Antisense Oligonucleotide to Cofilin Enhances Respiratory Burst and Phagocytosis in Opsonized Zymosan-stimulated Mouse Macrophage J774.1 Cells

Reiko Adachi, Kosei Takeuchi, and Kazuhiro Suzuki

From the National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan, and the Division of Biological Science, Graduate School of Science, Nagoya University, Furo-cho, Chigusa-ku, 464-8602 Nagoya, Japan

Phagocytes play a central role in the host defense system, and the relationship between the mechanism of their activation and cytoskeletal reorganization has been studied. We have previously reported a possible involvement of cofilin, an actin-binding protein, in phagocyte functions through its phosphorylation/dephosphorylation and translocation to the plasma membrane regions. In this work, we have obtained a new line of evidence showing an important role of cofilin in phagocyte functions using the mouse macrophage cell line J774.1 and an antisense oligonucleotide to cofilin. Upon stimulation with opsonized zymosan (OZ), cofilin was phosphorylated, and it accumulated around phagocytic vesicles. As the antisense oligonucleotide to cofilin, a 20-mer S-oligo corresponding to the sequence including the AUG translational initiation site was found to be effective. In the cells treated with the antisense oligonucleotide, the amount of cofilin was less than 30% of that in the control cells, and the level of F-actin was two or three times higher than that in the control cells before and throughout the cell activation. In the antisense oligonucleotide-treated cells, OZ-triggered superoxide production was three times faster than that in the control cells. Furthermore, phagocytosis of OZ was enhanced by the antisense. These results show that cofilin plays an essential role in the control of phagocyte function through regulation of actin filament dynamics.

Phagocytes, including neutrophils and macrophages, are at rest in the absence of any stimulant; but, when activated by invading microorganisms or harmful substances, they play a central role in host defense systems through chemotaxis, adhesion, phagocytosis, superoxide production, degranulation, and the release of cytokines or lipid mediators. The mechanism of phagocyte activation has been studied in terms of protein phosphorylation and cytoskeletal reorganization (1). Originally, we found that cofilin, an actin- and phosphatidylinositol 4,5-phosphate-binding protein, is rapidly dephosphorylated upon cell activation and translocated to plasma membrane regions in neutrophil-like HL-60 cells (2). These findings have been confirmed by other groups (3–5). Cofilin is a widely distributed protein highly conserved among various species and plays an essential role in the control of actin filament dynamics (6–8). It has been well investigated in, for example, Dictyostelium (9–11), yeast (12–14), Xenopus egg (15–17), mammalian neural cells (18–20), and immune cells (21–23). Cofilin can be phosphorylated at Ser-3, and only the unphosphorylated form of cofilin can bind actin and depolymerize or sever the actin filament. It is also proposed that cofilin is involved in the turnover of actin filament.

To date we have studied the relation between cofilin and phagocyte functions. We used opsonized zymosan (OZ) as a cell stimulant, a complement C3bi-coated insoluble polysaccharide, the receptor for which is a member of the β2 integrin family (CR3, CD11b/CD18), and have reported that cofilin and phagocyte functions such as superoxide production or phagocytosis were strongly correlated with each other under the regulation by Src family tyrosine kinase and phospholipase C (24–26). We also reported that cofilin was engaged in chemotaxis (27). Lately, by employing the method of LIM kinase 1 (LIMK1) transfection, we have demonstrated that the cofilin-dependent regulation of actin is implicated in superoxide production (28). LIMK is a kinase for Ser-3 of cofilin and regulates the activity of cofilin downstream of the Rho family small GTPases, which are major cytoskeletal regulators (29–32).

In this paper, we investigated changes in the phosphorylated state and intracellular distribution of cofilin upon cell activation in another cell line of phagocyte, the mouse macrophage cell line J774.1, and further studied the participation of cofilin in phagocyte functions using an antisense oligonucleotide to cofilin. We unexpectedly found that OZ induced an increase in the net phosphorylation of cofilin rather than dephosphorylation, in contrast to our previous reports on other kinds of phagocytes (2, 25), in addition to the translocation of cofilin to plasma membrane regions where phagocytic vesicles were generating. We have additional indications that treatment of the cells with an antisense oligonucleotide that we designed against cofilin elevated the level of filamentous actin (F-actin) and enhanced the functions of phagocytes, respiratory burst, and phagocytosis. This is the first report describing an effective antisense oligonucleotide sequence for cofilin expression. The relationship between phagocyte functions and the regulation of the actin cytoskeleton by cofilin will be discussed.

MATERIALS AND METHODS

Cells and Antibodies—The murine macrophage cell line J774.1 was obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank, and was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum.
Cofilin Antisense Enhances Phagocyte Functions

10% heat-inactivated fetal bovine serum. The rabbit anti-cofilin antibody was generously provided by Drs. I. Yahara and K. Iida (Tokyo Metropolitan Institute of Medical Science). Monoclonal anti-cofilin antibody (MAB22) was a kind gift from Drs. T. Obinata and H. Abe (Chiba University, Japan). The rabbit anti-phosphorylated cofilin antiserum was kindly donated by Drs. K. Mizuno and K. Ohashi (Tohoku University, Sendai, Japan). The antisera was derived from a rabbit that was immunized with a phosphorylated N-terminal peptide, (AcNH2-AS-(p)GVAVSDC-conjugated keyhole limpet hemocyanin, and reacts only with phosphorylated cofilin and not with unphosphorylated cofilin. The anti-actin monoclonal antibody was obtained from Cedarlane ( Hornby, Canada). The Cy3-conjugated monoclonal anti-actin antibody was purchased from Sigma. Fluorescein isothiocyanate (FITC)-conjugated keyhole limpet hemocyanin, and reacts only of phosphorylation, the staining intensities were determined using apple Fluor 208-labeled phalloidin. The stained cells were observed with a confocal laser-scanning microscope (ACAS Ultima Z, Meridian Instruments).

Reagents—Zymosan (Sigma) and FITC-conjugated zymosan (Molecular Probes, Eugene, OR) were opsonized with normal mouse serum (Chemicon International, Temecula, CA) as described previously (33). Phosphorothioated 20-mer oligonucleotides corresponding to the sequence that includes the AUG translational initiation site of the murine non-muscle-type cofilin mRNA (34) were obtained from Hokkaido System Science (Sapporo, Japan). The antisense oligonucleotide sequence was 5′-CCACACGGAGGGCATTT-3′, and the sequence did not have significant homology with any other sequences in the data base. Two control oligonucleotides, the sense sequence and the mismatch sequence in which five nucleotides of the antisense sequence were replaced with other nucleotides, were 5′-AACATGGCCCTGTGTTGGTG-3′ and 5′-CCAGACGAGTGGGCATCTTT-3′, respectively. FUGENE 6 transfection reagent was obtained from Roche Diagnostics. AlexaFluor 488-labeled phalloidin was a product of Molecular Probes. Diogenes, the cellular luminescence enhancement system for superoxide detection, was acquired from National Diagnostics (Atlanta, GA). All other chemicals used were commercial preparations of the highest purity.

Detection of OZ-induced Phosphorylation of Cofilin.—J774.1 cells (4 × 10⁵) cultured on the bottom (6.9 cm²) of upright tissue culture flasks (Falcon, Lincoln Park, NJ) were washed with HBSS. After the addition of 0.9 ml of HBSS, the cells were pre-incubated at 37°C for 10 min. The cells were then activated with OZ (0.1 ml of 10 mg/ml suspension, final 1 mg/ml) at 37°C and treated with ice-cold DFP-containing inhibitor solution as described previously (33). The cells were then washed with Tri-buffered saline, solubilized in 300 µl of 125 mM Tris-HCl (pH 6.8) containing 0.1% SDS and 2 µl MgCl₂ by sonication, and treated with 50 units of Benzoic nuclease (Merek) at room temperature for 10 min to break the cellular DNA. Each lysate was then mixed with 30 µl of 10% SDS solution containing 20 µm dithiothreitol, sonicated for 5 min, and incubated at 75°C for 5 min. One hundred microliters of each sample was subjected to SDS-PAGE using 12.5% gel. Each sample was then transferred to nitrocellulose membrane and probed with the antibody against cofilin. The bands were visualized by chemiluminescence detection (Amersham) with horseradish peroxidase-conjugated secondary antibody (ACAS Ultima Z, Meridian Instruments) with excitation and emission wavelengths of 488 and 500–560 nm, respectively.

Antisense Oligonucleotide Treatment.—For the assay of cofilin antisense, J774.1 cells were cultured in a 96-well microplate (4.5 × 10⁵ cells/well on day 0; culture volume, 100 µl). The oligonucleotide (antisense) was mixed with FuGENE 6 transfection reagent according to the manufacturer’s protocol, except that the amount of FuGENE 6 used was ~30% of the amount recommended by the protocol for plasmid transfection. The oligonucleotide mixture was added to the cells once a day for 3 days. On day 2, the culture medium was replaced with a fresh one before the third oligonucleotide treatment. On day 3, the amounts of concentrated of OZ were assayed by a protein assay reagent (Pierce), and the portion of each sample that contained the same amount of proteins was subjected to Western blotting as described above to evaluate the effect of cofilin antisense.

Staining of F-actin.—Cells were cultured in a Lab-Tek II chamber slide and activated with OZ (final 2 mg/ml) as described above. The reaction was terminated by the addition of paraformaldehyde (final 2%), and F-actin in the cells was stained as described previously (25) using AlexaFluor 488-labeled phalloidin. The stained cells were observed, and the fluorescence intensity of each cell was determined by a confocal laser scanning microscope (ACAS Ultima Z, Meridian Instruments).

Assay of Superoxide Production.—Cells were cultured in clear bottomed 96-well white plates (Corning, NY), washed with HBSS, and then mixed with Diogenes in HBSS. Diogenes is a chemiluminescent reagent to specifically detect superoxides. The cells were then activated with OZ (final 1 mg/ml), incubated at 37°C, and the time-dependent generation of chemiluminescence was monitored by a plate reader (ARVOx, PerkinElmer Life Sciences). Based on the data thus obtained, the accumulated superoxide was calculated by curve fitting and integration.

Phagocytosis.—Cells cultured in a Lab-Tek II chamber slide were activated with FITC-labeled OZ (final 0.2 mg/ml) at 37°C. After 15 min, the cells were fixed with formaldehyde (final 4%), and immunostaining for actin was performed using Cy3-conjugated monoclonal anti-actin antibody according to the method of cofilin staining described above. The stained cells were observed with a confocal laser-scanning microscope (LSM 510, Carl Zeiss). The green fluorescence of FITC-labeled OZ was observed at an excitation wavelength of 488 nm and an emission wavelength of 505–550 nm, and the red fluorescence of the Cy3-conjugated anti-actin antibody was monitored at an excitation wavelength of 543 nm and an emission wavelength longer than 560 nm. At least 400 cells were observed in each sample, and the cells that had phagocytosed OZ were counted.

RESULTS

OZ-Induced Phosphorylation of Cofilin and Its Translocation to the Plasma Membrane.—We have previously reported that, in neutrophil-like HL-60 cells and macrophage-like U937 cells, cofilin was dephosphorylated and translocated to the plasma membranes in response to cell activation with OZ, and we considered that cofilin is involved in the activation of phagocytes (2, 24–26). In the present work, cells of the mouse macrophage cell line J774.1 were activated with OZ, and the changes of cofilin in its phosphorylated state and intracellular distribution were investigated. OZ-induced changes in the phosphorylated state of cofilin were estimated by Western blotting using a specific anti-phosphorylated cofilin antibody. The results are shown in Fig. 1. Fig. 1A shows the antibody-stained protein band of phosphorylated cofilin and the total (both phosphorylated and unphosphorylated) cofilin. The staining intensities were determined, and the relative degrees of phosphorylation are shown in Fig. 1B. OZ enhanced phosphorylation of cofilin in J774.1 cells in a time-dependent manner. Ten minutes after the addition of OZ, the content of phosphorylated cofilin was 2.9 times higher than that of the unstimulated cells, and after 20 min the level of phosphorylated cofilin was then reduced.

The changes in intracellular distribution of cofilin in the OZ-activated J774.1 cells were studied by indirect immunostaining and a confocal laser scanning microscopy. As shown in Fig. 2, in the unstimulated cells cofilin was diffusely distributed in the cytosol and nuclear regions. One minute after the addition of OZ, the shape of the cells engulfing the OZ was remarkably changed, and cofilin accumulated around the generating phagocytic vesicles. After 5 min, a large proportion of cofilin had accumulated around the generated phagocytic vesicles. However, cofilin that had accumulated around the generated phagocytic vesicles had almost disappeared by 10 min after cell activation. Such changes in the distribution of cofilin have also been observed in neutrophil-like HL-60 cells and macrophage-like U937 cells activated by OZ (2, 25). It is likely that cofilin translocates to the phagosome-forming membranes and then diffusely returns to the cytosol when the formation of stable phagosomes has been accomplished.

Effect of Antisense Oligonucleotide on Cofilin Expression.—As described above, in J774.1 cells the phosphorylation and translocation of cofilin to the phagosome-forming area were observed

Downloaded from http://www.jbc.org/ by guest on July 26, 2018
in response to cell activation. As a tool for investigation of the role of cofilin in the cells, we employed an antisense oligonucleotide to cofilin. We designed 22 phosphorothioated oligonucleotide (S-oligo) as antisense sequences using a computer analysis, and ten of them were synthesized and assayed. The most effective antisense we tested was a 20-mer S-oligo corresponding to the sequence that included the AUG translational initiation site of the murine non-muscle-type cofilin mRNA (from 4-base upper to 13-base lower from AUG). As control oligonucleotides, the sense sequence, which was equivalent to the mRNA sequence targeted by the antisense, and the mismatch sequence, in which five nucleotides of the antisense sequence were replaced with others, were employed. These oligonucleotides were added to the cells in the presence of the transfection reagent, FuGENE 6, once a day for 3 days. This application had little effect on the viability of the cells, although their number was about one third of that of the non-treated cells. The sense and the mismatch oligonucleotides did not influence the growth of the cells very much. Observation of the cells treated with rhodamine-labeled oligonucleotides with a confocal laser scanning microscope showed that almost all cells incorporated the oligonucleotides (data not shown). Fig. 3 shows a typical result of Western blotting to evaluate the antisense effect. As a reference protein not influenced by the antisense, actin was also stained, and relative staining intensities were calculated. In the cells treated with the antisense oligonucleotide, the protein amount of cofilin was decreased to 70–90% (depending on experiments), although the sense and the mismatch oligonucleotides showed no or only a slight effect on cofilin expression. These results show that the oligonucleotide used in this study was extremely effective as the cofilin antisense and could be applicable to some other types of cells or tissues.

Effect of Cofilin Antisense on F-actin—Cofilin is an actin-depolymerizing and severing protein, and it is closely related to actin filament dynamics. We therefore examined the changes in intracellular filamentous actin (F-actin) in the antisense-treated cells with AlexaFluor 488-phalloidin, a specific F-actin-staining reagent. Fig. 4A shows the photographs obtained by confocal laser scanning microscopy. The intensity of fluores-
cence derived from F-actin in each cell was determined for at least 60 cells in one sample. The experiment was repeated three times, and the average of relative fluorescence intensities are shown in Fig. 4B. In the control cells, F-actin was found at the cell periphery when the cells were not stimulated. After the addition of OZ to the cells, the level of F-actin was rapidly increased, particularly around the phagocytic vesicles. This OZ-evoked increase in F-actin declined to the original level within 10 min, at which time phagocytosis was nearly accomplished. In the sense oligonucleotide-treated cells, the content and the time-dependent changing pattern of F-actin were very similar to those in the control cells. However, in the antisense oligonucleotide-treated cells, the level of F-actin was two or three times higher than that in the control cells before and throughout the cell activation, although the pattern of changes resembled that of the control cells. These results show that the cofilin antisense oligonucleotide induced overall augmentation of the intracellular content of F-actin.

Enhancement of Superoxide Production by Cofilin Antisense—We then investigated the effects of the antisense oligonucleotide to cofilin on one of the phagocyte functions, the production of superoxide. The antisense or control oligonucleotide-treated cells were stimulated with OZ, and the generated superoxide was assayed by the chemiluminescence method. The accumulated superoxide was calculated by integration, and the curves thus obtained are shown in Fig. 5. The antisense oligonucleotide-treated cells produced superoxide at a rate three times higher than the control cells, whereas the rates of superoxide production by the sense and mismatch oligonucleotide-treated cells were similar to that of control cells. These data clearly indicate that treatment of the cells with the cofilin antisense oligonucleotide enhanced the respiratory burst.

Effect of Cofilin Antisense on Phagocytosis—Next, we evaluated the effect of the antisense oligonucleotide on another phagocyte function, namely phagocytosis. After OZ-stimulation, the cells were stained with Cy3-conjugated anti-actin antibody to visualize cell shapes with red fluorescence. The phagocytotic activities of the cells were monitored by confocal laser scanning microscopy using FITC-labeled OZ. The results showed the photographs of phagocytosis. These pictures are merged images of green fluorescence, red fluorescence, and differential interference images. In intracellular actin is red, free OZ is green, and the engulfed OZ is yellow as a merged color. However, in the antisense oligonucleotide-treated samples, 63% of the cells showed phagocytotic activity. These results indicate that the antisense oligonucleotide for cofilin enhanced phagocytic activity in J774.1 cells.

DISCUSSION

In this paper, we first investigated the changes in the phosphorylated state and the intracellular distribution of cofilin upon cell activation in cells of the mouse macrophage cell line J774.1, and next we designed a useful antisense oligonucleotide to cofilin and examined its effects on phagocyte functions. We obtained the following results. 1) Upon activation by OZ, phosphorylation of cofilin was increased 3-fold. 2) Cofilin, which was distributed diffusely in the cytosol and nuclei, was translocated to plasma membrane regions by OZ stimulation. 3) The antisense oligonucleotide to cofilin markedly enhanced the OZ-triggered superoxide production. 4) Treatment with the antisense caused an increase in phagocytic activity against OZ.

We found that the antisense oligonucleotide to cofilin, a 20-mer S-oligo corresponding to the sequence that includes the AUG translational initiation site of cofilin mRNA, is a useful tool for decreasing specifically the level of cofilin. Because cofilin is a ubiquitous protein and has been widely studied, the

Fig. 4. 

Fig. 5. 

OZ-induced changes in intracellular F-actin in J774.1 cells. J774.1 cells treated with the antisense or the sense oligonucleotide were stimulated by OZ at 37 °C for the indicated times. The cells were fixed with paraformaldehyde, and the intracellular F-actin was stained with AlexaFluor 488-conjugated phalloidin. The fluorescence-stained F-actin in the individual cells is shown in panel A, and the time course of relative fluorescence intensities is shown in panel B. Square, control cells; circle, the antisense-treated cells; triangle, the sense-treated cells. A representative result of three experiments is shown.

Superoxide production by the antisense-treated J774.1 cells. The cells were stimulated with OZ, and superoxide production was monitored by chemiluminescence. The accumulation of superoxide produced by the control cells (a), the antisense-treated cells (b), the sense-treated cells (c), and the mismatch-treated cells (d) are shown.
antisense oligonucleotide presented here can be a valuable means to evaluate the role of cofilin in various types of cells. We observed that the number of the antisense-treated cells was lower than that of untreated or control oligonucleotide-treated cells. This was probably because cofilin is essential for cell proliferation (12, 35), and thus a low level of cofilin should inhibit cell division (12, 15, 21).

Because cofilin controls actin filaments by depolymerizing and severing, it is reasonable that the F-actin content was elevated when the protein expression of cofilin was inhibited by the antisense oligonucleotide. The pattern of the change in the level of F-actin upon cell activation in the antisense-treated cells was similar to that of the control cells. In general, it is known that stimulation triggers an increase of F-actin in phagocytes (25, 36, 37). It seems that, in the cells treated with the antisense oligonucleotide, the balance between assembly and disassembly of F-actin shifted toward filament formation, although a dynamic change in the F-actin level in response to cell stimulation occurred as well as in the control cells, probably through the contribution of the expressed cofilin and other actin-regulating proteins such as gelsolin (38, 39).

We have reported previously that wild-type LIMK1-overexpressing U937 cells showed increased superoxide production, whereas in the dominant negative LIMK1-expressing cells the respiratory burst was suppressed (28). The results shown in Fig. 5 coincide with the previous observation from the point of view that the increase in F-actin leads to enhancement of superoxide production. It has been elucidated that the superoxide-generating enzyme NADPH oxidase is integrated into the plasma membranes of phagocytes and is activated by assembly with cytosolic factors p47phox, p67phox, p40phox, and Rac (1). The translocation mechanisms of these factors have not been determined, whereas the possible role of the actin cytoskeleton in the translocation of the cytosolic factors and the activation of the oxidase has been reported (33, 40–43). In addition, Tamura et al. (44) reported that actin filaments prolonged the lifetime of NADPH oxidase. Granfeldt and Dahlgren (45) described a requirement for actin filament for prolonged superoxide production after phagocytosis was completed. Thus, it can be surmised that an appropriately higher level of F-actin is required to prolong the lifetime of NADPH oxidase, and this may be achieved by increasing the expression of cofilin in the antisense-treated cells.

In general, phagocytosis is thought to involve the spatial and temporal reorganization of the actin-based cytoskeleton at the sites of particle ingestion, and local polymerization of actin filaments supports the protrusion of pseudopodia that eventually engulf the particle (46). In our previous work, it was observed that the phagocytic activity of the dominant negative LIMK1-expressing macrophages was much lower than that of the control cells (28). Biene et al. (47) reported that cellular invasion by the pathogenic bacterium Listeria monocytogenes was inhibited in both cases of transfection, i.e., the transfection of wild-type LIMK and the transfection of dominant negative LIMK. Based on these observations, it is possible to consider that phagocytosis is controlled appropriately by both the activation and the deactivation of cofilin to form phagocytic vesicles. The antisense oligonucleotide to cofilin induced increased levels of F-actin, which are thought to be adequate for effective phagosome formation without causing unnecessary rigidity to the cells.

To date, it has been reported that cofilin was dephosphorylated in response to cell activation in phagocytes (2–5, 25), which is in contrast to the results in this paper. One possible explanation for this observation may be the difference in the types of cells. In neutrophil-like HL-60 cells, dephosphorylation of cofilin occurred very rapidly. More than 70% of cofilin was dephosphorylated within 1 min by various kinds of phagocyte activators (2). In the case of neutrophils derived from peripheral blood, the rate of dephosphorylation of cofilin was also very rapid (3–5). In contrast, about 60% of cofilin remained phosphorylated 10 min after cell activation in macrophage-like U937 cells (25). In general, neutrophils are cultured in suspension, although macrophages have adhesive properties. U937 cells are cultured in suspension, and they are induced to become macrophage-like adhesion cells by certain kinds of drugs. J774.1 cells show a strong adhesive character by themselves without any induction. It is possible to postulate that the conditions of the actin cytoskeleton in resting cells and the manner of its change in response to cell activation depend on the character of the cells. In fact, it has been reported that the level of total cofilin and the ratio of phosphorylated and unphosphorylated cofilin differ in various tissues or cells (2, 6, 23, 48). In addition, in the case of chemotaxis induced by nitric oxide, cofilin translocated to the plasma membrane regions but was neither phosphorylated nor dephosphorylated in neutrophil-like HL-60 cells (27). It can be thought that, upon cell activation, signals leading to both phosphorylation and dephosphorylation of cofilin are generated to control appropriately the dynamics of the actin cytoskeleton in each intracellular region and at each time point. In the case of J774.1 cells, cofilin translocated to plasma membrane regions upon cell activation, possibly because the proper regulation of actin filament by cofilin was necessary there, and by Western blotting, an increase in phosphorylation of cofilin, instead of dephosphorylation...
Cofilin Antisense Enhances Phagocyte Functions

45571

...tion, was observed in whole cells as a result of conditions of entire cofilin.

It is already known that LIMK is regulated under Rho family small GTPases, Cdc42, Rac, and Rho (29–32). Recently, it was reported that a protein phosphatase named Slingshot had the activity of dephosphorylate cofilin (49). If we have more information about the signal transduction pathway modulating the activity of cofilin and machinery of actin filament regulation, the relationship between the cytoskeletal system and cellular phagocyte function will be clarified.

REFERENCES

1. Babior, B. M. (1999) *Blood* 93, 1464–1476
2. Suzuki, K., Yamaguchi, T., Tanaka, T., Kawashima, T., Nishimaki-Mogami, T., Yamamoto, K., Tsuji, T., Irimura, T., Hayakawa, T., and Takahashi, A. (1995) *J. Biol. Chem.* 270, 19551–19556
3. Okada, K., Takano-Omuro, H., Obinata, T., and Abe, H. (1996) *Exp. Cell Res.* 237, 116–122
4. Djafarzadeh, S., and Niggli, V. (1997) *Exp. Cell Res.* 236, 427–435
5. Heyworth, P. G., Robinson, J. M., Ding, J., Ellis, B. A., and Badwey, J. A. (1997) *Histochem. Cell Biol.* 108, 221–235
6. Bamburg, J. R. (1999) *Annu. Rev. Cell Dev. Biol.* 15, 185–230
7. Maciver, S. K., and Hussey, P. J. (2002) *Genome Biol.* 3, 3007 (review)
8. McGeough, A., Pope, B., and Weeds, A. (2001) *Results Probl. Cell Differ.* 32, 135–154
9. Aizawa, H., Sutoh, K., and Yahara, I. (1996) *J. Cell Biol.* 132, 335–344
10. Ichetovkin, I., Han, J., Pang, K. M., Knecht, D. A., and Condeelis, J. S. (2000) *J. Biol. Chem.* 275, 35779–35782
11. Nagaishi, K., Adachi, R., Kasahara, T., and Suzuki, K. (2001) *Cell. Signal.* 13, 17–22
12. Adachi, R., Matsui, S., Kinoshita, M., Nishida, E., and Mizuno, K. (1998) *Nature* 393, 805–809
13. Okada, K., Blanchoin, L., Abe, H., Chen, H., Pollard, T. D., and Bamburg, J. R. (2002) *J. Biol. Chem.* 277, 628–636
14. Aizawa, H., Wakatsuki, S., Ishii, A., Moriyama, K., Sasaki, Y., Ohashi, K., Sekine-Aizawa, Y., Sekihara-Fushigawa, A., Mizuno, K., Goshima, Y., and Yahara, I. (2001) *Nat. Neurosci.* 4, 367–374
15. Samstag, Y., Dreizler, E. M., Ambach, A., Szakiel, G., and Meuer, S. C. (1996) *J. Immunol.* 156, 4167–4173
16. Nagaishi, K., Adachi, R., Kasahara, T., and Suzuki, K. (2002) *Mol. Cell. Biol.* 22, 774–783
17. Aizawa, H., Sutoh, K., Tsuji, T., Kawashima, S., Ishii, A., and Yahara, I. (2001) *J. Immunopharmacol.* 25, 855–864
18. Matsui, S., Matsumoto, S., Adachi, R., Kusui, K., and Takahashi, A. (2002) *J. Biol. Chem.* 277, 544–549
19. Arber, S., Barbayannis, F. A., Hansen, H., Schneider, C., Stanyon, C. A., Bernard, O., and Caroni, P. (1998) *Nature* 393, 805–809
20. Nishida, E., and Mizuno, K. (1998) *Nature* 393, 809–812
21. Okada, K., Blanchoin, L., Abe, H., Chen, H., Pollard, T. D., and Bamburg, J. R. (2002) *J. Biol. Chem.* 277, 628–636
22. Miyasaka, K., Matsumoto, S., Nishida, E., and Mizuno, K. (1998) *Nature* 393, 805–809
23. Okada, K., Blanchoin, L., Abe, H., Chen, H., Pollard, T. D., and Bamburg, J. R. (2002) *J. Biol. Chem.* 277, 628–636
24. Aizawa, H., Sutoh, K., Tsuji, T., Kawashima, S., Ishii, A., and Yahara, I. (1996) *J. Biol. Chem.* 270, 10923–10932
25. Nagaishi, K., Adachi, R., Kasahara, T., and Suzuki, K. (2001) *Cell. Signal.* 13, 17–22
26. Adachi, R., Matsui, S., Kinoshita, M., Nishida, E., and Mizuno, K. (1998) *Nature* 393, 809–812
27. Sumi, T., Matsumoto, R., Taki, Y., and Nakamura, T. (1999) *J. Cell Biol.* 147, 1519–1532
28. Ohashi, K., Nagata, K., Maekawa, M., Ishizaki, T., Narumiya, S., and Mizuno, K. (2000) *J. Biol. Chem.* 275, 3577–3582
29. Suzuki, K., Yamaguchi, T., Oshizawa, T., Yamamoto, Y., Nishimaki-Mogami, T., Hayakawa, T., and Takahashi, A. (1995) *Biochim. Biophys. Acta* 1266, 261–267
30. Nishida, E., and Mizuno, K. (1998) *Mol. Cell. Biol.* 18, 3053
31. Aizawa, H., Sutoh, K., Tsuji, T., Kawashima, S., Ishii, A., and Yahara, I. (1996) *J. Biol. Chem.* 270, 10923–10932
32. Greenberg, S., el Khoury, J., di Virgilio, F., Kaplan, E. M., and Silverstein, S. C. (1991) *J. Cell Biol.* 113, 757–767
33. Downey, G. P., Chan, C. K., Lea, P., Taki, Y., and Grinstein, S. (1992) *J. Cell Biol.* 116, 695–706
34. Sun, H., Yamamoto, M., Meijllano, M., and Yin, H. L. (1999) *J. Biol. Chem.* 274, 33179–33182
35. Aizawa, H., Sutoh, K., Tsuji, T., Kawashima, S., Ishii, A., and Yahara, I. (1995) *J. Biol. Chem.* 270, 10923–10932
36. Nagaishi, K., Adachi, R., Kasahara, T., and Suzuki, K. (2001) *Cell. Signal.* 13, 17–22
37. Downey, G. P., Chan, C. K., Lea, P., Taki, Y., and Grinstein, S. (1992) *J. Cell Biol.* 116, 695–706
38. Sun, H., Yamamoto, M., Meijllano, M., and Yin, H. L. (1999) *J. Biol. Chem.* 274, 33179–33182
39. Kwon, K. M., and Yeh, J. (2000) *Curr. Opin. Cell Biol.* 11, 103–108
40. Mammalian, M., Volpp, B. D., McCormick, S., Leidal, K. G., and Clark, R. A. (1991) *J. Biol. Chem.* 266, 5911–5917
41. el Benna, J., Ruedi, J. M., and Babior, B. M. (1994) *J. Biol. Chem.* 269, 6729–6734
42. Grogan, A., Reeves, E., Keep, N., Wijetjes, F., Totty, N. F., Burlingame, A. L., Hsuain, J. J., and Segal, A. W. (1997) *J. Biol. Chem.* 272, 6729–6734
43. Woodman, R. C., Ruedi, J. M., Jesaitis, A. J., Okamura, N., Quinn, M. T., Smith, R. M., Curnutte, J. T., and Babior, B. M. (1991) *J. Clin. Invest.* 87, 1345–1351
44. Tamura, M., Kanno, M., and Endo, Y. (2000) *Biochem. J.* 340, 369–375
45. Granfeldt, D., and Dahlgren, C. (2001) *Inflammation* 25, 165–169
46. May, R. C., and Machesky, L. M. (2001) *Curr. Opin. Cell Biol.* 13, 1061–1077
47. Bierne, H., Gouin, E., Roux, P., Caroni, P., Yin, H. L., and Cossart, P. (2001) *J. Cell Biol.* 155, 101–112
Antisense Oligonucleotide to Cofilin Enhances Respiratory Burst and Phagocytosis in Opsonized Zymosan-stimulated Mouse Macrophage J774.1 Cells
Reiko Adachi, Kosei Takeuchi and Kazuhiro Suzuki

J. Biol. Chem. 2002, 277:45566-45571.
doi: 10.1074/jbc.M207419200 originally published online September 23, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M207419200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 48 references, 20 of which can be accessed free at http://www.jbc.org/content/277/47/45566.full.html#ref-list-1