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REVIEW

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Complexin regulation of synaptic vesicle release: mechanisms in the central nervous system and specialized retinal ribbon synapses

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Abstract

Synaptic ribbons, recognized for their pivotal role in conveying sensory signals in the visual pathway, are intricate assemblages of presynaptic proteins. Complexin (CPX) regulates synaptic vesicle fusion and neurotransmitter release by modulating the assembly of the soluble NSF attachment protein receptor (SNARE) complex, ensuring precise signal transmission in the retina and the broader central nervous system (CNS). While CPX1 or CPX2 isoforms (CPX1/2) play crucial roles in classical CNS synapses, CPX3 or CPX4 isoforms (CPX3/4) specifically regulate retinal ribbon synapses. These isoforms are essential for sustaining synaptic plasticity related to light signaling, adapting to changes in circadian rhythms, and dynamically regulating visual function under varying light conditions. This review explores the regulation of synaptic vesicle release by CPX in both the CNS and retinal ribbon synapses, with a focus on the mechanisms governing CPX3/4 function in the retina. Additionally, by reviewing the role of CPX and ribbon synapse dysfunction in non-retinal diseases, we further hypothesize the potential mechanisms of CPX in retinal diseases and propose therapeutic strategies targeting CPX to address retinal and CNS disorders associated with synaptic dysfunction.

Keywords Complexin, Ribbon synapse, Photoreceptor synapse, SNARE complex, Retina, Central nervous system

Introduction

Communication between neurons specifically depends on the release of neurotransmitters, which are intricately regulated by synaptic structures. The soluble NSF attachment protein receptor (SNARE) complex facilitates neurotransmitter release into the synaptic cleft by enabling the efficient fusion of synaptic vesicles with the presynaptic membrane and binding to the receptors of the subsequent neuron, thereby ensuring accurate signal transmission [1]. Complexin (CPX) precisely regulates neurotransmitter release by promoting the assembly and enhancing the stability of the SNARE complex, as well as modulating vesicular transport among distinct vesicular pools within the synapse [2]. CPX1 is the primary isoform in the mammalian central nervous system (CNS). Conversely, the mammalian retina contains all known CPX isoforms [3]. In addition to classical synaptic structures, the retina comprises a unique synapse architecture known as the ribbon synapse. Within the ribbon synapse, a dynamic and protein-rich structure referred to as the ribbon binds synaptic vesicles in close proximity to the active zone via thin filaments, facilitating the rapid release of synaptic vesicles [4, 5]. Although the current understanding of CPX function is primarily derived from studies of CPX1 in the central nervous system, other

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isoforms of CPX—specifically CPX3 or CPX4 isoforms (CPX3/4)—in the retina provide new research perspectives. These isoforms exhibit distinct functions in retinal ribbon synapses, potentially further regulating vesicle transport between pools to optimize signal transmission during high-frequency stimulation [6]. Additionally, they can regulate circadian rhythms [7]. To date, these functions have not been demonstrated by CPX1, presenting new possibilities for exploring CPX function in depth from a novel perspective. In this review, we provide a comprehensive overview of the role of CPX in synaptic vesicle regulation across both CNS and retinal ribbon synapses, highlighting the functional importance of CPX3/4 in visual processing. Furthermore, we explore the potential of targeting CPX for treating synaptic transmission disorders in both the CNS and retina.

SNARE-CPX complexes regulate the release of synaptic vesicles in the central nervous system

During neurotransmitter release, the fusion of synaptic vesicles with presynaptic membranes requires the overcoming of electrostatic repulsive forces between negatively charged phospholipid membranes. This process primarily relies on the energy generated during the assembly of the SNARE complex, which serves as the central molecular mechanism facilitating nearly all types of intracellular fusion and extracellular secretion [8]. The presence of cell-specific SNARE components in various neuronal types, coupled with the binding of these proteins, ensures the precise delivery of vesicles to their target neurons. The role of CPX, particularly its isoform CPX1, as a regulator of the SNARE complex has been extensively investigated in the central nervous system. Here, we examine in detail the structure and function of the SNARE complex and CPX1, along with their regulatory mechanisms during synaptic vesicle fusion.

SNARE complex

Synaptobrevin/ VAMP2, syntaxin, and SNAP-25 collectively form a SNARE complex characterized by a highly stable four-helix bundle structure [9] (Fig. 1). Synaptobrevin on the vesicular membrane is called vesicular SNARE (v-SNARE), while syntaxin and SNAP-25 on the presynaptic membrane are called target SNAREs (t-SNAREs). Before the formation of the SNARE complex, syntaxin exists in a closed state, where the H3 domain of syntaxin interacts with the autonomously folded Habc domain at its N-terminal end.

Under the coordinated regulation of Munc18-1 and Munc13-1, syntaxin-1 transitions from a closed to an open conformation, facilitating SNARE complex assembly. Munc18-1 has an arched structure composed of three domains, into which the Habc region and H3 domain of syntaxin-1 are embedded, further stabilizing the closed conformation of syntaxin-1, thereby preventing premature SNARE assembly [10]. The MUN domain of Munc13-1 interacts with the hinge region of syntaxin-1 via its NF hydrophobic pocket, catalyzing the transition from its closed to open conformation and driving the conversion of the Munc18-1/syntaxin-1 complex into the SNARE complex [11, 12]. As members of the SM protein family, Munc18-1 and Munc13-1 interact with SNARE proteins to prevent premature disassembly of the SNARE complex by NSF/SNAPs, while also promoting SNARE complex assembly and membrane fusion [13–15]. Notably, the binding of Munc18-1 to syntaxin-1 in its closed conformation stabilizes the Munc18-1/syntaxin-1 complex, distinguishing Munc18-1 from other SM proteins. However, in Munc18-1 knockout mice, neurotransmitter release is completely abolished, indicating that Munc18-1 may also interact with the open conformation of syntaxin-1 to promote SNARE complex assembly and drive membrane fusion [16].

As SNARE complex assembly initiates, vesicle membranes gradually approach the presynaptic membrane. Precise recognition between the v-SNARE and t-SNARE proteins drives the formation of the trans-SNARE complex, whose coupled folding and assembly bring the two membranes into close proximity, providing the energy necessary to overcome the membrane fusion barrier [17]. When synaptic vesicles dock to presynaptic membranes, SNAP-25, syntaxin and synaptobrevin rapidly assemble into partially formed SNARE complexes. These partially assembled complexes bind to synaptotagmin and CPX and are stabilized in an activated state that is resistant to NSF/SNAP degradation. Excitatory signaling, which leads to changes in Ca^{2+} concentration, brings the vesicle membrane into tighter apposition with the presynaptic membrane, facilitating membrane fusion. Concurrently, the assembly of the cis-SNARE complex is completed, catalyzing the formation of intermembrane fusion pores. The size of the fusion pore is closely related to the number of SNARE proteins functioning cooperatively. Three or more SNARE proteins can induce the pore diameter to exceed 1.5 nm, facilitating indefinite expansion of the pore and promoting rapid neurotransmitter release [18]. Following neurotransmitter release, the cis-SNARE complex is removed by the ATPase NSF and its accessory protein, SNAP [19]. The NSF/SNAP complex also monitors the SNARE complex and intermediate components throughout the entire cycle, ensuring proper recycling of vesicles in subsequent cytosolic cycles.

Although SNARE-mediated membrane fusion is well understood, the molecular details of the fusion intermediates remain unclear. While SNARE complexes are known to bring membranes into close proximity, the

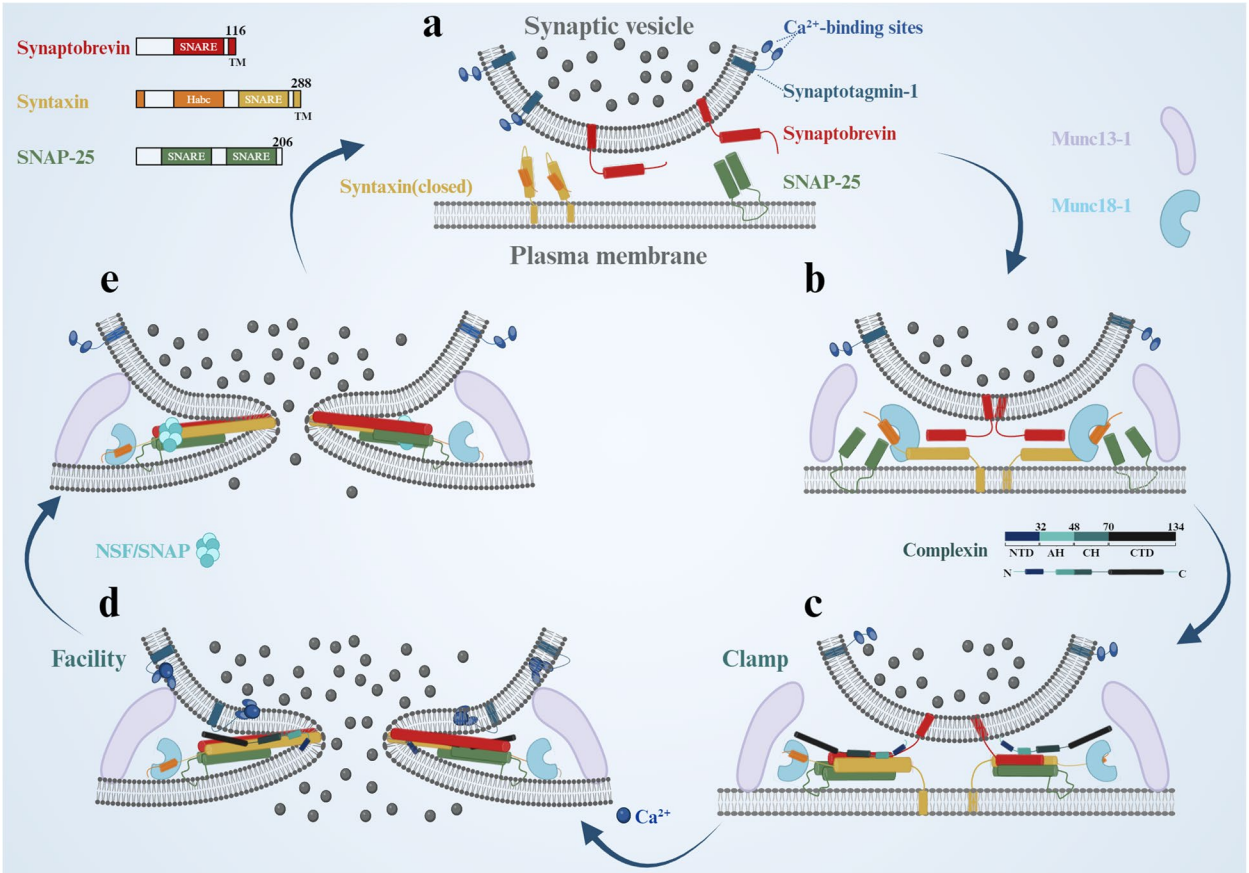

Fig. 1 SNARE complex-mediated synaptic vesicle fusion cycle. **a** The distribution of synaptobrevin, synaptotagmin-1, syntaxin, and SNAP-25 on the presynaptic and plasma membranes is depicted. The Habc domain of syntaxin (orange) binds to the SNARE motif (H3 domain) (yellow), thereby maintaining the closed state of syntaxin. **b** Munc18-1 and Munc13-1 collaboratively regulate the transition of Syntaxin-1 to its open state, facilitating the initiation of SNARE complex assembly. **c** The partial assembly of the SNARE complex promotes the close contact between the vesicle and the plasma membrane. Meanwhile, Complexin inserts into the groove between synaptobrevin and syntaxin, exhibiting its clamp function to inhibit premature fusion. **d** The interaction between Ca^{2+} and Synaptotagmin-1 triggers the binding of NTD of CPX to the fully assembled SNARE complex, facilitating its association with the plasma membrane. This interaction drives the fusion of phospholipid molecules within the membrane, ultimately leading to the formation of a fusion pore, highlighting Complexin's facilitative role. **e** NSF/SNAP disassembles the SNARE complex, thereby allowing the vesicles to be recycled and reused in the process of release

exact molecular events that drive the formation and expansion of the fusion pore are still not fully understood. The free energy released during SNARE complex assembly is predicted to be approximately 35–68 kBT, which is well above the estimated energy threshold for membrane fusion, typically around 20–30 kBT, necessary to overcome the fusion pore formation barrier [18, 20]. Key molecular events, including lipid rearrangement, SNARE zippering, and the transition from hemifusion to pore formation, remain poorly understood. Understanding these processes is crucial for elucidating how fusion proteins overcome the free energy barriers to complete membrane fusion. Advanced techniques such as high-resolution cryo-electron microscopy (cryo-EM) and single-molecule Förster resonance energy transfer (smFRET) studies could be employed to investigate the conformational changes in SNARE complexes during these stages [21, 22].

Structure and function of CPX

CPX is composed of four domains: the N-terminal domain (NTD), the accessory α-helix (AH), the central α-helix (CH), and the C-terminal domain (CTD) [23]. The distinct functional roles of these domains collectively contribute to the precise regulation of the synaptic vesicle release process by CPX (Table 1). The CH domain is highly conserved across species and tightly binds to Synaptobrevin and syntaxin-1 of the SNARE complex, establishing itself as a central component of CPX-regulated SNARE complex function [24]. The AH domain, located

Table 1 CPX domains and their functions

Domains of CPX	Effect on synaptic vesicle fusion	Main functions	Reference
CH	Enhance/Inhibit	• Binding site to the SNARE complex directly	[28–30]
AH	Inhibit	• Blocking the C-terminal assembly of the complex by insertion and replacement of part of the V-SNARE motifs • Improving integration with Synaptobrevin • Enhancing electrostatic repulsion between membranes and inhibiting synaptic vesicle fusion	[25, 26]
NTD	Enhance	• Removing the inhibitory effect of AH on synaptic vesicle fusion • Forming an amphipathic helix with the C-terminus of SNARE and promoting synaptic vesicle release by stabilizing the C-terminus of SNARE	[31, 32]
CTD	Enhance/Inhibit	• Focusing SNARE complex subunits at the cytoplasmic site through their amphipathic helical anchoring to synaptic vesicles effectively inhibits peripheral synaptic vesicle fusion • Folding to the AH region inhibits synaptic vesicle fusion release by enhancing AH inhibition of SNARE complex assembly • Stabilizing of the fusion pore of nascent vesicles and acceleration of synaptic vesicle fusion release	[33–37]

CH Central α-helix, *AH* Accessory α-helix, *NTD* N-terminal domain, *CTD* C-terminal domain

on the N-terminal side of the CH domain, plays a crucial role in inhibiting spontaneous synaptic vesicle fusion. It prevents SNARE complex assembly by inserting into the C-terminus of synaptobrevin and interacting with its subunits while enhancing intermembrane electrostatic repulsion [25, 26]. In contrast, the NTD promotes membrane fusion activity. The NTD can both counteract the inhibitory effect of the AH on vesicle release and facilitate vesicle release by stabilizing the C-terminus of SNARE [27]. (Fig. 2).

The CTD serves as a membrane curvature sensor, allowing CPX to be accurately targeted to the appropriate membrane region, thereby ensuring proper function [23]. The CTD remodels vesicle membranes by modulating membrane dynamics, slowing the rate of fusion pore closure, and stabilizing newly formed fusion pores. It also enhances the rate of fusion pore formation, thereby increasing the efficiency of vesicle release [33–35]. The CTD may inhibit vesicle fusion by anchoring its amphipathic helix to the synaptic vesicle membrane. This anchoring promotes the insertion of the CH domain into the groove between synaptobrevin and syntaxin, thereby indirectly preventing the full assembly of the SNARE complex [36]. Another hypothesis proposes that the CTD may fold onto the AH domain, facilitating the interaction of lipids and proteins with its amphipathic helix. This interaction may help to solidify the localization of the AH domain within the SNARE complex and enhance the inhibitory effect of the AH domain [37]. (Fig. 2).

CPX regulates synaptic vesicle fusion by balancing inhibition and facilitation, depending on its interaction with various partners. In its inhibitory role, CPX stabilizes vesicles in a release-ready state by preventing premature fusion. Upon an excitatory stimulus and an increase in intracellular Ca^{2+}, CPX shifts to a facilitory role, promoting synchronous vesicle release. This transition is mediated by the binding of NTD of CPX to the C-terminus of the fully assembled ternary SNARE complex, which displaces the inhibitory action of the AH domain [38]. Importantly, synaptotagmin-1 (Syt-1) competes with CPX for SNARE binding in a Ca^{2+}-dependent manner. At higher Ca^{2+} concentrations, Syt-1 displaces CPX from PI [4, 5]P2-enriched membrane regions, further facilitating vesicle fusion [39]. Meanwhile, CPX functions as a checkpoint, overseeing the quality control of synaptic vesicles to ensure that they are in the correct state prior to membrane fusion, thereby facilitating the efficient release of neurotransmitters [40].

CPX enhances the efficiency of synchronized synaptic vesicle fusion through its dual regulatory role, a function that has varied across species throughout evolutionary history. (Table 2). In the nervous system of *C. elegans* and at the *Drosophila* neuromuscular junction, CPX primarily inhibits vesicle release [41–44]. In contrast, in the nervous systems of mammals and in the auditory neurotransmission of bats, CPX predominantly acts as a facilitator of vesicle release [45, 46].These evolutionary differences likely result from several factors. First, structural adaptations of CPX across species, particularly in its interactions with the SNARE complex, may have driven functional divergence. Second, as nervous system complexity increased throughout evolution, CPX may have become more specialized, focusing on facilitating synchronized release in complex systems, while performing broader roles in simpler systems. Additionally, variations in calcium signaling sensitivity and regulation across species may impact CPX function, particularly in calcium-dependent synaptic fusion. Lastly, differences in neural

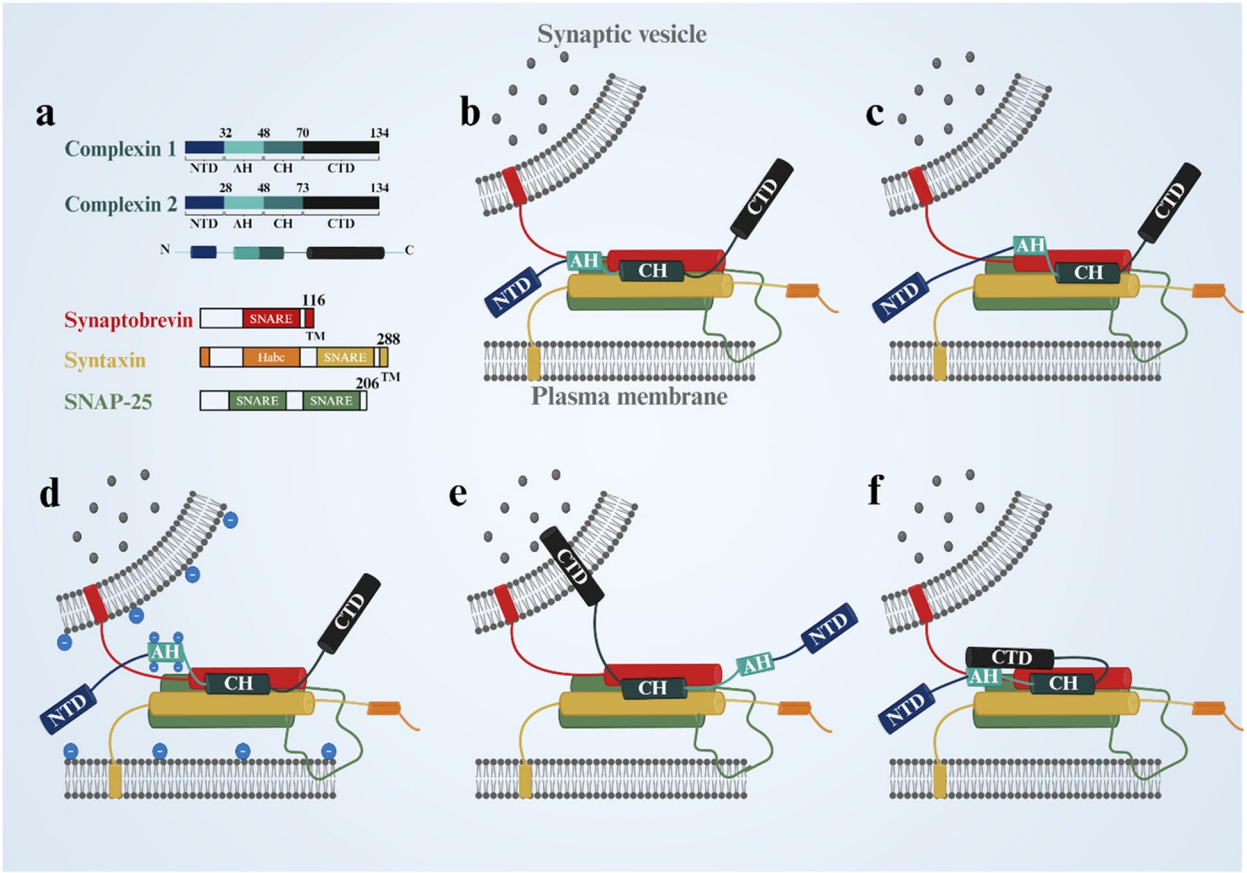

Fig. 2 CPX inhibits the assembly of the SNARE complex. **a** Schematic representation of different domains within CPX and the SNARE complex proteins. Numbers indicate the amino acid positions at the termination points of the respective domains. (b, c, d) Models illustrating the mechanism of AH in directly inhibiting SNARE complex assembly: **b** The AH inhibits SNARE complex assembly by inserting itself into the SNARE complex. **c** The AH binds to synaptobrevin, blocking the assembly of the SNARE complex. **d** The AH increases electrostatic repulsion between membranes, thereby inhibiting SNARE complex assembly. **e**, **f** Models illustrating the indirect inhibition of SNARE complex assembly by the CTD: **e** The CTD anchors to highly curved vesicle membranes, guiding the CH to insert into the groove between synaptobrevin and syntaxin, thereby indirectly preventing the complete assembly of the SNARE complex at its C-terminal. **f** The CTD folds back onto the AH, enhancing the functional activity of the AH and indirectly blocking the complete assembly of the SNARE complex. NT = N-terminal domain, AH = accessory helix, CH = central helix, and CTD = C-terminal domain

Table 2 CPX Functions in Central Synapses Across Species

Species	CPX Role	Observed Effects	Reference
Drosophila melanogaster	Primarily inhibition	CPX suppresses spontaneous and evoked synaptic vesicle release	[42–44]
Caenorhabditis elegans	Primarily inhibition	CPX suppresses spontaneous and evoked synaptic vesicle release	[41]
Mammals (Mouse)	Primarily facilitation	CPX enhances evoked release, prevents premature fusion, modulates ultrasound transmission	[45]
Microbats	Primarily facilitation	CPX improves synaptic efficiency in ultrasound transmission, enhancing echolocation	[46]
Megabats	Weaker facilitation	Lower CPX expression results in reduced neurotransmitter release compared to microbats	[46]

activity frequency and speed across species may influence the balance between CPX's facilitative and inhibitory roles in synaptic transmission.

The divergence and convergence between CPX1/2 and CPX3/4 within the retina

Retinal information processing is governed by intricate interactions between photoreceptors, bipolar cells,

horizontal cells, amacrine cells, and ganglion cells, which encode visual signals via specialized synaptic connections. Photoreceptors transduce light into biochemical signals and release glutamate to modulate ON/OFF bipolar cells [47, 48]. Horizontal cells fine-tune signals through feedback and feed-forward mechanisms, producing a center-surround effect that enhances contrast sensitivity, essential for visual processing [49–51]. Ribbon synapses play an essential role in this process, enabling precise communication between photoreceptors, bipolar cells, and horizontal cells [52–54]. These synapses ensure high-fidelity signal transmission, particularly along the photoreceptor-bipolar cell pathways [55]. Rod ribbon synapses involve interactions between AMPA-positive horizontal cell axons and mGluR6-positive bipolar dendrites, while cone ribbon synapses form more complex connections with multiple types of bipolar cells [56–58]. Gap junctions between cone pedicles and adjacent photoreceptors support signal integration, especially under mesopic conditions, thereby enhancing contrast and edge detection [59–62].

CPX can be divided into two subfamilies: CPX1/2 and CPX3/4. CPX1/2 and CPX3/4 exhibit distinct differences across multiple dimensions, including CPX1/2 and CPX3/4 differ in various aspects, including structure, expression location, synaptic function, evolutionary conservation, compensation mechanisms, and affinity for SNARE [3, 28, 63–65]. (Table 3) By elucidating the differences between CPX1/2 and CPX3/4, we can clarify their distinct regulatory roles in CNS synapses and retinal ribbon synapses. This understanding deepens our knowledge of CPX-mediated synaptic vesicle release and sheds light on the molecular pathologies of neurotransmitter dysfunction. These insights are crucial for developing targeted therapies and advancing the early diagnosis and treatment of synaptic disorders. Although the synaptic regulatory functions of CPX3/4 in the visual system are less understood, recent studies have begun to uncover their unique roles in retinal ribbon synapses. Lux et al. conducted a detailed analysis of CPX isoform distribution in the retina, revealing that CPX3/4 is primarily localized at ribbon synapses within photoreceptors and bipolar cells, whereas CPX1/2 is concentrated at conventional synapses of amacrine and ganglion cells [66]. Notably, CPX4 is specifically expressed in rod synaptic terminals, while CPX3 and CPX4 are co-expressed in cone synaptic terminals. Functionally, CPX3/4 exhibits a relatively lower affinity for the SNARE complex, primarily due to structural differences in their SNARE-binding domains, with CPX4 displaying particularly weak interactions with the SNARE complex. This reduced affinity may impair their ability to stabilize the SNARE complex, leading to decreased sensitivity [3]. Nevertheless, CPX3 and CPX4 possess highly conserved SNARE-binding regions, allowing them to compensate effectively for the loss of neurotransmitter release in CPX1 and CPX2 double knockout (DKO) neurons, restoring normal function when overexpressed [3].

Classical regulation of retinal synapses by CPX1/2

Although CPX1/2 are the earliest isoforms detected during retinal development, most current research has focused on the association between CPX3/4 and visual function [66, 67]. Thus, the specific contributions of each CPX isoform to visual processing remain unclear. CPX3/4 knockout (KO) mice present with visual impairments, including delayed light responses and abnormalities in electroretinogram (ERG) recordings. In comparison, CPX1 knockout mice display only isolated b-wave abnormalities in the ERG [66, 67]. CPX1 is predominantly expressed in the ganglion cell layer of the retina, while CPX2 is primarily localized in the inner nuclear layer [66]. Despite its distinct expression pattern, CPX2 knockout (KO) mice do not exhibit significant retinal dysfunction, indicating that role of CPX2 in the retina may be compensated by redundant mechanisms or other proteins. In contrast, CPX1 KO mice show profound alterations in retinal function, which are likely due

Table 3 Divergence and convergence between CPX1/2 and CPX3/4 in the retina

Characteristic	CPX1/2	CPX3/4
Structure	Lacks CAAX motif, no farnesylation	Contains CAAX motif, undergoes farnesylation
Affinity for SNARE	High affinity	Lower affinity, especially for CPX4
Expression Location	Conventional synapses, widely expressed in the brain	Retinal ribbon synapses, particularly in photoreceptors and bipolar cells
Synaptic Function	Stabilizes the SNARE complex, promotes efficient synaptic vesicle release	Farnesylation enhances synaptic targeting, compensating for CPX1/2 loss
Evolutionary Conservation	High homology with invertebrates	Unique to vertebrates, especially in ribbon synapses
Compensation Mechanism	Loss results in significant functional deficits	Partially compensates for the functional loss of CPX1/2 double knockout

to intrinsic retinal abnormalities rather than cortical dysfunction. This is because CPX1 is mainly expressed in the ganglion cell layer, where it directly modulates synaptic transmission in the retina [66]. Additionally, CPX1 KO mice display severe motor deficits, including ataxia and increased seizure susceptibility, which are closely related to widespread distribution of CPX1 in the central nervous system [64, 68]. These findings underscore the functional divergence between CPX1/2 and CPX3/4, with CPX3/4 having a specialized role in retinal ribbon synapses, while CPX1/2 are involved in broader synaptic transmission and neural functions.

However, the current gene knockout mouse models have not clarified the impact of CPX2 on retinal function, nor have they clarified the detailed molecular mechanisms of CPX1 [66]. Future research should prioritize the following aspects: First, it is essential to investigate the potential compensatory mechanisms of CPX2 in the retina by employing both gene knockout and overexpression models. Second, considering the homologous similarity between CPX1 and CPX2, investigating their interactions will clarify their collaborative roles. Finally, based on CPX1's established function in the CNS, further exploration of its specific role in the retina is necessary. Addressing these questions will significantly enhance our understanding of the mechanisms underlying visual processing and will clarify the integrated contributions of CPX1/2 to retinal function.

Specific regulation of retinal ribbon synapses by CPX3/4

The transmission of visual information depends on efficient, coordinated transport between multiple vesicle pools within the ribbon synapse. The vesicle release pool near the presynaptic membrane rapidly responds to excitatory signals by releasing vesicles. The vesicle storage pools connected to the ribbon quickly replenish the release pool with vesicles [69]. Additionally, a large cytoplasmic vesicle storage pool contains numerous immature vesicles, which are not yet fully primed for release. These vesicles must undergo further maturation and preparation processes before they can be integrated into the synaptic release cycle [70, 71]. At ribbon synapses, this rapid replenishment enables photoreceptor cells to transmit information efficiently, maintaining visual stability under varying light conditions. This entire process involves vesicle generation, transport, anchoring, fusion, and recycling, with each step tightly regulated to ensure the effective transmission of visual information [71, 72].

Ribbon synapses predominantly express CPX3/4, which optimizes visual information by regulating vesicle release [66]. Depending on Ca^{2+} sensitivity, ribbon synapses exhibit different modes of vesicle release, including spontaneous fusion (independent of calcium channels), transient release (time-locked to presynaptic calcium endocytosis), asynchronous release (driven by residual calcium ions after calcium channel closure), and synchronous release [73–75]. CPX3/4 function similarly to CPX1/2 by acting on the SNARE complex, inhibiting spontaneous synaptic vesicle fusion while lowering the free energy barrier for membrane fusion, thereby facilitating rapid temporal asynchronous vesicle release [65]. However, when the CT region of CPX3 is replaced with that of CPX1, the modified CPX3 no longer inhibits spontaneous vesicle release, suggesting distinct regulatory mechanisms between CPX3 and CPX1/2 [63, 76]. In the study of retinal rod bipolar cells, both mouse and zebrafish models were employed. Different approaches, including KO models and the use of specific inhibitory peptides, were utilized in mice to interfere with ACPX functions [77, 78]. In zebrafish, genetic manipulation techniques were applied to assess CPX-related disruptions in visual signaling pathways [79]. Deletion of CPX4 at the spherical end of the rod does not affect the number of vesicles in the release pool but results in increased vesicle release rate and delayed vesicle delivery. This indicates that CPX4 plays a crucial role in minimizing synaptic noise and expanding the dynamic range by modulating the dynamics of vesicle release, thereby enhancing contrast sensitivity and temporal resolution of the visual system [80]. In the retina treated with CPX-SBD (Complexin-SNARE binding domain inhibitory peptide), the cytoplasmic reserve vesicle pool is significantly reduced, while the number of vesicles attached to the ribbon structure remains unaffected by CPX-SBD [77].

Ribbon synapses primarily express CPX3/4, which optimize visual information processing by regulating vesicle release [66]. Depending on Ca^{2+} sensitivity, these synapses exhibit various modes of vesicle release, including spontaneous, transient, asynchronous, and synchronous release [73–75]. CPX3/4, like CPX1/2, interact with the SNARE complex to inhibit spontaneous vesicle fusion while lowering the energy barrier for membrane fusion, thereby promoting rapid asynchronous vesicle release [65]. However, replacing the CT region of CPX3 with that of CPX1 abolishes its ability to inhibit spontaneous vesicle release, indicating distinct regulatory mechanisms between CPX3 and CPX1/2 [63, 76].This distinction highlights the different roles of CPX3 and CPX4 at ribbon synapses. For instance, deletion of CPX4 at the rod synaptic terminal does not affect the size of the vesicle pool but significantly increases the vesicle release rate and delays vesicle replenishment. This suggests that CPX4 modulates vesicle release dynamics to minimize synaptic noise and expand the dynamic range, thereby enhancing contrast sensitivity and temporal resolution in the visual system [80]. In retinas treated with CPX-SBD,

the cytoplasmic reserve vesicle pool in ON bipolar cells is significantly reduced, while the number of vesicles attached to the ribbon synapse remains unaffected [77]. Both CPX3 and CPX4 regulate vesicle transport and release across the ribbon synapse, with CPX3 possibly more involved in regulating the number of vesicles in the storage pool, and CPX4 influencing vesicle volume and function. Together, they optimize visual information processing.

The regulation of CPX3/4 in visual signal conduction

A number of abnormalities in retinal function were observed in CPX3/4 knockout (KO) mice, including impaired visual acuity, delayed responses to light, and abnormal retinal electrical signals [78].CPX3/4 are critical for maintaining retinal plasticity and signal integrity by modulating specific neuronal subtypes involved in visual transmission.(Table 4). Photoreceptor ribbon synapses regulate the vesicular storage pool, allowing photoreceptors to adapt to varying light conditions and rapidly respond to changes in ambient illumination [81]. Under physiological conditions, synaptic vesicles self-regulate in response to light intensity, decreasing in bright environments and increasing in darkness. However, in CPX3/4 double knockout (DKO) mice, synaptic vesicles fail to respond to changes in light intensity, leading to impaired circadian rhythm regulation in the retina [6]. Circadian rhythms, tightly regulated by the endogenous biological clock, are essential for maintaining visual function stability and adaptability. CPX3 exhibits diurnal variation, with lower expression levels at night, highlighting its role in circadian rhythm regulation and downstream neuronal activity [7].

Studies have shown that CPX3/4 knockout increases the spontaneous neurotransmitter release of horizontal cells, reduces synaptic synchrony and precision, and enhances the amplitude of their response to light stimuli, as observed in CPX3/4 DKO mouse models [6]. In bipolar cells, CPX-SBD increases spontaneous vesicle release while reducing the reserve vesicle pool by approximately 20%, without affecting the vesicle number associated with ribbon synapses [77]. Moreover, in rod bipolar cells that exclusively express CPX3, knockdown of CPX3 decreases synaptic transmission synchrony, enhances asynchronous release, and inhibits retinal ganglion cell output, impairing the precise transmission of visual signals [78]. CPX3/4 regulate vesicle transport and release through their interactions with the SNARE complex, indirectly influencing neuronal function across multiple retinal layers. Current studies have largely focused on the effects of CPX3/4 on individual retinal neurons, providing valuable insights into their role at the cellular level. However, to fully understand how CPX3/4 modulate broader visual signaling pathways across retinal networks, future research should investigate system-level dynamics, potentially through methods such as conditional knockout (KO) models targeting specific retinal layers or circuit-level analyses.

Table 4 Effects of CPX3/4 on neurons at all levels of the retina

Types of retinal cells	Methods	Main findings	Reference
Cone	Gene knockout animal models	• CPX3 is a potential target for circadian rhythm regulation	[7, 82]
Rod	single-cell electrophysiological recordings	• CPX4 deficiency causes abnormal ribbon synapses • Increased Ca^{2+}-dependent synaptic vesicle release • Desensitization of phototransduction	[80]
BC	Single-cell electrophysiological recordings combined with peptide synthesis techniques	• CPX-SBD increases spontaneous vesicle release • Reduces Ca^{2+}-triggered vesicle fusion • No impact on ribbon-associated vesicle numbers • Significantly lowers reserve vesicle density	[77]
	Single-cell electrophysiological recordings combined with Gene knockout animal models	• CPX3 loss decreases transmission synchrony • Enhances asynchronous release • Inhibits retinal ganglion cell output • Impairs precise visual signal transmission	[78]
	single-cell electrophysiological recordings with electroretinograms	• CPX3/4 knockout reduces ON/OFF pathway responses • Disrupts rod-to-bipolar synapse transmission • Decreases synaptic synchrony and precision	[67]
HC	single-cell electrophysiological recordings combined with Gene knockout animal models	• CPX3/4 knockout increases neurotransmitter release • Reduces synaptic synchrony and precision •Increases light response amplitude •Affects both spontaneous and evoked release	[6]

BC bipolar cell, *CPX-SBD* Complexin-SNARE binding domain inhibitory peptide, *HC* horizontal cell

The potential role of CPX in retinal diseases

As a presynaptic protein, CPX is well-studied for its role in regulating vesicle release. However, research exploring the association between CPX and retinal diseases is still limited. By analyzing the mechanisms linking CPX to other non-retinal diseases, we aim to investigate its potential role in retinal diseases and evaluate its feasibility as a therapeutic target in the future.

In the central nervous system, CPX ensures efficient neurotransmitter release by influencing the formation of the SNARE complex. CPX1, in particular, is crucial for maintaining synaptic function and protecting synaptic integrity. Dysfunction of CPX1 has been linked to synaptic damage in Alzheimer's disease, where abnormal accumulation of β-amyloid and tau proteins in the brains of Alzheimer's patients leads to impaired synaptic function, and dysregulation of CPX may exacerbate this synaptic damage [83]. Additionally, CPX2 plays a vital role in cognitive function, particularly in regulating neural activity in the prefrontal cortex and hippocampus. Altered expression of CPX2 in patients with schizophrenia is thought to contribute to synaptic dysfunction, which ultimately impacts cognitive functions like working memory [84]. In CPX1 knockout animal models, while cognitive function is largely preserved, significant motor coordination deficits and impaired behavioral responses are observed. Given the similarities in synaptic transmission mechanisms between the visual and central nervous systems, it is hypothesized that CPX may affect retinal diseases by modulating synaptic transmission in various functional neurons [85].

Ribbon synapses, present not only in the retina but also in inner ear hair cells, provide insights into the potential pathogenic mechanisms of CPX3/4 in retinal diseases by comparing them with other systems [86]. In the auditory system, ribbon synapses connect inner hair cells to spiral ganglion neurons, transmitting sound signals via calcium-triggered vesicle release. Abnormalities in ribbon synapses can lead to hidden hearing loss, with normal hearing thresholds but reduced auditory brainstem response amplitudes [87]. With aging, the number of ribbon synapses decreases significantly, yet the remaining synapses increase in size and enhance their capacity for sustained vesicle release. However, this compensatory mechanism may result in hypersensitivity in auditory responses [88]. In the visual system, mutations in ribbon synapse proteins impact synaptic vesicle release and are closely associated with inherited retinal diseases [89]. A reduction in the number of ribbon synapses and abnormal localization impairs photoreceptor synaptic transmission, leading to vision loss—a key mechanism in early-stage AMD [90]. Thus, CPX dysfunction may not only disrupt synaptic transmission but also play a role in maintaining photoreceptor nutrition and function. Moreover, CPX expression in amacrine cells further indicates that CPX may be involved in complex signaling pathways between multiple neuronal layers in the retina, affecting overall retinal health.

Recent studies have identified the transducin subunits Gat1 and Gb1g1 as critical interactors in the CPX4-SNARE complex [80]. These subunits facilitate photoreceptor-specific signaling processes, including the translocation of transducin responses to light stimuli from cone outer segments and synaptic terminals to cone inner segments [91]. The translocation of transducin from the outer to the inner segment takes approximately 10 min, while in dark-adapted rod photoreceptors, the size of the readily releasable vesicle pool (RRP) starts to decrease after 10 to 15 min of light exposure. This temporal concordance indicates that the translocation of transducin and CPX4-regulated vesicle release share a significant temporal and spatial correlation, potentially co-regulating the light adaptation mechanism [6, 92].

The synergistic interaction between the CPX4-SNARE-transducin complex enhances the spatial and temporal precision of neural regulation, presenting great potential in precision medicine. CPX4, through its dual function of clamping and activation, reduces neuronal hyperactivity induced by light stimulation, ensuring that optogenetic tools target specific neuronal populations with precision rather than triggering widespread neural network responses. Additionally, the cooperation between CPX4 and transducin enables neurons to respond to light stimuli more synchronously and accurately, thereby facilitating refined control of synaptic activity. In retinal neuronal repair, optogenetic tools combined with CPX4 regulation can precisely control the formation of photoreceptor ribbon synapses, ensuring that newly formed synapses establish effective connections and restore normal visual signal transmission. This precise control mechanism significantly improves the efficiency of neural network reconstruction and enhances the likelihood of functional recovery.

Conclusion

Current research highlights the critical role of CPX in regulating synaptic vesicle release across the CNS and in retinal ribbon synapses. However, several aspects of CPX function are still unclear, particularly regarding its precise regulatory mechanisms and its viability as a therapeutic target. This modulatory role of CPX may vary significantly among different neuronal types. In photoreceptor cells, CPX appears to fine-tune synaptic vesicle release based on fluctuating light conditions, thus maintaining optimal sensitivity to light stimuli. Conversely, in bipolar cells, CPX's role seems more central to the precise timing and amplitude of synaptic release, thereby optimizing

complex signal processing. Despite evidence that CPX, particularly CPX1, is strongly influenced by intracellular calcium fluctuations, uncertainty persists regarding whether calcium alone can meet the sensitivity demands of photoreceptors under diverse light intensities or support the nuanced regulation required by bipolar cells for processing varied signals. Additionally, the involvement of additional regulatory factors or signaling molecules could contribute to a more flexible mechanism. Further research into CPX's regulatory mechanisms under various physiological conditions—such as stress, inflammation, or cellular adaptations—will be vital in assessing its potential as a drug target and exploring its practical therapeutic applications.

In the future, the retina could become a key focus for investigating potential drug targets of CPX. The retina contains all CPX isoforms that modulate synaptic structure and influence the release of synaptic vesicles, particularly playing a crucial role in ribbon synapses. Although ribbon synapses are present in inner ear cells, no auditory impairment has been observed in CPX2, CPX3, and CPX4 knockout mice, indicating that CPX may not be crucial in the inner ear or might be compensated by alternative physiological mechanisms [93]. In contrast, CPX demonstrates unique biological functions in the retina, highlighting the need for further investigation.

In summary, although the roles and regulatory mechanisms of CPX in various types of neurons require more thorough investigation, its potential in the nervous system is highly promising. With more intensive research, investigating the diverse regulatory mechanisms of CPX could lead to important advancements in the treatment of CNS disorders and retinal diseases.

Abbreviations

CPX	Complexin
SNARE	Soluble NSF attachment protein receptor
CNS	Central nervous system
CPX1/2	CPX1 or CPX2 isoforms
CPX3/4	CPX3 or CPX4 isoforms
v-SNARE	Vesicular SNARE
t-SNARE	Target SNARE
NTD	N-terminal domain
AH	Accessory α-helix
CH	Central α-helix
CTD	C-terminal domain
KO	Knockout
DKO	Double knockout
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ERG	Electroretinogram
Syt-1	Synaptotagmin-1
NSF	N-ethylmaleimide-sensitive factor
SNAP	Soluble NSF Attachment Protein
RRP	Readily releasable vesicle pool
AMD	Age-related macular degeneration
RDD	Retinal degenerative disease
PI(4,5)P2	Phosphatidylinositol 4,5-bisphosphate
FRET	Förster Resonance Energy Transfer
cryo-EM	Cryo-electron microscopy

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors did not use generative AI and AI-assisted technologies.

Authors' contributions

Y.-Z.L. and G.-Y.L. prepared the first draft of the manuscript. All authors edited the review article. G.-Y.L. approved the submission of the manuscript. All authors contributed to the writing and editing and agreed to the submission of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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