Mechanical loading by fluid shear is sufficient to alter the cytoskeletal composition of osteoblastic cells

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Jackson WM, Jaasma MJ, Tang RY, Keaveny TM. Mechanical loading by fluid shear is sufficient to alter the cytoskeletal composition of osteoblastic cells. Am J Physiol Cell Physiol 295: C1007–C1015, 2008.—Many structural modifications have been observed as a part of the cellular response to mechanical loading in a variety of cell types. Although changes in morphology and cytoskeletal rearrangement have been widely reported, few studies have investigated the change in cytoskeletal composition. Measuring how the amounts of specific structural proteins in the cytoskeleton change in response to mechanical loading will help to elucidate cellular mechanisms of functional adaptation to the applied forces. Therefore, the overall hypothesis of this study was that osteoblasts would respond to fluid shear stress by altering the amount of specific cross-linking proteins in the composition of the cytoskeleton. Mouse osteoblasts cell line MC3T3-E1 and human fetal osteoblasts (hFOB) were exposed to 2 Pa of steady fluid shear for 2 h in a parallel plate flow chamber, and then the amount of vimentin, α-actinin, filamin, and talin in the cytoskeleton was measured using Western blot analyses. After mechanical loading, there was no change in the amount of actin monomers in the cytoskeleton, but the cross-linking proteins α-actinin and filamin that cofractionated with the cytoskeleton increased by 29% (P < 0.01) and 18% (P < 0.02), respectively. Localization of the cross-linking proteins by fluorescence microscopy revealed that they were more widely distributed throughout the cell after exposure to fluid shear. The amount of vimentin in the cytoskeleton also increased by 15% (P < 0.01). These results indicate that osteoblasts responded to mechanical loading by altering the cytoskeletal composition, which included an increase in specific proteins that would likely enhance the mechanical resistance of the cytoskeleton.

MC3T3-E1; human fetal osteoblasts; α-actinin; filamin; cytoskeleton

Cells respond to changes in their mechanical environment by undergoing a variety of structural modifications, including shape change (40), cytoskeletal rearrangement (21, 49), and by altering their attachments to both the substrate (49, 55) and adjacent cells (9). The whole cell mechanical behavior (26) and local cytoskeletal stiffness (21, 39) may also be altered after exposure to mechanical loads. These cellular responses protect the cell from the imposed mechanical forces by reinforcing the plasma membrane (33) and cell adhesions (11, 17, 20). Furthermore, there is evidence that as the interconnected network of the cytoskeleton rearranges, it modulates the amount of force required to initiate mechanical signals (21, 25, 29) and the mechanotransductive pathways that transmit those signals to the nucleus (8, 15, 34). Although the effect of mechanical loading on cellular structure has been widely reported, considerably less is known about how the same loads affect the proteins that organize the network of cytoskeletal filaments. The microstructure of the cytoskeleton is defined by a wide variety of cross-linking proteins that are part of the cytoskeletal composition and make a substantial contribution to the whole cell mechanical behavior (24, 42). Therefore, an improved understanding of how the cytoskeletal composition responds to mechanical loading could yield valuable insight into the mechanisms used by cells to adapt to their mechanical environment.

Cells protect themselves from mechanical loads by rearranging and reinforcing the cytoskeletal network. Of the three types of filaments in the cytoskeleton, the actin microfilaments are the most sensitive to mechanical stimulus (30). Microfilaments have also been shown to make a substantial contribution to whole cell mechanical behavior (6, 53, 61), although there is evidence that intermediate filaments may also contribute to cytoskeletal toughness (32) and interact synergistically with actin (13). Cross-linking proteins, in particular α-actinin and filamin, organize the cytoskeleton and alter the mechanical properties of microfilament networks (57, 58, 60, 64). α-Actinin can organize the actin microfilaments in various orientations but usually forms parallel bundles (4). Filamin cross-links the filaments perpendicularly to form orthogonal networks (56). These cross-linking proteins are present throughout the cell (38) and play an important role in determining the structure of the actin cytoskeleton (32, 63).

Few studies have considered how the cytoskeletal composition changes in response to mechanical loads. Network polymer models of the cytoskeleton predict that recruitment of cross-linking proteins to the filament network could be an important mechanism of controlling cell stiffness (29, 47), and this has been supported by several studies. α-Actinin is involved in the cellular mechanoprotective response (52), and increasing the amount of α-actinin in the cytoskeleton is sufficient to increase the whole cell resistance to deformation (27). Filamin has also been implicated in the rearrangement and stiffening of cytoskeletal structures in regions that are exposed to localized mechanical stimulus (20). These studies suggest that cells alter the cytoskeletal composition to increase their whole cell mechanical resistance in response to loading, but it remains unknown how these cytoskeletal proteins contribute to changes in the cytoskeletal composition at the whole cell level following exposure to externally applied loads. Therefore, the overall hypothesis of this study was that osteo-

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blasts would respond to fluid shear stress by altering the amount of specific cross-linking proteins in the composition of the cytoskeleton. The specific objectives were to determine the effect of fluid shear stress on 1) the amount of polymerized actin microfilaments and vimentin intermediate filaments; 2) the amount of actin cross-linking proteins α-actinin and filamin associated with cytoskeleton; and 3) to observe how the localization of these cross-linking proteins in the cytoskeleton changes in response to fluid shear relative to the actin microfilaments.

MATERIALS AND METHODS

Antibodies and cytoskeletal probes. Mouse anti-β-actin monoclonal IgG (clone AC-15), mouse anti-α-actinin monoclonal IgM (clone BM-75.2), mouse anti-vimentin monoclonal IgM (clone VIM 13.2), mouse anti-talin monoclonal IgG (clone 8D4), and mouse anti-GAPDH monoclonal IgM (clone 17.1) were obtained from Sigma (St. Louis, MO). Mouse anti-filamin A monoclonal IgG (clone FLMN01 [PM6/317]) was obtained from ABCam (Cambridge, UK). Rabbit anti-ERK polyclonal IgG (clone C-14) was obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). All antibodies were reactive against human antigens, and all antibodies except anti-filamin were cross-reactive with mouse antigens. Donkey IgG raised against mouse IgG and IgM were obtained from Jackson ImmunoResearch (West Grove, PA) and conjugated to either horseradish peroxidase or FITC. Donkey IgG raised against rabbit IgG and conjugated with horseradish peroxidase was obtained from Santa Cruz Biotechnologies. Rhodamine Phalloidin, Alexa Fluor 488 conjugated DNase and Hoechst were obtained from Molecular Probes (Eugene, OR).

Cell culture and mechanical loading with fluid shear stress. Human fetal osteoblasts 1.19 (hFOBs) and murine osteoblasts (MC3T3-E1s) were obtained from ATCC (Manassas, VA). The hFOBs were cultured with 1:1 mixture of dMEM without phenol red and Ham’s F12 medium (GIBCO, Herndon, VA) supplemented with 10% fetal bovine serum (FBS, GIBCO) and 0.3 mg/ml G814 (Sigma) in an humidified incubator at 37°C and 5% CO2 (hFOBs have a temperature-sensitive promoter of osteogenic lineage commitment that is activated at 37°C). The MC3T3-E1s were cultured with α-MEM (Cellgro, Gathersburg, MD) supplemented with 10% FBS and 1% penicillin-streptomycin cocktail (GIBCO) in a humidified incubator at 37°C and 5% CO2. The cells were grown to confluency on either glass coverslips or polycarbonate plates. After exposure to flow, the cells were grown to confluency on either glass coverslips or polycarbonate plates. After exposure to flow, the cells were washed briefly with PBS and fractionated by detergent extraction. The MC3T3-E1s were cultured with CO2 independent media (GIBCO) supplemented with 10% FBS and 4 mM l-glutamine (GIBCO). A digitally controlled peristaltic pump (Cole-Palmer, Vernon Hills, IL) maintained a chamber (45, 51) maintained at 37°C for MC3T3-E1s and 34°C for hFOBs. The chamber was placed in a closed loop that was perfused with 5 mM EDTA and 0.5% Triton-X 100 insoluble cytoskeletons were then generated by incubation with 0.5% Triton-X 100 in Tris buffer for 7.5 min. The proteins that were soluble in the 0.5% Triton-X 100 were collected as the soluble fraction, and the Triton-X insoluble cytoskeletons were treated with 5 mM EDTA and 0.5% Triton-X 100 in Tris buffer, scraped off the surface, and collected as the cytoskeleton fraction. Differential centrifugation was performed by scraping cells off the surface in lysis buffer (0.5% Triton-X 100, 0.25% deoxycholic acid, and 5 mM EDTA in Tris buffer). The lysates were centrifuged at 10,000 g for 30 min, and the supernatant was removed as the soluble fraction. The pellet was dissolved in the lysis buffer with 0.1% SDS to make the cytoskeletal fraction. All the lysates were brought to 0.1% SDS and 5 mM EDTA, mixed vigorously, and sheared through a 23-gauge needle. All lysis buffers contained protease inhibitor cocktail for mammalian tissue (Sigma) and protease inhibitor cocktail 2 (Sigma). The concentration of protein in each fraction was quantified using the BCA kit (Pierce), and each sample was normalized to the sum of the concentrations for its soluble and cytoskeletal fraction.

Western blot analysis. The protein fractions were mixed with Laemmli sample buffer (37), separated by SDS-PAGE in a 7.5% gel, and transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA). The membranes were blocked in 4% milk and 1% BSA in Tris-buffered saline with 0.1% Tween-20 (TBST) and then incubated with primary antibodies for 90 min at room temperature in TBST with whole donkey IgG diluted 1:1,000. After washing in TBST was completed, the secondary antibody was applied for 30 min at room temperature in TBST with 0.5% milk. The blots were visualized with ECL chemiluminescence (GE Biosciences, Piscataway, NJ) on BioMax Light X-ray film (Kodak, Rochester, NY), and densitometric analysis was performed in ImageJ (NIH, Bethesda, MD).

Immunostaining. For immunostaining experiments, cells were grown on glass coverslips and washed once before fixing. The cytoskeletons were extracted by incubating for 5 min with 1 mM DSP (Pierce) in Tris buffer and then for 5 min with 0.5% Triton-X 100 in cytoskeleton buffer [in mM: 20 2-(N-morpholino)ethanesulfonic acid, 276 KCl, 6 MgCl2, 6 EGTA, and 320 sucrose, pH 6.1]. The Triton-X insoluble cytoskeletons were fixed with 1 mM 3,3′-dithiobis(sulfosuccinimidyl propionate) (DTSSP, Pierce), a water-soluble analog of DSP, in cytoskeleton buffer for 15 min, and the unbound cross-linkers were quenched with 100 mM glycine (Sigma) in PBS. Cells were fixed without extraction in 3% paraformaldehyde in cytoskeleton buffer for 20 min and permeabilized with 0.4% Triton-X 100 in cytoskeleton buffer for 10 min. Fixed cells were blocked in 2% BSA (Sigma) for 30 min. Primary antibodies were incubated in PBS with whole donkey IgG diluted 1:100 for 2 h at room temperature or overnight at 4°C. The FITC conjugated secondary antibodies incubated in PBS with rhodamine phalloidin and Hoechst for 30 min. The coverslips were then mounted to slides with VectaShield Hardset (Vector Labs, Burlingame, CA) and visualized at ×400 with a TE300 inverted florescence microscope (Nikon, Melville, NY). Images were captured in Simple PCI (Compix, Cranberry Township, CA) using an ORCA 100 cooled CCD camera (Hamamatsu, Japan).

Statistics. Each flow sample was matched with a static control, and all experimental procedures for each pair were performed simultaneously. The experiment was repeated 12 times with a single pair of samples in each experiment. The blots from the soluble and cytoskeletal fractions generated in each pair were analyzed using matched pair t-tests in JMP (Version 5, SAS Institute, Cary, NC). Statistical significance was assigned to α = 0.03 adjusted for six independent tests using the Simes procedure of controlling the false discovery rate (3). The mean percent increases were determined by dividing the mean difference in blot densities between the static and flow sample for each protein by the mean density of the static blot for that protein. Confidence intervals of 95% were calculated for the percent increase of each protein following exposure to flow to demonstrate whether the confidence interval contained zero.
fraction (ERK) can interact weakly and nonspecifically with the cytoskeleton (5, 23, 54), and some ERK cofractionated with the cytoskeletal following differential centrifugation. ERK was not detected in the cytoskeletal fraction after detergent extraction with cytoskeletal stabilization. β-Actin also appeared to be concentrated in the cytoskeletal fraction following differential centrifugation, whereas the amount of β-actin in each fraction generated by detergent extraction was similar to what was observed from the cells fractionated with differential centrifugation without cytoskeletal stabilization.

The amount of α-actinin (P < 0.01), filamin (P < 0.02), and vimentin (P < 0.01) in the cytoskeletal fractions was significantly different in hFOBs exposed to fluid shear compared with the static controls (n = 12, Fig. 2, A–C). There was no significant difference detected for any of the proteins in the soluble fraction or for β-actin and talin in the cytoskeletal fractions. The mean percent increase of α-actinin, filamin, and vimentin in the cytoskeleton was 29%, 18%, and 15%, respectively. There was also a significant change in the stoichiometric relationship between the cross-linking proteins and the actin filaments after exposure to fluid shear (Fig. 2D). In the cytoskeletal fractions, the ratio of α-actinin to β-actin increased 26% (P < 0.001), and the ratio of filamin to β-actin increased 16% (P < 0.05). There was no significant difference in the ratios of the cross-linking proteins to β-actin in the soluble fractions.

The monomeric unit of actin microfilaments g-actin was stained with DNase and used for a positive control for soluble proteins in fixed cells (Fig. 3). Cells that were fixed without stabilization contained a substantial amount of g-actin. However, after stabilization and extraction with detergent, only a trace amount of g-actin could be detected in the fixed cells. The method of fixation did not appear to alter the morphology of the f-actin cytoskeleton.

**RESULTS**

Cytoskeletal stabilization and fractionation by detergent extraction was sufficient to observe cytoskeletal cross-linking proteins in the cytoskeletal fraction without interfering with the extraction of soluble proteins. Without cytoskeletal stabilization, differential centrifugation was effective to fractionate the cytoskeletal filaments, but nearly all of the cytoskeletal cross-linking proteins appeared in the soluble fraction (Fig. 1A). Cytoskeletal stabilization was necessary to observe cytoskeletal cross-linking proteins in the cytoskeletal fraction (Fig. 1B). All of the positive control for the cytoskeletal fraction (vimentin) appeared in the cytoskeletal fraction regardless of the method of fractionation. The positive control for the soluble fraction (ERK) can interact weakly and nonspecifically with the cytoskeleton (5, 23, 54), and some ERK cofractionated with the cytoskeletal following differential centrifugation. ERK was not detected in the cytoskeletal fraction after detergent extraction with cytoskeletal stabilization. β-Actin also appeared to be concentrated in the cytoskeletal fraction following differential centrifugation, whereas the amount of β-actin in each fraction generated by detergent extraction was similar to what was observed from the cells fractionated with differential centrifugation without cytoskeletal stabilization.

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Treatment with cytochalasin D caused the actin cytoskeleton to depolymerize (Fig. 4). After treatment with cytochalasin D, cells that were fixed with detergent extraction did not appear to contain a substantial amount of \( \alpha \)-actinin. There were punctate regions of intense staining for \( \alpha \)-actinin that did not appear to localize with the cytoskeleton and may have been cellular debris or vesicle bound \( \alpha \)-actinin. \( \alpha \)-Actinin also appeared to colocalize with the actin microfilaments wherever they were not completely depolymerized. However, the \( \alpha \)-actinin staining was weak and diffuse throughout most of the cell. In cells that were treated with cytochalasin D and fixed without extraction, the \( \alpha \)-actinin staining in the body of the cell remained intense, but there were fewer regions where the \( \alpha \)-actinin appeared to colocalize with the cytoskeleton.

\( \alpha \)-Actinin and filamin were widely distributed throughout hFOBs and MC3T3-E1s after exposure to fluid shear. Filamin colocalized with f-actin bundles in the cells exposed to static cultures, and these structures are more highly concentrated at the periphery of the cells (Fig. 5). Additional distinct stress fibers formed throughout the cell in response to the fluid flow (Fig. 5, B vs. E), and filamin was also colocalized with these newly formed structures (Fig. 5, C vs. F). In static control cells, the \( \alpha \)-actinin was primarily localized to punctate regions (Fig. 6), which were often near the end of actin bundles (Fig. 6A). After exposure to fluid shear, stress fibers formed, and \( \alpha \)-actinin was more evenly distributed throughout the cell and along the stress fibers (i.e., it is no longer confined to the punctate regions, Fig. 6F). Although the stress fibers were dense, there were regions of diffuse \( \alpha \)-actinin staining that remained in the cytoskeletal residue following extraction but were not clearly colocalized with actin stress fibers (Fig. 6D).

**DISCUSSION**

A better understanding of the cytoskeletal composition could provide insight into a variety of cellular responses to mechanical loading, such as mechanotransduction and mechanoprotection. The cytoskeletal cross-linking proteins are an important component of the cytoskeleton that determines the organization, interconnectivity, and mechanical properties of the filament network (18, 24, 42). To investigate the cytoskeletal changes that likely contribute to this mechanical behavior, we sought to test the hypothesis that osteoblasts would respond to fluid shear stress by altering the amount of specific cross-linking proteins in the composition of the cytoskeleton. After exposure to fluid shear, there was a significant increase in the amount of \( \alpha \)-actinin and filamin that was available to interact with the cytoskeleton throughout the cell. Since there was no significant change in the amount of these proteins in the soluble fraction, the cells may have upregulated the synthesis of these cross-linking proteins in response to mechanical loading to account for the observed increase in the cytoskeletal fraction. Furthermore, there was no change in the amount of monomeric g-actin in either the cytoskeletal or soluble fraction. Taken together, these results suggest that the change in cytoskeletal structure and whole cell mechanical behavior in response to...
Fig. 4. Stabilization of α-actinin binding in the extracted cytoskeletons. α-Actinin remained bound to the actin filaments in the cytoskeletal reside after cytoskeletal stabilization and detergent extraction. Murine osteoblasts (MC3T3-E1s) were treated with cytochalasin D (D–F and J–L) or the DMSO vehicle (A–C and G–I). The cells were fixed with cytoskeletal stabilization and detergent extraction (A–F) or with paraformaldehyde (G–L). After fixation, the cells were stained with rhodamine phalloidin for f-actin (red in channel in A, D, G, and J; single channel images in B, E, H, and K), FITC conjugated antibodies raised against α-actinin (green channel in A, D, G, and J; single channel images in C, F, I, and L), and the nuclei were stained with Hoechst (blue channel in A, D, G, and J). The scale bar represents 25 μm.
fluid shear may be driven by the recruitment of cross-linking proteins to the cytoskeleton rather than by increasing the amount of actin filaments. There was also a significant increase in the amount of vimentin intermediate filaments in the cytoskeleton, which is likely a mechanoprotective response by the cells to minimize the risk of mechanical failure (32). These findings provide an improved understanding of how the composition of the cytoskeleton changes in response to the extracellular mechanical environment.

There are several features of this study that support the validity of our findings. First, we stabilized the interaction of the cytoskeleton and adjacent proteins before cytoskeletal extraction (2, 41). α-Actinin and filamin are highly dynamic cross-linking proteins and disassociate easily from the cytoskeleton (56, 60), and their availability to interact with the cytoskeleton is tightly regulated by a variety of mechanisms (16, 56). We performed a rigorous set of control experiments to ensure that soluble cellular components were extracted from the cell after detergent extraction (Figs. 1 and 3), and the proteins that remained in the cytoskeletal residue after extraction were in close proximity to the cytoskeleton (Figs. 1 and 4). This method made it possible to observe differences in the amount of cross-linking proteins that were available to interact with the cytoskeletal fraction in response to a particular treatment. Second, the cells were mechanically stimulated using a magnitude of fluid shear that is within the predicted range of in vivo mechanical loads (1, 35, 62) and sufficient to increase the whole cell stiffness in osteoblasts (26). Therefore, the changes that were observed in the cytoskeleton may represent a part of the physiological response to loading. Finally, to verify that the increase in cross-linkers could be considered a whole cell response, the extracted cytoskeletons were immunostained for α-actinin and filamin after exposure to fluid shear. Both cross-linking proteins were localized throughout the cytoskeletal structure, which confirmed that they had not been sequestered to specific regions of the cell. Talin, used to control for focal adhesion proteins (19), was not significantly increased in the cytoskeletal fraction after exposure to fluid shear. Therefore, it is unlikely that the concentration of α-actinin and filamin was increased in the cytoskeleton as a result of focal adhesion contact formation.

Despite these strengths, two caveats should be noted. First, the cells were exposed to a magnitude of fluid shear that was consistent with other studies, but the type of flow in this study was limited to steady fluid shear stress. The physiological responses of osteoblasts to fluid shear stress, such as the expression of prostaglandin E2, cyclooxygenase-2, and osteopontin are induced by both steady and oscillatory flow (43, 46, 50), although oscillatory flow might more closely emulate in vivo loading of osteoblasts by pericellular fluid flow (28). In contrast, the arrangement of stress fibers (43, 50, 65), viscoelastic response (36), and role of the cytoskeleton in mechanotransduction (43) is different after application of steady flow compared with oscillatory flow. Therefore, the
optimal cytoskeletal composition also likely depends on the type load being applied, and these effects should be investigated in future studies. Second, Western blot densities were normalized to the total protein concentration in each sample rather than to an internal control in each fraction, as was necessary to avoid a priori assumptions about which proteins would remain constant in the cytoskeletal composition between the static and flow samples (14). The measurement of protein concentration may not be completely reliable, but a large sample size was included in this investigation to offset any errors associated with this measurement. A more conservative analysis was also performed by normalizing the amount cross-linking protein to the amount of actin in each fraction (Fig. 2D). There was a significant change in the ratio of α-actinin to actin filaments ($P < 0.01$) after exposure to fluid shear, and this alteration to the stoichiometry of the cross-linker/filament interaction is sufficient to indicate a change in the cytoskeletal composition (58).

Though morphological changes occur to the structure of the actin cytoskeleton in response to mechanical loading, the amount of polymerized actin remained unchanged. This has been previously observed using histomorphometry on fluorescently stained f-actin in insoluble MC3T3-E1 osteoblast cytoskeletons before and after exposure to fluid shear stress (46). In addition, the total amount of β-actin monomer did not increase in osteoblasts exposed to biaxial stretch (44) or in 3T3 fibroblasts that were exposed to tensile loads through focal adhesion contacts (10, 11). Though neither study investigated whether there was a change in the amount of polymerized versus soluble actin, they corroborate the present study since the total amount of β-actin in both the soluble and cytoskeletal fractions did not change. These results imply that any changes in the mechanical behavior of the cell that occur in response to loading are due to other changes in the cytoskeletal composition, such as the cross-linking proteins that determine the microstructure of the cytoskeleton.

The present study does not address the mechanism used by the cells to increase the total concentration of cross-linking proteins in the cytoskeleton in response to fluid shear stress. However, several previous studies that corroborate these observations indicate that mechanical loading can stimulate a redistribution of existing cross-linking proteins in the cell as well as gene expression of these proteins. It has been demonstrated that α-actinin is recruited to stress fibers in MC3T3-E1 osteoblasts after exposure to 1 Pa of steady fluid shear for 1 h (49), though an increase in the total amount of β-actinin could not be detected under these conditions (7), possibly because there was insufficient time for protein synthesis and the change in α-actinin was undetectable. Since α-actinin is constitutively expressed in eukaryotic cells (12), translation of α-actinin could also have been upregulated rapidly after the onset of loading. Other investigators have observed an increase in filamin mRNA within 15 min in primary fibroblasts that were exposed to tensile loads through integrins (20). Furthermore,
there was a substantial increase in the amount of total filamin in the fibroblasts after 4 h, which suggest that the cells may also rapidly upregulate the synthesis of filamin (10, 11). Additional investigation in needed to determine the extent to which de novo synthesis of cross-linking proteins is necessary to reinforce the cytoskeleton at the whole cell level.

Based on the mechanical function of the proteins studied in this investigation, increasing their concentration in the cytoskeleton will likely increase the whole cell resistance to deformation. The concentration of α-actinin and filamin has a substantial effect on the mechanical behavior of gels made from reconstituted actin microfilaments (57, 58, 60, 64) and of whole cells (27, 58). There is additional evidence that cross-linking proteins interact synergistically to enhance the mechanical properties of the cytoskeletal network (18, 58, 59). Intermediate filaments also protect the cell against mechanical overloads (22) because they form networks that are tough and become very stiff at high magnitudes of strain (31). Vimentin and actin may also have a synergistic effect on the stiffness of the cytoskeletal network (48). Further investigation is needed to determine whether the observed change in cytoskeletal composition occurs to increase the stiffness of the cell at physiological load levels or to provide protection against mechanical rupture from pathological overloads.

In summary, this study demonstrated that osteoblasts respond to fluid shear by increasing the amount of α-actinin, filamin, and vimentin that is present in the cytoskeleton. Based on the mechanical function of these proteins, it is likely that this response would be sufficient to increase the whole cell stiffness, which has been observed to occur in osteoblasts exposed to identical mechanical loads (26). As such, this study adds to the wealth of evidence suggesting that α-actinin and filamin contribute to the whole cell response to mechanical loading. It also describes methods to facilitate further investigation into proteins that are redistributed in the cell by recruitment to or from the cytoskeleton in response to loading or other stimuli. Finally, this study indicates specific changes to the cytoskeletal composition that may be significant to mechanical stimuli. Finally, this study indicates specific changes to the cytoskeletal network (48).

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