BALD-2: A MUTATION AFFECTING THE FORMATION OF DOUBLET AND TRIPLET SETS OF MICROTUBULES IN *CHLAMYDOMONAS REINHARDTII*

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ABSTRACT

The mutant strain *bald-2* is unique among "flagellaless" strains of *Chlamydomonas reinhardtii* isolated to date, in that it possesses a mutant basal body: it is only capable of forming a ring of nine singlet microtubules, 180 nm in diameter, instead of the usual triplet basal body which is 225 nm in diameter. This singlet basal body lacks structural stability and the ability to associate with striated fiber material but retains two critical properties of basal bodies, namely, information specifying the length to which it should elongate and the ability to induce, albeit rarely, a flagellar transition region, a short, singlet-containing axoneme, and a specialized tunnel in the cell wall through which flagella normally emerge. The mutation seems to be specific for B- and C-microtubule synthesis or assembly since all other cytoplasmic sets of microtubules appear normal in numbers, orientation, and stability.

During the past 10 years, mutant strains of the biflagellate alga *Chlamydomonas reinhardtii* have contributed significantly to an understanding of the morphogenesis and function of the flagellum. At least 18 gene loci have been shown to influence normal flagellar length and motility (22, 23, 39), and certain "paralyzed" flagella have been found to lack the central pair of microtubules (28, 40). Such mutant strains have been subjected to genetic analysis (22, 33, 34) and have been the basis of studies on the mechanism of flagellar beating in vitro (1). More recently, conditional mutant strains have been isolated whose axonemes depolymerize at elevated temperature, and such strains have been mapped to several gene loci (17). None of these strains, however, exhibit structural defects in their triplet-containing basal bodies, which has led at least one group of investigators to speculate that any mutation affecting these microtubules might be lethal (40).

The present paper reports the structural properties of *bald-2*, a mutant strain of *C. reinhardtii* that is capable of normal rates of growth and cell division, forms a ring of nine singlet microtubules identical to an early stage in basal body development (3, 5, 6, 18, 19), but is unable to form triplet-containing basal bodies or doublet-containing axonemes. To our knowledge, this represents the first case that a mutation affecting doublet and triplet sets of microtubules has been described.

MATERIALS AND METHODS

Wild-type (strain 137c) and *bald-2* cells, both mating-type *plus*(mt^+), were grown in a minimal salts medium (37) in a 12-h light and 12-h dark synchronous cycle as previously described.[1] The *bald-2* strain was found to attain good synchrony under these conditions. In order to sample basal body morphology at various stages of the

[1] Hinckley, N. M., and U. W. Goodenough. 1975. Gametic differentiation in *Chlamydomonas reinhardi*. I. Production of gametes and their fine structure. Manuscript in preparation.

cell cycle and life cycle, cells were harvested for electron microscopy at 5 h into the light period of the synchronous cycle (when mitosis is not occurring), at 2 h into the dark period (when mitosis is occurring), and after being transferred to nitrogen-free medium and allowed to differentiate into gametes.[1] These three samples are referred to in the text as interphase, mitotic, and gametic.

Cells were fixed for electron microscopy by procedures described elsewhere (14, and footnote 1), and examined in a Hitachi HU-11C microscope. All micrographs have been printed at the same magnification.

RESULTS

Wild-Type Basal Apparatus

The fine structure of the wild-type *C. reinhardtii* basal apparatus has been described in detail by Ringo (31), Johnson[2] and Porter (18), and Cavalier-Smith (5, 29) and it is reviewed here only to provide the reader with a description of the normal cell and a set of micrographs with which to compare the phenotype of the mutant *bald-2* strain.

Fig. 1 shows many of the elements of the wild-type basal apparatus in a nondividing vegetative cell. Two mature basal bodies (labeled 1 and 2) lie at oblique angles to one another at the cell anterior. Four bands of "cortical" microtubules (*cmt*) originate in the vicinity of these mature basal bodies and radiate out from the cell anterior to form a basket-like array beneath the cell membrane (31). Within the two "V's" formed by the cortical microtubule bands lie two immature basal bodies (Fig. 1, labeled 3 and 4) that exist as short structures throughout most of the cell cycle (15) and elongate just before mitosis (18).

Fig. 2 depicts a more distal section of the wild-type basal apparatus. The proximal striated fiber connecting mature basal bodies (31) is shown in frontal section. Also visible is the banded flagellar collar which surrounds the flagellar tunnel (*T*) within the cell wall. The collar extends down as far as the transition region (*tr*) between flagellum and basal body, where it appears to make contact with the flagellar membrane. Within the tunnel lies the flagellum proper; several sets of outer doublets and the central pair are evident, as is the flagellar membrane with its fuzzy carbohydrate coat (34, and footnote 3). In wild-type interphase cells, the flagellum attains a length of 8–9 μm (29).

C. reinhardtii cells normally undergo two successive rounds of mitosis so that four daughter cells come to lie within the original mother wall. In such postmitotic daughter cells, immature basal bodies are occasionally found free in the cytoplasm, exhibiting no apparent association with any mature basal bodies. Various stages of assembly of these structures have been observed, starting with a ring of nine singlet (A) microtubules, 180 nm in diameter (18). Doublet (B) and triplet (C) microtubules are then added to this singlet ring as described in the legend to Fig. 1 (5, 18).

Fig. 3 depicts a young flagellum in a postmitotic wild-type cell. Evident are the two lateral V-shaped wedges that mark the transition region (*tr*) between the basal body and the axoneme proper. The length of the young flagellum, measured from the base of its transition region, is 0.75 μm. It has not yet acquired a flagellar collar, and the flagellar membrane is seen to bear a fuzzy coat. A thin layer of amorphous material (believed to be new wall) overlies the cell surface. Fig. 3 also includes a proximal striated fiber (*psf*). Two such fibers associate with the ends of the basal bodies at the level of the proximal cartwheel (31).

General Properties of the Bald-2 Mutant Strain

The *bald-2* mutant strain was obtained by ultraviolet irradiation of mt^+ cells and was selected on the basis of its inability to undergo flagellar agglutination with gametic mt^- cells (13, and footnote 4). The mutant cells are immotile as gametes as well as at all stages of the vegetative cell cycle, and they exhibit no flagella with the light microscope (hence the designation *bald*). Nonetheless, they grow at wild-type rates on agar plates and in a liquid minimal salts medium, have normal levels of chlorophyll and, except for their basal and flagellar apparatus, have normal cytoplasmic fine structure.

In liquid culture and on newly inoculated agar plates, *bald-2* cells are slow to release from the mother wall after mitosis, and clumps of 8, 16, 32, and 64 cells are common. This phenotype is, however, a property of most "flagellaless" strains of *C. reinhardtii*, including strains with normal basal bodies but defective flagellar elongation (23, B. Huang, personal communication; and our observations on six other *bald* strains). It therefore

[2] Previous surname of U. W. Goodenough.

[3] Bergman, K., U. W. Goodenough, D. A. Goodenough, J. Jawitz, and H. Martin. 1975. Gametic differentiation in *Chlamydomonas reinhardi*. II. Flagellar membranes and the agglutination reaction. Manuscript in preparation.

[4] U. W. Goodenough. Manuscript in preparation.

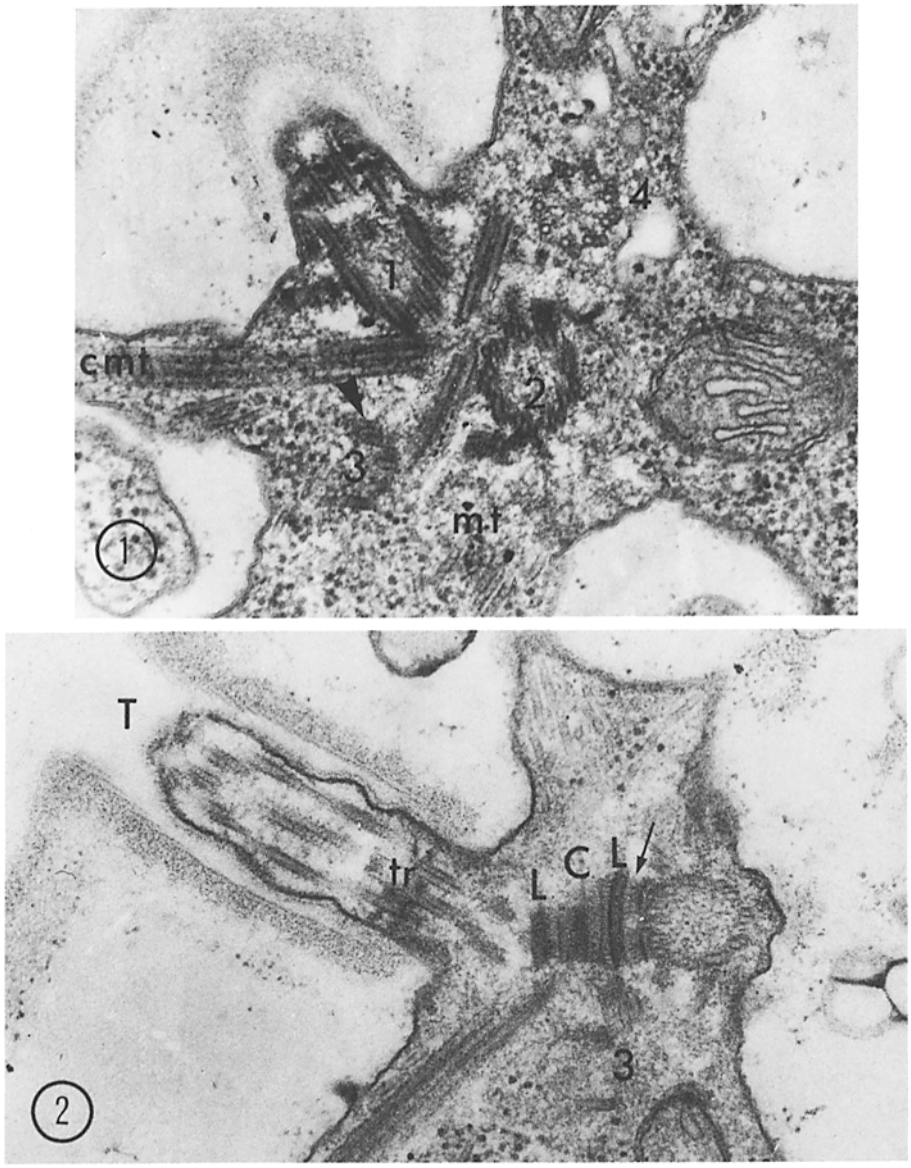


FIGURE 1 Wild-type basal apparatus in an interphase vegetative cell. Mature basal bodies (1 and 2), developing basal bodies (3 and 4), and three bands of cortical microtubules (*cmt*) are present. Probasal body 3 measures 90 nm in length and contains triplet microtubules (arrowhead). Probasal body 4 exhibits the cartwheel structure associated with the proximal end of basal bodies (12). Of the nine positions in the ring, four contain triplet sets of microtubules, two carry only the innermost (A) microtubule, two appear as A singlets associated with indistinct triplet material, and one is ill-defined. Linker fibers connect A with adjacent C (outermost) microtubules. Other microtubules (*mt*), lighter staining than the cortical microtubules, also occupy the region. × 60,000.

FIGURE 2 Wild-type basal apparatus in an interphase vegetative cell. The proximal striated fiber consists of a central element (*C*), 26 nm wide, and two lateral elements (*L*), each 33 nm wide. Thin striations lie between the central and each lateral element, and an additional thin striation lies close to the one basal body present in the section. Slender horizontal filaments (arrow) connect the vertical elements and attach to two of the basal body triplets. A longitudinal section of a short developing probasal body, 180 nm in diameter, is indicated at 3, and a band of cortical microtubules is evident. The flagellum and its associated transition region (*tr*) lie within the flagellar tunnel (*T*). The flagellar membrane exhibits a fuzzy surface coat. × 60,000.

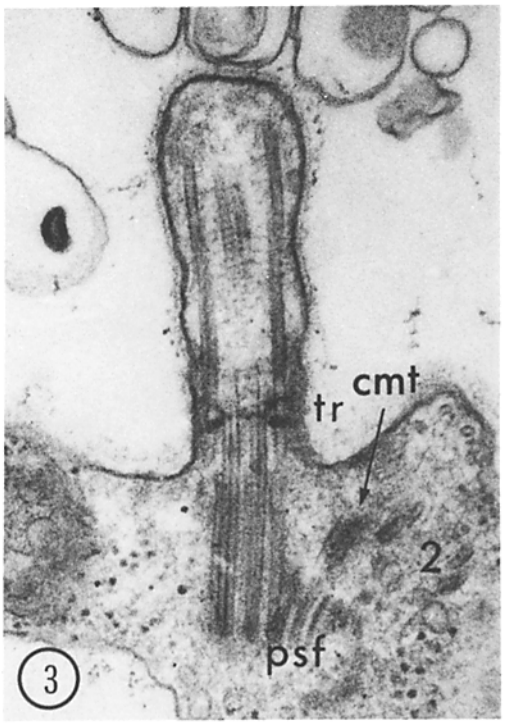


FIGURE 3 Wild-type postmitotic daughter cell showing a forming flagellum, its transition region (*tr*), its basal body, and associated cortical microtubules (*cmt*). A second basal body is apparent at 2. A proximal striated fiber (*psf*) is associated with the basal body; it lacks the consistent periodicity and horizontal filaments of the distal fiber (Fig. 2) and attaches to the basal body via wide rather than narrow striated elements. Arrow points to a crescent of material that may be adding to the proximal fiber. Vesicles above the flagellar tip may represent the addition of membrane to the growing flagellum (30). × 60,000.

seems unlikely that this phenotype is a direct consequence of the *bald-2* mutation.

Structure of the Singlet Basal Body Formed by Bald-2 Cells

The only type of basal body formed by *bald-2* cells is a ring of nine singlet microtubules, 180 nm in diameter. Figs. 4 and 5 show two such rings in cross section taken from various stages in the cell cycle. In all, 15 singlet basal bodies have been observed in *bald-2* cells and none exhibit triplets; since triplets are common and singlets very rare in sections of wild-type cells, we feel confident that we would have encountered triplets if they had been present in *bald-2* cells.

The *bald-2* singlet rings resemble the immature singlet rings of wild-type cells (5, 18) in several respects. First, their component A microtubules are less densely staining than those of mature triplets. Second, their proximal hub-and-spoke cartwheel material is less distinct than in mature basal bodies. Finally, they possess "linker" fibers connecting adjacent A microtubules (Figs. 4 and 5; and Fig. 30 of reference 5). Whether these relate to the A-C linkers found in mature basal bodies (12; see Fig. 1 of this paper) is not known.

Number of Basal Bodies per Bald-2 Cell

Except in one moribund cell with a number of aberrant features, only one basal body has been observed per section of a *bald-2* cell. This stands in marked contrast to wild-type cells, where two (or more) basal bodies per section are common (Figs. 1–3) and one per section relatively uncommon (except in postmitotic daughter cells).

Length of the Bald-2 Singlet Basal Body

Several singlet basal bodies encountered in *bald-2* cells have permitted estimates of the length of the organelle. A set of four consecutive serial sections included the singlet basal body shown in Fig. 5 in cross section, giving a minimum length of 200 nm (assuming a 50-nm section thickness). Moreover, two oblique sections of singlet structures have been obtained (Figs. 6 and 7) wherein the longest microtubules measure 230 and 270 nm, respectively. Thus, in three cases, the singlet basal body of *bald-2* cells is found to be at least half the length of a mature basal body (375 nm). In all three cases, the singlet structures were found to lie at the cell anterior but were not associated with the surface membrane.

In contrast, one example has been found (Fig. 8) where a *bald-2* singlet basal body, measuring 180 nm in diameter, has oriented perpendicular to the cell surface of a postmitotic daughter cell, elongated to the mature length of 375 nm, and attempted the induction of a transition region and axoneme. Both the transition region and the axoneme are aberrant in structure, as will be discussed below, but it is clear from Fig. 8 that the *bald-2* mutation does not prevent singlet basal body elongation at the appropriate (postmitotic) point in the *C. reinhardtii* cell cycle.

Flagellar Stubs Induced by Bald-2 Cells

Figs. 8–11 illustrate the four examples of flagellar "stubs" we have encountered after examining

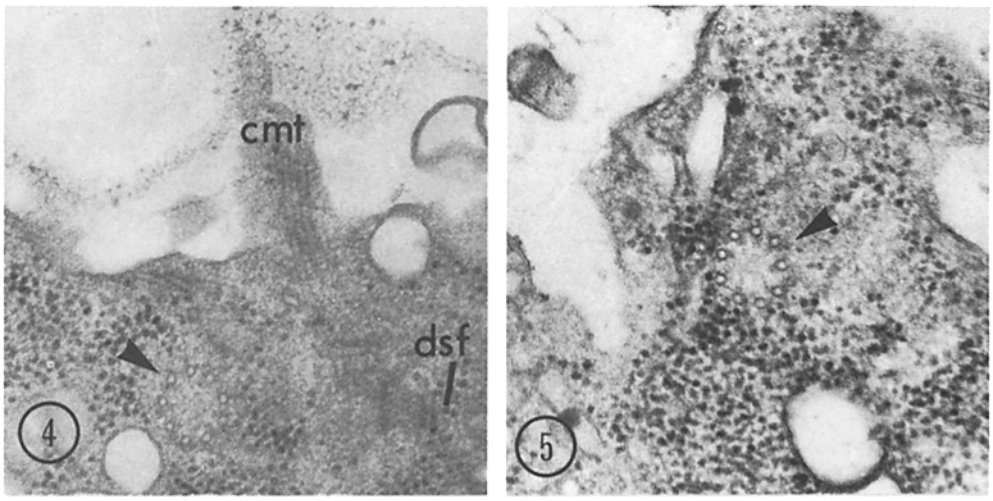


FIGURE 4 Interphase *bald-2* vegetative cell showing a singlet basal body (arrowhead). Faint A-A linker fibers are present between two pairs of microtubules. Also present is a portion of a distal striated fiber (*dsf*) being approached by a band of cortical microtubules (*cmt*). × 60,000.

FIGURE 5 Mitotic *bald-2* cell containing a singlet basal body (arrowhead). Three adjacent serial sections also carried this basal body; the final section contained a crescent of three singlets and thus represented the distal tip. A few radial spokes are present within the basal body section shown here, indicating that the proximal end has been sectioned. Thus the entire basal body probably occupied five to six sections. A distinct A-A linker connects one pair of microtubules. × 60,000.

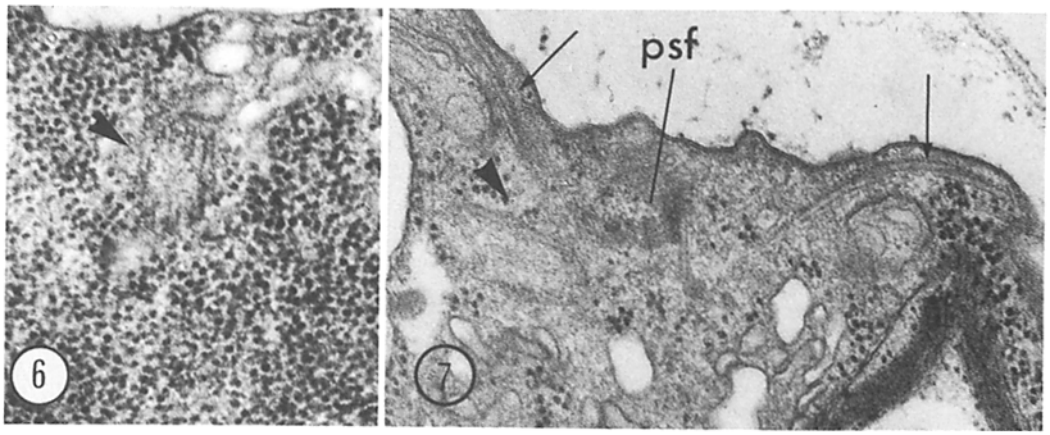


FIGURE 6 Mitotic *bald-2* cell showing an oblique section of a singlet basal body (arrowhead). The structure is 180 nm in diameter, and the longest microtubule measures 230 nm. × 60,000.

FIGURE 7 Gametic *bald-2* cell containing several elements of the basal apparatus. A singlet-containing basal body (arrowhead), 180 nm wide and 270 nm long, assumes an aberrant orientation with respect to the cell surface. Two bands of cortical microtubules (arrows) approach a proximal striated fiber (*psf*); the band on the left appears to make contact with the left edge of the fiber. The right edge of the fiber is associated with a lone microtubule. × 60,000.

thousands of *bald-2* cells in thin section. Our interpretation of these images is as follows. It appears that, at least rarely, the singlet basal body of postmitotic *bald-2* cells can elongate to a mature length and initiate flagellar formation. The resultant flagellar stubs exhibit a spectrum of deviant morphology. The "new" stub shown in Fig. 8 contains a disordered transition region,

short segments of single microtubules, and an accumulation of amorphous material that presumably represents unpolymerized tubulin. The "old" stubs found in vegetative cells fixed many hours after mitosis (Figs. 9–11) contain more or less disordered transition regions and singlet microtubules.

The most striking difference between "old" and "new" stubs is the absence of basal bodies beneath the "old" stubs. In Figs. 10 and 11 the basal body region is occupied by ribosome-containing cytoplasm (compare with Fig. 2, where one basal body is out of the plane of section but its "region" excludes ribosomes). In Fig. 9 a basal body-sized region of amorphous material lies beneath the stub; the presence of a proximal striated fiber (*psf*) at the proximal end of this ribosome-free area strongly suggests that a basal body once occupied the region and subsequently depolymerized.

Taken together, Figs. 9–11 indicate that on those apparently rare occasions when the *bald-2* singlet basal body elongates and attempts flagellar induction, the elongated structure is unstable and cannot be detected *in situ* after several hours. The figures also indicate that in the absence of a triplet basal body, a reasonably normal transition zone may develop (Fig. 10) and microtubules may enter a flagellar protruberance, but the flagellum cannot form doublet microtubules or elongate normally.

A comparison of Figs. 3 and 8 is particularly interesting. Comparable stages of flagellar development in wild-type and *bald-2* cells are depicted: in both cases, cell wall material is being laid down, a flagellar collar has not yet formed, and the basal bodies are associated with proximal striated fibers and cortical microtubules. Only the wild-type cell contains two basal bodies, however, and the *bald-2* basal body and axoneme are both clearly mutant in structure.

The three "old" *bald-2* flagellar stubs shown in Figs. 9–11 are all associated with flagellar collar material. This begins correctly at the level of the transition region and is seen in Fig. 9 to attain the normal length of about 0.9 μm. However, the collar material then grows over the top of the tunnel, presumably because the latter is unoccupied by a flagellar shaft which would normally prohibit such an overgrowth.

It is of interest to note that the membranes surrounding the *bald-2* flagellar stubs (Figs. 8–11) are completely devoid of the fuzzy carbohydrate coat that extends out from wild-type flagellar membranes (Figs. 2 and 3).

The Striated Fibers of Bald-2 Cells

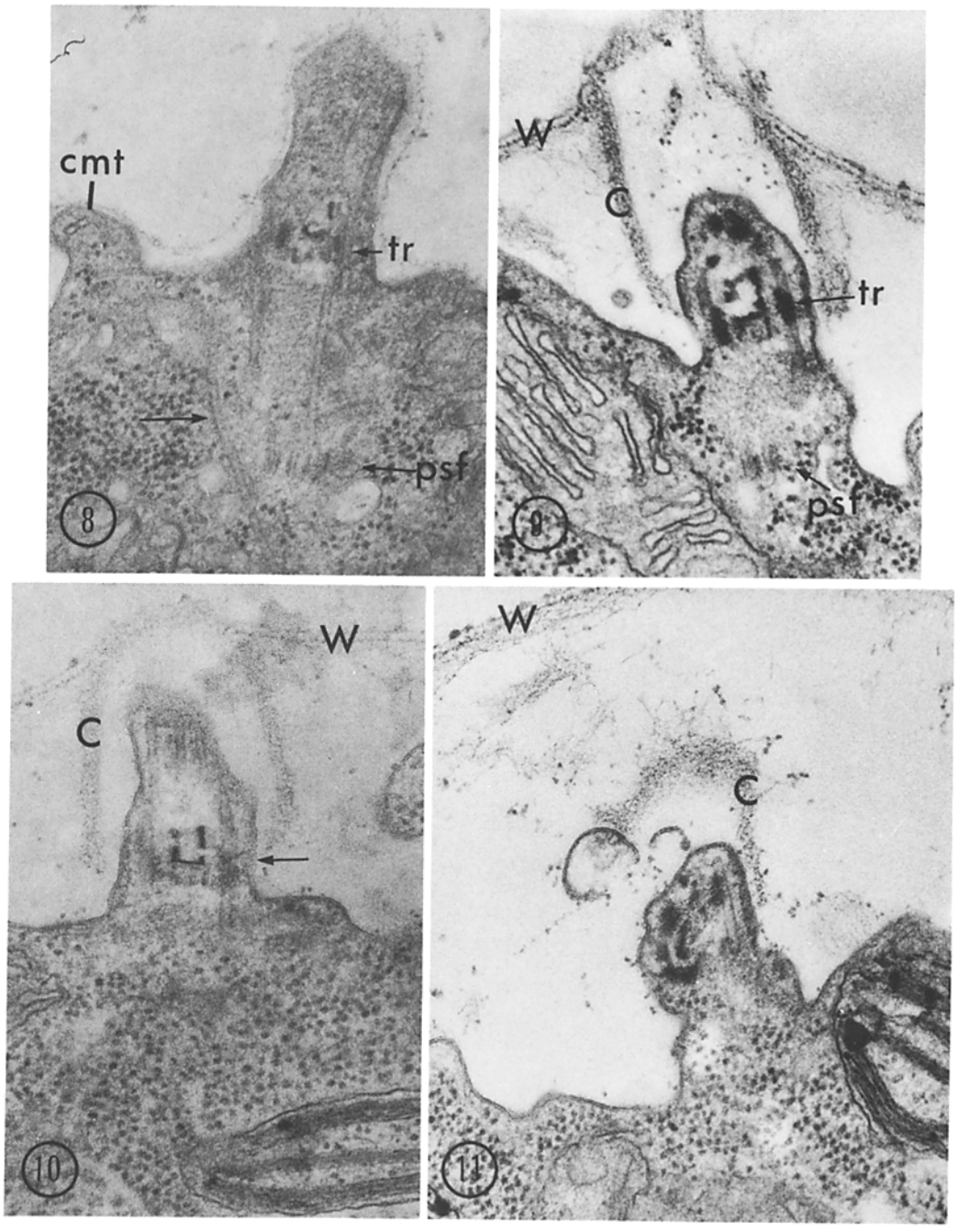
Striated fibers have been encountered frequently in *bald-2* cells. In most cases it has been possible to identify these as proximal (Figs. 7–9 and 12) or distal (Figs. 4 and 13) on the basis of their position and/or resemblance to wild-type structures. In several cases these fibers have been found in the vicinity of *bald-2* singlet basal bodies (Figs. 4, 7, and 8) but in no case have they been found in contact with the singlets: in Figs 8 and 9 the proximal fibers lie *beneath* the basal body regions, and in Fig. 4 the distal fiber is separated from the singlet by ribosome-containing cytoplasm. The probable proximal fiber in Fig. 7 makes contact with a microtubule, but this is not a member of any identifiable basal body. Many sections of striated fibers fail to include basal bodies (Figs. 12 and 13), and, in two cases, proximal striated fibers were found by consecutive serial sections to be devoid of basal body associations at either end.

The two examples of distal striated fibers that we have encountered (Figs. 4 and 13) have the same periodicity as, but are lighter staining than, their wild-type counterpart (Fig. 2) and exhibit aberrant positions with respect to the cell surface. Proximal fibers have been found more frequently and are more normal in appearance, but these too may be oriented incorrectly (Fig. 12).

The striated fibers of *bald-2* cells congregate in the same region of the cell: for example, two proximal striated fibers lie in the five serial sections adjacent to the distal striated fiber shown in Fig. 13, and a grazing section of a distal fiber is probably present in Fig. 12. Cortical microtubules (small arrowhead) converge on these regions, and flagellar collar material is present in Fig. 12, but no basal bodies can be recognized. More generally, it is apparent from our examination of *bald-2* that the mutation does not interfere with a cell's ability to localize the various structural elements of its basal and flagellar apparatus to a medial anterior region (e.g., Figs. 4, 7, 12, and 13), but it does interfere with the cell's ability to pattern them correctly with respect to one another.

Other Microtubule Systems in Bald-2 Cells

The bands of *bald-2* cortical microtubules depicted in Figs. 4, 7, 8, 12, and 13 are indistinguishable from those of the wild type (31) in that they converge on the cell anterior in groups of four tubules and progressively lose tubules as they near their site of termination (or origin). The focal



orientation of these microtubules toward striated fiber material rather than toward basal bodies is particularly evident in *bald-2* since the basal body singlets are often dissociated from the fibers (e.g., Figs. 4, 7, 12, and 13).

The intranuclear spindle microtubules and the cleavage furrow microtubules of mitotic *bald-2* cells are indistinguishable from those of wild type in their numbers, orientation, and time of appearance (18). In two instances a *bald-2* singlet basal body has been localized at the head of the cleavage furrow in a mitotic cell, just as in wild-type cells (18).

DISCUSSION

Nature of the Bald-2 Mutation

The present study demonstrates that the *bald-2* mutation prevents the assembly of stable B and C microtubules in basal bodies and flagella. Recent studies have established that all microtubules, including B and C microtubules, are composed of the same two general classes of tubulin monomers (8, 21, 25), but that B and C microtubules possess several unique properties: they contain 11 instead of 13 protofilaments (38); they "share" a wall with A microtubules (11, 31, 38); and they are more labile to heat or detergent treatment than A microtubules (4, 20, 36, 41). It is also clear from recent studies that the ability to form doublets and triplets is not an inherent property of nonflagellar tubulin. Thus, isolated axonemes (2) or basal bodies (32), presented with tubulin monomers derived from cytoplasmic brain microtubules, can act to nucleate their in vitro polymerization, but the microtubules so formed are always singlet, never doublet (2, and J. Rosenbaum, personal communication). It therefore appears that doublet-and triplet-forming tubulins are either (*a*) distinctive gene products that are closely related to nonflagellar tubulins; (*b*) tubulins that have undergone some posttranslational modification (e.g., differential glycosylation) (7); or (*c*) tubulins that associate with some other macromolecule(s) not available to brain or other cytoplasmic tubulins. Subclasses of tubulins 1 and 2 have been demonstrated by isoelectric focusing of flagellar tubulin from *C. reinhardtii* (42), but the origin and significance of these subclasses is not yet established (24).

FIGURE 8 Mitotic *bald-2* cell bearing a "new" flagellar stub. A singlet basal body 180 nm in diameter, whose longest microtubule measures 375 nm, underlies a disordered transition region (*tr*). The stub is surrounded by a membrane that lacks the fuzzy coat, and the membrane is overlain with a thin layer of presumed cell wall material that also covers the nonflagellar cell surface. The interior of the stub contains amorphous material, which resembles unpolymerized tubulin (18), and a few faint single microtubules. A proximal striated fiber (*psf*) lies beneath the singlet basal body, and an unusual long microtubule lies to its left (arrow). Two cortical microtubules are indicated at *cmt*. × 60,000.

FIGURE 9 Interphase *bald-2* cell bearing an "old" flagellar stub. The region once occupied by a basal body is represented by a region of amorphous material, at the base of which lies a proximal striated fiber (*psf*). The transition region (*tr*) contains a broken H-shaped structure and other dense material. Two single microtubules in the transition region appear to break down as they enter the stub. The membrane covering the stub lacks a fuzzy coat. A flagellar collar (*C*) (37) associates with the transition region, extends to near-normal length (0.9 nm), and seals off at the distal end of the tunnel. The mature cell wall is indicated at *W*. × 60,000.

FIGURE 10 Interphase *bald-2* cell bearing an "old" flagellar stub. Prominent is an H-shaped region of the transition zone which contains several nicks but is otherwise structurally intact. Arrow points to one V-shaped wedge at the transition zone periphery (compare with Fig. 3). Four single microtubules are present in the flagellar stub and one extends down into the basal body region which is represented by a thin layer of amorphous material. Ribosome-containing cytoplasm lies beneath this layer. The flagellar collar (*C*) seals off where it meets the cell wall (*W*). The stub membrane is devoid of a fuzzy coat. 60,000 ×.

FIGURE 11 Interphase *bald-2* cell exhibiting a particularly degenerate "old" flagellar stub containing one single microtubule and dense aggregates of what is presumably transition-region material. The basal body region has become largely occupied by ribosome-containing cytoplasm. A flagellar collar (*C*) initiates normally at the transition region, but grows over the stub well below the level of the wall (*W*). The stub membrane lacks a fuzzy coat. × 60,000.

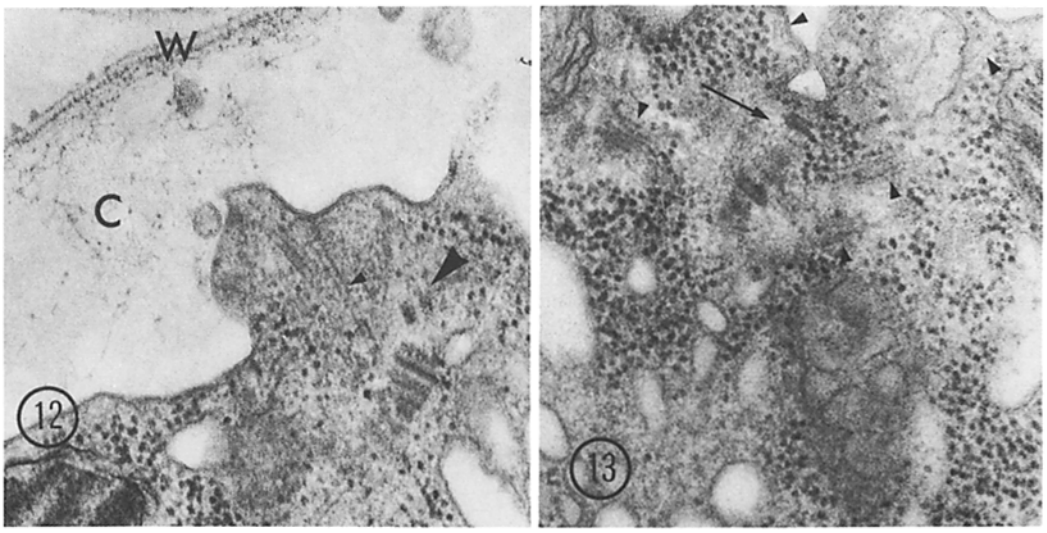


FIGURE 12 Interphase *bald-2* cell showing a prominent proximal striated fiber toward which cortical microtubules (small arrowhead) are focused. The periodicity of the striations and size of the fiber are identical to those of the fiber shown in Fig. 3, but the *bald-2* fiber lacks basal body associations. Large arrowhead indicates a probable grazing section of a distal striated fiber. Flagellar collar material (*C*) overlies the region, associating with the cell wall (*W*). × 60,000.

FIGURE 13 Interphase *bald-2* cell showing a prominent distal striated fiber toward which cortical microtubules (small arrowheads) are focused. Long arrow indicates a portion of the fiber in which the slender horizontal filaments can be seen to advantage. The periodicity of the striations and the dimensions of the two dense lateral and faint central elements are identical to those of the fiber shown in Fig. 2, but no basal body is associated with the *bald-2* fiber and the fiber is oriented perpendicular rather than parallel to the cell surface. The five sections adjacent to this contained two proximal striated fibers. × 60,000.

One possibility, therefore, is that the *bald-2* mutation affects a gene that is involved in producing stable doublet-forming tubulin monomers, be it directly (as a distinct gene product) or indirectly (by modifying or associating with "universal" tubulin gene products).

An alternate possibility is that the *bald-2* mutation interferes with the proper assembly of the A-microtubule "partition" (that portion of the A tubule onto which B microtubules nucleate). The partition is particularly resistant to disassociation by heat or detergent treatment (20, 24, 41, 42) and, according to Witman et al. (42), is composed of a single tubulin subclass in *C. reinhardtii*. Possibly, therefore, the three protofilaments that make up the partition are uniquely able to induce doublet formation and are somehow rendered defective by the *bald-2* mutation.

Function of the B and C Microtubules in Basal Bodies

In a recent review (10) Fulton concludes that the only firmly established function of basal bodies is to induce the formation of flagella (e.g., reference 30). A logical deduction from this axiom would be that mutant basal bodies should give rise to mutant flagella, and this is indeed found to be the case with *bald-2* cells. A more careful consideration of the mutant phenotype, moreover, gives additional insight into basal body function.

It has been possible to establish that B and C microtubules are not required for basal bodies to elongate. Previous studies had shown that, in numerous ciliated and flagellated cell types (including *C. reinhardtii*), basal bodies are initiated as very short (50–100 nm) rings of singlet microtubules (3, 5, 6, 18, 19); these soon acquire B and C microtubules, and only then elongate to their mature length (generally 400–600 nm). It might therefore be argued that the acquisition of B and C tubules is a prerequisite for elongation, but this is clearly not the case in *bald-2*: in the absence of B and C microtubules, the mutant singlet basal bodies proceed to elongate to near-normal if not normal length at the appropriate times in the cell cycle.

On the other hand, the present study does point to a role for the B and C microtubules in basal body stability. The singlet rings in *bald-2* are generally indistinct (as are immature rings of singlets in normal cells), suggesting that some additional, electron-dense substance may normally be added to the ring as singlets are converted to triplets. Whether such a postulated substance, or A-C linker fibers (12), or the triplet configuration itself is responsible, it is clear that elongated singlet basal bodies are not stable and may even depolymerize *in situ* (Fig. 9).

Finally, the present study indicates that the B and C microtubules are essential for the attachment of striated fiber material to basal bodies: such attachments have never been observed in *bald-2* cells. This feature of the mutant strain is considered in a later section.

The Rare Appearance of Bald-2 Singlet Basal Bodies

The 15 examples of singlet *bald-2* basal bodies that we have encountered in this study resulted from careful scrutiny of many thousands of cell sections. By comparison, examination of 50 random sections of cells carrying the *bald-4* mutation, a mutation affecting flagellar elongation beyond the level of the transition region but not triplet formation (J. Hoffman, unpublished observations), yielded five images of basal bodies. These very different statistics would be expected in three distinct situations: (*a*) the *bald-2* singlet rings might usually be very short and thus rarely caught in section; (*b*) the *bald-2* singlet rings might be very unstable so that they are present in only a few cells in a culture at any one time; or (*c*) only a few *bald-2* cells in a culture might manage to form singlet rings, the remainder producing no basal bodies at all. Our observations cannot rule out the third possibility but are more consistent with the first and second. It seems likely that singlet rings may be stable only as long as they remain short, and fall apart when or soon after they attempt to elongate.

Function of the Striated Fibers Associated with Basal Bodies

Basal bodies are commonly associated with striated material (reviewed in reference 43) whose composition and function is largely unknown. Steinman (35) proposed that the centriole itself is probably not an active participant in striated fiber formation, a conclusion supported by the present study: *bald-2* cells are found to produce both the proximal and distal forms of these fibers despite their aberrant basal body structures.

The distal striated fiber of *C. reinhardtii* is bilaterally symmetrical (Fig. 2; reference 31) and normally attaches to the two basal bodies so that its central element is precisely coincident with the cell midline (see Fig. 1 of reference 18). The fact that this fiber fails to attach to singlet basal bodies in *bald-2* may account for the fact that two singlet basal bodies are not found in the same section of a *bald-2* cell. More generally, it seems likely that the precise bilateral spacing of the two basal bodies in normal *C. reinhardtti* (an essential feature of a biflagellate cell if it is to swim in a straight path and not a circle) may be entrusted to the distal striated fiber: being of fixed length and periodicity and bearing sites at either end that apparently associate specifically with basal body triplets, this fiber may well bring basal body pairs together, particularly in cases where basal bodies are assembled separately and must "find" partners (5, 18).

Genetic Analysis of Bald-2 Cells

Even with theories of basal body "genetic autonomy" now in doubt (10), a genetic analysis of the *bald-2* mutation will clearly be of considerable interest. Genetic analysis of "flagellaless" strains of *C. reinhardtii* is frustrated by the fact that mating is usually initiated by an agglutination of the flagellar tips of mt^+ and mt^- gametes. We have found it possible to bypass this requirement by centrifuging gametic *bald-2* mt^+ cells together with wild-type mt^- gametes for 2 h. Occasional zygotes form under these conditions, perhaps because the specialized membrane regions responsible for zygotic cell fusion (9, and footnote 5) are occasionally brought into contact. Preliminary results indicate that *bald-2* and wild-type cells emerge from these zygotes in a 1:1 ratio (13), suggesting that the trait is determined by a single nuclear gene; additional crosses involving additional markers are presently being performed to confirm this conclusion. We are also exploring cell fusion techniques (16, 26, 27) with the goal of performing

[5] Goodenough, U. W., and R. Weiss. 1975. Gametic differentiation in *Chlamydomonas reinhardi*. III. Cell wall lysis and microfilament-associated mating structure activation in wild-type and mutant strains. Manuscript in preparation.

complementation analysis of *bald-2* and the other six *bald* strains we have isolated.

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