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DEPARTMENT OF MICROBIOLOGY & CELL SCIENCE

UNIVERSITY OF FLORIDA

1059 McCarty Hall
Gainesville, FL 32611
Phone (904) 392-1096

June 23, 1984

Dear Physarum Newsletter readers and contributors:

This is our second issue from Gainesville, and we are getting the bugs out of the production system. Ann Curry has been my capable assistant on both issues. We apologize for the mediocre quality of the Xeroxing on both of these issues; our departmental machine is not very good. We are getting a new one before the next issue. Hopefully, quality will improve.

Please let me know of any address changes, corrections, etc. in a timely manner so that issues are sent to the correct places. And PLEASE SEND ME ABSTRACTS AS SOON AS YOU HAVE THEM! It appears that we will be able to put out at least 2 and maybe 3 issues per year, and prompt receipt of abstracts here will assure that issues are up to date and useful. Also, you may occasionally find an abstract or two about something other than Myxomycetes. Some are things included because I think you may be interested in them; others may slip in inadvertantly due to sloppy editing and screening. Ignore them!

A brief word about the meeting in France. One of our colleagues, Ian Walker from Oxford University, became very ill on the bus back to Perpignan from Font-Romeu. He was flown to Paris with us and taken to the hospital, where he died two days later (30 April) of pericarditis. We were all shocked to learn of his death and extend sympathy to his colleagues and widow. Except for this sad note at the end, the Font Romeu meeting was a large success, well organized and executed by Michel Wright, Marie-Louise Oustrin, Yvette Tollon, Lluis Mir, Andre Moisand, and colleagues from Toulouse. The Lycee Climatique et Sportif is in a beautiful setting high in the Pyrenees. Michel was a slave-driver, keeping us in sessions from early till late, but we did manage an afternoon trip to several surrounding churches and a huge solar furnace. He had also arranged some excellent lectures on related cell biology topics by non-Physarum workers. All told, a memorable experience. A set of abstracts is included for U.S. Newsletter subscribers who did not attend the meetings in France.

Best wishes to all!

Henry Aldrich

Henry Aldrich, Editor

McARDLE LABORATORY
FOR CANCER RESEARCH
DEPARTMENT OF ONCOLOGY
MEDICAL SCHOOL, UNIVERSITY OF WISCONSIN

May 29, 1984

Dr. Henry C. Aldrich
Department of Botany
University of Florida
Gainesville, FL 32611

Dear Henry,

I enclose Abstracts from some current reprints and preprints from our laboratory, including that of the Ph.D. thesis of Tim Schedl in Molecular Biology. This thesis will be available from University Microfilms in Ann Arbor, Michigan. Anyone interested can also request a copy of this thesis from my laboratory.

The Physarum meeting in Madison will be held July 9-13, 1985 in the Friedrick Conference Center on campus. The meeting will be run as a Workshop, with all participants staying at the Center. There will be five themes - the cell cycle; differentiation; motility; genetics and genome structure; and technical feasibilities. For the first four themes, there will be a full morning session of prepared talks and then an informal afternoon session of discussions and posters. In the evening, an outside rapporteur will join with the morning's chairman to lead an open evaluation of what directions seem to be promising. The fifth theme, technical feasibilities, will be strictly related to Physarum biology.

To maintain the Workshop atmosphere, we hope to include active researchers from several countries and to emphasize the participation of new contributors. We hope to have some travel funds available to accomplish these goals. Persons wanting to participate should submit an abstract to me by February 1, 1985.

Sincerely,

William F. Dove
Professor of Oncology and Genetics

WFD:kjd

Encl.

450 North Randall Avenue, Madison, Wisconsin 53706 608-262-2177

Restriction Fragment Length Polymorphisms and Gene Mapping in Physarum

Thomas G. Laffler
Department of Microbiology and Immunology
Northwestern University Medical School
303 E. Chicago Ave.
Chicago, IL 60611
(312) 649-8074

At the recent Physarum meeting in Font Romeu a number of investigators have reported the isolation of various sorts of Physarum clones. While it appears that cDNA clones can be isolated without undue difficulty, genomic cloning may still present considerable problems. Nonetheless, significant numbers of unique sequence clones will soon be available. These clones will provide the needed material for developing something that Physarum geneticists have long dreamed of - a genetic map.

In Physarum, the major limitation to mapping has been the lack of routine nutritional mutants to provide landmarks for mapping other, more interesting mutations. Recombinant DNA materials can provide a wealth of these needed landmarks. Each unique sequence clone is a specific probe for a single genetic locus. When a unique sequence clone is used to probe a Southern transfer of an agarose gel on which a restriction digest of genomic DNA has been fractionated, one expects to see a single band of hybridization. Multiple bands can be generated if the restriction enzyme cleaves within the region complementary to the probe or if the two homologous chromosomes differ in the arrangement of the restriction sites flanking the region detected by the probe. The latter instance is a case of a restriction fragment length polymorphism (RFLP). Given the small fraction of the genome that actually codes for proteins and the redundancy of the genetic code, there is considerable latitude of silent variation in the genome. It is not uncommon to encounter these restriction site polymorphisms which are inherited as simple codominant alleles. It has been estimated that 500 well chosen RFLP markers could completely span the human genetic map (1), by extension about 50 markers could cover the Physarum map. Schedl and Dove (2) have reported a hinIII site polymorphism in M_3CV detected by an actin probe, and Schedl et al (3) report other polymorphisms detected by tubulin probes. In each case, while the diploid plasmodium showed multiple bands, each haploid amoebal segregant carried a subset of the diploid pattern defining a single haplotype. In the amoebal population, inheritance of each haplotype followed a simple 1:1 Mendelian pattern, behaving as a single gene - as a landmark.

All that this sort of analysis requires is a probe and DNA's from a diploid strain and several of its haploid segregants. The major limitation is the required set of amoebal DNA's. For the minimum genetic information, one must consider a set of at least 30 amoebal segregants and preparation of their DNA's represents a considerable effort. The Dove-Gull laboratories have already identified RFLP markers closely linked to actin and tubulin genes. We would like to propose that a standard set of strains be established with which we can collect segregation data for a number of polymorphisms. Statistically significant similarities of segregation patterns for two RFLP's will indicate linkage. The database will then reveal any additional linkage relationships as other markers are added. The appeal of such a system is that the database can be examined retrospectively as new

RFLP's are added, so that each probe need be used for only one set of experiments. Since all the different polymorphisms will be segregating in the progeny of a single cross, the same segregants can be analyzed with each probe as it is added to the collection.

It is important to select carefully the strains to be used in this database to maximize the liklihood of finding a polymorphism. Both CL and the various M_3 derivatives appear to descend from the WisI isolate. The WisII is quite distinct from WisI and should be considered an independent strain. A cross between WisI and WisII ought to have a maximum potential for heterozygosity. Jessica Gorman has sporulated WisII and isolated a set of haploid amoebal segregants. One of these, MA275, has been crossed to CLd to generate the "maxihet" plasmodium, MA275 x CLd (3). Burland has crossed MA275 with a number of benomyl-resistant mutations isolated in CLd and has maintained a set of about 45 segregants for RFLP mapping. I would like to propose to function as a repository for these strains and to maintain the planned database. I will gladly send CL and MA275 x CLd DNA to any interested party for preliminary blotting experiments with their clones. If they find evidence of a polymorphism after cleavage with a series of restriction endonucleases, they can contact me for further material or collaboration. With the new generation of blotting membranes, a single blot can be repeatably hybridized and washed. Perhaps one centrally maintained set of blots can meet the needs of several investigators.

I would be delighted to hear from any readers about their thoughts criticisms, and suggestions.

REFERENCES

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3. Schedl, T., J. Owens, W. Dove, and T. Burland (1984) Genetics of the tubulin gene families of Physrum. Genetics, in press.

Genet. Res., Camb. (1983), 42, pp. 285–296
Printed in Great Britain

A new type of plasmodium formation in *Physarum polycephalum*

By ROGER W. ANDERSON*

Department of Biochemistry, University of Oxford, Oxford OX1 3QU, England

AND CHRISTINE L. TRUITT†

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

(Received 7 April 1983 and in revised form 16 August 1983)

SUMMARY

Haploid amoebae of *Physarum polycephalum* may form plasmodia sexually by 'crossing', which involves cellular and nuclear fusion, or asexually by 'selfing', which occurs without nuclear fusion. In most amoebal strains, selfing is seen in clonal cultures only at very low frequency. In the present study, we have shown that selfing occurs at a similarly low frequency in mixtures of crossing-incompatible amoebae, but is stimulated in crossing-compatible mixtures. In certain compatible mixtures involving mutant strains, where crossing is temperature-sensitive, selfing may be stimulated even at a temperature that largely or wholly abolishes crossing. The extent to which selfing is stimulated appears to be influenced by *matB*, a locus which is known to affect the frequency of amoebal fusion. We have failed to detect any filter-transmissible factor that might be responsible for the effects we have observed. We suggest a sequence of events that might bring about 'stimulated selfing' as a consequence of abortive crossing.

J. Protozool., 30(4), 1983, pp. 617–624

The Structure and Composition of the Stalk of the Ciliated Protozoan *Sorogena stoianovitchae*[1]

RICHARD L. BLANTON,[2] STEVEN A. WARNER,[3] and LINDSAY S. OLIVE[4]
Department of Botany, University of North Carolina, Chapel Hill, North Carolina 27514

ABSTRACT. *Sorogena stoianovitchae* is an unusual ciliated protozoan with a life cycle characterized by the aggregation of individual trophic cells to form a multicellular sorogen that rises from the liquid culture medium surface by the secretion of a stalk. The noncellular stalk is a tapered, longitudinally furrowed structure composed of a fibrillar matrix that is initially hydrated, but with time dehydrates, the stalk becoming thin and brittle. This dehydration is of importance from the earliest stages of stalk formation since it results in the formation of the outer sheath-like region of the stalk that appears to provide much of the support of the stalk. Cytochemical tests of the stalk for polysaccharides (including acidic mucopolysaccharides) and proteins are positive. Proteolytic enzymes degrade the stalk. Lectins specific for glucose and N-acetyl-D-glucosamine bind to the stalk. Gas chromatography analysis detected the presence of fucose, glucose, glucosamine, and arabinose, as well as a variety of amino acids, predominantly glycine. The cytochemical and biochemical tests, the ultrastructural data, and the behavior of the stalk material suggest that the stalk is composed of a matrix of complex protein-polysaccharide molecules.

Localization and DNA sequence around the initiation site of ribosomal RNA transcription in *Physarum polycephalum*

Beat Blum[1], Thomas Seebeck[1], Richard Braun[1], Patrick Ferris[2] and Volker Vogt[2]

[1]Institut für Allgemeine Mikrobiologie, University of Bern, Baltzerstrasse 4, 3012 Bern, Switzerland, and [2]Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853, USA

Received 17 May 1983; Revised and Accepted 26 October 1983

ABSTRACT

We have used S1 nuclease to map the initiation site of ribosomal RNA transcription in the acellular slime mold Physarum polycephalum, and we have determined the sequence of 1011 nucleotides surrounding the start site. Consistent with others' observations, there is little homology with the comparable region of other species. As predicted by previous restriction mapping, direct repeats roughly 30 base pairs in length are present upstream of the initiation site and a 148 base pair duplication occurs in the external transcribed spacer. The results also suggest the presence of a processing site within the external transcribed spacer of the ribosomal transcription unit.

Journal of Microscopy, Vol. 133, *Pt* 3, *March* 1984, *pp.* 323–334
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Methylamine tungstate and tannic acid in fixation for high contrast and preservation of fine structure: application to myxomycete plasmodia

by MATTHEW H. CHESTNUT *and* ARTHUR L. COHEN, *Department of Botany, Washington State University, Pullman, WA* 99164, *U.S.A.*

KEY WORDS. Methylamine tungstate, sodium tungstate, TEM methods, tannic acid, slime mould, plasmodium, fixation, membrane visualization, contrast.

SUMMARY

The effects of several fixation methods for electron microscopy were compared, using macroplasmodia of *Badhamia utricularis* (myxomycete) as test objects. A brief osmium tetroxide (Os) exposure followed by a mixture of glutaraldehyde (GA) and tannic acid (TA) with subsequent treatment with methylamine tungstate (MAT) overcame most commonly encountered difficulties in fixing these structures. Lead staining of the sections was necessary to bring out the range of densities inherent in the delicate detail of ground cytoplasm, membranes, and organelle structure retained by Os/GA-TA/MAT. Microfilaments were well preserved with good contrast and little evidence of breakage. Although GA as the initial fixative gave good organelle preservation, Os pretreatment was required to prevent artefactual changes in plasmodial strand morphology. The several fixation procedures tested gave pronounced differences in mitochondrial appearance, in some cases giving a negative stained appearance to the cristae. Some advantages in interpretation may result from such reversed contrast. The high contrast and range of densities achieved following the Os/GA-TA/MAT schedule permitted routine use of thin (silver-grey) sections, thereby potentially increasing the resolution.

Mycologia, 76(2), 1984, pp. 366–369.

LIFESPANS AND SENESCENCE IN SIX SLIME MOLDS

JIM CLARK

T. H. Morgan School of Biological Sciences, University of Kentucky, Lexington, Kentucky 40506

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5-Methyldeoxycytidine in the *Physarum* minichromosome containing the ribosomal RNA genes

Craig A.Cooney*, Harry R.Matthews and E.Morton Bradbury

Department of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616, USA

Received 18 August 1983; Revised and Accepted 12 December 1983

ABSTRACT

5-Methyldeoxycytidine (5MC) was analyzed by high pressure liquid chromatography (HPLC) and by restriction enzyme digestion in rDNA isolated from Physarum polycephalum. rDNA from Physarum M3C strain microplasmodia has a significant 5MC content (about half that of the whole genomic DNA). This rDNA contains many C5MCGG sites because it is clearly digested further by Msp I than by Hpa II. However, most 5MC is in other sites. In particular, alternating CG sequences appear to be highly methylated. HPLC of deoxyribonucleosides shows that most of the transcribed regions contain little or no 5MC. Restriction digestion indicates that there is little or no 5MC in any of the transcribed regions including the transcription origin and adjacent sequences. Over 90% of the total 5MC is in or near the central nontranscribed spacer and most methylated restriction sites are in inverted repeats of this spacer. rDNA is very heterogeneous with respect to 5MC. The 5MC pattern doesn't appear to change with inactivation of the rRNA genes during reversible differentiation from microplasmodia (growing) to microsclerotia (dormant), showing that inactivation is due to changes in other chromatin variables. The 5MC pattern is different between Physarum strains. The possible involvement of this 5MC in rDNA chromatin structure and in cruciform and Z-DNA formation is discussed.

ISOLATION AND PURIFICATION OF TRANSCRIPTIONALLY ACTIVE RIBOSOMAL CHROMATIN FROM THE SLIME MOULD, *PHYSARUM POLYCEPHALUM*

MARTIN CUNNINGHAM *, THOMAS SEEBECK and RICHARD BRAUN

Institute of General Microbiology, University of Bern, Baltzer-Strasse 4, CH-3012 Bern (Switzerland)

(Received June 6th, 1983)
(Revised manuscript received October 26th, 1983)

Biochimica et Biophysica Acta, 781 (1984) 18–29
Elsevier

Key words: Ribosomal gene; Nucleoprotein particle; Active chromatin; (P. polycephalum)

In the acellular slime mold *Physarum polycephalum* the ribosomal genes are all located on linear, extrachromosomal DNA molecules which are clustered in the nucleolus. This report describes the isolation and purification of these ribosomal genes as functionally active chromatin particles. Nucleolar lysates are fractionated by gel filtration to remove ribosomal precursors and other soluble material. The ribosomal chromatin is subsequently separated from contaminating nuclear chromatin by a sucrose gradient centrifugation step. This procedure allows the isolation of the ribosomal genes as intact nucleoprotein particles, which are now amenable to a biochemical analysis of their structural and functional properties.

Biochem. J. (1984) 217, 805–811
Printed in Great Britain

Assay of adenosine 5′-P^1-tetraphospho-P^4-5‴-adenosine and adenosine 5′-P^1-tetraphospho-P^4-5‴-guanosine in *Physarum polycephalum* and other eukaryotes

An isocratic high-pressure liquid-chromatography method

Preston N. GARRISON and Larry D. BARNES
Department of Biochemistry, University of Texas Health Science Center, San Antonio, TX 78284, U.S.A.

(*Received 28 June 1983/Accepted 11 October 1983*)

A5′pppp5′A has been proposed to serve as a molecular signal that triggers DNA replication. When published methods proved to be inadequate for the assay of A5′pppp5′A in *Physarum polycephalum* by h.p.l.c. (high-pressure liquid chromatography), a set of purification procedures was developed that allowed assay of as little as 2pmol of A5′pppp5′A. A5′pppp5′A was purified from cellular extract by covalent boronate chromatography, treated with alkaline phosphatase to hydrolyse residual mononucleotides and analysed by isocratic ion-exchange h.p.l.c. The analysis was facilitated by a pre-column switching procedure that allowed early-eluted species to be diverted from the analytical column. By using this procedure A5′pppp5′A has been detected in *Physarum polycephalum* (1.4pmol/mg of protein), *Saccharomyces cerevisiae* (3.6pmol/mg of protein) and rat liver (3.3pmol/mg of protein). In each case a minor peak was also seen, which was identified as A5′pppp5′G. The identity of both peaks was confirmed by co-elution with standards on isocratic and gradient h.p.l.c. and treatment with enzymes, including a dinucleoside polyphosphate pyrophosphohydrolase from *Physarum polycephalum*.

SEQUENCE ORGANISATION IN NUCLEAR DNA FROM *PHYSARUM POLYCEPHALUM*

ARRANGEMENT OF HIGHLY-REPEATED SEQUENCES

LINDA M. GERRIE, JOHN HUMPHREYS, OLIVER P. PEOPLES and NORMAN HARDMAN *
Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen, AB9 1AS (U.K.)

(Received May 30th, 1983)

Biochimica et Biophysica Acta, 741 (1983) 214–223
Elsevier

Key words: DNA organisation; Repeated sequence; (P. polycephalum)

Recombinant plasmids containing highly repetitive *Physarum* DNA segments were identified by colony hybridisation using a radioactively-labelled total *Physarum* DNA probe. A large number of these clones also hybridised to a foldback DNA probe purified from *Physarum* nuclear DNA. The foldback DNA probe was characterised by reassociation kinetic analysis. About one-half of this component was shown to consist of highly repeated sequences with a kinetic complexity of 1100 bp and an average repetition frequency of 5200. Direct screening of 67 recombinant plasmids for foldback sequences using the electron microscope revealed that about one-half were located in segments of DNA containing highly repetitive sequences; the remainder were present in clones containing low-copy number repeated elements. Analysis of two DNA clones showed that they contained repetitive elements located in over half of all DNA segments containing highly repetitive DNA and that the foci containing these highly repetitive sequences had different sequence arrangements. The results are consistent with the hypothesis that the most highly repeated DNA sequence families in the *Physarum* genome are few in number and are clustered together in different arrangements in about one-sixth of the genome. Over one-half of the foldback DNA complement in the *Physarum* genome is derived from these segments of DNA.

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Direct repeats surrounding the ribosomal RNA genes of *Physarum polycephalum*

Masahira Hattori, Arar Ljljana* and Yoshiyuki Sakaki

Research Laboratory for Genetic Information, Kyushu University, School of Medicine, Fukuoka 812, Japan

Received 11 January 1984; Accepted 1 February 1984

ABSTRACT

Sequence homology was found between the external transcribed spacer and the terminal non-transcribed spacer of Physarum polycephalum rDNA. The homologous sequences were located 2kb upstream from the 18S rRNA gene and 0.3kb downstream from 26S rRNA gene, respectively, and were arranged in a direct repeat manner. Sequence analyses showed that the direct repeats consisted of two parts: one was sequences of about 130bp which showed over 90% sequence homology with each other. The other consisted mainly of many tandem repeats of a 50—52bp unit. The direct repeat-rRNA genes-direct repeat unit was found to be flanked by short direct repetitious sequences. Based on these findings, the significance of the direct repeat is discussed in terms of evolution of rDNA.

European Journal of Cell Biology 32, 67-74 (1983)

Demonstration of different patterns of microtubule organization in Physarum polycephalum myxamoebae and plasmodia using immunofluorescence microscopy

Jane C. Havercroft, Keith Gull[1])

Biological Laboratory, University of Kent, Canterbury, Kent/United Kingdom

Received March 7, 1983
Accepted August 13, 1983

Physarum — microtubules — mitosis — centrioles — immunofluorescence

We have used anti-tubulin antibodies and immunofluorescence microscopy to determine the overall distribution of microtubules during interphase and mitosis in both the myxamoebae and plasmodia of the slime mold Physarum polycephalum. We have paralleled these observations with electron microscopy of the same stages. The myxamoebae possess a network of cytoplasmic microtubules whilst the coenocytic plasmodium does not possess any cytoplasmic microtubules — at either interphase or mitosis. In plasmodia microtubules are, however, elaborated by an intranuclear microtubule organizing centre (MTOC) during prophase of mitosis and these microtubules proceed to form part of the mitotic spindle. There is little difference in the overall distribution and arrangement of microtubules during division of either the myxamoebal or plasmodial nuclei. These findings are discussed in relation to the synthesis of tubulin during the plasmodial cell cycle and the rearrangements of the nuclear envelope during mitosis.

Experimental Cell Research 149 (1983) 359–373

Variance of Ploidy in Mitochondrial Nucleus during Spherulation in *Physarum polycephalum*

S. KAWANO,[1] S. NISHIBAYASHI,[1] N. SHIRAISHI,[2] M. MIYAHARA[2] and T. KUROIWA[1]

[1]*Department of Cell Biology, National Institute for Basic Biology, Myodaijicho, Okazaki 444 and* [2]*Department of Medical Biology, Kochi Medical School, Nankoku, Kochi 781-51, Japan*

In *Physarum polycephalum*, microplasmodia differentiated into spherules when cultures were aged for 8–10 days. Respiration rates of the microplasmodia decreased rapidly with ageing to a 90% decrease in oxygen consumption over 9 days. We studied this phenomena by isolating and characterizing mitochondria from microplasmodia and spherules at different stages of spherulation. Oxygen uptake by the isolated mitochondria decreased with spherulation. Morphological and biochemical analyses showed that mitochondrial differentiation to inactive state was characterized by a decrease not only in dimension but also of content (DNA, RNA and protein). Diminutive mitochondria contained small particle-shaped mitochondrial nuclei. The DNA content, measured by microscopic fluorometry, was about 1.15 and 0.58×10^{-10} g, which corresponded to about 16 and 8 genome copies, respectively (e.g., 32 genome copies per mitochondrion at mitochondrial G1). Restriction endonuclease analysis showed that the physical structure and methylation pattern of the mtDNA had not changed although the DNA content per mitochondrion had decreased remarkably with spherulation. This showed that changes in the ploidy level of the mitochondrial nucleus during spherulation were due to reduction in the number of whole mitochondrial genomes.

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Cellular Clocks and Oscillators

R. R. KLEVECZ,* S. A. KAUFFMAN,† AND R. M. SHYMKO‡

*Departments of *Cell Biology and ‡Radiation Research, City of Hope Research Institute, Duarte, California, and †Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania*

Posttranslational control of ornithine decarboxylase by polyamine-dependent protein kinase[1,2]

GLENN D. KUEHN AND VALERIE J. ATMAR

Department of Chemistry, New Mexico State University, Las Cruces, New Mexico 88003

In nuclei and nucleoli of the slime mold *Physarum polycephalum*, ornithine decarboxylase (OrnDCase) (*M*, 70,000) is phosphorylated by a protein kinase reaction that is dependent on spermidine and spermine. Putrescine antagonizes the phosphorylation. Phosphorylation of OrnDCase inhibits its capacity to catalyze decarboxylation of ornithine. The protein kinase that catalyzes this phosphorylation has many properties similar to those of nuclear protein kinase II, or type G, which has been studied by other groups. The interaction of this protein kinase with OrnDCase resembles the behavior of the OrnDCase antizyme described by other investigators. Phosphorylated OrnDCase binds to purified, palindromic rDNA isolated from nucleoli. It also stimulates transcription of the ribosomal genes by RNA polymerase I in a chromatin form of rDNA. It does not stimulate transcription in a purified, homologous transcription system comprised of RNA polymerase I, rDNA, and phospho-OrnDCase. Thus, phospho-OrnDCase may have a function in promoting rRNA gene transcription but the detailed mechanism is yet unclear. The polyamine-dependent protein kinase and its natural substrate of 70,000 daltons have been demonstrated in other eukaryotic cells, including bovine spermatozoa and rat liver nuclei, and in Ehrlich ascites tumor cells, where the protein kinase is induced by interferon. This phosphorylation system appears to be widely distributed and conserved among eukaryotic species.—Kuehn, G. D.; Atmar, V. J. Posttranslational control of ornithine decarboxylase by polyamine-dependent protein kinase. *Federation Proc.* 41: 3078–3083; 1982.

ELECTRON MICROSCOPY SOCIETY OF SOUTHERN AFRICA – PROCEEDINGS – VOLUME 13 - 1983 23

Elektronmikroskopievereniging van Suidelike Afrika Johannesburg (1983)

MALACHITE GREEN AS A FIXATION ADDITIVE FOR PLANT CELLS

June R. Lawton

Electron Microscope Unit, University of Durban-Westville

Immunocytochemistry of the acellular slime mold *Physarum polycephalum*

IV. Differentiation and dynamics of the polygonal actomyosin system

Wallid Naib-Majani [1], Friedhelm Achenbach [1], Klaus Weber [2], Karl-Ernst Wohlfarth-Bottermann [1], and Wilhelm Stockem [1]

Differentiation (1984) 26:11–22

[1] Institute for Cytology, University of Bonn, Ulrich-Haberland-Str. 61a, D-5300 Bonn 1, Federal Republic of Germany
[2] Max-Planck-Institute of Biophysical Chemistry, Nikolausberg, Am Faßberg, D-3400 Göttingen, Federal Republic of Germany

Abstract. The polygonal arrangement of actomyosin fibrils in different stages of the acellular slime mold *Physarum polycephalum* is correlated with morphogenetic processes at the cell surface. Light and electron microscopic investigations on both endoplasmic drops and thin-spread small plasmodia demonstrate that the differentiation of a polygonal pattern depends on a transient deficiency of plasma membrane invaginations.

Glycerol-extracted specimens show condensation and drastic spatial changes in the organization of the polygonal net after addition of ATP, thus indicating contractile properties of this system. Observations with the polarizing microscope reveal rhythmic changes in fibrillar birefringence intensity corresponding to the protoplasmic streaming activity, i.e., birefringence increases during contraction and decreases during relaxation. Cell fusion experiments, local irradiation with blue light (450 nm), and chemical treatment by impeding the mitochondrial function with DNP (2,4-dinitrophenol) demonstrate morphological as well as physiological interdependences of the actomyosin system, the motive force generation, and the expression of a locomotor polarity in plasmodia of *Physarum polycephalum*.

Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 3163–3167, June 1983
Biochemistry

Complete nucleotide sequence of the 26S rRNA gene of *Physarum polycephalum*: Its significance in gene evolution

(DNA sequence analysis/origin of 5.8S rRNA gene/eukaryote-specific sequence)

TAKESHI OTSUKA*, HISAYUKI NOMIYAMA*†, HIDEAKI YOSHIDA*, TOSHIO KUKITA‡§, SATORU KUHARA‡, AND YOSHIYUKI SAKAKI¶

*Department of Biochemistry, Kyushu University School of Medicine; ‡Department of Agricultural Chemistry, Faculty of Agriculture, Kyushu University; and ¶Research Laboratory for Genetic Information, Kyushu University School of Medicine, Fukuoka, Japan

Communicated by Van R. Potter, January 24, 1983

ABSTRACT The complete nucleotide sequences of the 5.8S and 26S rRNA genes of *Physarum polycephalum* and the transcribed spacer between them were determined. Comparison of the sequences with those of the *Escherichia coli* 23S rRNA and yeast 26S rRNA genes showed that there are 16 highly homologous regions in the sequences of *Physarum* and *E. coli* and that eukaryotes have some eukaryote-specific extra sequences. The sequence immediately following the 5.8S-like region of *E. coli* 23S rRNA was found to be highly homologous to the 5′ terminus of *Physarum* 26S rRNA, indicating that the eukaryote-specific 5.8S rRNA gene is derived from the 5′-terminal region of the prokaryote large rRNA gene.

Food Deprivation Is Not a Prerequisite for the Amoebal to Plasmodial Transition in Physarum polycephalum

Developmental Genetics 4:117–127 (1983)

Dominick Pallotta, Serge Blanchard, and Hélène Larue

Département de Biologie, Université Laval, Québec, Canada

The effect of food supply on the onset of asexual and sexual plasmodium formation in Physarum polycephalum was studied. Asexual differentiation occurs readily in amoebae carrying the *mat*Ah mating type allele. The density at which these amoebae begin to differentiate is influenced by the *ind* locus, which controls the production of a diffusible inducer. The alleles *ind-1* and *ind-2* are known. Strains carring the *ind-1* allele begin plasmodium formation at a low amoebal density (rapid differentiation), while strains carring the *ind*-2 allele differentiate at a higher amoebal density (slow differentiation). The onset of differentiation is characteristic of the strain and did not change with a 20-fold variation in the number of food bacteria available. Sexual differentiation occurs between compatible amoebal strains. For a given pair of amoebal strains the onset of plasmodium formation occurs at a characteristic cell density that is determined by the genetic backgrounds of the strains. The *ind* locus is one of the genes that influences this cell density. Plasmodia are formed at a lower cell density in crosses involving compatible amoebae carrying the *ind-1* allele than they are in crosses with strains carrying the *ind-2* allele. As was found for asexual differentiation, an approximate 20-fold variation in the food supply did not affect the initiation of sexual plasmodium formation. These results suggest that in most cases starvation does not trigger the differentiation of amoebae into plasmodia. The time of onset of plasmodium formation is determined largely by genetic factors.

Key words: Physarum polycephalum, differentiation, food supply

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An abundant family of methylated repetitive sequences dominates the genome of *Physarum polycephalum*

Oliver P.Peoples and Norman Hardman

Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, UK

Received 8 September 1983; Accepted 19 October 1983

ABSTRACT

A significant portion (20%) of the Physarum genome can be isolated as a HpaII-resistant, methylated fraction. Cloned DNA probes containing highly-repeated sequences derived from this fraction were used to define the pattern of structural organisation of homologous repeats in Physarum genomic DNA. It is shown that the probes detect an abundant, methylated family of sequences with an estimated genomic repetition frequency greater than 2100, derived from a large repeated element whose length exceeds 5.8kb. Sequences comprising the long repetitive element dominate the HpaII-resistant compartment and account for between 4-20% of the Physarum genome. Detailed restriction/hybridisation analysis of cloned DNA segments derived from this compartment shows that HpaII/MspI restriction sites within some copies of the long repeated sequence are probably deleted by mutation. Additionally, segments of the repeat are often found in different organisational patterns that represent scrambled versions of its basic structure, and which are presumed to have arisen as a result of recombinational rearrangement in situ in the Physarum genome. Preliminary experiments indicate that the sequences are transcribed and that the structural properties of the repeat bear some resemblance to those of transposable genetic elements defined in other eukaryotic species.

SEQUENCE ORGANISATION IN NUCLEAR DNA FROM *PHYSARUM POLYCEPHALUM*

GENOMIC ORGANISATION OF DNA SEGMENTS CONTAINING FOLDBACK SEQUENCES

OLIVER P. PEOPLES, ARTHUR C. ROBINSON, PAUL A. WHITTAKER and NORMAN HARDMAN *

Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen, AB9 1AS (U.K.)

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Key words: DNA organisation; Foldback sequence; (P. polycephalum)

DNA clones containing foldback sequences, derived from *Physarum polycephalum* nuclear DNA, can be classified according to their pattern of hydridisation to Southern blots of genomic DNA. One group of DNA clones map to unique DNA loci when used as a probe to restriction digests of *Physarum* nuclear DNA. These cloned segments appear to contain dispersed repetitive sequence elements located at many hundreds of sites in the genome. Similar patterns of hybridisation are generated when these cloned DNA probes are annealed to DNA restriction fragments of genomic DNA obtained from a number of different *Physarum* strains, indicating that no detectable alteration has occurred at these genomic loci subsequent to the divergence of the strains as a result of the introduction or deletion of mobile genetic elements. However, deletion of segments of some cloned DNA fragments occurs following their propagation in *Escherichia coli*. A second, distinct group of clones are shown to be derived from highly methylated segments of *Physarum* DNA which contain very abundant repetitive sequences with regular, though complex, arrangements of restriction sites at their various genomic locations. It is suggested that these DNA segments contain clustered repetitive sequence elements. The results lead to the conclusion that foldback elements in *Physarum* DNA are located in segments of the genome which display markedly different patterns of sequence organisation and degree of DNA methylation.

Mycologia, 76(2), 1984, pp. 194–203.

FLUORESECENT ANTIBODY STAIN AND DISC ELECTROPHORESIS ANALYSIS OF REPRESENTATIVE MATING STRAINS IN *PHYSARUM POLYCEPHALUM* AND *DIDYMIUM IRIDIS*

ROBERT M. QUEEN

University of Florida Experiment Station, Agricultural Research and Education Center, Lake Alfred, Florida 33850

AND

JAMES L. KOEVENIG

Department of Biological Sciences, University of Central Florida, Orlando, Florida 32816

ABSTRACT

Antigenic differences associated with myxamoebal clones of different mating types in *Didymium iridis* and *Physarum polycephalum* were examined using fluorescent antibodies and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Immunofluorescence of heteroadsorbed anti-myxamoebal sera showed there is no mating strain-specific activity in either species but the sera were shown to be genus-specific. Intergeneric differences and similarities were revealed in the electrophoretic patterns of the myxamoebal protein extracts from *P. polycephalum*, *D. iridis*, and *Dictyostelium discoideum*. Intraspecific differences were noted in *D. iridis*.

Key Words: electrophoresis, myxomycete, fluorescent antibody, mating strains.

The microcyst wall of *Didymium iridis*: chemical analyses[1]

THOMAS J. RAUB[2]
Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, U.S.A. 32611

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RAUB, T. 1984. The microcyst wall of *Didymium iridis*: chemical analyses. Can. J. Microbiol. 30: 162–170.

Chemical analyses of the microcyst cell wall of *Didymium iridis* were done to compare it with other well-studied organisms, *Physarum polycephalum* and *Physarum flavicomum*. Large wall fragments were obtained by breakage in a Braun homogenizer. Chemical analyses of purified walls identified neutral sugars, protein, and hexosamine as the major components. Wall polysaccharides were mostly composed of galactosamine with smaller amounts of glucose and galactose. The protein component consisted of large quantities of threonine and aspartate–asparagine with trace amounts of the sulfur-containing amino acids. Most of the wall protein was soluble in alkaline urea. Sodium dodecyl sulfate – polyacrylamide gel electrophoresis was used to identify seven major bands, at least four of which are acidic glycoproteins. Most of the galactosamine was associated with the urea – hot alkali insoluble fraction comprised mostly of glucose. This galactosaminoglucan was partially sulfated and acetylated and arranged as microfibrils that maintain cell shape.

Identification of tubulin isoforms in the plasmodium of Physarum polycephalum by in vitro microtubule assembly

Anne Roobol, Marianne Wilcox, Eileen C. A. Paul[1]), Keith Gull

Biological Laboratory, University of Kent, Canterbury, Kent/United Kingdom

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Physarum plasmodium — in vitro microtubule assembly

The tubulins of the plasmodium of Physarum polycephalum have been identified by in vitro microtubule assembly from partially purified extracts of asynchronous microplasmodia and late G2 macroplasmodia. The plasmodial tubulin group comprised of 2 α tubulins (app. m.w. 51000 daltons) and 2β tubulins (app. m.w. 58000 daltons and 55000 daltons) and appeared to be identical with a group of polypeptides which are synthesized periodically in late G2. Two of the plasmodial tubulin subunits (one α and one β) were identical to the Physarum amoebal tubulin α and β subunits as characterised by 2D gel positions.

Isolation and Characterization of a High Molecular Weight Actin-binding Protein from *Physarum polycephalum* Plasmodia

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KAZUO SUTOH, MAKOTO IWANE, FUMIO MATSUZAKI, MASAKO KIKUCHI, and ATSUSHI IKAI
Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Hongo, Tokyo 113, Japan

ABSTRACT A high molecular weight actin-binding protein was isolated from the *Physarum polycephalum* plasmodia. The protein (HMWP) shares many properties with other high molecular weight actin-binding proteins such as spectrin, actin-binding protein from macrophages, and filamin. It has a potent activity to cross-link F-actin into a gel-like structure. Its cross-linking activity does not depend on calcium concentrations. Hydrodynamic studies have revealed that the protein is in the monomeric state of a polypeptide chain with molecular weight of ~230,000 in a high ionic strength solvent, while it self-associates into a dimer under physiological ionic conditions. Electron microscopic examinations of HMWP have shown that the monomer particle observed in a high ionic strength solvent is rod shaped with the two-stranded morphology very similar to that of spectrin. On the other hand, under physiological ionic conditions, the HMWP dimer shows the dumb-bell shape with two globular domains connected with a thin flexible strand.

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Unstable Activator Models for Size Control of the Cell Cycle

JOHN J. TYSON

Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, *U.S.A.*

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The unstable activator model of Wheals & Silverman (1982) is extended to account for the delay of nuclear division in the acellular slime mold, *Physarum polycephalum*, that is caused by pulse treatments with inhibitors of protein synthesis. The model is solved exactly to predict the delay as a function of the half-life of the activator. The Wheals–Silverman model is found to give results comparable, but not superior, to other unstable activator models of the cell cycle.

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Acetylation sites in histone H3 from *Physarum polycephalum*

Jaap H. Waterborg[+] and Harry R. Matthews*

Department of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616, USA

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Histone H3 from *Physarum polycephalum* was labelled with [^{3}H]acetate in G2 phase of the cell cycle. Only histones H3 and H4 were labelled and the H4 was removed by chromatography. Sequential Edman degradation of labelled H3 showed that acetate was incorporated into residues 9, 14, 18 and 23 which correspond to the sites of acetyl-lysine determined in histones H3 from other organisms. The results confirm the sequence conservation of H3 and support the notion that data on H3 acetylation, obtained with *Physarum*, can be extrapolated to higher eukaryotes.

Histone H3 *Acetylation site* *Acetyl-lysine* *H3 sequence conservation* *Higher eukaryote* (Physarum polycephalum)

Acetylation and methylation sites in histone H4 from *Physarum polycephalum*

Jaap H. WATERBORG, Sharon R. FRIED, and Harry R. MATTHEWS

Department of Biological Chemistry, School of Medicine, University of California, Davis

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Histone H4 has been isolated and purified from plasmodia of *Physarum polycephalum*. The four major fragments produced by hydrolysis of H4 by acetic acid were separated and the complete amino acid sequence of two of them was determined. By analogy with calf H4, these peptides are at the C-terminus and give the sequence from residue 68 to the C-terminus (residue 102). In this 35 residue sequence there are two minor differences from calf H4: (i) residue 77 is arginine in *Physarum* H4 and lysine in calf H4; (ii) lysine-79 is partially methylated in *Physarum*. Arginine occurs at position 77 in pea H4 but the occurrence of methylated lysine at position 79 has not been reported for other species. In the N-terminal region, amino acid compositions of acetic acid, tryptic and chymotryptic peptides indicate that *Physarum* H4 and calf H4 have identical sequences from the N-terminus to residue 47. There may be minor differences in the region from residue 46 to residue 67. The sites of acetylation were determined by Edman degradation of acetate-labelled peptide 4–17 of *Physarum* H4. Acetylation was observed at positions 5, 8, 12, and 16. The only other labelled peptide was the N-terminal peptide, which is not susceptible to Edman degradation and is thus probably α-*N*-acetylated as in most other organisms. The results confirm the conservation of H4 sequence and place *Physarum* H4 in an intermediate position between lower eukaryote H4, such as yeast or *Tetrahymena* H4, and higher eukaryote H4, such as mammalian H4 or pea H4.

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Accessibility of histone H4 gene of *Physarum polycephalum* to DNase I during the cell cycle

Marcelle L. Wilhelm, F. Xavier Wilhelm, Barbara Toublan* and Raymond Jalouzot*

Institut de Biologie Moléculaire et Cellulaire, Strasbourg and **Laboratoire de Biologie Cellulaire, Reims, France*

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DNase I was used as a probe to detect conformational changes of the H4 histone gene of *Physarum polycephalum* during the cell cycle. The degradation of histone genes was followed by gel electrophoresis and hybridization with a probe for the H4 histone gene. It was found that even during mitosis when chromatin is condensed into chromosomes, the histone genes are preferentially degraded by DNase I. The histone genes retain a characteristic structure which is recognized by DNase I during all stages of the cell cycle and thus independently of the biosynthesis of histones.

Physarum *Histone gene* *DNase I* *Cell cycle*
Chromatin *Southern blot*

A transposon-like DNA fragment interrupts a *Physarum polycephalum* histone H4 gene

Marcelle L. Wilhelm and François-Xavier Wilhelm*

Laboratoire de Biophysique, Institut de Biologie Moléculaire et Cellulaire du CNRS, 15 rue René Descartes, 67084 Strasbourg Cédex, France

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A recombinant DNA library was screened for histone H4 genes using a sea urchin probe. One recombinant was analysed by restriction enzyme mapping and Southern blotting. The complete DNA sequence of the H4 histone locus was determined. An 86 base pair interrupting sequence was found within the histone H4 coding sequence. The inserted DNA fragment has some characteristics of a transposable element.

Histone gene *Interrupting sequence* *Transposon* Physarum polycephalum

CELL STRUCTURE AND FUNCTION 8, 255–265 (1983)

Contractile and Structural Reactions to Impediments of Ca^{2+}-Homeostasis in *Physarum polycephalum*[1]

K.-E. Wohlfarth-Bottermann[2], Z. Shraideh[3] and Z. Baranowski[4]

Institute of Cytology, University of Bonn, Bonn, FRG

ABSTRACT. A combined application of 5 mM KCN and 19μM Ca++-ionophore A-23187 leads to pronounced contractures of plasmodial strands of *Physarum polycephalum*. The appearance of the contractures is independent of the amount of Ca++ in the external medium. Tensiometric registrations of longitudinal contraction activity (isometric regime) reveal an average tension increase of 50 mp compared with the preceding tension level before the addition of KCN and ionophore.

This high force output during the contracture coincides with a pronounced increase in the number of cytoplasmic actomyosin fibrils. Their ultrastructure is seen as a high lateral density of strictly parallel arranged F-actin filaments; the state of cytoplasmic actomyosin during this isometric contracture corresponds to the ultrastructure of isometrically contracted fibrils during the normal contraction-relaxation cycle of this organism.

A simultaneous impediment of respiration and Ca++ homeostasis strongly favours a shift of the actin equilibrium to the high polymeric side in the form of fibrils and may thus be used as a preparatory step improving the specimens in the context of other investigations, *e.g.*, for immunocytochemical investigations or for the preparation of cell-free models to be reactivated after extraction procedures.

A New Type of Plasmodium Formation in *Physarum polycephalum*

by ROGER W ANDERSON

Department of Biochemistry, University of Oxford, Oxford OX1 3QU, England

and CHRISTINE L TRUITT

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Haploid amoebae of *Physarum polycephalum* may form plasmodia sexually by "crossing", which involves cellular and nuclear fusion, or asexually by "selfing", which occurs without nuclear fusion. In most amoebal strains, selfing is seen in clonal cultures only at very low frequency. In the present study, we have shown that selfing occurs at a similarly low frequency in mixtures of crossing-incompatible amoebae, but is stimulated in crossing-compatible mixtures. In certain compatible mixtures involving mutant strains, where crossing is temperature-sensitive, selfing may be stimulated even at a temperature that largely or wholly abolishes crossing. The extent to which selfing is stimulated appears to be influenced by *matB*, a locus which is known to affect the frequency of amoebal fusion. We have failed to detect any filter-transmissible factor that might be responsible for the effects we have observed. We suggest a sequence of events that might bring about "stimulated selfing" as a consequence of abortive crossing.

In press Genetical Research

Genetic Analysis of Resistance to Benzimidazoles in Physarum: Differential Expression of Beta-tubulin Genes

Timothy G. Burland, Tim Schedl, Keith Gull & William F. Dove
McArdle Laboratory,
University of Wisconsin
Madison, WI 53706

(GENETICS, IN PRESS)

ABSTRACT

Physarum displays two vegetative cell types, uninucleate myxamoebae and multinucleate plasmodia. Mutant myxamoebae of Physarum resistant to the antitubulin drug MBC (methylbenzimidazole-2-yl-carbamate) were isolated. All mutants tested were cross-resistant to other benzimidazoles, but not to cycloheximide or emetine. Genetic analysis showed that mutation to MBC resistance can occur at any one of four unlinked loci, benA, B, C, or D. MBC resistance of benB and benD mutants was expressed in plasmodia, but benA and benC mutant plasmodia were MBC sensitive, suggesting that benA and benC encode myxamoeba-specific products. Myxamoebae carrying the recessive benD210 mutation express a β-tubulin with novel electrophoretic mobility, in addition to a β-tubulin with wild-type mobility. This and other evidence indicates that benD is a structural gene for β-tubulin, and that at least two β-tubulin genes are expressed in myxamoebae. Comparisons of the β-tubulins of wild-type and benD210 strains by gel electrophoresis revealed that, of the three (or more) β-tubulin genes expressed in Physarum, one, benD, is expressed in both myxamoebae and plasmodia, one is expressed specifically in myxamoebae and one is expressed specifically in plasmodia. However, mutation in only one gene, benD, is sufficient to confer MBC resistance on both myxamoebae and plasmodia.

Genetics of the Tubulin Gene Families of Physarum

Tim Schedl, Judi Owens, William F. Dove & Timothy G. Burland

McArdle Laboratory,
University of Wisconsin,
Madison, WI 53706

(GENETICS, IN PRESS)

ABSTRACT

The organization of the α- and β-tubulin gene families in Physarum was investigated by Mendelian analysis. Restriction endonuclease generated DNA fragments homologous to α- and β-tubulin show length polymorphisms that can be used as markers for genetic mapping. Analysis of meiotic assortment among progeny of heterozygotes allowed α- and β-tubulin sequence loci to be defined. There are four unlinked α-tubulin sequence loci (altA, B, C, and D), and at least three unlinked β-tubulin sequence loci (betA, B and C). The α-tubulin loci are not linked to the β-tubulin loci. — Segregation of tubulin sequence loci with respect to ben mutations that confer resistance to antitubulin benzimidazole drugs was used to investigate whether any members of the α- or β-tubulin gene families are allelic to ben loci. The β-tubulin sequence locus betB is allelic to the resistance locus benD, the betA locus is probably allelic to benA, and the α-tubulin sequence locus altC may be allelic to benC. The molecular implications of benzimidazole resistance phenotypes when only one of the expressed β-tubulin gene family members mutates to drug resistance are discussed in relation to tubulin function.

Cell Cycle Regulation of Tubulin RNA Level, Tubulin Protein Synthesis, and Assembly of Microtubules in *Physarum*

(JOURNAL OF CELL BIOLOGY, IN PRESS)

TIM SCHEDL, TIMOTHY G. BURLAND, KEITH GULL, and WILLIAM F. DOVE
McArdle Laboratory, University of Wisconsin, Madison, Wisconsin 53706. Dr. Gull's permanent address is Biological Laboratories, University of Kent, Canterbury, England.

ABSTRACT The temporal relationship between tubulin expression and the assembly of the mitotic spindle microtubules has been investigated during the naturally synchronous cell cycle of the *Physarum* plasmodium. The cell cycle behavior of the tubulin isoforms was examined by two-dimensional gel electrophoresis of proteins labeled in vivo and by translation of RNA in vitro. α1-, α2-, β1-, and β2-tubulin synthesis increases coordinately until metaphase, and then falls, with β2 falling more rapidly than β1. Nucleic acid hybridization demonstrated that α- and β-tubulin RNAs accumulate coordinately during G2, peaking at metaphase. Quantitative analysis demonstrated that α-tubulin RNA increases with apparent exponential kinetics, peaking with an increase over the basal level of >40-fold. After metaphase, tubulin RNA levels fall exponentially, with a short half-life (19 min). Electron microscopic analysis of the plasmodium showed that the accumulation of tubulin RNA begins long before the polymerization of mitotic spindle microtubules. By contrast, the decay of tubulin RNA after metaphase coincides with the depolymerization of the spindle microtubules.

GENETIC ORGANIZATION AND EXPRESSION OF ACTIN AND ALPHA- AND BETA-TUBULIN GENE FAMILIES IN *PHYSARUM POLYCEPHALUM*

Tim Schedl

Under the supervision of Professor William F. Dove

The organization of the actin, α-tubulin and β-tubulin gene families in *Physarum* was investigated by Mendelian analysis. Actin homologous sequences are found at four unlinked loci (*ardA* through *D*). α-tubulin sequences are found at four unlinked loci (*altA* through *D*), and β-tubulin sequences are found at a minimum of three unlinked loci (*betA* through *C*). There are multiple actin sequences at the *ardA* locus, and multiple α-tubulin sequences at the *altB* locus. The *alt* loci are unlinked to the *bet* loci while the *altD* locus maps about 2 cM from the *ardC* locus.

Segregation of tubulin sequence loci with respect to *ben* mutations that confer resistance to antitubulin benzimidazole drugs was used to investigate whether any members of the α- or β-tubulin gene families map to *ben* loci. Mapping suggests that the following pairs of sequence and resistance loci are probably identical: *betA* and *benA*, *betB* and *benD*, *altC* and *benC*. This and other data indicate that mutations in only one of two (or more) co-expressed members of the β-tubulin gene family is sufficient to confer benzimidazole resistance in haploids (epistasis) but not diploids (recessive).

The temporal relationship between tubulin expression and assembly of the mitotic spindle microtubules was investigated during the naturally synchronous cell cycle of the Physarum plasmodium. The cell cycle behavior of the tubulin isoforms was examined in two-dimensional gels of proteins labelled in vivo and by translation of RNA in vitro. Synthesis of α1, α2, β1 and β2 tubulin increases coordinately until metaphase, and then falls, with β2 falling more rapidly than β1. Nucleic acid hybridization demonstrated that α- and β-tubulin RNAs accumulate coordinately during G2, peaking at metaphase. Quantitative analysis demonstrated that α-tubulin RNA increases with apparent exponential kinetics, peaking with an increase over the basal level of greater than 40-fold. After metaphase, tubulin RNA levels fall exponentially, with a short half-life. Electron microscopic analysis of the plasmodium showed that the accumulation of tubulin RNA begins long before the polymerization of mitotic spindle microtubules. By contrast, the decay of tubulin RNA after metaphase coincides with the depolymerization of the spindle microtubules.

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APPROVED William F. Dove.

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