1977 *PHYSARUM* CONFERENCE: TAMPA, FLORIDA

The North American meeting this year will be in association with the Second International Mycological Congress, August 27 - September 3, 1977. Symposia of special interest to *Physarum* researchers have drawn the participation of a number of overseas associates, making this meeting particularly attractive and the co-scheduling of the 1977 *Physarum* conference quite compelling. Following discussions among Ned Holt, Henry Aldrich, Gene Goodman and Tom Evans, the following situation emerges:

1) There are many sessions within the Congress that will no doubt be of interest to us (a brief compilation prepared by Henry Aldrich is on p.). In particular are two symposia ("The Biology of *Physarum*" and "Biochemistry of Differentiation in the Cell Cycle") plus a round table ("Slime Mold Phylogeny"), all scheduled for Wednesday.

2) Contributed papers are to be presented in poster sessions. There will be a special *Physarum* poster session on Thursday or Friday of that week. Abstracts must be submitted by April 1, 1977. Abstract forms are part of a registration packet and are to be obtained by writing to Dr. M.S. Fuller, Dept. of Botany, Univ. of Georgia, Athens, Georgia 30602 (telephone: (404) 542-3732). Indicate on the registration material that you have been invited by Henry Aldrich to present your paper in the *Physarum* poster section. Please send a copy of submitted abstracts to the PNL for inclusion in the next newsletter.

3) A *Physarum* "workshop" is being organized by Gene Goodman. To be convened on Thursday evening, the format of this session will be rather informal resembling that of past *Physarum* meetings. To get on the agenda, you must submit a brief description of your presentation by July 1. These abstracts, along with the poster session abstracts, will be printed and available at the congress. ALL SUBMISSIONS AND QUESTIONS RELATING TO THIS SESSION MUST GO TO: Dr. Eugene Goodman, Division of Science, University of Wisconsin - Parkside, Kenosha, Wisconsin 53140 (telephone: (414) 553-2422).

4) To participate in this *Physarum* meeting, you must register as a member of the Second International Mycological Congress. Pre-registration material can be obtained by writing to Dr. Fuller. As is typical of international meetings these days, the cost is high (approx. $60); however, this congress affords us a find opportunity to meet with colleagues from around the world and should provide the basis for an excellent conference.

5) An informal *Physarum* dinner will be arranged for Friday evening, September 2, 1977.

Further details regarding this meeting will be sent out with the next PNL this spring. In the meantime, you've just got time to submit abstracts for the poster session.

THIRD EUROPEAN *PHYSARUM* WORKSHOP

This outstanding three-day conference at Ruttihubelbad, Switzerland, was organized by Richard Braun and colleagues. Abstracts from the meeting are included with this mailing of the PNL.

Symposia and Special Interest Meetings of Probable Interest to Physarologists

Attending Second International

Mycological Congress

Tampa, Florida, Aug. 27 - Sept. 3, 1977
University of South Florida

	Morning Symp.	Afternoon Symp.	Evening Spec. Int. Meeting
Mon, Aug. 29	RNA & Protein Reg. Alberghina Lovett Lacroute Huttermann	Dimorphism Stork Sypherd Cassone Clark-Walker Carbonell	Poly-A (J. Lovett, chm) Mycology & Analytical Technology (W. Hess)
Tues., Aug. 30	Wall biosynthesis Bracker Gooday Cantino Hori		
	RNA-containing viruses Lemke Buck Day Kleinschmidt Saksena	Cell Wall Growth Nickerson Wessels Young Streiblova	Dev. & Ecology of Cellular Slime Molds (H. Hohl, chm)
Wed., Aug. 31	Biology of Physarum Rusch Lestourgeon Braun Wohlfarth-Bottermann Haugli Lafontaine Daniel	Biochem of diff. in cell cycle Sauer Ashworth Cummins Halvorson	Slime mold phylogeny Alexopoulos Olive Raper Perkins
Thurs, Sept.1	Genetic & morphogen. of higher Basidios Day Butler Raper Wessels Stamberg	Mitosis Heath Forer Pringle Kubai	Physarum facts, flops, foibles, futures (Goodman, chm)
Fri, Sept. 2	DNA-containing fungal viruses Kazama Myers Esser Slonimski	Physiol of obligate parasitism Staples Coffey Ellingboe M. Shaw	Microtubules & mitosis (I.B.Heath, chm)
Sat, Sept. 3	Morphogenesis of fungal sex organs Beckett Ethnomycology A. Smith Wasson Schultes Lowy	Mushroom morphogenesis Esser Gooday Gruen Leonard Uno	

TITLES AND SUMMARIES IN PRINT

Apogamic Development of Plasmodia in the Myxomycete *Physarum polycephalum:* A Cinematographic Analysis

R. W. Anderson, D. J. Cooke [1] and Jennifer Dee

Department of Genetics, The University, Leicester, England

Protoplasma 89, 29–40 (1976)

Received November 10, 1975

Summary

Strain CL of *Physarum polycephalum* forms multinucleate plasmodia within clones of uninucleate amoebae. The plasmodia have the same nuclear DNA content as the amoebae. Analysis of plasmodial development, using time-lapse cinematography, showed that binucleate cells were formed as a result of nuclear division in uninucleate cells. Binucleate cells developed into plasmodia by further nuclear divisions and cell fusions. No fusions involving uninucleate cells were observed. A temporary increase in cell and nuclear size occurred at the time of binucleate cell formation.

Vol. 68, 561, 1976 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

ADENYLATE CYCLASES IN PHYSARUM POLYCEPHALUM: INHIBITION OF A NUCLEAR ENZYME BY POLYAMINES

V. J. Atmar[1], J. A. Westland, G. Garcia[2], and G. D. Kuehn[3]
Department of Chemistry
New Mexico State University
Las Cruces, New Mexico 88003

Received November 3, 1975

SUMMARY: Two distinct adenylate cyclase enzymes have been found in Physarum polycephalum. One is derived from isolated nuclei and is potently inhibited by an equimolar combination of the three polyamines, putrescine, spermidine, and spermine. The second enzyme is particulate, derived from the cytoplasmic compartment, and is insensitive to inhibition by the polyamines. These observations support a potential role for the polyamines in the control of adenosine 3',5'-monophosphate synthesis in P. polycephalum nuclei.

The Genetic Basis of the Incompatibility Reaction following Plasmodial Fusion between Different Strains of the Myxomycete *Physarum polycephalum*

By M. J. CARLILE

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

Journal of General Microbiology (1976), 93, 371–376

SUMMARY

Post-fusion incompatibility among plasmodia derived from *Physarum polycephalum* strain 29 is controlled by interactions between alleles at three loci, two of which are linked. A reaction will occur between a plasmodium which is heterozygous or a homozygous dominant at a locus, and one which is a homozygous recessive at the same locus. Depending on genotype with respect to the three loci, incompatibility reactions among plasmodia can be absent, unilateral or bilateral.

CONTROL OF CHEMOTAXIS IN *PHYSARUM POLYCEPHALUM*

A. C. H. DURHAM and E. B. RIDGWAY. From the Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143 and the Department of Physiology, Medical College of Virginia, Richmond, Virginia 23298. Dr. Durham's present address is Laboratoire des Virus des Plantes, Institut de Biologie Moleculaire et Cellulaire, 15, Rue Descartes, 67000 Strasbourg, France.

THE JOURNAL OF CELL BIOLOGY · VOLUME 69, 1976 · pages 218–223

Volume 61, 234 FEBS LETTERS January 1976

DIFFERENTIAL CLEAVATE OF *PHYSARUM* DNA FROM DISTINCT POINTS OF S PHASE BY RESTRICTION ENZYME Eco RI

Helmut FOUQUET and Helmut W. SAUER

Fachbereich Biologie der Universität, 775 Konstanz, West Germany

Temperature-Sensitive Mutants of the Slime Mould *Physarum polycephalum*

II. Mutants of the Plasmodial Phase

Molec. gen. Genet. 149, 115–119 (1976)

Elliot C. Gingold[1], William D. Grant[2], Alan E. Wheals[3], and Marian Wren
Department of Genetics, The University, Leicester LE1 7RH, England

Summary. Methods are described for the isolation and testing of temperature-sensitive plasmodial strains of *Physarum polycephalum*. Nineteen temperature-sensitive strains were found by screening plasmodia derived from mutagenised amoebae and the properties of these are described. A scheme is outlined for the detection of specific mitotic cycle lesions amongst temperature-sensitive strains, and the properties of a presumptive mitotic cycle mutant are described.

Synthesis of Ribosomal RNA during the Mitotic Cycle in the Slime Mould *Physarum polycephalum*

Leonard HALL and Geoffrey TURNOCK
Department of Biochemistry, University of Leicester

Eur. J. Biochem. *62*, 471–477 (1976)

1. An isotope dilution technique has been used to analyze the synthesis of metabolically stable nucleic acids during the mitotic cycle in surface plasmodia of the slime mould *Physarum polycephalum*. Microplasmodia that had been labelled with [^{3}H]uridine were used to prepare a surface culture, after a period of growth long enough to ensure that radioactivity was present only in tRNA, rRNA and DNA. The synthesis of rRNA or nuclear DNA during the growth of the surface plasmodium was then followed by measuring the specific activity of the nucleic acid.

2. Synthesis of rRNA during the mitotic cycle shows the following characteristics: (a) it is low during the immediate period of nuclear division, (b) synthesis is then continuous throughout interphase and (c) the rate of synthesis increases 5–6-fold between the beginning and end of interphase. These results are discussed in relation to the pattern of replication of the genes for rRNA.

3. Approximately 80% of the nuclear DNA replicates during the first 90 min of the mitotic cycle; completion of replication, however, occupies the remainder of interphase.

LEVELS OF RNA POLYMERASES DURING THE MITOTIC CYCLE OF *PHYSARUM POLYCEPHALUM*

ARMIN HILDEBRANDT and HELMUT W. SAUER
Fachbereich Biologie der Universität, Konstanz (G.F.R.)

Biochimica et Biophysica Acta, 425 (1976) 316–321

Summary

Two RNA polymerase activities were quantitatively solubilized in plasmodial homogenates from *Physarum polycephalum* by sonication at 0.5 M ammonium chloride concentration. The proportions of RNA polymerases A and B were determined by four different methods.

Equal activity levels of both enzyme A and enzyme B were detected throughout the synchronous mitotic cycle of *Physarum*.

ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 176,. 214-217 (1976)

Differential Template Specificities of Nuclear RNA Polymerases Isolated from *Physarum polycephalum*

ARMIN HILDEBRANDT AND HELMUT W. SAUER

Fachbereich Biologie der Universität Konstanz, Postfach 7733, 775 Konstanz, West Germany

Received March 2, 1976

RNA polymerases A and B from *Physarum* were more active on denatured homologous, calf thymus, or phage DNA than on the corresponding native templates. We obtained distinct patterns of template activities for various single- and double-stranded synthetic homopolymers and alternating copolymers. Some templates were copied asymmetrically. All dC-rich structures were highly active templates. Poly(dA) was efficiently transcribed only in combination with oligo(dT), not with poly(dT). Differential activities of enzymes A and B on several synthetic templates and phage DNA suggest different requirements for the RNA synthesis by the two RNA polymerases from *Physarum*.

Experimental Cell Research 97 (1976) 418–425

ADVANCE OF MITOSIS BY HISTONE PHOSPHOKINASE

R. J. INGLIS,[1] T. A. LANGAN,[2] H. R. MATTHEWS,[1] D. G. HARDIE[1] and E. M. BRADBURY[1]

[1]*Department of Physics, Portsmouth Polytechnic, Gun House, Hampshire Terrace, Portsmouth, UK, and* [2]*Department of Pharmacology, University of Colorado, School of Medicine, Denver, CO 80220, USA*

SUMMARY

The previous observation that growth-associated histone kinase (HKG) from Ehrlich ascites cells brings forward mitosis in *Physarum polycephalum* has been confirmed with more step 1 histone kinase and a more purified (step 2) histone kinase and the statistical significance of the results assessed. The mitosis appears normal in the phase contrast microscope and DNA synthesis is initiated after mitosis as usual. In vitro the growth-associated histone kinase phosphorylates chromatin, the phosphate appearing in F 1 histone. The results are interpreted as providing support for the hypothesis that growth-associated histone kinase controls the initiation of mitosis through F 1 histone phosphorylation and chromosome condensation.

Physarum Tropomyosin-Troponin Complex

Isolation and Properties[1]

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J. Biochem., 78, 583–588 (1975)

The relaxing protein (TM-TN complex) was isolated from plasmodia of *Physarum*. SDS-gel electrophoresis revealed that the relaxing protein consists of tropomyosin subunits with a molecular weight of 35,000, troponin subunits with molecular weights of 38,000 (T) and 24,000 (I) and several other components. No component corresponding to muscle troponin-C (MW=18,000) was detected in the plasmodium relaxing protein. The relaxing protein combined with muscle F-actin, and inhibited the ATPase [EC 3.6.1.3] activity and superprecipitation of reconstituted muscle actomyosin in the absence of Ca^{2+} ions. This inhibition was reversed by adding 1 μM Ca^{2+} ions.

CHANGE IN ATP-PYROPHOSPHOHYDROLASE ACTIVITY DURING SPHERULE FORMATION OF *PHYSARUM POLYCEPHALUM*

MASARU KAWAMURA, NOBUKO TONOTSUKA and KEI NAGANO
Department of Biology, Jichi Medical School, Yakushiji, Tochigi (Japan)

Biochimica et Biophysica Acta, 421 (1976) 195–202

Summary

The activity of Ca^{2+}-dependent ATP pyrophosphohydrolase was found to fluctuate during spherule formation of the acellular slime mold *Physarum polycephalum* under starving incubation. The enzyme activity increased up to 16-fold at the 3rd day of the starvation, then decreased drastically to less than its original level. Column chromatography of the enzyme preparation suggested that the increase in the activity was due to de novo synthesis of a new isozyme. Cycloheximide inhibited the synthesis. The two isozymes were different in their Ca^{2+} sensitivity, the new one being less sensitive.

THE JOURNAL OF CELL BIOLOGY · VOLUME 69, 1976 · pages 393-406

ACTOMYOSIN CONTENT OF *PHYSARUM* PLASMODIA AND DETECTION OF IMMUNOLOGICAL CROSS-REACTIONS WITH MYOSINS FROM RELATED SPECIES

DIETRICH KESSLER, VIVIANNE T. NACHMIAS, and ARIEL G. LOEWY

From the Department of Biology, Haverford College, Haverford, Pennsylvania 19041, and the Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19174

ABSTRACT

The content of myosin in plasmodia of the myxomycete *Physarum polycephalum* was measured by an immunological technique, quantitative microcomplement (C') fixation. Migrating plasmodia (starved after growth on rolled oats) contained 0.60 ± 0.08 (SD) mg myosin per g fresh plasmodia. Myosin comprised 0.77% ± 0.05 (SD) of the total plasmodial protein. When total plasmodial proteins were separated by electrophoresis on SDS-polyacrylamide gels, a large amount of protein appeared in a band comigrating with muscle actin. Densitometry performed after Coomassie blue staining indicated that as much as 15–25% of the total protein in the plasmodium could be actin. This gives an actin/myosin ratio by weight in the myxomycete plasmodium as high as 19–33, a very "actin-rich" actomyosin compared with rabbit skeletal muscle actomyosin with an actin/myosin ratio of 0.6. Starvation stimulates rapid migration and is correlated with a higher percent of both myosin and actin in the total protein of the plasmodium compared with normally growing cultures. Immunological cross-reaction of myosins from a variety of species was measured by C' fixation using an antiserum produced against purified native myosin from *P. polycephalum*. Although myxomycete and vertebrate striated muscle myosins have very similar morphological and biochemical properties, and apparently possess similar binding properties to F-actin, only myosins from myxomycetes in the order *Physarales*, rather closely related to *P. polycephalum*, gave detectable cross-reactions. This finding suggests that many amino acid sequences in myosin have been variable during evolution.

Chemotactic and Other Responses of Plasmodia of *Badhamia utricularis* to an Extract of *Stereum hirsutum* and to Certain Other Substances

By D. KNOWLES* AND M. F. MADELIN

Department of Botany, The University, Bristol BS8 1UG

Journal of General Microbiology (1975), **89**, 235–244

SUMMARY

An extract of the basidiomycete *Stereum hirsutum* attracted plasmodia of *Badhamia utricularis*. Attracted plasmodia moved at a fairly constant speed until they contacted the source of attractant. The plasmodial front was directed towards the source by the production and advance of lobes at the nearest point of the front, and the attenuation and withdrawal of lobes in more remote parts. Directly applied extract halted the normal reversals of protoplasmic streaming in plasmodia and induced one-way flow for up to 25 min. It also caused accumulation of protoplasm, and swelling and lengthening of the treated plasmodial strands. Benzamide, a non-volatile anaesthetic, also suppressed protoplasmic flow reversals and caused protoplasm to accumulate in swellings but did not cause chemotaxis. Extract from one other fungus, *Metarrhizium anisopliae*, possessed very similar activity to Stereum extract.

A method of isolation of mitochondrial nucleoid of *Physarum polycephalum* and evidence for the presence of a basic protein

T. KUROIWA, S. KAWANO and M. HIZUME, *Department of Biology, Faculty of Science, Okayama University, Okayama 700, Japan*

Exptl. Cell Res. 97, 435 (1976)

Summary. A large amount of nucleoids could be isolated from mitochondria of the slime mold *Physarum polycephalum* by treating the mitochondria successively with Triton X-100 and Nonidet P-40 followed by centrifugation. The preparation retained the ultrastructure characteristics of the intact mitochondrial nucleoid. The population of proteins extracted from the nucleoid preparation was analysed by polyacrylamide gel electrophoresis. The result indicated presence of at least one species of basic protein.

Nuclear behaviour during meiosis in the myxomycete Physarum polycephalum

MORTEN M. LAANE & FINN B. HAUGLI

Laane, M. M. & Haugli, F. B. 1976. Nuclear behaviour during meiosis in the myxomycete Physarum polycephalum. *Norw. J. Bot.* 23, 7–21.

Spore cleavage and meiotic events are not strictly coupled in the myxomycete *Physarum polycephalum*. Meiosis normally occurs about 20 hours after spore cleavage, but in rare cases nuclei may go through meiosis in the developing sporangium before spore delimination. More than one nucleus is often included in a single spore. Up to three of the four nuclei resulting from meiosis may degenerate. Thus, mature spores arise via different pathways. These conclusions are based on light- and electron microscopical techniques together with Feulgen fluorescence measurements of relative DNA content per nucleus.

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F. B. Haugli, Institute of Medical Biology, University of Tromsø, N-9000 Tromsø, Norway.

(Institute of Botany, University of Oslo, Oslo, Norway)

Examination of Fungal Nuclei with the Feulgen-Fluorescence Method

By Morten Motzfeldt LAANE[1]) and Thore LIE

„Mikroskopie" Bd. 31 (1975), S. 85—90

A simple modified Feulgen-fluorescence method is described which gives very bright fluorescing nuclei in several fungal species, making a detailed study of nuclear behaviour possible. The specimens are fixed in 3 parts absolute ethanol and 1 part glacial acetic acid for 4 minutes, then hydrolysed in 5 N HCl for 5 minutes at 20°C, rinsed in distilled water and placed in the Feulgen solution for 10 minutes. The samples are rinsed several times in SO_2-water, dehydrated with ethanol and embedded in Euparal. Although no staining can be seen by ordinary bright-field microscopy, an intense nuclear fluorescence is seen by incident green-light excitation (interference green filter combination Zeiss BP 546, dichroitic mirror FT 580, absorption filter LP 590). The method enables nuclear and cromosomal research in many fungal species that have been difficult or impossible to study by other staining methods.

Vol. 71, 789 , 1976 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

FLUCTUATIONS IN CYCLIC ADENOSINE 3':5'-MONOPHOSPHATE AND CYCLIC GUANOSINE 3':5'-MONOPHOSPHATE DURING THE MITOTIC CYCLE OF THE ACELLULAR SLIME MOULD *PHYSARUM POLYCEPHALUM*

James R. Lovely and Richard J. Threlfall

Department of Botany, Imperial College, London SW7 2AZ, U.K.

Received May 25, 1976

SUMMARY Cyclic adenosine 3':5'-monophosphate (cyclic AMP) and cyclic guanosine 3':5'-monophosphate (cyclic GMP) have been determined at half-hourly intervals throughout the mitotic cycle of *Physarum polycephalum*. Cyclic AMP was constant at 1pmole/mg protein throughout except for a transient peak of 17pmoles/mg protein in the last quarter of G2. Cyclic GMP was more variable (2-4pmole/mg protein) rising to 9.5pmole/mg protein during the 3 hour S period and to 7pmole/mg protein during the last hour of G2. The significance of these changes is discussed.

Attraction of Plasmodia of the Myxomycete, *Badhamia utricularis*, by Extracts of the Basidiomycete, *Stereum hirsutum*

By M. F. MADELIN, FIONA AUDUS* AND D. KNOWLES†

Department of Botany, University of Bristol, Bristol BS8 1UG

Journal of General Microbiology (1975), **89**, 229–234

SUMMARY

Pieces of the fruit body of *Stereum hirsutum* attracted the migrating plasmodia of *Badhamia utricularis* at a distance of 4 cm. Extracts of fruit bodies made with organic solvents could attract, but aqueous extracts could not. Cultured mycelium and extracts of cultured mycelium also attracted strongly, but activity was not detected in culture filtrate. Phase separation with organic and aqueous phases concentrated the attractive principle. Paper chromatography indicated the presence of a single substance of high activity which migrated in isopropanol–ammonia–water with an R_F of 0·83 to 0·88 and in butanol–acetic acid–water with an R_F close to 0·9. The active extracts from fruit bodies and cultured mycelium were thermostable. The attractant diffused through both aqueous and gaseous phases.

Defined and Semi-defined Media for the Growth of Amoebae of *Physarum polycephalum*

By CLARE H. R. McCULLOUGH AND JENNIFER DEE
Department of Genetics, The University, Leicester LE1 7RH

Journal of General Microbiology (1976), 95, 151–158

SUMMARY

Amoebae of the true slime mould *Physarum polycephalum* were cultured in two fully-defined liquid media containing amino acids, glucose, three vitamins and a buffered salts solution. Absolute requirements were demonstrated for methionine, haematin, thiamine and biotin, all of which were known to be specific requirements of the plasmodial stage. Methods are described for large-scale culture in three semi-defined media.

Antibody to *Physarum* myosin

I. PREPARATION AND FUNCTIONAL EFFECTS

Immunology 1976 30 419

V. T. NACHMIAS & D. KESSLER *Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia, and Department of Biology, Haverford College, Haverford, Pennsylvania, U.S.A.*

Summary. Preparation of antibody to *Physarum* myosin is described, and evidence is presented that the antibody is specific for this molecule. A diffusion coefficient of 1×10^{-7} cm²/s is estimated. The antibody interfered with myosin enzyme activity and with superprecipitation of actomyosin. It did not cross-react with rabbit striated muscle myosin.

OSCILLATIONS OF CALCIUM ION CONCENTRATIONS IN *PHYSARUM POLYCEPHALUM*

E. B. RIDGWAY and A. C. H. DURHAM. From the Department of Physiology, Medical College of Virginia, Richmond, Virginia 23298, and the Department of Biochemistry, University of California, San Francisco, California 94143. Dr. Durham's present address is the Laboratoire des Virus des Plantes, Institut de Biologie Moleculaire et Cellulaire, 67000 Strasbourg, France.

THE JOURNAL OF CELL BIOLOGY · VOLUME 69, 1976 · pages 223-226

Cytologische Untersuchungen zur Endo- und Exocytose bei acellulären Schleimpilzen[1]

ROLF STIEMERLING und WILHELM STOCKEM

Institut für Cytologie und Mikromorphologie, Universität Bonn, Bundesrepublik Deutschland

Mit 9 Abbildungen

Protoplasma 85, 243–260 (1975)

Summary

Cytological Studies on Endo- and Exocytosis in Acellular Slime Molds

Acellular slime molds (*Physarum confertum*) take up food particles by endocytosis. During the uptake of a special food mixture consisting of five components (Aerosil, pigment of inkfish, yeast, starch, and aleuron) the plasmodium looses its typical vein-like structure for a certain period by building a coherent plasma mass spread over the food. The different components of the food are ingested in the basal region of the plasma mass either singular and selectiv or as big composed portions. The diameter of the so-formed endosomes differs between 1 and 50 µm depending on the nature and size of the food particles.

After finishing food uptake the plasmodium rebuilds the characteristic network habitus with the shuttle streaming inside the veins as it is typical for acellular slime molds.

Food vacuoles are transported by the streaming endoplasm until its contents is digested. Some hours later endosomes with its indigestible rests assemble in the basal region of the veins where they are deposited in the stationary ectoplasm. The rests of the food particles are segregated into a peripheric system of cell membrane invaginations which separates endoplasm from ectoplasm and which opens to the external environment by numerous pores. Indigestible rests of food particles are defecated by exocytosis. The significance of the cisternlike cell membrane invaginations for the secretion of indigestible products and slime substances is discussed.

THE CONTROL OF MITOSIS IN *PHYSARUM POLYCEPHALUM*

The Effect of Lowering the DNA : Mass Ratio by UV Irradiation

P. E. SUDBERY[1] and W. D. GRANT

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

Experimental Cell Research 95 (1975) 405–415

SUMMARY

A model for the control of mitosis is presented and, along with four other models described previously, is tested by the response of *Physarum polycephalum* to UV irradiation. Plasmodia were irradiated following the second mitosis (M II) after fusion of microplasmodia. As shown by other authors, the onset of the next mitosis (M III) was delayed but the period M III–M IV was shortened relative to control plasmodia. It is shown that the period M III–M IV cannot be shortened beyond a minimum of 6 h despite increasing doses of UV. This minimum length is shown to be relatively independent of growth rate. If conditions were such that the length of M III–M IV was shortened to this minimum value the length of M IV–MV was also shorter than the corresponding control period. If the period M II–M IV was longer than the minimum following irradiation then the length of M IV–M V was not shortened. It is argued that only the latter situation allows models to be tested and it is shown how the observed result is consistent with only two of the five models considered. A further test compared the length of M III–M IV under these conditions with that predicted from the amount of DNA destroyed by the UV. This result was consistent only with the same two models.

PHYSICAL PROPERTIES AND CHEMICAL COMPOSITIONS OF CYTOPLASMIC AND MITOCHONDRIAL MALATE DEHYDROGENASE FROM *PHYSARUM POLYCEPHALUM*

W. MARTIN TEAGUE and HENRY R. HENNEY JR.
Department of Biology, University of Houston, Houston, Texas 77004 (U.S.A.)
Biochimica et Biophysica Acta, 434 (1976) 118–125

SUMMARY

The malate dehydrogenase isoenzymes from *Physarum polycephalum* have been purified to homogeneity as confirmed by gel filtration chromatography, polyacrylamide gel disc electrophoresis and analytical ultracentrifugation.

Certain physical and chemical parameters of the malate dehydrogenase isoenzymes reported here include sedimentation, molecular weight and subunit molecular weight. Most unique of the differences between the isoenzymes were the widely separate isoelectric points of 9.83 for mitochondrial malate dehydrogenase and 6.14 for the supernatant malate dehydrogenase. The amino acid analyses of each form were done revealing the isoenzymes were unquestionably unique proteins differing in the content of ten amino acids.

Thermotaxis in a Slime Mold, *Physarum polycephalum*

WUNG-WAI TSO and TAG E. MANSOUR[2]

Department of Pharmacology, Stanford University Medical School, Stanford, California 94305

Physarum polycephalum is thermotactic toward 29 ± 1°C avoiding both higher and lower temperatures. 29°C appears to be a combined optima for growth and locomotion. It is likely that thermotaxis is a more efficient way of avoiding unfavorable temperature than transforming into spherules.

BEHAVIORAL BIOLOGY, 14, 499-504 (1975)

Temperature-Sensitive Mutants of the Slime Mould *Physarum polycephalum*

I. Mutants of the Amoebal Phase

Molec. gen. Genet. 149, 111–114 (1976)

Alan E. Wheals[1], William D. Grant[2], and Brigitte M. Jockusch[3]
Max-Planck-Institut für Biologie, Abt. Melchers, D-7400 Tübingen, Federal Republic of Germany

Summary. A replica plating method for isolating *ts* amoebal mutants of *Physarum polycephalum* has been devised. Temperature-sensitive mutations occur at a frequency after nitrosoguanidine mutagenesis of 10^{-3} per survivor, are stable but are not usually expressed in the plasmodia formed from these amoebae in clones. Some of these mutants appear to be cell-cycle stage specific.

Cycling Aggregation Patterns of Cytoplasmic F-Actin Coordinated with Oscillating Tension Force Generation*

K.-E. Wohlfarth-Bottermann and M. Fleischer

Institut für Cytologie der Universität Bonn

Cell Tiss. Res. 165, 327–344 (1976)

Summary. Isometric contracting protoplasmic veins of *Physarum polycephalum* show cycling patterns of cytoplasmic F-actin, dependent on their oscillating contraction behaviour (minute rhythms). The process of fibrillogenesis represents a parallel arrangement of F-actin chains ("plasma filaments, microfilaments") during the *isometric contraction* phase. A part of the results of the present work corroborates previous results on stretch-activated veins which showed that the fibrillar form of F-actin reflects the isometric contracted state.

During *isometric relaxation* phase, a disaggregation of the fibrillar pattern takes place and is accompanied by a deparallelisation of F-actin chains. Therefore, the isometric relaxed state of cytoplasmic actomyosin is non-fibrillar in nature. Thus, the morphologically detectable fibrillar form of cytoplasmic actomyosin, according to physiological interpretation, is solely representative of the isometric contracted state.

The question whether assembly-disassembly processes, e.g. G⇌F-actin-transformation, play a role in the contraction-relaxation cycle is discussed.

C. R. Acad. Sc. Paris, t. 283 (8 novembre 1976) Série D 1361

PHARMACOLOGIE. *Mise en évidence de l'action du méthyl benzimidazole 2 yl carbamate* (MBC) *et du méthyl* [5 (2 *thiényl carbonyl*) 1 H *benzimidazole 2 yl carbamate*] (R17 934) *sur le noyau de* Physarum polycephalum (*Myxomycètes*). Note (*) de MM. **Michel Wright, André Moisand,** M^mes^ **Yvette Tollon** et **Marie-Louise Oustrin,** présentée par M. René Truhaut.

The toxicity of these compounds was determined in the amoebae and plasmodia. An electron microscopic study shows an increase in nuclear size which is in agreement with the increase of the total amount of DNA. Microtubules are present but they are not organized in a normal mitotic apparatus. Some other nuclear abnormalities are described.

TITLES AND SUMMARIES IN PRESS

NUCLEAR DNA CONTENT AND CHROMOSOME NUMBERS THROUGHOUT THE LIFE CYCLE OF THE COLONIA STRAIN OF THE MYXOMYCETE, *PHYSARUM POLYCEPHALUM*

JOYCE MOHBERG*

Genetics Department, University of Leicester LE1 7RH, England

SUMMARY

Nuclear DNA content and ploidy have been determined at different stages of the life cycle of the Colonia strain of the myxomycete *Physarum polycephalum*. Analyses at the plasmodial stage showed that (*a*) Burton and Feulgen DNA analyses agreed within 15 % with strains which ranged from 0·6 to 3·6 pg of DNA per nucleus; (*b*) S-phase in Colonia is during the early part of interphase as in the Wisconsin strain; (*c*) in heterothallic and heterothallic × Colonia crossed strains there are 1·0–1·2 pg of DNA and 70 chromosomes per nucleus and in Colonia 0·6 pg of DNA and 40 chromosomes.

Germinating spores of all strains contained one population of cells with about 0·5 pg of DNA and 40 chromosomes and another of larger cells with up to 2·5 pg of DNA and 200 chromosomes. The polyploid nuclei comprised 2–20 % of the total in heterothallic strains, 2–65 % in heterothallic × Colonia crosses and 25–75 % in Colonia.

A method was devised for making chromosome spreads of amoebae grown on bacterial lawns. Cells were first exposed to dilute formaldehyde at 26 C for 30 min, then spread on slides with hot lactic acid and stained. Such spreads of CLd (Colonia) and RSD4 (heterothallic) amoebae both contained about 40 chromosomes.

The data are consistent with the view that Colonia is haploid throughout its life cycle and that chromosome number is neither halved during sporulation nor doubled during plasmoidal formation. However, the possibility exists that an alternance of ploidy occurs by way of the few diploid nuclei present in the plasmodium.

J. Cell Science, in press

Cytochemical Studies on Intracellular Digestion in the Acellular Slime Mold *Physarum confertum*

Wilhelm Stockem und Rolf Stiemerling

Institut für Cytologie und Mikromorphologie
Universität Bonn, BRD

Summary

The acellular slime mold *Physarum confertum* possesses a digestive system which can be compared to that of normal animal cells. The system could be demonstrated morphologically and cytochemically by the localization of acid phosphatase activity. The Golgi apparatus and the endoplasmic reticulum proved to be involved in the formation of digestive enzymes. From these cell organelles the hydrolases are transported to food vacuoles by primary lysosomes. Primary lysosomes and food vacuoles confluate to secondary lysosomes in which digestion takes place. The hydrolyzed contents of the secondary lysosomes seem to be distributed throughout the cytoplasm by micropinocytotically formed vesicles. The application and absorption of substances differing in their degree of digestibility revealed that acid hydrolases are also released into vacuoles which contain indigestible material, i.e. silicon dioxide. However, the contents of these vacuoles are defecated soon after their formation has occurred; in contrast, the hydrolisation and resorption of digestible food particles (aleuron and yeast) could be observed over a period of at least 18-26 hours.

Protoplasma, in press

INTRACELLULAR SEGREGATION OF ENDOCYTOTICALLY INGESTED SUBSTANCES

W. Stockem und R. Stiemerling

Institute of Cytology
University of Bonn, FRG

Acellular slime molds and amebae have developed a mechanism for the intracellular segregation of endocytotically ingested substances: Food mixtures consisting of two (egg white and Aerosil) and five different components (starch grains, aleuron grains, pigment granules, yeast cells and Aerosil) respectively are absorbed into the cytoplasm by the formation of large food vacuoles (diameter 30 - 50 µ). Later, these vacuoles are divided into several smaller vesicles (diameter 3-5 µm) which contain only one or mostly one of the food components. The significance of the intracellular segregation of ingested substances in respect to the phenomenon of cell digestion is discussed.

Cytobiologie, in press

THE CONTROL OF MITOSIS IN *PHYSARUM POLYCEPHALUM*: THE EFFECT OF DELAYING MITOSIS AND EVIDENCE FOR THE OPERATION OF THE CONTROL MECHANISM IN THE ABSENCE OF GROWTH

BY P.E. SUDBERY AND W.D. GRANT

Department of Genetics, The Adrian Building, University Rd.
Leicester LE1 74R, UK.

Experiments were performed to test hypothetical mechanisms for the control of mitosis in *Physarum polycephalum*. The effect of delaying mitosis was shown to result in a single shortened intermitotic period, agreeing with a common prediction and substantiating a basic assumption that the DNA: Mass ratio is homeostatically controlled. When nuclei were destroyed by UV irradiation compensation occurred through a shortened intermitotic period in the complete absence of growth. This is consistent with only two of the five mechanisms considered.

J. Cell Science, in press

AN EXTRACELLULAR INDUCER OF ASEXUAL PLASMODIUM FORMATION IN PHYSARUM POLYCEPHALUM

Philip J. Youngman, Paul N. Adler , Thomas M. Shinnick and Charles E. Holt

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

ABSTRACT. Asexual conversion of amoebae to plasmodia was studied in the Colonia isolate of the myxomycete, Physarum polycephalum. When a culture of Colonia amoebae is grown on a bacterial lawn, a period of amoebal growth precedes the appearance of cells committed to the plasmodial state. The onset of plasmodium production appears to be related to amoebal nutrition since cultures supplied with fewer bacteria display earlier differentiation. For a period of time after differentiation is initiated, conversion of amoebae to plasmodia is rapid and proceeds as an exponential function of time. A filter-transmissible substance, apparently released by differentiating cells, is implicated in the control of this rapid conversion.

Proceedings of the National Academy of Sciences, in press.

Cell Motility

BOOK B
Actin, Myosin and Associated Proteins

edited by
R. Goldman
Carnegie-Mellon University

T. Pollard
Harvard University

J. Rosenbaum
Yale University

COLD SPRING HARBOR CONFERENCES ON CELL PROLIFERATION
VOLUME 3

Actin and Actinin from Myxomycete Plasmodia

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Nagoya University, Chikusa-ku, Nagoya, Japan

Studies on Motility in *Physarum polycephalum*

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Regulation and Polarity: Results with Myxomycete Plasmodium and with Human Platelets

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University of Pennsylvania, Philadelphia, Pennsylvania 19174

The Fungal Spore. Form and Function. Edited by Darrell J. Weber and Wilford M. Hess. Wiley-Interscience, a Division of John Wiley & Sons, New York, 1976, 895 p., $30.

This book is a transcript of the Second International Fungal Spore Symposium held in Provo, Utah during July 1974. The topics covered in the book relate to the morphology and physiology of the mature fungal spore and its germination. Quite understandably, fungal sporulation is not specifically covered; the magnitude and importance of these processes are such that a future meeting will deal directly with them.

The Fungal Spore is composed of eighteen extensive reviews written by invited specialists in the field of mycology. Areas of consideration include the major groups of organisms traditionally included among the fungi, as well as the two types of slime molds. The result of the obviously careful selection of invited speakers is that the volume provides a well-rounded review of the present status of fungal spore research. Short summaries of the discussions at the end of almost every chapter provide additional insight into areas of controversy and active research.

. . . from a review by D.A. Cotter and K.R. Dahlberg, ASM News 42, 428 (1976). The chapter "Resistant Structures in the Myxomycetes" by H.C. Aldrich and M. Blackwell was summarized earlier in the PNL (6, 41, 1974).

THE NEW BIOLOGY: II

The Cancer Puzzle

By ROBERT F. WEAVER, Ph.D.

Neither plant nor animal, the lowly slime mold (**left**) may offer scientists a clue to the Jekyll-and-Hyde transformation of a healthy cell to a runaway cancerous one. By learning how this simple one-celled organism changes function, researchers hope to uncover the mechanisms that alter human cells.

National Geographic 150, 396 (1976)

ADDITIONAL ARTICLES IN PRINT

G. Brand, A. Huttermann and F.B. Haugli
"Differential Expression of RNAse Activities in the Life Cycle of Physarum polycephalum"
Naturwissenschaften 62, 535 (1975). (PNL 8, 11, 1976)

J.R. Denbo and D.M. Miller
"Factors Affecting the Movement of Slime Mold Plasmodia"
Comp. Biochem. Physiol. 55a, 5 (1976). (PNL 8, 10, 1976)

H.H. Evans, S.R. Littman, T.E. Evans and E.N. Brewer
"Effects of Cycloheximide on Thymidine Metabolism and on DNA Strand Elongation in Physarum polycephalum"
J. Mol. Biol. 101, 169 (1976). (PNL 8, 12, 1976)

E.M. Goodman, B. Greenebaum, and M.T. Marron
"Effects of Extremely Low Frequency Electromagnetic Fields on Physarum polycephalum"
Radiat. Res. 66, 531 (1976). (PNL 8, 12, 1976).

R.J. Inglis, T.A. Langan, H.R. Matthews, D.G. Hardie, and E.M. Bradbury
"Advance of Mitosis by Histone Phosphokinase"
Exptl. Cell Res. 97, 418 (1976). (PNL 8, 10, 1976)

R. Nagai and T. Kato
"Cytoplasmic Filaments and their Assembly into Bundles in Physarum Plasmodium"
Protoplasma 86, 141 (1975). (PNL 7, 19, 1975)

V.M. Vogt and R. Braun
"Repeated Structure of Chromatin in Metaphase Nuclei of Physarum"
F.E.B.S. Letters 64, 190 (1976). (PNL 8, 13, 1976).

V.M. Vogt and R. Braun
"Structure of Ribosomal DNA in Physarum polycephalum"
J. Mol. Biol. 106, 567 (1976). (PNL 8, 13, 1976).

McArdle Sclerotia Collection

As many of you may know, Professor Harold Rusch is now serving as the Director of the Regional Cancer Center at the University of Wisconsin, and has closed his research laboratory. The collection of sclerotia is being transferred to Cleveland, where it will be maintained and will continue to be available to the scientific community. Address questions and requests to: Sclerotia Collection, c/o Tom Evans, Division of Radiation Biology, Case Western Reserve University, Cleveland, Ohio 44106.

THESIS

High Density Induction of a Quiescent Cell State in *Physarum polycephalum*

by

Linda Lee McAlister

(A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science (Biology) at Southern Methodist University, 1976)

Starvation-induced differentiation in the simple eukaryote *Physarum polycephalum* has been characterized by specific and reproducible changes in the complement of acidic nuclear proteins. When exponentially growing microplasmodia are subjected to conditions of high density, rpaid changes in the electrophoretic profile of the acidic nuclear proteins are observed which correspond in part to those induced by prolonged starvation. This suggests the possibility of a generalized mechanism for cellular transition from active growth to a non-proliferative cell state.

Short term high density conditions result in few changes in nuclear proteins other than the acidic protein fraction. All changes in nuclear protein components are completed within the 2 h after initiation of high density conditions as compared with 21 h when differentiation is induced by starvation. Incorporation of ^{3}H-thymidine into nuclear DNA decreases by 53.2% after 1 h at high density. DNA synthesis also decreases by more than 50% after 7 h of starvation. Similarly, high density results in a quantitative decrease in RNA synthesis comparable to reported decreases in RNA synthesis observed during prolonged starvation. Comparison of the ultrastructural morphology of microplasmodia at high densities with that of starved cultures shows some striking similarities between the two cell states. Nuclei in both cases show an increase in heterochromatic clumping relative to nuclei characteristic of exponential growth. Mitochondrial morphology and cytoplasmic organization are also similarly and distinctly altered by starvation and high density.

COMMUNICATION

A Simplified Growth Medium for *Physarum polycephalum*

E.N. Brewer and Amanda Prior

Division of Radiation Biology
Case Western Reserve University

We have devised a simplified version of the Danial and Rusch (J. Gen. Microbiol., 25, 47-59, 1961) growth medium. One liter of the modified medium contains 10 g tryptone, 3 g yeast extract, 9 g dextrose, 3.6 g citric acid, and 0.5 g $MgSO_4 \cdot 7H_2O$; pH is brought to 4.6 with 30% KOH, and sterile hematin solution added to the sterile medium as usual. Microplasmodial growth rate is about 10% faster for the modified medium, and interdivision times are reduced from 9 to about 8 h, and are less variable, for stationary macroplasmodia.

ABSTRACTS OF MEETING PRESENTATIONS

Union of Swiss Societies of Experimental Biology
8th Annual Meeting
Fribourg, April 9-10, 1976

Ribosomal RNA Synthesis during the Mitotic Cycle in *Physarum polycephalum*

L. Hall and G. Turnock
Institut für allgemeine Mikrobiologie der Universität, Altenbergrain 21, CH-3013 Bern

An isotope dilution technique has been used to analyze the synthesis of metabolically stable nucleic acids during the mitotic cycle in surface plasmodia of the slime mould *Physarum polycephalum*. Microplasmodia that had been labelled with ^{3}H uridine were used to prepare a surface culture, after a period of growth long enough to ensure that radioactivity was present only in tRNA, rRNA and DNA. The synthesis of rRNA during the growth of the surface plasmodium was then followed by measuring the specific activity of the nucleic acid. – Synthesis of rRNA during the mitotic cycle shows the following characteristics: (a) it is low during the immediate period of nuclear division, (b) synthesis is then continuous throughout interphase and (c) the *rate* of synthesis increases 5–6fold between the beginning and end of interphase. These results are discussed in relation to the pattern of replication of the genes for rRNA.

Experientia 32, 796 (1976)

Studies on two RNA Polymerases from the Nuclei of *Physarum polycephalum*

S. S. Smith and R. Braun
Institut für allgemeine Mikrobiologie der Universität, Altenbergrain 21, CH-3013 Bern

We have attempted to study the purification of the RNA polymerases from kilogram quantities of *Physarum* microplasmodia in some detail. Under the conditions we employ, both known polymerases chromatograph together at about 0.15 *M* $(NH_4)_2SO_4$ on DEAE-Cellulose, but elute as separate peaks on DEAE-Sephadex at 0.2 *M* (α-amanitin sensitive polymerase) and 0.3 *M* (α-amanitin resistant polymerase) $(NH_4)_2SO_4$. In addition, a batchwise exposure to DEAE-Sephadex in the presence of 0.35 to 0.5 *M* $(NH_4)_2SO_4$, or an adsorption and stepwise elution with 0.4 *M* $(NH_4)_2SO_4$ appears to be a prerequisite for subsequent separation during DEAE-Sephadex chromatography. We employ the batchwise method since, in addition to removing more than 50% of the ploysaccharide and more than 90% of the nucleic acid in the extract, it results in a large activation of polymerase activity. Our present purification procedure employs a DEAE-Sephadex batch step, followed by DEAE-Sephadex, DEAE-Cellulose and Phospho-Cellulose chromatography to give nearly homogenous enzymes in about 1% yield.

Experientia 32, 813 (1976)

Ribosomal DNA in *Physarum*

V. Vogt and R. Braun
Institut für allgemeine Mikrobiologie der Universität, Altenbergrain 21, CH-3013 Bern

The sequences coding for *Physarum* ribosomal RNA are localized on independently replicating, linear DNA molecules of a discrete size, 37×10^6 daltons. Restriction endonucleases $EcoR_1$ and $Hindiii$ each cut rDNA into one large and two small fragments. The latter are represented twice per intact molecule, once at each end. Sedimentation and electron microscopic analyses of intact rDNA that has been neutralized from alkaline solution indicate that the entire rDNA molecule has a rotational axis of symmetry near the center. Blocks of short, inverted repetitious sequences appear to be located at the center of the native rDNA and also at 3.7 to 11×10^6 daltons flanking the center.

Experientia 32, 814 (1976)

American Society of Biological Chemists
67th Annual Meeting
San Francisco, June 6-10, 1976

JOINING OF DNA REPLICATION INTERMEDIATES IN ISOLATED NUCLEI OF PHYSARUM. E.N. Brewer* (SPON: F.M. Sumpun). Case Western Reserve University, Cleveland, Ohio 44106

In homogenates prepared from S-phase cultures of Physarum polycephalum, approximately half of the progeny-strand DNA is synthesized as 10 S fragments while the remainder sediments in alkaline sucrose density gradients as a heavier species similar in size to that observed for newly-synthesized DNA of intact plasmodia (Biochim. Biophys. Acta, 402, 363 (1975)). The 10 S fragments are converted into ca. 3-fold longer DNA strands in isolated nuclei under conditions which support little or no DNA chain elongation. This conversion requires Mg^{++}, ATP, and a heat-stable "cytoplasmic" factor. Under the incubation conditions employed, no breakdown of the heavier DNA species or of parental-strand DNA is observed. The results support the suggestion that in this organism, the 10 S fragments represent DNA replication intermediates which eventually are ligated to form mature DNA progeny strands. (Supported by NSF grant GB-40299 and by contract E(11-1)2486 with the USERDA.

Fed. Proc. 35, 1418 (1976)

NUCLEOTIDE METABOLISM IN *PHYSARUM POLYCEPHALUM*: CYCLOHEXIMIDE EFFECTS. Helen H. Evans, Sandra R. Littman* and Thomas E. Evans*. Case Western Reserve Univ., Cleveland, Ohio 44106.

Treatment of *P. polycephalum* with cycloheximide results in increased pools of deoxyribonucleoside triphosphates (Bergter and Braun, Exp. Cell Res. 84, 436, 1974), as well as in an inhibition of DNA replication. We have measured the pool size and the specific activity of TTP following a 15-min incubation of S-phase plasmodia with labeled precursors ± cycloheximide, using the DNA polymerase method of Solter and Handschumacher (Biochim. Biophys. Acta 174, 585, 1969) as modified by Walters *et al.* (*ibid.* 319, 336, 1973). At a cycloheximide concentration of 10 µg/ml, the pool size of TTP doubled 5-10 min after drug addition and remained at a relatively constant level for at least 45 min. (DNA replication occurred at a normal rate for 10 min after drug addition but then stopped abruptly.) The specific activity of TTP following incubation of cycloheximide-treated plasmodia with [^{3}H]-thymidine was lower than the control. The decrease in specific activity 30 and 45 min after drug addition was too great to be accounted for by dilution by the expanded TTP pool. When [^{14}C]-formate was used as the labeled precursor, the presence of cycloheximide did not result in a decrease in the specific activity of TTP. Also, since the cycloheximide-induced expansion of the TTP pool was prevented by the addition of fluorodeoxyuridine, it is possible that treatment of S-phase plasmodia with cycloheximide causes an increase in the *de novo* synthesis of deoxyribonucleotides. Supported by NIH Grant CM19484 and ERDA Contract E(11-1)2486.

Fed. Proc. 35, 1496 (1976)

DEVELOPMENTAL CHANGES IN THE RELATIVE ABUNDANCES OF TWO FORMS OF RNA POLYMERASE II IN THE SLIME MOLD PHYSARUM POLYCEPHALUM. Robert F. Weaver*, Martha J. Shobe* and Chong-Gun Cho* (SPON: Phillip Nordin). Univ. of Kansas, Lawrence KS 66045

Two forms of DNA-dependent RNA polymerase II are selectively extracted from the vegetative plasmodium of the slime mold. One form (IIa) is readily released by mild sonication. The other (IIb) is extracted upon more vigorous sonication. Polymerase IIa predominates in vegetative plasmodia. It elutes from DEAE Sephadex at a relatively low ionic strength (≈0.10 M ammonium sulfate) and has a relatively high ionic strength requirement for optimal activity (0.08 M ammonium sulfate). Polymerase IIb elutes from DEAE Sephadex at a higher ionic strength (≈0.14 M ammonium sulfate) and exhibits a very low ionic strength optimum. (Ammonium sulfate concentrations as low as 0.01 M inhibit activity.) The slime mold sporulates in response to a period of starvation in the dark followed by illumination. During the starvation process, a shift in the IIa/IIb ratio takes place such that the predominant polymerase II in starved and sporulating plasmodia is IIb. (Supported by Grant #NP190 from the American Cancer Society.)

Fed. Proc. 35, 1637 (1976)

(Ca 3) *Nucleotide Metabolism in* Physarum polycephalum: *The Effect of Ionizing Radiation.* HELEN H. EVANS, SANDRA R. LITTMAN,* AND THOMAS E. EVANS,* Case Western Reserve University, Cleveland, Ohio 44106.

The effect of ionizing radiation on the incorporation of labeled precursors into DNA in *P. polycephalum* was found to vary with the precursor used. We therefore investigated changes in the pools and specific activities of the deoxynucleoside triphosphates according to the DNA polymerase method of Solter and Handschumacher (*Biochim. Biophys. Acta* 174, 336, 1969) as modified by Walters *et al.* (*ibid.* 349, 336, 1974). The pools of all four deoxynucleoside triphosphates were increased to a similar extent 15 min after the irradiation of early S-phase plasmodia with 10 krad. The specific activities of TTP and dCTP were not reduced in irradiated plasmodia as compared to controls following a 15 min incubation in the presence of (³H)deoxycytidine. The specific activities of the pyrimidine nucleoside triphosphates were reduced in the irradiated plasmodia, however, when either (³H)thymidine or (³H)deoxyuridine was used as the labeled precursor. Since irradiation appeared to reduce DNA synthesis (as indicated by (³H)deoxycytidine incorporation) at doses lower than those necessary to produce pool expansion, it is possible that the accumulation of deoxynucleoside triphosphates results from the inhibition of DNA synthesis. The results also suggest that the conversion of deoxycytidine to deoxynucleotides is increased following the radiation. A similar effect has been observed in CHO cells by Walters *et al.* (*Radiat. Res.* 60, 173, 1974). [Supported by USERDA contract E(11-1)2486.]

Radiat. Res. 67, 531 (1976)

1st International Congress on Cell Biology
Boston, Massachusetts, September 5-10, 1976

553. ORGANISATION OF GENES FOR RIBOSOMAL RNA IN PHYSARUM POLYCEPHALUM Harald V.Molgaard, Harry R. Matthews and E. Morton Bradbury. Physics Department, Portsmouth Polytechnic, King Henry 1 Street, Portsmouth PO1 2DZ, U.K.

Physarum polycephalum nucleolar satellite DNA (rDNA) has been analysed by restriction enzyme digests and hybridisation to ribosomal RNA (rRNA). The rDNA is isolated as molecules of molecular weight 39 Mdaltons, which may represent their size in the nucleolus. The rDNA was digested with the restriction enzymes Eco R1 and Hind III. Each enzyme gave three fragments that were separated and characterised for molecular weight and relative molarity by gel electrophoresis and analytical ultracentrifugation. Data from double digests and partial digests fixed the positions of the restriction enzyme sites on the rDNA. The fragments produced by the restriction enzymes were hybridised to the separated 26S rRNA and 19S rRNA from P. polycephalum. The restriction enzyme sites are arranged symmetrically, implying each molecule is a palindrome. The Eco R1 restriction sites are 3.82 Mdal and 5.61 Mdal from each end and the Hind III restriction sites are 5.74 Mdal and 9.08 Mdal from each end. The gene for 26S rRNA includes both Eco R1 sites and the first Hind III site. The gene for 19S rRNA includes the second Hind III site so the genes are also arranged palidromically. There is a large central region, 21 Mdaltons, of DNA not complementary to 26S or 19S rRNA as well as smaller "spacer" regions at each end. Supported by Science Research Council and NATO.

J. Cell Biol. 70, 185a (1976)

926. DNA REPLICATION IN PHYSARUM POLYCEPHALUM: ANALYSIS OF PRODUCTS MADE IN PRESENCE OF CYCLOHEXIMIDE Steinar Funderud and Finn Haugli, Institute of Medical Biology, University of Tromsø, Tromsø, Norway.

DNA replication in plasmodia of Physarum polycephalum is naturally synchronous and tightly coupled to mitosis. In wild type strains cycloheximide inhibits protein synthesis and causes a decrease in incorporation of ^{3}HTdR and ^{3}H-AdR into DNA. A ribosomal, cycloheximide-resistant strain show no inhibition of protein-synthesis and no decrease in total DNA synthesis with cycloheximide.

Product analysis of DNA replication intermediates on denaturing sucrose gradients show that primary, secondary and tertiary stages in the replication of DNA is depressed in wildtype in presence of cycloheximide, while unaffected in the cycloheximide resistant strain. The possibility that this inhibition of all stages of DNA synthesis is caused by inhibition of a stoichometric factor (histones, unwinding protein or similar) rather than a specific replication factor (initiation protein, polymerase etc.) is under investigation.

J. Cell Biol. 70, 309a (1976)

981. THE STRUCTURE OF rDNA CHROMATIN FROM PHYSARUM POLYCEPHALUM Robert M. Granger. Department of Biology, Yale University, New Haven, Ct. 06520 U.S.A.

By purifying the chromatin containing a single gene, one can study the specific proteins and their associations with a defined DNA sequence that are difficult to analyze in total nuclear chromatin. Nucleolar preparations from the plasmodial stage of the slime mold, Physarum polycephalum, are highly enriched in the chromatin containing the genes for ribosomal RNA (rDNA chromatin), providing such a model system. The rDNA of Physarum is localized in extrachromosomal, linear molecules 19 microns in length (described in detail by V. Vogt and R. Braun, personal communication). DNA isolated from nucleolar preparations and sized by electron microscopy is largely in intact rDNA units (75% of the DNA by weight). More than 90% of the DNA in such nucleolar preparations can be shown to be rDNA molecules by the characteristic buoyant density of Physarum rDNA in cesium chloride equilibrium gradients. Nucleolar preparations routinely yield 50-100 micrograms of rDNA, permitting preparative studies of rDNA chromatin proteins. The subunit structure characteristic of total chromatin from a variety of eukaryotes is also found in Physarum rDNA chromatin. Digestion of nucleoli with the enzyme micrococcal nuclease yields DNA fragments which are multiples of a discrete size, indicative of chromatin subunit structure. (Supported by N.I.H. Grant GM 12427-12 to J.G.Gall. R.M.G. is a Fellow of The Jane Coffin Childs Memorial Fund for Medical Research)

J. Cell Biol. 70, 327a (1976)

1084. AN ULTRASTRUCTURAL AND RADIOAUTOGRAPHIC STUDY OF THE EVOLUTION OF THE NUCLEUS DURING THE CELL CYCLE OF PHYSARUM POLYCEPHALUM André Lord, Louis Nicole and Jean G. Lafontaine. Department of Biology, Laval University, Quebec, Canada G1K 7P4.

Advantage has been taken of the natural synchrony which exists in macroplasmodia of Physarum polycephalum to undertake a detailed study of the organization of the chromosomes and the nucleolus during the cell cycle as well as of certain of their biosynthetic activities. High resolution radioautography reveals that DNA synthesis is initiated a few minutes only after the anaphase nucleus has given rise to two daughter nuclei. The chromatin is then aggregated into a continuous mass within which numerous bodies of persisting nucleolar material are observed. As the nucleus increases in size and takes on more regular contours, thymidine incorporation is seen to take place within the more transparent portions of the nuclear cavity. By the G_2 period, only the nucleolus incorporates this precursor. At that stage, the nucleolus consists of well developed granular zones and of numerous fibrillar ones which appear in the form of short, coarse threads or of ring-like structures. These nucleolar regions undoubtedly each contain a linear or circular DNA molecule of the type recently isolated by biochemical techniques. At late prophase these fibrillar zones disperse to give rise to numerous remnant bodies which accompany the anaphase chromosomes to the pole of the nucleus. The fact that these persisting bodies remain labeled with thymidine indicates that they contain DNA. The onset of nucleolar formation at telophase results from aggregation of these bodies. Labeling with uridine and lysine indicates, however, that both RNA and proteins are soon added to the growing nucleolus. These observations strongly suggest that the nucleolus, in this organism, consist of distinct units each containing DNA, RNA and proteins which persist during the division stages and contribute partly to formation of the new nucleolus.

J. Cell Biol. 70, 362a (1976)

1186. PHASE SPECIFIC DNA BINDING PROTEINS DURING THE MITOTIC CYCLE IN PHYSARUM John J. Wille, Jr. Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803

The role of DNA-binding proteins in the regulation of mitotic timing and control of temporal order of replication was investigated in mitotically synchronous plasmodia of the Myxomycete, Physarum polycephalum. Protein extracts were prepared from well-timed plasmodia at most possible phases of the mitotic cycle and fractionated by DNA-cellulose chromatography on double-stranded Physarum DNA. Binding proteins were further characterized by acrylimide gel electrophoresis. Proteins extracted from plasmodial extracts with 0.5 M NaCl were retained on DNA-cellulose at low ionic strength, and eluted at 200 mM NaCl. During the S period, a series of phase specific DNA-binding proteins appear in a fixed temporal order, those appearing in the early S showed preferrential binding to early S replicating DNA, those appearing later in S showed preferrential binding to late S replicating DNA. Hydroxylapatite column fractionation of 10 minutes pulse-labeled DNA, prepared at successively later times in S, exhibited characteristic elution profiles as a function of time in S. Nitrocellulose DNA-protein binding assay indicated that material eluted from the single-strand region contained sites responsible for phase-specific protein binding. During the G2 period, DNA-binding proteins showed little preferrential binding to replicating DNA. However, the amount of protein DNA binding increased progressively through G2. These results suggest that Physarum's mitotic cycle is separable into two distinct timing processes, one coincident with S, governing the replication order of DNA by sequential appearance of replication control proteins, and a later protein-accumulation phase.

J. Cell Biol. 70, 396a (1976)

ADDITIONS TO THE MAILING LIST

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