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# Genetic control of flagellar structure in *Chlamydomonas reinhardii*

BY J. R. WARR, A. McVITTIE, SIR JOHN RANDALL AND J. M. HOPKINS

*Department of Biophysics and Medical Research Council Biophysics Research Unit, University of London, King's College*, 26–29 *Drury Lane, London, W.C.*2

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## 1. INTRODUCTION

Studies on the genetic control of organelle structure and morphogenesis are of particular interest since they may help to provide a bridge between present knowledge of the control of protein structure and studies on the control of gross morphology and differentiation in multicellular organisms. It has been shown that the flagellum of *Chlamydomonas reinhardii* provides a promising experimental system for studies at this level of organization (Randall, Warr, Hopkins & McVittie, 1964), since it is a structurally well-defined organelle occurring in an organism which is genetically familiar (e.g. Levine & Ebersold, 1960; Ebersold, Levine, Levine & Olmsted, 1962; Sager, 1955, 1965).

*Chlamydomonas reinhardii* is a unicellular green alga with two flagella per cell. The structure of these flagella as seen in the electron microscope closely resembles that described previously for the flagella and cilia of a wide range of organisms (e.g. Gibbons & Grimstone, 1960; Fawcett, 1961). The principal features of this structure consist of two central fibres and nine outer fibres running almost the whole length of the flagellum and embedded in a matrix which is surrounded by a membrane (Plate I, Fig. 1). The outer nine fibres are doublets, thus appearing as figures of eight in transverse sections. They are continuous with the outer components of the basal body although the central two fibres terminate above the distal end of this structure (Plate I, Fig. 2). This arrangement is, of course, quite distinct from the relatively simple structure of bacterial flagella (Kerridge, Horne & Glauert, 1962; Lowy & Hanson, 1965).

In order to gain some understanding of the genetic control of flagella structure, our approach has been to search for mutant strains with structural abnormalities of these organelles. This is most conveniently achieved by screening for mutants with impaired motility, since a proportion of such strains may well possess concomitant structural abnormalities of the flagella. Several non-motile strains have previously been isolated in the related species, *C. moewusii*, although examination with the electron microscope of five of these mutant strains with full-length, paraly[illegible]d flagella revealed no abnormalities of the internal structure (Gibbs, Lewin



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& Philpott, 1958). More recently, a mutant of *C. reinhardii* with paralysed flagella has been isolated and shown to possess a clearly defined structural abnormality. In this mutant, the central pair of fibres has been replaced by an irregular core of apparently disorganized material (Randall *et al.*, 1964).

The present paper describes the isolation and genetic analysis of further non-motile mutants of *C. reinhardii*. Several of these mutants and of others isolated by Dr R. A. Lewin have been shown to possess a structural defect similar to that described in the previous paper. A partial suppressor of some of the mutations has been isolated and shown to act with differing degrees of efficiency on different mutations.

## 2. MATERIALS AND METHODS

### (i) *Strains*

Cultures of wild-type *Chlamydomonas reinhardii* were obtained from the Cambridge collection and originally from Professor R. P. Levine's laboratory. Cultures of several paralysed mutants of *C. reinhardii* isolated by Dr R. A. Lewin were provided by Professor Levine (cf. Table 1).

### (ii) *Culture conditions and media*

Experimental cultures were maintained at 25°C. at an intensity of illumination of 500 foot-candles provided by tubular fluorescent lamps. The culture medium was based on medium 1 described by Sager & Granick (1954) in which ferric chloride was replaced by 0·01 g./l. ferric citrate and 0·01 g./l. citric acid. Solid medium contained 10 g./l. and semi-solid medium 5 g./l. of Oxoid No. 3 agar.

### (iii) *Mutagenic treatment*

The technical details of the mutagenic treatment varied with the different mutagens employed and are briefly described below.

Acridine orange (G. T. Gurr Ltd.): approximately $10^5$ log phase cells were spread on plates containing 10 or 100 μg./ml. and incubated for 7 or more days. One hundred per cent of cells plated on 10 μg./ml. and over 15% of cells plated on 100 μg./ml. yielded colonies. It is probable that this mutagenic treatment involved the combined effects of acridine orange and light (Ritchie, 1964; Nakai & Saeki, 1964).

Aminopurine nitrate (K & K Laboratories, Plainview, New York) and proflavine hemisulphate (B.D.H. Ltd., Poole, Dorset): approximately $10^5$ cells/dish were plated on 200 μg./ml. or 250 μg./ml. respectively and incubated for 7 days.

Hydroxylamine hydrochloride (Eastman Organic Chemicals, Rochester, New York): log phase cells at a density of approximately $10^6$/ml. were exposed for 4 hours to an autoclaved 0·01 M solution of hydroxylamine hydrochloride adjusted to pH 7 with NaOH. The cells were then removed from the hydroxylamine by centrifuging and washing in distilled water or by filtering through a Millipore filter. Approximately 1% of cells survived.

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Ultra-violet irradiation: log phase cells at a density of approximately $6 \times 10^6$ cells/ml. were exposed to ultra-violet light for 2 or 4 hours in an experimental arrangement closely resembling that described by Lewin (1960). Control platings indicated that survival was approximately 5%.

### (iv) *Isolation of mutants with impaired motility*

After exposure to a mutagen, an enrichment procedure (Lewin, 1954) was used to increase the proportion of cells with impaired motility in relation to the majority of wild-type, motile cells. About $10^6$ cells were transferred to 150 ml. of liquid medium in a separating funnel and incubated, with illumination from above, for 3 to 5 days. Mutants with impaired motility multiplied at the bottom and wild-type cells at the top of these cultures. Usually, all but the bottom 2 ml. of the culture was withdrawn through a sterile pipette attached to an automatic pipetting unit (A. R. Howell Ltd., London, N.W.2) and replaced by 150 ml. of fresh medium. Since the non-motile cells at the bottom of the vessel were not removed by this washing process, it presumably resulted in further enrichment of non-motile relative to motile cells.

After further incubation for 1 to 3 days cells were withdrawn from the bottom of the culture and plated at approximately 150 cells/dish on semi-solid medium. On semi-solid agar, motile cells spread out from the edge of the colony by swimming through the surface film of liquid. The resulting colonies are larger and flatter than those produced by non-motile cells. Thus mutants with impaired motility may be identified on semi-solid medium by their smaller, darker and more heaped appearance. Lack of motility can be confirmed by direct microscopic examination or by transferring these putative mutants into tubes of liquid medium where growth will be confined to the bottom if the strain is unable to swim (Lewin, 1954). Only one strain with any particular mutant phenotype was isolated from each enrichment vessel to ensure that each mutant isolated arose from a separate mutational event.

### (v) *Crossing technique*

The crossing technique and methods for dissection of zygotes were similar to those described by Ebersold & Levine (1959). Zygotes were matured on medium solidified with 2% agar.

### (vi) *Electron microscopy*

Techniques for fixing and embedding specimens for electron microscopy were as previously described (Randall *et al.*, 1964). In order to increase the chance of seeing a reasonable number of true transverse sections of flagella per sample, more concentrated cell preparations were used. When quantitative estimates of proportions of flagella with different types of centre fibres were required, (as in cultures of leaky mutants and certain suppressed strains to be described), the nine outer fibres as seen in transverse section were first examined. Only if they were found to have

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good order and preservation was the state of the central fibres assessed. Cultures were always well shaken prior to fixation. Several blocks from the same culture gave consistent results.

### (vii) *Estimation of motile and spinning cells*

During the studies of leakiness and suppression, it was necessary to estimate the proportion of cells which were non-motile, spinning (i.e. moving in a circle or swimming on a spiral course) or fully motile. This was accomplished by examining freshly made suspensions of about $10^7$ cells/ml. in distilled water in a haemocytometer. The cells were from 2–4-day cultures on solid medium and were almost entirely flagellate. Cells for replicate counts were from growth on separate petri dishes to reduce variation due to environmental differences. The number of spinning and motile cells was usually counted in a ruled area containing at least 5000 cells, although in very leaky strains (over 4% spinning cells), a smaller area (including at least 700 cells) was examined.

## 3. RESULTS

### (i) *Types of mutant isolated*

Thirty-six mutants of *Chlamydomonas reinhardii* with impaired motility have been isolated using the techniques described above. These mutants have a range of phenotypes similar to that described previously for *C. moewusii* (Lewin, 1952, 1954). They may be broadly classified as follows:

(*a*) *Palmella mutants.* These are frequently occurring strains in which growth in liquid or on solid media under normal culture conditions is in the form of clumps of cells surrounded by transparent palmella coats. The flagella of such strains are either lacking or embedded within the capsule and consequently the cells are non-motile. Palmella mutants have not been studied during the present work and are not considered further.

(*b*) *Mutants with gross morphological abnormalities.* These strains have flagella which are either very short or which tend to develop small swellings and eventually round up completely. Many of these mutants have a tendency to become palmelloid and may initially be mistaken for palmella mutants. They are best observed in young (2- or 3-day) cultures on solid medium. Genetic and electron microscope studies on this class of mutants are at present incomplete and consequently they also will not be described in further detail. They are, however, a class of mutants of potential importance for studies of flagellar morphogenesis.

(*c*) *Mutants that swim abnormally.* In these strains the majority of cells have normal length flagella and are capable of swimming, but in a slow, jerky or otherwise abnormal manner. Electron microscope examination of the flagella of each strain of this class has so far not revealed any well-defined structural abnormality.

(*d*) *Non-swimming mutants.* The majority of cells of these strains are incapable of progression through liquid medium. However, although cell movement is rarely

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observed, some form of flagellar movement is commonly found. This varies widely between the different strains from almost complete inactivity, through sporadic twitching or gentle waving to rapid, ineffectual beating. Activity may also vary within a given strain according to prevailing conditions such as age of culture.

This heterogeneous group of mutants may be divided into three subgroups. Firstly, there are those strains in which the flagella tend to be held out in the form of a 'V' (Plate II, Fig. 3), with a variable amount of twitching. Electron microscope examination of these mutants has proved to be of particular interest and will be dealt with in a later section.

Table 1. *Frequency of various types of abnormal flagella mutants in* Chlamydomonas reinhardii

| | Isolated during the present work | Isolated by Dr R. A. Lewin |
|---|---|---|
| 1. Grossly abnormal | 11 | 1 |
| 2. Abnormal swimming | 4 | 3 |
| 3. Paralysed | | |
| (*a*) straight flagella | 8 | 6 |
| (*b*) curved flagella | 7 | 3 |
| (*c*) flagella in various positions | 6 | 4 |

Secondly there are strains in which the flagella tend to be curved. Strains of the third subgroup have flagella held in a wide variety of positions, none of which is really characteristic. Examination of the fine structure of flagella of each member of these two subgroups has so far not revealed any structural abnormality.

The frequency of different types of abnormal flagella mutants isolated during the present work and of others isolated by Lewin is recorded in Table 1. There was no apparent correlation between the type of mutant isolated and the mutagen employed. The procedure for the isolation of mutants does not permit any estimates of the mutation rates to be made and the possibility of spontaneous mutation cannot be excluded as the origin of any of these strains.

### (ii) *The centre-pair mutation*

The flagellar fine structure of one of the mutants with paralysed straight flagella has been described previously (Randall *et al.*, 1964). It was shown that the central pair of fibres was replaced by an irregular region of moderately densely staining material. The outer fibres appeared normal. A transverse section of a flagellum of this mutant is shown in Plate II, Fig. 4, and may be contrasted with the wild-type flagellum shown in Plate I, Fig. 1. Examination of thirteen other mutants with paralysed flagella held straight out from the cell has now shown that they all have a similar fine structural abnormality, i.e. they have flagella lacking a central pair of fibres in an organized form. We have called them centre-pair mutants. Since the arrangement of nine outer and two central fibres seen in electron micrographs of wild-type flagella is commonly termed the $9+2$ pattern, the arrangement of fibres in the centre-pair mutants may be conveniently termed the $9+0$ pattern.

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Six of these mutations were originally isolated by Lewin and it has been shown that they map at four unlinked loci (Ebersold *et al.*, 1962). The loci were designated *pf*15, *pf*18, *pf*19 and *pf*20, 'pf' being an abbreviation for 'paralysed flagella'. There are two pairs of alleles: *pf*15 and *pf*15*A*; *pf*19 and *pf*19*A*.

Table 2. *Location of newly isolated centre-pair mutations*

| Locus | Linkage group | Newly isolated allele | Mutagen employed for isolation |
|---|---|---|---|
| *pf*15 | III | None | — |
| *pf*18 | II | *pf*18*A* | Proflavine |
| | | *pf*18*B* | Proflavine |
| | | *pf*18*C* | Ultra-violet light |
| | | *pf*18*D* | Acridine orange |
| *pf*19 | X | *pf*19*B* | Proflavine |
| | | *pf*19*C* | Hydroxylamine |
| | | *pf*19*D* | Acridine orange |
| *pf*20 | IV | *pf*20*A* | Aminopurine |

Each of the eight newly isolated strains was found to involve mutation at a single gene by crossing to wild-type, and was then crossed to strains carrying representative alleles of each of the four mapped 9+0 loci (see above). Recombination with three of the mapped loci but not with the fourth was observed in each case, indicating that all the new mutations are either closely linked to or at a known locus. The

Table 3. *Number of tetrads examined from zygote platings and the consequent upper limits of percentage recombination*

| Cross | Number of cols. examined | Number of recombinant cols. | Upper limit percentage recombination |
|---|---|---|---|
| *pf*18 × *pf*18*A* | 412 | 0 | 0·37 |
| *pf*18 × *pf*18*B* | 342 | 0 | 0·44 |
| *pf*18 × *pf*18*C* | 266 | 0 | 0·56 |
| *pf*18 × *pf*18*D* | 472 | 0 | 0·32 |
| *pf*19 × *pf*19*B* | 1120 | 0 | 0·14 |
| *pf*19 × *pf*19*C* | 910 | 0 | 0·16 |
| *pf*19 × *pf*19*D* | 1020 | 0 | 0·15 |
| *pf*20 × *pf*20*A* | 1120 | 0 | 0·14 |

distribution of the eight new mutations amongst these loci and the symbols for the mutant alleles is recorded in Table 2. We have followed the symbol convention previously employed by Ebersold *et al.* (1962).

Relatively few zygotes were examined in these crosses (usually about twenty in each case) so that the possibility of rare recombinants being undetected could not be excluded. Much larger numbers of zygotes from the appropriate crosses were therefore examined using the zygote plating technique described by Levine & Ebersold (1960). Zygotes were plated at about 100 per dish on semi-solid medium

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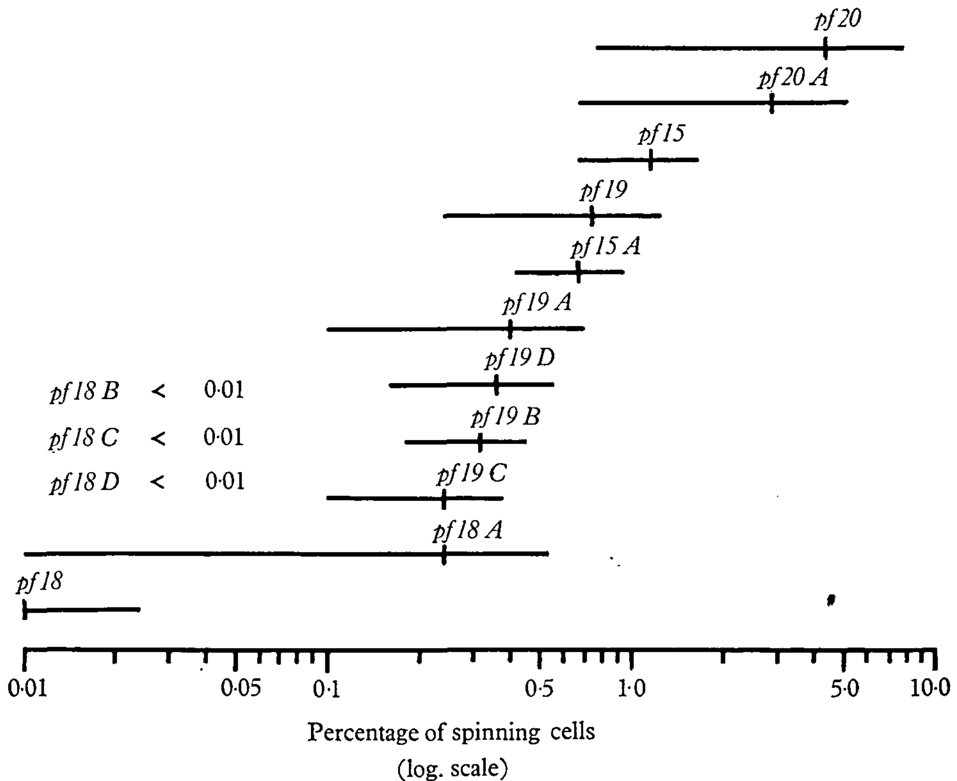
and germination allowed to proceed without dissection. Over 90% germination of zygotes in these platings could usually be inferred from parallel crosses of the same strains where smaller numbers of zygotes were dissected individually. In crosses where both parental strains carry mutant alleles for paralysed flagella, parental ditype (PD) zygotes will give rise to colonies containing only paralysed cells and




Text-fig. 1. Leakiness of centre-pair mutants as observed in the light microscope. The short vertical line represents the mean of five counts of the percentage spinning cells in young cultures and the horizontal line represents two standard deviations either side of the mean. A logarithmic scale has been used. Spinning cells have not been observed in cultures of *pf*18*B*, *pf*18*C* and *pf*18*D* and consequently these mutants are not represented in this figure.

consequently they will be small and dark. In contrast, non-parental ditype (NPD) and tetratype (T) tetrads will contain motile recombinant cells which will spread out to give larger colonies. The results of the zygote platings are summarized in Table 3. No recombinant colonies were observed in any of the crosses. The consequent upper limits for percentage recombination at the 5% probability level have been calculated from the formula for complete tetrads given by Horowitz, Fling, MacLeod & Sueoka (1961).

More extensive examination has shown that certain of the fourteen centre-pair mutants are leaky. Assessment of leakiness may be made in terms of either light or

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electron microscope observations. In the former case, leakiness is represented by the presence of a small minority of cells capable of either spinning like a top in one position or slowly swimming forward along a spiral course. It is likely that both these forms of abnormal movement result from the presence of one functional and one non-functional flagellum in the same cell. A very much smaller proportion of fully motile cells may also be present in some cases.

The proportion of spinning cells present in young cultures (2–4-day) of each mutant grown on solid medium has been estimated by direct observation in a haemocytometer (cf. Materials and Methods section). Five counts were made for each strain. The mean of the counts plus and minus two standard deviations is recorded for each strain in Text-fig. 1. A logarithmic scale has been used for this figure. Under the conditions used there is found to be some correlation between the degree of leakiness and the mutant locus. Three of the *pf*18 alleles (*pf*18*B*, *pf*18*C* and *pf*18*D*) are completely non-leaky and a fourth (*pf*18) is almost so. The fifth allele at this locus (*pf*18*A*) has been found to have a rather higher degree of leakiness. All the *pf*15 and *pf*19 alleles show intermediate values, whereas the two *pf*20 alleles are found to be exceptionally leaky.

At the fine structural level, leakiness is manifested by the presence of a certain proportion of flagellar transverse sections showing either wild-type (i.e. $9+2$) arrangement of fibres or a new arrangement with one fibre organized and the other disorganized ($9+1$ pattern; see Plate II, Fig. 5). Examination of twenty flagellar transverse sections from strains carrying any of the mutations at the *pf*15, *pf*18 or *pf*19 loci has revealed only $9+0$ flagella. In contrast, strains carrying the *pf*20 or *pf*20*A* alleles were both found to have $9+2$ and $9+1$ flagella in addition to those of the $9+0$ type. Two hundred and three true transverse sections of *pf*20 flagella revealed 57% $9+2$, 9% $9+1$ and 34% $9+0$ flagella types. A preliminary survey indicates that similar proportions are found in the presence of the *pf*20*A* allele. In longitudinal sections of *pf*20 flagella, the central fibres have been found in the plane of section for distances of 1·0–1·5 $\mu$. In all cases, these fibres are organized or disorganized over the entire region in which they are visible, suggesting that the state of the central fibres as seen in a single transverse section may well represent their condition along the whole length of the flagellum.

Thus *pf*20 and *pf*20*A* are the most leaky mutations as judged by the proportion of spinning cells seen in the light microscope and the only leaky mutations as judged by the presence of intact central fibres as seen in the electron microscope. However, since only twenty flagella transverse sections have been examined for each strain, the possibility of rare flagella with intact central fibres in any of the strains carrying *pf*15, *pf*18 or *pf*19 alleles has not yet been excluded.

Although there is a qualitative correlation between light and electron microscope data concerning the mutants in general, there is a quantitative discrepancy in the data concerning *pf*20. Approximately 5% of cells of this strain are seen to be spinning (cf. Text-fig. 1). Assuming that in spinning cells only one flagellum of the pair is functional and neglecting the rare fully motile cells ($<0{\cdot}5\%$), it may be inferred that about 2·5% of flagella are functional. Electron microscope evidence

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suggests the presence of over 50% 9+2 flagella, which presumably should all be functional. The reason for this lack of correlation between the estimates of functional flagella deduced from the two sets of data is not clear at present.

Experiments have been performed to test the possibility that the minority of motile and spinning cells seen in cultures of leaky mutants in fact represent the accumulation of revertants or some form of partial revertants respectively. Six-day cultures of *pf*20 and *pf*20*A* strains were grown in tubes of liquid medium. Such cultures consist predominantly of paralysed cells forming a pellet at the bottom of the tube and a minority of motile or spinning cells at the top of the tube. Cells were withdrawn from the top of the culture and plated on semi-solid medium. Cells from twenty colonies arising from platings of each of these two mutants were examined in the light microscope and in each case found to comprise a mixture of cells similar to that found in the original culture, i.e. mostly paralysed with a few spinning and occasional motile cells. Cells from three colonies of one mutant (*pf*20) were also cloned and examined in the electron microscope. In each case, there were similar proportions of 9+2, 9+1 and 9+0 flagella as described previously. These results are to be expected if the two mutants are leaky, but are inconsistent with the hypothesis that spinning and motile cells are revertants.

### (iii) *Isolation of a suppressor mutation*

Interactions between genes are likely to represent a basic feature of organelle morphogenesis and so it seemed possible that a search for suppressors of the centre-pair mutations would be profitable. Consequently, an attempt was made to select motile revertants from one of the centre-pair mutants. Cells of strain *pf*19*B* were grown in the presence of 250 mg./l. proflavine for one week and then selection was made for swimming cells by successive subcultures from the surface layers of a liquid culture. One of the strains isolated by this procedure was a partial revertant which had a far higher proportion of spinning and swimming cells than observed in *pf*19*B* cultures. It was purified from a single colony and found to be stable on repeated subculture. Twenty counts of cell types in cultures of this revertant strain gave a mean of 6·20% spinning cells (standard deviation 1·44) and 0·26% fully motile cells (s.d. 0·18). This compares with a mean of 0·32% spinning cells (s.d. 0·07) and less than 0·01% fully motile cells in cultures of *pf*19*B*.

Electron microscope examination of flagella of the revertant reveals three distinct types of fine structure, similar to those occurring in cultures of very leaky mutants (*pf*20, *pf*20*A*). That is to say, there are 9+0, 9+1 and 9+2 flagella present, whereas only 9+0 flagella have been found in cultures of *pf*19*B*. Examination of 118 flagella transverse sections showed 40%, 17% and 43% of the three types respectively. Longitudinal sections of flagella of the revertant showed that individual fibres are either organized or disorganized over a considerable distance and probably the whole length of the flagellum (Plate II, Fig. 6).

On crossing the revertant to wild-type, approximately a quarter of the segregants were phenotypically similar to strains carrying *pf*19*B* alone, approximately a

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quarter of the segregants showed the revertant phenotype and the remaining half were phenotypically wild-type. These results suggested that the revertant phenotype was due to interaction between the *pf*19*B* allele and an unlinked suppressor (*su*1). The classification of members of each type of tetrad in this cross and their

Table 4. *Proportions of segregant types in different classes of tetrad obtained on crossing the revertant strain to wild-type*

| | | Segregant types | | | |
|---|---|---|---|---|---|
| | Number of tetrads | Motile $pf19B^{+};su1^{+}$ | Motile $pf19B^{+};su1$ | Revertant phenotype $pf19B;su1$ | Mutant phenotype $pf19B;su1^{+}$ |
| PD | 8 | 2 | 0 | 2 | 0 |
| NPD | 4 | 0 | 2 | 0 | 2 |
| T | 15 | 1 | 1 | 1 | 1 |

corresponding genotype is shown in Table 4. The observed ratio of 8PD : 4NDP : 15T tetrads provides no evidence of linkage between the *pf*19 and *su*1 loci (cf. Perkins, 1953). It may be predicted that all the motile segregants in NPD tetrads carry the mutant suppressor allele although they are phenotypically wild-type (cf. Table 4). Two such strains were crossed to strains carrying *pf*19*B* and suppressed segregants obtained in the expected proportions, thus confirming the suppressor hypothesis.

(iv) *Suppression of other centre-pair mutations*

Motile strains carrying *su*1 were crossed to strains carrying each of the centre-pair mutations and the segregants examined for the occurrence of suppression. The extent of suppression by *su*1 varies somewhat between the centre-pair loci.

All mutant alleles at the *pf*15 and *pf*19 loci are suppressed to a similar degree to *pf*19*B*, described above. Suppressed segregants have about 10% spinning cells and 1% fully motile cells. Counts of these suppressed strains made on different occasions have varied from 4–20% spinning cells and there are indications that slight environmental differences may influence the level of suppression. However, there is invariably over a fivefold increase in the percentage of spinning cells compared to unsuppressed segregants, permitting unambiguous classification in all cases. $9+0$, $9+1$ and $9+2$ flagella have been found to be present in strains carrying each of the *pf*15 and *pf*19 alleles with the suppressor. In all cases, the $9+1$ type is the least common although extensive data on proportions of flagella types has only been obtained for *pf*19*B*; *su*1 (see above).

Suppressed segregants have not been detected in crosses between the two most leaky mutants (*pf*20 and *pf*20*A*) and strains carrying the suppressor. However, the considerable proportion of motile and spinning cells in cultures of these mutants would make the detection of a slight degree of suppression difficult.

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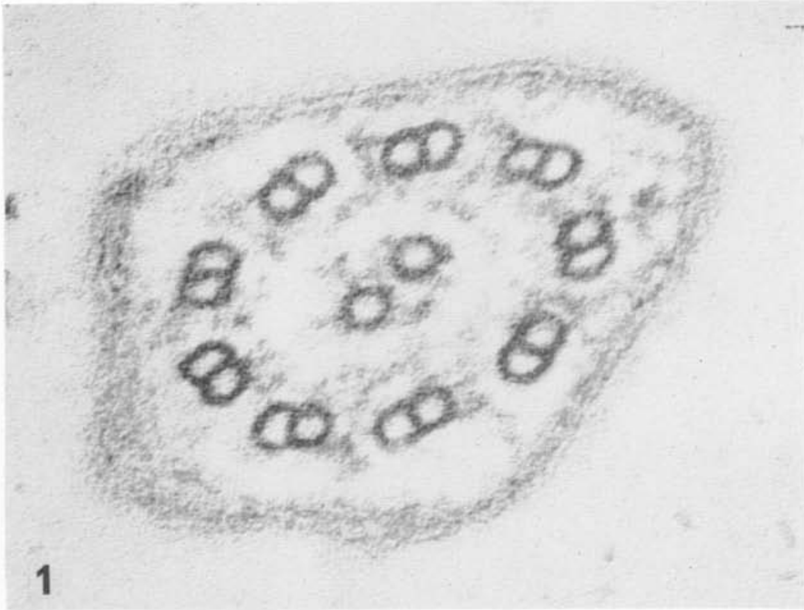

EXPLANATION OF PLATES

Plate I

Fig. 1 (*top*). Transverse section through a wild-type flagellum, showing typical 9 + 2 arrangement of fibres. Electron micrograph × 260,000.

Fig. 2 (*bottom*). Longitudinal section through the anterior region of wild-type *Chlamydomonas reinhardii* showing the basal bodies of the two flagella. Note that the outer fibres, but not the central ones, are continuous with the basal structure in the left-hand flagellum. The right-hand flagellum passes out of the plane of section above the basal body. Electron micrograph × 62,200.



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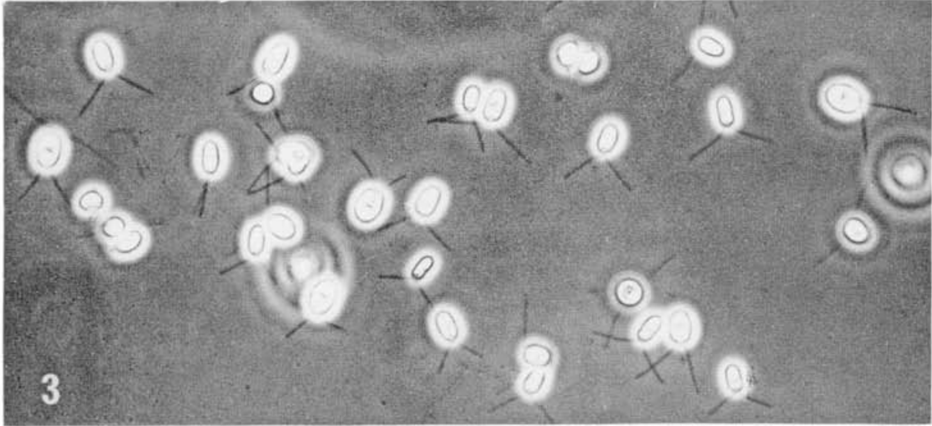


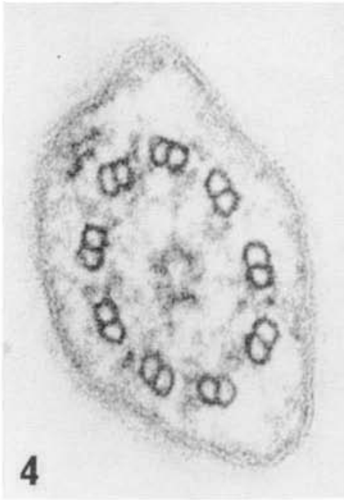


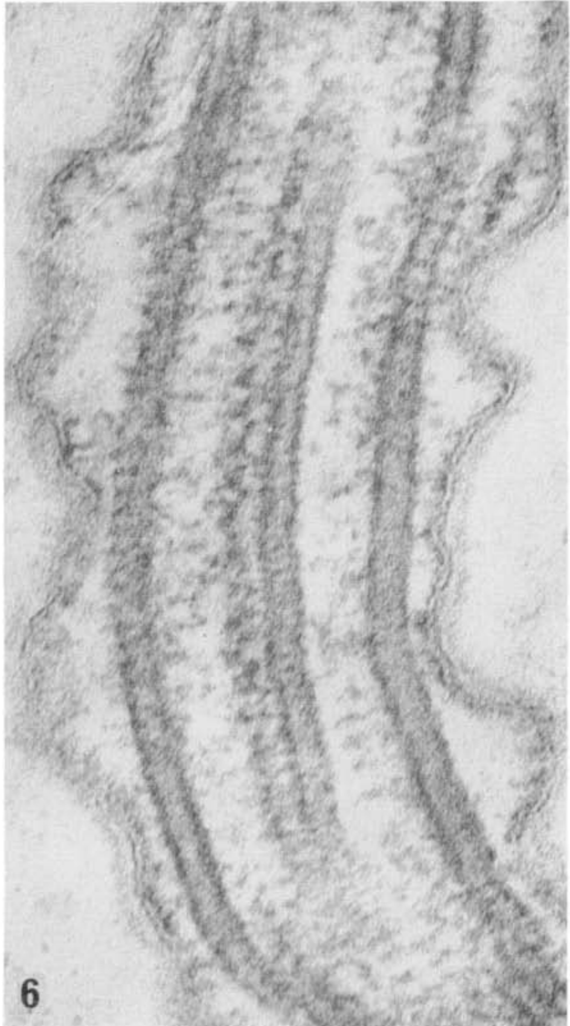


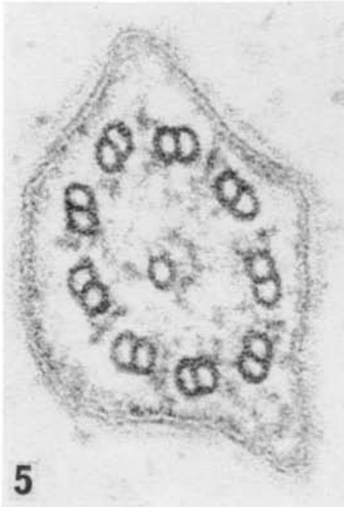


Plate II

Fig. 3 (*top*). Phase contrast photomicrograph of a culture of a paralysed mutant with flagella held in the form of a 'V'. × 840.

Fig. 4 (*centre left*). Transverse section through a flagellum of a centre-pair mutant, showing the 9 + 0 arrangement of fibres. Note the presence of some disorganized material in the centre of the flagellum. Electron micrograph × 139,000.

Fig. 5 (*bottom left*). Transverse section through a flagellum of a very leaky mutant (*pf*20) showing the 9 + 1 arrangement of fibres. Electron micrograph × 172,000.

Fig. 6 (*bottom right*). Longitudinal section through a 9 + 1 flagellum of strain *pf*19*B*;*su*1 showing one central fibre intact and the other disorganized along the entire length that they are visible. Electron micrograph × 172,000.

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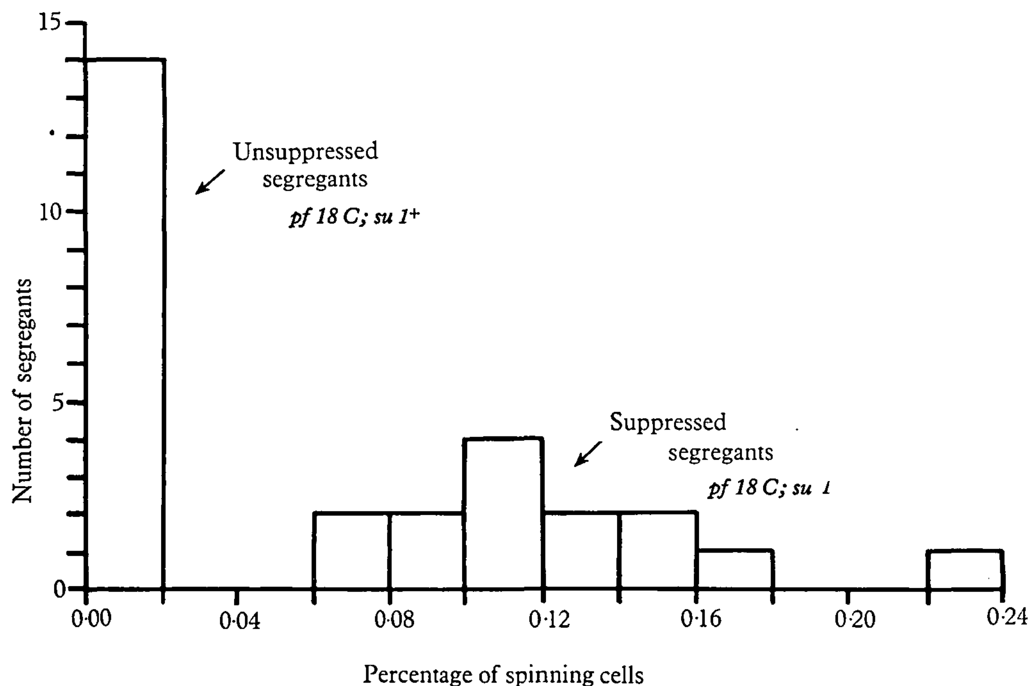
Crossing *su*1 into strains carrying each of the *pf*18 alleles did not yield any segregants suppressed to a similar extent as the *pf*15 and *pf*19 alleles. However, it will be recalled that three out of five alleles at this locus are completely non-leaky, i.e. spinning cells have not been detected amongst them (*pf*18*B*, *pf*18*C* and *pf*18*D*), and a fourth allele has less than 0·01% spinning cells (*pf*18). This situation has permitted the detection of a very slight degree of suppression of these four alleles




Text-fig. 2. Percentage spinning cells of segregants from the cross *pf*18*C*;*su* × *pf*18*C*$^{+}$;*su*1. A quarter of the segregants are entirely paralysed and have no spinning cells (*pf*18*C*;*su*1$^{+}$) and a quarter of the segregants have around 0·1% spinning cells (*pf*18*C*;*su*1). The remaining half of the segregants (*pf*18*C*$^{+}$;*su*1 and *pf*18C$^{+}$;*su*1$^{+}$) have entirely motile cells and are not represented on this figure.

which would be unrecognizable with any of the more leaky mutations. Suppressed segregants have 0·1–0·2% spinning cells which permits clear-cut distinction from unsuppressed segregants (less than 0·01%). This may be seen from the counts of spinning cells of twenty-eight segregants from the cross *pf*18*C*;*su*1$^{+}$ × *pf*18*C*$^{+}$;*su*1, which are recorded in Text-fig. 2. Wild-type segregants are not included in this figure.

Twenty flagella from strains carrying *pf*18, *pf*18*B*, *pf*18*C* or *pf*18*D* in conjunction with *su*1 have been examined in the electron microscope. Only 9 + 0 flagella have so far been found.

Data concerning partial and very slight suppression of centre-pair mutations are summarized in Table 5. Tetrad ratios are also given in this table and show that there is no evidence of linkage between *su*1 and the *pf*15, *pf*18 and *pf*19 loci. The

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abnormal segregation ratios recorded for cross *pf*15;*su*1$^+$ × *pf*15$^+$;*su*1, are a feature of all crosses involving our stock cultures of *pf*15 and are not connected with the presence of *su*1.

Table 5. *Partial and very slight suppression of centre-pair mutations by* su1

| Mutant allele | Level of suppression | Tetrad ratios in crosses *pfx*;*su*1$^+$ × *pfx*$^+$;*su*1 PD:NPD:T |
|---|---|---|
| *pf*15 | + + | (Abnormal) |
| *pf*15*A* | + + | 10:7:15 |
| *pf*18 | + | 5:1:9 |
| *pf*18*A* | − ? | — |
| *pf*18*B* | + | 2:4:11 |
| *pf*18*C* | + | 3:3:8 |
| *pf*18*D* | + | 1:1:8 |
| *pf*19 | + + | 4:2:5 |
| *pf*19*A* | + + | 2:2:6 |
| *pf*19*B* | + + | 7:6:14 |
| *pf*19*C* | + + | 3:1:5 |
| *pf*19*D* | + + | 2:1:3 |
| *pf*20 | − ? | — |
| *pf*20*A* | − ? | — |

+ + partial suppression by *su*1.
+ very slight suppression by *su*1.
− ? suppression not detected, although the possibility of slight suppression has not been excluded.

Finally, it may be useful to summarize the phenotypes resulting from the various gene combinations described above. This has been done in terms of light microscope observations in Table 6. Electron microscope studies on small samples of flagella

Table 6. *Approximate percentage of spinning cells in strains carrying the centre-pair mutations alone and in conjunction with* su1

| Locus | Number of alleles | Percentage spinning cells: Unsuppressed | Percentage spinning cells: Suppressed |
|---|---|---|---|
| *pf*18 | 4 | 0·0 | 0·1–0·4 |
| | 1 | 0·0–0·5 | (not suppressed) |
| *pf*15 | 2 } | 0·2–1·0 | 4–20 |
| *pf*19 | 5 } | | |
| *pf*20 | 2 | 1–10 | (not suppressed) |

(twenty transverse sections) have revealed only 9+0 arrangements of fibres in all strains except those involving *pf*15 and *pf*19 alleles in conjunction with *su*1 or *pf*20 alleles alone.

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## 4. DISCUSSION

Fourteen independently isolated mutations have been found to affect the formation of the central fibres of *Chlamydomonas reinhardii* flagella. Structural abnormalities of cilia and flagella have been described previously in several organisms but are either the result of rare developmental errors (e.g. Pitelka, 1962; Satir, 1962; Afzelius, 1963) or specialized products of differentiation occurring in multicellular organisms (e.g. Barnes, 1961; Afzelius, 1962) and these systems have been inappropriate for studies of the genetic aspects of flagella morphogenesis.

The range of mutants isolated on the basis of impaired motility in *C. reinhardii* merits discussion. In the majority of cases, no apparent fine structural abnormalities have been detected. This result is to be expected since a range of metabolic processes is likely to be involved in flagellar activity. It has already been found that in comparison with wild-type, certain paralysed mutants of *C. moewusii* have an altered rate of potassium uptake (Ronkin & Buretz, 1960) and a lower ATPase activity (Brokaw, 1960). Furthermore, the present electron microscope examination of the mutants has not eliminated the possibility that relatively slight structural deformities of the visible components of *C. reinhardii* flagella or basal bodies may be revealed by more extensive study.

Although mutations leading to disruption of the central fibres were isolated relatively frequently, mutations affecting the outer nine fibres have not yet been found. It may be significant in this context that the outer fibres are continuous with the basal body structure while the centre fibres terminate near the distal end of it. In addition, there is a well-known homology between basal bodies and centrioles (see review by Fawcett, 1961). Any mutation affecting the outer fibres might have effects on basal body and centriole structure, and such effects might be lethal.

The fourteen centre-pair mutations have been found to map at four unlinked loci. It would be of interest to know whether each locus represents a single cistron or several adjacent, functionally related cistrons. This question and also that of dominance relationships of the mutants may be amenable to future study, using the recently developed technique for obtaining diploids in *C. reinhardii* (Gillham, 1963).

Detailed speculation on the mode of action of these genes would be premature on the basis of results presented here. In general terms it is apparent that any of the centre-pair loci could either specify the structure of the protein components of the central fibres or affect fibre structure in an indirect manner. The number of protein species in the central fibres of *Chlamydomonas* flagella is at present unknown. In the case of *Tetrahymena pyriformis* cilia, electrophoretic studies have suggested at least five polypeptide species are present in the entire bundle of nine outer and two central fibres (Watson & Hynes, 1965), whilst serological studies suggest at least three antigens (Alexander, 1965). Favre, Boy de la Tour, Segrè & Kellenberger (1965) have recently discussed possible mechanisms of indirect action of genes controlling head formation in T-even phage and suggestions similar to some of

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theirs may be applicable in the present case. Some possible products of genes exerting indirect control of fibre structure are allosteric effectors (Monod, Changeux & Jacob, 1963), enzymes involved in the formation of centre-fibre components, flagellar components outside the centre fibres but necessary for their stability, and templates or enzymes involved in assembly of fibres.

Mutation at a fifth locus leads to partial suppression of all the mutant alleles at two of the $9+0$ loci and very slight suppression of four alleles at a third. Current theories of suppression fall broadly into two groups. Firstly, there is evidence that certain suppressors act by forming an altered component of the protein synthesizing system (e.g. an sRNA) resulting in the misreading of the mutant codon (e.g. Brody & Yanofsky, 1963; Brenner, Stretton & Kaplan, 1965; Capecchi & Gussin, 1965). This may lead to the formation of a certain proportion of functional protein molecules. Such suppressors are likely to be codon specific rather than cistron specific. The second group of theories of suppression includes all those involving interactions of primary gene products rather than changes in their initial synthesis. Often such suppressors are thought to alter the micro-environment of the cell as so to compensate for a defect inherent in the mutant protein (e.g. Suskind & Kurek, 1959; McDougall & Woodward, 1965; see also Dorn, 1965). On the basis of the present evidence, we favour a mechanism of action for the centre-pair suppressor involving the interaction of gene products rather than translational errors. This view rests on the observation that all seven alleles at the *pf*15 and *pf*19 loci are suppressible. It seems unlikely that all these mutant sites involve the same codon, as would be predicted on the translational hypothesis. Contrary to this view, it may be argued that a suppressor of mutant alleles at several distinct loci is also unexpected on the gene product interaction hypothesis, but this need not be so if the functions of these loci are closely integrated as in the present case. It will be of interest to test the effect of the suppressor on other mutations which result in non-motility without having any apparent effect on the centre fibres.

Although it is not yet possible to interpret the action of these genes in biochemical terms, certain features of the present work may provide clues for the ultimate solution of this problem. Firstly, it should be noted that some electron dense material is present along the axis of all $9+0$ flagella and this suggests that at least one major component of the centre fibre is present in the appropriate region of the flagellum although not properly organized. Furthermore the presence of normal fibres in $9+1$ and $9+2$ flagella of the very leaky mutants and of certain suppressed strains implies that the abnormality of the other fibres does not involve any very fundamental lesion of fibre morphogenesis. The organization of a considerable proportion and possibly the whole length of individual fibres in leaky and suppressed strains implies an 'all or none' control of fibre assembly and the occurrence of $9+1$ flagella demonstrates that the two fibres may be organized separately. However, the proportions of flagella types seen (i.e. $9+0$, $9+1$ and $9+2$) are incompatible with complete independence of formation of the two central fibres of a flagellum. If the probability of formation of a single fibre is $x$, complete independence would be reflected in an excess of $9+1$ flagella (probability $2x(1-x)$) over either $9+2$

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(probability $x^2$) or 9 + 0 flagella (probability $(1-x)^2$). In fact both 9 + 0 and 9 + 2 flagella were in all cases found to be in excess of the 9 + 1 type.

The significance of these observations will only be apparent when we have biochemical data to complement the genetic and electron microscope results presented here. In addition, for a full understanding of the genetic control of flagella structure, further mutants are required with a wide range of abnormalities. Consequently, we are studying the mutants with gross structural abnormalities briefly described in the early part of this paper and screening for further mutations affecting flagellar components other than the central fibres. Studies on cytoplasmic inheritance in *C. reinhardii* by Sager (1962, 1965) and Gillham (1965) and the demonstration of DNA in basal bodies of *Tetrahymena* cilia (Randall & Disbrey, 1965) suggest that a search for cytoplasmic mutations affecting the flagellum may also be fruitful.

## SUMMARY

Seventeen non-motile strains of *Chlamydomonas reinhardii* isolated by Dr Lewin and thirty-six newly isolated strains have been examined in the electron microscope for structural abnormalities of the flagella. Fourteen of them have straight, paralysed flagella in which the central fibres are replaced by an irregular core of disorganized material. The fourteen mutations map at four unlinked loci. Some of them are leaky; light and electron microscope observations on leakiness are described. In the former case leakiness is measured by the proportion of motile cells, and in the latter by the proportion of intact centre fibres seen in transverse sections. The degree of leakiness is to some extent characteristic of particular loci.

A partial suppressor of some of these mutations has been isolated which acts on all the mutant alleles at two loci and to a much lesser extent on four out of five alleles at a third locus.

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