Katanin localization requires triplet microtubules in Chlamydomonas reinhardtii

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Katanin Localization Requires Triplet Microtubules in Chlamydomonas reinhardtii

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Abstract

Centrioles and basal bodies are essential for a variety of cellular processes that include the recruitment of proteins to these structures for both centrosomal and ciliary function. This recruitment is compromised when centriole/basal body assembly is defective. Mutations that cause basal body assembly defects confer supersensitivity to Taxol. These include bld2, bld10, bld12, uni3, vfl1, vfl2, and vfl3. Flagellar motility mutants do not confer sensitivity with the exception of mutations in the p60 (pf19) and p80 (pf15) subunits of the microtubule severing protein katanin. We have identified additional pf15 and bld2 (β-tubulin) alleles in screens for Taxol sensitivity. Null pf15 and bld2 alleles are viable and are not essential genes in Chlamydomonas. Analysis of double mutant strains with the pf15-3 and bld2-6 null alleles suggests that basal bodies in Chlamydomonas may recruit additional proteins beyond katanin that affect spindle microtubule stability. The bld2-5 allele is a hypomorphic allele and its phenotype is modulated by nutritional cues. Basal bodies in bld2-5 cells are missing proximal ends. The basal body mutants show aberrant localization of an epitope-tagged p80 subunit of katanin. Unlike IFT proteins, katanin p80 does not localize to the transition fibers of the basal bodies based on an analysis of the uni1 mutant as well as the lack of colocalization of katanin p80 with IFT74. We suggest that the triplet microtubules are likely to play a key role in katanin p80 recruitment to the basal body of Chlamydomonas rather than the transition fibers that are needed for IFT localization.

Introduction

Taxol is a natural product that binds to β-tubulin and stabilizes microtubules in eukaryotic cells. Taxol is used for treatment of a variety of cancers as it blocks mitosis [1]. In the unicellular green alga, Chlamydomonas reinhardtii, Schöler and Huang showed that a mutation in β-tubulin (tub2-1) confers resistance to colchicine and supersensitivity to Taxol. They concluded that the microtubules in tub2-1 cells are hyperstabilized, which causes the Taxol supersensitive phenotype [2]. Katanin is a microtubule severing protein [3,4]. It is composed of two subunits; the p60 subunit is a catalytic AAA ATPase and the p80 subunit targets the heterodimer to the centrosome in metazoans. Katanin influences microtubule dynamics through its ability to sever microtubules. This property is observed in Drosophila S2 mitotic spindles [5] and C. elegans meiotic spindles [6]. Sharma and colleagues [7] showed that loss of either the catalytic (p60) or the targeting (p80) subunit of katanin in Tetrahymena results in short cilia and a knockout allele of p60 confers Taxol supersensitivity. Thus, there appear to be several pathways in cells that when mutated confer Taxol sensitivity.

Because katanin localizes to the centrosome, this localization seems likely to require intact centrioles/basal bodies. Centrioles are a component of the metazoan centrosome and help to recruit pericentriolar material (PCM) that nucleates both cytoplasmic and spindle microtubules [8,9]. Functional centrioles and intact subdistal appendages are required for the recruitment of PCM proteins in animals. SPD-2, SPD-5 and SAS-4, which were first identified in C. elegans and localize to centrioles by immunoelectron microscopy, play essential roles in centriole biogenesis and they are needed to recruit γ-tubulin and aurora kinase to the PCM [9–11]. Sas-4 is thought to play a key role as it shows binding to αβ-tubulin dimers [12] as well as with Sas-5 and Cep152/Asl. Recruitment in Drosophila requires Asl (Cep152) and D-Spd2 (Cep192) [13]. The SPD-2/Cep192 homolog and centrosomin (Cnn), localizes to the centrioles and spd2 mutants show significantly reduced concentrations of several centrosomal proteins that include Cnn, γ-tubulin, Del1/Dgrp91, and D-TACC [8,14]. Subdistal appendages on mature centrioles contain ninein [15]. Tissue culture cells depleted of ninein by siRNA show a significant reduction in γ-tubulin and the complete absence of the protein, astrin, at the centrosome [16]. Thus, defective centrioles or depletion of centriolar proteins prevent recruitment of some or all pericentriolar proteins.

When a centriole matures and converts to a basal body, it recruits intraflagellar transport (IFT) proteins and motors [17,18]. Just as centriolar proteins are required to recruit PCM compo-
ments for cytoplasmic and spindle microtubules, defective basal bodies disrupt localization of IFT proteins. In wild-type *Chlamydomonas* cells, IFT proteins accumulate around the basal body in a horseshoe-shaped structure [19,20]. The *bld2*-1 mutant has an incompletely assembled basal body [21], and although the IFT proteins are present, their localization is diffuse rather than in the horseshoe-shaped pattern.

We reasoned that *Chlamydomonas* mutants with basal body defects that lack the ability to dock intraflagellar transport proteins correctly could also fail to localize PCM components properly. We report a new phenotype that is associated with basal body biogenesis defects; these mutant strains show increased sensitivity to the microtubule-stabilizing drug, Taxol. We hypothesize that lack of localization or mislocalization of PCM-targeted proteins contributes to the Taxol supersensitivity phenotype.

Strains with basal body integrity defects fall into several classes. The first class shows defects in the assembly of the microtubule blades and includes *bld2*, *bld10*, *bld12*, *uni2* and *uni3*. The *bld2*, *bld10*, and *bld12* mutants lack complete microtubule blades, and *uni3* mutants lack triplet microtubules [22–25]. The *uni1* and *uni2* mutants show a defect in the transition zone [26,27] and the *uni1*; *uni2* double mutant affects the change from triplet to doublet microtubules [28]. The second class of mutants shows defects in the fibers that are required to maintain proper basal body orientation and segregation and include *vfl1*, *vfl2*, and *vfl3* [29–31].

We screened existing basal body and flagellar mutants for increased sensitivity to the microtubule-stabilizing drug, Taxol, as well as performed several screens for additional mutants that confer Taxol sensitivity. We find that katanin mutants in *Chlamydomonas* confer Taxol sensitivity and that mutants with basal body defects confer Taxol sensitivity, and have abnormal recruitment of the p80 katanin subunit to the basal bodies.

**Materials and Methods**

**Cell Culture, Genetic and Phenotypic Analyses**

*Chlamydomonas reinhardtii* growth conditions [32], matings [33], and revertant isolation using ultraviolet irradiation [34] were performed as previously described. Aflagellate strains were mated with 100 mM dibutyryl cyclic AMP (Sigma-Aldrich, St. Louis, MO) and 30 mM isobutyl 1-methylxanthine (Aldrich, Milwaukee, WI) [33]. For each sample, 200 cells were counted after fixation with 1% glutaraldehyde in phosphate buffer (pH 7.4). Taxol (paclitaxel, Sigma-Aldrich) was tested at concentrations varying with 1% glutaraldehyde in phosphate buffer (pH 7.4). Taxol was assayed for their ability to oppose gravity.

To differentiate between mutations resulting from chromosome loss or mitotic recombination, dominant enhancers, and new alleles we took advantage of the absence of a *Fod* restriction enzyme site in the *bld2*-2 allele. PCR amplification of the region around the mutation and digestion with the *Fod* restriction enzyme produces both the digested wild-type product and uncut *bld2*-2 product in the heterozygous parental diploid. If mitotic recombination or chromosome loss occurs, only the *bld2*-2 fragment would be amplified by PCR [34].

To isolate new alleles in haploid strains, an insertional mutant collection with 3000 independent strains, which was a kind gift from Dr. Lauren Mets (University of Chicago), was used. It was constructed in the CC-125 strain by transformation with the ble gene [40]. The collection was screened by replica plating using RepliPlate pads (FMC, Rockland, ME) onto solid R medium with 8 μM Taxol at 25°C. Plates containing Taxol were maintained in yellow Lucite boxes [35].

**PCR and Sequencing**

Genomic DNA from *bld2*-5 and *bld2*-6 cells was isolated using a modified protocol of the Genisol Maxi-Prep Kit (Abgene, Rockford, IL). Between 10^5–10^6 cells were suspended in 50 μl of 1X Tris-EDTA buffer with a 100-fold reduction in the suggested volumes. Primers for sequencing were described previously and are available upon request. REDTaq DNA polymerase (Sigma-Aldrich) was used with the following conditions: 31 cycles of 1 min at 95°C, 1 min at 57°C and 1 min at 72°C followed by a 10 min extension at 72°C. PCR products were column purified (QIA-GEN, Valencia, CA) and then cycle sequenced using the following conditions: 2 min at 96°C and 32 cycles of 96°C for 10 sec, 50°C for 5 sec and 60°C for 4 min. Sequencing reactions were precipitated with the addition of 125 mM EDTA and 100% ethanol and incubated for 15 min at room temperature (RT). Reactions were centrifuged, washed with 70% ethanol and dried before the addition of Hi- Di formamide (Applied Biosystems, Foster City, CA). After 2 min incubation at 95°C, the reactions were loaded onto a 3100 Genetic Analyzer (Applied Biosystems). Sequenced data were aligned and analyzed with Sequencher (Gene Codes, Ann Arbor, MI). Genomic DNA from the eight intragenic revertants was isolated and sequenced as described above with primers that produce a 367 bp fragment (m-tsn-28-1f; TGTCACACCGGGAACATTGAC and m-tsn-28-1r-GA-CAGCTGCTGCATTGTGAT). TAIL PCR determined the
insertion site of the ble gene in the bld2-6 allele [41,42] using primers in Table S2.

Construction of PF15 Transgene by Knitting PCR

The pPF15 vector, provided by Elizabeth Smith (Dartmouth University), was used to amplify two fragments of 304 and 195 bp for knitting PCR [13] using the primers PF15 FRAG F/PF15 NOT R (CCCTCCTGCGCCAGGTGATG, CTAGCGGCCGCGCTGCGCCAGCCTG) and PF15 NOT F/ PF15 FRAG R (CAGCTGGCGCAGCGCGGCCGCTAG, CATTGTCCTGCAGGGCCAC). The PF15 NOT primers contain the Nol restriction enzyme site and it changes the last amino acid of PF15 from a leucine to an asparagine. These fragments were amplified using Klentaq Long and Accurate polymerase using the following conditions: 30 cycles of 1 min at 95°C, 1 min at 56°C and 1 min at 68°C, followed by a 30 min extension at 68°C. The fragments were purified from a 2% agarose gel (Gel Purification Kit; MO BIO Laboratories Inc., Carlsbad, CA). The above PCR was repeated using equal quantities of each PCR product and the PF15 FRAG primers to incorporate a Nol restriction site that was used to clone the HA tag into the gene (Figure S1). The resulting approximately 500 bp fragment was gel purified and cloned into the pCR4-TOPO vector (Invitrogen, Carlsbad, CA). Transformed colonies were verified by colony PCR and used to isolate plasmid DNA with the

Figure 1. Basal body mutant strains show supersensitivity to Taxol. (A) Serial dilution of mutant, rescued, and intragenic revertant strains on control medium and (B) 8 μM Taxol-containing medium. Phase images of cells on media with different Taxol concentrations. (C, G) Wild-type, (D, H) pf15-1, (E, I) bld2-6 and (F, J) bld2-6, pf15-1 double mutant on 10 μM (C–F) or 6 μM Taxol (G–J) containing medium. The bld2-6, pf15-1 double mutant is unable to grow on 6 μM Taxol containing medium compared to the single mutant strains. Scale bar in Panel C equals 10 μm. Panels C–J are at the same magnification.

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Wizard Plus SV Minipreps DNA Purification System (Promega, Madison, WI). The PF15 fragment with the incorporated Not I site (pPF15-N) was digested with Eco RI, gel purified as described above, and ligated to the LITMUS 28i vector (New England Biolabs, Ipswich, MA). Transformed colonies were verified by colony PCR using the PF15 FRAG primers. The HA tag was ligated separately into pPF15-N plasmids. Positive colonies were assayed for number of tags and orientation by PCR and digestion. Multiple tagged PF15 genes were transformed into the pf15 mutant strain by electroporation [44,45] and transformants were screened by their ability to oppose gravity.

Preparation of Cells for Electron Microscopy

Cells were prepared for electron microscopy using methods described in O’Toole et al. [46,47]. Briefly, aliquots of cells grown in suspension were spun at 500 x g and then resuspended in 150 mM mannitol. The samples were spun again at 500 x g and the resulting loose cell pellet was then transferred to aluminum sample holders and rapidly frozen in a Balzers HPM010 high pressure freezer (BAL-TEC, Technotrade International, Manchester, NH). The frozen cells were freeze-substituted for three days at −90°C in 1% OsO4 and 0.1% uranyl acetate in acetone, warmed to room temperature and embedded in epon/araldite resin.

Serial thin (50–70 nm) or thick (250–400 nm) sections were cut using an Ultracut-E microtome (Leica, Germany) and the section ribbons were collected onto Formvar-coated copper slot grids. The

| Strain       | Temperature (°C) | 0  | 1  | 2  | >3 |
|--------------|------------------|----|----|----|----|
| BLD2         | 25               | 5  | 3.5| 91.5| 0  |
| BLD2         | 32               | 16 | 7  | 77  | 0  |
| bld2-1       | 25               | 100| 0  | 0   | 0  |
| bld2-1; BLD2TG| 25             | 5.5| 7.5| 87  | 0  |
| bld2-6       | 25               | 100| 0  | 0   | 0  |
| bld2-6; BLD2TG| 25             | 22.5| 3.5| 72.5| 1.5|
| bld2-5       | 14               | 100| 0  | 0   | 0  |
| bld2-5       | 21               | 100| 0  | 0   | 0  |
| bld2-5       | 25               | 95 | 4  | 1   | 0  |
| bld2-5       | 32               | 95 | 4.5| 0   | 0.5|
| bld2-5; BLD2TG| 25             | 6.3| 14.8| 80.3 | 0 |
| bld2-7       | 25               | 2.5| 4  | 93.5| 0  |
| bld2-9       | 25               | 7  | 3  | 90  | 0  |
| BLD2/BLD2    | 25               | 15 | 9  | 76  | 0  |
| bld2-5/BLD2  | 25               | 10.3| 3.9| 84.5| 0.81|

Gametic

bld2-5       | 25               | 79 | 14 | 6.5| 0 |
| bld2-5       | 32               | 74 | 15.5| 10.5| 0|
| bld2-6       | 25               | 100| 0  | 0   | 0  |
| bld2-7       | 25               | 10 | 18.5| 71.5| 0 |
| bld2-9       | 25               | 8.5| 12 | 79.5| 0 |

*BLD2TG indicates the ε-tubulin transgene described previously [24].

Figure 2. Centrin localization varies in the bld2-5 and bld2-6 strains. (A, B) Wild-type cells with an extended pattern of centrin. (C) Approximately 41% of bld2-5 cells have centrin that collapses on the
sections were post-stained in 2% uranyl acetate in 70% methanol followed by Reynold’s lead citrate. For tomography, 15 nm colloidal gold particles were used (Sigma-Aldrich).

Electron Microscopy

Serial thin sections were imaged in a Philips CM10 EM (FEI, Mahwah, NJ) operating at 80 kV. Serial sections of the basal body through the transition zone from 13 cells were collected to

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**Figure 3. Rootlet microtubules are disorganized in the bld2-5 and bld2-6 strains.** (A, B) Rootlet microtubules in wild-type cells form a cruciate pattern. (C, D) bld2-5 cells show an aberrant number and placement of rootlet microtubules. (E, F) The bld2-5; BLD2 strain shows a wild-type rootlet microtubule phenotype (N = 15). Pseudorevertants bld2-7 (G, H) and bld2-9 (I, J) have a nearly wild-type rootlet microtubule phenotype but splaying occurs at the ends of the microtubules (arrow). (K, L) The tub2-1 strain has increased acetylated $\alpha$-tubulin staining. (M, N) The bld2-6 cells have a severe disorganization of rootlet microtubules. Scale bar in Panel A equals 5 $\mu$m. Panels A–N are at the same magnification. doi:10.1371/journal.pone.0053940.g003

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nucleus (n = 70). (D) bld2-5 cells with a wild-type centrin pattern. (E, F) The rescued transformant, bld2-5; BLD2, and (G, H) the pseudorevertants, bld2-7 and (I, J) bld2-9 have extended centrin. (K, L) All bld2-6 cells show centrin collapses on or around the nucleus. Scale bar in panel L equals 10 $\mu$m. Panels A–L are at the same magnification. doi:10.1371/journal.pone.0053940.g002
document the phenotype and aid in the interpretation of
tomographic data.

Electron tomography was carried out essentially as described
[47,48]. The specimens were placed in a tilt-rotate specimen
holder (Gatan, Pleasanton, CA) and tomographic data sets
recorded using a TECNAI F30 intermediate-voltage electron
microscope (FEI, The Netherlands) operated at 300 kV. Images
were captured every 1° over a ±60° range using a Gatan
2 K×2 K CCD camera at a pixel size of 1 nm. The grid was
rotated 90°, and a second tilt series was acquired. Dual-axis
tomographic reconstruction was carried out using the IMOD
software package as previously described [46,49,50]. Briefly, the
tilted views were aligned using the positions of the colloidal gold
particles, and tomograms were calculated using an R-weighted
back projection algorithm. The two tomograms were then aligned
to each other and combined. Finally, dual-axis tomograms from
serial sections were aligned and combined using the methods
described by O’Toole et al. [46]. A total of 7 dual-axis tomograms
were reconstructed to examine the 3-D fine structure of the
bld2-5 basal bodies.

**Indirect Immunofluorescence**

Interphase cells were treated with autolysin to remove cell walls
[51,52] and resuspended in MT buffer [53] and 12% hexylene
glycol (Sigma-Aldrich) for 5 min at RT and dried. Slides were
incubated in methanol prechilled to −20°C for 10 min. Slides
were rehydrated in 1× PBS and incubated in blocking solution
(12.5% BSA, 0.01% cold water fish gelatin (Sigma-Aldrich)) in
1× PBS for 30 min. Slides were transferred to blocking solution
with 10% newborn goat serum (Accurate Chemical, Westbury,
NY) and incubated for 30 min at RT without agitation. Primary
antibodies were diluted in blocking solution and incubated
overnight at 4°C with the following dilutions; anti-acetylated α-
tubulin (1:1000, Sigma-Aldrich), anti-centrin (1:1000; kindly
provided by Dr. Jeff Salisbury, Mayo Clinic), anti-HA (500 ng/
μl; Roche, Indianapolis, In), anti-IFT74 (1:600; kindly provided by
Dr. Carlo Iomini), and anti-γ-tubulin (1:1000; Sigma-Aldrich).
Slides were washed in 10% blocking solution three times with
agitation, 10 min each, at RT and incubated in secondary
antibody for 1 hr at RT. Alexa 594 and Alexa 488 mouse and
rabbit secondary antibody (Invitrogen) were used at 1:1000
dilution in blocking solution. Slides were washed three times with
agitation, 10 min each, at RT and mounted with Vectashield
(Vector Laboratories, Burlingame, CA). Images were collected on
an Axiohot microscope modified with a Lambda DG-4 light
source (Sutter Instrument Company, Novato, CA) equipped with a
Photometrics Cascade 512B camera (Roper Scientific, Tucson,
AZ) and a Physick Instrument piezoelectric stage (Karlsruhe,
Germany). Slidebook Digital Software was used for deconvolution
of the images (Intelligent Imaging Innovations, Denver, CO) on a
Dell dual processor computer (Round Rock, TX). Images were
exported to Adobe Photoshop CS2 (Adobe Systems, Mountain
View, CA).

**Immunoblotting**

Protein extracts from intact cells were prepared from equal
numbers of cells of each strain and mixed with Laemmli sample
buffer (Biorad, Hercules, CA) and 2% 2-mercaptoethanol. The
samples were boiled for 5 min and centrifuged for 1 min to pellet cell debris before loading supernatant onto the gel. Proteins from intact cells were size-fractionated on SDS-PAGE minigels (1.0 mM thick, 10% acrylamide; 29:1 with Bis-acrylamide) and transferred to Immobilon-P membranes (Millipore; Billerica, MA) in 20% methanol at 50 V for 1 hour. The rat anti-HA high-affinity antibody (200 ng/ml) and chicken anti-PbsA (1:10,000, Agrisera; Sweden) were diluted in 5% milk in PBS. Secondary antibodies, donkey anti-rat HRP (1:10,000, Jackson Labs) and rabbit anti-chicken IgY, HRP (1:10,000, Promega) were diluted in 5% milk in PBS. Lumi-Light Western blotting substrate (Roche) was used for detection and exposure to Super RX x-ray film (Fujifilm, Stamford, CT). Using Image J, the expression of katanin p80 was normalized to the PbsA control by measuring the pixels within a constant area and calculating a ratio for each to be compared to pf15; PF15HA.

Table 2. Average number of viable cells after 48 hr exposure to 8 μM Taxol.

| Strain | Rich Medium | Rich Medium+Taxol | p value |
|--------|-------------|-------------------|---------|
| BLD2   | 245 ± 12.2  | 224 ± 27          | 0.16    |
| bld2-5 | 271 ± 20.8  | 263 ± 17.2        | 0.17    |
| bld2-6 | 270 ± 27    | 264 ± 40.6        | 0.85    |
| tub2-1 | 245 ± 32.6  | 243 ± 25.2        | 0.91    |
| pf15-1 | 266 ± 15.2  | 246 ± 18.2        | 0.16    |
| pf15-3 | 232 ± 15.8  | 222 ± 19.7        | 0.67    |

Cells were treated with 0.4% Trypan blue and examined by phase microscopy. 300 cells from three independent samples were counted. For each strain, the number of the viable cells in media (failing to stain with Trypan blue) with and without Taxol was compared by a Student's t-test to establish a p value.

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Figure 5. Mature basal bodies in the bld2-5 strain contain defects in microtubule blade organization. Selected tomographic slices show the progression from the proximal (A) to the distal end (F) of the basal body. (A) Probasal bodies contain a ring of amorphous material at their proximal base. (B–F; arrowheads) Mature basal bodies (BB1) contain amorphous material that extends distally rather than in a proximal ring. (D–F) The assembly of microtubule blades is also incomplete with singlet, doublet and sometimes triplet microtubules present. (C, D; arrows) The cartwheel structure is observed distally. BB1, mature basal body 1; BB2, mature basal body 2; rMT, rootlet microtubules. Scale bar equals 100 nm and Panels A–F are at the same magnification. Schematic representation of the structure of a wild-type basal body and the defects in bld2-5 basal bodies as one moves from the proximal to distal region of the basal body.

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Results

Screening Existing Chlamydomonas Mutants for Taxol Supersensitivity

Missense mutations in pf15 mutant, which encodes the katanin p80 subunit and pf19-1, which encodes the katanin p60 subunit, were originally identified as paralyzed flagellar mutants with a defect in the loss of the p60 katanin subunit in *Tetrahymena* [7], these mutants confer sensitivity to Taxol (Figure 1). Wild-type cells arrest as swollen cells on 18 μM Taxol medium, while pf15-1 and pf19-1 mutant cells become swollen and do not divide on 8 to 18 μM Taxol media (Figure 1C, G). We screened other mutants with defects in axonemal substructures needed for motility (pf2-, pf9-, pf14, pf16, pf17, pf18*, odu2, oda2) [55,57,58] and only the two katanin mutants confer Taxol supersensitivity.

We next examined the role of basal bodies in this phenotype. The bld2-1 [34], uni1-2 [22], bld10-1 [23], bld12-1 [25], vfl1 [30], vfl2-1 [31], and pf2 [29] mutations confer Taxol supersensitivity (Figure 1A, B), while the uni1-2 [26] and the uni2-2 [27] mutants do not confer supersensitivity. Since a majority of these strains have a high proportion of aflagellate cells, we asked if the Taxol phenotype was related to the aflagellate phenotype or the basal body phenotype. Mutants that lack flagella due to defects in IFT phenotype were originally identified as paralyzed flagellar mutants with a synthetic phenotype should be observed using the two null alleles. If there are other proteins that must be recruited, then an additive or of double mutants is more severe than either mutant alone. If there was observed that generates a nonsense codon at amino acid Q59. The gene was sequenced and a C to T change [66].

It is likely that multiple proteins may require intact basal bodies for localization and some of these may influence microtubule dynamics. Thus, the Taxol phenotype of the basal body mutants could arise from a failure to recruit katanin or a failure to recruit katanin and other proteins. This can be tested in double mutants of null mutants of katanin and basal body proteins. Therefore, we sought to identify null alleles of pf15 and bld2. *bld2*; pf15 Double Mutants have an Additive Phenotype

The Taxol phenotype of the *bld2* alleles and the *pf15* strains is similar and both are unable to grow on 8 μM Taxol containing medium. Double mutants were constructed to ask if the phenotype of double mutants is more severe than either mutant alone. If there are other proteins that must be recruited, then an additive or synthetic phenotype should be observed using the two null alleles. Wild-type, *pf15-3*, *bld2-6*, *pf15-3; bld2-6*, *bld2-6*; *pf15-1*, and *bld2-6*; *pf15-3* were grown on 0, 4, 6, 10 μM Taxol containing medium. All strains grow on 4 μM and only the *bld2-6*, *pf15-1* and the *bld2-6*; *pf15-3* double mutant strains fail to grow on 6 μM Taxol containing medium while the single mutant strains form colonies on this concentration (Figure 1K). This additive phenotype suggests that the recruitment of other proteins besides katanin p80 may affect microtubule dynamics. This is not surprising in that over 100 proteins have postulated to reside at the centrosome [66].

Characterization of the *bld2-4* allele

We had previously reported that ε-tubulin is an essential gene based on the *bld2-4* allele [34]. The *bld2-4* allele is an insertional transfectants. The rescued strains have flagella with normal length and motility. The new allele is likely to be a null allele and the phenotype of the new *pf15-3* strain has similar phenotypes to the original *pf15-1* allele, although the presence of central pair microtubules has not been determined in the *pf15-3* mutant.

Isolation of a Taxol Supersensitive *bld2-6* Allele

A collection of 3000 mutant strains made by insertional mutagenesis with the *ble* gene, which confers Zeocin resistance, [40] was screened for the failure to form colonies on 8 μM Taxol medium. Thirty-five strains showed Taxol supersensitivity and were crossed with a wild-type strain (CC-124) to determine if the insertional *ble* marker cosegregates with the Taxol phenotype. Cosegregation of Zeocin resistance and Taxol supersensitivity was observed in only one strain (strain ble12), which suggests that the selection on Zeocin medium was highly mutagenic as this high frequency of a lack of cosegregation is not observed in other insertional collections. In the ble12 strain, the Taxol phenotype (Figure 1A, B) and resistance to Zeocin cosegregates in 370 tetrads.

This strain has a third phenotype; it completely lacks flagella (Table 1) and this phenotype cosegregates as well. The three phenotypes map to linkage group III near the *NT2* locus based on crosses to CC-1952. The ble12 strain fails to complement the *bld2-2* and *bld2-5* mutations (see below) for the Taxol and flagellar phenotypes in 8 independent diploid strains for each allele. It also fails to complement the *bld2-2* mutation for the meiotic phenotype; no viable progeny were recovered from 104 tetrads of this cross. PCR with 21 primer pairs in this region revealed that ble12 carries a 12.6 kb deletion that removes the ε-tubulin gene and the coding region of the *PRMT1* gene, which encodes a protein methyltransferase protein (Table S2). The ble12 mutant allele is named *ble2-6*.

Isolation of *pf15-3* as a Taxol Supersensitive Mutant

Wild-type cells were mutagenized with ultraviolet light and 100 independent, nonmotile strains were isolated and 12 of these confer Taxol supersensitivity. One of these strains had immotile flagella. Three lines of evidence show that it carries a new *pf15* allele. The strain was mapped and is tightly linked to the *PF15* locus in 36 tetrads. The gene was sequenced and a C to T change was observed that generates a nonsense codon at amino acid Q59. The immotile cilia defect and the Taxol supersensitivity is rescued by the *PF15::HA* transgene (described below) in 17 independent
mutation that has a dominant lethal meiotic phenotype. Genetic analysis showed that the bld2-4 allele could only be recovered in a disomic background, which suggested that \( \varepsilon \)-tubulin was an essential gene in Chlamydomonas. The isolation of the bld2-6 brought into question if \( \varepsilon \)-tubulin is an essential gene. To ask if the BLD2 transgene is sufficient to rescue the lethal phenotype [34], we used the disomic bld2-1; bld2-4 in a series of crosses (Figure S2). We found that two copies of the BLD2 gene are necessary to rescue the meiotic lethal phenotype and that the BLD2 transgene fails to rescue the mitotic lethality. To ask if the insertion is associated with a deletion and to determine the extent of the deletion, progeny obtained from a cross with the polymorphic strain, CC-1952, were used. Progeny were scored by the presence of the NIT2 allele from the CC-1952 parent, the absence of the bld2-1 allele, the presence of the BLD2 transgene. The extent of the deletion was estimated by the presence/absence of heterozygosity of physical markers surrounding the BLD2 gene (Table S1). The deletion extends from position 4125635 on chromosome 3 to between 4026909 and 4021900, which removes 28 predicted genes. The dominant meiotic lethality is likely to be due to the loss of multiple genes given that the transgene rescues the meiotic phenotype of bld2-6 (see below). Rescue of the mitotic lethality of bld2-4 is likely to require additional flanking DNA and thus \( \varepsilon \)-tubulin is not an essential gene as reported previously [34].

Isolation and Identification of the bld2-5 Allele

Concurrently, we identified an additional bld2 allele using a noncomplementation screen. A screen of phenotypically wild-type heterozygous BLD2/bld2 diploid strains produced six strains that failed to swim. Non-complementation screens produce several outcomes in addition to new alleles, which include mitotic recombination, chromosome loss and unlinked dominant enhancers [34]. Two of the mutant strains resulted from mitotic recombination or chromosome loss based on the loss of the FokI restriction site (see Material and Methods). The remaining four strains remained heterozygous at the BLD2 locus and were mated to a bld2 strain to determine if the new mutations were new alleles or unlinked dominant enhancers. Two of the mutants produced swimming progeny that suggest an unlinked dominant enhancer. The other two strains did not produce swimming progeny, which suggests new alleles. One of these strains (4-1) was characterized.

The 4-1 mutant strain was backcrossed twice to wild-type cells to remove unlinked mutations and to restore euploidy as judged by greater than 86% meiotic viability. The backcrossed 4-1 strain failed to oppose to gravity and lacked flagella and the aflagellate phenotype was used for mapping. The mutation failed to recombine with the bld2-1 allele in 210 complete tetrads. We analyzed 1571 progeny from the cross of 4-1 to the polymorphic strain, CC-1952 [67] using dCAPs markers (Table S2). The 4-1 mutation maps to a 54.1 kb region that includes the BLD2 gene [68] and gives a value of 102 kb per map unit for this region.

We sequenced \( \varepsilon \)-tubulin from the 4-1 strain and found a T to A transition that changes an isoleucine to an asparagine at amino acid 163 (I_{163}N). Introduction of a wild-type copy of BLD2 into the 4-1 strain through a cross with a bld2-1 strain with an unlinked...
Figure 8. IFT74 localization is aberrant in the bld2 alleles. (A–C) IFT74 and katrin p80 do not colocalize. (D–F) IFT74 (green) localizes to the base of the flagella in wild-type cell as a band as well as to the proximal region of the flagella and partially colocalizes with centrin (red) in the striated fiber at the distal end of the basal body but not along the nuclear basal body connectors at the proximal end (red). (G–I) bld2-5 cells show staining at the base of the flagella, however the localization appears reduced compared to wild-type cells. (J–L) IFT74 localizes throughout the cytoplasm in bld2-6 cells. In about one-half of the cells examined, IFT74 localizes near the aberrant centrin staining at the nucleus. DNA (blue) is stained with DAPI. Scale bar in Panel A equals 5 µm. Panels A–L are at the same magnification.

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BLD2 transgene (BLD2 TG) produced tetrads with two aflagellate progeny and two swimming progeny (n = 57). The F0Δ restriction enzyme digest assay (see Materials and Methods) differentiates between swimming progeny with the BLD2 transgene and the bld2-1 allele (heterozygous) or the 4-1 allele (homozygous). Approximately one-half of the swimming progeny show the homozygous digestion pattern predicted for the 4-1 strain and the remainder shows the heterozygous pattern of wild-type and bld2-1 (n = 6; data not shown). Thus, the transgene rescues the flagellar phenotype of the 4-1 allele to the same extent as it rescues the bld2-1 allele (see Table 1). Unlike the other bld2 alleles, the 4-1 allele does not display a meiotic phenotype in four homoallelic or 16 heteroallelic meiotic crosses of independent meiotic progeny.

The isoleucine is not highly conserved in α-tubulin from a diverse range of organisms (7 of 28), therefore we screened for reversion of the aflagellate phenotype to provide further evidence that this change was responsible for the phenotypes. Cells were mutagenized with ultraviolet light and 25 independent strains that swim were isolated. In crosses of these swimming strains to wild-type cells, the aflagellate phenotype was not recovered in 8 of the 25 strains in at least 10 tetrads; these are likely to be intragenic revertants or tightly linked suppressors. The other 17 strains contain extragenic suppressor mutations. The characterization of these extragenic suppressors will be reported elsewhere. To determine if the mutation in the 4-1 strain is changed in the revertants, a 367 bp fragment containing the I163N mutation was sequenced. Four of the strains convert the asparagine back to isoleucine; these are true revertants. The remaining four strains are pseudorevertants. Two strains (T42 and T16) change the asparagine to serine (N163S) and the other two strains (T33 and T29) convert the serine at position 144 to a glycine (S144G, I163N), but retain the asparagine. The amino acids from asparagine to serine (N163S) and the other two strains (T33 and T29) convert the asparagine back to serine (S144G, I163N) and rootlet microtubules phenotypes of the 4-1 strain have wild-type rootlet microtubules (Figure 3E–J), but lack the centrin localization (Figure 2E–J). The transgene containing the wild-type centrin localization (Figure 2D). It appears that the presence of an extended nucleo-basal body connector does not guarantee flagellar assembly.

In wild-type strains, centrin is a component of the distal striated fiber that connects the distal ends of the basal bodies, the stellate fibers of the transition zone, and extends as fibers from the basal body to the nucleus (the nucleo-basal body connector) [71] (Figure 2A, B). In bld2-1 strains, centrin collapses on or around the nucleus [34,72]. Collapsed centrin occurs in 41% of bld2-5 cells (n = 50, Figure 2C) while the remainder has a wild-type localization pattern (Figure 2D). It appears that the presence of an extended nucleo-basal body connector does not guarantee flagellar assembly.

In wild-type strains, acetylated α-tubulin labeling of rootlet microtubules forms a crisscross pattern [32,73] (Figure 3A, B). The bld2-5 strain, like other bld2 strains, shows disorganized rootlet microtubules in 95% of the cells (Figure 3C, D). The 5% of cells with wild-type rootlet microtubules are likely to have intact microtubule blades at the proximal end of the basal bodies [74]. Proper placement of the cleavage furrow depends on both centrin and rootlet microtubules [75]. Defects in these cytoskeletal structures cause aberrant cleavage furrow placement [76]. The area of newly divided daughter cells was measured to determine if the cleavage furrows were properly placed, since wild-type cells produce daughters with equal sizes [34]. Based on measurements of 100 pairs of cells, the bld2-5 strain produces daughters with significantly different areas (p = 0.001), which indicates a defect in cleavage furrow placement (Figure 4A). The sum of bld2-5 sister cells’ areas is significantly smaller than wild-type (p = 5×10-14) (Figure 4B), which may suggest that the cells divide earlier than in wild-type cells. Both rescued transformants (bld2-5; BLD2 TG) and intragenic revertants (bld2-7 and bld2-9) assemble comparable numbers of flagella as wild-type cells (Table 1). They display wild-type centrin localization (Figure 2E–J). The transgene containing strain has wild-type rootlet microtubules (Figure 3E, J), but approximately 50% of rootlet microtubule bundles (n = 15) in the intragenic revertants appear to be slightly splayed at their ends (Figure 3H).

The bld2-6 allele also shows centrin collapsed on the nucleus (Figure 2K, L) and disorganized rootlet microtubules (Figure 3M, N). bld2-6 cells are 100% aflagellate as vegetative or gametic cells (Table 1). A single copy of the α-tubulin gene rescues the flagellar, Taxol, and meiotic defects as well as centrin localization (n = 20) and the rootlet microtubules phenotypes of the bld2-6 allele (n = 15).

Phenotypic Analysis of the bld2-5 and Pseudorevertant Strains

The bld2-5 strain exhibits a less severe flagellar assembly defect than observed in previously described bld2 alleles. Ninety-five percent of the bld2-5 cells are aflagellate, but 4% of the cells have one flagellum and 1% has two flagella in logarithmically grown cells (n = 200) (Table 1). Surprisingly, when bld2-5 cells are deprived of nitrogen and arrested in G1 of the cell cycle as gametic cells, 20.5% of the cells assemble at least one flagellum. Changing the cell cycle time by growing cells at 14°C or 32°C does not change the number of flagellated cells (Table 1), which suggests the bld2-5 allele is not temperature-sensitive, but may be modulated by nutritional cues. This is the only bld2 allele that shows this phenotype.

In wild-type strains, centrin is a component of the distal striated fiber that connects the distal ends of the basal bodies, the stellate fibers of the transition zone, and extends as fibers from the basal body to the nucleus (the nucleo-basal body connector) [71] (Figure 2A, B). In bld2-1 strains, centrin collapses on or around the nucleus [34,72]. Collapsed centrin occurs in 41% of bld2-5 cells (n = 50, Figure 2C) while the remainder has a wild-type localization pattern (Figure 2D). It appears that the presence of an extended nucleo-basal body connector does not guarantee flagellar assembly.

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bld2-5 Cells have Staggered Microtubule Blades Lengths in Mature Basal Bodies

Each mature basal body is associated with a probasal body that will elongate during the next mitotic cycle [77,78]. In wild-type
cells, the probasal bodies are roughly 80 nm in length and consist of a proximal ring of amorphous material, a nine-spoked cartwheel and nine triplet microtubule blades [79]. In bld2-5 cells, the proximal ends of the probasal bodies maintain a ring of amorphous material and a cartwheel, however microtubule blades may be incomplete (Figure 5; Movie S1). Unlike the probasal bodies, the amorphous material in the mature bld2-5 basal body is not present as a thin ring rather it can extend to over 200 nm, which is variable from cell to cell. Most tomographic reconstructions have both amorphous material and microtubule blades (Figure 5A–E; arrowheads; Figure S3; arrowheads). The assembly of microtubule blades is also incomplete with singlet, doublet and sometimes triplet microtubules present as one moves from the proximal base of the basal body to the distal end (Figure 5; right; Figure S3). Incomplete basal bodies were also observed with only 7 or 8 blades are present at the distal tip (Figure S3A). In some cells the cartwheel structure assemblies further from the proximal base than in wild-type basal bodies (Figure 5 C, D; arrow; Figure S3C; arrow). Basal bodies competent to template flagella assemble ectopic transition zone material in the basal body proper (Figure S3B, D; arrow), which resembles the ectopic transition zone present in the uni3-1 strain [47]. Probasal bodies in the bld2-5 strain assemble with minor defects; however, as the basal body matures, it loses its integrity, which indicates that Bld2p is needed to maintain basal body structure. The collapsed centrin fibers and aberrant rootlet microtubules observed by immunofluorescence are confirmed by the tomography in which the fibers are misplaced in many of the cells.

Taxol Supersensitive Strains Recover from Taxol Treatment

Mutant and wild-type cells were treated with 8 μM Taxol for 48 hours, washed twice in rich medium and stained with Trypan blue to determine the number of dead cells in each replicate sample (n = 3). There was no significant difference between the average number of live cells in treated and untreated cultures of bld2-5, bld2-6, tub2-1, pf15-1, pf15-3, and pf15 strains (Table 2). These data suggest that the Taxol supersensitivity of these mutants does not arise from a basal body integrity checkpoint but rather from an arrest.

Katanin is Mislocalized in the bld2 Mutants

Katanin influences microtubule dynamics by severing microtubules. The PF15 gene encodes p80 katanin [56] and pf15 strains show Taxol supersensitivity (Figure 1A, B). We constructed a PF15-HA epitope tagged vector that places the HA tag at the terminal amino acid, which was changed from a leucine to an asparagine, transformed the plasmid into the PF15 strain, and screened for rescue of the paralyzed flagellar phenotype. Twenty-one independent swimming strains were isolated and each transgenic strain transformed the plasmid into the PF15 strain [20]. We examined localization of another component of the IFT B complex, IFT74. As shown previously [61], IFT74 localizes to the flagellar base as a punctate dot and in the proximal region of the flagella in wild-type cells (Figure 8D–F). IFT74 and katanin p80 do not colocalize in wild-type cells (Figure 8A–C). This result supports the uni1-2 result that katanin p80 is not present on the transition fibers.

Like the katanin p80 localization, the IFT74 pattern in bld2-5 is more diffuse than in wild-type cells (n = 40, Figure 8G–I). In bld2-6 cells, there is increased staining throughout the cytoplasm of all cells with dots that are not at the basal body region (n = 40) (Figure 8J–L). Sixteen cells show IFT74 localization near the basal bodies. The increased cytoplasmic staining in bld2-6 cells may reflect an increased level of IFT74 in the cytoplasm that would normally be present in the flagella.

Discussion

Loss of Basal Body Integrity Perturbs Katanin Localization

The pf15 and pf19 alleles are unique among the flagellar motility mutants tested in Chlamydomonas; they confer supersensitivity to Taxol (Figure 1). The mutants with basal body integrity defects (bld2, bld10, bld12, uni3) or basal body fiber defects (pf11, pf12, pf13) also confer Taxol supersensitivity and suggest a simple hypothesis that we tested. These organelles help to recruit proteins for spindle assembly and function. Specifically, we showed that a loss of basal body integrity results in a failure to recruit and localize katanin and this failure mimics the loss of function phenotype of the pf15 and pf19 strains. Acetylation, a post-translational modification of α-tubulin, is associated with more stable microtubules [81], and the Taxol supersensitivity phenotype of a β-tubulin mutant [2]

Katanin Localization Requires Triplet Microtubules, but not the Transition Zone

To further determine which structures in the basal body apparatus are needed for katanin p80 localization, we examined p80 localization in uni1-2 cells, which lack a transition zone on the daughter basal body [20] in uni3-1 cells, which assemble doublet but not triplet microtubules [22,80]; and in bld10 cells, which lack any microtubule blades [23]. Katanin p80 localization in uni1-2 cells appears as two dots (n = 6), which suggests it does not require the transition zone for proper localization (Figure 6Q, R). In bld10-1 cells, there is no localization of katanin p80 (n = 30), which further supports the need for the basal body for localization of katanin. Localization of katanin p80 in the uni3 cells shows a variety of phenotypes that include the complete absence (40/50), diffuse staining (5/50) or increased signal (5/50) (Figure 6S–V). Because uni3-1 cells lack triplet microtubule blades except at the distal tip of the basal body [22], it seems likely that triplet microtubules are important for katanin p80 localization but are not sufficient.

IFT52 localizes to transition fibers and localizes aberrantly in the bld2-1 allele [20], we examined localization of another component of the IFT B complex, IFT74. As shown previously [61], IFT74 localizes to the flagellar base as a punctate dot and in the proximal region of the flagella in wild-type cells (Figure 8D–F). IFT74 and katanin p80 do not colocalize in wild-type cells (Figure 8A–C). This result supports the uni1-2 result that katanin p80 is not present on the transition fibers.

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or in the katanin mutants in Tetrahymena [7] is associated with increased levels of acetylated α-tubulin. The bld2 alleles do not have increased acetylation of interphase microtubules (Figure 3).

We identified viable null alleles in the PF13 and BLD2 genes, which suggest that these two genes are not essential in Chlamydomonas. The bld2-6; gf15 double mutant confers a more severe defect than in either single mutant on Taxol medium. This more severe phenotype indicates that recruitment of additional proteins at basal bodies affects microtubule dynamics. None of the mutants block spindle function in mitosis in the absence of Taxol. Given that the bld2-1 and bld2-6 alleles have a recessive meiotic defect, there may be a stronger requirement for recruitment of proteins to the meiotic spindle than to the mitotic spindle in Chlamydomonas. Although mutants with basal body integrity defects can still build spindles and progress through the cell cycle, microtubules in these cells may be inherently unstable as judged by Taxol sensitivity.

Electron tomography of the bld2-5 allele suggests that α-tubulin is necessary for basal body assembly/elongation. This staggered phenotype of bld2-5 is also observed in the bld2-1; gnl-1 strain, where gnl-1 is a partial suppressor of the bld2-1 allele [34], and it is similar to the knockdown phenotype observed in Paramecium [82]. Since the probasal body structure is not affected to a large degree, this allele suggests that this mutant α-tubulin is able to participate in the initiation events needed to build the probasal body, but not in the elongation of the probasal body to the daughter basal body. Based on tomographic reconstruction of duplicating basal bodies, these two events are separated in time. The existing probasal body elongates to become the daughter basal body at prophase and the new probasal body forms in metaphase (O’Toole and Dutcher, in preparation).

Rasi and colleagues reported that katanin p60 is an essential gene, is required for release of the basal bodies from the flagella using an RNA interference strategy to the p60 gene, and that katanin localizes to the basal bodies [83]. However, Dymek and Smith suggest that katanin p60 is encoded by the PF19 gene [84]. Based on two pf19 alleles, this gene does not play a role in release of flagella, and the anti-p60 serum reported by Rasi does not recognize the tagged p60-GFP protein in whole cell extracts [84], which makes our tagged gene the only means to examine localization of the katanin heterodimer. Our phenotypes for pf15 and pf19 are similar and suggest that katanin p60 and p80 behave similarly in Chlamydomonas as in other organisms.

Localization of Katanin Requires Intact Basal Bodies

The transition fibers are required for IFT localization, and subdistal appendages are required for PCM localization in animal cells, several of our observations were unexpected. In uni1-2 cells, which lack the transition zone and transition fibers on the younger of the two basal bodies, two dots of katanin are observed rather than one. Additionally, IFT74 and katanin p80 do not colocalize. Thus, we suggest that the transition fibers are not required for katanin p80 localization and that the requirements at the basal bodies for IFT and katanin p80 recruitment and localization are different.

We suggest that the recruitment requires triplet microtubules as the uni3 mutant shows defects in p80 localization but has normal transition fibers. The uni3 mutant fails to assemble triplet microtubules, but 25% of the cells assemble two flagella, 25% assemble one flagellum, and 50% have no flagella. We have hypothesized that the age of the basal bodies influences this distribution [22,47]. As the basal bodies age, they may acquire additional proteins or post-translational modifications that allows for recruitment. Thus, the katanin localization phenotype is intriguing in that there are three localization phenotypes in uni3 mutants. These may correspond to the age of the older basal body. The triplet microtubules are known to be important for the localization of centrin and rosette microtubules via specific microtubule blades [47]. We cannot rule out the model that the triplet microtubules do not directly recruit p80, but may play an indirect role via intermediary proteins. Basal bodies in C. elegans and Drosophila lack triplet microtubules, but still recruit PCM. Like Chlamydomonas, basal body mutants in these organisms also fail to recruit PCM and suggest the mechanism of PCM recruitment may depend on the basal body structure of an organism.

Other Proteins that Moderate Taxol Sensitivity

Besides basal body defects, work in other systems has shown multiple modes to confer Taxol sensitivity. Interestingly, two smoothened antagonists sensitize cells to Taxol in ovarian cancer cell lines [85]. It is interesting to consider that localization of the hedgehog pathway in the cilia could modulate recruitment of other proteins to the basal bodies. Increased expression of Nek4, a NIMA-like kinase is associated with Taxol sensitivity [86]. Its targets remain unknown. Finally, changes in expression of Septin10 and Bub3 alter Taxol sensitivity. Increased Sept10 expression is associated with sensitivity while decreased expression is associated with resistance to Taxol [87,88]. Modifiers that confer Taxol sensitivity have been identified in HapMap lymphoblastoid cell lines; the predominant class of genes with variants encodes solute carriers (SLC) [89]. Resistance to Taxol has been observed in a large number of clinical samples following treatment with this drug. Mutations in over 20 amino acids in β-tubulin have been observed in Taxol resistant cell lines [90]. There may be many targets and mechanisms by which Taxol sensitivity is modulated. Further screens for the Taxol supersensitivity phenotype may provide a new class of variants that will be useful for cancer therapeutics.

Supporting Information

Figure S1 Schematic drawing of the katanin p80 epitope-tagged transgene. The last amino acid and the stop codon were mutated to a NotI restriction site by knitting PCR. The 3X hemagglutinin (HA) tag was introduced into the engineered site. (TIF)

Figure S2 Rescue of the meiotic phenotype requires two wild-type copies of the BLD2 gene and the BLD2 transgene does not rescue the mitotic lethality. Cross I is a repeat of the results obtained previously [34] showing that the disomic strain (red and black chromosomes) produces no viable progeny when crossed by wild-type strain (CC-124, blue) (n = 120 tetrads). Cross II involves a wild-type CC-1952 parent (green chromosome) carrying the BLD2 transgene (purple) that is unlinked to the BLD2 locus. The presence of the transgene is sufficient to rescue the meiotic phenotype (75% of the progeny from 40 tetrads survive). This result is reinforced by results in Cross III that uses progeny from Cross II that lack the bld2-1 chromosome, which were eliminated from consideration using PCR and digestion with FokI (Materials and Methods). This leaves six possible genotypes. No allagulate progeny were recovered, which strongly suggests that the bld2-4 allele is lethal (indicated by 0 viable progeny for strains iii and iiil). dCAPS markers described in Tables S1 and S2 were used to determined which strains carried CC-1952, bld2-4 chromosomes, and the BLD2 transgene. Ten strains were used for Cross III. Three of them had the CC-1952 chromosome with the transgene gave greater than
92% viable progeny in 25 tetrads. Two of them had the CC-1952 chromosome and no transgene and gave 89% viability in 25 tetrads. Three progeny had the bld2-4 chromosome, the CC-1952 chromosome, and the transgene and gave 60% viability in 125 tetrads, but no allaggellate progeny were recovered, which suggests that the bld2-4 allele is lethal. Two progeny had the bld2-4 chromosome and the CC-1952 chromosome and gave 24% viability in 100 tetrads. Again no allaggellate progeny were recovered.

Figure S3 Serial thin sections through bld2-5 basal bodies show structural variation. A–C. Serial, 80 nm sections of bld2-5 basal bodies shown in cross section from three different cells (proximal-distal, left-right). The proximal basal body contains dark, amorphous material surrounding partial microtubule bladles (A, arrowheads). The assembly of blades can be incomplete with singlet, doublet and triplet blades as one moves from the proximal to the distal tip. (C) The cartwheel is abnormally assembled in the middle of the basal body rather than the proximal base (arrow). (B, D) Some cells show ectopie transition zone material assembled in the basal body proper, shown in cross section (B, arrow) and longitudinal view (D, arrow). Scale bar equals 200 nm.

Table S1 Primers used for mapping bld2-4 to a 100 kb region and bld2-5 to a 54.1 kb region of Chlamydomonas reinhardtii (JGI version 5.3).

Table S2 Primers used to delineate the deletion in the bld2-6 strain.

Movie S1 A movie of serial, tomographic slices through the complete volume of bld2-5 basal bodies.

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Author Contributions
Conceived and designed the experiments: JME ETO AJA SKD. Performed the experiments: JME ETO LL THG BK AJA SKD. Analyzed the data: JME EOT LL THG BK AJA SKD. Contributed reagents/materials/analysis tools: JME EOT LL THG BK AJA SKD. Wrote the paper: JME EOT AJA SKD.

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