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Protection Against Osmotic Stress by cGMP-Mediated Myosin Phosphorylation

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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 of intracellular guanosine 3',5'-monophosphate was shown to mediate phosphorylation of three threonine residues on the myosin tail, which caused a relocation 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.

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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 mhcaAA 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 mhcaAA cells showed the same sensitivity to high concentrations of glucose as mhca cells (Fig. 1A), which suggests that phosphorylation of myosin II at its tail 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 chemotactic adenine 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 mhcaAA, mhca-, and mhcaAA strains (Fig. 1B). No increase of cGMP levels was found in the nonchomotic mutant KJ-8 in which gua...
Whereas the survival of XP55, mhcw', mhc-,
and mhcAAA was not influenced by 1 mM
8Br-cGMP. Results are the means
and SD of three independent experiments with triplicate determinations.

To investigate the role of cGMP in osmoreg-
ulation, we tested the survival of this mutant under conditions of osmotic stress. Mutant
KI-8 cells proved to be very sensitive to os-
motic stress; survival of these cells was re-
duced by an amount similar to that seen in mhc- mutants (Fig. 1A). Moreover, 300 mM
glucose induced the transient phosphoryla-
tion of myosin II in wild-type XP55 cells,
which was already detectable after 2 min,
whereas mutant KI-8 showed no response
(Fig. 1C). A cell-permeable cGMP analog,
8-bromoguanosine 3',5'-monophosphate
(8Br-cGMP), was added to these mutants
in combination with 300 mM glucose.
Whereas the survival of XP55, mhcw', mhc-
and mhcAAA was not influenced by 1 mM
8Br-cGMP, the analog restored the survival
of mutant KI-8 (Fig. 2A). Half-maximal
restoration was induced by ~100 mM 8Br-
cGMP and was specific for this cell-perme-
able 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 gua-
nylyl cyclase mutant but not in the myosin
mutants.

The distribution of the cytoskeletal pro-
teins actin and myosin was analyzed by con-
focal fluorescence microscopy with labeled
phallolidin to stain actin filaments and
monoclonal antibodies to label myosin II
(Fig. 3). Control cells were highly motile and
elongated with many pseudopodial exten-
sions. Actin filaments were localized primar-
ily in the extending pseudopodia (20),
whereas myosin II was found throughout the
cytosol and enriched in pseudopodia. At
10 min after the osmotic shock with 300 mM
glucose, cells shrank by ~50% (21), assum-
ing a rigid state in which a more spherical
core region was surrounded by flattened ex-
tensions. 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 primar-
ily in a layer beneath the actin-rich cortex.

Thus, we propose the following model:
Osmotic stress induces the activation of gua-
nyl cyclase. The main function of the pro-
duced cGMP is to mediate myosin II phos-
phorylation at three threonine residues on its
tail, which enhances the disassembly of myo-
sin 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 mecha-
nisms because chemotactic signal transduc-
tion is ablated in mutants lacking the het-
ero- trimeric guanosine triphosphate binding
protein (G protein) subunits Ga2 (22) or
Gβ (23), which show a normal response to
osmotic stress (24). In yeast, osmosensing is
mediated by a two-component system com-
posed of a histidine kinase and its receiver
domain (25). In the yeast system, the sensor
mediates osmoregulation through the pro-
duction of glycerol. Recently, a gene encod-
ing a putative histidine kinase has been iden-
tified in D. discoideum; interestingly, a mu-
tant 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 sys-
tem shown here.

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Regulation of PHO4 Nuclear Localization by the PHO80-PH085 Cyclin-CDK Complex

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PHO4, a transcription factor required for induction of the PHO5 gene in response to phosphate starvation, is phosphorylated by the PHO80-PH085 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-PH085 turns off PHO5 transcription by regulating the nuclear localization of PHO4.

The transcription of PHO5, which encodes a secreted acid phosphate, 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 induction 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 PHO81 (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 PHO4 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 PHO5 promoter in vivo under inducing conditions (3), the phosphorylation pattern of PHO4 is suggested to be affected 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 PHO4 is not phosphorylated (2) and which express PHO5 constitutively (I), PHO4 was concentrated in the nucleus, even when the strains were grown in phosphate-rich medium (6). In contrast, PHO4 was predominantly cytoplasmic in a pho81Δ strain grown in low or high phosphate conditions (6). In this strain, PHO4 is phosphorylated even when yeast are starved for phosphate (2),...