New structural motifs in the ligand-binding module of cytokinin receptors

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Cytokinin receptors belong to a large family of membrane spanning sensor histidine kinases found in most prokaryotes and some eukaryotes. They are a part of two-component systems which use a sensor kinase and a response regulator for the specific signal transduction up to primary cellular targets [1-3]. Cytokinins are ubiquitous plant hormones controlling plant growth and development. The cytokinin-binding domain of receptors was delineated, like some other sensing domains from pro- and eukaryotes, as CHASE domain [4, 5]. CHASE-containing proteins are widespread in the plant kingdom. In higher plants, CHASE domains are almost exclusively a part of the hybrid histidine kinases. The CHASE and adjacent domains are flanked at both sides with transmembrane helices. The alignment of sequences adjacent to CHASE domain revealed unexpected high level of sequence conservation. In the short (10 residues) linker (D-linker) between CHASE domain and downstream transmembrane (D-TM) helix almost half of residues are highly conserved resulting in core motif CR(F/Y)x(Q/H)K(P/A)P. The motifs of orthologs groups, especially of AHK3 [(CRFKQK(P/A)], are even more conserved. The conservation of the left part of the D-linker can be explained by the fact that it belongs to the proximal PAS-like subdomain in the binding module. The right part of the D-linker evidently participates together with D-TM helix in the interdomain signal transmission. The upstream linker (U-linker) sequence between the upstream transmembrane (U-TM) helix and the CHASE domain appears to be extremely conserved, especially by its right part adjacent to CHASE. This U-linker contains 13 fully conserved residues of 50; this proportion (26%) greatly exceeds the same proportion in CHASE domain (about 9%). Additional 15 residues strongly prevail and altogether these conserved letters constitute core motif of this receptor region:
(S/N)MCD(E/Q)RARMLQDQF(N/S)VS(M/V)NHV(H/Q)A(L/M)(A/S)IL(V/I)STFH(H/Y)

According to 3D-structure of receptors, this region almost entirely corresponds to the long (pivot) α1 helix preceding the CHASE domain. From 3D structure of the binding module, it was evident that this long α-helix holds two subdomains of the CHASE domain [6]. However, the holding function does not necessarily require a high level of amino acid conservation. The recovered conserved motifs suggest more important role for this pivot α-helix in the receptor functioning. This conserved helix may fix the appropriate conformation of the PAS domain and direct its movement upon ligand binding. Also this pivot α-helix together with α2 helix forms a dimerization interface, though conserved N-terminal parts of pivot helices of neighboring receptors do not interact directly. The function of one of the conserved residues of the α-helix is more obvious, that is conserved cysteine which forms disulfide bridge with another conserved cysteine. Both reacting cysteines are located beyond CHASE domain and bring the flanking transmembrane helices into close proximity [3, 6]. The highest degree of conservation of these two cysteines implies an important role of this disulfide bridge in the receptor function.

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1. G.A.Romanov (2012) Cytokinins, *McGraw Hill Encyclopedia of Science & Technol*., **5**:205-207.
2. S.N.Lomin et al. (2012) Receptor properties and features of cytokinin signaling, *Acta Naturae,* **4**(3):31-45.
3. M.Steklov et al. (2013) Structural basis for cytokinin receptor signaling: an evolutionary approach, *Plant Cell Rep*., **32**, in press.
4. V.Anantharaman, L.Aravind (2001) The CHASE domain: a predicted ligand-binding module in plant cytokinin receptors and other eukaryotic and bacterial receptors, *Trends Biochem. Sci.*, **26**:579-582.
5. C.Mougel, I.B.Zhulin (2001) CHASE: an extracellular sensing domain common to transmembrane receptors from prokaryotes, lower eukaryotes and plants, *Trends Biochem. Sci.*, **26**:582-584.
6. M.Hothorn et al. (2011) Structural basis for receptor recognition by *Arabidopsis thaliana* histidine kinase 4, *Nature Chem. Biol*., **7**:766-768.