

It takes two to tango: oligomerization of neurotransmitter transporters and ER export

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Abstract. Neurotransmitter transporters are constitutive oligomers. The working hypothesis underlying the current work postulates that oligomerization supports ER export (and trafficking through subsequent compartments) because it allows for the efficient recruitment of coat proteins such as the COPII proteins Sec24/Sec23. Here we identified motifs that direct the export from the ER and the intermediate compartment (ERGIC). (www.actabiomedica.it)

Key words: GABA-transporter-1, Sec24, COPII, intermediate compartment

Introduction

By using FRET (Foerster resonance energy transfer) microscopy, we have previously shown that members of the Na⁺/Cl⁻-dependent family of neurotransmitter transporters are constitutive oligomers; this was initially explored with the serotonin transporter/SERT) and the GABA-transporter-1 (GAT-1) (1), but the finding have been confirmed with many other transporters of this family. Mutations which disrupt the capacity of the transporter to form oligomers result in intracellular retention of the transporters (2-4). The C-terminus of GAT-1 contains motifs that specify COPII-dependent ER-export and exocyst-dependent membrane insertion (5). Based on these observations, we surmised that ER-export of transporters was contingent on their oligomeric state because the oligomeric arrangement allowed for the rapid assembly of coat components on the nascent export vesicle.

Materials and Methods

The experiments relied on stable and transient

transfections of HEK293 cells with plasmids driving the expression of wild type and mutant versions of GAT1 and SERT. Oligomer formation was assessed by FRET microscopy (1), inward and outward transport by uptake and superfusion experiments (6), the nature of the intracellular compartment by costaining with appropriate markers (5), insertion into the plasma membrane by colocalization with trypan blue fluorescence (7).

Results and Discussion

The last three amino acids of GAT1 recruit the exocyst via a type II-PDZ motif. The proximal motif which directs ER-export is, in fact, comprised of two separate motifs: ER-export of GAT1 was contingent on the binding of Sec24 to the C-terminus of GAT1 (i.e. to ⁵⁶⁶RL⁵⁶⁷).

However, after reaching the ERGIC, trafficking of GAT1 was dependent on a distinct, tri-hydrophobic motif in the C-terminus (⁵⁶⁹VMI⁵⁷¹). Our observations show for the first time that export of a protein from the ERGIC is dependent on amino acid residues provided by the cargo molecule.

References

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