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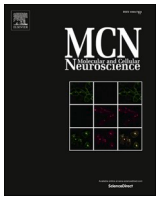

Review

Turnover of synaptic adhesion molecules

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SUMMARY

Molecular interactions between pre- and postsynaptic membranes play critical roles during the development, function and maintenance of synapses. Synaptic interactions are mediated by cell surface receptors that may be held in place by trans-synaptic adhesion or intracellular binding to membrane-associated scaffolding and signaling complexes. Despite their role in stabilizing synaptic contacts, synaptic adhesion molecules undergo turnover and degradation during all stages of a neuron's life. Here we review current knowledge about membrane trafficking mechanisms that regulate turnover of synaptic adhesion molecules and the functional significance of turnover for synapse development and function. Based on recent proteomics, genetics and imaging studies, synaptic adhesion molecules exhibit remarkably high turnover rates compared to other synaptic proteins. Degradation occurs predominantly via endolysosomal mechanisms, with little evidence for roles of proteasomal or autophagic degradation. Basal turnover occurs both during synaptic development and maintenance. Neuronal activity typically stabilizes synaptic adhesion molecules while downregulating neurotransmitter receptors based on turnover. In conclusion, constitutive turnover of synaptic adhesion molecules is not a necessarily destabilizing factor, but a basis for the dynamic regulation of trans-synaptic interactions during synapse formation and maintenance.

1. Introduction

Synaptic adhesion molecules are cell surface receptors that play roles during synapse development or function based on adhesive trans-synaptic interactions (de Wit and Ghosh, 2016; Klein and Pasterkamp, 2021; Sanes and Zipursky, 2020; Sudhof, 2021). The idea of trans-synaptic adhesion is most easily associated with synaptic stability, yet all synaptic proteins undergo turnover, i.e. replacement through recycling or degradation and de novo synthesis (Cohen and Ziv, 2019; Dorrbaum et al., 2018; Fornasiero et al., 2018). Whereas turnover of many synaptic proteins and transmitter receptors has been studied in some detail, comparably little is known about the mechanisms and roles of turnover of synaptic adhesion molecules. Here we review recent experimental evidence for relatively high turnover rates compared to other synaptic protein classes. We further discuss ideas how high levels of turnover affect the notion of synaptic adhesion as a mechanism for synaptic stability and plasticity, both during synapse development and function.

Synaptic adhesion molecules constitute a heterogeneous group of several protein families that serve a remarkable array of diverse functions, most prominently development roles that contribute to functional adult synaptic connectivity (Agi et al., 2020; Sanes and Zipursky, 2020). The classification of synaptic adhesion molecules is not always straightforward, as some 'outgroup' membrane proteins, including neurotransmitter receptors, have been found to contribute through trans-synaptic interactions to synapse development or maintenance (Fossati and Charrier, 2021). Conversely, some proteins previously considered as cell adhesion molecules have more recently been referred to as cell recognition molecules, or even more generally, as cell surface molecules in light of their divergent functions (Hassan and Hiesinger, 2015; Hiesinger, 2021; Sanes and Zipursky, 2020). A hallmark of these membrane proteins are extracellular domains with adhesive properties (e.g. immunoglobulin or Cadherin domains); yet, the proteins' functions may also depend on signaling or other protein interactions, and these functions may only make sense as contributors to collaborative molecular and cellular processes (Hiesinger, 2021). Throughout early development, a membrane receptor likely executes different context-dependent roles that precede its function as a synaptic adhesion molecule in synapse development or function. During development, only few cell surface receptors have been shown to function at filopodial tips at the precise moment when filopodial interactions 'decide' on stabilizing synaptic contacts (Wit and Hiesinger, 2023). In adult neurons, trans-synaptic interactions play roles in organizing pre- and postsynaptic protein complexes and thus synaptic properties (Gomez et al., 2021; Sudhof, 2021). These trans-synaptic interactions often persist throughout the adult life and play important roles in synaptic plasticity (Arikkath and Reichardt, 2008; Kania and Klein, 2016; Keable et al., 2020; Lai and Ip, 2009). Hence, turnover of synaptic adhesion molecules must be analyzed at the time and place the molecules function, in a context-dependent manner.

Trans-synaptic interactions, either during development or function, appear at odds with turnover at first sight: either the interaction must

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break or the membrane-embedding of an entire complex on one side of the trans-synaptic complex must break in order for turnover to take place. Counterintuitively, membrane proteins that engage in trans-synaptic interactions undergo no less turnover than many other proteins at the synapse – and in some cases more (Dorrbaum et al., 2018; Fornasiero et al., 2018; Kokotos et al., 2018). Turnover is therefore interesting from two perspectives: first, the mechanisms that allow to replace the proteins or complexes without disrupting the roles ascribed to their adhesion; second, the actual functions served by turnover that would not be possible if the adhesive complexes stayed in place stably.

While neurotransmitter receptor turnover has been studied in considerable detail (both mechanistically and functionally), surprisingly little is known about the mechanisms and functional consequences of how synaptic adhesion molecules are turned over. In this review we focus on the mechanisms and roles of turnover of those membrane receptors that have been shown to confer trans-synaptic interactions at synapses during development and function. Of these, turnover mechanisms and roles have been characterized best for three families: Cadherins, Neurexins/Neuroligins and ephrins/Eph receptors, as discussed in the following sections. The shared mechanisms and functional roles of turnover identified for these families suggest commonalities with the many other synaptic adhesion proteins for which little data is currently available regarding their turnover.

2. Synaptic adhesion proteins exhibit both constitutive and neuronal activity-induced turnover

Turnover at synapses relies on multiple factors, including the abundance and affinity of extracellular receptors that bind in *trans* (across cells) and intracellular scaffolds and interacting receptors that bind in *cis* (within the same cell) (Chamma et al., 2020). Plasma membrane-associated proteins have repeatedly been found in proteomics studies to constitute a group of relatively short-lived proteins compared to other synaptic proteins (Cohen and Ziv, 2019). Several recent proteomics studies further provided quantitative assessments of the turnover of cell surface receptors. A recent analysis of purified bulk endosomes from primary cultures of cerebellar granule neurons identified turnover rates for many classes of proteins following activity-dependent bulk endocytosis (Kokotos et al., 2018). Remarkably, cell adhesion molecules have one of the highest rankings in the study, followed by other synaptic proteins, including cytoskeletal, GTP-binding and mitochondrial proteins. Similarly, cell adhesion molecules were found to be amongst the most short-lived proteins in another unbiased proteomics study using dynamic SILAC (stable isotope labeling with amino acids in cell culture) to determine half-lives of more than 5000 proteins in rat primary hippocampal cultures under basal activity conditions (Dorrbaum et al., 2018). In a follow-up study, the same group found that the up- or downregulation of neuronal activity led to a downregulation of adhesion molecules while synaptic vesicle proteins were upregulated (Dorrbaum et al., 2020); most, but not all, adhesion molecules exhibited a slowdown of turnover upon chemical 'upscaling' of neuronal activity. A systematic and unbiased proteome analysis of the aging rat brain based on pulse-chase isotope labeling followed by mass spectrometry found that 99 % of long-lived proteins function as histones, nuclear pore complexes, structural proteins, myelin sheath or enzymes (Toyama et al., 2013); a notable exception amongst the remaining 1 % of long-lived surface proteins was IgSF8, a cell surface protein that plays a role in synaptic connectivity and function (Apostolo et al., 2020; Toyama et al., 2013). Another isotope labeling approach aimed to provide lifetime measurements for ~3500 'brain proteins' in different tissues and subcellular fractions (Fornasiero et al., 2018). Here, cell adhesion molecules exhibited lifetimes that were similar to neurotransmitter receptors as well as cytosolic synaptic proteins. Finally, a recent study of neuronal and synaptic consequences of acute block of protein synthesis identified what protein classes are most sensitive to a disruption in protein supply, an indicator of turnover (Cohen et al., 2022). Adhesion molecules and regulators of synapse organization were amongst the proteins exhibiting the strongest downregulation after 8 h of protein synthesis inhibition, suggesting that they are amongst the proteins with highest turnover (Cohen et al., 2022). Collectively, these data suggest that most synaptic adhesion molecules do not exhibit increased stability compared to other synaptic proteins and, if anything, undergo relatively high turnover both under basal and activity-induced conditions (Fig. 1).

3. Synaptic adhesion molecules are predominantly turned over via the endolysosomal system

What local degradation mechanisms underlie the turnover of synaptic adhesion molecules? The ubiquitin-proteasome system (UPS) preferentially degrades short-lived proteins (Cohen and Ziv, 2019). However, a systematic study of synaptic protein degradation and turnover under conditions of pharmacologically blocked UPS activity revealed that most synaptic proteins appear not to be degraded by the UPS at least under basal conditions (Hakim et al., 2016). The UPS is currently not considered a likely mechanism involved in the turnover of synaptic adhesion molecules.

Two endomembrane degradation systems are known to degrade plasma membrane receptors: endolysosomal degradation and autophagy. Macroautophagy (hereafter referred to as autophagy) is a major endomembrane turnover mechanism in all eukaryotic cells whereby cargo is engulfed by a double membrane phagophore, formation of a double membrane autophagosome, and degradation after fusion with lysosomes (Klionsky and Emr, 2000; Mizushima and Komatsu, 2011; Stavoe and Holzbaur, 2019). In neurons, autophagy has been implicated in neuronal maintenance based on the observation of adult onset degeneration in mutant neurons (Hara et al., 2006; Komatsu et al., 2006). Autophagosomes form at axon terminals in various neuron types and are retrogradely trafficked to the cell body, suggesting a role in the turnover of presynaptic proteins in neurons; yet, it has been difficult to pinpoint autophagic cargo proteins that are selectively degraded on the presynaptic side (Andres-Alonso et al., 2021; Decet and Verstreken, 2021; Stavoe and Holzbaur, 2019; Vijayan and Verstreken, 2017). On the postsynaptic side, autophagic degradation of membrane receptors has been shown for neurotransmitter receptors, including AMPAR and GABAR (Lieberman and Sulzer, 2020; Rowland et al., 2006; Shehata et al., 2012). The role of autophagy for the turnover of synaptic adhesion receptors is less clear than for transmitter receptors. This is despite the demonstration of various roles of autophagy during synapse formation and maintenance in flies, worms and mice (Kiral et al., 2020; Shen and Ganetzky, 2009; Stavoe et al., 2016; Tang et al., 2014). In flies, loss of autophagy leads to the aberrant stabilization of synaptogenic filopodial contact, resulting in an increase of synapse numbers (Kiral et al., 2020). Amongst synaptic proteins tested in this study were the synaptic seeding factors Syd-1 and Liprin-alpha as well as the synaptic adhesion protein Lar; all were shown to be required for normal synapse development, but only the seeding factors, not Lar, were found in autophagosomes (Kiral et al., 2020). In mice, exuberant synapses initially form in the absence of autophagy, but a subsequent pruning step fails (Lieberman et al., 2019; Tang et al., 2014). Since pruning of dendritic spines requires the removal of a plethora of proteins, and absence of autophagy does not alter the postsynaptic morphology (Andres-Alonso et al., 2021), the effect on synaptic adhesion proteins, if any, is likely indirect. We conclude that, at the time this review was written, synaptic adhesion molecules have not been shown to be specific cargo proteins of autophagic degradation.

In contrast to the UPS and autophagy, endolysosomal degradation has commonly been implicated in the degradation of adhesion molecules both during development and maintenance of neurons and synapses. However, endolysosomal degradation and turnover may encompass more than one mechanism. In many cases, well-known and ubiquitous endolysosomal trafficking regulators of the rab GTPase

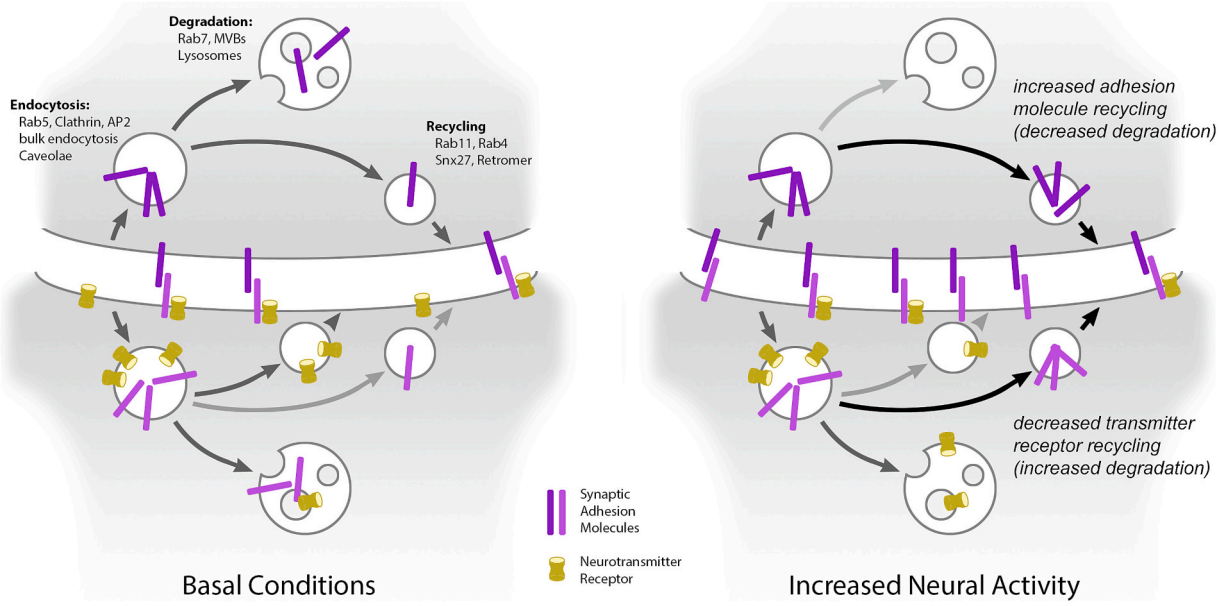

Fig. 1. Synaptic adhesion molecules and neurotransmitter receptors undergo different rate changes of recycling and degradation in response to neural activity. Under basal conditions, some (and maybe all) synaptic adhesion molecules and transmitter receptors are constitutively turned over via endocytosis followed by recycling or degradation. When neural activity is increased, most synaptic adhesion molecules stabilize or increase in numbers, whereas transmitter receptor numbers typically decrease under elevated activity levels. Both effects can be reconciled by mechanisms that include endocytosis on shared endosomes, followed by endosomal sorting and quantitatively tuned, receptor-specific rates of recycling versus degradation.

family have been implicated, including Rab5 for early endosomes, Rab7 for late endosomes and Rab11 for recycling endosomes. However, a systematic analysis of null mutants for *rab* genes in *Drosophila* has recently highlighted that numerous endolysosomal associated Rab GTPases may function in the modulation of trafficking at synapses without affecting development or viability under laboratory conditions (Kohrs et al., 2021). In the same system, at least two parallel endolysosomal degradation mechanisms have been shown at synaptic terminals, a neuron-specific pathway implicated in synaptic vesicle protein degradation, and a ubiquitous pathway for plasma membrane proteins (Jin et al., 2018). These findings suggest that more neuron-specific endolysosomal trafficking mechanisms remain to be discovered. Developmental turnover of different adhesion molecules occurs at the neuronal cell body or at synapses, depending on the place of action (Williamson et al., 2010). A block of degradation can lead to the accumulation of adhesion proteins on signaling endosomes and thereby gain-of function defects (Williamson et al., 2010). These data suggest the existence of specialized endolysosomal compartments at axon terminals and synapses that employ specialized molecular machinery to modulate the degradation of synaptic adhesion molecules as well as other membrane-bound synaptic proteins.

Degradation via local endolysosomal mechanisms can occur constitutively through continuous turnover; alternatively, degradation can be induced by neuronal activity or another trigger (Fig. 2). Constitutive turnover allows modulation of cell surface presentation through fine-tuning of endosomal sorting and the choice between degradation and recycling. As highlighted in the next section on the synaptic adhesion proteins of the Cadherin, Neurexin/Neuroligin and Ephrin/Eph families, both constitutive turnover and activity-induced turnover are common both during development and the maintenance of synaptic plasticity and function.

4. Mechanisms and roles of turnover: Cadherins, Neurexins/Neuroligins, Ephrins/Eph receptors and beyond

The cadherin superfamily represents a diverse group of transmembrane receptors that have been implicated in a plethora of cell-cell adhesion-based mechanisms. Of the two major types of classic cadherins, the ultrastructurally well-defined adherens junctions (E-Cadherin) and more diffuse adhesive contacts (N-cadherin), only the N-cadherin type and related family members have been characterized as synaptic adhesion molecules. N-Cadherin (N-Cad) turnover and its function at the synapse have been studied both during development and in mature neurons. During development, N-Cadherin is continuously turned over (Thoumine et al., 2006; Williamson et al., 2010). During early maturation of mouse cortical neurons, N-Cadherin turnover was found to be required for correct neurite formation and migration behavior. N-Cadherin turnover in these immature neurons was shown to be Caveolin-1-dependent but clathrin-independent. The authors proposed a regulatory mechanism that ensures spatiotemporally restricted functions required for the correct timing of N-Cadherin's roles during development (Shikanai et al., 2018). N-Cadherin trafficking is required for neuronal migration and depends on Rab5 and Rab11 (Kawauchi et al., 2010). In developing *Drosophila* photoreceptor neurons, the small GTPase Rab6 and its effector protein Rich are required for N-Cadherin localization to synaptic terminals and proper neuronal connectivity (Tong et al., 2011), while the loss of Rab7 leads to loss of synaptic maintenance (Cherry et al., 2013). The role of N-Cadherin trafficking for synaptogenesis has been studied extensively and is reviewed elsewhere (Arikkath and Reichardt, 2008).

In adult neurons, N-cadherin is required for long-term stabilization of synapses (Mendez et al., 2010). N-Cadherin undergoes constitutive turnover in the absence of changes to neuronal activity, i.e. under basal conditions (Brigidi and Bamji, 2011; Tai et al., 2007). While the role of constitutive turnover in adult neurons is largely unclear, the effects of activity-dependent turnover of synaptic adhesion molecules on synapse function and morphology have been studied in some detail. Endocytosis of N-Cadherin is attenuated through binding of catenins, a class of proteins with essential roles in cell-cell adhesion that link cadherins to the actin cytoskeleton (Brigidi and Bamji, 2011). The roles of catenins in the stability and turnover of cadherins have been established in multiple studies (Abe et al., 2004; Ribeiro et al., 2018; Tai et al., 2007; Thoumine et al., 2006). Specifically, turnover of N-Cadherin can be regulated by β-catenin (Thoumine et al., 2006) or δ-catenin binding (Brigidi et al.,

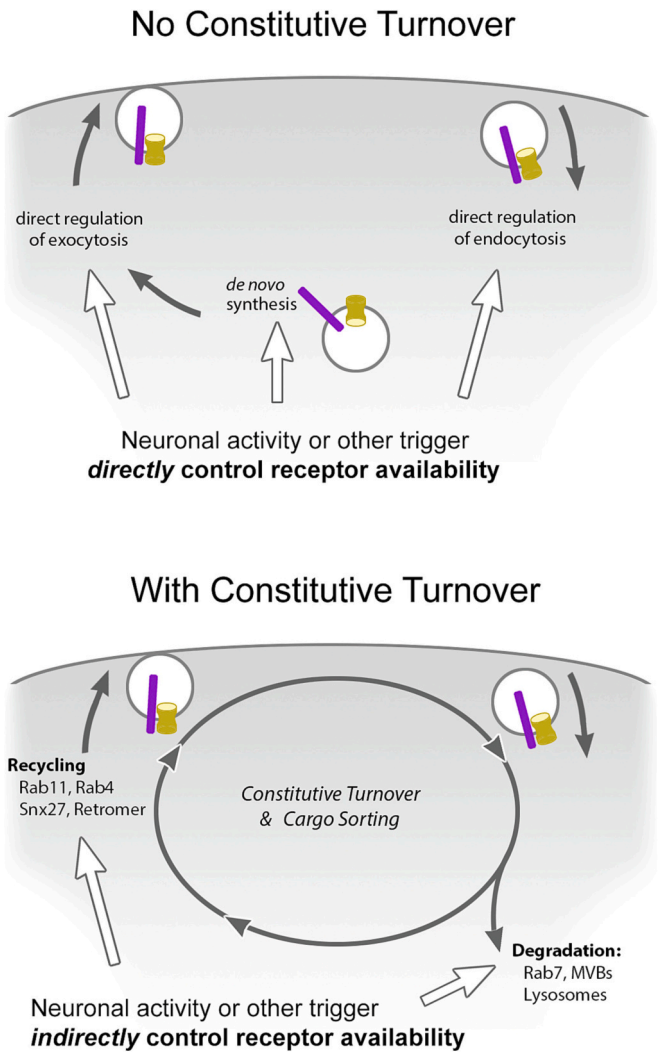

Fig. 2. Constitutive turnover of synaptic adhesion molecules and neurotransmitter receptors allows for fine-tuning of surface localization through modulation of the recycling/degradation ratio.
In the absence of constitutive turnover, effects of neuronal activity or other triggers have to impinge directly on endocytosis or exocytosis to change receptor localization on the surface. By contrast, under conditions of constitutive turnover, neural activity or other triggers can change the surface availability of receptors through altered rates of recycling versus degradation.

2015). NMDA receptor activation at synapses can cause a reduction in the rate of N-Cadherin turnover through a reduction in endocytosis via reduced phosphorylation of β-Catenin (Tai et al., 2007). When reduced endocytosis is not counterbalanced by increased recycling, N-Cadherin levels increase at the membrane, likely stabilizing synaptic adhesion (Arikkath and Reichardt, 2008; Brigidi and Bamji, 2011; Mendez et al., 2010; Tai et al., 2007; Tanaka et al., 2000). Similarly, a β-Catenin mutant that exhibits increased N-Cadherin binding reduced N-Cadherin turnover and thereby adhesion, stimulated the formation of new presynaptic release sites, increased vesicle recycling and anchored presynaptic vesicles more firmly to stabilize synapses (Chen et al., 2017). Activity-dependent changes to dendritic spines of hippocampal neurons were also shown to be partly regulated by a mechanism that implicates a protocadherin (arcadlin), activation of a kinase cascade and site-specific phosphorylation of a protein that ultimately triggers N-Cadherin endocytosis (Yasuda et al., 2007). This protocadherin/kinases/N-Cadherin pathway thereby provides a mechanism for the transduction of neuronal activity into changes of spine morphology through turnover of N-Cadherin.

Synaptic adhesion proteins of *the Neurexin (Nrx) and Neuroligin (Nlg)* families are well-characterized trans-synaptic binding partners that regulate functional synaptic properties (Gomez et al., 2021; Sudhof, 2017). Neurexins are the presynaptic partners that bind to Neuroligins on the postsynaptic membrane; both partners undergo turnover in their respective compartments during development and throughout adult synaptic function (LaConte et al., 2016; Lagardere et al., 2022; Neupert et al., 2015; Schapitz et al., 2010). The internalization and turnover of Neuroligins depend on two factors: the regulation of their association with the postsynaptic scaffold as well as trans-synaptic binding to presynaptic Neurexins, both during development and in adulthood (Jeong et al., 2019; Lagardere et al., 2022). The mobility of postsynaptic Nlg1 in cultured rat hippocampal neurons decreases throughout development, and has been proposed to do so as a consequence of synapse maturation (Lagardere et al., 2022). Absence of postsynaptic Nlg1 results in an increase of activity-dependent endocytosis in the presynaptic terminal (Luo et al., 2021). Nlg1 is turned over based on endolysosomal degradation in an activity- and Dynein-dependent manner (Schapitz et al., 2010). Nlg1 turnover persists into adulthood in cultured rat and mouse hippocampal neurons, where increased activity (induction of long-term potentiation) leads to an increase of Nlg1 on the membrane and causes increased spine heads and an increased size of the postsynaptic density (PSD length) (Schapitz et al., 2010). Correspondingly, levels of Nlg1 depend on stimulation of synapses in adult neurons of rats, mice and bees (Biswas et al., 2010; Schapitz et al., 2010). In another example of Nlg1 turnover-dependent regulation of the strength of an excitatory synapse, trafficking of Nlg1 and Syt4 via Syx4 regulates bouton numbers in developing *Drosophila* NMJ (Harris et al., 2016). Similar to Nlg1 at excitatory synapses, Nlg2 at inhibitory synapses is constitutively turned over during development and under basal conditions. Nlg2 is internalized in endosomes, where it co-localizes with SNX27, a member of the retromer complex and facilitator of membrane protein recycling; loss of SNX27 leads to lower Nlg2 levels at the membrane and subsequent synaptic destabilization and decreased inhibitory signaling (Binda et al., 2019; Halff et al., 2019). Retromer-dependent regulation of the rate of recycling is a mode of synaptic modulation that requires constitutive turnover, as discussed in more detail below.

Similar to Neuroligins, Neurexins exhibit surface mobility and turnover throughout synaptic development and adult maintenance (Chamma et al., 2020). During development, α-Nrxs are more mobile than β-Nrxs, despite their larger extracellular domain (Neupert et al., 2015). Similarly, at adult cortical perisomatic inhibitory synapses, α-Nrx and β-Nrx exhibit different turnover rates; specifically the turnover of Nrx1β is attenuated by neuronal activity (Fu and Huang, 2010). Conversely, block of neuronal activity in cortical cultures via TTX leads to a downregulation of Nrx1α and Nrx1β based on increased protein turnover, following increased phosphorylation by CASK (LaConte et al., 2016). CASK is a scaffolding protein and kinase that had previously been shown to phosphorylate Neurexins in an activity-dependent manner (Mukherjee et al., 2008) and whose loss leads to reduced synaptic function (Atasoy et al., 2007). Neurexin transcription appears to be unaffected by loss of neuronal activity at the timescale when synaptic Neurexin is reduced, suggesting increased turnover (LaConte et al., 2016). In developing cultured rat and mouse hippocampal neurons, endocytosis is mediated by SorCS1, which maintains a balance between axonal and dendritic Nrx surface levels in the same neuron. SorCS1-Rip11 interaction facilitates Nrx1α sorting from early endosomes (EEs) to recycling endosomes (REs). In the absence of SorCS1, Nrx1α accumulates in EEs and mispolarizes to the dendritic surface, impairing synaptogenesis onto Nlg1-expressing postsynapses (Ribeiro et al., 2019; Savas et al., 2015). Nrx is transported to the synapse via synaptic vesicle protein transport vesicles (STVs) in a fast-microtubule-dependent manner in developing hippocampal neurons in rats and mice (Neupert et al., 2015).

Ephrin and their Eph receptors constitute a large class of interacting membrane proteins that are best known for their roles during brain

development through adhesion and signaling (Kania and Klein, 2016; Lai and Ip, 2009). However, ephrins and Eph receptors are also found in mature neurons and dendritic spines where they regulate neurotransmission and plasticity, as exemplified for ephrinB2, ephrinB3 and EphA4 (Essmann et al., 2008; Murai et al., 2003). While EphB signaling is a positive regulator of spine formation and maturation, activation of EphA4 in adult mouse hippocampal neurons leads to spine retraction, triggered by binding of ephrinB3 on astrocytic processes (Murai et al., 2003). EphA4 intracellular cleavage and signaling was further shown to be triggered by neuronal activity; the increased availability of the EphA4 intracellular domain leads to increased dendritic spine numbers. Multiple links between Ephs/ephrins and transmitter receptors can directly affect synaptic plasticity. For example, a cleavage product of the EphB2 receptor can directly phosphorylate the NMDA receptor (Litterst et al., 2007) and ephrinB2 stabilizes AMPA receptors through interaction with the EphB4 receptor in mouse hippocampal neurons, thereby strengthening synapses (Essmann et al., 2008). Also in this study, conditional ephrinB2 knockouts revealed enhanced constitutive internalization of AMPA receptors underlying the reduced synaptic transmission, suggesting fine-tuning of constitutive turnover. Ephrins and Ephs can simultaneously act as receptor and ligand, either in *cis* or in *trans*, as reviewed in detail elsewhere (Kania and Klein, 2016). Both proteins are degraded via the endolysosomal system during development and function (Cowan et al., 2005; Deininger et al., 2008; Hoogenraad et al., 2005; Nievergall et al., 2010).

Similar to other membrane receptors, molecularly controlled turnover shapes spatially and temporally restricted functions of Eph receptors. During neuronal development, the phosphatase PTP1B regulates EphA3 surface availability through direct interaction and ligand-stimulated receptor internalization and turnover via endosomes (Nievergall et al., 2010). In mature glutamatergic neurons of the mouse amygdala, Eph activation leads to phosphorylation of Rin1, which in turn mediates EphA4 endocytosis via Rab5-positive endosomes after engagement with ephrinB3 (Deininger et al., 2008). Neuronal activity has been proposed to regulate synaptic strength directly via regulation of ephrinB3 phosphorylation, which reduces PSD-95 interaction and increases PSD-95 turnover (Hruska et al., 2015). Hence, in this case a trans-synaptic adhesion molecule has been proposed to destabilize synapses, in contrast to the increase in trans-synaptic stabilization observed for N-Cadherin, Neurexins and Neuroligins.

A central role for endolysosomal degradation underlying continuous or activity-induced turnover is also supported by findings on other synaptically localized cell surface molecules. For example, NCAM (neural cell adhesion molecule) is required for synapse strengthening in an activity-dependent manner and has been studied intensively in the context of long-term potentiation (LTP), synaptic remodeling and plasticity (Bukalo et al., 2004; Diestel et al., 2007; Dityatev et al., 2000). Similar to the synaptic adhesion molecules discussed above, NCAM turnover depends on endolysosomal mechanisms. Different NCAM isoforms undergo Rab5-dependent endocytosis in developing and mature neurons by clathrin- and caveolae-dependent pathways (Diestel et al., 2007). NCAM recycling is Rab4- and Rab11-dependent and regulated via ubiquitylation as an internalization signal, but without changing the rate of degradation. NCAM has also been shown to regulate endocytosis of one of its ligands, FGFR1 (Francavilla et al., 2009). A similar phenomenon has been observed for the cell adhesion molecule L1, whose endocytosis leads to increased ß1-integrin endocytosis, albeit in a non-synaptic context (Caswell and Norman, 2006; Panicker et al., 2006). Similar to vertebrate NCAM, the *Drosophila* homolog Fasciclin 2 (Fas2) functions in synapse formation and plasticity through the regulation of pre- or postsynaptic levels (Ashley et al., 2005; Schuster et al., 1996a, 1996b). Fas2 is continuously recycled in and out of the synaptic membrane by an endolysosomal mechanism that depends on the protein MICAL-like. In MICAL-like mutants, Fas2 levels are decreased, and synaptic bouton numbers are increased (Nahm et al., 2016). Synaptic activity causes downregulation of Fas2, which is necessary for synaptic sprouting (Schuster et al., 1996b), consistent with the phenotype of higher bouton numbers in MICAL-like mutants. Turnover of Fas2 depends on endocytosis and recycling; a mutant of the endosomal adapter-protein amphiphysin leads to a decrease of synaptic Fas2 levels due to defective recycling and re-insertion of Fas2 into the membrane (Mathew et al., 2003). In a final example considered here, Neuroglian (Nrg), a member of the immunoglobulin superfamily and a homolog of mouse neural adhesion molecule L1, is also required for synapse formation and stability (Enneking et al., 2013; Godenschwege et al., 2006). Nrg can homophilically interact on the pre- and postsynaptic site or heterophilically, e.g. with Semaphorin1a (Godenschwege and Murphey, 2009). Endocytosis of Nrg is mediated by Rab5/ESCRT to enable dendritic pruning in *Drosophila*; here, Nrg is co-trafficked in Rab5-positive endosomes with Roundabout (Robo) and N-Cad in these neurons (Zhang et al., 2014). Recycling of Nrg occurs via retromer in a Rab4/Rab11-dependent manner in the context of synaptic function and maintenance (Walsh et al., 2021).

In sum, synaptic adhesion molecules, and in particular the families of N-Cadherins, Neurexins/Neuroligins and ephrins/Eph receptors all modulate surface presentation via endolysosomal and recycling mechanisms and in response to neuronal activity. In some cases (N-Cadherin, Nlg2) there is evidence that this modulation of synaptic localization is affected through modulation of constitutive turnover (Fig. 2). Furthermore, the surface localization of synaptic adhesion molecules is often stabilized by increased neuronal activity. These two aspects are discussed in the two following sections.

5. Comparison and co-regulation of turnover of synaptic adhesion molecules and neurotransmitter receptors

Compared to the synaptic adhesion proteins discussed above, the turnover of neurotransmitter receptors has been studied extensively and is reviewed elsewhere (Chater and Goda, 2022; Diering and Huganir, 2018; Keable et al., 2020; Lorenz-Guertin and Jacob, 2018; Malinow and Malenka, 2002). However, several synaptic adhesion proteins and transmitter receptor subunits can directly interact and these interactions have roles in synapse development, plasticity and maintenance. Both inhibitory GABA receptors and excitatory AMPA receptors have been shown to undergo constitutive endolysosomal turnover (Ehlers, 2000; Kittler et al., 2000; Luscher et al., 1999). In rat hippocampal neurons, AMPA receptors, but not NMDA receptors, are continuously endocytosed and blockade of endocytosis specifically leads to increased responses of AMPA receptors (Ehlers, 2000; Luscher et al., 1999). Insulin, which activates clathrin- and dynamin-dependent endocytosis, causes rapid removal of AMPA receptors out of the postsynaptic membrane (Man et al., 2000). Both AMPA and GABA receptors are constitutively endocytosed in a clathrin-dependent manner via the AP2 complex (Carroll et al., 1999; Kittler et al., 2000; Lee et al., 2002). Compared to AMPA receptors, insulin has the opposite effect on GABA receptors: It induces an increase of the receptors on the membrane, without affecting protein synthesis (Wan et al., 1997).

Similar to synaptic adhesion molecules, synaptic activity affects the turnover rate of neurotransmitter receptors through endocytosis, degradation and retromer-dependent recycling (Ehlers, 2000; Luth et al., 2021; Temkin et al., 2017; Yong et al., 2021). However, as schematized in Fig. 1, transmitter receptors are initially downregulated in response to increased neuronal activity, whereas synaptic adhesion receptors are more typically stabilized in response to activity, as discussed above for N-Cadherin. (Tai et al., 2007; Tanaka et al., 2000), Neuroligin1 (Schapitz et al., 2010) and Neurexin1β (Fu and Huang, 2010). Little is known about the co-regulation of these opposite effects of activity on the trafficking of the two receptor types. Remarkably, both synaptic adhesion molecules and transmitter receptors have been shown to co-traffic on the same vesicles and to be regulated by similar mechanisms; for example, the sorting receptor SorCS1 is responsible for regulation of AMPARs, Nrx, and Nlg (Savas et al., 2015). AMPARs are

also co-trafficked with N-Cad by GRIP1 in a KIF5C-dependent manner (Heisler et al., 2014). However, AMPAR trafficking is a multifaceted process involving differential trafficking of different subunits and the long-term insertion of more AMPA receptors following LTP, as reviewed elsewhere (Diering and Huganir, 2018; Huganir and Nicoll, 2013; Malinow and Malenka, 2002). A recent 'surface proteome' analysis in cultured neurons found that increased surface abundance of AMPARs correlates with increased abundance of a few cell adhesion molecules, including the potential synaptic adhesion molecule Slitrk1, following chemical LTP induction (van Oostrum et al., 2020). More detailed and functional analyses of these cell adhesion molecules and their trafficking will be required to make sense of this data, especially since cell adhesion molecules can exert very different and sometimes opposing, context-dependent functions.

Finally, the main classes of synaptic adhesion molecules discussed in this review have all been linked to functional transmitter receptor turnover, in particular for AMPA receptors. Stabilization of Cadherins at the adult synapse has been shown to completely block cocaine-induced changes in AMPA receptor localization and long-term potentiation (Mills et al., 2017). Stabilization of AMPA receptors is also regulated by ephrinB2 in mouse hippocampal neurons, thereby strengthening synapses (Essmann et al., 2008). And finally, increased interaction of a mutant form of Neuroligin3 with AMPA receptors increases AMPA receptor internalization, while the same mutation in Neuroligin4 causes increased AMPA receptor-mediated synaptic responses (Chanda et al., 2016). Recent reviews on the functional interactions of synaptic adhesion molecules and transmitter receptors are available elsewhere (Chamma et al., 2020; Fossati and Charrier, 2021; Keable et al., 2020). While mechanisms of co-trafficking versus opposite effects of activity on turnover at the synapse remain to be elucidated, the principal role of constitutive turnover as a basis for modulation in response to activity or other triggers may highlight a common theme, as discussed in the final section.

6. Constitutive turnover of synaptic adhesion molecules as a basis for regulatory mechanisms of synapse development, function and maintenance

Neuronal activity or other triggers can alter the synaptic localization of synaptic adhesion molecules either by acute exo−/endocytosis, proteolytic cleavage, or modulation of constitutively turned over receptors through changes in the degradation vs recycling ratio (Fig. 2). Constitutive turnover of synaptic adhesion proteins allows for fast and fine-tuned changes of synaptic adhesion, and thereby synaptic strength. Instead of acutely regulating endocytosis or exocytosis of adhesion receptors, constitutive turnover allows to regulate surface levels through endolysosomal sorting mechanisms that fine-tune the rates of lysosomal degradation versus recycling to the plasma membrane (Fig. 2). At adult synapses, the ratio of degradation versus recycling of synaptic adhesion receptors can be fine-tuned by an endolysosomal sorting mechanism based on the function of the retromer or retriever complexes (Cullen and Steinberg, 2018). The retromer and retriever pathways provide independent mechanisms that regulate the sorting of integral membrane proteins for degradation or recycling following endocytosis; both are based on similar, but molecularly distinct multi-protein complexes that utilize different adaptor proteins (McNally et al., 2017). Retromer utilizes the adaptor protein sorting nexin 27 (snx27) to rescue membrane receptors from lysosomal degradation (Gallon and Cullen, 2015). As described above, snx27 directly binds to Neuroligin2, and reduced snx27 leads to increased Nlg2 degradation and thereby weakening of inhibitory synapses (Halff et al., 2019). This fine-tuning effect of snx27-dependent modulation of the rate of recycling of a postsynaptic adhesion protein depends on the 'running machinery' of constitutively turned over Nlg2.

The fundamental concept of modulation based on constitutive membrane receptor turnover was influentially described in the developmental context of axonal midline crossing for turnover of the Robo receptor in *Drosophila* nervous system development (Keleman et al., 2002). Constitutive turnover of Robo allows for regulation through a short burst of an intracellular, endolysosomal 'divergence' receptor (commissureless) that interferes with the conveyor-belt-like recycling of Robo for only a brief period of time, during which Robo gets degraded immediately without reaching the membrane, thus allowing a short time window for a single event of midline crossing (Keleman et al., 2002). A similar mechanism has only recently been shown for midline crossing in vertebrates (Gorla et al., 2019) and little is known about the sorting of synaptic adhesion molecules based on constitutive turnover in adult neurons. In theory, sorting based on constitutive turnover could reconcile the observed downregulation of neurotransmitter receptors and upregulation or stabilization of synaptic adhesion molecules in response to neuronal activity with the finding that both types of proteins can be found on the same endosomal compartments; differential sorting and opposite fates at the degradation/recycling choice point could quantitatively tune surface localization (Fig. 1). The retromer and retriever complexes are particularly well poised for this kind of receptor turnover 'tuning' underlying spatio-temporally specific roles (Cullen and Steinberg, 2018). While retromer has been implicated in both synaptic adhesion molecules and neurotransmitter receptor recycling (Binda et al., 2019; Choy et al., 2014; Gallon and Cullen, 2015; Halff et al., 2019; Luth et al., 2021; Temkin et al., 2017; Walsh et al., 2021), the role of retriever remains yet to be studied in more detail in neurons. To what extent these types of mechanisms will be validated depends both on the characterization of specific cargo adaptors as well as live analyses in an in vivo context.

Declaration of competing interest

The authors declare no competing interests.

Data availability

No data was used for the research described in the article.

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