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Cell Biology of the BLOC-1 Complex Subunit Dysbindin, a Schizophrenia Susceptibility Gene

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Abstract

There is growing interest in the biology of dysbindin and its genetic locus (*DTNBP1*) due to genetic variants associated with an increased risk of schizophrenia. Reduced levels of dysbindin mRNA and protein in the hippocampal formation of schizophrenia patients further support involvement of this locus in disease risk. Here, we discuss phylogenetically conserved dysbindin molecular interactions that define its contribution to the assembly of the biogenesis of lysosome-related organelles complex-1 (BLOC-1). We explore fundamental cellular processes where dysbindin and the dysbindin-containing BLOC-1 complex are implicated. We propose that cellular, tissue, and system neurological phenotypes from dysbindin deficiencies in model genetic organisms, and likely individuals affected with schizophrenia, emerge from abnormalities in few core cellular mechanisms controlled by BLOC-1-dysbindin-containing complex rather than from defects in dysbindin itself.

Keywords

Dysbindin; *DTNBP1*; Hermansky–Pudlak; BLOC-1; AP-3; Schizophrenia

Dysbindin, the product of the gene *DTNBP1*, was first identified in 2001 as a coiled-coil domain containing protein enriched in muscle and brain [1, 2]. Information related to this gene product has grown in the last 10 years—ranging from molecular studies in diverse cell

types to functional brain imaging of humans carrying defined alleles of *DTNBP1* [2]. Our interest in *DTNBP1* and its product, dysbindin, emerges from two seminal observations linking this gene and its products to schizophrenia. First, Straub reported an association of genetic variants in the gene *DTNBP1*, located on chromosome 6p22.3, with an increased risk of schizophrenia [3, 4]. Second, Talbot et al. described reduced dysbindin protein expression in the hippocampal formation of schizophrenia patients [5]. These observations positioned *DTNBP1* genetic polymorphisms and dysbindin protein content at the forefront of disease pathogenesis. *DTNBP1* stands out in genetic meta-analyses as a disease susceptibility gene among more than 1,000 genes reported with either positive or negative association with schizophrenia [6, 7]. In this perspective, we will summarize the evidence linking dysbindin with schizophrenia and discuss fundamental cellular mechanisms regulated by dysbindin and dysbindin-containing protein complexes. We emphasize that dysbindin exists preponderantly as a member of the BLOC-1 complex, and that the structural integrity of this complex strictly depends upon dysbindin expression. We therefore advocate for a paradigm change in the interpretation of dysbindin-related phenotypes in model genetic organisms and humans as emerging from impaired function of the whole BLOC-1 protein complex. Unraveling the contributions of the *DTNBP1* locus to schizophrenia requires a comprehensive understanding of phylogenetically conserved dysbindin molecular interactions. Thus, recognizing dysbindin contributions to disease within the BLOC-1 complex immediately puts on the table interactions conserved from invertebrates to humans. More importantly, it brings our attention to fundamental cellular mechanisms regulated by BLOC-1 that could be at the core of schizophrenia pathogenesis.

Association of Dysbindin and Schizophrenia: Genetic Evidence

A central feature of schizophrenia is the strong genetic component associated with disease development as concluded from monozygotic twin studies, which indicate a heritability of ~80% [8–10]. Rare highly penetrant genetic deficiencies have led to possible disease mechanisms. These include copy number variations such as chromosome microdeletions or microduplications [11–17]; chromosomal translocations comprising the *DISC1* locus [18]; and mutations affecting the postsynaptic scaffolding protein Shank3 [19] or a kinesin motor isoform [20]. In contrast to these genetic variants, the vast majority of schizophrenia cases fit into a polygenic model where principal contributions to disease are believed to result from convergence of multiple genes of small to moderate effect size [21–23]. These genetic features of schizophrenia have hampered the progress in the understanding of disease pathogenesis.

Genome-wide analyses of schizophrenia-affected individuals have uncovered multiple haplotypes that strongly associate with disease. In these studies, *DTNBP1* ranks 20th in a group of 45 genes selected as strongly associated with disease risk out of a total pool 1,008 genes studied thus far [6, 7]. For example, a defined allele in the *DTNBP1* gene (rs1011313) was associated with susceptibility to schizophrenia in a meta-analysis comparing 2,696 Caucasian patients with schizophrenia with 2,849 controls [6, 7]. Based on the Ioannidis guidelines for the analysis of cumulative evidence in genetic association studies, the *DTNBP1* association with disease is considered with a strong degree of epidemiologic reliability [24]. However, attempts to identify mutations in the exome of *DTNBP1* in schizophrenia patients have been negative so far [25]. Moreover, the only human case reported carrying a loss-of-function allele in *DTNBP1* lacked psychiatric manifestations. This patient carried a homozygote 307C-T transition in the *DTNBP1* gene resulting in the substitution of glutamine 103 to a stop codon (Q103X). The patient, the daughter of consanguineous parents, was affected by Hermansky-Pudlak syndrome type 7 (HPS7; OMIM 203300) exhibiting oculocutaneous albinism, ease of bruising, and a bleeding tendency, yet there were “no apparent behavioral abnormalities in the [affected] individual”

nor a report of disease in her consanguineous relatives [26]. The absence of evidence of psychiatric illness in this patient, her family, and an individual affected by Hermansky–Pudlak type 8 (HSP8; OMIM 203300; see below) raises doubts about the involvement of *DTNBP1* in disease. This uncertainty about *DTNBP1* in disease susceptibility is further enhanced by the inconsistent association of defined *DTNBP1* polymorphisms with schizophrenia [3, 27, 28] and the inconsistent association of the *DTNBP1* locus with disease across multiple patient cohorts of diverse ethnicities [29–33]. However, it should be kept in perspective that even when the genome between individuals is identical, as is the case with monozygotic twins, not all individuals develop schizophrenia arguing for other factors necessary to trigger disease. The polygenic character of schizophrenia raises the issue that individuals possessing susceptibility alleles in genes, such as *DTNBP1*, may express psychiatric phenotypes only when these genetic variants occur in a propitious genome and when environmental factors come to play [34]. A propitious genome for disease development would contain additional susceptibility alleles in other loci. Each allele in isolation would not trigger disease. However, only a combination of susceptibility alleles in different genetic loci would trigger disease.

Biochemical, Anatomical, and Functional Consequences of Carrying *DTNBP1* Polymorphisms Associated to Disease

The genetic evidence for the involvement of *DTNBP1* in schizophrenia may be considered ambiguous. However, a stronger case for *DTNBP1* involvement on disease emerges when considering molecular, anatomical, and systems/behavioral phenotypes associated with *DTNBP1* polymorphisms in humans.

DTNBP1 polymorphisms associated with disease are found in noncoding regions of the *DTNBP1* locus [35]. However, the reduced levels of expression of *DTNBP1* mRNA and protein in postmortem schizophrenia brains suggest that these noncoding polymorphisms could affect transcript or protein levels. Pioneer quantitative immunocytochemistry and immunoblot studies by Talbot indicate that 73% to 93% of schizophrenia cases displayed dysbindin reductions in the hippocampal region. Reductions averaged 18% to 42% in two clinical case cohorts totaling 32 patients [5]. These findings contrast with the normal levels of the synaptic vesicle protein synaptophysin in the same cases arguing against a loss of synapses. These findings have been replicated using biochemical analysis of dysbindin levels in homogenates of the dorsolateral prefrontal cortex of schizophrenic patients in two independent studies [5, 36, 37]. Reduction in hippocampal protein levels is mirrored by reduced levels of dysbindin mRNA in the hippocampal formation of patients with schizophrenia [38–40]. Similarly, patients with schizophrenia had decreased dysbindin mRNA levels in multiple layers of the dorsolateral prefrontal cortex, whereas synaptophysin mRNA levels seemed unaffected [40]. Importantly, *DTNBP1* polymorphisms associated with increased disease risk may influence the content of *DTNBP1* messenger RNA [38]. *DTNBP1* polymorphisms associated to increased schizophrenia risk correlate with a reduction in *DTNBP1* mRNA expression in human cerebral cortex, whereas putative “protective” polymorphisms associate with high *DTNBP1* expression [38]. Although these observations need replication in other patient cohorts, they provide good circumstantial evidence supporting the hypothesis that noncoding polymorphisms in *DTNBP1* regulate its transcript expression and thus dysbindin content and function in specific areas of the brain.

DTNBP1 risk polymorphisms associated with reduced levels of mRNA in patients also correlate with reduced gray matter volume in the dorsolateral prefrontal and occipital cortex [41]. These observations fit well to the behavioral findings of impaired spatial working memory [42] and electrophysiological data consistent with impaired visual processing also observed in carriers of this same *DTNBP1* risk polymorphisms [43]. Reduced volume of

gray matter has been recapitulated in other patient cohorts with a different *DTNBP1* risk polymorphism [44] further supporting the model that *DTNBP1* susceptibility variants may affect specific cortical regions in schizophrenia. Furthermore, evidence is mounting supporting the idea that *DTNBP1* risk polymorphisms associate with differences in brain function encompassing changes in prefrontal brain function [45], cortical activation during verbal tasks [46], visual processing [43, 47], and emotional working memory [48]. Collectively, correlative biochemical, genetic, anatomical, and functional evidence argues that carriers of *DTNBP1* alleles associated with disease display phenotypes of progressive complexity that may contribute to schizophrenia susceptibility. Unraveling the contributions of the *DTNBP1* locus to schizophrenia requires a comprehensive understanding of phylogenetically conserved dysbindin molecular interactions, the fundamental cellular pathways modulated by dysbindin and its associated proteins, and cell–cell interactions influenced by dysbindin and its interactors. These fundamental questions are the central focus of this manuscript.

The Eight Musketeers: “All for One, One for All,” that is BLOC-1's Motto

A common occurrence in the psychiatric literature related to dysbindin is to consider this protein in isolation. Instead, genetics and comparative phylogeny define a conserved core of dysbindin molecular interactions. These dysbindin interactions define a stable protein complex known as the BLOC-1 complex (biogenesis of lysosome-related organelles complex-1). Most brain dysbindin, if not all, exists as part of BLOC-1, a large molecular weight complex with a Stokes' radius of ~95 Å and a native molecular size of 200±30 kDa. BLOC-1's molecular weight far exceeds dysbindin's predicted molecular weight of ~39.5 kDa [49, 50]. The BLOC-1 complex is a heterooctamer comprising of dysbindin, pallidin, muted, cappuccino, BLOS1, BLOS2, BLOS3/reduced pigmentation, and snapin polypeptides [51] (Fig. 1a; Fig. 1b shows three BLOC-1 subunits coprecipitating with recombinant dysbindin). The molecular architecture of binary interactions between BLOC-1 complex subunits is conserved from *Drosophila* to mammals as determined by two-hybrid analysis [50–53] (Fig. 1a depicts interactions among BLOC-1 subunits in mammals). This illustrates that the BLOC-1 organization was selected early on in evolution and its conservation from fly to human argues for the functional significance of this architecture. The phenotype of pallidin, muted, BLOS3/reduced pigmentation, cappuccino, and dysbindin-deficient mice further supports dysbindin incorporation into this complex. First, loss of one BLOC-1 subunit triggers downregulation of other complex subunits. For example, dysbindin-null mice *sandy* (*DTNBP1*^{sd/sd}) express reduced levels of muted, pallidin, and snapin polypeptides. For example, Fig. 2 illustrates the absence of pallidin in *Pldn*^{pa/pa} mouse hippocampus, a phenotype similarly observed in *Dtnbp1*^{sd/sd} hippocampus. Concurrently, dysbindin is reduced in null mouse models of Blos3/ reduced pigmentation (*BLOCIS3*^{rp/rp}), muted (*Muted*^{mu/mu}), and pallid (*Pldn*^{pa/pa}) [26, 50, 54]. These molecular/genetic associations and biochemical phenotypes of loss-of-function alleles converge again in the systemic phenotypes of pallidin, muted, BLOS3/reduced pigmentation, cappuccino, and dysbindin-deficient mice. They all share phenotypes including oculocutaneous pigment dilution, bleeding diathesis, and pulmonary fibrosis that are hallmarks of a human autosomal recessive disorder—the Hermansky–Pudlak syndrome [51, 55, 56]. A phenotypic trait—pigment dilution—is also observed in flies carrying null mutations in Blos1 (CG30077) [52]. Similarly, patients with mutations in dysbindin (Hermansky–Pudlak syndrome type 7, HPS7) and Blos3/reduced pigmentation (Hermansky–Pudlak syndrome type 8, HPS8) share all or part of the phenotypic triad found in BLOC-1-deficient mice [26, 57]. Together, these phenotypes from model genetic organisms and humans strongly support a role for dysbindin in functions integral to the BLOC-1 complex.

Interestingly, and somewhat surprisingly, there are no reports of neuropsychiatric phenotypes in the two patients bearing mutations in BLOC-1 subunits: dysbindin (HPS7) and Blos3/reduced pigmentation (HPS8) [26, 57]. In contrast, mice and flies lacking BLOC-1 subunits possess well-defined neurological phenotypes. There is a growing literature documenting neurobehavioral phenotypes in *sandy* mice (*DTNBP1^{sd/sd}*) [58] despite the fact that the initial description of the *sandy* mutation did not detect neurobehavioral abnormalities [26]. Deficiencies in the dysbindin and Blos1 *Drosophila* orthologs trigger synaptic electrophysiological phenotypes and neurobehavioral abnormalities [52, 59]. Similarly, dysbindin- and snapin-null mice share common synaptic electrophysiological phenotypes (see below). Biochemically defined synaptic vesicle and hippocampal phenotypes are also shared in three BLOC-1 null mutations namely *muted*, *pallid*, and *sandy* (Larimore, unpublished observations and [60, 61]). This phenotypic convergence is extended to behavioral phenotypes shared in *muted*, *pallid*, *reduced pigmentation*, and *cappuccino*-deficient mice [60]. Collectively, these results from *Drosophila* and mice argue for a phylogenetically conserved role of BLOC-1 in synaptic function. Significantly, studies by Talbot, in brain tissue samples from schizophrenia patients, point to an association of dysbindin reduction and synaptic vesicle composition as exemplified by the increased level of the vesicular glutamate transporter 1 (Vglut1) in presynaptic terminals of schizophrenia brains [5]. Importantly, Vglut1 forms a complex with BLOC-1 in brain synaptic fractions and neuronal cell lines [60].

It is reasonable to hypothesize that a role for dysbindin in the risk of schizophrenia pathogenesis is as an integral subunit of the BLOC-1 complex. This hypothesis makes two predictions. Firstly, alleles in BLOC-1 subunit genes should influence disease risk. In fact, significant association between *BLOC1S3* alleles and schizophrenia and epistatic genetic interactions between *DTNBP1* and *MUTED* contributing to schizophrenia support the first prediction [62]. However, a genetic association between *DTNBP1* and *MUTED* in schizophrenia patients is controversial [63]. Secondly, reduction of dysbindin protein in schizophrenia should be associated with reduced levels of other BLOC-1 proteins. Among those proteins, Talbot has found reductions not only in dysbindin but also in pallidin, snapin, and especially BLOS3 in the inner molecular layer of the dentate gyrus of schizophrenia cases (Talbot, personal communication). Similarly, we have observed reduced levels of pallidin in the hippocampal formation of *sandy* mice (Fig. 2). These are suggestive leads that need further development to properly implicate the BLOC-1 complex in schizophrenia and other psychiatric disorders.

What Fundamental Cellular Processes Are Affected in Dysbindin/BLOC-1 Loss-of-Function?

There is an expansion in the reported interactions and putative functions of dysbindin and its complex, BLOC-1 [35, 64–66]. However, we are lacking quantitative and unbiased identification of the molecular interactors of dysbindin/BLOC-1 such as those offered by genetic screens and quantitative proteomics. Definition of stoichiometric dysbindin/BLOC-1 interactors is needed to prioritize them and assess their potential impact on the dysbindin/BLOC-1 loss-of function phenotypes. We discuss these interactions beginning this section with promising emerging data hinting to roles of dysbindin–BLOC-1 on transcriptional and cytoskeletal regulation. We finish discussing BLOC-1/dysbindin interactions relevant to membrane protein sorting and membrane fusion/secretion, which are the best documented as defined either by data replication or by the convergence of multiple biochemical and genetic approaches.

Transcriptional and Cytoskeletal Regulation

The roles of BLOC-1 subunits in transcriptional and cytoskeletal regulation are not yet thoroughly defined. Transcriptional control by dysbindin is suggested by reports of a nuclear localization of this protein [67–69]. Dysbindin binds the nuclear transcription factor Y beta (NF-YB) and modulates transcription via this protein interaction. Similarly, the entry of dysbindin into the nucleus has been proposed to increase transcription of synapsin I gene and synapsin I protein content [67, 70]. Synapsin I in turn reversibly tethers synaptic vesicles to the actin cytoskeleton [71]. Synapsin I transcript and protein are decreased in the cortex and hippocampal formation of dysbindin-null mice [67]. Whether these transcriptional effects of dysbindin occur in the context of BLOC-1 complexes or reflect the isolated activity of dysbindin remains unknown. Since deficiencies in other BLOC-1 subunits, *Blos3*/reduced pigmentation (*BLOC1S3^{rp/rp}*), muted (*Muted^{mu/mu}*), and pallid (*Pldn^{pa/pa}*) reduce the cellular levels of dysbindin [26, 50, 54], it is plausible that the levels of synapsin I are also down-regulated in other BLOC-1 deficiencies. These changes in the content of transcripts likely reflect the tip of the iceberg, and unbiased analyses of the transcriptome of BLOC-1-deficient brains are needed to fully uncover the potential of dysbindin and BLOC-1 in regulating transcription.

It is interesting that dysbindin controls the levels of synapsin I, a protein with capacity to bind and bundle actin filaments [71]. The activity of dysbindin controlling actin dynamics may extend beyond transcriptional control of an actin-binding protein such as synapsin I. Dysbindin interacts with actin regulatory proteins WAVE2 and Abi1 [72] and pallidin co-sediments with polymerized actin [73]. Downregulation or the absence of dysbindin alters the architecture of actin cytoskeleton in neuroblastoma cells and in growth cones of dysbindin-null cultured hippocampal neurons [74]. Moreover, dysbindin siRNA alters the morphology of dendritic spines, a phenotype postulated to involve local alterations of the actin cytoskeleton [72]. To what extent these growth cone and dendritic spine phenotypes are due to contribution of other cellular mechanisms, such as transcription, is presently unknown. Whether these effects of dysbindin on the actin cytoskeleton are either functional properties of the BLOC-1 complexes or the isolated activity of dysbindin has not been resolved.

Membrane Protein Sorting

The first suggestion that BLOC-1 may play a role in membrane protein sorting came from the phenotypic similarities between BLOC-1 deficiencies and loss-of-function alleles affecting subunits of the clathrin-adaptor complex 3 (AP-3). AP-3 is a heterotetramer constituted by δ , β 3A or β 3B, μ 3A or μ 3B, and σ 3A or σ 3b subunits [75, 76]. AP-3 recognizes sorting signals in the cytosolic domain of selected membrane proteins destined from endosomes to lysosomes, lysosome-related organelles—such as melanosomes and platelet dense granules—and synaptic vesicles [75, 77, 78]. Like other clathrin adaptors, AP-3 orchestrates the concentration of membrane proteins into nascent vesicles destined to these locations as well as deformation of the lipid bilayer to generate a vesicle [77, 78]. The precise role that BLOC-1 may play in membrane protein sorting and vesiculation is not clear, yet substantial evidence supports a shared role of BLOC-1 and AP-3 in sorting and vesiculation processes.

AP-3 and BLOC-1 deficiencies possess common phenotypes in mouse and humans triggering the diagnostic triad that characterizes the Hermansky–Pudlak syndrome. Deficiencies in the human *Ap3b1* locus, encoding the β 3A polypeptide, lead to Hermansky–Pudlak syndrome type 2 (HPS2). Natural and engineered mouse mutants affecting the AP-3 subunits δ or β 3A recapitulate main features of human HPS2 [51, 75]. The close association between BLOC-1 and AP-3 is further highlighted by the reduced immunoreactivity of AP-3

in the dentate gyrus of BLOC-1-deficient mice muted (*Muted^{mu/mu}*) and pallid (*Pldn^{pa/pa}*) [60] and the decreased dysbindin immunostaining in the dentate gyrus of AP-3-null mocha mice (*Ap3d1^{mh/mh}*, see Fig. 2) [79]. Predictably, AP-3 or BLOC-1 deficiency phenotypic similarities are shared at a cellular level where a hallmark phenotype is increased cell surface levels of membrane proteins known to bind to AP-3 [79–84]. Interestingly, this phenotype has been reported for dopa-mine D2 receptors (DRD2) and NMDA receptors in neuronal cells lacking or downregulated for pallidin, muted, or dysbindin BLOC-1 subunits [85–88]. The phenotypic similarities extend to regulated secretory organelles where the absence of the BLOC-1 subunits dysbindin or snapin leads to enlarged chromaffin granules and synaptic vesicles, phenotypes in part recapitulated in AP-3 null mice (*Ap3d1^{mh/mh}*) [60, 89, 90]. The commonality of systemic and cellular phenotypes between AP-3 and BLOC-1 loss-of-function mutants is explained in part by the coexistence of BLOC-1 and AP-3 on the same vesicles [61, 79], a reflection of a biochemical interaction between BLOC-1 and AP-3 [60, 80, 91]. In fact, the interaction between dysbindin and/or BLOC-1 with the adaptor complex AP-3 has been documented independently by ten publications [60, 61, 66, 69, 79, 80, 91–94].

What role does BLOC-1 play once on membranes? A plausible hypothesis is that BLOC-1 could act as adaptor/accessory adaptor recognizing membrane protein cargoes. Adaptors are recruited to membranes by the action of small GTP-binding proteins of the ras superfamily and phosphoinositol phospholipids [95]. Arf GTPases recruit clathrin adaptors. Such is the case of AP-3, which is recruited to endosome membranes by Arf1 in its GTP conformation [96, 97]. However, whether BLOC-1 is recruited to membranes by Arf-dependent mechanisms is not yet defined. Suggestive data indicate that the association of AP-3 with the BLOC-1 complex in membranes is enhanced by the addition of nonhydrolysable GTP analogs [80]. Whether this represents BLOC-1 independently being recruited by Arf GTPases to endosomal membranes or BLOC-1 “piggybacking” on the Arf dependency of an AP-3 membrane recruitment mechanism remains to be resolved. Once recruited to membranes by Arf family members, adaptors, and accessory adaptors recognize sorting signals in the cytosolic domain of membrane proteins (cargoes) and concentrate them into nascent vesicles [77, 78, 95]. Vesicles bud off and shed their attached coat [80].

A predictable consequence of an adaptor/accessory adaptor deficiency is the absence of cargoes in target organelles. This could result from either a vesicle carrier not being made or a family of membrane proteins not being included in a vesicle. BLOC-1 can be found in vesicles coated with AP-3 or clathrin-coated vesicles both in neuronal and nonneuronal cells lines, suggesting a role as an adaptor/accessory adaptor [79, 91, 98, 99]. Does BLOC-1 deficiency affect cargo content of target organelles? In brain, BLOC-1 is required for the targeting of synaptic vesicle proteins to the nerve terminal [60, 61]. For example, BLOC-1 regulates the synaptic targeting of the AP-3 cargo phosphatidylinositol-4-kinase type II alpha (Larimore, unpublished results and [91, 100]). This kinase is targeted to presynaptic and postsynaptic compartments (Larimore, unpublished results). Thus, it could be speculated that a downstream consequence of reduced levels of dysbindin in schizophrenia brain may be related to either defective targeting of synaptic vesicle proteins or presynaptic receptors, such as DRD2, to or from nerve terminals of affected individuals [101–108] as well as defective targeting to or from postsynaptic compartments [87]. BLOC-1-null *muted* or *pallid* brains possess reduced levels of the synaptic vesicle protein SNARE VAMP7/TI-VAMP [60]. These phenotypes correlate with changes in the content of VAMP7/TI-VAMP in synaptic vesicles [61]. Similarly, BLOC-1 loss-of-function affects targeting of cargoes in other cells derived from the neural crest like melanocytes. *Muted* BLOC-1-null melanocytes possess defective targeting of the Menke's copper transporter to melanosomes, a targeting event independent of AP-3 [109]. Collectively, although circumstantial, these results argue

for a role of BLOC-1 as an adaptor/accessory adaptor that either operates in an AP-3 dependent or independent manner.

Membrane Fusion–Secretion

Membrane fusion is specified by successive mechanisms, which involve rab GTPases, single- or multisubunit tethers, SM (Sec1/Munc18-like) proteins, and SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) [110–113]. SNAREs are necessary and sufficient to mediate membrane fusion. However, eukaryotic cells possess tethering mechanisms to bring in close proximity membranes before SNARE-mediated fusion takes place. Multisubunit tethering complexes in target membranes bind SNAREs, and they recognize rab GTPases present in the incoming vesicle [112]. The interaction between the tether and the GTPase brings vesicle and target in close proximity for fusion. Downstream of tethering, SNARE residing in a vesicle (v- or R-SNARE) and a target membrane (t- or Q-SNARE) “zipper” from their N-terminal ends toward their C-terminal membrane anchoring domains, forming a tetrahelical parallel bundle. SNARE zippering brings membranes in close apposition to initiate fusion. The prevalent model is that SNAREs directly function as fusion catalysts [110, 111]. Once fusion occurs, the resulting cis SNARE tetrahelical parallel complex is resolved into individual SNAREs, which are targeted back to their resident membranes. [110–112]. BLOC-1 likely participates in multiple stages of this elaborated sequence of events. In fact, BLOC-1 subunits either biochemically or genetically interact with key molecules required at different stages of fusion which include rabs [52], SNAREs [49, 114, 115], SM proteins [93], and subunits of tethers such as the exocyst [65, 66, 116]. However, the precise mechanism and the subcellular sites of action of BLOC-1 in fusion remain to be explored in detail. In this section, we will summarize the molecular associations and functional studies implicating BLOC-1 subunits in membrane fusion.

The BLOC-1 subunit pallidin interacts with early endosome SNARE syntaxin13 whereas snapin with the late endosome SNARE syntaxin 8, and the plasma membrane t-SNAREs SNAP23-25, respectively [49, 114, 115, 117, 118]. BLOC-1 deficiencies alter the distribution of SNAREs and/or decrease the cellular content of SNAREs [79, 115]. Also, BLOC-1 *mutated* null-mice possess altered content of the lysosomal/synaptic vesicle SNARE VAMP7/TI-VAMP in synaptic vesicles [60, 61]. Furthermore, overexpression of the BLOC-1 subunit dysbindin increases the expression of SNAP25, a phenotype that correlates with increased basal and induced glutamate secretion in cultured neurons [115]. The best evidence implicating BLOC-1 subunits in secretion/fusion comes from the analysis of regulated secretion in snapin and dysbindin-null mice. Snapin regulates association of the putative Ca²⁺-sensor synaptotagmin with the synaptic SNARE complex, and the absence of snapin decreases calcium-regulated exocytosis of chromaffin granules [117, 119]. In addition, snapin-null neurons have reduced frequency of miniature excitatory postsynaptic events, smaller release-ready vesicle pool size, and desynchronized synaptic vesicle fusion [120]. Similarly, dysbindin-null mice are characterized by larger vesicle size both in synaptic vesicles from hippocampal neurons and chromaffin granules from adrenomedulla cells, slower quantal vesicle release, a reduced frequency of miniature excitatory postsynaptic events, and smaller total population of the readily releasable vesicle pool [121]. These findings may account for *in vivo* microdialysis results of dysbindin-null mice, which reveal a decreased depolarization-induced dopamine release in the prefrontal cortex [122]. Although, neither of these studies has interpreted their results as derived from deficiencies in the whole BLOC-1 complex, the similarity of these secretory phenotypes in snapin- and dysbindin-null neuronal/neuroendocrine cells argues in favor of a BLOC-1 complex-dependent phenotype rather than phenotypes emerging from a single subunit deficiency. A recent and exciting development is the finding in *Drosophila* that dysbindin participates

presynaptically in adaptive, homeostatic modulation of vesicle release modulating the calcium dependency of vesicle release [59]. These findings are exciting as they suggest that specialized synaptic vesicle pools or variations in the coupling of calcium sensors and the fusion machinery may be linked to dysbindin and possibly the BLOC-1 complex.

How can these secretory phenotypes be interpreted? A pressing question is the precise mechanism(s) by which the absence of BLOC-1 subunits, snapin and dysbindin, triggers secretory phenotypes. In the case of snapin, the coupling with the calcium sensor synaptotagmin 1 is a strongly supported mechanism. However, secretory phenotypes could be additionally interpreted as emerging from the capacity of BLOC-1 to bind individual SNAREs as part of a sorting mechanism. Thus, defective concentration of individual SNAREs into vesicles or target membranes could account for secretory phenotypes. Alternatively, BLOC-1 could bind to SNARE tetrahelical complexes. BLOC-1 binds to SNAP25 in vitro, yet when this SNARE is presented in a tetrahelical SNARE complex, binding of BLOC-1 is impaired [49]. However, there is low level of BLOC-1 association to the tetrahelical complex [49]. This suggests that BLOC-1 could be involved either in regulating assembly, clamping of trans-SNARE tetrahelical complexes, or affecting either the targeting or resolution of cis-SNARE tetrahelical complexes. These proposed BLOC-1 mechanisms could operate in isolation or in concert. The mild character of the neuronal secretory phenotypes in dysbindin- and snapin-null neurons as compared with core SNARE deficiencies [123, 124] suggest that these BLOC-1 subunits play modulatory roles in synaptic vesicle fusion and/or affect a subpopulation of vesicles where SNAREs, other than VAMP2 and SNAP25, determine the fusion event.

Discussion and Perspective

Deficiencies of the BLOC-1 subunits pallidin or dysbindin modify neuronal architecture affecting process extension, growth cone morphology, or spine length [49, 72, 74]. Each one of the fundamental cellular processes previously described—sorting, fusion, transcription, and cytoskeletal regulation—could either by itself or in combination account for morphological phenotypes observed in dysbindin/BLOC-1 loss-of-function. Higher order cellular, tissue, and system phenotypes are likely to result from a combination of molecular defects defined by the protein–protein interactions engaged by dysbindin. It is plausible that there will be functions of dysbindin that occur independently of the BLOC-1 complex. However, since the most evolutionarily conserved, better documented, and higher stoichiometry dysbindin interactions are those that position dysbindin within the BLOC-1 complex, we advocate for an expansion of dysbindin studies to consider deficiencies in other BLOC-1 subunits null alleles or loss-of-function approaches. This strategy has already been pioneered [85] to expand the repertoire of molecules to buttress suspected cellular mechanisms contributing to the genesis of schizophrenia.

The precise understanding of the molecular interactions and cellular functions engaged by molecules implicated in schizophrenia, such as dysbindin, will shed light into mechanisms that when impaired contribute to disease pathogenesis to a certain degree. However, it is likely that multiple cellular processes in different combinations may be affected in order to trigger schizophrenia. Thus, it is imperative to expand our knowledge of multiple molecular candidates of disease susceptibility. A monochromatic focus in just few molecules is unlikely to unravel this complex disease.

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Fig. 1.

Molecular architecture of the BLOC-1 complex. **a** Diagram represents the molecular associations between the eight subunits of the BLOC-1 complex as determined from yeast two hybrid analyses and information in curated databases (<http://thebiogrid.org/>) [50–52]. **b** Dysbindin coimmunoprecipitates with BLOC-1 subunits. Cell extracts from HEK293 cells expressing muted tagged with a triple Flag tag (**b**) or untransfected (**c**) were immunoprecipitated (IP) with beads coated with Flag antibodies (*lanes 2, 3, and 5*) or control mouse IgG (*lane 4 and 6*). As controls, immunoprecipitations were outcompeted with triple Flag peptide to prevent binding of muted Flag and associated proteins to beads (*lane 2*) or homogenates (*Hom*) were excluded from reactions carrying Flag antibodies (*lane 3*). The presence of the BLOC-1 subunits dysbindin, pallidin, and Blos3 was determined by immunoblot of SDS–PAGE resolved immune complexes. Note the selective precipitation of BLOC-1 subunits only in *lane 5*

Fig. 2.

Levels of the BLOC-1 subunits pallidin and dysbindin in brains of wild type and BLOC-1 or AP-3 mutant mice. **a** Depicts double immunofluorescent confocal microscopy images of the dentate gyrus from wild type (C57B) and BLOC-1-null *sandy* (*Dtnbp1^{sd/sd}*) and *pallid* (*Pldn^{pa/pa}*) mice stained with antibodies against the synaptic vesicle SNARE VAMP2 (*red channel*) and the BLOC-1 subunit pallidin (*green channel*). Note the absence of the BLOC-1 subunit pallidin both in *pallid* mouse and the dysbindin-null *sandy* mouse. **b** Images of the CA1 region of the hippocampus from wild type (*Ap3d1^{+/+}*) and mocha (*Ap3d1^{mh/mh}*) mice stained with antibodies against dysbindin. Antibody–antigen complexes were revealed with an immunoperoxidase reaction. Note the reduction in the levels of dysbindin in AP-3 null *mocha* hippocampal tissue