Osmotic stress-dependent serine phosphorylation of the histidine kinase homologue DokA

ABSTRACT

A stimulus-dependent serine phosphorylation of a eukaryotic histidine kinase homologue was demonstrated for the first time in vivo. That implies that DokA, although showing typical structural features of a bacterial two-component system, might be part of a eukaryotic signal transduction pathway that involves serine/threonine kinases.

INTRODUCTION

Background Two-component systems are central elements of the bacterial signaling circuitry. Signal transduction by these systems usually involves autophosphorylation of a histidine kinase on a conserved histidine residue and subsequent transfer of the phosphoryl group to a conserved aspartate on a receiver domain. Until recently, two-component systems had only been found in bacteria. In the past few years, genes coding for histidine kinase homologues and their corresponding receivers have also been discovered in eukaryotic organisms [for a review see]. Most of the corresponding eukaryotic gene products are part of a phosphoryl relay, which consists of a hybrid histidine kinase with a kinase and a receiver domain on the same polypeptide, a histidine phosphotransfer protein and a second receiver as part of a response regulator. The function of eukaryotic two-component systems as histidine kinases was questionable until Posas et al. showed that the Saccharomyces cerevisiae gene product Sln1 acts as a histidine kinase in vitro and in vivo. More recently, histidine kinase activity of the ethylene receptor Etr1 from Arabidopsis was demonstrated in vitro. Further studies showed, however, that eukaryotic two-component systems do not function as independent pathways, but are often connected to serine/threonine- and tyrosine kinase cascades. Thus, the yeast Sln1-Ypd1-Ssk1 phosphoryl relay acts as an osmosensor, which activates a MAP-kinase cascade when cells are exposed to high osmolarity. The Dictyostelium discoideum protein RegA consists of a N-terminal receiver domain and a C-terminal phosphodiesterase domain. Phosphorylation of the RegA response regulator via a two-component phosphoryl relay in turn activates the RegA phosphodiesterase thereby causing a decrease in the intracellular cAMP level. Eukaryotic phytochromes, another class of histidine kinase homologues, were shown to act as light-regulated serine/threonine kinases in vitro instead of acting according to the histidine kinase paradigm. These results suggest that eukaryotic two-component systems, although being homologues of bacterial histidine kinases and receivers, might show post-translational modifications found in the already established eukaryotic signal transduction systems. In the amoeba Dictyostelium discoideum, several genes coding for histidine kinases have been described []. Deletions of individual histidine kinase genes cause different developmental phenotypes such as rapid aggregation, disproportioned fruiting body and stalk ratios or impaired spore formation. Moreover, cells lacking the histidine kinase gene dokA are osmosensitive, i.e. the viability of these cells is decreased when exposed to high osmolarity for up to two hours. Given the evidence that DokA is part of the osmotic response system of Dictyostelium, we have examined whether DokA shows kinase activity in an osmolarity-dependent manner. In this paper, we present evidence that the histidine kinase homologue DokA is phosphorylated on a serine residue in vivo when Dictyostelium cells are exposed to a high osmolarity medium. We further demonstrate that the phosphorylation site is located in a domain homologous to bacterial

histidine kinases and that mutation of the conserved histidine does not affect the serine phosphorylation of DokA.

CONCLUSION

Conclusions We have demonstrated an osmotic stress-dependent serine phosphorylation of the eukaryotic histidine kinase homologue DokA in vivo. The phosphorylation does not depend on the conserved histidine residue, which is essential for the function of two-component systems and is not due to an autophosphorylation reaction. This confirms the idea that eukaryotic homologues of bacterial signal transduction systems might be integrated in signaling pathways involving serine/threonine kinases.