Genet. Res., Camb. (1971), **18**, *pp.* 107–113
With 1 *plate*
Printed in Great Britain

The flagella of temporary dikaryons of *Chlamydomonas reinhardii*

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(*Received* 24 *April* 1971)

SUMMARY

The structure and function of flagella are genetically determined and single gene mutants – for example, lacking in motility or of abnormal flagellar length – have previously been investigated. When such mutants are crossed with wild-type, temporary dikaryons – prozygotes – are formed with two nuclei and a common cytoplasm. The properties of the four flagella – two originally abnormal – have been observed as a function of time. In wild-type × non-motile mutant crosses, restoration of motility has been observed in a number of cases. If the dikaryons are deflagellated regeneration occurs, together with restoration of motility or of normal length to the previously abnormal pair. Complementation at the cytoplasmic level has been found in paired mutants.

This paper is part of a longer study (Randall *et al.* 1964, 1967; Warr *et al.* 1966; Cavalier-Smith, 1967; Hookes, Randall & Hopkins, 1968; Randall, 1969; Jacobs, Hopkins & Randall, 1969; Jacobs & McVittie, 1970; Chasey, 1969; Hopkins, 1970; Starling, 1969; McVittie, 1969*a*, *b*; Starling, 1970) of the morphopoiesis of a eukaryotic organelle and gives a preliminary account of the properties of the flagella of temporary dikaryons of *Chlamydomonas reinhardii*. We have recognised for some time that the application of heterokaryon techniques (Ephrussi & Weiss, 1965; Harris & Watkins, 1965; Harris 1970) to our own system might be of value, especially in the elucidation of control mechanisms and in more rapid complementation analysis. As a temporary expedient for the production of cell fusion in *C. reinhardii*, use has been made of the mating process which occurs naturally as part of the sexual cycle of this organism (Levine & Ebersold, 1960). Prozygotes are produced from the mixing of wild-type cells of opposite mating types, a process which can achieve an efficiency of some 80%. For our purpose, the mating of wild-type cells with flagellar mutants is of greater interest; unfortunately in such matings the efficiency is frequently very much less, but has nevertheless been sufficient for our initial purpose.

When flagella are removed from haploid cells of *C. reinhardii* (Randall *et al.* 1967; Randall, 1969; Rosenbaum & Child, 1967; Rosenbaum, Moulder & Ringo, 1969) new flagella are formed over a period of a few hours. The essence of our

experiments derives from the fact that for some hours after union of the gametes the *Chlamydomonas* prozygote retains both pairs of flagella, the two nuclei remain separate (Cavalier-Smith, 1967) and the cell possesses a 'mixed' cytoplasm. It has thus been convenient to refer generally to these early prozygotes as temporary dikaryons. In particular we have used the terms homokaryon and heterokaryon to describe the dikaryons produced by the mating of cells homozygous and heterozygous respectively for genes controlling motility and flagellar length. During this short period it has proved possible to remove some or all of the four flagella from the prozygote and to observe their regeneration before the normal regression of early maturation begins. Technical details for the culture (Sueoka, 1960) and gametogenesis (Sager & Granick, 1954) of *C. reinhardii* are well known and will not be dwelt on here. Similarly it will be assumed that the general features of the sexual life-cycle of this organism are familiar (Levine & Ebersold, 1960).

1. THE FLAGELLA OF HOMOKARYONS

(i) *Early movement*

When wild-type gametes of opposite mating type are mixed, single quadriflagellate, binucleate prozygotes are duly formed. These cells are not immediately motile. For some 10 min after completion of cell fusion flagellar movement is uncoordinated and some irregular backward movement of the cell is often noticed. The forward movement of the quadriflagellate prozygote begins subsequent to the observable coordination of the flagella pairs.

(ii) *Regression*

The full-length flagella of homokaryons persist for some 2–3 h and then begin to regress, the length decreasing linearly with time at a velocity at 25 °C of $\sim 2 \times 10^{-9}$ ms^{-1}; the whole process is complete within about 1·5 h. This phenomenon of linear regression is also exhibited by the intact flagellum of a haploid cell following the amputation of its partner (Randall, 1969; Rosenbaum & Child, 1967; Rosenbaum *et al.* 1969). In this instance the pattern of regeneration is variable; sometimes it begins before regression is complete, sometimes not. Similar patterns of complementary regeneration and regression have been observed in prozygotes when less than four flagella have been removed. In these experiments selective flagellar amputation was achieved by the use of an ultraviolet microbeam (Starling, 1970). Neither in haploid cells nor prozygotes is there any evidence from electron micrographs of withdrawal of the intact flagellum into the body of the cell (Cavalier-Smith, 1967).

(iii) *Flagellar regeneration in homokaryons (prozygotes)*

When populations of prozygotes are deflagellated, the subsequent regeneration of the flagella can be followed photographically (Randall, 1969; Rosenbaum *et al.* 1969). In dikaryons – as in haploids and diploids – all flagella (four, two and two respectively) regenerate. In dikaryons the mean length of each of the four flagella

has not been observed to exceed about 8 μm, whereas the two flagella of a haploid gamete would normally reach a length of 10–12 μm. If prozygotes with regenerated flagella are not interfered with, the next stage in the life cycle, namely regression of all four flagella, proceeds normally.

Work on flagellar regeneration in normal vegetative cells (Rosenbaum *et al.* 1969) has shown by the use of cycloheximide that the necessary protein is derived both from a residual pool and *de novo* synthesis; this result has been confirmed by Starling (1970). With a concentration of cycloheximide sufficient to inhibit the synthesis of TCA-insoluble protein, it has been shown that the haploid cells contain a residual pool of protein precursors sufficient to make two approximately half-length flagella. Under the same conditions the four flagella of homokaryons also regenerate to about half-length. In broad terms such a result is to be expected, since each gamete presumably contributes equally to the general pool of the homokaryon. Also on general grounds it could be inferred that the total amount and the concentration of precursor protein would both be significant. In normal regeneration (i.e. no cycloheximide present) the mean flagellar lengths of homokaryotic prozygotes are about 80 % of the mean haploid value and well above that compatible with the known size of the total residual pool. It is therefore inferred that synthesis of some flagellar protein takes place during regeneration of prozygote flagella.

2. THE FLAGELLA OF HETEROKARYONS

Important characteristics of the flagellar mutants used in our initial crosses with wild-type gametes are listed in Table 1.

Owing to the low mating efficiency of these crosses, preparations of heterokaryotic prozygotes were invariably contaminated with haploid cells. However, because

Table 1. *Flagellar characteristics of certain mutants of* C. reinhardii

		Phenotypic characteristics	
Mutant	Linkage group	Light microscope	Electron microscope (axoneme)
pf 16	IX	Straight flagella; some movement; variable angle between flagella	9+2
pf 17	VII	Curved flagella; normal length	9+2
pf 18C	II	Rigid, straight, V-shaped flagella pair	9+0 (non-leaky)
pf 19B	X	Rigid, straight, V-shaped flagellar pair	9+0 (slightly leaky)
Lf 1	II	Long, generally unequal flagella; slow regeneration	9+2

of characteristic differences in size and shape it was always possible to distinguish between the two types of cell.

(i) *Changes in the motility of temporary heterokaryons*

Experiments have been made to discover whether the paralysed flagella of a heterokaryon can have their motility restored without recourse to the regenerative process. For this purpose crosses of the mutants listed in Table 1 (Randall *et al.* 1964; Warr *et al.* 1966; Starling, 1969; McVittie, 1969*a*, *b*) have been made to wild-type strains. Individual prozygotes were then observed continuously for overall motility of the cell and with a view to the detection of motility in the hitherto paralysed pair. The following results were obtained.

(*a*) *Restoration of cell motility*

Temporary heterokaryons were formed by crossing wild-type with pf 16, pf 18C and pf 19B. In all cases the heterokaryons were able to swim after about 10 min. This period is comparable with that observed in homokaryons and there is thus no abnormal lag in the restoration of cell motility.

(*b*) *The behaviour of the originally paralysed flagellar pair*

The fact that heterokaryons formed from the union of wild-type and mutant cells can swim is no guarantee that all four flagella are necessarily active, as the results in Table 2 demonstrate.

Table 2. *Flagellar motility in undeflagellated heterokaryons*

Heterokaryon	Approximate value of time (min) required for swimming to begin using: Two flagella	Four flagella
1. Wild-type × pf 16	—	10
2. Wild-type × pf 18C	10	No observed in 40
3. Wild-type × pf 19B	10	20

(1) *Wild-type × pf 16.* All four flagella are used in swimming 10 min after cell fusion. This is virtually identical with the homokaryon Wt × Wt cited above and there is no stage in the development of motility when only two flagella are used. Restoration of motility to the paralysed pair is thus quite rapid.

(2) *Wild-type × pf 18C.* The heterokaryon swims using two flagella only in about 10 min after cell fusion. Over a period of 40 min, motility in the originally paralysed pair has not been detected.

(3) *Wild-type × pf 19B.* Motility appears in two stages. The heterokaryon begins to swim in 10 min using the presumptively normal pair of flagella. Only after 20 min are all four flagella used. Restoration of motility in the original paralysed flagella of heterokaryons involving wild-type cells is thus common, but not apparently universal.

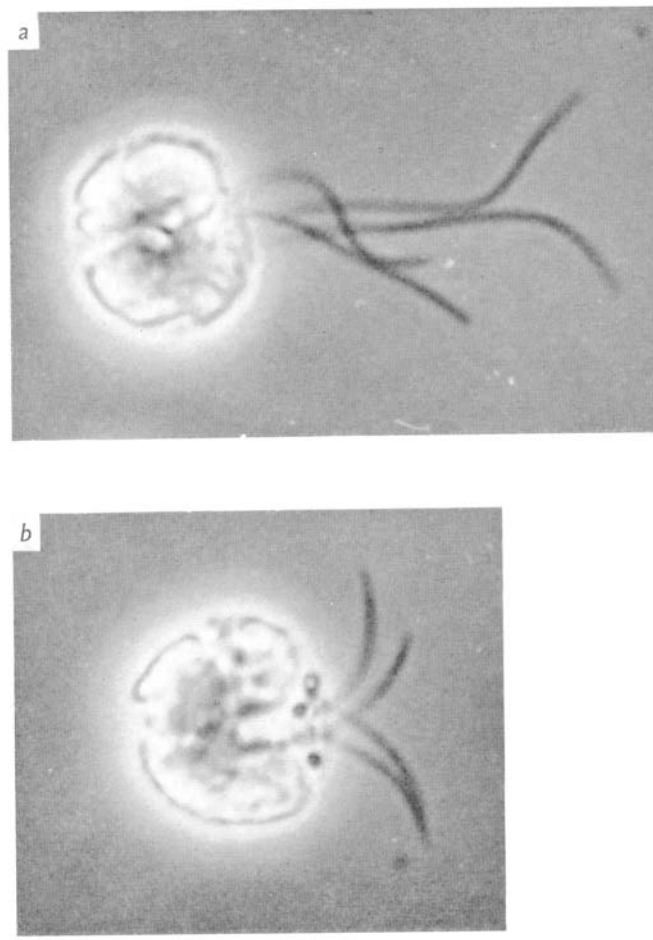

Fig. 1. (*a*) Photomicrograph (× 2500) of fixed prozygote formed by crossing Wt with Lf 1. Thirty minutes after mating the flagella are those of the original haploid cells and the long ones of Lf 1 are clearly distinguishable from Wt flagella. (*b*) A similar prozygote from which the original flagella have been removed, thus allowing regeneration to take place, photographed 70 min after deflagellation. There is now virtually no difference in the lengths of the two pairs of flagella, which are on average about 80% of the wild-type value.

https://doi.org/10.1017/S0016672300012465 Published online by Cambridge University Press

(*c*) *The behaviour of regenerated flagella in heterokaryons*

Heterokaryons 1 and 2 of Table 2 have been deflagellated and the flagella allowed to regenerate. In both examples the heterokaryons use all four flagella for swimming. It appears from the results on the Wild-type × pf 18C heterokaryon that restoration of quadriflagellate motility is easier when the flagella are being newly formed. The wild-type × pf 19B heterokaryon has not yet been stated.

(*ii*) *The flagella of dikaryons containing the long-flagella mutant Lf 1*

(*a*) *Regeneration*

The very much longer period required by Lf 1 for complete regeneration is already known (McVittie, 1969*a*, *b*; Starling, 1970). Fig. 1(*a*) illustrates how readily the long flagella of this mutant can be distinguished from those of the wild-type in the heterokaryon Wt × Lf 1 prior to deflagellation. After deflagellation, regeneration of all four flagella takes place at approximately the same rate as for wild-type haploid cells. Moreover, the lengths of all four regenerated flagella are now approximately equal in length, although rather shorter than those of wild-type cells (Fig. 1*b*). It again appears that the wild-type genome dominates the phenotypic characteristics of the heterokaryon flagella.

(*b*) *Complementation at the cytoplasmic level*

By the use of artificially induced diploids (Ebersold, 1967) it has been already established that complementation takes place between unlinked flagellar mutants of *C. reinhardii* (Starling, 1969). Corresponding studies have now been carried out on suitable temporary heterokaryons of paired mutants. As to be expected, the yields of prozygotes were low; it has, however, been possible to establish that complementation takes place. Differences in the rate of restoration of motility to particular pairs of flagella have again been detected. One example will suffice:

pf 17 × pf 19B. The flagellar behaviour follows a distinct pattern with respect to time. (*a*) At first all four flagella of the prozygote are non-motile. (*b*) After a short interval swimming prozygotes with two motile flagella and a pair of straight trailing flagella were seen. Since the pf 19B flagella are straight and those of pf 17 curved, it can be inferred that the flagella of pf 17 recover their motility more rapidly. (*c*) At a later stage prozygotes swam with all four flagella.

3. DISCUSSION

An important result of these preliminary experiments with *C. reinhardii* lies in the dominance of wild-type gene products in the flagella of heterokaryons. So far, the analysis of this dominance has been limited to phenotypic characteristics observable in the light microscope. These observations are now being amplified by studies in the electron microscope, both of the original flagella of the heterokaryons and of those reformed after deflagellation. *A priori* it seems probable that the central pair of tubules of defective flagella in Wt × pf18C and in Wt × pf19B

heterokaryons will become structurally normal as motility is restored. However, if this does not happen, the functional importance of the central pair becomes questionable. The mechanism of any restoration of structure is of great interest and could obviously involve breakdown or repair of existing subunits. In heterokaryons involving 9+2 mutants, such as pf 16, pf 17 and Lf 1, no obvious structural difference from wild-type is as yet known to exist. (Further experiments on diploids and heterokaryons involving the Lf 1 mutant are in progress.) If such differences cannot be found, it would seem that non-structural components of the flagella are involved.

The application of existing cell-fusion techniques (Harris & Watkins, 1965; Harris, 1970; Power, Cummins & Cocking, 1970; Poole, Howell & Lucy, 1970) and the exploration of new possibilities is essential if the full potential of our approach is to be realised. For example, we cannot at present form interspecific heterokaryons between cells of *C. reinhardii* and *C. moewusii*, which has a different pattern of gene dominance. It would be most interesting to follow up early work on *C. moewusii* (Lewin, 1954), by the development of interspecific heterokaryons. Without further evidence, however, the application of existing techniques to organisms surrounded by cell walls must be in doubt; but there is some reason to be hopeful of success with gametes in which the wall is believed to be absent from the anterior parts. Work on these lines is already in progress.

Corresponding information for heterokaryon flagella regenerated in the presence of cycloheximide would be valuable. To take one hypothetical example: in a *C. reinhardii* heterokaryon of the type Wt × mutant we would expect wild-type dominance to be expressed. If, therefore, the mutation has resulted in the synthesis of a defective limiting protein, the heterokaryon in the presence of cycloheximide would have sufficient protein to make only four quarter-length flagella. On the other hand, if the mutant flagellar protein is normal, and some 'accessory' – but necessary – substance abnormal, the wild-type accessory would be dominant and four half-length flagella would be expected. We are at present engaged on apparent pool measurements for a variety of mutants, homokaryons, heterokaryons and homo- and heterozygous diploids.

Our experiments have also provided a means of measuring the regression of flagella in populations of cells as part of the natural life-cycle, but do not at this stage add to our knowledge of the mechanism of this process. It is noticeable that the regression rate already quoted is not very different in magnitude from the normal rate of regeneration in its early stages. Later of course regeneration slows down, thus leading to a characteristic value for flagellum length.

Much of the work described in this report was carried out in the Department of Biophysics, University of London King's College and the Medical Research Council Biophysics Unit and has been continued in the Department of Zoology, University of Edinburgh. We are indebted to Professor J. M. Mitchison and Professor P. M. B. Walker for provision of facilities and the Medical Research Council for continued support.

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