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PRELIMINARY REPORT

CONTROL OF FLAGELLUM FORMATION IN *NAEGLERIA GRUBERI*

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Naegleria gruberi, a group of soil-water amoebae, is capable of transforming from an amoeboid form to a temporary flagellate form, when suspended in dilute aqueous solution. Shuster (1963) and Dingle and Fulton (1966) observed by electron microscopy that the flagellar apparatus, consisting of flagella, their basal bodies (centriole-like structures) and rhizoplasts, are newly formed during amoebo-flagellate transformation. They could not find any centriole-like structure or its precursor in amoebae or even in mitotic amoebae. The origin of the centriole-like basal bodies during transformation has not been clarified. Many biologists have suggested that centrioles are " self-replicating organelles " containing DNA, but the idea that the centriole-like structure of basal bodies arises *de novo* in *Naegleria* has been generally accepted (Fulton, 1971). Thus, the amoebo-flagellate transformation of *Naegleria* provides a most attractive system for studies on cellular differentiation and regulation at the organella level.

Several factors which influence the formation of flagella have been reported. Factors such as a high ionic concentration, deoxycorticosterone, progesterone, pentamethonium, yeast extract and bacteria as a food are all potent agents in keeping *Naegleria* as an amoeba and preventing the change to the flagellate phase (Perkins and Jahn, 1970; Willmer, 1970; Fulton, 1970). However no definite mechanism triggering off the transformation has yet been found. Actinomycin D and cyclohexamide also inhibit the formation of flagella (Fulton, 1970). Amoebae possessing the necessary genetic information seem to be able to respond to certain changes in their environment by producing the flagellar apparatus. This communication reports the effects of cyclic adenosine-3′, 5′-phosphate (cAMP) and temperature on the transformation. *N. gruberi*, AB-2, was isolated by us from soil. Methods for its cultivation and for measurement of the percentage of flagellates and of flagella per flagellate were as described by Fulton and Dingle (1967).

1. *Effect of cAMP on amoebo-flagellate transformation*

Recent studies have shown that cAMP acts as a regulatory agent at the level of transcription and translation, and also at the post-translational level in unicellular organisms (Robison, Butcher and Sutherland, 1971; Riedel et al., 1973). We examined the effect of cAMP on the induction of flagella formation in *N. gruberi*. Fig. 1 shows results on the effects of adding cAMP at various times during incubation. cAMP completely inhibited the

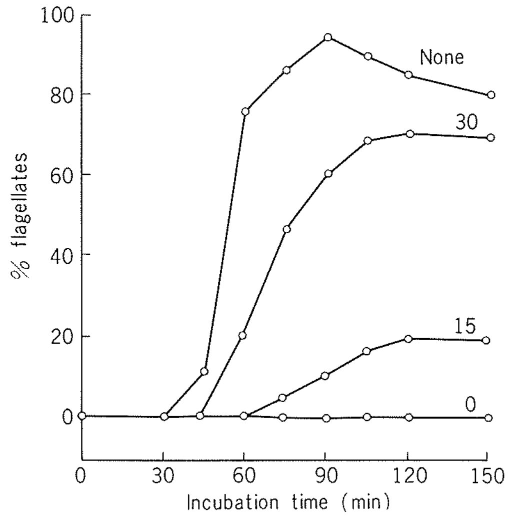

FIGURE 1. *Effect of cAMP on a transforming population. Amoebae grown on a nutrient agar plate with Aerobacter aerogenes at 33 C overnight were suspended in 0.002* M *Tris-(hydroxymethyl) aminomethane-HCl buffer at pH 7.4, washed almost free of Aerobacter by differential centrifugation and resuspended in Tris buffer at a final concn of approximately* 2×10^6 *cells per ml. Volumes of 2 ml of the suspension were put in test tubes and shaken at 28 C. Cyclic AMP was added at a final concn of 1* mM *after incubation for 0, 15 and 30 min. The progress of transformation was evaluated by counting the percentage of flagellates (cells with flagella) in samples fixed at 15 min intervals in Lugol's iodine.*

appearance of flagella when added at the beginning of incubation. When added after 30 min incubation, however, its inhibitory effect was no longer observed. cAMP seems to act on the early step of transformation. The inhibitory action of cAMP is reversible. Removal of cAMP from the cell suspension induces flagella formation with the normal latent period. When actinomycin D is added at the time of removal of cAMP, the transformation remains completely inhibited. This result could be interpreted to mean that cAMP acts at the transcriptional level. However, an alternative explanation is that cAMP acts directly on a precussor of the flagellar apparatus, such as microtubules and prevents the appearance of flagella and their basal bodies, as microtubular protein can be phosphorylated *in vitro* by cAMP-dependent protein kinase (Goodman et al., 1970). The amoeboflagellate transformation is not affected by cGMP at a concn of 1 mM. At present no conclusions can be drawn on the inhibitory mechanism of cAMP on the amoeboflagellate transformation, but in further studies the inhibitory actions of the various factors listed above might be shown to be due to that of cAMP. Electron microscopic observations revealed that no centriole-like structure is formed in the presence of cAMP. Recently, Rubin and Filner (1973) reported that cAMP influences both flagella function and flagella regeneration (elongation) in *Chlamydomonas reinhardtii*. However, they did not study the *de novo* formation of basal bodies.

2. *Effect of temperature on the number of flagella*

Flagellates of *N. gruberi* usually have two flagella. Dingle (1970) described the production of multiple basal bodies and flagella (average number 4.5 per cell) on exposing amoeboid cells of *N. gruberi* NB-1 to the sublethal temperature of 38–39 C for the first 50 min of incubation of the transformation procedure. We repeated this temperature-shock experiment on strain AB-2, but could not confirm their finding even at 38–39 C or at 42 C. Incubation of amoebae at 45 C caused irreversible rounding of cells. Strain AB-2 could grow at 40 C on nutrient agar with *Aerobacter aerogenes* for food, whereas strain NB-1 used by Dingle could not. So, we tried to isolate temperature-sensitive (*ts*) mutants of AB-2 which do not grow at 40 C. The method for isolation of these *ts* mutants was as follows.

Amoebae at a concn of approximately 2×10^6/ml in 0.002 M Tris-HCl buffer at pH 7.4 were irradiated with about 2500 ergs/mm^2 of ultraviolet light which resulted in 1–5% cell survival. The surviving cells were plated on nutrient agar with enough bacteria to form a confluent lawn. When the amoebae had cleared the bacterial lawn, they were suspended

TABLE 1. *Effect of temperature shock on number of flagella per flagellate*

Strain	Incubation	Number of cells having n flagella				
		n=1	2	3	4	>5
AB–2	28 C for 120 min	11	187	1	0	0
AB–2	40 C for 60 min, and then 28 C for 60 min	8	188	4	0	0
ts–1	28 C for 120 min	10	190	0	0	0
ts–1	40 C for 60 min, and then 28 C for 60 min	12	188	0	0	0
ts–2	28 C for 120 min	3	187	4	6	0
ts–2	40 C for 60 min, and then 28 C for 60 min	3	57	52	76	12

in Tris-HCl buffer, appropriately diluted and again spread on nutrient agar plates to give about 20 " plaques " per plate. These plates were incubated at 28 C for 2 days, and then replica-plated with " velveteen " onto plates spread with bacteria. The replicated plates were incubated overnight at 40 C. Then they were compared with the original plates to locate missing plaques on the replicas. In this way we have so far isolated seven *ts* mutants.

As shown in Table 1, one of those *ts* mutants, *ts*-2, underwent temperature shock as described by Dingle (1970) and produced more than two flagella (average 3.3) when incubated at 40 C for the first 60 min during the transformation procedure. Other *ts* mutants did not show the temperature shock. Hence, the formation of multiple flagella by exposing amoebae to sublethal temperature does not seem to be a usual reaction in *Naegleria* species, but rather an abnormal process caused by a mutation. At present, however, we do not know whether single gene mutation in *ts*-2 causes both the temperature-sensitive growth and the formation of multiple flagella, because of the lack of a good method for genetic analysis of *N. gruberi*.

The results presented here are one example that mutation and the environment can act together to influence the development of cell character.

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