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Review

Functional mechanisms of neurotransmitter transporters regulated by lipid–protein interactions of their terminal loops☆

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ABSTRACT

The physiological functions of neurotransmitter:sodium symporters (NSS) in reuptake of neurotransmitters from the synapse into the presynaptic nerve have been shown to be complemented by their involvement, together with non-plasma membrane neurotransmitter transporters, in the reverse transport of substrate (efflux) in response to psychostimulants. Recent experimental evidence implicates highly anionic phosphatidylinositol 4,5-biphosphate (PIP_2) lipids in such functions of the serotonin (SERT) and dopamine (DAT) transporters. Thus, for both SERT and DAT, neurotransmitter efflux has been shown to be strongly regulated by the presence of PIP_2 lipids in the plasma membrane, and the electrostatic interaction of the N-terminal region of DAT with the negatively charged PIP_2 lipids. We examine the experimentally established phenotypes in a structural context obtained from computational modeling based on recent crystallographic data. The results are shown to set the stage for a mechanistic understanding of physiological actions of neurotransmitter transporters in the NSS family of membrane proteins. This article is part of a Special Issue entitled: Lipid–protein interactions.
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1. Introduction

In the past decade the mechanistic evaluation of the structural basis for the function of the neurotransmitter:sodium symporters (NSS) proteins has been enriched greatly by the availability of crystallographically determined molecular models, starting with very first X-ray structure of Leucine transporter (LeuT) [1], the bacterial homolog of the neurotransmitter transporters [2] that rapidly became a prototype for extensive structure-function studies. The initial structure of LeuT revealed a transmembrane (TM) bundle composed of 12 helical segments that incorporated a centrally located primary substrate binding site (S1) and two Na^+ binding sites, Na1 and Na2. These sites were occluded from both intracellular (IC) and extracellular (EC) vestibules exposed to the aqueous environment. Subsequent crystallographic studies

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[3–9] captured LeuT and its mutant constructs (mostly dictated by crystallization requirements, or an engineered Cl^- site [10]), in various other conformations (including outward- and inward-facing), and in complex with distinct ligands in the S1 site, as well as with tricyclic antidepressants in the EC vestibule. A variety of yet additional states that can be accessible to the proteins that share LeuT-like architecture, were inferred from forms of LeuT in the presence and absence of ligands and ions [11], and crystallographic models of other bacterial transporters with similar architecture (the "LeuT fold") [12–21].

The availability of such a rich repertoire of structures has prompted various formulations of mechanistic models of the transport cycle in NSS proteins directly from structure [22–25], consistent with the alternating access mechanism [23]. Not surprisingly, however, experimental and computational studies of the dynamic properties of the LeuT prototype under various conditions showed that these direct approximations from structure were incomplete (see, for example, Refs. [26–28]). Indeed, ensemble and single-molecule level spectroscopic measurements on LeuT [28–31] and on Mhp1 [26], as well as enlightening atomistic scale computational simulations of LeuT [9,27–39] and of homology models of mammalian dopamine (DAT) [40–44] and serotonin (SERT) [45,46] NSS transporters, provided valuable mechanistic insights into ion- and ligand-dependent conformational dynamics in NSS proteins. Together, these findings suggest that the alternating access is achieved through concerted dynamic rearrangements in specific structural motifs of the protein [47]. Indeed, extensive computational modeling and simulations of the system [27,32–38] focused on the detailed manner in which binding of substrate and ions from the extracellular side of the transporter induces conformational changes in the protein leading to the translocation of substrate and ions towards the intracellular vestibule. Furthermore, ligand binding and uptake measurements in LeuT together with computational modeling [32,48,49] have suggested a mechanistic model of allosteric Na^+-coupled symport in which intracellular release of the S1-bound substrate is triggered by the binding of a second substrate molecule in the extracellular vestibule located ~11 Å above the S1 site, termed the S2 site.

Recently, the very first X-ray structure of eukaryotic NSS protein, DAT from *Drosophila melanogaster* (dDAT) in complex with tricyclic antidepressant nortriptyline, has been reported [50]. In this structure, dDAT is in outward-open state, similar to LeuT model stabilized by an inhibitor tryptophan [4], providing novel insights into relationships between the bacterial prototype LeuT, and the eukaryotic neurotransmitter transporters.

Members of the NSS family, in addition to DAT and SERT, include the transporters of the neurotransmitters γ-aminobutyric acid, glycine, and norepinephrine (respectively known as GAT, GlyT, and NET), which are responsible for the clearance of the neurotransmitters from the synaptic cleft and their translocation back into the presynaptic nerve terminal [51–56]. In the function of these NSS, neurotransmitter reuptake into the presynaptic cell is powered by coupling to the transmembrane sodium gradients. Their functions in neuronal signaling have made these transporters primary targets for medications [57], and they have been implicated in the mechanisms of action of abused psychostimulants, such as cocaine and amphetamine (AMPH), and in various psychiatric and neurological disorders that include drug addiction, schizophrenia, and Parkinson's disease [58].

Interestingly, while there have been several reports regarding a role for the membrane in which the NSS are embedded, in their functional mechanisms (e.g., see [59–61]), until recently there had been no experimental evidence to suggest a role for plasma membrane components in specific modes of regulation of the NSS proteins. This was changed by *in vitro* and *in vivo* studies on DAT [62] and SERT [63] that have unambiguously established a direct involvement of PIP_2 (phosphatidylinositol 4,5-biphosphate) lipids in the transport mechanisms of these NSS proteins.

PIP_2 lipids are strongly anionic (with a net charge of −4e at neutral pH) and represent only a minor fraction of the phospholipid composition of the cytoplasmic leaflet of plasma membranes [64,65]. Nevertheless, they regulate many cellular processes by affecting function and organization of both peripheral and integral membrane proteins. For example, PIP_2 lipids anchor various proteins to the cell membrane through interactions with specific sensor domains, such as the pleckstrin homology (PH) domain [66] and have been shown to regulate the function of various channels and enzymes (reviewed in Ref. [67]). The evidence implicating PIP_2 lipids, for the first time, in the function of the NSS is thus much more recent [62,63].

Here we review the recent findings regarding NSS/PIP_2 lipid interactions and discuss their significance to the physiology of the NSS transporters. The evaluation of the mechanisms at the molecular-level that has emerged from combined experimental and computational studies of the phenotypes, is shown to produce novel mechanistic insights regarding the role of the long N- and C-terminal regions of the mammalian NSS members in functional mechanisms regulated by PIP_2 lipids. As the mechanisms of regulation involve direct association of these terminal domains with PIP_2 lipids, we provide a structural context, from computational modeling, for the dynamics of the interactions between PIP_2 lipids and the N-terminus of the NSS proteins and the manner in which functional properties of specific physiological actions of the NSS are affected.

2. Terminal loops as functional units of neurotransmitter transporters

While the crystallographic data shows that the TM bundle of LeuT and dDAT share remarkable similarity, as would be expected for closely related members of the LeuT-fold protein family [1], one of the major structural differences between the bacterial transporters and the neurotransmitter transporters (NT-s) is the presence in NT proteins of long intracellular N-terminal (N-term) and C-terminal (C-term) segments. This difference is expected to be of significant mechanistic importance as intracellular loop regions of membrane proteins are often identified as functional domains. For example, as substrates for kinases in EGFR, and connections to the signalosome of GPCRs (e.g., see [68,69]). In the structures of the NT-s, the N-term and C-term segments contain numerous putative phosphorylation sites (Fig. 1), and various protein kinases have been implicated in the regulation of the function of the transporter proteins on this basis [70–73]. For DAT, specifically, the phosphorylation of the Ser residues distal (farthest from the TM bundle) in the N-terminal segment (Fig. 1) has been shown to be carried out by Ca(2+)/calmodulin-dependent protein kinase II (CaMKII) and to require binding of CaMKII to the C-term of the DAT [74]. Notably, this phosphorylation is involved in the interesting phenotype of amphetamine-dependent efflux whereby the substrate, e.g., DA, is

hDAT

^{1}MSKSKCSVGLMSSVVAPAKEPNAVGPKEVELILVKEQNGV^{40}
^{41}QLTSSTLTNPRQSPVEAQ^{58}

hSERT

^{1}METTPLNSQKQLSACEDGEDCQENGVLQKVVPTPGDKVES^{40}
^{41}GQISNGYSAVPSPGAGDDTRHSIPATTTLVAELHQGERET^{81}

hNET

^{1}MLLARMNPQVQPENNGADTGPEQPLRARKTAELLVVKERN^{40}
^{41}GVQCLLAPRDGDAQ^{54}

hVMAT2

^{1}MALSELALVRWLQESRRSRKLILFIVFLAL^{30}

Fig. 1. Sequences of the N-terminal regions of the human dopamine transporter (*hDAT*), serotonin transporter (*hSERT*), norepinephrine transporter (*hNET*), and vesicular monoamine transporter 2 (*hVMAT2*); positively charged Arg/Lys residues are indicated in red, Ser/Thr residues that are potentially targeted for phosphorylation, are in green.

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