

Bioinformatic analysis of plant microtubule and cell cycle regulating kinases

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Protein kinases regulate a number of processes in eukaryotic cells through controlling of protein phosphorylation levels. The genome of model plant *Arabidopsis* contains more than 1000 protein kinase genes and this information is used intensively for clarifying of different peculiarities of plant kinome organization. Recently we shown that tubulin phosphorylation (Blume et al., 2008a) including phosphorylation on tyrosine residues (Blume et al., 2008b) is involved efficiently in plant microtubule functioning.

Because the situation with presence of pure tyrosine kinases in plant genome is still unclear, bioinformatic homology analysis of their animal genes and respective *Arabidopsis* sequence pool was conducted out. Analysis of plant gene similarity to the phosphate-binding regions of animal tyrosine kinases shown, that genes for Zap70 tyrosine kinase family is the most homological to potential plant homologues. tBLASTn (<http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=3702>) scanning of *Arabidopsis* genome against mouse Zap70 catalytic domain detected 503 consensus regions corresponding to 494 protein kinases. It means that only ~ 50% of *Arabidopsis* kinases are homological to animal kinases. These genes are distributed between different chromosomes: 150 - on I, 71 - on II, 94 - on III, 65 - on IV and 114 - on chromosome V. SMART (<http://smart.embl-heidelberg.de/>) analysis shown the correspondence of their products to the models of catalytic domains of S_TKc, STYKc, TyrKc, Pkinase, Pkinase_Tyr, S_TKc, D1phk, D1pme, D1qpc, D1fgka, D1ir3a, D1b6cb, 1a06, 1byg, 1cm8, 1e1v, 1f3m, 1fgi, 1fot, 1fpu, 1fvr, 1g3n, 1gol, 1ia8, 1ian, 1jpa, 1koa, 1pme, 1qcf, 1ql6, 1qpd, 2ptk, 2src, 3lck and d1b6cb kinases. It is interesting, that among potential products, we identified proteins corresponding to HMMs and patterns of catalytic domains of non-typical Tyr-specific kinases (Pkinase_Tyr) and canonical animal and human TyrKc.

To investigate potential role of plant microtubule and cell cycle protein phosphorylation, we bioinformatic search of potential plant homologues of such human kinases, as SLK, PAK5, PAK6, PAK7, MARK1, MAST2, TTBK1, TTBK2, AURKA, PLK1, PLK4, PASK and NEK1 was done. SIB-BLAST ([blastp: http://www.expasy.org/tools/blast/](http://www.expasy.org/tools/blast/)) query against the UniProt Knowledgebase (Swiss-Prot + TrEMBL) was run in the database subsection "Viridiplantae" with standard options (Comparison Matrix – "Auto-select", E threshold=10, Filter the sequence for low-complexity regions – "On", Gapped alignment – "On" and Identity BLAST – "On"). As the target sequences we used the amino acid sequences of the catalytic (Kinase) domains of human kinases, which was identified with the help of the SMART tool. Following-up identification was based on the data of the local and multiplaer aliments, cladistic analysis (N-J), correspondence to the patterns and HMMs, analysis of the databases information. Also we took into account similarity and hits of the residues in the functional important positions: phosphate-binding regions, active center, etc. Basing on these approaches we have identified plant homologues of the human serine-threonine kinases of the SLK (*Vitis vinifera*: A7P2E2), MAST2 (*V. vinifera*: A7PHB5, A7NTE9, A7NXD3, A5BWH0; *A. thaliana*: Q9MB45, Q9LV15, Q94F38, Q8GZ40; *Physcomitrella patens* sp. patens: A9TQ65, A9TUB0, A9T694) and AURORA2 (*Oryza sativa* sp. japonica: Q5SNH4, Q4R1K7; *O. sativa* sp. indica: A2WLL4; *Populus balsamifera* sp. trichocarpa: A9PFI9; *V. vinifera*: A7P4F7, A5BPE0, A7PY12; *Zea mays*: B4F8A1; *A. thaliana*: AUR2, AUR2 Isof.2, AUR1, AUR3) families.

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Ya. Blume, A. Yemets, C. Lloyd (2008) Plant tubulin phosphorylation and its role in cell cycle progression. In: *The Plant Cytoskeleton: Key Tool for Agro-Biotechnology* (Ya.B. Blume, W.V. Baird, A.I. Yemets, D. Breviario, Eds), Springer: Dordrecht, p. 145-159.

Ya. Blume, A. Yemets, V. Sulimenko, T. Sulimenko, J. Chan, C. Lloyd, P. Dráber (2008) Tyrosine phosphorylation of tubulin in plant cells. *Planta*, doi: 10.1007/S00425-008-0816-z.