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Neurodevelopmental Disease Mechanisms, Primary Cilia, and Endosomes Converge on the BLOC-1 and BORC Complexes

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Abstract

The Biogenesis of Lysosome-related Organelles Complex-1 (BLOC-1) and the BLOC-One-Related Complex (BORC) are cytosolic protein complexes required for specialized membrane protein traffic along the endocytic route and for the spatial distribution of endosome-derived compartments, respectively. BLOC-1 and BORC complex subunits and components of their interactomes have been associated with the risk and/or pathomechanisms of neurodevelopmental disorders. Thus, cellular processes requiring BLOC-1 and BORC interactomes have the potential to offer novel insight into mechanisms underlying behavioral defects. We focus on interactions between BLOC-1 or BORC subunits with the actin and microtubule cytoskeleton, membrane tethers, and SNAREs. These interactions highlight requirements for BLOC-1 and BORC in membrane movement by motors, control of actin polymerization, and targeting of membrane proteins to specialized cellular domains such as the nerve terminal and the primary cilium. We propose that the endosome-primary cilia pathway is an underappreciated hub in the genesis and mechanisms of neurodevelopmental disorders.

Keywords

BLOC-1; dysbindin; BORCS7; cilium; schizophrenia

The Biogenesis of Lysosome-related Organelles Complex-1 (BLOC-1) and the BLOC-One-Related Complex (BORC) are two cytosolic protein complexes necessary for membrane traffic and organelle positioning. BLOC-1 is required for early/recycling endosome membrane protein sorting and tubulation whereas BORC is necessary for lysosome positioning in mammalian cells (Ryder and Faundez, 2009; Ghiani and Dell’angelica, 2011; Mullin et al., 2011; Pu et al., 2015; Guardia et al., 2016; Farias et al., 2017).

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Structural Complexity of the BLOC-1 and BORC Complexes

BLOC-1 and BORC are stable complexes in diverse mammalian cells and *Drosophila* neurons (Ghiani et al., 2009; Ghiani and Dell’angelica, 2011; Mullin et al., 2011; Pu et al., 2015). Recombinant BLOC-1 and BORC complexes can be assembled as stable complexes with a precise stoichiometry of eight monomeric subunits in bacteria (Lee et al., 2012; Pu et al., 2015). BLOC-1 is formed by eight subunits BLOC1S1 to 6, DTNBP1, and SNAPIN (Ghiani and Dell’angelica, 2011; Mullin et al., 2011) (Figure 1A–B, Table I). An additional binding partner, herein referred to as the non-obligate BLOC-1 constituent, KXD1, has been proposed to be part of the BLOC-1 complex (Fig. 1A). KXD1 is a protein with unknown function that interacts in vitro with four of the BLOC-1 subunits BLOC1S1, BLOC1S2, BLOC1S4 and DTNBP1 (Hayes et al., 2011; Yang et al., 2012). KXD1 also co-precipitates with dysbindin from *Drosophila* cerebral ganglia extracts (Fig. 2A). The non-obligate BLOC-1 constituent designation stems from the fact that KXD1 null mouse mutant phenocopies other BLOC-1 mutants in their pigmentation and platelets defects. However, elimination of KXD1 does not destabilize other BLOC-1 subunits, a common molecular phenotype for other BLOC-1 subunits mutant alleles (Yang et al., 2012). The BORC complex is a recently identified cytosolic heterooctamer. BORC is constituted by BORCS5, BORCS6, BORCS7, BORCS8, the non-obligate BLOC-1 constituent KXD1, plus three subunits common with the BLOC-1 complex: SNAPIN, BLOC1S1 and BLOC1S2 (Figs. 1C and 2B, Table I) (Pu et al., 2015).

BLOC-1 and BORC complexes have been conceptualized as independent molecular species (Figs. 1C and 2B), however evidence from several interactomes suggest that there may exist different BLOC-1 and BORC subunit combinatorial arrangements (Figs. 1C–D and 2B). This idea is supported by the following observations: first dysbindin co-precipitates with BORCS6, a finding first described in the original article discovering the BORC complex (Fig. 2B) (Pu et al., 2015). Moreover, the BioPlex human interactome identified dysbindin coprecipitating with BORCS6 and BORCS8 (Fig. 1D) (Hutlin et al., 2015). The complexity of these binary interactions extends to BLOC1S5 which coprecipitates with BORCS6 in mammalian cells (Fig. 1D) (Huttlin et al., 2015). Our own studies in *Drosophila* indicate that dysbindin coprecipitates with BORCS6/CG17180 and KXD1/CG10681 (Fig. 2A). Collectively, these observations suggest that the subunit composition of the BLOC-1 and BORC complexes may be more fluid than anticipated. The evidence suggests the existence of stable BLOC-1/BORC hybrid complexes or complexes where some BLOC-1 or BORC subunits dynamically exchange to confer novel functionality to stable complexes.

Loss of a BLOC-1 subunit induces down-regulation of other BLOC-1 complex polypeptides. Dysbindin-null mice *sandy* (*Bloc1s8dy/dy*) lacking dysbindin polypeptide also express reduced levels of Bloc1s5, Bloc1s6, and Snapin (Li et al., 2003; Starcevic and Dell’Angelica, 2004; Feng et al., 2008). This interdependency of BLOC-1 subunits for stability appears to be universal, as evidenced by reduced pallidin expression in *Drosophila* Bloc1s1/blos1 and Bloc1s8/dysbindin mutants (Chen et al., 2017). Similarly, dysbindin is reduced in null mouse models of Bloc1s3 (*Bloc1s3p/p*), Bloc1s5 (*Bloc1s5mu/mu*) and Bloc1s6 (*Bloc1s6pa/pa*) (Li et al., 2003; Starcevic and Dell’Angelica, 2004; Feng et al., 2008). These changes in protein expression in BLOC-1 deficiencies also affect the steady
state levels of BLOC-1 subunit transcripts. The expression of Bloc1s2 and 3, Bloc1s6 and Snapin mRNAs are reduced in *Bloc1s6mut/mut* hippocampus (Larimore et al., 2014). If BLOC-1 and BORC subunits assemble into hybrid complexes or are dynamically exchanged, then the expression of BORC subunits could be modified by mutations in BLOC-1 subunits or the non-obligate KXD1 polypeptide. Bloc1s5 expression is greatly increased in *Kxd1−/−* mouse kidney tissue (Yang et al., 2012). Finally, the dysbindin null mutation *Bloc1s8dy/dy* increases the expression of the mouse orthologue of Borcs7 in mouse hippocampus (2010012O05Rik) (Gokhale et al., 2016). These data collectively argue that genetic defects in subunits of either the BLOC-1 or BORC complexes can modify the expression of subunits belonging to both complexes. These expression phenotypes further support the hypothesis that additional BLOC-1/BORC hybrid complexes may exist (Fig. 1D).

These considerations about BLOC-1/BORC complexes and their molecular phenotypes are important to interpret genome wide gene-disease associations studies defining the risk of neurodevelopmental disorders such as schizophrenia. We argue that the interactomes of risk loci are a fertile ground for the identification of cellular mechanisms of neurodevelopmental disorders.

**Neurodevelopmental Disorders Associated to BLOC-1 and BORC Complexes**

Neurodevelopmental disorders are a group of behavioral afflictions whose onset occur early in life or adolescence. These disorders include autism and schizophrenia spectrum disorders, intellectual disability, and attention deficit hyperactivity disorder (Rapoport et al., 2005; American Psychiatric Association. and American Psychiatric Association. DSM-5 Task Force., 2013). Frequently, these disorders coexist in the same individual. Furthermore, the same genetic defect can cause different afflictions in distinct subjects, indicating that these disorders exist in a disease continuum, referred as developmental brain dysfunction (Moreno-De-Luca et al., 2013; Kirov, 2015).

DTNBP1, the gene encoding the BLOC-1 subunit dysbindin/BLOC1S8 received great attention as polymorphisms in DTNBP1 were implicated as risk factors for schizophrenia (Straub et al., 2002; Schwab et al., 2003; Williams et al., 2004; Gornick et al., 2005; Talbot et al., 2009; Fatjo-Vilas et al., 2011; Mullin et al., 2011). However, the present consensus is that DTNBP1 polymorphisms do not increase the risk of schizophrenia (Farrell et al., 2015). This current view developed from genome wide association studies in large patient populations that failed to identify DTNBP1 polymorphisms or mutations associated with schizophrenia above statistical noise (Fig. 3) (Xu et al., 2011; Fromer et al., 2014; Purcell et al., 2014; Schizophrenia Working Group of the Psychiatric Genomics, 2014; Genovese et al., 2016; Singh et al., 2017). DTNBP1 alleles initially considered to be significant schizophrenia susceptibility alleles, turned out to be inconsistently associated with disease risk across patient cohorts of diverse ethnicities (Morris et al., 2003; Mutsuudi et al., 2006; Turunen et al., 2007; Peters et al., 2008; Strohmaier et al., 2010). Finally, there are no reports of schizophrenia or psychotic disorders in patients bearing mutations in the BLOC-1 subunits dysbindin, BLOC1S3 (HPS8), and BLOC1S6 (Li et al., 2003; Morgan et al., 2006; Badolato et al., 2012; Bryan et al., 2017). However, one patient carrying a mutation in
DTNBP1 was reported to have delayed motor and language development without additional neurological symptoms (Bryan et al., 2017). Despite this lack of genetic association between DTNBP1 polymorphisms and schizophrenia, the evidence supports a role of DTNBP1 and dysbindin in cognitive function. In fact, genetic association studies show that DTNBP1 polymorphisms influence cognitive and neuroanatomical traits in humans (Ghiani and Dell’angelica, 2011; Mullin et al., 2011; Papaleo et al., 2014). Moreover, mouse and Drosophila dysbindin mutants have pronounced neurological, behavioral, learning, plasticity, and synaptic phenotypes (Cox et al., 2009; Dickman and Davis, 2009; Talbot et al., 2009; Tang et al., 2009; Carlson et al., 2011; Ghiani and Dell’angelica, 2011; Karlsgodt et al., 2011; Shao et al., 2011; Gokhale et al., 2015; Mullin et al., 2015; Petit et al., 2017).

Although the human genetic association data are compelling arguments against subunits of the BLOC-1 complex, we believe that, particularly with dysbindin, its status as a risk factor for psychotic disorders is more nuanced, as we describe below.

Contrary to the human genetic evidence, a powerful argument supporting the participation of the BLOC-1 complex in schizophrenia pathogenic mechanisms is the seminal findings by K. Talbot (Talbot et al., 2004; Tang et al., 2009; Talbot et al., 2011). Talbot found that dysbindin is enriched in synaptic fields of the human hippocampal formation (Talbot et al., 2004). Seventy to ninety percent of schizophrenia cases have reduced dysbindin expression in the hippocampus by immunocytochemistry. Decreased dysbindin expression ranged between 18 to 42% in 32 patients as compared to matched control pairs (Talbot et al., 2004). These findings have been replicated biochemically in homogenates of the dorsolateral prefrontal cortex of schizophrenic patients in two independent studies by the group (Talbot et al., 2004; Tang et al., 2009). Similarly, dysbindin mRNA expression is reduced in the hippocampal formation of schizophrenia patients (Weickert et al., 2004; Bray et al., 2005; Weickert et al., 2008).

How can we reconcile the discordant population genetic and dysbindin expression data in schizophrenia subjects? We have argued that biochemical and genetic interactomes of BLOC-1 complex subunits may hold the key to understand how the BLOC-1 may participate in neurodevelopmental disorder mechanisms (Gokhale et al., 2012; Mullin et al., 2013; Gokhale et al., 2016). Genes encoding the BLOC-1 complex subunits may be spared from polymorphisms or mutations in neurodevelopmental disorders, yet the expression of BLOC-1 complex subunits may be upstream or downstream of other loci that themselves are genetic risk factors or causative of neurodevelopmental disorders. The following observations support this contention. First, Dysbindin interacts with DISC1 at the synapse (Furukubo-Tokunaga et al., 2016; Furukubo-Tokunaga et al., 2016). DISC1 is a gene mutated in neuropsychiatric and neurodevelopmental disorders, and it is required for neuronal development and synapse function (Blackwood et al., 2001; Kilpinen et al., 2008; Crepel et al., 2010; Porteous et al., 2011; Wen et al., 2014). Second, we have reported that Bloc1s8dy/dy increases the expression of mouse Borcs7 (Gokhale et al., 2016). This observation is relevant as a small nucleotide polymorphism in the chromosomal schizophrenia risk locus 10q24.32 increases the expression of BORCS7 (rs7085104, Fig. 3). The 10q24.32 locus encompasses the AS3MT and the BORCS7 genes, which are the principal schizophrenia genetic risk factors within this locus (Duarte et al., 2016; Li et al., 2016).
The BLOC-1 biochemical interactome offers additional insights. The actin polymerization complex Arp2/3, components of the SNARE-NSF fusion machinery, subunits of the COG and exocyst tethers, and the AP-3 adaptor associate with the BLOC-1 complex (Mullin et al., 2011; Gokhale et al., 2012; Gokhale et al., 2016). These BLOC-1 associated cytosolic complexes have been implicated in neurodevelopmental disorders (research database described by Mirzaa et al. (Mirzaa et al., 2014) can be found at https://www.dbdb.urmc.rochester.edu/home) (Mirnics et al., 2000; Foulquier, 2009; Han et al., 2013; Zanni et al., 2013; Climer et al., 2015; Fairfield et al., 2015; Ammann et al., 2016; Assoum et al., 2016; Anazi et al., 2017). Importantly, rare de novo mutations in the coding regions of subunits belonging to the Arp2/3, COG, BORC and AP-3 complexes are significantly enriched in schizophrenia patients despite the fact that mutations in BLOC-1 subunits and dysbindin are not enriched in the same patient population (Fig. 3) (Purcell et al., 2014).

Of particular interest is the interaction of the BLOC-1 complex with the Arp2/3 actin polymerization machinery. Arp2/3 is widely expressed in all cells, and it plays a central role in the polymerization of branched actin networks (Rotty et al., 2013). The Arp2/3 complex co-isolates with dysbindin/BLOC1S8 and other subunits of the BLOC-1 complex. Arp2/3 and BLOC-1 interact at the synapse to modulate pre- and post-synaptic morphology as well as adaptive synaptic plasticity. Arp2/3 complex subunit expression is reduced in synaptic fractions of Bloc1s8^sdy/sdy brains (Gokhale et al., 2016). This finding is important as it parallels the reduced mRNA content of Arp2/3 complex subunits in the dorsolateral prefrontal cortex layer 3 and 5 pyramidal cells in schizophrenia patients, implicating the Arp2/3 pathway in schizophrenia and neurodevelopmental disorder pathogenesis (Datta et al., 2016). The following evidence further supports Arp2/3 complex function involvement in the pathogenesis of schizophrenia and other neurodevelopmental disorders. First, rare de novo deleterious mutations in the coding sequence of the Arp2/3 complex subunit gene ARPC3 are significantly enriched in schizophrenia patients as compared to unaffected individuals (Fig. 3, p =0.0378) (Purcell et al., 2014). Direct evidence of the Arp2/3 complex requirement for maintaining activity in a neuronal circuit comes from a conditional mouse loss-of-function mutation of ArpC3 as well. This conditional ArpC3 mouse mutant causes psychomotor agitation and stereotypical behaviors, which are relieved by antipsychotics (Kim et al., 2013; Kim et al., 2015). Finally, the involvement of the Arp2/3 complex in neuropsychiatric disorders is not restricted to loss-of-function defects but also to Arp2/3 gain-of-function as demonstrated in a SHANK3 overexpression mouse model (Han et al., 2013). This mouse overexpression model was generated to explore the mechanisms downstream of chromosomal duplications in 22q13 locus which span the gene encoding the post-synaptic scaffold SHANK3. The 22q13 duplication associates with a range of neurodevelopmental disorders from Asperger syndrome, attention deficit-hyperactivity disorder, to schizophrenia (Durand et al., 2007; Failla et al., 2007; Moessner et al., 2007; Rahikkala et al., 2013; Ahn et al., 2014). Overexpression of SHANK3 causes manic-like behavior in mice and at the synapse level SHANK3 overexpression increased Arp2/3 complex expression and F-actin at dendritic spines (Han et al., 2013). These findings strongly suggest that the Arp2/3 and, by extension, the mechanisms that control actin dynamics participate in the pathogenesis of diverse neurodevelopmental disorders. We
emphasize that even when genetic evidence may not support the role of a molecule in a particular neurodevelopmental disorder, the interactome of that molecule may hold key mechanistic connections with genes genetically implicated in neurodevelopmental disorder risk (Barabasi et al., 2011; Mullin et al., 2013; Boyle et al., 2017).

**BLOC-1 and BORC Complex Subunits and their Cellular Mechanisms**

In this section, we discuss recent developments in the biology of the BLOC-1 and BORC complexes that could explain neuronal phenotypes in organisms carrying mutations in components of the BLOC-1 complex, in particular dysbindin. We refer readers to previous comprehensive reviews about the BLOC-1 complex (Wei, 2006; Raposo and Marks, 2007; Ghiani and Dell’angelica, 2011; Mullin et al., 2011). The precise molecular mechanisms wherein BLOC-1 and its interactome are required are still in development. However, there are major cellular processes requiring the BLOC-1 complex and its interactome that merit attention. These cellular processes are membrane protein sorting, actin cytoskeleton dynamics, and primary cilia function. These cellular mechanisms are described independently for analytic purposes. However, these cellular mechanisms are integrated into higher order processes such as the case of BLOC-1 complex requirement for membrane protein sorting from endosomes to the primary cilium membrane.

**Synaptic Vesicle Trafficking Mechanisms and the BLOC-1 and BORC Complexes**

Mammalian and *Drosophila* BLOC-1 complex subunits dysbindin/BLOC1S8, BLOC1S6, and SNAPIN have been localized to dendrites, dendritic spines, axons, axon terminal and synaptic vesicles by light and electron microscopy (Talbot et al., 2004; Talbot et al., 2006; Dickman and Davis, 2009; Talbot et al., 2011; Dickman et al., 2012; Larimore et al., 2013; Chen et al., 2017). BLOC-1 subunits are required for the proper targeting of membrane cargoes including synaptic vesicle proteins and post-synaptic receptors (Mullin et al., 2011). BLOC-1 binds some of these membrane proteins and aids in their sorting into endosome-derived vesicles and tubules bound to specialized cellular domains and secretory organelles. This BLOC-1 sorting function may be accomplished by BLOC-1 either in isolation or by association with the AP-3 adaptor complex (Di Pietro et al., 2006; Mullin et al., 2011; Gokhale et al., 2012; Lee et al., 2012). Here we define BLOC-1 subunit cargoes as membrane proteins that either interact with BLOC-1 subunits, or their subcellular location and/or expression is altered by BLOC-1 subunits mutations. The membrane proteins expressed in neurons that fulfill some or all of these BLOC-1 cargo criteria are: diverse SNAREs (VAMP7, SNAP25, syntaxin 1A, and syntaxin 13), phosphatidyl-inositol 4 kinase type 2 alpha (PI4K2A), the Menkes copper transporter (ATP7A), Notch1, NR2A-containing NMDA receptors, and the dopamine D2 and D3 receptors (Numakawa et al., 2004; Di Pietro et al., 2006; Salazar et al., 2006; Setty et al., 2007; Setty et al., 2008; Ji et al., 2009; Tang et al., 2009; Marley and von Zastrow, 2010; Karlsodt et al., 2011; Larimore et al., 2011; Dickman et al., 2012; Papaleo et al., 2012; Ryder et al., 2013; Saggu et al., 2013; Gokhale et al., 2015; Dennis et al., 2016; Schmieg et al., 2016; Sinclair et al., 2016). Of these receptors, dopamine D2 receptor is of particular interest as this neurotransmitter receptor localizes to the primary cilium, a cellular compartment whose membrane protein composition, morphology and function are sensitive to mutations in the BLOC-1 subunits BLOC1S6 and

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dysbindin/BLOC1S8 (Monis et al., 2017). We will later describe the hypothesis that defective targeting of membrane protein receptors, due to BLOC-1 genetic defects, contributes to the pathogenesis of neurodevelopmental disorders endophenotypes.

VAMP7 and PI4K2A are present in synaptic vesicles. Genetic defects in BLOC-1 complex subunits impair the delivery of VAMP7 and PI4K2A to synaptic vesicles (Newell-Litwa et al., 2009; Newell-Litwa et al., 2010; Morgan et al., 2013). These synaptic vesicle cargo defects are selective as synaptic vesicle numbers and vesicular levels of generic synaptic vesicle proteins, such as synaptophysin and VAMP2, are normal in BLOC-1 subunit mutant axon terminals (Newell-Litwa et al., 2009; Newell-Litwa et al., 2010; Morgan et al., 2013). How do genetic defects in BLOC-1 subunits alter synaptic vesicle composition? There are three vesicular delivery mechanisms that require the integrity of BLOC-1 and BLOC-1/BORC subunits in neurons. BLOC-1 complex subunits participate in anterograde delivery of membrane proteins from the cell body to axon terminals in mammalian neurons (Larimore et al., 2011). A similar defect in anterograde movement of synaptic vesicle proteins has been recently documented in BORC subunit mutants in C. elegans (Niwa et al., 2017). Evidence in Drosophila demonstrates that the BLOC-1-BORC complex subunit Blos1 and the BLOC-1 complex subunits pallidin/BLOC1S6 and dysbindin/BLOC1S8 participate in sorting between axon terminal endosomes and synaptic vesicles (Mullin et al., 2015; Chen et al., 2017). This Drosophila sorting mechanism is revealed only during conditions of high synaptic activity (Mullin et al., 2015; Chen et al., 2017). In contrast, spontaneous and evoked neurotransmission under basal conditions appears unaffected (Mullin et al., 2015; Chen et al., 2017). This suggests an important requirement for BLOC-1 when membrane trafficking demands must be sustained during conditions of stress. Interestingly, electrophysiological, imaging, and ultrastructural evidence implicates the Rab5 GTPase interacting with pallidin/BLOC1S6 to rapidly recycle synaptic vesicles through early endosomes during elevated synaptic activity (Chen et al., 2017). Further, there is evidence that BLOC1 in yeast regulates the activity of the Rab5 homolog (John Peter et al., 2013). Thus, BLOC1 may work with Rab5 to enable rapid membrane trafficking and sorting of synaptic vesicles to rapidly replenish the vesicle pool during conditions of high activity. Finally, the BLOC-1/BORC subunit SNAPIN participates in retrograde cargo delivery from axon terminals to cell bodies (Zhou et al., 2012; Ye and Cai, 2013; Di Giovanni and Sheng, 2015). These three mechanisms have been documented in different neuronal cellular systems and species and with different cargoes, yet we postulate they may operate simultaneously in neurons and other polarized cell types. We speculate that a combination of these three mechanisms would fine-tune the composition of synaptic vesicle and possibly other presynaptic organelles without affecting the overall abundance.

Cytoskeletal Mechanisms and the BLOC-1 and BORC Complexes

Subunits of the BLOC-1 complex interact or colocalize with components of the actin and microtubule cytoskeleton (Talbot et al., 2006; Chen et al., 2017). BLOC1S6 was first described as a protein that co-sediments with polymerized actin in vitro (Falcon-Perez et al., 2002). Later, dysbindin/BLOC1S8 and other subunits of the BLOC-1 complex were demonstrated to interact with the Arp2/3 activators WAVE2, Abi1, annexin A2, and the WASH complex in addition to the Arp2/3 complex itself (Ryder et al., 2013; Abazyan et al.,...
BLOC-1, Arp2/3 activators, the Arp2/3 complex itself, and actin polymerization act on localized domains of endosomes to define the formation of vesicles/tubules required for membrane protein sorting (Ryder et al., 2013; Delevoye et al., 2016; Gokhale et al., 2016). Localized Arp2/3 dependent actin polymerization is a requirement for multiple vesicle formation steps. Membrane invagination, scission and release of a vesicle from its donor membrane all require Arp2/3-dependent actin polymerization (Carreno et al., 2004; Kaksonen et al., 2005; Merrifield et al., 2005; Yarar et al., 2005; Kaksonen et al., 2006; Collins et al., 2011; Picco et al., 2015). The precise role of BLOC-1 in controlling Arp2/3 activity remains unexplored, but we postulate that BLOC-1 resides upstream of Arp2/3 itself or the Arp2/3 actin nucleation activators. BLOC-1 would constrain Arp2/3 activity temporally or spatially, and/or prevent the removal of Arp2/3 dependent factors from membranes. This model explains why Arp2/3 mutants rescue BLOC-1 mutant defects at the Drosophila synapse and the decreased actin exchange on the limiting membrane of BLOC-1-deficient endosomes. This model also accounts for the defective cargo delivery observed in melanocytes lacking the Arp2/3 activator annexin A2 or treated with a drug that block Arp2/3 function (Delevoye et al., 2016; Gokhale et al., 2016). Vesiculation events controlled by the BLOC-1-Arp2/3 machinery may turn into tubulation events depending of the participation of kinesin motors and the microtubule cytoskeleton. In melanocytes, the motor is kinesin 13A and the formation of tubules requires the activity of BLOC-1, the Arp2/3 activator annexin A2, and the Arp2/3 complex (Delevoye et al., 2016).

The BLOC-1 subunit interacting Arp2/3 activators WAVE2, Abi1, annexin A2, and WASH have been functionally analyzed (Ryder et al., 2013; Abazyan et al., 2014; Jia et al., 2014; Delevoye et al., 2016; Gokhale et al., 2016). Genetic elimination or downregulation of one of these Arp2/3 activators results in disrupted trafficking or morphology of a subcellular compartment. Dysbindin/BLOC1S8 is required to stabilize dendritic protrusions by a CAMK2A- and Abi1/WAVE2-dependent mechanism (Abazyan et al.; Jia et al., 2014). Dendritic spines form, retract, and these unstable spines interconvert between mature and immature protrusions at a higher frequency in Bloc1s8dy/dy neurons compared to wild type cells. These dysbindin/BLOC1S8 mutant spine dynamics and morphology phenotypes are rescued by expression of Abi1, thus demonstrating a BLOC-1-Abi1 pathway (Jia et al., 2014). It remains unresolved whether these dendritic phenotypes result solely from impaired actin dynamics shaping spine architecture or are caused by defective actin-dependent vesicle/tubule formation from endosomes via BLOC-1 dependent mechanisms.

Down-regulation of the Arp2/3 activators WASH or annexin A2 disrupt the targeting of membrane proteins destined to the late endosomes (VAMP7 and PI4K2A) or cargoes targeted to melanosomes (VAMP7, TYRP1) demonstrating that vesicle/tubule formation events require localized activity of the Arp2/3 complex (Ryder et al., 2013; Delevoye et al., 2016; Dennis et al., 2016). The WASH complex and annexin A2 activate the Arp2/3 complex in endosomes yet only annexin A2 is required in melanosome biogenesis whereas the strumpellin subunit of the WASH complex is dispensable for melanosome biogenesis (Delevoye et al., 2016; Tyrrell et al., 2016). The interaction between the BLOC-1 subunit dysbindin/BLOC1S8 and Arp2/3-dependent mechanisms is also documented at the Drosophila neuromuscular junction by genetic interactions between Arp2/3 and BLOC-1.
subunits loss-of-function alleles (Gokhale et al., 2016). The morphology and adaptive synaptic plasticity defects caused by dysbindin/BLOC1S8 mutations are phenocopied by Arp2/3 complex haploinsufficiency. Importantly, combined Arp2/3 and BLOC-1 genetic defects suppress these phenotypes suggesting an inhibitory effect of BLOC-1 on Arp2/3 activity (Gokhale et al., 2016). WAVE2, Abi1, annexin A2, and the WASH localize to different subcellular compartments suggesting that the BLOC-1-Arp2/3 machinery functions in diverse subcellular compartments (Rotty et al., 2013). Experiments in Drosophila indicate that Arp2/3 and BLOC-1 subunit mutations alter the dendritic arbor of sensory neurons. However, and in clear contrast with the fly neuromuscular junction, there is no suppressor effect of Arp2/3 haploinsufficiency on dysbindin/BLOC1S8 loss-of-function dendritic arbor phenotypes (Gokhale et al., 2016). These differences point to BLOC-1 binding different Arp2/3 activators in different cellular compartments, cells types, or combinations of thereof.

BLOC-1/BORC complex subunits associate with the microtubule motors dynein, kinesin 1, 3, and kinesin 13A. Dynein interacts with SNAPIN while other BORC complex subunits bridge target organelles with an Arl8b-Kinesin-1 or 3 complexes (Di Giovanni and Sheng, 2015; Pu et al., 2015; Delevoye et al., 2016; Dennis et al., 2016; Guardia et al., 2016; Farias et al., 2017). If the boundaries of the BLOC-1 and BORC complex are more fluid than just two independent complexes, as suggested by the molecular interactions between BLOC-1 and BORC subunits, then it would be possible for BLOC-1 and BORC complex subunits to specify the motor and directionality of movement of organelles whose biogenesis requires BLOC-1/BORC activity. A test case for this speculative model would be neurons in which BLOC-1 complex subunits are required for three trafficking steps: first, anterograde cell body to axon terminal targeting of BLOC-1 cargoes and late endosome markers (Larimore et al., 2011; Farias et al., 2017). Second, the removal of cargos from axon terminal endosomes back to the cell body via dynein (Di Giovanni and Sheng, 2015). Lastly, BLOC-1 is required locally at the synapse for synaptic vesicle membrane protein sorting, adaptive synaptic plasticity, and sustained neurotransmission (Dickman and Davis, 2009; Dickman et al., 2012; Mullin et al., 2015; Chen et al., 2017). Interestingly, BLOC-1 components show distinct subsynaptic localizations at the Drosophila synapse, with dysbindin and SNAPIN being localized to synaptic vesicles while pallidin is localized to the cytoskeleton (Dickman and Davis, 2009; Dickman et al., 2012; Chen et al., 2017). Indeed, BLOC-1 and BORC complexes subunit combinations could vectorially couple sorting events with specific movement by engaging different motors. These are universal principles that are particularly evident in the targeting of cargoes by BLOC-1 and BORC complexes to the axon and the primary cilium. We discuss these processes in detail below (Larimore et al., 2011; Farias et al., 2017; Monis et al., 2017).

**Membrane Tethers, Fusion, and the Primary Cilium**

Membrane fusion proceeds through steps involving a cascade of rab GTPases, tethering factors, SM (Sec1/Munc18-like) proteins, and SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptor). Distance between fusing membranes is bridged sequentially by tethering factors, which bring membranes in proximity spanning distances up to 200 nm. Tethers are followed by SNAREs, which undergo pairing across opposing membranes separated up to 20 nm. SNARE embedded in vesicle and target membranes form

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a tetrahelical bundled complex that determines specific fusion of vesicles to their target organelles. After fusion and once on the target membrane, SNARES tetrahelical bundles are resolved by the activity of the ATPase N-ethylmaleimide-sensitive factor NSF for SNARE recycling back to the vesicle donor compartment for a next round of fusion (Jahn and Scheller, 2006; Wickner and Schekman, 2008; Sudhof and Rothman, 2009; Brocker et al., 2010).

BLOC-1 interacts with rabs, SM proteins, SNAREs, NSF (Huang et al., 1999; Hikita et al., 2009; Lu et al., 2009; Cheli et al., 2010; Ghiani et al., 2010; Gokhale et al., 2015), and the tethering complexes exocyst and the COG (Gokhale et al., 2012; Monis et al., 2017). These multimeric tethers participate in post-Golgi vesicle fusion events and in intra-Golgi fusion events, respectively (Bao et al., 2008; Rodriguez-Fernandez and Dell’Angelica, 2009; Mead et al., 2010). The BLOC-1 subunit BLOC1S6 binds the early endosome SNARE syntaxin13 whereas SNAPIN binds the late endosome SNAREs syntaxin 8, and the plasma membrane t-SNAREs SNAP23-25, respectively (Huang et al., 1999; Ilardi et al., 1999; Buxton et al., 2003; Lu et al., 2009; Ghiani et al., 2010). The SNARE VAMP7 binds the BLOC-1 complex, yet the subunit responsible for this interaction has not been identified (Ryder et al., 2013). In addition to biochemical evidence, BLOC-1 components genetically interact with SNAP-25 and NSF (Gokhale et al., 2015; Chen et al., 2017). Disruption of SNARE-BLOC-1 interactions manifest as alterations of SNAREs subcellular localization and/or decrease in the cellular content of SNAREs in neuronal cells and melanocytes (Salazar et al., 2006; Newell-Litwa et al., 2010; Ryder et al., 2013; Dennis et al., 2016).

Interactions between BLOC-1 complex subunits NSF, exocyst and the COG complex have been functionally tested (Dickman et al., 2012; Gokhale et al., 2012; Gokhale et al., 2015; Gokhale et al., 2016; Chen et al., 2017; Monis et al., 2017). In this section, we focus on an exciting and novel BLOC-1 function that emerges from interactions between BLOC-1 complex subunits with the vesicle tether exocyst and the intraflagellar transport factor 20 (IFT20) (Gokhale et al., 2012; Monis et al., 2017). The exocyst is an octameric protein complex required for tethering and spatial targeting of post-Golgi vesicles to the plasma and ciliary membranes for fusion (He and Guo, 2009; Das and Guo, 2011). Exocyst interacts with the BLOC-1 complex, IFT88, and IFT20 (Fogelgren et al., 2011; Monis et al., 2017). IFT20 is a subunit of the IFT particle, a group of 22 polypeptides that contain binding sites for various cargos or engage cargo adaptors that need to be transported from the cytoplasm for concentration into the cilium (Fig. 4A) (Follit et al., 2006; Taschner and Lorentzen, 2016; Ishikawa and Marshall, 2017). Proteome wide interactomes reveal that the protein-protein interactions between BLOC-1, exocyst and IFT particle are multipronged (Fig. 4A) (Camargo et al., 2007, Huttlin, 2015 #921; Wang et al., 2011; Havugimana et al., 2012; Wan et al., 2015; Monis et al., 2017). Disruption of the BLOC-1-exocyst-IFT20 pathway selectively impair the targeting of the ciliary membrane protein polycystin 2 from endosomes to primary cilia while sparing other cilia targeted membrane proteins (Monis et al., 2017). Defects in the BLOC-1-exocyst-IFT20 pathway alter ciliary morphology and collective tubule diameter in the nephron in Boc1s8 and Boc1s8 null mice (Monis et al., 2017). These phenotypes highlight the functional relevance of BLOC-1 complexes to primary cilium function. The BLOC-1-exocyst-IFT20 pathway has profound implications for neurodevelopmental disorders. This BLOC-1-dependent primary cilium sorting
mechanism could provide a cellular substrate and nexus between three chief pathogenesis hypotheses of schizophrenia: the neurodevelopmental, dopaminergic, and GABAergic hypotheses (Tandon et al., 2008; Howes and Kapur, 2009; Tandon et al., 2009; Rapoport et al., 2012). In the next section, we will discuss these hypotheses from the angle of primary cilium biology.

Primary Cilia and its Implications for the Dopaminergic and GABAergic Hypotheses of Schizophrenia

Primary cilia, the immotile tubular protrusions found on cell surface, have come to be recognized as an important center for a wide range of cellular functions such as signal transduction, cell fate determination, and differentiation (Guemez-Gamboa et al., 2014). Cilia are required for G-protein-coupled receptor dependent signaling as well as sonic hedgehog- and planar cell polarity-dependent neuronal specification and tissue morphogenesis (Guemez-Gamboa et al., 2014). Among the receptors localized to cilia are neurotransmitter and neuropeptide G-protein-coupled receptors such as dopamine receptors (DRD1, DRD2, DRD5), serotonin receptors (HTR6), neuropeptide receptors that control appetite, puberty onset, mood, pain (GALR2 and 3, KISSR1, MCHR1, NMUR1, NPFRR1, NPY2R-5R, PRLHR, SSTR3) (Marley and von Zastrow, 2010; Avasthi et al., 2012; Leaf and Von Zastrow, 2015; Hilgendorf et al., 2016). Of this growing list of receptors several ligand/receptors pairs have been considered in schizophrenia mechanisms (Caceda et al., 2007). One receptor family that requires particular attention are dopamine receptors. Dopaminergic pathogenic hypotheses of schizophrenia center on altered dopaminergic tone across different brain regions. In some brain regions, such as the frontal cortex, there is decrease dopaminergic tone whereas in the striatum there is an increased dopaminergic activity (Howes and Kapur, 2009). The dopamine hypothesis is supported by strong genetic evidence implicating DRD2 dopamine receptors (Schizophrenia Working Group of the Psychiatric Genomics, 2014). The dopamine receptor DRD2 is targeted from endosomes by the BLOC-1 complex raising the interesting prospect that dopamine receptor targeting to cilia could be impaired in BLOC-1 mutants. DRD2 receptor levels increase in the cell surface of BLOC-1 mutant cells (~2–3 fold) (Iizuka et al., 2007; Ji et al., 2009; Marley and von Zastrow, 2010; Kobayashi et al., 2011) yet we do not know if DRD2 receptor levels decrease in cilia from BLOC-1 mutant neurons. BLOC-1 could act as a rheostat controlling the magnitude and place of DRD2 receptor signaling either from plasma membrane or primary cilium compartments (Fig. 4B). Changes in the balance between ciliary and non-ciliary dopaminergic cell surface signaling could explain why dopamine receptor expression studies in schizophrenia patients show modest elevation of DRD2/3 receptor density (1.1–1.2 fold) (Laruelle, 1998; Zakzanis and Hansen, 1998; Kestler et al., 2001; Howes and Kapur, 2009).

The second hypothesis of schizophrenia we discuss is the GABAergic hypothesis. Patient phenotypes supporting this hypothesis are observed in BLOC-1 complex mutant mice. The GABAergic hypothesis is founded on the observation that schizophrenia patients have reduced expression of the GABA-synthesizing enzyme, GAD67 protein and the calcium buffering protein parvalbumin (Akbarian et al., 1995; Volk et al., 2000; Hashimoto et al.,
Parvalbumin marks a subpopulation of GABAergic interneurons sensitive to BLOC-1 genetic defects. \textit{Bloc1s8\textsuperscript{sdy/sdy}} mice have reduced expression of parvalbumin in interneurons and reduced interneurons numbers in the hippocampus (Carlson et al., 2011; Larimore et al., 2017). The cause of these \textit{Bloc1s8\textsuperscript{sdy/sdy}} hippocampal phenotypes is unknown yet defective primary cilium function is a plausible hypothesis. This hypothesis is founded on the observation that the primary cilium is required for the migration and placement of GABAergic interneurons in the developing cerebral cortex, axonal growth and guidance, and dendritic arborizations (Baudoin et al., 2012; Higginbotham et al., 2012; Guo and Anton, 2014; Guo et al., 2015). These phenotypes are in line with the neurodevelopmental and GABAergic hypotheses of schizophrenia. In addition, primary cilium signaling is required for post-migration developmental processes in interneurons, such as connectivity and circuit activity (Guo et al., 2017). In fact, selective loss of a ciliary-specific GTPase in interneurons alters GABAergic neuronal morphology and synaptic connectivity, therefore altering excitatory/inhibitory activity balance (Guo et al., 2017). This unbalance in excitatory/inhibitory activity is a central mechanism of neurodevelopmental disorders and it is a phenotype shared by \textit{Bloc1s8\textsuperscript{sdy/sdy}} and other mouse models of neurodevelopmental disorders (Gogolla et al., 2009; Carlson et al., 2011; Yizhar et al., 2011). Thus, primary cilia modulate neurotransmission in mature circuits even after participating in neurodevelopment.

A model where cilia participate in neurodevelopmental disorder mechanisms makes testable predictions (Fig. 4B). First, ciliary gene products or components of their interactomes should be among risks factors for schizophrenia. The 108 high confidence schizophrenia risk loci identified by the Schizophrenia Genomics Consortium support this idea. These 108 loci include 22 genes whose products participate in primary cilia biology (Fig. 3C) (Schizophrenia Working Group of the Psychiatric Genomics, 2014). DISC1 gene defects were identified in a large pedigree affected by psychiatric pathology (Furukubo-Tokunaga et al., 2016; Furukubo-Tokunaga et al., 2016). Mutations on DISC1 alter synaptic vesicle recycling and generate schizophrenia endophenotypes in mice (Porteous et al., 2011; Wen et al., 2014; Tomoda et al., 2016). DISC1 is required for formation and/or steady state maintenance of primary cilia that enrich dopamine receptors at the primary cilium membrane (Marley and von Zastrow, 2010). DISC1 localizes at the base of primary cilia and interacts with TRAF3IP1/MIP-T3/IFT54 a component of intraflagellar transport particle (IFT) subcomplex B (Fig. 4A) (Li et al., 2008; Marley and von Zastrow, 2010; Berbari et al., 2011). None of the components of the IFT machinery have an increased burden of deleterious mutations in schizophrenia patients. However, genes encoding molecules belonging to the IFT particle interactome have an increased frequency of mutations in schizophrenia patients. CEP290 interacts with IFT components (Fig. 4A), and it is required for tethering the flagellar membrane to the transition zone microtubules, which is essential for defining a barrier at the base of the cilium to maintain flagellar protein composition (Craige et al., 2010; Shimada et al., 2017). CEP290 carries an enriched burden of predicted deleterious mutations 2.3 times higher in schizophrenia patients as compared to controls (p<0.0012, Fig. 3) (Purcell et al., 2014). Ciliary barrier mechanisms may be enriched deleterious mutations. Septins are required to maintain the separation of the primary cilium membrane and the plasma membrane (Hu et al., 2010). The SEPT6 encoding septin 6 carries...
an enriched burden of deleterious mutations 2.5 times higher in schizophrenia cases than controls and it is near a significant difference as compared with unaffected individuals (p<0.0539, Fig. 3) (Purcell et al., 2014). SEPT6 is not the only septin gene with potential implications for neurodevelopmental disorders. Septin 5, encoded by the SEPT5 gene, interacts with septin 6 (Fig. 4a) (Corominas et al., 2014; Huttlin et al., 2015; Wan et al., 2015). The SEPT5 gene resides in the 22q11.2 chromosomal segment, a genomic domain that when deleted on one copy confers the highest risk for schizophrenia and other neurodevelopmental disorders (International Schizophrenia, 2008; Karayiorgou et al., 2010; Guna et al., 2015; McDonald-McGinn et al., 2015; Van et al., 2017). The hemideletion of the 22q11.2 locus spans 1.5 to 3 megabases harboring 46 protein-coding genes, 41 of which express in the human brain (Guna et al., 2015). This 22q11.2 hemideletion increases the risk of schizophrenia by 20 to 25 times as compared to the lifetime general population risk of 1% (International Schizophrenia, 2008; Karayiorgou et al., 2010; Guna et al., 2015; McDonald-McGinn et al., 2015; Van et al., 2017). As we will discuss below, there may be additional loci within the 22q11.2 deleted region that participate in primary cilium formation and maintenance such as the transcription factor TBX1 (Marley and von Zastrow, 2012). Primary cilia function has not been studied in 22q11.2 microdeletion syndrome, yet some features of the microdeletion syndrome suggest that there may be unexpected connections with cilia. 22q11.2 microdeletion affected patients frequently experience conotruncal anomalies, including tetralogy of Fallot, ventricular septal defect, truncus arteriosus, and interrupted aortic arch (Momma, 2010; Agergaard et al., 2012). These cardiovascular congenital defects are also found in patients affected with classic ciliopathies (Koefoed et al., 2014; Reiter and Leroux, 2017).

The model of primary cilia as a subcellular compartment where neuropsychiatric risk genes and mechanisms converge predicts that neurodevelopmental disorder genes should influence cilia morphogenesis (Fig. 4B). Marley and Von Zastrow, who screened 41 candidate genes associated with schizophrenia, bipolar affective disorder, autism spectrum disorder and intellectual disability have tested this idea. (Marley and von Zastrow, 2012). They identified 23 candidates that affect cilia morphology in NIH3T3 cells. Three of these genes increased cilia length when down-regulated. These 23 genes include the DISC1 interactor CEP63 encoding the centrosomal protein 63, the neurodevelopmental gene NRXN1 encoding neurexin 1, TBX1 -a gene present in the 22q11.2 chromosomal segment, FOXP1, NOTCH4, and ANK3. All of these genes associated with neurodevelopmental syndromes were not previously known to affect primary cilium morphology (Mirzaa et al., 2014). These data support the concept that primary cilia interactomes contain genes that associate with neurodevelopmental disorder risk. We propose that primary cilia dysfunction resulting from multiple partial loss-of-function hits on primary cilia targets is a novel neurodevelopmental disorder pathogenesis mechanism (Fig. 4B). A recent article reviews genes implicated in diverse neuropsychiatric disorders from a left-right patterning organ perspective (Trulioff et al., 2017).
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Figure 1. The BLOC-1 and BORC Interactomes

A. Diagram of the proposed structure of the BLOC-1 complex according to Lee et al. (Lee et al., 2012). The non-obligate component KXD1 has been included. B-C depict Interactomes of the BLOC-1 and BORC complexes in isolation and D presents all molecular interactions between the BLOC-1 and BORC complexes. Interactomes presented in B-D are derived from six published proteome-wide interactomes datasets described in the following references (Camargo et al., 2007; Huttlin, 2015 #921; Wang et al., 2011; Havugimana et al., 2012; Corominas et al., 2014; Wan et al., 2015). These six datasets were analyzed and curated using the Genemania platform (Warde-Farley et al., 2010). Binary interactions obtained in Genemania were plotted using Cytoscape 3.5.1 (Shannon et al., 2003).
Figure 2. Dysbindin/BLOC1S8 Coprecipitates with Subunits of the BORC Complex
A. Immunoprecipitation of the BLOC-1 complex assembled in *Drosophila* neurons with anti-GFP antibodies. Extracts were prepared from *Drosophila* heads expressing recombinant UAS-Dysbindin-Venus driven by the pan-neuronal GAL4 driver C155 as described (Mullin et al., 2015). Controls were performed from heads of animals expressing GAL4 driver C155 alone. Note that the *Drosophila* orthologues of BORCS6 and KXD1 coprecipitate with Dysbindin-Venus. B. Depicts a heat map of the number of peptide identified by mass spectrometry in experiments performed by Pu at al. (Pu et al., 2015). Note that precipitations with BORCS6 as a bait precipitate dysbindin/BLOC1S8 (1-2 peptides).
Figure 3. Deleterious Mutations in Genes from BLOC-1, BORC, and Primary Cilia Interactomes in Schizophrenia Patients

Graphs show the log10 p value of the frequency of predicted deleterious mutations comparing schizophrenia affected individuals and non-affected controls. P-values are empirical, uncorrected one-sided burden tests. A. presents components of the BLOC-1/BORC interactome. B. presents genes from the primary cilia interactome. In A, KYNU, UFL1 and SHKBPI1 are presented as comparisons since they were identified as genes with increased burden in schizophrenia patients (Fromer et al., 2014; Purcell et al., 2014). Data were obtained with the GeneBook engine [http://bit.ly/2fkvgLq](http://bit.ly/2fkvgLq) that summarizes multiple exome studies (Fromer et al., 2014; Purcell et al., 2014). C presents genes that affect cilia found within the 108 high risk genomic loci identified by the Schizophrenia Working Group of the Psychiatric Genomics, Consortium (Schizophrenia Working Group of the Psychiatric Genomics, 2014).
Figure 4. Primary Cilium Interactome and the Primary Cilium Hypothesis of Neurodevelopmental Disorders and Schizophrenia
A. Presents an interactome of the BLOC-1 interactome, primary cilia complexes involved in protein targeting and barrier formation. Interactome was generated as indicated in figure 1A. The barrier components CEP290, SEPT5, and SEPT6 associate with schizophrenia risk (see figure 3) and connect with multiple components of the primary cilium interactome. B. Presents a diagram of the primary cilium hypothesis of neurodevelopmental disorders and schizophrenia. Primary cilium is depicted as a red rod emanating from the cell body of a neuron. Red indicates the presence of dopamine receptors targeted and concentrated into the primary cilium. Mutations that cause neurodevelopmental disorders or schizophrenia affect primary cilium morphology and redistribute dopamine and/or other receptors away from the primary cilium and into the plasma membrane, which are represented as a thin red line delimiting the cell body and dendrites. Small triangles represent GABAergic terminals.
whose number are reduced in BLOC-1 and cilia deficiencies. An unbalance between primary cilium and plasma membrane signal transduction is postulated to be pathogenic for neurodevelopmental disorders and schizophrenia endophenotypes.
## Table 1

### BLOC-1 and BORC Nomenclature

Table presents the official gene names in humans (all caps), mouse, and *Drosophila* as well frequently used names to designate BLOC-1 and BORC subunits.

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<th>Gene Drosophila</th>
<th>Protein Name</th>
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