity.** T47D human breast carcinoma cells (*A*) or mouse ES cells (*B*) were incubated with 2 μg/ml cytochalasin D (*Cyto D*), 5 μM taxol, 0.5 μg/ml nocodazole (*Nocod*), or 400 mM sorbitol for the indicated times. JNK was assayed using GST-c-Jun as a substrate. For analysis of ERK and p38 activity cell lysates were prepared, resolved by SDS-polyacrylamide gel electrophoresis, and immunoblotted with either anti-phospho-ERK or anti-phospho-p38 antibodies. The experiment shown is representative of several experiments analyzing time courses and activation of MAPK pathways in response to these stimuli.

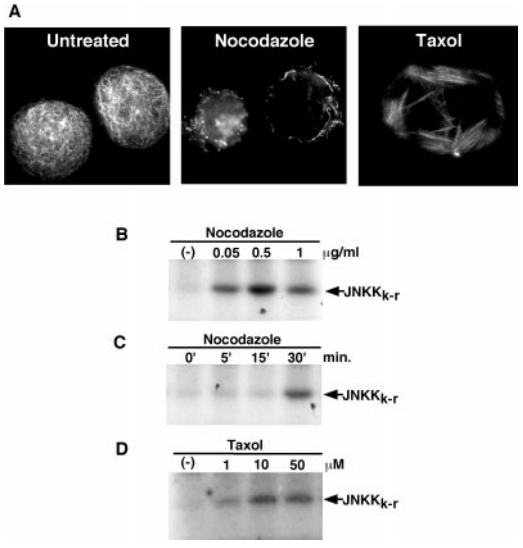


FIG. 2. **MEKK1 is activated in response to treatment of cells with nocodazole or taxol.** *A*, T47D cells exposed to 0.5 μg/ml nocodazole for 30 min or 10 μM taxol for 16 h have an altered microtubule cytoskeleton. Shown are immunofluorescence images of representative control, and nocodazole- and taxol-treated cells stained with anti-tubulin antibodies. T47D cells were treated with the indicated concentrations of nocodazole (*B*) or taxol (*D*) for 30 min or for different times with 0.5 μg/ml nocodazole (*C*). Cells were lysed and MEKK1 immunoprecipitated and assayed measuring *in vitro* kinase activity using JNKK$_{k-r}$ as substrate. The experiment is representative of 2–4 independent determinations for each condition.

lized with 0.2% Triton X-100 and incubated with an anti-tubulin antibody, followed by an incubation with 1.5 mg/ml Cy^3-conjugated affinity-purified donkey anti-rabbit Ig (Jackson ImmunoResearch Laboratories, West Grove, PA). Coverslips were mounted and analyzed as described previously (14).

RESULTS

JNK Is the Dominant MAPK Activated by Microtubule Toxins—Exposure of cells to conditions and drugs that alter the cytoarchitecture strongly activate the JNK pathway. Fig. 1, *A* and *B*, shows that with T47D human breast carcinoma cells and ES cells, respectively, microtubule toxins (taxol and nocodazole), actin fiber disruption (cytochalasin D), and hyperosmolarity (sorbitol) strongly activate the JNK pathway. In these studies modest concentrations of these stimuli were used for short times where the cells were not adversely affected, demonstrating that activation of the JNK pathway is an early major response to these stimuli.

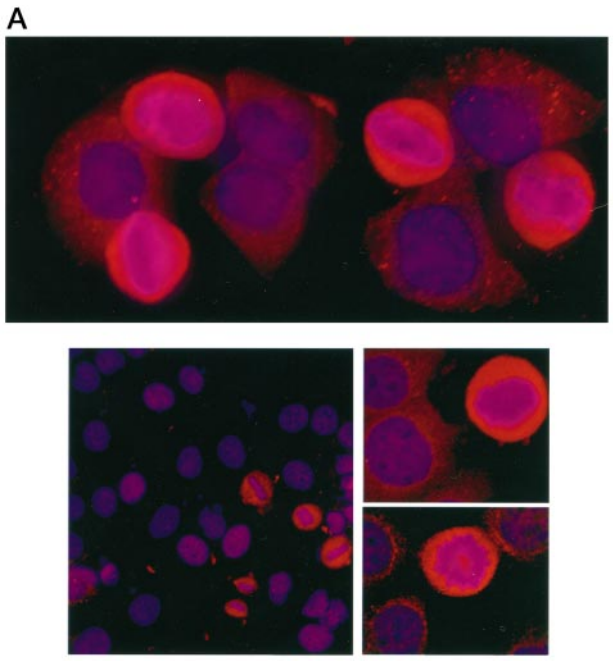
Analysis of the ERK and p38 pathways indicates that the different treatments differentially activate MAPK pathways, even though each stimulus significantly activates JNK. As predicted, hyperosmolarity achieved with sorbitol addition to the growth medium activated p38 in both cell types. Cytochalasin D and nocodazole activated p38 similarly in T47D cells but not in ES cells. ERK activation is weak in ES cells in response to most stimuli[2] and not measurably activated in response to the compounds used to alter cell shape. In contrast, for T47D cells, cytochalasin D and nocodazole modestly activated ERK, whereas taxol was a very weak activator of the ERK pathway. Sorbitol-induced hyperosmolarity stimulated significant ERK activation. Interestingly, taxol did not significantly activate p38 in either cell type; JNK activation is the primary response to taxol treatment. This finding indicates microtubule poisoning by taxol is sensed differently by the cell than nocodazole-induced depolymerization of microtubules.

MEKK1 Is Activated in Response to Stimuli That Alter Microtubules—MEKKs regulate the JNK pathway (15–17), suggesting that a specific MEKK could mediate the JNK activation in response to microtubule reorganization of the cytoarchitecture. We found that MEKK1 is activated by stimuli that alter microtubule dynamics. Nocodazole disrupts microtubules in T47D cells (Fig. 2*A*) and activates MEKK1 in a concentration- and time-dependent manner (Fig. 2, *B* and *C*). Taxol treatment of T47D cells, which stabilizes microtubule structures (Fig. 2*A*), activates MEKK1 (Fig. 2*D*). Thus, the microtubule toxins, nocodazole and taxol, activate MEKK1.

MEKK1 Protein Expression Is Increased during the M Phase of the Cell Cycle—During the cell cycle the greatest change in microtubule structure occurs in mitosis. Analysis of T47D breast carcinoma cells proliferating in normal growth conditions demonstrated that the expression of MEKK1 is increased in the M phase of the cell cycle. Fig. 3*A* shows T47D cells, co-stained using an antibody recognizing MEKK1 and the DNA stain, DAPI. Immunofluorescence microscopy readily demonstrated that mitotic cells have significantly higher levels of MEKK1 expression than non-mitotic cells. To quantitate differences in anti-MEKK1 immunofluorescence in mitotic *versus* non-mitotic cells, deconvolved confocal three-dimensional images were constructed. Images of mitotic and non-mitotic cells were quantitated for anti-MEKK1 immunofluorescence. Fig. 3*B* shows that mitotic T47D cells express approximately 3-fold higher MEKK1 protein levels than non-mitotic cells. Similar results have been observed in other cell types (not shown).

To biochemically confirm the immunofluorescence analysis, T47D cells were treated with microtubule toxins to arrest cells at G_2/M in the cell cycle. The G_2/M block of treated cells was confirmed by cell cycle analysis using flow cytometry (not shown). Immunoblotting with the anti-MEKK1 antibody used for immunofluorescence demonstrated that MEKK1 protein levels were increased in drug-treated cells accumulated at G_2/M, compared with untreated cell populations having cells randomly in all phases of the cell cycle (Fig. 3*C*, *upper left panel*). As a control, treatment of cells with etoposide, which induces DNA damage in S phase and blocks cells in G_1/S of the

[2] T. Yujiri and G. L. Johnson, unpublished observations.



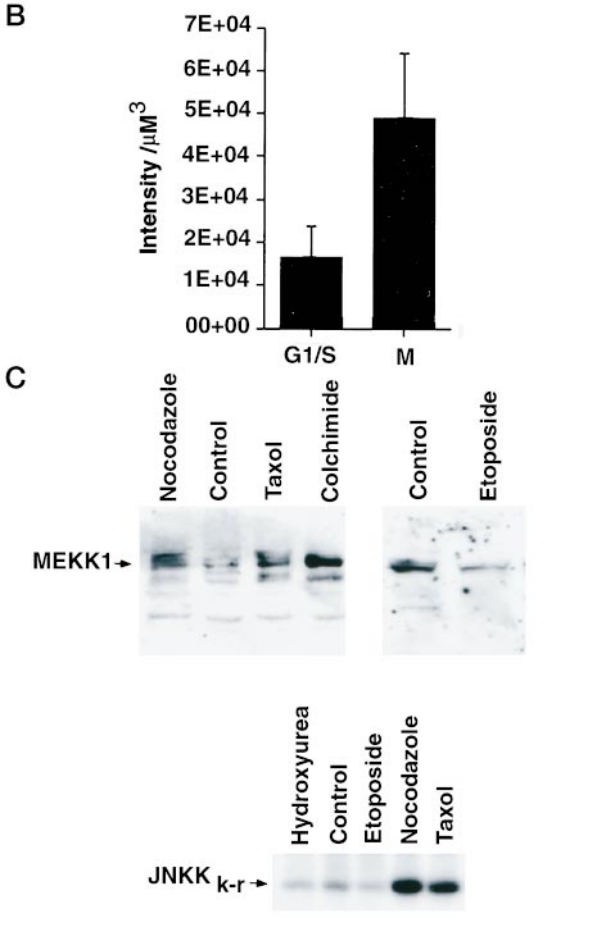


FIG. 3. **MEKK1 expression is increased in the M phase of the cell cycle.** *A*, proliferating T47D breast adenocarcinoma cells were fixed and fluorescently stained with an anti-MEKK1 antibody followed by a Cy^3-tagged secondary antibody. DNA was stained with DAPI. Metaphase cells, evident by chromosome condensation, were stained more intensely with the anti-MEKK1 antibody relative to G_1/S phase T47D cells. Shown are 0.5-μm deconvolved confocal images of the thickest region of each the cell. *B*, for quantitation, 10 M phase and 10 G_1/S phase cells were analyzed by three-dimensional reconstruction of 0.5-μm confocal sections of the cell. The fluorescence signal over the entire thickness of the cell was summed. Shown is the mean ± S.D. of the summed MEKK1 immunofluorescence of G_1/S *versus* M phase T47D cells. The difference in immunofluorescence between G_1/S and M phase cells is significant at a $p < 0.05$ using the Student's *t* test. *C*, *upper panels*, T47D cells were incubated for 20 h in 10% serum without (control) or with 0.05 μg/ml nocodazole, 1 μM taxol, 1 μM colchimide, or 0.1 μM etoposide. Flow cytometry analysis verified that taxol, nocodazole, and colchimide arrested cells at G_2/M and etoposide-treated cells were blocked in G_1/S. Cells were harvested and analyzed by immunoblotting for MEKK1 expression. The blot showing control and etoposide (*right*) was exposed longer than the blot showing MEKK1 expression (*left*) in cells exposed to microtubule toxins. *Lower panel*, MEKK1 was immunoprecipitated and assayed from lysates prepared from T47D cells treated with 1 mM hydroxyurea, 0.1 μM etoposide, 1 μM taxol, 0.05 μg/ml nocodazole, or no drug for 20 h. MEKK1 activity was assayed using $JNKK_{k\text{-}r}$ in an *in vitro* kinase assay.

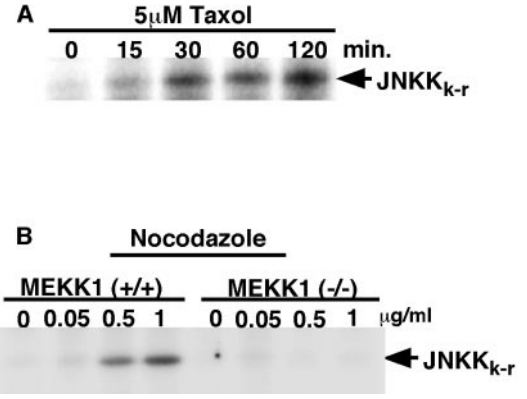


FIG. 4. **Specificity of the MEKK1 *in vitro* kinase assay from microtubule poison-treated cells.** ES ($MEKK1^{+/+}$ or $MEKK1^{-/-}$) cells were treated with 5 μM taxol for the indicated times or with different concentrations of nocodazole for 30 min. Cells were lysed and MEKK1-immunoprecipitated. $JNKK_{k\text{-}r}$ was added with [γ-^{32}P]-ATP to the immunoprecipitate. MEKK1-dependent phosphorylation of $JNKK_{k\text{-}r}$ was determined by resolving the proteins in the assay using SDS-polyacrylamide gel electrophoresis followed by autoradiography.

cell cycle, did not increase MEKK1 expression (Fig. 3*C*, *upper right panel*). In fact, etoposide, which acutely activates MEKK1, induces a loss of MEKK1 expression in T47D cells after several hours of drug exposure, resulting from the induction of caspase 3 cleavage of the 196-kDa MEKK1 protein (18, 19). We have found that taxol and nocodazole treatment of cells does not induce the cleavage of MEKK1 like that observed for DNA-damaging drugs. Thus, the regulation of MEKK1 protein levels and caspase cleavage in response to microtubule toxins and DNA-damaging drugs is different. Consistent with the increased levels of MEKK1 in T47D cells blocked at G_2/M, the total MEKK1 activity is also increased by the microtubule toxin-induced block at G_2/M (Fig. 3*C*, *lower panel*). In contrast, hydroxyurea and etoposide, which act in S phase of the cell cycle do not cause an increase in MEKK1 activity following prolonged cellular exposure to these drugs. Cumulatively, the findings demonstrate increased MEKK1 expression at the G_2/M phase of the cell cycle and that microtubule toxins activate MEKK1. Thus, MEKK1 regulation is responsive to changes in microtubule organization in the cell.

Targeted Disruption of MEKK1 Expression Causes Loss of JNK Activation in Response to Microtubule Reorganization—We have targeted the disruption of MEKK1 expression by homologous recombination (20). $MEKK1^{-/-}$ ES cells do not express the MEKK1 protein. Treatment of wild type ES cells with either taxol or nocodazole activates MEKK1 measured by immunoprecipitation and *in vitro* kinase assay (Fig. 4, *A* and *B*). Importantly, no kinase activity is observed in immunoprecipitates from lysates of $MEKK1^{-/-}$ ES cells treated with nocodazole (Fig. 4*B*). This finding unequivocally demonstrates that our antibodies selectively immunoprecipitate MEKK1 and

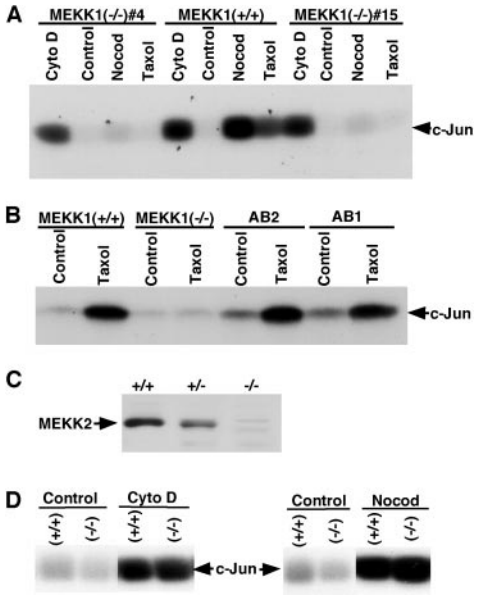


FIG. 5. **MEKK1 but not MEKK2 is required for JNK activation in response to exposure of ES cells to microtubule poisons.** *A*, wild type ES cells (MEKK1$^{+/+}$) and two ES cell clones having targeted disruption of MEKK1 expression (*MEKK1*$^{-/-}$ *#4 and #15*) were exposed to 4 μg/ml cytochalasin D (*Cyto D*), 5 μM taxol, 0.5 μg/ml nocodazole (*Nocod*) or growth medium only (*Control*) for 2 h. Cells were lysed and JNK activity was measured using GST-c-Jun as substrate. *B*, the cDNA encoding the full-length 196-kDa MEKK1 in pCEP4 was stably transfected into MEKK1$^{-/-}$ cells. Hygromycin-resistant, MEKK1-expressing clones were characterized by immunoblotting. Two re-expression clones (*AB1* and *AB2*) and MEKK1$^{-/-}$ and MEKK1$^{+/+}$ ES cells were challenged with 5 μM taxol for 1 h, lysed, and assayed for JNK activity. *C*, immunoblot of MEKK2$^{+/+}$, MEKK2$^{+/-}$, and MEKK2$^{-/-}$ cells, showing the loss of MEKK2 expression in ES cells having targeted disruption of the MEKK2 gene. *D*, cytochalasin D (2 μg/ml) and nocodazole (0.5 μg/ml) were incubated with wild type (+/+) and MEKK2$^{-/-}$ (−/−) ES cells for 2 h. Cells were lysed and assayed for JNK activity using GST-c-Jun as substrate.

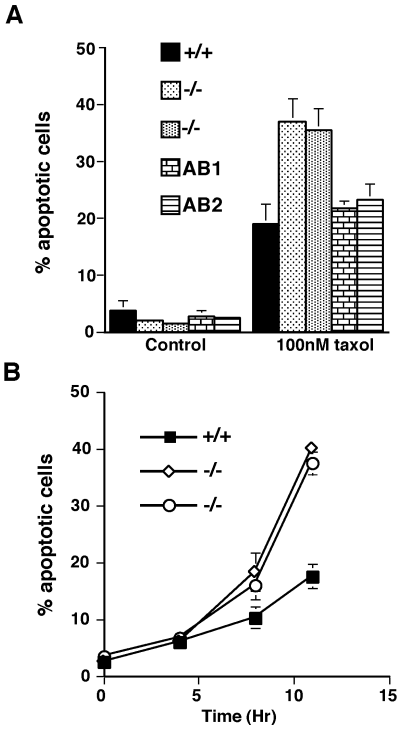


FIG. 6. **MEKK1 expression protects cells from taxol-induced apoptosis.** *A*, ES cells, wild type (+/+), MEKK1$^{-/-}$ (−/−), and MEKK1 re-expression (*AB*) clones were incubated in growth medium without (*control*) or with 100 nM taxol for 12 h. *B*, the designated ES cell clones were incubated with 100 nM taxol for 4, 8, or 12 h. Apoptotic cells were determined by quantitating acridine orange staining of condensed nuclei. The results represent the mean ± S.E. and are representative of three independent experiments.

that MEKK1 is responsible for the JNKK phosphorylation in the *in vitro* kinase assay. As predicted from this result, JNK activation in response to the microtubule toxins nocodazole and taxol is lost in two independent MEKK1$^{-/-}$ clones (Fig. 5*A*). Interestingly, the disruption of the actin cytoskeleton with cytochalasin D activates the JNK pathway in MEKK1$^{-/-}$ and MEKK1$^{+/+}$ ES cells (Fig. 5*A*). Thus, MEKK1 is absolutely required for JNK activation in response to microtubule but not actin fiber disruption. Re-expression of the 196-kDa MEKK1 protein by stable transfection of MEKK1$^{-/-}$ with a plasmid encoding the full-length MEKK1 cDNA reconstituted the regulation of the JNK pathway by taxol (Fig. 5*B*). The reconstitution of JNK activation in response to taxol demonstrates that this response is specifically mediated by MEKK1.

We have also disrupted the expression of MEKK2 by homologous recombination in ES cells (Fig. 5*C*).[3] Both microtubule and actin fiber disruption by nocodazole and cytochalasin D, respectively, activated the JNK pathway similarly in MEKK2$^{+/+}$ and MEKK2$^{-/-}$ ES cells (Fig. 5*D*). Thus, MEKK2 is not involved in the regulation of the JNK pathway in response to changes in the microtubule and actin cytoskeleton; the microtubule response involves MEKK1. This finding demonstrates the selectivity of different MEKKs for regulation by specific stimuli.

Targeted Disruption of MEKK1 Expression Increases Apoptosis in Response to Microtubule Toxins—We have shown that MEKK1$^{-/-}$ ES cells do not activate JNK following treatment with nocodazole or taxol. Prolonged shape change and microtubule disruption induce apoptosis in many cell types (3, 20). Fig. 6*A* shows that MEKK1$^{-/-}$ ES cells have a significantly greater apoptotic index in response to taxol, relative to MEKK1$^{+/+}$ ES cells. Fig. 6*B* shows that temporally MEKK1$^{-/-}$ cells become apoptotic more rapidly than MEKK1$^{+/+}$ cells. At a significantly slower rate, MEKK1$^{+/+}$ cells will reach the same apoptotic index as taxol-treated MEKK1$^{-/-}$ cells. Thus, MEKK1 activation has a protective function in response to microtubule poisoning. To prove this fact, the two MEKK1$^{-/-}$ ES cell lines having MEKK1 expression re-established by stable transfection of a MEKK1 expression plasmid were tested for their sensitivity to taxol-induced apoptosis (Fig. 6*A*). Just as in the reconstitution of JNK activation, the re-expression of MEKK1 expression rescued the survival of cells exposed to taxol similar to wild type ES cells.

Cytochalasin D disrupts the actin cytoskeleton but has little effect on microtubule integrity. Cytochalasin D treatment of T47D cells strongly stimulates MEKK1 and JNK activities (Fig. 7*A*). Similarly, cytochalasin D treatment of wild type ES cells activates MEKK1 (Fig. 7*B*) and strongly activates JNK in both MEKK1$^{+/+}$ and MEKK1$^{-/-}$ ES cells (Fig. 5*A*). The activation of JNK, independent of MEKK1 in ES cells, allowed the question of whether MEKK1 or JNK mediates the cell survival response to actin cytoskeleton poisoning. Fig. 7, *C* and *D,* shows that the sensitivity of ES cells to undergo cytochalasin D-induced apoptosis is virtually identical in a MEKK1$^{-/-}$ or MEKK1$^{+/+}$ background. The increased apoptotic response of MEKK1$^{-/-}$ cells is specific to microtubule toxins and not actin cytoskeleton toxins. Thus, the enhanced sensitivity of MEKK1$^{-/-}$ cells to undergo apoptosis in response to microtu-

[3] T. P. Garrington, T. Yujiri, S. Gibson, and G. L. Johnson, manuscript in preparation.

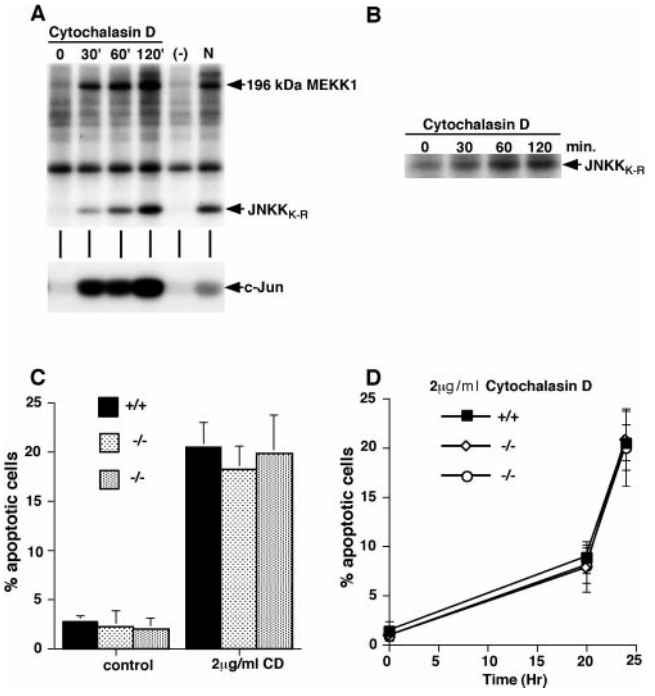


FIG. 7. **MEKK1 is activated but is not required for JNK stimulation following exposure of ES cells to cytochalasin D.** *A*, T47D cells were exposed to 8 μg/ml cytochalasin D for the indicated times, 0.5 μg/ml nocodazole (*N*) for 2 h, or buffer with Me_2SO for 2 h (−). Cells were lysed and MEKK1-immunoprecipitated with anti-MEKK1 antibody. Immunoprecipitates were incubated with recombinant $JNKK_{k-r}$ and [γ-^{32}P]-ATP, resolved by SDS-polyacrylamide gel electrophoresis, and analyzed by autoradiography. Autophosphorylation of MEKK1 and phosphorylation of $JNKK_{k-r}$ is shown by *arrows*. For assaying JNK activity, GST-c-Jun was added to aliquots of the same cell lysates used for immunoprecipitation. *B*, wild type ES cells were incubated with 8 μg/ml cytochalasin D for the indicated times. Cells were lysed, MEKK1 antibody was added to lysates for immunoprecipitation, and MEKK1 activity was assayed as described in *A*. *C* and *D*, wild type (+/+) and MEKK1$^{-/-}$ (−/−) ES cells were incubated without or with 2 μg/ml cytochalasin D (*CD*) for 24 h (*C*) or 20 or 24 h (*D*). Apoptotic cells were quantitated by acridine orange staining. The results represent the mean ± S.E. of triplicate determinations and are representative of three independent experiments.

bule toxins may involve the loss of JNK activation regulated by MEKK1.

DISCUSSION

Changes in cytoskeletal dynamics stimulate signal transduction pathways. Several studies indicate that the microtubules play an integral part in regulating signaling pathways. We have shown that JNK is the dominant MAPK activated by microtubule toxins, including taxol and nocodazole. MEKK1 is absolutely required for JNK activation when cells are exposed to nocodazole and taxol. MEKK1 is similarly required for JNK activation in response to mild hyperosmolarity and cold stress, both of which alter the integrity of the microtubule cytoskeleton and cell shape. Targeted disruption of MEKK1 expression unequivocally defined its role by being the MAPK kinase kinase regulating the JNK pathway in response to changes in microtubule integrity. How might microtubule restructuring activate MEKK1? One hypothesis would involve the GTP-binding protein Rac1 (21). Rac1 has been shown to bind β-tubulin in a GTP-dependent manner (22). The binding of Rac1·GTP did not influence tubulin polymerization, and it was proposed that tubulin-Rac1·GTP complexes would control Rac1 signaling. MEKK1 binds Rac1 in a GTP-dependent manner (14). Rac1 activates the JNK pathway as does MEKK1 (23, 24), and inhibitory MEKK1 mutants block Rac1 activation of JNK (14). If Rac1 functions as a sensor for cytoskeletal changes it could stimulate MEKK1, resulting in JNK activation and protection of cells from apoptosis. The increased expression of MEKK1 during the G_2/M phase of the cell cycle is consistent with its involvement in sensing the dramatic microtubule changes that occur during mitosis. The protective properties of MEKK1 would prevent cells from defaulting into apoptosis during progression through the G_2/M phase. The increased apoptotic response of MEKK1$^{-/-}$ ES cells to microtubule toxins is consistent with this hypothesis. Confirmation of this hypothesis will require the use of an inducible dominant-negative Rac1 construct to determine the role of Rac1 in microtubule disruption-stimulated JNK activity. In the future, cell lines expressing an inducible dominant-negative Rac1 will be generated to test this hypothesis.

The targeted gene disruption of MEKK1 expression unequivocally defined the function of MEKK1 in responding to changes in microtubule dynamics in mouse ES cells, namely the protection of ES cells from committing to apoptosis. The role of MEKK1 is specific in that loss of its expression causes complete loss of JNK activation in response to microtubule disruption. Targeted disruption of MEKK2 expression had no effect on the JNK response to microtubule disruption in ES cells. Thus, each MEKK will be predictably found to respond to very specific upstream stimuli. Support for this prediction is observed with the loss of specific receptor activation of the JNK pathway in MEKK2 knockouts that is not observed with MEKK1 knockouts.[3]

The role of MEKK1 in cell survival is contrary to its pro-apoptotic functions when cleaved by caspases (18, 25). MEKK1 is a substrate for caspase 3 and is cleaved at Asp^{874}. The cleavage of MEKK1 at Asp^{874} releases a 91-kDa activated kinase domain that amplifies the activation of caspases. The activated full-length 196-kDa MEKK1 does not activate caspases or apoptosis (18, 19, 25). Thus, there is a dual role of MEKK1 that is controlled by caspases. Activation of MEKK1 promotes cell survival; targeted disruption of MEKK1 expression unequivocally defines this function of MEKK1. Caspase cleavage of MEKK1 causes loss of the survival response and conversion to a pro-apoptotic response of the newly generated 91-kDa MEKK1 COOH-terminal kinase domain. If the survival response mediated by MEKK1 could be abrogated, then microtubule toxins like taxol would have greater efficacy as chemotherapeutic drugs. From a practical standpoint, defining proteins and genes specifically regulated by the MEKK1-activated JNK pathway might define new drug targets for the treatment of cancer and other diseases.

Acknowledgment—We thank Nancy Lassignal Johnson for expert assistance in digital confocal immunofluorescence imaging and figure preparation.

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