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# Transcription in ultra violet-irradiated plasmodia of *Physarum polycephalum*

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**Abstract.** In *Physarum polycephalum*, transcription is significantly inhibited for the whole of the ultra violet-induced, extended G2 phase. Although, there is some increase in the rate of transcription closer to the first post-irradiation mitosis, thereafter, ovrall synthesis continues to be low. Also, the plasmodia developed significant resistance to actinomycin D during this period. The resistance is both in terms of mitosis and RNA synthesis. The overall depression in transcription and the drug resistance are apparently related phenomena.

**Keywords.** Ultra violet-irradiation; transcription; actinomycin-resistance.

## Introduction

Nuclear divisions in the multinucleate, syncytial plasmodia of *Physarum polycephalum*, a myxomycete, are synchronous and rhythmic in nature. Fusion of several microplasmodia grown in agitated cultures would yield large disc-shaped surface plasmodium (macroplasmodium) of several centimetres in diameter, containing millions of nuclei in a single mass of cytoplasm. A huge surface plasmodium, for all practical purposes, is a single (multinucleate) giant cell and because of the large size is ideal for biochemical analysis. Moreover, synchrony of mitosis is also exhibited between sister macroplasmodia made from pooled microplasmodial cultures and this is yet another advantage offered by this organism. The plasmodium is extraordinarily resistant to radiations because of the multinucleated state. This fact and the synchronous and rhythmic mitotic cycles make the organism ideal for studying cycle-dependent radiation effects other than mere cell killing and also in providing information on mitotic triggering mechanisms. Several investigations have already been carried out to understand the role of macromolecular synthesis in the control of mitotic cycles in *Physarum*, employing ultra violet light as a perturbing agent (Sachsenmaier, 1966; Devi *et al.*, 1968a; Sachsenmaier *et al.*, 1970; Devi and Guttes, 1972; Dworzak and Sachsenmaier, 1973; Sachsenmaier and Dworzak, 1976; Tyson *et al.*, 1979; Valsala Kumari and Nair, 1981). Some of the above studies as well as those by Sudbery and Grant (1975, 1976) have helped in the formation of various models on the rhythmicity of the mitotic cycles in this organism (Reviewed by Winfree, 1980).

Abbreviations used: UV, Ultra violet; PFM, post-fusion mitosis; PIM, post-irradiation mitosis; SDM, semi-defined medium; SDS, sodium dodecyl sulphate; CR, control ribosome; CN, control nuclei; IR, irradiated ribosome, IN, irradiated nuclei.

Ultra violet (UV)-irradiation of the plasmodia causes the degeneration of large number of nuclei and the nucleo-cytoplasmic ratio is thus altered. Because of this, the delayed first post-irradiation mitosis (PIM) is followed by several shorter-than-normal mitotic cycles (Devi *et al.,* 1968a; Sachsenmaier *et al.,* 1970; Devi and Guttes, 1972). It is also known that the irradiated plasmodia become largely resistant to actinomycin in terms of mitosis (Devi and Guttes, 1972) and RNA synthesis (Dworzak and Sachsenmaier, 1973; Sachsenmaier and Dworzak, 1976). With these findings in view, we have now made further studies on transcription in the irradiated plasmodia during the extended G2 phase prior to the first post-irradiation mitosis and also during the following short mitotic cycle.

## Materials and methods

### *Culturing the organism*

The Wisconsin strain ($M_3$ C) of *Physarum polycephalum* was grown as microplasmodia in suspension cultures on a semi-defined medium (SDM) (Daniel and Baldwin, 1964), at a temperature of 24° C. Mitotically synchronous surface plasmodia (macroplasmodia) were made by fusion of a large number of microplasmodia according to the method of Guttes and Guttes (1964). Sectors from the same plasmodium or sister plasmodia made from pooled microplasmodial suspension, and hence showing synchronous mitosis among themselves, are used in the different experiments. Surface plasmodia in G2 prior to the second post-diffusion mitosis (PFM) or in G2 prior to the third PFM are used, especially in regard to DNA replication synchrony is best after the first PFM.

### *Determination of mitotic stages*

The mitotic stages were determined by observation of ethanol fixed smear preparations made from explants of plasmodia removed at different times in the mitotic cycle, under the oil immersion lens of a phase microscope (Guttes *et al.,* 1961).

### *Irradiation, actinomycin treatment and labelling*

For irradiation, a Philips 15-watt germicidal lamp was employed which emits approximately 90% of its UV energy at 2537 Å at a dose rate of 9.37 $Jm^{-2}$ $Sec^{-1}$. Total dose of irradiation was 1400 $Jm^{-2}$ except where otherwise mentioned.

Actinomycin D (Sigma Chemical Co., St. Louis, Missouri, USA) was used at a concentration of200 µg/ml of SDM. It is known from earlier studies that, in *Physarum*, actinomycin D is required to be used at these rather high concentrations for any significant effect in, *in vivo* experiments (Mittermayer *et al.,* 1965), although much lower concentrations are sufficient *in vitro,* while working with isolated plasmodial nuclei (Mittermayer *et al.,* 1966b; Grant, 1972). [$^3$H]-Uridine (BARC, India; Specific activity, 20.5 Ci/mmol) was used for *in vivo* labelling of RNA and details are provided as legends to the respective figures and tables.

### *Autoradiography*

Small explants from labelled plasmodia were fixed in acetic acid : alcohol (1:3) or Champy's fixative and autoradiography was carried out on 3 µ sections of the material Acid soluble components were removed by washing the slides with 5%

cold trichloroacetic acid for 15 min. Kodak AR-10 stripping film was used in the preparation of autoradiographs. Exposure was for 12 days at 4° C and development was for 12 min in Kodak D-19 Developer at 20° C. Visual grain counting under the oil immersion lens of a phase contrast microscope was carried out for obtaining quantitative data. In determining the number of grains over the nucleus, all grains over the nucleus and those within a distance of approximately 1 μ from the nuclear periphery, were counted as nuclear label. Slides used for grain counting had very low background. Nevertheless, background corrections have been made to obtain the values given in the different figures.

*Preparation of nuclei and ribosomes*

Nuclei and ribosomes were prepared by adopting the techniques used by Mittermayer *et al.* (1966a) as well as some aspects of the methods by Zellweger and Braun (1971). The plasmodia, after labelling with [$^3$H]-uridine at desired times during the mitotic cycle, were given a wash in SDM and were homogenised in an electrically operated teflon-pestled-glass homogeniser. The homogenisation medium used was the following: 0.2 M sucrose, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 M Tris-HCl pH 7.2. The homogenate was centrifuged at 50 *g* in a SS 34 rotor of a Sorvall RC2-B Centrifuge for 10 min. The nuclear pellet obtained after centrifugation of the supernatant at 2800 *g* was washed by spinning through 1 M sucrose in 0.01 M $MgCl_2$ , 0.01M Tris-HCl pH 7.2 and later kept frozen. The supernatant got after removal of the nuclei was again centrifuged at 5000 *g* for 15 min in order to remove mitochrondria, membrane fragments etc. To the supernatant obtained after this centrifugation, sodium dodecyl sulphate (SDS) was added to give a final concentration of 0.1% and mixed thoroughly. This was then centrifuged through a discontinuous gradient of a 20% sucrose solution (10 ml) layered over 10 ml of a 40% sucrose solution at 100,000 *g* for 2 h in an SW 27 rotor of a Beckman L5-50 ultracentrifuge. The sucrose solutions were prepared in the following buffer: 0.8 M KCl, 10 mM $MgCl_2$, 10 mM Tris-HCl pH 7.2. The ribosomal pellets thus obtained were then stored frozen.

Before extracting RNA from the nuclear and ribosomal fractions, each was treated with 0.25 M cold (4° C) perchloric acid for 15 min. Following this, the suspension containing nuclei were centrifuged at 2800 *g* in a SS 34 rotor of a Sorwall RC 2-B centrifuge for 15 min. The suspension containing ribosomes were centrifuged at 100,000 *g* for 1 h in an SW 27 rotor of a Beckman L5-50 model ultracentrifuge. Washing with 0.25 M perchloric acid in the cold was repeated once more. The supernatant from the second wash was used for obtaining background counts.

*Extraction and estimation of RNA*

For the removal of acid soluble components and extraction of acid-insoluble RNA from the plasmodia, the procedure of Mittermayer *et al.* (1964) was followed. Plasmodial sectors were shaken in cold (4° C) 4% trichloroacetic acid in acetone-water (1:1) mixture for about 10 min on a vortex. The suspension was then left in the cold at 4° C for 12 h. After this the plasmodial pieces were resuspended by shaking again on a vortex and centrifuged at 200 *g* in an IEC PR-6 centrifuge. The residue obtained was treated once more with the above trichloroacetic acid: acetone

mixture, followed by 3 washes in 0.25 M perchloric acid at 4° C. The supernatant from the last wash was used for obtaining background counts.

RNA was extracted from whole plasmodial residue obtained after the above acid washes or from the nuclear and ribosomal fractions respectively, by using bovine pancreatic ribonuclease. For this extraction, for every 1.9 ml of 0.06 M $KHCO_3$, 0.1 ml of a 1 mg/ml solution of RNase (RNase used here was a product of Boehringer, W. Germany and the stock solution, prepared in distilled water, was kept in boiling water bath for 10 min to inactivate contaminating DNase) was added to the respective smaples. Incubation was carried out at 30° C for 1.5 h. The reaction was stopped by adding 0.5 ml of a solution of 0.5 M trichloroacetic acid for every 2 ml of the extract. From each sample, aliquots were taken for radioactivity assay and for RNA estimation. For radioactivity assay, aliquots were spotted on millipore filters and counted in a Beckman LS 100 C Liquid Scintillation Counter, using a toluene, PPO, POPOP mixture. Quantitative estimations on RNA were made by employing the orcinol test (Ceriotti, 1958).

## Results

Both autoradiographic (figure 1) and biochemical (table 1) studies show that nuclei of irradiated plasmodia incorporate much less [$^3$H]-uridine, following a pulse-labelling schedule, when compared to control. For example, autoradiographs show that in the irradiated plasmodia, there is a shift in the labelling pattern

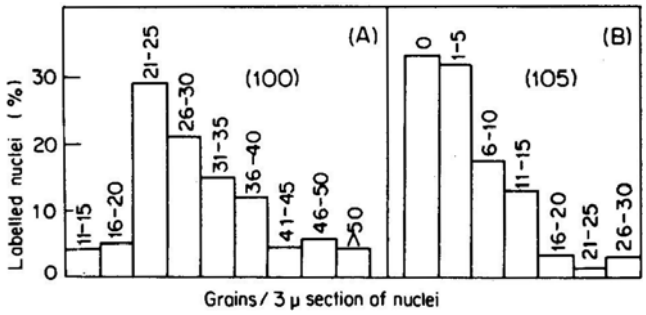


**Figure 1.** RNA synthesis in irradiated and control plasmodia as measured by autoradiography (dose of irradiation: 1267 $Jm^{-2}$). Abscissa: grains/3 μ section of nuclei: ordinate: % of labelled nuclei. Pulse labelling for 10 min with [$^3$H]-uridine, 30 μCi/ml of salts medium (Daniel and Baldwin, 1964). A and B are sectors of the same macroplasmodium.

A - Control, labelled 49 min prior to the second PFM.

B - Irradiated 49 min prior to the second PFM and labelled immediately after that. Value given in parenthesis is the number of nuclei counted.

from the higher grain classes of nuclei to the lower and there is considerable increase in the percentage of nuclei with no grains as well (figure 1). While this is the situation for most of the extended G2 phase, nuclear labelling increases considerably closer to the first PIM and thereafter (figure 2B and table 1). Ribosomal labelling also increases following the first PIM (table 1). However, the ratio of the specific activity of RNA from ribosomes to that of nuclear RNA in the irradiated plasmodia (IR/IN), taken during this period, indicates that the increase in ribosomal labelling is not proportional to the increase in nuclear label. This ratio was even lower before the first PIM (table 1).

**Table 1.** Specific activity of RNA in nuclear and ribosomal fractions from UV-irradiated Plasmodia of *Physarum polycephalum.*

| Time of labelling | cpm/µg RNA after pulse and chase | | | | CR/CN | IR/IN |
|---|---|---|---|---|---|---|
| | Nuclei | | Ribosomes | | | |
| (column 1) | Control (column 2) | Irradiated (column 3) | Control (column 4) | Irradiated (column 5) | (column 6) | (column 7) |
| 1 h 45 min before third PFM (15 min after irradiation) | 45786 | 15526 (66.09) | 14886 | 4658 (68.71) | 0.33 | 0.30 |
| 45 min before third PFM (1 h 15 min after irradiation) | 55966 | 13605 (75.69) | 16335 | 5330 (67.37) | 0.29 | 0.39 |
| 3 h. before first PIM (6 h after irradiation) | | 38319<br>28011 | | 6331<br>3930 | | 0.17<br>0.14 |
| 4 h 45 min after first PIM or 3 h 30 min before second PIM (13 h 45 min after irradiation) | | 36719<br>56246 | | 9492<br>14774 | | 0.26<br>0.26 |

CR, Control ribosome; CN, control nuclei; IR, irradiated ribosome; IN, irradiated nuclei.

Controls are given for the first two experimental material. No strictly comparable controls are possible for others. Values given in parenthesis denote % inhibition in relation to respective controls. Pulse labelling with 100 µCi/ml of [$^3$H]-uridine for 30 min followed by 1 h chase in SDM.

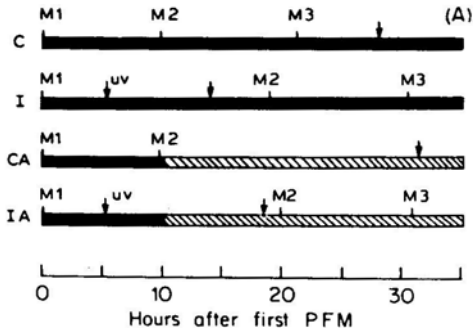


**Figure 2. A.** The schedule of irradiation, actinomycin treatment and [$^3$H]-uridine labelling, in relation to mitotic cycle, in the Plasmodium, the labelling data of which are given in figure 2B.

C, Unirradiated sector on SDM; I - irradiated sector on SDM; CA – unirradiated sector on actinomycin; IA - irradiated sector on actinomycin; Ml - first PFM, M2 - second PFM (first PIM in the case of irradiated sector) M3, third PFM (second PIM in the case of irradiated sector) ↓ UV – marks the time of irradiation. ■ – marks the duration of actinomycin treatment. Marks the beginning of labelling with [$^3$H]-uridine.

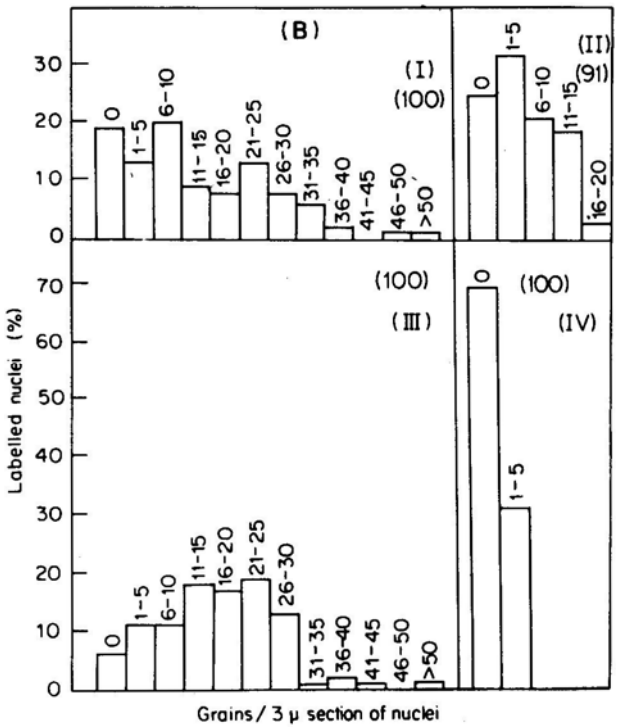


**Figure 2B.** Effect of actinomycin D on RNA synthesis in irradiated plasmodia as measured by autoradiography (dose of irradiation: 1267 $Jm^{-2}$). Abscissa: grains/3 μ section of nuclei: Ordinate: % of labelle d nuclei. Concentration of actinomycin– 200 μg/ml of SDM. Pulse labelling– 10 min; Concentration of [$^3$H]-uridine-30 μCi/ ml in salts medium. I, II, III and IV are sectors of the same plasmodium.

I, Irradiated on SDM; II, irradiated on actinomycin; III, control on SDM; IV, control on actinomycin.

Value given in parenthesis is the number of nuclei counted.

In support of our earlier studies (Devi and Guttes, 1972) and that of Sachsenmaier (Dworzak and Sachsenmaier, 1973; Sachsenmaier and Dworzak, 1976), we have observed that the process of mitosis and the duration of the mitotic cycle in the irradiated plasmodia is not susceptible to rather high doses of actinomycin D (200 μg/ml), to which control plasmodia are highly susceptible. For example, it is seen that additional mitotic delay due to the effect of this drug is much less when plasmodia irradiated during early G2 are placed on the drug a few hours after irradiation (figures 2A and 3A). This cannot be due to the lack of time for the action of the drug, as in these experiments, the plasmodia are continuously exposed to the drug for the whole of the UV induced, extended G2 phase of over 8 h. In unirradiated control plasmodia, similar treatment leads to several hours of delay towards mitosis (figures 2A and 3A and Mittermayer *et al.*, 1965) followed by abnormal mitosis (a kind of abortive mitosis) in majority of the nuclei (Devi *et al.*, 1968b; Guttes *et al.*,1969). Furthermore, in the irradiated plasmodia, the mitotic cycle following the first PIM is fully resistant to 200 μg/ml of this antibiotic (figures 2A and 3A). This effect is highly significant, as in the unirradiated plasmodia, once a

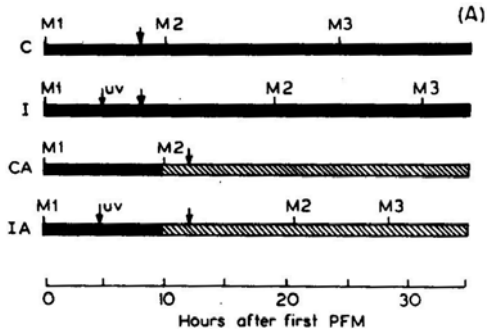


**Figure 3A.** The schedule of irradiation, actinomycin treatment and [$^3$H]-uridine labelling, in relation to the mitotic cycle, in the plasmodium, the data of which are given in figure 3B. The explanation for the symbols are as same as for figure 2A.

mitosis occurred in the presence of actinomycin D, another mitosis did not occur in the continued presence of the drug (figure 3 A). Yet another observation made is that, in cases where the UV induced, extended G2 phase is further increased by actinomycin D, the duration of the following short mitotic cycle is decreased correspondingly, such that the overall period remains about the same, but subject to a certain minimum for the short mitotic cycle (figure 3A). Also, the large number of abortive mitotic figures induced by this antibiotic in control plasmodia (Devi *et al.*, 1968b; Guttes *et al.*, 1969), are largely absent in similarly treated irradiated plasmodia.

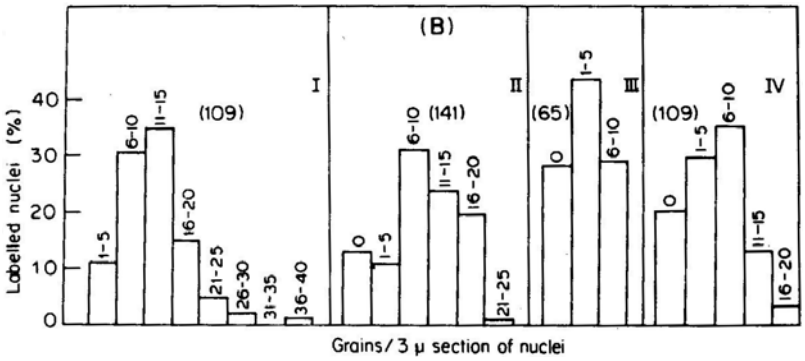


**Figure 3B.** Effect of actinomycin D on RNA synthesis in irradiated plasmodia as measured by autoradiography (dose of irradiation: 1267 $Jm^{-2}$). Abscissa: grains/3 μ section of nuclei; Ordinate: % of labelled nuclei. Concentration of actinomycin– 200 μg/ml of SDM: Pulse labelling – 10 min; concentration of [$^3$H]-uridinen – 30 μCi/ml in salts medium. I, II, III and IV are sectors of the same plasmodium.

I, Control on SDM; II, irradiated on SDM; III, control on actinomycin; IV, irradiated on actinomycin.

Value given in parenthesis is the number of nuclei counted.

The significantly decreased sensitivity of mitosis to actinomycin D in the irradiated system is accompanied by a corresponding decrease in the action of this drug on the incorporation of [$^3$H]-uridine into RNA. For example, when unirradiated plasmodia in G2 show from 60% to 70% inhibition of [$^3$H] -uridine incorporation after about 2 h of drug treatment, similarly treated irradiated plasmodia

show only 26% to 44% inhibition, when compared to untreated irradiated plasmodia (table 2). However, as we can see, irradiation itself causes varying degress of inhibition of transcription in these plasmodia (tables 1 and 2).

**Table 2.** Effect of actinomycin D on RNA synthesis in irradiated plasmodia of *Physarum polycephalum.*

| Time of collection of samples | cpm/μg RNA | | | |
|---|---|---|---|---|
| | SDM | | Actinomycin D | |
| | Control | Irradiated | Control | Irradiated |
| 1 h 30 min before third PFM | $AC_1$-1188 | $AI_1$-939 (21) | $AC_2$-470 (60) | $AI_2$-692 (26) |
| 55 min before third PFM | $BC_1$-1022 | $BI_2$-816 (20) | $BC_2$-268 (74) | $BI_2$-455 (44) |
| 9 h after irradiation, during the extended G2 phase prior to first PFM | — | $AI_3$-389 | — | $AI_4$-585 (no inhibition) |
| | — | $BI_3$-704 | — | $BI_4$-849 (no inhibition) |
| 5 h 50 min after first PIM | — | $AI_5$-390 | — | $AI_6$-737 (no inhibition) |
| | — | $BI_5$-323 | — | $BI_6$-351 (no inhibition) |

Plasmodia A and B were irradiated 4 h before third PFM. $AC_1$ and $AC_2$, control sectors from plasmodium A; $AI_1$ to $AI_6$, irradiated sectors from plasmodium A; $BC_1$ and $BC_2$, control sectors from plasmodium B. $BI_1$ to $BI_6$ , irradiated sectors from plasmodium B.

Dose of actinomycin D-200 μg/ml; labelling for 30 min with 50 μCi/ml of [$^3$H]-uridine. Values given in parenthesis denote percentage inhibition either due to irradiation or due to actinomycin. For computing percentage inhibitions in irradiated plasmodia treated with actinomycin, counts obtained from irradiated are taken as 100%. For computing percentage inhibitions due to irradiation alone, counts obtained from control are taken as 100%.

Another observation which we have made is that, although, biochemical analysis made on total cellular RNA show that the UV-irradiated system is completely resistant to actinomycin D (200 μg/ml), from the later part of the extended G2 phase onwards (table 2), autoradiographs show that, nuclear labelling shows some susceptibility to the drug even during this period. Some of the higher grain classes of nuclei are absent in the autoradiographs prepared from the drug-treated, irradiated plasmodia, with a corresponding increase in the lower grain classes (figures 2B and 3B). In the biochemical analysis, where specific radioactivity of total cellular RNA is assayed, these smaller level effects, observable in a cytological study of individual nuclei are apparently not detected.

## Discussion

In the present studies, we have taken specific activity of RNA following pulse labelling schedule with [$^3$H] -uridine, as an indicator of transcription, for the following reasons, (i) The rate of [$^3$H]-uridine incorporation into RNA is likely to

represent true differences in the rate of RNA synthesis in this organism. Using such a method of assay, it has been possible to show the biphasic pattern of overall RNA synthesis during the mitotic cycle of *Physarum* in an *in vivo* system (Mittermayer *et al.,* 1964; Braun *et al.,* 1968; Sauer *et al.,* 1969) which has later been confirmed in *in vitro* studies (Mittermayer *et al.,* 1966b; Grant, 1972). (ii) RNA precursor pools do not markedly alter during the cell cycle, with the possible exception of a short period round about mitosis (Grant, 1973). (iii) Following UV-irradiation, there is a significant decrease in total nuclear DNA, as a result of degeneration of many nuclei in this syncytial system (Devi *et al.,* 1968a; Devi and Guttes, 1972). Since the number of degenerating nuclei varies in the different plasmodia, evaluating transcription in terms of nuclear DNA would not be as meaningful as it should be. (iv) Our autoradiographic studies, where assay is in terms of incorporation of [$^3$H]-uridine into invidiual nuclei, corroborate much of what is found biochemically.

The resistance to actinomycin D (C1) (200 µg/ml) shown here and elsewhere (Devi and Guttes, 1972; Dworzak and Sachsenmaier, 1973; Sachsenmaier and Dworzak, 1976) could only be partial, as it was observed by Sachsenmaier and Dworzak (1976) that nuclei in the irradiated plasmodia get arrested in metaphase at 500 µg/ml of actinomycin (they had used actinomycin C), an effect seen in control plasmodia at 100 µg/ml to 250 µg/ml of the drug. In control plasmodia, 500 µg/ml of the drug prevents the nuclei even from entering mitosis. In this context, it may be noted that the concentration of actinomycin D required for inhibition of RNA synthesis and mitosis is extraordinarily high even in control plasmodia (Mittermayer *et al.,* 1965) and may very well be, in part at least, due to permeability barriers, as RNA synthesis by isolated nuclei in *Physarum* is blocked by actinomycin D at a concentration as low as 0.1 µg/ml (Mittermayer *et al.,* 1966b). If this is so, it is possible that irradiation might have increased these barriers and is responsible for the above observed results. We had checked this point in an earlier study using [$^3$H]-actinomycin D incorporation (12.5 µCi/ml; specific activity–2.9 Ci/mmol; incubation *in vivo* for 1 h), followed by quantitative autoradiography and found no evidence to support this. Also, some of our preliminary *in vitro* RNA synthesis studies employing isolated nuclei from irradiated plasmodia show that these nuclei are less susceptible to actinomycin D inhibitions, when compared with control nuclei (P. A. Valsala Kumari and Vimala R. Nair, unpublished). However, it may still be worthwhile to repeat the *in vivo* incorporation experiments (Devi and Guttes, 1972) for longer incubation periods with higher concentrations of [$^3$H]-actinomycin D.

It is known that actinomycin D reacts preferentially with derepressed regions of DNA (Geuskens, 1977). Any change in the conformation of the DNA molecule should change the degree of affinity of the drug to it, as for maximum binding, there should be perfect matching of the symmetries of either of these molecules (Stryer, 1975). Some conformational changes are to be expected in the DNA of the irradiated system because of the presence of photoproducts such as thymidine dimers formed as a result of UV action (Smith and Hanawalt, 1969). In view of the above, a certain amount of decrease in the effect of actinomycin D on the irradiated plasmodia is expected, as we have shown that UV has considerably inhibited transcription in this system (figure 1 and tables 1 and 2). In fact, it is seen that, if the percentage inhibitions due to UV alone are added up to that obtained by actinomycin

in the irradiated system, the combined value is about the same as that obtained by actinomycin alone in the control plasmodia (table 2). It is perhaps relevant here to note that, not only UV, but ionizing radiation also decreases the sensitivity of RNA synthesis and nuclear division to actinomycin C in *Physarum* (Sachsenmaier and Dworzak, 1976).

In a sucrose density gradient analysis of [$^3$H] -uridine pulse-labelled RNA from *Physarum,* we had shown that, there is a decrease in the production of 26 *SS* nd 19 *S* rRNA types in the irradiated plasmodia with a corresponding increase in the synthesis of low molecular weight RNAs including 4 *S* RNA (Valsala Kumari and Vimala Nair, 1981). The lower (in comparison to control in G2) specific activity of total cellular RNA (the bulk of which would be accounted for by rRNA) in the irradiated plasmodia during the extended G2 phase (table 2) even at a time when nuclear labelling has increased (table 1 column 3) and the decreased ribosomes/ nuclei label seen during this period (table 1 column 7) are meaningful when viewed in this context.

It is known that, just as in the case of other eukaryotes (Penman *et al.,* 1968), rRNA synthesis in *Physarum* is preferentially susceptible to the action of actinomycin D (Mittermayer *et al.,* 1964; Grant, 1972). The rDNA in this organism is nearly exclusively contained (except for 5 S genes) in the nucleolus as free extrachromosomal DNA. It has a density of 1.712±0.002 g/ml corresponding to 54% guanine plus cytosine (Seebeck and Braun, 1980). In view of this, the preferential action of actinomycin D on rDNA transcription in *Physarum* is expected as actinomycin is known to interact with the guanine residues of DNA, each of the cyclic peptides of the drug forming hydrogen bonds with the 2-amino group of guanine in the DNA (Stryer, 1975). A consequent decrease in the action of this antibiotic on the irradiated plasmodia is also expected because of the apparent inhibition of rRNA production in this system.

It would appear that the decrease in overall growth (Devi and Guttes, 1972), the decrease in the production of 26 *S* and 19 *S* rRNA types (Valsala Kumari and Vimala Nair, 1981) and the reduced susceptibility to actinomycin D seen in the irradiated plasmodia are all related phenomena.

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## References

Braun, R., Mittermayer, C. and Rusch, H. P. (1966) *Biochim. Biophys. Acta,* **114,** 27.

Ceriotti, G. (1958) *J. Biol. Chem.,* **214,** 59.

Daniel, J. W. and Baldwin, H. H. (1964) in *Methods in Cell Physiology,* ed. D. M. Prescott (NewYork: Academic Press, Inc.) p. 9.

Devi, V. R. and Guttes, E. (1972) *Radial. Res.,* **51,** 410.

Devi, V. R., Guttes, E. and Guttes, S. (1968a) *Exp. Cell Res.,* **50,** 589.

Devi, V. R., Guttes, E. and Guttes, S. (1968b) *19th Annual AIBS Meeting, Columbus, Ohio,* Sept. 3–7.

Dworzak, E. and Sachsenmaier, W. (1973) *Proceedings of the 2nd Europ. symp. on the cell cycle, Innsbruck/ Austria, University of Innsbruck,* vol. 779, p. 15.

Geuskens, M. (1977) in *Principles and Techniques of Electron Microscopy*, ed. M.A. Hayat (NewYork: Van Nostrand Reinhold Company) vol. 7, p. 163.
Grant, W. D. (1972) *Eur. J. Biochem.*, **29,** 94.
Grant, W. D. (1973) in *The Cell Cycle in Development and Differentiation*, eds M. Balls and F. S. Billet (London: Cambridge University Press) p. 77.
Guttes, E. and Guttes, S. (1964) in *Methods in Cell Physiology*, ed. D. M. Prescott (NewYork: Academic Press, Inc.) p. 43.
Guttes, E., and Guttes, S. and Devi, V. R. (1969) *III Int. Congs. on Protozoology, Leningrad*, July2–9.
Guttes, E. Guttes, S. and Rusch, H. P. (1961) *Dev. Biol*, **3,** 588.
Mittermayer, C., Braun, R., Chayka, T. C. and Rusch, H. P. (1966a) *Nature* (*London*), **210,** 1133.
Mittermayer, C., Braun, R. and Rusch, H. P. (1964) *Biochim. Biophys. Acta*, **91,** 399.
Mittermayer, C., Braun, R. and Rusch, H. P. (1965) *Exp. Cell Res.*, **38,** 34.
Mittermayer, C., Braun, R. and Rusch, H. P. (1966b) *Biochim. Biophys. Acta*, **114,** 536.
Penman, S., Vesco, C. and Penman, M. (1968) *Mol. Biol.*, **34,** 49.
Sachsenmaier, W. (1966) in *Probleme der Biologischen Reduplikation*, ed. B. Sitte (NewYork: Springer Verlag) p. 139.
Sachsenmaier, W., Dönges, K. H., Rupf, H. and Czihak, G. (1970) *Z. Naturf.*, **25b,** 866.
Sachsenmaier, W. and Dworzak, E. (1976) in *Radiation and Cellular Control Processes*, ed. J. Kiefer (Berlin, Heidelberg, New York: Spinger Verlag) p. 229.
Sauer, H. W., Goodman, E. M., Babcock, K L. and Rusch, H. P. (1969) *Biochim. Biophys. Acta*, **195,** 401.
Seebeck, T. and Braun, R. (1980) *Adv. Microb. Physiol.*, **21,** 1.
Smith, K. C. and Hanawalt, P. C. (1969) *Molecular Photobiology. Inactivation and Recovery*, (London, New York: Academic Press).
Stryer, L. (1975) *Biochemistry* (San Francisco: W. H. Freeman and Company; Tokyo, japan: Toppan Company Limited) p. 614.
Sudbery, P. E. and Grant, W. D. (1975) *Exp. Cell Res.*, **95,** 405.
Sudbery, P. E. and Grant, W. D. (1976) *J. Cell Sci.*, **22,** 59.
Tyson, J., Garcia-Herdugo, G. and Sachsenmaier, W. (1979) *Exp. Cell Res.*, **119,** 87.
Valsala Kumari, P. A. and Vimala R. Nair. (1981) *Radiat. Res.*, **88,** 37.
Winfree, A.T. (1980) *The Geometry of Biological Time. Biomathematics* (NewYork, Heidelberg, Berlin: Springer-Verlag) p. 8.
Zellweger, A. and Braun, R. (1971) *Exp. Cell Res.*, **65,** 413.