PTI1-LIKE AND PTK-LIKE PROTEIN KINASES


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Comparative analysis of whole sequenced genomes from model eukaryotes shows that roughly 3% of genes encode protein kinases, while functional evidence on phosphoproteomes demonstrates that >30% of cellular proteins is phosphorylated by kinases. So far, most of the >900 putative protein kinases detected in silico in the proteome of Arabidopsis thaliana have been tentatively attributed to the Ser/Thr class; in addition, a few histidine kinases have been identified and recent bioinformatic evidence suggests the presence in Arabidopsis of a complement of putative protein tyrosine kinases (PTKs).

However, in all organisms, complete biochemical characterization concerns only a limited part of the kinase complement, as well as evidence on the involvement of kinases in cell pathways is often incomplete and/or controversial. For this reason, an approach to as yet uncharacterized kinases may contribute to shed light on (or even to unravel) multiple pathways, eliciting further investigations.

We have started cloning of roughly 40 genes from Arabidopsis encoding putative kinases, a number of which – based on bioinformatic markers – is expected to show either tyrosine or dual (Ser/Thr and Tyr) specificity. More recently, we have started also a comparative analysis aimed at identifying, cloning and characterizing homologous kinase genes from Vitis.

So far, cDNAs encoding four such Arabidopsis kinases have been cloned in binary pYES vectors for the expression in S. cerevisiae and subsequent purification as 6xHis-tagged chimerae; cloning of further kinase cDNAs from Arabidopsis and Vitis is in progress.

Preliminary evidence is reported here on (i) the purification and biochemical characterization of a PTI1-like kinase, showing peculiar biochemical properties; (ii) two-hybrid screens and analysis of kinase-interacting proteins, suggesting an involvement and a possible regulatory role in pathogen/stress response pathways and (iii) in silico evidence on receptor-tyrosine kinase (RTK)-like sequences, suggesting that RTKS might play a pivotal role in plant development, in agreement with our previous and unpublished (Floriduz M. et al., submitted) biochemical and cellular data.

References: