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NF-κB Activation by Disruption of Microtubule Array during Myogenesis of L6 Cells

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We have previously reported that NF-κB is involved in the regulation of nitric oxide synthase gene expression during differentiation of chick embryonic myoblasts. However, how NF-κB is timely activated during myogenesis remains elusive. One of the most prominent events in myogenesis is myoblast membrane fusion, which is accompanied with massive cytoskeletal reorganization. Here we show that the activity of NF-κB markedly increases in L6 rat myogenic cells that have just initiated morphological changes by treating nocodazole, a microtubule-disrupting agent. Furthermore, the induction of NF-κB activation was closely correlated with the myoblast fusion. In addition, a variety of agents that disrupt microtubules stimulated the myoblast fusion as well as the induction of NF-κB activation. In contrast, taxol, a microtubule-stabilizing agent, suppressed the induction of NF-κB activation and inhibited spontaneous differentiation of L6 cells as well. In addition, we found that the NF-κB in the cells consists of p50/p65 heterodimers. These results support the idea that reorganization of microtubule at early stages of differentiation plays a role as a signal for NF-κB activation during myogenesis.

During the differentiation of skeletal muscle cells, mononucleated myoblasts undergo a cellular fusion process to form multinucleated myotubes. In the early stages of differentiation, myoblasts start to elongate their shape. As the process proceeds, the highly elongated myoblasts align with neighboring cells in an appropriate orientation, and then eventually fuse with their neighbors to form multinucleated myotubes (Nameroff and Munar, 1976; Knudsen and Horwitz, 1977). These extensive morphological changes observed in myoblasts are accompanied with massive reorganization of cytoskeletal proteins including dramatic changes in microtubule dynamics (Gundersen et al., 1989).

Changes in cell shape have been recognized as signals for alteration in the program of gene expression (Bissell et al., 1982; Ben-Ze'ev, 1991; Doyle and Botstein, 1996). For example, transcriptions of several genes, including the ones that encode urokinase type plasminogen activator (uPA) and interleukin-1β (IL-1β), are activated by the disruption of microtubule in fibroblasts (Botteri et al., 1990; Manie et al., 1993; Lee et al., 1994). In addition, the disruption of microtubule is recently proposed to serve as a signal for NF-κB activation and induction of NF-κB-dependent gene expression in HeLa cells (Rosette and Karin, 1995).

NF-κB/Rel is a transcription factor that induces the expression of a wide variety of genes, including interleukin-2 (IL-2) receptor gene, class I and class II major histocompatibility antigen genes, and several viral enhancers (Lenardo and Baltimore, 1989; Liebermann and Baltimore, 1990; Shimizu et al., 1990). NF-κB/Rel is a dimer composed of proteins that belong to the Rel family. The members of this family in mammalian cells include the proto-oncogene product c-Rel, p50/p105, p65 (RelA), and RelB (Baeuerle, 1991; Nolan and Baltimore, 1992; Liou and Baltimore, 1993). NF-κB is typically localized in the cytoplasm as an inactive complex with an inhibitor protein called IκB (Blank et al., 1992; Gilmore and Morin, 1993; Liou and Baltimore, 1993; Grumont and Gerondakis, 1994). In response to NF-κB inducers, Iκ B is degraded through ubiquitinationproteasome system allowing dissociation of NF-κB from IκB, and NF-κB moves to the nucleus, where it directly binds to the decameric cis-acting κB DNA motif and induces a transcription. So far a wide variety of extracellular signals, such as tumor necrosis factor-α (TNF-α), IL-1, phorbol esters, and lipopolysaccharides, have been identified to induce the activation of NF-κB (Sen and Baltimore, 1986; Lowenthal et al.,

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1989; Osborn et al., 1989; Shirakawa and Mizel, 1989).

Nitric oxide (NO) acts as a messenger for the membrane fusion in chick skeletal embryonic myoblasts. NO is generated by nitric oxide synthase (NOS), an enzyme that catalyzes the stoichiometric conversion of L-arginine to NO and L-citrulline, and the expression of NOS increases transiently during myogenesis (Lee et al., 1994). Previously, we and others demonstrated that NF-κB is involved in the induction of NOS expression during chick myogenesis (Xie et al., 1994; Lin et al., 1996; Lee et al., 1997). Then, we have attempted without success to identify extracellular signals that induce the NF-κB activity. However, in light of massive cytoskeletal reorganization which is experienced by myoblasts during myogenesis, it is possible that microtubule reorganization rather than extracellular signal molecules is a key factor that may lead to NF-κB activation. Here we describe evidence that the disruption of microtubule is implicated in the NF-κB activation during myogenesis.

Materials and Methods

Materials

Culture media, fetal bovine serum (FBS), and horse serum (HS) were obtained from Gibco BRL. Isotope and enhanced chemiluminescence reagent (ECL) were purchased from Amersham Corp. Poly(dI-dC)·poly(dI-dC) was from Pharmacia LKB Biotechnology, Inc. Decameric NF-κB consensus oligonucleotide, anti-IκB-α antibody and affinity-purified rabbit IgGs against the following members of NF-κB/Rel family, p50, p65, c-Rel, and RelB, were purchased from Santa Cruz Biotechnology. Nocodazole, colchicine, taxol, vinblastine, podophyllotoxin, and all other reagents were obtained from Sigma Chemical Co.

Myogenic cell culture

L6 myogenic cell line (Yaffe, 1968) was obtained from American Type Culture Collection. The cells were plated on plastic tissue culture dishes at a concentration of 1.8×10^4/ml. Growth media consisted of Dulbecco's modified essential media (DMEM) supplemented with 10% FBS. Three days after the cell seeding, the culture media was changed with differentiation media, which contained 5% HS instead of 10% FBS. For measurement of myoblast fusion, the cells were fixed with 1% glutaraldehyde for 30 min, stained with 0.1% hematoxyllin for 30 min, and with 0.5% eosin for 30 sec. Extent of myoblast fusion was expressed as the percentage of the number of nuclei in fused cells to the total number of nuclei within more than 10 randomly chosen fields under a microscope. Cells were considered as fused only if there were clear cytoplasmic continuity and at least three nuclei were present in each myotube.

Electrophoretic mobility shift assay

An oligonucleotide probe containing the decameric consensus NF-κB binding site sequence was end-labeled with [γ-^{32}P]ATP using T4 kinase. The nuclear extracts of L6 cells were prepared as described by Schreiber et al. (1989). Binding reactions (20 μl total) were performed by incubating 10 μg nuclear extract with reaction buffer [20 mM Hepes, pH 7.9, 1 mM EDTA, 60 mM KCl, 12% glycerol, 4 μg poly(dI-dC) · poly(dI-dC)] in the presence or absence of antibody for 10 min at 4℃, followed by a 20~25 min incubation at 4℃ with the probe (~80,000 cpm). Reaction mixture was electrophoresed at 30 mA for 3 h on 5% polyacrylamide gels in high ionic strength buffer (50 mM Tris, 380 mM glycine, 2 mM EDTA, pH 8.5). The gels were then dried and subjected to autoradiography.

Immunoblot analysis

Immunoblot analysis was done as described by Burnette (1981) with minor modifications. The cell extracts (30 μg) were electrophoresed on 10% slab gels containing SDS, and the proteins in the gels were transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were incubated with 5% (w/v) nonfat milk in PBST [137 mM NaCl, 2.7 mM KCl, 16.3 mM $Na_2HPO_4 \cdot 12H_2O$, 1.5 mM KH_2PO_4, 0.1% (v/v) Triton X-100]. They were then reacted with the monoclonal antibody (MF-20) raised against myosin heavy chain or the polyclonal antibody against IκB-α. After washing three times with PBST, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG. Immunochemical detection was performed by ECL method by following the manufacturer's recommendation.

Results

Microtubule dynamics affect myoblast fusion

To examine whether cytoskeletal reorganization affects myoblast fusion, microtubule-stabilizing agent, taxol (0.5 μM), or microtubule-disrupting agent, nocodazole (0.1 μM), were treated to L6 cells at 3 days after the cell plating, and assessed for cell fusion. As shown in Fig. 1, stabilization of microtubule by taxol inhibited myoblast fusion as well as the synthesis of myosin heavy chain. On the contrary, disruption of microtubule by nocodazole induced a precocious myoblast fusion along with increased synthesis of myosin heavy chain. These results indicate that microtubule dynamics affect not only myoblast fusion but also myosin heavy chain synthesis. At the concentration of taxol treated, the cells were found not to affect proliferation capacity and viability, indicating that inhibition of membrane

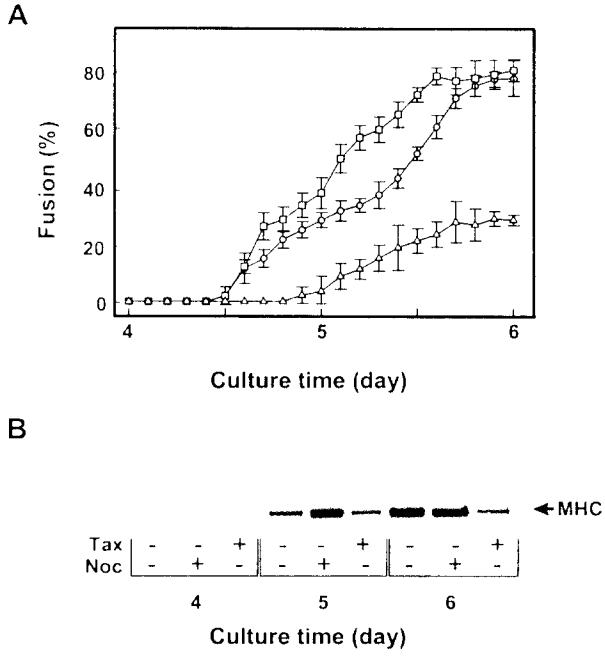

Fig. 1. Nocodazole induces precocious myoblast fusion and early muscle-specific gene expression. (A) L6 cells in differentiation media were incubated without (○) and with 100 nM nocodazole (□) or 500 nM taxol (△) for various periods. Each point represents the mean + S.E. of three separate experiments. (B) Expression levels of myosin heavy chain (MHC) in the cells treated with various combinations of nocodazole and taxol were estimated by immunoblotting analysis with anti-MHC antibody, MF 20.

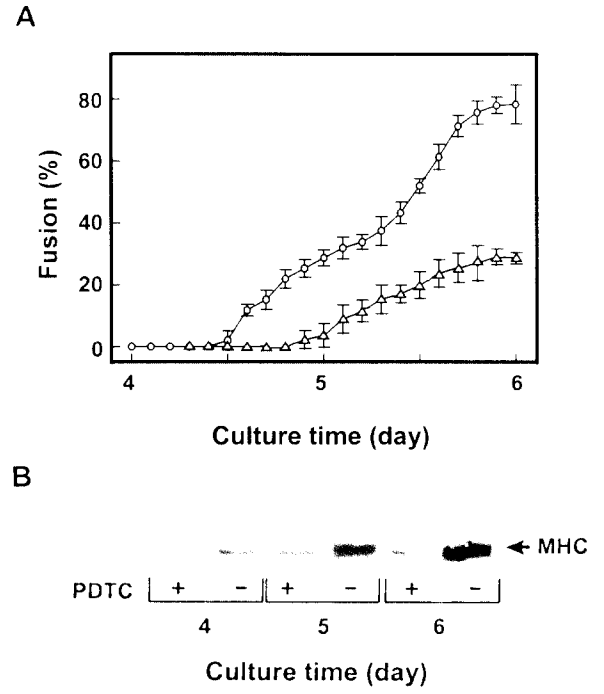

Fig. 2. PDTC inhibits the myoblast fusion and muscle-specific gene expression. (A) L6 cells in differentiation media were treated with (△) or without (○) 1 μM PDTC for various periods. The extent of myoblast fusion was scored as percentage of the number of nuclei in fused cells to total number of nuclei within more than 10 randomly chosen fields under a microscope. Data represent the mean + S.E. of three separate experiments. (B) Expression levels of myosin heavy chain (MHC) with (+) or without (-) PDTC were estimated by immunoblot analysis with anti-MHC antibody, MF 20.

fusion and myosin heavy chain gene expression were not due to nonspecific cytotoxic effect of taxol. There is no direct evidence, however, that the taxol effect we observed was resulted from the delay in microtubule reorganizing kinetics or the defect in microtubule arrays. Thus, all these results suggest that microtubule reorganization is likely to be involved in the myoblast differentiation.

Activation of NF-κB in L6 myoblast differentiation

One important question to be answered is how the reorganization of microtubule leads to myoblast fusion. A plausible way by which the cytoskeleton can affect nuclear gene expression is by modulating the activity of transcriptional factor that resides in the cytoplasm of unstimulated cells as an inactive form. The inactive form of transcriptional factor can be activated and then migrated to the nucleus in response to various stimuli (Hunter and Karin, 1992). One of the well characterized examples is NF-κB and we have established that transcriptional regulation of NOS gene is modulated by NF-κB in chick embryonic myoblasts (Lee et al., 1997).

To test whether the activation of NF-κB is also involved in myogenesis of L6 cells, we measured the extent of myoblast fusion following exposure to pyrrolidine dithiocarbamate (PDTC), an antioxidant that acts as a specific inhibitor of NF-κB activation (Schreck et al., 1992). PDTC (1 μM) dramatically reduced the myoblast fusion as well as myosin heavy chain synthesis (Fig. 2). The reduction of myoblast fusion by PDTC was quite similar to that obtained by taxol treatment.

If NF-κB activation is somehow correlated with the myoblast fusion, *de novo* activation of NF-κB should occur in the course of myoblast differentiation. Therefore, using a palindromic NF-κB binding site as a probe, electrophoretic mobility shift assay was performed for the cells 3 days after switching the growth media to differentiation media. As shown in Fig. 3, the result indicated that NF-κB activity was induced within 24 h, maintained for 48 h, and diminished thereafter.

Depolymerization of microtubule induces NF-κB activation

Since NF-κB was shown to be activated during myoblast differentiation, how NF-κB is timely activated for the myoblast fusion to occur has to be documented. Considering an extensive morphological change and massive cytoskeletal reorganization experienced by myoblasts during myogenesis, we examined a possible link between the microtubule reorganization and NF-κB activation. When L6 cells

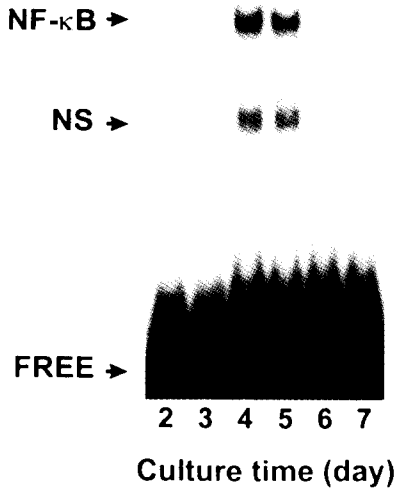

Fig. 3. Induction of NF-κB activation during differentiation of L6 cells. Differentiation of L6 cells was induced by changing growth media (DMEM with 10% FBS) with differentiation media (DMEM with 5% HS) at day 3 after plating. Nuclear extracts were prepared at indicated times after plating and analyzed for NF-κB binding activity by electrophoretic mobility shift assay using palindromic NF-κB-binding site as a probe. Note that the DNA binding activity of NF-κB markedly increased after the media change at day 3. NF-κB, NF-κB · DNA complex; NS, nonspecific complex; FREE, unbound probe.

at day 3 in differentiation media were exposed to 0.1 μM nocodazole, NF-κB activation was significantly

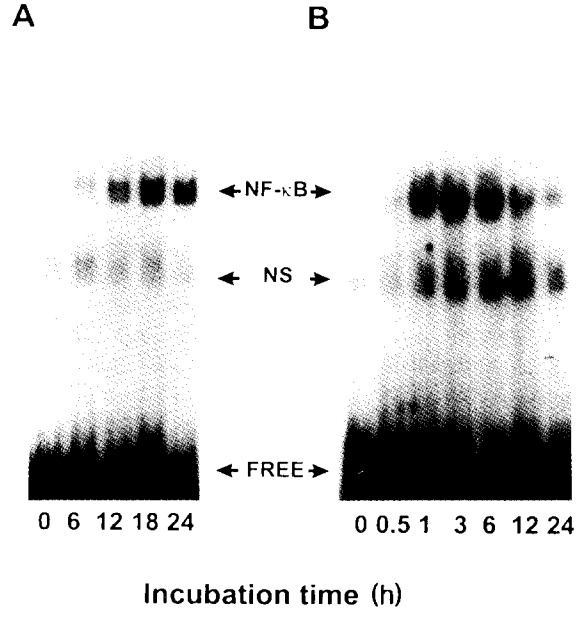

Fig. 4. Nocodazole induces NF-κB activity. L6 cells in differentiation media were treated with (B) or without (A) 100 nM nocodazole at day 3 after plating. Nuclear extracts were prepared at indicated times following the nocodazole treatment and analyzed for NF-κB binding activity by electrophoretic mobility shift assay with palindromic NF-κB-binding site as a probe.

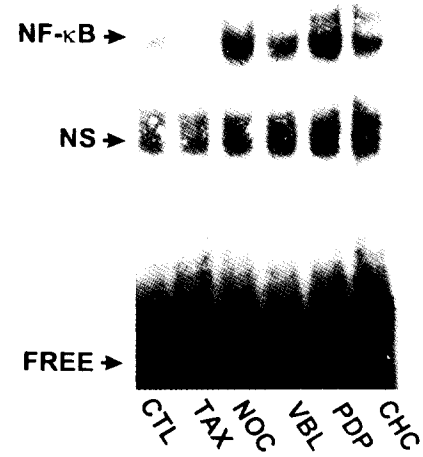

Fig. 5. Disruption of microtubule induces NF-κB activity in L6 cells. L6 cells in differentiation media were treated with either nocodazole (*NOC*; 100 nM), taxol (*TAX*; 500 nM), podophyllotoxin (*PDP*; 500 pM), vinblastin (*VBL*; 500 pM), or colchicine (*CHC*; 10 nM) for 5 h. Nuclear extracts were analyzed for NF-κB binding activities by electrophoretic mobility shift assay using palindromic NF-κB-binding site as a probe. CTL, control.

induced within 1 h and lasted for about 12 h. The early induction of NF-κB activation was significant compared to the untreated cells (Fig. 4). This early induction of NF-κB activation by nocodazole was likely to be attributed to the disruption of microtubule, in view of the fact that several other microtubule-disrupting agents, such as colchicine, podophyllotoxin, and vinblastin, exerted a similar effect as nocodazole. Furthermore, taxol, a microtubule-stabilizing agent, effectively suppressed NF-κB activation as reasoned (Fig. 5).

It has been well documented that induction of NF-κB binding activity is closely correlated with the degradation of IκB-α (Henkel et al., 1993). Therefore, the degradation of IκB-α provides another indicator for the NF-κB activation. Thus, the levels of IκB-α were quantified by immunoblot analysis for the cells that were treated with varying concentrations of nocodazole. Nocodazole reduced the level of IκB-α but concurrently induced the NF-κB activity at concentrations exceeding 0.1 μM (Fig. 6). Thus the levels of IκB-α and NF-κB were reciprocally correlated. It was noteworthy, however, that nocodazole, at 1 μM concentration, neither reduced the level of IκB-α nor induced NF-κB activity. These results suggest that the microtubule disruption affects NF-κB activation process via an intermediate step, such as IκB degradation.

Identification of NF-κB/Rel family in myoblasts

NF-κB/Rel family is known to be composed of four distinct DNA-binding subunits, called c-Rel, p50/p105,

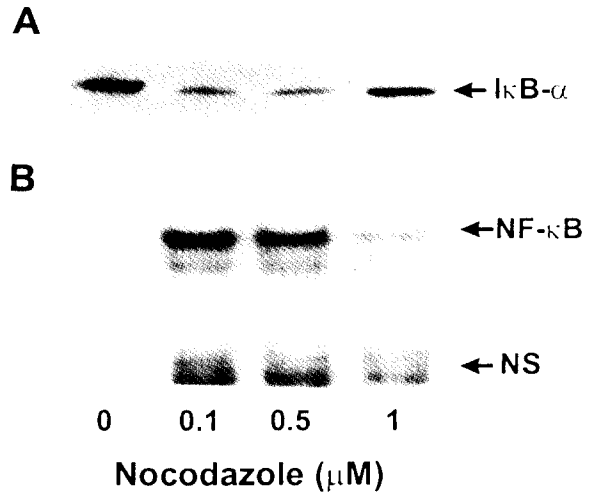

Fig. 6. Nocodazole stimulates degradation of I-κB and induction of NF-κB activation. L6 cells in differentiation media were treated with nocodazole for 5 h and analyzed for cytoplasmic and nuclear extracts. (A) Amounts of IκB-α in cytoplasmic extracts (30 μg) were estimated by immunoblotting assay with anti-IκB-α serum. (B) Nuclear extracts were assayed for NF-κB binding activity.

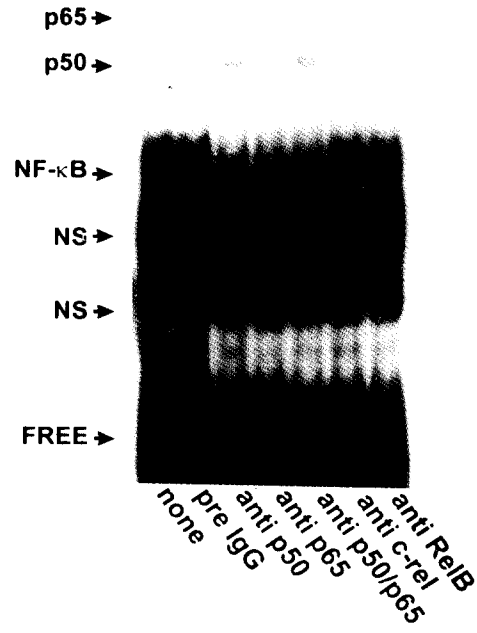

Fig. 7. Characterization of the NF-κB · DNA complex in L6 cells. Nuclear extracts were pretreated with either normal rabbit serum (*pre IgG*) or affinity-purified IgGs against p50, p65 (RelA), c-Rel, RelB or the combination of p50/p65, followed by electrophoretic mobility shift assays. The mobility of the NF-κB · DNA complex was further shifted by the anti-p50 antibody or anti-p65 antibody. NF-κB, NF-κB · DNA complex; p50, anti-p50 antibody · NF-κB · DNA complex; p65, anti-p65 antibody · NF-κB · DNA complex; NS, nonspecific complex; FREE, unbound probe.

p60 (RelA), and RelB. These members have pivotal roles in the generation of tissue differentiation or immune response through their various formation of homo- or heterodimers. However, NF-κB/Rel isoforms in myoblasts have not been identified as of yet. Therefore, we tried to identify the NF-κB/Rel isoform in the L6 cells. Nuclear extracts of the cells were exposed to various antibodies that specifically recognize the members of NF-κB family and then subjected to electrophoretic mobility shift assays. As shown in Fig. 7, anti-p50 and anti-p65 antibodies were further shifted to DNA-protein complex, whereas normal rabbit IgG or antibodies against c-Rel or RelB had no effect on the DNA-protein complex. These results demonstrate that the activated NF-κB is most likely the p50/p65 heterodimer.

Discussion

Previously we reported that the expression of NOS during differentiation of chick embryonic myoblasts is under the control of NF-κB (Lee et al., 1997). The report has proposed that NF-κB may serve as a transcription factor that modulates the expression of a number of genes during myogenesis. In the present study, we show that NF-κB is activated likewise during myogenesis of L6 cells. When proliferating L6 myoblasts were exposed to differentiation media that lacks mitogen, NF-κB activity dramatically increased within 12 h and gradually decreased thereafter (Fig. 1). Possible involvement of NF-κB in differentiation process was further supported by the suppressive effect of PDTC, a specific inhibitor of NF-κB activation, on differentiation of L6 cells.

However, little is known about how NF-κB is timely activated prior to membrane fusion. Activity of NF-κB has been known to be induced through two quite different mechanisms. One is by a large array of extracellular inducers including endotoxin, TNF-α, and IL-1β, whose effects are mediated by membrane receptors (Brown et al., 1993; Sun et al., 1993; Xie et al., 1994). Although we have performed an extensive survey to identify the inducers that activate NF-κB in cultured L6 myoblasts, none of the inducers tested had any effect on the activation of NF-κB. The other is by the changes in cell shape. It has been demonstrated that cell shape changes are able to exhibit specific effects on gene expression (Ben-Ze'ev, 1991). Particularly, a variety of agents and conditions that depolymerize microtubules are reported to activate NF-κB and induce NF-κB-dependent gene expression in HeLa cells (Rosette and Karin, 1995). Skeletal muscle differentiation represents one of the most dramatic morphological changes, in which mononucleated myoblasts are transformed into elongated, multinucleated myotubes. Dramatic alterations in the cytoskeleton are commonly believed to evoke morphogenetic events, and there are some evidence that reorganization of cytoskeleton is involved in the morphogenetic events of myogenesis (Gundersen et al., 1989).

A major observation made in the present study is that a correlation exists between the induction of NF-κB and disruption of microtubule during L6 cell differentiation. Treatment of nocodazole, a reversible inhibitor of tubulin polymerization, to the cells induced the NF-κB activation within 1 h. This rapid induction of NF-κB activity by nocodazole was about 11 h faster than that of the mock-treated control. A crucial role of NF-κB activation by disruption of microtubule was further substantiated by the observation that a precocious induction of NF-κB activation resulted in a precocious cell fusion. Similar results were consistently observed with several other microtubule-disrupting agents, such as colchicine, podophyllotoxin, and vinblastine. These results suggest that the observed induction of NF-κB activation is a direct and specific response to microtubule disruption (Fig. 4).

An important question to be answered is how the disruption of microtubule leads to the induction of NF-κB activation. It has been reported that a considerable disruption of microtubules is detected within 15 min of exposure to nocodazole (Rosette and Karin, 1995). Despite of this finding, our results indicated that the induction of NF-κB activation was not detectable until 1 h after the exposure of the cells to nocodazole (Fig. 3B). This time lag between the exposure of the cells to nocodazole and the induction of NF-κB activation possibly implies that microtubule disruption does not directly induce NF-κB activity, but via some intervening intermediate steps necessary for NF-κB activation. Furthermore, the microtubule disruption induced by nocodazole resulted in the degradation of IκB (Fig. 5A). Referring to the fact that a critical step leading to IκB degradation is its phosphorylation (Lux et al., 1990; Zabel and Baeuerle, 1990), it is likely that so far unidentified kinase(s) which phosphorylate IκB might be activated upon microtubule depolymerization. It is, therefore, conceivable that NF-κB is implicated in the secondary event in gene expression which is activated by the reorganization of microtubule that serves as a primary event for the muscle cell differentiation.

Acknowledgements

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References

Baeuerle PA (1991) The inducible transcription activator NF-kappa B: regulation by distinct protein subunits. *Biochim Biophys Acta* 1072: 63-80

Ben-Ze'ev A (1991) Animal cell shape changes and gene expression. *Bioassay* 13: 207-212.

Bissell MJ, Hall HG, and Parry G (1982) How does the extracellular matrix direct gene expression? *J Theor Biol* 99: 31-68.

Blank V, Kourilsky P, and Israel A (1992) NF-κB and related proteins: Rel/dorsal homologies meet ankyrin-like repeats. *Trends Biochem Sci* 17: 135-140.

Botteri FM, Ballmer-Hofer K, Rajput B, and Nagamine Y (1990) Disruption of cytoskeletal structures results in the induction of the urokinase-type plasminogen activator gene expression. *J Biol Chem* 265: 13327-13334.

Brown K, Park S, Kanno T, Franzoso G, and Siebenlist U (1993) Mutual regulation of the transcriptional activator NF-κ B and its inhibitor, IκB-α. *Proc Natl Acad Sci USA* 90: 2532-2536.

Burnette WN (1981) "Western blotting": Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal Biochem* 112: 195-203.

Doyle T and Botstein D (1996) Movement of yeast cortical actin cytoskeleton visualized *in vivo*. *Proc Natl Acad Sci USA* 93: 3888-3891.

Gilmore TD and Morin PJ (1993) The I kappa B proteins: members of a multifunctional family. *Trends Genet* 9: 1019-1029.

Grumont RJ and Gerondakis S (1994) Alternative splicing of RNA transcripts encoded by murine p105 NF-κB gene generates IκB-γ isoforms with different inhibitory activities. *Proc Natl Acad Sci USA* 91: 4367-4371.

Gundersen GG, Khawaja S, and Bulinski CJ (1989) Generation of a stable, posttranslationally modified microtubule array is an early event in myogenic differentiation. *J Cell Biol* 109: 2275-2288.

Henkel T, Machleidt T, Alkalay I, Kronke M, Ben-Neriah Y, and Baeuerle PA (1993) Rapid proteolysis of I kappa B-alpha is necessary for activation of transcription factor NF -κB. *Nature* 365: 182-185.

Hunter T and Karin M (1992) The regulation of transcription by phosphorylation. *Cell* 70: 6231-6240.

Knudsen KA, and Horwitz AF (1977) Tandem events in myoblast fusion. *Dev Biol* 58: 328-338

Lee JS, Von der Ahe D, Kiefer B, and Nagamine Y (1993) Cytoskeletal reorganization and TPA differently modify AP-1 to induce the urokinase-type plasminogen activator gene in LLC-PK1 cells. *Nucleic Acids Res* 21: 3365-3372.

Lee KH, Baek MY, Moon KY, Song WK, Chung CH, Ha DB, and Kang M-S (1994) Nitric Oxide as a messenger molecule for myoblast fusion. *J Biol Chem* 289: 14371-14374.

Lee KH, Kim DG, Shin NY, Song WK, Kwon H, Chung CH, and Kang M-S (1997) NF-κB-dependent expression of nitric oxide synthase for membrane fusion of chick embryonic myoblasts. *Biochem J* (in press).

Lenardo MJ, and Baltimore D (1989) NF-κB: a pleiotropic mediator of inducible and tissue-specific gene control. *Cell* 58: 227-229.

Liebermann T and Baltimore D (1990) Activation of IL-6 gene expression through the NF-κB transcription factor. *Mol Cell Biol* 10: 2317-2324.

Lin AW, Chang CC, and McCormick CC (1996) Molecular cloning and expression of an avian macrophage nitric-oxide synthase cDNA and analysis of the genomic 5'-flanking region. *J Biol Chem* 271: 11911-11919.

Liou H-C and Baltimore D (1993) Regulation of the NF-κB/rel transcription factor and IκB inhibitor system. *Curr Opin Cell Biol* 5: 477-487.

Lowenthal J, Ballard D, Bogerd H, Bohnlein E, and Greene W (1989) Tumor necrosis factor-alpha activation of the IL-2 receptor alpha gene involves the induction of κB-specific DNA binding proteins. *J Immunol* 142: 31221-31228.

Luduena RF and Roach MC (1991) Tubulin sulfhydryl groups as probes and targets for antimitotic and antimicrotubule agents. *Pharmacol Ther* 49: 133-152.

Lux SE, John KM, and Bennett V (1990) Analysis of cDNA

for human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation and cell-cycle control proteins. *Nature* 344: 36-42.

Manie S, Schmid-Alliana A, Kubar J, Ferrua B, and Rossi B (1993) Disruption of microtubule network in human monocytes induces expression of interleukin-1 but not that of interleukin-6 nor tumor necrosis factor-alpha: Involvement of protein kinase A stimulation. *J Biol Chem* 268: 13675-13681.

Nameroff M and Munar E (1976) Inhibition of cellular differentiation by phospholipase C. *Dev Biol* 49: 288-293

Nolan GP and Baltimore D (1992) The inhibitory ankyrin and activator Rel proteins. *Curr Opin Genet Dev* 2: 211-220.

Osborn L, Kunkel S, and Nabel GJ (1989) Tumor necrosis factor α and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor κB. *Proc Natl Acad Sci USA* 86: 2336-2341.

Rosette C and Karin M (1995) Cytoskeletal control of gene expression: disruption of microtubules activates NF-κB. *J Cell Biol* 128: 1111-1119.

Schreck R, Meier B, Mannel DN, Droge W, and Baeuerle PA (1992) Dithiocarbamates as potent inhibitors of nuclear factor κB activation in intact cells. *J Exp Med* 175: 1181-1194.

Schreiber E, Matthias P, Muller MM, and Shaffner W (1989) Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells. *Nucleic Acids Res* 17: 6419-6420

Sen R and Baltimore D (1986) Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* 46: 705-716.

Shimizu H, Mitomo K, Watanabe T, Okamoto S, and Yamamoto K-I (1990) Involvement of a NF-κB like transcription factor in the activation of the interleukin-6 gene by inflammatory lymphokines. *Mol Cell Biol* 10: 561-568.

Shirakawa F, and Mizel S (1989) *In vitro* activation and nuclear translocation of NF-κB catalyzed by cAMP-dependent protein kinase and protein kinase C. *Mol Cell Biol* 9: 2424-2430.

Sun SC, Gaunch PA, Ballard DW, and Greene WC (1993) NF-kappa B controls expression of inhibitor I kappa B alpha: evdence for an inducible autoregulatory pathway. *Science* 259: 1912-1915.

Xie Q-W, Kashiwabara Y, and Nathan C (1994) Role of transcription factor NF-κB/Rel in induction of nitric oxide synthase. *J Biol Chem* 169: 4705-4709.

Yaffe D (1968) Retention of differentiation potentialities during prolonged cultivation of myogenic cells. *Proc Natl Acad Sci USA* 61: 477-483.

Zabel U and Baeuerle PA (1990) Purified human IκB can rapidly dissociate the complex of the NF-κB transcription factor with its cognate DNA. *Cell* 61: 255-265.

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