Structure of GDNF Family Ligand Artemin Complexed with Its GFRα3 Receptor

The glial cell line-derived neurotrophic factor (GDNF), neurturin (NRTN), artemin (ARTN), and persephin (PSPN) are GDNF family ligands (GFLs) that are crucial for the development and maintenance of many neurons [1, 2]. The trophic effect of GFLs on the dopamine and motor neurons has stimulated interest in their use for the treatment of neurodegenerative diseases such as Parkinson's. These structurally related neurotrophic factors signal by forming a ternary complex with a nonsignaling, ligand-specific GFRα receptor and a signaling and shared receptor tyrosine kinase RET. Four different GFRα receptors (GFRα1-4) have been identified. The preferential interactions between GFLs and GFRα receptors have also been established as GDNF to GFRα1, NRTN to GFRα2, ARTN to GFRα3, and PSPN to GFRα4 [3]. Given the importance of GFLs in basic neurobiology and their potential therapeutic value, it is a compelling goal to understand the molecular basis of the interactions between GFLs and their receptors.

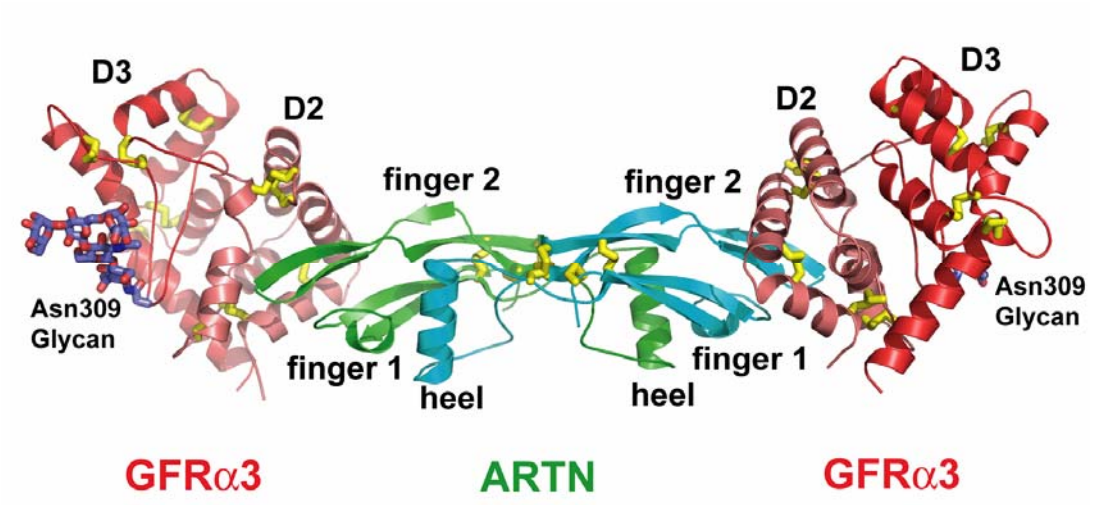

Figure 1. Overall structure the ARTN-GFRα3 complex in ribbon representation. One ARTN homodimer (monomers in cyan and green) binds two truncated GFRα3 receptors (D2 in deep salmon and D3 in red). The observed N-linked carbohydrates at Asn-309 position of GFRα3 are shown as sticks in dark blue. (From Wang *et al*., 2006)

The structures of ARTN-GFRα3 binary complex and unbound ARTN in two crystal forms have been determined by a combination of heavy atom and molecular replacement methods using data collected at SSRL Beam Line 11-1 and at the ALS. The binary complex is composed of one ARTN homodimer and two truncated GFRα3 receptors consisting of the D2 and D3 domains (Figure 1). Instead of being two independent domains as people previously thought, the D2 and D3 domains were packed together to form a globular structure. Both D2 and D3 domains are folded as a triangle spiral, having disulfide bonds in the corners of the triangle to fix the fold. The ARTN monomer structure has two β sheet fingers, a cysteine-knot core motif, and an α-helical heel. Two ARTN monomers form a symmetric homodimer with an inter-chain disulfide bond.

The complex structure of ARTN with GFRα3 revealed a convergent recognition mode for all GFLs. In the ARTN/GFRα3 binding interface, the tip ends of fingers 1 and 2 of ARTN insert

into a pocket in the center of GFRα3 D2 domain surrounded by helices α1, α2, and α5 (Figure 2a). The ARTN/GFRα3 interface has two contact patches in the center, one hydrophobic and one hydrophilic, which are conserved in all GFL-GFRα pairs. The hydrophobic patch is composed of residues Met-199 and Trp-205 of ARTN and Tyr-182, Gly-183,

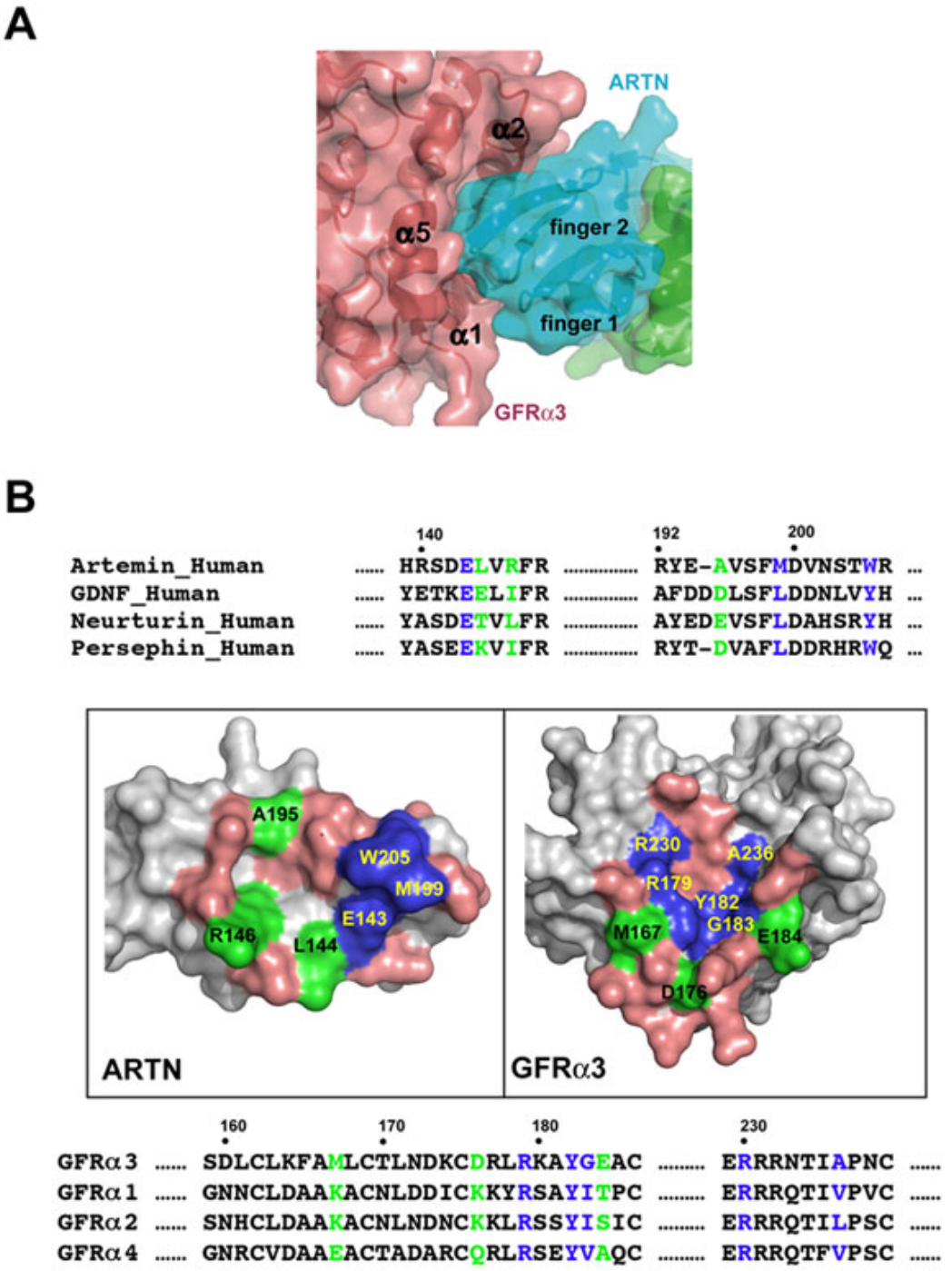

Figure 2. Ligand-receptor contacts between Artemin and GFRα3. (A) Molecular surfaces highlight the knob-in-hole complementarity between the protruding ARTN finger region (green and cyan) and the recessed center of GFRα3 D2 domain (deep salmon) formed by helices α1, α2, and α5. (B) Residues in the common anchor points are colored in blue, and potential binding specificity determinants are colored in green on a background of the total buried surface (light brown). The same colors are applied to the residues in the sequence alignment. (From Wang *et al.*, 2006)

and Ala-236 of GFRα3. All these positions are conserved as hydrophobic residues in other GFLs and GFRα receptors (Figure 2b). Residues Glu-143 of ARTN and Arg-179, Arg-230 of GFRα3 in the hydrophilic patch are strictly conserved in all GFL-GFRα pairs (Figure 2b). Mutations of the conserved positions in GDNF resulted in a completed loss of its binding activity for GFRα1 receptor [4]. While these residues clearly serve as common anchor points, the surrounding non-conserved residues may be responsible for the binding specificity between GFLs and GFRα receptors.

Based on the complex structure and other information, we have proposed two composite RET binding surfaces on the ARTN-GFRα3 binary complex, which would facilitate the recruitment of two RET receptors, leading to the close proximity of RET intracellular tyrosine kinase domains required for the signaling.

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