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Leishmania MAP kinases – Familiar proteins in an unusual context

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Abstract

Mitogen-activated protein kinases are well-known mediators of signal transduction of higher eukaryotes regulating important processes like proliferation, differentiation, stress response and apoptosis. In *Leishmania*, the typical three-tiered module of MAP kinase signal transduction pathways is present. However, typical activators like cell surface receptors and substrates such as RNA polymerase II transcription factors are missing. Here, I describe the set of 15 putative mitogen-activated protein kinases encoded in the *Leishmania* genome and discuss their potential function.
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1. Introduction

Protein kinases and their opposing regulatory proteins, protein phosphatases, are key regulatory molecules in eukaryotic cells coordinating intracellular processes and relaying signals recognised after environmental changes. In particular, mitogen-activated protein (MAP) kinases and the associated signalling cascades are highly conserved in all eukaryotes. They regulate many important cellular processes such as differentiation, proliferation, cell shape and motility, stress response and apoptosis.

Leishmania parasites undergo profound biochemical and morphological changes when they pass through their life cycle. There is evidence that reversible phosphorylation plays a significant role in the adaptation of the parasite to its different environments in the gut of the sandfly or in the macrophage of the infected mammal (Mukhopadhyay et al., 1988; Dell and Engel, 1994). The insect-stage promastigotes are spindle-shaped cells 10–20 μm in length with a motile flagellum protruding from the flagellar pocket at the anterior end of the cell. Different morphological forms of promastigotes can be distinguished in the insect vector (Gossage et al., 2003; Kamhawi, 2006). The slightly motile, short and ovoid, procyclic promastigotes develop from amastigotes within the first 48 h in the sandfly while the bloodmeal is still enclosed by the peritrophic membrane (PM). In the subsequent 24 h they transform into the non-dividing long slender nectomonad forms which escape from the PM and start to migrate from the abdominal to the thoracic midgut. By day four they change shape again to short, broad, dividing leptomonad forms which produce the promastigote secretory gel filling the thoracic midgut. Finally, after day five the leptomonads differentiate to non-dividing, infectious metacyclic promastigotes that accumulate at the stomodeal valve. Simultaneously, haptomonads which are non-motile, small, broad, leaf-like parasites with short flagella appear. They attach to cuticular surfaces of the gut via hemidesmosome-like expansions of their flagellar tip and form a parasite plug at the stomodeal valve. The metacyclic promastigotes are the highly infective forms which are pre-adapted to the mammalian host. Once taken up by the macrophage the metacyclics differentiate to amastigotes by melting down the flagellum, changing overall shape to a spherical cell and reducing cell volume in a process that likely involves autophagy (Besteiro et al., 2006). This differentiation can be mimicked in vitro by raising the incubation temperature from 27 °C to 34 °C and lowering the pH in the medium from pH 7.5–5.0 (Bates, 1994). The transition from one developmental stage to

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the other is likely regulated by the coordinated action of protein kinases and phosphatases.

The genome project of *Leishmania major* revealed 179 eukaryotic protein kinases (Parsons et al., 2005). This number represents approximately 2% of the *Leishmania* genome and 30% of the human kinome which is the entire complement of the human protein kinases. A striking difference between the *Leishmania* kinome and the human kinome is the lack of receptor-tyrosine kinases and tyrosine kinase-like kinases. However, life stage-specific tyrosine-phosphorylation has been found in *Leishmania* and in related kinetoplastids (Mukhopadhyay et al., 1988; Parsons et al., 1991, 1993, 1995; Dell and Engel, 1994) and protein kinases homologous to dual-specificity kinases are encoded in the respective genomes (Parsons et al., 2005; Li et al., 1996; Wiese et al., 2003a; Kuhn and Wiese, 2005). Kinases of the NEK (*n*ever-in-mitosis/Aspergillus NIMA-related kinase), the STE (homologues of yeast Sterile 7, Sterile 11, and Sterile 20 kinases) and the CMGC groups (*c*yclin-dependent kinases CDK, *M*AP kinases, *g*lycogen-synthase kinases 3, *c*ell *d*ivision *c*ycle-dependent (cdc)-like kinases) represent 56% of the *Leishmania* kinome. Nine MAP kinase homologues have been identified recently in *Leishmania mexicana* using degenerate oligonucleotides encoding two conserved motifs of MAP kinases (Wiese et al., 2003b). In the genome of *L. major* which became available on GeneDB a little later (Ivens et al., 2005) 15 MAP kinase homologues could be identified using the amino acid pattern (TXYXXXRXYRXPE) in the motif search algorithm of GeneDB (http://www.genedb.org). This motif contains two typical features of MAP kinases (Miyata and Nishida, 1999), the dual-phosphorylation motif TXY and the (P + 1)-specificity pocket determining the site-directed substrate phosphorylation at serine/threonine followed by proline. The six putative MAP kinase genes that were initially missed were cloned and sequenced from *L. mexicana* using DNA-probes derived from the corresponding *L. major* homologues to screen a genomic DNA library (Wiese et al., 1995). In addition, two atypical MAP kinases from *L. major* have been identified in the GeneDB database (Parsons et al., 2005) despite the fact that they lack the required (TXYXXXR XYRXPE) signature sequence. These MAP kinases (Accession No.: LmjF03.0210; LmjF13.0780) are thus not considered as bona fide MAP kinases here. Table 1 compiles the potential MAP kinases found in the different trypanosomatid species using the motif search algorithm described above. The 15 MAP kinase homologues present in the completed genome of *L. major* could also be identified in *L. mexicana*, *Leishmania infantum* and *Leishmania braziliensis*. However, as long as the genome sequences of these species have not been completed, no final statement about the number of MAP kinase genes in these organisms could be made. There is no homologue for MPK7 and MPK8 present in the completed genome of *Trypanosoma brucei*. Moreover, the yet incomplete genome of *Trypanosoma cruzi* also seems to lack these kinases. Therefore, it is likely that MPK7 and MPK8 play a role in a developmental stage specific for *Leishmania* and/or may be an adaptation of this parasite to specific environmental conditions.

Fig. 1 shows a sequence alignment of the 15 MAP kinases cloned and sequenced from *L. mexicana*. The proteins vary in length from 358 amino acids for LmxMPK1 to 1579 amino acids for LmxMPK8. All molecules reveal the typical 12 kinase subdomains indicated by roman numerals, however, the core kinase domain which usually consists of around 300 amino acids is extended by a number of insertions in four of these kinases, LmxMPK7, LmxMPK8, LmxMPK12 and LmxMPK15. The insertions are located in hinge regions between secondary structural elements in the typical kinase fold which consists of an amino-terminal lobe comprised of seven β-sheets and two α-helices and a carboxy-terminal lobe which is largely α-helical. The alignment shown here is a complemented complete version of the alignment previously published (Wiese et al., 2003b). Predicted secondary structural elements are aligned to the respective elements known from the crystal structure of p38 MAP kinase (Wang et al., 1997). Gaps produced by the primary sequence alignment were manually corrected to avoid interruption of predicted secondary structural elements. The kinase amino-terminal extensions comprise 3–52 amino acids. In addition, these 15 *L. mexicana* MAP kinases possess carboxy-terminal extensions comprising 52–1,186 amino acids. Long carboxy-terminal extensions have also been found in mammalian MAP kinases ERK5 (400 amino acids), ERK7 and ERK8 (Zhou et al., 1995; Lee et al., 1995; Abe et al., 1999, 2002). The extension is likely to play a regulatory role in ERK5 as only a truncated version shows in vitro activity (Zhou et al., 1995). In ERK7 the carboxy-terminal domain is required for the cellular localization and functions as a negative regulator of growth (Abe et al., 1999). Nothing is known yet about the function of these extensions in *Leishmania*. However, it has been shown in *T. brucei* TbECK1, which is the trypanosome homologue of LmxMPK6, that the carboxy-terminal extension is involved in the regulation of the kinase activity in procyclic trypanosomes. Expression of a mutant protein lacking large parts of this extension in the wild type background led to slow growth associated with the appearance of cells with aberrant karyotypes and morphologies (Ellis et al., 2004). Further mutagenesis known to disrupt the kinase activity reversed the phenotype caused by the expression of the short TbECK1, indicating that its uncontrolled kinase activity caused the phenotype.

Fig. 2 shows an unrooted phylogenetic tree of the 15 MAP kinase homologues from *L. mexicana*. The relationship of the kinases was determined on the basis of the homology of their kinase domains which are depicted in Fig. 1 using ClustalW (http://www.ebi.ac.uk/clustalw/) and the TreeView software (http://taxonomy.zoology.gla.ac.uk/rod/rod.html) (Page, 1996). The two kinases with the longest carboxy-terminal extensions, LmxMPK6 and LmxMPK8, fall into one branch. Likewise,

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