PHYSARUM NEWSLETTER

DECEMBER, 1974

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Please mail contributions and correspondence to
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TWO-DAY GAINESVILLE CONFERENCE TO PRECEDE PUERTO RICO A.S.C.B. MEETING

Plans for the 1975 *Physarum* meeting are well under way, according to conference coordinator Henry Aldrich. Participants should plan to arrive in Gainesville on Sunday, November 9. The first session of the meeting will be held on the campus of the University of Florida on Monday morning. A concluding conference dinner is planned for Tuesday evening. The Cell Biology meeting is scheduled to begin Wednesday evening, leaving ample travel time Wednesday for those continuing on to Puerto Rico. While in Gainesville, you can obtain accomodations at the student union at an approximate cost of $10 per person per night.

Details on abstract formats, submission dates, etc., will be included in the next PNL. Further meeting information may be mailed out independently as well. Direct requests, questions and comments to:

Dr. Henry Aldrich
Department of Botany
University of Florida
Gainesville, Florida 32611
(Telephone: 904-392-3261 or 904-392-1184)

REPORT FROM LEICESTER

From September 9 to 13 the second European *Physarum* Meeting was held in Leicester, bringing together some 60 colleagues from Northern and Western Europe. Unfortunately there were no participants from Eastern Europe and only few were able to attend from U.S. labs. The whole gathering was superbly organized and took place at a hall of residence with comfortable rooms, a cafeteria, an excellent lecture hall and even a bar.

Three full days were taken up by talks and discussions, ranging from physiology and biochemistry to genetics of *Physarum*. Abstracts of the material presented have been or will be sent to all recipients of the *Physarum* Newsletter.[1] What is particularly encouraging is that the biochemists and geneticists are starting to find common ground: fortunately there are now several labs where both arts are practiced. It seems likely that cell cycle mutants or developmental mutants will become available within a year or two.

On the last day of the meeting Jennifer Dee and her colleagues gave a practical course on techniques for *Physarum* genetics. This was excellently presented and allowed one to geel that one might learn to master *Physarum* genetics without being a magician after all. Course outlines are available on request.[2]

Many thanks are due to Jennifer Dee, Bill Grant and Geoff Turnock for creating a stimulating and congenial atmosphere at the Leicester meeting.

Richard Braun

Editor's footnotes:

1. Abstracts are included with this mailing of the PNL (except for attendees).
2. For the booklet "Techniques and strains for *Physarum* genetics" by J. Dee and D.J. Cooke, write to Dr. Jennifer Dee, Department of Genetics, University of Leicester, Leicester LE1 7RH, England.

THANK YOU FOR REGISTERING AND MAKING YOUR CONTRIBUTIONS TO THE MAILING FUND

We have received over $200 which will keep us in stamps for a couple of years. The fresh mailing list will be included with the next issue, since registrations are still being received at a significant rate! Please note: All those requesting to be put on our mailing list are expected to make contributions to the mailing fund.

TITLES AND SUMMARIES IN PRINT

THE THERMOSTABILITY OF SOME INDICES OF VITALITY IN THE PHYSARUM POLYCEPHALUM PLASMODIUM

V. A. Bernstam and S. Arndt

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

SUMMARY

The dynamics of manifestations of thermal injury in the Physarum polycephalum plasmodium reveals that the leakage of pigments and the reduction of protoplasmic streaming flow rate are the earliest signs of the thermal injury among those examined. The complete, but reversible cessation of protoplasmic streaming and the leakage of products of nucleic acid and protein metabolism are brought about by 10 minutes' heat shocks at 37, 38 and 41° C, resp. Pulse heating at 47—50° results in the complete inhibition of respiration and the maximal level of leaked substances.

Tsitologiya 15, 1091 (1973)

EFFECTS OF HEATING ON THE INCORPORATION OF RNA AND PROTEIN SYNTHESIS PRECURSORS IN VIVO IN THE PLASMODIUM PHYSARUM POLYCEPHALUM

V. A. Bernstam

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

SUMMARY

Effects of supraoptimal temperatures on incorporation of labelled precursors of RNA and protein synthesis into acid soluble and acid insoluble products have been studied in plasmodia of Physarum polycephalum grown in axenic culture on semidefined medium. It is shown that under conditions which do not preclude entry of precursors into soluble pools, the incorporation of C^{14}-leucine into protein displays lower thermostability than incorporation of H^3-uridine into RNA.

Tsitologiya 16, 160 (1974)

EFFECTS OF STARVATION AND CYCLOHEXIMIDE ON THE REPAIR OF HEAT INDUCED IN THE MYXOMICETE PHYSARUM POLYCEPHALUM PLASMODIUM

V. A. Bernstam and S. Arndt

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

SUMMARY

In the experiments with the inhibitor of protein synthesis—cycloheximide it has been shown that the thermal injury of Physarum polycephalum plasmodia produced by a single 10 minutes heating at 41° C, which does not induce spherulation, can be repaired without concommittant protein synthesis. Resumption of the protoplasmic streaming halted by the heating pulse served as an indicator of repair of thermal injury. Repair of the thermal injury which involves transformation of the heated plasmodia into sclerotia following a 10 minutes pulse at 43° C is blocked by cycloheximide as well as by transfer of the heated cultures to a «starvation» medium (peptone and yeast extract excluded). Three hour «starvation» period, as well as inhibiton of protein synthesis by cycloheximide prior to heating, accellerates the heat induced transformation of plasmodia into sclerotia. It also lowers the temperature threshold for thermally induced metabolic changes in plasmodia leading to spherulation. It is supposed that a certain triggering role in the process of spherulation is given to alteration (suppression?) of the translation process.

Tsitologiya 16, 381 (1974)

Arch. Microbiol. 95, 347–356 (1974)

Effects of Supraoptimal Temperatures on the Myxomycete *Physarum polycephalum*

II. Effects on the Rate of Protein and Ribonucleic Acid Synthesis

V. A. Bernstam

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

Received May 23, 1973

Summary. The effects of supraoptimal temperatures on the incorporation of precursors of RNA and protein synthesis *in vivo*, on the incorporation of ^{3}H-UTP into isolated nuclei, and on the ribonuclease activity were studied in *Physarum polycephalum* plasmodia. Elevated temperatures inhibited the *in vivo* incorporation of ^{14}C-leucine into protein and accelerated the ^{3}H-UTP incorporation into RNA under conditions of unimpaired uptake of the labelled precursors into the soluble pools. The increased synthesis of RNA at higher temperatures was also observed in nuclei isolated from growing plasmodia. The elevated temperatures caused an increase of the rate of RNA degradation, and a significantly higher ribonuclease activity was found in heated plasmodia.

Arch. Microbiol. 95, 357–363 (1974)

Effects of Supraoptimal Temperatures on the Myxomycete *Physarum polycephalum*

III. Effects of Starvation and Cycloheximide on Repair of Thermal Injury in Plasmodia

V. A. Bernstam and S. Arndt

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

Received May 23, 1973

Summary. In experiments using an inhibitor of protein synthesis cycloheximide it was shown that the thermal injury of the *Physarum polycephalum* plasmodia produced by 10 min heatings at 41° C can be repaired without involvement of concomittant protein synthesis. Resumption of the protoplasmic streaming halted by heat shocks served as an indicator of the repair of thermal injury. Repair of the thermal injury attainable only through sclerotization of plasmodia following 10 min heat shocks at 43° C is blocked by cycloheximide as well as by transfer of the heated cultures to a "starvation" medium (no peptone and yeast extract). Three h period of starvation as well as inhibition of protein synthesis for 3 h prior to heating accelerates the heat-induced transformation of plasmodia into sclerotia and lowers the temperature threshold of metabolic transformations required for sclerotization.

Trans. Br. mycol. Soc. **62** (1), 213 (1974)

THE MYXOMYCETE *PHYSARUM NUDUM*: LIFE-CYCLE AND PURE CULTURE OF PLASMODIA

M. J. CARLILE

Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ

Trans. Br. mycol. Soc. **62** (2), 401. (1974)

INCOMPATIBILITY IN THE MYXOMYCETE *BADHAMIA UTRICULARIS*

M. J. CARLILE

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Cytobios 10/49 193-205

Ultrastructure of pure cultures of *Physarum flavicomum* 1 Conversion of a plasmodium to microplasmodia and microsclerotia, and the process of slime secretion

Lena Cheung, Henry R. Henney Jr, and Wallis H. Clark Jr

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Abstract

A plasmodium of *Physarum flavicomum* variety 1, grown in pure culture on a solid medium, has numerous nuclei and mitochondria in the peripheral cytoplasm of the advancing fan, and lacks an ectoplasmic region. A growing microplasmodium in liquid shake culture is more vacuolated than the plasmodium, and contains a large ectoplasmic region which is usually separated from the rest of the cytoplasm by a fibrillar region. When the growth rate declines, the microplasmodium loses its large ectoplasmic and fibrillar regions, becomes highly vacuolated, and increases the secretion of extracellular slime by vacuolar exocytosis. A starved microplasmodium, differentiating into a dormant microsclerotium, forms an outer wall and inner cytokinetic 'furrows', which arise by the coalescence of vacuoles. Inner walls, formed within the 'furrows', segregate the microsclerotium into individual spherules.

Plasmodium formation without change in nuclear DNA content in *Physarum polycephalum*

BY D. J. COOKE AND JENNIFER DEE

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

Genet. Res., Camb. (1974), 23, *pp.* 307–317

SUMMARY

The Colonia isolate of *Physarum polycephalum* produces plasmodia within amoebal clones. Wheals demonstrated genetically that amoebae of the *C*50 strain of this isolate, when crossed with heterothallic amoebae, yielded recombinant progeny. He concluded that nuclear fusion and meiosis occurred in these crosses and suggested that nuclear fusion was also involved in plasmodia formation in clones. He thus designated the strain 'homothallic'.

In the present work genetic evidence is presented which indicates that the Colonia strain *CL*, when crossed with heterothallic strains, also yields recombinant progeny and thus undergoes nuclear fusion and meiosis. Microdensitometric measurements of nuclear DNA content are reported which indicate that *CL* amoebae are haploid like heterothallic amoebae, and crossed plasmodia are diploid. However, clonally formed *CL* plasmodia were found to have the same G_2 nuclear DNA content as *CL* amoebae. This observation excludes the possibility of nuclear fusion when plasmodia form within clones of *CL* amoebae and therefore the strain cannot be homothallic. Two alternatives, apogamy and coalescence, are proposed as the most likely mechanisms for clonal plasmodium formation in strain *CL*.

AN EXTRACELLULAR RENNIN-LIKE ENZYME PRODUCED BY *PHYSARUM POLYCEPHALUM*

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(Received July 30th, 1973)

Biochimica et Biophysica Acta, 334 (1974) 410–416

SUMMARY

Three new proteases have been detected in the culture fluid of the myxomycete *Physarum polycephalum*. Production of these enzymes reached a maximum after 120 h. The main protease has been isolated and purified by $(NH_4)_2SO_4$ fractionation and DEAE cellulose chromatography. The enzyme has a pH optimum of 4.5–5.0 and a temperature optimum of 35 °C. The enzyme has been shown to have a specificity similar to that of rennin (chymosin, EC 3.4.4.3) when acting upon cow κ-casein.

ISOLATION OF ADENYLATE-RICH RNA FROM *PHYSARUM POLYCEPHALUM*

H. FOUQUET[a], R. BÖHME[a], R. WICK[a], H.W. SAUER[a] and R. BRAUN[b]

[a]*Universität Konstanz, Fachbereich Biologie, D-775 Konstanz, (G.F.R.) and* [b]*Institut für allgemeine Mikrobiologie, Universität Bern, Altenbergrain 21, CH-3013 Bern (Switzerland)*

(Received January 29th, 1974)

Biochimica et Biophysica Acta, 353 (1974) 313—322

Summary

RNA of *Physarum* was labeled in vivo with uridine or adenosine, extracted, purified and fractionated in Peaks I and II by chromatography on oligo-(dT)-cellulose or Sigma Cell 38. Peak I contained ribosomal RNA, Peak II poly(A)-rich RNA with a heterogeneous sedimentation pattern. Both types of RNA were detected in S phase or G_2 phase of the cell cycle, however, the labeling of ribosomal RNA was depressed in early S phase.

Metabolism during differentiation in the slime mold *Physarum polycephalum*[1]

E. M. GOODMAN AND T. BECK

University of Wisconsin, Parkside Campus, Kenosha, Wisconsin 53140

Accepted October 22, 1973

GOODMAN, E. M., and T. BECK. 1974. Metabolism during differentiation in the slime mold *Physarum polycephalum*. Can. J. Microbiol. **20**: 107–111.

The introduction of growing microplasmodia into a balanced salt solution induces the formation of hard-walled units (spherules) within 24 h to 36 h. The respiration rate was followed throughout differentiation and was found to decrease from a starting value of 62 μl O_2/mg protein per hour to 17.5 μl O_2/mg protein per hour after 40 h in the salt solution. The use of exogenous glucose and protein catabolism was also studied, and the results indicate that proteins are a major energy source during spherulation. On the basis of the data from this study and the results of other investigators, the process of spherulation has been divided into three developmental periods.

A Method of Indirect Mutant Selection in *Physarum polycephalum* Using the Antibiotic Netropsin

Jessica A. Gorman and William F. Dove

McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin, USA

Molec. gen. Genet. 133, 345—351 (1974)

Summary. A method of indirect mutant selection, analogous to the bacterial penicillin technique, has been developed for *Physarum polycephalum.* The antibiotic netropsin was found to be lethal to growing but not to nongrowing (cycloheximide inhibited) myxamoebae. In reconstruction experiments, a 200 fold enrichment of cycloheximide sensitive over cycloheximide resistant cells was obtained. This technique was used to isolate temperature sensitive mutants. The mutant frequency among survivors was increased at least 40 fold by netropsin selection.

Ultrastructural Effects of Griseofulvin on the Myxomycete *Physarum polycephalum*

Inhibition of Mitosis and the Production of Microtubule Crystals

K. Gull [1] und A. P. J. Trinci

Microbiology Department, Queen Elizabeth College, Kensington, London, England

Protoplasma 81, 37—48 (1974)

Summary

The antifungal antibiotic griseofulvin inhibits the growth of the myxomycete *Physarum polycephalum.* Mitosis in the microplasmodium is inhibited by griseofulvin. Griseofulvin treated microplasmodia show many ultrastructural abnormalities. Nuclei of such microplasmodia are extremely large, often up to 30 µm in diameter and are presumably polyploid. Griseofulvin induces the formation of crystalline-like inclusions of microtubules in the nucleoplasm. Organization of the plasmodial surface is also affected by griseofulvin. Griseofulvin treated plasmodia show blebbing of the peripheral cytoplasm.

Growth of the haploid and diploid phases of *Physarum flavicomum* in the same partially defined media[1]

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

Accepted March 7, 1974

Henney, H. R., Jr., M. Asgari, and M. R. Henney. 1974. Growth of the haploid and diploid phases of *Physarum flavicomum* in the same partially defined media. Can. J. Microbiol. 20: 967–970.

The haploid phase (myxamoebae-swarm cells) of the myxomycete *Physarum flavicomum* variety I grows readily in partially defined liquid media, which were developed for the culture of the diploid plasmodial phase. Cell yields of between 2×10^7 and 4×10^7 cells/ml are obtained in these media in aerobic shake culture. Growth rates are more rapid in shake culture than in stationary culture but growth does not occur in anaerobic conditions. The simpler of the two media contains salts, glucose, biotin, thiamine, hematin, and casein hydrolysate. Haploid cells consume about half as much oxygen per milligram protein per hour as the diploid microplasmodia growing in the same medium.

Can. J. Microbiol. 20, 967 (1974)

Vitalmikroskopische Darstellung cytoplasmatischer Actomyosin-Fibrillen

In vivo-demonstration of cytoplasmic actomyosin-fibrils

Norbert Hülsmann [1]), *Martin Habercy und Karl Ernst Wohlfarth-Bottermann*

Institut für Cytologie und Mikromorphologie der Universität Bonn

MICROSCOPICA ACTA
BAND 76 · HEFT 1
Seiten 38—47 · 1974

Summary

Nearly colorless plasmodia of a pigment-defective strain of *Physarum polycephalum* that hardly contain any optically perturbing inclusions have been investigated on a thin layer of agar by contrast enhancing microscopic methods. Under the conditions of increasing pressure by the coverglass and incubation with 0.1% Knop's solution anisotropic fibrillar differentiations of the protoplasm, identical with actomyosin fibrils, could be demonstrated by phase contrast- and differential-interference contrast-microscopical methods.

Longitudinally orientated fibrillar bundles traversing the entire diameter of the protoplasmic vein are formed in interconnecting strands of the plasmodial network. These bundles can appear branched as well as overlapping.

Circular and longitudinal arrangements of fibrils that must be attributed to the ectoplasm were found in terminal strands. The experimental conditions for this first in vivo-demonstration of actomyosin fibrils in *Physarum* are discussed. The fibrils are functionally interpreted as a mechanical stress dependent, holding structures of the plasmodium.

By the described simple preparation live observation of protoplasmic actomyosin fibrils is made possible and their functional analysis can be facilitated.

Studies on the Regulation of Carbohydrate Synthesis during Growth and Differentiation of *Physarum polycephalum*

I. Activity of UDPGpyrophosphorylase during Spherulation

A. Hüttermann, M. Gebauer, G. Brand, and I. Wessel

Forstbotanisches Institut der Universität Göttingen

Arch. Microbiol. 98, 215—223 (1974)

Abstract. During the starvation-induced spherulation of the true slime mold *Physarum polycephalum*, a metabolic shift occurs from glycogen synthesis during growth to slime (polygalactose) secretion during this differentiation. The synthesis rate of glycogen during growth is 3.5 spec. mU, of slime during spherulation 2.1 spec. mU. The specific activity of UDPGpyrophosphorylase (EC 2.7.7.9) increases during the first nine h of starvation by about 40% and then decreases. After 24 h of starvation only 30% of the specific activity present during growth is left. During differentiation induced by 0.5 mannitol its specific activity decreases continuously, without an initial increase, to about 25% of the initial value. The specific activity of UDPGpyrophosphorylase is not influenced by the addition of cycloheximide or actinomycin-C during the differentiation.

Inorganic pyrophosphatase during differentiation (spherulation) of Physarum polycephalum

ALOYS HÜTTERMANN [1]), and MARLIES GEBAUER

Forstboranisches Institut der Universität Göttingen

CYTOBIOLOGIE
VOLUME 7 · NO. 4
Pages 383–392 · 1973

Two active bands of an alkaline inorganic pyrophosphatase in crude extracts from *Physarum polycephalum* could be demonstrated, which had similar kinetic properties as the purified enzyme from *Escherichia coli*. Their relative activity did not change during the differentiation (spherulation) nor their specific activity. From inhibitor experiments a very low rate of turnover of this enzyme could be concluded.

Methods for determining the pyrophosphatase activity in crushed gels and fractions of CsCl-gradient centrifugations are described.

The Preparation and Preliminary Characterisation of Chromatin from the Slime Mould *Physarum polycephalum*

Brigitte M. JOCKUSCH and Ian O. WALKER
Max-Planck-Institut für Biologie, Abteilung Melchers, Tübingen

Eur. J. Biochem. 48, 417–425 (1974)

A method is described for the preparation of nuclei from plasmodia of the slime mould, *Physarum polycephalum*. The nuclei can be gently lysed by suspending them in 10 mM EDTA. A method is described for the preparation of chromatin in high yield from nuclei lysed in EDTA. Between 60 and 70% of the total nuclear DNA may be recovered in the chromatin fraction which has the following chemical composition, DNA : RNA : protein = 1.0:1.0:4.0.

The acid-soluble protein fraction was present in equal weight proportion to the DNA and consisted almost entirely of the five major *Physarum* histones resolvable by polyacrylamide-gel electrophoresis in urea. The molecular weights of these histones, determined by polyacrylamide-gel electrophoresis in sodium dodecylsulphate, were as follows: P1, 24500; P3, 14000; lysine-rich P4, 14500; arginine-rich P4, 14500; P5, 13000; P6, 10500. The lysine-rich *Physarum* histone, P1, differed significantly in molecular weight from the corresponding calf thymus histone, f1 (mol. wt 21000).

The acid-insoluble protein fraction from the chromatin was resolved into over 30 different fractions by polyacrylamide-gel electrophoresis in dodecylsulphate. The majority of the proteins had molecular weights between 14000 and 60000. No significant differences were observed between the polyacrylamide-gel patterns of chromatin proteins isolated from chromatin prepared from the synchronised nuclei of plasmodia in the middle of S and the middle of the G2 phases of the cell cycle.

Differentiation of Physarum polycephalum: inhibition by alcohols

Hans Kleinig [1])
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Lehrstuhl für Zellbiologie

CYTOBIOLOGIE
VOLUME 9 · NO. 2
Pages 240–243 · 1974

The differentiation of plasmodia into sphérules in *P. polycephalum* is seriously affected by alcohols. Methanol and ethanol delayed this process in a concentration of 0.5 % for 12 and 24 h, respectively. n-Butanol was a reversible inhibitor at a concentration of 0.05 %. Growth of *P. polycephalum* was not affected by these agents.

STUDIES ON MITOCHONDRIAL STRUCTURE AND FUNCTION IN *PHYSARUM POLYCEPHALUM*

III. Electron Microscopy of a Large Amount of DNA Released from a Central Body in Mitochondria by Trypsin Digestion

THE JOURNAL OF CELL BIOLOGY
VOLUME 63, 1974 · pages 299–306

TSUNEYOSHI KUROIWA. From the Department of Biology, Faculty of Science, Okayama University, Okayama 700, Japan

GENETIC CONTROL OF SOMATIC CELL FUSION IN A MYXOMYCETE

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HEREDITY, Volume 32, Part 1, pp. 95-104, February 1974

Somatic cell fusion in the myxomycete *Didymium iridis* is controlled by at least 11 loci. Each locus has a pair of alternate alleles and diploid cells (plasmodia) must be phenotypically identical in order to fuse freely with each other. Cells with unknown fusion alleles can be tested against genetically defined testers. Thus identification of alleles at known fusion loci is relatively easy. The study of new fusion alleles is much more complex since no testers exist. However, F_1's from a given cross can be analysed simultaneously for several new fusion loci without resorting to an additional breeding programme. Theoretical considerations for this procedure are presented. Of the 11 loci, seven have been identified as strong loci; these loci do not permit any plasmodial fusion. Four fusion loci have been identified as weak loci; they allow a partial, temporary fusion which is terminated within 2 to 3 minutes by cytoplasmic coagulation. Possible biochemical mechanisms in these cell recognition phenomena are discussed.

Effect of light on the carbohydrate metabolism of *Physarum flavicomum* plasmodia

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Microbios 10, 39 (1974)

Plasmodial shake cultures of *Physarum flavicomum* exposed to light, metabolize glucose at a reduced rate compared to light protected cultures. The activities of both the Embden-Meyerhof-Parnas and the pentose phosphate pathways are equally depressed by light, and therefore, neither pathway is preferentially inhibited. The light induced inhibition of glucose metabolism is not due to an interference with glucose uptake, since transport is unaffected by light exposure.

Analysis of Isoaccepting tRNAs during the Growth Phase Mitotic Cycle of *Physarum polycephalum*†

P. W. Melera,*·‡ C. Momeni, and H. P. Rusch

ABSTRACT: A reverse phase chromatography study of *Physarum polycephalum* isoaccepting tRNAs isolated during the growth phase mitotic cycle was undertaken. No significant quantitative or qualitative changes were noted in the 20 tRNA families during the mitotic cycle, although some question remains as to a possible quantitative change in the seryl tRNA population. These data combined with aminoacylation studies reported previously, which showed essentially complete quantitative stability in aminoacylation levels for 20 amino acids throughout the mitotic cycle (Melera, P. W., and Rusch, H. P. (1973), *Biochemistry 12*, 1307), strongly suggest that while under restrictive quantitative and qualitative control itself the possible involvement of tRNA in the active control of growth phase mitotic cycle events or growth phase protein synthesis appears to be minimal. The RPC-2 chromatograms revealed the presence of 44 isoaccepting tRNA species during the growth phase mitotic cycle. Single acceptors were found for seven amino acids, four of which, Asp, His, Ile, and Trp, are coded for by three or less codons, while the remaining three, Ala, Gly, and Val, are coded for by four codons. Only two isoacceptors were found for Leu.

PROPERTIES OF *PHYSARUM* MYOSIN PURIFIED BY A POTASSIUM IODIDE PROCEDURE

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THE JOURNAL OF CELL BIOLOGY · VOLUME 62, 1974 · pages 54-65

Myosin has been purified free of actin from *Physarum* actomyosin by a two step adaptation of the classical potassium iodide method for depolymerizing actin. On 12% sodium dodecyl sulfate (SDS) gels, the single major slowly moving protein band present in the calcium activated adenosine triphosphatase peak (90% pure) is associated with two fast moving bands of molecular weights of approximately 17,000 and 21,000 daltons, respectively. Densitometry shows the molar ratio of heavy chains to the 21,000 and 17,000 dalton chains on the gels to be 1:2:1.

The highly purified myosin forms filaments up to 2.5 μm long in the presence of 5 mM magnesium and 0.05 M KCl. Calcium ions were not required for the formation of long filaments from this highly purified myosin.

At low ionic strength (0.05 M KCl) the magnesium ATPase of the highly purified myosin is activated four- to tenfold by muscle actin. The extent of activation is a function of the actin concentration and levels off at high levels of actin. In 0.1 mM calcium salts the ATPase activity is approximately 60% of that in 1 mM EGTA.

In summary, *Physarum* myosin is similar to a number of muscle myosins as well as to platelet and fibroblast myosin, which all possess light chains of two different molecular weights associated with the heavy chains. Under ionic conditions close to those in vivo, highly purified *Physarum* myosin aggregates into long filaments.

THE RAPID INTRANUCLEAR ACCUMULATION OF PREEXISTING PROTEINS IN RESPONSE TO HIGH PLASMODIAL DENSITY IN *PHYSARUM POLYCEPHALUM*

C. NATIONS,[1] W. M. LeSTOURGEON, B. E. MAGUN[2] and H. P. RUSCH

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

Experimental Cell Research 88 (1974) 207–215

When actively growing microplasmodia of the lower eukaryote *Physarum polycephalum* are gently pelleted and allowed to stand at high plasmodial densities for 45 min, three specific nuclear acidic proteins undergo dramatic quantitative changes. Two major proteins of molecular weight 46 000 and 94 000 increase 110 and 320 %, respectively. The increase in these two proteins is not markedly attenuated during periods when 88 % total protein synthesis is blocked by cycloheximide, and the specific radioactivities of these proteins from prelabeled and continuously labeled control and pelleted plasmodia are essentially identical. A third protein of molecular weight 34 000 decreases by 51 % during the 45 min period and when cycloheximide is present, a 36 % decrease in this protein still occurs. The rapid changes which occur in these three proteins in response to high plasmodial density also develop, together with many other changes, during plasmodial differentiation, but only after about 6 h of starvation. It is concluded that the rapid increase in the 46 000 and 94 000 mol. wt proteins results from protein transfer phenomena rather than de novo synthesis and that these proteins perhaps function in the early reorganization of cell metabolism rather than in structural differentiation. In further comparative studies it has been observed that mature spherules of *P. polycephalum* contain a major acidic protein not present in growing or differentiating plasmodia and also that the complement of residual acidic proteins differs in starvation-induced vs cold-induced spherules.

CULTURE OF *PHYSARUM GYROSUM*

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Mycologia, Vol. 66, 349, 1974

COMPARATIVE MEASUREMENTS OF NUCLEAR DNA IN A HETEROTHALLIC AND A SELF-FERTILE ISOLATE OF THE MYXOMYCETE, DIDYMIUM IRIDIS[1]

C. D. Therrien and John J. Yemma

Department of Biology, Pennsylvania State University, University Park 16802, and Department of Biology, Youngstown State University, Youngstown, Ohio 44503

Amer. J. Bot. 61(4): 400–404. 1974.

Comparative measurements were made of the nuclear Feulgen-DNA content of a heterothallic and a self-fertile isolate of the myxomycete *Didymium iridis*. Plasmodial nuclei of both isolates contain the diploid amount of DNA. The replicated diploid (4C) values for the heterothallic and the self-fertile isolates are 5.66 and 5.95, respectively. Myxamoebae, however, are quite dissimilar in their nuclear DNA content. Those of the heterothallic isolates, Honduran 1-2 (A^1) and Panamanian 2-4 (A^7), have mean values of 3.81 and 3.69, whereas myxamoebae of the self-fertile Philippine-1 isolate were found to have a mean value of 6.07. Myxamoebae of the Ph-1 isolate are, therefore, at the same ploidy level as the Ph-1 plasmodium. Mean DNA values for Ph-1 sporangial nuclei were in category 4C. Measurement of the DNA content of mitotic metaphases in sporangia at T = 6 hr confirmed that the mean DNA content of both Ph-1 myxamoebae and plasmodial nuclei is equivalent to 4C. It is concluded that nuclear phase alternance is lacking in the Ph-1 isolate and that the plasmodium of this isolate develops by apogamy.

CYTOPLASMIC INHERITANCE OF THE SELFING FACTOR IN THE MYXOMYCETE *DIDYMIUM IRIDIS*

JOHN J. YEMMA

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Heredity (1974), 32 (2), 231-239

The mechanism by which the selfing factor is inherited in the heterothallic myxomycete *Didymium iridis* has been investigated. A non-selfing isolate, Honduran A^8 was crossed with two selfing isolates, Panamanian 2-4 and Panamanian 2-7. The latter two isolates bear the mating alleles A^7 and A^8 respectively. Meiotic segregants were then analysed for selfing frequency. Mating types were used as a nuclear marker. The capacity to self was inherited by all segregants, with the A^8 segregant selfing at a higher frequency than either the A^7 or A^8 segregant. The incidence of selfing increases in the A^8 segregant as it is successively recloned. In the F_8 generation the frequency of selfing is observed to be 100 per cent. A cytoplasmic factor is postulated with the Honduran A^8 mating type being more susceptible to its action than either of the other two mating types.

Studies on the Mechanism of DNA Replication in *Physarum polycephelum*

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The synthesis of single-stranded DNA subunits (4×10^7 daltons) in *Physarum polycephalum* was studied by alkaline sucrose density gradient centrifugation. The results were compared with the synthesis of the double-stranded DNA molecules ($2{\cdot}3 \times 10^8$ daltons) which they comprise, as determined from neutral sucrose density gradient centrifugation patterns. Although the initiation of synthesis of most double-stranded DNA molecules takes place relatively early in the S period, synthesis of the subunits within them is initiated throughout at least the first two hours of this period. Similarly, replicating (presumably forked) DNA molecules appear to split into daughter DNA molecules prior to the completion of synthesis of the subunits therein. The average rate of DNA chain elongation within subunits is $0{\cdot}3 \times 10^6$ dalton/minute. It is suggested that alkaline sucrose density gradient centrifugation may be a more sensitive method for determining the time required for the completion of replication than other methods based solely on the incorporation of radioactive DNA precursors into an acid-insoluble product.

Journal of Molecular Biology, in press

Methods for the isolation and analysis of plasmodial mutants in *Physarum polycephalum*

By D. J. Cooke and Jennifer Dee

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(*Received* 30 May 1974)

SUMMARY

Amoebae of the Colonia isolate of *Physarum polycephalum* produce plasmodia within individual amoebal clones.

This paper reports the derivation from the Colonia strain *C*50 of a strain *CL* (Colonia Leicester) which produces plasmodia in clones with high efficiency and which completes the life cycle reliably and repeatedly in single clones. The derivation of a line *CLd* (CL delayed plasmodium formation) is described and, using *CLd*, the construction of the isogenic heterothallic strains mt_1;*CL* and mt_2;*CL*.

The above strains provide a system for the isolation and genetic analysis of mutants of *P. polycephalum* within a uniform genetic background, in particular mutants expressed in the plasmodium.

Using this system two auxotrophic mutants have been isolated. Preliminary genetic analysis has shown that they are due to single gene mutations.

Genetical Research, in press

Slime moulds in biological research

Jennifer Dee

The cellular slime moulds (Acrasiales) and the acellular slime moulds (Myxomycetes) provide valuable tools for biological research. In the Myxomycetes, which form the main subject of the article, there are two separate growth phases consisting of microscopic, uninucleate amoebae and a macroscopic, acellular, multinucleate 'plasmodium'. The development of plasmodia from amoebae presents problems similar to those of differentiation in higher organisms and is now being studied by a variety of techniques including genetical analysis. The Myxomycete plasmodium is a giant homogeneous cell, mixed by vigorous protoplasmic streaming, and the mitotic cycle and two types of differentiation occur synchronously in it. Biochemical studies of these processes have been extensive, particularly in the species Physarum polycephalum, *but genetical analysis is also necessary to investigate their control. This should now be possible, since methods have been developed for the isolation and genetical analysis of mutants in the 'Colonia' strain of* P. polycephalum *which apparently remains haploid throughout the life-cycle.*

Science Progress, Oxford, in press

Some New Myxomycete Records for the Neotropics and Some Taxonomic Problems in the Myxomycetes

MARIE L. FARR[1]

FARR, MARIE L. (Mycology Laboratory, Plant Protection Institute, U. S. Department of Agriculture, Beltsville, Maryland 20705). Some New Myxomycete Records for the Neotropics and Some Taxonomic Problems in the Myxomycetes. *Proc. Iowa Acad. Sci.* 81(1): 37-40, 1974.

New myxomycete records for the neotropics and certain taxonomic problems are discussed. Attention is focused on the genus ***Diachea*** and particularly *D. bulbillosa*, ***Badhamia obovata***, ***Didymium leominum***, and *D. floccosum*.

INDEX DESCRIPTORS: Neotropical Mxyomycetes, Myxomycetes, Myxomycetes Taxonomy, Diachea.

Proceedings of the Iowa Academy of Science, in press

SLIME MOULD ACTIN: HOMOLOGY TO VERTEBRATE ACTIN AND PRESENCE IN THE NUCLEUS.

R. N. Jockusch, M. Becker, I. Hindennach and F. Jockusch.

Max-Planck-Institut für Biologie, Abteilung Melchers, Correnssstr. 41, D-7400 Tübingen.

Sequence homology between muscle actin of a mammal (rabbit) and cytoplasmic actin of a slime mould (Physarum polycephalum) is revealed by tryptic finger-printing. The same technique was used to demonstrate actin in the nucleus of Physarum.

Experimental Cell Research, in press

Spherulation of Physarum polycephalum II. Alterations in Lipid Composition

by Hans Kleinig, Ulrika Lempert, and Kurt Zaar

In Physarum polycephalum several qualitative and quantitative alterations in the lipid pattern during the differentiation process from growing microplasmodia into hard-walled spherules in a 96-h-period were described. These alterations comprised e. g. a decrease in the total amount of phospholipids, a decrease of the phosphatidyl choline to phosphatidyl ethanolamine ratio which increased again at later stages, an increase of the sterol to phospholipid ratio which decreased again at later stages, a relative accumulation of triglycerides, ubiquinone, and sterol esters. A considerable turnover of phospholipids was also observed. No changes were found in the sterol pattern and in the relative proportion of ether-analogues of phospholipids. The results were discussed in relation to the numerous ultrastructural changes that occur during spherulation as described in the preceeding publication.

Cytobiologie, in press

NOTES OF INTEREST

Schleicher and Schuell (Keene, New Hampshire 03431) will provide its #576 paper in 8.2 cm diameter. The charge is the same as for the 9 cm size.

From Jennifer Dee: " . . . the following theses are now lodged in the Leicester University library:

D.J. Cooke: "Studies on the Colonia Isolate of *Physarum polycephalum*" (September 1974)

P.E. Sudbery: "Studies on the Control of Mitosis in the Plasmodium of *Physarum polycephalum*" (September 1974).

" . . . If anyone wants to read the theses, they will have to purchase a copy (or part of a copy) from the library. They should write to Interlibrary Loans, University Library, University of Leicester, University Road, Leicester."

Covalent Linkage Between RNA and Nascent DNA in the Slime Mold, Physarum Polycephalum

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When α-^{32}P-deoxyribonucleoside triphosphates are injected into plasmodia of the eukaryotic slime mold, Physarum polycephalum, they are incorporated initially into strands of DNA which are mostly less than 300 nucleotides long. Sixty minutes after injection the incorporated triphosphate is found in much longer strands. If the short strands found two minutes after injection are centrifuged to equilibrium in a Cs_2SO_4 density gradient, they migrate to a density slightly greater than that of single-stranded Physarum DNA. When these short strands are treated with alkali to hydrolyze RNA, their length is not significantly reduced but a small fraction of the incorporated ^{32}P is made acid-soluble and is identified as a mixture of the four ribonucleoside 2', 3'-monophosphates. Such transfer of ^{32}P to ribonucleotides occurs when any of the 4 α-^{32}P-deoxyribonucleoside triphosphates is used for injection, but the transfer is greatest with α-^{32}P-dGTP. We conclude that very short stretches of RNA are found linked through phosphodiester bonds to the 5' ends of nascent DNA chains in Physarum polycephalum and that any of the 16 possible combinations of ribo-and deoxyribonucleotides can occur at the RNA-DNA junction.

Proceedings of the National Academy of Sciences, U. S. A., in press

Extensive fibrillar protoplasmic differentiations and their significance for protoplasmic streaming.
X. The arrangement of actomyosin-fibrils in experimentally unaffected protoplasmic veins of Physarum in situ.

By K.E. Wohlfarth-Bottermann

S u m m a r y

Protoplasmic veins of the plasmodial phase of Physarum with diameters of 1 mm and more were fixed by immersion in situ, embedded together with the substrate (filter paper) in styrole-methacrylate and were investigated by phase-contrastmicroscopy of semithin sections (thickness 2-4 µm). Analysis of serial sections of the veins in situ revealed the arrangement of cytoplasmic actomyosin fibrils within the ectoplasmic region of the strands. Fibrils and fibrillar sheets of the cytoplasmic actomyosin system are intimately connected with plasmalemma-invaginations.

According to the arrangement and direction of the fibrils relative to the long axis of the strands, three different systems are present:

1) Longitudinal fibrils within the peripheral region of the ectoplasmic strand wall.

2) Circular fibrils near the borderline of ecto- and endoplasm.

3) Radial and/or irregular running fibrils within the ectoplasm.

The longitudinal system and the circular system presumably are responsible for longitudinal and radial contraction activities respectively which can be registered in the plasmodium by tensiometric measurements under physiological conditions.

Protistologica, in press

Spherulation of Physarum polycephalum I. Ultrastructure

Kurt Zaar and Hans Kleinig

The ultrastructure of growing and spherulating microplasmodia of the mycomycete Physarum polycephalum has been studied after fixation with glutaraldehyde. Main features of the ultrastructural organisation of growing and early differentiating microplasmodia are extended plasma membrane invaginations which are continuous with vacuoles of a conspicuous plasmatic vacuolar system. In the growing and differentiating microplasmodia a Golgi apparatus is evident consisting of small dictyosomes and large secretary vesicles. As judged by the morphological observations the Golgi apparatus is engaged in the synthesis and the extrusion of the polysaccharid slime covering the organism.

By nutrient deprivation during a 48 h period, microplasmodia differentiate into hard walled spherules. Alterations in the ultrastructural organization during this period were followed additionally by stereological measurements. In particular, we observed an increase in the number of cell organelles per test volume with a concomitant increase in the electron density of the cytoplasm, an increase in the vacuolar membrane system, and a decrease in the extent with a subsequent loss of plasma membrane invaginations. After approximately 32 h the microplasmodia cleave into oligonucleated plasma portions. This is consistent with a significant rise in the surface density of plasma membranes and a simultaneous decline in the surface density of vacuolar membranes.

On the base of ultrastructural observations we postulate three major events during spherulation of P. polycephalum: a condensation of the cytoplasm through desiccation by enhanced synthesis and extrusion of a highly hydrated polysaccaride slime by the Golgi apparatus in an early period of differentiation, the cleavage of the syncytial plasmodia into oligonucleated portions mainly through incorporation of the vacuolar membranes into the separating plasma membranes, and the elaboration of a wall consisting of two different fibrillar layers by these plasma portions.

Cytobiologie, in press

Acidic Proteins of the Nucleus

Edited by

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ACADEMIC PRESS New York San Francisco London 1974

Chapters with special interest to Physarologists:

Chapter 3: Extraction and Characterization of the Phenol-Soluble Acidic Nuclear Proteins. W.M. LeStourgeon and W. Wray.

Chapter 5: Characterization of Nuclear Phosphoproteins in Physarum polycephalum. B.E. Magun.

Chapter 6: The Nuclear Acidic Proteins in Cell Proliferation and Differentiation. W.M. LeStourgeon, R. Totten and A. Forer.

Chapter 8: Acidic Nuclear Proteins and the Cell Cycle. J.R. Jeter and I.L. Cameron.

"Resistant Structures in the Myxomycetes"

H.C. Aldrich and M. Blackwell

("Proceedings of the Second International Fungal Spore Symposium", Edited by D. Weber and W.M. Hess)

"There may be some hesitation among mycologists to embrace Myxomycetes as true fungi, and we do not pretend to have any definitive answer to the question of their true relationships. J.S. Olive maintains that their affinities lie among the Protozoa, and there are persuasive arguments to support this view. Martin and Alexopoulos continue to consider them fungi, and make a case in favor of this position. The most reasonable justification for including Myxomycetes in a symposium on fungal spores is, however, that mycologists have been the most persistent and successful students of the Myxomycetes and the groups loosely referred to as "slime molds", and it is upon this latter foundation that we present a review of the structures formed by these organisms to resist environmental adversities. . . ."

ADDITIONAL ARTICLES IN PRINT

D. Bersier and R. Braun
"Pools of Deoxyribonucleoside Triphosphates in the Mitotic Cycle of Physarum"
Biochim. Biophys. Acta 340, 463 (1974)
(For summary, see PNL 6, 11, 1974)

W.R. Jeffery and H.P. Rusch
"Induction of Somatic Fusion and Heterokaryosis in Two Incompatible Strains of Physarum polycephalum"
Developmental Biology 39, 331 (1974)
(For summary, see PNL 6, 12, 1974)

N. Kislev and I. Chet
"Scanning Electron Microscopy of Freeze-Fractured Sclerotia of Physarum polycephalum"
Tissue and Cell 6, 209 (1974)
(For summary, see PNL 6, 12, 1974)

C. Nations, W.M. LeStourgeon, B.E. Magun and H.P. Rusch
"The Rapid Intranuclear Accumulation of Preexisting Proteins in Response to High Plasmodial Density in Physarum polycephalum"
Experimental Cell Research 88, 207 (1974)
(For summary, see PNL 6, 13, 1974)

U. Ryser and R. Braun
"The Amount of DNA Coding for rRNA During Differentiation (Spherulation) in Physarum polycephalum"
Biochim. Biophys. Acta 361, 33 (1974)
(For summary, see PNL 6, 14, 1974)

K.E. Wohlfarth-Bottermann
"Plasmalemma Invaginations as Characteristic Constituents of Plasmodia of Physarum polycephalum"
J. Cell Science 16, 23 (1974)
(For summary, see PNL 6, 14, 1974)

(The following articles were referred to the PNL
by Dr. A. Oplatka, Editor of *J. Mechanochem. Cell Motility*)

S. Hatano and T. Totsuka
"The Polymerization of Plasmodium Actin in the Presence of Divalent Cations"
J. Mechanochem. Cell Motility 1, 67 (1972)

S. Hatano
"Conformational Changes of Plasmodium Actin Polymers Formed in the Presence of Mg^{++}"
J. Mechanochem. Cell Motility 1, 75 (1972)

S. Fujime and S. Hatano
"Plasmodium Actin Polymers Studied by Quasielastic Scattering of Laser Light"
J. Mechanochem. Cell Motility 1, 81 (1972)

D. Kessler
"On the Location of Myosin in the Myxomycete Physarum polycephalum and its Possible Function in Cytoplasmic Streaming"
J. Mechanochem. Cell Motility 1, 125 (1972)

ABSTRACTS OF MEETING PRESENTATIONS

Botanical Society of America
Arizona State University, June 16-20, 1974

C. DALE THERRIEN* and JOHN J. YEMMA, Department of Biology, Pennsylvania State University, University Park, Pa. and Department of Biology, Youngstown State University, Youngstown, Ohio - Differential Gene Activity in Myxomycete Swarmers and Myxamoebae.

Quantitative cytochemical determinations were made of RNA, DNA and protein bound lysine in both swarmers and myxamoebae of the heterothallic myxomycete Didymium iridis. Total cytoplasmic RNA values for swarmers and myxamoebae were 0.021 and 0.043. The observed decrease in swarmer RNA content may be related to the relatively higher nuclear lysine content recorded for the swarmers. The values for nuclear lysine were 0.076 and 0.037 for the swarmers and myxamoebae. The ratio cytoplasmic RNA/nuclear lysine was calculated for both cell types, and found to be 0.92 and 3.89 for the swarmers and myxamoebae respectively. These values are consistent with a hypothesis that lysine-rich histones are more efficient inhibitors of RNA transcription. Lower nuclear DNA values and a decreased RNA/DNA ratio in the swarmers also suggest a decreased template activity for swarmer nuclei. The calculated RNA/DNA ratios were 0.021 for the swarmers and 0.043 for the myxamoebae. The increased ratio noted in the myxamoebae is due predominantly to an increase in the RNA in those cells. The virtually identical cytoplasmic RNA/cytoplasmic lysine ratios recorded for the swarmers and myxamoebae, 1.13 and 1.15, suggests that most of the observed increase in cytoplasmic RNA is ribosomal.

JOHN J. YEMMA* and C. DALE THERRIEN, Department of Biology, Youngstown State University, Youngstown, Ohio, and Department of Biology, Pennsylvania State University, University Park, Pa. - Quantitative Cytochemical Analysis of Nuclear DNA in Zygote Nuclei of the Myxomycete Didymium iridis.

The nuclear DNA content of swarmers and myxamoebae of the isolates Honduran 1-2 (A^1) and Panamanian 2-7 (A^8), and zygotes resulting from the cross Hon 1-2 (A^1) X Pan 2-7 (A^8) was measured by Feulgen-cytophotometry. The mean nuclear DNA content for both swarmers and myxamoebae of the Honduran isolate was found to be 3.8. However, in the case of the Panamanian isolate, myxamoebae were found to have a mean DNA content of 4.3, whereas swarmer nuclei had a mean DNA content of 3.7. Histograms representing nuclear DNA in the mixed mating type populations, Hon 1-2 (A^1) X Pan 2-7 (A^8), show a bimodal distribution for the swarmer nuclei, whereas similar populations of myxamoebae do not show a bimodal distribution. The mean DNA content was, however, similar in both cases, being 5.3 for the swarmer population and 5.9 for the amoeboid population. Since mean values tend to obscure bimodal distribution patterns, exclusive use of such values in the analysis of data such as these is cautioned against. Histograms show that swarmer nuclei are either in class 2C, prefusion, or class 4C, postfusion, with almost no nuclei in the intermediate or synthetic class. Conversely, most amoeboid nuclei were in the intermediate class. It is concluded that amoeboid zygote nuclei are actively dividing, whereas swarmer zygote nuclei are in G_2 arrest.

Am. J. Bot. 61 (#5, Supplement), 26 (1974)

Canadian Federation of Biological Societies
Hamilton, Ontario, June 25-28, 1974

T.S. Bilkey and J.E. Cummins (Introduced by A.W. Day)

Single-Strand DNA Breakage Is Caused By Dimethylmercury Treatment During DNA Replication in Physarum polycephalum.

DNA from the plasmodial slime mold Physarum polycephalum (a primitive eukaryote) was examined by alkaline-sucrose gradient sedimentation following nuclear isolation. Late log-phase cultures were incubated 12-15 hours (1-1.5 generations) in a medium containing ^{3}H-thymidine. Nuclei were isolated, lysed in alkali, placed on alkaline-sucrose gradients and run for 1.5 hours at 25 thousand rpm in the SW 25.2 rotor of the spinco ultracentrifuge. Analysis of the radioactivity in DNA extracted from control cultures indicated that there was a single DNA peak of 100s. Cultures treated with 10^{-4} molar dimethylmercury (DMM) showed two peaks, one at 100s and the other at 45s. The extent of DMM breakage was comparable to or greater than the effect of a kilorad dose of X-ray on Physarum DNA. This was evidenced by the relative amounts of radioactivity in the slowly sedimenting DNA peak.

Fifth International Congress of Radiation Research
Seattle, Washington, July 14-20, 1974

D-24-1 *UV- and X-Ray Effects on Nucleoside Kinase Production During the Mitotic Cycle of* Physarum polycephalum. W. SACHSENMAIER, E. DWORZAK, H. MADREITER, AND W. LINSER, University of Innsbruck, Innsbruck, Austria.

Thymidine (I)— and deoxycytidine (II)- kinase activity in *Physarum polycephalum* undergo cyclic variations related to the synchronous 10 hr mitotic cycle. Both enzymes exhibit a distinct maximum in early S-phase. UV light (λ_{max} 254 nm, 5–10,000 ergs/mm²) applied during early and middle interphase delays the enzyme maximum parallel to the delay of the next mitosis without affecting the yield of enzyme production. Treatment during late interphase or during mitosis partially inhibits the increase of (I) but stimulates excess production of (II) beyond the time of the control maximum. X-irradiation (25 kV, 1 kr) during a narrow period (pro-metaphase) largely stimulates production of (I) and (II) at the time of the next (delayed) mitosis. Sensitivity to actinomycin of irradiated plasmodia is markedly reduced probably due to repair processes. A model is discussed suggesting that (I) and (II) are controlled by gene activation (A) at the end of G_2-phase followed by a "shut-off" mechanism (B) in early S-phase. UV light appears to affect the (B)-process of (II) more than (A) allowing excess enzyme production. X-rays on the other hand may interfere with the formation of a repressor acting on the translation level. (Supported by the Fund of Austrian Cancer Research Institutes).

Radiat. Res. 59, 211 (1974)

Also, "Temporal Order of DNA Replication Following γ-Radiation of Physarum polycephalum"
T.E. Evans & H.H. Evans (PNL 6,22,1974)

Radiat. Res. 59, 209 (1974)

Society of Protozoologists
Wesleyan University, August 14-16, 1974

Relationship of Na^+ flux and concentration to motility of Physarum polycephalum, JOHN M. SIMS • EUGENE C. BOVEE, Univ. of Kansas, Lawrence, 66045

Cinematographic studies are presented demonstrating that as the external Na^+ concentration decreases oscillatory flow of protoplasm in Physarum is interrupted. At external concentrations approximating the internal concentration no interruption is observed. At concentrations of 10^{-3}M or less there is shut down and recovery within 4.5 min. Recovery starts in the leading edge proceeds through the non-channeled and channeled protoplasmic regions, with the trailing tube region recovering last. In regions where tubular and non-tubular protoplasmic masses are closely associated and contiguous, the non-tubular masses recover first. Recovery in the larger trailing tubes starts in the ectoplasm as small streamlets. Their numbers and sizes increase until the streamlets join, leading to the normal flow pattern. Neutron activation analysis of 1mg. specimens shows that the Na^+ concentration of large trailing tubes ranges from 63 to 75 meq/Kg. Smaller tubes in association with non-tubular protoplasm in association with the smaller tubes range from 6 to 24 meq/Kg., while the leading edge concentration was 1 to 9 meq/Kg. Efflux of Na^+ from the slime mold is dependent upon the nature of the external environment. Efflux into distilled water is very rapid with all of the Na^+ which will leave, being gone within the first minute. Efflux into Na-free growth medium is 50% by 3 to 5 min. depending on the specimen type used. Specimens consisting of leading edge and channeled regions lose 50% of the effluxable Na^+ in 3.5 min. while specimens consisting of tubes lose 50% in 4.5 min. The efflux curves are very similar, but the amount of Na^+ lost by tubes is approximately 3 times that of leading edge-channeled specimens. From neutron activation analysis the average Na^+ concentration for the tubular region is 3 times that of leading edge-channeled region. (Supported by Univ. of Kansas Biomedical Grant 4948-6706-7 and by NSF grant GB-16616 to E.C. Bovee.)

J. Protozool. 21, 432 (1974)

Federation of European Biochemical Societies
Budapest, August 25-30, 1974

VARIABLE LEVELS OF RNA POLYMERASES A AND B IN GROWTH AND DIFFERENTIATION OF PHYSARUM

A. Hildebrandt and H.W. Sauer, FB Biologie der Universität Konstanz, Germany

We have purified and characterized RNA polymerases A and B from Physarum with respect to subunit structure, ∝ amanitin sensitivity metal ion requirements and salt dependence. We find an unchanging pattern of both enzymes in the naturally synchronous cell cycle, although variable synthesis of RNA classes was indicated. During differentiation we observe a decrease in enzyme A (80 %), in agreement with a drop in stable RNA content. DNA extracts contain a RNA polymerase inhibitor, tentatively identified as polyphosphate. In addition, we find a new enzyme of the "A type" but sensitive to ∝amanitin.

ROLLING CIRCLES IN NUCLEOLAR rDNA FROM MITOTIC NUCLEI OF PHYSARUM POLYCEPHALUM

H.W.Sauer, FB Biologie der Universität Konstanz, Germany, and H.-J.Bohnert, Botanisches Institut der Universität Düsseldorf, Germany

We detected DNA structures which resemble rolling circles with a contourlength of 5 - 50 μ and attached linear pieces 4 - 5 μ long. Such DNA was found in the heavy satellite DNA (1.712 g/cm^3) from nuclei and in nucleolar DNA (1.712 g/cm^3). Purified ribosomal RNA was hybridized selectively to the DNA fractions containing rolling circles. We propose that the mitotic nucleolus of Physarum is an " episome " like the amplified nucleoli in Xenopus oocytes.

G. Brand, W. Hoflmann and A. Hüttermann

Changes in the Specificity of RNA- and Protein-Degrading Enzymes – a Possible Point of Control during Differentiation

The starvation-induced differentiation (spherule formation) of the true slime mold *Physarum polycephalum* is characterized by very pronounced changes in its metabolism (for a review see[1]). One point of a possible control of these metabolic events was found to be differential protein synthesis[1]. Since the amount of molecules in a cell is a function of both synthesis and degradation, the RNA- and protein-degrading enzymes were studied during this differentiation. In growing plasmodia, three different isoenzymes of ribonuclease were found by isoelectric focusing; after 6 h of starvation a fourth ribonuclease appeared. After 24 h of total starvation, the time when the first spherules are visible, only one of the four ribonucleases is left, which is an endonuclease specific for uridine and inosine bonds.

Of the protein-degrading enzymes, aminopeptidases have been studied so far in detail: during growth and differentiation, one very active isoenzyme was found with a broad substrate specificity (virtually all unsubstituted 4-nitroanilides are hydrolyzed). In addition to this isoenzyme, two more bands with a narrower specificity were found in extracts from growing plasmodia which disappeared during differentiation. In differentiating plasmodia, three new, very active isoenzymes appeared, which were not found during growth. This change in isoenzyme pattern results in a pronounced change in overall aminopeptidase specificity.

Since both ribonucleases and aminopeptidases exhibit a high specific activity and are very likely not bound to cell compartments, those observed changes in specificity, which are under control at the level of synthesis, may well play an important role in the control of RNA and protein content during cell differentiation.

[1] Hüttermann, A. (1973) in *Physarum polycephalum* – Object of Research in Cell Biology (Hüttermann, A., ed.) pp. 55–76, Fischer-Verlag, Stuttgart.

H-S Z. Physiol. Chem. 355, 1181 (1974)

U. Guntermann, I. Tan and A. Hüttermann

Induction of α-Glucosidase During the Cell Cycle of Myxobacter Al-1

The successful synchronization of *Myxobacter* Al-1 by zonal centrifugation[1] enabled us to study the inducible enzyme α-glucosidase (EC 3.2.1.20) during the cell cycle of this organism. The enzyme can be induced by adding maltose as the sole carbon source to a defined medium. This induction is the result of a true de novo synthesis of the enzyme protein, as was demonstrated using the technique of density labelling with deuterated amino acids[2] and subsequent analysis in metrizamide gradients[3]. The mechanism of this induction is not based on catabolite repression.

During the cell cycle of cultures continuously growing on maltose, α-glucosidase activity exhibits a peak with a maximum towards the end of the division cycle. The same pattern was observed in cultures pregrown on glucose and induced asynchronously for only a quarter of the generation time. Thus the mechanism of "oscillatory repression" (comp.[4]) as a means of control for the synthesis of this enzyme during the cell cycle can be excluded.

[1] Hartmann, H. (1974) Thesis Univ. Karlsruhe.

[2] Hu, A. S. L., Bock, R. M. & Halvorson, H. O. (1962) *Anal. Biochem.* 4, 489–504.

[3] Hüttermann, A. & Guntermann, U. (1974) *Anal. Biochem.*, submitted.

[4] Mitchison, J. M. (1971) The Biology of the Cell Cycle, Cambridge University Press.

H-S Z. Physiol. Chem. 355, 1200 (1974)

A. Hildebrandt and H. W. Sauer

An Inhibitor of RNA Polymerase A from Physarum

Nucleolar RNA polymerase A from *Physarum* can be distinguished from the nucleoplasmic RNA polymerase B in terms of: resistence to α-amanitin, low salt optimum, subunit structure, template specificity and the high sensitivity towards a high molecular weight substance, previously described as "polyphosphate-like material"[1].

DNA extracted from nuclei isolated in stationary growth phase is a poor template for the in vitro assay of RNA polymerase A. Purification of DNA on cesium chloride or sucrose gradients increased its template activity (factor 10–15). The contaminating material is free of nucleic acids, protein or slime and inhibits in vitro RNA synthesis by RNA polymerase A at low concentrations (10^{-6} M phosphate). RNA polymerase B from *Physarum* and *E. coli* RNA polymerase are inhibited only at a higher concentration (factor 20) and other phosphorus compounds: phosphate buffer, pyrophosphate and authentic polyphosphate, affect the enzymes only at still higher concentrations (factor 1000). Upon transition from exponential to stationary growth phase, activity of RNA polymerase A drops significantly (factor 20) while the activity of enzyme B remains practically unchanged. RNA synthesis was reduced while the "polyphosphate-like material" increased 5-fold[1].

Therefore we postulate that the material described here is involved in vivo in a coarse regulation of ribosomal RNA synthesis. This notion is supported by 2 other findings: a slow turnover of the RNA polymerases of *Physarum* and the inhibition by "polyphosphate-like material" of endogeneous RNA polymerase A activity in isolated nucleoli.

Furthermore RNA polymerases of *Physarum* can be readily distinguished by the different sensitivities to this material.

[1] Goodman, E.M., Sauer, H.W., Sauer, L. & Rusch, H.P. (1969) *Can. J. Microbiol.* 15, 1325–1331.

H-S Z. Physiol. Chem. 355, 1206 (1974)

A. Hüttermann and U. Guntermann

Metrizamide in D_2O – a Novel Solute for the Isopycnic Banding of Proteins

The density labelling of proteins with heavy isotopes with subsequent analysis using equilibrium density gradient sedimentation was introduced by Hu et al.[1]. Although this method can be used to demonstrate elegantly the de novo synthesis of a given enzyme without prior purification, one disadvantage is that many enzymes are not stable enough for 40 h centrifugation in a 3M CsCl or RbCl solution. Metrizamide[2-(3-acetamido-5-*N*-methylacetamido-2,4,6-triiodobenzamido)-2-deoxy-D-glucose] dissolved in D_2O was found to be a very suitable medium for isopycnic banding of proteins: it is inert, and has little influence on the activity of enzymes[2]. Solutions in the density range of 1.3–1.45 $g \times cm^{-3}$ have low viscosities. Since the spontaneous equilibrium gradient, which is dependent on the angular velocity, occurs only after a long period of centrifugation in metrizamide solutions, the isopycnic banding of proteins can be performed at the highest available speed with any preformed gradient. Thus the centrifuge times for such studies can be shortened considerably and the method can be extended to proteins with lower molecular weights. Examples for the separation of proteins of different densities are given.

[1] Hu, A. S. L., Bock, R. M. & Halvorson, H. O. (1962) *Anal. Biochem.* 4, 489–504.

[2] Mullock, B. M. & Hinton, R. H. (1973) *Biochem. Soc. Trans.* 1, 27–29.

H-S Z. Physiol. Chem. 355, 1210 (1974)

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233. LONG TERM EFFECTS OF ELECTROMAGNETIC FIELDS ON PHYSARUM POLCEPHALUM
E.M. Goodman, M. Marron* and B. Greenebaum*. Science Division, University of Wisconsin-Parkside, Kenosha, Wisconsin.

The purpose of this study was to ascertain the long term effects of extremely low frequency electromagnetic fields (ELF-EMF) on growth and development in the myxomycete Physarum polycephalum. To date, microplasmodia have been continuously exposed to an ELF-EMF of 75Hz 2.0 G, 0.7 V/m for 701 days. Another set of cultures has been exposed to 60Hz, 2.0 G, 0.7 V/m for 434 days. The time between successive mitoses in the cultures exposed to ELF-EMF was 1-2 hours longer than controls. This delay appeared after approximately 90-120 days of continuous exposure. The reproducibility of the effect has been confirmed when new cultures introduced into these ELF-EMF environments began displaying a delay after 90 days exposure. Removal of a culture from the ELF-EMF results in slow dissipation of the delay with the culture returning to control levels after about 60 days. The ability to complete both the sexual (sporulation) or asexual (spherulation) life cycles was not affected, however subtle changes in gross morphology, and a general slowdown of protoplasmic streaming was detected. Experiments are currently in progress to elucidate threshold levels and to ascertain whether the electric, magnetic or both acting in concert are responsible for these effects. This work was supported by contract N00014-67-A-0128-0021 administered by the Office of Naval Research.

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312 NUCLEO-CYTOPLASMIC INTERACTIONS AND THE CONTROL OF NUCLEAR EVENTS IN THE CELL CYCLE OF PHYSARUM POLYCEPHALUM.
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Since nuclear proteins have been implicated as controlling transcription during the cell cycle in eukaryotes, we have studied their synthetic and migratory characteristics throughout the cell cycle of P. polycephalum. The proteins of wild type and polyploid plasmodia of Physarum were prelabeled with ^{14}C-Leucine or ^{3}H-Leucine. These plasmodia were fused for two hours. The nuclei were isolated and the smaller recipient cell nuclei (wild type) were separated from the larger donor cell nuclei (polyploid) by sedimentation through a sucrose gradient. The proteins were extracted from the recipient cell nuclei using: (1) 0.14 M NaCl, (2) 0.25 N HCL, (3) buffered phenol and (4) 5% SDS-5% 2-mercaptoethanol. Ratios of ^{14}C/^{3}H show that differential transfer of labeled preformed donor cell proteins into the recipient cell nucleus occurs during fusion. The quantity of proteins transferred varies among the different fractions and with the phase of the cell cycle. For example, when both plasmodia are in S very few donor cell proteins are found in the salt soluble fraction of the recipient cell nucleus. However, if the donor cell is in G_2 and the recipient cell in S, the amount of prelabeled protein appearing in the salt soluble fraction is significantly increased. In additional experiments we found that the low quantity of salt soluble proteins transferred during S was due to the high rate of synthesis and turnover of these proteins occurring at that time. These experiments show that cytoplasmic proteins from donor cells differentially migrate into recipient cell nuclei during the different phases of the cell cycle. In addition this fusion system should enable us to obtain relatively large quantities of these mobile proteins so that they can be characterized by electrophoresis and other procedures. (Supported by NIH Grants TO1-CA-5002 and CA-07175)

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382. IDENTIFICATION OF CONTRACTILE PROTEINS IN NONHISTONE NUCLEAR PROTEIN FRACTIONS. Wallace M. LeStourgeon, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee.

Two of the major nonhistone proteins from Physarum polycephalum have been purified under nondenaturing conditions from 1.0 M KCl nuclear extracts and identified as actin and myosin. A third major protein (mol wt 34,000) has been purified from crude nuclear actomyosin complexes and found to bind nuclear actomyosin in the presence of Mg^{++}. Based on gel electrophoresis and amino acid composition these proteins are also present in nuclei of HeLa cells and mouse embryo fibroblasts. Physarum nuclear actin comigrates with rabbit muscle actin during electrophoresis and like other actins contains the unusual residue N^{τ}-methylhistidine (1 mole/mole). Nuclear actin reacts with rabbit muscle heavy meromyosin to form the "arrowhead" structures characteristic of actin-HMM complexes. Nuclear actomyosin contracts on the addition of ATP and hydrolyses ATP with efficiencies similar to muscle actomyosin. The nucleus specific Mg^{++} dependent actomyosin binding component inhibits the Ca^{++} stimulated ATPase by 30% while the Mg^{++} ATPase is unaffected. This protein is similar to the regulatory protein tropomyosin with respect to molecular weight and Mg^{++} dependence yet the amino acid composition indicates that this protein is not directly analogous to muscle tropomyosin. Control experiments with radioactive protein, sequential nuclear purification studies, and studies on purified chromosomes indicate that these proteins exist in nuclei in vivo. When non-dividing states are induced in the above cells, the Mg^{++} dependent actomyosin binding protein disappears from the nuclei while actin increases several fold in intranuclear concentration; associated with these changes is a generalized condensation and inactivation of chromatin. Isolated metaphase chromosomes from Physarum show a several fold enrichment in myosin content and an altered ratio of actin to the 34,000 mol wt protein. These observations suggest that contractile proteins may play a role in the condensation of chromatin during mitosis and cell differentiation.

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409. NUCLEAR PHOSPHOPROTEINS OF EUKARYOTIC CELLS Bruce Magun*. Department of Anatomy, University of Tennessee Medical Units, Memphis, Tennessee. (Introduction by G. Gordon Robertson)

In an effort to determine the role of nuclear protein phosphorylation in the control of gene regulation in eukaryotes, the nuclear phosphoproteins of the slime mold Physarum polycephalum have been characterized using ^{32}P and ^{33}P isotopic labeling followed by electrophoresis on SDS-polyacrylamide gels. After labeling continuously in ^{3}H-amino acids and $^{32}P_i$, scintillation counting permitted calculation of phosphates per polypeptide in individual gel slices. The results, which represent minimum values, varied between 1 and 2 phosphates per polypeptide. Gel autoradiography demonstrated that most nuclear proteins were apparently not phosphorylated. Pulse-labeling with $^{32}P_i$ or labeling of nuclei in vitro with γ-^{32}P-ATP revealed markedly different autoradiographic profiles from those obtained after continuous labeling in $^{32}P_i$. Differences in phosphorus metabolism among phosphoproteins was demonstrated by $^{32}P_i$ pulse experiments, which demonstrated that ^{32}P was stable in some proteins and actively turning over in others. During the process of differentiation (starvation) the phosphate concentration of all phosphoproteins decreased markedly, and incorporation of phosphate was reduced to low values.

The nuclear phosphoproteins of hamster BHK21 and monkey Vero cells were also examined by similar techniques. Nuclear proteins were recovered as four fractions, comprising 96% of the total nuclear protein. The rate of ^{32}P incorporation into nuclear sap proteins, histones, acidic nuclear proteins and cytoplasmic proteins was similar. ^{32}P gel autoradiographic profiles revealed marked qualitative differences among the different protein fractions. As BHK21 cells attained confluency, incorporation of ^{32}P into all protein fractions was reduced to 20% of that found in exponentially-growing cells. From these studies we conclude that nuclear acidic proteins are similar to other nuclear and cytoplasmic proteins in their degree of phosphorylation.
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459. REGULATION OF ORNITHINE DECARBOXYLASE ACTIVITY IN PHYSARUM John L.A. Mitchell Department of Biology, Northern Illinois University, DeKalb, Illinois.

Ornithine decarboxylase (ODC), which is essential for the synthesis of polyamines, is extremely sensitive to growth conditions and exhibits rapid four-fold fluctuations in activity associated with the different phases of a mitotic cycle. Previous experiments using protein synthesis inhibitors have suggested that this enzyme is unusually unstable (T ½ 14 min) and thus undergoes a rapid turnover. We have examined alternative mechanisms of ODC activity regulation by comparing the physical and kinetic properties of the enzyme at various phases in the naturally synchronous mitotic cycle of Physarum polycephalum. The Km of the activation of this enzyme by pyridoxal-5'-phosphate (PLP) varies over a ten-fold range, during this cycle, in direct correlation with the radical changes in activity previously reported. This enzyme activity pattern was reduced to a regular step-doubling during the S period when assays were performed at a coenzyme level which assured saturation for the enzyme at all phases. Inhibitors of protein and RNA synthesis also cause an increase in the PLP Km from about 1.0μM to 10μM within 40 min, thus the apparent half-life of this enzyme is calculated to be about 10 min when measured at coenzyme levels reduced to less than saturating by extraneous Schiff base formation with Tris buffer or excess substrate. However, at assay PLP concentrations of greater than 50μM the half-life of the core enzyme was found to be greater than 120 min. The data suggest that this variation in enzyme affinity for coenzyme, which may be due to a simple subunit disaggregation or the dissociation of a small polypeptide factor necessary for coenzyme binding, is the natural mechanism for the sensitive regulation of this enzyme activity in the presence of the low PLP levels within a cell. (Supported by grant #74-11 of the Illinois Division of the American Cancer Society.)

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474. ACTIN-LINKED REGULATION OF ACTOMYOSIN IN PHYSARUM POLYCEPHALUM V. Nachmias, A. Asch* and M. Plaut*, Department of Anatomy, University of Pennsylvania, Philadelphia 19174, Pennsylvania

A calcium sensitive ATP splitting fraction has been isolated from this myxomycete by differential centrifugation. The ratio of ATP split in 0.1mM $CaCl_2$ to that in 1mM EGTA was from 1.5 to 6.6 in different preparations. Addition of desensitized muscle actin (no tropomyosin or troponin bands on gels) increased the rate of hydrolysis in EGTA so that the extent of inhibition approached or equalled zero. Rabbit heavy meromyosin did not affect EGTA inhibition. The ATPase in 0.1mM $CaCl_2$ was inhibited by increased ionic strength. The fraction was negative for glycogen. A sensitive complement fixation test with antibody specific for this myosin showed 2-3% myosin in the fraction. SDS gels showed both actin and myosin to be present in the fraction, together with polypeptides in the size range of 14,000 to 39,000 daltons. A low salt extract of Physarum precipitated with muscle myosin also showed some evidence of calcium sensitivity and several bands on SDS gels with molecular weights below actin, together with a 56,000 dalton band. This ratio of these bands to that of actin was 1:20 or less instead of 1:3 with platelets in identical experiments. We conclude that the enzymatic data support actin-mediated regulation of the actomyosin of this primitive organism, and that the gel results suggest that either regulatory proteins are loosely associated with actin or that a part of the actin occurs in a form unassociated with such proteins.

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585. SYNTHESIS OF MITOTIC PROTEINS DURING G_2 IN PHYSARUM POLYCEPHALUM
Ronald C. Rustad, Eugene N. Brewer, and Nancy L. Oleinick. Division of Radiation Biology, Department of Radiology, Case Western Reserve University, Cleveland, Ohio, 44106

Approximately 30 min before metaphase the syncitial slime mold Physarum polycephalum becomes insensitive to the inhibition of mitosis by cycloheximide (i.e., passes the CH marker) suggesting that the synthesis of all proteins necessary for mitosis has been completed. Mitosis is delayed by exposure to γ-radiation at any time prior to about 20 min before metaphase (γ marker) (Oleinick, Radiat. Res. 52, 638, 1972). We report here the appearance of a new CH marker following exposure of Physarum to γ-radiation within 30 min of metaphase, i.e., after the normal CH marker. Cultures were exposed to 700 rads of ^{60}Co γ-radiation, and sectors of irradiated plasmodia were transferred to cycloheximide-containing medium (50 μg/ml) at various times thereafter. As controls, other sectors of each plasmodium were either (A) left untreated, (B) irradiated without subsequent CH treatment, or (C) exposed to cycloheximide but not irradiated. When cultures were irradiated between the times of the original CH and γ markers mitosis could be prevented by CH treatment initiated shortly after irradiation. At later times, mitosis in irradiated plasmodial sectors was insensitive to cycloheximide (having passed the new CH_γ marker). A new CH_γ marker could be demonstrated even when the cultures had already passed both the original CH and γ markers at the time of irradiation. These data do not support the hypothesis that irradiation at these times in late G_2 delays mitosis by interfering with the synthesis of mitotic proteins. Rather, these results could be explained by radiation-induced damage to mitotic proteins or to other structures (e.g., chromatin) which requires protein synthesis for replacement or repair. The effects may be qualitatively or quantitatively different before and after the γ marker. (Supported by USAEC Contract No. W-31-109-ENG-78).

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597 COINCIDENCE OF PROTOPLASMIC STREAMING AND BIREFRINGENT STRUCTURE IN PLASMODIUM FRAGMENT.
Hidemi Sato, Tadashi Hatano*, Fumio Matsumura*, Harunori Ishikawa* and Yukiko Sato*. Department of Biology, University of Pennsylvania; Institute of Molecular Biology, Nagoya University and Department of Anatomy, School of Medicine, Tokyo University.

10^{-2}M Caffeine treatment of the actively growing slime mold, Physarum polycephalum, produces small plasmodium fragments ("The PC-drop") which retain the ability to undergo protoplasmic streaming in the presence of calcium ion. The streaming site shows transitory birefringence correlated with the streaming. This birefringence could be the result of the rapid reversible association and orientation of fibrous molecules which are distributed in the hyaline zone. Improved fixation with 3% gluturaldehyde-3% formalin-10^{-2}M Tris malleate buffer solution at pH 7.0, allows us to preserve this structure without altering its initial birefringence. Electron micrography clearly reveals well oriented thin filaments, which have a diameter of 6.0±0.5 nm and dimensionally coincide with actomyosin, are the predominant structures of the birefringent zone. The volume fraction of these filaments is theroretically calculated as 4% minimum and 10% maximum. We confirmed that myosin B extracted from the PC-drops shows a typical reversible change of viscosity correlated with the amount of ATP given. Protoplasmic streaming in PC-drops is controlled by the amount of free calcium ion available and the threshold value is about 10^{-6}M. The transitory birefringence correlated with the initiation and the continuation of streaming could reflect the amount of the oriented actomyosin molecules in this zone. *Supported by grants from NIH-CA10171, NSF-GB31739X and GF34908.

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DNA REPLICATION, THE NUCLEAR MEMBRANE, AND OKAZAKI FRAGMENTS IN EUKARYOTIC ORGANISMS. J.A. Huberman, A. Waqar, H. Horwitz, F. Feldman, R.A. Deich and A. Tsai. Dept. of Biology, Massachusetts Institute of Technology, Cambridge, Mass. 02139, U.S.A. When CHO cells synchronized by Colcemid reversal are pulse-labeled for 30 seconds with ^{3}H-thymidine, then fixed, thin-sectioned, autoradiographed, and examined in the electron microscope, grains are seen over sites of DNA replication within the nuclei. In early S phase these sites are distributed throughout the nucleus while in late S phase they are clustered over condensed chromatin around the nuclear membrane and in other discrete sites within the nucleus. The distance of early S phase sites from the nuclear membrane is great enough so that a role of the membrane in DNA replication can be excluded. Instead, our results are consistent with the idea that DNA is replicated wherever it happens to be located inside the nucleus; euchromatin is replicated in early S and heterochromatin is replicated in late S.

We have also started a systematic study of the role of Okazaki fragments in DNA replication, both in CHO cells and in the syncitial slime mold Physarum polycephalum. Our conclusions to date are: 1) such fragments are present in both organisms, 2) many of the fragments contain stretches of ribonucleotides, 3) smaller oligodeoxynucleotides ($\sim$28) are also labeled in short pulses with ^{3}H-thymidine.

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3Sa 5 DISCONTINUOUS REPLICATION OF EUKARYOTIC DNA. J.A. Huberman, H. Horwitz, R. Minkoff, and M.A. Waqar. Dept. of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, U.S.A.

We have investigated the size and kinetics of synthesis of the single strands of DNA formed during DNA replication in two eukaryotic organisms-mammalian cells grown in tissue culture and the slime mold, Physarum polycephalum, grown as a syncytial plasmodium. In both cases radioactive precursor molecules (usually ^{3}H-thymidine) are first incorporated into very short single strands. In the case of mammalian cells the radioactive label from the short strands is subsequently found in strands of molecular weight from about 10^{7} to about 10^{8} daltons. These intermediate-sized single strands correspond to the growing daughter strands of each bidirectional replication unit (previously detected by DNA fiber autoradiography). As adjacent replication units fuse, the intermediate strands increase in size to that of parental DNA strands ($>10^{8}$ daltons). The shortest nascent single strands from both mammalian cells and Physarum polycephalum contain stretches of attached RNA. In the case of Physarum, at least, this attached RNA is bound to the 5' end of the DNA through a standard 3',5' phosphodiester linkage. This mode of attachment suggests that the RNA serves as a primer for new DNA strand synthesis. Work is in progress on the nucleotide sequences at the RNA-DNA junction. Present results were obtained by injecting α-^{32}P-deoxyribonucleoside triphosphates directly into the Physarum plasmodium, isolating the labeled short DNA strands, hydrolyzing them with alkali, and then analyzing the released ribonucleoside monophosphates for transferred ^{32}P. These results show clearly that the nucleotide sequences at the RNA-DNA junction are not random ones.