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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

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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 inter-

actions 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 lead 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 de-

creased 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* ($DTNBP1^{sdy/sdy}$) 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 $Dtnbp1^{sdy/sdy}$ hippocampus. Concurrently, dysbindin is reduced in null mouse models of Blos3/reduced pigmentation ($BLOC1S3^{rp/rp}$), 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

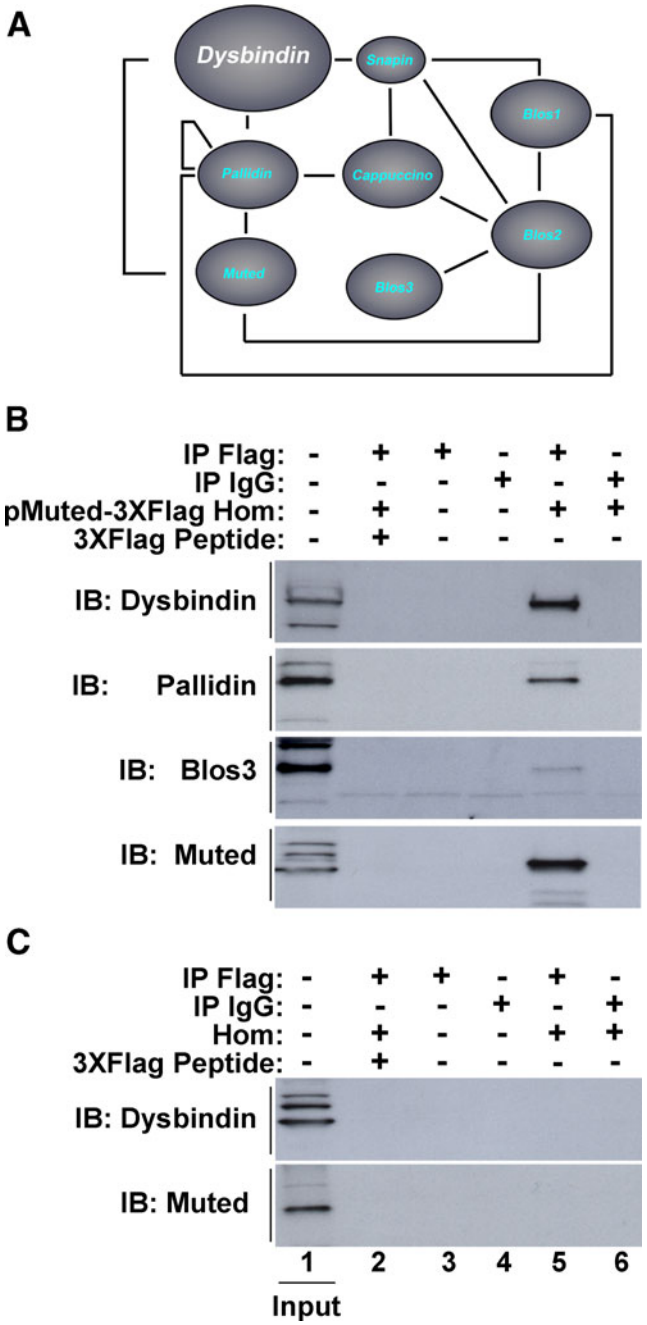

Fig. 1 Molecular architecture of the BLOC-1 complex. **a** Diagram represents the molecular associations between the eight subunits of the BLOC-1 complex as determined from yeast two hybrid analyses and information in curated databases (http://thebiogrid.org/) [50–52]. **b** Dysbindin coimmunoprecipitates 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*

literature documenting neurobehavioral phenotypes in *sandy* mice (*DTNBP1*$^{sdy/sdy}$) [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

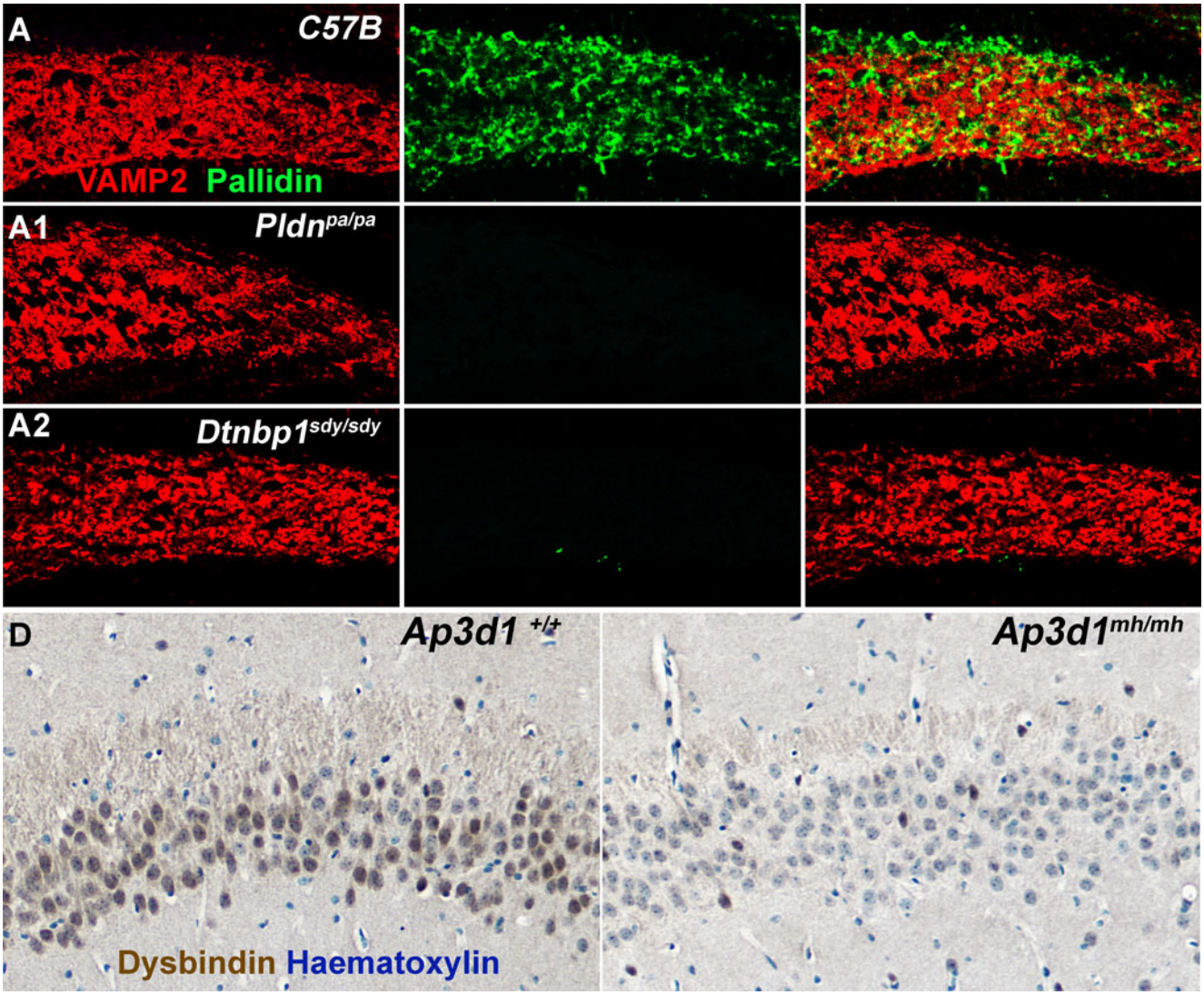

Fig. 2 Levels of the BLOC-1 subunits pallidin and dysbindin in brains of wild type and BLOC-1 or AP-3 mutant mice. **a** Depicts double immunofluorescent confocal microscopy images of the dentate gyrus from wild type (C57B) and BLOC-1-null *sandy* (*Dtnbp1*$^{sdy/sdy}$) and *pallid* (*Pldn*$^{pa/pa}$) 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

[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 downregulated 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, β3A or β3B, μ3A or μ3B, and σ3A or σ3b 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 membranes 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 β3A polypeptide, lead to Hermansky–Pudlak syndrome type 2 (HPS2). Natural and engineered mouse mutants affecting the AP-3 subunits δ or β3A 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 dopamine 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 an 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 to 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 posses tethering mechanisms to bring in close proximity membranes before SNARES-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 *muted* null-mice posses 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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References

1. Benson MA, Newey SE, Martin-Rendon E, Hawkes R, Blake DJ (2001) Dysbindin, a novel coiled-coil-containing protein that interacts with the dystrobrevins in muscle and brain. J Biol Chem 276(26):24232–24241
2. Talbot K, Ong WY, Blake DJ, Tang D, Louneva N, Carlson GC et al (2009) Dysbindin-1 and its protein family, with special attention to the potential role of dysbindin-1 in neuronal functions and the pathophysiology of schizophrenia. In: Kantrowitz Ja (ed) Handbook of Neurochemistry and Molecular Neurobiology, vol. 27. Springer Science, New York, pp 107–241
3. Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV et al (2002) Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. Am J Hum Genet 71(2):337–348

4. Straub RE, MacLean CJ, O'Neill FA, Burke J, Murphy B, Duke F et al (1995) A potential vulnerability locus for schizophrenia on chromosome 6p24-22: evidence for genetic heterogeneity. Nat Genet 11(3):287–293
5. Talbot K, Eidem WL, Tinsley CL, Benson MA, Thompson EW, Smith RJ et al (2004) Dysbindin-1 is reduced in intrinsic, glutamatergic terminals of the hippocampal formation in schizophrenia. J Clin Invest 113(9):1353–1363
6. Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ et al (2008) Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. Nat Genet 40(7):827–834
7. Schizophrenia_Research_Forum. 2010 13 March 2010 [cited; Available from: http://www.schizophreniaforum.org/res/sczgene/default.asp
8. Cardno AG, Marshall EJ, Coid B, Macdonald AM, Ribchester TR, Davies NJ et al (1999) Heritability estimates for psychotic disorders: the Maudsley twin psychosis series. Arch Gen Psychiatry 56(2):162–168
9. Cannon TD, Kaprio J, Lonnqvist J, Huttunen M, Koskenvuo M (1998) The genetic epidemiology of schizophrenia in a Finnish twin cohort. A population-based modeling study. Arch Gen Psychiatry 55(1):67–74
10. Sullivan PF, Kendler KS, Neale MC (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 60(12):1187–1192
11. Consortium IS (2008) Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature 455 (7210):237–241
12. Stefansson H, Rujescu D, Cichon S, Pietilainen OP, Ingason A, Steinberg S et al (2008) Large recurrent microdeletions associated with schizophrenia. Nature 455(7210):232–236
13. Moreno-De-Luca D, Mulle JG, Kaminsky EB, Sanders SJ, Myers SM, Adam MP et al (2010) Deletion 17q12 is a recurrent copy number variant that confers high risk of autism and schizophrenia. Am J Hum Genet 87(5):618–630
14. Karayiorgou M, Simon TJ, Gogos JA (2010) 22q11.2 microdeletions: linking DNA structural variation to brain dysfunction and schizophrenia. Nat Rev Neurosci 11(6):402–416
15. Mulle JG, Dodd AF, McGrath JA, Wolyniec PS, Mitchell AA, Shetty AC et al (2010) Microdeletions of 3q29 confer high risk for schizophrenia. Am J Hum Genet 87(2):229–236
16. Ingason A, Rujescu D, Cichon S, Sigurdsson E, Sigmundsson T, Pietilainen OP et al (2010) Copy number variations of chromosome 16p13.1 region associated with schizophrenia. Mol Psychiatry 16(1):17–25
17. McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, Yoon S et al (2009) Microduplications of 16p11.2 are associated with schizophrenia. Nat Genet 41(11):1223–1227
18. Brandon NJ, Millar JK, Korth C, Sive H, Singh KK, Sawa A (2009) Understanding the role of disc1 in psychiatric disease and during normal development. J Neurosci 29(41):12768–12775
19. Gauthier J, Champagne N, Lafreniere RG, Xiong L, Spiegelman D, Brustein E et al (2010) De novo mutations in the gene encoding the synaptic scaffolding protein SHANK3 in patients ascertained for schizophrenia. Proc Natl Acad Sci USA 107 (17):7863–7868
20. Tarabeux J, Champagne N, Brustein E, Hamdan FF, Gauthier J, Lapointe M et al (2010) De novo truncating mutation in kinesin 17 associated with schizophrenia. Biol Psychiatry 68(7):649–656
21. Gottesman II, Shields J (1967) A polygenic theory of schizophrenia. Proc Natl Acad Sci USA 58(1):199–205
22. Risch N (1990) Linkage strategies for genetically complex traits. I. Multilocus models. Am J Hum Genet 46(2):222–228
23. Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF et al (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460 (7256):748–752
24. Ioannidis JP, Boffetta P, Little J, O'Brien TR, Uitterlinden AG, Vineis P et al (2008) Assessment of cumulative evidence on genetic associations: interim guidelines. Int J Epidemiol 37 (1):120–132
25. Dwyer S, Carroll L, Mantripragada KK et al. Mutation screening of the DTNBP1 exonic sequence in 669 schizophrenics and 710 controls using high-resolution melting analysis. Am J Med Genet B Neuropsychiatr Genet 153B(3): 766–774
26. Li W, Zhang Q, Oiso N, Novak EK, Gautam R, O'Brien EP et al (2003) Hermansky-Pudlak Syndrome Type 7 (HPS-7) results from mutant dysbindin, a member of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). Nat Genet 35(1):84–89
27. Schwab SG, Knapp M, Mondabon S, Hallmayer J, Borrmann-Hassenbach M, Albus M et al (2003) Support for association of schizophrenia with genetic variation in the 6p22.3 gene, dysbindin, in sib-pair families with linkage and in an additional sample of triad families. Am J Hum Genet 72(1):185–190
28. Williams NM, Preece A, Morris DW, Spurlock G, Bray NJ, Stephens M et al (2004) Identification in 2 independent samples of a novel schizophrenia risk haplotype of the dystrobrevin binding protein gene (DTNBP1). Arch Gen Psychiatry 61 (4):336–344
29. Morris DW, McGhee KA, Schwaiger S, Scully P, Quinn J, Meagher D et al (2003) No evidence for association of the dysbindin gene [DTNBP1] with schizophrenia in an Irish population-based study. Schizophr Res 60(2–3):167–172
30. Mutsuddi M, Morris DW, Waggoner SG, Daly MJ, Scolnick EM, Sklar P (2006) Analysis of high-resolution HapMap of DTNBP1 (dysbindin) suggests no consistency between reported common variant associations and schizophrenia. Am J Hum Genet 79 (5):903–909
31. Peters K, Wiltshire S, Henders AK, Dragovic M, Badcock JC, Chandler D et al (2008) Comprehensive analysis of tagging sequence variants in DTNBP1 shows no association with schizophrenia or with its composite neurocognitive endophenotypes. Am J Med Genet B Neuropsychiatr Genet 147B(7):1159–1166
32. Strohmaier J, Frank J, Wendland JR, Schumacher J, Jamra RA, Treutlein J et al (2010) A reappraisal of the association between dysbindin (DTNBP1) and schizophrenia in a large combined case-control and family-based sample of German ancestry. Schizophr Res 118(1–3):98–105
33. Turunen JA, Peltonen JO, Pietilainen OP, Hennah W, Loukola A, Paunio T et al (2007) The role of DTNBP1, NRG1, and AKT1 in the genetics of schizophrenia in Finland. Schizophr Res 91(1–3):27–36
34. Abazyan B, Nomura J, Kannan G, Ishizuka K, Tamashiro KL, Nucifora F et al (2010) Prenatal interaction of mutant DISC1 and immune activation produces adult psychopathology. Biol Psychiatry 68(12):1172–1181
35. Guo AY, Sun J, Riley BP, Thiselton DL, Kendler KS, Zhao Z (2009) The dystrobrevin-binding protein 1 gene: features and networks. Mol Psychiatry 14(1):18–29
36. Tang J, LeGros RP, Louneva N, Yeh L, Cohen JW, Hahn CG et al (2009) Dysbindin-1 in dorsolateral prefrontal cortex of schizophrenia cases is reduced in an isoform-specific manner unrelated to dysbindin-1 mRNA expression. Hum Mol Genet 18 (20):3851–3863
37. Talbot K, Louneva N, Cohen JW, Kazi H, Blake DJ, Arnold SE (2011) Synaptic dysbindin-1 reductions in schizophrenia occur in an isoform-specific manner indicating their subsynaptic location. PLoS ONE 6(3):e16886
38. Bray NJ, Preece A, Williams NM, Moskvina V, Buckland PR, Owen MJ et al (2005) Haplotypes at the dystrobrevin binding

protein 1 (DTNBP1) gene locus mediate risk for schizophrenia through reduced DTNBP1 expression. Hum Mol Genet 14 (14):1947–1954
39. Weickert CS, Rothmond DA, Hyde TM, Kleinman JE, Straub RE (2008) Reduced DTNBP1 (dysbindin-1) mRNA in the hippocampal formation of schizophrenia patients. Schizophr Res 98(1–3):105–110
40. Weickert CS, Straub RE, McClintock BW, Matsumoto M, Hashimoto R, Hyde TM et al (2004) Human dysbindin (DTNBP1) gene expression in normal brain and in schizophrenic prefrontal cortex and midbrain. Arch Gen Psychiatry 61(6):544–555
41. Donohoe G, Frodl T, Morris D, Spoletini I, Cannon DM, Cherubini A et al (2010) Reduced occipital and prefrontal brain volumes in dysbindin-associated schizophrenia. Neuropsychopharmacology 35(2):368–373
42. Donohoe G, Morris DW, Clarke S, McGhee KA, Schwaiger S, Nangle JM et al (2007) Variance in neurocognitive performance is associated with dysbindin-1 in schizophrenia: a preliminary study. Neuropsychologia 45(2):454–458
43. Donohoe G, Morris DW, De Sanctis P, Magno E, Montesi JL, Garavan HP et al (2008) Early visual processing deficits in dysbindin-associated schizophrenia. Biol Psychiatry 63(5):484–489
44. Narr KL, Szeszko PR, Lencz T, Woods RP, Hamilton LS, Phillips O et al (2009) DTNBP1 is associated with imaging phenotypes in schizophrenia. Hum Brain Mapp 30(11):3783–3794
45. Fallgatter AJ, Herrmann MJ, Hohoff C, Ehlis AC, Jarczok TA, Freitag CM et al (2006) DTNBP1 (dysbindin) gene variants modulate prefrontal brain function in healthy individuals. Neuropsychopharmacology 31(9):2002–2010
46. Markov V, Krug A, Krach S, Whitney C, Eggermann T, Zerres K et al (2009) Genetic variation in schizophrenia-risk-gene dysbindin 1 modulates brain activation in anterior cingulate cortex and right temporal gyrus during language production in healthy individuals. Neuroimage 47(4):2016–2022
47. Mechelli A, Viding E, Kumar A, Pettersson-Yeo W, Fusar-Poli P, Tognin S et al (2010) Dysbindin modulates brain function during visual processing in children. Neuroimage 49(1):817–822
48. Wolf C, Jackson MC, Kissling C et al. (2011) Dysbindin-1 genotype effects on emotional working memory. Mol Psychiatry 16(2):145–155
49. Ghiani CA, Starcevic M, Rodriguez-Fernandez IA, Nazarian R, Cheli VT, Chan LN et al (2010) The dysbindin-containing Complex (BLOC-1) in brain: developmental regulation, interaction with SNARE proteins and role in neurite outgrowth. Mol Psychiatry 15(2):115, 204–215
50. Starcevic M, Dell'Angelica EC (2004) Identification of snapin and three novel proteins (BLOS1, BLOS2, and BLOS3/reduced pigmentation) as subunits of biogenesis of lysosome-related organelles complex-1 (BLOC-1). J Biol Chem 279(27):28393–28401
51. Di Pietro SM, Dell'Angelica EC (2005) The cell biology of Hermansky-Pudlak syndrome: recent advances. Traffic 6 (7):525–533
52. Cheli VT, Daniels RW, Godoy R, Hoyle DJ, Kandachar V, Starcevic M et al (2010) Genetic modifiers of abnormal organelle biogenesis in a drosophila model of BLOC-1 deficiency. Hum Mol Genet 19(5):861–878
53. Nazarian R, Starcevic M, Spencer MJ, Dell'Angelica EC (2006) Reinvestigation of the dysbindin subunit of BLOC-1 (biogenesis of lysosome-related organelles complex-1) as a dystrobrevin-binding protein. Biochem J 395(3):587–598
54. Feng YQ, Zhou ZY, He X, Wang H, Guo XL, Hao CJ et al (2008) Dysbindin deficiency in sandy mice causes reduction of snapin and displays behaviors related to schizophrenia. Schizophr Res 106(2–3):218–228
55. Li W, Rusiniak ME, Chintala S, Gautam R, Novak EK, Swank RT (2004) Murine Hermansky-Pudlak syndrome genes: regulators of lysosome-related organelles. BioEssays 26(6):616–628
56. Wei ML (2006) Hermansky-Pudlak syndrome: a disease of protein trafficking and organelle function. Pigment Cell Res 19 (1):19–42
57. Morgan NV, Pasha S, Johnson CA, Ainsworth JR, Eady RA, Dawood B et al (2006) A germline mutation in BLOC1S3/reduced pigmentation causes a novel variant of Hermansky-Pudlak Syndrome (HPS8). Am J Hum Genet 78(1):160–166
58. Talbot K (2009) The sandy (sdy) mouse: a dysbindin-1 mutant relevant to schizophrenia research. Prog Brain Res 179:87–94
59. Dickman DK, Davis GW (2009) The schizophrenia susceptibility gene dysbindin controls synaptic homeostasis. Science 326 (5956):1127–1130
60. Newell-Litwa K, Chintala S, Jenkins S, Pare JF, McGaha L, Smith Y et al (2010) Hermansky-Pudlak protein complexes, AP-3 and BLOC-1, differentially regulate presynaptic composition in the striatum and hippocampus. J Neurosci 30(3):820–831
61. Newell-Litwa K, Salazar G, Smith Y, Faundez V (2009) Roles of BLOC-1 and AP-3 complexes in cargo sorting to synaptic vesicles. Mol Biol Cell 20(5):1441–1453
62. Morris DW, Murphy K, Kenny N, Purcell SM, McGhee KA, Schwaiger S et al (2008) Dysbindin (DTNBP1) and the biogenesis of lysosome-related organelles complex 1 (BLOC-1): main and epistatic gene effects are potential contributors to schizophrenia susceptibility. Biol Psychiatry 63(1):24–31
63. Gerrish A, Williams H, Moskvina V, Owen MJ, O'Donovan MC, Williams NM (2009) An examination of MUTED as a schizophrenia susceptibility gene. Schizophr Res 107(1):110–111
64. Li W, Feng Y, Hao C, Guo X, Cui Y, He M et al (2007) The BLOC interactomes form a network in endosomal transport. J Genet Genomics 34(8):669–682
65. Rodriguez-Fernandez IA, Dell'Angelica EC (2009) A data-mining approach to rank candidate protein-binding partners-The case of biogenesis of lysosome-related organelles complex-1 (BLOC-1). J Inherit Metab Dis 32(2):190–203
66. Mead CL, Kuzyk MA, Moradian A, Wilson GM, Holt RA, Morin GB (2010) Cytosolic protein interactions of the schizophrenia susceptibility gene dysbindin. J Neurochem 113 (6):1491–1503
67. Fei E, Ma X, Zhu C et al. (2010) Nucleocytoplasmic shuttling of dysbindin-1, a schizophrenia related protein, regulates synapsin I expression. J Biol Chem 285(49):38630–38640
68. Okuda H, Kuwahara R, Matsuzaki S, Miyata S, Kumamoto N, Hattori T et al (2010) Dysbindin regulates the transcriptional level of myristoylated alanine-rich protein kinase C substrate via the interaction with NF-YB in mice brain. PLoS ONE 5(1): e8773
69. Oyama S, Yamakawa H, Sasagawa N, Hosoi Y, Futai E, Ishiura S (2009) Dysbindin-1, a schizophrenia-related protein, functionally interacts with the DNA-dependent protein kinase complex in an isoform-dependent manner. PLoS ONE 4(1):e4199
70. Numakawa T, Yagasaki Y, Ishimoto T, Okada T, Suzuki T, Iwata N et al (2004) Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia. Hum Mol Genet 13(21):2699–2708
71. Cesca F, Baldelli P, Valtorta F, Benfenati F (2010) The synapsins: key actors of synapse function and plasticity. Prog Neurobiol 91(4):313–348
72. Ito H, Morishita R, Shinoda T et al. Dysbindin-1, WAVE2 and Abi-1 form a complex that regulates dendritic spine formation. Mol Psychiatry 15(10): 976–986
73. Falcon-Perez JM, Starcevic M, Gautam R, Dell'Angelica EC (2002) BLOC-1, a novel complex containing the pallidin and

muted proteins involved in the biogenesis of melanosomes and platelet-dense granules. J Biol Chem 277(31):28191–28199
74. Kubota K, Kumamoto N, Matsuzaki S, Hashimoto R, Hattori T, Okuda H et al (2009) Dysbindin engages in c-Jun N-terminal kinase activity and cytoskeletal organization. Biochem Biophys Res Commun 379(2):191–195
75. Newell-Litwa K, Seong E, Burmeister M, Faundez V (2007) Neuronal and non-neuronal functions of the AP-3 sorting machinery. J Cell Sci 120(Pt 4):531–541
76. Dell'angelica EC (2009). AP-3-dependent trafficking and disease: the first decade. Curr Opin Cell Biol 21(4):552–559
77. Bonifacino JS, Traub LM (2003) Signals for sorting of transmembrane proteins to endosomes and lysosomes. Annu Rev Biochem 72:395–447
78. Robinson MS (2004) Adaptable adaptors for coated vesicles. Trends Cell Biol 14(4):167–174
79. Salazar G, Craige B, Styers ML, Newell-Litwa KA, Doucette MM, Wainer BH et al (2006) BLOC-1 complex deficiency alters the targeting of adaptor protein complex-3 cargoes. Mol Biol Cell 17(9):4014–4026
80. Di Pietro SM, Falcon-Perez JM, Tenza D, Setty SR, Marks MS, Raposo G et al (2006) BLOC-1 interacts with BLOC-2 and the AP-3 complex to facilitate protein trafficking on endosomes. Mol Biol Cell 17(9):4027–4038
81. Setty SR, Tenza D, Truschel ST, Chou E, Sviderskaya EV, Theos AC et al (2007) BLOC-1 is required for cargo-specific sorting from vacuolar early endosomes toward lysosome-related organelles. Mol Biol Cell 18(3):768–780
82. Baust T, Anitei M, Czupalla C, Parshyna I, Bourel L, Thiele C et al (2008) Protein networks supporting AP-3 function in targeting lysosomal membrane proteins. Mol Biol Cell 19 (5):1942–1951
83. Janvier K, Bonifacino JS (2005) Role of the endocytic machinery in the sorting of lysosome-associated membrane proteins. Mol Biol Cell 16(9):4231–4242
84. Le Borgne R, Alconada A, Bauer U, Hoflack B (1998) The mammalian AP-3 adaptor-like complex mediates the intracellular transport of lysosomal membrane glycoproteins. J Biol Chem 273(45):29451–29461
85. Iizuka Y, Sei Y, Weinberger DR, Straub RE (2007) Evidence that the BLOC-1 protein dysbindin modulates dopamine D2 receptor internalization and signaling but not D1 internalization. J Neurosci 27(45):12390–12395
86. Ji Y, Yang F, Papaleo F, Wang HX, Gao WJ, Weinberger DR et al (2009) Role of dysbindin in dopamine receptor trafficking and cortical GABA function. Proc Natl Acad Sci USA 106 (46):19593–19598
87. Tang TT, Yang F, Chen BS, Lu Y, Ji Y, Roche KW et al (2009) Dysbindin regulates hippocampal LTP by controlling NMDA receptor surface expression. Proc Natl Acad Sci USA 106 (50):21395–21400
88. Marley A, von Zastrow M (2010) Dysbindin promotes the post-endocytic sorting of G protein-coupled receptors to lysosomes. PLoS ONE 5(2):e9325
89. Asensio CS, Sirkis DW, Edwards RH (2010) RNAi screen identifies a role for adaptor protein AP-3 in sorting to the regulated secretory pathway. J Cell Biol 191(6):1173–1187
90. Grabner CP, Price SD, Lysakowski A, Cahill AL, Fox AP (2006) Regulation of large dense-core vesicle volume and neurotransmitter content mediated by adaptor protein 3. Proc Natl Acad Sci USA 103(26):10035–10040
91. Salazar G, Zlatic S, Craige B, Peden AA, Pohl J, Faundez V (2009) Hermansky-Pudlak syndrome protein complexes associate with phosphatidylinositol 4-kinase type II alpha in neuronal and non-neuronal cells. J Biol Chem 284(3):1790–1802
92. Hashimoto R, Ohi K, Okada T, Yasuda Y, Yamamori H, Hori H et al (2009) Association analysis between schizophrenia and the AP-3 complex genes. Neurosci Res 65(1):113–115
93. Hikita T, Taya S, Fujino Y, Taneichi-Kuroda S, Ohta K, Tsuboi D et al (2009) Proteomic analysis reveals novel binding partners of dysbindin, a schizophrenia-related protein. J Neurochem 110 (5):1567–1574
94. Taneichi-Kuroda S, Taya S, Hikita T, Fujino Y, Kaibuchi K (2009) Direct interaction of dysbindin with the AP-3 complex via its mu subunit. Neurochem Int 54(7):431–438
95. Bonifacino JS, Glick BS (2004) The mechanisms of vesicle budding and fusion. Cell 116(2):153–166
96. Faundez V, Horng JT, Kelly RB (1998) A function for the AP3 coat complex in synaptic vesicle formation from endosomes. Cell 93(3):423–432
97. Ooi CE, Dell'Angelica EC, Bonifacino JS (1998) ADP-ribosylation factor 1 (ARF1) regulates recruitment of the AP-3 adaptor complex to membranes. J Cell Biol 142(2):391–402
98. Borner GH, Harbour M, Hester S, Lilley KS, Robinson MS (2006) Comparative proteomics of clathrin-coated vesicles. J Cell Biol 175(4):571–578
99. Salazar G, Craige B, Wainer BH, Guo J, De Camilli P, Faundez V (2005) Phosphatidylinositol-4-kinase type II alpha is a component of adaptor protein-3-derived vesicles. Mol Biol Cell 16(8):3692–3704
100. Craige B, Salazar G, Faundez V (2008) Phosphatidylinositol-4-kinase type II alpha contains an AP-3 sorting motif and a kinase domain that are both required for endosome traffic. Mol Biol Cell 19:1415–1426
101. Perreault ML, Hasbi A, Alijaniaram M, Fan T, Varghese G, Fletcher PJ et al (2010) The dopamine D1-D2 receptor heteromer localizes in dynorphin/enkephalin neurons: increased high affinity state following amphetamine and in schizophrenia. J Biol Chem 285(47):36625–36634
102. Howes OD, Kapur S (2009) The dopamine hypothesis of schizophrenia: Version III—the final common pathway. Schizophr Bull 35(3):549–562
103. De Mei C, Ramos M, Iitaka C, Borrelli E (2009) Getting specialized: presynaptic and postsynaptic dopamine D2 receptors. Curr Opin Pharmacol 9(1):53–58
104. Mengual E, Pickel VM (2002) Ultrastructural immunocytochemical localization of the dopamine D2 receptor and tyrosine hydroxylase in the rat ventral pallidum. Synapse 43 (3):151–162
105. Wang H, Pickel VM (2002) Dopamine D2 receptors are present in prefrontal cortical afferents and their targets in patches of the rat caudate-putamen nucleus. J Comp Neurol 442(4):392–404
106. Bamford NS, Zhang H, Schmitz Y, Wu NP, Cepeda C, Levine MS et al (2004) Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. Neuron 42 (4):653–663
107. Levey AI, Hersch SM, Rye DB, Sunahara RK, Niznik HB, Kitt CA et al (1993) Localization of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. Proc Natl Acad Sci USA 90(19):8861–8865
108. Sesack SR, Aoki C, Pickel VM (1994) Ultrastructural localization of D2 receptor-like immunoreactivity in midbrain dopamine neurons and their striatal targets. J Neurosci 14 (1):88–106
109. Setty SR, Tenza D, Sviderskaya EV et al. (2008) Cell-specific ATP7A transport sustains copper-dependent tyrosinase activity in melanosomes. Nature 454(7208):1142–1146
110. Jahn R, Scheller RH (2006) SNAREs—engines for membrane fusion. Nat Rev Mol Cell Biol 7(9):631–643
111. Wickner W, Schekman R (2008) Membrane fusion. Nat Struct Mol Biol 15(7):658–664

112. Brocker C, Engelbrecht-Vandre S, Ungermann C (2010) Multisubunit tethering complexes and their role in membrane fusion. Curr Biol 20(21):R943–R952
113. Sudhof TC, Rothman JE (2009) Membrane fusion: grappling with SNARE and SM proteins. Science 323(5913):474–477
114. Huang L, Kuo YM, Gitschier J (1999) The pallid gene encodes a novel, syntaxin 13-interacting protein involved in platelet storage pool deficiency. Nat Genet 23(3):329–332
115. Lu L, Cai Q, Tian JH, Sheng ZH (2009) Snapin associates with late endocytic compartments and interacts with late endosomal snares. Biosci Rep 29(4):261–269
116. Bao Y, Lopez JA, James DE, Hunziker W (2008) Snapin interacts with the Exo70 subunit of the exocyst and modulates GLUT4 trafficking. J Biol Chem 283(1):324–331
117. Ilardi JM, Mochida S, Sheng ZH (1999) Snapin: a SNARE-associated protein implicated in synaptic transmission. Nat Neurosci 2(2):119–124
118. Buxton P, Zhang XM, Walsh B, Sriratana A, Schenberg I, Manickam E et al (2003) Identification and characterization of snapin as a ubiquitously expressed SNARE-binding protein that interacts with SNAP23 in non-neuronal cells. Biochem J 375(Pt 2):433–440
119. Tian JH, Wu ZX, Unzicker M, Lu L, Cai Q, Li C et al (2005) The role of snapin in neurosecretion: snapin knock-out mice exhibit impaired calcium-dependent exocytosis of large dense-core vesicles in chromaffin cells. J Neurosci 25(45):10546–10555
120. Pan PY, Tian JH, Sheng ZH (2009) Snapin facilitates the synchronization of synaptic vesicle fusion. Neuron 61(3):412–424
121. Chen XW, Feng YQ, Hao CJ, Guo XL, He X, Zhou ZY et al (2008) DTNBP1, a schizophrenia susceptibility gene, affects kinetics of transmitter release. J Cell Biol 181(5):791–801
122. Nagai T, Kitahara Y, Shiraki A, Hikita T, Taya S, Kaibuchi K et al (2010) Dysfunction of dopamine release in the prefrontal cortex of dysbindin deficient sandy mice: an in vivo microdialysis study. Neurosci Lett 470(2):134–138
123. Schoch S, Deak F, Konigstorfer A, Mozhayeva M, Sara Y, Sudhof TC et al (2001) SNARE function analyzed in synaptobrevin/VAMP knockout mice. Science 294(5544):1117–1122
124. Washbourne P, Thompson PM, Carta M, Costa ET, Mathews JR, Lopez-Bendito G et al (2002) Genetic ablation of the t-SNARE SNAP-25 distinguishes mechanisms of neuroexocytosis. Nat Neurosci 5(1):19–26