PHYSARUM NEWSLETTER

JULY, 1974

Volume 6 No. 1

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3227 3/72

1975 NORTH AMERICAN PHYSARUM MEETING TO BE HELD IN GAINESVILLE, FLORIDA

Henry Aldrich has generously volunteered to coordinate the next (post Leicester) Physarum meeting to be held in November, 1975, the exact dates and details to be announced later in the PNL (the conference will be timed to complement the annual meeting of the American Society for Cell Biology, which is to be held in Puerto Rico that year). If you have any questions and/or suggestions regarding the above, please contact: Dr. Henry C. Aldrich, Dept. of Botany, University of Florida, Gainesville, Florida 32611, U.S.A.

LARGE ATTENDANCE EXPECTED IN LEICESTER . . . As of April, over 60 Physarologists had registered an intent to participate in the Second European Physarum Meeting. To be held at the University of Leicester September 9-13, the conference schedule calls for paper presentations during the mornings of September 10-12 with informal group discussions on the afternoons of Tuesday and Thursday, and features a one-day course in "Techniques for Physarum Genetics" to be held on Friday the thirteenth . . . Indications are clear that the excellant organizational work of Jennifer Dee, Bill Grant and Geoff Turnock will result in an outstanding meeting.

MYXOMYCETE FILM BIBLIOGRAPHY . . . Several fine myxomycete films are known to have been produced, such as the two on D. nigripes by Norman Kerr (PNL 3, 22, 1971) and the P. polycephalum film of the McArdle Laboratory. Certainly other such products exist, although they may or may not be so sophisticated! With the cooperation of those of you who have (or know of) films of specific interest to those working on myxomycetes, we will assemble an up-to-date bibliography for publication in the PNL. Initially, we need the following information: title and brief summary; duration; date; size; and any special features, costs, etc. Direct your responses to the editor and watch for the dynamic bibliography of our collective cinematographic accomplishments in future issues of "The Physarum Newsletter".

CORRECTION . . . The next European Cell Cycle meeting will be held in September, 1975, rather than 1974 as reported previously in the PNL.

PNL READERS TAKE NOTICE! Unless you register for our new mailing list, this will be the last issue that you will receive. Minimum contributions of $1.00 to help defray mailing costs are likewise required (unless precluded by governmental policy). As long as you're sending in the registration form below, why not include references to articles overlooked by the PNL, Titles and Summaries of papers in press and abstracts of papers to be presented at scientific meetings?

. . . . . .

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TITLES AND SUMMARIES IN PRINT

Arch. Mikrobiol. 92, 251—261 (1973)

Effects of Supraoptimal Temperatures on the Myxomycete *Physarum polycephalum*

I. Protoplasmic Streaming, Respiration and Leakage of Protoplasmic Substances

Victor A. Bernstam and Stephan Arndt

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of the Komarov Botanical Institute, the USSR Academy of Sciences, Leningrad

Received February 19, 1973

Summary. The effects of heat shock on the protoplasmic streaming, respiration and leakage of plasmodial constituents absorbing at 260 nm (products of nucleic acid metabolism), 280 nm (products of protein metabolism), and 415 nm (the yellow pigments of the plasmodia) were studied in plasmodia of the myxomycete *Physarum polycephalum*.

Plasmodia grown on a semidefined medium displayed a lower primary thermoresistance of the protoplasmic streaming, and had a lower Q_{10} coefficient of the heat injury of this function compared to those grown on rolled oats. They are able to repair thermal injuries during heating. The primary thermoresistance of the protoplasmic streaming is not changed during the mitotic cycle.

A 10 min heating at 32°C lowers the rate of protoplasmic streaming and results in a leakage of plasmodial pigments. After a 10 min exposure at 37—38°C the protoplasmic streaming is stopped, the respiration reduced, and products of nucleic acid metabolism are detectable in the heating fluid. Leakage of protein metabolits was observed after 10 min heatshocks at 41°C. A heating of the plasmodia to 47—50°C caused the highest level of leaked substances and the complete cessation of respiration.

In contrast to higher plants, the respiration and leakage of the pigments are thermolabile indicators of the condition of *Physarum polycephalum* plasmodia.

Eur. J. Biochem. 33, 131—139 (1973)

Phosphorylation of Very-Lysine-Rich Histone in *Physarum polycephalum*

Correlation with Chromosome Condensation

E. Morton Bradbury, Robert J. Inglis, Harry R. Matthews, and Nitza Sarner

Biophysics Laboratories, Physics Department, Portsmouth Polytechnic, Portsmouth

(Received June 13/November 10, 1972)

Physarum polycephalum histones have been analysed by acrylamide gel electrophoresis. Two of the five major bands had electrophoretic mobilities identical with the mobilities of two bands from calf thymus histone. The *P. polycephalum* pattern is qualitatively the same at all stages of the synchronous mitotic cycle. Quantitative changes in the relative proportion and relative mobility of the very-lysine-rich histone are reported. In particular, a dramatic increase in phosphate content of this histone occurred in late G2 phase with a peak where chromosome condensation is seen to be occurring in the phase contrast microscope. Phosphate content is low during S phase and the peaks of RNA synthesis.

SUB-NUCLEAR COMPONENTS OF *PHYSARUM POLYCEPHALUM*

E. M. BRADBURY, H. R. MATTHEWS, J. McNAUGHTON and H. V. MOLGAARD

Portsmouth Polytechnic, Biophysics Laboratory, Physics Department Portsmouth, Hants (Great Britain)

(Received August 13th, 1973)

Biochimica et Biophysica Acta, 335 (1973) 19–29

SUMMARY

Methods are presented for isolating *Physarum polycephalum* nuclei in such a way that they can be broken by standard procedures. Preparation of sub-nuclear components, particularly nucleoli, metaphase plates, interphase chromatin and nuclear membranes is then described. The chromosome number in one strain, $a \times l$, was 22. No nuclei at higher ploidy were observed. Nucleoli were purified in an A zonal rotor to a new standard of purity such that the content of satellite DNA was 55% of the total "nucleolar DNA". The satellite DNA was finally obtained free from detectable main band DNA in milligram quantities. A partial purification procedure for interphase chromatin is described.

Control of Cell Division by Very Lysine Rich Histone (F1) Phosphorylation

E. M. Bradbury, R. J. Inglis & H. R. Matthews

Biophysics Laboratory, Department of Physics, Portsmouth Polytechnic, Portsmouth

F1 histone phosphorylating activity correlates closely in the cell cycle with the behaviour of the mitotic trigger. It is proposed here that the phosphorylation of F1 histone is the initiation step for mitosis.

Nature Vol. 247 February 1 1974

257–261

Molecular basis of control of mitotic cell division in eukaryotes

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Nature Vol. 249 June 7 1974

553–556

Purification and Properties of Two RNA Polymerases from *Physarum polycephalum*

(slime mold/polypeptide subunits)

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Communicated by Kenneth B. Raper, January 7, 1974

ABSTRACT **Two RNA polymerases have been purified from the slime mold *Physarum polycephalum*, one sensitive and one resistant to α-amanitin. Both enzymes are more active with denatured DNA than native DNA as a template and prefer Mn^{++} rather than Mg^{++} as a divalent cation. The α-amanitin-sensitive enzyme shows maximum activity at 0.15 M KCl, whereas the resistant enzyme is most active at very low ionic strength. Analysis of the resistant enzyme on polyacrylamide gels containing sodium dodecyl sulfate shows two subunits present in a 1:1 ratio with molecular weights of 205,000 and 125,000.**

Proc. Nat. Acad. Sci. USA
Vol. 71, No. 4, pp. 1174–1177,
April 1974

EFFECT OF ANAEROBIC CONDITIONS ON PLASMODIA OF THE MYXOMYCETE *PHYSARUM POLYCEPHALUM*

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Department of Biochemistry, Imperial College of Science and Technology, London, S.W.7

586-588

Trans. Br. mycol. Soc. 61 (3), (1973)

I. CHET and NAOMI KISLEV

SCANNING ELECTRON MICROSCOPY OF SPHERULES OF *PHYSARUM POLYCEPHALUM*

ABSTRACT. The ultrastructure of starvation-induced sclerotia and mannitol-induced spherules was studied by a scanning electron microscope (SEM). Each spherule in the berry-like sclerotium is not rounded and has lobe-like forms, whereas the separate spherules are significantly bigger and rounded. Bead-like granules were observed inside and on the spherules. Broken spherules observed in the SEM show ultrastructure of cell wall, cytoplasm, vacuoles and granules, nuclei and nucleoli.

TISSUE & CELL 1973 5 (4) 545-551

Trans. Amer. Micros. Soc., 92(1): 65–79. 1973.

ULTRASTRUCTURAL OBSERVATIONS ON CAPILLITIAL TYPES IN THE TRICHIALES (MYXOMYCETES)[1]

TIM T. ELLIS, RAYMOND W. SCHEETZ,[2] and CONSTANTINE J. ALEXOPOULOS
The Cell Research Institute and Department of Botany,
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Ellis, T. T., Scheetz, R. W. & Alexopoulos, C. J. 1973. Ultrastructural observations on capillitial types in the Trichiales (Myxomycetes). *Trans. Amer. Micros. Soc.*, 92: 65–79. Phase contrast micrographs and scanning and transmission electron micrographs are presented for eleven species of the Trichiaceae and two species of the Dianemaceae. Ultrastructural evidence supports the recognition of five types of capillitium based on the presence or absence of a lumen and on the specific combination and organization of amorphous (lacking distinct structure) and fibrillar components.

Eur. J. Biochem. *44*, 407–410 (1974)

Reassociation Kinetics of Nuclear DNA from *Physarum polycephalum*

Helmut Fouquet, Brigitte Bierweiler, and Helmut Wilhelm Sauer
Universität Konstanz, Fachbereich Biologie, and Universität Heidelberg, Zoologie II

(Received November 16, 1973/February 18, 1974)

Nuclear DNA of *Physarum* is made up of 45% repeated ($c_0 t_{1/2} = 0.07-0.7$ mol · l^{-1} · s) and 55% non-repeated base sequences ($c_0 t_{1/2} = 500-1100$ mol · l^{-1} · s) and has a complexity of 130 times the *Escherichia coli* genome. DNA replicated early in the S phase of the cell cycle, contains few repeated base sequences (below 10%).

Purification and Properties of Deoxyribonucleic Acid-dependent Ribonucleic Acid Polymerases from the Slime Mold *Physarum polycephalum**

The Journal of Biological Chemistry
Vol. 249, No. 6, Issue of March 25, pp. 1792–1798, 1974 (Received for publication, July 30, 1973)

Stella Z. Gornicki,‡ Susan B. Vuturo, Timothy V. West, and Robert F. Weaver
From the Department of Biochemistry, McCollum Laboratories, University of Kansas, Lawrence, Kansas 66044

SUMMARY

The nuclear DNA-dependent RNA polymerases have been partially purified from the slime mold *Physarum polycephalum*. Two major forms of the enzyme are readily separated upon phosphocellulose chromatography. One of these (R) is resistant to the mushroom toxin α-amanitin, has a low salt optimum, and has a relatively low preference for denatured DNA over native DNA. This enzyme is therefore probably identifiable with RNA polymerase I from the higher eukaryotes. The other enzyme (S) is sensitive to α-amanitin, has a relatively high salt optimum, and has a relatively high preference for denatured DNA over native DNA. This enzyme is apparently analogous to RNA polymerase II from the higher eukaryotes. Slime mold RNA polymerase R has been purified to near homogeneity. This enzyme has a sedimentation coefficient of approximately 13.1 S. Polyacrylamide gels run under denaturing conditions reveal a probable subunit structure as follows: $200{,}000_1$; $135{,}000_1$; $45{,}000_1$; $24{,}000_2$; $17{,}000_1$.

CONTINUOUS NUCLEOLAR DNA SYNTHESIS IN LATE-INTERPHASE NUCLEI OF *PHYSARUM POLYCEPHALUM* AFTER TRANSPLANTATION INTO POST-MITOTIC PLASMODIA

E. GUTTES

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J. Cell Sci. 15, 131–143 (1974)

SUMMARY

In the myxomycete, *Physarum polycephalum*, nuclear DNA synthesis commences immediately upon completion of mitosis. While the synthesis of extranucleolar DNA is completed within a few hours, nucleolar DNA synthesis occurs during most of the *S*-phase and the entire G_2 phase of the intermitotic period. When large (polyploid), late-interphase nuclei were allowed to bypass mitosis by transplantation into recipient plasmodia which were at early interphase and which belonged to a strain having smaller nuclei, the nucleolar DNA of the transplanted nuclei continued to be labelled (autoradiographs) after incubation of the host plasmodia with [^{3}H]thymidine until they entered prophase along with the nuclei of the host plasmodium, approximately one intermitotic period later. This labelling was DNase-sensitive and RNase-resistant. When late-interphase nuclei were labelled with [^{3}H]thymidine just prior to transplantation, there was no decrease of label after transplantation during the additional intermitotic period. We conclude from these experiments that there is no obligatory alternation between nucleolar DNA duplication and mitosis in *Physarum polycephalum* and that nucleolar DNA replication might exhibit amplification during an experimentally prolonged intermitotic period.

JOURNAL OF BACTERIOLOGY, May 1974, p. 761–763 Vol. 118, No. 2

Activity of Uridine Diphosphate *N*-Acetylglucosamine-4-Epimerase During Spherulation of *Physarum polycephalum*

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Received for publication 4 February 1974

The specific activity of uridine diphosphate *N*-acetylglucosamine-4-epimerase increases during spherulation of *Physarum polycephalum*, a process that involves the synthesis of galactosamine walls. This increase is prevented by the addition of cycloheximide.

FILAMENT FORMATION BY SLIME MOULD MYOSIN ISOLATED AT LOW IONIC STRENGTH

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J. Cell Sci. 15, 113–129 (1974)

SUMMARY

Myosin was isolated and purified from plasmodia of the slime mould *Physarum polycephalum* by a new method. This method is based on actomyosin extraction at low ionic strength after extensive washing, followed by the selective precipitation of myosin at pH 6·1 under relaxing conditions. The yield of myosin was 3–5 times higher than reported for other methods.

In contrast to earlier studies a remarkably strong tendency to filament formation was found for slime mould myosin, probably due to a better preservation of some structural properties during preparation. Conditions were worked out under which numerous filaments up to 4 μm in length can be produced. It was established that not only a gradual decrease of ionic strength may influence filament formation, but also pH, ATP concentration and the presence of divalent cations. Compared to the current filament model, a difference exists in the structure of the filaments. No central bare zone can be found, and thus they lack an apparent bipolarity. Along the entire filament there are lateral projections representing the head portion of myosin molecules. A clear periodicity with an axial repeat of about 14 nm was observed, indicating a highly ordered arrangement of these projections.

In this paper it is shown for the first time that myosin from one of the primitive motile systems is able to form aggregates of high structural order, indicating that the contraction of non-muscular actomyosin systems is not necessarily effected with oligomeric or randomly aggregated myosin. The possible role of myosin aggregation *in vivo* and the similarity of filament structure to that recently reported for myosin from vertebrate smooth muscle and striated muscle are discussed.

Cytological Studies on Mitosis of Plasmodium of *Physarum polycephalum*[1]

Cytologia 38: 165–175, 1973

Eiko Hosoda and Nobunori Tanaka[2]

Laboratory of Genetics, Department of Botany,
Faculty of Science, University of Tokyo, Tokyo, Japan

Recieved August 26, 1970

Summary

In the multinucleated plasmodium of *Physarum polycephalum* all nuclei are completely synchronized in mitotic cycle. The duration of a mitotic cycle was 9 hours at 25°C and those of M, G_1, S and G_2-phase were 0.5 hours, less than 1 minute, 2.5 hours and 6.0 hours, respectively. M-phase was further divided into 15 minutes of prophase, 10 minutes of prometaphase, 5 minutes of metaphase, less than 1 minute of anaphase and 4 minutes of telophase.

In the first, incorporation of arginine-^{3}H into total protein of plasmodium was investigated. There were three main peaks: the first one in early S-phase, the second in early G_2-phase, and the third in late G_2-phase.

From the inhibitory effect of cycloheximide on protein syntheses just before and after the start of mitosis, it is considered that protein required for initiation of mitosis is synthesized until before prophase, in the other words, at a phase just before prophase or a little more earlier phase, and another protein that required for shift of mitotic phase from metaphase to anaphase is synthesized probably in prophase and in prometaphase in a certain synthetic rate. Besides, from the inhibitory effect of actinomycin D on mitosis, the RNA required for this mitotic shift is synthesized in premitotic phase about 40 minutes before the start of prophase.

N. KISLEV and I. CHET*

SCANNING ELECTRON MICROSCOPY OF SPORULATING CULTURES OF THE MYXOMYCETE *PHYSARUM POLYCEPHALUM*

ABSTRACT. Scanning electron microscopy of sporulating cultures of *Physarum polycephalum* shows in detail the fine structure of the stalk, peridium, capillitia and spores. The peridium has a wrinkled structure and funnel-like cavities (5–10 μ in diameter) which are the capillitia openings. The capillitia are a network of tubes with differing diameters. The capillitia are covered with fine granules, and contain various amounts of bead-like granules of up to 2 μ in diameter. The spherical spores (10 μ in diameter) are covered with fine granules similar to those covering the capillitia.

TISSUE & CELL 1973 5 (3) 349–357

PHYSICAL SEPARATION OF NUCLEI FROM TWO INDEPENDENT PLASMODIA OF *PHYSARUM POLYCEPHALUM* AFTER FUSION

J. J. McCORMICK. From the Michigan Cancer Foundation, Detroit, Michigan 48201

THE JOURNAL OF CELL BIOLOGY · VOLUME 62, 1974 · pages 227–231

Experimental Cell Research 82 (1973) 197–209

A CHARACTERIZATION OF RIBONUCLEIC ACID IN THE MYXOMYCETE *PHYSARUM POLYCEPHALUM*

P. W. MELERA[1] and H. P. RUSCH

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

SUMMARY

This report deals with the quantitative extraction of total nucleic acid (TNA) containing undegraded RNA from the slime mold *Physarum polycephalum*. With the use of a three-step phenol extraction technique, approx. 95 % of the nucleic acid optical density and 90 % of the ^{3}H-uridine incorporated radioactivity were routinely recovered in the extracts. With the use of this technique it was shown that (1) the TNA mg dry wt of the mold did not change throughout the mitotic cycle, even though the dry wt doubled; this indicates a continual net synthesis of nucleic acid throughout the cycle; (2) the relative proportions of the various nucleic acid components did not change significantly during the cycle and were found to be DNA, 6 %; rRNA, 82 %; and sRNA, 12 %; (3) RNA molecules with mol wts of 4.1 m and 1.9 m, which exhibit properties of rRNA precursors were found in plasmodia labeled for 20 min with ^{3}H-uridine. Furthermore, there appears to be an RNA fraction, found only in nucleic acid preparations presumably enriched in nuclear RNA components, which is heat-labile, does not enter 2.6 % acrylamide gels during 4 h of electrophoresis, and has a uridine/methyl ratio different from the presumed rRNA precursors and mature rRNA.

Mycologia 65, 1284-1295 (1973)

A MYXOMYCETE WITH A SINGULAR MYXAMOEBAL ENCYSTMENT STAGE

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AND

CONSTANTINE J. ALEXOPOULOS

Department of Botany, University of Texas, Austin, Texas 78712

SUMMARY

An aberrant culture of what is probably *Didymium nigripes* is described in which free-living myxamoebae seemingly constitute the primary trophic phase. Following vegetative growth, most of the myxamoebae collect into simple aggregations and form mounds or erect tapered columns prior to encystment. Such columns consist of similar microcysts throughout and show no demarcation into areas of supportive *vs.* propagative cells. Microcysts germinate and release myxamoebae which may promptly renew vegetative growth, or in aqueous media develop flagella and form swarm cells. Plasmodia appear rarely in the myxamoebal cultures, but when formed these can be cultivated by frequent transfer to fresh substrates preinoculated with *Escherichia coli* or *Aerobacter aerogenes*. When not retransferred, such plasmodia either produce poorly formed sporangia within 6–8 da or, failing to fruit (as often occurs), collect into darkly pigmented moribund masses and disintegrate. Sclerotia are not formed naturally and attempts to induce sclerotization have been unsuccessful. Of special significance is the consistent reappearance after an additional week (±) of the myxamoebal stage at the sites where fructification was effected or attempted. The origin of the emergent myxamoebae is not known, but their presence, renewed growth, and subsequent encystment serve to insure continuity of the slime mold even in the absence of normal sporangia and spores.

Sexual and somatic cell fusions in the heterothallic slime mould *Didymium iridis* 1 Fusion assay, fusion kinetics and cultural parameters

Ian K. Ross, G. L. Shipley and R. J. Cummings

Department of Biological Sciences, University of California, Santa Barbara, California 93106, U.S.A.

Microbios 1973 7 149–164

Abstract

A biological assay for measuring the rate and numbers of both sexual and somatic cell fusions in the heterothallic slime mould *Didymium iridis* was described. Various cultural parameters that affected these cell fusions were examined. A model system of cell differentiation leading to sexual fusion competence was presented and the significance of the assay and the model to cell fusions in general was discussed.

Sexual and somatic fusion in the heterothallic slime mould *Didymium iridis* 2 Effects of actinomycin D, cyclohexamide and lysosome stabilizers

Ian K. Ross and Gregory L. Shipley

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Microbios 1973 7 165–171

Abstract

Preliminary experiments with the sexual and somatic fusion assay system were performed. Effects of actinomycin D, cyclohexamide and three lysosome stabilizers were studied. The results were discussed in relation to the model system of cell fusion proposed for *Didymium iridis* and to general theories of cell fusion.

Experimental Cell Research 82 (1973) 315–318

ANNULAR STRUCTURES IN ISOLATED NUCLEI OF *PHYSARUM POLYCEPHALUM*

J. H. N. SCHEL and F. WANKA

Department of Chemical Cytology, University of Nijmegen, Toernooiveld, Nijmegen, The Netherlands

SUMMARY

Whole-mount preparations of isolated interphase nuclei of *Physarum polycephalum* show annular structures. The outer and inner diameters are about 1 080 and 580 Å respectively. Ultrastructural features of the annuli are described; an association of the rings with fibers of various diameters, probably of chromatin nature, is also reported.

DEVELOPMENTAL BIOLOGY VOLUME 37, 213-218 (1974)

Changes in the Activity of Selected Enzymes during Starvation of *Physarum polycephalum*

R. L. TAYE[1]

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Accepted October 24, 1973

The levels of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, lactate dehydrogenase, and cyclic phosphodiesterase activities were examined in growing and starving plasmodia of *Physarum polycephalum*. The activities of lactate dehydrogenase, 6-phosphogluconate dehydrogenase, and glucose-6-phosphate dehydrogenase decreased whereas that of cyclic phosphodiesterase increased. The change in activity of lactate dehydrogenase was the result of the variation of the activity of a single enzyme quite similar to the lactate dehydrogenases of higher animals.

TITLES AND SUMMARIES IN PRESS

POOLS OF DEOXYRIBONUCLEOSIDE TRIPHOSPHATES IN THE MITOTIC CYCLE OF *PHYSARUM*

Danielle Bersier and Richard Braun

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SUMMARY

The pool sizes of ribonucleoside and deoxyribonucleoside triphosphates were determined in the synchronous mitotic cycle of the acellular slime mold *Physarum polycephalum*. Averaged over the whole mitotic cycle the pool of all four ribonucleoside triphosphates was found to be 32.0 µmol/g protein and that of the deoxyribonucleoside triphosphates 0.63 µmol/g protein. For the eight nucleoside triphosphates the following average pool sizes were found, expressed in µmol/g protein: ATP 18.6, GTP 5.37, CTP 2.77, UTP 5.20, dATP 0.028, dGTP 0.077, dCTP 0.18, dTTP 0.33.

At the beginning of the S-phase and shortly before it, the pools of all four deoxyribonucleoside triphosphates are higher than in the middle of the G2-period. This pattern is particularly noticeable for dATP and dCTP, for which the pools expand by a factor of more than five.

Biochim. Biophys. Acta, in press

TAXONOMIC STUDIES IN THE MYXOMYCETES. IV. *PROTOPHYSARUM PHLOIOGENUM* GEN. ET. SP. NOV. (PHYSARACEAE)

Meredith Blackwell and Constantine J. Alexopoulos

Cell Research Institute and Department of Botany
The University of Texas at Austin, Austin, Texas 78712

SUMMARY

A new genus and species of Myxomycetes (order Physarales) is described. The organism, which has been maintained in laboratory culture for seven years, appears to form a link between the Echinosteliales and the Physarales, with closer affinities to the latter. Its distribution is confined to the type locality (Boulder, Colo., U. S. A.) and it is known only from moist chamber culture on the bark of *Ulmus americana*. In general appearance it resembles *Lamproderma*, *Comatricha* and *Macbrideola* but the subhypothallic development of its sporophore and its phaneroplasmodial assimilative stage preclude its inclusion in the Stemonitales.

Mycologia, in press

INDUCTION OF SOMATIC FUSION AND HETEROKARYOSIS IN TWO INCOMPATIBLE STRAINS OF PHYSARUM POLYCEPHALUM

William R. Jeffery and Harold P. Rusch

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

Abstract

These studies describe a method for the induction of somatic fusion and production of heterokaryons between two incompatible strains of the myxomycete Physarum polycephalum. It was shown that plasmodia disrupted into small nucleated pieces by homogenization undergo fusion and plasmodial reorganization when incubated under defined conditions. Homogenization and reorginization of two incompatible strains of P. polycephalum resulted in the formation of some heterokaryotic plasmodia which showed the characteristic nuclei of both original strains. Analysis of the fusion characteristics of the reorganized plasmodia and the original strains indicated that the genes which control somatic compatibility were expressed in both nuclear types within the heterokaryotic plasmodia. These studies support the hypothesis that somatic fusion is controlled at the level of the plasmodial surface.

Developmental Biology, in press

Scanning Electron Microscopy of Freeze-Fractured Sclerotia of Physarum polycephalum

N. Kislev and I. Chet

Department of Plant-Pathology and Microbiology
The Hebrew University, Faculty of Agriculture, Rehovot, Israel

Summary

The ultrastructure and architecture of freeze-fractured sclerotia of Physarum polycephalum was studied by a scanning electron microscope (SEM). The sclerotia are built of many spherules grouped together in a common outer coat. Each spherule has hard walls which separate it from its neighbors. The spherules are rounded in 2 day old sclerotia, and have a lobe-like structure three weeks later. A new simple technique for obtaining freeze-fractured biological material is described and compared with freeze-fracture in a freeze-etching apparatus. The ultrastructural details of the fractured sclerotia are described.

Tissue and Cell, in press

The Rapid Intranuclear Accumulation of Preexisting Proteins in Response to High Plasmodial Density in Physarum polycephalum

Claude Nations, Wallace M. LeStourgeon, Bruce E. Magun and Harold P. Rusch

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

Summary

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

Experimental Cell Research, in press

Activities of DNA Degrading Enzymes in the Slime Mold *Physarum polycephalum*

Evidence for Five Different Enzymes

B. J. J. Polman, H. M. J. Janssen and Ch. M. A. Kuyper

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Received August 20, 1973

Abstract. Crude extracts of *Physarum polycephalum* contain five DNA degrading enzyme activities. One enzyme activity degrades native DNA with a maximum activity at pH 3.2. Four others degrade heat-denatured DNA and have their maximum activity at pH's 3.4, 4.0, 7.6 and 8.5 respectively. The five DNA degrading activities react in different ways to administration of divalent cations and show different stabilities towards heat inactivation or incubation conditions.

Archiv fUr Mikrobiologie, in press

THE AMOUNT OF DNA CODING FOR rRNA DURING DIFFERENTIATION (SPHERULATION) IN *PHYSARUM POLYCEPHALUM*

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(Received February 5th, 1974)

Summary

The amount of DNA coding for rRNA in growing and starved plasmodia of *Physarum polycephalum* was determined by RNA · DNA hybridization: nuclear DNA was extracted from macroplasmodia and from starved microplasmodia (spherules) and hybridized with 19 and 26 S rRNA. No difference was found between the two DNA preparations; both hybridized at saturation to about 0.2% with rRNA.

Biochimica et Biophysica Acta, in press

Plasmalemma Invaginations as Characteristic Constituents in Plasmodia of Physarum polycephalum

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Abstract

The protoplasmic organization in plasmodia of Physarum polycephalum grown on agar or filter paper, with oats or with a partially defined medium as food, was studied by light- and electron microscopy. Phase contrast microscopical observation of unstained 1 µm sections of plasmodia fixed in situ revealed an extensive system of plasmalemma invaginations in all parts of the plasmodium, irrespective of the cultivation method. In plasmodial strands involved in shuttle streaming, the endoplasmic channel is surrounded by an extensive labyrinth system which is continuous with peripheral plasmalemma invaginations. Thus, an intricate system of extracellular clefts characterizes the stationary ectoplasmic wall of the vein while in the streaming endoplasmic core the invagination system is not present.

Cytobiologie, in press

THE FUNGI

VOLUME IV B

A Taxonomic Review with Keys: Basidiomycetes and Lower Fungi

AN ADVANCED TREATISE

G.C. Ainsworth, F.K. Sparrow and A.S. Sussman, eds., Academic Press, New York, 1973

CHAPTER 3

Myxomycetes[1]

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I. GENERAL CHARACTERISTICS

The assimilative stage of this class is a free-living, acellular, multinucleate, amoeboid mass of protoplasm, the plasmodium, enclosed by an amorphous gelatinous sheath, varying from microscopic to an extensive system of branching and anastomosing veins in which the protoplasm streams rhythmically and reversibly. Under favorable conditions the plasmodium becomes converted into one or many fruiting bodies (sporophores). Reproduction occurs by spores: in the exosporous forms they are borne singly on hairlike stalks, in the endosporous forms they are found inside stalked or sessile spore cases which are seated on a horny, membranous, spongy, or calcareous base, the hypothallus. Spores on germination produce one to four naked myxamoebae or flagellated swarm cells, the latter with one or two (rarely more) anterior whiplash flagella, invariably with at least two basal bodies. Asexual reproduction occurs by binary fission of myxamoebae or fragmentation of the plasmodium; sexual reproduction takes place by fusion of compatible myxamoebae or swarm cells forming zygotes which grow into plasmodia. Myxomycetes are homothallic or heterothallic. Mitosis is astral in myxamoebae, intranuclear in zygotes, and intranuclear and synchronous in plasmodia. Under conditions unfavorable for growth or metabolism, the myxamoeba encysts forming a microcyst, and the plasmodium either fragments into a number of disconnected cysts or becomes transformed into a horny mass, the sclerotium, consisting of usually multinucleate cell-like units, the spherules, capable of reforming the plasmodium under favorable conditions.

[1]Supported by National Science Foundation Grant GB-6812X. I would like to thank Dr. Raymond W. Scheetz for executing the line drawings that appear in this chapter.

CONTRACTILE PROTEINS FROM THE MYXOMYCETE PLASMODIUM

Advan. in Biophys., Vol. 5, pp. 143–176 (1973)

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SUMMARY

Contractile proteins, myosin B, myosin A, and actin have been isolated and purified from the myxomycete plasmodium, which shows a vigorous protoplasmic streaming. Native tropomyosin has been also extracted from plasmodium. Generally speaking, though the taxonomical positions of the two organisms are very distant, the properties of plasmodium actin and myosin A are very similar to those of corresponding proteins from rabbit striated muscle, especially when they are compared with each other in the monomer state. For example, the molecular weight of plasmodium actin is 44,000, which is almost the same as that of actin from muscle. The molecule of plasmodium myosin A has a rod-like structure with a globular head at one end just like that of muscle myosin A. The amino acid compositions of actin and myosin A are very similar in the two organisms. Plasmodium G-actin can polymerize with muscle G-actin to form a copolymer of F-actin. Plasmodium F-actin can make the hybrid actomyosin with muscle myosin A (or *vice versa*). It is highly probable that the actin and myosin A could have existed in a more primitive form of organism before it evolved to myxomycetes and higher organisms.

However, there are some remarkable differences in the state of polymers of actin and myosin A in the two organisms. Plasmodium myosin A forms only oligomers, up to, perhaps, pentamers at low salt concentrations including the physiological concentration of plasmodium (0.03 M KCl, pH 7.0). Plasmodium G-actin can polymerize to a different polymer state from F-actin in the presence of Mg^{2+} which seems very flexible on electron micrographs and shows ATPase activity. This flexible polymer of plasmodium actin shows a reversible conformational change under the physiological conditions of plasmodium (0.4 mM ATP) varying with the increase and decrease of ATP concentration. The rate of change in the length of the polymer is estimated to be more than 30% of the original length of the polymer.

The gel layer of peripheral parts of plasmodium shows contraction and relaxation, each lasting 2 or 3 min, to push out or suck in the inner sol. It has been reported that many bundles of actin filaments exist in the outer gel layer of plasmodium although thick filaments have not been observed in plasmodium. The fact that the rate of contraction of the peripheral parts is less than 10% of the contraction or relaxation of the gel layer is explained by the folding or unfolding of actin filaments which is induced by the change of the inner conditions in plasmodium.

DNA DEGRADING ENZYMES OF THE SLIME MOLD PHYSARUM POLYCEPHALUM SCHWEIN.

by

Bernardus Jacobus Johannes Polman

(A thesis presented towards the degree of Doctor of Natural Sciences at the Catholic University of Nijmegen, June, 1974)

GENERAL DISCUSSION AND SUMMARY

The acellular slime mold Physarum polycephalum Schwein. possesses several DNA degrading enzymes. In crude extracts these enzymes show maximal activity at different pH's. One enzyme activity degrades native DNA with a maximum activity at pH 3.2. Four others degrade heat denaturated DNA, and have their maximum activities around pH 3.4, 4.0, 7.6 and 8.5 respectively.

These activities are affected by divalent cations in different ways. The acid activities were inhibited by Mg^{2+}, Mn^{2+}, Ca^{2+} and Co^{2+}; however, to different extents. Zn^{2+} inhibited the pH 4.0 activity, but stimulated the pH 3.2 activities. Also EDTA inhibited the acid activities. Probably these enzymes need the presence of specific divalent cations in the crude enzyme extracts to attain their maximal activity (chapter IV).

The activities at pH 7.6 and pH 8.5 were inhibited by Ca^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+} and EDTA. Addition of Co^{2+}, or EDTA suppressed their activity completely. On the contrary, the alkaline activities are stimulated by Mg^{2+} and Mn^{2+}. Mg^{2+} had the greatest effect on the pH 7.6 activity; 5 mM $MgCl_2$ produced the highest stimulation at pH 7.6, while at pH 8.5 already 1 mM $MgCl_2$ gave maximum stimulation. On the other hand, Mn^{2+} stimulated most the pH 8.5 activity and 5 mM $MnCl_2$ had the highest effect (chapter IV).

Crude enzyme extracts (pH 7.1) were kept at different temperatures. The enzyme activity decreased in dependency on the increasing temperature in different ways. The pH 3.2 activities, especially the pH 3.2 activity on denaturated DNA, were most thermolabile. The activities at pH 4.0 and 8.5 were most thermostable (chapter IV).

Unlike the other activities the pH 3.2 activities decreased during the incubation, probably due to the low pH, because in the crude enzyme extract (pH 7.1) the activities were stable at 30°C. The pH 3.2 activity on denaturated DNA became inactivated most strongly during the incubation (chapter IV).

From the results mentioned above - different effect of divalent cations, different stability towards higher temperatures, different stability of the 3.2-DNases during the incubation - was concluded that the activities consist of different enzymes (chapter IV). Only Hiramaru et al. (1969) described the existence of DNA degrading activities in slime molds. These authors showed in crude enzyme extracts of Physarum polycephalum only one DNase activity on denaturated DNA with maximum activity at pH 4.5. After purification the activity turned out to be

an aspecific nuclease. However, they prepared the crude enzyme extracts in a way completely different from ours in phosphate buffer (Hiramaru et al., 1969a). We found phosphate buffers to be strongly inhibitory on the alkaline activities.

From the distribution of the five DNase activities over several cell fractions and from the different pattern of bands after electrophoretic separation of the activities more evidence was obtained that the activities belong to different enzymes (chapter V).

After removing unbroken microplasmodia and other large particles from the homogenate with centrifugation at 90 g during 5 minutes the homogenate was divided into five successive fractions, namely a 500 g pellet, a 2500 g pellet, a 25.000 g pellet, a 100.000 g pellet and a 100.000 g supernatant. The greater part of all enzyme activities was localized in the cytosol, the 100.000 g supernatant. But the DNase activities were to a greater extent associated with the particle fractions than the RNase activities (chapter V).

The distribution of the DNase activities over the four particle fractions was compared to the distribution of the RNase activities over these fractions. Namely because the RNase activity with yeast RNA as substrate possessed only one pH optimum at pH 3.8 - 4.0 (chapter V).

The distributions of the RNase activities measured at pH 3.2, 4.0, 7.6 and 8.5 were highly correlated. On the other hand, the distributions of the DNase activities correlated badly compared to the RNase activities, except the distributions of the DNase activities at pH 7.6 and 8.5 which correlated very well. The distribution of the DNase activity compared to that of the RNase activity at the same pH was badly correlated, except the DNase and RNase activities at pH 4.0. The distribution of these two activities over the four cell fractions was highly correlated (chapter V).

After electrophoretic separation of the proteins of the crude enzyme extract on polyacrylamide gels, DNase activity in the gels was detected according to Boyd and Mitchell (1965) and the RNase activity according to Wilson (1969).

After incubation of the gel at the acid pH's 4 - 5 bands were visible, at the alkaline pH's 7 - 9 bands. The relative intensity of the bands differed between the various activities. The RNase activities showed only two bands at any pH. However, after incubation of the gel at pH 7.6 or pH 8.5 one of the bands was hardly visible (chapter V).

From the cell fractionation experiments and from the electrophoretic separation of the enzymes it was concluded that the DNase activities consisted of a mixture of different enzymes with maximum activity at different pH's.

This conclusion was supported by the data obtained by the chromatographic separation on DEAE-cellulose columns of the acid-soluble products from salmon sperm DNA after incubation with crude enzyme extracts. At every pH mixtures of mono- and oligonucleotides were obtained. The activities at the various pH's could not be divided into exo- or endonucleases. Probably every activity consisted of mixtures of these two types of enzymes (chapter V).

Polman - DNA Degrading Enzymes of P. polycephalum (continued)

The behaviour of the DNase activities under changing physiological conditions was studied. The acid DNases as a group behave in a way different from the alkaline DNases (chapter VI).

1. In growing microplasmodia in shaken cultures the specific activities of the acid DNases increased after about 25 hours after inoculation. The specific activities of the alkaline DNases decreased during the first 10 hours after inoculation, remained more or less constant until about 25 hours and increased after that.
2. The alkaline activities showed during the mitotic cycle 3 maxima in specific activity, namely during the early S-phase (0 - 1 hour after mitosis), the late S-phase (2 - 3 hours after mitosis) and the mid G-phase (5 - 7 hours after)mitosis). Although in some experiments the acid activities showed maxima in specific activity, these maxima were not reproducible. They were not observed in all experiments.
3. During spherulation the DNase specific activities increased until about 10 hours after the start of the starvation, the acid activities remained more or less constant until about 30 hours of starvation and decreased afterwards. The alkaline specific activities remained increasing during the spherulation.
4. After supplying complete medium to spherules the acid specific activities increased, the alkaline specific activities, however, decreased and started to increase only at about 25 hours after germination.

The fluctuations of the DNase specific activities were correlated with the fluctuations in the DNA and RNA contents relative to the protein contents in the shaken cultures during the different physiological conditions. The DNase activities could be connected with the breakdown of nucleic acids entering the cell for example by phagocytosis, or with the synthesis of RNA and DNA. Firm conclusions, however, could not be drawn from these data (chapter VI).

We tried to demonstrate DNase activities in nuclei isolated from microplasmodia of Physarum polycephalum. Previously, however, the nuclear isolation method was studied and several nuclear isolation methods were compared. The procedure of Mohberg and Rusch (1971) showed to yield best nuclear preparations as judged by phase contrast microscopy of these preparations. However, an isolation medium containing only salts was in some cases preferable to the sucrose medium of Mohberg and Rusch. As every isolation medium causes artefacts the salt medium could be used as a good alternative for the sucrose medium (chapter VII).

Nuclear preparations from Physarum polycephalum possessed DNase activity with maximum activity at pH 4.0. The activities, however, differed from experiment to experiment. The specific activities of the DNases in the crude enzyme extract were 5 - 50 times higher than in the nuclear preparations. The fraction of total acid phosphatase activity in the nuclear preparations was higher than the fraction of total DNase activity in these preparations. Probably the DNase activity of the nuclear preparations was mainly brought about by contamination of these preparations with cytoplasmic DNases (chapter VIII).

ADDITIONAL ARTICLES IN PRINT

D. Bersier and R. Braun
"Effect of cycloheximide on pools of deoxyribonucleoside triphosphates"
Experimental Cell Research 84, 436 (1974)
(For summary, see PNL 5, 38, 1973)

R. Braatz and H. Komnick
"Vacuolar calcium segregation in relaxed Myxomycete protoplasm as revealed by combined electrolyte histochemistry and energy dispersive analysis of x-rays"
Cytobiologie 8, 158 (1973)
(For summary, see PNL 5, 38, 1973)

H. Fouquet and R. Braun
"Differential RNA Synthesis in the Mitotic Cycle of Physarum polycephalum"
F.E.B.S. Letters 38, 184 (1974)
(For summary, see PNL 5, 39, 1973)

D.N. Jacobson and C.E. Holt
"Isolation of Ribosomal RNA Precursors from Physarum polycephalum"
Arch. Biochem. Biophys. 159, 342 (1973)
(For summary, see PNL 5, 39, 1973)

W.M. LeStourgeon, C. Nations and H.P. Rusch
"Temporal Synthesis and Intranuclear Accumulation of the Nuclear Acidic Proteins During Periods of Chromatin Reactivation in Physarum polycephalum"
Arch. Biochem. Biophys. 159, 861 (1973)
(For summary, see PNL 5, 40, 1973)

ABSTRACTS OF MEETING PRESENTATIONS

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NUCLEOTIDE SEQUENCES AT THE JUNCTION BETWEEN RNA AND NASCENT DNA IN THE SLIME MOLD, PHYSARUM POLYCEPHALUM. M. Anwar Waqar* and Joel A. Huberman. Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

When α-^{32}P-deoxyribonucleoside triphosphates are injected into living plasmodia of the slime mold, Physarum polycephalum, they are first incorporated into short strands on the order of 100 nucleotides long and then, with longer pulse times, into much longer strands comparable in size to those of non-replicating DNA. When the short ^{32}P-labeled strands are purified and then hydrolyzed with alkali, a small fraction of the ^{32}P-label is released as 2',3'-ribonucleotides. By labeling with all 4 α-^{32}P-deoxyribonucleoside triphosphates in separate experiments and by separating the labeled ribonucleotides with paper electrophoresis and chromotography, we have determined that all 16 possible combinations of ribo- and deoxyribonucleotides exist at the junctions between RNA and DNA in these short strands. Control experiments demonstrate that all ^{32}P label is incorporated on the 5'-side of deoxyribonucleosides, that the label released after alkaline hydrolysis is in ribonucleotides (not deoxyribonucleotides), and that the amount of P^{32} label transferred to ribonucleotides is consistent with a chain length on the order of 100 nucleotides.

Fed. Proc. 33, 1352 (1974)

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FURTHER STUDIES ON SLIME MOLD ACTIN. M. R. Adelman. Duke University, Durham, N.C. 27710. Actomyosin partially purified from Physarum polycephalum plasmodia (Biochem. 8: 4964 & 4976) contains, besides actin and myosin polypeptides (45,000 and 225,000, respectively, by SDS-gel electrophoresis), several low MW ones (10-40,000); these are absent from actomyosin further purified by methods expected to stabilize actin filaments. However, much of the actin is unstable (behaves as a monomer) during such processing. Extracts prepared in 10 mM Tris-maleate, 5 mM β-MSH, pH 7.0 (TM) contain actin (but not myosin) which sediments slowly (low n-mer) and is only converted to a fast-sedimenting form (polymer) in the presence of $\sim$1 mM ATP. This specific "stabilizing" effect of ATP is enhanced by KCl ($\sim$100 mM) and $CaCl_2$ ($\sim$1 mM). The impure actin which can thus be sedimented from a TM extract forms symmetric polymers (low solution viscosity). Chromatography on Sephadex G-200 yields a pure preparation which forms asymmetric polymers (high viscosity). Extracts made with TM plus ATP, KCl, and $CaCl_2$ contain as much actin as TM extracts and, when the actin is purified under such "stabilizing" conditions, no low MW polypeptides co-purify with it. Since slime mold actin is very easily extracted and exists in solution as a stable polymer only under restricted conditions, the filaments may be metastable in situ. The exact form of the actin polymers may be a function of non-actin proteins. If the plasmodium contains tropomyosin- and/or troponin-like proteins, they must differ significantly from their muscle counterparts. Supported by NIH Grant #'s 5-504-RR-6148 and 1-R01-GM-20141.

Fed. Proc. 33, 1522 (1974)

Abstract of a paper presented at the Sixth Annual Miami Winter Symposia ("Membrane Transformation in Neoplasia"), January 14-18, 1974, p. 106.

THE REPLACEMENT OF LIGHT BY c-AMP FOR THE STIMULATION OF DIFFERENTIATION INDUCING PHOTOMETABOLIC ION TRANSPORT.

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Light induces sexual differentiation (sporulation) of the plasmodial slime mold *Physarm polycephalum*. During the light induction period, proton uptake and concomitant K^+ release is reflected in the alkalinization of the culture medium. This ion exchange response, accelerated with the use of the cyclic decapetide antibiotic gramicidin S has been studied in suspensions of microplasmodia where light sensitizes this reaction (1). Exogenous Ca^{2+} suppresses this proton uptake and light partially reverses it (2). By electron microscopy extensive calcium accumulation has also been observed in certain vacuoles and in mitochondria during illumination (3). Ruthenium red and La^{3+} at optimum concentrations, stimulate, or at higher concentrations, suppress, this response indicating the involvement of the plasmodial surface in this calcium-controlled response. These inhibitors, particularly ruthenium red, probably act on a mucopolysaccharide layer containing galactose sulfate polymers (4) coating the outer surface of the plasma membrane (5). The role of this layer is thus implicated in the availability and transport of Ca^{2+} into the plasmodium and is possibly related to a similar function suggested for the external lamina of heart cells in calcium transport (6). Of particular significance is the ability of caffeine in the dark to mimic the action of light. Analysis of this caffeine effect reveals that dibutyryl adenosine 3',5' - monophosphate (dib c-AMP) at 1μM in the dark replaces light. Since theophylline and sodium fluoride also promote sensitization it is suggested that an early event in this differentiation is a light-driven elevation of the c-AMP level. Of coordinate importance is the probable implication of this mechanism in the rapid light *inhibition* of P. *polycephalum* growth. The comparative effects of several inhibitors on the ion movement, on sporulation and on growth support the specifity of the dib c-AMP action and suggest the function of a common primary photometabolism for both the induction of sporulation and the inhibition of growth. The effect of dib c-AMP so far observed is generally

Daniel - "The Replacement of Light . . . " (continued)

compatible with its effect on other differentiating systems whether induction of sporulation (e.g., 7, 8, 9), control of morphology (e.g., 10, 11), or control of growth (e.g., 12, 13) and may also be related specifically to the effect on the visual receptor mechanism (14) and on calcium transport (15, 16).

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Abstract of a paper presented at the Fifth International Congress of Radiation Research, Seattle, Washington, July 14-20, 1974.

"Temporal Order of DNA Replication Following γ-Radiation of Physarum polycephalum." Thomas E. Evans and Helen H. Evans, Case Western Reserve University, Cleveland, Ohio 44106, USA.

Sensitivity to ionizing radiation in Physarum polycephalum (as measured by mitotic delay) is most marked at the beginning of the S period; however, DNA replication occurs at almost the normal rate following irradiation (Nygaard, et al., in Advances in Radiation Research, Duplan and Chapiro, eds., Gordon and Breach, London, in Press). In order to determine if the temporal sequence of DNA replication is altered following radiation, density shift experiments were carried out (c.f. Braun and Will, Biochim. Biophys. Acta, 174, 246, 1969). It was found that the administration of 10 KR either at the beginning of S or in mid-S (5 and 60 min following metaphase, respectively) had no effect on the ordered progression of DNA replication. Thus, neither premature synthesis of late replicating species nor premature re-initiations at the temporal origin were observed. We conclude that the mitotic delay caused by irradiation of Physarum during the S period does not result from an alteration in the rate or order of DNA synthesis. Supported by U.S.A.E.C. Contract W-31-109-ENG-78.

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