REPORT FROM TAMPA BAY . . . .

Our ninth meeting was held in association with the 2nd International Mycological Congress on the campus of the University of South Florida. Participants in the Congress exceeded 1200.

The traditional dinner was organized by Ned Holt. Soon after the meeting, Ned sent in a letter describing some of the conference happenings. Excerpts:

> "The dinner went very well. The number of individuals who planned to attend kept going up and up; the actual number was 53! Quite a group, when one considers that we had originally envisaged a dinner for about 20! . . . One amusing incident; after I made a few remarks at the end of the meal, Henry did the same. A Polish colleague sitting beside me said, "He (Henry Aldrich) speaks a very different kind of English." Henry overheard this and was amused; he said he speaks "Teaxan" or some such word!
>
> "The meetings were very well organized; Henry was everywhere taking care of details. . . Gene Goodman's evening session was well attended and conducted in high spirits. There was standing room only at the Olive/Raper/Alexopoulos session on slime mold phylogeny.
>
> "I felt it was the best meeting we have had. There were numerous interesting papers and a variety of modes of presentation: symposia, informal evening talks, and posters. The poster room reserved for slime mold posters on Friday was a beehive of activity."

Henry Aldrich deserves special thanks for all his hard work both with the congress and the slime mold portions of it. A set of selected abstracts are included with this mailing of the PNL.

FOURTH EUROPEAN CONFERENCE - SPRING, 1979

Innsbruck, Austria, will be the site for the next *Physarum* meeting. Details will be forthcoming from Dr. Wilhelm Sachsenmaier, Inst. fÜr Biochemie und Exp. Krebsforschung, Universitat Innsbruck.

QUEBEC SITE OF NEXT NORTH AMERICAN MEETING

A 2-3 day meeting will be held on the campus of Laval University in mid-to-late August, 1979. According to Jean Lafontaine, room and board will be available at reasonable rates. August is a lovely month in Quebec, so you might wish to consider combining our meeting with a family vacation.

Further information regarding the Quebec conference will be forthcoming through the PNL and direct mailings. Individuals outside the North American continent who wish to be on the direct mailing list for this meeting should so inform Dr. Lafontaine. Any questions or comments? Contact Dr. J.G. Lafontaine, Biology Department, Faculty of Science, Laval University, Quebec P.Q. G1K 7P4, Canada.

Physarum Actin. Observations on Its Presence, Stability, and Assembly in Plasmodial Extracts and Development of an Improved Purification Procedure†

Biochemistry, (1977) 16, 4862

Mark R. Adelman

ABSTRACT: Actin is readily extracted from plasmodia of *Physarum polycephalum* by low ionic strength solutions which do not solubilize the plasmodial myosin. The actin in such extracts exists predominantly as a monomer which slowly denatures, apparently via removal of bound nucleotide, and is subsequently proteolyzed. However, the native monomeric actin can be induced to assemble into polymeric arrays under appropriate solvent conditions. Actin assembly is dependent on the addition of ATP and is a function of KCl and $CaCl_2$ concentrations. These observations have allowed the development of an improved actin purification scheme which is simple, rapid, and efficient, yielding ~60 mg of protein from 100 g of plasmodium. The actin thus obtained is pure, stable, and comparable to that obtained by previously described procedures. Furthermore, the observations suggest that actin polymers may be metastably assembled in vivo and raise the possibility that actin assembly, and plasmodial movements, could be regulated via alterations in intracellular concentrations of nucleotide and/or divalent cation.

Isolation and Analysis of Amoebal–Plasmodial Transition Mutants in the Myxomycete *Physarum polycephalum*

By R. W. ANDERSON and JENNIFER DEE

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Genet. Res., Camb. (1977), 29, *pp.* 21–34

SUMMARY

Plasmodium formation in the Myxomycete *Physarum polycephalum* normally involves fusion of haploid amoebae, carrying different alleles at the mating type (*mt*) locus, to give diploid plasmodia. Strains carrying the mt_h allele are capable of undergoing the amoebal–plasmodial transition with high efficiency within amoebal clones, resulting in the formation of haploid plasmodia. NMG mutagenesis of mt_h amoebae, followed by an enrichment procedure, was used to isolate mutants in which such clonal plasmodium formation was either delayed or absent. Thirteen mutants of the second type were analysed. Three of these were temperature-sensitive for plasmodium formation. All thirteen mutants were able to form diploid crossed plasmodia when mixed with a mt_1 strain. Three new genes were identified and designated *npf*A, *npf*B and *npf*C. A mutant allele of *npf*A rendered *clonal* plasmodium formation temperature-sensitive, but did not prevent crossing at the non-permissive temperature with derived strains carrying the same mutant allele. No recombination was detected between *npf*B or *npf*C and *mt*, but *npf*A was unlinked to *mt* and a locus (*apt*-1) shown in a previous study to be involved in plasmodium formation. The genes *npf*B and *npf*C were distinguished by complementation analysis. Strains of the genotype *npf*B$^-$; *npf*C$^+$ behaved in the same way as strains carrying the mt_a allele. The nature of the mutants and the role of the mating-type locus in the initiation of plasmodium formation are discussed.

Size and structure of mitochondrial DNA from *Physarum polycephalum*

H. J. BOHNERT, *Botanisches Institut I, Universität Düsseldorf, 4000 Düsseldorf, Germany*

Summary. One band of DNA with a buoyant density of 1.688 g cm^{-3} is found when isolated mitochondria of the slime mold *Physarum polycephalum* are incubated with deoxyribonuclease prior to lysis. The DNA consisted mainly of linear molecules up to about 20 μm in length. As many as 10% of the molecules were, however, of open circular conformation with a circumference of 19.1±0.5 μm. Mild lysis conditions favoured the isolation of DNA/protein complexes with no visible free ends. The data suggest that the mitochondrial DNA (mtDNA) of *Physarum* is a circle and that circularity may be maintained by DNA/protein interaction.

Exp. Cell Res. 106, 426 (1977)

A Nuclear Elongation Factor of Transcription from *Physarum polycephalum in vitro*

Gerold Hans ERNST and Helmut Wilhelm SAUER

Fachbereich Biologie der Universität Konstanz

Eur. J. Biochem. *74*, 253–261 (1977)

Homogenates of *Physarum* plasmodia contain a factor which stimulates UMP incorporation on native DNA by solubilized homologous RNA polymerases *in vitro*. The factor is a heat-sensitive protein and has been located in nuclei. It does not alter the template activity of DNA nor the initiation frequency of transcription. The factor interacts with free or bound RNA polymerase molecules (only at 37 °C and at low ionic strength) and yields larger transcripts *in vitro*.

The level of the factor *in vitro* fluctuates: it is gradually reduced during spherulation and reaches its maximum in mid S phase of the cell cycle of *Physarum*.

JOURNAL OF CELLULAR PHYSIOLOGY *91*: 297-304, 1977

The Relation of Cycling of Intracellular pH to Mitosis in the Acellular Slime Mould *Physarum polycephalum*

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ABSTRACT The relation between intracellular pH and the mitotic cycle of *Physarum polycephalum* was studied by two independent techniques. Both techniques revealed a long term cycling of intracellular pH which has the same period as the mitotic cycle. Qualitative detection of the changes in intracellular pH was made by measuring the changes in fluorescence of 4-methylesculetin which had been absorbed by the plasmodium. Quantitative measurements of intracellular pH were made throughout the mitotic cycle with antimony micro pH electrodes. The cycle of intracellular pH is sinusoidal in appearance. The maximum intracellular pH (pH 6.6) occurred at, or very near to, mitosis, and was approximately 0.6 pH units higher than the minimum pH, which occurred near the middle of the mitotic cycle.

J. CELL. PHYSIOL., 92: 447-456.

Adaptation to Trichodermin and Anisomycin in *Physarum polycephalum*

JESSICA A. GORMAN
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ABSTRACT The effects of the protein synthesis inhibitors trichodermin and anisomycin on the growth of the eucaryotic myxomycete *Physarum polycephalum* have been examined. When either of these drugs is added to log phase monoxenic cultures of myxamoebae, cell division is immediately arrested, but on continued incubation, growth resumes at a rate only slightly lower than that of drug free cultures. The length of the drug induced growth lag is roughly proportional to drug concentration. When adapted cells are transferred to fresh drug containing medium, growth is not inhibited. However, if the drug concentration is increased, transient inhibition is again exhibited. Measurement of the antibiotic concentration in used media demonstrates no significant external inactivation of either drug during adaptation. The resumption of growth cannot be attributed to the selection of stable drug-resistant mutants: single amoebal colonies arising on drug plates are found to be as drug-sensitive as control colonies when retested after subculture. In addition, when adapted cells are transferred to drug free medium, the phenotypic drug-resistance is completely lost after several generations of growth. As recovery occurs in the continuous presence of drug and is not due to the accumulation of drug-resistant mutants, this response appears to be an example of drug adaptation. Cross adaptation between anisomycin and trichodermin is also demonstrated, suggesting a common system is involved in adaptation to these structurally dissimilar, but functionally similar, drugs.

The Organisation of Genes for Transfer RNA and Ribosomal RNA in Amoebae and Plasmodia of *Physarum polycephalum*

Len HALL and Richard BRAUN

Institute for General Microbiology, University of Bern

Eur. J. Biochem. 76, 165–174 (1977)

1. Using hybridisation techniques nuclei from both amoebae and plasmodia of *Physarum polycephalum* were found to contain 275 genes each coding for 5.8-S, 19-S and 26-S rRNA, 685 genes for 5-S rRNA and 1050 genes for tRNA.

2. Hybridisation of these RNA species to both amoebal and plasmodial DNA fractionated on CsCl gradients reveal that the 5.8-S, 19-S and 26-S rRNA genes are located at a satellite position ($\varrho = 1.714$ g/cm^3) with respect to the main band of DNA, whereas 4-S and 5-S RNA genes are located exclusively in the main band of DNA ($\varrho = 1.702$ g/cm^3).

3. This result was confirmed by demonstrating that only the 5.8-S, 19-S, and 26-S rRNA species hybridise to purified plasmodial ribosomal DNA.

4. The 19-S and 26-S rRNA genes of amoebae are located on extrachromosomal DNA molecules of a discrete size ($M_r = 38 \times 10^6$) with identical properties to plasmodial ribosomal DNA.

Verh.Dtsch.Zool.Ges. 1976, 286, Gustav Fischer Verlag, Stuttgart, 1976

Zur Entwicklungsbiologie von Physarum: Modifikationen des RNA Polymerase Musters während des Wachstums und der Differenzierung

Developmental Biology of Physarum: Modifications of RNA Polymerase profiles during growth and differentiation

A. HILDEBRANDT und H. W. SAUER

Fachbereich Biologie der Universität, Konstanz

Als Beispiel eines einfachen Eukaryonten stellt der Myxomycet *Physarum* ein Modellsystem dar, das für Untersuchungen der Regulation des Mitosezyklus aufgrund einer natürlichen Teilungsynchronie von bis zu 10^8 Zellkernen besonders geeignet ist. Darüber hinaus lassen sich an diesem Organismus während der reversiblen Differenzierung zu Zysten und der irreversiblen Differenzierung der Fruchtkörper grundlegende entwicklungsbiologische Fragen, wie diejenige nach der Regulation der Genaktivität, analysieren. Es wurde bereits gezeigt, daß zur Bildung von Fruchtkörpern sowohl spezifische RNA- als auch Protein-Synthese notwendig ist (SAUER, H. W., und RUSCH, H. P.: Zool. Anz., Suppl. *33*, 350, 1969). Wir haben inzwischen die für höhere Zellen typischen multiplen RNA Polymerasen nachgewiesen (HILDEBRANDT, A., and SAUER, H. W.: FEBS Lett. *35*, 41, 1973) und möchten nun über Änderungen dieses Enzymmusters berichten, die Zusammenhänge zwischen Transkription und morphologisch sichtbarer Differenzierung wahrscheinlich machen.

1. Im Zellzyklus finden wir unveränderte Level an extrahierbarer nukleolärer (A) und nukleoplasmatischer (B) RNA Polymerase (HILDEBRANDT, A., and SAUER, H. W.: Biochim. Biophys. Acta *425*, 316, 1976). Betrachten wir jedoch den Anteil an transkribierendem Enzym B in isolierten Kernen, finden wir ein Aktivitätsmaximum in der S-Phase, welches bei einer Blockade der DNA Synthese nicht erscheint.

Somit könnten die Zellkerne durch die Neubesetzung ihrer DNA mit RNA Polymerasemolekülen in jedem Zellzyklus erneut programmiert werden.

2. Der Prozeß der Enzystierung wird durch ungünstige Wachstumsbedingungen ausgelöst, wobei es zur Reduktion des Stoffwechsels kommt. Wir beobachten eine deutliche Verringerung der Aktivität der RNA Polymerase A entsprechend der Abnahme der ribosomalen RNA Synthese aufgrund einer Inaktivierung des Enzymes durch eine kurzfristig synthetisierte und im Zellkern angehäufte Substanz.

Wir nehmen an, daß dieses «Polyphosphat» Signalwirkung hat wie Guanosintetraphosphat bei Bakterien («stringent control») oder zyklische Nukleotide in höheren Tieren («pleiotypic programme»).

3. Zur Sporulation ist die Synthese von zusätzlicher mRNA notwendig. Auf diesem Entwicklungsstadium beobachten wir eine weitere RNA Polymerase, welche sich anhand folgender Merkmale von den bereits bekannten RNA Polymerasen A, B und C unterscheidet: a) Elutionsverhalten an DEAE-Sephadex, b) Empfindlichkeit gegen hohe Ionenstärke, c) Template-Spezifität.

Aufgrund der vorhandenen Sensibilität dieses Enzyms gegen Amatoxin halten wir diese RNA Polymerase für ein modifiziertes Enzym vom Typ B, d. h. einen möglichen Kandidaten zur Synthese der für die Differenzierung spezifischen mRNA Fraktionen.

Unsere Beobachtungen ähneln den Befunden zur Transkriptionsregulation bei der Bakteriensporulation und lassen erstmalig für einen Eukaryonten eine Korrelation modifizierter RNA Polymerase mit einer definierten Differenzierungsleistung erkennen.

(Mit Unterstützung durch die Deutsche Forschungsgemeinschaft, Hi 194, Sa 139)

Vol. 74, No. 2, 1977 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

TRANSCRIPTION OF RIBOSOMAL RNA IN THE LIFE CYCLE OF PHYSARUM MAY BE REGULATED BY A SPECIFIC NUCLEOLAR INITIATION INHIBITOR

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Universität Konstanz
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SUMMARY
Physarum nucleoli contain an inhibitor of in vitro transcription with homologous RNA polymerase A. A strict negative correlation has been established of RNA polymerase A activity and amount of inhibitor during differentiation of Physarum. Location and concentration of the inhibitor as well as selective, yet reversible, binding to and inactivation of RNA polymerase A and in vitro reactivation of enzyme A preparation obtained during differentiation - but not during growth - suggest that the inhibitor might act in vivo to restrict rRNA transcription.

Wilhelm Roux's Archives 183, 107–117 (1977)

Discrimination of Potential and Actual RNA Polymerase B Activity in Isolated Nuclei During Differentiation of *Physarum polycephalum* *

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Summary. Isolated nuclei of *Physarum* contain endogenous RNA polymerase activity. We provide evidence for four different states of RNA polymerase B: 1. free enzyme (85%); 2. weakly bound enzyme (10%) and 3. tightly bound enzyme (0–4%), which can be solubilized from isolated nuclei with 0.5 M and 1.5 M NaCl respectively; 4. "initiated" enzyme. The latter fraction (1–5% of the total RNA polymerase B) is not soluble in salt extractions, does not accept external templates, shows high salt optimum for transcription (0.4 M NaCl) and produces by elongation RNA molecules of mainly 10 S. Treatment of isolated nuclei from differentiating cultures with Triton X-100 increases the proportion of the "initiated" enzyme at the expense of the tightly bound enzyme fraction. This indicates a potential transcription control mechanism which operates at the chromatin level and results in variable proportions of silent and transcribing RNA polymerase B molecules during differentiation of Physarum.

Viability of *Physarum polycephalum* Spores and Ploidy of Plasmodial Nuclei

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JOURNAL OF BACTERIOLOGY, Vol. 131, p. 473–476, 1977

Amoebae of *Physarum polycephalum* carrying the *m*th mating-type allele may differentiate into plasmodia in the absence of mating. Such plasmodia are haploid and, upon sporulation, produce mainly inviable spores. We have asked whether the viable spores arise from meiotic or mitotic divisions. Using a microfluorometric measurement of the deoxyribonucleic acid content of individual nuclei, we found the fraction of viable spores to be correlated with the proportion of rare, diploid nuclei contained in the generally haploid plasmodium. When homozygous diploid plasmodia were created by heat shocking, spore viability increased dramatically. We suggest that viable spores are produced via meiosis in *m*th plasmodia, that the *m*th allele has no effect on sporulation per se, and that the normal source of viable haploid spores is a small fraction of diploid nuclei ubiquitous in haploid plasmodia.

Onset Time of Signal for Mitosis Estimated from Mitotic Delays in UV-Irradiated Plasmodia of *Physarum polycephalum*

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CELL STRUCTURE AND FUNCTION 2, 101~ 109 (1977)

ABSTRACT. Mitotic delay by UV-irradiation was examined in G2 or M phase in naturally synchronous plasmodia of *Physarum polycephalum*. The mitotic delay curve indicated a linear decrease against the time of irradiation in G2. Extrapolation of the delay line in G2 to zero delay gave an intersection point which probably corresponded to the transition point from G2 to M, that is, the time of the signal for mitosis. The point was at 50 min prior to metaphase. The slope change of the delay curve suggests that UV-irradiation inhibits the production of a substance responsible for triggering mitosis rather than inactivate the function of a preexisting triggering substance. The delay was partially cancelled by adding the crude lysate of non-irradiated cells. This effect was strongest when the lysate was from late G2 cells.

AND THE LIST GROWS ON . . . .

Europe	70
Far East	10
North America	150

The Mailing Fund now stands at approx. $375, which means that we shouldn't have to solicit from current PNL readers for two years!

HIGH-DENSITY INDUCTION OF A QUIESCENT CELL STATE IN *PHYSARUM POLYCEPHALUM*

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J. Cell Sci. **25**, 179–190 (1977)

The non-histone protein complement of *Physarum polycephalum* changes rapidly when microplasmodia are subjected to conditions of high density. The changes in these proteins induced by high density are similar to the changes observed during starvation-induced encystment. A 50% decrease in DNA synthesis, observed after 7 h of starvation, is observed after only 1 h of high density. High density also results in a decrease in RNA synthesis comparable to decreases induced by prolonged starvation. Total heterochromatin increases in response to either high density or starvation. Increased heterochromatization is preceded by an increase in nuclear actin. Mitochondrial morphology and cytoplasmic organization are also similarly altered by starvation and high density. These observations suggest the possibility of a generalized mechanism for cellular transition from active growth to a non-proliferative cell state.

Genetic analysis of a cross between two homothallic strains of *Physarum polycephalum*

BY R. T. M. POULTER AND N. K. HONEY
Department of Biochemistry, University of Otago, Dunedin, New Zealand

Genet. Res., Camb. (1977), **29**, *pp.* 55–63

SUMMARY

The homothallic amoebal clones of *Physarum polycephalum* are of potential use in understanding the developmental genetics of this organism. Such an application requires that complementation and recombination analysis be possible between pairs of homothallic clones. This paper is a report of the formation of mixed plasmodia by pairs of homothallic amoebal clones. In order to detect such mixed plasmodia use was made of two marker genes involved in plasmodial fusion, *fus*A and *fus*B. Sporulation of a mixed plasmodium formed from two homothallic (delayed) amoebal clones yielded progeny amoebae which were genetically recombinant. It is deduced from the ratios of various genotypes in these progeny clones that the mixed plasmodium was diploid and that meiosis was associated with sporulation. There is therefore no impediment to the use of the homothallic strains for genetical analysis. The progeny amoebal clones were observed to be showing segregation for the characters homothallic (rapid) and homothallic (delayed). This observation, taken together with other related observations, suggests that the homothallic (delayed) character is produced by mutation of the homothallic (rapid) character. The rare plasmodia formed by a homothallic (delayed) amoebal clone are the result of reversion of this mutation. Amoebal clones of the homothallic (delayed) type are therefore developmental mutants unable to perform the differentiation from amoeba to plasmodium.

Enzymes for RNA Sequence Analysis

PREPARATION AND SPECIFICITY OF EXOPLASMODIAL RIBONUCLEASES I AND II FROM *PHYSARUM POLYCEPHALUM**

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 253, No. 2, Issue of January 25, pp. 437-445, 1978

DANIEL PILLY, AMANDA NIEMEYER, MAURICE SCHMIDT, AND J. PIERRE BARGETZI‡

From the ‡Department of Biochemistry, School of Chemistry, University of Geneva, 1211 Geneva 4, Switzerland

The common slime mold *Physarum polycephalum* secretes a variety of hydrolytic enzymes when grown in submersed cultures. Initiated by the isolation in 1969 of a rather crude RNase, proposed by Braun and Behrens as a useful analytical endonuclease, an extended search was conducted in our laboratory, which revealed that actually two distinct RNases occur, largely outweighed by a yet unknown acid phosphatase (Schmidt, M., Pipy, D., Wolny, M., and Bargetzi, J. P. (1972) *Experientia* 28, 739-740). Being all very acidic proteins, the three enzymes could be isolated in a single procedure. Purified RNase *Phy* I is characterized by a M_r of 25,000, a pI at 4.3, and optimal activity at pH 4.5, while RNase *Phy* II has a M_r of 24,500, a pI at 3.8, and optimal activity at pH 3.1. Both enzymes are reversibly inactivated at pH 8.0. The specificities have been explored systematically, first by means of the 16 usual dinucleoside monophosphates, and secondly with purified low M_r yeast-RNA. Although both RNases lack a clear all or none specificity, they exhibit wide rate differences. RNase *Phy* I was found to elicit a regular pattern of decreasing susceptibility, uridine being always the most labile residue and cytidine the least affected. Although the base attached to the C3′-oxygen has a dominant influence, the base present on the C5′-oxygen of the same phosphodiester also imparts a strong interaction. Thus, resistance to hydrolytic attack is maximum when cytidine is in the first location and when uridine occupies the second one. The pattern culminates in a nearly complete resistance of -CpC- and -CpU- pairs. On the other hand, RNase *Phy* II shows an outstanding preference for -GpN- pairs, while conversely the -ApN- pairs and the -Pyr-p-Pyr- doublets are left practically unsplit. None of these RNases appears to fit with the properties assigned by Egami's group (Hiramaru, M., Uchida, T., and Egami, F. (1969) *J. Biochem. (Tokyo)* 65, 693-700) to the three intraplasmodial RNases discovered in the same organism.

Synthesis and Transport of Myosin in *Physarum polycephalum*

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Eur. J. Biochem. *80*, 43–50 (1977)

Immunological techniques have been used to study the rate of synthesis and intracellular transport of myosin in the slime mould *Physarum polycephalum*. Quantitative precipitation of myosin in homogenates of *Physarum* was achieved using an antimyosin antibody produced in rabbit in response to purified *Physarum* myosin. Dodecylsulphate-gel electrophoresis revealed that about 50% of the precipitated material is myosin.

The rates of synthesis of total cellular protein and myosin were measured over the mitotic cycle. Both were found to increase exponentially or linearly between two successive nuclear divisions. Similarly, no difference in the proportion of myosin-synthesising polysomes, assayed by precipitation with antimyosin serum, could be detected between the S phase and G2 phase of the mitotic cycle.

Myosin makes up nearly 2% of total plasmodial proteins. Its transport into the nucleus occurs predominantly during the G2 phase.

The Replication of Ribosomal DNA in *Physarum polycephalum*

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and Institute of General Microbiology, University of Bern

Eur. J. Biochem. *80*, 557–566 (1977)

The DNA coding for ribosomal RNA in *Physarum polycephalum* exists as a collection of extrachromosomal molecules of molecular weight 37×10^6. We have investigated the replication of rDNA, with the following results. (a) Replication of rDNA is unscheduled. This means that molecules that are replicated at any particular time in one mitotic cycle have an equal probability of replicating again in each time interval in the subsequent cycle. Similarly, in a single cycle, some molecules replicate more than once, and some not at all. (b) Replication forks appear to move bidirectionally from points 45% or 33% from one end of the DNA. Replicating molecules observed by electron microscopy are all linear.

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ENDONUCLEASE ACTIVITY IN NUCLEI OF *PHYSARUM POLYCEPHALUM*

PARTIAL PURIFICATION AND CHARACTERIZATION

JAAP H. WATERBORG and CHARLES M.A. KUYPER

Department of Chemical Cytology, Faculty of Science, Toernooiveld, Nijmegen (The Netherlands)

Biochimica et Biophysica Acta, 478 (1977) 224—233

An endonuclease, present in the microplasmodia of *Physarum polycephalum*, has been partially purified from isolated nuclei by DEAE-cellulose and Sephadex G-75 chromatography.

1. The endonuclease produced single-strand scissions in double-stranded DNA which resulted in the generation of 5′-phosphoryl and 3′-hydroxyl termini. No activity was observed with single-stranded DNA as substrate.

2. The pH optimum was approximately 8.5.

3. Divalent cations were essential for enzyme activity. $MnCl_2$ and $MgCl_2$ gave maximal activity. $CaCl_2$, $ZnCl_2$ or $CoCl_2$ did not activate the enzyme.

4. The endonuclease activity was highly sensitive to monovalent cations.

5. Endonuclease activity was found in two forms after gel filtration: an activity in a homogeneous peak with a molecular weight of approx. 20 000, and an activity that had a heterogeneous molecular weight and which was isolated in a complex with DNA.

A possible function of the endonuclease in DNA replication is discussed.

OSCILLATING CONTRACTIONS IN PROTOPLASMIC STRANDS OF *PHYSARUM*: SIMULTANEOUS TENSIOMETRY OF LONGITUDINAL AND RADIAL RHYTHMS, PERIODICITY ANALYSIS AND TEMPERATURE DEPENDENCE

BY K. E. WOHLFARTH-BOTTERMANN

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J. exp. Biol. (1977), 67, 49–59

1. The construction of a 'twin-tension transducer' allows the simultaneous measurement of the same or different contraction rhythms at any selected sites of living plasmodia of *Physarum polycephalum*. This method has been used to analyse the relation of longitudinal and radial contraction activity within migrating plasmodia and plasmodial veins, under isometric as well as under isotonic conditions of measurement.

2. A periodicity analysis of the oscillating contraction rhythms revealed average period values for the longitudinal contraction cycle of 2·1 min and for the radial contraction cycle of 1·3 min at a temperature of 22 °C.

3. The periods of longitudinal contraction depend on the environment of the strands. The mean value under submerged conditions was 2·9 min.

4. The temperature dependences for both longitudinal and radial contraction cycles were determined to provide reliable values for the normal reaction range of the contractile system (cytoplasmic actomyosin). The values for radial contraction activity are 2·0 min at 16 °C, 1·5 min at 20 °C, and 1·2 min at 24 °C. The range between 16° and 24 °C can be regarded as physiological.

5. The possibility is discussed that only one 'genuine' contraction frequency of cytoplasmic actomyosin exists in *Physarum*.

TITLES AND SUMMARIES IN PRESS

MUTATIONS INCREASING ASEXUAL PLASMODIUM FORMATION in *Physarum polycephalum*

Paul N. Adler and Charles E. Holt

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ABSTRACT

Rare plasmodia formed in clones of heterothallic amoebae were analyzed in a search for mutations affecting plasmodium formation. The results show that the proportion of mutants varies with both temperature (18°, 26° or 30°) and mating type allele (mt1, mt2, mt3, mt4). At one extreme, only one of 33 plasmodia formed by mt2 amoebae at 18° is mutant. At the other extreme, three of three plasmodia formed by mt1 amoebae at 30° are mutant. The mutant plasmodia fall into two groups, the GAD (greater asexual differentiation) mutants and the ALD (amoeba-less life cycle) mutants. The spores of GAD mutants give rise to amoebae that differentiate into plasmodia asexually at much higher frequencies than normal heterothallic amoebae. Seven of 8 gad mutations analyzed genetically are linked to mt and one (gad-12) is not. The gad-12 mutation is expressed in strains with different alleles of mt. The frequency of asexual plasmodium formation is heat sensitive in some (e.g. mt3 gad-11), heat insensitive in two (mt2 gad-8 and mt2 gad-9) and cold sensitive in one (mt1 gad-12) of twelve GAD mutants analyzed phenotypically. The spores of ALC mutants give rise to plasmodia directly, thereby circumventing the amoebal phase of the life cycle. Spores from 5 of the 7 ALC mutants give rise to occasional amoebae as well as plasmodia. The amoebae from one of the mutants carry a mutation (alc-1) that is unlinked to mt and is responsible for the ALC phenotype in this mutant. Like gad-12, alc-1 is expressed with different mt alleles. Preliminary observations with amoebae from the other four ALC mutants suggest that two are similar to the one containing alc-1, one gives rise to revertant amoebae, and one gives rise to amoebae carrying an alc mutation and a suppressor of the mutation.

Genetics, in press

A plasmodial colour mutation in the Myxomycete *Physarum polycephalum*

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Genet. Res., Camb. (1977).

SUMMARY

A spontaneous mutation conferring white plasmodial colour on the Myxomycete *Physarum polycephalum* has been analysed. The mutant *whi*-1 allele is recessive to *whi*$^+$ in both heterokaryotic and heterozygous plasmodia. The *whi* locus is unlinked to *mt*, *npfA*, *fusA* and *leu*.

Deoxyribonucleic Acid Polymerase from *Physarum Polycephalum* Properties of the major cytoplasmic activity in exponentially growing microplasmodia

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DNA polymerase was purified 1000-fold from the cytoplasm of microplasmodia of the myxomycete Physarum polycephalum. The activity was found in two forms exhibiting molecular weights of 204 000 and 116 000 respectively. Both forms eluted together from DNA-cellulose and DEAE-Sephadex columns. The Stokes radii were 6.5 and 5.5 nm. The sedimentation coefficients were 7.6 and 5.2 S. The frictional ratios of 1.69 suggest a highly hydrated and/or an asymmetric structure of the molecule. The enzyme catalyzed reaction was sensitive to N-ethylmaleimide (60% inhibition by 1mM). Unlike DNA polymerase α from mammalian cells the Physarum enzyme was stimulated by 30 mM NaCl. Activated DNA was the preferred template. Poly(A)•(dT)$_{12}$ was not accepted. The "K_m-value" for deoxynucleoside triphosphates was 3 µM, for activated DNA 50 µg/ml and for Mg^{++} at the optimum [K^+] of 150 mM about 0.6 mM.

Europ. J. Biochem., in press

Ribosomal DNA in Spores of *Physarum polycephalum*

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DNA was isolated from plasmodia, spores and newly hatched amoebae of the slime mould *Physarum polycephalum*. The DNA preparations were fractionated in CsCl gradients and each fraction hybridised to combined 19S + 26S rRNA. In all three DNA preparations hybridisation was found to be limited to satellite DNA (ρ = 1,714 g ml^{-1}) and at saturation was found to reach a level of 0.16 to 0.18% of total DNA. The main band of nuclear DNA (ρ = 1,702 g ml^{-1}) did not hybridise appreciably. Further experiments using analytical CsCl gradients revealed that the ratio of satellite to main band DNA was similar in all three preparations. It is concluded that the genes for ribosomal RNA are equally reiterated in spores, hatching amoebae and in plasmodia. They appear to be similarly organised in all stages of the life cycle examined so far.

Biochim. Biophys. Acta, in press

A Gene Unlinked to Mating Type Affecting Crossing between Strains of *Physarum polycephalum*

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Two alleles of a gene (*rac*) unlinked to the mating-type locus (*mt*) have been identified in strains of *Physarum polycephalum* from different laboratories. Heterothallic strains differing in *mt* alleles cross more rapidly if they differ also in their *rac* alleles. The recovery of hybrid plasmodia from crosses between apogamic (mt_h) and heterothallic strains is more likely to be achieved if strains of different *rac* genotype are used.

Genet. Res., in press

Metabolic Stability of the Extrachromosomal Ribosomal RNA Genes in the Slime Mould *Physarum polycephalum*

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The rRNA genes of the slime mould *Physarum polycephalum* are located on free, linear DNA molecules of a discrete size, 38 x 10^6 daltons. Using an isotope dilution technique we have examined the metabolic stability of these extrachromosomal genes during active, balanced growth. Microplasmodia, prelabelled with [^{3}H] thymidine, were used to prepare synchronous surface plasmodial cultures which were subsequently grown on unlabelled medium. The gross synthesis of ribosomal DNA was then determined over three consecutive mitotic divisions from the ratio of ^{3}H to ^{14}C in a hybrid formed between the extracted [^{3}H] ribosomal DNA and a [^{14}C] rRNA probe. It was found that ribosomal DNA, like chromosomal DNA, is completely stable during active growth.

Preparation of Polysomes from Synchronous Macroplasmodia of *Physarum polycephalum*

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A simple method is described for preparing undegraded polysomes from synchronous plasmodia of *Physarum*. The method involves extraction at temperatures below 0°C in a buffer containing 44% glycerol.

Biochim. Biophys. Acta, in press

The reduction of radiation-induced mitotic delay by caffeine: A test of the cyclic AMP hypothesis†

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INT. J. RADIAT. BIOL.

1. Introduction

One of the well-known biochemical effects of caffeine is the inhibition of cyclic nucleotide phosphodiesterases (Butcher and Sutherland 1962). Thus, it seemed plausible to propose that the reduction in X-radiation-induced mitotic delay by caffeine may be the result of an elevated cyclic AMP content (Scaife 1971, Boynton, Evans and Crouse 1974, Walters, Gurley and Tobey 1974). To test this hypothesis, we have characterized the caffeine effect on radiation-induced mitotic delay in the naturally-synchronous plasmodial slime mould *Physarum polycephalum* during late G_2 and early prophase, and have compared the action of that compound with others of similar structure and/or physiological function. Our results indicate that the reduction of mitotic delay is not a result of altered cyclic AMP levels.

THE HETEROGENEITY OF CYTOPLASMIC DEOXYRIBONUCLEIC ACID POLYMERASE FROM PHYSARUM POLYCEPHALUM

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Cytoplasmic DNA polymerase activity (DNA deoxynucleotidyltransferase, EC 2.7.7.7) was partially purified from Physarum polycephalum. The procedure was based in a first step on the anomalous gel filtration behaviour of the enzyme; the second step was either ion-exchange chromatography or sucrose density centrifugation. The purification procedure revealed a considerable heterogeneity of the DNA polymerase activity. At least four species with different sedimentation coefficients (5.5S, 7.2S, 8.6S and 11.5S) were detected. Calculated molecular weights indicated a tendency for stoicheiometric polypeptide aggregation, accompanied by an alteration of the three-dimensional structure from a compact spheroid to a more open ellipsoid form. Sodium dodecylsulphate/polyacrylamide-gel electrophoresis and computed molecular weights suggest an active protomer in the range of 113,000 daltons; all data presented pertain to I 0.045, maintained during the whole procedure.

Biochem. J., in press

INTRANUCLEAR MITOSIS IN MACROPLASMODIA OF THE SLIME MOLD, PHYSARUM POLYCEPHALUM

(b + w film 16 mm, length 185 ft , projection time 5 min at 24 frames/s)

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Physarum macroplasmodia were obtained by fusion of microplasmodia of strains M3c VIII (diploid) or CL (haploid). They were cultured on black filter paper in liquid growth medium for films at low magnification or in tissue culture chambers on nutritive agar for films at high magnification (taken by Zeiss Neofluar 100/1,3, frame width 50 or 65 µm). Time lapse (0,5 - 720 frames/min), phase contrast and interference contrast techniques have been employed.

The following observations can be made:

1. First, growth and migration of a macroplasmodium or "giant cell" are demonstrated as a general overview. Veins can be clearly observed throughout the plasmodium; they contract rhythmically. The protoplasm including interphase nuclei exhibits typical shuttle streaming within the veins. Single cytoplasmic granules moving within the intense periodic streaming show characteristic saltatory motions.
2. First sign of mitosis is the displacement of the central nucleolus towards the nuclear membrane. Then the nucleolus dissolves and chromatin condenses into many small chromosomes. The chromosomes move towards the center of the nucleus.
3. During metakinesis the chromosomes swing perpendicularly with respect to the equatorial plane. The nuclei - whose membranes seem intact - rotate at least partially and the shuttle streaming is somewhat reduced. Anaphase movement begins synchronously and the chromosomes move collectively as two compact plates. During telophase the nuclei are stretched and constricted, thereby assuming typical dump-bell shape.
4. The daughter nuclei contain several compact bodies which gradually fuse to form a lobed nucleolar mass. Within one hour after metaphase, typical interphase nuclei develop each containing one central nucleolus.

A frame by frame analysis is currently made to determine the exact duration of mitotic phases in Physarum as well as a study of heat shocked plasmodia.

A copy of the film with accompanying text can be obtained from Dr. R. Wolf at cost price including air mail postage (110.-DM in Europe, 54 dollars overseas according to the present exchange rate).

THESES

CHEMOTAXIS IN THE ACELLULAR SLIME MOLD, *PHYSARUM POLYCEPHALUM*

Randall Lawrence Kincaid

Department of Pharmacology, Stanford University, August, 1977

The present study of chemotactic behavior in Physarum polycephalum introduces an objective and quantitative method for the measurement of chemotaxis in this organism and describes various factors which affect this system. A number of chemicals, primarily sugars and amino acids, have been tested for their chemotatic effects and the results indicate that the nutritional usefulness of a chemical is neither necessary nor sufficient for chemoattraction. D-fructose, which supports growth, is a poor attractant while several sugars which are not utilized for growth (2 deoxy d-glucose, 3-0-methyl d-glucose and d-xylose) are good attractants. In the case of carbohydrate chemotaxis, a general "structure-activity" relationship favoring the "chair" form of the pyranose ring is suggested. For the majority of the compounds, including all of the amino acids examined, their maximum chemotatic responses are less than those for the most potent carbohydrate attractants (d-glucose, d-galactose, 2 deoxy d-glucose and 3-0-methyl d-glucose).

Comparison of chemotactic behavior to different spatial distributions of the attractant, 3-0-methyl d-glucose, suggests that optimal movement occurs toward a steep, "continuous" gradient of this chemical. By comparison, movement toward a "step" gradient of attractant, at the same concentration which produces maximum effects as a continuous gradient, is slower and consistently yields poorer quantitative responses. Differences in the morphology and distribution of plasmodial mass are observed for responses to these two types of gradient.

The effect of starvation on the chemotatic behavior of plasmodia has been examined. Exponentially-growing cells which are transferred to non-nutrient buffer for various times produce experimental individuals with progressively faster chemotactic response times. Addition of glucose, but not fructose, to the "starvation medium" results in longer individual response times than seen for the corresponding buffer-incubated cells.

Several inhibitors of the enzyme cyclic nucleotide phosphodieterase (PDE) as well as cyclic adenosine 3'5' monophosphate (cAMP) and its dibutyryl analogue are attractants in this system while adenosine, 5' AMP, and purine ribonucleotide lack such an effect. For several of these chemicals, concentrations above those required for maximal chemoattraction often produce negative chemotaxis. Studies of the kinetic properties of phosphodiesterase and of the effects of various inhibitors on this enzyme activity have been carried out. An "intracellular" phosphodiesterase has been examined which has a Km of about 3×10^{-3} M and is inhibited by three methyl xanthines and a flavonoid (morin) but not by three other inhibitors (6, 7-dimethoxy 4 ethyl quinazoline ="quazodine", 4-(3-butoxy, 4 methoxy benzyl)-2-imidizoladinone ="Roche 20-1724" and etazolate hydrochloride ="Squibb 20009"). "Particulate" PDE activity exhibits non-Michaelian kinetics and is inhibited, to varying extents, by all seven of the inhibitors tested. An "extracellular" activity has been partially purified which yields a Michaelis constant of 0.7×10^{-3} M. It is inhibited in a manner resembling that observed for the particulate activity. A comparison of the chemotatic responses to the various inhibitors with their effects on enzyme activity suggests that attractant potentcy may be correlated with inhibition of extracellular and/or particulate phosphodiesterase.

(These studies were carried out in Dr. Tag E. Mansour's laboratory. Dr. Kincaid states that copies of his thesis can be obtained by writing to the Office of Graduate Studies, Stanford University, Stanford, California 94305.)

OBSERVATIONS ON INTERPHASE NUCLEI IN RELATION TO DNA REPLICATION

Johannes Hendrikus Nicolaas Schel

(A Thesis presented towards the degree of Doctor of Natural Sciences at the Catholic University of Nijmegen, October, 1977)

SUMMARY

This thesis mainly deals with fine structural observations made on interphase nuclei of two various eukaryotic organisms, the lower eukaryote Physarum polycephalum and the higher eukaryotic bovine liver cells, both cultered in vitro. The slime mold Physarum was used because of the synchrony in nuclear divisions, naturally occurring in the macroplasmodia. This allows the determination of the stage of a sample in the nuclear cycle and makes a comparison with other - e.g. biochemical - data possible. We have made use of this property in the chapters 4, 6 and 7. The bovine liver cells could be cultured in larger quantities and were therefore more suited for experiments in which extraction procedures with much loss of material were carried out (chapter 5).

Because of the possible involvement of nuclear membrane or related structures in eukaryotic DNA replication (chapter 1), much attention was paid to nuclear fine structure in relation to an ordered arrangement of chromatin during interphase. In this context, the occurrence and ultrastructure of nuclear pore complexes were emphasized (chapters 2 and 3).

In the first chapter a survey is given of literature about nuclear fine structure in relation to DNA replication, the literature cited being covered until May, 1977. The second chapter shows annular structures being present in whole-mount preparations of isolated interphase nuclei of Physarum. Ultrastructural features of these annuli were described, while often an association of the rings with fibrils was observed.

The ultrastructure of the nuclear pore complex in bovine liver cells was investigated in chapter 3. By use of whole-mount technique the presence of eight annular subunits could be demonstrated unequivocally. The use of Triton X-100 obviously caused a loosening of the annular structures from the nuclear surface. This was also indicated by the fact that carbon replicas of the surface of nuclei, isolated in buffer without Triton X-100, showed annular projections which were not present after isolation with Triton. Often, a second smaller circular component was observed. A proposal for the arrangement of these components was given in a model.

The replica technique was also used to analyze the number of pore/nucleus during the nuclear cycle in Physarum (chapter 4). It was shown that the main increase in nuclear size took place during the S-phase, a period in which also a strong increase in nuclear pore number was observed. The results were compared with others obtained by freeze-etching technique. By use of this technique also the pore formation or breakdown process could be made visible. Based on the experimental data, a possible function of the nuclear pore complex in eukaryotic DNA synthesis was discussed.

Large-scale isolation of bovine liver cell nuclei allowed the electron microscopic visualization of nuclear ghosts remaining after a series of extractions (chapter 5). In these preparations annular structures could still be recognized, often associated with thin fibrils which were not observed after combined DNase/RNase treatment. It was suggested that an association of deproteinized chromatin with these nuclear ghosts exists, and the function of such a complex was discussed in relation to DNA replication.

A direct demonstration of replicating nuclear DNA in Physarum was given in the next chapter (chapter 6). By use of a modified Kleinshmidt spreading technique some replicating DNA molecules, all from the first part of the S-phase, could be observed. Using these pictures, an estimation of the elongation rate in Physarum was made. This was also done in the last chapter (chapter 7), which describes some results obtained from Physarum by use of DNA fiber autoradiography. Visualization of replication clusters proved to be possible with this technique. The first preliminary results seem to indicate an elongation rate of 1.5-3 µm/min/fork.

(Dr. Schel writes that a limited number of copies of his thesis are available. Write to Dr. J.H.N. Schel, Department of Botany, Agricultural University, Wageningen, Arboretumlaan 4, THE NETHERLANDS.)

ADDITIONAL ARTICLES IN PRINT

I. Chet and A. Huttermann
"Melanin Synthesis During Morphogenesis of the Slime Mold *Physarum polycephalum*"
Biochim. Biophys. Acta 499, 148 (1977). (PNL 9, 16, 1977)

I. Chet and A. Huttermann
"Germination-Inhibitor in Slime Mould *Physarum polycephalum*"
F.E.M.S. Microbiol. Letters 1, 149 (1977). (PNL 9, 16, 1977)

I. Chet, A. Naveh and Y. Henis
"Chemotaxis and Movement of *Physarum polycephalum* and Its Responses to Some Neurotransmetters and Psychomimetic Compounds"
J. Mechanochem. Cell Motil. 4, 177 (1977). (PNL 9, 17, 1977)

L.S. Davidow and C.E. Holt
"Mutants with Decreased Differentiation to Plasmodia in *Physarum polycephalum*"
Molec. gen. Genet. 155, 291 (1977). (PNL 9, 17, 1977)

A. Lunn, D. Cooke and F. Haugli
"Genetics and Biochemistry of 5-Bromodeoxyuridine Resistance in *Physarum polycephalum*"
Genet. Res. 30, 1 (1977). (PNL 9, 18, 1977)

T.M. Shinnick and C.E. Holt
"A Mutation (*gad*) Linked to *mt* and Affecting Asexual Plasmodium Formation in *Physarum polycephalum*"
J. Bact. 131, 247 (1977). (PNL 9, 18, 1977)

P. Sudbery, K. Haugli and F. Haugli
"Enrichment and Screening of Heat Sensitive Mutants of *Physarum polycephalum*"
Genet. Res. 31, 1 (1977). (PNL 9, 18, 1977)

K.E. Wohlfarth-Bottermann and K.G. von Olenhusen
"Oscillating Contractions in Protoplasmic Strands of *Physarum*: Effects of External Ca^{++}-Depletion and Ca^{++}-Antagonistic Drugs on Intrinsic Contraction Automaticity"
Cell Biol. Int. Reports 1, 239 (1977). (PNL 9, 19, 1977)

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Part B. Typical Aspects of Cell Differentiation

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Methylmercury Induced DNA Damage and Its Repair

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ABSTRACT

Dimethylmercury (DMM) produced a radiomimetic breakage of DNA in a slime mould, *Physarum polycephalum*, Ontario strain, but another strain, M3C of the same organism, was refractory toward this kind of DNA damage. DNA breakage was detected using alkaline sucrose gradient centrifugation. Single strand nicks were the main kind of DNA damage in sensitive slime mould, as double strand DNA breaks were not detected following neutral sucrose gradient analysis of DMM-treated DNA. These single strand nicks were repaired by an enzymatic excision repair mechanism. The sensitivity to DMM radiomimesis was relatively uninfluenced by the stage of the cell cycle during which treatment was conducted. Finally, current experiments suggest that DMM-induced DNA nicking was the consequence of a direct attack of that compound on the DNA duplexes followed by endonucleatic cleavage at the binding site and not due to an inhibition of the enzymes of a DNA repair pathway.

The significance of these results is discussed in relation to the possibility that methylmercury (MeHg) may produce birth defects and cancer in humans and the need to screen populations for differences in sensitivity to MeHg induced gene damage.

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ORGANIZATION AND FUNCTION OF THE RIBOSOMAL GENES IN *PHYSARUM POLYCEPHALUM*

Vincent G. Allfrey, Edward M. Johnson, Irene Y.-C. Sun,
Virginia C. Littau, Harry R. Matthews and E. Morton Bradbury

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Zur Entwicklungsbiologie von Physarum: Reduktion der Synthese Protein-codierender RNA durch Blockade Chromatin-gebundener RNA-Polymerase B während der Differenzierung

Developmental biology of Physarum: Reduction of DNA-like RNA synthesis by blocking chromatinbound RNA polymerase B during differentiation

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Abstract

Während der Entwicklung eines *Physarum*-Plasmodiums vom Wachstum zur Sporulation nimmt die spezifische Syntheserate Proteincodierender RNA in vivo stark ab, während die extrahierbare Gesamtmenge desjenigen Enzyms, das für diese RNA-Klasse zuständig ist (RNA-Polymerase B) sich wenig verändert (Hildebrandt, A. und Sauer, H. W.: Biochem. Biophys. Res. Comm. 74, 466, 1977). Es läßt sich zudem nachweisen, daß nur maximal 5 % der in Physarumkernen insgesamt vorhandenen RNA-Polymerase B im transkriptionsfähigen Komplex mit der DNA vorliegen. Bei einem so großen Überschuß an freiem Enzym war zu vermuten, daß die mRNA-Synthese bei Physarum am Chromatin selbst reguliert wird. In der Tat spiegelt die in isolierten Kernen meßbare endogene Aktivität der Polymerase B die in-vivo-Verhältnisse wider; d. h. die Aktivität isolierter Kerne beträgt im Sporulations-kompetenten Zustand nur $^1/_5$ des Maximalwertes wachsender Kulturen.

Differenzierungs-kompetente Kerne können jedoch durch Extraktion mit dem Detergenz Triton X 100 (2 %) unter Bedingungen, die eine Neuinitiation von RNA-Polymerasen ausschließen, ebenfalls auf den Maximalwert von Kernen der Wachstumsphase gebracht werden. Produktanalysen zeigen, daß die künstliche Aktivierung auf einer Zunahme an transkribierenden B-Enzymmolekülen beruht. Es muß demnach geschlossen werden, daß in Kernen der Sporulationsphase am Chromatin sitzende Polymerasemoleküle durch (zu dieser Zeit vorhandene) Blocker an der Transkription gehindert werden, die mittels Detergenz herausgewaschen werden können. Diese Annahme wird durch den Nachweis solch blockierender Moleküle im Triton-Überstand reaktivierter Kerne gestützt. Es handelt sich um niedermolekulare Nichthistonproteine, die nur auf Kerne wachsender Kulturen hemmend wirken, nicht jedoch auf Kerne sporulierender Kulturen oder auf RNA-Polymerase B im reinen in vitro-Ansatz an Thymus-DNA. Da diese Elongationsblocker auf Kerne der Differenzierungsphase nicht additiv wirken, muß auf eine selektive Hemmung der mRNA-Synthese am Physarum-Chromatin während der Sporulation geschlossen werden.

Poly(A)-haltige RNA im Lebenszyklus von Physarum polycephalum

Poly(A)RNA during the life cycle of Physarum polycephalum

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Abstract

Der Myxomycet *Physarum* dient als Modellorganismus für den Mitosezyklus und für einfache Differenzierungsvorgänge, wie Enzystierung oder Sporulation (Sauer, H. W.: Konstanzer Universitätsreden 68, G. Hess (ed.), 1974). Wir untersuchten die Syntheseraten und die Menge der *in vivo* von der RNA-Polymerase B transkribierten RNA, und zwar die Fraktion, die durch eine poly(A)-Sequenz (p(A)RNA) gekennzeichnet und daher mittels Affinitätschromatographie zu isolieren ist (Fouquet, H. et al.: Biochim. Biophys. Acta 353, 313, 1974).

1. Zellzyklus: Die Syntheseraten der p(A)RNA wurde durch radioaktive Markierung mit ^{3}H-Uridin, die Menge durch Titration mit ^{3}H-poly(U) gemessen. Für Hybridisierungsexperimente wurden frühreplizierende DNA-Moleküle isoliert und mit radioaktiv markierter p(A)RNA aus verschiedenen Stadien des Zellzyklus vereinigt.

Deutsche Zoologische Gesellschaft (continued)

In der S-Phase, die bei *Physarum* unmittelbar nach der Telophase einsetzt, liegt das Maximum des *Gehaltes* an p(A)RNA. In der G_2-Phase bleibt der Spiegel dieser RNA-Fraktion konstant, während das Minimum in der Metaphase bestimmt wurde. Im Einklang damit stehen die Befunde über die *Syntheserate* von p(A)RNA, die in der Metaphase nicht nachweisbar ist. Am höchsten ist sie bereits in der frühen S-Phase; eine geringe Syntheseaktivität besteht während der G_2-Phase. In der Prophase finden wir wieder erhöhte Werte, die aber nicht das Maximum der frühen S-Phase erreichen. Bei der Analyse der p(A)RNA aus Zellkern und Zytoplasma zeigt sich eine rasche Abnahme der Kern-RNA um 30–50 %, während der Rest erst im Laufe von mindestens 6 h aus dem Kern verschwindet; die Kinetik der p(A)RNA aus dem Kern ist also zweiphasisch. Im Zytoplasma steigt die Menge der p(A)RNA innerhalb von 3 h allmählich an, wobei jedoch nur ein Drittel der ursprünglich im Kern vorhandenen Radioaktivität wiedererscheint.

Wir haben früher gezeigt, daß die hohe Transkriptionsaktivität der S-Phase nur dann zu beobachten ist, wenn auch die Replikation abläuft (Fouquet, H. et al.: J. Cell Sci. *18*, 27, 1975). Weiterführende Experimente weisen nun auf eine direkte «Replikations-Transkriptions-Kopplung» hin. In diesen Versuchen wird frühreplizierte DNA nach Dichte-shift-Markierung *in vivo* im präparativen Cäsiumchloridgradienten isoliert. Radioaktiv markierte p(A)RNA aus der frühen S-Phase (Kontrolle) oder aus blockierter früher S-Phase wird mit der isolierten DNA hybridisiert. Die Hybridisierungsrate der DNA mit jener RNA, die bei blockierter Replikation gewonnen wurde, ist um 40–80 % vermindert; wir schließen daraus, daß frühreplizierte DNA unmittelbar transkribiert wird.

2. Differenzierung: Es ist bekannt, daß bei der Enzystierung von *Physarum* der Gehalt an stabiler RNA abnimmt. Aufgrund von Inhibitorversuchen wurde vermutet, daß zum Auskeimen der Zysten eine RNA-Neusynthese dennoch nicht nötig ist (Sauer, H. W. et al.: Wilh. Roux'Arch. *165*, 110, 1970). Wir konnten nun zeigen, daß bei der Enzystierung von Mikroplasmodien auch die Syntheserate der p(A)RNA abnimmt. Bei vergleichender Analyse von Zellkern und Zytoplasma stellen wir aber fest, daß, anders als in der Wachstumsphase, der Gehalt an p(A)RNA im Kern zunächst vermindert, dann deutlich wieder erhöht ist. Die bei der Enzystierung im Zellkern angehäufte p(A)RNA könnte, in Analogie zu den Bedingungen in Oozyten, einen Informationsspeicher darstellen, der für das Auskeimen der Zysten benötigt wird.

Im Gegensatz dazu läßt sich aus früheren Experimenten schließen, daß für die Sporulation eine Neusynthese von RNA notwendig ist (Sauer, H. W. et al.: Zool. Anz. Suppl. *33*, 350, 1969); tatsächlich konnten wir durch DNA-RNA-Hybridisierungen eine Zunahme der p(A)RNA (60–80 %) bei der Sporulation beobachten.

CELL REPRODUCTION: Honoring Daniel Mazia
An ICN-UCLA Symposium organized by E.R. Dirksen and D. Prescott
Keystone, Colorado
March 19-24, 1978

792 CONTROL OF THE NUCLEAR DIVISION CYCLE IN PHYSARUM POLYCEPHALUM, Wilhelm Sachsenmaier, John Tyson and Gregorio Garcia-Herdugo, Inst f Biochem & Exp Krebsforschung, Univ. Innsbruck, A-6020 Innsbruck, Austria.
Mitosis in multinuclear plasmodia of the myxomycete P.polycephalum are naturally synchronous. Mixed plasmodia containing different sets of nuclei can be prepared by fusion of plasmodial pieces representing different stages of the mitotic cycle. Nuclei with a different history become synchronized rapidly after plasmodial fusion. A model is proposed suggesting that the timing mechanism of mitosis involves the stoichiometric interaction of a cytoplasmic initiator with nuclear receptor sites. The initiator appears to accumulate proportional to plasmodial growth whereas the number of nuclear sites doubles stepwise during each mitosis. Nuclear division is triggered at a critical ratio of initiator/nuclear sites which is reflected by a corresponding ratio of mass/DNA. This "titration model" characterizes the control mechanism of mitosis as a relaxation oscillator. The onset of mitosis functions as an essential component of the oscillator as opposed to alternative concepts based on the assumption of continuous limt cycle oscillators. Studies with inhibitors of DNA- and protein synthesis (5-fluoro-2'-deoxyuridine, cycloheximid, anisomycine), temperature shocks, UV- and X-irradiation further suggest that the mitotic initiator is unstable (half life 1-2 hrs) and cannot be synthesized during the S-period. (Supported by the Fund of Austrian Cancer Research Institutes, NIH-grant No 5 F32 CA 05152-02, and Fundacion Juan March)

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RT551 THE RELATIONSHIP BETWEEN POLY(A) TURNOVER AND RNA TRANSLATION IN PHYSARUM David S. Adams* and William R. Jeffery. Department of Zoology, University of Texas, Austin, Texas.

Poly(A) turnover and its relationship to translation in Physarum polycephalum was studied by pulse-chase experiments. The newy-synthesized RNA of post-mitochondrial fractions, which includes 90% of the total cytoplasmic poly(A), contains adenylate sequences averaging 16 and 90 nucleotide residues. Poly(A) degradation begins immediately after cytoplasmic entry and shows an initial turnover phase(5 hr. halflife) involving the loss of poly(A)$_{90}$ and a final phase(29 hr, halflife) characterized by the disappearance of oligo(A)$_{16}$. Changes in electrophoretic mobility and total quantity of poly(A)$_{90}$ show that degradation occurs by both a shortening process, in which it is eroded from its 3' terminus, and a destruction process in which the entire sequence is destroyed. Both processes begin simultaneously but shortening is terminated prior to destruction since poly(A)$_{50}$ accumulates during the chase and is later destroyed without a further detectable size reduction. A 37°C heat shock, which inhibits translation through polysome disruption, does not affect either degradative process, whereas cycloheximide, which blocks protein synthesis without releasing mRNA,does not affect shortening but severely supresses destruction. Thus poly(A) shortening occurs independent of translation while mRNA liberation from the polysome is required for the destructive process. The turnover of poly(A) in the post-mitochondrial fraction is not regulated by its association with structural proteins since it is not adsorbed to nitrocellulose filters at low ionic strength, does not sediment more rapidly than 5S in density gradients, and lacks the major 78,000 dalton polypeptide bound to poly(A) sequences in mammalian cells. About 10% of the total cytoplasmic poly(A) of Physasum is associated with a membrane enriched fraction and is metabolically stable during an 18 hr. chase. This poly(A) fraction, in contrast to that found in the post-mitochondrial supernatant, may be associated in a complex with specific proteins.

J. Cell Biol. 75, 339a (1977)

RT757 BIOCHEMICAL, BIOPHYSICAL AND IMMUNOLOGICAL CHARACTERIZATION OF THE MAMMALIAN HnRNP PROTEIN HOMOLOGUE FROM PHYSARUM POLYCEPHALUM Mark Christensen*, Ann Beyer*, Steve Poupore*, Barbara Walker* and Wallace LeStourgeon. Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235 U.S.A.

The lower eukaryote Physarum polycephalum possesses a single 34,000 molecular weight (MW) protein which is homologous to several of the proteins of mammalian 40S nuclear hnRNP particles [BBRC 74, 621 (1977) & Cell 11, 127 (1977)]. The protein may represent an evolutionary ancestor of mammalian hnRNP proteins A1, A2 and B1 in that its properties constitute a composite of those of the latter three hnRNP proteins. The Physarum protein possesses the cell state dynamic aspects of A1 (present only in rapidly-dividing cells), a MW and isoelectric point nearly identical to those of A2, and a methylated amino acid, N^G, N^G-dimethylarginine, as does the hnRNP protein B1. For these reasons we believe that the Physarum protein has great potential as a model nuclear RNA-binding protein. Biochemical procedures have been developed for purifying milligram quantities of this protein in a non-denatured state. The protein is soluble in water and preliminary evidence suggests that it may interact to form high MW oligomers. Circular dichroism studies on the purified protein in solution have provided an estimation that the protein contains 10-11% α-helix and 21-33% β-structure. The large percentage of β-structure is consistent with the protein's high content of glycine (22 Mole %). Mammalian hnRNP proteins A1, A2 and B1 also contain high glycine and they too probably contain significant amounts of β-structure. Recently, antibodies to the Physarum protein have been obtained, and strong evidence for their monospecificity has come from experiments involving specific antigen detection in SDS gels. Ouchterlony tests have provided evidence that HeLa hnRNP proteins will cross-react with antibody to the Physarum homologous protein.
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J. Cell Biol. 75, 342a (1977)

CU1031 REGULATION OF AMOEBAL CELL MOVEMENT IN PHYSARUM POLYCEPHALUM D.N. Jacobson, Department of Anatomy, Duke University Medical Center, Durham, North Carolina 27710.

Clones of P. polycephalum amoebae growing on E. Coli B form colonies with one of two characteristic morphologies as a function of the pH of the culture medium and the presence of live or formalin-killed bacteria (J. Cell Biol., 70:183a,1976). Time lapse microcinematographic studies of the movement of wild type and mutant cells have resulted in the identification of two kinds of cell behavior that are partially responsible for the generation of these forms. One involves regulation of the rate of cell movement over a twenty-fold range from 0.1 to 2 μ/min. The rate observed for wild type cells is dependent on the presence of live bacteria, the pH of the culture medium (pH 5 or pH 6), the quanity of bacteria in contact with the amoebae (5×10^8 - 6×10^9 live bacteria per plate or 10^9 live +10^{10} formalin-killed bacteria per plate), and the age of the culture. In contrast, only the presence of live bacteria is required for cells carrying the mutation mov-1 to move at high rates (1.0-2.5μ/min.). Genetic analysis of mov-1, using an easily scored mov-1 phenotype (large colonies at pH 6), shows that it is a typical Mendelian mutation and unlinked to a cycloheximide resistance marker. The observation of a constitutive high rate of movement for mov-1 cells suggests that the low rate of movement of wild type cells under some conditions (pH 6 or early stages of colony formation at pH 5) is the result of a specific regulatory mechanism rather than non-specific deleterious effects on cell metabolism. The second cell behavior involves regulation of the orientation of cell movement. At borders of the bacterial lawn moving amoebae change direction to avoid leaving the bacteria. These results show that P. polycephalum amoebae display complex forms of behavior that can be analyzed with a combination of phenomenological and genetic techniques. (Supported by NSF Grant #43510 and USPHS Grant #2-RO1-GM-20141).

J. Cell Biol. 75, 92a (1977)

CR351 LOCALIZATION OF 19S AND 26S rDNA SEQUENCES IN AN ALTERED NUCLEOSOME CONFIGURATION IN MICROPLASMODIA OF PHYSARUM POLYCEPHALUM. Edward M. Johnson*, Harry R. Matthews*, E. Morton Bradbury*, and Vincent G. Allfrey. The Rockefeller University, New York, NY, 10021.

The localization of rDNA sequences in chromatin subunits was studied by hybridization of purified 19S and 26S RNA to DNA from Physarum chromatin fragments prepared by Staphylococcal nuclease digestion and separated on sucrose gradients. The labeled rRNA hybridized to DNA from nucleosome monomers, dimers, trimers and higher oligomers indicating that a significant portion of the rDNA is packaged into repeating nucleosome structures. However, the distribution of rDNA sequences in the nucleosome fractions differed from the distribution of bulk DNA in that the rDNA sequences were recovered primarily in monomer-sized DNA lengths (140-160 base pairs). At early digestion times the fraction with the highest percentage of DNA hybridizing to the rRNA was a slowly-sedimenting peak clearly resolved from the heavier monomers and partially resolved from smaller fragments near the top of the gradient. This peak (peak A) contained DNA of predominantly 140 base pairs. Peak A had a higher RNA:DNA ratio and a lower overall protein:DNA ratio than did monomers and higher oligomers. The nonhistone protein:histone ratio was considerably higher for peak A than for monomers and higher oligomers, which possessed few nonhistone proteins. Sedimentation velocity analysis yielded values of 5S and 11S for peak A and monomers, respectively. As prepared from nuclear digests on 5% to 20% sucrose gradients containing 0.35M NaCl, peak A differed from monomer and higher oligomer fractions in relative proportions of the different histones. Presence of ribosomal cistrons in an altered nucleosome configuration may be related to changes in functional states of rDNA chromatin. Supported by the National Science Foundation (PCM76-19926) and NATO. (Intr. by V.C. Littau.)

J. Cell Biol. 75, 129a (1977)

CC693 CAFFEINE-RADIATION INTERACTIONS AND MITOTIC DELAY Nancy L. Oleinick, Eugene N. Brewer and Ronald C. Rustad. Division of Radiation Biology, Department of Radiology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106.

Caffeine increases the effects of radiation and certain chemicals on cell lethality, chromosome aberrations, mutations, and transformation, while it can inhibit post-replication repair of UV-damage and reduce the mitotic delay caused by ionizing radiation. We find that 0.1-10 mM caffeine reduces and can eliminate the mitotic delay caused by gamma-irradiation of the slime mold, Physarum polycephalum. For example, the mitotic delay resulting from a dose as high as 10 krad (261 min) was reduced (to 43 min delay) by 3 mM caffeine. Maximal reduction in mitotic delay occurs when caffeine is present throughout the postirradiation period. Late addition or premature removal of the drug leads to a diminished effect. Treatment with caffeine and subsequent drug removal prior to irradiation does not modify the radiation response. Theophylline and theobromine, which differ from caffeine only by a single methyl group each, mimic caffeine in reducing mitotic delay. However, three other known inhibitors of cyclic-AMP phosphodiesterase (methylisobutylxanthine, Ro-20-1724 [4(-3-butoxy-4-methoxybenzyl)-2-imidazolidinone], and papaverine [6,7-dimethoxy-1-veratrylisoquinoline]) increase rather than decrease the mitotic delay at otherwise non-toxic concentrations. Thus, it appears that in Physarum cyclic nucleotide metabolism may not be the basis for the "healing" effect of caffeine on radiation-induced mitotic delay. Caffeine is known to bind to single-stranded regions of DNA. Since caffeine amplifies most of the deleterious effects of radiations, its reduction of radiation-induced mitotic delay may reflect a by-pass or prevention of repair through DNA-caffeine interaction and lead to a decrease in the amount of premitotic time that is normally available for the repair of lethal and/or mutagenic radiation damage. (Supported by U.S.E.R.D.A.)

J. Cell Biol. 75, 10a (1977)

CR700 SUPERCONDENSED CHROMATIN FIBRILS OR CHROMOSOMES IN INTERPHASE NUCLEI OF PHYSARUM POLYCEPHALUM L. R. Troncale, L. X. Oakford,* R. Daly,* and S. Peterson* Dept. of Biology, California State Polytechnic University, Pomona, California, 91768.

Two predominant chromatin fibril organizations (250A & 100A) have become widely recognized after earlier workers demonstrated that larger diameter chromatin fibers were artifacts. We have found a 683 ± 119A diameter fibril (n=69) in interphase nuclei of Physarum using three different techniques (Kleinschmidt whole mount, Miller whole mount, and thin sections). These fibers, when organized into the next hierarchical level of 1121A fibers, apparently correspond to previously reported "dense bodies" which were presumed to be non-fibrillar in nature. Short trypsin digestion of these large-diameter fibrils yields 250A subfibrils, longer trypsin digestion reveals more 100A subfibrils, and short detergent treatments reveal highly convoluted 250A subfibrils still in situ. After treatment with Miller whole mount, nubodies are found associated with partially dispersed regions of the large-diameter fibrails which under this treatment appear 1121 ± 304A in diameter (n=69). Several different procedures & DNase controls were used to eliminate the possible alternative explanations of this fiber as non-nuclear, or as artifacts caused by beam contamination, whole mount preparation, stain contamination, use of aqueous stain, and polyploidy. Advanced eucaryotes treated with these techniques do not show this large-diameter chromatin fibril which is in agreement with previous workers. We suggest, therefore, this abnormally large fibril is found in interphase only in some intranuclear division organisms (i.e. primitive eucaryotes). This system may be used to study; (i) how nubodies are packed into higher order chromatin fibrils, (ii) as a direct test of Comings suggestion that interphase chromatin is maintained in interphase nuclei in pre-chromosomal packets (Am. J. Human Gen., 20, 440, 1968), & (iii) that this large-diameter chromatin fibril or "permanently condensed chromosome" is an evolutionary intermediate stage in the evolution of the chromosomal cycle which could be added to the schema developed by Kubai (Intern. Rev. Cytol., 43, 167, 1975).

J. Cell Biol. 75, 138a (1977)

MF692 INCORPORATION OF FLUORESCENTLY LABELED ACTIN INTO LIVING CELLS. Yuli Wang* and D. Lansing Taylor. The Biological Laboratories, Harvard University, Cambridge, Ma. 02138.

Fluorescently labeled actin has been incorporated into living cells including Chaos carolinensis and Physarum polycephalum in order to determine the distribution, supramolecular form and functional activity of actin in vivo. Vertebrate striated muscle actin and Physarum actin have been purified and labeled with 5-Iodoacetamidofluorescein. The labeled actin was competent to polymerize and to activate myosin ATPase in vitro. Functional labeled actin was utilized as part of the cellular actin pool while denatured labeled actin or labeled BSA was excluded from the cellular actin pool based on several control experiments: (1) "Flare streaming" models (Taylor et al. 1973 J. Cell Biol. 59: 378) prepared from preloaded giant amoebae exhibited fluorescence in the "flare loops" only with functional labeled actin, (2) the contraction of bulk extracts containing labeled proteins exhibited parallel increases in birefringence and fluorescence in the fibrils only with functional labeled actin, and (3) the injection of a contraction solution into living cells (Taylor 1977 Expt. Cell Res. 105: 413) preloaded with labeled proteins produced an intracellular contracted "knot" only with functional labeled actin. The intracellular fluorescence was quantitated with a microscope photometer system. The fluorescence was maximal in the ectoplasm of Physarum which contained birefringent, fluorescent bundles whose presence fluctuated with time. In contrast, the fluorescence was more uniform in Chaos carolinensis where no obvious fibrils were observed. The present results suggest that molecular cytochemistry will yield valuable information on the distribution, and dynamic activity of specific proteins in vivo with the potential for identifying the supramolecular structures using microspectroscopic methods. (Supported by NIH grant AM18111).

J. Cell Biol. 75, 261a (1977)

CC1178 A NECESSARY EVENT FOR MITOSIS IN PHYSARUM John J. Wille and Charles Hebert*. Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA.

The time of formation and role of the intranuclear microtubular organizer region (MOR) in mitotic timing was investigated by exposure of surface plasmodia of Physarum polycephalum to various concentrations of the drug, griseofulvin (GF), for varying durations at different phases of the mitotic cycle. At concentrations below 2.5 x 10^{-5}M, GF neither retards growth or delays mitosis, but selectively abolishes light-induced sporulation. At 50 ug/ml GF, plasmodial growth is slightly retarded, but mitosis is delayed 4-6 hours, if added in S or early G2 phases, 2-4 hours if added during G2 prior to early prophase, and negligible delay if added within 2 hours of metaphase. Mitotic delay with continuous treatment is accompanied by uniform entry of all nuclei into an abortive ring-chromosome metaphase, yielding polyploid daughter nuclei. Correlated EM studies reveal that GF induces multifocal MOR development and multiple nucleoli. Two-hour pulse treatment of plasmodia with 50 ug/ml GF at most phases of the cycle produces no excess mitotic delay. However, pulses beginning at early prophase and coinciding with the onset of nucleolar migration reversibly prevent MOR and yield total delays equivalent to pulse duration. We conclude that the initiation of MOR is a necessary condition for completion of the mitotic sequence and for mitotic timing as transient blockage of its formation results in permanent phase-shifts of the mitotic clock.

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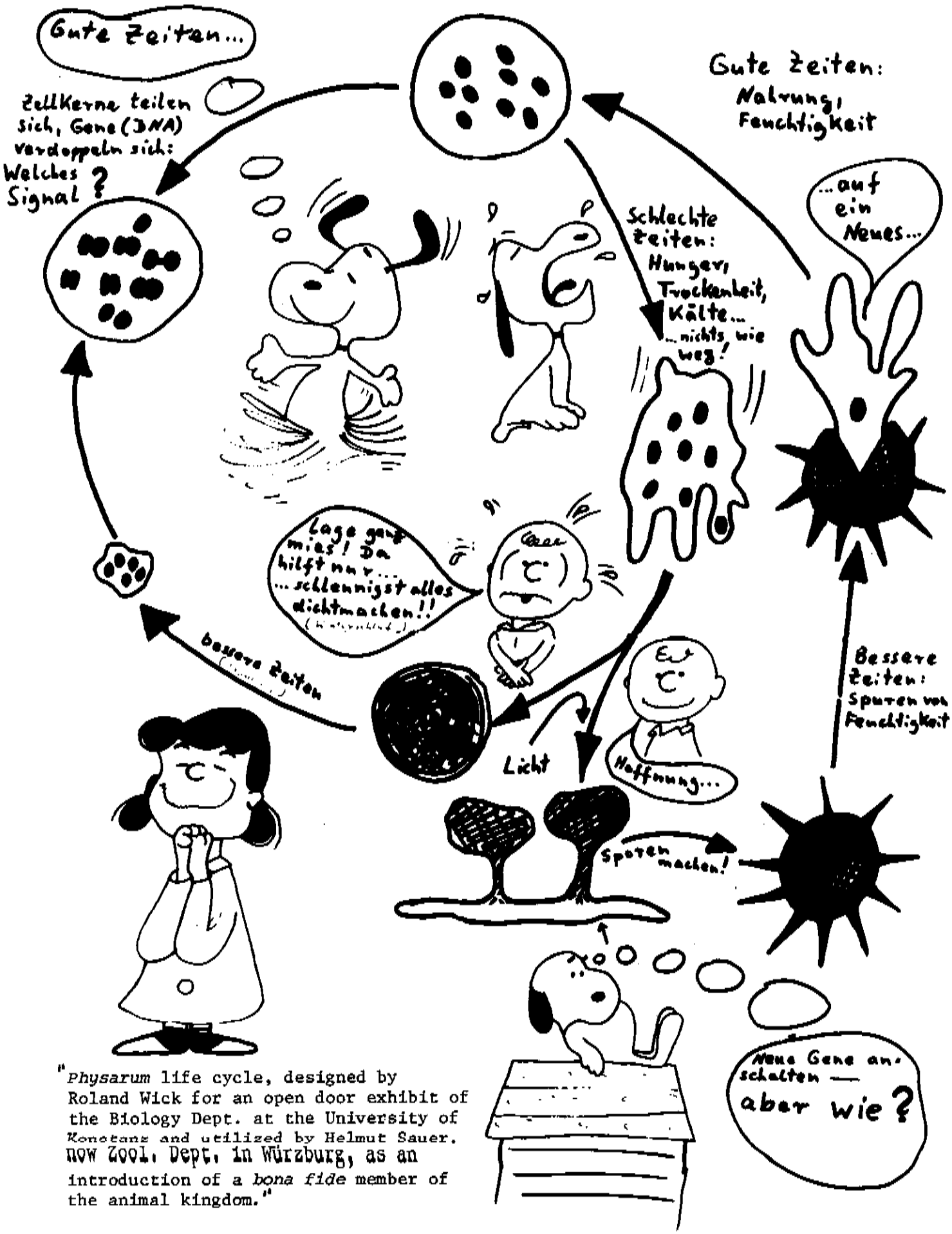

"*Physarum* life cycle, designed by Roland Wick for an open door exhibit of the Biology Dept. at the University of Konstanz and utilized by Helmut Sauer, now Zool. Dept. in Würzburg, as an introduction of a *bona fide* member of the animal kingdom."