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 (MAPK) 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–Ššk1 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...
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 glyocalyx. 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

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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 osmestress, 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 osmestress in yeast [10] (Figure I).

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**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).
of ∼80% of the total mass. Mucin glycans typically share the Ser/Thr-O-GalNAc structure. The mucin family contains secreted or membrane-bound forms. Classical gel-forming mucins are secreted proteins and contain Cys-rich von Willebrand-like domains and C-terminal Cys knot domains involved in the oligomerization required for gel formation. Each of the secreted mucin proteins (MUC2, MUC5AC, MUC5B, MUC6 and MUC19) displays a characteristic tissue expression pattern [11]. The high density of hydroxyl amino acids and the nature of the polypeptide-O-GalNAc in membrane-bound glycoproteins render the extracellular domain of the proteins highly extended and rigid [12]. In gel-forming mucins, these characteristics – together with their oligomerization properties – are essential for the rheological properties of mucus and for ensuring a luminal microenvironment in which epithelial surfaces are hydrated and protected from interactions with microorganisms. Conditions such as extracellular pH, redox milieu and protein–protein interactions (at the peptide or glycan levels) can affect the organization of the glyco-calyx layer covering epithelia [13] and hydration is crucial for maintaining the physiological role of mucins. MUC1 (the first human epithelial transmembrane mucin to be identified) was found in breast cells and a further 12 transmembrane mucins have been cloned and shown to be expressed in other epithelial tissues [14]. They all share STR domains, also organized as tandem repetitive sequences. Unlike gel-forming mucins, they contain cytosolic domains. Today a key defining feature for ‘mucins’ in mammals is the presence of extracellular STR domains, often with tandem repeats, containing a high number of O-GalNAc linked glycan chains.

Over the past few years it has become clear that there is more to mucins than was initially thought. The identification of transmembrane mucins with an intracellular domain – together with the notion that the extended mucin-like extracellular domain can project outwards to sense the environment – led to the hypothesis that mucins can sense and signal. Until now, only the roles of MUC1 and MUC4 in signaling have been explored. The evidence accumulated indicates that mucins can signal through at least four major mechanisms: (i) as coactivators for gene expression (i.e. the cleaved C-terminal domain of MUC1 can be found at promoters regulated by p53 and estrogen receptor α); (ii) through modulation of the activity of Src-kinase family members; (iii) as modulators of Wnt signaling through binding to β-catenin and p120; and (iv) by interacting with – and modulating the effects of – ErbB receptor tyrosine kinases and downstream signaling to the MAPK pathway [15]. The distinct distribution of transmembrane mucins and receptors at the luminal versus the basolateral membranes of epithelial cells provides for additional regulatory mechanisms. Proteolytic events leading to the release of the extracellular or cytoplasmic domains also enable regulation of ‘sensor’ and ‘signal’ events.

**Conclusion: a new role for mucins in eukaryotic cells?** Mucins display attractive features as osmosensor candidates: some are transmembrane proteins with intracellular signaling abilities, they have heavily glycosylated extracellular domains and as described above the structural organization and physical properties of these polymers change dramatically when alterations in the extracellular environment occur. In addition, they have been shown to bind to and detect the presence of cells, proteins and microorganisms. However, a role for mammalian mucins in sensing osmotic imbalances has not yet been proposed. The yeast model has provided unique evidence for a new role for mucins in osmostress sensing. Hkr1 and Msb2 are transmembrane mucin-like proteins coupled to the HOG intracellular signaling pathway, a prototypical SAPK pathway similar to the p38 pathway which is known to be activated in response to osmостress in mammals. The observation that alterations in the STR domains in the extracellular region results in changes of the sensing and signaling abilities of these proteins suggests that glycosylation might be the crucial element responsible for sensing alterations on extracellular osmolarity. Notwithstanding the differences in O-glycosylation of yeast and mammalian cells, a role for mucins in osmostress sensing and signaling in eukaryotic cells is a new and attractive hypothesis.

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