Protection against osmotic stress by cGMP-mediated myosin phosphorylation
Kuwayama, H; Ecke, M; Gerisch, G; van Haastert, Petrus

Published in:
Science

DOI:
10.1126/science.271.5246.207

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1996

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
10.1126/science.271.5246.207

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Protection Against Osmotic Stress by cGMP-Mediated Myosin Phosphorylation

Hidekazu Kuwayama, Maria Ecke, Günther Gerisch, Peter J. M. Van Haastert*

Conventional myosin functions universally as a generator of motive force in eukaryotic cells. Analysis of mutants of the microorganism Dictyostelium discoideum revealed that myosin also provides resistance against high external osmolarities. An osmo-induced increase in intracellular guanosine 3',5'-monophosphate was shown to mediate phosphorylation of three threonine residues on the myosin tail, which caused a relocalization of myosin required to resist osmotic stress. This redistribution of myosin allowed cells to adopt a spherical shape and may provide physical strength to withstand extensive cell shrinkage in high osmolarities.

Myosin II filaments bind to actin filaments more effectively than myosin monomers (10, 11). Phosphorylation of myosin II at three threonine residues of its tail region inhibits filament formation (10, 12) and thus weakens the interaction with actin filaments. Mutant mhcAAAA produces a myosin II heavy chain in which the phosphorylatable threonines are replaced by alanine residues. As a consequence of this mutation, myosin II exists predominantly in the filamentous state, and the assembly and disassembly rate are probably strongly reduced (9, 10). These mhcAAAA cells showed the same sensitivity to high concentrations of glucose as mhc− cells (Fig. 1A), which suggests that phosphorylation of myosin II at its tail was required to protect cells against osmotic stress (13).

Guanosine 3',5'-monophosphate (cGMP) acts as a universal second messenger in eukaryotic cells (14). In D. discoideum, cGMP levels increase upon stimulation with the chemotactant adenosine 3',5'-monophosphate (cAMP) (15). Intracellular cGMP regulates the assembly and disassembly of myosin filaments by inducing the phosphorylation of the threonine residues (16). Osmotic stress also activates guanylyl cyclase in wild-type D. discoideum cells; the cGMP concentration in amoeboid cells is pariially disordered in the structure, and Glu5, which is located in a tight turn. A three-dimensional, one-dimensional profile (27) shows a better-than-average score for a protein of this size, with no negative values (29). The only region with a somewhat low score is the flexible loop. A specific-sequence marker is provided by the single mercury position per subunit in the heavy atom derivative, which is close to the side chain nitrogen of Trp260 (there are neither histidines nor cysteines in Mi-cpn10). Crystals of selenomethionine-Mi-cpn10 were obtained but did not result in significant peaks in difference Pattersons and difference Fourier maps. The model was refined by using MOLSCRIPT and RASTER3D (29).


cGMP-Mediated Myosin Phosphorylation

Myosin II filaments bind to actin filaments more effectively than myosin monomers (10, 11). Phosphorylation of myosin II at three threonine residues of its tail region inhibits filament formation (10, 12) and thus weakens the interaction with actin filaments. Mutant mhcAAAA produces a myosin II heavy chain in which the phosphorylatable threonines are replaced by alanine residues. As a consequence of this mutation, myosin II exists predominantly in the filamentous state, and the assembly and disassembly rate are probably strongly reduced (9, 10). These mhcAAAA cells showed the same sensitivity to high concentrations of glucose as mhc− cells (Fig. 1A), which suggests that phosphorylation of myosin II at its tail was required to protect cells against osmotic stress (13).

Guanosine 3',5'-monophosphate (cGMP) acts as a universal second messenger in eukaryotic cells (14). In D. discoideum, cGMP levels increase upon stimulation with the chemotactant adenosine 3',5'-monophosphate (cAMP) (15). Intracellular cGMP regulates the assembly and disassembly of myosin filaments by inducing the phosphorylation of the threonine residues (16). Osmotic stress also activates guanylyl cyclase in wild-type D. discoideum cells; the cGMP concentration increased after 1 min and reached a peak at ~10 min after the onset of stimulation (17) (Fig. 1B). A transient accumulation of cGMP levels upon addition of 300 mM glucose was also observed in wild-type XP55 and in the mhc−, mhc−, and mhcAAAA strains (Fig. 1B). No increase of cGMP levels was found in the nonc electromutant KI-8 in which gua...
Osmotic stress induces the activation of guanylyl cyclase. The main function of the produced cGMP is to mediate myosin II phosphorylation and was specific for this cell-permeable cGMP analog (19). In addition, 8Br-cGMP in the presence of glucose induced myosin II phosphorylation in KI-8 cells (Fig. 2B). Thus, a cell-permeable cGMP analog restored osmosensitivity in the guanylyl cyclase mutant but not in the myosin II mutants.

The distribution of the cytoskeletal proteins actin and myosin was analyzed by confocal fluorescence microscopy with labeled phalloidin to stain actin filaments and monoclonal antibodies to label myosin II (Fig. 3). Control cells were highly motile and elongated with many pseudopodial extensions. Actin filaments were localized primarily in the extending pseudopodia, whereas myosin II was found throughout the cytoplasm and enriched in pseudopodia. At 10 min after the osmotic shock with 300 mM glucose, cells shrank by ~50% (21), assuming a rigid state in which a more spherical core region was surrounded by flattened extensions. Filamental actin remained in the extensions. Myosin II moved within 10 min toward the cortex of the core; dual labeling revealed that myosin II was localized primarily in a layer beneath the actin-rich cortex.

Thus, we propose the following model: Osmotic stress induces the activation of guanylyl cyclase. The main function of the produced cGMP is to mediate myosin II phosphorylation at three threonine residues on its tail, which enhances the disassembly of myosin II filaments and thereby recruits myosin for deposition below a peripheral layer of actin filaments. Dictyostelium discoideum cells respond chemotactically to cAMP. Guanylyl cyclase and myosin II phosphorylation are essential for both osmo- and chemosensory transduction. Activation of guanylyl cyclase by these signals must use different mechanisms because chemotactic signal transduction is ablated in mutants lacking the heterotrimeric guanosine triphosphate binding protein (G protein) subunits Ga2 (22) or GB (23), which show a normal response to osmotic stress (24). In yeast, osmosensing is mediated by a two-component system composed of a histidine kinase and its receiver domain (25). In the yeast system, the sensor mediates osmoregulation through the production of glycerol. Recently, a gene encoding a putative histidine kinase has been identified in D. discoideum; interestingly, a mutant with a disruption of this gene is sensitive to high osmolarities (26). Possibly, this two-component system is a common constituent of the osmosensory pathways, including the one that controls the actin and myosin system shown here.

**REFERENCES AND NOTES**

Regulation of PHO4 Nuclear Localization by the PHO80-PHO85 Cyclin-CDK Complex

Elizabeth M. O’Neill, Arie Kaffman, Emmitt R. Jolly, Erin K. O’Shea*

PHO4, a transcription factor required for induction of the PHO5 gene in response to phosphate starvation, is phosphorylated by the PHO80-PHO85 cyclin-CDK (cyclin-dependent kinase) complex when yeast are grown in phosphate-rich medium. PHO4 was shown to be concentrated in the nucleus when yeast were starved for phosphate and was predominantly cytoplasmic when yeast were grown in phosphate-rich medium. The sites of phosphorylation on PHO4 were identified, and phosphorylation was shown to be required for full repression of PHO5 transcription when yeast were grown in high phosphate. Thus, phosphorylation of PHO4 by PHO80-PHO85 turns off PHO5 transcription by regulating the nuclear localization of PHO4.

The transcription of PHO5, which encodes a secreted acid phosphatase, is tightly repressed when Saccharomyces cerevisiae are grown in phosphate-rich medium, and its induction is more than 100-fold increased when yeast are starved for phosphate (1). Transcriptional inactivation of PHO5 requires the transcription factor PHO4, and preliminary data suggest that PHO4 activity is negatively regulated by phosphorylation (2). When yeast are grown in phosphate-rich medium, the PHO80-PH085 cyclin-CDK complex phosphorylates PHO4 (2) and transcription of PHO5 is repressed. When yeast are starved for phosphate, the kinase activity of the PHO80-PH085 complex is down-regulated by the CDK inhibitor PH081 (3). This reduced activity results in the appearance of an underphosphorylated form of PHO4 (2) that, in combination with a second transcription factor, PHO2, binds to the PHO5 promoter and activates PHO5 transcription (4).

We wished to determine how changes in phosphate availability affect PHO4 function. Phosphate starvation does not have a large effect on PH04 stability; no difference in the amount of PHO4 is observed between yeast grown in low versus high phosphate medium (2). Because PHO4 occupies binding sites in the PH05 promoter in vivo under inducing, but not repressing, conditions (5), the phosphorylation of PHO4 appears to be a key step in the regulation of PH05 transcription at the level of DNA binding or some prior step, such as nuclear localization. Preliminary data suggest that phosphorylated and unphosphorylated PHO4 have a similar affinity for DNA (6).

We therefore examined the subcellular localization of PHO4 in wild-type cells grown in low or high phosphate medium. PHO4 is concentrated in the nucleus when yeast are starved for phosphate and is largely cytoplasmic when yeast are grown in phosphate-rich medium (Fig. 1, A to D). In pho80Δ and pho85Δ strains, in which PH04 is not phosphorylated (2) and which express PHO5 constitutively (1), PH04 was concentrated in the nucleus, even when the strains were grown in phosphate-rich medium (6).

Department of Biochemistry and Biophysics, University of California at San Francisco, School of Medicine, San Francisco, CA 94143-0448, USA.

*To whom correspondence should be addressed.