AUGUST 28 - SEPTEMBER 2

SECOND INTERNATIONAL MYCOLOGICAL CONGRESS AND 1977 *PHYSARUM* MEETING

This year an informal *Physarum* Conference will be held in conjunction with the Mycological Congress (IMC^2) on the campus of the University of South Florida, Tampa. The general daily format of the congress is as follows: five to seven symposia will be presented each morning and each afternoon (total = 72); poster sessions will run each day (360 contributed papers grouped in 17 categories); there will be three special lectures (late afternoon); and, 38 evening meetings have been organized (mini-symposia, round-tables, paper sessions, etc.).

Of the symposia, many will include presentations dealing primarily with *P. p.*; one of these symposia, organized by Henry Aldrich, will be entirely devoted to the "Biology of *Physarum*". A special Invited Poster Session on Slime Molds will include 26 paper (see list on p. 2). A workshop entitled "Techniques in Physarology" has been organized by Gene Goodman (see p. 2). Friday evening there will be a dinner for those IMC^2 Members interested in slime molds. PNL readers in North America will find a reservation form inserted in this issue.

Registration and Housing forms for the IMC^2 are available from Dr. Melvin S. Fuller, Department of Botany, University of Georgia, Athens, Georgia 30601 (telephone: 404-542-3732). According to the IMC^2 final circular, "Registration also will be possible upon arrival at the Congress, but there can be no guarentee of housing accommodations on the campus for registrations received in the July-August period. . . . A list of motels and campgrounds convenient to the campus may be obtained by writing to Prof. Fuller. Off-campus housing arrangements will not be handled by the Congress organization."

TECHNIQUES IN PHYSAROLOGY

Thursday Evening Workshop - September 1, 1977

2nd International Mycological Congress

H. Turner	Ultrastructural Studies of Cell Wall and Surface Membranes of *Physarum polycephalum*
D.N. Jacobson	Genetic Analysis with Colony Morphology Markers in *Physarum polycephalum*
H. Sauer	At Long Last, Native Chromatin from *Physarum*
A. Hüttermann	Biochemical and Physiological Differences Between Amoebae and Plasmodia
R. Braun	Myosin in the Mitotic Cycle, Labelling of Proteins, and Isolation of Polysomes From Surface Cultures
S. Funderud and F. Haugli	Replicon Topology and Size Maturation of Newly-Replicated DNA in *Physarum polycephalum*
T. Laffler	Temperature Sensitive Plasmodial Mutants

INVITED POSTER SESSION ON SLIME MOLDS, 9 AM-9 PM, Sept. 2

Mitochondrial configurations in Mycetozoa. Dykstra.
Cell wall and surface membranes of Physarum polycephalum. Turner/Hogan.
Mitosis in haploid plasmodia of Colonia. Steffens/Wille.
Plasma membrane of Dictyostelium during mating. Erdos/Aldrich.
Nuclear pore frequency in Physarum. Aldrich/Pendland.
Development and cytology of two acrasids. Spiegel/Olive.
Development in Planoprotostelium. Spiegel/Olive.
Ca and Si in peridia. Schoknecht/Keller.
Spore ornamentation in Myxomycetes. Kalyanasundaram.
SEM in Myxomycete systematics. Rammeloo.
SEM on Trichiaceae. Frederick/Roth.
Aggregating Polysphondylium violaceum: Role of esterases. Clark/McCoy.
Prostelid with compound nucleolus. Blanton/Olive/Stoianovitch.
Effect of light on migration in Physarum. Bialczyk/Rakoczy.
Phleomycin resistance in Physarum. Evans/Biehler/Evans.
Isolation of the plasma membrane of D. iridis. Yemma/Selanik.
Proteases of Physarum polycephalum. Nuske/Huttermann.
Conformational change in surface membrane of plasmodia. Kobatake.
The spherule wall of Physarum polycephalum. Zaar/Beyer/Kleinig.
Griseofulvin on mitotic timing. Hebert/Wille/Steffens.
Developmentally regulated mating type recognition. Shipley/Ross.
Cycloheximide not specific inhibitor of protein synthesis. Wendelberger-Schieweg/Huttermann/Haugli.
Methyl mercury DNA damage repair system. Nagainis/Cummins.
Plasmodial incompatibility in Didymium iridis. Clark.
Inducer of plasmodium formation in Physarum. Youngman/Smith/Hosler/Holt.
Apogamic development in Echinostelium. Therrien/Haskins.

The Uptake and Metabolism of Uridine by the Slime Mould *Physarum polycephalum*

Barbara BIRCH and Geoffrey TURNOCK

Department of Biochemistry, University of Leicester

Eur. J. Biochem. 69, 257–263 (1976)

1. Uridine is taken up by microplasmodia of *Physarum polycephalum via* a saturatable transport system with an apparent K_m of 29 μM. An intracellular concentration significantly higher than that in the growth medium is attained, suggesting that the uptake is an active process. Both deoxyribonucleosides and ribonucleosides are competitive inhibitors of the uptake of uridine.

2. In contrast, the rate of entry of uridine into surface plasmodia is a linear function of the concentration of the nucleoside in the growth medium, and the uptake is not inhibited by other nucleosides.

3. As well as serving as a source of pyrimidine nucleotides for the synthesis of nucleic acids, uridine is also catabolised by *P. polycephalum*. Uracil accumulates in the growth medium and there is also significant conversion of C-2 of the pyrimidine ring to CO_2. The proportion of uridine subject to catabolism in surface plasmodia is less than that observed for microplasmodia.

Vol. 74, 621 , 1977 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

IDENTIFICATION OF N^G, N^G-DIMETHYLARGININE IN A NUCLEAR PROTEIN FROM THE LOWER EUKARYOTE PHYSARUM POLYCEPHALUM HOMOLOGOUS TO THE MAJOR PROTEINS OF MAMMALIAN 40S RIBONUCLEOPROTEIN PARTICLES

Mark E. Christensen, Ann L. Beyer, Barbara Walker, and Wallace M. LeStourgeon
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Received November 22,1976

SUMMARY: A nuclear protein apparently homologous to the two major proteins of 40S heterogeneous nuclear ribonucleoprotein particles from mammalian cells has been isolated from the lower eukaryote Physarum polycephalum, purified, and found to contain a substantial amount of the unusual amino acid N^G, N^G-dimethylarginine. The apparent homology is based on similar molecular weights, basic isoelectric points and amino acid compositions including the dimethylarginine and a high content of glycine. The implications of the presence of this protein in Physarum polycephalum and the possible significance of the N^G, N^G-dimethylarginine are discussed.

Vol. 74, 941, 1977 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

DNA REPLICATION IN PHYSARUM POLYCEPHALUM: CHARACTERIZATION OF REPLICATION PRODUCTS MADE IN ISOLATED NUCLEI

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9000 Tromsø, Norway

Received December 6,1976

SUMMARY

Nuclei isolated from synchronous S-phase plasmodia of the myxomycete Physarum polycephalum were competent in production of low molecular weight DNA replication intermediates. Furthermore, these nuclei showed some competence in joining these fragments into DNA of intermediate molecular weight. The DNA molecules made in vitro could be correlated with products made in vivo.

DNA replication in Physarum polycephalum: characterization of DNA replication products made in vivo in the presence of cycloheximide in strains sensitive and resistant to cycloheximide

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Volume 4 Number 2 February 1977 Nucleic Acids Research

ABSTRACT

Synchronous plasmodia of cycloheximide-sensitive and cycloheximide-resistant strains of Physarum polycephalum were labelled with ^{3}[H]-deoxyadenosine in pulse and pulse-chase experiments in presence and absence of cycloheximide. The replication products were studied with alkaline sucrose gradient sedimentation analysis. We show that the action of cycloheximide on DNA replication in Physarum is mediated through the ribosome, since the ribosomally located resistance also makes the plasmodial DNA replication refractile to the action of cycloheximide. Cycloheximide caused inhibition of three stages in DNA replication in the wild type: first, the formation of primary replication units ("Okazaki" size fragments), secondly, the ligation of primary units into secondary ("Replicon" size) units and thirdly, the ligation of secondary units into mature DNA.

The Organisation of Genes for Transfer RNA and Ribosomal RNA in Amoebae and Plasmodia of *Physarum polycephalum*

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Eur. J. Biochem. 76, 165–174 (1977)

1. Using hybridisation techniques nuclei from both amoebae and plasmodia of *Physarum polycephalum* were found to contain 275 genes each coding for 5.8-S, 19-S and 26-S rRNA, 685 genes for 5-S rRNA and 1050 genes for tRNA.

2. Hybridisation of these RNA species to both amoebal and plasmodial DNA fractionated on CsCl gradients reveal that the 5.8-S, 19-S and 26-S rRNA genes are located at a satellite position ($\varrho = 1.714$ g/cm^3) with respect to the main band of DNA, whereas 4-S and 5-S RNA genes are located exclusively in the main band of DNA ($\varrho = 1.702$ g/cm^3).

3. This result was confirmed by demonstrating that only the 5.8-S, 19-S, and 26-S rRNA species hybridise to purified plasmodial ribosomal DNA.

4. The 19-S and 26-S rRNA genes of amoebae are located on extrachromosomal DNA molecules of a discrete size ($M_r = 38 \times 10^6$) with identical properties to plasmodial ribosomal DNA.

Cell-Cycle Dependence of Two Nuclear Histone Kinase Enzyme Activities

D. Graham HARDIE, Harry R. MATTHEWS, and E. Morton BRADBURY
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Eur. J. Biochem. 66, 37–42 (1976)

Growth-associated histone kinases have been extracted from *Physarum polycephalum* nuclei and resolved into two components by ion-exchange chromatography. The two component activities have different substrate specificities and different times of appearance in the cell cycle. It is proposed that the enzyme(s) phosphorylate H1 histone *in vivo* in G2 phase, possibly sequentially in time at different sites in the H1 amino acid sequence.

Characterization of Foldback Sequences in *Physarum polycephalum* Nuclear DNA Using the Electron Microscope

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Eur. J. Biochem. 74, 275–283 (1977)

An examination of the foldback fraction of nuclear DNA from *Physarum polycephalum* has been carried out using the electron microscope. Results show that the inverted repeat sequences responsible for the formation of foldback DNA range from 150–3000 bases in length, with a number-average size of 340 bases. About one-half of the inverted sequences form looped structures with loop sizes averaging 1200 bases in length. The distance between adjacent foldback sequences is estimated to be in the range 100–1500 bases.

Differentiation of *Physarum flavicomum*: Metabolic Patterns and the Role of Amino Acids in the Control of Encystment

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EXPERIMENTAL MYCOLOGY 1, 41–51 (1977)

HENNEY, H. R., JR., AND CHU, P., 1977. Differentiation of *Physarum flavicomum*: Metabolic patterns and the role of amino acids in the control of encystment. *Experimental Mycology* 1, 41–51. Haploid myxamoebae-swarm cells of *Physarum flavicomum*, grown in semidefined medium, differentiated in a nonnutrient salts solution to produce dormant microcysts. Over 90% of the original cell population converted to microcysts after 72 h of incubation. The intracellular content of protein, neutral hexose and RNA decreased significantly during encystment, but the DNA content was relatively stable. A change in metabolic patterns occurred during differentiation since encysting cells, but not growing cells, actively catabolized protein amino acids. Whether encystment progressed or not was determined by the extracellular availability of certain amino acids. The condition of amino acid imbalance and not glucose availability initiated the differentiation of the vegetative cells to microcysts. Only the branched chain aliphatic amino acids, leucine, isoleucine, and valine (in that order), were significant determinants of the encystment process. The compounds which inhibited encystment also inhibited intracellular proteolysis. Radioactive amino acids were actively taken up from the salts solution by encysting cells and incorporated into protein. Proteins formed in the early stages of encystment were degraded to a certain extent as encystment proceeded. However, [^{14}C]leucine-labeled proteins produced by encysting cells were degraded and catabolized less than those labeled with [^{14}C]-isoleucine and [^{14}C]valine.

INDEX DESCRIPTORS: *Physarum flavicomum*; myxamoebae-swarm cells; encystment; microcysts; differentiation; nutritional control; myxomycete.

Chemical Analyses of Cell Walls from Microcysts and Microsclerotia of *Physarum flavicomum*; Comparison to Slime Coat from Microplasmodia

HENRY R. HENNEY, JR. AND PHILIP CHU

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

EXPERIMENTAL MYCOLOGY 1, 83–91 (1977)

HENNEY, H. R., JR., AND CHU, P., 1977. Chemical analyses of cell walls from microcysts and microsclerotia of *Physarum flavicomum*; comparison to slime coat from microplasmodia. *Experimental Mycology* 1, 83–91. Haploid microcysts and diploid microsclerotia of *Physarum flavicomum* Berk. are dormant phases of the life cycle surrounded by rigid cell walls. Microplasmodia are the actively growing diploid phase of the life cycle. Microplasmodia lack a cell wall but are covered by a constantly replenished slime coat which is secreted and accumulates in liquid growth medium. Chemical analyses of purified walls from microcysts and microsclerotia revealed the presence of polysaccharide, lipid, and protein as major components. The polysaccharide of microcyst walls was composed of galactosamine with smaller amounts of glucose, galactose, and ribose. The polysaccharide of microsclerotial walls was primarily composed of galactosamine, with small amounts of glucose and galactose. The slime coat of microplasmodia consisted of a galactose-containing polysaccharide and protein. The protein components of microcyst and microsclerotial walls and the slime coat protein had strikingly similar amino acid compositions. Lysine was present in the greatest quantity, with large amounts of aspartic acid, alanine, glutamic acid, and glycine; the sulfur amino acids occurred in the smallest proportion.

INDEX DESCRIPTORS: *Physarum flavicomum*; myxomycete; thallophytes; microcysts; microsclerotia; cell walls; slime coat; microplasmodia; chemical identity.

Vol. 74, 466, 1977 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

TRANSCRIPTION OF RIBOSOMAL RNA IN THE LIFE CYCLE OF PHYSARUM MAY BE REGULATED BY A SPECIFIC NUCLEOLAR INITIATION INHIBITOR

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Received November 11, 1976

SUMMARY
Physarum nucleoli contain an inhibitor of in vitro transcription with homologous RNA polymerase A. A strict negative correlation has been established of RNA polymerase A activity and amount of inhibitor during differentiation of Physarum. Location and concentration of the inhibitor as well as selective, yet reversible, binding to and inactivation of RNA polymerase A and in vitro reactivation of enzyme A preparation obtained during differentiation - but not during growth - suggest that the inhibitor might act in vivo to restrict rRNA transcription.

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

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ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 176, 214–217 (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*.

8

Transformation of Cytoplasmic Actin

Cell Tiss. Res. 173, 495–528 (1976)

Importance for the Organization of the Contractile Gel Reticulum and the Contraction — Relaxation Cycle of Cytoplasmic Actomyosin

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Summary. (1) Within the low viscous flowing endoplasm of *Physarum polycephalum* a considerable amount of actin is in the non-filamentous state. This can be demonstrated by applying poly-L-lysin to surface spreads of native protoplasm. (2) It has been shown that in protoplasmic drops the endoplasm -ectoplasm transformation is accompanied by an actin polymerization from the non-filamentous state to F-actin. (3) The actual state of the labile G-F-actin equilibrium determines the varying consistency (viscosity) of the cytoplasm. (4) Increasing viscosity can be interpreted as being brought about by a) shifting of the G-F-actin equilibrium to the filamentous side, and (b) increased myosin-mediated binding sites between actin filaments. (5) Polymerization and depolymerization processes are involved in the rhythmically occurring contraction-relaxation cycle of cytoplasmic actomyosin in *Physarum*. (6) Cytoplasmic actin and myosin represent the architectural proteins of the contractile gel reticulum in eukaryotic cells. (7) The importance of the regulation of actin polymerization as a basic control mechanism of the eukaryotic cell is discussed.

Key words: Cytoplasmic actomyosin – Actin transformation – Contractile gel reticulum – Cytoplasmic viscosity – Cell motility – Poly-L-lysin – Phalloidin.

Volume 62, number 3 FEBS LETTERS March 1976

SUBUNIT STRUCTURE OF *PHYSARUM POLYCEPHALUM* CHROMATIN

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The subunit structure of chromatin from Physarum polycephalum

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Volume 3 no.12 December 1976 Nucleic Acids Research

ABSTRACT

Nucleosome DNA repeat lengths in *Physarum* chromatin, determined by nuclease digestion experiments, are shorter than those observed in most mammalian chromatin and longer than those reported for chromatin of certain other lower eukaryotes. After digestion with staphylococcal nuclease for short periods of time an average repeat length of 190 base pairs is measured. After more extensive digestion an average repeat length of 172 base pairs is measured. Upon prolonged digestion DNA is degraded to an average monomer subunit length of 160 base pairs, with only a small amount of DNA found in lengths of 130 base pairs or smaller. Mathematical analysis of the data suggests that the *Physarum* nucleosome DNA repeat comprises a protected DNA segment of about 159 base pairs with a nuclease-accessible interconnecting segment which ranges from 13 to 31 base pairs. The spacing data are compatible with measurements from electron micrographs of *Physarum* chromatin.

Glycosidases from the Culture Medium of *Physarum polycephalum*

By D. C. KILPATRICK* and J. L. STIRLING
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Biochem. J. (1977) **161**, 149–150

Eight exo-glycosidase activities were detected in the axenic culture medium of the myxomycete, *Physarum polycephalum*. The secretion of each enzyme examined followed the growth curve and continued during the stationary phase after the cessation of growth. Two or more forms of each enzyme were detected after electrophoretic separation. The β-*N*-acetyl-D-hexosaminidase activity was readily separated into its two electrophoretic forms, X and Y, which were purified 145- and 306-fold respectively. These β-*N*-acetyl-D-hexosaminidases had several similar characteristics. Evidence is presented that the major electrophoretic form of α-D-galactosidase is heterogeneous. The possible functions of extracellular glycosidases in the life-history of the organism are discussed in the light of their occurrence and properties.

STUDIES ON MITOCHONDRIAL STRUCTURE AND FUNCTION IN *PHYSARUM POLYCEPHALUM*

V. Behavior of Mitochondrial Nucleoids throughout Mitochondrial Division Cycle

THE JOURNAL OF CELL BIOLOGY · VOLUME 72, 1977 · pages 687–694

TSUNEYOSHI KUROIWA, SHIGEYUKI KAWANO, and MASAHIRO HIZUME

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ABSTRACT

The fine structure of mitochondria and mitochondrial nucleoids in exponentially growing *Physarum polycephalum* was studied at various periods throughout the mitochondrial division cycle by light and electron microscopy. The mitochondrial nucleoid elongates longitudinally while the mitochondrion increases in size. When the nucleoid reaches a length of approximately 1.5 μm the mitochondrial membrane invaginates at the center of the mitochondrion and separates the mitochondrial contents. However, the nucleoid does not divide even when the mitochondrial sections are connected by a very narrow bridge. Just before division of the mitochondrion, the nucleoid divides by constriction of the limiting membrane of the dividing mitochondrion. After division, one end of the nucleoid appears to be associated with the inner mitochondrial membrane. The nucleoid then again becomes situated in the center of the mitochondrion before repeating these same processes.

ULTRASTRUCTURAL AND RADIOAUTOGRAPHIC INVESTIGATION OF THE NUCLEOLAR CYCLE IN *PHYSARUM POLYCEPHALUM*. CHARACTERIZATION OF DNA-CONTAINING SUBUNITS

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J. Cell Sci. 23, 25–42 (1977)

SUMMARY

The present study has been mainly focused on the nucleolar cycle in the slime mould *Physarum polycephalum*. The ultrastructural characteristics of the interphase nucleolus, in this species, are quite similar to those of nucleoli in other organisms: it is essentially constituted of large particulate zones surrounding denser regions which are predominantly fibrillar in texture. The latter nucleolar zones, following fixation with osmium tetroxide, are characterized by the presence of opaque granules approximately 25 nm in diameter. Contrary to the situation which generally prevails in other eukaryotes, the late prophase nucleolus fragments into numerous globular bodies which are recognizable by the presence of opaque particles. These fibrillo-granular nucleolar fragments persist during mitosis and are observed to become incorporated in the newly formed nucleolus. High-resolution radioautographic observations reveal that these nucleolar remnants contain DNA. The present observations together with recent biochemical data from other authors on the characteristics and mode of duplication of nucleolar DNA in *P. polycephalum* have led us to the hypothesis that the nucleolus, in this organism, contains several distinct globular subunits each containing ribosomal DNA as a key component. The existence of such morphological subunits appears to account for the unusual behaviour of the nucleolus during the cell cycle.

CYTOPLASMIC DNA-BINDING PHOSPHOPROTEINS OF *PHYSARUM POLYCEPHALUM*

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Experimental Cell Research 103 (1976) 219–231

SUMMARY

The cytoplasmic DNA-binding proteins of *Physarum polycephalum* were recovered by chromatography of cytosol extracts on sequential columns of native and denatured calf thymus DNA-cellulose. 5.4% of the total cytosol protein was bound to native DNA-cellulose, while 4.4% was bound to denatured DNA-cellulose. Stepwise salt gradient elution of the columns separated the DNA-binding proteins into 9 fractions which were analysed by acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Several hundred discrete polypeptide bands were identified, with many more high molecular weight polypeptides (greater than 100 000 D) binding to native than to denatured DNA. Continuous in vivo labelling of microplasmodia in $KH_2[^{32}P]O_4$ and [^{3}H]leucine was used to determine which of the DNA-binding proteins were phosphorylated, and to approximate their phosphorus content. About 30–40 phosphoproteins were resolved among the DNA-binding proteins. Most phosphoproteins contained less than 3 phosphates per polypeptide, but a small number of low molecular weight phosphoproteins (less than 50 000 D) contained from 5 to 10 phosphates per polypeptide. The majority of high molecular weight DNA-binding phosphoproteins bound to native DNA and were eluted with 0.25 M NaCl. As a group, the DNA-binding proteins were enriched in protein-bound phosphorus when compared with the cytosol proteins which did not bind to DNA. The phosphorus content of the cytoplasmic DNA-binding proteins was similar to that of the acidic nuclear proteins.

THE JOURNAL OF CELL BIOLOGY · VOLUME 72, 1977 · pages 502-505

Ca^{++} REGULATION IN CAFFEINE-DERIVED MICROPLASMODIA OF *PHYSARUM POLYCEPHALUM*

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ADVANCED INITIATION OF THE FIRST SYNCHRONOUS MITOSIS FOLLOWING COALESCENCE OF STARVED, UV-IRRADIATED MICROPLASMODIA OF *PHYSARUM POLYCEPHALUM*

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Experimental Cell Research 104 (1977) 279–285

SUMMARY

The microplasmodia of the slime mold, *Physarum polycephalum*, coalesce readily upon contact. The nuclei of the resulting macroplasmodia divide in synchrony approx. 6–8 h after coalescence. If prior to coalescence the microplasmodia are maintained on non-nutrient salts solution, followed by continued starvation of the resulting macroplasmodia, the nuclei also will eventually divide, although at a much later time. This mitosis occurs earlier if the starved microplasmodia are irradiated with UV light prior to coalescence. The most pronounced advancement of mitosis was found in plasmodia which were obtained by coalescence of irradiated, starved microplasmodia with non-irradiated ones.

Elemental Sulfur: Accumulation in Different Species of Fungi

Abstract. *Sulfur, in elemental form, is present in several fungi especially in self-inhibited and resting structures such as dormant spores and sclerotia. The possible importance of sulfur in fungal spore dormancy is discussed.*

SCIENCE, VOL. 196 428 22 APRIL 1977

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NUCLEOPROTEIN CHROMATIN SUBUNIT FROM *PHYSARUM POLYCEPHALUM*

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Biochimica et Biophysica Acta, 475 (1977) 131—138

Summary

The nucleoproteins resulting from digestion of the nuclei of the true slime mold *Physarum polycephalum* with micrococcal nuclease have been resolved according to the size classes in linear sucrose gradients containing 0.5 M NaCl, and analysed for DNA, RNA and protein content.

The basic nucleoprotein subunit has been found to contain a DNA fragment of about 150—170 base pairs complexed with an approximately equal amount, on a weight basis, of basic proteins and a relatively small amount of non-histone proteins (about 35% of the amount of DNA).

Higher nucleoprotein oligomers were shown to contain spacer DNA fragments between adjacent subunits and a considerably higher ratio of non-histone proteins to DNA than the basic subunit.

Both the basic subunit and higher nucleoprotein oligomers of *Physarum* chromatin contain some amount of tightly bound RNA. However, in contrast to the distribution of the non-histone proteins, the ratio of RNA to DNA is similar in both fractions.

Growth of *Physarum gyrosum* on Agar Plates and in Liquid Culture[1]

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ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1976, p. 613-617 Vol. 10, No. 4

The physical and nutritional requirements of the antibiotic-producing slime mold *Physarum gyrosum* were examined to develop a liquid medium for this myxomycete. Liquid culture is desired to expedite a useful scale of production of antibiotic materials for ease of isolation and structure study. Culture conditions were selected to favor antibiotic production rather than maximum growth. The medium devised consisted of 0.010 M potassium phosphate buffer (pH 6.0), 2% bakers' yeast, and 0.2% glucose and was supplemented with either 10^{-7} M hemoglobin (preferred) or 2.0 ml of live *Escherichia coli* per 100 ml of culture medium grown to a steady-state population in nutrient broth. The slime mold, which contained some *E. coli* carried along with the inoculum, was allowed to grow as a surface plasmodium at 20°C in the dark with weekly subculturing for stocks or for 10 days for antibiotic production. *P. gyrosum* produced the same antibiotic materials when grown in liquid medium as it did when grown on agar plates. A seeded plate disk assay against *Bacillus cereus* was employed to follow antibiotic activity.

Physarum flavicomum Malate Dehydrogenase Isozymes

The Physical, Chemical, and Kinetic Analyses of Mitochondrial and Supernatant Forms

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

EXPERIMENTAL MYCOLOGY 1, 30–40 (1977)

TEAGUE, W. M., AND HENNEY, H. R., JR., 1977. *Physarum flavicomum* malate dehydrogenase isozymes: The physical, chemical, and kinetic analyses of mitochondrial and supernatant forms. *Experimental Mycology* 1, 30–40. The structure and function of malate dehydrogenase isozymes isolated from *Physarum flavicomum* have been investigated. Two proteins, one localized in the cytosol and one in the mitochondria, demonstrated malate dehydrogenase activity. These proteins were purified by a combination of acetone fractionation, chromatography, and isoelectric focusing. Total purity was defined by electrophoretic, hydrodynamic, and conformational properties. Biochemical and biophysical characteristics established for both isozymes include sedimentation coefficients, molecular weight, subunit molecular weight, isoelectric point, total amino acid contents, and thermal stability. Both isozymes exhibited similar functional properties in regard to optimum pH, optimum substrate concentration, Michaelis constants, and response to certain substrate analogs. The effects of nucleoside phosphates were tested, also revealing a sensitivity of the mitochondrial form to adenosine phosphates, while the supernatant form was relatively unaffected. The extensive analyses offered here are compared with data on malate dehydrogenase isozymes from vertebral sources. The data suggest a strong conservation of the functional properties for these isozymes.

Physarum polycephalum malate dehydrogenase: inhibitor analyses of the mitochondrial and supernatant isozymes

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Accepted February 3, 1977

TEAGUE, W. M., and H. R. HENNEY, JR. 1977. *Physarum polycephalum* malate dehydrogenase: inhibitor analyses of the mitochondrial and supernatant isozymes. Can. J. Microbiol. 23: 589–595.

The effects of naturally occurring metabolites were tested on the malate dehydrogenase (L-malate: NAD^+ oxidoreductase, EC 1.1.1.37) isozymes from the eucaryotic protist *Physarum polycephalum*. Several of the Krebs cycle intermediates were inhibitors for each isozyme indicating that a similar catalytic process was involved for both forms. The metabolites ATP, ADP, and AMP were inhibitors competitive with NAD for the mitochondrial isozyme but not the supernatant form. Several other nucleoside phosphates had no effects. Tests of protein sulfhydryl, arginine- and tyrosine-modifying reagents revealed a similar functional sensitivity by both isozymes to these reagents. Those results are compared with data on isozymes from more complex tissue with comments on the physiological significance of those combined data.

Apogamic Induction of Haploid Plasmodia in a Myxomycete, *Didymium iridis*

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DEVELOPMENTAL BIOLOGY 49, 283–287 (1976)

Interisolate crosses between haploid (mean DNA = 0.32) CR 5-5 (A^2) myxamoebae and polyploid (mean DNA = 1.80) CR 2-25 (A^4) myxamoebae of the myxomycete *Didymium iridis* result in plasmodia that have the haploid (mean DNA = 0.32) DNA content rather than the predicted polyploid value. F_1 clones possess the mating type allele of the CR 5-5 clone only, and they also have the same mean DNA content as CR 5-5 myxamoebae. Crosses between these F_1 clones and CR 2-25 myxamoebae again resulted in the production of haploid plasmodia. Hence, the polyploid CR 2-25 clone appears to induce the CR 5-5 clone to produce plasmodia without involving itself in nuclear fusion.

ISOZYME BULLETIN 10:32 (1977)

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MALATE DEHYDROGENASE ISOZYMES IN PHYSARUM

Two isozymes of malate dehydrogenase have been demonstrated for Physarum polycephalum and P. flavicomum vegetative cells as well as P. flavicomum haploid cells by the techniques of polyacrylamide-gel disc electrophoresis and isoelectric focusing. Analyses by polyacrylamide-gel electrophoresis of mitochondria from these sources revealed only the more cathodal protein associated with these organelles (M-MDH). The other, more anodal form was found in the soluble cytoplasm (S-MDH). Crude extracts of organisms grown on carbon sources other than glucose have isoelectric patterns of MDH isozymes equivalent to glucose grown cells. The isozymes from the plasmodial stage of both species have been purified to homogeneity as confirmed by gel filtration chromatography, polyacrylamide-gel disc electrophoresis and analytical ultracentrifugation. Final specific activities as measured by oxaloacetate reduction for P. polycephalum were 1,020 for M-MDH and 810 for S-MDH. The specific activities for the P. flavicomum isozymes were 1,080 for m-MDH and 839 for S-MDH.

The isozymes from both species were composed of dimeric, size equivalent subunits as demonstrated by sodium dodecyl sulfate gel electrophoresis. The molecular weights as established by sedimentation equilibrium were 69,000 for both P. polycephalum isozymes, 70,000 for P. flavicomum M-MDH and 65,000 for P. flavicomum S-MDH. Total amino acid analyses revealed the isozymes from each species differed in the content of 10 amino acids. Moreover, the identities of the 10 amino acids were not the same for each species.

The determination of optimum pH and substrate concentrations as well as Michaelis constants revealed the Physarum isozymes to be similar to each other as well as to a variety of malate dehydrogenases from other sources. Analyses of the functional activity by inhibition with substrate analogues indicated a striking similarity among the isozymes. Inhibition was restricted to the carboxylic acids such as citrate, fumarate, isocitrate, α-keto-glutarate and others of a similar structure. No inhibition could be demonstrated using monocarboxylic acids or saturated dicarboxylic acids. The M-MDH isozymes from Physarum were selectively inhibited by the adenosine phosphates; ATP gave the highest level of inhibition. The S-MDH isozymes were not inhibited. Other nucleoside phosphates had no effect on either isozyme.

The results suggest the conservation of the malate dehydrogenase characteristics from the simple eucaryotic myxomycetes to the more complex mammalian sources. The indications are that the isozymes are intimately involved in energy equilibrium. The differential effects of the adenosine nucleotides on the isozymes was the first indication of a possible selective control on their activity.

Timing of mitosis in Physarum polycephalum: Effects of agents affecting cyclic AMP Concentrations

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EXPERIENTIA 33, 28 (1977)

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Summary. Cultures of *Physarum polycephalum* incubated with caffeine or theophylline for over 100 min prior to mitosis exhibited mitotic delay proportional to the time of treatment before 100 min. Starved cultures exhibited mitotic delay at times of starvation longer than 180 min and slight stimulation from 100–180 min. Dibutyryl cAMP appeared to accelerate reconstruction of the nucleus following mitosis.

CHEMOTAXIS IN *PHYSARUM POLYCEPHALUM*

Effects of Chemicals on Isometric Tension of the Plasmodial Strand in Relation to Chemotactic Movement

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Experimental Cell Research 100 (1976) 337–344

SUMMARY

The effects of chemicals were examined on the isometric tension of the plasmodial strand of the true slime mold *Physarum polycephalum*, and chemotactic motive forces were compared with the contractile properties of the strand. The results were:

1. Isometric tension changed rhythmically within a period of 3–4 min and a few mg in amplitude. Application of attractants (glucose, galactose, maltose, $Ca(H_2PO_4)_2$, and $K(H_2PO_4)$ led to a decrease in the amplitude of the tension. Contrary to this, repellents (sucrose, inorganic salts) increased the amplitude of the tension. The base line of the tension did not change appreciably unless the concentration of chemicals applied was not too high as compared with respective thresholds.

2. Changes in the isometric tension, F, induced by application of chemicals were analysed quantitatively in terms of integral of isometric tension with respect to time during a period as defined by $S = \int F\,dt$. The values of S changed gradually with increase of concentration of chemicals above their respective thresholds.

3. The threshold concentrations of chemicals determined by measurements of the isometric tension agreed with those obtained from chemotactic motive force and from membrane potential changes.

4. The plasmodium of *Physarum polycephalum* moved away vigorously from high osmolarity by producing a large transient increase of motive force of the protoplasmic movement. Similarly, the isometric tension increased transiently with a high peak when the concentration of sugars and glycerol exceeded 0.2 M. The maximum tension was linearly proportional to the diameter of the strand.

These results indicate that contraction or relaxation of the plasma gel is the primary cause of the negative and positive chemotaxis in the slime molds.

Photoreceptor pigment that induces differentiation in the slime mold *Physarum polycephalum*

(sporulation/absorption spectra/microinjection)

Proc. Natl. Acad. Sci. USA
Vol. 73, No. 11, pp. 3896–3899, November 1976
Biochemistry

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Communicated by Charles D. Mitchener, August 13, 1976

ABSTRACT **An extract of small molecules (molecular weight <500) of the slime mold *Physarum polycephalum* undergoes a shift in ultraviolet-visible absorption spectrum upon illumination. This illumination also confers on the extract the ability to induce sporulation when injected into a starved, unilluminated slime mold. The spectral shift and the appearance of the sporulation-inducing activity both occur regardless of whether the illumination is carried out on an intact slime mold or on the plasmodium-free extract itself. Thin-layer chromatography resolves the slime mold extract into four major visible fractions. One of these has high sporulation-inducing activity after illumination *in vitro*.**

TITLES AND SUMMARIES IN PRESS

Melanin Synthesis During Morphogenesis of the Slime Mold *Physarum polycephalum*

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Melanin synthesis is taking place during sporulation but not during spherule formation in the myxomycete *Physarum polycephalum*. Melanin-like pigment was extracted from spores. An almost identical substance of polyphenols was extracted from spherules and was characterized by its U.V. and I.R. absorbance spectra. Polyphenol oxidase activity was very low in spherules and showed only one weak band of isoenzyme in isoelectric focusing polyacrylamide gels. In sporulating cultures a much higher activity, and increasing numbers of isoenzymes, were detected after illumination during the differentiation process. The addition of melanin precursors resulted in synthesis of brownish yellow spherules, probably containing dopachrome whereas addition of polyphenol oxidase inhibitors resulted in yellow sporangia. The results indicate that melanogenesis is the final stage in maturation rather than an essential part of the morphogenetic process itself.

Biochim. Biophys. Acta, in press

Germination-Inhibitor in Slime Mould *Physarum polycephalum*

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Spherulation, one type of differentiation in the slime mould Physarum polycephalum, is characterized by the cleavage of the plasmodium to numerous resistant structures called spherules (1). Spherulation was induced by subjecting the myxomycete to adverse conditions such as starvation (2), or, without involving starvation, by 0.5M mannitol (3). Spherules induced via both ways can germinate not only in any growth promoting medium but even in distilled water (3). In a further study, Chet and Rusch (4) suggested that stable RNA messengers code for different proteins during germination.

Very little attention has been diverted so far to the germination of sperules (5,6). In this study a method for the precise determination of the time of spherule germination was developed and evidence is presented for the existence of a germination inhibiting factor which is produced by sporulating cultures.

F.E.M.S. Microbiol. Letters, in press

Chemotaxis and Movement of *Physarum polycephalum* and Its Responses to Some Neurotransmitters and Psychomimetic Compounds

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A method was developed for studying the effect of some neurotransmitters and drugs on the rate of movement and the chemotactic value of the plasmodium of *Physarum polycephalum*. Epinephrine (adrenaline) at a concentration of 1 mg/ml, reduced the rate of movement and shortened the length of the cycles of shuttle streaming, but did not affect the chemotactic response. The drugs DL-amphetamine, cannabinol and heroin diminished the rate of movement, whereas Na-barbitol misled the chemotactic response.

J. Mechanochem. Cell Motil., in press

MUTANTS WITH DECREASED DIFFERENTIATION TO PLASMODIA IN PHYSARUM POLYCEPHALUM

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Mutant ("APT") amoebae that display reduced ability to form plasmodia asexually were isolated by the use of an enrichment procedure. The results of reconstruction experiments show that the procedure enriches only for mutants blocked early in the pathway from amoeba to plasmodium. Mutants were isolated from four parents, two of which produce plasmodia asexually because they carry the allele mth of the mating type locus, and two because they carry gad (greater asexual differentiation) mutations. The APT mutants varied widely in the frequency of residual plasmodium formation, which occurred, in some cases, by reversion. The mutants, called apt (amoeba to plasmodium transition), were recessive in diploids and linked to the mating type (mt) locus. Mutants derived from the gad parents, unlike the parents themselves, crossed readily with heterothallic amoebae. Progeny analysis from such crosses indicates that both gad mutations are linked to mt. The mutants derived from one of the mth parents fell into two groups on the basis of their ability to cross with the mutants derived from the mt2 gad-8 parent. The result suggests that the mth-derived mutants represent two or more complementation groups. Mutants derived from the mt2 gad-8 parent crossed with mt2 amoebae and hence display an altered mating specificity.

Accepted for publication, Molec. and Gen. Genetics.

Genetics and Biochemistry of 5-Bromodeoxyuridine Resistance in *Physarum polycephalum*

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5-Bromodeoxyuridine (BrdU) resistant mutants of *Physarum polycephalum* were isolated as colonies of myxamoebae growing on BrdU-substituted bacteria after exposure to long-wave ultraviolet light (UV). Twenty-four such mutants were studied. They all show Mendelian segregation in crosses with wild type. Plasmodia constructed from mutant amoebae were all deficient in deoxythymidine incorporation. Extracts made from selected plasmodia showed that all except one had low thymidine kinase activity.

Genetical and biochemical complementation studies revealed 2 complementation groups: 23 mutants, bur A, had low thymidine kinase while 1 mutant, bur B, had normal thymidine kinase levels.

Genetical Research, in press

A MUTATION (GAD) LINKED TO MT AND AFFECTING ASEXUAL PLASMODIUM FORMATION IN PHYSARUM POLYCEPHALUM

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Amoebae of the acellular slime mold Physarum polycephalum convert to plasmodia both asexually and sexually. Genetic analysis of a mutant that exhibits enhanced asexual plasmodium formation is reported. The mutant carries a single lesion (gad-11) located 12.3 map units from mt, a gene that controls mating specificity in sexual plasmodium formation. The mutation, which was isolated in an mt3 strain, is also expressed in mth and mt4 strains.

In press, J. Bact., July, 1977.

Enrichment and Screening of Heat Sensitive Mutants of *Physarum polycephalum*

Peter Sudbery, Kari Haugli and Finn Haugli

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Myxamoebae of the selfing, apogamic Colonia strain of *Physarum polycephalum* were exposed to nitrosoguanidine in logarithmic growth phase and subjected to conditional suicide at high temperature using 5-bromodeoxyuridine with ultraviolet irradiation and/or netropsin as suicide agents.

Heat sensitive mutants among survivors were screened for in the amoebal and/or plasmodial state and frequencies compared. A preliminary characterization of mutants was attempted.

Genetical Research, in press

Oscillating Contractions in Protoplasmic Strands of *Physarum*: Effects of External Ca^{++}-Depletion and Ca^{++}-Antagonistic Drugs on Intrinsic Contraction Automaticity

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1. The minimal requirement of external Ca-concentration for continuance of contraction activity lies in the range of 10^{-4}M. 2. As in mammalian smooth muscle, the Ca antagonistic drugs verapamil and D 600 ($5 \cdot 10^{-4}$M) suppress the minute-rhythms. The action of the drugs is inhibited by 5 mM Ca, La or Mn. De novo generation of contraction automaticity is not inhibited by external Ca depletion or by Ca antagonists. 3. It is concluded that rhythmical Ca fluxes across the cortical plasmalemma are not a precondition for triggering continuance or de novo generation of contraction automaticity.

Cell Biology Internat. Reports 1, 1977, in press

ADDITIONAL ARTICLES IN PRINT

J. Mohberg
"Nuclear DNA Content and Chromosome Numbers Throughout the Life Cycle of the Colonia Strain of the Myxomycete, *Physarum polycephalum*"
J. Cell Science 24, 95 (1977). (PNL 8, 40, 1976)

W. Stockem and R. Stiemerling
"Cytochemical Studies on Intracellular Digestion in the Acellular Slime Mold *Physarum confertum*"
Protoplasma 89, 117 (1976). (PNL 8, 40, 1976)

W. Stockem and R. Stiemerling
"Intracellular Segregation of Endocytotically Ingested Substances"
Cytobiologie 13, 158 (1976). (PNL 8, 41, 1976)

P.E. Sudbery and W.D. Grant
"The Control of Mitosis in *Physarum polycephalum*: The Effect of Delaying Mitosis and vidence for the Operation of the Control Mechanism in the Absence of Growth"
J. Cell Science 22, 59 (1976). (PNL 8, 41, 1976)

P.J. Youngman, P.N. Adler, T.M. Shinnick and C.E. Holt
"An Extracellular Inducer of Asexual Plasmodium Formation in *Physarum polycephalum*"
Proc. Natl. Acad. Sci. USA 74, 1120 (1977). (PNL 8, 41, 1976)

CHAPTER 1

HISTONES, CHROMATIN STRUCTURE, AND CONTROL OF CELL DIVISION

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Current Topics in Developmental Biology, Volume 9

THE MYXOMYCETES – SOME PROBLEMS AND UNANSWERED QUESTIONS

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Referee: Lindsay S. Olive
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A brief history of the development of myxomycete studies and some personal reminiscences during the past four decades are presented. Special emphasis is placed on the chronology of the developments that led to the relatively easy cultivation of *Physarum polycephalum* in quantity and the subsequent overemphasis that has been placed on this atypical species. A wish is expressed for less work on this species and more work on other species in order that a more balanced picture of the myxomycetes may be presented. This is followed by brief discussions of several basic problems, the solutions of which are necessary for a more complete understanding of the group.

CRC Critical Reviews in Microbiology
Volume 4

THESES

Genetic Investigations of Differentiation in *Physarum polycephalum*

by

Lance Steven Davidow

(Submitted January 10, 1977, to the Department of Biology, Massachusetts Institute of Technology, in partial fulfillment of the requirements for the degree of Doctor of Philosophy)

Abstract. The thesis concerns the asexual conversion of uninucleate amoebae to multinucleate plasmodia in an acellular slime mold, Physarum polycephalum. The major topics are the isolation and analysis of mutants deficient in the amoeba to plasmodium transition (apt mutants), the analysis of diploid amoebae, and the isolation of temperature-sensitive plasmodial mutants.

An enrichment procedure was used in the isolation of apt mutants. Reconstruction experiments show that the procedure gives a 1000-fold enrichment for the mutants, but no enrichment for a mutant defective in a vegetative plasmodial function. Amoebae of the parental strains produce plasmodia at very high frequency. Amoebae of the 109 mutants do so over a continuous wide range of strain-dependent frequencies. Even though the mutants were chosen for their deficient asexual differentiation, the lesions they contain are tightly linked to mt, a locus that controls mating specificity. The apt mutations are recessive, since diploid amoebae of the genotype mth apt/mt3 apt$^+$ display the same, unusually frequent differentiation of mth apt$^+$/mt3 apt$^+$ diploid amoebae. The mutations are divided into two complementation groups, aptE and aptF, on the basis of plasmodium formation by mixtures of apt amoebae. Mutants isolated from a mt2-derived parent can cross with mt2 wild type strains and display, thereby, altered mating specificity. A mutation, called rap1, that increases the rate of asexual differentiation, was characterized and is found to be unlinked to mt.

Diploid amoebae are used in genetic studies of dominance and complementation (see above). Earlier studies showed that a fraction of the amoebal progeny of a sexually formed plasmodia are diploid rather than haploid. The mode of generation and genetic properties of these diploid amoebae were investigated. All sexually formed plasmodia generate diploid progeny, heterozygous for mt at frequencies between 10^{-3} and 10^{-1}. Segregation studies of heterozygotic plasmodia support the hypothesis that amoebal diploids are meiotic products of rare tetraploid plasmodial nuclei. An unusual class of mating type heterozygous amoebae were recovered which appear to be triploid or aneuploid.

Biochemical and morphological differences between plasmodia and amoebae have previously been documented. I looked for genetic evidence that the amoeba to plasmodium transition includes a change in gene expression. Using improved methods, I isolated temperature-sensitive plasmodial mutants. Some mutations expressed in plasmodia are not expressed in amoebae.

An appendix, describing experiments on amoebal nutrition and the plasmodial cell cycle, is included.

ABSTRACT OF B.Sc. PROJECT REPORT

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TEMPERATURE-SENSITIVE MUTANTS OF PHYSARUM POLYCEPHALUM AMOEBAE

S.B. HOUGHTON

Gorman and Dove's (1974) method for the isolation of temperature sensitive mutants of the amoebal stage of Physarum polycephalum using the antibiotic netropsin as the selective agent was used to yield 20 temperature-sensitive mutants out of one hundred and sixty-four survivors. The nitrosoguanidine killing rate was 85%; the netropsin killing rate was 97%. Netropsin selection appears to reduce the proportion of "leaky" mutants that are isolated when compared with the unselective isolation methods of Wheals et al. (1976). It does not appear to enhance the isolation of cell cycle mutants, although one of the mutants isolated may have a lesion affecting cell division. Fifteen of the mutants were studied in microscope slide cultures. The phenotypes observed at the non-permissive temperature suggest, in some cases, the nature of the lesions producing them. The loss of viability when maintained at the non-permissive temperature is strain dependent. The mutants are all specific to the amoebal stage, with possibly one exception, in which temperature sensitivity is not reproducible.

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WHEALS, A.E., GRANT, W.D. & JOCKUSCH, B.M. (1976). MGG 149, 111.

Any correspondence to Dr. A. WHEALS.

ABSTRACTS OF MEETING PRESENTATIONS

21st Annual Meeting of the Biophysical Society
New Orleans, Louisiana, February 15-18, 1977

F-PM-A10 REGULATION OF PHYSARUM ACTIN POLYMERIZATION. M. R. Adelman, Department of Anatomy, Duke University Medical Center, Durham, N.C. 27710

Since actin is readily extracted from Physarum plasmodia and exists in crude extracts (CX) in a monomeric form which will only polymerize under appropriate conditions, plasmodial movements might in part be controlled via regulation of actin assembly. Therefore the requirements for polymerization of pure actin were compared with those for the actin in CX. Our actin purification has been improved by the inclusion in all buffers of 5mM PP_i since PP_i inhibits the proteolytic production of the actin fragment BP (MW ~37,000). BP is involved in the Mg polymer of Hatano, et al since only actin preparations devoid of BP polymerize (in 100mM KCl) to the same extent w/wo 1mM $MgCl_2$. The polymerization in CX of actin (endogenous or exogenous) requires optimal levels of KCl (~100mM), ATP (1-2mM), and $CaCl_2$ ($>10^{-5}$ M). The polymerization rate of purified actin is optimal at 100 mM KCl and falls off sharply at higher or lower [KCl]; however polymerization at 100mM KCl proceeds equally well over broad ranges of ATP (0.1 to 5mM) and calcium (10^{-8} to 10^{-3}M). The requirement for ATP in actin assembly in CX may be explained by the finding that ATP is rapidly degraded. There is an initial, calcium-dependent, conversion of ATP to AMP (presumably by the pyrophosphohydrolase of Kawamura and Nagano) followed by a slower reaction in which AMP disappears and ADP appears. PP_i inhibits both reactions, the second somewhat more strongly, and this may explain its prevention of actin proteolysis. We are now studying the extent to which the adenosine nucleotide metabolizing enzymes and/or associated factors play a role in regulating actin assembly. The calcium effects demonstrated in CX could reflect an indirect regulation of the G⇌F equilibrium; this would lead to control of motility either because actin filaments are required to transmit force or because F-, but not G-, actin activates myosin ATPase. Supported by NIH #s 2-R01-GM-20141 and 5-S04-RR-6148.

Biophys. J. 17, 269a (1977)

61st Annual Meeting
Federation of American Societies for Experimental Biology
Chicago, Illinois, April 1-8, 1977

EFFECTS OF POLYAMINES ON ADENYLATE CYCLASE ACTIVITY AND ACIDIC NUCLEAR PROTEIN PHOSPHORYLATION IN PHYSARUM POLYCEPHALUM. V.J. Atmar* and G.D. Kuehn. New Mexico State University, Las Cruces, NM 88003

A combination of three polyamines (spermine, spermidine, and putrescine) in concentrations of 0.33 mM each, strongly inhibits a soluble adenylate cyclase enzyme isolated from nuclei of Physarum polycephalum but has no effect on a membrane-bound cytoplasmic adenylate cyclase. The addition of the same three polyamines to intact nuclei of P. polycephalum causes a six to ten-fold increase in the phosphorylation of nonhistone acidic nuclear proteins. The nuclear nonhistone protein fraction is separable by SDS polyacrylamide gel electrophoresis into 30-35 proteins. However, the stimulation of phosphorylation in the presence of polyamines is due to increases in the phosphorylation of only four proteins of molecular weights 25,000, 43,500, 76,500, and 94,700. The polyamine-mediated increase in acidic nuclear protein is decreased 80% by the addition of 1 mM cyclic AMP and 70% by the addition of 0.5 mM dibutyryl cyclic AMP. Control experiments in which plasmodia were labelled with [^{3}H]-thymidine and [^{3}H]-uracil demonstrated that no DNA or RNA coextracted with the acidic nuclear protein fraction. These results suggest a mechanism whereby the polyamines might mediate changes in nuclear protein phosphorylation by effecting nuclear cyclic AMP levels. (This research was funded by PHS fellowship grant 5 F32 CA05199 to V.J.A. and research grant GM18538 to G.D.K.)

Fed. Proc. 36, 686 (1977)

TURNOVER OF POLY(A) IN THE CYTOPLASM OF PHYSARUM POLYCEPHALUM. David S. Adams* and William R. Jeffery* (SPON: J. Eichberg). Dept. of Biophys. Sci., Univ. of Houston, Houston, TX. 77004

Polyadenylic acid [poly(A)] turnover and its relationship to translation was studied in Physarum polycephalum by pulse-chase experiments. Poly(A) sequences with average lengths of 18 and 125 nucleotides are found in newly synthesized RNA in the post-mitochondrial fractions of microplasmodia. Poly(A) degradation begins immediately after cytoplasmic entry and shows biphasic kinetics. The initial degradation phase (half-life 5 hrs.) involves the turnover of $poly(A)_{125}$, while the final phase (half-life 29 hrs.) involves that of $oligo(A)_{18}$. Changes in electrophoretic mobility and total quantity of $poly(A)_{125}$ show that degradation occurs by both a shortening process, in which poly(A) is gradually eroded from its 3' terminus, and a destruction process, in which the entire sequence is rapidly degraded. Although both processes begin simultaneously, shortening is terminated prior to destruction since $poly(A)_{65}$ accumulates during the chase and is later destroyed without a further detectable size reduction. Heat shock and puromycin, which inhibit translation, disrupt polysome structure, and release mRNA, do not affect either degradative process. In constrast, cycloheximide and emetine, which inhibit protein synthesis without disrupting polysome structure, do not effect shortening but severely suppress destruction. Thus poly(A) shortening may occur independent of translation, while initial uncoupling of the mRNA from the polysome may be a prerequisite for poly(A) destruction. (Supported by Amer. Cancer Soc. Grant NP-188).

Fed. Proc. 36, 769 (1977)

PURIFICATION OF ANTIBIOTICS FROM PHYSARUM GYROSUM BY HIGH PRESSURE LIQUID CHROMATOGRAPHY. Richard L. Taylor* and M. Frank Mallette, The Pennsylvania State University, University Park, PA 16802.

The myxomycete Physarum gyrosum has been shown to produce antibiotic materials active against gram positive and gram negative bacteria and in addition the yeasts Saccharomyces cerevisiae and Torulopsis sphaerica. 1-Butanol extract of P. gyrosum cultures grown on agar or in liquid culture medium were partially purified by two paper chromatography steps: 1) ethyl acetate:pyridine:water (2:2:1) and 2) methanol: water:ammonium hydroxide (20:40:1). Purification of the active materials was accomplished by reverse phase column chromatography using a Waters Associates high pressure liquid chromatograph with a preparative scale column, 7 mm x 121 mm, packed with Bondapack C_{18}/Porasil B. The active materials were eluted with a methanol:water gradient and were rechromatographed in different methanol:water solvent systems to obtain separate pure components. Purities of these active materials were checked by thin layer chromatography. Antibiotic activity was demonstrated by the inhibition of growth of Bacillus cereus in nutrient broth. (Supported in part by the Pennsylvania Agricultural Experiment Station. Hatch 2161.)

Fed. Proc. 36, 772 (1977)

PARTIAL PURIFICATION AND CHARACTERIZATION OF A PROTEIN LYSINE METHYLASE FROM PLASMODIA OF PHYSARUM POLYCEPHALUM. M. Venkatesan* and I.R. McManus, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Plasmodia of Physarum polycephalum contain an active soluble protein lysine methylase that catalyses the transfer of methyl groups from S-adenosyl-L-methionine to methylate the ε-amino group of lysine residues with formation of ε-N-mono-di- and tri-methyllysine. This enzyme has been partially purified and characterized. It is localized in the 100,000g supernatant fraction from plasmodial homogenates, and is inactive in the absence of exogenous protein acceptors. It has been purified 40 fold by ammonium sulfate fractionation and by chromatography on DEAE-cellulose and Sephadex-G-200. The rate of protein methylation is dependent on time of incubation and enzyme concentration and requires the presence of sulfhydryl reducing agents. It has optimal activity at pH 8 and is inhibited by S-adenosyl-L-homocysteine and EDTA. It loses 80% of its activity when heated for 3 min at 50°C. Proteins able to act as methyl group acceptors include histones H3 and H1 and skeletal muscle myofibrillar proteins. Lysine, polylysine, bovine serum albumin, ribonuclease A, and cytochrome c are not methylated. Plasmodial myosin contains ε-N-mono, di-, and trimethyllysine, as well as N^G,N^G-dimethylarginine in a ratio of 1:1:2:2 and actin has low levels of N-methylated lysines in addition to 3-methylhistidine. The relation of this lysine methylase to methylation of these contractile proteins is being studied. (Supported in part by NIH AM15826 and from the MDAA.)

Fed. Proc. 36, 831 (1977)

9th Annual Meeting
Union of Swiss Societies of Experimental Biology
Zurich, April 1–2, 1977

Ribosomal DNA in spores in Physarum polycephalum

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Using RNA-DNA hybridization techniques spores of the true slime mold Physarum polycephalum were found to contain 320 genes, each coding for 19 S and 26 S rRNA. Hydridization of rRNA to spore DNA fractionated on CsCl density gradients shows that the sequences coding for 19 S and 26 S RNA are located at a satellite position (1.714 g/cm³) of greater density than the main band DNA (1.702 g/cm³). The data demonstrate that in spores ribosomal DNA is not degraded and that no amplification of these genes takes place in hatching amoebae. The DNA content of spores (0.6 pg/spore) and the number of extrachromosomal rRNA genes present suggest that spores are in G2 phase.

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The organization of genes for transfer RNA and ribosomal RNA in amoebae and plasmodia of Physarum polycephalum

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Using RNA-DNA hybridization techniques nuclei from both amoebae and plasmodia of the true slime mould Physarum polycephalum were found to contain 275 genes each coding for 5.8S, 19S and 26S rRNA, 685 genes for 5S rRNA and 1050 genes for tRNA. Hybridization of these RNA species to both amoebal and plasmodial DNA fractionated on isopycnic CsCl gradients reveal that the 5.8S, 19S and 26S rRNA genes are located at a satellite position (ϱ = 1.714 g/cm³) with respect to the main band of DNA, whereas 4S and 5S RNA genes are located excluaively in the main band peak of DNA (ϱ = 1.702 g/cm³). This result was confirmed by demonstrating that only the 5.8S, 19S and 26S rRNA species hybridize to purified plasmodial ribosomal DNA. The 19S and 26S rRNA genes are localized on extrachromosomal DNA molecules of a discrete size (38 million daltons) in amoebae as well as in plasmodia.

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Subunit structure of chromatin from Physarum polycephalum

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Limited digestion of isolated nuclei from Physarum with micrococcal nuclease reveals DNA fragments which are multimers of a repeating subunit. The size of the subunit was calculated from acrylamide-agarose gels calibrated with rat liver DNA fragments. The subunit structure differs from that of higher eukaryotes: each subunit contains only 170–175 base pairs as compared to 190–200 base pairs in higher eukaryotes. However, a quasi limit digest of 140 base pairs is obtained with Physarum chromatin as with higher eukaryotes. The basic repetitive structure of chromatin from diploid plasmodia and haploid amoebae is the same. The extrachromosomal ribosomal DNA located in the nucleoli, is arranged in a similar chromatin structure. Ribosomal chromatin is somewhat more slowly digested by micrococcal nuclease than bulk chromatin as determined by acid soluble products after different digestion times. Isolated DNA fragments after chromatin digestion hybridize equally well with 19 + 26S rRNA as does unfragmented DNA.

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