The plant homologue of MAP kinase is expressed in a cell cycle-dependent and organ-specific manner

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Summary

In animals, MAP kinase plays a key role in growth factor-stimulated signalling and in mitosis. The isolation of a Medicago sativa cDNA clone MsK7 which shows 52% identity to animal MAP kinases is reported. The deduced protein sequence shows all the important structural features of MAP kinases and also contains the highly conserved Thr-183 and Tyr-185 residues. Neighbouring amino acid residues within the regulatory domain of MPF into immature oocytes leads to activation of MAP kinase (Gotoh et al., 1991) and S6 kinase II (Haccard et al., 1990).

Two forms of MAP kinase have recently been isolated and designated according to their molecular weights p42\(^\text{mapk}\) and p44\(^\text{mapk}\) (Sturgill and Wu, 1991). In p42\(^\text{mapk}\) the phosphorylation sites have been mapped to Thr-183 and Tyr-185 (Payne et al., 1991). Boulton et al. (1990, 1991) have cloned several MAP kinases and provided evidence for the existence of a MAP kinase gene family of more than three members.

Two very similar kinases (FUS3 and KSS1) have been cloned from budding yeast. They play a pivotal role in the mating pathway. In the presence of a mating factor, the pathway is activated and leads to arrest of cell division. FUS3 transduces signal to SAP1, Cdc42, Cdc-28 and...
Figure 1. Nucleotide and deduced amino acid sequence (single letter code) of MsK7 cDNA.
MsK7 encodes a homologue to MAP kinase

Comparison of the alfalfa MsK7 protein with current protein sequence libraries revealed highest homologies to rat ERK1 (52%) and yeast FUS3 and KSS1 (50 and 49%, respectively). Alignment to the three sequences (Figure 2) shows all four proteins to have a similar structural arrangement of the 11 conserved kinase domains. With the exception of the first 20 amino acids, the sequences are highly similar including the very carboxy terminus. Considerable homology of the MsK7 kinase was also observed to the different homologues of the fission yeast cdc2 kinase (30–40%). Interestingly, the MAP kinases including MsK7 and the yeast FUS3 and KSS1 kinases look like amino and carboxy terminally extended versions of the cdc2 kinases. At each end, 30–40 amino acids are extending the basic cdc2 kinase structure.

MsK7 is expressed in a cell cycle-dependent manner

After washing out the cells, the drug was added to the synchronous division cycle. At different time points, aliquots were removed and analysed by flow cytometry (Figure 3). In asynchronous cultures (Figure 3.0) approximately 70% of the cells were in G1 phase. After treatment with aphidicolin for 24 h, 85% of the cells accumulated at the G1/S transition point (Figure 3.1). When the drug was washed out, the cells were allowed to grow in fresh medium, nearly 60% of the cells passed through S phase synchronously (Figure 3.3) and entered G2 phase after 6.5 h (Figure 3.4). After 10 h, according to microscopic examination, about 30% of the cells were in mitosis. After 15 h, more than 60% of the cells were in G1 again. At the indicated time points in Figure 3 (0 to 8), aliquots were removed and analysed by Northern hybridization with a 32P-labelled MsK7 fragment (Figure 4, upper panel). In asynchronously growing and aphidicolin-arrested G1/S cells the kinase transcript levels were hardly detectable (Figure 4, upper panel, lanes 0 and 1, respectively). However, after release from the aphidicolin block for 5 h, when cells had entered S phase, MsK7 kinase transcript levels had accumulated (Figure 4, upper panel, lane 3). Throughout S and G2 phases, considerable amounts of MsK7 transcripts were observed (Figure 4, upper panel, lanes 3 to 6). When cells entered G1 phase of the next cell cycle (Figure 4, lane 7), MsK7 kinase mRNA levels had decreased to undetectable levels. As a control, the same set of RNAs were hybridized to the alfalfa homologue of the human translationally controlled tumour protein (P53).

Stem- and root-specific expression of MsK7

To investigate the expression pattern of the MsK7 kinase in different organs, RNA was prepared from the following alfalfa organs: young and mature roots, stems, parallel to those of the MsK7 kinase and indicate that the pattern of the MsK7 transcript levels represents true cell cycle-dependent fluctuation.

| MsK7 | 1 | KEGGAPADDTVSDAAPPPQMPGIENIPAVLSHGGPFQYNIFGIVFEVTAKYPPIPMIPPGAYGICSAHSETNEHAVKKIANFQ |
| ERK1 | 1 | EPRGTAGVVPFFGEVEVK.QP,D.GPR,-TQLQY.E...M.S.-YDVRKTR.I...=SP.E |
| FUS3 | 1 | MPKRYVINSSDFQSL-L.E...V...THK.G.I...=EP. |
| KSS1 | 1 | MARTIT.DIPSQ...LVDL.E...T...IHKPSGIK...|=Q.P.S |
| MsK7 | 92 | KIADKRTLREIKLLRH-HHENVVAIRDIVPPQREVNDYIAELMTDLH011RSNQ-------ALSEEHQYFLQLRLGLKHYHANV |
| ERK1 | 66 | HQYTCQ....Q.....I.QGF-R....IG....LRA...TL.AMR...VQ..E...YKLLK.Q....=..ND.I. |
| FUS3 | 49 | KPLF.L....I.K.P-K....ITT..FN.QR...DSP...N....E..IQ....Q....RV...=....M...DD..I...I...AV...VL.GL |
| KSS1 | 49 | KLIYVF...I....YFHE...IIS.LKR...VISDKL.A...LVE...E...QYQ...NRQNGST...FD...V...T....I...A...S...Q. |
| MsK7 | 178 | LHRDKLPSMLLAINCDLKYIDCFLAR-VT---SETO-------F----MTEYUYVTRWAPELLNNSDYYAIVDSVGIFICMELMDR |
| ERK1 | 151 | ITTQ....I.....I.TAPDHEG.Q...=....I...A.....LA...KG...KS....LA...MLSN |
| FUS3 | 134 | I....I....I....I....V....V....V....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I....I...
**Figure 3.** Synchronization of an alfalfa suspension culture.

Flow cytometric analysis of cell synchrony. (0) Asynchronous cells; (1) cells after 24 h of aphidicolin arrest; (3 to 8) synchronized cells after 2.5, 5, 6.5, 8.5, 10.5, 15 and 24 h after release from the aphidicolin block. Samples were taken at the indicated time points and analysed by flow cytometry. The profiles represent the distribution of the cells in the different phases: the left peak consists of cells with 2C levels of DNA, the right peak consists of cells with 4C levels; the area between 2C and 4C peaks represents cells with an intermediate DNA content.

**Figure 4.** Cell cycle-dependent MsK7 transcript levels.

Cells were synchronized by aphidicolin for 24 h and then released from the block. Aliquots were taken after 2.5, 5, 6.5, 8.5, 10.5, 15 and 24 h. From 100 µg total RNA poly(A)⁺ RNA was selected and analysed by Northern hybridization with MsK7 (upper panel) or Msc27 (bottom panel). Lanes represent the following phases: 0, asynchronous cells; 1, aphidicolin-arrested G1/S cells; 2 and 3, S phase; 4 and 5, G2 phase; 6, M phase; 7 and 8, G1 phase.

Nodes, young and mature leaves, flowers and suspension cultured cells. Northern hybridization with a ³²P-labelled MsK7 fragment (Figure 5, upper panel) showed high transcript levels in suspension cultured cells (Figure 5, upper panel, lane 8) and low levels in old roots and stems only (Figure 5, upper panel, lanes 2 and 3, respectively), but not in nodes, leaves or flowers (Figure 5, upper panel, lanes 4 to 7, respectively). The same filter was also hybridized with a ³²P-labelled Msc27 fragment as a control (Figure 5, bottom panel). Clearly, transcript levels of Msc27 are not constant in the different organs. Compared with the transcript levels found in suspension cultured cells (Figure 5, bottom panel, lane 8), relatively high Msc27 mRNA levels were detected in most organs (Figure 5, bottom panel, lanes 1 to 7, respectively) indicating that the organ-specific expression pattern of the MsK7 gene was not due to different amounts of loaded RNA.
cycle phase-specific transcript levels were observed. This disagrees with data reported from *Xenopus*, where no change of MAP kinase transcript levels was observed during mitotic cycles (Gotoh et al., 1991). However, since it is now clear that a family of MAP kinase genes exists in higher plants which are regulated by distinct modes of expression, it will be important to elucidate the tissue-specific and stage-specific expression pattern of these genes and to compare them with the data obtained in this study.

Figure 5. Expression of MsK7 in alfalfa organs.
RNA was extracted from young roots, mature roots, stems, nodes, young leaves, mature leaves, flowers and suspension cultured cells (tanes 1 to 8, respectively). Of each sample, poly(A)+ RNA was selected from 100 μg total RNA and analysed by Northern hybridization with MsK7 (upper panel) or MsC27 (bottom panel).

Discussion

MAP kinases are a family of protein kinase genes involved in re-entry into the cell cycle at G0/G1 and in entry to mitosis. So far, animals and yeast have been shown to contain members of this family. For the first time, we report here the presence of a MAP kinase gene in plants. Using a different approach, another MAP kinase-like gene has been isolated in our laboratory from tobacco (Wilson et al., manuscript submitted). Furthermore, when this work had been completed, a cDNA clone encoding apparently the same MAP kinase was isolated from alfalfa (Jacob, personal communication). The alfalfa and the tobacco gene show about the same homology scores to the animal and yeast kinases, but lack the kinase domain. It is interesting to note that in higher plants, where a large number of MAP kinases have been cloned (Hyams et al., 1995), all of them show a similar organization and domain structure. It is likely that the kinase domain is a key element in the regulation of these kinases. It is expected that the different members of this family show different expression patterns in different tissues and stages of development. In order to address this issue, we are presently preparing antisera to the MsK7 kinase to study the activity of this protein in different organs and cells of alfalfa.
Southern analysis

DNA (10 µg) of M. sativa was digested with HindIII and separated on 0.7% agarose gels. After blotting, the nylon filters were hybridized to the coding sequence of the MsK7 clone. Washing of the filter was done with 2 x SSC, 0.1% SDS for 30 min at room temperature for low stringency. After exposure of the filter, it was washed with 0.1 x SSC, 0.1% SDS for 30 min at 60°C for high stringency.

RNA extraction and Northern analysis of transcript levels

Suspension cultured cells or plant tissue was frozen in liquid nitrogen and extracted by grinding in a mortar as described (Györgyey et al., 1991). Total RNA was quantified by gel electrophoresis and ethidium bromide staining and by spectrophotometry. At each sample 100 µg of total RNA were poly(A)+-purified by Dynabeads according to the manufacturer’s instructions (Dyna, Oslo, Norway). Poly(A)+ RNAs were separated on denaturing agarose gels, blotted to nylon filters and hybridized with a 32P-labelled MsK7 cDNA fragment according to standard procedures.

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References


EMBL Data Library, GenBank and DDBJ databases accession number X66469.