## Prophase-like Chromosome Condensation Induced by Actinomycin D in the Slime Mold, *Physarum polycephalum*[1]

Since the discovery that actinomycin D specifically inhibits, *in vitro*[2] and *in vivo*[3], the DNA-dependent formation of RNA, this compound has been employed extensively for investigating the formation of messenger RNA and its role in cell growth and differentiation[4–12] and in mitosis[4,5,8,11]. During an investigation of differential sensitivities of macromolecular syntheses toward actinomycin D at different times of the mitotic cycle in *Physarum polycephalum*, we found that the drug caused morphological alterations of the nuclei which, in plasmodia that were placed on the actinomycin during early and mid-interphase, were different from that produced in plasmodia which were exposed to the drug at a later period. The following report deals with these morphological alterations.

Plasmodia of the slime-mold, *Physarum polycephalum*, were prepared by allowing microplasmodia growing in agitated submersed culture[13] to coalesce on filter paper as described elsewhere[14,15]. Such plasmodia, since their nuclei undergo mitosis in synchrony[14,16], are comparable, for practical purposes, to giant multinucleated 'cells'. We shall apply in the following the term 'mitotic cycle' to that period in the life cycle of a plasmodium which elapses from any stage of synchronous mitosis to the same stage of the next mitosis.

We employed for the experiments plasmodia which had completed the second synchronous post-fusion mitosis. In such plasmodia, the completion of a full mitotic cycle required approximately 12–14 h. At different times after the second synchronous post-fusion mitosis, sectors were removed, in duplicate, from the plasmodia. One of the sectors ('experimental sector') was placed on growth medium to which actinomycin D had been added, with ethanol (final concentration 2%) as carrier (see [4]), to give a final concentration of 125 μgm/ml. This comparatively high concentration was found necessary, in preliminary experiments, to inhibit the incorporation of uracil-$H^3$ into RNA (see [5], and unpublished experiments) and to produce significant morphological alterations of the nuclei. The other sector ('control sector') was transferred to a culture dish containing growth medium of identical composition as that used for the experimental sector, and containing the same amount of ethanol as above, but without actinomycin. The experimental sectors remained on the actinomycin-medium until their nuclei entered metaphase. In each experiment, one sector was left in the original culture dish. This sector was used to determine later, in retrospect, at which time in relation to the whole mitotic cycle the exposure of the experimental sectors to actinomycin had begun. The control sectors were used to determine, by comparison with the corresponding experimental sectors, the effect of the drug upon the nuclear morphology and upon the onset of mitosis. Both these determinations could be made with precision and without need for statistical analysis, since it was previously established[17] that the nuclei of different plasmodial pieces which are removed simultaneously from one plasmodium and placed on different culture dishes containing growth media of identical composition, will undergo the next following mitosis almost simultaneously. They enter the next prophase within $\pm 10$ min, when the sectors were removed at the beginning of the intermitotic period, and, in pieces which were separated at a more advanced stage of the intermitotic period, the degree of synchrony is even better. Hence, any difference larger than 20 min between the experimental pieces and the control pieces with regard to the morphology of the nuclei or to the timing and duration of mitosis was considered as a result of the addition of the actinomycin to one of them. In order to determine the stage of the mitotic cycle at which a plasmodium, or a plasmodial sector, had arrived at any time prior to, or during an experiment, ethanol-fixed smear preparations, obtained from the plasmodial periphery, were examined, unstained, under phase-contrast.

Figure 1 shows nuclei of a plasmodial sector which was placed, at 6 h after mitosis, on growth medium containing actinomycin D for a total of 2 h. Figure 2 shows nuclei of the control sector at the same time, i.e. 8 h after mitosis. For comparison, Figure 8 shows nuclei of a plasmodium which had been starved on non-nutrient balanced salt solution[13] for more than 36 h. The nuclei of the actinomycin-treated plasmodium are similar to those of the starved plasmodium and both differ from those of the control piece in nuclear and nucleolar size, and in the more coarse-granular appearance of the chromosomes in the former.

Figure 3 shows the prophase of the same plasmodium as in Figure 1, 6 h and 25 min later, and Figure 4 shows the prophase of the control piece (same as in Figure 2), which began approximately 20 min prior to that in the corresponding experimental piece of Figure 3. For comparison, Figure 7 shows the prophase of a plasmodium which was starved on non-nutrient balanced salt solution for approximately 36 h[15]. The prophase of the actinomycin-plasmodium was morphologically similar to that in the starved plasmodium, and both differed from that in the growing control piece mainly in nucleolar and nuclear size, as before, and in the more central position of the nucleoli in the actinomycin-treated and in the starved plasmodia as compared to the peripheral position of the large nucleoli in the control piece.

The same result as in Figure 1 was obtained in all plasmodia which were placed on the actinomycin medium at any time of the intermitotic period more than 5 h prior to the next mitosis. Their nuclei entered prophase shortly (20–45 min) after those of the controls did.

A different picture was obtained when the plasmodia were placed on actinomycin at a time less than 5 h prior to mitosis (Figure 5). The nuclei shown in Figure

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5 are from a plasmodial piece which had been placed, at $3^1/_2$ h before mitosis, on actinomycin D for approximately $1^1/_2$ h. For comparison, Figure 6 shows nuclei of the untreated control piece at the same time. The morphological appearance of the actinomycin nuclei was similar to that in Figures 3 and 7, both of which represent 'true' prophases. This actinomycin-induced 'prophase' lasted for a little more than 2 h, and it was followed by metaphase approximately 10 min after the controls entered metaphase. In the untreated plasmodia, prophase did not require more than approximately 15 min preceding metaphase. A 'prophase'-like condensation effect of actinomycin was found in 9 out of 10 different plasmodia which were placed on the actinomycin medium at a time not more than 5 h, and not less than 2 h prior to mitosis. In these plasmodia, the chromosome condensation began between $1^1/_4$ and 2 h after they were placed on the actinomycin medium, and it lasted until a few minutes after metaphase began in the controls. When placed on the actinomycin medium less than 2 h prior to mitosis, both the experimental and the control plasmodia entered prophase approximately simultaneously.

The striking morphological similarity between the advanced actinomycin-induced chromosome condensation and true prophase poses an interesting problem. The fact that the actinomycin-induced chromosome condensation lasted until after the termination of prophase in the controls, does not necessarily indicate that its morphological similarity to true prophase is fortuitous. ANDERSON[18,19] has shown, years ago, that prophase-like chromosome condensation can be induced, e.g. by polyamines, and it is possible that our observation is related to his findings. However, we should like to propose an alternative hypothesis, namely, that the prophase-like chromosome condensation obtained by actinomycin at late interphase might be an indirect result of the known inhibitory effect of actinomycin upon DNA-dependent RNA synthesis[2,3]. It has been reported that, in synchronized cultures of *Tetrahymena*, the levels of nucleoside triphosphates[20,21], reduced pyridine nucleotides[22], and ATP[23] rise sharply

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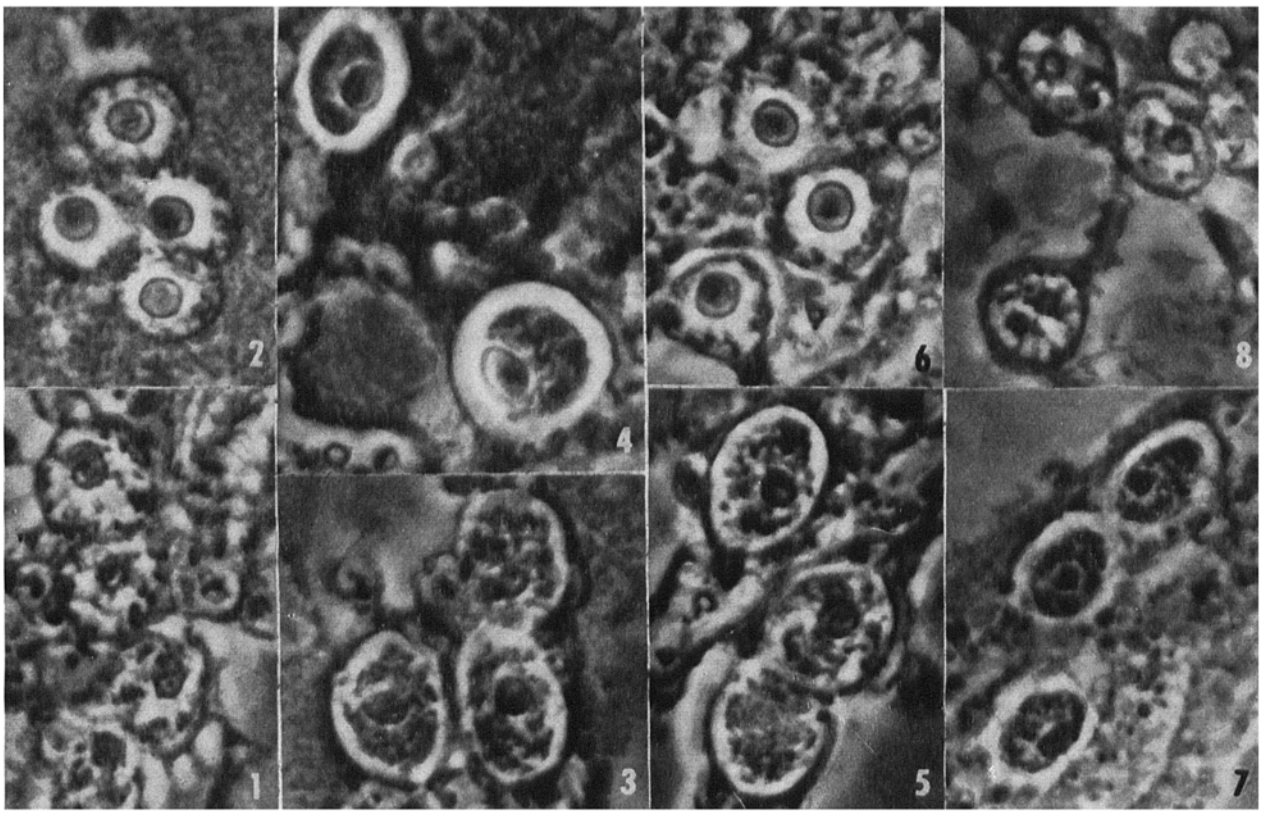


Smear preparations of plasmodial explants, ethanol-fixed, unstained, phase-contrast; magnification ×2900. Fig. 1. Nuclei of a mid-interphase plasmodium, after 2 h on actinomycin D. Fig. 2. Nuclei from the control sector at the same time. Fig. 3. Nuclei from the same plasmodium as in Figure 1, $6^1/_2$ h later: Prophase. Fig. 4. Prophase-nuclei from the control sector. Fig. 5. Actinomycin-induced chromosome condensation. This plasmodium was placed on actinomycin for a period of $1^1/_2$ h, beginning at $3^1/_2$ h prior to mitosis. Fig. 6. Nuclei from the control sector at the same time. Fig. 7. Prophase in a plasmodium that was starved for approximately 36 h. Fig. 8. Interphase-nuclei of a plasmodium that was starved more than 36 h.

prior to mitosis, and the importance of the maintenance of a critical level of ATP in cleaving sea urchin eggs for continued occurrence of mitoses has been recently demonstrated[24]. Actinomycin, by inhibiting energy-utilizing macromolecular syntheses, might, at late interphase, induce prematurely a situation similar to that reported for premitotic *Tetrahymena*. A comparison of the energy metabolism in actinomycin-treated plasmodia with that of premitotic plasmodia should be of interest under this point of view[25].

*Zusammenfassung.* Actinomycin D (125 µg/ml) bewirkt prophaseartige Kernveränderungen in den Plasmodien des mitotisch synchronen Schleimpilzes *Physarum polycephalum* bei etwa zweistündiger Einwirkung, im Zeitraum von weniger als etwa 5 h vor Mitosebeginn.

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## Adaptation of the Guinea-pig to Histamine. Sensitivity of the Guinea-pig's Ileum to Histamine and Acetylcholine[1]

The process of physiological adaptation of an organism develops on different functional levels including nervous and humoral elements. In consequence this process manifests itself by changes in responsiveness of the respective effectors.

Guinea-pigs may be adapted to histamine; it is an open question whether this process involves the bronchi only, on which this adaptation has been demonstrated[2]. We thought it interesting to see whether other smooth muscle structures were also involved, and we therefore studied the guinea-pig ileum.

*Methods.* Isolated pieces of ileum of 25 guinea-pigs, males and females, 300–450 g of body weight were used. Of these animals, 16 had been adapted to histamine (H) by daily intraperitoneal injections beginning with a dose of 0.24 mg/kg of histamine[3], which was raised every 5 days by 0.03 mg of H to the terminal adaptation dose of 0.42 mg/kg. This took 6 to 8 weeks. Prolongation of tolerance time of an animal in histamine aerosol[4] under conditions previously described[5] served as a criterion of histamine adaptation.

Isolated ilea of 9 non-adapted guinea-pigs were used as controls. Every isolated ileum was suspended in oxygenated Tyrodé's solution without atropine, according to Magnus's method as modified by Maśliński and Czekaliński[6]. The temperature of the bath was maintained at 33°C. Histamine and acetylcholine added to the bath were in contact with the ileum for 10–15 sec before rinsing. Sensitivity of isolated pieces of ileum to the doses of 0.0012, 0.0024, 0.0048, 0.0096, and 0.0192 µg of histamine in 1 ml of the bath, and 0.005, 0.01, 0.02, 0.04, and 0.08 µg of acetylcholine chloride[7] in 1 ml of the bath, was tested. A photooptic method of registration was employed.

Histaminase activity in homogenated ileum samples was determined by the method described by Kapeller-Adler[8].

*Results.* The dose-response curves of the ileum of adapted and control guinea-pigs to H are presented in Figure 1. The responsiveness of the ileum to acetylcholine is presented in Figure 2. The histaminase activities were as follows: Ileum of 9 adapted guinea-pigs = 4.0, 5 = 0.98 PU/0.125 g fresh tissue[9]. Ileum of 5 non-adapted guinea-pigs = 4.1, = 5 1.1 PU/0.125 g fresh tissue.

*Discussion.* The data presented reveal a change in histamine reactivity of ileal smooth muscle from guinea-pigs adapted to H. The change in reactivity is expressed by a significant decrease of contraction height. The change of H reactivity of the guinea-pig ileum is not paralleled by a significant change in acetylcholine sensitivity, as shown in Figure 2.

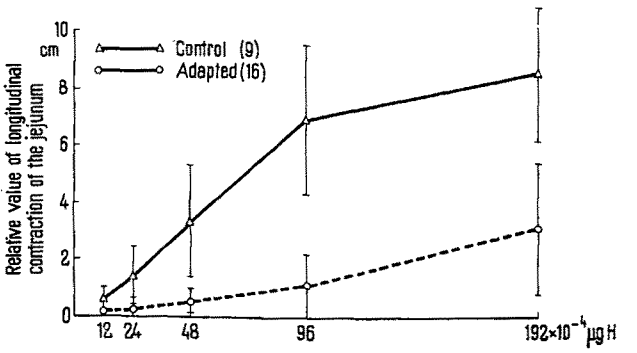


Fig. 1. The points on the curves represent mean values with standard deviations in cm of light beam deflection on administering histamine. The difference of mean values in two groups of the experiment on administering the dose of 0.0096 µg of histamine is statistically significant, $p = 0.001$. In parentheses number of guinea-pigs used in the experiment are shown.

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