NOTES FROM THE EDITOR

This is being written in Marburg, West Germany, where I am collaborating with Prof. R.K. Thauer's group studying methanogenic bacteria. Many of you are aware of my second interest, necessitated by funding problems in the slime mold field. Others of us have the same problem: Richard Braun also works on trypanosome membranes, Aloys Huettermann on Fomes. I hope to attend the Canterbury meetings, and may see some of you there before you read this. Below are some bits of news:

C.J. Alexopoulos died in Austin, Texas, on May 15, 1986. He had been in declining health from recurring brain tumors and spinal arthritis. I last saw him just after Christmas, 1985. He was bedridden and not very alert. His friends and students will miss him, but are relieved that his suffering has ended. Anyone wishing to do so may contribute to the Mycological Society of America Fund for the Alexopoulos Prize. This is a small endowment established in 1978 by his friends and students to finance an annual award by the society for outstanding research by a young mycologist. It now earns some $400 annually. Checks may be made to "MSA Alexopoulos Fund" and sent to Dr. Meredith Blackwell, Dept. of Botany, LSU, Baton Rouge, LA 70803 USA. Mrs. Alexopoulos is being advised of these gifts. An obituary will appear in a future issue of Mycologia.

EUROPEAN MEETINGS: To be (or were, depending when this gets mailed from Florida!) held at University of Kent, Canterbury, England, July 13-18, 1986. Contact Keith Gull for details. His phone is 227-66822 ext. 7582.

NORTH AMERICAN MEETINGS: Scheduled at Berkeley, California, July 6-9, 1987. Ray Collins and Harry Matthews are organizing. They will mail abstract and registration information to everyone on our Newsletter mailing list. Others should write O.R. Collins, Botany, University of California, Berkeley, CA 94720.

IT'S CONTRIBUTION TIME AGAIN!:
The PNL publication kitty has run out. For those of you unaware of the way this operation works, the Dept. of Microbiology & Cell Science at the University of Florida pays copying and mailing expenses for the PNL. The publication fund, maintained at the UF credit union, buys covers, copy paper, and mails the European issues to Richard Braun air parcel post, who then mails them to European subscribers courtesy University of Bern. These are the reasons why we need so little money.
RATE AND METHOD OF PAYMENT: The current rate is $10, which pays for an indeterminant period, probably about 4 years, until the kitty runs out again. You can send checks (dollars only, please) made out to Henry Aldrich/Physarum Newsletter. Or you can wait until the next European or North American meeting and pay me personally. If for some reason you cannot pay due to currency exchange or other problems, just drop me a note explaining the difficulty. If you have paid me $10 within the past 2 years, ignore this request. Wait and pay the next time we run short.
THANKS! KEEP THOSE REPRINTS ROLLING IN, FOLKS!

Henry Aldrich

POSTDOCTORAL POSITION

To study the role of protein glycosylation in the development of the cellular slime mold Dictyostelium discoideum. The project involves biochemical, immunochemical, genetic and immunocytochemical approaches to the problem. Initial appointment is for one year with possible renewal for up to three years. Available July 1, 1986.

For details contact:
Dr. Christopher M. West
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Box J-235 J. Hillis Miller Health Center
University of Florida, Gainesville, FL 32610.
Phone: (904) 392-3329.

Quantitative Cytochemie mit dem interaktiven Bildanalyse-System IBAS II am Beispiel von Calcium-Bindungestellen im Zellkern des Schleimpilzes Physarum polycephalum.

Zeiss Inform., Oberkochen, 28, 42–44 (1985), Heft 97

Friedhelm Achenbach[1],
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J Mol Evol (1985) 22:220–229

Tubulin Evolution: Ciliate-Specific Epitopes Are Conserved in the Ciliary Tubulin of Metazoa

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Summary. In spite of their overall evolutionary conservation, the tubulins of ciliates display electrophoretic and structural particularities. We show here that antibodies raised against *Paramecium* and *Tetrahymena* ciliary tubulins fail to recognize the cytoplasmic tubulins of all the metazoans tested. Immunoblotting of peptide maps of ciliate tubulins reveals that these antibodies react with one or very few ciliate-specific epitopes, in contrast to polyclonal antibodies against vertebrate tubulins, which are equivalent to autoantibodies and recognize several epitopes in both ciliate and vertebrate tubulins. Furthermore, we show that the anti-ciliate antibodies recognize ciliary and flagellar tubulins of metazoans ranging from sea urchin to mammals (with the exception of humans). The results support the conclusion that although duplication and specialization of tubulin genes in metazoans may have led to distinct types of tubulins, the axonemal one has remained highly conserved.

Key words: Microtubules — Tubulin antibodies — Immunoblotting — Immunocytology — Autoantibodies — Gene duplication

Cell Tissue Kinet. (1985) 18, 623–630.

Effects of the free radical generator paraquat on differentiation, superoxide dismutase, glutathione and inorganic peroxides in microplasmodia of *Physarum polycephalum*

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(*Received 24 October* 1984; *revision accepted* 13 *May* 1985)

Abstract. The herbicide paraquat was used to investigate the effects of oxidative stress on the spherulation of *Physarum polycephalum* microplasmodia. The responses of a white non-differentiating strain of *Physarum* were compared with those of a common yellow strain that readily spherulates in salts-only starvation medium. The addition of paraquat to the salts medium increased the specific activity of superoxide dismutase in both strains; it also induced an increase in the intracellular inorganic peroxide concentration in both strains. Glutathione concentration was higher in the paraquat-treated yellow strain than in the controls. Paraquat had no effect on glutathione concentration in white microplasmodia. Paraquat accelerated spherulation in yellow microplasmodia. The white microplasmodia responded to the herbicide by cleaving into structures similar to immature spherules; however, these structures were not viable. The results of this study support the hypothesis that free radicals are involved in cell state transitions.

Cell Biol Int Rep (1985) 9(1), 85-90

Consequences of impeding in mitochondrial functions in Physarum polycephalum. III. Reversible cessation of the contraction-relaxation cycle and the temperature sensitivity of the alternate respiratory pathway

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Received June 24, 1985
Accepted October 30, 1985

Physarum polycephalum — alternate respiration — temperature sensitivity — oscillations

The tensiometric technique has been used to corroborate that mitochondria of Physarum polycephalum exhibit two pathways of electron transport: a cyanide-sensitive cytochrome pathway, and an alternate pathway that is KCN-resistant but salicylhydroxamic acid (SHAM)-sensitive. Extracellularly applied solutions containing both malate and pyruvate as well as ketoglutarate are efficient substrates that sustain the alternate pathway. When cytochrome oxidase and glycolysis are inhibited, the efficiency of these substrates is demonstrated by the maintenance of both cell integrity and the contraction-relaxation cycle of plasmodial actomyosin. Blockage of both respiratory pathways by KCN with SHAM, but without inhibition of glycolysis, leads to the reversible cessation of the contraction-relaxation cycle, probably due to a specific inhibition of the relaxation phase. The integrity of the cell supported solely by substrates for the alternate respiration is destroyed by increasing the temperature from 19–20 °C to 23–24 °C, a result demonstrating the temperature sensitivity of the alternate respiratory pathway in these plasmodia. The results presented emphasize the role of respiration in the contraction-relaxation cycle of plasmodial contractile systems.

EVOLUTION AND PATTERNS OF EXPRESSION OF THE *PHYSARUM* MULTI-TUBULIN FAMILY ANALYSED BY THE USE OF MONOCLONAL ANTIBODIES[1]

Molecular Genetics of Filamentous Fungi, pages 265–275

Christopher R. Birkett, Kay E. Foster and Keith Gull

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Canterbury, Kent CT2 7NJ, England

ABSTRACT We have used a panel of seven well defined monoclonal antibodies to probe blots of the myxamoebal and plasmodial tubulin isotypes. Differential reactivity of isotypes to these antibodies suggests a) the plasmodial α1 tubulin isotype is complex. The monoclonal antibody KMP-1 reveals a heterogeneity of α1 sub-types that focus within this one 2D gel spot. b) The β1 group of isotypes appear to be very related tubulins. c) The plasmodial specific β2 tubulin differs significantly from the β1 isotypes in its reactivity to anti-β tubulin monoclonals. Further, the plasmodial specific β2 tubulin is recognised by the normally α tubulin specific monoclonal YL1/2, suggesting that this β tubulin carries an α tubulin-like carboxyl terminus. It may be that the plasmodial specific α2 and β2 tubulin isotypes have arisen in evolution via a recombination event involving the regions of the α1 and β1 structural genes encoding the carboxyl termini of these tubulins.

J. Protozool., 32(4), 1985, pp. 740–741

Cellulose Detected in the Stalk of *Protostelium irregularis* (Eumycetozoea)[1]

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[aa]*Centre de Recherches sur les Macromolécules Végétales (C.N.R.S.),[3] B.P. 68, 38402 Saint-Martin-D'Hères, France*

ABSTRACT. The stalk of *Protostelium irregularis* (Eumycetozoea) has been studied with light and electron microscopy and selected-area electron diffraction. The stalk is positively birefringent and fibrillar. Diffraction patterns obtained from stalks indicate that crystalline cellulose I is one component of the stalk.

RADIATION RESEARCH 104, 242–261 (1985)

Absence of a 45 or 60 Hz Electric Field-Induced Respiratory Effect in *Physarum polycephalum*

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BRAYMAN, A. A., MILLER, M. W., COX, C., CARSTENSEN, E. L., AND SCHAEDLE, M. Absence of a 45 or 60 Hz Electric Field-Induced Respiratory Effect in *Physarum polycephalum*. *Radiat. Res.* 104, 242–261 (1985).

Microplasmodia of *Physarum polycephalum* strain M_3C were exposed to 60 Hz continuous wave electric fields of 0.7 and 70 V/m for more than 13 weeks and to 45 Hz CW fields of the same strengths for nearly 7 weeks. At a particular field strength, duplicate subcultures of sham-exposed and field-exposed microplasmodia were independently maintained and measured to allow comparison of variation between subcultures within an exposure treatment group. Microplasmodial oxygen uptake rates were measured using a Clark-type oxygen electrode system, and respiratory rates were expressed on a unit protein basis. Few consistent respiratory rate differences between sham- and field-exposed subcultures were detected at either 0.7 or 70 V/m. These findings are in contrast to earlier reports of significant microplasmodial respiratory rate depression in response to 45 or 75 Hz, combined 0.7 V/m, 2.0 G electric and magnetic fields and in response to 75 Hz, 0.7 V/m electric field exposure [Greenebaum *et al.*, DHEW Report FDA-77-8010, Vol. I, pp. 449–459 (1977); Goodman *et al.*, *Radiat. Res.* 78, 485–501 (1979)]. Growth rate data are also presented and similarly fail to show an exposure treatment effect.

Volume 189, number 1 FEBS 2868 September 1985

Nonhistone proteins of the transcriptionally active chromatin fraction of *Physarum polycephalum*, associated with nucleosome linker DNA instead of histone H1

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Received 21 June 1985

It was established that 2 nonhistone proteins preferentially associated with the transcriptionally active chromatin fraction of *Physarum polycephalum* (M_r = 37000 and 39000) are bound to nucleosome linker DNA instead of histone H1. This observation suggests the possibility that 37 and 39 kDa proteins are important structural elements of active chromatin, involved in maintaining its open conformation.

Physarum polycephalum *Nonhistone protein* *Histone H1* *Transcriptionally active chromatin*

Biochimica et Biophysica Acta 826 (1985) 95–100
Elsevier

BBA 91511

Chromatin reorganization during early differentiation of *Physarum polycephalum* *

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(Received May 28th, 1985)

Key words: Differentiation; Transcription; Chromatin structure; Histone H1; (*P. polycephalum*)

When microplasmodia of *Physarum polycephalum* are induced to differentiate by starvation, the rate of RNA synthesis measured in vitro with endogenous RNA polymerase B drops rapidly, decreasing after 12 h of starvation to 30% of the initial value. Transcriptional inactivation is accompanied by: (a) 60% decrease in the amount of the chromatin fraction solubilizable by light DNAase I digestion; (b) increase in the content of histone H1 and several nonhistone proteins in the DNAase-I-solubilized chromatin; and (c) formation of a nucleosome-like structure that is absent from this fraction during normal growth. No marked change in the structure and composition of the total chromatin on starvation is evident. These data suggest that the repression of transcription during differentiation in *Physarum* is correlated with structural reorganization of active chromatin regions.

SHORT NOTE

Fluctuations in S-adenosyl-L-methionine (AdoMet) during Mitotic Cycle of the Myxomycete *Physarum polycephalum*

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A sensitive radioisotope dilution method was used to measure the S-adenosyl-L-methionine (AdoMet) content in macroplasmodia of the slime mold *Physarum polycephalum* during the mitotic cycle. The AdoMet pool had two maxima, one during mitosis, the other in the middle of G2 phase.

Macroplasmodia of the myxomycete *Physarum polycephalum* are an ideal system for studying the biochemistry of nuclear division and growth, since it is a syncytium in which all nuclei undergo natural synchronous mitosis [1]. In the present investigation this system is used to study the size of the S-adenosyl-L-methionine (AdoMet) pool during mitotic cycle. The energy-rich compound AdoMet is not only an active methyl donor in many enzymatic or non-enzymatic reactions [2] but it also has a key position in polyamine biosynthesis. The knowledge of its pool size should contribute to understanding the extent of methylation reactions and polyamine biosynthesis during cell cycle.

Gene, 39 (1985) 203–211
Elsevier

GENE 1430

Nucleotide sequence of the central non-transcribed spacer region of *Physarum polycephalum* rDNA

(Recombinant DNA; methylcytosine; replication origin; repetitious sequences; concerted evolution; palindromes)

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(Received November 14th, 1984)
(Accepted August 8th, 1985)

SUMMARY

In *Physarum polycephalum* the rRNA genes are present on linear extrachromosomal molecules, each containing two transcription units arranged as a giant palindrome. In the center of the molecule, between the two transcription units, is the 23-kb central spacer, previously shown to contain the replication origins and several regions of direct and inverted repeats. Segments of all the repeats in the spacer have been sequenced and their overall organization determined. The entire spacer consists of reiterations of only 1200 bp of different DNA sequences. The sequence surrounding the transcription start point is not repeated in the spacer, as it is in *Xenopus* and *Drosophila*. Sequencing of purified, uncloned rDNA localized some of the methylcytosine residues in the spacer. The repetitious sequences appear to be undergoing concerted evolution.

JOURNAL OF ULTRASTRUCTURE RESEARCH 91, 127–137 (1985)

Centriole Size Modifications during the Cell Cycle of the Amoebae of the Myxomycete *Physarum polycephalum*

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Received March 25, 1985, and in revised form September 10, 1985

Anterior and posterior centrioles of *Physarum* amoebae are indistinguishable by their size during interphase but there is a correlation between the size of the two centrioles in the same amoeba. The interphase length of centrioles in diploid amoebae possessing only one pair of centrioles was 11% longer than in the case of the haploid strain. Treatment with taxol led to a 23 and 32% increase of the mean length in interphase and blocked mitosis, respectively. Conversely, during control mitosis the parental centrioles showed a 12% decrease of their mean length while the size of the daughter centrioles increased progressively. Neither nocodazole nor cold treatment induce a decrease of centriole length. The mean length of the cartwheel structure (internal proximal part) although constant during mitosis could be increased 24% in the presence of taxol. Similarly there was a correlation between the number of anterior satellites and the centriole length.

Biochem. J. (1985) 232, 21–24 (Printed in Great Britain)

ADP-ribosyltransferase in isolated nuclei during the cell cycle of *Physarum polycephalum*

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ADP-ribosyltransferase was measured in isolated nuclei of *Physarum polycephalum*. Activity was determined with and without exogenous DNA and histones. During the synchronous cell cycle the activity measured with exogenous substrates exhibited a typical peak enzyme pattern with a maximum of activity in S-phase, whereas activity measured without exogenous substrates displayed a step enzyme pattern. Both activities doubled in each cell cycle.

Vol. 134, No. 2, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 29, 1986 Pages 469-476

ROLE OF mRNA 5'-TERMINAL CAPS IN TRANSLATIONAL DORMANCY OF PHYSARUM POLYCEPHALUM

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Received November 14, 1985

Translational regulation of protein synthesis accompanies sclerotization in Physarum polycephalum. Plasmodial and sclerotial poly(A)$^+$RNA were translated in a message-dependent wheat germ lysate in the presence of the cap analogue 7-methylguanosine-triphosphate to determine whether 5' structural alterations in mRNA accompany translational repression. The translation of plasmodial and sclerotial poly(A)$^+$RNA was reduced to identical levels suggesting that both RNA populations are capped. The 5'-termini of plasmodial and sclerotial poly(A)$^+$RNA were identified as $m^7G^{5'}ppp^{5'}C_m$. Alterations in the 5'-cap of mRNA during sclerotization do not appear to be responsible for translational dormancy.

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 260, No. 30, Issue of December 25, pp. 16106-16113, 1985
Printed in U.S.A.

Phosphorylation of Histidine in Proteins by a Nuclear Extract of *Physarum polycephalum* Plasmodia*

(Received for publication, May 20, 1985)

Verena D. Huebner and Harry R. Matthews

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A high salt nuclear extract from the true slime mold *Physarum polycephalum* was used as a source of kinase activity for the incubation of calf thymus histones with [γ-^{32}P]ATP. A major proportion of the ^{32}P incorporated into histones was acid-labile and alkali-stable. The nature of the alkali-stable phosphorylated component was analyzed by subjecting the phosphorylated protein to total alkaline hydrolysis and separating the resultant phosphoamino acids by anion exchange chromatography. The ^{32}P-labeled material co-chromatographed with phosphohistidine standards and did not co-chromatograph with phosphoserine, phosphothreonine, or phosphotyrosine standards. In similar experiments using reversed phase high-performance liquid chromatography to separate the phosphoamino acids, the ^{32}P-labeled phosphoamino acid behaved like the 1-isomer of phosphohistidine, in not being retained by the column, and unlike 3-phosphohistidine, phosphoserine, phosphothreonine, phosphotyrosine, and phosphoarginine, which were all retained on the column. Histone H4 was a good substrate for the histidine kinase activity and the location of the phosphorylated histidine residue was probed by peptide mapping using chymotrypsin or V8 protease. Both maps were consistent with labeling of histidine 75 and inconsistent with labeling of histidine 18. The data show that *Physarum* nuclei contain a major kinase activity which produces phosphohistidine. The methods we have developed for studying this kinase activity provide the basis for a complete characterization of the structure and function of the *Physarum* enzyme and can be applied to the study of similar kinase activities in other systems.

Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 6475–6479, October 1985
Biochemistry

Replication timing of the H4 histone genes in *Physarum polycephalum*

(DNA replication/cell cycle/gene expression)

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Communicated by James F. Bonner, June 6, 1985

ABSTRACT The time of replication of the two H4 histone genes (H41 and H42) was determined during the naturally synchronous mitotic cycle of *Physarum polycephalum*. 5-Bromo-2'-deoxyuridine labeling and density gradient centrifugation was used to isolate newly synthesized DNA from defined periods of S phase. The DNA was analyzed by Southern hybridization with a cloned probe containing one of the H4 histone genes of *Physarum*. The results indicate that the two H4 histone genes are replicated in the first 30 min of S phase but not exactly at the same time. H41 is replicated during the first 10 min of S phase, when only 15% of the genome is duplicated, whereas H42 replicates between 20 and 30 min after the onset of S phase. The possible relationship between the periodic expression of the genes and the timing of their replication is discussed.

Experimental Cell Research 161 (1985) 460–472

Isolation and Characterization of a Membrane–DNA Complex in the Mitochondria of *Physarum polycephalum*

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A membrane–DNA complex was isolated by centrifugation of sheared lysate of isolated mitochondria in 20–60% sucrose step solution. Analyses using Hoechst 33258/CsCl density gradient centrifugation and restriction endonuclease treatment showed that DNA in the membrane–DNA complex was AT-rich compared with total mitochondrial DNA (mt DNA) and contained Eco RI fragments of E-4, 5 and 8, which were localized on the right hand of *Physarum* mitochondrial genome. Phenethyl alcohol (PEA) and ethidium bromide (EB) could disrupt the membrane–DNA complex to release DNA fragments from their complex in vitro. Addition of 0.5% or more PEA, which released 80–90% of the DNA from the membrane–DNA complex in vitro, inhibited not only mitochondrial nuclear division but also mitochondrial division in vivo. EB treatment at more than 1 mg/ml disrupted the membrane–DNA complex in vitro to release 77% of the total DNA in the complex. Addition of 10 μg/ml EB induced unequal mitochondrial nuclear division in the microplasmodia, e.g., a dividing dumbbell-shaped mitochondrion had the mt-nucleus in one side and as a result formed then one nucleated and one enucleated mitochondrion. From the EB-pretreated mitochondria, a lesser amount of the membrane-DNA complex was isolated than from the control. These findings mean than the unequal mt-nuclear division is due to dissociation of DNA and the membrane system in the membrane-DNA complex. They strongly suggested that the DNA region (E-4, 5 and 8), where the mitochondrial nucleus is associated with the mitochondrial membrane system plays an important role in mitochondrial nuclear division.

Inhibitory Ca^{2+}-Control of Movement of Beads Coated with *Physarum* Myosin Along Actin-Cables in *Chara* Internodal Cells

Brief Report

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Received June 21, 1985
Accepted August 9, 1985

Summary

The actin-activated ATPase activity *Physarum* myosin was shown to be inhibited at μM levels of Ca^{2+}. To determine if Ca^{2+} regulates ATP-dependent movement of *Physarum* myosin on actin, latex beads coated with *Physarum* myosin were introduced into *Chara* cells by intracellular perfusion. In perfusion solution containing EGTA, the beads moved along the parallel arrays of *Chara* actin filaments at a rate of 1.0–1.8 μm/sec; however, in perfusion solution containing Ca^{2+}, the rate reduced to 0.0–0.7 μm/sec. The movement of beads coated with scallop myosin, whose actin-activated ATPase activity is activated by Ca^{2+}, was observed only in the perfusion solution containing Ca^{2+}, indicating that myosin is responsible for the inhibitory effect of Ca^{2+} on *Physarum* myosin movement. The involvement of this myosin-linked regulation in the inhibitory effect of Ca^{2+} on the cytoplasmic streaming observed in *Chara* internodal cell and *Physarum* plasmodium was discussed.

Keywords: Motility; Calcium control; *Chara; Physarum polycephalum;* Myosin; Actin filaments.

Abbreviations: ATP, adenosine 5'-triphosphate; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethyleneglycol-bis(β-aminoethylether) N,N,N',N'-tetraacetic acid; PIPES, piperazine-N,N'-bis(2-ethanesulfonic acid).

European Journal of Cell Biology 39, 278-282 (1985) © Wissenschaftliche Verlagsgesellschaft mbH · Stuttgart

Isolation of highly purified and more native nuclei of Physarum polycephalum utilizing Surfynol, hexylene glycol and Percoll

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Received August 26, 1985
Accepted October 28, 1985

Electron microscopy — RNA synthesis — reinitiation

For the preparation of nuclei from the slime mold Physarum the method of Mohberg and Rush [12] is commonly used. In comparison to this method we demonstrate some advantages of a new preparation method using as detergent 0.8% Surfynol (instead of 0.1% Triton X-100), 0.5 M hexylene glycol (instead of 0.25 M sucrose) and a self-generated Percoll gradient by a 25-min centrifugation (48000*g*), starting with 25% Percoll dilution (instead of a centrifugation into a 1 M sucrose cushion). Nuclei prepared by the new method have the following characteristics: *1)* Cross sections of nuclear pellets show high purity and undisrupted double membranes in the electron microscope. *2)* The contamination by cytoplasmic proteins (malate dehydrogenase measured) and by slime is ½ and 1/10 that of the former preparation respectively. *3)* Protein content per nucleus is 1.8 times higher. *4)* Endogenous RNA synthesis is more than 8 times higher and shows an increase over 60 min with a value of plus 40% to the 20-min value. *5)* This late increase in newly synthesized RNA is due to reinitiation of RNA polymerase II as shown by the RNA-initiation inhibitor 5,6 dichloro-1-β-D-ribofuranosyl-benzimidazole (DRB). *6)* The nuclei contain in addition to RNA-polymerase I and II also RNA-polymerase III, which is inhibited by 100 µg/ml α-amanitin.

Experimental Cell Research 161 (1985) 533–540

Analysis of *Physarum* Proteins throughout the Cell Cycle by Two-dimensional PAGE

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The accumulation of several hundred proteins during the nuclear division cycle of *Physarum polycephalum* was measured by digital image processing of silver-stained two-dimensional (2D) polyacrylamide gels. In contrast to previous studies, we have used an organism with a naturally synchronous cell cycle, so there are no uncertainties concerning synchronization artifacts or cell-sorting artifacts, and we have measured the specific amounts of each protein rather than the rate of synthesis. Since one-dimensional SDS–PAGE shows no significant fluctuations in the most abundant plasmodial proteins, we have loaded 2D gels so that proteins of low-to-moderate abundance appear in the linear range of the silver stain standard curve. Only five proteins showed reproducible, measurable fluctuations during the cell cycle. One of these proteins was tubulin. Full quantitative information was obtained by analysing the digital images of silver-stained gels by a general image processing system.

The EMBO Journal vol.4 no.13A pp.3557–3562, 1985

Transposon-like properties of the major, long repetitive sequence family in the genome of *Physarum polycephalum*

Douglas H.Pearston, Mairi Gordon and Norman Hardman

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Communicated by D.Sherratt

A family of long, highly-repetitive sequences, referred to previously as '*Hpa*II-repeats', dominates the genome of the eukaryotic slime mould *Physarum polycephalum*. These sequences are found exclusively in scrambled clusters. They account for about one-half of the total complement of repetitive DNA in *Physarum*, and represent the major sequence component found in hypermethylated, 20–50 kb segments of *Physarum* genomic DNA that fail to be cleaved using the restriction endonuclease *Hpa*II. The structure of this abundant repetitive element was investigated by analysing cloned segments derived from the hypermethylated genomic DNA compartment. We show that the '*Hpa*II-repeat' forms part of a larger repetitive DNA structure, ~8.6 kb in length, with several structural features in common with recognised eukaryotic transposable genetic elements. Scrambled clusters of the sequence probably arise as a result of transposition-like events, during which the element preferentially recombines in either orientation with target sites located in other copies of the same repeated sequence. The target sites for transposition/recombination are not related in sequence but in all cases studied they are potentially capable of promoting the formation of small 'cruciforms' or 'Z-DNA' structures which might be recognised during the recombination process.
Key words: Physarum/repetitive DNA/transposon

Postfusion incompatibility in *Physarum polycephalum*: changes in protein pattern of a heterokaryon

J. A. M. SCHRAUWEN

Department of Botany, University of Nijmegen, The Netherlands

Accepted May 21, 1985

SCHRAUWEN, J. A. M. 1985. Postfusion incompatibility in *Physarum polycephalum*: changes in protein pattern of a heterokaryon. Can. J. Microbiol. 31: 778–781.

The polypeptide patterns of two different strains of *Physarum polycephalum*, sensitive and killer, showed only minor differences on two-dimensional electrophoretograms. After heterologous fusion of the sensitive and killer plasmodia, newly formed proteins could be demonstrated which were not detectable in homologous fused plasmodia. The lethal reaction did not occur until after the aforementioned protein synthesis.

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Growth and differentiation of wild type amoebae of *Physarum polycephalum* in liquid culture

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A method for growing wild type amoebal strains of *Physarum polycephalum* in two-membered liquid culture is presented. The medium is a simple buffered salts solution. We found that a minimal level of divalent cation was required for growth. All amoebal strains tried to date have grown under our conditions in stationary culture. Growth under gyratory conditions was only successful at 60 rpm or less and consistent growth required a period of adaptation over several transfers. Differentiation of two apogamic strains, CL and CH1, were compared. Contrary to the results seen on agar plates, the time of onset for the first committed amoebae was identical for both strains in liquid culture. Attempts to demonstrate mating between two genetically compatible amoebal strains grown together in liquid culture were not successful.

amoebae; liquid culture; growth; differentiation

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A FURTHER ASSESSMENT OF THE SUB-NUCLEOSOMAL PRODUCT, "PEAK A", FROM PHYSARUM POLYCEPHALUM

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Nuclease digestion studies of Physarum polycephalum nuclei (1-3) and nucleoli (4) over the past few years have been centred on a number of modified nucleosomal products which have been related to active-gene regions of the genome. We have re-investigated one such particle, peak A, using the techniques of differential melting and polyacrylamide gel electrophoresis and show that this material is unlikely to be a specific histone:DNA complex as suggested by earlier authors.

Electrophoresis 1986, 7, 107–113

Original papers

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Computer analysis of two-dimensional gels by a general image processing system

In order to survey changes in accumulation of several hundred proteins during the naturally synchronous nuclear division cycle of *Physarum polycephalum*, we have developed methods for analyzing two-dimensional (2-D) gel electrophoretograms using the general image processing system developed by the Spatial Data Analysis Laboratory at Virginia Tech. In this paper we describe fast and accurate methods for removing non-homogeneous background intensity from a 2-D gel image, for resolving overlapping protein spots, and for estimating the total integrated intensity in a protein spot by Gaussian modeling.

Experimental Cell Research 162 (1986) 486–494

Spatial and Temporal Organization of Intracellular Adenine Nucleotides and Cyclic Nucleotides in Relation to Rhythmic Motility in *Physarum* Plasmodium

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Spatio-temporal organization of a migrating plasmodium was studied both by analysing intracellular concentrations of adenine and cyclic nucleotides and by applying image processing for recording oscillatory changes in thickness with use of microcomputers. ATP and ADP concentrations were about twice as high in the front as in the rear, while AMP distributed uniformly. On the other hand, cAMP and cGMP concentrations were several times higher in the rear than in the front, showing oscillations in between. The cAMP concentrations at the front oscillated with a phase advancing about one-third of the period with respect to the phase of the thickness oscillation, while cGMP concentration there varied only little. ATP concentration oscillated concomitantly with H^+. A feedback control loop consisting of (ATP-H^+)-cAMP-Ca^{2+} is proposed. The possible mechanism of rhythmic contractions involving mitochondria which may excrete pulses of Ca^{2+} and induce cell polarization is discussed.

Identification of an autonomously replicating sequence near a histone gene of *Physarum polycephalum*

Curr Genet (1986) 10:459–462

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Summary. Fragments of DNA wich function as autonomous replication sequences in yeast were cloned from *Physarum polycephalum*. The ars activity is located in a 1.2 kbp fragment extanding 1.5 kbp to 2.7 kbp upstream of the 5′ end of a histone H4 gene. Our recent finding that a replication origin is located at a distance less than 3 kbp of this histone gene suggests that the ars element identified coincides with a specialized replication origin and can be used to direct chromosome replication in *Physarum polycephalum*.

Key words: Ars sequence – Histone gene – Replication origin – *Physarum polycephalum*

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Identification of Histone H1° in *Physarum polycephalum*

ITS HIGH LEVEL IN THE PLASMODIAL STAGE INCREASES IN AMOUNT AND PHOSPHORYLATION IN THE SCLEROTIAL STAGE*

(Received for publication, June 10, 1985)

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An antiserum specific for the globular domain of the bovine very lysine-rich histone subfraction H1° cross-reacted with a single protein band in the chromosomal proteins isolated from microplasmodia of the true slime mold *Physarum polycephalum*. Its amino acid composition was characteristic of a very lysine-rich histone which supported its identification as *Physarum* H1°. Unlike *Physarum* H1, which is 50% larger than mammalian H1, *Physarum* H1° migrated very close to bovine H1° on sodium dodecyl sulfate gels.

In microplasmodia, the ratio of H1° to H1 was 0.66, whereas in sclerotia H1°/H1 was 1.33. Furthermore, both H1 and H1° in sclerotia were highly phosphorylated. The high level of H1° in the mitotically active microplasmodia argues against the proposed role of H1° just as an inhibitor of DNA replication. More probable is an association of H1° with quiescent but transcriptionally competent chromatin which could also include cell cycle genes. Hyperphosphorylation of H1 and H1° in sclerotia is probably required to maintain an inactive condensed state which can be reversed by dephosphorylation to allow transcriptionally competent chromatin to become available for expression.

54th Annual Meeting of the Genetics Society of America
Boston, Mass. August 11-15, 1985

Genetics 110 (3 part 2) 1985 S4-S5

(*1.10*) **Carrino, J. and T. Laffler, Northwestern University, Chicago, Illinois.** ***Cell cycle periodic transcription of tubulin and histone genes.*** —We are studying the molecular mechanisms involved in the cell cycle regulation of gene expression. In the myxomycete, *Physarum polycephalum*, the synthesis of both tubulin and histone genes is cell cycle regulated. The majority of tubulin synthesis occurs in a 2.5-hr period directly preceding mitosis, whereas the synthesis of histones is confined to S-phase. We have examined α-tubulin and histone H4 RNA accumulation over the cell cycle by both RNA dot hybridization and *in vitro* nuclear transcription. Tubulin mRNAs begin to accumulate approximately 30' prior to mitosis, peak at M-45', and decline rapidly at mitosis, returning to basal levels by early S. Surprisingly, H4 transcripts display a similar inductive profile. Analysis of transcription rates *in vitro* confirms these results. Peak levels of both α-tubulin and histone H4 gene transcription occur late in G_2 and are five- to eight-fold greater than basal levels. Although histone protein synthesis is restricted to S-phase, transcription of the histone genes is primarily a late G_2 event. These results suggest that the tubulin and histone genes of Physarum are part of a set of genes whose transcription is coordinately expressed in late G_2 phase and that the timing of histone protein synthesis is likely due to a posttranscriptional mechanism.

3rd Joint Meeting of the Biochemical Societies of France, Germany and Switzerland. Basel, Switzerland Sept 30-Oct 2, 1985

Biol Chem Hoppe-Seyler 366 (9) 1985

A. Hildebrandt

Methylation is an early and necessary step in the cell differentiation programme of the slime mould Physarum polycephalum

Illumination of starved macroplasmodia of Physarum induces sporangia morphogenesis 9 hours after light effect (a.l.e.). This differentiation process can be prevented (but only in a sensitive one hour period) by competitive inhibitors of methyltransferases, applied either by direct injection into the plasmodium or by addition to the medium (5 mM S-adenosyl-homocysteine, 10 mM L-ethionine, internal or external same concentrations). The injection technique allows the determination of the needed minimal internal concentration and of the moment of methylation; i.e. between the 3rd and 4th hour a.l.e. (inhibitor addition after this point of time has no effect). By the fact that 5 mM 5-azacytidine or 5 mM 5-aza-2'-deoxycytidine (given before the 4th hour a.l.e.) do also prevent the sporulation (in contrast to cytidine, 8-azaguanine or 6-azauridine) we suppose that DNA could be the substrate for methylation.

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G.Meyer, F.Tomalik and A.Hildebrandt

Two dimensional gel analysis of Physarum polycephalum DNA restriction fragments: Unusual repetitive sequences. Degradation of ribosomal genes during development

Physarum nuclear DNA was digested with 8 restriction endonucleases. The DNA fragments were analysed by two dimensional gel electrophoresis (first dimension according to molecular weight, second dim. acc. to mol. weight and base composition) (1).
a) It is shown that in all patterns exist one to two highly repetitive fragments that differ heavily from the rest of sequences with respect to their high (A+T)-content and to their lack of methylation. They are probably all interspersed repetitive sequences and one of them (≈ 1000 bp-fragment in the AluI-pattern) seems to be transcribed as judged by hybridization to nuclear RNA.
b) Comparsion of the restriction patterns of DNA from logarithmically growing microplasmodia and from starving, sporulation competent macroplasmodia shows a significant decrease of the number of the extrachromosomal nucleolar ribosomal genes during starvation. Hybridization of Southern blots and dot blots to labeled rRNA demonstrates that the reduction is to about 37% of the original copy number. The phenomenon is similar to the starvation-induced degradation of ribosomal genes in Tetrahymena (2, for review).

1) Müller,W., Hattemohl,I., Schütz, H.-J., Meyer, G. Nucl. Acids Res. 9, 95-119 (1981).
2) Engberg, J. Eur. J. Cell Biol. 36, 133-151 (1985).

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Eighteenth Annual Meeting of the Japanese Society of Developmental Biologists Nagoya, Japan May 9-11, 1985 in Development, Growth and Differentation 27(4): 1985 500

IIA-16 Sporulationless oat-growth declining variants in successively cultured Physarum polycephalum CL plasmodia. Eiko Hosoda Tokyo Metropolitan Isotope Res. Inst., Tokyo

The previous report of the sporulationless variants (oatl$^-$) obtained from successively oat-cultured K and KH11 plasmodia (Hosoda, 1984) was further analized by introducing haploid CL strain (Dee, 1982 Review).

Clonal isolate of CL plasmodia was successively maintained on oats as plasmodia and in liquid growth medium as microplasmodia, with serial examination of sporulating ability. Then, 60, 380, 27, and 67 clonal isolates derived from spores of 58, 136 days' oat-cultures and 78, 198 days' shaker cultures were feeded on oats and illuminated for sporulation induction after the previous methods (Hosoda, 1981). The rate of sporulation competent (sp$^+$)ones in the total isolates depended on the time of isolation (visual development of plasmodia), or more exactly, on that of oat-culture start. It was maximum among the earliest isolates, 100-80%, and were 80-70% in total isolation period of 9-25 days after spore inoculation. All the sp$^-$ plasmodia showed retarded growth, excreted exessive amount of slime, were liable to break into pieces when starved, and often melt down in the light. No culture method specific characteristics were detected thusfar.

After about 1 year, oat-cultured plasmodia were found sporulationless, and simultaneously, less vigorous in growth with similar appearances with some of above described sp$^-$ isolates, though microplasmodia kept sporulation competent and much the same growth rate as before in the liquid medium.

The results suggest that, in case of oat-culture, sp$^-$ variants had arisen in plasmodia and accumulation of them caused variation of plasmodia.

58th General Meeting of the Japanese Pharmacological Society Tokyo, Japan, March 26-29, 1985 in Jpn J Pharmacol 39 (Suppl.) 1985 p. 265

P2B0313) Effect of dephosphorylation by acid phosphatase on assembly and ATPase activities of Physarum myosin.

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Myosin was purified from plasmodia of slime mold, Physarum polycephalum to a purity of homogeneity (J. Biochem. 90, 1829). Total phosphate determination after ashing myosin revealed that myosin was in the phosphorylated form (4-5 mol/mol of 500,000 Mr myosin). Autoradiography of myosin prepared from Physarum cultured in the medium containing radioactive phosphate showed that only heavy chain is phosphorylated at the multiple sites.

Incubation of myosin with potato acid phosphatase at neutral pH dephosphorylated about 2 mol/mol myosin. However, appreciable changes were not detected in Mg-activated, K-EDTA-activated, actin-activated, and Ca-activated ATPase activities. After dialyzing myosin against assembly buffer (30 mM KCl 10 mM Mg^{2+} at pH 6.8), myosin was separated into monomeric and polymeric forms by the centrifugation. Ratio of monomer to polymer was increased from about 3:97 to about 30:70 by the dephosphorylation, indicating that dephosphorylation of heavy chain shifts the monomer-polymer equilibrium to monomeric myosin.

Dephosphorylated myosin was further subjected to analytical ultracentrifugation and electron microscopy. This myosin showed folded 8S form in the assembly buffer and conventional extended 6S form in the high salt, suggesting that this conformational change of heavy chain involved in the shift of the monomer-polymer equilibrium.

Part of this work was carried out at the MRC Laboratory of Molecular Biology at Cambridge, U.K. The authors wish their cordial thanks to Dr. John Kendrick-Jones for his advice and support.

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PHYSARUM NEWSLETTER Mailing List Changes, 5/14/86

Nadia Smulian: Remove

Khalid Choudry: Remove (deceased 1979)

Charles Cantor: Remove (addressee unknown)

Charles Rodi: Remove (left)

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