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Activation of a Trk receptor is mediated by a disordered extracellular juxtamembrane region

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Motivation and Aim: Tropomyosin receptor kinases (Trks) include TrkA, TrkB and TrkC receptors. They specifically bind neurotrophins to regulate neuronal differentiation, survival and growth. Trks are a prospective target to treat various neurodegenerative and neurological diseases. Recently, allosteric regulation of the TrkB receptor by antidepressant drugs was observed [1]. Further search of potential agonists and antagonists of Trks requires the knowledge of the spatial structure of these receptors and mechanism of activation.

To date, multiple high-resolution NMR and X-ray structures of separate extracellular and intracellular domains of Trk receptors have been obtained, including complexes with ligands. Structure of the dimeric transmembrane domain of human TrkA was resolved. It is commonly considered that Trk receptors dimerize and transduce signal upon ligand binding, but recently it was reported that TrkA additionally requires the mutual rotation of transmembrane domains in the dimer to be activated [2]. The ligand-binding domain is connected to the transmembrane helix through an extracellular juxtamembrane region (eJTM) with a low propensity to form a stable secondary structure, which has not yet been examined. Thus, ligand binding should be somehow conformationally coupled to the transmembrane domain by the eJTM, but the mechanics of this coupling remain obscure. In the present work we aimed to make a structural investigation of the eJTM region and to test the effect of its mutations on the behavior of the TrkA receptor.

Methods and Algorithms: For cellular assays HeLa, PC12 and PC12nnr5 cell lines were used. For NMR studies we produced the construct containing the juxtamembrane region and transmembrane domain of human TrkA (TrkA-eJTM-TMD) using a cell-free expression system based on the E.coli lysate. To assign the protein backbone signals in NMR spectra we employed the standard set of triple resonance experiments along with relaxation measurements to assess the mobility and ordering and diffusion spectroscopy to evaluate the particle radii.

Results: We demonstrated that eJTM is intrinsically disordered and interacts with neither lipid bilayer nor divalent cations. According to cellular assays, replacement of 5 conservative prolines to glycines strongly reduced the affinity of TrkA to its ligand. NMR spectroscopy showed that this replacement made the eJTM on average more compact and helical, but left it disordered. The activating mutation, K410C [2], led to

the formation of a transmembrane dimer different from the previously observed, which should correspond to the active state of TrkA.

Conclusion: Neurotrophin binding to Trk receptors is mechanically coupled to the transmembrane domain through an intrinsically disordered region. Together, our findings strongly support the hypothesis that in addition to the ligand-binding domain, neurotrophins directly bind the disordered eJTM region of Trk receptors.

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