Research Focus

Mucins, osmosensors in eukaryotic cells?

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The molecular mechanisms required for sensing high osmolarity in the extracellular environment are not well defined in eukaryotes. A recent study showed that yeast Msb2 and Hkr1, which are related to mammalian mucins, are excellent candidates for sensing osmostress and for activating the HOG stress-activated protein kinase pathway involved in osmostress adaptation. Transmembrane mucins activate several signaling cascades in mammals and could therefore be important for sensing osmotic imbalances in higher eukaryotes.

Sensing and signaling osmostress in yeast

Adaptation to environmental stress requires changes in many aspects of cellular behavior. In eukaryotic cells, high osmolarity results in the activation of a conserved module of three consecutively activated tiers of kinases: the stress-activated protein kinases (SAPKs), including the mammalian p38 and the yeast Hog1 [1,2]. In *Saccharomyces cerevisiae*, osmostress activates the HOG pathway, which elicits an extensive program for osmostress adaptation [2–4]. Two upstream branches of the HOG pathway can lead to the activation of the central core of the pathway, which comprises the mitogen-activated protein kinase kinase (MAPKK) Pbs2 and the Hog1 MAPK (Box 1). The first branch involves the Sln1 'two-component' osmosensor, composed of the Sln1 histidine kinase and the Ypd1–Ssk1 phospho-transfer proteins [5–7]. Similar osmosensing systems are in use in bacterial cells, *Dictyostelium*, other fungi and plants but they are not present in mammalian cells despite the conservation of the SAPK signaling cascade in higher eukaryotes. It was well known that in addition to the Sln1 'two-component' osmosensor the HOG pathway had a second mechanism for activating Pbs2 [8]. This second branch of the pathway involves the adaptor transmembrane protein Sho1 and several proteins that, once stimulated by osmostress, participate in the activation of the Ste11 MAPKKK and subsequently the activation of Pbs2 (Box 1) [9]. However, genetic evidence from several laboratories clearly suggested that action of Sho1 alone was not sufficient to explain how osmotic imbalances were detected and indicated that additional components must exist for sensing osmostress. A recent report shows that the mucin-like proteins Msb2 and Hkr1 are involved in activation of the HOG pathway [10]. Given that mucins can activate intracellular signaling cascades in mammalian cells this report suggests a potential mechanism for osmosensing in higher eukaryotes.

Mucin-like proteins Msb2 and Hkr1 activate the HOG pathway

Through a series of elegant genetic studies Saito and colleagues have shown that in yeast the mucin-like transmembrane proteins Hkr1 and Msb2 are the potential osmosensors that activate the Sho1-branch of the HOG pathway in response to high osmolarity [10]. Hkr1 and Msb2 are related proteins with a redundant function in the activation of the HOG pathway. Deletion of the two is required to abolish osmostress sensing, possibly the factor that made their identification using genetic approaches difficult. Hkr1 and Msb2 localize at the plasma membrane, as does the adaptor protein Sho1. They contain a single transmembrane segment and a large extracellular region with a stretch of Ser- and Thr-rich amino acids (STR domain) that contains a tandem of Ser-, Thr- and Pro-rich repeats. These domains, highly conserved among mucins, are the regions that are modified by glycosylation (Figure 1). The STR region of Hkr1 and Msb2 determines their ability to sense osmostress: partial deletion of the STR domains results in hyperactive Hkr1 and Msb2 proteins. These observations, together with the fact that mutations in the Hkr1 STR domain result in altered intracellular signaling, suggest an important role for the glycosylated domains in sensing changes in extracellular osmolarity. It is well known that glycosylated polymers change their properties depending on the degree of water accessibility, raising the possibility that high osmolarity might produce significant volume changes in the STR domains that are then transmitted to the rest of the protein. In addition to the STR domains, a unique conserved region found near the transmembrane extension of Hkr1 and Msb2 (HMH domain) seems to be essential for the function of these proteins, whereas the cytoplasmic domains are mainly dispensable in terms of signaling functionality.

Epistasis analyses clearly demonstrated that Hkr1 and Msb2 are upstream of any known component of the HOG pathway and that they might activate the HOG pathway by more than one mechanism. Activation of Hkr1 and Msb2 requires contact with Sho1 and its transmembrane domains. In addition, Msb2 can signal in response to osmostress via an alternative mechanism that involves its cytoplasmic domain and that is independent of the transmembrane domains of Sho1. Sho1 therefore has two important functions: one, as an adaptor protein to

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Box 1. Schematic diagram of the HOG signaling pathway

There are two branches of the HOG pathway leading to the phosphorylation and activation of the central core of the pathway that comprises the MAPKK Pbs2 and the Hog1 MAPK [16,2–4]. The first branch involves a 'two-component' osmosensor, composed of the Sln1–Ypd–Ssk1 proteins [5–7]. The Sln1 transmembrane protein has intrinsic histidine kinase activity and, using a phospho-relay mechanism involving Ypd1 and Ssk1, it controls the activity of Ssk1, which in turn interacts with and regulates the Ssk2 and Ssk22 MAPKKKs. In addition to the Sln1 'two-component' osmosensor, a second mechanism can activate Pbs2 in the HOG pathway [8]. This was known to involve the transmembrane proteins Sho1 and Msb2 (a mucin-like protein with a single transmembrane segment) although the role of the latter protein had not been mechanistically defined [8,17,18]. Sho1-dependent signaling also requires the small G-protein Cdc42 and the PAK (p21-activated protein kinase) family members Ste20 and Cla4. The transmembrane protein Opy2 (which targets Ste50 to the membrane) and the Ste11-interactor protein, Ste50, are also key components of this branch [19–24]. Once stimulated by osmostress, they participate in the activation of the Ste11 MAPKKK and subsequently Pbs2 [9]. In addition to Sho1, the mucin-like proteins Hkr1 and Msb2 are required for sensing osmostress in yeast [10] (Figure I).

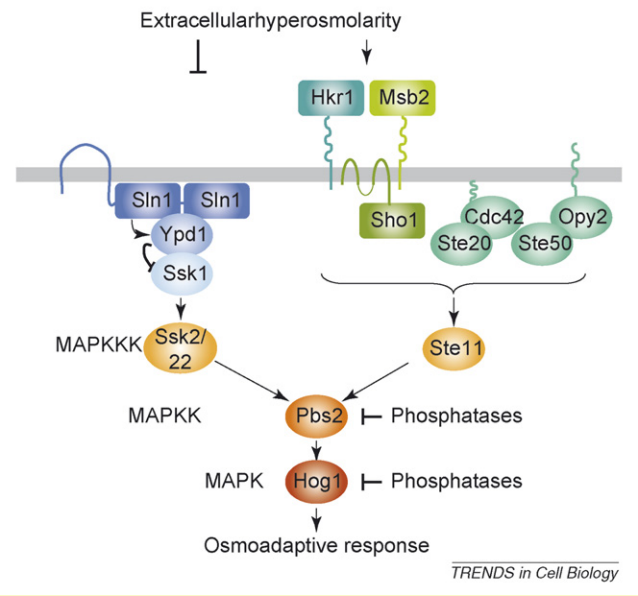

Figure I.

tether several components of the Hog1 pathway at the plasma membrane and another to actively transmit the signal from the upstream sensors. Although the *in vivo* relevance of the alternative mechanisms for activation of the Sho1 branch of the HOG pathway is not fully elucidated and requires further investigation, it is clear that yeast use a complex osmosensing mechanism to sense and transmit changes in the extracellular osmolarity.

Mucins in mammals

Mucins are the main components of mucus, an adhesive, viscoelastic gel covering the surface of internal epithelia and the glycocalyx. Similar to Hkr1 and Msb2 these glycoproteins are characterized by high Ser and Thr residue (STR) content, with these residues being organized as tandem repeats of unique sequences. They are also heavily glycosylated with a carbohydrate content in the range

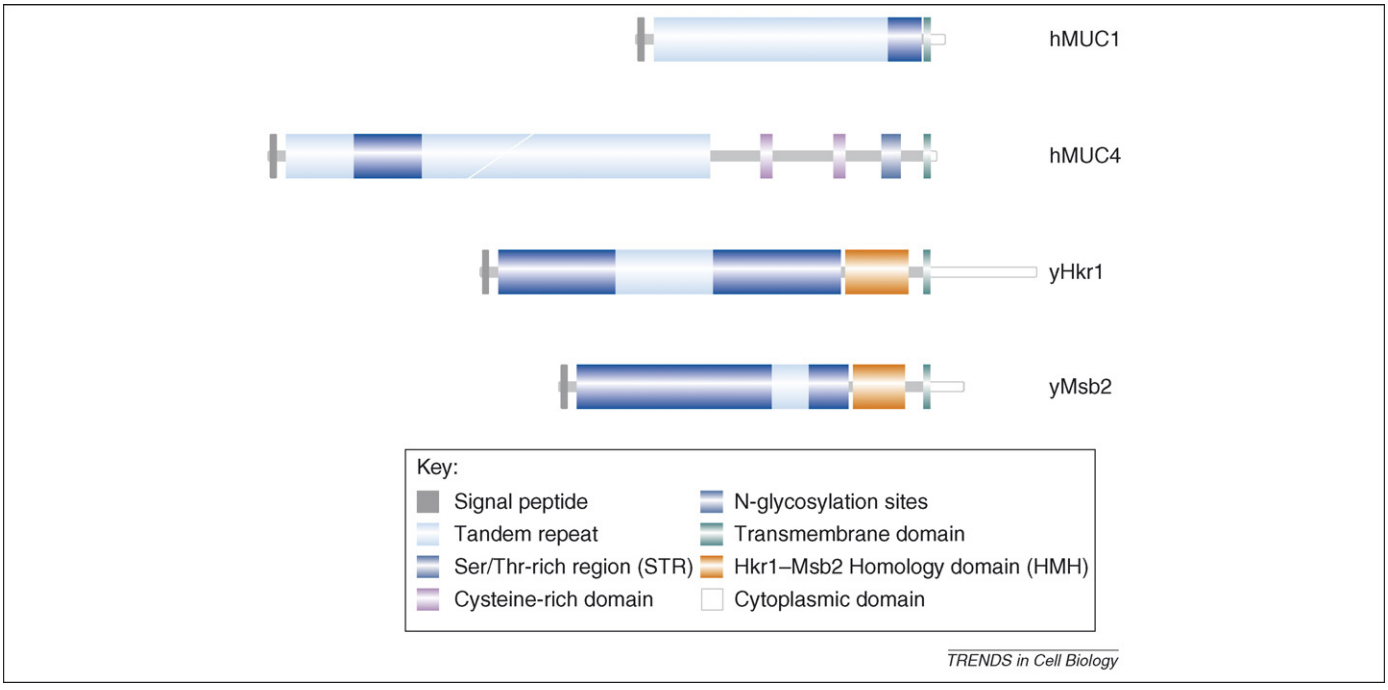

Figure 1. Schematic representation of the structure of the eukaryotic membrane-bound mucins. Comparison of the MUC1 and MUC4 members of the transmembrane mucin family with the yeast Hkr1 and Msb2 mucin-like proteins. The membrane-bound class of mucins are type I proteins with a single transmembrane domain and different lengths of cytoplasmic tails at the C-terminus. Extracellular domains carry a central Ser- and Thr-rich domain (STR), a tandem of Ser- Thr- and Pro-rich repeats (tandem domain) that is highly glycosylated. In addition, Hkr1 and Msb2 have a highly conserved region between them (HMH domain).