The MsK family of alfalfa protein kinase genes encodes homologues of shaggy/glycogen synthase kinase-3 and shows differential expression patterns in plant organs and development

Aniko Pay1, Claudia Jonak1, Laszlo Bogre1,2, Irute Meskieni1,2, Theresa Mairinger1, Aladar Szalay2, Erwin Heberle-Bors1 and Heribert Hirt1,2
1Institute of Microbiology and Genetics, University of Vienna, Dr. Bohrgasse 9, 1030 Vienna, Austria, 2Plant Molecular Genetics, 6-30 Medical Science Building, University of Alberta, Edmonton, Canada T6G 2H7, and 3Institute of Botany, Lithuanian Academy of Science, Vilnius 2039, Lithuania

Summary

This paper reports on the isolation of a novel class of plant serine/threonine protein kinase genes, MsK-1, the positional information to the involved cells (Martinez-Arias et al., 1988). The predicted protein sequences of some of the segment-polarity genes are consistent with a function in a signal transduction process involved in the determination of cell identity within the segmental unit (Hooper and Scott, 1989; Nakano et al., 1989; Van den Heuvel et al., 1989).

Intercellular interactions also appear to be involved in the determination of cell fates during Drosophila development. For example, the central nervous system arises from a number of neuroblasts that segregate from initially ectodermal cells. If a neuroblast is removed experimentally, a neighbouring cell will change its fate and become a neuroblast (Doe and Goodman, 1985). A process called lateral inhibition normally prevents neighbouring cells from becoming the same in an organiser, but this inhibition fails in the absence of a shaggy-like protein (Bouhafs et al., 1988).

Drosophila shaggy mutants show two seemingly unrelated phenotypes with characteristics similar to both of the above described processes. One type of shaggy mutation results in the loss of growth control of neuroblasts in the embryo and bristle mother the imago (Bouhafs et al., 1989; Simpson et al., 1990). Functional shaggy has been shown to be necessary for the intercellular communication process which regulates the formation of only one bristle mother cell (Simpson and Carteret, 1989). The other type of shaggy mutants abnormal embryos in that all cells develop neural characteristics (Bouhafs et al., 1989). The molecular identity of shaggy has revealed that the gene encodes several serine/threonine protein kinases which are developmentally regulated (Bouhafs et al., 1990; Siegfried et al., 1990). Although the different kinases have identical domains, different amino-terminal extensions are by differential processing.

Shaggy shows 75% identity to glycogen synthase-3/Factor A (Woodgett, 1990). Glycogen synthase-3/Factor A is a multifunctional enzyme that phosphorylates glycogen synthase (Embry et al., 1980; Hemmings et al., 1982). GS-3 was also shown to be identical to Factor A, the activator protein for inorganic pyrophosphate-phosphatase-1 (Hemmings et al., 1982; Vandenheede et

Introduction

In Drosophila, the segment-polarity genes are required for the establishment of the metameric pattern of the embryo, specifying positions of cells within the segments (Nusslein-Volhard and Wieschaus, 1980). The number, position and polarity of the metameric units is dictated by the products of interacting maternal and zygotic segmentation genes. The activity of the segment-polarity genes has been suggested to control the cellular interactions which convey the positional information to the involved cells (Martinez-Arias et al., 1988). The predicted protein sequences of some of the segment-polarity genes are consistent with a function in a signal transduction process involved in the determination of cell identity within the segmental unit (Hooper and Scott, 1989; Nakano et al., 1989; Van den Heuvel et al., 1989).

Intercellular interactions also appear to be involved in the determination of cell fates during Drosophila development. For example, the central nervous system arises from a number of neuroblasts that segregate from initially ectodermal cells. If a neuroblast is removed experimentally, a neighbouring cell will change its fate and become a neuroblast (Doe and Goodman, 1985). A process called lateral inhibition normally prevents neighbouring cells from becoming the same in an organiser, but this inhibition fails in the absence of a shaggy-like protein (Bouhafs et al., 1988).

Drosophila shaggy mutants show two seemingly unrelated phenotypes with characteristics similar to both of the above described processes. One type of shaggy mutation results in the loss of growth control of neuroblasts in the embryo and bristle mother the imago (Bouhafs et al., 1989; Simpson et al., 1990). Functional shaggy has been shown to be necessary for the intercellular communication process which regulates the formation of only one bristle mother cell (Simpson and Carteret, 1989). The other type of shaggy mutants abnormal embryos in that all cells develop neural characteristics (Bouhafs et al., 1989). The molecular identity of shaggy has revealed that the gene encodes several serine/threonine protein kinases which are developmentally regulated (Bouhafs et al., 1990; Siegfried et al., 1990). Although the different kinases have identical domains, different amino-terminal extensions are by differential processing.

Shaggy shows 75% identity to glycogen synthase-3/Factor A (Woodgett, 1990). Glycogen synthase-3/Factor A is a multifunctional enzyme that phosphorylates glycogen synthase (Embry et al., 1980; Hemmings et al., 1982). GS-3 was also shown to be identical to Factor A, the activator protein for inorganic pyrophosphate-phosphatase-1 (Hemmings et al., 1982; Vandenheede et
GSK-3 was reported to phosphorylate the transcription factors c-jun and c-myc. While phorbol esters cause specific dephosphorylation of c-jun, phosphorylation by GSK-3 inhibits its DNA binding activity (Woodgett, 1990).

We report here the isolation of a novel class of plant serine/threonine protein kinases (MsK-1, MsK-2 and MsK-3) which are 65–70% identical to the shaggy and GSK-3 proteins. All of these genes belong to the superfamily of cdc2-like genes. GSK-3, shaggy and the plant genes share a highly conserved catalytic protein kinase domain. Different amino-terminal extensions distinguish different family members. In contrast to shaggy, the plant transcripts do not originate from differential processing of the same gene but are encoded by different members of a gene family. Individual members of this family of plant kinases were found to undergo different transcriptional regulation in various organs and during flower development. Since different shaggy transcripts perform strikingly different functions in Drosophila, the transcription pattern of the different plant homologues suggests a similar diversity of roles in plant development.

Results

Isolation of MsK-1, MsK-2 and MsK-3

To isolate sequences encoding the cdc2 protein kinases from the soybean, we performed one hybridization screen with an alfalfa suspension culture cDNA library probed with the oligonucleotide (K-P-Q-N-L), one clone containing more than 16 nucleotides was isolated. The cDNA was sequenced and then inserts were ligated into an expression vector. Three different classes of cDNA were isolated: a) 1.1 kb (MsK-1), 1.5 kb (MsK-2) and 1.8 kb (MsK-3).

Alignment to a family of highly related protein kinases

Three clones were analysed for homology with sequences of 411, 411 and 412 amino acids for MsK-1, MsK-2 and MsK-3, respectively. Within the open reading frames of MsK-2 amino acid sequence, highly conservation was found in all three clones. Alignment of the predicted protein sequences of the three clones showed a co-linear arrangement of the amino acids over the entire length, as shown in Figure 2. The open reading frames of all MsK clones were very similar. Overall, identities of 83% for MsK-1/2-3 and 88% for MsK-2/3 and 83% for MsK-1/3 were obtained. Although the majority of amino acid differences were found at the N- and C-terminal ends, the presence of conserved domains within these regions was still observed. Analysis of the predicted amino acid sequences revealed that all three genes contain the conserved sequence domains of serine/threonine protein kinases (Hanks et al., 1988).

MsK-1, MsK-2 and MsK-3 are homologues of GSK-3/shaggy and belong to the cdc2 superfamily of protein kinases

A homology search in current protein sequence databanks showed highest similarity to the rat GSK-3 and to the Drosophila shaggy proteins. All predicted MsK proteins were found to have identities of 65–70% to different members of the GSK-3 and shaggy kinases. Significantly homology was also observed to the cdc2 kinases (35–40%) from yeast (Hindley and Phear, 1989; Lörincz and Reed, 1984), humans (Lee and Nurse, 1986), and plants (e.g. Hirt et al., 1991) and to the yeast protein kinases KSS1 (Courchesne et al., 1989), FUS3 (Elion et al., 1990). The close relationship of these soybean clones to established protein kinases found in other organisms indicated that these are the first reported members of a novel superfamily of plant protein kinases.
Alfalfa protein kinases homologous to shaggy/GSK-3

(a) MsK-1

GAG CAC ATG CTT TGG TGG TCT GCC TGG TGG GTA TGG TGG TTA AAA ATT GAA AGG GGT TCA

GTT GCA ATG GGG TGG GTC GGT GCC CCA ACT TCA GGT TTT AGA GAA GTC ATT GAT GGT GAT

GTT GAA ATT GGT GAT GAT GAT GAT AAA ATT AGG GAT

GAT AGA GAA ATG GAA GCC ACC GTT GAT GAC GGC AAT GGA AGC GAG ACA CAA GAT ATC ATT

D R M E M E T V V D G N R T G H I

GTC ACT ACT ATT GTG GTG AGA ATT GTT CAC CAA AAG CAG ACT ATA AGC TAT ATG GCA GAG

V T T I G R N G Q O K Q T I S Y M A E

CGT CTT GTA GGA CAT GTA TCA TGT TCT CAG GCT AAG TGC TGG GTA ACT GCT

R V V G H G S F G V V F Q A K C L E T G

GAA ACC GTG CCT ATC AAA AAG TGT CTT CAA GAC AAG AGG TAC AAG ACC CGG GAA TGG CAA

E T V A I K V L Q D K R Y K N R E L O

NGA ACT ATG CAG CCG TCT GAT CAC CCG AAT GTG GCT GCT TTA AAG CAT GCT TAT TTA TGT TCT TAC

N V V S L K H C F F S T

510

G AAT TTT GTA CTT GAG TAT GTT CTT GAA ACA GTT CAT

N L V L I V P E T V H

570

G TGG AAC CAA AGG ATG CCA ATG ATT TAT GTG AAG GTC

L N I Q R H M P I Y V K L

630

G TTA ATT CAT ATC CAT GTC ATT GAA GTC TGT CAT

L S Y I H R C I G V C H

690

G TGG GTC AAT CCA CAC ACC CAC GAG GCT AAA TTA TGC

L V N P H T H Q V K L C

750

G GAT AAA GGC GAA CCA AAT ATA TCG TAT ATA TGT TCT

V K G E P N I S Y I C S

810

G ATT TTT GGA GCA ACT GAA TAT ACT ACT GCT ATT GAT

I F G A T E Y T T A I D

870

G GCT GAG CTG CTT GGA CAA CGT TCC CCA GTG

A L L L L G Q P L F P F

930

G PAG ATC ATC AAG GTT CTG GCT CAC ACA AGA AAA TTA TGC

E I I K V L G T P T E

990

G TAT ACC GAA TTT AAA TCT CCT CAA ATC AAA GCA CAT

Y E F R F P Q I K A H

1050

G CGG ATG CCT GCA GAA GCT GTT GTT GTA TCA AGA

R M P A E A V D L V S R

1110

G SGG TGC CAA GCT TTA GAT TGC TGG ACC CAT CCT TCT

R C Q A L D C L T H P F

1170

G CCT CGG TTG CCA ACT GGC CCT GTC CTC CCA CCC CGT

A R L P T G R F L P P E

1230

G GAA GGA GTT CCA GTC GAG ACC TATG AAA CGT GTT

K G V P V E T L M K L V

1290

G CCG TTT TTG GCC TGG TTA TAT GTG GTA AAA TGG ACG

F P L G L

1350

G TGA ACG TTC TAT TGG ATG ATA TGA TAT TTA GTA

G A T A G A A A T T A G A T A T G C T A C C A A T A T T A

1410

G AAG TAC CCT TTT CTC GTA CAA CAG ATG ATT GTA ACA

A T C A T C A A T G T T A T C A T T T A A A T T A T T

1470

G AAT ATT GCG TAA AAA AAA AAA AAA AAA AAA AA

The alfalfa MsK cDNA clones.

Figure 1. Nucleotide and predicted amino acid sequences of the alfalfa MsK cDNA clones.

(a) MsK-1; (b) MsK-2; (c) MsK-3.
Figure 1. Continued.
Alfalfa protein kinases homologous to shaggy/GSK-3

<table>
<thead>
<tr>
<th>580</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MsK-3</strong></td>
</tr>
<tr>
<td>TTT TTT TTT TTT TTT TTT TTT TAA AAT ATT TTC CAT TCG TTT CTT CTT TCT TCT TCT CTA</td>
</tr>
<tr>
<td>ATT CCG AAT CCC AGC GAG TCC ACT TCC ATT CAA AGT CAT AGC TAG ACG CAA TCC ATT CCA</td>
</tr>
<tr>
<td>TCC CTC TCT TGG AGT TGA AGA GGT GAT TGG GGG GGT GGA TCT GTA TCA GTC TGT TGT</td>
</tr>
<tr>
<td>AGC TGC TCA TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC</td>
</tr>
<tr>
<td>7.5</td>
</tr>
<tr>
<td>A S G V A P A S G V A P A S G V A P A S G V A P A S G V A P A S G V A P A S G V</td>
</tr>
<tr>
<td>270</td>
</tr>
<tr>
<td>GCA TCA GGT GGT GCT GCA CCT GCT TCT GGA TTT ATA GAT AAG AAT GTA CCA TGC GGT</td>
</tr>
<tr>
<td>ACC TAT AAT ATT GGA GAG AAT AGT GCT GAG GAG TGG GAG GTA GAT CAA GGG GAG AAT AGG</td>
</tr>
<tr>
<td>300</td>
</tr>
<tr>
<td>GAA GTA GGA GGA GGA GGA GGA GGA GGA GGA GGA GGA GGA GGA GGA GGA GGA GGA GGA</td>
</tr>
<tr>
<td>360</td>
</tr>
<tr>
<td>V K E L P E M N D M K I R D D K E M E</td>
</tr>
<tr>
<td>390</td>
</tr>
<tr>
<td>GCC GCT ACC ATT GTA GAT GGA AAT GGG ACC GAA ACC GGA CAT ATA ATT GTC ACG ACC ATT</td>
</tr>
<tr>
<td>420</td>
</tr>
<tr>
<td>A T A T I V D G N E T E T G H I I V I I</td>
</tr>
<tr>
<td>450</td>
</tr>
<tr>
<td>GGT GGT AAA AAT GGC CAG CCA AAG CAG ACA ATA AGT TAC AGG GCC GCT GGT GTA GCT</td>
</tr>
<tr>
<td>GKK KNG QPK QTK TIS YMA ERV VG</td>
</tr>
<tr>
<td>480</td>
</tr>
<tr>
<td>CAT GGA TCT TTT GGT GTA TCT TTT CAG GCA AAG TGT TGG GAG ACT GGA AAG ACT GGT</td>
</tr>
<tr>
<td>510</td>
</tr>
<tr>
<td>HGS FPG V V V Q A K C L E T G E T V A</td>
</tr>
<tr>
<td>540</td>
</tr>
<tr>
<td>570</td>
</tr>
<tr>
<td>ATA AAG AAG GTT CTC CAA GAT AAG AGG TAC AAG AAC CGG CAA TGG CAA ACT ATG CGG CT</td>
</tr>
<tr>
<td>600</td>
</tr>
<tr>
<td>630</td>
</tr>
<tr>
<td>660</td>
</tr>
<tr>
<td>CTG GAC CAC CCT AAT GGT GTA TCT TGT AAG CAC TGC TGC TGC TGC TCA AGC ACT GAA AAG GAC</td>
</tr>
<tr>
<td>L DH N P V S L K H C F F S T E K D</td>
</tr>
<tr>
<td>690</td>
</tr>
<tr>
<td>720</td>
</tr>
<tr>
<td>GAG CTT TAT CTT AAT CTC GTG CTT GAA GAT TAT GGT CTC GAG ACT GTC ACC GCG GTG AGT</td>
</tr>
<tr>
<td>750</td>
</tr>
<tr>
<td>780</td>
</tr>
</tbody>
</table>

**Figure 1.** Continued.
terminal regions as shown in Figure 3. Also within the MsKs, homology is much reduced in the amino-terminal regions (Figure 2). Including these divergent amino-terminal regions, amino acid identities between the MsK and shaggy/GSK-3 kinases amount to scores between 65 and 70% for different pairs.

Figure 3. Alignment of the predicted alfalfa MsK-2, rat GSK-3 and Drosophila shaggy proteins.
Amino acids are represented in the single letter code. Identical amino acids are indicated by dots. Where necessary, gaps were introduced and are denoted by hyphens.

Highly different transcript levels of MsK-1, -2 and -3 in vegetative alfalfa organs and suspension culture cells
To determine the size of the mRNAs of the MsK-1, -2 and -3 genes, Northern analysis was performed with poly(A)^+ RNA from alfalfa suspension cultures. To avoid cross-hybridization, 3' end specific fragments were synthesized by PCR. MsK-1, -2 and -3 genes were found to encode mRNAs of approximately 1.6 kb, 1.5 kb and 1.8 kb, respectively. Whereas MsK-1 was found to be strongly expressed, MsK-2 and MsK-3 transcript levels were very low (data not shown).

To analyse the expression of the three MsK genes in various vegetative organs of alfalfa plants, RNA was prepared from leaves, petioles, stems, roots and nodes. Northern analysis with both total RNA or poly(A)^+ RNA resulted in detection of constitutive transcript levels of MsK-1. However, no transcripts could be observed for MsK-2 or MsK-3, even after 2 weeks of exposure (data not shown). Since we knew from Northern analysis of suspension cultured cells that MsK-2 and MsK-3 transcript levels were very low, transcript levels of all three genes were quantitatively determined by reverse transcribing the RNA samples and PCR amplification with specific primers. The following controls were performed. First, to certify that equal amounts of total RNA were used in the reverse transcription reactions, the RNA samples were quantified by spectrophotometry and by ethidium bromide staining of denaturing agarose gels, as shown in Figure 4a. Second, all reactions were performed at the same time in parallel.

Third, to test whether the RNA preparations contained contaminating genomic DNA traces, PCR was performed with and without the use of reverse transcriptase on the
Figure 4. Reverse transcribed PCR amplification.
(a) To prove that equal amounts of RNA were used per organ, total RNA was isolated from alfalfa leaves, stems, roots, nodes, flower buds, developing flowers, unopened mature flowers, green seed pods and globular, heart-shaped and torpedo-shaped somatic embryos (lanes 1 to 11, respectively). After spectrophotometric quantification, 10 µg of each sample was loaded onto a 1% denaturing agarose gel and stained with ethidium bromide.
(b) To prove that reverse transcription is necessary for PCR amplification of transcripts, total RNA was isolated from suspension cultured alfalfa cells and treated with or without DNase (lanes 1, 2 and 3, 4, respectively) and with or without reverse transcriptase before amplification with PCR (lanes 1, 3 and 2, 4, respectively). 32P-end-labelled cdc2 primers were used. PCR products for the cdc2 gene were separated on a 5% polyacrylamide gel.
(c) To prove linearity of the amplification system, 0.1, 0.5, and 1 µg of total RNA was used for each reaction (lanes 1 to 3, respectively). PCR products for the cdc2 gene were separated on a 2% agarose gel, blotted to nylon filters and hybridized with gene-specific probes. The result of such an analysis is shown in Figure 5. MsK-1 appeared to be constitutively expressed in all five organs (Figure 5a, lanes 1 to 5). In contrast, MsK-2 was found to be absent in leaves and petals but expressed at similar levels in stems, roots and nodes (Figure 5b, lanes 1 to 5, respectively). MsK-3 transcripts were completely absent in leaves and petals, hardly detectable in stems and roots and moderately expressed in nodes (Figure 5c, lanes 1 to 5, respectively). The amplification products of the internal control cdc2 gene (Figure 5d) show that the observed differences in the MsK transcript levels are representative of the steady-state levels of the mRNAs within the different organs.

Changing pattern of MsK-1, -2 and -3 transcript levels during flowering

To analyse whether MsK-1, -2 and -3 genes may play a temporal role during flower development, northern blots of stages of flowers were quantitatively analysed. For this purpose, total RNA was isolated from flower buds (corolla enclosed in the sepals), developing flowers (corolla protruding 2 mm from sepals), unopened mature flowers and green seed pods (lanes 1 to 5 in Figure 6, respectively). MsK-1 appeared to be constitutively expressed as in vegetative tissues (Figure 6a). MsK-2 levels were only

Figure 5. Organ distribution of MsK-1, -2 and -3 transcripts.
Total RNA was isolated from alfalfa leaves, petals, stems, roots and nodes (lanes 1 to 5, respectively). After first strand synthesis, MsK-1, -2 and -3 or cdc2 transcripts were amplified by PCR. PCR products were separated on 2% agarose gels, blotted to nylon membranes and hybridized to 3' probes specific for: (a) MsK-1; (b) MsK-2; (c) MsK-3; (d) cdc2.
present in flower buds (Figure 6b, lane 1). MsK-3 transcript levels were high in early flower development, gradually decreased in later stages and were absent after fertilization (Figure 6c, lanes 1 to 5, respectively).

MsK-1, -2 and -3 transcripts in somatic embryos

In Drosophila, shaggy is developmentally transcribed during embryogenesis, giving rise to different mRNAs in each stage. To analyse the situation of the MsK genes in plants, we determined mRNA levels of the three MsK genes in various stages of alfalfa somatic embryos: globular embryos (lane 1), heart-shaped embryos (lane 2) and torpedo-shaped embryos (lane 3). MsK-1 transcripts were only observed in globular embryos (Figure 7b, lane 1) while MsK-2 and MsK-3 transcripts were moderate in globular embryos and decreased in the later stages (Figure 7c, lanes 1 to 3, respectively).

Discussion

Shaggy belongs to the class of segment-polarity genes, necessary for correct pattern formation within each segment. It also plays a role in epidermal cell differentiation in the adult fly (Bourois et al., 1989; Simpson et al., 1988). Mutations in segment-polarity genes lead to deletions or duplications of parts in each segment. They are thought to act co-ordinately by means of intercellular communication. The transcription factors c-jun and c-myb, glycogen synthase and phosphoprotein phosphatase-1 are substrates of GSK-3. Since both GSK-3 and shaggy are mediators of extracellular signals, they can be envisioned to integrate diverse extracellular signals into a conserved pathway of signal transduction.

In this paper, we report on the isolation of several cDNA clones which encode a novel family of serine/threonine protein kinases in plants. Comparison with current data banks revealed highest homology to the Drosophila shaggy and rat GSK-3 protein kinases (65–70% identities). Significant identity scores were obtained with several other protein kinase genes (30–35%), all of which belong to the cdc2/CDC28 superfamily of protein kinases.
Alfalfa protein kinases homologous to shaggy/GSK-3

Comparison of the predicted protein sequences of the two mammalian GSK-3 genes and the two characterized Drosophila shaggy transcripts revealed high conservation over most of the sequence. However, sequence similarity of the two GSK-3 proteins disappeared in the N-terminal direction at the same conserved amino acid motive where the differentially processed shaggy gene products diverge from each other. Alignment of the MsK proteins with the GSK-3 and shaggy sequences revealed a similar picture.

obtained clear evidence that the MsK genes are differentially transcribed in various vegetative organs and during flowering, it will be important to identify the particular tissues or cells that actively synthesize the kinases. Finally, by expression of mutant versions of the MsK genes, we hope to interfere with their normal function in plants. It will be seen whether, in analogy to Drosophila or mammals, embryogenesis, organogenesis or specific metabolic pathways.
heating the reaction mixture for 3 min at 65°C. The 3' primers for the reverse transcription were: for 3' Ms k-1 (CTA TCA AG TCA TCA TAT GGA A), for 3' Ms k-2 (TAG TAA CAA TTG TCC TAG T), for 3' Ms k-3 (AAC TGG AGA CAT ACG ATT AGA T) and for 3' cdc2 (GCC TGC AGC AAA CTG AAG TGG CT). Annealing of the primers to 0.5 μg of DNase-treated RNA was done by heating to 100°C for 5 min, then slowly cooling to 70°C on a thermocycler, and gradually cooling to 55°C. PCR was performed with the above 3' end primers and the following 5' end primers: for 5' Ms k-1 (AGG AAT TCG AGA GAC CTT GT), for 5' Ms k-2 (ATA AGC TTA TGC AGA TGG TTA GTG T), for 5' Ms k-3 (TGG TGC AGC CTT GTG TTA T), and for 5' cdc2 (GGA AGC TTA GTG AAT GTG T). PCR was done for 20 cycles of 1 min at 92°C, 2 min at 69°C, and 3 min at 72°C. The amplified fragments were run on 2% agarose gels, blotted to nylon membranes and hybridized to 32P-labelled 3'-specific Ms k-1, Ms k-2, Ms k-3 and cdc2 probes.

Acknowledgements

We thank Zarko Hrzenjak for technical assistance. This work was supported by grants S6004 BKO and P8883-MOB from the Österreichischer Fonds zur Förderung der Wissenschaften.

References


EMBL Data Library accession numbers X68411 (Ms k-1), X68410 (Ms k-2) and X68409 (Ms k-3).