

Molecular evolution of cytokinin receptors: a phylogenetic analysis

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Cytokinins are ubiquitous plant hormones; their signal is perceived by sensor histidine kinases – cytokinin receptors [1]. Receptor histidine kinases are multidomain proteins combining sensor and effector modules; the sensor domain was named CHASE and serves for cytokinin binding. A phylogenetic analysis using available plant genomes showed that plant CHASE-containing histidine kinases has monophyletic origin, and the number of these receptors is species-specific, rising from 2-3 in lycophyte (*Selaginella*) and moss (*Physcomitrella*) to at least 7 (maize) or 8 (soybean) in angiosperms. CHASE-containing receptors of flowering plants fall into three branches of the phylogenetic tree corresponding to the Arabidopsis cytokinin receptors CRE1/AHK4, AHK3 and AHK2 [2, 3]. Within a single branch, CHASE domains are quite similar, the proportion of fully conserved amino acids exceeds 30%. But if we consider the whole set of cytokinin receptors, this proportion falls drastically to only 8.9%. Interestingly, receptors from primitive plants (*Physcomitrella*, *Selaginella*) do not correspond to these branches [2, 3]. This means that common ancestors of CHASE-containing receptor orthologs appeared in land plants after separation of primitive plants (mosses, lycophytes) from the general evolutionary tree but before its split into monocots and dicots [2, 4].

Not every plant species encodes representatives of all three evolutionary branches of receptors. Only one CHASE-containing hybrid histidine kinase belonging to the orthologs of CRE1/AHK4 and AHK3, respectively, was found in some dicotyledonous plants: potato (*Solanum tuberosum* var. Phureja) and the common monkey-flower *Mimulus guttatus*. Potato has another protein StHK3b belonging to AHK3 ortholog group. This protein consists of CHASE and histidine kinase domains but has no RR-domain. The canonical receptor from potato contains phenylalanine residue instead of the conserved tyrosine (Tyr318 in CRE1/AHK4). Note that tomato (*Solanum lycopersicum*), a close relative of potato, encodes the normal representatives of receptors belonging to all three evolutionary branches. The CRE1/AHK4 ortholog is duplicated in the Fabaceae; four orthologs of CRE1/AHK4 have

been identified. The remaining 4 soybean receptors belong pairwise to the two other evolutionary branches. In lucerne *Medicago truncatula* the only ortholog of CRE1/AHK4 was identified. The common bean *Phaseolus vulgaris* and the legume *Lotus japonicus* have two representatives of the ortholog of CRE1/AHK4 each, but lack orthologs of AHK2 or AHK3. It should be noted that in the receptor PvHK4a from common bean the highly conserved leucine (Leu284) is substituted with tryptophan. Species owing mutated cytokinin receptors harbor also their normal paralogs belonging as a rule to CRE1/AHK4 evolutionary branch. Thus, the general feature of all studied dicotyledonous species (except the common monkey-flower) is the mandatory presence of orthologs of the CRE1/AHK4 receptor.

The monocotyledonous species rice and maize have representatives of two evolutionary branches of receptors, the orthologs of AHK3 and AHK4. The AHK4 group can be divided into two subgroups, corresponding to ZmHK1a and ZmHK1b maize receptors. In corn, two receptors belong to each of these subgroups. However, the foxtail millet (*Setaria italica*) has no ortholog in one of the subgroups. Sorghum (*Sorghum bicolor*) and *Brachypodium distachyon* have 3 receptors but no orthologs of AHK2. Thus, all the known genomes of monocotyledonous plants encode at least one of the representatives of CRE1/AHK4 orthologs which are present predominantly in two versions. According to evolutionary analysis, the putative importance of receptor orthologs may be ranged as follow: CRE1/AHK4>AHK3>AHK2 [3, 4].

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