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MEETING ABSTRACT

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Exocyst-dependent trafficking of the wild-type dopamine transporter

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Background: The transfer of material between organelles is mediated by carrier vesicles. Each vesicle transport reaction can be divided into four essential steps: vesicle budding, transport, tethering, and fusion. The exocyst is a multiprotein complex required by many membrane proteins for delivery to and insertion into the plasma membrane. Uptake through the dopamine transporter (DAT) represents the primary mechanism used to terminate dopaminergic transmission in the brain. However, little is known about the specialized trafficking of DAT towards the target membrane. DAT requires an intact C-terminal PDZ-binding motif to reach the cell surface, whereas the closely related serotonin transporter SERT does not. Here, we tested the hypothesis that DAT requires the exocyst for reaching the cell surface.

Methods: HEK 293 or CAD cells were transiently co-transfected with plasmids encoding the wild-type dopamine transporter (DAT) and serotonin transporter (SERT) along with different components of the exocyst, *i.e.* Exo70, Sec6 and Sec8, using jetPRIME (Polyplus). Radioligand uptake, confocal laser scanning microscopy and immunoprecipitation experiments were performed 48 h after transfection to study the effect of exocyst components on trafficking of DAT and SERT.

Results: DAT relied on the exocyst to reach the cell surface. Surprisingly, SERT did not require the exocyst complex to reach the cell surface, regardless of whether the experiments were performed in HEK 293 cells (a cell line of fibroblast origin) or in CAD cells (a Cath.a-cell-derived line of neuronal origin) membrane. We found that three components of the exocyst complex, Sec6, Sec8 and Exo70, separately control trafficking of DAT. Immuno blots also showed the effect of exocyst components on trafficking of DAT as compared to SERT as control.

Discussion: The exocyst mediates DAT targeting to the presynaptic membrane. Identification of proteins as DAT-interactors along with the molecular bases and physiological significance of such interactions will result in a better understanding the role DAT plays in regulating dopamine homeostasis in the brain.

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