Timing of mitosis in Physarum polycephalum: Effects of agents affecting cyclic AMP Concentrations

J. R. TREVITHICK and R. BRAUN[1]

Department of Biochemistry, University of Western Ontario, London (Ontario, Canada N6A 5C1); and Institute of General Microbiology, University of Bern, Altenbergrain 21, CH-3013 Bern (Switzerland), 10 June 1976

Summary. Cultures of *Physarum polycephalum* incubated with caffeine or theophylline for over 100 min prior to mitosis exhibited mitotic delay proportional to the time of treatment before 100 min. Starved cultures exhibited mitotic delay at times of starvation longer than 180 min and slight stimulation from 100–180 min. Dibutyryl cAMP appeared to accelerate reconstruction of the nucleus following mitosis.

A large number of studies have indicated that cyclic AMP has a function in the regulation of the process of mitosis[2–7]. All of these studies are consistent with the view that mitosis is inhibited by high concentrations of cyclic AMP. In addition, it seems likely that a 'spike' of cAMP occurs before mitosis can be initiated. The phosphodiesterase inhibitors caffeine and theophylline are known to cause increases in intracellular cyclic AMP levels and retardation of mitosis[7–10]. One of the organisms best suited for studies of physiological aspects of mitosis is the myxomycete *Physarum polycephalum*. When grown under defined conditions nearly all nuclei of one single, macroscopic plasmodium undergo synchronous mitosis[11–13].

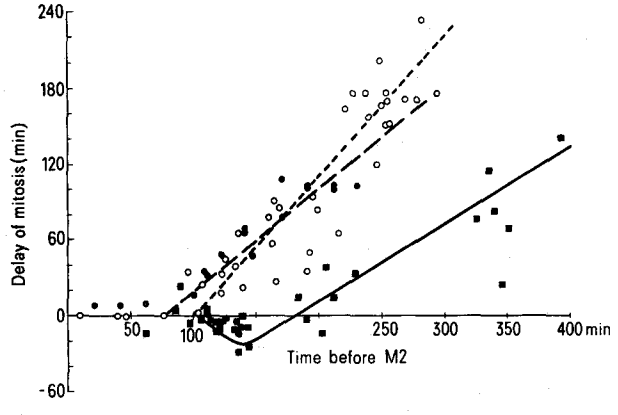

Fig. 1. Effect of caffeine, theophylline or starvation on timing of mitosis in *Physarum*. Treatments: --○--, caffeine (3 m*M*); --×--, theophylline (3 m*M*); —■—, starvation.

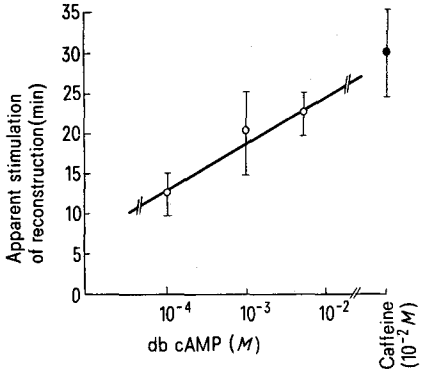

Fig. 2. Apparent stimulation of reconstruction in *Physarum* by dibutyryl cyclic AMP and caffeine. Cultures were placed on medium containing dibutyryl cyclic AMP or caffeine (10 m*M*) for 46 min prior to mitosis and observed at 20 min following mitosis in the control cultures. The point for caffeine (10 m*M*) is plotted for comparison: —●—.

Experiments were done prior to the second synchronous mitosis, using *Physarum polycephalum* grown on N+C−P medium[14]. When cultures obtained by plating up to 0.9 ml of microplasmodial suspension as a ring on a filter paper were cut into 10 pieces and these pieces replaced on the old medium, all pieces of culture went through mitosis at an identical time, within 5 min of each other.

Whole cultures or segments of cultures were always transferred to new media while still on their original filter paper supports. At every transfer, the culture was first blotted on a paper towel, then immersed in new medium, blotted again and finally placed on a larger filter paper supported by a stainless steel grid over the new medium. In starvation experiments, a segment of the culture was transferred as a control to new N+C medium, while the rest of the culture was twice submerged in, and then kept on, salts medium. This is a N+C growth medium lacking sugar, citrate, phosphate, tryptone and yeast extract. To facilitate taking of samples, the treated culture was sometimes cut into small segments which were then placed far apart on filter paper to prevent fusion.

Mitosis was determined microscopically, using fixed smears. The stages of reconstruction were determined with reference to a standard set of slides. This permitted determination of the time of mitosis with a certainty of approximately 5–10 min.

Cultures were treated with a concentration of caffeine (3 m*M*) found in preliminary experiments to inhibit the timing of mitosis but not to have a strong inhibitory effect on growth of the cultures over a 21-hour period. This concentration of caffeine did not have any effect

[1] Acknowledgments. The authors wish to express their appreciation to Dr. Volker Vogt and Dr. Richard Threlfall for helpful discussions, to Kathryn Behrens for able technical assistance and to the European Molecular Biology Organization for a short-term fellowship to J. R. T. during the course of this work. Supported by grant 3.501.75 of the Swiss National Science Foundation.

[2] B. M. BOMBIK and M. M. BURGER, Expl Cell Res. *80*, 88 (1973).
[3] R. H. RIXON, J. F. WHITFIELD and J. P. MACMANUS, Expl Cell Res. *63*, 110 (1970).
[4] G. TISMAN and V. HERBERT, In vitro *9*, 86 (1973).
[5] R. A. HARPER, B. A. FLAXMAN and D. P. CHOPRA, J. Invest. Dermat. *62*, 384 (1974).
[6] L. VON SALLMAN and P. GRIMES, Invest. Ophthalm. *13*, 210 (1974).
[7] I. YASUMASU, A. FUJIWARA and K. ISHIDA, Biochem. biophys. Res. Commun. *54*, 628 (1973).
[8] C. E. ZELLEG, R. A. JOHNSON, D. L. FRIEDMAN and E. W. SUTHERLAND, J. Cell Biol. *55* (2, pt 2); 296a Abstr. (1972).
[9] M. J. BUETTNER, E. SPITZ and H. V. RICKENBERG, J. Bact. *14*, 1068 (1973).
[10] P. K. WAYNE and O. M. ROSEN, Proc. natn. Acad. Sci., USA *71*, 1436 (1974).
[11] F. L. HOWARD, Am. J. Bot. *18*, 116 (1931).
[12] E. GUTTES, S. GUTTES and H. P. RUSCH, Devel. Biol. *3*, 588 (1961).
[13] H. C. ALDRICH, Am. J. Bot. *56*, 290 (1969).
[14] D. BERSIER and R. BRAUN, Biochim. biophys. Acta *340*, 463 (1974).

in inhibiting mitosis when added to cultures within a period of approximately 100 min prior to mitosis (Figure 1). Before this time, incubation on caffeine for increasing time appeared to result in a delay of mitosis which was approximately a linear function of time. Cultures similarly incubated with theophylline at the same concentration (3 m*M*) showed an almost identical pattern of delay of mitosis (Figure 1).

When cultures were starved for a long time, some asynchrony appeared to develop. Usually it was possible to evaluate the time of mitosis if the smear was taken from the growing edge of an advancing lobe[15]. Cultures starved for a period of time longer than 180 min showed a delay of mitosis which increased for longer periods of starvation (Figure 1). Between about 100 min prior to mitosis and 180 min, a small but consistent acceleration of mitosis by starvation occurred.

Preliminary experiments have shown a marked increase in cyclic AMP levels in cultures starved for 6.5 h prior to the expected time of mitosis, which is accompanied by marked delays in mitosis. Other cultures at shorter times of starvation, in which mitosis is not inhibited, showed a small spike of cAMP (only double normal levels at its maximum) at approximately 75 min prior to mitosis. These data are consistent with a model in which the peak in cAMP is a determining step in mitosis.

Cultures which were dipped in salts medium appeared to gain water and when scraped off the papers appeared more slimy than control cultures. When such cultures were compared to those of controls not exposed to salts medium, in 5 different experiments the weight of the frozen starved plasmodia was 86.1 mg/mg protein, while that of controls was 67.9 mg/mg protein, a 27% increase in wet weight as a result of the starvation treatment. The effect was noticeable already after 15 min.

No consistent delay of mitosis was observed in several preliminary experiments using dibutyryl cyclic AMP in the medium. It is possible that endogenous levels of the cyclic nucleotide phosphodiesterase are high enough in G2 to prevent significant accumulation of cyclic AMP under these conditions. It was observed, however, that dibutyryl cyclic AMP at high levels, when added to cultures near mitosis, appeared to speed up the overall process of mitosis and reconstruction (Figure 2). The stimulation of the overall process appears to be a linear function of the log of the concentration of cAMP (Figure 2). In caffeine (10 m*M*), an even greater acceleration of reconstruction after mitosis was found than that shown by db-cAMP.

[15] E. Guttes and S. Guttes, Expl. Cell Res. *30*, 242 (1963).

Mode of action of Acorus calamus L. oil vapours on adult male sterility in red cotton bug

O. Koul, K. Tikku and B. P. Saxena

Regional Research Laboratory, Council of Scientific and Industrial Research, Jammu Tawi 180 001 (India), 1 March 1976

Summary. In normal freshly moulted insects Spermotozoa are present and pass into the vas deferens after 24 h. They show morphological changes when inside the female genital tract. Sterility caused by Acorus calamus oil vapours is due to agglutation, malformation and immobility of sperms, which makes the sperms unable to undergo morphological differentiation in the female tract.

Acours calamus oil vapours have been found to sterilize male house flies[1] (Musca domestica L.); females of Thermobia domestica (Packard)[2]; Dysdercus koenigii, F[3]; and females of five stored product beetles[4]. Unlike classical chemosterilants, Tepa and related compounds which have high mammalian-toxicity and phytotoxicity[5], the oil is a non-toxic compound. The investigations into the mode of male sterilization have been made by taking D. koenigii as the test insect.

Material and methods. The bugs were reared as described earlier[6]. Male and female adult insects were separated immediately after moulting into separate jars and provided with food and water. 0–4-hold males were exposed to oil vapours for 4 days by the method already described[3]. Random samples containing 10 insects were taken from the control and the thrice replicated experiments each day. For various studies, Aceto orcein stained squashes of testes, vas deferens and oviduct were prepared.

Results. In normal insects, the spermatogenesis was maximum in freshly moulted adult insects, where the meiotic figures were quite apparent. The number of sperms varied in different testes; but no individual lacked such structures. No sperm was found in the vas deferens in such individuals. In 24–48-h-old insects, the bundles of sperms were seen in the testes and the vas deferens. Numerous sperms were seen separating from each other (figure 1), with their bodies in complete swirling waves (figure 2). The sperms entered the vas deferens only 24 h after moulting and their maximum number was found in 72-h-old adults. Copulations were observed 24 h after moulting. The maximum number of sperms traced in the female tract was found 24 h after copulation. In the female tract, the sperms showed definite morphological changes, particularly in the head or nuclear region. This end becomes broad and flattened (figure 3) where the body shows normal swirling waves.

In treated insects, after 24 h of the treatment, the meiotic divisions were normal but the sperms appeared to be unable to move. In 48-h-old treated insects, the sperms in the testes and vas deferens were very elongated structures (figure 4). Most of them were adhered to each other. The head was either highly elongated (figure 5) or com-

1 A. C. Mathur and B. P. Saxena, Naturwissenschaften *62*, 576 (1975).
2 B. P. Saxena and E. B. Rohdendorf, Experientia *30*, 1298 (1974).
3 B. P. Saxena and A. C. Mathur, Experientia *32*, 315 (1976).
4 B. P. Saxena, *in press*.
5 D. G. Campion, Bull. ent. Res. *61*, 577 (1972).
6 B. P. Saxena and J. B. Srivastava, Experientia *28*, 112 (1972).