beeinflusst damit nicht nur den Transport von K^+ selbst, sondern auch indirekt die damit gekoppelten Reaktionen, den Protonentransport und die Phosphorylierung im Licht. Durch Erhöhung des passiven K^+-Einstromes in Gegenwart von Dinactin muss wie beschrieben bei obligater Kopplung des Protonenonfluxes zum K^+-Efflux und der Phosphorylierung zum ersteren in Anwesenheit bestimmter Konzentrationen von Dinactin eine Stimulierung der Phosphorylierung durch K^+, gleichzeitig aber auch eine Reduktion des messbaren Protonenflusses durch die Verringerung des Ladungsgradienten sichtbar werden. Die Beeinflussung der untersuchten photosynthetischen Reaktionen wäre dann auf eine Verschiebung der K^+-Konzentration innerhalb bzw. ausserhalb der Chloroplasten zurückgeführt. Anderseits ist auch eine direkte Einwirkung auf den lichtabhängigen Ionentransport selbst denkbar. Noch liegen heute keine Untersuchungen vor, die eindeutig nachweisen, über welche Mechanismen die einzelnen Reaktionen sich gegenseitig beeinflussen (vgl. auch GREVILLE[9]).

Summary. The macrotetralid antibiotic Dinactin uncouples phosphorylation from electron transport in illuminated chloroplasts in the presence of Na^+ at lower concentrations than in K^+, while the light-induced proton uptake is more inhibited in a medium with K^+ than with Na^+. The large volume changes of whole chloroplasts in the light and after addition of Dinactin are parallel to the amount of K^+ in the chloroplasts.

R. BACHOFEN[10]

Institut für Allgemeine Botanik der Universität, CH-8006 Zürich (Schweiz), 14. Dezember 1970.

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[10] Die Resultate der vorliegenden Arbeit entstanden zusammen mit ELISABETH BOSSHARD-HEER, H. R. HOHL, CH. PFLUGSHAUPT und INGRID SPECHT-JÜRGENSEN. Wir danken dem Schweizerischen Nationalfonds für die grosszügige Unterstützung.

Continuous Nucleolar DNA Synthesis after Inhibition of Mitosis in *Physarum polycephalum*[1]

Nuclear DNA synthesis in *P. polycephalum* follows immediately upon mitosis. In most nuclei the replication of the extranucleolar DNA is completed within the first 3h of the intermitotic period ([2–4]), whereas replication of the nucleolus-associated DNA, as determined by exposure to short pulses of thymidine-^{3}H, occurs throughout the remaining approximately 6–7h preceding the next mitosis[5]. The results described in the following lend support to the hypothesis that the replication of nucleolus-associated DNA is a continuous process which may go beyond the level of duplication during the intermitotic period[6].

Material and methods. Culture methods and experimental procedure. The organism was grown in the form of microplasmodia in agitated culture[7]. Mitotically synchronized surface plasmodia were prepared as described previously[8]. At a stage just prior to or during the time at which the previously central nucleolus gradually begins to move toward the nuclear membrane in preparation for mitosis (henceforth referred to as the 'acentric stage'), plasmodia were covered with a circular piece of wet filter paper of approximately the same size and shape as the plasmodia. This caused the plasmodia to spread from underneath the filter paper. If the beginning of mitosis was imminent at that time, a phase difference with regard to the time of mitosis developed between the nuclei in the peripheral areas of the plasmodia which had spread from under the filter paper and those in the covered parts of the plasmodia. In the free areas of the plasmodia the nuclei advanced with almost no delay toward, or through, mitosis, whereas mitosis was delayed in the covered areas. While the plasmodia continued to spread, the nuclei from the covered areas were carried, by protoplasmic streaming, into the more advanced, peripheral areas. As a result, the peripheral, postmitotic regions contained a few nuclei which were either in the acentric stage or in early prophase[9]. The number of these nuclei in the postmitotic regions was kept small by excising the central, covered, parts as soon as a few, either acentric or early-prophase nuclei were found among the postmitotic nuclei in smear preparations.

Cytological methods. For determination of mitotic stages[10], ethanol-fixed smear preparations from small explants of the plasmodial periphery were inspected under phase contrast[10]. For autoradiography, plasmodial pieces containing a small number of either acentric or early-prophase nuclei were incubated for periods of 30 min at different times during the S period following mitosis, with 50 µc/ml of thymidine-^{3}H from Schwarz BioResearch, Inc., Orangeburg, New York, specific activity 11.0 c/mmole. Squash preparations[11] were processed for autoradiography[5]. The preparations were incubated in metal desiccators, under nitrogen atmosphere, for periods of up to 5 months. Control slides were treated with DNAse[5] prior to autoradiography.

Results. Both acentric and early-prophase nuclei retained their morphological appearance for several hours before they gradually assumed the morphological appearance of interphase nuclei. Figure 1 shows a representative autoradiograph of an acentric nucleus in an S-phase environment, from a plasmodium which was incubated with thymidine-^{3}H for 30 min, beginning approximately 2.5h after metaphase of the advanced nuclei. In the acentric nucleus only the nucleolus-associated DNA is labelled. Figure 2 shows an autoradiograph of an early-prophase nucleus surrounded by heavily labelled post-mitotic (=S phase) nuclei. This plasmodium was incubated

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for 30 min, beginning 90 min after metaphase of the surrounding, early-interphase nuclei. As in the acentric nucleus, the nucleolus-associated DNA of the early-prophase nucleus is labelled, whereas the extranucleolar DNA remained unlabelled. In thin areas of our preparations there were no gradual transitions with regard to the number of silver grains between the overexposed. heavily labelled nuclei and the lightly labelled, premitotic nuclei. No label was found after treatment with DNAse prior to autoradiography.

In contrast to the highly-labelled, overexposed nuclei, the number of silver grains found over the delayed, premitotic nuclei in Figures 1 and 2 was quite low, and some nuclei appeared to be unlabelled. In order to determine if their number was within the range that was statistically expected, we calculated the Poisson distribution of the grain counts[12]. An example is given in Figure 3. In this preparation, 7.8% of the nuclei were premitotic, and 87.5% of all silver grains over these nuclei were located above the nucleoli while 8.3% of the silver grains were located within a distance of 2 µm from the nucleoli. No grain count could be obtained from the other, overexposed nuclei. The Poisson distribution for grain counts over the premitotic nuclei shows that the number of nuclei having no silver grains (17 out of 316) was within the range of statistical expectation. The absence of silver grains over these nuclei therefore does not indicate that they did not incorporate thymidine-^{3}H.

Discussion. We were unable to determine the stage of the overexposed nuclei in the autoradiographs. However, since the plasmodia were in S phase at the time of fixation, and since there was no gradual transition, with regard to the number of silver grains, between lightly-labelled, premitotic nuclei and the nuclei which were overexposed, we assume that the overexposed nuclei were postmitotic nuclei. We cannot exclude the possibility that the late incorporation of thymidine-^{3}H into nucleolus-associated DNA was a result of a slow-down of DNA synthesis caused by the method used for desynchronization. However, we find this unlikely, since the plasmodia remained covered with filter paper for only approximately 30 min, during which time the delayed nuclei were carried, by protoplasmic streaming, from the covered, premitotic parts into the more advanced early-interphase environment of the free areas of the plasmodia. Since transplantation of G_2-phase nuclei into S-phase plasmodia does not cause reinitiation of DNA replication in the extrachromosomal DNA[4], we assume that the presence of some silver grains over the extranucleolar areas of premitotic nuclei might be due to lack of resolution, rather than to extranucleolar label, in the overexposed preparations. We propose the hypothesis that the incorporation of thymidine-^{3}H into the nucleolar DNA of the delayed, premitotic nuclei is due to the extension of continuous DNA synthesis beyond the level of duplication. It is likely that the nucleolus-associated DNA in *P. polycephalum* represents ribosomal cistrons, as in other organisms[13], since a heavy satellite[11,14] has

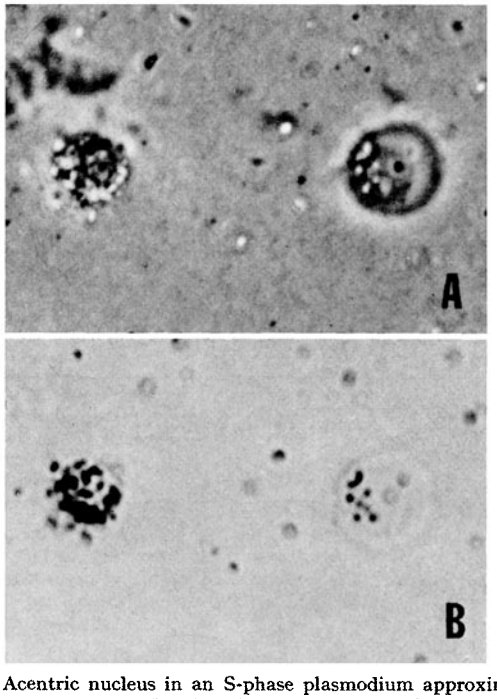

Fig. 1. Acentric nucleus in an S-phase plasmodium approximately 3 h after metaphase. Incubated with thymidine-^{3}H for 30 min immediately preceding fixation. Squash preparation. With A) and without B) phase contrast. Magnification: ×2200.

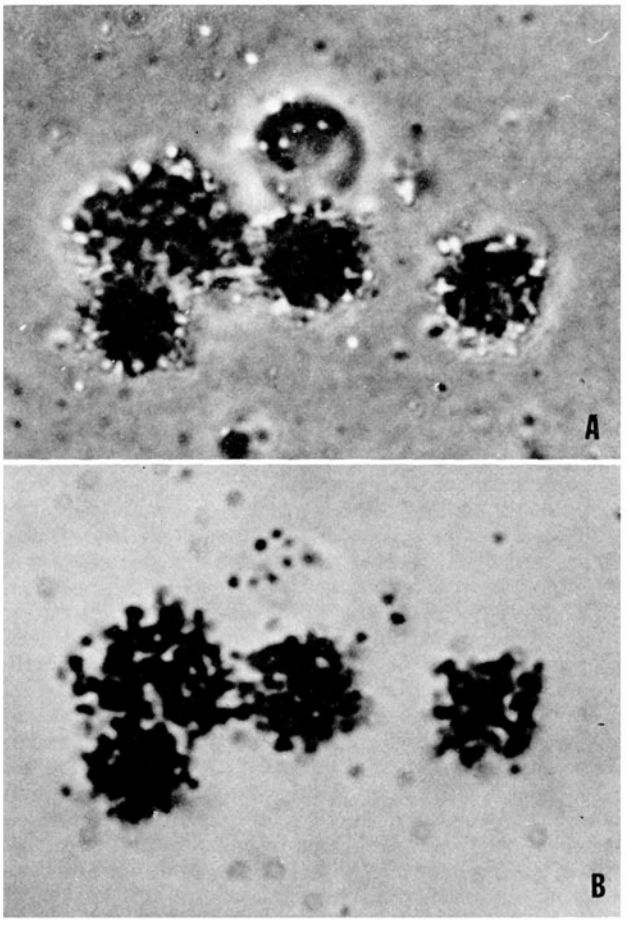

Fig. 2. Early-prophase nucleus in an S-phase plasmodium, 2 h after metaphase. Incubation with thymidine-^{3}H for 30 min immediately preceding fixation. Squash preparation. With A) and without B) phase contrast. Magnification: ×3000.

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been found which incorporates thymidine-^{3}H during G_2 phase and which hybridizes with RNA[14]. Replication beyond the level of duplication(= amplification[15]) of nucleolar DNA cistrons coding for ribosomal RNA[13] has been found in oocytes[16]. It will be of interest to study nucleolar DNA replication in nuclei which were prevented from dividing by similar methods, but for longer periods of time than it was possible in the present experiments. Such an investigation is now in progress.

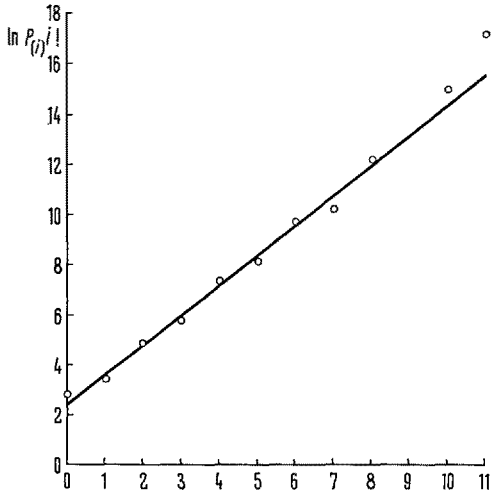

Fig. 3. Poisson distribution of grain counts over premitotic nuclei in postmitotic plasmodia (same preparation as in Figure 2). The following formula was used, $P = N A^i \cdot e^{-A}/i!$. A, mean value of grain counts (= 3.35); i, number of grains found (0, 1, 2, 3, etc.); P, probability of finding i grains over nuclei; N, actual number of nuclei counted (316).

Zusammenfassung. Wird bei einem künstlich synchronen Plasmodium des Myxomyceten *Physarum polycephalum* die Mitose promitotischer Kerne durch deren Verlagerung in postmitotische (=S phase) Zonen des gleichen Plasmodiums verhindert, so behält die nucleoläre DNS, im Gegensatz zur extranukleolären DNS, weiterhin die Fähigkeit, ^{3}H-Thymidin einzubauen. Es wird die Hypothese vorgeschlagen, dass die Synthese der nukleolären DNS einem anderen Kontrollmechanismus unterliegt als die der extranukleolären DNS.

E. GUTTES and MAUREEN M. TELATNYK

Division of Biology, University of Texas at Dallas, Box 30365, Dallas Texas 75230, USA),
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Inhibition of Monoamine Oxidase by 3-Amino-2-Oxazolidinone and 2-Hydroxy-Ethylhydrazine

3-Amino-2-oxazolidinone was recognized in a mouse behavior screen in these laboratories as a compound having activity like that of known inhibitors of monoamine oxidase (MAO), and further study showed that it had dopa-potentiating activity typical of MAO inhibitors in mice. We found that the compound did not inhibit MAO in vitro but that brain homogenates from treated mice showed nearly complete inhibition of MAO. 3-Amino-2-oxazolidinone is a known metabolite of furazolidone[1]; neither agent inhibits MAO in vitro, but both cause in vivo inhibition[1,2] suggested to be due to their conversion to 2-hydroxy-ethylhydrazine (HEH)[1]. We describe here some comparisons of 3-amino-2-oxazolidinone and HEH as inhibitors of MAO.

MAO activity was assayed by the method of WURTMAN and AXELROD[3] except that C^{14}-phenethylamine ($8 \times 10^{-5} M$) was used as substrate in place of tryptamine. Male albino mice (16–20 g) obtained from a local supplier were given i.p. injections of the drugs. The mice were then killed by decapitation, and the organs were rapidly removed and frozen on dry ice. Whole homogenates were used for the assay of MAO activity. Homogenates of tissues from untreated mice were used for in vitro studies with the inhibitors.

The Table shows that 3-amino-2-oxazolidinone did not inhibit MAO activity in vitro, whereas HEH inhibited in a manner typical of irreversible inhibitors, that is it required preincubation with enzyme prior to substrate addition for maximum inhibition.

Figure 1 shows the in vivo inhibition of MAO by these compounds. Maximum inhibition occurred rapidly, within 60 min or less, after the compounds were injected into mice. The onset of inhibition by 3-amino-2-oxazolidinone, which apparently acts indirectly, was as rapid as the onset of action of HEH, which can act directly. Both compounds caused greater inhibition of MAO in liver than in brain, but this difference was more pronounced with HEH.

In vitro inhibition of MAO

Inhibitor	Concentration (M)	Inhibition (%)	
		No preincubation	30 min preincubation
Brain			
3-Amino-2-oxazolidinone	10^{-3}	4	0
2-Hydroxyethylhydrazine	10^{-3}	96	99
	10^{-4}	61	99
	10^{-5}	22	98
	10^{-6}	3	47
	10^{-7}	0	2
Liver			
3-Amino-2-oxazolidinone	10^{-3}	9	8
2-Hydroxyethylhydrazine	10^{-3}	99	100
	10^{-4}	60	99
	10^{-5}	14	96
	10^{-6}	4	68
	10^{-7}	0	9

Where indicated, enzyme and inhibitor were preincubated in buffer at 37 °C prior to addition of substrate. Control values, in nanomoles of substrate oxidized/min/g tissue were 34 and 32 for brain, respectively, without and with the 30 min preincubation and 609 and 600 for liver, respectively, without and with the 30 min preincubation.