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Proteins Interacting with Monoamine Transporters: Current State and Future Challenges

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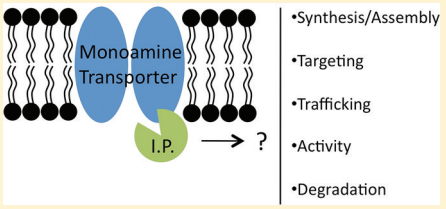
Supporting Information

ABSTRACT: Plasma membrane and vesicular transporters for the biogenic amines, dopamine, norepinephrine, and serotonin, represent a group of proteins that play a crucial role in the regulation of neurotransmission. Clinically, monoamine transporters are the primary targets for the actions of many therapeutic agents used to treat mood disorders, as well as the site of action for highly addictive psychostimulants such as cocaine, amphetamine, methamphetamine, and 3,4-methylenedioxymethamphetamine. Over the past decade, the use of approaches such as yeast two-hybrid and proteomics has identified a multitude of transporter interacting proteins, suggesting that the function and regulation of these transporters are more complex than previously anticipated. With the increasing number of interacting proteins, the rules dictating transporter synthesis, assembly, targeting, trafficking, and function are beginning to be deciphered. Although many of these protein interactions have yet to be fully characterized, current knowledge is beginning to shed light on novel transporter mechanisms involved in monoamine homeostasis, the molecular actions of psychostimulants, and potential disease mechanisms. While future studies resolving the spatial and temporal resolution of these, and yet unknown, interactions will be needed, the realization that monoamine transporters do not work alone opens the path to a plethora of possible pharmacological interventions.



The biogenic amines, norepinephrine, serotonin, and dopamine, play a critical role in the control of motor function, endocrine secretion, appetite, and the complex mechanisms associated with cognition, emotion, mood, and reward behaviors.[1−5] Not surprisingly, dysfunction in the control of monoamine homeostasis has been linked with a variety of psychiatric and neurological disorders, including depression,[6] attention deficit hyperactivity disorder,[7] schizophrenia,[8] Tourette's syndrome,[9] post-traumatic stress disorder,[10] anxiety,[11,12] Parkinson's disease,[13] and drug addiction.[14] Despite early evidence indicating biogenic amines were produced in neuronal tissue, the appreciation for monoamine molecules as bona fide neurotransmitters stemmed from a series of classic experiments examining the fate of labeled norepinephrine in sympathetic nerve terminals.[15] In these early studies, Hertting and Axelrod proposed that neurons were able to store neurotransmitters in nerve endings as a mechanism for regulated release. Specifically, they demonstrated that norepinephrine was recaptured by the presynaptic nerve ending and used for further release.[15] Subsequent experiments also demonstrated similar storage and uptake mechanisms for dopamine and serotonin.[16] Decades of extensive research have shown that these two cellular functions, vesicular storage and plasma membrane reuptake, depend upon the existence of transporter proteins that recognize specific monoamines and selectively generate a pathway for these molecules to cross neuronal lipid bilayers.

Although pharmacological data proposed the existence of neuronal monoamine reuptake, the molecular identity of the proteins responsible for monoamine transport was resolved in the early nineties with the cloning of the genes encoding plasma membrane and vesicular monoamine transporters.[17−25] Plasma membrane transporters for dopamine [dopamine transporter (DAT)], norepinephrine [norepinephrine transporter (NET)], and serotonin [serotonin transporter (SERT)] are encoded by separate genes and belong to a family that also contains transporters for GABA, glycine, proline, and betaine.[26] On the other hand, vesicular monoamine transporters represent a distinct gene family, containing two genes encoding vesicular monoamine transporter-1 (VMAT1)[24] and vesicular monoamine transporter-2 (VMAT2).[25] The gene products of DAT, NET, and SERT share a high degree of homology at the amino acid sequence, and each contains 12 putative transmembrane domains (TMDs), intracellular amino and carboxy termini, and a large, heavily glycosylated, extracellular loop between TMD 3 and 4.[26] VMAT1 and VMAT2 also contain 12 TMDs with amino and carboxy termini extending into the cytosol, as well as a large intralumenal loop between TMD1 and TMD2.[24,25] Despite having similar topologies, VMAT1 and VMAT2 share no sequence homology with plasma membrane monoamine transporters and are more closely related to the vesicular transporter for acetylcholine.[27] At the functional level, plasma membrane monoamine transporters translocate substrates through a sodium- and chloride-dependent mechanism,[28,29] whereas vesicular transporters use the proton electrochemical

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gradient across the vesicular membrane to transport monoamines.[30] Several alternatively spliced NET mRNAs have been identified, differing in the exons that encode residues within the carboxy-terminal domain.[31,32] These variations affect the maturation process and the trafficking of the transporter protein to the plasma membrane.[32] Recently, alternative splicing within intron 3 of DAT was demonstrated in human tissue.[33] Although the physiological significance is unknown, these DAT splice variations result in the introduction of premature stop codons. In addition, multiple single-nucleotide polymorphisms (SNPs) have been found in the coding region of monoamine transporters, some of which have been shown to affect the structure, function, and/or regulation of the transporter.[34]

The identification of the genes encoding the monoamine transporters allowed for the generation of molecular probes and specific antibodies for examination of the expression pattern of the mRNAs, as well as the subcellular localization of the transporter proteins. Within the brain, the anatomical localization of the transcripts revealed plasma membrane monoamine transporters are expressed exclusively in neurons that produce their cognate neurotransmitter[17−23] (Figure 1). In contrast, all monoaminergic neurons within the central nervous system express the VMAT2 gene, while the expression of VMAT1 is restricted to chromaffin cells and the endocrine system.[24,25,35] Immunocytochemical studies localized DAT, NET, and SERT not only to presynaptic nerve terminals but also to somatodendritic compartments[36−38] (Figure 1), suggesting multiple sites of action for monoamine transporters. DAT and SERT molecules are not located at the synaptic active site but, instead, are confined to perisynaptic areas[39,40] (Figure 1), implying that specific targeting mechanisms exist for the proper localization of these transporters. In the case of NET, electron microscopy studies revealed a predominant intracellular localization of the transporter in the nerve terminals of norepinephrine neurons innervating the mouse prefrontal cortex[41,42] (Figure 1). Although the generalizability of these observations needs to be examined, they suggest differential regulation in the trafficking and/or targeting of monoamine transporters.

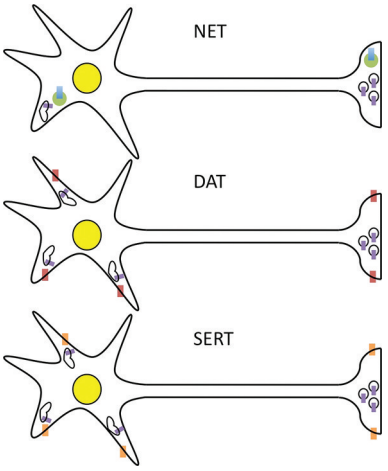

Figure 1. Subcellular localization of monoamine transporters. All three types of monoaminergic neurons in the central nervous system express the vesicular monoamine transporter (VMAT2, purple). VMAT2 is localized to both synaptic vesicles in the axon terminal and to tubulovesicular structures in dendrites. Each type of monoaminergic neuron expresses a distinct plasma membrane transporter specific to their cognate neurotransmitter. The norepinephrine transporter (NET, blue) is localized to endosomal structures in the axon terminals. Both the dopamine transporter (DAT, red) and the serotonin transporter (SERT, orange) are localized to the plasma membrane in both the axon terminal and somatodendritic compartment of their respective neuron.

Cloning of the genes encoding monoamine transporters also allowed for the use of heterologous expression systems to examine the basic biophysical properties and cellular regulation of monoamine transporters. Findings derived from these studies include the presence of channel activity within the transporter molecule,[43−46] the identification of residues involved in substrate translocation,[47,48] the elucidation of the molecular mechanisms associated with reverse transport,[49−52] and the discovery of dynamic trafficking mechanisms.[53−57] Moreover, these observations revealed a far more complex regulation of transporters than previously anticipated and changed the original perception that monoamine transporters existed as static "vacuum cleaners" to a view in which they are seen as highly organized and regulated protein complexes. The past 10 years have seen the undertaking of a systematic search to identify the proteins responsible for the regulation of synthesis, assembly, targeting, trafficking, function, and degradation of transporters. As a result of this intensive research, the number of identified proteins interacting with transporters has grown considerably. In this review, we will describe the life cycle of monoamine transporters and the protein interactions that occur within each distinct phase and discuss the proposed and potential mechanistic implications for these interactions. For the sake of clarity, DAT, NET, and SERT will be termed "plasma membrane transporters", whereas VMAT2 will be termed a "vesicular transporter". The majority of studies have focused on DAT, and as such, the bulk of our discussion will describe experiments using DAT. In addition, a brief section regarding the few VMAT2 interacting proteins that have been identified will be included. We will conclude with a discussion of potential directions and challenges to further the study of plasma membrane and vesicular monoamine transporter interacting proteins.

SYNTHESIS AND ASSEMBLY OF PLASMA MEMBRANE TRANSPORTERS

Synthesis and assembly of plasma membrane transporters occur within the endoplasmic reticulum (ER) and Golgi complex of neurons. Several protein interactions have been identified as being critical for the proper synthesis and assembly of transporters. One of the first proteins to interact with a nascent transporter molecule is another newly produced transporter. There is compelling evidence indicating that monoamine transporters exist as oligomeric complexes in cells,[58−64] and the data are consistent with a model in which oligomerization is required for the efficient exit of transporters from the ER[63,64] (Figure 2A). Several TMDs have been postulated to be involved in the assembly of the transporter oligomeric complex.[63,65−67] Although there are no structural data at the atomic-resolution level for any of the plasma membrane transporters, a recent crystal structure from a bacterial leucine transporter (LeuT) homologue supports the formation of transporter oligomers. LeuT crystallizes as a dimer with subunits adopting a parallel orientation,[68] supporting the

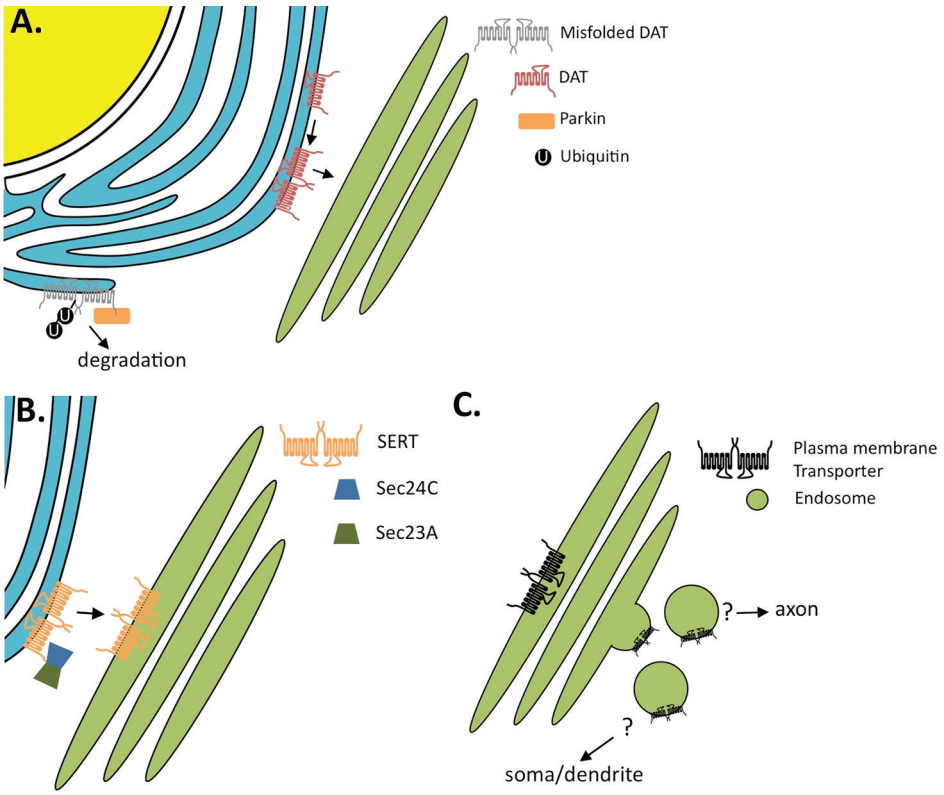

Figure 2. Synthesis and assembly of monoamine transporters. (A) Following synthesis of the nascent transporter, monoamine transporters oligomerize within the endoplasmic reticulum before being exported to the Golgi apparatus. Misfolded DAT is proposed to be degraded by parkin. (B) The COPII component protein, Sec24C, binds to the carboxy terminus of SERT and is proposed to be necessary for ER to Golgi transport. SERT has also been shown to bind Sec23A. (C) Following passage through the Golgi complex, transporters are targeted to axons and the somatodendritic compartments of neurons. The interactions dictating this differential targeting have not been identified.

hypothesis that plasma membrane transporters exist and function as oligomers at the cell surface.

The Parkinson's disease-related E3 ubiquiti ligase, parkin, has been shown to interact with DAT[69] and to regulate the early events involved in the assembly of the oligomeric transporter complex.[70] Overexpression of parkin in SH-SY5Y or HEK-293 cells resulted in increased transporter activity and cell surface expression of DAT.[70] Surprisingly, this effect was accompanied by an enhanced ubiquitination of the transporter. Further investigation revealed that parkin was targeting misfolded oligomers for ubiquitination.[70] These results support a role for parkin in transporter quality control within the ER (Figure 2A). Interestingly, the Parkinson's disease-linked T240R parkin mutant did not mimic the effect of the wild-type parkin protein, suggesting the accumulation of misfolded DAT may be involved in the development of neurodegenerative disorders.

Following proper folding and assembly within the ER, transporter oligomers are exported to the Golgi complex for glycosylation. Both SERT and DAT have been shown to interact with components of the coat protein complex II (COPII), and these interactions are required for export from the ER to the Golgi apparatus.[71−73] Initial experiments with HEK-293 cells showed that the carboxy terminus of SERT was sufficient for the precipitation of Sec24D, and that mutations in the putative Sec24D binding motif resulted in retention of the transporter in the ER.[71,72] More recently, a proteomic study revealed that the carboxy terminus of SERT was able to recruit Sec24C and Sec23A from brain tissue and that depletion of Sec24C from both HeLa and JAR cells resulted in the intracellular accumulation of the transporter (Figure 2B).[73] Interestingly, the cell surface delivery of SERT was shown to be dependent on the expression of Sec24C, not Sec24D. However, the plasma membrane expression of DAT and NET did require Sec24D.[73] Although these multiple Sec24−transporter interactions have not been investigated in neuronal systems, these observations suggest that plasma membrane transporters may use different components of the COPII system for vesicular passage through the ER−Golgi complex.

TARGETING TRANSPORTERS TO THE MEMBRANE

Following assembly and export from the Golgi apparatus, transporters are targeted to the plasma membrane (Figure 2C). As mentioned above, plasma membrane transporters have been found localized to both presynaptic and somatodendritic compartments,[36−38] yet almost no information is available regarding the transporter motifs or the interacting proteins responsible for targeting transporters to these different subcellular localizations. It is tempting to speculate that the differential targeting of transporters to nerve terminals versus somatodendritic compartments is regulated by distinct protein−protein interactions.

Using the carboxy terminus of DAT as bait in a yeast two-hybrid screen, we identified the PDZ domain-containing protein interacting with C-kinase 1 (PICK1) as a DAT-interacting protein.[74] Overexpression of both PICK1 and DAT in HEK-293 cells resulted in colocalization of the two proteins in a clustered pattern and an enhancement of dopamine uptake that was associated with increased levels of plasma membrane DAT. The

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PICK1 binding site was mapped to the last three residues of DAT (LKV), a class II PDZ binding consensus sequence. Interestingly, deletion of these last three residues of DAT impaired the targeting of the transporter to presynaptic structures in cultured neurons.[74] These findings were the first to demonstrate that neurotransmitter transporters are regulated by PDZ domain-containing proteins and suggested that PDZ-mediated interactions might have a role in the presynaptic targeting of DAT. Subsequent studies by Bjerggaard and colleagues also showed that deletion of the PDZ binding consensus sequence dramatically impaired proper maturation and export of the transporter in HEK-293 cells and identified additional carboxy-terminal sequences of DAT that might also participate in transporter targeting.[75] However, when the PDZ-binding domain of DAT was mutated so that the last three residues were replaced with alanine residues (LKV-AAA), the mutant transporter failed to interact with PICK1 but was still expressed in the cell membrane of HEK-293 cells. The authors' interpretation of these observations was that the canonical PDZ binding site was not required for proper targeting of DAT.[75] However, a further study demonstrated that mice expressing the same DAT LKV-AAA mutant, where the PDZ binding domain residues were substituted with alanine, compromised the presynaptic targeting of the transporter in brain,[76] supporting a crucial role of the canonical PDZ binding site in the presynaptic targeting of DAT. The discrepancies in these findings highlight the caution that must be taken when interpreting results from studies using heterologous expression systems in the examination of neuronally specific mechanisms such as synaptic targeting.

TRAFFICKING INTO AND OUT OF THE PLASMA MEMBRANE

Plasma membrane transporters undergo highly regulated trafficking between the cell surface and endosomal compartments (Figure 3). Recently, an immunofluorescent study using rat dopamine neurons in culture, combined with brain tissue fractionation, demonstrated a differential distribution pattern of endosomal markers between somatodendritic compartments and axons.[77] In presynaptic structures, DAT was found predominantly at the plasma membrane and in recycling endosomes, whereas in somatodendritic compartments, DAT was detected in early, late, and recycling endosomal compartments[77] (Figure 3). These findings suggest differential pathways and/or protein−protein interactions mediate DAT trafficking mechanisms in these two cellular compartments. The majority of studies have examined the trafficking of plasma membrane transporters by using heterologous systems, which makes it difficult to determine whether identified trafficking mechanisms are analogous to somatodendritic and/or presynaptic compartments of neurons. In several reports, the use of synaptosomal preparations has provided information relevant to plasma membrane trafficking mechanisms associated with presynaptic transporters.

In general, trafficking of plasma membrane transporters has been shown to occur under basal conditions[78] and can also be induced by transporter substrates,[79−82] inhibitors,[83,84] activation of second-messenger systems,[53−57] and protein−protein interactions.[51,85−101] In the case of DAT, substrates including dopamine and amphetamine induce a biphasic effect on DAT trafficking in heterologous systems. At short exposure times (seconds to minutes), amphetamine and dopamine cause an upregulation of DAT surface levels,[82] whereas longer incubation times (>10 min) induce internalization of the transporter through a clathrin-dependent mechanism.[80,81] Cocaine, a DAT inhibitor, has been shown to upregulate DAT cell surface levels in cells in culture.[83,84]

In addition, second-messenger systems that regulate the trafficking of monoamine transporters include protein kinase A, protein kinase C (PKC), cGMP-dependent protein kinase (PKG), p38MAPK, mitogen-activated protein kinase phosphatase 3 (MKP3), tyrosine kinases, phosphatases, calcium- and calmodulin-dependent kinases, and arachidonic acid.[53−57] Of these, the most studied effect is the internalization of transporters by PKC activation, which is mediated through a clathrin-dependent mechanism in heterologous cells.[102,103] Mutagenesis studies have revealed that phosphorylation of DAT is not required for the PKC-induced internalization of the transporter,[104] but rather for the amphetamine-induced reversal of DAT.[105] Instead, a nonclassical endocytic signal was shown to be necessary and sufficient for both constitutive and

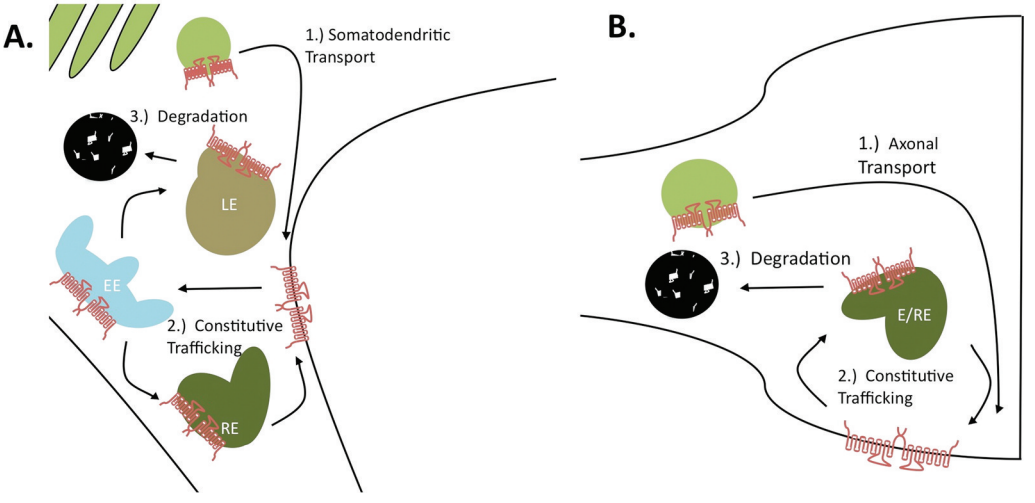

Figure 3. Plasma membrane trafficking of the dopamine transporter. (A) Within the somatodendritic compartment of midbrain dopamine neurons, DAT colocalizes with makers for early (EE), recycling (RE), and late endosomes (LE). (B) In axonal varicosities of striatal-projecting dopamine neurons, DAT colocalizes primarily with recycling endosome markers and is proposed to use an abbreviated trafficking route between the plasma membrane and "early and recycling" endosomes.

Table 1. Interacting Proteins Affecting the Trafficking of Monoamine Transporters

interacting protein	DAT	SERT	NET
PICK1	co-immunoprecipitation (co-IP); overexpression: increased activity and plasma membrane levels in HEK-293 cells	weak yeast two-hybrid	co-IP
SCAMP2	overexpression: decreased activity and plasma membrane levels in HEK-293 cells	overexpression: decreased activity and plasma membrane levels in HEK-293 cells	not determined
PP2A	co-IP	co-IP; pharmacological inhibition of PP2A decreases SERT activity in HEK-293 cells and midbrain synaptosomes and weakens the SERT−PP2A interaction.	co-IP; pharmacological inhibition of PP2A or PKC activation decreases NET activity in slices of vas deferens and weakens the NET−PP2A interaction
PKGIα	not determined	co-IP; downregulation of PKGIα from HeLa cells with small interfering RNAs eliminates the 8-Br-cGMP-induced regulation of SERT activity	not determined
nNOS	weak co-IP	co-IP; overexpression of nNOS decreases SERT uptake and plasma membrane levels in HEK-293 cells; nNOS knockout increases SERT activity and plasma membrane levels	not determined
Hic-5	co-IP; overexpression: decreased activity and plasma membrane levels in HEK-293 cells	co-IP	yeast two-hybrid
syntaxin1A	co-IP; proteolytic cleavage of syntaxin1A increases DAT activity. overexpression of syntaxin1A decreases DAT activity and plasma membrane levels	co-IP; proteolytic cleavage of syntaxin1A decreases SERT activity and plasma membrane levels in thalamocortical neurons	co-IP; proteolytic cleavage of syntaxin1A decreases NET activity in CAD cells and cortical synaptosomes
D2DR	co-IP; overexpression of D2DR increases DAT uptake and plasma membrane levels in HEK-293 cells	not determined	not determined
GPR37	co-IP; GPR37 knockout increases activity and plasma membrane levels in striatal synaptosomes	not determined	not determined
α-synuclein	co-IP; overexpression results in changes in DAT plasma membrane levels in heterologous cells	co-IP; overexpression of α-synuclein decreases SERT activity and plasma membrane levels in Ltk(−) cells	co-IP; overexpression of α-synuclein decreases NET activity and plasma membrane levels in Ltk(−) cells
PKCβ	co-IP; knockout of PKCβ in mice decreases DAT activity and plasma membrane levels in striatal synaptosomes, alters the amphetamine biphasic effect on DAT trafficking	not determined	not determined

PKC-induced internalization of DAT, although different residues within that sequence are more important for one mechanism than the other.[106]

Protein−protein interactions also affect the trafficking of transporters, and the vast majority of the identified transporter interactions have been studied at this level of regulation (Table 1). These interactions can be grouped into (a) signaling molecules, including protein phosphatase 2A (PP2A),[85] the β isoform of protein kinase C (PKCβ),[51,86] cGMP-dependent protein kinase Iα (PKGIα),[87] and the neuronal nitric oxide synthase (nNOS);[88] (b) G protein-coupled receptors, including the dopamine D2 receptor (D2DR)[89] and orphan G protein-coupled receptor 37 (GPR37);[90] (c) presynaptic proteins, including syntaxin1A[91−93] and α-synuclein;[94−97] and (d) miscellaneous proteins, including Hic-5,[98] SCAMP2,[99,100] and flotillin-1.[101]

All three transporters have been shown to co-immunoprecipitate with the catalytic subunit of PP2A (PP2Ac) from neuronal preparations.[85] In midbrain synaptosomes and SERT-transfected HEK-293 cells, inhibition of PP2A with okadaic acid resulted in decreased uptake activity concomitant with a reduction in the amount of PP2Ac interacting with the transporter. Activation of PKC with phorbol esters also decreased the amount of PP2Ac interacting with SERT in HEK-293 cells. Similarly, in vas deferens slices, a NET-enriched preparation, β-PMA, or okadaic acid treatment resulted in a reduction in transporter activity and a decrease in the amount of PP2Ac that co-immunoprecipitated with NET.[85] Thus, signaling pathways that activate PKC lead to internalization of SERT or NET concomitant with the dissociation of the SERT−PP2A interaction, which suggests the PP2A−transporter interaction occurs at the plasma membrane. Interestingly, in the case of SERT, the effect of PKC activation was blocked by serotonin,[85] leading the authors to suggest that the SERT−PP2Ac interaction could be regulated in an activity-dependent manner.

More recently, an interaction between DAT and PKCβ has been demonstrated by co-immunoprecipitation.[51] Analysis of the PKCβ knockout mouse showed a decreased level of dopamine uptake, a reduced level of amphetamine-induced dopamine efflux, and a decreased level of cell surface DAT.[86] Furthermore, the effects of amphetamine on DAT trafficking were opposite to those observed in wild-type mice. In striatal synaptosomes of PKCβ knockout mice, amphetamine caused DAT internalization with short incubations and upregulation of DAT cell surface levels with longer incubation periods.[86] This aberrant trafficking of DAT in response to amphetamine was mimicked in synaptosomes from wild-type animals by preincubation with the PKCβ inhibitor, LY379196. Although it is not clear whether PKCβ is involved in the PMA-induced internalization of DAT, the observations of Chen and colleagues suggest that PKCβ is a regulator of amphetamine-induced trafficking of DAT in vivo.

Motivated by studies in which the activation of the adenosine A3 receptor (A3R) led to an increase in SERT activity through a mechanism involving cGMP and PKG activation,[107,108] Steiner and co-workers demonstrated a physical and functional interaction between SERT and PKGIα.[87] In the immortalized serotonergic raphe neuronal cell line RN46A, SERT colocalized and co-immunoprecipitated with PKGIα. Blockade of PKGIα with the specific inhibitor DT-2 prevented both the 8-Br-cGMP stimulation of SERT in RN46A cells[87] and the increased SERT

activity observed after A3R activation in brain synaptosomes and in the intact hippocampus.[108] Interestingly, several SNPs altering the coding sequence of SERT (T4A, G56A, E215K, K605Q, and P612S) were insensitive to PKG activation.[109] Likewise, a SERT protein containing the I425V mutation, which has been associated with obsessive-compulsive disorder, did not show the increase in uptake activity as a result of PKG activation.[110] These observations suggest that psychiatric conditions associated with plasma membrane transporter SNPs may, in part, be explained by alterations in trafficking mechanisms.

Using a proteomic strategy, Chanrion and co-workers identified an interaction between the carboxy terminus of SERT and several proteins, including nNOS.[88] In HEK-293 cells, overexpression of nNOS decreased SERT activity through a mechanism involving a reduction of transporter cell surface levels. Consistently, SERT activity was increased in synaptosomes from nNOS deficient mice and synaptosomes, from wild-type mice, in which the SERT−nNOS interaction was disrupted with the use of an interfering peptide.[88] The authors also showed that in HEK-293 cells, activation of the transporter with serotonin enhanced the activity of nNOS, demonstrating a reciprocal, functional interaction between SERT and nNOS. Because NO produced by nNOS activation can increase the intracellular level of cGMP through activation of guanylyl cyclase, and cGMP can activate PKGIα, it is possible that changes in the trafficking of SERT by nNOS may be mediated via PKGIα. Nevertheless, these results further demonstrate the importance of interactions between transporters and signaling molecules in the regulation of trafficking mechanisms and the potential implications these interactions might have in psychiatric disorders.

G protein-coupled receptors (GPCRs) also modulate transporter cell surface levels through protein−protein interactions. The first evidence suggesting a functional coupling between GPCRs and plasma membrane transporters came from experiments in which activation or inhibition of D2DR resulted in changes in the activity of DAT.[111,112] However, it was unclear from these studies whether this was due to a direct interaction between DAT and D2DR or a consequence of the activation of second-messenger cascades. The first evidence that DAT and D2DR form a protein complex came from Lee and co-workers.[89] Using a set of GST fragments, Lee and co-workers found that the first 26 residues of the amino terminus of DAT precipitated the D2DR from rat striatal lysate, and conversely, residues 242−344 from the third intracellular loop of the D2DR were sufficient to precipitate DAT. Cotransfection of DAT with D2DR resulted in an increase in the level of dopamine uptake in HEK cells, which was unaffected by pretreatment of these cells with the D2 antagonist, haloperidol. This increase in the level of uptake correlates with increased levels of DAT at the plasma membrane. The in vivo relevance of this interaction was investigated by using interfering peptides to disrupt the DAT−D2DR interaction in mice.[89] Injection of a cell membrane permeant peptide fused to the first 15 residues of DAT decreased the amount of transporter that co-immunoprecipitated with D2DR and resulted in a reduction in DAT uptake activity. At the behavioral level, these animals showed an increase in locomotor activity, in terms of distance traveled and rearing.[89] Interestingly, a follow-up study, using post-mortem tissue from several human patient groups, revealed a significant decrease in the amount of D2DR that co-immunoprecipitated with DAT in patients diagnosed with schizophrenia, compared to control subjects.[113] These results highlight the importance of DAT protein−protein interactions not only in normal brain function but also in the pathology of psychiatric disorders like schizophrenia.

The orphan G protein-coupled receptor, GPR37, has also been found to regulate DAT trafficking through a protein−protein interaction with the transporter.[90] While studying *Gpr37* null mice, Marazziti et al.[114] observed a reduction in the striatal dopamine level, locomotor defects, increased sensitivity to amphetamine, and decreased responsiveness to the DAT inhibitor, cocaine. Subsequent experiments showed an increase in the level of dopamine uptake in brain synaptosomes from the *Gpr37* knockout compared to wild-type mice that correlated with an increase in the amount of DAT at the plasma membrane.[114] Using transfected HEK cells, an interaction between GPR37 and DAT was demonstrated by co-immunoprecipitation.[90] Whether this interaction is regulated by a GPR37 ligand, such as the neuropeptide head activator, remains to be investigated.

All three plasma membrane transporters have been shown to form protein complexes with syntaxin1A,[91−93,115] a neuronal membrane protein that plays a crucial role in membrane trafficking and neurotransmitter release.[116] Early experiments with the related GABA transporter (GAT) suggested a complex effect of syntaxin1A on transporter rates and trafficking mechanisms.[117] In hippocampal neurons where syntaxin1A was shown to co-immunoprecipitate with GAT, cleavage of syntaxin1A with botulinum toxin C1 (BoNT/C1) produced a seemingly paradoxical effect, an increase in the level of GABA uptake accompanied by a decrease in the cell surface levels of the transporter.[117] The syntaxin1A interaction site was mapped to the amino terminus of GAT, a domain involved in the regulation of substrate translocation. By binding the amino terminus, syntaxin1A prevents the effect of this domain on the translocation process and, as a result, inhibits uptake.[117] In parallel, interaction of syntaxin1A with the transporter increases the level of protein expression at the plasma membrane,[117] consistent with the role of syntaxin1A as a positive regulator of membrane trafficking and fusion. In the case of SERT, immunoprecipitation experiments with neurons in culture demonstrated a physical interaction with syntaxin1A.[91] The SERT−syntaxin1A interaction was disrupted by BoNT/C1 incubation and resulted in decreases in SERT uptake activity as a result of decreased cell surface expression levels. These results are interesting in comparison to the interaction between GAT and syntaxin1A. While syntaxin1A is capable of altering both the plasma membrane levels and catalytic activity of GAT, it only regulates the expression of SERT at the plasma membrane without changing the intrinsic catalytic activity. The interaction between NET and syntaxin1A also presents similarities and differences with respect to that described for GAT.[92,118] Among the similarities is the fact that the amino terminus of NET (residues 2−42) is required for the interaction with syntaxin1A. However, in contrast to the stimulatory effect of BoNT/C on GAT activity, cleavage of syntaxin1A with the toxin decreased NET activity in several preparations, including synaptosomes from brain cortex, slices from vas deferens, and heterologous cells in culture.[92] A role of syntaxin1A in the trafficking of NET was suggested by experiments in which several pharmacological manipulations known to alter the trafficking of NET (activation of PKC, changes in intracellular calcium, or amphetamine) were shown to

alter the amount of syntaxin1A co-immunoprecipitating with NET.[92,118,119] BoNT/C treatment not only prevented the internalization of NET induced by either activation of PKC or inhibition of PP2A but also disrupted the NET−syntaxin1A interaction.[92] An increasing intracellular calcium level also resulted in a loss of the NET−syntaxin1A interaction concomitant with an increase in NET activity and cell surface levels of the transporter.[118] Amphetamine, which also induces NET internalization, strengthened the NET−syntaxin1A interaction at the plasma membrane.[119] In contrast to the effect of BoNT/C on NET activity, experiments in striatal slices revealed that cleavage of syntaxin1A increased DAT activity but did not alter the PKC-induced transporter internalization.[93] In the non-neuronal LLCPK1 cell line, overexpression of syntaxin1A decreased DAT activity and transporter cell surface levels without alterations in the PKC-induced downregulation of transporter activity. Together, these observations support a model in which syntaxin1A regulates the delivery and/or recycling of plasma membrane transporters to the cell surface in a transporter-specific manner.

An additional interaction that modulates the trafficking of monoamine transporters involves α-synuclein.[94−97] Synucleins make up a family of cytosolic proteins containing an α-helical amino-terminal domain that binds phospholipid vesicles and are found predominantly in presynaptic nerve terminals.[120] Mutations in α-synuclein have been linked to familial cases of Parkinson's disease, and aggregates containing α-synuclein immunoreactivity are found in post-mortern brain tissue from both Alzheimer's and Parkinson's disease patients.[120] A physical interaction between α-synuclein and the last 22 residues of DAT was first reported by Lee and colleagues.[94] Overexpression of α-synuclein resulted in increased DAT activity in LTK− cells concomitant with increased cell surface levels of the transporter.[94] This interaction was confirmed by Werstinger and Sidhu who reported that overexpression of α-synuclein resulted in decreased activity and cell membrane levels of DAT in several cell lines.[95] These findings suggest that α-synuclein affects the trafficking of the transporter; however, the direction of the effect appears to depend on the heterologous system of choice and/or levels of protein expression. In a follow-up study, Sidhu's group showed that the inhibitory effect of α-synuclein on DAT trafficking was dependent on the integrity of the microtubule structure.[121] Pharmacological agents that disrupt microtubule dynamics prevented the effect of α-synuclein overexpression on DAT uptake and cell surface levels.[121] Additional studies have shown that trafficking of NET and SERT is also modulated by α-synuclein.[96,97] Experiments examining DAT activity in α-synuclein knockout mice have produced conflicting results. In one study, DAT uptake activity in striatal synaptosomes from α-synuclein knockout mice was not affected,[122] whereas in a different study, measurements of dopamine uptake in dorsal striatum of α-synuclein knockout mice by voltammetry showed a reduction in DAT activity.[123] Although the data supporting the physical interaction between DAT and α-synuclein are compelling, the discrepancies obtained from different model systems make it difficult to interpret the true functional consequences of this protein−protein interaction.

Plasma membrane transporters have also been shown to interact with the LIM domain-containing, focal adhesion protein, Hic-5.[98] This interaction was first identified with the carboxy terminus of DAT and NET using the yeast two-hybrid system and confirmed by co-immunoprecipitation experiments in both transfected HEK cells and neuronal tissue. Using GST precipitation assays, the carboxy-terminal LIM domain of Hic-5 and residues 561−590 of DAT were sufficient for this interaction. Functionally, overexpression of Hic-5 in HEK-293 cells decreased the level of dopamine uptake through a reduction in the amount of DAT at the plasma membrane.[98] A similar interaction between SERT and Hic-5 was also described in human platelets.[124] Serotonin, SERT inhibitors, and PKC activators modulated the strength of the interaction between SERT and Hic-5. Activation of PKC with phorbol esters caused an increase in the amount of SERT that co-immunoprecipitated with Hic-5, as well as a decrease in the amount of the transporter that could be detected at the plasma membrane.[124] Although the Hic-5−SERT interaction needs to be examined in neuronal tissue, these results suggest that Hic-5 may serve as a scaffolding protein involved in the regulation of transporter endocytosis.

Additionally, both DAT and SERT have been shown to interact with secretory carrier-associated membrane protein-2 (SCAMP2),[99,100] a protein involved in exocytotic trafficking. Overexpression of SCAMP2 in HEK-293 cells transfected with SERT or DAT resulted in a reduction in transporter activity with a concomitant decrease in the cell surface levels of the transporters.[99,100] In the immortalized serotoninergic rat raphe cell line RN46A-B14, SERT and SCAMP2 colocalized to intracellular compartments that also contain a lipid raft marker, flotillin-1, and syntaxin1A. These findings led the authors to suggest that these intracellular compartments containing SERT and SCAMP2 could represent recycling endosomes, as these compartments have been shown to be enriched in lipid raft markers, including flotillin. Recently, an interaction between DAT and flotillin-1 was demonstrated by co-immunoprecipitation in HEK-293 cells.[101] Two lines of evidence suggest that flotillin-1 is required for the PKC-induced internalization of DAT: the amount of DAT co-immunoprecipitating with flotillin-1 increased after PKC activation, and depletion of flotillin-1 from HEK-293 cells abolished the PKC-induced internalization of DAT.[101]

PROTEINS REGULATING TRANSPORTER FUNCTION

Plasma membrane transporters carry three intrinsic activities: inward substrate translocation, substrate efflux, and ion conductances.[53] At the physiological level, the significance of monoamine uptake in the termination of monoamine transmission and recycling of neurotransmitter has been extensively studied. In the case of DAT, deletion of the DAT gene in mice leads to a dramatic reduction in the size of intracellular dopamine stores despite an increase in the rate of dopamine synthesis.[125,126] Consequently, in the absence of DAT, de novo synthesis cannot maintain physiological levels of intracellular dopamine, suggesting a major role for the transporter not only in the termination of dopamine signaling but also in vesicular dopamine replenishment.

Using the ubiquitin-split yeast two-hybrid system to search for DAT-interacting proteins, we identified the synaptic vesicle protein synaptogyrin-3, suggesting a physical link between plasma membrane dopamine uptake and vesicular refilling.[127] In vitro studies showed that the amino termini of both DAT and synaptogyrin-3 were sufficient to observe the interaction. In PC12 and MN9D cells, overexpression of synaptogyrin-3 increased DAT activity without changing cell surface levels of

the transporter. Conversely, siRNA-mediated knockdown synaptogyrin-3 decreased DAT activity. Interestingly, the effect of synaptogyrin-3 overexpression was abolished in the presence of the VMAT2 inhibitor reserpine, suggesting a functional link between plasma membrane uptake and the vesicular storage system (Figure 4). In support of this hypothesis, the amino

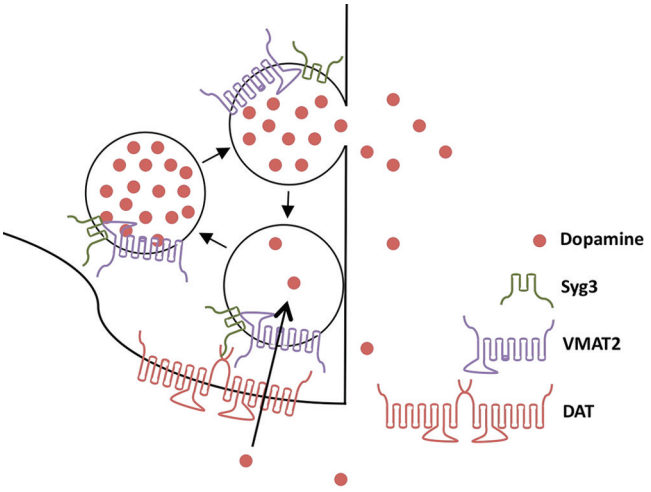

Figure 4. Proposed model of vesicular docking at the plasma membrane transporter. We hypothesize that the interaction between the synaptic vesicle protein, synaptogyrin-3, and the dopamine transporter brings synaptic vesicles into the proximity of sites of dopamine uptake. Following uptake of dopamine at the plasma membrane by DAT, dopamine would be loaded into synaptic vesicles docked at DAT.

terminus of DAT was able to pull down intact synaptic vesicles from a vesicle-enriched fraction.[127] These findings suggest a model in which a physical interaction between DAT and synaptic vesicles, through synaptogyrin-3, is important for the rapid and efficient clearance and loading of dopamine into vesicles after release (Figure 4). For this mechanism to be used, two distinct membrane compartments have to come together: plasma membrane (expressing DAT) and synaptic vesicle membranes (expressing synaptogyrin-3). Several reports have shown that DAT is not part of synaptic vesicle membranes,[77,128] which argues in favor, rather than against,[77] this potential association. Further experiments will be needed to examine whether a preexisting pool of synaptic vesicles resides close to the plasma membrane compartment containing DAT or, alternatively, if synaptic vesicles come in contact with DAT following neurotransmitter release. Of note, synaptogyrin-3 did not interact with NET,[127] suggesting different roles for the distinct monoamine transporters in the control of vesicular monoamine content.

The physiological significance of substrate efflux and ion conductances through transporters are less understood. Efflux of monoamines through plasma membrane transporters has been shown to be the primary mechanism used by psychostimulants such as amphetamine, methamphetamine, and 3,4-methylenedioxymethamphetamine (MDMA). Once inside the synaptic terminal, these drugs cause redistribution of vesicular monoamines into the cytosol, reversal in the direction of neurotransmitter transport, and increases in the extracellular levels of monoamines.[129] In the case of DAT, protein−protein interactions that regulate efflux include syntaxin1A,[50] PKCβ,[51,86] calmodulin kinase IIα (CamKIIα),[52] and flotillin-1.[101]

In addition to the effect of synatxin1A on DAT trafficking, syntaxin1A also promotes amphetamine-induced dopamine efflux. Interestingly, the strength of the interaction between DAT and syntaxin1A, mapped to the amino terminus of DAT, was increased following treatment with amphetamine, in both transfected cells and mouse synaptosomes.[50] There is also evidence suggesting a role for PKCβ in the amphetamine-induced dopamine efflux through DAT.[51] Inhibition of PKCβ abolishes dopamine efflux in brain slices, and the effect of amphetamine is significantly enhanced in heterologous cells upon overexpression of PKCβ. The increased effect of amphetamine by PKCβ overexpression was not due to increased DAT uptake activity or cell surface levels. Taken together, the data support a role for PKCβ not only in DAT trafficking but also in substrate efflux (Figure 5).

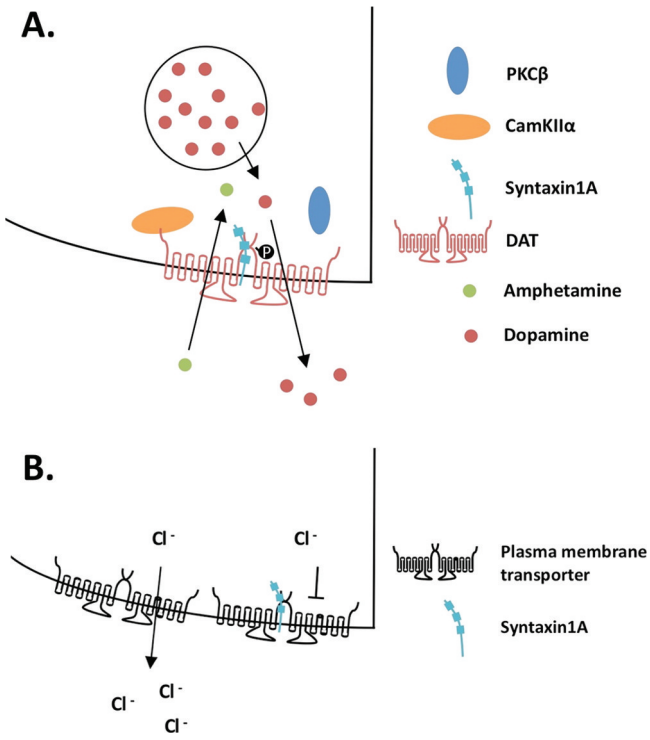

Figure 5. Interactions involved in amphetamine-induced substrate efflux and channel activity. (A) Following the uptake of amphetamine, dopamine is released into the cytoplasm and subsequently effluxed into the extracellular space by reversal of DAT. The efflux of dopamine through DAT is dependent upon the interactions between DAT and CamKIIα, syntaxin1A, and PKCβ. CamKIIα binds the C-terminus of DAT and induces phosphorylation of the N-terminus. Syntaxin1A interacts with the N-terminus of DAT, following phosphorylation. It is unknown where PKCβ binds to promote dopamine efflux. (B) The binding of syntaxin1A to the N-terminus of plasma membrane transporters inhibits the passage of chloride ions.

Another kinase, CamKIIα, has also been shown to bind to DAT and regulate amphetamine-induced dopamine release.[52] The interaction site was mapped to the carboxy terminus of the transporter, and the data support a model in which the binding of CamKIIα to the carboxy terminus induces phosphorylation of serine residues at the amino terminus. Substitution of these

serine residues at the amino terminus abolished the effect of amphetamine.[52,105] Interestingly, a functional link has been established between CamKIIα activation and plasma membrane transporter−syntaxin1A interaction;[50,130,131] inactivation of CamKIIα decreases the amount of syntaxin1A that co-immunoprecipitates with plasma membrane transporters. It is tempting to speculate that an interaction between CamKIIα and the carboxy terminus of DAT promotes the binding of syntaxin1A and PKCβ to induce phosphorylation of the transporter's amino terminus and dopamine efflux. Thus, the data suggest a model in which substrate efflux is regulated by a complex interplay involving several protein−protein interactions (Figure 5). Further studies will be required to examine how multiple proteins interact with the transporter and/or with each other to promote efflux.

In addition to their basic transmitter transport function, plasma membrane transporters also exhibit channel-like activity.[43−46] When expressed in heterologous systems, all three plasma membrane transporters exhibit several leak conductances that cannot be explained by the charge movement predicted by substrate translocation. There is some evidence of the physiological significance of these conductances in the dopamine and serotonin systems.[132,133] In dopamine neurons in culture, activation of a Cl^- conductance by dopamine or amphetamine resulted in an increased neuronal excitability,[132] and in thalamocortical neurons, SERT-mediated currents act to depolarize the cell membrane potential.[133] The only protein that has been shown to regulate transporter-associated conductances is syntaxin1A. In all three cases, syntaxin1A binds to the intracellular amino terminus of the transporters and inhibits channel activity.[91,92,134] In oocytes expressing SERT, as well as serotonin neurons in culture, the interaction between syntaxin1A and the transporter resulted in a reduction in SERT-mediated conductances.[91,133] Deletion of the syntaxin1A binding site in the amino terminus of NET results in an increase in transporter-associated leak currents.[46,92] Similarly, in *Caenorhabditis elegans*, the syntaxin1A homologue associates with DAT and suppresses transporter channel properties.[134] These results demonstrate the crucial role of protein−protein interactions in regulating plasma membrane transporter channel activity.

TRANSPORTER DEGRADATION

Very little is known about the molecular mechanisms associated with monoamine transporter degradation. Using the yeast two-hybrid system, Zhang and co-workers identified an interaction between the carboxy terminus of DAT and carboxypeptidase E (CPE).[135] This interaction was confirmed with a GST-based precipitation assay and by co-immunoprecipitation from rat striatal lysates. Overexpression of CPE resulted in an increase in the level of dopamine uptake in HEK cells transfected with DAT and increased DAT levels, both at the membrane and within the total lysate. This was proposed to be the result of an increase in the transporter half-life, suggesting a potential role for CPE in stabilizing or protecting DAT from degradation.[135]

In an effort to improve our understanding of the molecular mechanisms involved in DAT endocytosis, Sorkin's group developed a fluorescence-based assay designed to visually differentiate between intracellular and membrane pools of DAT. Using this assay in HeLa cells, Sorkin and co-workers conducted a siRNA screen against 52 human genes involved in endocytosis to identify proteins involved in DAT internalization.[136] As a result, three proteins were identified to be important for DAT internalization: the clathrin coat accessory proteins, Epsin and Eps15/Eps15R, and an E3 ubiquitin ligase, Nedd4-2.[136] These proteins were shown to co-immunoprecipitate with DAT from both HeLa cells and rat striatal lysates. The identification of Nedd4-2 suggested that the transporter was degraded by ubiquitination. Indeed, there was a decrease in the amount of ubiquitinated DAT in response to PKC activation in cells treated with Nedd4-2 siRNA.[136] These results provide further evidence that PKC-induced internalization may be linked to transporter degradation as previously suggested.[102] In support, three lysine residues at the amino terminus of DAT (Lys19, Lys27, and Lys35) were shown to be ubiquitinated in response to PKC activation.[137] Eliminating ubiquitination, by substituting these lysines, abolished the internalization of the transporter in response to PKC. Interestingly, Nedd4-2 knockdown also increased the steady state levels of DAT at the plasma membrane, suggesting Nedd4-2 may also be a regulator of constitutive endocytosis of DAT[136] (Figure 3).

VESICULAR TRANSPORTERS

No information is available regarding the pathways and/or proteins involved in biosynthesis, assembly, and export of VMAT2. However, VMAT2 is also differentially targeted to both presynaptic terminals and somatodendritic compartments.[138,139] In nerve terminals, VMAT2 is localized to small synaptic vesicles, whereas in dendrites, the vesicular transporter localizes to a population of tubulovesicular structures. A carboxy-terminal leucine motif appears to be required for the sorting of VMAT2;[140] however, the proteins involved in the differential targeting of VMAT2 have not been elucidated.

At the functional level, very little is known about what proteins regulate the activity of vesicular transporters. The G protein Gαo_2 has been shown to decrease the level of vesicular uptake in heterologous cells and central neurons,[141] although it is not known whether this effect is the result of a direct interaction between the vesicular transporter and the G protein. Furthermore, in platelet cells, the reduction of VMAT2 activity by G proteins was dependent on the vesicular content of monoamines.[142] In platelets from mice lacking tryptophan hydroxylase-1, the enzyme responsible for the synthesis of serotonin, the effect of the G protein on VMAT2 was lost and only recovered when the cells were preloaded with monoamines. Site-directed mutagenesis identified the first luminal domain of VMAT2 as being responsible for the G protein downregulation, leading the authors to propose this domain as a monoamine level sensor. α-Synuclein has also been shown to interact with VMAT2 by GST precipitation assays.[143] In SH-SY5Y cells, overexpression of α-synuclein results in decreased VMAT2 protein and mRNA levels. No information is available regarding the impact of the α-synuclein interaction on transporter catalytic activity or subcellular distribution.

Using a proteomic approach, our group identified Hsc70 as a VMAT2-interacting protein.[144] Hsc70 is a chaperone protein that plays an important role in synaptic vesicle endocytosis in nerve terminals.[145] We demonstrated that Hsc70 colocalizes with VMAT2 in synaptic vesicles and interacts with the transporter to downregulate its activity. Because Hsc70 has been implicated in a physical and functional coupling mechanism between the vesicular GABA transporter and the

enzymes responsible for GABA synthesis,[146] we hypothesized that a similar mechanism might operate in the dopamine system.

In a subsequent study, we reported that VMAT2 and the enzymes responsible for synthesis of dopamine, tyrosine hydroxylase (TH) and amino acid aromatic decarboxylase (AADC), are physically and functionally coupled at the synaptic vesicle membrane.[147] Traditionally, dopamine synthesis by TH and AADC, and its subsequent transport into vesicles through VMAT2, have been regarded as two separate and independent processes. A physical and functional coupling between dopamine synthesis and vesicular loading may serve to limit increases in dopamine levels to the local area surrounding the synaptic vesicle membrane (Figure 6), allowing dopamine to be transported more efficiently into synaptic vesicles and minimizing its intracellular diffusion, potential oxidation, and toxicity. The elucidation of a coupling mechanism within the dopaminergic pathway could have implications for our understanding and treatment of neurodegenerative diseases such as Parkinson's disease.

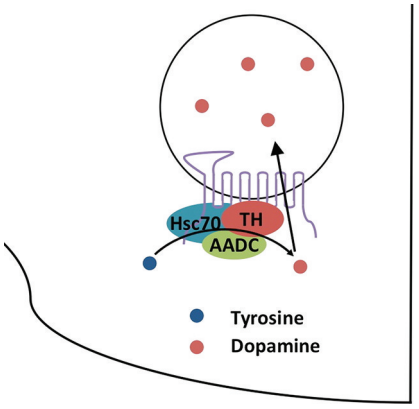

Figure 6. Proposed coupling of dopamine synthesis with vesicular transport. We propose a model in which the chaperone protein, Hsc70, serves to promote the formation of a complex between VMAT2 and the dopamine synthetic enzymes, TH and AADC. In this model, synthesis of dopamine, from tyrosine, would take place at the synaptic vesicle membrane and would be immediately followed by loading of newly synthesized dopamine into synaptic vesicles by VMAT2.

■ CONCLUSION AND FUTURE DIRECTIONS

The regulation of monoamine transporters is far more complex than previously anticipated. While the basic transporter function is encoded within the respective genes, it is the interactions that dictate the temporal and spatial regulation of transporter function. Without the identification and proper characterization of transporter protein−protein interactions, our knowledge of the mechanistic rules governing transporter synthesis, assembly, targeting, trafficking, degradation, and function will remain limited. As described in this review, many proteins interacting with monoamine transporters have been identified; however, the majority of the approaches currently used to characterize these interactions have taken static snapshots. Furthermore, most of the studies investigating monoamine transporter regulation by protein−protein interactions have relied on the use of heterologous expression systems. While these approaches have produced valuable information, the full complement of interacting proteins remains unknown, as well as when, where, and how these interactions are physiologically relevant. For instance, we know that many proteins affect the cell surface levels of monoamine transporters, yet it remains to be resolved whether these interactions mediate novel targeting mechanisms, constitutive or regulated trafficking, and/or degradation pathways. It will also be interesting to know which proteins form stable complexes versus transient interactions. Current and new live cell imaging techniques will be needed to increase the resolution of the spatial and temporal dynamics of these interactions to address these questions.

A potential level of complexity with respect to our understanding of transporters and their interacting proteins is caused by the fact that plasma membrane and vesicular transporters are expressed in distinct subcellular locations. For instance, plasma membrane transporters have been shown to be expressed in nerve terminals as well as dendrites[36−38] (Figure 1). While most studies have focused on the presynaptic transporters, very little is known about the function and regulation of transporters in the somatodendritic compartment. It is possible that the proteins interacting with transporters at these different locations may be distinct given the differential molecular structure of these two compartments. Indeed, a recent study examining the proteins expressed in the somatodendritic compartment of dopamine neurons in the substantia nigra noted the absence of several proteins normally found in presynaptic structures, including syntaxin1A.[148] In addition, an immunofluorescence study of dopamine neurons in culture showed a differential distribution of endocytic markers along with a different pattern of DAT localization between axons and dendrites[77] (Figure 2). These observations suggest that differences may exist when comparing the trafficking mechanisms, and subsequently transporter interacting proteins, between plasma membrane transporters localized to axons and dendrites.

A different level of complexity is caused by the heterogeneity of nerve terminal populations as a result of subpopulations of monoamine neurons in the brain. For instance, dopamine is produced in at least two distinct groups of neurons from the midbrain, the substantia nigra (SN) and the ventral tegmental area (VTA).[149] SN neurons project primarily to the dorsal striatum, whereas VTA neurons project primarily to the nucleus acumens, prefrontal cortex, and amygdala. Nerve terminals arising from these anatomically distinct groups of neurons might contain a different complement of transporter-interacting proteins (Figure 7A). Indeed, the dopamine transporter protein expressed in the nerve terminals of neurons in the nucleus accumbens has a larger apparent molecular weight than DAT expressed in the nerve terminals innervating the striatum due to differential post-translational processing.[150] Again, these observations suggest that these two sets of neuronal populations might have different complements of interacting proteins involved in the processing and maturation of the transporter. Furthermore, individual neurons make synaptic contacts with many different neurons, and individual neurons make contacts with distinct subcellular compartments, including cell bodies, dendrites, and axons (Figure 7B). To what degree are the interactions of the transporter defined by the synaptic identity? While many of these questions will require the development and refinement of techniques to study transporters at individual terminals, a good starting point might be to differentiate between transporter populations in nerve terminals versus dendrites and

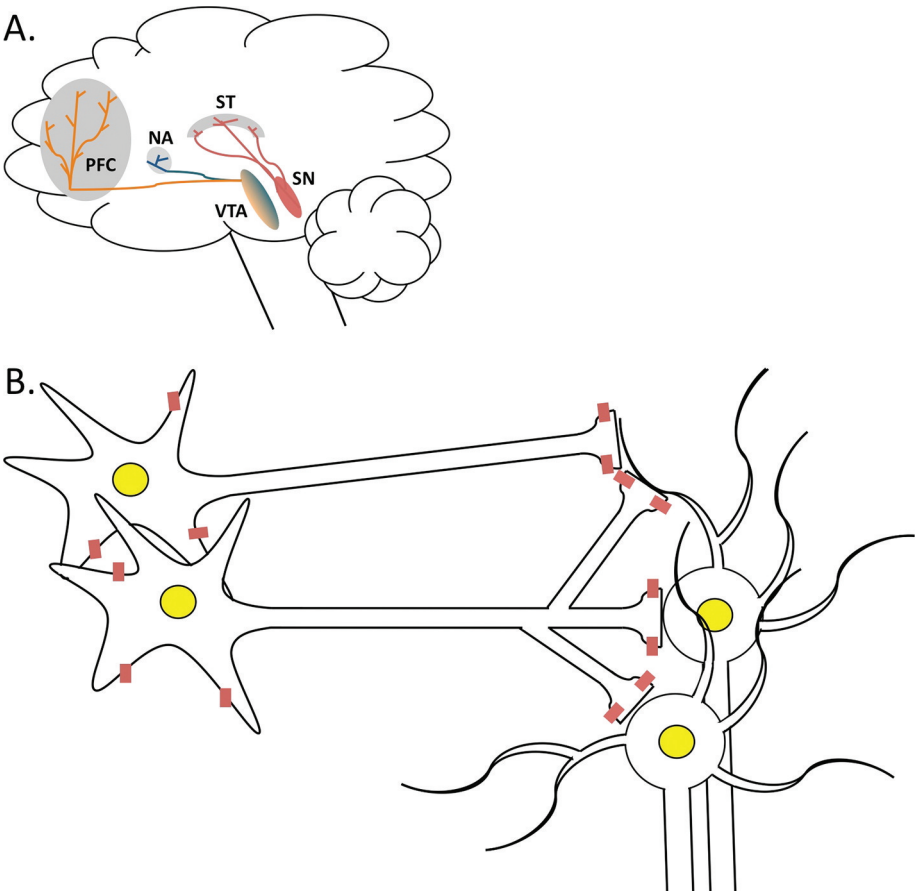

Figure 7. Potential sources of heterogeneity in monoamine transporter complexes. (A) The spatial and temporal regulation of DAT by interacting proteins might be different in subpopulations of dopamine neurons. Dopaminergic cell bodies reside in two main cell groups in the midbrain: the substantia nigra (SN) and the ventral tegmental area (VTA). Neurons in the SN project primarily to the dorsal striatum, whereas neurons in the VTA project primarily to the prefrontal cortex (PFC), the nucleus accumbens (NA), the hippocampus (not shown), and the amygdala (not shown). (B) Heterogeneity in DAT protein–protein interactions might also be caused by the molecular identity of the synapse where the transporter is located. Within neurons, monoamine transporters reside in the membrane of both the somatodendritic compartment and the axonal terminals. At the presynaptic level, individual neurons make synaptic contacts with several different neurons, and nerve terminals can synapse on both the soma and dendrites of these neurons.

transporter populations from nerve terminals reaching two different brain regions (i.e., striatum vs prefrontal cortex in the case of DAT).

The data, thus far, suggest a very important role for protein–protein interactions in the life cycle of plasma membrane and vesicular transporters. In addition, the identification and characterization of these interactions are beginning to elucidate mechanisms dealing not only with transporter function but also with monoamine signaling. Nevertheless, many challenges must be overcome to develop a complete picture of the physiological role played by protein–protein interactions in the regulation of monoamine transporter function. More importantly, future studies should address how disruptions of these interactions might be related to disease states.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Interacting proteins affecting the trafficking of monoamine transporters (Table 1). This material is available free of charge via the Internet at http://pubs.acs.org.

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