PHYSARUM NEWSLETTER

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PHYSARUM NEWSLETTER

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From The Editor:

I have just returned from the U.S. Physarum meetings in Berkeley, hosted by Ray Collins and Harry Matthews. We all felt that the meetings were very successful and thank Ray and Harry for showing us a good time. The winery tour in the Napa Valley and the Joffrey Ballet performance were non-scientific highlights. Scientifically, Finn Haugli brought the disappointing news that myxamoeba transformation is apparently not working. Eggehard Holler reported some exciting news about DNA polymerases. The tubulin work at Madison continues apace. Mike Madelin provided some surprising data about soil-borne Myxomycetes, including the news that they like to fruit on cabbage stems.

Following a tradition begun by Keith Gull in Madison in 1985, Greg Shipley presented the third annual GOLDEN SLIME AWARDS. Some of them were: Gerard Pierron, for presenting the first life cycle slide. Most exacting moderator, Volker Vogt. Most unbelievable data, Eggehard Holler. Most slippery data, Ray Collins. Most distracting noise, Jaap Waterborg. Best bath attire, Helmut Sauer. The "You should have seen the one that got away" award, Sylvia Kerr: "There was no film in the camera!" Most absurd question, Greg Shipley.

Anyone who did not attend the meeting who would like an abstract book should contact Henry Aldrich for a copy.

Volker Vogt has invited us to Ithaca, New York, for the next U.S. meeting in two years.

The next European Meeting will be in Regensburg, W. Germany, about July 18, 1988. Eggehard Holler is the organizer. Newsletter subscribers will receive details by mail. All Physarum workers worldwide are naturally welcome at both meetings.

Joyce Mohberg somehow found time to sort out all of her reprints. In doing so, she discovered some duplicates which she is willing to give away. There is a listing of those at the end of this issue. She also says that she needs original copies of Stewart and Stewart and of Rhea's plasmodium paper, if anyone has duplicates they could give her.

Finally, thanks for keeping the reprints coming in and making my job easier! Sorry this issue is a little late, but I was hospitalized in early April with a heart problem (all OK now!) and was delayed getting it out. Ms. Julie Bell has helped tremendously with the production process. Let me also remind you that original articles for the Newsletter are welcome at any time. Send them camera-ready, typed single-spaced.

Henry Aldrich

FILMS

by the Institut für den Wissenschaftlichen Film
Nonnenstieg 72
D-3400 Göttingen

Biol. 17/2 – C 1543

FILME FÜR FORSCHUNG UND HOCHSCHULUNTERRICHT

FRIEDHELM ACHENBACH, KARL ERNST WOHLFARTH-BOTTERMANN, Bonn, und INSTITUT FÜR DEN WISSENSCHAFTLICHEN FILM, Göttingen:

Film C 1543

Zellbiologische Studien an Physarum polycephalum Morphogenese und Differenzierung im Protoplasmatropfen

Verfasser der Publikation: FRIEDHELM ACHENBACH

Summary of the Film:

Cellbiological Studies on Physarum polycephalum – Morphogenesis and Differentiation in Protoplasmic Drops. Cytomorphogenetic processes responsible for plasmalemma regeneration and formation of the contractile apparatus consisting of plasmalemma invaginations in conjunction with the actomyosin system are described using time-lapse and animation.

Biol. 17/3 – C 1576

FILME FÜR FORSCHUNG UND HOCHSCHULUNTERRICHT

FRIEDHELM ACHENBACH, KARL ERNST WOHLFARTH-BOTTERMANN, Bonn, und INSTITUT FÜR DEN WISSENSCHAFTLICHEN FILM, Göttingen:

Film C 1576

Zellbiologische Studien an Physarum polycephalum Ein Modell zur Untersuchung cytoplasmatischer Actomyosine

Verfasser der Publikation: FRIEDHELM ACHENBACH

Summary of the Film:

Cellbiological Studies on Physarum polycephalum – Model Object for the Analysis of Cytoplasmatic Actomyosin. Following general considerations of the light- and electronmicroscopic fine structure of *Physarum polycephalum*, the film demonstrates different techniques to investigate rhythmic contractions of this organism which represent the basis of plasmodial locomotion.

Successive contraction-relaxation cycles experimentally induced in cell-free models of Physarum polycephalum

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European Journal of Cell Biology 42, 111-117 (1986)

Physarum polycephalum — cell-free models — reactivation of cytoplasmic actomyosin — oscillating contractions — DMSO extraction — calcium

Repeated contraction-relaxation cycles can be induced in 30-min-old endoplasmic drops of Physarum treated in 50% dimethylsulfoxide (DMSO) for 30 min. Contractions are triggered by the addition of MgATP in the presence of less than 10^{-6} M Ca^{++}, i.e., 10^{-7} M and in the absence of Ca^{++}. The contractions are N-ethylmaleimide (NEM) sensitive and are inhibited in the absence of Mg^{++} as well as in the presence of more than 10^{-3} M Ca^{++}. Moreover, a DMSO treatment exceeding 1 h prevents reactivation as well. At *p*H 7.2, relaxation of the models can be induced by removal of ATP in the presence of *p*Ca 7, and by changing *p*Ca from 7 to 5 in the presence of 0.1 mM ATP possibly due to the intrinsic elasticity of the models. During DMSO treatment and subsequent washing (0.5–24 h) the models retain their "compact" appearance, i.e., the density of the cytoplasm characteristic for its normal aspect in the phase-contrast microscope.

Plasmodial strands subjected to 50% DMSO treatment during tensiometric measurement are reactivated under isotonic and isometric regime by MgATP at low calcium concentration after washing for more than 2 h.

DIFFERENCES IN THE CELL SURFACES OF TWO COMPATIBLE *PHYSARUM POLYCEPHALUM* MYXAMOEBAE[1]

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Mycologia, 79(2), 1987, pp. 216–227.

ABSTRACT

The cell surface chemistry of *Physarum polycephalum* myxamoeba strains RSD4 and MA185, both capable of axenic growth, was studied. Myxamoebae were either washed with a low molarity phosphate/salt buffer or were left unwashed. The freeze-fractured, deep-etched cell surface of RSD4 cells was normally smooth, but after washing in buffer it was fibrous and rough. Similarly treated cells of strain MA185 were smooth under both circumstances. Several lectins were tested for their ability to bind to the cell surfaces of both strains. While concanavalin A bound only to washed cells of RSD4, it labeled both washed and unwashed cells of MA185. Wheat germ agglutinin bound in a patchy pattern to washed cells of both strains. A rosette test determined the ability of washed and unwashed myxamoebae of both strains to bind erythrocytes. Sheep red blood cells bound to the surface of unwashed cells of strain MA185. Analysis of the material washed from the surface of strain RSD4 cells with the phosphate/salt buffer indicated both protein and carbohydrate components. Between 16 and 20 polypeptides, indicated by polyacrylamide gel electrophoresis, were found in ethanol and ammonium sulfate precipitates of the cell surface material, in addition to four concanavalin A binding glycoproteins.

Belyavskii M A; Beilina S I; Beronfeld B S; Rashevskaya E P; Fateeva L A., 1986.
Light-stimulated changes in calcium exchange and their relation to the cell cycle control in Physarum polycephalum plasmodium.
Inst. Chemical Physics, Kosygin Str. 4, USSR-117977 Moscow.
STUD BIOPHYS 116 (3): 205-215.

It was shown, that white light in the physiological range of intensities causing mitotic block, when applied in the last quarter of the G2-phase of the Physarum plasmodium cell cycle, leads to: 1) inhibition of calcium uptake in the first calcium-binding compartment, *maximal in the last quarter of the G2-phase*; 2) *activation of the output processes*, presumably the ejection of the Ca++-binding vacuoles, in the same interval of the cell cycle; 3) increase in the rate of Ca++ uptake in the second compartment. The assumption is made that the action of light on the cell cycle is due to a lowering in the cytoplasmic free calcium concentration and activation of phosphoinositol turnover.

Reactivation of cytoplasmic actomyosin in *Physarum* plasmodia extracted with glycerol and dimethylsulphoxide

RENATE BELL and FRIEDHELM ACHENBACH

J. Cell Sci. 87(2). 1987. 231-240.

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Summary

Thin-spread plasmodia of *Physarum* were subjected to extraction procedures using 50% glycerol or DMSO (dimethylsulphoxide) followed by labelling of actin with fluorescent phallotoxins.

During the reactivation of the actomyosin system by 2 mM-MgATP fluorescent actin fibres contract isotonically, which results in numerous fluorescent 'contraction beads'.

After short-term extraction 1 mM-Ca^{2+} has an inhibitory effect on the reactivation. This calcium sensitivity is abolished after long-term extraction with glycerol.

Calcium at 10 mM irreversibly inhibits reactivation, irrespective of the duration of extraction. The inhibitory effect of 10 mM-calcium is prevented by phallotoxin labelling prior to incubation in Ca^{2+}.

The DMSO model shows an improvement in structural preservation when compared with the glycerol models. However, reactivation is inhibited by prolonged treatment with DMSO.

Key words: *Physarum polycephalum*, reactivation of cytoplasmic actomyosin, calcium, fluorescence microscopy.

Volume 14 Number 8 1986 Nucleic Acids Research

Processing in the external transcribed spacer of ribosomal RNA from *Physarum polycephalum*

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Received 19 March 1986; Accepted 2 April 1986

ABSTRACT

The rDNA of the myxomycete Physarum polycephalum is transcribed to give a 13.3 kb precursor of ribosomal RNA. At 1.7kb downstream of the primary initiation site there is a processing site or a second initiation site. This site was studied by S1-mapping, DNA sequencing and electron microscopy. None of these methods could conclusively distinguish between the two formal possibilities. However, capping experiments indicate that rapid processing is taking place at this site rather than reinitiation. In addition, primary transcripts and processed molecules were assayed throughout the synchronous mitotic cycle. During all interphase stages newly initiated transcripts of rDNA and products of the first processing step are present in similar amounts, indicating control of initiation and not of maturation as being the main regulatory step for the accumulation of mature rRNAs. During the brief period of mitosis the level of newly initiated rRNA precursors is lowered.

Bevlina, S I; Belyavskii M A. 1986. Calcium exchange in Physarum polycephalum plasmodium at different stages of the cell cycle. Inst. Biological Physics, Academy Sciences of the USSR, USSR-142 292 Puscino.
Stud Biophys 116 (3):195-203.

Using radioisotope method, kinetic analysis of Ca++ uptake and output was made and the dynamics of calcium exhange at different stages of Physarum Plasmodium cell cycle was studied. Based on the difference in the Ca++ uptake rates, 4 calcium - binding compartments with the exchange times of about 5, 15, 60 and more then 180 min and the volumes of a few mmoles altering about 1.5-2 times depending on the physiological state of the plasmodium were shown to exist. The changes in the volume of the first and third compartments supposely due to the ejection and appearance of newly formed Ca2+-binding vacuoles followed by changes in plasmalemma surface were observed in the last quarter of the G2 phase of the plasmodium cells cycle.

176 The Conserved Nucleolar Protein, B-36, is a Major Protein Constituent of a Ribonucleoprotein Complex. M. E. Christensen, Texas A & M University, College Station, Texas.

The nucleoli of eukaryotic cells contain a conserved 34 kD protein, B-36, which is similar to structural proteins which bind to pre-mRNA (forming hnRNP particles) and have a topological role in RNA splicing. It is hypothesized that B-36, due to its nucleolar location, may have a similar function, but in association with pre-rRNA. Previous results based on immunofluorescence showed that the localization of B-36 in the nucleolus of the slime mold Physarum polycephalum is sensitive to RNase digestion suggesting that the protein is bound to nucleolar RNA. A mild nuclear extraction method has been developed which releases greater than 80% of the newly-synthesized RNA together with an equivalent proportion of B-36 protein. Sucrose gradient analysis of this extract shows that B-36 sediments as the major protein constituent of a heterodisperse, 12-45S complex. This complex is extremely sensitive to RNase treatment, but is unaffected by DNase treatment. Differential RNase digestion reveals a rapid accumulation of a 10-12S substructure which is resistant to further RNA digestion. The 12-45S complex cosediments with newly-synthesized RNA (labeled during a 10 min pulse in vivo), while the 10-12S substructure lacks cosedimenting label. These results are tentatively interpreted to indicate that B-36 is the major protein constituent of a "core" structure which is itself associated loosely with an RNA species. The latter is lost during RNase digestion, leading to exposure of the resistant core. Such an interpretation is reminiscent of the organization of hnRNP complexes and supports the hypothesis that B-36 may be functionally analogous to the hnRNP core particle proteins. Work is currently underway to isolate and characterize the specific RNA component of the B-36 ribonucleoprotein complex.

Characterization of the Nucleolar Protein, B-36, Using Monoclonal Antibodies

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A panel of nine monoclonal antibodies has been produced against a major nucleolar protein, B-36, purified from the slime mold *Physarum polycephalum*. B-36, a 34 kD protein biochemically similar to the major structural proteins of mammalian hnRNP particles, was previously shown to be largely associated with the nucleolus. Eight of the monoclonal antibodies are specific for B-36 protein in *Physarum* and at least three different epitopes are represented among these eight. Using the monoclonal antibodies B-36 has been shown to be localized exclusively to the nucleolus in actively-growing *Physarum* cultures. The nucleolar localization of B-36 is dependent on the presence of intact RNA, but not DNA, supporting the hypothesis that B-36 is associated with nucleolar RNA, possibly in some analogous manner to the interaction of the related proteins within heterogeneous nuclear ribonucleoproteins (hnRNP) particles. B-36 is apparently a highly conserved nucleolar protein in eukaryotes as all eight of the monoclonal antibodies specific for B-36 in *Physarum* are also specific for a 34.5 kD nucleolar protein in rat liver. This indicates that a minimum of three distinct epitopes are conserved in B-36 protein from slime mold to rat.

Experimental Cell Research 166 (1986) 77–93

Morphological evidence for the existence of a more complex cytoskeleton in *Amoeba proteus*

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Summary. Various stabilization and extraction procedures were tested to demonstrate the ultrastructural organization of the cytoskeleton in normal, locomoting *Amoeba proteus*. Most reliable results were obtained after careful fixation in glutaraldehyde/lysine followed by prolonged extraction in a polyethylene glycol/Triton X-100 solution. Before dehydration in a graded series of ethanol and critical-point drying, the amoebae were split by the sandwich-technique, i.e., by mechanical cleavage of cells mounted between two poly-L-lysine-coated glass slides. Platinum-carbon replicas as well as thin sections prepared from such cell fragments revealed a cytoskeleton composed of at least four different types of filaments: (1) 5–7-nm filaments organized as a more or less ordered cortical network at the internal face of the plasma membrane and probably representing F-actin; (2) 10–12-nm filaments running separately or slightly aggregated through the cytoplasm and probably representing intermediate filaments; (3) 24–26-nm filaments forming a loose network and probably representing microtubules; and (4) 3–4-nm filaments as connecting elements between the other cytoskeleton constituents. Whereas microfilaments are responsible for protoplasmic streaming and other motile phenomena, the function of intermediate filaments and cytoplasmic microtubules in amoebae is still obscure.

Cell Tissue Res (1986) 246:163–168

Key words: Cytoskeletal organization – Filaments – Triton extraction – Replica technique – Electron microscopy – *Amoeba proteus*

THE MECHANICS OF MOTILITY IN DISSOCIATED CYTOPLASM

BIOPHYSICAL JOURNAL VOLUME 50 December 1986 1165 1183

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ABSTRACT We stimulate the dynamical behavior of dissociated cytoplasm using the Reactive Flow Model (Dembo, M., and F. Harlow, 1986, *Biophys. J.*, 50:109–121). We find that for the most part the predicted dynamical behavior of the cytoplasm is governed by three nondimensional numbers. Several other nondimensional parameters, the initial conditions, and boundary conditions are found to have lesser effects. Of the three major nondimensional parameters, one (D^*) controls the percentage of ectoplasm, the second (C^*) controls the sharpness of the endoplasm–ectoplasm boundary, and the third (R^*) controls the topological complexity of the endoplasm–ectoplasm distribution.

If R^* is very small, then the cytoplasm contracts into a single uniform mass, and there is no bulk streaming. If R^* is very large, then the cytoplasmic mass breaks up into a number of clumps scattered throughout the available volume. Between these clumps the solution undergoes turbulent or chaotic patterns of streaming. Intermediate values of R^* can be found such that the mass of cytoplasm remains connected and yet undergoes coherent modes of motility similar to flares (Taylor, D.L., J.S. Condeelis, P.L. Moore, and R.D. Allen, 1973, *J. Cell Biol.*, 59:378–394) and rosettes (Kuroda, K., 1979, Cell Motility: Molecules and Organization, 347–362).

Distribution of Acetylated Alpha-Tubulin in *Physarum polycephalum*

The Journal of Cell Biology, Volume 104, February 1987 303–309

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Abstract. The expression and cytological distribution of acetylated alpha-tubulin was investigated in *Physarum polycephalum*. A monoclonal antibody specific for acetylated alpha-tubulin, 6-11B-1 (Piperno, G., and M. T. Fuller, 1985, *J. Cell Biol.*, 101:2085–2094), was used to screen for this protein during three different stages of the *Physarum* life cycle—the amoeba, the flagellate, and the plasmodium. Western blots of two-dimensional gels of amoebal and flagellate proteins reveal that this antibody recognizes the α3 tubulin isotype, which was previously shown to be formed by posttranslational modification (Green, L. L., and W. F. Dove, 1984, *Mol. Cell. Biol.*, 4:1706–1711). Double-label immunofluorescence demonstrates that, in the flagellate, acetylated alpha-tubulin is localized in the flagella and flagellar cone. Similar experiments with amoebae interestingly reveal that only within the microtubule organizing center (MTOC) are there detectable amounts of acetylated alpha-tubulin. In contrast, the plasmodial stage gives no evidence for acetylated alpha-tubulin by Western blotting or by immunofluorescence.

Localization of Actin in Spores and Swarm Cells of Myxomycetes

TRANS. AM. MICROSC. SOC., 105(4): 387–395. 1986.

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Abstract. Myxomycete spores possessed intense fluorescent foci when stained with nitro-benzo-oxadiazole-phallacidin (NBD-Ph). Fluorescent foci concentrated at the spore periphery prior to germination were lacking once protoplasts emerged. Unstained controls or controls extracted with potassium iodide (KI) showed no fluorescence. Acid phosphatase activity was demonstrated in spores through the Gomori reaction, and the hypothesis that actin filaments are associated with lysosomes is explored. Several species of myxomycete swarm cells displayed fluorescence in the posterior region when treated with NBD-Ph. The anterior region, encompassing the basal bodies and nucleus, exhibited little or no fluorescence. The presence of actin was confirmed using a KI extracted control. Disrupted negatively-stained preparations viewed with a transmission electron microscope revealed a dense filamentous network that bound heavy meromyosin.

A mutant β-tubulin confers resistance to the action of benzimidazole-carbamate microtubule inhibitors both *in vivo* and *in vitro*

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Eur. J. Biochem. *163*, 449–455 (1987)

The mutant BEN210 of *Physarum polycephalum* is highly resistant to a number of benzimidazole carbamate agents, including methylbenzimidazole-2-yl-carbamate and parbendazole. The resistance is conferred by the *benD210* mutation in a structural gene for β-tubulin. This mutant allele encodes a β-tubulin with novel electrophoretic mobility. We have used this strain to determine whether the mutant β-tubulin is used in microtubules and whether this usage permits microtubule polymerisation in the presence of drugs both *in vivo* and *in vitro*.

In vitro assembly studies of tubulin purified from the mutant strain have shown that microtubules are formed both in the absence of drugs and in all drug concentrations tested (up to 50 μM parbendazole). In contrast, the assembly of microtubules from wild-type tubulin *in vitro* is totally inhibited by 2–5 μM parbendazole. Thus the resistance of BEN210 to parbendazole observed *in vivo* has been reproduced *in vitro* using tubulin purified from the mutant strain. Electrophoretic analysis of the microtubules formed *in vitro* has shown that both the wild-type and the mutant β-tubulin are incorporated into the microtubules and that the proportion of mutant to wild-type β-tubulin appears to remain constant with increasing drug concentration.

This is the first demonstration of a single mutation in a tubulin structural gene causing an altered function of the gene product *in vitro*.

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CHANGES IN PHOSPHORYLATION OF NONHISTONE PROTEINS DURING DIFFERENTIATION OF A LOWER EUKARYOTE PHYSARUM POLYCEPHALUM

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SUMMARY: During starvation-induced differentiation of a slime mold Physarum polycephalum several changes in the phosphorylation of nuclear proteins occur. The overall content of serine- and threonine-bound phosphate drops by 50% and de novo phosphorylation of a number of nonhistone proteins is drastically altered. On the contrary, no selective dephosphorylation of nuclear proteins phosphorylated under normal growth accompanies differentiation. © 1987 Academic Press, Inc.

Experimental Cell Research 168 (1987) 173–181

Flow Cytometry of the Differentiation of *Physarum polycephalum* Myxamoebae to Cysts

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Myxamoebae of *Physarum polycephalum*, strain Cld, were grown on agar lawns on live bacteria. Myxamoebae were harvested, fixed and stained with propidium iodide. Flow cytometry showed that, as in the case of *Physarum* plasmodia, there is no G1 phase during rapid exponential growth. However, an apparent G1 phase was observed at the end of exponential growth when the culture arrested with the G1 DNA content for about a day between growth and differentiation. Most myxamoebae differentiated into cysts, but some formed microplasmodia and others appeared to lose DNA. The cysts possessed the G2 phase DNA content and there was an S phase connecting the G1-arrested state with the encysted state. Encystment was blocked by hydroxyurea (HU) suggesting that DNA synthesis is essential for encystment. The natural temporary synchronization in G1 phase may provide the basis of a method for selecting mutants with a conditional block in G2 or M phases. © 1987 Academic Press, Inc.

The histones of *Dictyostelium discoideum*

Experientia 43(2), 1987, 147-151.

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Department of Biology, University of Southampton, Medical and Biological Sciences Building, Southampton SO9 3TU (England), 1 April 1986

Summary. The histones of the cellular slime mould *Dictyostelium discoideum* have been separated by electrophoresis using both acid urea and sodium dodecyl sulphate systems, and the gel pattern compared with that of histones from *Physarum polycephalum* and calf thymus. *Dictyostelium* is found to possess a full complement of H1, H2A, H2B, H3 and H4.

Key words. Dictyostelium; slime mould; histone; electrophoresis.

Cycloleucine relieves the adenine-induced inhibition of microcyst formation

Cytobios 49 31–37 1987

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Abstract

Myxamoebae-swarm cells of the eukaryote *Physarum flavicomum* convert to dormant, cell wall bound microcysts in the appropriate medium and environmental conditions. However, the addition of 5 mM adenine to the medium results in an increased intracellular concentration of S-adenosylmethionine (SAM) which produces a disruption of metabolic controls, an inhibition of microcyst formation, and cell death. Cycloleucine (1-aminocyclopentanecarboxylic acid) is known to be a specific inhibitor of ATP: L-methionine S-adenosyl transferase which catalyses the synthesis of SAM. Cells incubated with adenine and 5 mM cycloleucine are able to convert to microcysts at a rate approaching that of untreated control cells. Cells incubated with 5 mM adenine are not relieved of the inhibition with 1 mM cycloleucine while 10 mM cycloleucine provides no better relief than does the 5 mM concentration. Incubation of cells with adenine and methionine produces an inhibition of encystment comparable to that produced by adenine alone. Methionine by itself has no effect on microcyst formation.

Influence of polyamines and inhibitors of polyamine synthesis on the formation of microcysts

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Microbios 50 17–27 1987

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Abstract

Certain polyamines (PA), the PA-related diamines, and inhibitors of PA biosynthesis influence the ability of myxamoebae-swarm cells of *Physarum flavicomum* to differentiate to microcysts in the absence or the presence of adenine (an encystment inhibitor). Putrescine produces a moderate inhibition of encystment, but spermidine, spermine, or 1,3 diaminopropane are strong inhibitors and cell death ensues. Combining those compounds, with or without adenine, results in a strong inhibition. Methylglyoxal *bis*-(guanylhydrazone) produces rapid cell death in the absence or the presence of adenine. Difluoromethylornithine (DFMO) does not affect microcyst formation and does not relieve the inhibition caused by adenine. However, encystment in the presence of 5 mM cycloleucine is stimulated by DFMO. Cadaverine stimulates microcyst formation and increasingly relieves the inhibition induced by adenine up to a certain concentration. Cyclohexylammonium sulphate greatly stimulates microcyst formation, even in the presence of adenine.

Methylation Is an Early and Necessary Step in the Sporulation Programme of the Slime Mold *Physarum polycephalum*

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Exp. Cell Res. 167, 1986. 271-275.

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Formation of sporangia can be induced in starved macroplasmodia of *Physarum polycephalum* by illumination. This process of morphogenesis which starts 9 h after the needed period of illumination can be prevented by the competitive inhibitors of methyltransferases S-adenosyl-homocysteine or L-ethionine, applied either directly by microinjection into the plasmodia or by addition to the medium. Because 5-azacytidine (aza-C) or 5-aza-2'-deoxycytidine (aza-dC) also prevent sporulation (in contrast to cytidine, 8-azaguanine (aza-G) or 6-azu-uridine (azu-U) it is suggested that DNA is the substrate for methylation. The injection technique allows one to determine the period of methylation (i.e. between the 3rd and 4th h of the cell differentiation process after the induction by illumination). Based on the correlation between methylation and genome expression, it is suggested that some genes must be repressed by methylation during this period. © 1986 Academic Press, Inc.

Experimental Cell Research 167 (1986) 453–457

A Morphogen for the Sporulation of *Physarum polycephalum* Detected by Cell Fusion Experiments

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The light stimulus, which under conditions of starvation induces the development of sporangia in the slime mold *Physarum polycephalum*, can be transferred from the light-exposed part to the unexposed part of a plasmodium by means of plasma circulation. A small quantity of protoplasm from a sporulating donor plasmodium, which had passed through the premorphogenetic phase, was transferred by a short period fusion with a briefly starved, light-induction-incompetent acceptor plasmodium. This led to sporulation and even to a reduction of the premorphogenetic phase from 9 down to 3 h in the acceptor plasmodium. After fusion with a sporulating plasmodium, a highly starved plasmodium from a non-sporogenic culture line or a growing plasmodium from a normal line prevents further morphogenesis of sporangia in the sporulating partner. © 1986 Academic Press, Inc.

A DNA polymerase with unusual properties from the slime mold *Physarum polycephalum*

Eur. J. Biochem. *163*, 397 – 405 (1987)

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(Received October 13, 1986) – EJB 86 1088

Two forms of a DNA polymerase have been purified from microplasmodia of *Physarum polycephalum* by poly(ethyleneimine) precipitation and chromatography on DEAE-Sephacel, phosphocellulose, heparin Sepharose, hydroxyapatite, DNA-agarose, blue-Sepharose. They were separated from DNA polymerase α on phosphocellulose and from each other on heparin-Sepharose. Form HS1 enzyme was 30–40% pure and form HS2 enzyme 60% with regard to protein contents of the preparations. Form HS2 enzyme was generated from form HS1 enzyme on prolonged standing of enzyme preparations. The DNA polymerases were obtained as complexes of a 60-kDa protein associated with either a 135-kDa (HS1) or a 110-kDa (HS2) DNA-polymerizing polypeptide in a 1:1 molar stoichiometry. The biochemical function of the 60-kDa protein remained unknown. The complexes tended to dissociate during gradient centrifugation and during partition chromatography as well as during polyacrylamide gradient gel electrophoresis under nondenaturing conditions at high dilutions of samples. Both forms existed in plasmodia extracts, their proportions depending on several factors including those which promoted proteolysis.

The DNA polymerases resembled eucaryotic DNA polymerase β by several criteria and were functionally indistinguishable from each other. It is suggested that lower eucaryotes contain repair DNA polymerases, which are similar to those of eubacteria on a molecular mass basis.

Dynamic Aspects of the Contractile System in *Physarum* Plasmodium: I. Changes in Spatial Organization of the Cytoplasmic Fibrils According to the Contraction-Relaxation Cycle

Mitsuo Ishigami

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Dynamic changes in the spatial organizations of cytoplasmic fibrils (microfilament bundles) related to the contraction-relaxation cycle in thin-spread plasmodia of ***Physarum polycephalum*** were investigated by fluorescence microscopy, where NBD-phallacidin was used to stain the fibrils, combined with polarizing light microscopy.

The fibrillar organization in the anterior region, which consists of a fanlike spreading plasmodial sheet, strikingly changed according to the phase of the cycle. In the early stage of the contraction, as the endoplasm began to stream backward, the fibrils developed into a number of slender and flabby fibrils emanating from the inside of the cell membrane and the nodes. They became thicker and more straightforward fibrils running parallel to each other at the middle stage, and finally formed a thick framework consisting of a "polygonal network" near the tip of the migrating front and a "parallel array" in the inner part. In the relaxation phase, as the endoplasm streamed forward, the fibrillar framework disintegrated gradually and finally disappeared almost completely, remaining only around the nodes in some cases.

The fibrillar patterns in the posterior region, which consists of ramified strands, showed no conspicuous rhythmic change with alternation of the streaming direction.

Cell Motility and the Cytoskeleton 6:439–447 (1986)

Dynamic Aspects of the Contractile System in *Physarum* Plasmodium: II. Contractility of Triton Cell Models in Accordance With the Contraction and Relaxation Phases of the Plasmodia

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The contractility of *Physarum* plasmodium was investigated using cell models that were prepared by treating thin-spread plasmodia with ice-cold 0.2% Triton X-100. Cell models obtained from the anterior regions of the thin-spread plasmodia in the contraction phase retained many birefringent cytoplasmic fibrils. The fibrils vigorously contracted on addition of ATP, inducing simultaneous contraction of the whole cell models. In contrast, cell models prepared from the anterior regions in the relaxation phase scarcely contained the birefringent fibrils and exhibited only weak contractility on addition of ATP. The posterior regions of the thin-spread plasmodia, which were composed of ramified plasmodial strands, always retained many fibrils when treated with the Triton solution and showed intensive contraction on addition of ATP.

SDS-polyacrylamide gel electrophoresis showed that the model was enriched for actin and myosin. About 40% of the actin was extracted from the plasmodium by the Triton treatment, while scarcely any myosin was extracted.

Fragmin, a F-actin-fragmenting factor, caused the birefringent fibrils to diminish in the presence of Ca^{2+}, but more than 30 minutes was required for their complete disappearance. The birefringent fibrils weakened by 30-minute fragmin treatment disappeared immediately on addition of ATP or AMP-PNP.

Cell Motility and the Cytoskeleton 6:448–457 (1986)

Observations on the Ultrastructural Preservation of the Nucleus in the Myxomycete *Physarum polycephalum* as Observed in Resinless Sections

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KEY WORDS Reversible embedding, Nuclear preservation

ABSTRACT The ultrastructural preservation of the nucleus in both myxamoebae and macroplasmodia of the slime mold *Physarum polycephalum* was investigated using specimens processed with diethylene glycol distearate or Plexiglass. Following specimen embedding in either of these media, the polymers were dissolved from the semithin sections, which were then dried by the critical point process. In these resinless preparations, nuclei appeared to be pervaded by a complex network of fibrils of various diameters among which granules of different sizes were scattered. At least five different types of fibrils were detected in plasmodial as well as in amoebal nuclei. Resinless preparations obtained from diethylene glycol distearate processed specimens showed a number of easily recognized artifacts, some of which were noticeably less conspicuous when samples were embedded in Plexiglass. In both cases, no indication was obtained that extensive extraction of material had occurred during resin removal by solvents. The various artifacts that were produced following processing with these two media thus seem to have mostly developed during infiltration and hardening, rather than during dissolution of the polymers or critical point drying of the sections.

JOURNAL OF ELECTRON MICROSCOPY TECHNIQUE 5:227-241 (1987)

WIDESPREAD OCCURRENCE OF ANTI-TROPONIN T CROSSREACTIVE COMPONENTS IN NON-MUSCLE CELLS

J Cell Sci 85:1-19 (1986)

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SUMMARY

Using a monoclonal antibody generated against striated muscle troponin T, we previously noted the presence of crossreactive components in smooth muscle and non-muscle cells. Since the presence of troponin T in tissues other than striated muscle is controversial, we sought to establish the nature of the crossreaction and to determine the extent of its occurrence. For this study, indirect immunofluorescence microscopy and immunoblot analyses were performed. Crossreactive material was found in diverse cells from the animal, plant and fungal kingdoms. On the basis of morphological distributions, both microtubule-associated and non-microtubule-associated components were revealed. Microtubule-associated components of animal cell lines included a $35\times10^3 M_r$ protein, similar in electrophoretic mobility to skeletal troponin T ($37\times10^3 M_r$). Reactive components of comparable mobility were observed in immunoblots of brain and cerebellar homogenates. Filamentous staining was observed in a variety of mammalian cells in culture and in cells of vertebrate tissues. Chick cerebellar tissue showed reactions in the neurites of the molecular layer and granule cell bodies. In the plant kingdom, examination of the onion root-tip cells indicated an association of crossreactive components with interphase cortical microtubules, preprophase bands, the mitotic spindle and phragmoplast microtubules. In the fungal kingdom, both interphase and mitotic spindle microtubules in a cellular slime mould were reactive. Non-microtubule-associated components were observed in the centrosphere regions of mitotic sea-urchin eggs, in mitotic and interphase plasmodia of *Physarum polycephalum*, and in trichocysts and basal bodies of *Paramecium tetraurelia*. In all systems examined, the troponin T crossreactive components were located in regions or on structures of possible Ca^{2+} or calmodulin activity, suggesting a possible functional similarity to troponin T.

Intramolecular Localization and Effect on Conformational Stability in Vitro of Irreversible Interphase Phosphorylation of *Physarum* Histone H1†

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Biochemistry 1986, 25, 0495-0501

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ABSTRACT: To elucidate the intramolecular localization of irreversible interphase phosphorylation of *Physarum* histone H1 [Jerzmanowski, A., & Maleszewski, M. (1985) *Biochemistry 24*, 2360–2367] and its effect on H1's conformational properties, the circular dichroism spectra, the pH- and salt-dependent folding, and the products of trypsin digestion for the interphase phosphorylated (with five to nine phosphates per molecule) and enzymatically dephosphorylated H1 were compared. Both phosphorylated and dephosphorylated H1 show similar amounts (6.2 and 5.5%, respectively) of helicity at high ionic strength and upon limited digestion with trypsin form identical trypsin-resistant peptides of the size slightly larger than the analogous peptide from calf thymus H1. The circular dichroism analysis of the pH-dependent folding of *Physarum* H1 in water shows a strong effect of phosphorylation on the folding process in both the acidic and alkaline pH region. The analysis of the products of trypsin digestion of [^{32}P]PO_4-labeled *Physarum* H1 before and after enzymatic dephosphorylation is consistent with the interpretation that the interphase phosphorylation occurs predominantly within the 50–70 amino acid sequence directly adjacent to the trypsin-resistant peptide on its C-terminal side and that this sequence is itself involved in some kind of loose folding at high ionic strength. The studies of the formation of the trypsin-resistant peptide (the globular domain) as a function of salt concentration show that it is induced at 300 mM lower NaCl concentration for phosphorylated than for dephosphorylated H1. These results indicate that the stable, interphase phosphorylation of *Physarum* H1 enhances the salt-induced formation of the folded globular region in vitro. This conclusion together with our finding that only nonphosphorylated H1 occurs in the DNase I solubilized fraction of *Physarum* chromatin may be relevant for a mechanism of chromatin activation in *Physarum*.

Kawano S, Kuroiwa R, Anderson R. A third multiallelic mating-type locus in Physarum polycephalum. In press, J. Gen. Microb. Dept. Cell Biol., National Institute for Basic Biology, 38 Nishigonaka, Myodaijicho, Okazaki 444, Japan, and Dept. Genetics, University of Sheffield, Sheffield S10 2TN, UK.

Sexual development (crossing) in Physarum polycephalum occurs when two haploid amoebae fuse to form a diploid plasmodium. The imz locus influences the maximum pH at which crossing can occur. A new allele of imz has been identified, bringing the total number of alleles to three. Contrary to earlier findings, it has been shown that all homoallelic combinations of imz alleles display a similar pH limit for crossing, which is lower than that for imz-heteroallelic combinations. It is concluded that imz is a mating compatibility locus; thus the mating-type system of P. polycephalum comprises three multiallelic loci: matA, matB and imz. It is proposed that imz be renamed matC.

Kolosha, V O; Fodor I I. High homology of nucleotide sequences of Citrus limon and Saccharomyces cerevisiae ribosomal RNA cistron 26S.
Dokl Akad Nauk USSR 290 (4). 1986. 1006-1011. Inst. Biochem. Physiol. Microorg., Acad. Sci. USSR, Pushchino, USSR.

Comparative data were presented on the exact location of insertion sequences in C. limon and S. cerevisiae rRNA 26S. Data were also given on conservative sequences in C. limon, S. cerevisiae, Physarum polycephalum rRNA 26S and Escherichia coli rRNA 23S. The evolutionary relationship between higher plants and yeast is more significant than between higher plants and Physarum . The comparison of 2 sequences of rRNA 26S indicates that except for the insertion 10 in yeast rRNA or its disappearance from rRNA 26S of higher plants, no other significant changes occurred in the structure of 2 RNA 26S during the long period of divergence of higher plants and yeast from their common evolutionary predecessor.

Nuclear matrix proteins of *Physarum polycephalum*

Molec. Biol. Rep. 11: 219–223, 1986

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The proteins of nuclear matrix preparations from *Physarum polycephalum* were compared with analogous mammalian fractions by gel electrophoresis, DNA-binding studies and immunological tests. Polypeptides of 28 and 36 K dalton, which dominate in Physarum preparations, differed from calf thymus matrix proteins in that they were basic and showed low affinity to DNA. These polypeptides were present at about 1.2 mg per mg of nuclear DNA. Polypeptides of higher molecular weight occurred in the preparation at about 0.5 mg per mg of nuclear DNA. At least some of the latter proteins showed high affinity to DNA and cross-reacted with the antiserum against calf thymus matrix proteins.

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Spatio-Temporal Organization of Intracellular ATP Content and Oscillation Patterns in Response to Blue Light by *Physarum polycephalum*

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Protoplasma 135, 1986, 31-37.

Phototactic responses in a giant amoeboid cell of *Physarum* plasmodium were studied both by analyzing intracellular ATP content and by applying image processing for recording oscillatory changes in thickness with use of a microcomputer. The ATP content fluctuated and deviated from the initial level upon exposure to light with varying wavelength from 400 to 600 nm. The maximum decrease in the integrated value $\int_0^T ATP\,dt$ with $T \sim 9$ minutes occurred at the wavelength 450 nm. The ATP in a migrating plasmodium distributed twice as high in the front as in the rear. This polar pattern became unstable, and a new wavy pattern appeared by stimulating a local frontal part with blue light. In a concentrically extending plasmodium, peripheral and inside regions oscillated in opposite phase to each other. When part of this organism was exposed to light, stimulated and unstimulated regions began to oscillate in opposite phase, and phase waves propagated inward the stimulated region. And the protoplasm there became thinner, the strongest avoidance reaction occurring to 450 nm light as in ATP response. Phototactic behavior in *Physarum* is discussed in terms of bifurcation in spatio-temporal organization appearing in a self-organizing system.

Keywords: Blue light; Intracellular ATP; Oscillation pattern; *Physarum*

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INCORPORATION OF RADIOACTIVE MANNOSE INTO GLYCOLIPIDS AND GLYCOPROTEINS IN GROWING AND ENCYSTING PLASMODIA OF A SLIME MOLD

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SUMMARY. Plasmodia of the slime mold Physarum polycephalum incorporated radioactive mannose into the carbohydrate moiety of lipid-oligosaccharide conjugates and glycoproteins. When the pulse-labeled plasmodia were transferred to a non-radioactive medium, the radioactivity in the glycolipid decreased rapidly and it appeared in the glycoprotein fraction. This was not the case when the chase was performed in the presence of cycloheximide. These results suggest that transfer of the oligosaccharide unit from the lipid to protein is involved in the synthesis of glycoprotein in the slime mold. The transfer of the oligosaccharide appeared to be blocked in encysting plasmodia, suggesting that these glycoproteins are characteristic to actively growing plasmodia.

Murakami-Murofushi K; Minowa Y; Yamada R; Ohta J, 1986.
Mannosyl glycoproteins function in the conjugation of haploid amoebae of Physarum polycephalum.
Dep. of Biol., Fac. of Sci., Ochanomizu Univ., Ohtsuka, Bunkyo-ku, Tokyo 112, JAPAN.
Cell Struct Funct 11 (3): 219-226.

Concanavalin A at a concentration of 1.0 mg/ml completely inhibited the conjugation of haploid cells of Physarum polycephalum. This inhibitory effect was counteracted by alpha-methyl-D-mannoside, alpha-Mannosidase also inhibited conjugation. Neither wheat germ agglutinin nor lentil lectin had any apparent inhibitory effects. When Physarum cells were cultured in the presence of tunicamycin at a concentration of 1 .mu.g/ml, a similar inhibitory effect on conjugation was observed. The plasma membrane from the amoebae partially inhibited conjugation. On the basis of these results, the mannosyl glycoprotein(s) located on the cell surface is considered to function in conjugation process. Inhibitory compounds could be extracted from the cell surface with the detergent, Triton CF-54, and could be separated by concanavalin A-Sepharose column chromatography.

Structure of *Physarum* actin gene locus *ardA*: a nonpalindromic sequence causes inviability of phage lambda and *recA*-independent deletions

Gene, 48 (1986) 133–144

(Recombinant DNA; exon; intron; chloroacetaldehyde; exonuclease I and V; *recBC*⁻ *sbcB*⁻ mutants; S1-hypersensitive sites; slime mold)

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Previously we reported that approx. 80% of the genome from the plasmodial slime mold *Physarum polycephalum*, including all the actin genes, can be cloned only in *recBC*⁻ *sbcB*⁻ *Escherichia coli* hosts [Nader et al., Proc. Natl. Acad. Sci. USA 82 (1985) 2698–2702]. We have now sequenced the actin gene locus *ardA*. The nucleotide sequence of its coding region is flanked by the typical putative regulatory sequences for transcription initiation and polyadenylation. The coding region is interrupted by five introns, all located at novel positions with regard to those of previously analysed actin genes. Within the *ardA* gene we have located a 360-bp fragment which comprises exon V and parts of its flanking introns. This region suppresses plaque formation of recombinant λ phages and causes *recA*-independent deletions in phages and plasmids. In contrast to our previous hypothesis, this sequence is not a DNA palindrome, but consists of five (dA)·(dT)- and (dG)·(dC)-homopolymers. Both termination of replication and partial unwinding of duplex DNA under torsional stress were detected within the unstable 360-bp region in vitro.

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626 Changes in Antioxidant Defenses and Free Radical By-products During the Transition to a Non-proliferative Cell State in Physarum polycephalum. C. Nations, Southern Methodist University, Dallas.

Changes in the level of antioxidant defenses and the concentration of free radical by-products were examined in differentiating (MycVII and LU897xLU863), non-differentiating (LU897xLU897), and heterokaryon microplasmodia of Physarum polycephalum during sphérulation in salts-only medium. As differentiation proceeded, superoxide dismutase activity increased by as much as 4o-fold; GSH concentration and the rate of oxygen consumption decreased; cyanide-resistant respiration, hydrogen peroxide and organic peroxide concentrations were significantly increased. The white, non-differentiating culture failed to exhibit any of these changes. A heterokaryon obtained by the fusion of differentiating and non-differentiating strains was observed to differentiate at a very retarded rate and to exhibit the changes, observed in the sphérulating strains, at a correspondingly slower rate. The results support the hypothesis that a free radical mechanism is involved in the transition of microplasmodia to sphérules.

J. Cell Biol. 103(5 Part 2), 1986, 170a.

Superoxide dismutase activity and glutathione concentration during the calcium-Induced differentiation of Physarum polycephalum microplasmodia. In press, J. Cell Physiol

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ABSTRACT

Microplasmodia of Physarum polycephalum differentiate into spherules when the $CaCl_2$ concentration of their nutrient medium is increased to 54 mM (high-calcium). The salts medium that is routinely used to induce differentiation contains 8 mM $CaCl_2$ will not induce sphérulation in the absence of a calcium salt; no other metal is essential. High-calcium also induces the sphérulation of a strain of Physarum that had not previously been observed to sphérulate. The striking increase in superoxide disumutase activity (SOD) and the decrease in glutathione concentration (GSH) that are characteristic of salts-induced sphérulation do not occur in salts media containing high-calcium: both the SOD increase and the GSH decrease are retarded. In the absence of calcium no significant change in SOD is observed and very little change in GSH occurs. The immediate effect of the oxidative stress associated with sphérulation may be the release of calcium stores into the cytosol. The parameters modulating this stress are, in turn, sensitive to exogenous calcium concentrations.

Molecular cloning of stage specific mRNAs from amoebae and plasmodia of *Physarum polycephalum*

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Received April 29, 1986

Pallotta, D., Laroche, A., Tessier, A., Shinnick, T. & Lemieux, G. (1986) Molecular cloning of stage specific mRNAs from amoebae and plasmodia of *Physarum polycephalum*. *Biochem. Cell Biol.* *64*, 1294–1302

We constructed cDNA libraries from plasmodia and amoebal poly(A)$^+$ RNA of *Physarum polycephalum*. The libraries were screened by differential hybridization with labeled poly(A)$^+$ RNA of amoebae and plasmodia. The 136 plasmodial specific clones that gave the strongest hybridization signals were analysed in detail. From this group six different cDNA sequences were found. Four of the cDNAs each accounted for between 1 and 4.8% of all the clones in the library and represented abundant mRNAs. Two other clones constituted 0.2 and 0.4% of the total library. Seventeen clones in the amoebal library were amoebal specific. From these clones, seven different sequences were found. One of the sequences was present in nine clones (1.2%) of the library and considered abundant. The other six sequences were each found in only one or two clones. The specificity of these amoebal and plasmodial mRNAs was confirmed by Northern hybridization. Our results show that amoebae and plasmodia have different mRNA populations, which are most likely the result of differential gene expression in these two developmental stages.

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Histone Acetylation in Replication and Transcription: Turnover at Specific Acetylation Sites in Histone H4 from *Physarum polycephalum*

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Histone H4 from growing cells is partially acetylated at lysines 5, 8, 12, and 16. The turnover rate at each of these sites was investigated by pulse-labeling plasmodia of *Physarum polycephalum* with [^{3}H]acetate for 55 min in either S phase or G2 phase of the cell cycle. Labeled histone H4 was purified and digested with a protease which cleaves on the carboxyl side of arginine residues. The peptide containing the acetylation sites was purified by high-performance liquid chromatography. Subfractions of the peptide were obtained due to differences in acetyllysine content. Each subfraction was subjected to automated Edman degradation and the radioactivity released after each cycle was determined. Histone H4 was acetylated uniformly *in vitro* and acetylated peptide 1-23 was used as a control. The results show a very striking preference for turnover on lysine-5 in the "low acetyl" subfraction from cells in S phase; the "high acetyl" subfraction showed turnover at all four sites. The peptides labeled in G2 phase showed turnover mainly at positions -8, -12, and -16. The data imply that the patterns of histone acetyl turnover associated with replication and transcription are nonrandom and distinct. The results have implications for nucleosome structure particularly the possible role of lysine-5 in chromosome maturation and for the design of experiments to test chromatin function *in vitro*. © 1986 Academic Press, Inc.

ADP-ribosylation of histones and acid-soluble, high mobility group like chromosomal proteins from *Physarum polycephalum*

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POIRIER, G. G., S. COTÉ, and D. PALLOTTA. 1987. ADP-ribosylation of histones and acid-soluble, high mobility group-like chromosomal proteins from *Physarum polycephalum*. Biochem. Cell Biol. 65: 81–85.

Nuclei from the cellular slime mould *Physarum polycephalum* were incubated with [^{32}P]NAD. The chromosomal basic proteins were acid extracted and separated by two-dimensional polyacrylamide gel electrophoresis. After autoradiography the poly(ADP-ribosylated) proteins were identified. Histone H1 was the major acceptor. Histones H2B and H2A were significantly modified, although to a lesser extent than H1. In addition, the acid-soluble, high mobility group-like proteins AS-2 and AS-3 and the protein A-24 showed some modification. Histones H3 and H4 were not modified. The pattern of ADP-ribosylation did not change with NAD concentrations between 1 and 100 μM NAD.

Changes in plasmodial pigments of *Physarum polycephalum* in relation to the age of the culture

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RAKOCZY, L., MAJCHERCZYK, A., and HÜTTERMANN, A. 1987. Changes in plasmodial pigments of *Physarum polycephalum* in relation to the age of the culture. Can. J. Microbiol. 33: 217–220.

Plasmodia of the myxomycete *Physarum polycephalum* were grown in the dark on a semidefined medium and pigments were assayed from cultures of different ages. The absorption of the crude pigment extracts, measured at the maximum, increased with an increase in the growth rate of the organisms, but with a time lag of about 2 days when compared to increases in the plasmodial protein content. Anaylses of pigments were performed with a newly elaborated HPLC method, and eight pigments, monitored at 382 nm, were obtained from the different aged plasmodia. Throughout the culture, quantitative changes in the pigments were found which could be described as periodical. Individual pigments varied in their quantitative changes. In prolonged culture the amount of two pigments significantly increased, in comparison with the other colored compounds, and attained their maximum values after plasmodial growth ceased.

Acetylated α-Tubulin in *Physarum*: Immunological Characterization of the Isotype and Its Usage in Particular Microtubular Organelles

J. Cell Biol. 104. 1987. 41-50.

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Abstract. We have used monoclonal antibodies specific for acetylated and unacetylated α-tubulin to characterize the acetylated α-tubulin isotype of *Physarum polycephalum*, its expression in the life cycle, and its localization in particular microtubular organelles. We have used the monoclonal antibody 6-11B-1 (Piperno, G., and M. T. Fuller, 1985, *J. Cell Biol.*, 101:2085–2094) as the probe for acetylated α-tubulin and have provided a biochemical characterization of the monoclonal antibody KMP-1 as a probe for unacetylated tubulin in *Physarum*. Concomitant use of these two probes has allowed us to characterize the acetylated α-tubulin of *Physarum* as the α3 isotype. We have detected this acetylated α3 tubulin isotype in both the flagellate and in the myxameba, but not in the plasmodium. In the flagellate, acetylated tubulin is present in both the flagellar axonemes and in an extensive array of cytoplasmic microtubules. The extensive arrangement of acetylated cytoplasmic microtubules and the flagellar axonemes are elaborated during the myxameba-flagellate transformation. In the myxameba, acetylated tubulin is not present in the cytoplasmic microtubules nor in the mitotic spindle microtubules, but is associated with the two centrioles of this cell. These findings, taken together with the apparent absence of acetylated α-tubulin in the ephemeral microtubules of the plasmodium suggest a natural correspondence between the presence of acetylated α-tubulin and microtubule organelles that are intrinsically stable or cross-linked.

1175 Differential Replication of Cell Type Specific Genes in Physarum polycephalum. H.W. Sauer[1] and G. Pierron[2]. [1]Dept. of Biology, Texas A&M University, College Station, TX and [2]Institut de Recherches Scientifiques sur le Cancer, Villejuif, France.

We are interested in the concept that the replication of the genome may be a prerequisite for differential gene expression of a eukaryotic cell. The life cycle of Physarum allows for a test of the replication-transcription-coupling concept, because of its naturally synchronous mitotic cycle and distinct alternative cell types. We have shown that the activity of RNA polymerase B in early S-phase depends on DNA replication (J. Cell Sci. 41, 105, 1980) and demonstrated, by EM chromatin spreads, that active transcription units occur predominantly within early replicons (Eur. J. Cell Biol. 29, 104, 1982). Recently we have detected the invariant chronology of replication of the 4 unlinked actin loci of Physarum during its cell cycle (Proc. Natl. Acad. Sci. 81, 6393, 1984). We have now determined the timing of replication of cell type specific genes, utilizing cDNA clones of genes selectively expressed in the plasmodium or in the amoeba or during differentiation into dormant cysts (spherules), which were generously provided by D. Pallotta, Laval University, Quebec. By gene dosage determination and DNA density-shift experiments done during the synchronous S-phase of the plasmodium, we observed early replication of the plasmodial specific and late replication of the single copy genes specific for amoebae and spherules. Preliminary estimates of mRNA levels of plasmodial specific genes indicate that their replication is required for their elevated expression. We conclude that cell type specific genes of Physarum must replicate early if they are to be expressed, and postulate that commitment of a eukaryotic cell to selective gene expression is contingent upon the positioning of the respective genes in an early temporal compartment of the S-phase of its cell cycle.

J. Cell Biol. 103(5 Part 2), 1986, 317a.

Evidence That a low-M_r Diffusible Factor is Involved in Communication between Compatible Mating-types of the Slime Mould *Physarum polycephalum*

Journal of General Microbiology (1986), 132, 3491-3495.

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Two compatible mating-types of *Physarum polycephalum*, RSD4 and MA185, have recently been shown to have different surface properties by using aqueous two-phase partition. Changes in the surface properties of RSD4 amoebae were detected when in contact with cell culture medium from MA185 amoebae. The ability of MA185 culture medium to alter the surface of RSD4 amoebae was not affected by autoclaving or passage through a filter that removed molecules of M_r greater than 10000. Activity was detected in medium from MA185 amoebae that had not previously been in contact with RSD4 amoebae. These results provide evidence for the existence of a low-M_r factor produced by MA185 amoebae which affects the surface of RSD4 amoebae.

PHOSPHORYLATION OF MUSCLE AND NON-MUSCLE ACTINS BY CASEIN KINASE 1 IN VITRO

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Summary: Skeletal muscle and Physarum actins were markedly phosphorylated by casein kinase 1, but not by casein kinase 2. The amount of radioactive phosphate incorporated into muscle actin with 110 units of casein kinase 1 was approx. 0.2 mol per mol of actin, which was significantly greater than those catalyzed using the same number of enzyme units of protein kinase A or protein kinase C. The Km values of casein kinase 1 for muscle and Physarum actins were 0.270 and 0.667 mg/ml, respectively.

Amino-acid sequence data of β-tubulin from *Physarum polycephalum* myxamoebae

Eur. J. Biochem. *161*, 669–679 (1986)

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Starting with 7.7 mg of a β-tubulin isolated from myxamoebae of the slime mould *Physarum polycephalum*, 90% of the sequence has been determined by the Edman degradation of peptides generated by cyanogen bromide, trypsin and *Staphylococcus aureus* protease. Differences to other β-tubulins are mainly conservative and spread evenly throughout the chain except for a high concentration at the C-terminus. The *Physarum* β-tubulin shows most homology to *Chlamydomonas* β-tubulin (90.5%) and least homology to yeast β-tubulin (*S. cerevisiae*, 73.4%). Two tryptic peptides were isolated in approximately equal quantities which were identical except in one position (S/ALTVPELTQRMFDA) showing that at least two β-tubulins are present in myxamoebae. However, since this was the only heterogeneity found, these β-tubulins are probably very similar.

Amino Acid Sequence Data of α-Tubulin from Myxamoebae of *Physarum polycephalum*

J. Mol. Biol. (1986) 192, 919–924

Monika Singhofer-Wowra, Melvin Little, Lesley Clayton, Peter Dawsson, Keith Gull

About 96% of the amino acid sequence of an α-tubulin from the slime mould *Physarum polycephalum* has been determined. Of 430 sequenced amino acids, 30 differ from the deduced amino acid sequence of a recently published α-tubulin complementary DNA from the plasmodial form of *P. polycephalum*. The myxamoebal α-tubulin differs from all other known α-tubulins in one of the last three C-terminal amino acids that are Gly-Glu-Tyr instead of the usual Glu-Glu-Tyr. These last three amino acids are preceded by 11 residues that appear to be particularly susceptible to mutation. No heterogeneity was found whilst sequencing the myxamoebal α-tubulin, indicating that only one type of α-tubulin is present in myxamoebae. This α-tubulin appears to be less conserved than the previously described plasmodial α-tubulin, supporting the hypothesis that the structural constraints on tubulin in axonemes have a significant effect on its rate of mutation.

Volume 15 Number 1 1987 **Nucleic Acids Research**

Nucleotide sequence of *Physarum* U6 small RNA

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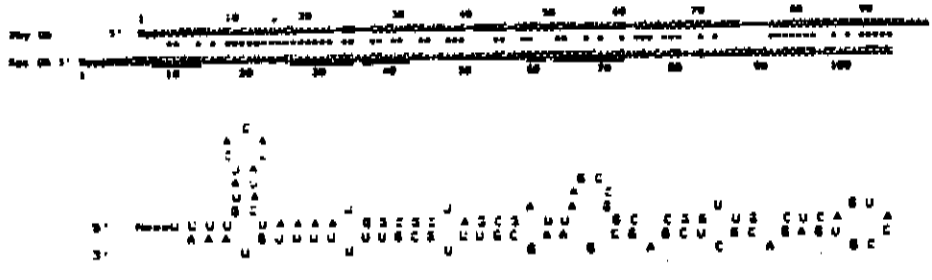
SEQUENCE ORIGIN:
Small RNA molecule U6 was isolated from the total cellular RNA of vegetative Physarum microplasmodia (1) by preparative polyacrylamide electrophoresis (2,3). The molecule was radiolabeled in vitro to high-specific-activity using [^{32}P]-pCp and RNA-ligase, and sequenced by direct chemical methods as we previously described (3). In this sequencing process, pseudouridine scores as a uridine, methylcytosine scores as a cytosine, and methyladenosine scores as an adenosine.



COMMENTS:
The primary sequence of Physarum U6 RNA is shown compared to the published (4) sequence of rat U6. Physarum U6 is 99 N long. One region, 93 N long, shows 65.6% homology (61/93) with nucleotides 10 to 108 of the rat U6 molecule. One 15 N subdomain, corresponding to nucleotides 18-32 of the rat molecule, is 100% conserved. Those rat U6 sequence domains proposed to base-pair with rat U4 RNA (5) are underlined in the Figure. Overall, these "functional" regions show 58.3% (21/36 N) homology with the rat sequence. Potential secondary structures of the Physarum U6 molecule were analyzed by computer programs (6). The programs predicted a secondary structure similar to the proposed structure for vertebrate U6 (4), basically, a simple hairpin structure with a single large single-stranded "bulge" in the center. Within the proposed stem regions, 86.7% (26/30) of the base changes preserved the hairpin structure. Interestingly, in those domains where primary structure shows poor homology, secondary structure is highly conserved.

OXYGEN FREE RADICALS PLAY A ROLE IN CELLULAR DIFFERENTIATION: AN HYPOTHESIS

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Abstract—Evidence from a variety of sources supports the view that oxygen free radicals play a role in cellular differentiation. It is postulated that cellular differentiation is accompanied by changes in the redox state of cells. Differentiated cells have a relatively more prooxidizing or less reducing intracellular environment than the undifferentiated or dedifferentiated cells. Changes in the redox balance during differentiation appear to be due to an increase in the rate of $O_2^{\cdot}$ generation. Differentiated cells, in general, exhibit higher rates of cyanide-resistant respiration, cyanide-insensitive SOD activity, and peroxide concentration and lower levels of GSH as compared to undifferentiated cells. The effects of free radicals on cellular differentiation may be mediated by the consequent changes in ionic composition.

Keywords—Cellular differentiation, Development, Oxygen metabolism, Free radicals, Oxidative stress, Redox state

Polyamines permit the preparation of stable *Physarum* core particles which have a structure similar to those from higher eukaryotes

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Biochimica et Biophysica Acta 908 (1987) 34–45

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Key words: Nucleosome core; Nuclease digestion; Neutron scattering; Circular dichroism; (*P. polycephalum*)

The inherent instability of *Physarum* nucleosome core particles prepared by micrococcal nuclease digestion in Na^+/Ca^{2+} buffers can be overcome by the addition of 0.15 mM spermine and 0.5 mM spermidine. Neutron scattering, circular dichroism, nuclease digestion and thermal denaturation studies carried out on these stable monosomes show them to be very similar to those obtained from higher eukaryotes.

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Differential gene expression during the amoebal-plasmodial transition in *Physarum*

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ABSTRACT

We have prepared cDNA libraries for amoebae and plasmodia of the acellular slime mould, **Physarum polycephalum**. Differential screening was used to isolate cell-type-specific cDNA clones (in bacteriophage M13) and both libraries yielded approximately 5% of such sequences. The amoebal- and plasmodial-specific clones were used to assay changes in transcription during the amoebal-plasmodial transition. The results obtained substantiate the view that the switch from amoebal to plasmodial characteristics occurs over several nuclear divison cycles. With one exception, the specific cDNAs came from single-gene families. Southern blotting experiments also showed that they hybridised to identical restriction fragments from amoebal and plasmodial DNAs indicating that genomic rearrangements are unlikely to be involved in the regulation of these genes.

Experimental Cell Research 169 (1987) 191–201

Patterns in the Distribution of Intracellular ATP Concentration in Relation to Coordination of Amoeboid Cell Behavior in *Physarum polycephalum*

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The *Physarum* plasmodium reacts tactically to external stimuli. The cell behavior of this giant amoeboid cell was studied by analysing intracellular ATP concentration. The two-dimensional (2D) spatial distribution of ATP depended on cell shape: a polar pattern for a unidirectionally migrating plasmodium, a bowl shape for a circular plasmodium, a hump shape for an oval plasmodium, or a wavy pattern for plasmodia stimulated with blue light or confined in a small chamber, etc. Local external stimulation brought about new patterns of ATP distribution. The ATP concentrations around the stimulated frontal region were reduced by about a half by stimulation with KCl (repellent) or casamino acids (attractant). In both cases, migration was inhibited. Migration velocity increased almost linearly with increasing concentration of intracellular ATP above the threshold (about 20 ug mg protein). Under anaerobic conditions or at low temperatures, the intracellular ATP oscillated slowly with a periodicity of about 30 min. Pattern formations in the intracellular ATP concentration and amoeboid coordination are discussed in terms of coupled chemical oscillators in a self-organizing system. © 1987 Academic Press, Inc.

Experimental Cell Research 169 (1987) 74–84

Myosin Switching during Amoebo-plasmodial Differentiation of Slime Mold, *Physarum polycephalum*

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We reported previously that myosins from amoebal and plasmodial stages in the life cycle of *Physarum polycephalum* differ in the primary structure of heavy chains and phosphorylatable 18000 *M*, light chains, while Ca-binding 14000 *M*, light chains are common to both myosins (Kohama & Takano-Ohmuro, Proc Jpn acad 60B (1984) 431; Kohama et al., J biol chem 260 (1986) 8022) [15, 16].

We have carried out immunofluorescence microscopical studies upon differentiating cultures of amoebic colonies, which show apogamic amoebo-plasmodial differentiation as follows: Typical amoebae differentiate into mono-nucleate intermediate cells with swollen nuclei and then into two or multi nucleate young plasmodia (Anderson et al., Protoplasma 89 (1976) 29 [4]). Antibodies against plasmodial myosin heavy chain (PMHC) and 18000 *M*, plasmodial myosin light chain (PMLC 18) stained intermediate cells and young plasmodia, but not typical amoebae. On the other hand, antibody against amoebal myosin heavy chain (AMHC) stained typical amoebae and intermediate cells—but not young plasmodia. Thus staining was detected using antibodies against both PMHC and AMHC in intermediate cells. Intermediate cells were also stained by antibody against another plasmodium-specific cytoskeletal protein, viz., high molecular weight actin-binding protein (HMWP).

We propose that synthesis of myosin subunits switches immediately from amoebal to plasmodial type in mono-nucleate cells with swollen nuclei. This myosin switching is associated with the initiation of HMWP synthesis. © 1987 Academic Press, Inc.

CITATIONS FOR WHICH WE RECEIVED NO ABSTRACTS

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Joyce Mobbery writes that she has a number of duplicate reprints that she would be willing to share. They are listed below. Contact her at Division of Science, CAS, Governor's State University, University Park, IL 60466. Thanks, Joyce!

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from Wright's Lab:

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