Vertebrate Nonmuscle Myosin II Isoforms Rescue Small Interfering RNA-induced Defects in COS-7 Cell Cytokinesis*

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RNA interference (RNAi) treatment of monkey COS-7 cells, a cell line that lacks nonmuscle myosin heavy chain II-A (NMHC II-A) but contains NMHC II-B and II-C, was used to investigate the participation of NMHC isoforms in cytokinesis. We specifically suppressed the expression of NMHC II-B or II-C using 21 nucleotide small interfering RNA (siRNA) duplexes. Down-regulation of NMHC II-B protein expression to 10.2 ± 0.7% inhibited COS-7 cell proliferation by 50% in the RNAi-treated cells compared with control cells. Moreover, whereas 8.7 ± 1.0% of control cells were multinucleated, 62.4 ± 8.8% of the NMHC II-B RNAi-treated cells were multinucleated 72 h after transfection. The RNAi-treated cells had increased surface areas and, unlike control cells, lacked actin stress fibers. Treatment of the COS-7 cells with NMHC II-C siRNA decreased NMHC II-C expression to 5.2 ± 0.1% compared with the endogenous content of II-C; however, down-regulation of NMHC II-C did not cause increased multinucleation. Immunoblot analysis using a pan-myosin antibody showed that the content of NMHC II-C was less than one-twentieth the amount of NMHC II-B, thereby explaining the lack of response to II-C siRNA. Introducing green fluorescent protein (GFP)-tagged NMHC II isoforms into II-B siRNA-treated cells resulted in reduction of multinucleation from 62.4 ± 8.8% to 17.8 ± 2.2% using GFP-NMHC II-B, to 29.8 ± 7.4% using GFP-NMHC II-A, and to 34.1 ± 8.6% using GFP-NMHC II-C-GFP. These studies have shown that expression of endogenous NMHC II-C in COS-7 cells is insufficient for normal cytokinesis and that exogenous NMHC II-A and NMHC II-C can, at least partially, rescue the defect in cytokinesis due to the loss of NMHC II-B.

Nonmuscle myosin II belongs to the class II myosin superfamily and is expressed in both muscle and nonmuscle cells. Similar to skeletal and smooth muscle myosin II isoforms, it is composed of one pair of heavy chains (200 kDa) and two pairs of light chains (20 and 17 kDa) (1). Three isoforms of nonmuscle myosin heavy chain (NMHC)2 II, termed NMHC II-A, NMHC II-B, and NMHC II-C, have been identified in mammalian cells (2–5) and are known to be encoded by three different genes, MYH9, MYH10, and MYH14, respectively, in humans (6, 7).

The three isoforms are well conserved throughout the whole molecule with a 64–80% identity in amino acids among the various isoforms (5), suggesting that they might share some cellular functions.

On the other hand, studies from a number of laboratories show that the different isoforms have distinct tissue distributions and play different cellular roles (5, 8–12). For example, murine cardiac myocytes contain NMHC II-B and NMHC II-C and lack NMHC II-A expression. Ablation of NMHC II-A or II-B in mice resulted in markedly different phenotypes, indicating a different role for these isoforms during embryonic development (13–15). In addition, recent studies show that the isoforms have different kinetics when examined by assays of actin-activated MgATPase activity and in vitro motility (16, 17).

Cytokinesis, a cellular process that follows mitosis, creates two daughter cells from one parent cell and can be divided into several stages according to morphological changes; division site positioning, cleavage furrow formation and ingestion, midbody formation, and cell separation (18, 19). Nonmuscle myosin II plays a major role in cleavage furrow formation and ingestion by assembling with actin to form an actomyosin-based contractile ring. This important function of nonmuscle myosin II in cytokinesis has been studied extensively (20–23). However, it is not yet known whether the three different isoforms of the NMHC can substitute for one another in mammalian cells.

To investigate the participation of NMHC II isoforms in regulating cytokinesis, we specifically down-regulated NMHC II-B or II-C expression by using small interfering RNA (siRNA) in COS-7 cells, a cell line that lacks NMHC II-A but contains NMHC II-B as well as II-C. We found that down-regulation of NMHC II-B, but not NMHC II-C, induced a defect in cytokinesis, but not karyokinesis, which is characterized by decreased cell numbers with increased multinucleation. This defect in COS-7 cells can be rescued by NMHC II-B and to a lesser extent by NMHC II-A and II-C.

EXPERIMENTAL PROCEDURES

DNA Constructs and RNA Oligonucleotides—GFP-tagged NMHC II-A and II-B plasmids were obtained from Dr. Qize Wei (Laboratory of Molecular Cardiology, NHLBI, NIH). NMHC II-C expression vector with GFP tagged at the N or C terminus was constructed by insertion of full-length mouse NMHC II-C into SalI/BamHI sites of enhanced GFP-C3 or enhanced GFP-N3 vector (Clontech, Palo Alto, CA). In the course of these experiments, we were informed by Dr. Thomas Egelhoff (Case Western Reserve University, Cleveland) of a number of mutations in our original GFP-NMHC II-A and II-B constructs, originally reported by Wei and Adelstein (24). We, therefore, with the help of Dr. Egelhoff, corrected both constructs and rehid the rescue experiments with the corrected constructs. We found no difference in our results. The uncorrected constructs have been used by a number of investigators (24–26) and found to localize properly and also to rescue phenotypes caused by depletion of NMHC II-B. NMHC II-B siRNA duplexes (directed at both the human, RefSeq accession number NM_005964, and monkey sequence, AAGGAUCGCUACUAUCAAGGA) and NMHC II-C

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1 The abbreviations used are: NMHC, nonmuscle myosin heavy chain; siRNA, small interfering RNA; GFP, green fluorescent protein; DAPI, 4',6'-diamidino-2 phenylindole.
siRNA (directed at the mouse sequence, RefSeq accession number NM_028021, UCCGUCAGCAGCUGUCUUUAU) were chemically synthesized by Dharmacon, Inc. (Lafayette, CO) and Qiagen (Valencia, CA), respectively. Control siRNA nonsense duplex (GGCGCGCUUUU-GUAGGAUUCG) and fluorescein-labeled control siRNA (AAUUCUTC-GGAACUGUCACGU) were purchased from Dharmacon, Inc. and Qiagen, respectively.

**RNA Isolation and RT-PCR**—Total RNA was extracted from a monkey COS-7 cell line using the RNeasy mini kit (Qiagen). Reverse transcription was carried out on 2 μg of total RNA in a final volume of 50 μl using random hexamer primers and the GeneAmpRNA PCR core kit (Applied Biosystems, Branchburg, NJ). PCRs were performed on 2 μl of reaction mixture in the presence of 1 μM dNTPs, 10 pmol primers, and 2.5 units of Pfu Turbo DNA polymerase (Stratagene, La Jolla, CA). The primers for NMHC II-B were designed according to the human MYH10 sequence (RefSeq accession number NM_005964), and Blast searches showed that they should not anneal with MYH9 (RefSeq accession number NM_002473) or MYH14 (RefSeq accession number NM_024729). The primers used for NMHC II-C were forward primer, 5'-ATGTCTGCAGATGTCGAGGACC, and reverse primer, 5'-ATGTCGCCAATCGGAGGAG. The gel-purified PCR products were subcloned using a PCR-Script Amp cloning kit (Stratagene), and different clones were isolated for sequence analysis.

**Cell Culture and Transfection**—COS-7 cells were obtained from ATCC (Manassas, VA) and were cultured in high glucose Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. Transient transfections with siRNA and DNA constructs were carried out using Nucleofector kits according to the manufacturer’s instruction (amaxa GmbH, Koeln, Germany) when cells were 60–70% confluent. By using fluorescein-conjugated siRNA, we observed that more than 95% of the siRNA cells were multinucleated compared with 8.7 ± 1.0% of control cells. At 96 h, 69.0 ± 3.7% were multinucleated compared with 12.1 ± 2.2% of control cells. Data are presented as the mean ± S.D. from four independent experiments. In each experiment, at least 100 cells were counted. Scale bar, 20 μM.
buffer (50 mM Tris-HCl, pH 8.0, 0.3 mM NaCl, 1% Nonidet P-40, 5 mM EDTA, 1 mM diethiothreitol, 0.5 mM phenylmethylsulfonyl fluoride) and 1% by volume protease inhibitor mix containing 4-(2-aminophenyl) benzenesulfonyl fluoride, pepstatin A, E-64, bestatin, leupeptin, and aprotinin (Sigma) at 4 °C. The lysates were sedimented at 10,000 × g for 10 min, and the supernates were analyzed by immunoblotting as previously described (27). The antibodies were anti-rabbit polyclonal antibodies against the C terminus of human NMHC II-A, II-B, and both human and mouse II-C, which were described previously (5), and the monoclonal pan-anti-myosin II antibody (M2.42), which recognizes an epitope in the globular head of Acanthamoeba myosin II and was a generous gift from Don Kaiser and Thomas D. Pollard (Yale University School of Medicine, New Haven, CT). We confirmed its equal reactivity to all three NMHC II isoforms by immunoblotting using baculovirus-expressed purified heavy meromyosin fragments (data not shown). Monoclonal antibodies to β-actin were from Sigma and α-tubulin from Santa Cruz. Peroxidase-conjugated goat anti-rabbit or anti-mouse IgGs (Pierce, Rockford, IL) were used as secondary antibodies. The proteins were visualized by SuperSignal West Pico chemiluminescent substrate (Pierce). Immunoblots were quantified using a Personal Densitometer SI (Amersham Biosciences).

Cell Growth Analysis—24 h after transfection, II-B siRNA-treated or control COS-7 cells were replated at a density of 5 × 10^4 cells/well in 12-well culture plates. The cells were trypsinized and harvested every 24 h, and the number of cells was counted using a hemocytometer in the presence of 0.4% trypan blue.

Immunofluorescence Microscopy—Cells grown on glass coverslips were washed several times in phosphate-buffered saline and fixed with 4% paraformaldehyde followed by permeabilization with 0.1% Triton X-100. For antibody staining, the samples were blocked with phosphate-buffered saline containing 0.1% bovine serum albumin and 4% normal goat serum for 30 min at 23 °C, incubated with affinity-purified rabbit polyclonal antibodies to NMHC II-A, II-B, or II-C at 4 °C overnight, followed by incubation with the appropriate Texas Red- or fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Jackson Immunoresearch, West Grove, PA) for 1 h at room temperature. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA). Phalloidin conjugated to rhodamine (Molecular Probes, Eugene, OR) was used to visualize F-actin. The images were collected using a Leica SP confocal microscope.

RESULTS

NMHC II-B siRNA Reduces II-B Expression—To down-regulate NMHC II-B expression in COS-7 cells, we treated the cells with 21-nucleotide siRNA that were specific for mRNA encoding monkey NMHC II-B. Control cells were transfected with a nonsense duplex (see “Experimental Procedures”). Using fluorescein-labeled control siRNA, we estimated that more than 95% of the cells were transfected. Scanning of immunoblots showed that NMHC II-B protein was decreased at 48 h and that the lowest content was found at 96 h following transfection (Fig. 1, A and B). After that, the protein level increased and was restored to control levels by 144 h. The data from immunoblot analysis are quantitated in Fig. 1B. Fig. 1C shows that there was no compensatory increase in NMHC II-A expression and no change in the protein expression level of NMHC II-C at 72 and 96 h.

NMHC II-B siRNA Inhibits Cytokinesis—To determine whether there was a change in cytokinesis in NMHC II-B down-regulated cells, we first investigated their replication (Fig. 2A). Following replating of equal numbers of nonsense or II-B siRNA-treated cells 24 h after transfection, the cells were counted every 24 h until 144 h. Between 96–144 h, there was a 2-fold or greater decrease in the number of II-B siRNA-treated cells compared with nonsense siRNA-treated cells (Fig. 2A).

When NMHC II-B siRNA-treated cells were replated at a later time, such as 144 h after transfection (when NMHC II-B expression had returned to control levels), there was no obvious difference in cell number between II-B siRNA-treated and nonsense siRNA-treated cells (data not shown), indicating that the decrease in cell proliferation is due to the decrease in II-B expression (Fig. 1B). Results from confocal microscopy (Fig. 2B) revealed a significant increase in multinucleated cells in the NMHC II-B siRNA-treated cells as quantitated at 72 and 96 h (Fig. 2C). These data suggest that NMHC II-B siRNA treatment induces a defect in cytokinesis, but not karyokinesis, similar to the finding by De Lozanne and Spudich (28) for Dictyostelium discoideum in myosin II-ablated cells.

Loss of Actin Stress Fibers in NMHC II-B siRNA-treated Cells—In contrast to nonsense siRNA-transfected cells, the NMHC II-B siRNA-treated cells lost their actin stress fibers except possibly in the cortical areas. Confocal microscopy showed that 72 h after transfection, NMHC II-B siRNA-treated cells had increased surface areas (Fig. 3, g and h) compared with nonsense siRNA-treated cells (c and d) and, instead of forming stress fibers, small filaments of actin were diffusely distributed throughout the cell (compare panels g and c). There was no difference in α-tubulin localization between the II-B siRNA-treated and nonsense siRNA-treated cells (data not shown).

NMHC II-C siRNA Reduces II-C Expression but Does Not Induce Multinucleation—We then studied the effect of using siRNA to reduce NMHC II-C in COS-7 cells, because this was the only other NMHC II isoform present in these cells. Fig. 4A shows that, unlike the results found using NMHC II-B siRNA, reducing NMHC II-C protein to 5.2 ± 0.1% of that of nonsense siRNA-treated cells did not result in increased multinucleation (Fig. 4B). In addition, unlike II-B siRNA-treated cells, which lost actin stress fibers (see above), there was no loss of stress fibers in II-C siRNA-treated cells (data not shown). We inter-
Rescue of Multinucleation by Myosin II Isoforms

NMHC II-C siRNA reduces II-C protein but does not induce multinucleation. A, COS-7 cells were transiently transfected with NMHC II-C siRNA or nonsense (Control) siRNA and were subjected to immunoblotting using anti-human II-C and anti-actin antibodies 72 h after transfection. Two different amounts of total cell lysates were loaded for each sample for SDS-PAGE and immunoblot analysis. B, quantitation of multinucleation in II-C siRNA-treated and nonsense (Control) siRNA-treated cells 72 h after transfection. Data are presented as the mean ± S.D. from four independent experiments. In each experiment, at least 100 cells were counted. C, immunoblot analysis shows that the amount of endogenous NMHC II-C is substantially less than NMHC II-B in COS-7 cells. A monoclonal pan-myosin antibody was used to detect all NMHC II isoforms in nonsense siRNA-treated (Control) and II-B siRNA-treated and II-C siRNA-treated COS-7 cells. The same blot was also probed with anti-NMHC II-B or II-C antibodies to confirm the decrease in each isoform in these cells. The blot using tubulin antibody was used to standardize sample loading and came from a second immunoblot loaded with the same amount of each sample.

Fig. 4. NMHC II-C siRNA reduces II-C protein but does not induce multinucleation. A, COS-7 cells were transiently transfected with NMHC II-C siRNA or nonsense (Control) siRNA and were subjected to immunoblotting using anti-human II-C and anti-actin antibodies 72 h after transfection. Two different amounts of total cell lysates were loaded for each sample for SDS-PAGE and immunoblot analysis. B, quantitation of multinucleation in II-C siRNA-treated and nonsense (Control) siRNA-treated cells 72 h after transfection. Data are presented as the mean ± S.D. from four independent experiments. In each experiment, at least 100 cells were counted. C, immunoblot analysis shows that the amount of endogenous NMHC II-C is substantially less than NMHC II-B in COS-7 cells. A monoclonal pan-myosin antibody was used to detect all NMHC II isoforms in nonsense siRNA-treated (Control) and II-B siRNA-treated and II-C siRNA-treated COS-7 cells. The same blot was also probed with anti-NMHC II-B or II-C antibodies to confirm the decrease in each isoform in these cells. The blot using tubulin antibody was used to standardize sample loading and came from a second immunoblot loaded with the same amount of each sample.

siRNA to II-B lowered the amount of total myosin II substantially more than did the siRNA directed at NMHC II-C. Probing the immunoblot with antibodies to NMHC-II-C and II-B confirmed that the siRNAs used were effective in lowering the protein levels of each isoform. By measuring the intensity of each band, we estimated that the amount of NMHC II-C in COS-7 cells is less than 5% of the total NMHC II. This could explain the inability of NMHC II-C siRNA to cause multinucleation in the COS-7 cells. To further examine this possibility as well as to address the ability of each isoform to effect multinucleation, we carried out rescue experiments.

Rescue of Multinucleation in NMHC II-B-deficient Cells by Various NMHC II Isoforms—We evaluated the ability of each NMHC II isoform to rescue the defect in cytokinesis caused by NMHC II-B down-regulation. We did this by introducing each of the full-length NMHC II isoforms tagged with GFP into the cells that had been previously treated with NMHC II-B siRNA for 24 h. Because this was our initial use of GFP-labeled NMHC II-C in transfection experiments, we used constructs having GFP at both the N terminus (GFP-NMHC II-C) and C terminus (NMHC II-C-GFP). Following transfection of the NMHC II-B siRNA-treated cells with the GFP-labeled NMHC II isoforms, the COS-7 cells were cultured for an additional 48 h and then the extent of multinucleation was quantitated. These experiments were carried out under conditions that normalized the transfection efficiency of all three GFP-NMHC isoforms to 30% of the cells transfected, using the GFP signal viewed by confocal microscopy as an indicator. Fig. 5A depicts images from confocal microscopy showing COS-7 cells following transfection.

These data confirm the absence of NMHC II-B at 72 h in the siRNA-treated cells that were subsequently transected with GFP alone (Fig. 5A, a–d), whereas the GFP-II-B panels (e–h) show the expression of the newly introduced NMHC II-B as detected by both GFP and an antibody to NMHC II-B. GFP II-A panels (i–l), GFP II-C (N-terminal; m–p), and II-C GFP (C-terminal; q–t) show the results of transfecting these constructs into the siRNA NMHC II-B-treated cells as detected by the GFP signal. Of note is the absence of NMHC II-B in each of these siRNA-treated cells at this time with the exception of those cells that were transfected with GFP-NMHC II-B (panel g). Unlike NMHC II-C GFP (C-terminal), which showed a normal filamentous structure in COS-7 cells (panel r), GFP II-C (N-terminal) exhibited a punctate structure (panel n), leading us to conclude that GFP at the N terminus of NMHC II-C forms aggregates of myosin.

Fig. 5B shows quantitation of the extent of multinucleation in the siRNA-treated cells that were subsequently transfected with GFP alone or GFP-NMHCs. Control cells transfected with nonsense siRNA displayed low numbers of multinucleated cells (8.7%), similar to untreated cells. On the other hand, NMHC II-B siRNA-treated cells transfected with GFP alone displayed an increased number of multinucleated cells (66% of the COS-7 cells found to contain the GFP signal were multinucleated). NMHC II-B siRNA-transfected cells subsequently transfected with GFP-NMHC II-A showed a decrease in multinucleation to 30% compared with 66% of the cells transfected with GFP alone and a decrease to 18% for the GFP-NMHC II-B-transfected cells at 72 h. Interestingly, NMHC II-C-GFP (C-terminal) decreased II-B siRNA-induced multinucleation to 34%. In contrast, GFP-NMHC II-C (N-terminal) was ineffective in rescuing the defect in cytokinesis, as 62% of these cells remained multinucleated following transfection. The inability of GFP-NMHC II-C (N-terminal) to rescue multinucleation was consistent with its failure to localize to filaments in COS-7 cells (Fig. 5A, panel n).
One explanation for the inability of endogenous NMHC II-C to prevent multinucleation (see above) and for the ability of NMHC II-C GFP to partially rescue the siRNA II-B-treated cells is that the amount of endogenous NMHC II-C is considerably smaller than the amount of NMHC II-C-GFP introduced to rescue the COS-7 cells. Fig. 5 shows that this is the case using immunoblot analysis. The figure shows that the expression level of NMHC II-C in the cells transfected with NMHC II-C-GFP is at least 10-fold greater (lane 3) compared with endogenous NMHC II-C (controls, lane 1), taking into account the 30% transfection efficiency. Note that introducing NMHC II-B siRNA/H11001 GFP did not alter II-C expression (lane 2).

We examined the formation of actin stress fibers in the cells transfected with each GFP-tagged NMHC II isoform. As shown in Fig. 6, both GFP-NMHC II-A, II-B, and II-C with actin in NMHC II-B siRNA-treated cells. 24 h following transfection with NMHC II-B siRNA, COS-7 cells were transfected with GFP-NMHC II-A, GFP-NMHC II-B, GFP-NMHC II-C (N-terminal), or NMHC II-C-GFP (C-terminal). 48 h later, these cells were stained and were visualized by GFP (a, d, g, and j) and rhodamine-phalloidin (b, e, h, and k). Panels c, f, i, and l are merged images of panels a and b, d and i, g and h, and j and k, respectively. Nuclei are stained with DAPI (blue). Note that NMHC II-C appears aggregated in panel g but is filamentous in panel j. Scale bar, 20 μm.

One explanation for the inability of endogenous NMHC II-C to prevent multinucleation (see above) and for the ability of NMHC II-C GFP to partially rescue the siRNA II-B-treated cells is that the amount of endogenous NMHC II-C is considerably smaller than the amount of NMHC II-C-GFP introduced to rescue the COS-7 cells. Fig. 5C shows that this is the case using immunoblot analysis. The figure shows that the expression level of NMHC II-C in the cells transfected with NMHC II-C-GFP is at least 10-fold greater (lane 3) compared with endogenous NMHC II-C (controls, lane 1), taking into account the 30% transfection efficiency. Note that introducing NMHC II-B siRNA + GFP did not alter II-C expression (lane 2).

We examined the formation of actin stress fibers in the cells transfected with each GFP-tagged NMHC II isoform. As shown in Fig. 6, both GFP-NMHC II-A and II-B, as well as NMHC II-C-GFP (C-terminal), introduced into the COS-7 cells led to the formation of actin stress fibers (panels b, e, and h). However, GFP-NMHC II-C (N-terminal) did not cause formation of stress fibers (panel h). Taken together, the data indicate that NMHC II-A, II-B, and II-C can at least partially rescue the defect in cytokinesis induced by II-B siRNA. These data also show that the amount of endogenous NMHC II-C following NMHC II-B siRNA treatment is not sufficient to support normal cytokinesis in COS-7 cells.

**DISCUSSION**

In this study, we have shown that NMHC II-A and II-C can partially rescue a defect in cytokinesis induced by down-regulation of NMHC II-B. We also provided evidence that the total
amount of myosin II appears to play a role in cytokinesis: we observed no increase in multinucleation following the down-regulation of NMHC II-C, but when we introduced substantially more of the NMHC II-C isoform into cells that were depleted for NMHC II-B we were able to decrease multinucleation from 66 to 34%. Under these conditions, NMHC II-C localizes to the contractile ring (data not shown). Thus, decreasing endogenous NMHC II-C using siRNA would not affect cytokinesis because the amount of NMHC II-C is only a small fraction of total NMHC II. We confirmed this by using siRNA directed against NMHC II-B and II-C to lower both isoforms at fraction of total NMHC II. We confirmed this by using siRNA correcting endogenous NMHC II-C using siRNA would not affect localizes to the contractile ring (data not shown). Thus, de-
				
tially more of the NMHC II-C isoform into cells that were
amount of myosin II appears to play a role in cytokinesis: we
Our results demonstrate for the first time that different vertebrate NMHC II isoforms can substitute for one another with respect to cytokinesis in a cultured cell system. We are presently investigating whether the same is true during mouse embryonic development by introducing NMHC II-A into NMHC II-B-ablated mice.

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References

1. Sellers, J. R. (1999) Myosins, Oxford University Press, Oxford
2. Katsuragawa, Y., Yanagisawa, M., Inoue, A., and Masaki, T. (1989) Eur. J. Biochem. 184, 611–616
3. Shohet, R. V., Conti, M. A., Kawamoto, S., Preston, Y. A., Brill, D., and Adelstein, R. S. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 7726–7730
4. Takahashi, M., Kawamoto, S., and Adelstein, R. S. (1992) J. Biol. Chem. 267, 17864–17871
5. Golomb, E., Ma, X., Jana, S. S., Preston, Y. A., Kawamoto, S., Shoham, N. G., Goldin, E., Conti, M. A., Sellers, J. R., and Adelstein, R. S. (2004) J. Biol. Chem. 279, 2800–2808
6. Simon, M., Wang, M., McBride, O. W., Kawamoto, S., Yamakawa, K., Gudla, D., Adelstein, R. S., and Weir, L. (1991) Circ. Res. 69, 530–537
7. Leal, A., Endele, S., Stengel, C., Huehne, K., Loetterle, J., Barrantes, R., Winterpacht, A., and Rutenstrauss, B. (2003) Gene (Amst.) 312, 165–171
8. Maupin, P., Phillips, C. L., Adelstein, R. S., and Pollard, T. D. (1994) J. Cell Sci. 107, 3077–3090
9. Kelley, C. A., Sellers, J. R., Gard, D. L., Bui, D., Adelstein, R. S., and Baines, I. C. (1996) J. Cell Biol. 134, 657–687
10. Kolega, J. (1998) J. Cell Sci. 111, 2085–2095
11. Murakami, N., Trenker, E., and Elzinga, M. (1993) Dev. Biol. 157, 19–27
12. Kolega, J. (2003) Mol. Biol. Cell 14, 4745–4757
13. Tullio, A. N., Accili, D., Ferraraz, V. J., Yu, Z., Takeda, K., Grinberg, A., Westphal, H., Preston, Y. A., and Adelstein, R. S. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 12407–12412
14. Tullio, A. N., Bridgman, P. C., Tresser, N. J., Chan, C., Conti, M. A., Adelstein, R. S., and Han, Y. (2001) J. Comp. Neurol. 435, 62–74
15. Conti, M. A., Even-Ram, S., Liu, C., Yamada, K. M., and Adelstein, R. S. (2004) J. Biol. Chem. 279, 41263–41266
16. Kovacs, M., Wang, F., Hu, A., Zhang, Y., and Sellers, J. R. (2005) J. Biol. Chem. 280, 38132–38140
17. Wang, F., Kovacs, M., Hu, A., Limouze, J., Harvey, E. V., Chen, I., Westwood, N. J., Sellers, R. S., and Mitchison, T. J. (2003) J. Cell Biol. 161, 93–201
18. Wei, Q., and Adelstein, R. S. (2000) Mol. Biol. Cell 11, 3617–3627
19. Lo, C. M., Buxton, D. B., Chu, C. C., Dembo, M., Adelstein, R. S., and Wang, Y. L. (2004) Mol. Biol. Cell 15, 982–989
20. Meshel, A. S., Wei, Q., Adelstein, R. S., and Sheetz, M. P. (2005) Nat Cell Biol. 7, 157–164
21. Buxton, D. B., Golomb, E., and Adelstein, R. S. (2003) J. Biol. Chem. 278, 15449–15455
22. De Lozanne and Spudich (28) showed that ablation of non-muscle myosin II results in failure of cytokinesis, but not kar-
yokinesis in Dictyostelium discoideum, provided they are
grown in suspension culture. Grown on a surface, the cells can
undergo division or cytofission by pulling themselves apart.
23. COS-7 cells resemble mammalian cardiac myocytes in that
they contain NMHC II-B and II-C, but not NMHC II-A. We have
previously shown in vivo experiments that ablation of NMHC II-B results in a partial defect in cytokinesis in mouse cardiac myocytes, but in the nonmyocyte cells in the heart, which do contain NMHC II-A (29). The loss of NMHC II-B results in a 70% decrease in the number of cardiac myocytes at embryonic day 12.5 (E12.5) and an increase in the number of binucleated myocytes from 1 to 23%. The results reported here support the ability of myosin II-C alone to prevent a complete failure in cytokinesis in the NMHC II-B-ablated cardiac myocytes.
24. Of note was the difference between whether the GFP was
attached to the C- or N-terminal end of the NMHC. A number of
laboratories, including ours, have noted that N-terminal GFP-tagged-NMHC II-A or II-B localizes with the endogenous NMHC when transfected into cells (24–26). However, we noted that this was only the case for NMHC II-C when GFP was attached to the C-terminal residue and not when it was fused to the N-terminal end. Moreover, although the former construct was able to reduce multinucleation in COS-7 cells treated with NMHC II-B siRNA from 66 to 34%, the GFP-N-terminal construct was not able to reduce multinucleation to any significant extent.
25. De Lozanne and Spudich (28) showed that ablation of non-
muscle myosin II results in failure of cytokinesis, but not kar-
yokinesis in Dictyostelium discoideum, provided they are
grown in suspension culture. Grown on a surface, the cells can
undergo division or cytofission by pulling themselves apart.
26. COS-7, a monkey kidney cell line, apparently does not use this
alternative form of cytokinesis because 68% of the cells became
multinucleated following NMHC II-B siRNA treatment even
though they were grown on a surface.
27. Why do NMHC II-A and II-C not rescue multinucleation to
the same extent that NMHC II-B does? This may simply reflect
the differences in the biochemical properties among the iso-
forms. Recent work has shown that all three isoforms differ with respect both in their ability to propel actin filaments in an
in vitro motility assay and in their actin-activated MgATPase
activities (16, 17, 34).
28. De Lozanne, A., and Spudich, J. A. (1987) Science 236, 1086–1091
29. Takeda, K., Kishi, H., Ma, X., Yu, Z.-X., and Adelstein, R. S. (2003) Circ. Res. 93, 330–337
30. Schroeder, T. E. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 1688–1692
31. Fujikura, Y., Porter, M. E., and Pollard, T. D. (1978) J. Cell Biol. 79, 268–275
32. Fukui, Y., and Inoue, S. (1991) J. Cell Biol. 18, 41–54
33. Lippincott, J., and Li, R. (1998) J. Cell Biol. 140, 355–366
34. Kim, K.-Y., Kovacs, M., Kawamoto, S., Sellers, J. R., and Adelstein, R. S. (April
20, 2005) J. Biol. Chem. 10.1074/jbc.M503488200

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Activation of mitogen-activated protein kinase kinase (M KK) 3 and MKK6 by type I interferons.

Yongzhong Li, Sandeep Batra, Antonella Sassano, Beata Majchrzak, David E. Levy, Matthias Gaestel, Eleanor N. Fish, Roger J. Davis, and Leonidas C. Platanias

Due to an inadvertent error, the wrong immunoblots were included in Fig. 8, E and F. Fig. 8 should appear as shown below. The figure legend and text remain unchanged.

![FIGURE 8](image-url)

We suggest that subscribers photocopy these corrections and insert the photocopies in the original publication at the location of the original article. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.
Vertebrate nonmuscle myosin II isoforms rescue small interfering RNA-induced defects in COS-7 cell cytokinesis.
Jianjun Bao, Siddhartha S. Jana, and Robert S. Adelstein

Under “Experimental Procedures,” subheading “DNA Constructs and RNA Oligonucleotides,” the last sentence that begins on page 19594 is incorrect. It should read as follows: “NMHC II-B siRNA duplexes (directed at both the human, RefSeq accession number NM_005964, and monkey sequence, AAGGAUCGCUACUAUUCAGGA) and NMHC II-C siRNA (directed at the human C1-inserted sequence, UCCGUCACCGUGUCUUAAU (S. S. Jana, unpublished data)) were chemically synthesized by Dharmacon, Inc. (Lafayette, CO) and Qiagen (Valencia, CA), respectively.”