PHYSARUM NEWSLETTER

Volume 4 No. 2

CONTENTS

Please mail contributions and correspondence to
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PLAN NOW FOR THE 1973 PHYSARUM CONFERENCE

Miami, Florida, is the site for the next Physarum meeting, and host Jack Daniel is making preparations for the event. The date will be either immediately before or after the Thirteenth Annual Meeting of the American Society For Cell Biology, November 15-17, 1973. According to Jack, November is a beautiful time of the year in Miami, due both to fine weather and the pre-season accomodation rates. The meetings will likely be held at the Papanicolaou Institute or at the University of Miami Medical School. Early indications are that this conference will be well-attended, including many participants from Europe. Dr. Daniel solicits any comments and suggestions you might have for this meeting. In particular, he would like to know whether you would prefer to have the conference before or after the Cell Biology Meeting. Address all inquiries, comments, etc., to:

Dr. John W. Daniel
The Papanicolaou Cancer Research Institute
University of Miami
Miami, Florida 33152 (USA)

LATE SUMMER PHYSARUM MEETINGS DRAW STRONG PARTICIPATION

Madison, August 21 and 22, 1972. Fifteen papers were presented to a group of about 40 Physarologists. The meetings were held in the Bowman Room of the McArdle Laboratory. Abstracts are enclosed with this mailing of the PNL for those who did not attend. Special thanks are due to Dr. Joyce Mohberg, whose efforts were in large part responsible for the success of the conference.

Göttingen, September 11-14, 1972. Thirty-four papers, 60 participants and the organizational work of Dr. Aloys Hüttermann combined to make the First European Physarum Conference an outstanding success. Programs from this meeting, which include abstracts of most of the presentations, have been mailed out by Dr. Hüttermann.

NEW MAILING LIST TOPS 100

The number of PNL recipients has grown to 105 from the initial 35 who received Volume One, Number One in December, 1968. Also increased are the preparation and mailing costs. Contributions toward the defrayment of these expenses will be greatly appreciated.

KEEP THOSE PAPERS AND ABSTRACTS COMIN' IN, FOLKS

We'll take all the Physarum News That's Fit To Print: Titles and summaries of papers accepted for publication, Thesis abstracts, abstracts of papers to be presented at meetings, bibliographies, methodological notes and hints, reports of findings that are not to be submitted for publication elsewhere . . . in short, any Physarum-related communication that you would like to see in the PNL.

Biochim. Biophys. Acta, 277 (1972) 639–645

POLYSOME PROFILES, AMINO ACID INCORPORATION *IN VITRO*, AND POLYSOME REAGGREGATION FOLLOWING DISAGGREGATION BY HEAT SHOCK THROUGH THE MITOTIC CYCLE IN *PHYSARUM POLYCEPHALUM*

E. N. BREWER

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(Received April 12th, 1972)

SUMMARY

Polysome profiles and amino acid incorporation were examined in cell-free preparations obtained from *Physarum polycephalum*. It was found that both the size distribution and the specific activity of the polysome preparations were essentially constant throughout the mitotic cycle, in contrast to the depression of protein synthesis known to occur *in vivo* in this organism during mitosis and again during interphase. It is suggested that the rate of protein synthesis *in vivo* is regulated by means of soluble cytoplasmic substances rather than by changes in the size distribution or functional capacity of the polysomes themselves.

Polysomes were disaggregated by heat shock during either the S or G_2 period. Polysome reaggregation occurred readily in cultures heat-shocked during G_2, but not in those heat-shocked during the S phase of the division cycle.

NATURE NEW BIOLOGY VOL. 239 SEPTEMBER 27 1972

108–110

Correlation between Unrepaired Radiation-induced DNA Strand Breaks and Mitotic Delay in *Physarum polycephalum*

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O. F. NYGAARD

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Journal of General Microbiology (1972), 71, 93–101

Stimulation of Mitosis Following Fusion of Plasmodia in the Myxomycete *Physarum polycephalum*

By B. CHIN, P. D. FRIEDRICH AND I. A. BERNSTEIN

Cellular Chemistry Laboratory, Department of Environmental and Industrial Health, The University of Michigan, Ann Arbor, Michigan, 48104, U.S.A.

(*Accepted for publication* 10 *January* 1972)

SUMMARY

Rapid fusion after contact between multinucleate, naturally synchronous plasmodia of the same strain of *Physarum polycephalum* was demonstrated by autoradiography. Fusion between plasmodia of equal size at the same stage in the mitotic cycle resulted in synchronous mitosis with a delay. This delay was slight if the nuclei were about to undergo mitosis at the time of fusion, but increased in a roughly linear fashion to about 4 h in plasmodia fused at an early stage in the mitotic cycle. Fusion between plasmodia at different stages in the mitotic cycle also resulted in synchronous mitosis, division of nuclei late in the cycle being delayed and of those early in the cycle being accelerated. When both plasmodia were in mid-interphase, the amount of acceleration was proportional to the difference in stages at the time of fusion, a 5 h phase difference giving about 3 h acceleration. However, non-synchronous mitosis resulted when a plasmodium about to undergo mitosis was fused with one earlier in the nuclear cycle, the nuclei in the former plasmodium presumably having reached a stage at which retardation could not occur.

Genetics 71: 63–71 May, 1972.

GENETICS OF SOMATIC FUSION IN *PHYSARUM POLYCEPHALUM:* THE PpII STRAIN[1]

O'NEIL RAY COLLINS

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AND

EDWARD F. HASKINS

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ABSTRACT

Plasmodial (somatic) fusion in a strain of *Physarum polycephalum*, a true slime mold, is controlled by four loci, each of which displays simple dominance. Two diploid plasmodia fuse with each other only if they are phenotypically or genotypically identical for all four fusion loci.

Reprinted from AMERICAN JOURNAL OF BOTANY
Vol. 59, No. 4, April 1972
pp. 337–340

GENETICS OF SOMATIC CELL FUSION IN TWO ISOLATES OF DIDYMIUM IRIDIS[1]

O'NEIL RAY COLLINS AND HUBERT LING

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Department of Biological Sciences, University of Delaware, Newark

ABSTRACT

Analysis of progeny from a cross between a Honduran clone and a Panamanian clone of *Didymium iridis* has revealed existence of five new plasmodial fusion loci in the Panamanian isolate. These are in addition to six which were previously known.

TISSUE & CELL 1972 4 (1) 15-36

J. W. DANIEL and U. JÄRLFORS*

PLASMODIAL ULTRASTRUCTURE OF THE MYXOMYCETE *PHYSARUM POLYCEPHALUM*

ABSTRACT. When plasmodia of *P. polycephalum* are fixed simultaneously with osmium tetroxide and glutaraldehyde, the cytoplasm is so preserved that a system of microchannels, resembling pinocytosis channels, and numerous discrete vacuoles can be observed. With either fixative alone, the cytoplasm appears to contain large irregular vacuoles or a vacuolar continuum. The microchannels, approximately 1 μ in diameter, arising as invaginations of the plasma membrane are each surrounded by a sheath of thin filaments which in areas of invagination at the plasmodial surface seems to merge with a well-defined cortex underlying the plasma membrane. Coating the plasma membrane is a structured slime layer continuous with the microchannel contents. The location and structure of the microchannel-cortex system strongly suggests it as the site for localization of contractile function implicated in cyclosis and motility. Mitochondrial structures of special interest are also described.

* Papanicolaou Cancer Research Institute and the Departments of Biology and Medicine, University of Miami, Miami, Florida 33136.

RADIATION RESEARCH 51, 410–430 (1972)

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Macromolecular Syntheses and Mitosis in uv-Irradiated Plasmodia of *Physarum polycephalum*[1,2]

VIMALA R. DEVI AND EDMUND GUTTES

Division of Biology, University of Texas at Dallas, Dallas, Texas

DEVI, VIMALA R. AND GUTTES, E. Macromolecular Syntheses and Mitosis in uv-Irradiated Plasmodia of *Physarum polycephalum*. *Radiat. Res.* 51, 410–430 (1972).

In the multinucleated plasmodia of the myxomycete *Physarum polycephalum*, irradiation with ultraviolet light delays the first postirradiation mitosis and the intervals between several subsequent mitoses are shorter than in unirradiated controls. In order to obtain more information on the factors involved, plasmodia were placed, at different times after irradiation, on media containing actinomycin (inhibition of RNA synthesis), cycloheximide (inhibition of protein synthesis), 5-fluoro-2'-deoxyuridine (inhibition of DNA synthesis), or nonnutrient balanced salt solution (total starvation). Sensitivity of the plasmodia to mitotic delay produced by actinomycin decreased rapidly after uv irradiation and reached approximately zero at the time when the nuclei would have divided if the plasmodia had not been irradiated. Following the first postirradiation mitosis, the plasmodia remained almost insensitive to continuous exposure to actinomycin for several mitotic cycles. Starvation of the irradiated plasmodia by transfer to nonnutrient balanced salt solution likewise failed to affect mitosis appreciably, whereas mitosis was considerably delayed by the same treatment in nonirradiated plasmodia. Sensitivity to cycloheximide and 5-fluoro-2'-deoxyuridine was approximately the same in the irradiated plasmodia as in unirradiated controls. At the time of the first postirradiation mitosis in the irradiated plasmodia, total protein and RNA per plasmodium were greater than the amounts

[1] This work was supported by U.S. Public Health Service Grant No. GM 11949.

[2] Reports of these data were presented at First Physarum Meeting, Madison, Wisconsin, Abstracts (1968); and IVth International Congress of Radiation Research, Evian, France, June 29–July 4, Abstract No. 337 (1970).

THE JOURNAL OF CELL BIOLOGY · VOLUME 54, 1972 · pages 179–184

SUBCELLULAR LOCALIZATION OF CALCIUM REPOSITORIES IN PLASMODIA OF THE ACELLULAR SLIME MOLD *PHYSARUM POLYCEPHALUM*

EARL ETTIENNE. From the Department of Biological Sciences, State University of New York at Albany, Albany, New York 12203. Dr. Ettienne's present address is the Department of Biological Sciences, Oakland University, Rochester, Michigan 48063.

Eur. J. Biochem. 29, 94—98 (1972)

The Effect of α-Amanitin and $(NH_4)_2SO_4$ on RNA Synthesis in Nuclei and Nucleoli Isolated from *Physarum polycephalum* at Different Times during the Cell Cycle

William D. Grant

Department of Genetics, University of Leicester

(Received March 20/May 15, 1972)

Nuclei isolated from various points in the mitotic cycle were examined for RNA synthesis in the presence of Mg^{2+} or Mn^{2+} + $(NH_4)_2SO_4$. A peak of activity 2.5—3 h after mitosis was only slightly increased by Mn^{2+} + $(NH_4)_2SO_4$ over that in the presence of Mg^{2+}, and inhibited some 50% by low levels of α-amanitin in the presence of either Mg^{2+} or Mn^{2+} + $(NH_4)_2SO_4$. A G2 peak of activity 2.5—3 h before mitosis was 100% increased by Mn^{2+} + $(NH_4)_2SO_4$ over that observed in the presence of Mg^{2+}, the increase being completely sensitive to low levels of α-amanitin, whereas the Mg^{2+} activity remained insensitive. Nucleoli isolated some 2.5—3 h before mitosis exhibited RNA synthesis which was unstimulated by Mn^{2+} + $(NH_4)_2SO_4$ and insensitive to α-amanitin. Similarities in RNA synthesis in isolated nucleoli and isolated G2 nuclei in the presence of Mg^{2+} with respect to pH optima and actinomycin-D sensitivity were observed. It is concluded that there is a high basal level of α-amanitin-insensitive RNA synthesis activity which is nucleolar and therefore probably ribosomal, rising to a peak some 2.5—3 h before mitosis, and a major peak of α-amanitin-sensitive nucleoplasmic RNA synthesis activity some 2.5—3 h after mitosis, thereafter dropping to a low level.

Actin in isoliertem Grundplasma von Physarum polycephalum

Actin in isolated ground plasm of Physarum polycephalum

Horst Hinssen [1])

Institut für Cytologie und Mikromorphologie der Universität Bonn

CYTOBIOLOGIE
BAND 5 · HEFT 2
Seiten 146–164 · 1972

Abstract

A fraction largely free of cell organelles was obtained by ultracentrifugation of the protoplasm of *Physarum polycephalum*. No actin filaments could be detected by electron microscopy in this fraction, but by formation of paracrystals it could be shown that actin was present in large amounts. Two different kinds of paracrystals exist under various conditions. Transformation of the actin to the normal F-form is not yet possible.

From the fact that the isolated groundplasm of the slime mold contained no filaments – though actin was present – it was concluded that the actin was in a nonfilamentous state, either as monomeric G-actin or as small oligomers of F-actin which cannot be identified as filaments, or as another form which is not yet known. Control experiments showed, that this was not an artefact of preparation, especially by the high centrifugal force. Therefore, it must be taken into account that part of the actin is present in such a nonfilamentous state in vivo, too. This is in contradiction to the conditions of striated muscle where only F-actin exists. It is possible that the special state of actin described here plays a functional role in the protoplasmic streaming of the slime mold, and that the ectoplasm-endoplasm transformation is connected with a transformation of actin itself. The nonfilamentous state of actin may be a form of transport, which dominates in the passively flowing endoplasm, whereas F-actin is present mainly in the ectoplasm and its fibrillar differentiations where active contractions take place.

Arch. Mikrobiol. 85, 91—94 (1972)

Differential Protein Synthesis during Differentiation (Spherulation) of *Physarum polycephalum*: Lack of Synthesis of Glucose-6-phosphate Dehydrogenase

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Forstbotanisches Institut der Universität Göttingen, Germany

Filament Formation by Purified *Physarum* Myosin

Proc. Nat. Acad. Sci. USA
Vol. 69, No. 8, pp. 2011–2014, August 1972

VIVIANNE NACHMIAS

Haverford College, Haverford, Pennsylvania 19041

Communicated by Kenneth V. Thimann, May 3, 1972

ABSTRACT ***Physarum*** **myosin can be separated from actomyosin by ultracentrifugation, and purified by gel filtration. Unlike actomyosin, myosin is soluble in 0.05 M KCl in the pH range of 6–7. However, in the absence of actin, the slime mold myosin can be precipitated in 0.05 M KCl by the addition of millimolar concentrations of $CaCl_2$. The precipitates consist of aggregated, short bipolar filaments. Magnesium has a similar effect, but results in the precipitation of more loosely packed aggregates.**

The length of the compact filaments is 0.45 μm; thus, predominantly tail-to-tail, but also some head-to-tail, interactions occur under these conditions. Since the size and shape of these thick filaments are close to those seen in fixed and sectioned ameboid cells and in platelets, all of these filaments are probably composed of myosins.

THE EFFECTS OF STARVATION AND LIGHT ON INTRA-MITOCHONDRIAL GRANULES IN *PHYSARUM POLYCEPHALUM*

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J. Cell Sci. 10, 1–14 (1972)

SUMMARY

The presence of spherical or rod-shaped electron-dense particles was noted within the mitochondrial cristae of *Physarum polycephalum* plasmodia. When sporulation was stimulated by starvation and subsequent illumination it was found that the numbers of such particles present per mitochondrion increased during the starvation phase, reaching a maximum after about 4 days, and then decreased rapidly during exposure of the plasmodium to light. Few particles were present in the mitochondria of spores. It is suggested that the particles may be similar to the mitochondrial granules of other organisms in providing a repository of calcium, which in *Physarum* is deposited in the capillitium during sporulation.

Additional Articles in Print

E. N. Brewer
"DNA Replication in Physarum polycephalum"
Journal of Molecular Biology 68, 401 (1972)
(For summary, see PNL 4, 14, 1972)

E. M. Goodman
"Axenic Culture of Myxamoebae of the Myxomycete Physarum polycephalum"
Journal of Bacteriology 111, 242 (1972)
(For summary, see PNL 4, 15, 1972)

A. Hüttermann
"Isoenzyme Pattern and de novo Synthesis of Phosphodiesterase during Differentiation (Spherulation) in Physarum polycephalum"
Archiv für Mikrobiologie 83, 155 (1972)
(For summary, see PNL 4, 15, 1972)

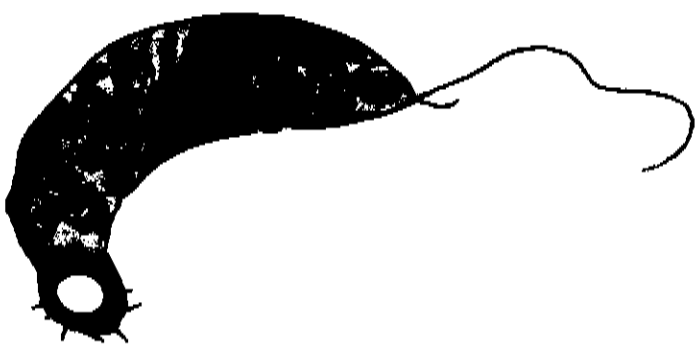
N. L. Oleinick
"The Radiation-Sensitivity of Mitosis and the Synthesis of Thymidine Kinase in Physarum polycephalum: A Comparison to the Sensitivity to Actinomycin D and Cycloheximide"
Radiation Research 51, 638 (1972)
(For summary, see PNL 4, 16, 1972)

W. Sachsenmaier, U. Remy and R. Plattner-Schobel
"Initiation of Synchronous Mitosis in Physarum polycephalum. A Model of the Control of Cell Division in Eukariots"
Experimental Cell Research 73, 41 (1972)
(For summary, see PNL 4, 17, 1972)

Forthcoming Review Articles on Physarum

W. D. Grant
"RNA synthesis during the cell cycle in Physarum polycephalum", in the British Society for Developmental Biology Symposium The Cell Cycle in Growth and Differentiation (edited by M. Balls and F.S. Billett), Cambridge University Press. (To be published in the Spring of 1973)

J. Mohberg
"The Nucleus of the Plasmodial Slime Molds", in The Cell Nucleus (edited by H. Busch), Academic Press. (To be published in early 1973)



Extensive fibrillar protoplasmic differentiations and their significance for protoplasmic streaming.

IX. Aggregation states of myosin and conditions for myosin filament formation in the plasmodia of Physarum polycephalum

Axel Alléra and Karl-Ernst Wohlfarth-Bottermann

Institut für Cytologie und Mikromorphologie der Universität Bonn

Eingegangen 24. Mai 1972

Summary

Thick filaments measuring 130 - 260 Å in width and up to 0,7 µ in length are present in the fibrils of glycerinated plasmodia of Physarum polycephalum. They consist of 20-25 Å thin subfilaments and occasionally are connected with actin filaments by cross-bridges. Unlike actin filaments, they do not form arrowhead structures with muscle heavy meromyosin. However, after myosin extraction and subsequent fixation in aldehydes, thick filaments are lacking. These results indicate that the thick plasma filaments of Physarum represent myosin filaments.

In addition to myosin filaments, thin filaments measuring 20-50 Å in diameter are found. They occur free within the groundplasm and plasmafibrils as well as in close structural relation to myosin filaments. Since they do not bind muscle heavy meromyosin, they are assumed to represent monomeric and oligomeric myosin.

In glycerinated plasmodial drops myosin filaments are found after fixation with glutaraldehyde or osmium tetroxide, whereas in unglycerinated drops they are only present after application of slowly fixing aldehydes.

The assumption that myosin filaments form by aggregation of oligomeric myosin during convulsive contractions unspecially induced by glycerination or slow fixations is confirmed by the observation that abundant thick filaments are present in "contraction islets" when the drops are fixed in paraformaldehyde solution containing ATP.

Regarding the high tendency of myosin to aggregate in the plasmodium it seems likely that myosin forms aggregates of various sizes functionally connected with contractions in vivo. However, this aggregation is apparently transitory and cyclic in relation to the contraction-relaxation cycle underlying the protoplasmic shuttle streaming. In particular, transformation of stationary ectoplasm into streaming endoplasm may involve disaggregation of larger myosin aggregates into monomeric or oligomeric units for transportation.

This investigation provides further indications that contraction in Physarum is caused by a sliding mechanism. The myosin aggregates normally involved in contraction seem to be smaller than myosin filaments.

Cytobiologie, in press

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Rifampicin-sensitive RNA and Protein Synthesis by Isolated Mitochondria of *Physarum polycephalum*

William D. Grant and Russell T.M. Poulter

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Leicester LE1 7RH, England

(Received 5 May, 1972)

Summary. A new method has been developed for the isolation of mitochondria from the Myxomycete Physarum polycephalum. Isolated mitochondria were found to incorporate [^{3}H] UTP into an acid-insoluble product sensitive to ribonuclease, the incorporation being inhibited by low levels of actinomycin D, and rifampicin. Isolated mitochondria also incorporated [^{14}C] leucine into an acid-insoluble product, the incorporation being uninhibited by cycloheximide, but inhibited by low levels of both chlorampenicol and rifampicin. In the presence of rifampicin, the major part of the acid-insoluble radioactivity derived from the incorporation of [^{3}H] UTP disappeared, with a half-life of 2-3 minutes. A parallel decrease in [^{14}C] leucine incorporating ability was noted in mitochondria preincubated for increasing periods in the presence of rifampicin, suggesting that the major part of the acid-insoluble radioactivity derived from the incorporation of [^{3}H] UTP was likely to have a messenger function in [^{14}C] leucine incorporation. The product of [^{3}H] UTP incorporation was found to run on polyacrylamide gels as a relatively homogeneous peak, immediately preceding E.coli 16$ ribosomal RNA, and clearly distinguishable from Physarum polycephalum mitochondrial ribosomal RNA, which has been extracted and characterised on gels for the first time.

J. Mol. Biol., in press

Molec. gen. Genet. ■■■. ■■■–■■■ (1972)

Mutagenesis and Mutant Selection in *Physarum polycephalum*

Finn B. Haugli and William F. Dove
McArdle Laboratory for Cancer Research, Medical School
University of Wisconsin, Madison, Wisconsin

Received March 13, 1972

Summary. Physarum polycephalum myxamoebae were exposed to ultraviolet irradiation and plated in the absence and presence of caffeine. Caffeine reduces the shoulder on the UV^1 dose-survival curve, thereby increasing the UV-sensitivity for survival. Caffeine alone is a moderate mutagen. Used in conjunction with UV a strong mutagenic action is observed. Active growth is required for both of these mutagenic actions.

Populations of *Physarum* myxamoebae mutagenized with NMG or EMS could be enriched for two classes of mutants by incubating at high temperature (30°C) with 5-bromodeoxyuridine—substituted bacteria followed by irradiation with long wave UV light and recovery at low temperature (23°C). One class of mutants was obtained in high yields after repeated cycles of light inactivation. These are not heat sensitive. Rather they are defective in utilization of DNA precursors provided by the bacteria. The other mutant class, obtained in low yields after limited selection, are heat sensitive. Three independent mutants of this kind, all leaky, were obtained. Reconstruction experiments show that all are selectants.

Molec. gen. Genet. ▮▮▮, *1—11* (19▮▮)
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Genetics and Biochemistry of Cycloheximide Resistance in *Physarum polycephalum*

Finn B. Haugli and William F. Dove

McArdle Laboratory for Cancer Research, Medical School, University of Wisconsin, Madison, Wisconsin

Antonio Jimenez

Department of Biochemistry, College of Agriculture and Life Sciences, University of Wisconsin, Madison, Wisconsin

Received March 13, 1972

Summary. Cycloheximide-resistant mutants of *Physarum polycephalum* were induced in the haploid myxamoebae by the combined action of UV[1] and caffeine (Haugli and Dove, 1972) or by treatment with NMG[2]. Eight independent mutants segregated in a Mendelian fashion (Table 1). Crosses between 6 of the mutants revealed 2 loci, *actA* and *actB*, for cycloheximide resistance (Table 2).

All mutants are expressed in the plasmodium and are recessive in heterozygotes (Fig. 1 and 2). One mutation, conferring resistance to high levels of cycloheximide, was studied in heterokaryons and found to be incompletely recessive.

An *in vitro* peptide synthesizing system was constructed from ribosomes from *Physarum* and supernatant factors from *Saccharomyces cerevisiae*. Cycloheximide strongly inhibited the activity of ribosomes derived from either wild type or mutants at the *actB* locus. In contrast, ribosomes from mutants at the *actA* locus were resistant to cycloheximide. Thus, the *actA* locus operates through the ribosomes.

Regulation of Polyamine Synthesis in Physarum polycephalum During Growth and Differentiation

John L.A. Mitchell and Harold P. Rusch

McArdle Laboratory for Cancer Research
Madison, Wisconsin 53706

Summary. The levels and synthesis of polyamines were investigated in Physarum polycephalum to obtain information about their regulation during growth and differentiation in a lower eukaryote. Putrescine pools rapidly increased 4-5 fold during the change from dormant spherules to growing plasmodia. The activity of ornithine decarboxylase (EC 4.1.1.17), which converts ornithine to putrescine, reflected this rapid change in the level of putrescine. Spermidine levels were closely correlated with protein concentrations during differentiation due to variations in the activity of S-adenosyl-L-methionine decarboxylase which is involved in the conversion of putrescine to spermidine. This enzyme was not stimulated by putrescine, unlike the similar enzyme in other eukaryotes, thereby permitting independent regulation of putrescine and spermidine levels. The high levels of both putrescine and spermidine suggest separate functions for these polyamines in Physarum.

The half lives of ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase were 14 and 21.5 min respectively. These short half-lives keep the polyamine metabolism under a very tight control as illustrated by the rapid fluctuations in enzyme activity during differentiation and the synchronous mitotic cycle. The step patterns of these unstable enzymes during the mitotic cycle suggests that these enzyme levels are limited by gene dosage.

Biochim. Biophys. Acta, in press.

Abstracts of papers presented at the Twelfth Annual Meeting of The American Society For Cell Biology, Nov. 8-11, St. Louis, Mo.

267. EFFECT OF CYTOCHALASIN B ON CYTOPLASMIC STREAMING IN THE SLIME MOLD PHYSARUM POLYCEPHALUM Dietrich Kessler, Department of Biology, Haverford College, Haverford, Pa.

Cytochalasin B has been found to arrest cytoplasmic movement in a variety of cell types. Since the myxomycete Physarum polycephalum has been used extensively in studies of the molecular basis of cytoplasmic streaming, it is of interest to study the effect of cytochalasin on movement in this organism. These studies were done with light microscopy by observation of streaming and migration in plasmodia freshly cut to a size of $4mm^2$ from larger surface cultures which had been growing for 24h on the standard tryptone-yeast extract medium. The small plasmodial squares were placed on the surface of a solution containing 10mM tris-maleate buffer, pH 7.0, with the addition of various amounts of a stock solution of cytochalasin B dissolved in dimethyl sulfoxide (DMSO). At cytochalasin concentrations of 100-125ug/ml extrusion of the cytoplasm at the edge of the plasmodial squares began immediately, while the cytoplasm in the interior of these squares continued normal shuttle streaming. The extruded cytoplasm was no longer capable of movement and gradually lost pigment. After 3h in the cytochalasin solution, 80-100% of the plasmodia had been completely drained of cytoplasm and were dead. The surviving plasmodia began migrating normally onto the surface of the medium after 3h with no further cytoplasmic extrusion. The presence of 5-10mM $CaCl_2$ or $MgCl_2$, but not KCl or NaCl, prevented extensive extrusion and death. At lower cytochalasin concentrations extrusion was not as prevalent. Control plasmodia placed on buffer with up to 2.5% DMSO continued to stream normally with no cytoplasmic extrusion. (Supported by SNSF and NSF.)

J. Cell Biol. 55, 134a (1972)

305. LOCALIZATION OF NUCLEOLAR AND CHROMATIN ACIDIC PROTEIN CHANGES DURING DIFFERENTIATION IN PHYSARUM POLYCEPHALUM Wallace M. LeStourgeon* and Harold P. Rusch. McArdle Laboratory for Cancer Research, Medical Center, University of Wisconsin, Madison, Wisconsin.

Two distinct classes of residual acidic nuclear proteins based on selective solubility in buffer saturated phenol (pH 8.2) and hot SDS have been extracted from both isolated nucleoli and nuclei during synchronous growth and differentiation in the slime mould Physarum polycephalum. Quantitative comparisons of electrophoretically separated proteins of both fractions from nucleoli and nuclei reveal that during differentiation leading to meiosis numerous changes occur in the phenol soluble fractions primarily in the chromatin proteins ranging in molecular weight from 50,000 to 160,000. Only 2 nucleolar proteins, comprising 80% of the total nucleolar proteins, with molecular weights of 37,000 and 41,000, are extractable with phenol and are unchanged during differentiation. However, two nucleolar proteins of molecular weight 76,500 and 86,500 not extractable with phenol are extracted with hot SDS and the protein of molecular weight 86,500 disappears during differentiation. The phenol extraction procedure solubilizes only 20% of the total residual nuclear proteins and dissociates 20% of the bound DNA into the aqueous extraction phase. Incorporation studies with ^{14}C-amino acids demonstrate that the new proteins which appear during differentiation are newly synthesized and that there are two periods (mid S and late G_2) of increased label incorporation during the 8 hour mitotic cycle. The nucleolar acidic proteins as compared to the acidic proteins of chromatin, are high in glycine, serine, and aspartic acid but low in valine and isoleucine.

J. Cell Biol. 55, 153a (1972)

339. DNA REPAIR AFTER UV IRRADIATION IN PLASMODIA OF PHYSARUM POLYCEPHALUM J. Justin McCormick. Michigan Cancer Foundation, Detroit, Michigan.

Plasmodia of P. polycephalum were irradiated with 14 500 ergs/mm^2 of ultraviolet irradiation during early G2 period and the subsequent repair synthesis of DNA was studied using neutral and alkaline cesium chloride gradients. It was found that the G2 period, which is normally 5 h in length, is prolonged to 18 h by such a dose of irradiation and that repair synthesis occurs almost entirely during the last 9 h of this period. Following the extended G2 period, the nuclei undergo mitosis in a normal manner and during the DNA synthesis period which follows mitosis all the nuclear DNA is replicated semiconservatively as shown by density label centrifugation studies. During the extended G2 period, normal replication of the nuceleolar satellite DNA and trace amounts of nuclear DNA occurs and the total amount synthesized is equal to what would have been synthesized in a G2 period of normal length. Caffeine shows a strong inhibitory effect on DNA repair synthesis without interfering with DNA replication or the length of the intermitotic period. Similar repair studies with smaller doses of UV show shorter delays in the mitosis following irradiation and smaller amounts of repair synthesis of DNA.

J. Cell Biol. 55, 170a (1972)

437. MICROSPECTROPHOTOMETRIC COMPARISON OF THE NUCLEI IN UV-IRRADIATED AND NONIRRADIATED PLASMODIA OF PHYSARUM POLYCEPHALUM Tom D. Rogers. Dept. of Biology, North Texas State University, Denton, Texas, and E. Guttes, Division of Biology, University of Texas at Dallas.

Plasmodia of the myxomycete, Physarum polycephalum were irradiated with ultraviolet light (14,500 ergs/mm^2) at late interphase. Nuclei at different stages of the mitotic cycle of irradiated plasmodia and of unirradiated controls were scanned, at 260 nm, with the Zeiss Universal Microspectrophotometer (UMSP-I), using 0.5 μ-diam. measuring spots at 0.5 μ-intervals. Just prior to mitosis, the average extinction per nucleus was approx. 80% higher in the nuclei of irradiated plasmodia than in the control nuclei. In the irradiated plasmodia, even 2.0 hr after the first delayed post-UV mitosis, the average extinction by the nuclei was approx. 10% higher than that by premitotic nuclei of nonirradiated plasmodia. Treatment with actinomycin (100 μg/ml) for a period of 6.0 hr beginning 2.0 hr after mitosis, increased the average total extinction per nucleus in the irradiated plasmodia by approx. 23%, whereas the same treatment had no effect in non-irradiated plasmodia. The irradiated plasmodia contained large numbers of pycnotic, degenerating nuclei, and the total number of nuclei in the irradiated plasmodia was up to 28% lower than in unirradiated plasmodia. We have previously found that the mitotic cycles following the first delayed post-irradiation mitosis are shorter and less sensitive to actinomycin and starvation than in nonirradiated plasmodia (Devi et al., Exptl. Cell Res. 50, 589, 1968; Guttes and Devi, IV. Internatl. Congress Radiation Research, Evian, France, June 29-July 4, Abstr. No. 337, 1970). It is possible that the increased quantity of UV-absorbing substances found in the nuclei of irradiated plasmodia before and after the first, delayed, postirradiation mitosis is related to this behavior. (Supported by NASA Grant NGR 44-027-005, Suppl. 1 and by NSF Grant No. GB 29311.)

J. Cell Biol. 55, 219a (1972)

523. RELATIONSHIPS BETWEEN CHROMATIN, ANNULI, AND THE NUCLEAR ENVELOPE
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Often, chromatin-nuclear envelope (NE) associations have been shown to occur at the annulus. We have investigated two alternatives: either that the "annular ring" of the NE is a membraneous component, or that it consists of a ring of chromatin. Pure nuclear pellets of P. polycephalum isolated during interphase according to the method of Mohberg and Rusch (1971. Exp. Cell Res. 66:305) were waterspread and either exposed to detergents, or digested with trypsin, DNase, or RNase for varying times, then observed on a Zeiss 9S-2. Several morphological relationships were found: (1) chromatin was observed to be associated with the NE at the site of annular "remnants," "rings," and also undifferentiated portions of the NE. Identity of components was confirmed by digestion studies. Stretching forces of the Kleinschmidt technique suggested that attachments were not artifacts; (2) ring-like assemblies of the nuclear pores appeared to pull away from the NE leaving "remnants" of varying diameters in the membrane; (3) comparative biochemical digestion (0-10 min) revealed trypsin-resistant biconvex structures in the chromatin after long treatment times; (4) both detergent and trypsin-treated NE revealed a fibrillar network substructure. Careful analysis of 300 grids suggested that additional inquiry into the nature of the "annular ring" might further support the contention that it is chromatin, since: (i) frequently the "ring" maintained its structural integrity after violent expulsion from the NE while membraneous "remmants" did not, (ii) "ring" ultrastructure showed the same modifications after detergent treatment as did the chromatin, while membrane ultrastructure showed uniquely different modifications, (iii) digestion studies of isolated "annular rings" revealed trypsin-resistant cores with linkers too short to be cistrons called "structural DNA." These results can be summarized by a model of the nuclear pore with DNA strands interlocked by repetitive DNA into a ring-like structure covered by non-histone proteins.

J. Cell Biol. 55, 262a (1972)

Abstracts of papers presented at the Fourth Annual Meeting of The Union of Swiss Societies for Experimental Biology, Geneva.

Acid-Soluble Deoxyribonucleoside Triphosphates Pools and DNA Synthesis in Physarum

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Acid-soluble nucleoside triphosphates (NTPs) of *Physarum polycephalum*, an acellular Myxomycete with a synchronous mitotic cycle, were determined after ^{32}P label and separation by thin layer chromatography. Their levels, expressed in counts per minute, were related to protein content.

The distribution of individual triphosphates was calculated in percent of the sum of their activities. The deoxyribonucleoside triphosphates (dNTPs) vary from 3% to less than 1% of the total activity between the beginning and the end of S period and the ribonucleoside triphosphates between 97 and 99%. The pool sizes of dNTPs decreased in the following order: dTTP, dCTP, dGTP, dATP. During DNA replication, dTTP and dGTP decreased by a half, dCTP dropped tenfold and dATP became too small to be measurable. These low levels remained rather constant throughout G2 and increased again at the onset of the following S period.

This shows a parallelism between the supply of dNTPs and the rate of chromosomal DNA synthesis, but does not indicate whether there is a direct interdependence between the two. dCTP seems the most representative of DNA replication. The low remaining amounts of dNTPs in G2 could be related to mitochondrial and satellite DNA replication which takes place throughout the cycle.

Supported by SNSF, grant 3.183.69.
Experientia 28, 734 (1972)

A New Acid Phosphatase (Exoplasmodial) from *Physarum polycephalum*

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Investigations on nucleolytic enzymes appearing in the broth of *Physarum polycephalum* cultures revealed a second ribonuclease, besides RNAse I discovered by Braun, and a new phosphomonoesterase. A purification procedure was worked out in order to supply all three enzymes in high yields and purity. The exoplasmodial acid phosphatase has a MW of 49,000 daltons, as established by equilibrium sedimentation, amino acid composition and gel exclusion. Its isoelectric point is 4, as ascertained by gel electrofocusing. It is highly soluble in water, but extensive dilution impairs activity, suggesting a possible dissolution into subunits. Freezing inactivates, but glycerol or BSA afford some protection, more efficiently provided by non-ionic detergents. EDTA, other chelating agents or thiols have no effect on activity, but NaF strongly inhibits. Main features: Highly active on *p*-nitrophenyl phosphate, with a K_M of $2.5 \times 10^{-3} M$ established by a double regression best fit program on the hyperbolic Michaelis function, and by Eadie plots, this phosphatase is nonetheless just as active against 3'CMP, and somewhat less against other 3'-nucleotides, with a distinctive base specificity. 5'-nucleotides are dephosphorylated at half the rate of the 3' (or 2')-N, however, these substrates cannot qualify as 'activated esters' as compared with PNP. Compounds having a *vic. hydroxyl* on the alkyl moiety of the ester, when sterically favorable, are preferred substrates, perhaps due to cyclization by H-bonding. Citrate is a strong activator and significantly shifts the optimum of the pH profile curves from 4.5 for other buffers to below pH 3.0.

Supported by SNRF, grant 3.420.70, and by CIBA-GEIGY.

Experientia 28, 739 (1972)

Discussion Group F 3, Friday 8th September 1972, 14oo.
Internat. Congress of Cell Biology, Sussex (England).

S. HATANO, Nagoya (Japan)

Conformational change of actin polymer from the myxomycete plasmodium.

Plasmodium actin polymerizes to a different state of polymer from F-actin in the presence of Mg^{++} (Mg-polymer), Mg-polymer is a flexible filament diameter of which is about 100 Å and shows ATPase activity. Recently we found that this polymer shows the reversible conformational change (folding and unfolding) when 0.5 mM ATP is added to it under the physiological salt conditions of plasmodium (0.03 M K^{+}, 7 mM Mg^{++}, pH neutral). The maximum change of the overall length of the polymer is estimated to be more than 30 %. The role of this comformational change in vivo will be discussed.

H. KOMNICK, Bonn (Germany)

(1) Contraction and fine structure of plasmodial actomyosin gels.

Actomyosin extracted from Physarum by 0.5 M KCl, purified, and precipitated at 0.03 M KCl in the form of a thread contracts on the addition of ATP. Fine structural analysis of thin sections and negatively stained suspensions of the actomyosin gels reveals the presence of both actin and myosin filaments. Comparative studies on isolated skeletal muscle actomyosin provide evidence that a sliding mechanism apparently can operate by either myosin filaments or oligomeric myosin units.

(2) Involvement of a calcium pump in control of cytoplasmic streaming.

Myxomycete plasmodia as well as amebae possess a calcium pumping membrane system, which is capable of accumulating Ca^{++} from concentrations of 10^{-6} M. In plasmodial strands, calcium containing vacuoles are encountered in the relaxed front pole of protoplasmic influx. The results suggest that the calcium pump locally controls the free Ca^{++} and thereby triggering the contraction-relaxation cycle.

Discussion Group F 3, Friday 8th September 1972, 14oo.
Internat. Congress of Cell Biology, Sussex (England). (Continued)

V. T. NACHMIAS, Haverford (USA)

Thick filaments from Physarum myosin in vitro.

Electron microscope studies, in close conjunction with other approaches lead to the conclusion that microfilaments, rather than microtubules, may provide the structural basis for cytoplasmic streaming in many ameboid cells and in shuttle streaming. Certain microfilaments from amebae and from Physarum polycephalum have been shown to be biochemically similar to actin when isolated, and to combine with heavy meromyosin or subfragment one from vertebrate striated muscle to form a complex that possesses the axial periodicity and the polarity characteristic of the actomyosin complex of muscle. This polarity occurs also in the complex formed when both actin and myosin components are from Physarum or when the actin is from muscle and the myosin from Physarum. Biochemical studies likewise demonstrate that at the molecular level there are strong similarities between cytoplasmic streaming and muscle contraction.

In amebae a second distinct type of filament has been consistently found both in fixed cells and in a cytoplasmic supernatant fraction.

It has also been reported in Physarum, but only during a certain stage of the life cycle. This filament is 0.5 μ long and 150-250 Å in diameter. We present here circumstantial evidence to support the view that these "short thick filaments" are composed of myosin-like protein. We have used Physarum myosin purified by ultracentrifugation and Agarose gel filtration, to produce "short thick filaments" in vitro in the absence of actin. Physarum myosin is soluble in 0.05 M KCl but aggregates to form typical bipolar 0.45 μ long filaments when 1-10 mM calcium salts are added. The implications for assembly of myosin filaments and their role in cytoplasmic streaming will be discussed.

K. E. WOHLFARTH-BOTTERMANN, Bonn (Germany)

Identification of Physarum actin and myosin filaments in situ.

The 80 Å wide plasmafilaments are identified in situ as F-Actin by decoration with muscle heavy meromyosin. Under conditions of high motive force generation, they form fibrillar bundles visible in thin sections.

Short and thick filaments of Myosin-nature can be revealed and identified in glycerinated and unglycerinated plasmodia under various conditions of pretreatment and fixation. The appearence of myosin-filaments is interpreted as a result of either convulsive contration or relaxation.

The aggregation state of myosin in vivo and its interaction with F-actin filaments will be discussed.

PUBLICATIONS ON PHYSARUM

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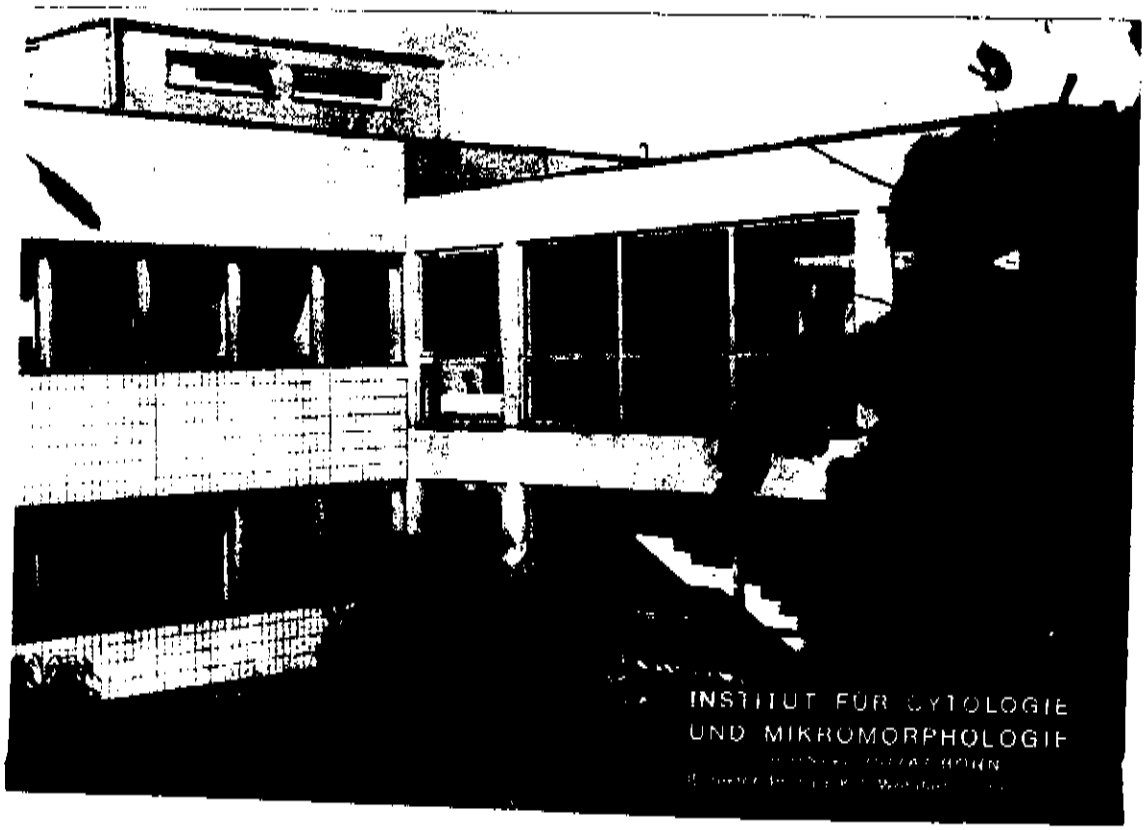

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