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Experimental Cell Research 154 (1984) 394–401

Newly-Initiated DNA Isolated from *Physarum* in Early S Phase Consists of Nascent–Nascent Duplexes

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We have studied the nature of newly initiated DNA released during DNA isolation at the beginning of S phase of *Physarum polycephalum*. The released DNA was separated from the bulk DNA by sedimentation through sucrose gradients. Gentle shearing strongly enhanced the release of newly initiated DNA. The additionally released material had a larger average molecular weight. Buoyant density analysis after labelling with bromodeoxyuridine (BrdU) revealed that the released DNA consisted of nascent–nascent duplexes for more than 90%. This indicates that the release of newly initiated DNA occurs by branch migration. We conclude that shearing enhances branch migration by destabilization of the double helix.

Journal of General Microbiology (1985), 131, 195–200. *Printed in Great Britain*

Oscillations in Intracellular ATP, cAMP and cGMP Concentration in Relation to Rhythmical Sporulation under Continuous Light in the Myxomycete *Physarum polycephalum*

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(*Received 4 July 1984*)

The times of sporulation in populations of plasmodia derived from single starving plasmodia and the variation in intracellular ATP, cAMP and cGMP concentrations were determined from the time when the plasmodia were exposed to continuous light. Sporulation occurred from about 10 h after illumination, with further intermittent sporulation at 5 h or 10 h intervals. Intracellular ATP, cAMP and cGMP concentrations oscillated, usually in phase, with a period of 4–5 h until the irreversible commitment to sporulation occurred. The ATP concentration stopped oscillating, remained at the same level for a few hours and decreased gradually during sporangium formation. Oscillations in cAMP and cGMP concentrations continued, little affected by commitment to sporulate or subsequent sporulation. The period of ATP oscillation did not differ over a wide range of temperature. In plasmodia which were starved only for 1 d and therefore unable to sporulate, light failed to induce ATP oscillation. The concentrations of cAMP and cGMP oscillated but not in phase.

Cell Biology International Reports, Vol. 9, No. 1, January 1985

Alternative pathway of respiration in Physarum polycephalum plasmodia

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Abstract

The external application of inhibitors of glycolysis in the presence of KCN shows a lethal effect on plasmodia of Physarum polycephalum. However, α-ketoglutarate, but not succinate, maintains the contraction-relaxation cycle of plasmodial actomyosin in spite of the fact that glycolysis and cytochrom oxidase are inhibited. The oscillations supported by ketoglutarate disappear in the presence of SHAM, an inhibitor of alternative oxidase. These results imply the existence of KCN-resistant, alternative pathway of electron transport in the mitochondria of Physarum polycephalum.

Volume 11 Number 23 1983 — Nucleic Acids Research

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

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Received 17 May 1983; Revised and Accepted 26 October 1983

ABSTRACT

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

The Effect of Heat Shock on the Cell Cycle Regulation of Tubulin Expression in *Physarum polycephalum*

JOHN J. CARRINO and THOMAS G. LAFFLER
THE JOURNAL OF CELL BIOLOGY · VOLUME 100 FEBRUARY 1985 642–647
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ABSTRACT In the myxomycete *Physarum polycephalum*, tubulin synthesis is subject to mitotic cycle control. Virtually all tubulin synthesis is limited to a 2-h period immediately preceding mitosis, and the peak of tubulin protein synthesis is accompanied by a parallel increase in the level of tubulin mRNA. The mechanism by which the accumulation of tubulin mRNA is turned on and off is not clear. To probe the relationship between tubulin regulation and cell cycle controls, we have used heat-shocks to delay mitosis and have followed the pattern of tubulin synthesis during these delays. Two peaks of tubulin synthesis are observed after a heat shock. One occurs at a time when synthesis would have occurred without a heat shock, and a second peak immediately precedes the eventual delayed mitosis. These results are clearly due to altered cell cycle regulation. No mitotic activity is detected in delayed plasmodia at the time of the control mitosis, and tubulin behavior is shown to be clearly distinct from that of heat shock proteins. We believe that the tubulin family of proteins is subject to regulation by a thermolabile mitotic control mechanism but that once the cell has been committed to a round of tubulin synthesis the "tubulin clock" runs independently of the heat sensitive system. In delayed plasmodia, the second peak of synthesis may be turned on by a repeat of the commitment event.

Plasma Membrane Regeneration in the Myxomycete *Badhamia utricularis*

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Protoplasma 124, 52—64 (1985)

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Received January 3, 1984
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Summary

Plasmodial strands of the myxomycete *Badhamia utricularis* were injured and, after fixation and sectioning, examined using light and transmission electron microscopy. Injury results in the formation of a cytoplasmic droplet that rapidly regenerates a plasma membrane by the apparent fusion of vesicles at its surface. During the same time several structurally distinct layers form within the droplet. Some of these internal layers, which persist for several minutes after injury, may also arise from vesicle fusion. These results have differences from those reported for the related *Physarum polycephalum*.

Keywords: Cytoskeleton; Myxomycete; Plasma membrane regeneration; Transmission electron microscopy; Vesicle fusion.

Mapping of restriction enzyme cuts by a new two-dimensional procedure*

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Experientia 40 (1984), Birkhäuser Verlag, CH-4010 Basel/Switzerland

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Summary. A new procedure has been worked out to establish restriction maps. The method is fast, does not in general require labeled DNA and has been applied to map the linear palindromic rDNA of *Physarum* with the restriction enzyme *Bst*EII.
Key words. Physarum; ribosomal DNA; restriction; method, two-dimensional; gel electrophoresis.

Mycologia, 77(2), 1985, pp. 300–307.
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GENETICAL RELATEDNESS OF A FORMER APOMICT AND A HETEROTHALLIC ISOLATE IN *DIDYMIUM IRIDIS* (MYXOMYCETES)[1]

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ABSTRACT

Several lines of the Pan 4 isolate of *Didymium iridis* (Ditmar) Fries have been made to convert from an apomictic to a heterothallic life cycle mode. With the single exception of the Ky 1 isolate, clones from the converted lines are either incompatible or only partially compatible with all available naturally occurring heterothallics. This indicates that during its existence as an apomict Pan 4 diverged from those heterothallic counterparts to the point where it is now reproductively isolated from them. In the case of the Ky 1 isolate, however, sexual compatibility is relatively good. This is especially intriguing because Ky 1 is incompatible with all other known heterothallics. Analyses of Pan 4 × Ky 1 F_1 hybrids show that recombination did occur, as evidenced by recovery of both parental mating types, by segregation of two vegetative (plasmodial) fusion classes, and by increased sexual compatibility in crosses of F_1's × parents as compared to parent × parent. These results show that Pan 4 and Ky 1 are more related to each other than each is to any other isolate. Whether mating types of these two isolates constitute a separate multiple allelic series remains somewhat uncertain, however, because of partial barriers between Pan 4 and Ky 1.

Key Words: apomixis, heterothallism, genetical divergence.

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

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

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

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

Journal of General Microbiology

(1985) Vol.131, part 5, p. 1167–1179.

The Effect of Ploidy on the Stability of Plasmodial Heterokaryons in *Physarum polycephalum*

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(*Received 31 August 1984; revised 7 December 1984*)

The stability of actively growing heterokaryons made by fusing haploid and diploid plasmodia of *Physarum polycephalum* has been investigated with the aid of genetic markers affecting plasmodial colour and amino acid requirements. All heterokaryons initially expressed the dominant alleles present in both component plasmodia, but after a few subcultures every heterokaryon synthesized from a haploid plus a diploid plasmodium changed to express the recessive alleles carried by the haploid component. In contrast, heterokaryons synthesized from combinations of haploid plasmodia remained stable throughout many subcultures. No evidence was found for the segregation of incompatibility alleles responsible for heterokaryon instability, although the possibility could not be excluded that a gene closely linked to *matA* was involved. A direct test of the effect of ploidy on heterokaryon stability was carried out, in which the use of isogenic haploid and diploid plasmodia allowed the effect of incompatibility genes to be eliminated. Again the phenotype of every haploid plus diploid heterokaryon changed to that of the haploid component, whereas haploid plus haploid heterokaryons remained stable. Variations in the relative sizes of the haploid and diploid plasmodia altered the speed, but not the direction, of the changes observed. Analysis of progeny indicated that the changes in phenotype were due to loss of diploid nuclei from the heterokaryons.

Amer. J. Bot. 72(3): 376–382. 1985.

A MORPHOMETRIC ANALYSIS OF CYTOLOGICAL CHANGES DURING SPORE MATURATION IN THE MYXOMYCETE DIDYMIUM IRIDIS[1]

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ABSTRACT

Ultrastructure of spore maturation in the myxomycete *Didymium iridis* was investigated using morphometric analytical techniques. Changes in actual volume (μm^3) and relative volume (Vv) of nuclei, autophagic vacuoles, mitochondria, microbodies, lipid droplets, and spore wall were described for spores in three stages of development. Stage I spores were newly formed, surrounded only by the cell membrane. Stage II spores were approximately 1 hr older than Stage I spores and possessed surface spines, but little if any additional wall material. Stage III spores were 24 hr old and possessed a fully formed, multilayered wall. The results of this study indicate that spore maturation in *D. iridis* is a multistep process involving a decrease in spore volume and coordinated changes in specific organelle compartments. From Stage I to Stage III, mean spore volume decreased by more than 50%. Percent volume data (Vv) showed that Stage I spores allocated volume equally to all measured organelles except microbodies and the spore wall, the latter of which had not yet begun to develop. By Stage II, only the nucleus and spore wall showed significant changes in Vv values, both increasing. In terms of actual volume, the nucleus, autophagic vacuole and spore wall increased by Stage II. Between Stages II and III the cell wall was the only component to increase in volume, all others decreased in volume. Our data indicate a close relationship between a decrease in organelle volume and an increase in cell wall volume in the Stage III spore. The autophagic vacuole and the cell wall dominated the volume of the Stage III spore while the remaining volume was allocated unequally to the other components.

Trans. Br. mycol. Soc. 82 (2), (1984)

TRICHIA FERNBANKENSIS SP.NOV., A SECOND SPECIES OF *TRICHIA* WITH OPERCULATE SPORANGIA

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A new species, *Trichia fernbankensis*, with operculate sporangia is described and illustrated. It differs from the most closely related taxon, *T. crateriformis*, principally by the reticulate spores and non-cellular operculum.

Mycologia, 76(6), 1984, pp. 1123–1125.

SYNAPTONEMAL COMPLEXES IN AN APOMICTIC LINE OF *STEMONITIS FLAVOGENITA* (MYXOMYCETES, STEMONITALES)[1]

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An Immunological Approach to Enrich a Mitotic Stimulator and to Reveal G_2-Phase–specific Proteins in *Physarum polycephalum*

THE JOURNAL OF CELL BIOLOGY · VOLUME 100 JUNE 1985 1930–1933

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ABSTRACT Purified antibodies from an antiserum against S-phase proteins of the myxomycete *Physarum polycephalum* were attached to protein-A-Sepharose CL-4B. A late G_2-phase extract that contained a mitosis-stimulating protein was applied to this immunoadsorbent, and the mitosis-stimulating protein was enriched by a factor of ten. This protein, which is present in the cell in low amounts, is synthesized in late G_2 phase and obviously degraded in a later stage of the cycle. Immunoadsorption of a G_2-phase extract with anti–S-antibodies decreased the 700 main proteins to 20 as demonstrated by two-dimensional gel electrophoresis. No difference in protein pattern could be observed on two-dimensional gels between S-phase and G_2-phase extracts before and after immunoadsorption with anti–S-antibodies. This indicates that there are no G_2-phase–specific proteins among the 700 most abundant proteins of *Physarum polycephalum*.

J. interdiscipl. Cycle Res. 1984, Vol. 15 Nr. 4, pp. 251-266

Phase Relations of Oscillatory Contraction Cycles in *Physarum* Plasmodia: II. Occurrence of Type 0 Resetting

by

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ABSTRACT

The radial contraction-relaxation activity was recorded in Physarum by the infrared technique either at distant sites or along a linear array of 5 or 10 sites, each 2.5 mm distant from each other. There is a tendency in the plasmodium to maintain the same period throughout the whole plasmodium, although the period may change with time. The recording at a single site represents aggregated rhythms, the mode of which is either synchronous, or twinned or of the shoulder type. The last two modes refer to a split rhythm. There is also a tendency toward synchrony at neighbouring sites. However, there is often a gradient of phase along the array of recorded sites. The gradient is not stable and often shows a reversion. This reversion displays the type 0 resetting: the resetting occurs spontaneously. Usually the resetting takes place only in a part of the oscillating system at a certain site. The local synchronous mode may thus be changed into the twinned or the shoulder mode.

Change of Contractile Behavior in Plasmodia of *Physarum polycephalum* During Mitosis

Protoplasma 124, 71—79 (1985)

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Summary

Oscillations of ectoplasmic contraction in plasmodia of the myxomycete *Physarum polycephalum* growing on agar containing semidefined medium were studied to determine if the contractile force is altered during the synchronous mitosis. In interphase the regular oscillations of contraction in the plasmodial sheet had an average period of 0.93 minutes in plasmodia growing at 24 °C. During mitosis the amplitude of these oscillations gradually decreased, ceasing for an average time of 2.7 minutes in 74% of the 23 plasmodia studied. Cessation of oscillating contractions in mitosis was accompanied by a decrease in the width of the channels embedded in the plasmodial sheet, and a decrease in the velocity of endoplasmic shuttle streaming usually to a complete standstill. Of 13 plasmodia in which the mitotic stage was very accurately determined, the stop in oscillating contractions occurred during metaphase in 10 plasmodia, and in prometaphase, anaphase, telophase in the 3 others. The cessation of contractile oscillations or of streaming did not occur absolutely simultaneously during mitosis in widely separated locations within one plasmodium, indicating mitotic asynchrony over a period of a few minutes within each plasmodium. We suggest that the halt of plasmodial migration during mitosis reported by others is caused by a decrease or cessation at slightly different times in the amplitude of ectoplasmic contractile oscillations in different areas of a plasmodium in mitosis resulting in an overall lack of coordination of endoplasmic flow throughout the plasmodium, thus temporarily halting migration. Possible physiological mechanisms linking a decrease in actomyosin contraction with the metaphase stage of mitosis are discussed.

Keywords: Contractile activity; *Physarum*; Synchronous mitosis.

Reprinted from *J. Mol. Biol.* (1984) 179, 651–666

Regions in the Ribosomal Minichromosome of *Physarum polycephalum* are Protected from Restriction Nucleases; Protection is Insensitive to High Salt in the G Phase and Sensitive in the M Phase of the Cell Cycle

Peter Künzler, Urs Pauli and Richard Braun

Immunocytochemistry of the acellular slime mold *Physarum polycephalum*

IV. Differentiation and dynamics of the polygonal actomyosin system

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Differentiation (1984) 26:11–22

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

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

European Journal of Cell Biology 36, 1-7 (1985) © Wissenschaftliche Verlagsgesellschaft mbH · Stuttgart

Immunocytochemistry of the acellular slime mold Physarum polycephalum.

V. Cryosectioning now allows analysis of non-extracted plasmodia of any and all stages

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Dedicated to Prof. Dr. Kurt Mühlethaler, Zürich, on the occasion of his 65th birthday

Received August 22, 1984
Accepted October 11, 1984

Physarum polycephalum — cryosectioning — immunocytochemistry — phallotoxins — cytoplasmic actin

The spatial distribution of cytoplasmic actin in endoplasmic drops as well as in plasmodial strands can be demonstrated in cryosections by fluorescently labelled phallotoxins and actin antibodies. Our results on cryosections show an identical fibrillar actin distribution as revealed in semithin sections after conventional fixation and embedding.

Thus, it is now possible to apply immunocytochemical analysis to any and all plasmodial stages with or without prior fixation and without using extraction procedures. Consequentially the loss of soluble compounds during processing is avoided. The most protective pretreatment of the living specimens before freezing is a 15 min incubation in 1.5 M sucrose containing 50 mM KCl, 10 mM EGTA and 10 mM PIPES buffer, pH 7.0, at 4 °C.

Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6393–6397, October 1984
Cell Biology

Invariant temporal order of replication of the four actin gene loci during the naturally synchronous mitotic cycles of *Physarum polycephalum*

(sequential DNA replication/cell cycle)

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Communicated by David M. Prescott, June 25, 1984

ABSTRACT The chronological sequence of replication for the four unlinked actin gene loci of *Physarum* has been established. Southern hybridization analysis of density-labeled, bromodeoxyuridine-substituted DNA isolated from defined periods of S phase demonstrates that three actin loci (*ardB*, *ardC*, *ardD*) are duplicated early, corresponding to the first 10% of the genome. The fourth locus (*ardA*) replicates later, between 80 and 100 min into S phase and after 75% of DNA synthesis is completed. Gene-dosage determinations, based on the quantitation of hybridization signals from DNAs isolated from various times during S phase, confirm the results obtained with bromodeoxyuridine-substituted DNA and increase the temporal resolution. The chronological order of replication in the macroplasmodium appears constant through two consecutive cell cycles and after prolonged growth in suspension culture. The precise chronology of DNA synthesis at the gene level extends to the coordinate replication of allele pairs.

Enrichment of fibrillar cytoplasmic actomyosin in protoplasmic strands of *Physarum polycephalum* for the production of cell-free models

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Cell Tissue Res (1985) 239:365–374

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Summary. The treatment of isolated protoplasmic strands of *Physarum polycephalum* with 2.5% ethanol in a physiological salt solution under isometric conditions induces the formation of a large amount of mostly longitudinally organized actomyosin fibrils in the endoplasmic channel, a region normally free of actomyosin fibrils. The quantity of fibrillogenesis as well as the concomitant force output during the induced contractures are dependent on the Ca^{++}-content and the temperature of the test solution. The method was developed to optimize the structure of the plasmodial strands before their subsequent transformation into cell-free models by permeabilization and extraction of the strands.

Cryosections of plasmodial strands containing cytoplasmic actomyosin fibrils stained with fluorescently labeled phallotoxins offer a further assay for the study of their contraction physiology under cell-free conditions.

Key-words: Cytoplasmic actomyosin – Fibrillogenesis – Ethanol – Ca^{++} – Low temperature – *Physarum polycephalum* – Cryosectioning – Cell-free models

Cell Biology International Reports, Vol. 8, No. 12, December 1984 1065-1068

REACTIVATION OF NBD-PHALLACIDIN-LABELLED ACTOMYOSIN FIBRILS IN CRYOSECTIONS OF PHYSARUM POLYCEPHALUM: A NEW CELL-FREE MODEL

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ABSTRACT

Application of ATP to cryosections of plasmodial strands from Physarum polycephalum leads to an isotonic contraction of the cytoplasmic actomyosin fibrils: when the fibrils are labelled with NBD-phallacidin, their contraction can be observed in the fluorescence microscope.

While performing contraction, the fibrils separate into many small units and the formerly continuous fibrils exhibit the appearance of beaded chains.

The possibility of visualizing directly the contraction of cytoplasmic actomyosin fibrils in the fluorescence microscope represents a favourable condition for the study of their physiological contraction mechanism, because this new and convenient cell-free model offers in situ contractile structures that are non-denatured and non-extracted.

Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 7117–7121, November 1984
Cell Biology

Photomorphogenesis in *Physarum*: Induction of tubulins and sporulation-specific proteins and of their mRNAs

(slime mold/differentiation/cDNA/*in vitro* translation)

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ABSTRACT Sporangiophore formation in *Physarum* plasmodia starts about 10 hr after photoinduction. It is characterized by the induction of two tubulins and of at least 15 major sporangiophore morphogenetic proteins. *In vitro* translation of extracted mRNA revealed that differential gene expression is based on a highly synchronous temporal program of loss of plasmodial and induction of sporulation-specific mRNA species. Using a cloned cDNA encoding part of a sporangiophore morphogenetic protein from *Physarum* as a probe it was found that the induction of the complementary mRNA activity is due to the induction of the mRNA itself. The results suggest that light induces, with a lag phase of about 10 hr, the transient activation of sporulation-specific genes.

J. interdiscipl. Cycle Res. 1984, Vol. 15, Nr. 4, pp. 241-250

Phase Relations of Oscillatory Contraction Cycles in *Physarum* Plasmodia: I. A Serial Infrared Registration Device and its Application to Different Plasmodial Stages

by

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ABSTRACT

A new and inexpensive electronic device is described which enables serial registrations of the oscillatory contraction activities at closely neighbouring sites within plasmodia of Physarum polycephalum. At the same time, this new construction allows the immediate automatic transformation of the original sinusoidal contraction curves into multiple phase graphs.

The application on different stages of plasmodia reveals phase gradients as a characteristic phenomenon especially in isolated objects. The investigation of plasmodial strands without endoplasm demonstrates that the radial activities represent active contractile phenomena of the ectoplasmic tube.

CELL STRUCTURE AND FUNCTION 7, 275–283 (1982)
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Primary Oscillator of Contractional Rhythm in the Plasmodium of *Physarum polycephalum*: Role of Mitochondria

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ABSTRACT. Various inhibitors were microinjected into the cytoplasm of the plasmodium of *Physarum polycephalum*, and their effects were monitored by measurements of contractile activity, and the intracellular ATP and cytoplasmic Ca^{++} concentrations. Fluoride and monoiodoacetate (glycolytic inhibitors) reduced the ATP by 50%, but the contractional rhythm was unaffected. In contrast, azide and arsenate (respiratory inhibitors) and 2,4-dinitrophenol (uncoupler) induced a transient decrease of 50% in the ATP value and a 2–3-fold transient prolongation of the rhythm. A combined injection of glycolytic and respiratory inhibitors reduced the ATP to 10% and led to complete cessation of the rhythm. P-chloromercuribenzoate (SH-blocking reagent) had similar effects as those of a respiratory inhibitor, but N-ethylmaleimide had no affect. Ruthenium red (inhibitor of mitochondrial Ca^{++}-uptake) did not affect the ATP value, but did bring about gradual prolongation of the rhythm accompanying an increase in the Ca^{++} efflux into the cytoplasm.

These results are evidence that the time-keeping of contractional rhythm in *Physarum* plasmodium is related primarily to mitochondrial activity.

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Partial Purification and Characterization of Microtubular Protein from *Trypanosoma brucei**

(Received for publication, October 18, 1983)

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The tubulin proteins of the parasitic hemoflagellate *Trypanosoma brucei brucei* were purified and characterized. Cytoskeletal microtubules of trypanosomes do not disrupt under conditions used to solubilize brain tubulins. Trypanosomal tubulins, solubilized by extensive sonication, were partially purified from the crude cell extracts by taxol-mediated polymerization. Taxol-induced microtubules were identified by electron microscopy and analyzed biochemically. They consist predominantly of two proteins of about 52,000 and 56,000 Da. Their mobilities on sodium dodecyl sulfate gels differ slightly from those of bovine brain tubulins. Immunological cross-reactivity with antibodies raised against bovine brain tubulins confirmed the nature of the trypanosomal proteins. Peptide mapping of bovine and trypanosomal α- and β-tubulins was performed by enzymatic digestion with staphylococcal protease V8 and chemical cleavage with *N*-chlorosuccinimide. In both cases, the peptide patterns generated from the trypanosomal α- and β-tubulins were closely related to each other. This suggests that the trypanosomal α- and β-tubulins may have remained more conserved during evolution than the tubulins from higher eukaryotes.

The trypanosomal α-tubulin is post-translationally modified *in vivo* by the reversible addition of a tyrosine residue at its COOH terminus. As in higher eukaryotes, this reaction is completely specific for the α-polypeptide chain. Our observation represents the first documentation of the occurrence of COOH-terminal tyrosinolation of α-tubulin in an eukaryotic microorganism.

Two Steady States in Membrane Potential Deflection in Relation to Chemoreception and Chemotaxis by *Physarum polycephalum*

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Summary

In the plasmodium of *Physarum polycephalum* application of various monovalent cation salts elicited either a depolarization or a hyperpolarization of the membrane potential. The hyperpolarization was restricted to phosphates and bicarbonates of large cations (Na^+, Li^+, NMe_4^+, NEt_4^+). More than 50 other combinations of cations (K^+, Rb^+, Cs^+, NH_4^+) and anions (Cl^-, NO_3^-, SO_4^{2-}, acetate, lactate, citrate, etc.) induced the depolarization. In both cases the magnitude of the deflection in membrane potential ($\Delta\varphi$) varied linearly with logarithm of concentration above the threshold C_{th} ($\sim 10^{-4}$ M for all monovalent cation salts examined) according to the following equation:

$$\Delta\varphi = \pm R \log (C/C_{th}).$$

The value of R was 10–15 mV, and plus and minus signs correspond to depolarization and hyperpolarization, respectively. Depolarizing and hyperpolarizing agents competed with each other and exhibited a sharp transition between the two states of the membrane which were characterized by — R and R in the above equation or displayed a strong hysteresis, depending on which agents had first been applied to the plasmodial membrane. This transition in the membrane potential corresponded to the transition between positive and negative taxis at the behavioral level.

Keywords: Chemoreception; Chemotaxis; *Physarum polycephalum*.

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Patterns of histone acetylation in *Physarum polycephalum*

H2A and H2B acetylation is functionally distinct from H3 and H4 acetylation

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Histone acetylation has previously been correlated with both chromosome replication and transcription. We present evidence that (a) confirms both correlations in the true slime mold, *Physarum polycephalum* and (b) shows that quite a different pattern of acetate turnover is associated with replication compared with transcription. The pattern asssociated with replication involves turnover of acetate on all four core histones on species containing one or two acetates per molecule. This pattern was resolved from the transcription-associated pattern by three different procedures: (a) detailed analysis of gels of histones pulse-labelled with acetate; (b) the pattern of acetylation of histones pulse-labelled with [^{3}H]lysine; and (c) the pattern of acetylation of soluble histones. The pattern associated with transcription is restricted to histones H3 and H4 and occurs mostly on highly acetylated species. This pattern was resolved by (a) analysis of gels of histones pulse-labelled with acetate; (b) the pattern of histone acetylation in G2 phase of the cell cycle; and (c) the pattern of histone acetylation in the presence of cycloheximide.

THE ORGANIZATION OF REPLICONS

J.H. Waterborg and S. Shall

In: "The Cell Division Cycle in Plants"
Soc. Exp. Biol. Semin. Series, Vol. 26, pp. 15-35.
Eds. J. A. Bryant and D. Francis.
Cambridge University Press, Cambridge, 1985.

INTRODUCTION

This paper is a collection of ideas related to the concept of a unit of DNA replication, the replicon. The various levels of organisation of chromatin and nucleus together ensure the correct regulation, both temporal and spatial, of the process of replication of all the DNA molecules, and their equal distribution to the two daughter cells during mitotic division.

The experiments with which we will illustrate this discussion are mainly taken from studies with the lower eukaryote Physarum polycephalum. This organism has a very high natural mitotic synchrony which allows many experimental approaches to the regulation of DNA replication that are impossible in other cell systems.

Experimental Cell Research 155 (1984) 171–177

High Diadenosine Tetraphosphate (Ap_4A) Level at Initiation of S Phase in the Naturally Synchronous Mitotic Cycle of *Physarum polycephalum*

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Levels of the diadenosine tetraphosphate Ap_4A are high during exponential growth of *Physarum*, decrease during encystment (spherulation) and increase again during excystment. Consistently, a rapid 8–30-fold increase in Ap_4A level occurs at entry into S phase of the mitotic cycle and is maintained during the first half of genome replication. The elevated Ap_4A level depends significantly on ongoing DNA replication and is completely sensitive to the protein synthesis inhibitor cycloheximide administered either before or after initiation of S phase. © 1984 Academic Press, Inc.

Adenine Inhibits Microcyst Formation in *Physarum flavicomum* and Affects the Cellular Level of S-Adenosylmethionine

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Summary

The effect of various purines, pyrimidines and nucleosides on the encystment of haploid cells of *Physarum flavicomum* was determined. Of the compounds tested guanine, guanosine, cytidine, cytosine, 5-methylcytosine and uracil had no effect on encystment. Adenosine, thymine, uridine and 3-methyladenine only slightly delayed encystment and protein degradation. Adenine and, to a lesser extent, hypoxanthine produced a significant inhibition of encystment and greatly increased rates of autolytic protein and RNA degradation, which eventually led to about 75% cell death in the adenine-exposed cells. The inhibition of microcyst formation by adenine was concentration dependent. The incubation of cells with adenine resulted initially in elevated intracellular levels of S-adenosylmethionine up to 3.5 times the level of untreated control cells.

Keywords: Adenine; Encystment; Microcysts; Myxomycete; *Physarum flavicomum;* S-adenosylmethionine.

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Histone H4 gene is transcribed in S phase but also late in G_2 phase in *Physarum polycephalum*

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Communicated by G. Dirheimer

The myxomycete *Physarum polycephalum* contains two types of H4 histone genes. Southern blotting of restriction endonuclease fragments of *P. polycephalum* DNA and hybridization to a cloned probe labelled by nick-translation indicate that there are only one or two copies of each H4 gene per haploid genome. A cloned homologous genomic probe was used to study the cellular abundance of H4 mRNA during the cell cycle. We report that the H4 mRNA is not only transcribed in S phase as previously described for other organisms but that transcription of the H4 gene also occurs at the end of G_2 phase. Since no translation of the histone messenger was observed in G_2 phase this suggests that the histone mRNA synthesized in G_2 constitutes a pool of molecules in anticipation of the next S phase.

Key words: histone gene transcription/cell cycle/*Physarum polycephalum*

An Electron Microscope Thick Section Study of Endomembrane Organization in the Myxamoebae of *Physarum polycephalum*

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Summary

The post-fixation of *Physarum polycephalum* myxamoebae with a zinc iodide-osmium tetroxide mixture resulted in the accumulation of an electron dense deposit in the lumen of the nuclear envelope, the endoplasmic reticulum, and most of the cisternae of the Golgi apparatus. In double-stained thin sections of impregnated myxamoebae the preservation of other cytoplasmic organelles was excellent. Examination of thick sections (0.25 µm) at 120 kV revealed the complexity of the endomembrane system and continuities between both the ER and the nuclear envelope, and the ER and the Golgi apparatus. No direct continuities were observed between the cisternae of the Golgi apparatus and the nuclear envelope. A three-dimensional view of membrane organization was obtained from stereopairs, while tilting the sections at much steeper angles revealed whether any of the apparent continuities seen were real or were simply the result of overlap. A morphologically distinct region of the ER, which bears similarities to the GERL region described in other organisms, was found in continuity with the remaining ER, the Golgi apparatus, and the nuclear envelope.

Keywords: Endoplasmic reticulum; Golgi apparatus; Myxamoebae; *Physarum polycephalum;* Zinc impregnation.

Abstracts of the Annual meeting for Belgian Society for Cell Biology
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Reactivation of cell-free models derived from glycerol-extracted endoplasmic drops of Physarum polycephalum.

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Endoplasmic drops of Physarum polycephalum, glycerol-extracted in the presence of EDTA are reactivated by MgATP. No reactivation is achieved without Mg^{++} or with ADP or AMP and inorganic phosphate, respectively. Furthermore, contraction can be reversibly inhibited by organic mercury compounds and irreversibly by NEM, probably indicating the inhibition of the myosin ATPase (1).
In the absence of ATP, condensation of the models occurs upon replacement of EDTA at millimolar concentrations of divalent cations in spite of the presence of NEM. This condensation reaction is probably performed by the superfine filament system. The preservation of actin after long-term glycerol extraction is demonstrated by fluorescence microscopy of whole-mount preparations. The structural preservation is controlled by phase contrast and electron microscopy.
Supported by the Deutsche Forschungsgemeinschaft (Wo 20-22/1).
Reference: (1) Wohlfarth-Bottermann, K.E.: Cell-free models from Physarum polycephalum - Pitfalls and improvements. In: Cell Motility, Mechanism and Regulation. Proceedings of the 10th Yamada Conference 1984. Eds. H. Ishikawa, H. Sato, S. Sato, University of Tokyo Press (1985).

Physarum polycephalum - model object in contraction physiology of cytoplasmic actomyosin.

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Plasmodia of Physarum polycephalum are composed of a network of protoplasmic strands and a leading front zone in the direction of migration. The contractile apparatus of this organism is represented by a complex system of plasmalemma invaginations sandwiched by cytoplasmic actomyosin. Whole plasmodia as well as cut portions are accessible to different techniques used to register oscillating contractions under experimental conditions. The infrared-reflexion technique (1) and the photo-cell registration (2) are applied as non-invasive techniques. The tension transducers (3) allow introduction of isometric and isotonic measuring conditions. Perfusion chambers are used for the application of test solutions. An isolated strand mounted as a "trapeze" serves to study signal transmission within the giant cell.
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Morphogenesis and differentiation in protoplasmic drops of Physarum polycephalum.

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Endoplasmic drops generated by puncturing plasmodial strands of the acellular slime mould Physarum polycephalum undergo several cytomorphogenetic processes during the transformation of undifferentiated endoplasm to a migrating plasmodium (1). Within the first 10 sec the regeneration of the plasma membrane occurs by fusion of peripherally located vesicles (2). The combination of intra- and exocytotic processes results in generation of an extensive invagination system characteristic of plasmodial ectoplasm. Simultaneously, a sol-gel transformation occurs due to actin polymerisation and subsequent formation of actomyosin fibrils (3). In collaboration with plasmalemma invaginations the actomyosin system represents the contractile apparatus of this organism, performing oscillating contractions which cause endoplasmic shuttle streaming that results in locomotion.
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Calcium and cytoskeletal function in Physarum polycephalum

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During the past decades the knowledge about cytoskeletal elements in Physarum polycephalum has increased considerably. The actomyosin system as well as the tubuline have been investigated intensively. Recently, a new class of "superfine" filaments has been proposed to be compared of a titin-like protein. The properties and functions of the three protein systems seem to be calcium dependent.
The localization of calcium binding sites by means of the pyroantimonate technique (1) is presented in connection with reports on the role of calcium in regulating cytoskeletal elements and associated regulatory proteins.

Reference: (1) Achenbach, F., Achenbach, U., Kessler, D. (1984). Calcium binding sites in Physarum polycephalum as revealed by the pyroantimonate technique. J. Histochem. Cytochem. 32, 1177-1184.

Gravisensitivity of the acellular slime mold Physarum polycephalum.

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Changing the gravity-vector, by turning a cell about 180° or by simulating weightlessness with the aid of a fast rotating clinostat, is one important tool for testing the gravisensitivity of a single cell. Under "functional zero gravity- (0 g-) conditions" Physarum shows initially a transient frequency increase in the oscillating contraction automaticity as well as in cytoplasmic shuttle streaming. 15-30 minutes after the onset of 0 g, the slime mold starts to regulate - with typical overshoots - these parameters back to normal values which is achieved at the earliest 40 minutes after the beginning of the 0 g-simulation. Within this regulation period the velocity of the shuttle streaming increases. Cessation of the rotation again induces strong reactions, the most prominent of which is a strong decrease in the velocity of the shuttle streaming.
Blockage of respiration with KCN reduces the 0 g-reaction and the regulation is inhibited. Similar reactions as during the 0 g simulation-experiments are observed when horizontally positioned specimens are turned 180°.

Reference: Block, I., W. Briegleb, K.-E. Wohlfarth-Bottermann: Influence of simulated weightlessness on the motility of the acellular slime mold Physarum polycephalum. Proc. of the Second Europ. Symp. on Life Sciences Research in Space, ESA SP-212, 27-30 (1984).

Demonstration of the microfilament system in fixed cell fragments and microplasmodia of Physarum under normal and experimental conditions

K. Brix, J. Kukulies and W. Stockem, Institute for Cytology, University of Bonn, Federal Republic of Germany.

More than 10 different fixation and extraction procedures were tested to preserve and visualize the microfilament system in cell fragments and microplasmodia of Physarum polycephalum by rhodaminyl-phalloidin staining. Relaxed and experimentally contracted small cell fragments display a thin microfilament layer below the plasma membrane and at the hyalo-granuloplasma border, respectively. Larger cell fragments develop a more complicated microfilament system by the formation of distinct fibrils running across the cytoplasm. The external application of different substances affecting the motile activities and morphology of the cell fragments induces characteristic changes in the spatial organization of the microfilament system. A complicated arrangement of microfilaments was also observed in microplasmodia grown in axenic shuttle cultures. In addition to a continuous cortical actin layer bordering the cytoplasmic site of the plasma membrane invagination system, a conspicuous fibrillar actin system is found. The participation of the microfilament system in the generation of different movement phenomena of Physarum cell fragments and microplasmodia is discussed.

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Electron microscopical demonstration of the cytoskeleton in Physarum by the replica technique

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The plasma membrane properties of free living organisms and normal tissue cells are distinctly different. This is demonstrated by extraction experiments on the acellular slime mold Physarum polycephalum. Living plasmodial fragments produced by caffeine treatment and extracted with different concentrations (0.1-5%) of Triton-X-100 supplemented by other agents (EGTA, EDTA, KCl, $MgCl$, PEG, glycerol, DTT, PMSF) show extensive motile phenomena (protoplasmic streaming, changes in shape) several minutes before they die. Inorder to avoid drastic artefactual alterations the cytoskeleton preparations were done on specimens carefully fixed in methanol, methanol/glycerol mixtures, various aldehydes or $Os/HgCl_2$ before extraction. After freeze-drying or critical-point-drying the platin/carbon replicas of mechanically fractured material revealed a very extensive system of filaments in different cytoplasmic regions. Cytochemical staining for polysaccharides delivered clear evidence for the slime nature of one class of filaments ranging in diameter between 2 nm and 50 nm. These filaments are not only restricted to the exoplasmic space but also occur in the nuclear and cytoplasmic matrix outside of vacuoles (compare Horisberger et al., Biochem. Biophys. Acta 542, 308, 1978). The second class of filaments is localized at the internal face of the plasma membrane or at the hyalo-granuloplasma border and represents actin.

Supported by a grant of the DFG (Sto 126/1-4).

IMMUNOCYTOCHEMICAL ANALYSIS OF NON-EXTRACTED AND NON-DENATURED PHYSARUM CYTOPLASMIC ACTOMYOSIN IN CRYOSECTIONS.

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The spatial distribution of cytoplasmic actin in endoplasmic drops as well as in plasmodial strands can be demonstrated in cryosections by fluorescently labelled phallotoxins and actin antibodies (1). The results show an identical fibrillar actin distribution as revealed in semithin sections after conventional fixation and embedding (2). Thus, it is now possible to apply immunocytochemical analysis to any and all plasmodial stages with or without prior fixation and extraction procedures. This advance avoids the loss of soluble compounds and opens the way for the cytochemical localization of regulatory and accessory proteins involved in the contraction of cytoplasmic actomyosin in Physarum. The most protective pretreatment of the living specimens before freezing is a 15-min incubation in 1.5 M sucrose containing 50 mM KCl, 10 mM EGTA and 10 mM PIPES buffer, pH 7.0, at 4° C (1).

Supported by the Deutsche Forschungsgemeinschaft (Wo 20-22/1).

Ref.: (1) Naib-Majani, W., Wohlfarth-Bottermann, K.E.: Europ. J. Cell Biol.(in press).
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Actin and tubulin expression during photoinduced sporulation in Physarum.

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Light-induced sporulation in Physarum plasmodia is a highly synchronous process (1,2) and is a model system to study differential gene expression during development of lower eucaryotes (3). Among the various proteins induced during sporangiophore morphogenesis two tubulins (α- and β-type) have been identified. Using a homologous α-tubulin cDNA it has been shown that α-tubulin induction is caused by the transient induction of the corresponding mRNA sequences at a distinct time point of development. Possible functions of the tubulins in the differentiating plasmodia are discussed. The synthesis of 3 plasmodial actin variants is inhibited during sporangiophore formation. By in vitro translation of mRNA, Northern blot hybridization and transcription in isolated nuclei it is shown that the inhibition of actin synthesis is based on the almost complete degradation of cellular actin RNAs (1.6, 1.9 and 3.3 kb) in spite of continued transcription of actin-specific RNA sequences. These studies strongly suggest post-transcriptional control of actin expression during Physarum differentiation.

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Amino acid sequence of a Physarum polycephalum α-tubulin.

Monika Singhofer-Wowra, Günter Krämmer and Melvyn Little, Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, FRG. Tim Schedl, McArdle Institute for Cancer Research, Wisconsin, U.S.A.

We have elucidated the nucleotide sequence of a cDNA which appears to code for all but the last 25-30 C-terminal amino acids of an α-tubulin expressed in the plasmodium of the slime mold Physarum polycephalum. Amino acid sequence differences to other α-tubulins are distributed fairly evenly throughout the sequence, although a small region in position 299-308 contains a cluster of amino acid residues unique to Physarum α-tubulin. The most conserved past of the α-tubulin sequence is in positions 396-426. The sequence is 70 % homologous to two yeast α-tubulins from Schizosaccharomyces pombe and about 83 % homologous to five animal α-tubulins from chick, pig, human, rat and sea urchin.

Cell Tissue Kinet. (1985) 18, 217–232.

Abstracts of the 10th International Cell Cycle Conference

24–28 April 1984 at the Banff Centre, Alberta, Canada

Characteristic growth and nuclear nonhistone protein patterns of the white mutant (LU 887 × LU 897) strain of *Physarum polycephalum* microplasmodia

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A study of the growth of white (LU 887 × LU 897) microplasmodia was made by subculture from quiescent *v.* log cultures. Based on weight, dry weight and protein content routines for subculturing the white strain are presented. A comparison of growth characteristics was made by subculture during log growth phase of the white *v.* yellow (Mc3 VII) strains of microplasmodia. Both strains show generally sigmoidal growth curves with the yellow proliferating more rapidly than the white strain. Both strains reach approximately the same wet weight mass though the white strain shows a disproportionate increase in wet:dry weight ratio suggesting greater glycoprotein slime accumulation. The yellow microplasmodia show a relatively constant wet:dry weight ratio and a greater protein content than the white strain; these results lead to the suggestion that the two strains differ in the pattern of expenditure of metabolic energy. During a 6-day culture period glucose content of the media decreased linearly from 90 mg/ml to zero. For either culture strain, initial media pH (4·6) increased to 5·5 by day 6 while the lactic acid content was unchanged. In the yellow strain, protein estimation by the Bradford and biuret methods gave similar results: values by the method of Lowry *et al.* were consistently higher. The complement of nuclear nonhistone proteins of white microplasmodia as qualitatively and quantitatively quite similar to that of yellow microplasmodia after 2 days of growth; numerous quantitative differences were observed after 3 days of culture. Cell-cycle time for the two strains was quite similar.

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