Detection of a surface-exposed PEST like sequence in the metabotropic glutamate receptor mGluR1α

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Abstract

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PHD protein secondary structure analysis software is a neural-network based method, rating at an expected average accuracy $\sim 72\%$ for the three states helix, strand and loop when using multiple alignment input (Rost and Sander, 1993a,b, 1994). Using the PHD also allows the prediction of solvent accessibility (i.e. surface exposure) of individual amino-acid residues of a protein. PEST sequences are defined as hydrophilic stretches of polypeptide sequences rich in proline, glutamic acid, serine and threonine, and flanked by positively charged residues and are an important feature of eukaryotic proteins with intracellular half-lives of less than 2 h (Rogers et al., 1986; Rechsteiner et al., 1987; Rechsteiner and Rogers, 1996). The data accumulated since 1986 indicate strongly that PEST sequences serve as signals for proteolysis and their presence correlates well with ubiquitination, fast endocytosis and rapid protein degradation (by both proteasome and lysosomal proteases) but not with protein compartmentation or functional activity (Rechsteiner and Rogers, 1996, and references therein). Presence of PEST motifs do not necessarily lead to constitutive degradation of the protein, unless these sequences are surface exposed and not masked by the interacting proteins. If no structural information is available for a protein under study it is therefore necessary to also check for the surface accessibility of the predicted PEST sequences.

The metabotropic glutamate receptors belong to the class of seven transmembrane receptors, which are coupled to G-proteins and mediate intracellular signal transduction. Unusually long C-terminus of the mGluR1α receptor appears to contain various sorting motifs which are critical for correct sorting and targeting of the receptor in neurones and their retention in the plasma membrane (Ciruela et al., 1999a). Recently a family of Homer proteins have been identified among the genes which are induced in neurones of the hippocampus and cortex by excitatory synaptic activity and during development (Brakeman et al., 1997; Kato et al., 1997). The Homer proteins are produced from three genes and a number of various splicing forms of the Homer proteins have been recently described (Soloviev et al., 2000). Homer proteins bind to the Pro.Pro.x.x.Phe.Arg motif found in the C-terminus of mGluR1α receptor and in other proteins. Recently an evidence for the role of Homer proteins in cell surface targeting and anchoring of mGluR1 receptors have been provided (Ciruela et al., 1999b, 2000). Moreover, Ciruela et al. have also reported a significant increase in the amount of total mGluR1α protein detected upon its co-expression with the Homer-1a protein in HEK-293 cells. The mechanism responsible for the increased stability of the mGluR1α proteins is not yet clear. Transcriptional and/or translational control by Homer proteins could be responsible for such changes in the
amount of mGluR1α receptor produced. This is unlikely, however, as no such changes were reported by Ciruela et al. for similarly co-expressed mGluR1β receptor, a splicing variant of the mGluR1α, lacking the Homer binding domain in its C-terminus. Another mechanism for the rapid increase in the amount of mGluR1α protein may be a reduction in the degradation rate of the receptor. Since rapid protein degradation is frequently related to the presence of the PEST sequences, the sequence of the mGluR1α protein have been analysed using ‘PESTfinder’ as well as ‘PHD’ software. The results of the analysis indicate that mGluR1α protein contains a highly scoring PEST sequence in its C-terminus, which is also predicted to be exposed on the protein surface (Figure 1). Strikingly, the PEST sequence overlaps with the Homer-binding motif (boxed sequence, Figure 1). Because the Homer-binding site must be exposed on the surface of the mGluR1α protein, this also confirms surface accessibility of the PEST sequence motif as predicted by the PHD. Overlapping of the PEST and the Homer-binding sites indicates for a new role of Homers as blockers of the mGluR1α internalisation and proteolysis in addition to their role in cell surface targeting and anchoring of the receptor. Fast mGluR1α receptor degradation, which may be downregulated by Homer proteins, may play an important role in neuronal development and plasticity, memory acquisition and learning.

References