PHYSARUM NEWSLETTER

JUNE, 1973

Volume 5 No. 1

CONTENTS

Please mail contributions and correspondence to
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Cleveland, Ohio 44106 (USA)

3227 3/72

Papanicolaou Cancer Research Institute

AT MIAMI/1155 N. W. 14th STREET MIAMI, FLORIDA 33136 305/371-5572, x59

Dear Myxomycetoia:

Greetings to our far flung and often migratory members. We invite you to Miami to attend the Fourth Physarum Conference now planned for November 19-20. A myxomycete field trip is also planned for the afternoon of November 18, to be led by Dr. Alexopoulos. A number of European enquiries have already been received and we want particularly to encourage attendance by our overseas colleagues. The conference will be held at the Fairchild Tropical Gardens in Miami. The American Society of Cell Biology meetings are being held in nearby Miami Beach, November 15-18.

It is hoped that the conference will provide an opportunity not only for the presentation of new information, but also for the expansion of recent material presented at the ASCB or elsewhere, and above all, for free and informal discussion. Each paper will be scheduled, as in the past, for a 20 minute presentation followed by 10 minutes of discussion. More than one paper may be presented. Please use the format indicated on p. 2 of this Newsletter; limit to one 8 1/2" x 11" page per paper. Send abstracts to: J.W. Daniel, Papanicolaou Cancer Research Institute, 1155 NW 14th Street, Miami, Florida 33136, to arrive by September 15. The format and deadline are to facilitate abstract duplication and assembly and program scheduling. Every effort will be made to include abstracts received later than September 15 provided program time is still available. When sending in abstracts or otherwise communicating with me, please let me know whether you plan to have a car at our conference.

Housing will be available at three nearby motels where reasonable off season rates are effective in November and accomodations obtained with reasonable notice. Approximate rates and addresses are given on p. 2.

While southern Florida is a well known vacation area and very pleasant in mid-November it is also worthwhile mentioning some of the unique and more notable features in the immediate area. In addition to the well known Everglades National Park, the Pennekamp State Park, an underwater area in the upper Florida Keys (just south of Miami) includes a living coral reef and associated sea life and is accessible to swimmers, divers and by transparent bottomed boat. The Miami Seaquarium (and nearby laboratories of the University of Miami School of Marine and Atmospheric Sciences) is located on Virginia Key accessible by causeway in Miami. Finally, the Fairchild Tropical Gardens (the site of our meeting) embraces an outstanding and extensive collection of palms, tropical and subtropical plants and an orchid house.

We look forward to seeing you in November.

Sincerely yours,

Jack

John W. Daniel

Format for Abstracts: 1 "

METHYLATION OF PARENTAL DNA IN PHYSARUM AND OTHER EUKARYOTIC CELLS. T. E. Evans, H. H. Evans and S. R. Littman. Division of Radiation Biology, Case Western Reserve University, Cleveland, Ohio.

Although 5-methylcytosine comprises . . . . . .

* * * * * *

Housing Information:

The University Inn 1390 South Dixie Highway Coral Gables, Florida 33146 Telephone: 305-667-2554	Single: Double: Kitchenette: (for two) Extra Bed:	21.00 24.00 27.00 2.00
Holiday Inn 1350 South Dixie Highway Coral Gables, Florida 33146 Telephone: 305-667-5611	Single: Double: Kitchenette: (for two) Extra Bed:	16.00, 18.00, 20.00 18.00, 20.00, 22.00 25.00 4.00
Howard Johnson's Motor Lodge 1430 South Dixie Highway Coral Gables, Florida 33146 Telephone: 305-665-7501	Double Bed: for one for two Twin Beds: Two Double Beds: Extra Bed	 16.00 17.00 18.00 20.00 4.00

← 1 3/8 " →

← ad lib

Transportation will be provided from the motels to the Fairchild Tropical Gardens. Public bus service is also available.

The Field Trip is planned to start at about 2:00 P.M., November 18 and will last two to three hours. We will leave from the motel area.

Myxomycotina

Myxogastromycetes

Physaromycetidae

Physarales

Physaraceae

Physarum

polycephalum

1 "

Inheritance of plasmodial valine requirement in *Physarum polycephalum*

By JENNIFER DEE, A. E. WHEALS* and C. E. HOLT†

Department of Genetics, University of Leicester, Leicester LE1 7RH, England

Genet. Res., Camb. (1973), **21**, *pp.* 87–101

SUMMARY

A defined medium for *Physarum polycephalum* plasmodia has been devised containing glutamic acid, glycine, methionine, biotin, thiamine, glucose, salts and haematin, which supports good growth on agar plates of many different strains. Tests on this medium have revealed a requirement for valine in some plasmodia formed by homothallic progeny (mt_h *apt-1*$^+$) from the cross *a* (mt_l *apt-1*$^+$) × *APT1* (mt_h *apt-1*$^-$). The valine requirement was also inherited among heterothallic progeny of this cross and its segregation was followed in several heterothallic crosses. To explain the results it is proposed that valine synthesis requires the presence of dominant alleles at either of two unlinked loci and that only plasmodia homozygous for recessive alleles at both loci are valine dependent. In some crosses studied only one pair of alleles is segregating and valine requirement thus provides a useful genetic marker, and the first reported nutritional marker in *P. polycephalum*. The value of crosses with *apt*$^-$ mutants for both the detection and analysis of plasmodial markers is demonstrated and discussed.

J. Mol. Biol. (1973) **74**, 563–572

Methylation of Parental and Progeny DNA Strands in *Physarum polycephalum* †

Helen H. Evans, Thomas E. Evans and Sandra Littman

Departments of Radiology, Biochemistry and Microbiology Case Western Reserve University, Cleveland, Ohio 44106, U.S.A.

(*Received 7 August 1972, and in revised form 4 December 1972*)

Although 5-methylcytosine comprises 4 to 8% of the cytosine residues in the major nuclear DNA of *Physarum polycephalum* (Evans & Evans, 1970), only 1% of the cytosine residues of progeny DNA become methylated during replication. Further methylation occurs during the same and subsequent mitotic cycles, so that 6 to 7 cycles after its synthesis, 5-methylcytosine comprises 5 to 7% of the DNA-cytosine residues of a single generation of DNA. The extent of methylation occurring during the S period has been measured by the determination of the specific activity of the precursor (*S*-adenosylmethionine) and the product (DNA-5-methylcytosine) and by comparison of the radioactivity in DNA-cytosine and DNA-5-methylcytosine after incorporation of [^{14}C]deoxycytidine. Continuing methylation of parental DNA has been shown by density shift experiments and by the conversion of prelabeled DNA-cytosine to DNA-5-methylcytosine. The DNA-5-methylcytosine once formed was found to be stable.

Isolation and Purification of the Extracellular Ribonuclease from *Physarum polycephalum*

D. R. Farr, H. Amster, and M. Horisberger

R & D Department of Nestlé Products,
Technical Assistance Co. Ltd., Lausanne, Switzerland

Arch. Mikrobiol. 85, 249—252 (1972)

Summary. The myxomycete *Physarum polycephalum* has been reported to produce an extracellular ribonuclease. A quick procedure is described for the isolation and purification of the ribonuclease from the culture supernatant. It involves an initial concentration of the culture supernatant by slow freezing, followed by ammonium sulfate precipitation. The enzyme was adsorbed onto DEAE-cellulose and eluted with a sodium chloride gradient.

Dependence of the Nucleolar Structure on DNA and RNA Synthesis

M. Gontcharoff and B. Rao

Laboratoire de Biologie cellulaire et Centre de Biologie et Biochimie du Développement, Faculté des Sciences, Reims

Chromosoma (Berl.) 38, 441—457 (1972)

Abstract. The dependence of nucleolar structure on DNA and RNA synthesis in synchronous cultures of the slime mold *Physarum polycephalum* was traced through the mitotic cycle. The blockage of RNA synthesis produces a characteristic abnormality of the nucleolar structure when imposed at any time during interphase. But differences in the function of the early and late replicating DNA molecules were observed. The blockage of DNA synthesis causes abnormality of nucleolar structure only when imposed during the early part of the S-period.

BBA 97493

THYMIDINE PHOSPHORYLATION IN THE CELL CYCLE OF *PHYSARUM POLYCEPHALUM* AND THE EFFECT OF 5-FLUORO-2'-DEOXYURIDINE AND HYDROXYUREA

A. HILDEBRANDT* AND H. W. SAUER

Zoologisches Institut der Universität Heidelberg (W. Germany)

(Received July 6th, 1972)

Biochimica et Biophysica Acta, 294 (1973) 8–14

SUMMARY

Exogenous [*Me*-^{3}H]thymidine was taken up into G_2 phase plasmodia of *Physarum polycephalum* but no sizeable pools of thymidine phosphates were detected, even at a high level of thymidine kinase activity during a blockade of DNA synthesis. We confirmed cyclic changes in thymidine kinase and demonstrated steady thymidylate kinase activities in the cell cycle. When DNA synthesis was blocked by hydroxyurea or fluorodeoxyuridine (dFUrd) the elevated thymidine kinase activity of control plasmodia was found. Upon reversal of the inhibition, a low activity of thymidine kinase was observed after a blockade by hydroxyurea, while dFUrd treatment had resulted in an enzyme activity that was even higher than in S phase controls.

Expil Cell Res 76 (1973)

Myosin-like protein in *Physarum* nuclei

B. M. JOCKUSCH,[1] U. RYSER[2] and O. BEHNKE,[3]
[1]*Max-Planck-Institut für Biologie, Abt. Melchers, Tübingen, BRD,* [2]*Institut Suisse de Recherches Expérimentales sur le Cancer, Switzerland, and* [3]*Department of Anatomy C, University of Copenhagen, Denmark*

464

MYCOLOGIA, VOL. 64, 1972

DIGESTION AND THE DISTRIBUTION OF ACID PHOSPHATASE IN THE MYXAMOEBAE OF PHYSARUM FLAVICOMUM [1, 2]

FREDERICK Y. KAZAMA [3] AND HENRY C. ALDRICH

Department of Botany, University of Florida, Gainesville, Florida 32061

SUMMARY

During the intracellular digestion process of *Escherichia coli* by *Physarum flavicomum*, acid phosphatase activity can be localilized in food vacuoles, in dictyosomes, in membrane-bound vesicles, and, tentatively, in smooth endoplasmic reticulum. Digestive events similar to that postulated to occur in various protozoans appear to be operative in *P. flavicomum*.

529

ON THE LOCATION OF MYOSIN IN THE MYXOMYCETE *PHYSARUM POLYCEPHALUM* AND ITS POSSIBLE FUNCTION IN CYTOPLASMIC STREAMING

DIETRICH KESSLER

Department of Biology, Haverford College, Haverford Pennsylvania 19041

J. Mechanochem. Cell Motility
1972, Vol. 1, pp. 125–137

Thick, tapered filaments up to a length of about 0.4 um in thin sections are observed with the electron microscope within cytoplasmic fibrils after glycerol treatment of starved slime mold plasmodia. Since *Physarum* myosin can aggregate into similar thick filaments *in vitro*, these observations indicate that myosin is probably present in the ectoplasmic fibrils of the living plasmodium and is visualized as thick filaments after glycerination. Thin microfilaments of actin are also located in these fibrils (Allera *et al.*, 1971). That these actomyosin-containing fibrils have the ability to contract is suggested by the observation of deformed membranes of nuclei and vacuoles at the site of fibril attachment.

The contraction of ectoplasmic fibrils attached at both ends to the plasmalemma could function in cytoplasmic streaming by producing a constrictive force on the fluid endoplasm and causing it to flow. The orientation of the microfilaments in fibrils which are attached to distended membranes is compatible with the hypothesis that the fibrils shorten by a sliding filament mechanism.

CELL CYCLE VARIATION IN CYCLIC ADENOSINE 3', 5'-MONOPHOSPHATE-DEPENDENT INHIBITION OF A PROTEIN KINASE FROM PHYSARUM POLYCEPHALUM

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New Mexico State University
Las Cruces, New Mexico 88003

Vol. 49, No. 2, 1972 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

SUMMARY

A protein kinase present in the acellular slime mold, Physarum polycephalum, has been found to exhibit cell cycle dependence with respect to inhibition by cyclic adenosine 3',5'-monophosphate (cyclic AMP). The capacity of 1×10^{-5} M cyclic AMP to inhibit the kinase was maximal (~80%) during G2 phase. At the onset of mitosis, a sharp rate of decrease occurred in the inhibitory response to cyclic AMP which continued for approximately one hour to mid-S phase. For approximately one hour during mid-S phase, the kinase activity was independent of cyclic AMP concentration. Thereafter, the capacity of cyclic AMP to inhibit the protein kinase was restored over a two-hour interval. Complete restoration coincided with the termination of S phase. Protein kinase levels measured in these same preparations in the absence of cyclic AMP remained invariant throughout the cell cycle. Thus, control of the protein kinase is apparently achieved through coordinate action of cyclic AMP plus other unidentified factors rather than by differential synthesis and destruction of a single kinase enzyme.

Experimental Cell Research 78 (1973) 351–359

STUDIES ON MITOCHONDRIAL STRUCTURE AND FUNCTION IN *PHYSARIUM POLYCEPHALUM*

I. *Fine Structure, Cytochemistry, and ^{3}H-Uridine Autoradiography of a Central Body in Mitochondria*

T. KUROIWA

Health Physics Laboratory, Tokyo Metropolitan Isotope Research Center, 2-11-1 Fukazawa, Setagaya-ku, Tokyo, Japan

SUMMARY

The fine structure, cytochemistry and autoradiography of the rod-shaped central body in the mitochondria of the slime mold, *Physarum polycephalum*, has been investigated. The central bodies are stained with Feulgen stain and, like the nucleoli, are stained metachromatically with azure B. At the ultrastructural level, they are composed of a semi-electron-dense axial region which is sensitive to treatment with DNase and an electron-dense peripheral region which surrounds the axial region and is sensitive to treatment with RNase. With electronmicroscopic autoradiography it has been shown that the central body and its peripheral region, after short exposure to ^{3}H-uridine, incorporate ^{3}H-uridine into a form, possibly RNA, which is insoluble in trichloroacetic acid and can be extracted with RNase though not with DNase. It is suggested that the central body is composed of an axial component which contains primarily DNA and a peripheral component which contains primarily RNA and that the RNA is synthesized in the central body.

ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 153, 144–156 (1972)

Localization of Nucleolar and Chromatin Residual Acidic Protein Changes During Differentiation in *Physarum polycephalum*

WALLACE M. LESTOURGEON AND HAROLD P. RUSCH

McArdle Laboratory for Cancer Research, University of Wisconsin Medical Center, Madison, Wisconsin 53706

Received September 21, 1972

The total nuclear proteins (30% by nuclear dry weight) of the plasmodial slime mould *Physarum polycephalum* have been quantitatively separated into four distinct solubility classes possessing characteristic molecular weight ranges and electrophoretic profiles. The residual acidic proteins of nuclei and nucleoli have been extracted during synchronous growth and during two forms of differentiation and separated into two fractions with buffer-saturated phenol, pH 8.2, and hot sodium dodecyl sulfate (SDS). Quantitative comparisons of electrophoretically separated proteins of both residual fractions from nucleoli and nuclei reveal numerous changes in the phenol-soluble phosphoproteins during both forms of starvation-induced differentiation, and most of the changes occur in the chromatin-associated proteins ranging in molecular weight from 32,000 to 160,000. Reversion of the changes induced by starvation is complete within 8–10 hr after refeeding and precedes the first post-feeding mitotic division by 2 hr.

Only two nucleolar proteins with molecular weights of 37,000 and 41,000 are extractable with phenol and are unchanged during differentiation. Five additional residual nucleolar proteins remaining after phenol extraction can be extracted with hot SDS and one of these proteins with a molecular weight of 86,500 disappears during differentiation. Incorporation studies with ^{14}C labeled amino acids demonstrate that the new proteins which appear during differentiation are newly synthesized, that proteins which disappear during differentiation incorporate no label during development of the new cell states, and that there are two periods (mid S and late G_2) of increased isotope incorporation during active growth. The residual nucleolar proteins as compared to the residual nuclear proteins are high in serine, glycine, and aspartic acid but low in valine and isoleucine.

Tsitologiia 14, 463–471 (1972)

THE INFLUENCE OF NUTRITION ON THE THERMORESISTANCE OF PHYSARUM POLYCEPHALUM

A. G. Lomagin, V. A. Bernstam and *L. I. Zheleznyak*

Laboratory of Cytophysiology and Cytoecology, Botanical Institute of the Academy of Sciences of the USSR, Leningrad

SUMMARY

Influence of nutrition on the «primary» and the «general» (Protoplasma, 1970, 69 : 417) thermoresistance of the myxomycete Physarum polycephalum has been studied. Using different nutrient agar media, the repair of thermal injury was found to be retarded as compared with that on non-nutrient media. The general thermoresistance (the survival) of plasmodia also decreased. The primary thermoresistance determined by the cessation of protoplasmic streaming was the same whatever medium was used. Under conditions of better efflux of metabolic products, the nutrition exerted slight, if any, effect on both the duration of reparation of thermal injury repair, and the general thermoresistance [illegible] medium. The retardation of the repair observed on nutrient agar media in contrast to the repair on non-nutrient ones, and the decrease in the general thermoresistance in the former case is suggested to be due to the influence of products of myxomycete metabolism accumulating in agar.

Using the liquid semi-defined nutrient medium of Daniel and Baldwin, the retardation of the repair of thermal injury brought about by short-term heating at 38–41° C was delayed as opposed to the repair on non-nutrient medium. However, a higher heating (43–45° C) of plasmodia kept on the nutrient medium induced a transition of the plasmodium into vital sclerotia thus increasing significantly the general thermoresistance of the myxomycete as compared with that on the non-nutrient media. No sclerotia were formed during heating on any other nutrient media studied.

Arch. Mikrobiol. 90, 189—198 (1973)

Carbohydrate Metabolism during Differentiation (Sclerotization) of the Myxomycete *Physarum flavicomum*

Thomas J. Lynch and Henry R. Henney, Jr.

Department of Biology, University of Houston, Houston, Texas 77004

Received November 3, 1972

Summary. The metabolism of carbohydrates during differentiation (sclerotization) of *Physarum flavicomum* was studied using the radiorespirometric technique. After about 36 h in a sclerotization (starvation) medium the metabolism declined to a level characteristic of the dormant state. Sclerotia incubated in complete growth medium quickly reverted to a metabolically active state and by 9 h they regained about 50% of their metabolic potential.

Sclerotia metabolize carbohydrates by the Embden-Meyerhof-Parnas (EMP)-tricarboxylic acid and the pentose phosphate (PP) pathways. Compared to growing plasmodia the activity of the EMP is reduced to a greater extent than the PP in sclerotia. Also, EMP-produced triose phosphates are not well equilibrated: there is a greater yield of $^{14}CO_2$ from the C-4 of glucose than from the C-3; the C-3 is incorporated into the lipid fraction to a greater extent than the C-4.

The metabolism of carbohydrates by sclerotia is stimulated by cyclic-3′-5′-adenosine monophosphate.

FEBS Letters 7, 80-81 (1970)

CHEMICAL COMPOSITION OF SLIME FROM THREE SPECIES OF MYXOMYCETES

Henry L.SIMON and Henry R.HENNEY, Jr.

Department of Biology, University of Houston, Houston, Texas 77004, USA

BIOCHEMISTRY, VOL. 12, NO. 7, 1973 1307

Aminoacylation of Transfer Ribonucleic Acid *in Vitro* during the Mitotic Cycle of *Physarum polycephalum*†

P. W. Melera*·‡ and H. P. Rusch

ABSTRACT: *In vitro* aminoacylation of purified transfer RNA was studied during the mitotically synchronous growth phase of the myxomycete *Physarum polycephalum*. Transfer RNA was prepared from early-cycle (1-hr postmitosis), mid-cycle (4-hr postmitosis), and late-cycle (1–2 hr premitosis) stationary plasmodia, and aminoacylated with homologous and heterologous synthetase. The relative amount of tRNA/unit amount of total nucleic acid did not vary during the cycle. The total *in vitro* amino acid acceptor activity/A_{260} unit of tRNA remained constant at 75% (picomoles of amino acid/nanomole of tRNA), and the relative acceptor activity for each amino acid was strikingly uniform throughout the cycle. The data show that in terms of *in vitro* aminoacylation ability neither the tRNAs nor the synthetase enzymes change during the mitotic cycle of *Physarum* and demonstrate a tight quantitative control on the synthesis of tRNA during the mitotically synchronous growth phase of the organism.

BBA 97467

DNA REPAIR AFTER ULTRAVIOLET IRRADIATION IN SYNCHRONOUS PLASMODIA OF *PHYSARUM POLYCEPHALUM*

J. JUSTIN MCCORMICK*, CAROL MARKS AND H. P. RUSCH

McArdle Laboratory for Cancer Research, University of Wisconsin Medical Center, Madison, Wisc. (U.S.A.)

(Received June 30th, 1972)

Biochim. Biophys. Acta, 287 (1972) 246–255

SUMMARY

Plasmodia of *Physarum polycephalum* were irradiated with 14 500 ergs/mm² of ultraviolet irradiation during early G2 period and the subsequent repair synthesis of DNA was studied. It was found that the G2 period, which is normally 5 h in length, is prolonged to 18 h by such a dose of irradiation and that repair synthesis occurs almost entirely during the last 9 h of this period. Following the extended G2 period, the nuclei undergo mitosis in a normal manner and during the DNA synthesis period which follows mitosis all the nuclear DNA is replicated semiconservatively as shown by density label centrifugation studies. During the extended G2 period, normal replication of the nucleolar satellite DNA and trace amounts of nuclear DNA occurs and the total amount synthesized is equal to what would have been synthesized in a G2 period of normal length. Caffeine shows a strong inhibitory effect on DNA repair synthesis without interfering with DNA replication or the length of the intermitotic period.

Experimental Cell Research 78 (1973) 89–97

LOCALIZATION OF RIBOSOMAL RNA GENES BY HIGH RESOLUTION AUTORADIOGRAPHY

U. RYSER,[1] S. FAKAN and R. BRAUN[2]

Department of Cell Biology, Swiss Institute for Experimental Cancer Research, 1011 Lausanne, Switzerland

SUMMARY

In the myxomycete *Physarum polycephalum*, the genes for ribosomal RNA replicate to a large extent in the G2 phase of the mitotic cycle. In mitotically synchronous plasmodia this fact allows the rRNA genes to be localized by electron microscopic autoradiography. After labelling with thymidine in the G2 phase, silver grains were concentrated over the fibrillar and not over the granular regions of the nucleoli. This shows the presence of DNA in these fibrillar regions or their immediate vicinity. Following labelling and chase during the G2 period, nucleoli are dispersed during mitosis and silver grains concentrate over some regions of condensed chromatin.

Chromosoma (Berl.) 37, 101—116 (1972)

Electron Microscopy of Dividing Cells

IV. Behaviour of Spindle Microtubules during Nuclear Division in the Plasmodium of the Myxomycete, *Physarum polycephalum*

Aiko Sakai and Michio Shigenaga

Biological Laboratories, Nara Women's University, Nara, Japan

Received December 31, 1971 / Accepted January 10, 1972

Abstract. During intranuclear mitosis in plasmodia of *Physarum polycephalum* the primordium of spindle microtubules which is a somewhat electron opaque, amorphous structure with fibrous or granular elements, occurs in the center of early prophase, 20 to 30 minutes before metaphase. Then, the primordium seems to divide into two parts. Spindle microtubules develop radially from the primordia of spindle microtubules. These spindle microtubules increase in number and length during prophase. Spindle microtubules are completed in about five minutes before metaphase. The nuclear envelope remains intact during prophase, but after metaphase it breaks at the polar regions. The nuclear envelope of the daughter nucleus is re-formed from the original nuclear envelope.

THE JOURNAL OF CELL BIOLOGY · VOLUME 57, 1973 · pages 220-224

INTRANUCLEAR MICROTUBULE ORGANIZING CENTER IN EARLY PROPHASE NUCLEI OF THE PLASMODIUM OF THE SLIME MOLD, *PHYSARUM POLYCEPHALUM*

KENJI TANAKA. From the Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan

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EVIDENCE FOR THE ATTACHMENT OF RNA TO PULSE-LABELED DNA IN THE SLIME MOLD, PHYSARUM POLYCEPHALUM

M.Anwar Waqar and Joel A. Huberman

Department of Biology, Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Vol. 51, No. 1, 1973 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

SUMMARY

When Physarum polycephalum is pulse-labeled for up to 20 minutes with ^{3}H-thymidine and the shortest labeled DNA strands are partially purified by sedimentation through a neutral aqueous sucrose gradient and then through a formamide-sucrose gradient, these short strands band in Cs_2SO_4 isopycnic density gradients at a density greater than that of bulk single-stranded DNA. Their density is brought partially or nearly completely back to that of single-stranded DNA by hydrolysis with pancreatic RNase A or alkali, respectively. Therefore the dense material attached to the short pulse-labeled DNA strands consists at least partially of RNA.

BBA 97469

THE EFFECT OF CYCLOHEXIMIDE ON THE SYNTHESIS OF MAJOR AND SATELLITE DNA COMPONENTS IN *PHYSARUM POLYCEPHALUM*

P. A. TH. J. WERRY AND F. WANKA

Department of Chemical Cytology, University of Nijmegen, Nijmegen (The Netherlands)

(Received July 10th, 1972)

Biochim. Biophys. Acta, 287 (1972) 232–235

SUMMARY

Exposure of macroplasmodia to 50 μM cycloheximide during the S-phase strongly suppresses the labeling of the major nuclear DNA component of *Physarum polycephalum*.

In contrast, [^{3}H]thymidine incorporation into mitochondrial and nucleolar DNA during the G2-phase is only slightly affected by the inhibitor. The results suggest that only synthesis of the chromosomal DNA requires simultaneous formation of specific proteins.

Developmental mutants in a homothallic strain of *Physarum polycephalum*

By A. E. WHEALS*

Department of Genetics, University of Leicester, Leicester LE1 7RH, England

Genet. Res., Camb. (1973), **21**, *pp.* 79–86

SUMMARY

A derivative line of the homothallic Colonia strain of *Physarum polycephalum* has been isolated which produces plasmodia with high efficiency within clones of amoebae. Using the synergistic effect of ultraviolet light and caffeine, mutants of this line have been isolated which fail to undergo the developmental transition between haploid amoebae and diploid plasmodia (*apt* mutants). They are isolated by selecting for amoebae which fail to produce plasmodia within clones. Complementation tests of four mutants have shown that they are mutants of four different loci and they are recessive to wild-type. A further analysis of one mutant reveals that the *apt-1* locus is unlinked to three other known markers. Crosses of this mutant with heterothallic strains yield progeny which are homothallic indicating that the lesion is not a revertant from a homothallic to a heterothallic mating-type. The use of this system in isolating developmental mutants is discussed.

Amer. J. Bot. 59(8): 828–835. 1972.

QUANTITATIVE MICROSPECTROPHOTOMETRY OF NUCLEAR DNA IN SELFING STRAINS OF THE MYXOMYCETE DIDYMIUM IRIDIS[1]

JOHN J. YEMMA[2] AND C. DALE THERRIEN
Department of Biology, Pennsylvania State University, University Park

ABSTRACT

Genetic and cytochemical investigations of the origin, development, nuclear activity, and ploidy level of plasmodia obtained from selfed clones S-2 and B_1P-33 of the heterothallic myxomycete, *Didymium iridis*, are presented. To demonstrate that selfing did not result from contamination of the clones, or mutations at the mating-type locus, crosses were made between F_1 clones and clones of known mating types. The data were inconsistent with these two possibilities. DNA was quantified by Feulgen-DNA microspectrophotometry. All cellular phases studied (logarithmic amoebae, swarmers, and encysted amoebae) appear to be haploid, with the nuclear DNA being in the replicated (2C) state. The plasmodia are in all cases diploid; however, the data indicate that the selfed plasmodia are in an extended G_1 condition. The nuclear DNA content of these is therefore 2C, whereas that of the cross plasmodium is 4C. Sporangial nuclei exhibit DNA in diploid replicated (4C) category.

Additional Articles in Print

A. Allera and K. Wohlfarth-Bottermann
"Extensive fibrillar protoplasmic differentiations and their significance for protoplasmic streaming. IX. Aggregation states of myosin and conditions for myosin filament formation in the plasmodia of Physarum polycephalum"
Cytobiologie 6, 261 (1972)
(For summary, see PNL 4, 34, 1972)

W.D. Grant and R.T.M. Poulter
"Rifampicin-sensitive RNA and Protein Synthesis by Isolated Mitochondria of Physarum polycephalum"
Journal of Molecular Biology 73, 439 (1973)
(For summary, see PNL 4, 35, 1972)

F.B. Haugli, W.F. Dove and A. Jimenez
"Genetics and Biochemistry of Cycloheximide Resistance in Physarum polycephalum"
Molecular and General Genetics 118, 97 (1972)
(For summary, see PNL 4, 35, 1972)

F.B. Haugli and W.F. Dove
"Mutagenesis and Mutant Selection in Physarum polycephalum"
Molecular and General Genetics 118, 109 (1972)
(For summary, see PNL 4, 35, 1972)

TITLES AND SUMMARIES IN PRESS

Functional Homologies of Acidic Chromatin Proteins in Higher and Lower Eukaryotes

Wallace M. LeStourgeon, Wayne Wray, and Harold P. Rusch

SUMMARY

Within a specific fraction of acidic chromatin-associated proteins from HeLa and the lower eukaryote Physarum polycephalum numerous similarities exist. Several of the similar polypeptides in both cell types are synthesized and appear in the residual chromatin material while still others disappear in response to starvation, a common and universal stimulus. Proteins which incorporate no radioactive amino acids during starvation and ultimately disappear from the residual chromatin material are resynthesized upon refeeding. This resynthesis must be complete before mitosis will again occur. These observations suggest that within the complement of acidic chromatin proteins functional homologies exist in diverse eukaryotes.

Experimental Cell Research, in press

Time of Synthesis of Genes for Ribosomal Ribonucleic Acid in *Physarum*†

Carol Shaw Newlon,‡ Gail E. Sonenshein,§ and Charles E. Holt*

ABSTRACT: The time in the cell cycle when the genes for ribosomal RNA are synthesized was determined in the plasmodial stage of *Physarum polycephalum*. Three approaches were used. (1) Plasmodia were exposed to [^{3}H]thymidine during the G2 phase of the cell cycle; nuclear satellite DNA, which is preferentially labeled under these conditions, was isolated and hybridized in solution with ribosomal RNA (rRNA). Analysis of the hybridization mixture revealed that 10–25% of the labeled DNA was in DNA–RNA hybrids, which demonstrates synthesis of rDNA during the G2 phase. (2) The per cent of nuclear DNA hybridizable with saturating amounts of rRNA was measured for DNA isolated from plasmodia at different times in the cell cycle. The per cent hybridization increased about 50% during the G2 phase, which also demonstrates synthesis of rDNA during this period. (3) Plasmodia were incubated with the DNA density label iododeoxyuridine, nuclear DNA was isolated, and the buoyant density profile of rDNA was determined. A fraction of the rDNA was shown to be of increased density when the density label was applied during either the S phase, the first half of the G2 phase, or the second half of the G2 phase (there is no G1 phase in *Physarum* plasmodia). Thus, rDNA synthesis occurs during all phases of the mitotic cycle in this organism.

Biochemistry, in press

CARBOHYDRATE METABOLISM IN THE PLASMODIUM OF THE MYXOMYCETE *Physarum flaviconum*

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Abstract

Carbohydrate metabolism in the growing plasmodial phase of *Physarum flavicomum* was studied in partially defined media using the radiorespirometric technique and specifically labeled ^{14}C-substrates. The Embden-Meyerhof-Parnas (EMP)-tricarboxylic acid cycle (TCA) and the pentose phosphate pathways are the routes by which glucose is utilized by this Myxomycete. The replacement of the usual citrate-phosphate buffer by succinate-phosphate results in a decreased uptake of ^{14}C-glucose from the medium and a corresponding decline in the rate of interval $^{14}CO_2$ evolution. The addition of an inorganic nitrogen source (ammonium nitrate) to the medium, also decreases the rate of carbohydrate metabolism and alters the relative participation of the pathways by favoring the EMP-TCA. Supplementing the medium with cyclic-3'-5'-adenosine monophosphate produces a transient stimulation of the rate of metabolism by the EMP-TCA. The plasmodium is relatively impermeable to gluconate and pyruvate and does not readily metabolize amino acids.

Canadian J. Microbiology. in press (June. 1973)

ABSTRACTS OF MEETING PRESENTATIONS

Abstract of paper presented at the Twelfth Annual Meeting of The American Society For Cell Biology, Nov. 8-11, 1972 (omitted from PNL 4, # 2).

431. CAPILLARY SUCTION AS TESTING PRESSURE GRADIENTS IN PROTOPLASMIC MOTION
R. A. Rinaldi, G. Kirby and I. Cameron. Biophysics Lab.,NYU, N.Y.; Dept.Mech. Eng., Texas Tech U., Lubbock, Texas; Anatomy Dept.,U.Texas, San Antonio,Texas.

Protoplasmic reaction to applied differential pressure has been classically demonstrated by many investigators. A recent report in Science (174, R. D. Allen,et al, 1971) indicates that advancing pseudopodia are not altered by the application of suction pressure to protoplasm. In repeating these experiments we confirmed that capillary pipettes (indicating a high negative pressure compared to environmental fluid pressure) had no discernible effect on the course of psuedopodial extension. Further, we did comparable experiments on Physarum, which all investigators agree flows by means of hydraulic pressure forces developed from its contracting tubes, and noted that there was no effect on protoplasmic flow from suction pressure. Upon analysis of our motion picture films which are presented here, it was obvious that the pressure recorded by our gauges did not indicate the pressure at the pipette tip but instead the pressure at the vacuum source. Also gauge vacuums of 25mm Hg did not alter the flow pattern of Amoeba or Physarum. By calculating the drop of pressure in the capillary pipette it was found that the tip pressure was much lower. The indicated pressure was not transmitted to the cell. However, when suction was applied to the cell, the protoplasm could easily seal the bore of the pipette or at higher pressures flow down the pipette. An analogy would be a sealed rubber glove filled with a fluid. If suction were placed on one finger and a hole made in another, the fluid would flow out of the hole independently of the applied suction due in part to the forces generated by the elasticity of the glove. A similar situation is conceivable in cells with protoplasmic gel cortices which can generate contractile forces produc ing internal turgor pressure of protoplasm. Thus, those experiments suggesting that applied pressures do not affect protoplasmic movement are inconclusive.

J. Cell Biol. 55, 216a (1972)

Abstracts of papers presented at the "Sommertagung der Gesellschaft für Biologische Chemie", May 23-25, 1972.

A. Hildebrandt und H. W. Sauer

Über die Bedeutung der Thymidinphosphorylierung für die S-Phase des Zellzyklus von Physarum polycephalum

Wir betrachten die synchronen Kernteilungen des Myxomyceten *Physarum* als zyklische Differenzierungsvorgänge während des Zellwachstums und die S-Phase als einen biochemischen Marker der Differenzierungsleistung. Wir untersuchten die Frage, ob die Mitose durch eine sequentielle Transkription programmiert ist oder durch einen bestimmten Zustand des Zellstoffwechsels induziert wird. Die lineare Transkription einer Anzahl von Genen könnte die bekannte Korrelation der S-Phase mit dem Maximum der Thymidin-Kinaseaktivität (EC 2.7.1.21) durch nur einen Kontrollmechanismus erklären[1].

Wir konnten zeigen, daß die Aktivität der Thymidin-Kinase auch dann erhöht ist, wenn die DNA-Synthese blockiert war. Allerdings war die Wirkung von Inhibitoren (Hydroxyharnstoff, HU 5 mg/ml; 5-Fluor-2'-desoxyuridin, FdUrd, 10 µg/ml plus Uridin 200 µg/ml) auf die Thymidin-Kinase-Aktivität unterschiedlich. a) FdUrd bewirkte einen Anstieg der Enzymaktivität noch über das Niveau der S-Phase hinaus. b) Nach Auswaschung des Hydroxyharnstoffes war erneut DNA-Synthese zu beobachten, obwohl die Enzymaktivität auf das Niveau der G_2-Phase abgesunken war und nicht wieder anstieg. Die Entkoppelung der DNA-Synthese von der Zunahme der Enzymaktivität macht eine gemeinsame Regulation der S-Phase und der Thymidinphosphorylierung unwahrscheinlich. Dagegen sprechen auch zwei weitere Befunde: 1. Obwohl der Enzymspiegel variabel ist, lassen sich keine Unterschiede in den Poolgrößen der aus [^{3}H]Thymidin in vivo gebildeten Thymidinphosphate erkennen. 2. Die im Hauptweg der TTP-Bildung gelegene Thymidinmonophosphat-Kinase (EC 2.7.4.9) zeigt keine Aktivitätsschwankungen im Zellzyklus.

Da die verwendeten Inhibitoren die DNA-Synthese nur indirekt hemmen und die Transcription nicht deutlich beeinflussen, weisen die Zunahme der Thymidin-Kinase-Aktivität unter FdUrd und ihre Abnahme durch Hydroxyharnstoff auf die Bedeutung hin, die veränderte Poolgrößen für die Enzymaktivitäten in der Zelle haben können.

[1] Sachsenmaier, W. & Ives, D. H. (1965) *Biochem. Z.* 343, 399–405.

Adresse: Dr. H. W. Sauer, Zoologisches Institut der Universität, D-69 Heidelberg, Berliner Str. 15.

H-S Z. Physiol. Chem. 353, 715 (1972).

W. Schiebel und U. Bamberg

Charakterisierung einer löslichen DNA-Polymerase aus isolierten Kernen des synchron wachsenden Myxomyceten Physarum polycephalum

Der einfach organisierte Eukaryont *Physarum polycephalum* erlaubt die Gewinnung einheitlicher, von Natur aus synchroner Zellkerne[1], die Desoxyribonucleotide aus zugefügten Triphosphaten dann in ein säureunlösliches Produkt einbauen, wenn sie während der DNA-Synthesephase isoliert worden sind[2].
Bei erhöhter Salzkonzentration (2M KCl) läßt sich aus dieser Kernfraktion nach Zentrifugieren (20 min 35000×*g*) ein Extrakt erhalten, der nach Dialyse den Einbau von [^{3}H]dTTP in säureunlösliches Material stimuliert. Die Aktivität des Kernextraktes wird durch Wärmedenaturierung (46°C 10 min) auf 50% vermindert. Anwesenheit von $Mg^{2\oplus}$ (30mM) und DNA im Inkubationsansatz ist für den Einbau essentiell. Mit pankreatischer Desoxyribonuclease (EC 3.1.4.5) partiell abgebaute DNA ist etwa 10mal wirksamer als native DNA. Für optimalen Einbau werden außer freien 3'-OH-Gruppen auch Doppelstrangbereiche benötigt, denn einzelsträngige DNA stimuliert weniger stark als doppelsträngige DNA. Die Einbaugeschwindigkeit bleibt für 60 min konstant und ist der Proteinkonzentration zwischen ungefähr 1 und 10 µg proportional. Als Puffer wurde 0,1M Morpholinopropansulfonsäure/KOH verwendet, das pH-Optimum liegt bei 6.8. Pyrophosphat, Desoxyribonuclease, Pronase sowie der SH-Gruppen-Blocker *p*-Hydroxymercuriphenylsulfonat hemmen auf weniger als 7% der Kontrolle. Zentrifugation im Saccharosegradienten (8–34% G/G) ergab eine Aufteilung der Polymeraseaktivität. Ob es sich hierbei um verschiedene Enzyme oder Assoziation von Enzymprotein mit wechselnden Mengen DNA handelt, konnte bisher nicht entschieden werden. Die langsamer sedimentierenden Anteile zeigten S-Werte zwischen 7 und 10. Vorläufige Untersuchungen zur Aktivität innerhalb des Mitosezyklus deuten darauf hin, daß die extrahierbare Enzymaktivität/Plasmodium in der ersten Hälfte des etwa 11stdg. Zyklus konstant bleibt.

[1] Mohberg, J. & Rusch, H. P. (1971) *Exp. Cell Res.* **66**, 305–316.
[2] Brewer, E. N. & Rusch, H. P. (1965) *Biochem. Biophys. Res. Commun.* **21**, 235–241.

Adresse: Dr. W. Schiebel, Max-Planck-Institut für Biochemie, D-8 München 2, Goethestr. 31.

H-S Z. Physiol. Chem. 353, 753 (1972).

MYXOLANEOUS NEWS

ALOYS HUTTERMANN is now visiting at the Univ. of Tromso (Norway) and will be there until January, '74 . . . JENNIFER DEE is presently working with NED HOLT at M.I.T. (Cambridge, Mass.). She'll be heading back to Leicester in September . . . HELEN and TOM EVANS will be spending one year with BILL DOVE, et al., at the McArdle Labs (U. of Wisc.) starting Sept. 1, 1973 . . . . ODDVAR NYGAARD is completing his first year as Managing Editor of "Radiation Research".

A. Hüttermann

Differentielle Proteinsynthese während der Differenzierung von Physarum polycephalum: Nachweis durch Dichtemarkierung

Beim echten Schleimpilz *Physarum polycephalum* kann die Bildung von Sklerotien durch Überführen von wachsenden Plasmodien in ein nährstofffreies Medium induziert werden, nach 24 h sind dann die ersten Mikrosklerotien sichtbar und nach weiteren 12 alle Plasmodien in Sklerotien differenziert.
Während der ersten 24 h nach der Induktion wurde ein sehr unterschiedliches Verhalten im Aktivitätsverlauf verschiedener Enzyme beobachtet: Glutamat-Dehydrogenase und Phosphodiesterase (EC. 3.1.4.1) steigen auf etwa das neunfache des Ausgangswertes an, während Glucose-6-phosphat-Dehydrogenase (EC 1.1.1.49) auf ein Drittel der ursprünglichen Aktivität absinkt.
Durch Einbau deuterierter Aminosäuren und anschließende Analyse im CsCl-Gradienten konnte gezeigt werden, daß:

Phosphodiesterase während der gesamten Differenzierung,

Glutamat-Dehydrogenase nur während der letzten 12 h nach Beginn der Induktion und

Glucose-6-phosphat-Dehydrogenase nicht synthetisiert wird,

Somit konnte für diese Differenzierung eine differentielle Proteinsynthese mit unterschiedlichem Zeitverlauf nachgewiesen werden.

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H-S Z. Physiol. Chem. 353, 718 (1972).

Abstract of paper presented at
the 57 th FASEB Meeting
April 15-20, 1973

1286 BIOCHEMISTRY

ISOLATION OF A DNA REPLICASE FROM PHYSARUM POLYCEPHALUM, E.N. Brewer* (SPON: Paul S. Lavik). Case Western Reserve Univ., Cleveland, O. 44106

Nuclei isolated from S-phase cultures of Physarum polycephalum are capable of supporting the incorporation of ^{3}H-dATP into acid-insoluble product at ca. 5% of the in vivo rate. Mg^{++}, spermine, ATP, and all four deoxyribonucleoside triphosphates are required for maximal activity. Alkaline sucrose density gradient centrifugation data (Brewer, J. Mol. Biol., 68, 401, 1972) suggest that the labeled precursor is incorporated into daughter DNA strands, but not into parental strands, indicating that this in vitro activity represents replicative rather than repair synthesis. The level of incorporation can be reduced by washing the nuclei, and restored by adding back the nuclear extracts. These extracts exhibit DNA polymerase activity when assayed with native salmon sperm DNA as template. Addition of cycloheximide to the growth medium of Physarum inhibits DNA replication in vivo (Muldoon et al., Biochem. Biophys. Acta, 247, 310, 1971). In the present study, exposure of the intact organism to cycloheximide (10 µg/ml) resulted in decreased DNA synthesis by isolated nuclei, and in decreased polymerase activity of nuclear extracts. The results suggest that the "DNA polymerase" extracted from nuclei isolated from Physarum is involved in the DNA replication process in this organism. (Supported in part by NIH Grant GM-19484, American Cancer Society Institutional Grant IN 57-K, and AEC Contract W-31-109-ENG-78.)

Fed. Proc. 32, 452 (1973)

2nd EUROPEAN SYMPOSIUM ON THE CELL CYCLE

University of Innsbruck, 2 – 4 April 1973

PROGRAMME

THE CELL CYCLE IN NORMAL AND MALIGNANT CELLS

sponsored by the
European Cell Biology Organization (ECBO) and the Austrian Cancer Society, Section Tirol

REPLICATION OF RIBOSOMAL DNA IN PHYSARUM

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In plasmodia of the myxomycete Physarum polycephalum more than 95% of the 10^9 nuclei go through mitosis at the same time. In cesium chloride gradients three fractions of Physarum DNA can be distinguished: main nuclear DNA; mitochondrial DNA (about 10% of total DNA) and nuclear satellite DNA (about 2% of total DNA). The main nuclear DNA replicates in the first 3 hours (S-phase) of the 9 hour intermitotic time. Both satellite DNA fractions replicate in the G2 phase as well as in the S-phase, but no isotope incorporation into nuclear satellite DNA is seen in the first hour after mitosis, a time during which nucleoli are reformed following their apparent disintegration in mitosis.

It is shown here that preparations of nucleoli contain most of the nuclear satellite DNA and that contaminating main band DNA can be removed in preparative cesium chloride gradients. The purified nucleolar satellite DNA hybridizes to an extent of about 30% with excess ribosomal RNA. Thus most of this DNA consists of genes for ribosomal RNA. It can be calculated that ribosomal genes are reiterated 1000 to 2000 times per nucleus and that each gene is transcribed, on average, two to four times per minute.

By electron microscope autoradiography the ribosomal DNA has been localized in the fibrillar and not the granular regions of the nucleoli.

ACTOMYOSIN-LIKE PROTEINS IN THE NUCLEI OF PHYSARUM POLYCEPHALUM

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Actomyosin-like proteins were found among the proteins which can be extracted from nuclei isolated from plasmodia of the slime mold Physarum polycephalum. The nuclear actin was characterized by comparing it with actin purified from the cytoplasm of P.p. Molecular weights, solubility, aggregation properties, and tryptic fingerprints of the two proteins were very similar, but different from the data obtained for rabbit actin. The possibility that cytoplasmic actin contaminated the nuclei during nuclear isolation was excluded in a mixing experiment. The nuclear actin is located in the nucleolus, accumulating there in late G_2 phase and comprising 27 % of the total nucleolar protein.

A myosin-like protein was extracted with the nuclear proteins soluble in buffers of high ionic strength. This protein contains polypeptides with a molecular weight (200 000) similar to the large polypeptides of rabbit myosin. Myosin-like fibers were seen with the electron microscope in mitotic nuclei of thinsectioned plasmodia.

There is evidence that nuclear actin is not restricted to nuclei performing an intranuclear mitosis, as found in the plasmodia of Physarum polycephalum, but is present in other organisms with different types of mitosis. On the basis of this evidence, a general function of these contractile proteins in mitotic events will be discussed.

ADVANCED INITIATION OF SYNCHRONOUS NUCLEAR DIVISION IN PHYSARUM POLYCEPHALUM FOLLOWING UV-IRRADIATION: EFFECTS OF ACTINOMYCIN C.

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Macroplasmodia of the myxomycete Physarum polycephalum contain several millions of nuclei which divide in full synchrony every 8 to 10 hours. Initiation of synchronous nuclear mitosis probably is controlled by a cytoplasmic factor which accumulates gradually during interphase. A model has been described (1) suggesting that the initiator molecules react with nuclear receptor sites in a stoichiometric way. As soon as all nuclear sites are covered by initiator molecules, which are formed proportional to the increase of the total plasmodial mass, all nuclei start to divide and a new set of nuclear receptors is formed. The interdivision time thus is determined by the time required to "titrate out" a given number of nuclear receptors with a gradually increasing number of initiator molecules.

This model has been derived in part from the observation that nuclear mitosis can be advanced by reducing the number of nuclei in a plasmodium by UV-irradiation (2). The immediate effect after irradiation of plasmodia with UV-light (λ max. 254 mμ, ca. 7500 ergs/mm^2) at any time during the first 2/3 of the nuclear division cycle is a delay of the next mitosis by 2 to 3 hours. All nuclei enter the delayed mitosis synchronously, however, a considerable number of them (ca. 30%) become pycnotic during the following S-phase and disappear within a few hours probably by lysis. As a consequence, the nucleo-cytoplasmic ratio (reflected by the ratio of DNA/protein) is decreased. The second mitosis occurs after a shortened interval almost making good the initial delay of the first division. Subsequent mitoses occur up to two hours earlier compared to the untreated controls. According to our model, mitosis in a plasmodium with a reduced nucleo-cytoplasmic ratio enter mitosis prematurely since the diminished number of nuclear receptor sites are covered with cytoplasmic initiator molecules faster than in a normal plasmodium.

Interestingly, the onset of synchronous mitosis in irradiated plasmodia becomes less sensitive to actinomycin C. Nuclear division in normal plasmodia is inhibited by 250 μg/ml actinomycin C if added to the medium as late as 1 hour prior to the expected prophase (3). The timing of the second post-irradiation mitosis in UV-treated plasmodia, however, is little or not effected by this concentration of the drug (Table 1). The loss of sensitivity to actinomycin has been interpreted recently by Devi and Gutes (4) to mean that the preparation of postirradiation mitoses in UV-treated plasmodia no longer depends on the synthesis of new RNA since some nuclear material essential for mitosis is released from degrading nuclei and revitalized by the surviving nuclei. Our experiments (Table 1) however indicate that RNA synthesis in irradiated plasmodia also becomes largely resistant to actinomycin C (100 and 250 μg/ml), which easily explains the loss of the antimitotic effect of the drug. Increasing the concentration up to 500 μg/ml partially overcomes this resistance and causes inhibition of mitosis similar to the effect observed with lower concentrations of actinomycin in unirradiated plasmodia. We conclude therefore that the onset of premature postirradiation mitoses does depend on newly synthesized RNA (and presumably on protein) like normal mitoses. A rough calculation suggests that our model can fully account for the observed advancing effect on mitosis only by the loss of 20 to 40% of the nuclei without assuming a vitalizing effect by nuclear material liberated from degrading nuclei.

	Act.C	Spec. radioactivity of RNA			
	μg/ml	cpm/mg RNA		%	
		A	B	A	B
Control	0	14.1	9.9	100	100
	100	9		19	
	250	6.3		13	
	500		1.3		13
UV-irr.	0	8.6	5.6	100	100
	100	4.6		54	
	250	5.8	6.2	68	110
	500		3.4		61

Table 1. Effect of actinomycin C on RNA synthesis in normal and UV-irradiated plasmodia of Physarum polycephalum.
A macroplasmodium (8 4 cm) was dissected into two equal parts 3.5 hrs prior to mitosis (M_1) and one segment was irradiated immediately with UV light (λ max = 254 mμ, 7500 ergs/mm^2).
Small pieces (2 cm^2) of each moiety were transferred onto medium containing various concentrations of actinomycin C 6 hrs (experiment A) of 7 hrs (experiment B) after control mitosis (M_2).
After 30 minutes the plasmodial pieces were pulse labelled with ^{14}C-uridine (0.2 μCi/ml, 52 mCi/mM) in the presence of actinomycin for 30 minutes and the specific radioactivity of total RNA was determined by liquid scintillation counting.

EFFECTS OF IONIZING RADIATION ON THE CYCLE DEPENDENT FLUCTUATIONS OF THYMIDINE KINASE IN PHYSARUM POLYCEPHALUM

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Irradiation of synchronous plasmodia of the myxomycete Physarum polycephalum with X-rays (1000 r) delays the onset of the next mitosis up to about 3 hours. This effect strongly depends on the stage of the nuclear division cycle at the time of treatment (3). The maximum delay of mitosis (M) is observed if irradiation occurs during the preceding mitosis (M-1). A second peak of sensitivity exists at the end of the G_2-period, however, about 10 times larger doses are required to cause the same effect as seen after irradiation during mitosis (M-1). Enzyme activity of thymidine kinase increases sharply just prior to the onset of synchronous mitosis and reaches a peak during the early S-phase (1). The peak of enzyme activity in X-ray treated plasmodia is shifted parallel to the delay of nuclear mitosis (3).

A striking stimulatory effect on thymidine kinase production is observed if X-rays are applied during a narrow period from prophase to telophase of mitosis (M-1): enzyme activity rises to a peak at the time of the delayed mitosis (M^*) which is 2 - 3 times higher than in unirradiated controls. Irradiation at other times in the cycle, including the second radiosensitive period at the end of G_2, does not cause any stimulatory effect. Fig. 1 indicates that the maximum stimulatory effect on enzyme activity is achieved exactly at the same point of the cycle where X-irradiation induces a maximum delay of the next mitosis. Mixing of extracts prepared from normal and irradiated plasmodia as well as inhibitor studies with cycloheximide suggest that the observed stimulation of enzyme activity reflects an increase of enzyme production rather than activity modification or similar changes, like thymidine kinase in normal and irradiated plasmodia. On the other hand, non periodic enzymes (thymidinemonophosphat kinase, thymidinediphosphate kinase, glucose 6 phosphate dehydrogenase) were not influenced by X-ray treatment.

Irradiated plasmodia become remarkably resistant to the antibiotic actinomycine C. The transition point for the inhibitory effects on mitosis as well as on the induction of thymidine kinase and deoxycytidine kinase is located about 1 - 5 hours prior to the onset of mitosis in normal plasmodia (2,4). Irradiated plasmodia become refractive to actinomycine 250 mg/ml) at the same time as the controls, since mitosis is delayed by 2.5 hours in the irradiated culture, the refractive period is prolonged to 4 hours (Fig. 2). At first sight this might be interpreted to mean that X rays interfere with the timing mechanism of mitosis and with the induction of periodic enzymes at the level of translation rather than at the level of transscription of the corresponding genetic information. However, labelling with 14C uridine reveals that RNA synthesis in irradiated plasmodia becomes less sensitive to actinomycine C simultaneously with the onset of the refractory period of mitosis and enzyme induction (Fig. 2A). Increasing the drug concentration up to 500 mg/ml largely overcomes the radiation induced resistance with respect to all 3 parameters. The reduced sensitivity to actinomycine C of irradiated plasmodia may be linked to DNA repair synthesis.

As a working hypothesis we assume that X rays applied during the high sensitive period just prior to the onset of S phase interfere with nuclear DNA possibly by causing single strand breaks. The damage is "fixed" during DNA replication before fast repair processes become effective. One of the consequences is a delay of the formation of gene products required for the initiation of nuclear mitosis. Other consequences involve the timing as well as the yield of periodic enzyme production. The stimulatory effect of X-rays on thymidine kinase and deoxycytidine kinase might result from the interference with the formation of a repressor as discussed previously (3).

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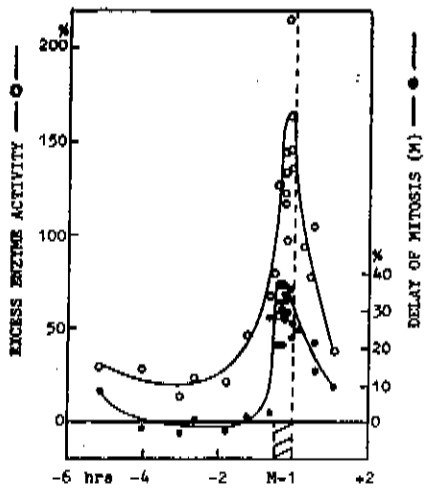

Fig. 1. X - ray induced delay of mitosis and stimulation of thymidine kinase production.
Individual plasmodia from a series of replicate synchronous cultures were irradiated at various points of the nuclear division cycle indicated on the abscissa. The delay of mitosis (M) was observed compared to unirradiated controls and thymidine kinase activity was determined at the expected peak within 30 min after telophase. The ordinate indicates
a) delay of mitosis (M) in % of the normal cycle length (•——•)
b) [illegible] enzyme activity in % over control peak (o——o).

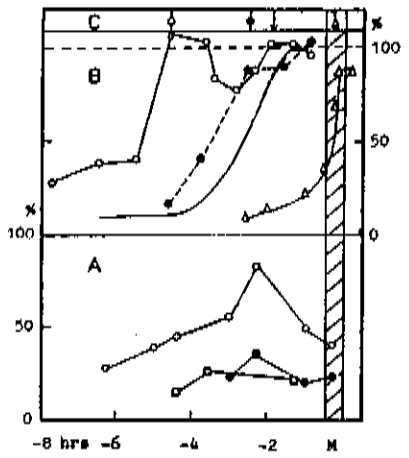

Fig. 2. Effect of actinomycin C on RNA synthesis, thymidine kinase production and nuclear mitosis following X ray treatment.
Synchronous macroplasmodia were irradiated with 1000 R during metaphase of mitosis (M - 1), i.e. 10.5 hrs prior to control mitosis (M).

A) Normal and irradiated plasmodial segments, ca. 2 cm^2 were dissected and transferred onto medium containing 250 or 500 µg/ml actinomycin C at various time points indicated on the abscissa. After 30 min they were pulse labelled with 0.2 µCi/ml ^{14}C-uridine (62 mCi/mM) for 30 min in the presence of actinomycin and the incorporation of label into RNA was determined by liquid szintillation counting (2).
The ordinate indicates specific radioactivity of RNA relative to controls (= 100%), not treated with actinomycin C.

o——□ not irradiated, 250 µg/ml act.C,
o——o irradiated, 250 µg/ml act.C,
•——• irradiated, 500 µg/ml act.C.

PERIODICITY OF NAD METABOLISM IN THE CELL CYCLE

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Two enzymes of NAD metabolism are confined to the chromosomes. This fact suggests that they participate in some way in chromosomal physiology. The two enzymes are NAD pyrophosphorylase (NAD PPase) and poly ADP-ribose polymerase (P-ADPR-P). NAD pyrophosphorylase catalyses the synthesis of NAD from ATP and nicotinamide mononucleotide. It is a reversible reaction. Since this enzyme is confined exclusively to the chromosomes, NAD biosynthesis is unique among all the coenzymes; all the other coenzymes are synthesized in the cytoplasm. No direct involvement of NAD in either DNA or RNA biosynthesis is known at the moment. The second chromosomal enzyme, poly ADP-ribose polymerase catalyses the synthesis of the polymer-poly ADP-ribose. The substrate for this reaction is NAD, and the reaction is entirely DNA dependent. The polymer is covalently bonded to chromosomal proteins. The function of this enzyme is quite obscure at this time.

We have examined the specific activity of these two enzymes through the cell cycle. The specific activity of NAD pyrophosphorylase in isolated nuclei of Physarum polycephalum increased between 1 and 1/2 hour before mitosis and reached a peak at mitosis. The activity remained high throughout the S-phase, which follows mitosis directly in this organism, and then declined to a stable, basal level which was maintained throughout the G2 period. This fluctuation in activity is similar to that observed for DNA polymerase and thymidine kinase, but is unlike that of other metabolic enzymes in Physarum.

The peak specific activity was about 8 times the basal value.

The specific activity of the poly ADP-ribose polymerase in isolated Physarum nuclei also varied during the cell cycle. The fluctuation was the mirror image of that observed with NAD pyrophosphorylase. During G2 a constant high specific activity was observed. After mitosis a sharp drop in specific activity lead to a minimum in late S phase. The minimum activity was about half the G2 specific activity.

We have also estimated the cellular NAD^+ content during the cell cycle in LS, mouse fibroblast cells. The cells were synchronized by the velocity sedimentation method in a sucrose gradient. The synchronized cells showed a peak of NAD^+ content at 15 to 20 hours; this corresponds approximately to the S phase. The magnitude of the fluctuation was not very large.

We observe a close temporal association between NAD pyrophosphorylase activity, poly ADP-ribose activity and DNA synthesis. We have also demonstrated a temporal relationship between growth rate and these two enzyme activities and NAD^+ levels. We construct therefore the working hypothesis that NAD metabolism and these two chromosomal enzymes in particular serve to directly integrate DNA and cell replication with energy metabolism and growth rate.

REGULATION OF THYMIDINE PHOSPHORYLATING ENZYMES IN THE SYNCHRONOUS NUCLEAR DIVISION CYCLE OF PHYSARUM POLYCEPHALUM

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Enzyme activities of thymidine kinase (I) and deoxycytidine kinase (II) undergo periodic fluctuations during the synchronous nuclear mitotic cycle of the myxomycete Physarum polycephalum. The activity peaks of both enzymes coincide with early S phase which immediately follows nuclear division in this organism. The enzymes phosphorylating thymidine monophosphate and thymidine diphosphate as well as various other enzymes not related to DNA metabolism remain at essentially constant levels (enzyme units/mg soluble protein) throughout the division cycle. The average relative specific activities of the periodic enzymes are considerably lower compared to the nonfluctuating enzymes. Inhibitor studies with actinomycin C and cycloheximide suggest that the sharp increase of enzyme activities (I) and (II) observed just prior to the onset of mitosis reflects true increase of enzyme protein. Inhibition of DNA synthesis with hydroxyurea, 5 fluoro 2' deoxyuridine, or 5' fluorothymidine stimulates unscheduled excess production of enzyme. A similar effect is observed during the treatment with actinomycin C at intermediate concentrations (30 µg/ml) which cause only a moderate inhibition of RNA synthesis. The paradox effect of actinomycin on enzyme production is most pronounced under conditions which block dividing nuclei in metaphase for several hours.

A model is discussed suggesting that the production of enzymes (I) and (II), and possibly of other enzymes not yet tested, is stimulated at the end of G_2 by an endogenous inducer at the transcription level. Enzyme synthesis is turned off as soon as the corresponding structural genes are replicated during the early S period. Interference with DNA replication or arresting mitotic nuclei in metaphase with actinomycin C prevents repression of the activated genes, thus allowing excess enzyme production.

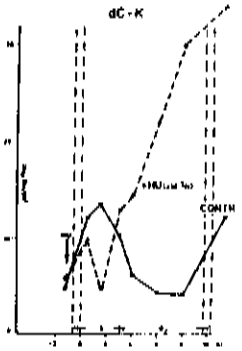

Fig. 1

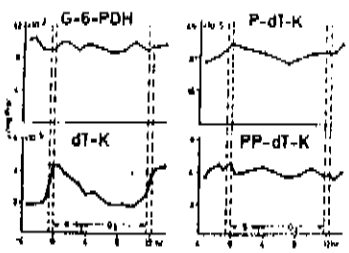

Fig. 2

	Compound	Conc. (mM)	dT-K	dC-K	DNA-Synth.
Control	Control		100 %	100 %	100 %
	Hydroxyurea	1	190	240	42
		3	240	800	33
		10	100	n.t.	8
	5 Fluorodeoxyuridine + Uridine	0.43 0.4	150	350	
	5' Fluorothymidine	0.8	200	580	
	Amethopterin + Adenosin	0.002 0.01	140	240	
		µg/ml			
	Actinomycin C	10	70	150	
		30	90	220	
		50	60	170	
		100	20	70	
	Cycloheximide	5	20	20	

Tabelle I: Relative enzyme activities (%) of thymidine kinase (dT-K) and deoxycytidine kinase (dC-K) in non synchronous suspension cultures (S) of Physarum polycephalum after 16 hrs treatment with various inhibitors.

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Fig.1. Specific activities of various enzymes in high speed supernatants of plasmodial homogenates prepared at various points of the mitotic cycle of Physarum polycephalum.
dT-K: Thymidine kinase (1.3). P-dT-K: Thymidinemonophosphate kinase (4). PP dT K: Thymidinediphosphate kinase (5).
G-6-PDH: Glucose 6 phosphate dehydrogenase (1).

Fig.2. Specific enzyme activity of deoxycytidine kinase (dC-K) in synchronous plasmodia of Physarum polycephalum. Enzyme activity was assayed analogous to thymidine kinase (1.3) using ^{14}C-deoxycytidine as substrate. O ----O normal plasmodium, Δ -----Δ plasmodium transferred onto medium containing 3.10^{-3}M hydroxyurea (HU).

Abstracts of papers presented at the 73rd Annual Meeting of the American Society for Microbiology, May 6-11, 1973.

G 72 Accommodation to Low Levels of Environmental Stressors: Protection against Cadmium-Induced Mitotic Delay in Physarum polycephalum. B. Chin*, G. Lesowitz, and I.A. Bernstein. Univ. Michigan, Ann Arbor.

The following data demonstrate that a cell has the ability to accommodate to low levels of environmental stressors by developing a protective mechanism against subsequent exposure of greater magnitude:

(1) Exposure of a cell in early-G_2 to a subthreshold dose of cadmium (10^{-4} M for 30 min) had no effect upon the timing of mitosis; exposure of a cell in late-G_2 to a suprathreshold dose (5×10^{-4} M for 30 min) resulted in a significant delay of 2 hr in a 14 hr cell cycle; no measurable delay in the timing of mitosis was detected when a cell was exposed first to a subthreshold dose in early-G_2 and then to a suprathreshold dose in late-G_2.

(2) A study of the nature of this protective response showed that the level of protection was dependent upon the concentration of the first exposure, the protection raised the threshold in late-G_2 by almost one order of magnitude, the protective response could be elicited when exposure occurred within the greater part of the cell cycle, and the development of protection was time-dependent.

(3) Similar protective responses were also induced with low levels of mercury and nickel against cadmium and against themselves, respectively.

P 209 Purification and Properties of Cytoplasmic and Mitochondrial Malate Dehydrogenases of Physarum polycephalum. W. M. TEAGUE,* and S. S. HENNEY, JR. Univ. of Houston, Houston, Tex.

The cytoplasmic and mitochondrial forms of malate dehydrogenase (L-malate:NAD oxidoreductase, EC 1.1.1.37) were separated and purified from P. polycephalum plasmodia using acidification, acetone precipitation, ammonium sulfate fractionation, DEAE- and Se-cellulose chromatography, and Sephadex gel filtration. The mitochondrial (M-MDH) and cytoplasmic (S-MDH) forms were also distinguished by their relative electrophoretic mobility, thermolability, and catalytic properties. The M-MDH was the more cathodal of the two isoenzymes and thermally more labile. Both the S-MDH and M-MDH exhibited a pH optimum of 7.6 for oxaloacetate (OAA) reduction and 10 for malate oxidation. The optimal OAA concentration for S-MDH and M-MDH was 0.25 mM and 0.35 mM, respectively. The malate optimum was 5 mM for S-MDH and 6 mM for M-MDH. Michaelis constants and ability to utilize nicotinamide adenine dinucleotide analogues served to further distinguish between S-MDH and M-MDH. Many properties of the P. polycephalum isoenzymes were similar to those of more complex organisms.

A Mysterious Blob Stirs Scare in Texas

DALLAS (AP) — It's something right out of an aftermidnight television horror movie—a mysterious, encompassing ooze, dubbed "The Blob." Sor far it appears to be friendly. There are those who toy with the notion that it may be a long-dormant mutation from outer space.

"People fear the unknown," said Arnold Dittman, a member of Growth International, a company that recycles waste. "If they don't know what it is, they naturally fear it. We all dream, and we probably all would like to see something from outer space. But I doubt if this is anything like that."

To him it's just a harmless bacteria, perhaps a mutation.

Reports of the blob came from various areas after it first made its debut two weeks ago, oozing up in the backyard of a suburban Garland housewife, Marie Harris.

The Dallas Times Herald heralded the birth of the blob this way;

"The mysterious membrane still pulses . . . It has multiplied itself 16 times over in two weeks . . . blackish mucous inside. . . . reddish with thick bubbles on top . . . foamy like shaving cream . . . turns colors when punctured . . . when the bubbles burst, it appears to be bleeding red and purplish inside." That did it. From an area east of Dallas Edna Smith reported seeing the blob climbing a telephone pole.

"It was red and pulsating, like the one I read about it," she added. "For heaven's sake, what is it?"

Then a North Dallas woman was heard from. She refused to be identified, but said: "I'm scared to death. I have the same thing on my hedge. I can't kill it."

Where could the blob have come from?

To the northwest at Aurora, Tex., a small graveyard is said by villagers to hold the remains of a mysterious creature whose spacecraft crashed there in 1897. Dallas newspaper accounts of the day noted the reports.

Could the blob have come along for the ride? Not in the opinion of Dittman, who said his Growth International is concerned with the recycling of waste, including the use of bacteria to digest it.

"The blobs appear to be a combination of various bacteria," said Dittman. "But we don't know what that combination is at this time because the samples we've picked up have died.

Script writers customarily call up the Army with tanks and deadly rays, and the air force with atomic bombs as a last resort against a fictional blob from outer space, or the blob from the depths of the Black Lagoon.

Mrs. Harris had a more conventional solution. She got a call from a Dallas woman, who recommended using tobacco mixed with water, an old-time remedy for killing garden insects. It seems to have worked, drying the blob into a white, crusty material at the edge of the garden.

That Blob Was a Fungus

DALLAS (AP)—The blob in Marie Harris' back yard is dead and probably won't return, two Texas scientists said yesterday. Dr. Fannie Hurst, botanist at Baylor University and Jerry Flook, herbarium botanist at Southern Methodist University's science library, agreed that the so-called "blob" was a common slime mold or a lower fungus.

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