PHYSARUM NEWSLETTER

VOLUME 20, NUMBER 1

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THE *PHYSARUM* NEWSLETTER

August, 1988

Volume 20, Number 1

From the editor:

This message is not a very pleasing one for two reasons. First, as you will see on pages i and ii, Harold Rusch died early this summer. His passing will be felt by everyone in our field. The biography/obituary was contributed by Bill Dove and Helmut Sauer.

Second, since I no longer have funding for *Physarum* research and have little or no work on the organism going on in my lab, I must ask to end my term as Newsletter editor. This is to solicit volunteers to take over. Write or phone me at 904-392-1096 to discuss it. Hopefully someone will emerge in time to take over at the next U.S. meeting next summer. I am sorry that I cannot continue, but most of my work now is on bacteria. My interest in *Physarum* continues, but without funding, as many of you also know, research is hard to keep going.

Bill Dove reports that the Japanese meetings this past summer were very good. He has funding to prepare and mail copies of the abstracts of those meetings to all workers on our mailing list. We will be mailing those to you as soon as they are available.

Henry Aldrich

Future Meetings

The next U.S. *Physarum* meeting is scheduled for summer of 1989 at Cornell University in Ithaca, New York, hosted by Volker Vogt. When details are available, we will pass them on. I have no word on the next European meeting site as yet.

Contributions to this publication:

Original contributions are welcome at any time. Send them to the editor, single spaced, well typed, ready for reproduction. Please also send abstracts of published papers as soon as they are available, or a copy of the abstract from the page proof is fine if available.

Harold Paul Rusch was born on July 15, 1908, in Merrill, Wisconsin, the son of Henry Albert and Olga (Brandenburg) Rusch. After a boyhood spent mainly in Wausau, he studied at the University of Wisconsin in Madison, where he received the B.A. degree in 1931 and the M.D. degree in 1933. After his internship he began his career in cancer research as a Bowman Research Fellow at the University of Wisconsin Medical School in 1935, and from that time on his energies were devoted to this field. Dr. Rusch founded and served as the Director of the McArdle Memorial Laboratory for Cancer Research from its beginnings in 1940 until 1972. It is generally acknowledged that during this time it was the finest cancer research institute in the world. In 1972 Dr. Rusch founded and became Director of the new Wisconsin Clinical Cancer Center at the University of Wisconsin-Madison. He remained at that post until his retirement in 1978.

The contributions of Dr. Rusch to the establishment of national and international policy with respect to cancer were of major importance. He served as President of the American Association for Cancer Research from 1954 to 1955 and as President of the Association of American Cancer Institutes from 1972 to 1974. He was also a member of the Commission on Cancer Research of the International Union Against Cancer from 1958 to 1966. From 1950 to 1964 he served as Editor-in-Chief of CANCER RESEARCH, the official publication of the American Association for Cancer Research, and he was also on the editorial boards of a number of other scientific journals. He was appointed to President Kennedy's Committee on Heart Disease and Cancer in 1961 and to the U.S. Senate National Panel of Consultants on the Conquest of Cancer in 1970.

Dr. Rusch was very active in the American Cancer Society as a member of its Research Advisory Council (1962-1965), Board of Directors (1965-1974), and several committees. In 1970 he received the Annual Wisconsin Divisional Award of the American Cancer Society, and in 1972 its Annual National Award. Other honors included election as a Fellow of the American Academy of Arts and Sciences in 1959; the Distinguished Service Award of the University of Wisconsin Alumni Association in 1970; and the UW Medical Alumni Citation in 1973. In 1981 he received the Papanicolaou Award from the Papanicolaou Institute in Miami for his contributions to the understanding of cancer.

The underlying goal of Dr. Rusch's studies was the elimination of cancer. In 1941, he pioneered the identification of those wavelengths of sunlight that are responsible for causing skin cancer. In the early 1940s he also showed that overeating, which results in obesity, increases the risk of cancer by reducing the activity of the adrenal glands. Dr. Rusch was especially interested in the concept that cancer does not occur as the result of a single event, but rather as a series of biochemical changes. This approach led to subsequent work on the stages of tumor formation, a field in which the McArdle Laboratory achieved eminence in the subsequent decades. Beginning in 1955, his research focused on the biochemical events that control growth and differentiation in a model system, the primitive mold *Physarum polycephalum*; as a consequence, he established that this organism is a useful model for studies of cell biology. The *Physarum* experiments directed by Rusch that seem most germinal are the analyses of the conservation of the replication cycle (Braun, Mittermayer, and Rusch, 1965) and of the accumulation of mitogenic activity in the cytoplasm (Rusch, Sachsenmeier, Behrens, and Gruter, 1966). These investigations grew out of the technical foundation established by Daniel and Rusch (1961) — a defined growth medium for *Physarum* plasmodia. The vitality of Rusch's *Physarum* laboratory during the 1960s was due in major ways to the skill and interest of Dr. Joyce Mohberg. Those of us who enjoyed his company in *Physarum* research knew him as eternally curious, a man who enjoyed each day of discussions in the laboratory. And we note the conviction with which he followed the possibility of differentiation-inducing substances — a foresight of the concept of "tumor-suppressor genes" that is being so actively pursued in the late 1980s.

A thoughtful listener, an exemplar of courtesy and honesty, an extraordinary judge of people, Harold Rusch was admired and loved by a wide circle of friends, colleagues, and students. He was devoted to his family and proud of their accomplishments.

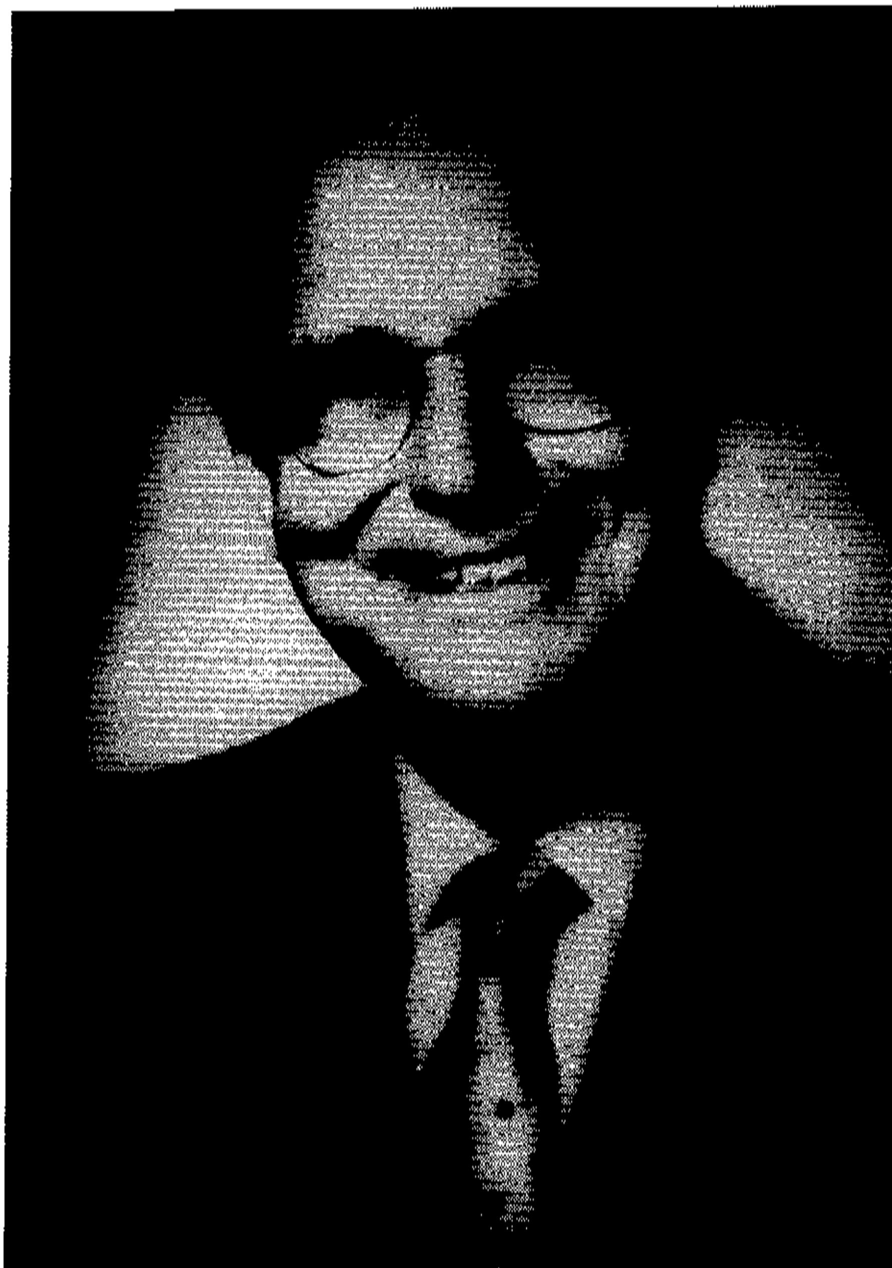
Dr. Rusch was preceded in death by his daughter, Judith Ann Tyler, in 1976, and by his first wife, Lenore Robinson Rusch, in 1978. He is survived by his wife, Louise Turner Van Wart Rusch; his daughter, Carolyn Elizabeth (Mrs. George) Schlotthauer; two grandchildren, Kristina and William Schlotthauer; a brother, William Rusch; and two nieces. Memorial contributions may be

made to the McArdle Laboratory for Cancer Research, University of Wisconsin-Madison, or to the Wisconsin Clinical Cancer Center, University of Wisconsin-Madison.

Braun, R., Mittermayer, C., and Rusch, H. P. (1965) Sequential temporal replication of DNA in *Physarum polycephalum*. Proc. Natl. Acad. Sci. U.S.A. *53:* 924-931.

Daniel, J. W., and Rusch, H. P. (1961) The pure culture of *Physarum polycephalum* on a partially defined soluble medium. J. Gen. Microbiol. *25:* 47-59.

Rusch, H. P., Sachsenmaier, W., Behrens, K., and Gruter, V. (1966) Synchronization of mitosis by the fusion of the plasmodia of *Physarum polycephalum*. J. Cell. Biol. *31:* 204-209.

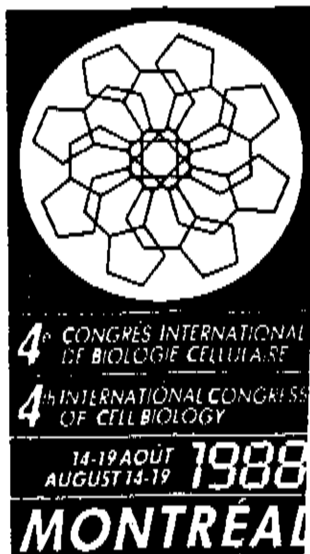


ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS
Vol. 261, No. 1, February 15, pp. 205–211, 1988

Superoxide Dismutase Induces Differentiation in Microplasmodia of the Slime Mold *Physarum polycephalum*

R. G. ALLEN,*,[1] ARTHUR K. BALIN,* R. J. REIMER,* R. S. SOHAL,† AND CLAUDE NATIONS†

Evidence is presented that supports a role for the enzyme superoxide dismutase (SOD) in the differentiation of the slime mold, *Physarum polycephalum*. SOD activity increases 46-fold during differentiation. A strain of *Physarum* that does not differentiate exhibits no change in SOD activity. Addition of SOD, via liposomes, to the nondifferentiating strain induces differentiation; this effect is enhanced by an inhibitor of glutathione synthesis. Other antioxidants selected for study failed to induce differentiation. Conversely, oxidative treatments including introduction of D-amino acid oxidase, via liposomes, induced differentiation. Cellular oxidation is the probable cause of the SOD effect. © 1988 Academic Press, Inc.



ELECTRON MICROSCOPIC AND MICROPROBE ANALYSES OF CALCIUM-INDUCED DIFFERENTIATION OF WHITE (LU887XLU897) AND YELLOW (LU863XLU897) MICROPLASMODIA OF *PHYSARUM POLYCEPHALUM*.

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A recent study has shown that the addition of 54mM $CaCl_2$ to microplasmodial cultures of *Physarum polycephalum* will induce the organism to differentiate into clusters of spherules (Nations *et al.*, *J. Cell. Physiol.* 133:186, 1987). The effects of this treatment were first observed for a yellow strain that readily spherulates in response to starvation; however, the $CaCl_2$ treatment was subsequently found to induce the spherulation of a white strain that had not previously been observed to spherulate. The present study reveals that the white strain will spherulate in the salts-only medium if the $CaCl_2$ content of its nutrient medium is increased by 4mM. Electron microscopic and microprobe analyses of the 0-, 6-, 30-, and 72-hour stages of differentiation reveal that: a) the white strain begins differentiation as early as 30 hours in salts with spherule formation occurring within 72 hours; b) differentiation in the white strain is not as synchronized as in the yellow strain; c) the absence of calcium in media for both strains prevents differentiation. Evidence is presented for the role of mitochondria in the sequestering and release of calcium and a unique cytoplasmic inclusion resembling contractile protein is described.

The EMBO Journal vol.6 no.13 pp.4149–4157, 1987

The F-actin capping proteins of *Physarum polycephalum*: cap42(a) is very similar, if not identical, to fragmin and is structurally and functionally very homologous to gelsolin; cap42(b) is *Physarum* actin

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Communicated by M. van Montagu

We have carried out a primary structure analysis of the F-actin capping proteins of *Physarum polycephalum*. Cap42(b) was completely sequenced and was found to be identical with *Physarum* actin. Approximately 88% of the sequence of cap42(a) was determined. Cap42(a) and fragmin were found to be identical by amino acid composition, isoelectric point, mol. wt, elution time on reversed-phase chromatography and amino acid sequence of their tryptic peptides. The available sequence of cap42(a) is >36% homologous with the NH_2-terminal 42-kd domain of human gelsolin. A highly homologous region of 16 amino acids is also shared between cap42(a), gelsolin and the *Acanthamoeba* profilins. Cap42(a) binds two actin molecules in a similar way to gelsolin suggesting a mechanism of F-actin modulation that has been conserved during evolution.

BIOCHEMICAL SOCIETY TRANSACTIONS Vol. 15, No. 5, 1987, pp. 844–845

Expression of cell-type-specific mRNAs in developmental mutants of *Physarum*

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In apogamic strains of *Physarum polycephalum*, uninucleate amoebae differentiate into large multinucleate plasmodia; neither cell fusion nor nuclear fusion is involved (Anderson *et al.*, 1976), so that amoebae and plasmodia are genetically identical. At first, enlarged uninucleate cells arise which are irreversibly committed to plasmodium formation (Burland *et al.*, 1981). These cells then undergo rounds of nuclear division, without cytoplasmic division, eventually giving rise to plasmodia.

Changes in gene expression during plasmodium formation can be studied using cell-type-specific cDNA clones (Sweeney *et al.*, 1987). This approach can be combined with the isolation and characterization of mutant strains unable to complete normal development. Here we describe two newly isolated developmental mutants and present preliminary data on the expression of developmentally regulated genes in these mutants.

Apogamic amoebae were mutagenized and screened for mutants unable to form plasmodia (R. W. Anderson, unpublished work). The first mutant we will describe is designated RA612. Time-lapse filming reveals that early development in RA612 is similar to that in its apogamic parent, but instead of developing into mature plasmodia, abnormal multinucleate cells accumulate, with defects in motility, fusion and cytoplasmic streaming. Northern blots were carried out with total RNA from amoebae, uninucleate committed cells and developmentally arrested multinucleate cells of RA612. RNA from plasmodia of the apogamic strain CL was used as a control. Uninucleate committed cells were isolated as described by Blindt *et al.* (1986). Northern blotting with cell-type-specific cDNA probes was as described by Sweeney *et al.* (1987).

The results of the Northern blots are shown in Fig. 1. As expected a probe prepared from the constitutively expressed clone P210 hybridized to an mRNA present in all cell types. The early-activating plasmodial-specific clones P46 and P325 detected mRNA species absent in amoebae of RA612, but present in uninucleate committed cells and multinucleate cells. P46 hybridized much less strongly to RA612 RNA than to CL plasmodial RNA. In CL, mRNA homologous to P46 is undetectable in amoebae, but present in developing cell types, though at a lower concentration than in mature plasmodia (Sweeney *et al.*, 1987). The late-activating plasmodial-specific probe P57 hybridized only to RNA from CL plasmodia. The amoebal-specific probes A195 and A273 hybridized strongly to RNA from RA612 amoebae and weakly to RNA from RA612 committed cells and plasmodia. In CL these probes hybridize weakly to RNA from developing cell types, but not to RNA from plasmodia. For each probe, therefore, the result for RA612 multinucleate cells is the same as given by intermediate cell types of CL. This agrees with the filming analysis in suggesting that early differentiation is not markedly abnormal, and that the terminal phenotype of the multinucleate cells resembles a late stage of development.

Time-lapse filming of a second mutant, RA614, shows that amoebae give rise to enlarged uninucleate cells resembling those that form in CL. The committed-like cells enlarge, but do not undergo nuclear division. Northern blotting data from RA614 are shown in Fig. 1. As expected, P210 hybridized to an RNA species present in all cell types. The results with the early-activating plasmodial-specific probes were conflicting: P325 hybridized to a message present in enlarged cells but not in amoebae, while P46 failed to detect any homologous RNA in either mutant cell type. Although A195 hybridizes strongly only to amoebal RNA of CL, the concentration of RNA homologous to A195 was similar in amoebae and in enlarged cells of RA614. The pattern of results for RA614 is thus rather complex, but suggests an earlier developmental arrest than that of RA612.

Protoplasma (1987) 141: 101–111

Growth and Development in Relation to the Cell Cycle in *Physarum polycephalum*

JULIET BAILEY, R. W. ANDERSON[1], and JENNIFER DEE*

Summary

In strain CL of *Physarum polycephalum*, multinucleate, haploid plasmodia form within clones of uninucleate, haploid amoebae. Analysis of plasmodium development, using time-lapse cinematography, shows that binucleate cells arise from uninucleate cells, by mitosis without cytokinesis. Either one or both daughter cells, from an apparently normal amoebal division, can enter an extended cell cycle (28.7 hours compared to the 11.8 hours for vegetative amoebae) that ends in the formation of a binucleate cell. This long cycle is accompanied by extra growth; cells that become binucleate are twice as big as amoebae at the time of mitosis. Nuclear size also increases during the extended cell cycle: flow cytometric analysis indicates that this is not associated with an increase over the haploid DNA content. During the extended cell cycle uninucleate cells lose the ability to transform into flagellated cells and also become irreversibly committed to plasmodium development. It is shown that commitment occurs a maximum of 13.5 hours before binucleate cell formation and that loss of ability to flagellate precedes commitment by 3–5 hours. Plasmodia develop from binucleate cells by cell fusions and synchronous mitoses without cytokinesis.

Gene, 59 (1987) 265–277

Gene families encode the major encystment-specific proteins of *Physarum polycephalum* plasmodia

François Bernier[a], Gérald Lemieux[a] and Dominick Pallotta[b]

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SUMMARY

The encystment of *Physarum polycephalum* plasmodia, also called spherulation, involves the synthesis of many specific mRNAs and proteins. Most of these molecules accumulate at the onset of the major morphological and physiological changes typical of this differentiation pathway and are not present during the other two transitions leading to dormancy in *Physarum*, namely sporulation and encystment of amoebae. The nucleotide sequences of apparently full-length cDNA copies of the four major encystment-specific mRNAs were determined. The four sequences included the entire coding regions and at least 26 nucleotides of the 5′-nontranscribed leaders. The encoded proteins were named spherulins. We found that spherulins 1a and 1b are 81% homologous and are thus members of a gene family. They both possess putative signal peptides and *N*-glycosylation sites, suggesting that they are cell-wall glycoproteins. Spherulin 2a and spherulin 3a are non-homologous proteins. The absence of signal peptides suggests that they are intracellular structural proteins. Low-stringency Southern hybridizations showed that each also belongs to a two-member gene family.

Curr Genet (1988) 13:151–157

Different developmental programs for amoebal and plasmodial encystment in *Physarum polycephalum*

François Binette[1], Dominick Pallotta[1], and Gérald Lemieux[2]

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Summary. Amoebal and plasmodial encystment (spherulation) of *Physarum polycephalum* were compared. Encystment of amoebae was induced by the addition of glucose to a dense culture. A change in cellular proteins during the amoeba to microcyst transition was demonstrated by SDS-polyacrylamide gel electrophoresis. We also observed that the encystment of amoebae did not involve the accumulation of the proteins which accompany plasmodial encystment. A cDNA library was constructed from poly(A)$^+$ RNA of encysted amoebae and was screened by differential hybridization. Two different mRNAs, specific to mature microcysts were identified. These mRNAs were not found in encysted plasmodia. Likewise, four encysted plasmodial mRNAs were absent from microcysts. These results show that different developmental programs are used for amoebal and plasmodial encystment.

Cell Biology International Reports, Vol. 12, No. 1, January 1988 29

Cyanide-resistant respiration of Physarum polycephalum in course of starvation

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Abstract

The inhibition of respiration of Physarum polycephalum microplasmodia by KCN varies between 30 % and 70% of initial rate of O_2 uptake, depending on the time of starvation. The kinetics of the development of cyanide-resistant respiration and its sensitivity toward salicylhydroxamic acid (SHAM) point out that CN-resistant respiration represents the activity of the alternative pathway of the electron transport. There is no evidence that during starvation the alternative pathway of respiration is active in the absence of cyanide.

Cell Biology International Reports, Vol. 11, No. 11, November 1987 803

CHEMICALLY INDUCED CHANGES IN THE MORPHOLOGY, DYNAMIC ACTIVITY AND CYTOSKELETAL ORGANIZATION OF PHYSARUM CELL FRAGMENTS

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ABSTRACT

Spherical cell fragments derived from Physarum polycephalum by caffeine-treatment were used as an experimental system to investigate the influence of 15 externally applied substances on the general morphology, motile behavior and cytoskeletal organization of the acellular slime mold. In comparison to controls, the most obvious changes observed after chemical stimulation proved to be cytokinetic activities, ameboid-like movement phenomena, intense cell surface dynamics and formation of cytoplasmic actin fibrils. The results demonstrate the high adaptability of the microfilament system in Physarum even when subjected to extrem conditions in the external environment.

MOLECULAR AND CELLULAR BIOLOGY, Mar. 1988, p. 1275–1281 Vol. 8, No. 3

A Gene Encoding the Major Beta Tubulin of the Mitotic Spindle in *Physarum polycephalum* Plasmodia

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The multinucleate plasmodium of *Physarum polycephalum* is unusual among eucaryotic cells in that it uses tubulins only in mitotic-spindle microtubules; cytoskeletal, flagellar, and centriolar microtubules are absent in this cell type. We have identified a β-tubulin cDNA clone, β105, which is shown to correspond to the transcript of the *betC* β-tubulin locus and to encode β2 tubulin, the β tubulin expressed specifically in the plasmodium and used exclusively in the mitotic spindle. *Physarum* amoebae utilize tubulins in the cytoskeleton, centrioles, and flagella, in addition to the mitotic spindle. Sequence analysis shows that β2 tubulin is only 83% identical to the two β tubulins expressed in amoebae. This compares with 70 to 83% identity between *Physarum* β2 tubulin and the β tubulins of yeasts, fungi, alga, trypanosome, fruit fly, chicken, and mouse. On the other hand, *Physarum* β2 tubulin is no more similar to, for example, *Aspergillus* β tubulins than it is to those of *Drosophila melanogaster* or mammals. Several eucaryotes express at least one widely diverged β tubulin as well as one or more β tubulins that conform more closely to a consensus β-tubulin sequence. We suggest that β-tubulins diverge more when their expression pattern is restricted, especially when this restriction results in their use in fewer functions. This divergence among β tubulins could have resulted through neutral drift. For example, exclusive use of *Physarum* β2 tubulin in the spindle may have allowed more amino acid substitutions than would be functionally tolerable in the β tubulins that are utilized in multiple microtubular organelles. Alternatively, restricted use of β tubulins may allow positive selection to operate more freely to refine β-tubulin function.

MYCOTAXON

Vol. XXX, p. 197 October-December 1987

MYXOMYCETES FROM THAILAND - II

B.ING

Department of Biology, Chester College of Higher Education, Cheyney Road, Chester CH1 4BJ, U.K.

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Department of Biology, Chiang Mai University, Chiang Mai, Thailand

The most recent account of myxomycetes in Thailand (Siwasin & Ing, 1982) listed 34 species, mainly from the northern hill forests. This added sixteen species to those recorded by Reynolds & Alexopoulos (1971.) In June 1981 S.Samarnpan collected seventeen species in dipterocarp forest at Nakhornnayok, north-east of Bangkok. None are new to Thailand but represent a cross-section of species found in tropical rain-forest. Specimens were determined by B.Ing and are housed in the herbarium at Chiang Mai and in Hb.B.Ing. In the list that follows N signifies a species previously found in the northern hill forest and S signifies a species previously found in rain-forest or other lowland sites.

Arcyria cinerea (Bull.)Pers. N,S.
A. denudata (L.)Wettst. N,S.
Collaria arcyrionema (Rost.)Nann.-Brem. N,S.
Cribraria cancellata (Batsch)Nann.-Brem. N,S.
C. microcarpa (Schrad.)Pers. S.
Didymium squamulosum (Alb. & Schw.)Fr. N,S.
Hemitrichia calyculata (Speg.)Farr N,S.
Physarella oblonga (Berk. & Curt.)Morg. S, including specimens of f. alba Alexop.
Physarum flavicomum Berk. N,S.
P. melleum (Berk. & Br.)Massee S.
P. nutans Pers. N,S.
P. stellatum (Massee)Martin N.
P. tenerum Rex S.
P. viride (Bull.)Pers. S.
Stemonitis smithii Macbr. N.
S. splendens Rost. N,S.
Stemonitopsis typhina (Wiggers)Nann.-Brem. S.

Journal of Cell Biology

Vol. 105, No. 4 Part 2, 1987, p. 68A

375 Immunoprecipitation of Ribonucleoprotein Complexes Containing the Conserved Nucleolar Protein, B-36. M.E. Christensen, K.E. Fuxa, and M.A. Junio, Department of Biology, Texas A & M University, College Station, Texas 77843.

B-36 is a major, highly-conserved protein which is localized exclusively in the fibrillar region of the nucleolus of eukaryotic cells. B-36, a 34 kD basic protein rich in glycine and dimethylarginine, shares many features with structural proteins which organize mRNA precursors into RNP particles (ie. hnRNP proteins A1, A2, and B1). Since B-36 is nucleolar, it has been suggested that B-36 may have an analogous role, but in association with newly-synthesized rRNA precursors. In support of this idea we have previously demonstrated that B-36 can be extracted from isolated nuclei of Physarum polycephalum as the major protein component of a heterodisperse set of RNP complexes. In order to characterize the RNA component, gradient-separated complexes have been immunoprecipitated using protein A-sepharose beads to which B-36 polyclonal antibodies are bound. Immunoprecipitated RNA is enriched 15-fold for pulse-labeled RNA as compared to that of control precipitates. Analysis for the presence of specific pre-rRNA sequences has been done by hybridizing Northern transfers with cloned probes spanning the transcribed portion of the Physarum ribosomal genes. Results indicate that the immunoprecipitated RNA contains rRNA sequences and is enriched for a sequence within the External Transcribed Spacer region upstream of the 5' end of the mature 19S rRNA. Hybridization with more restricted probes is currently being done to determine the specificity of the association of B-36 with the rRNA precursor. Supported by NSF Grant #DCB-8417078.

Biochimica et Biophysica Acta 910 (1987) 111–115

A charged contaminant prevents *Physarum* chromatin from aggregating

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Unlike typical chromatins, the soluble *Physarum* chromatin can not be precipitated by salt. Using the reconstituted system based on the H1/H5-depleted chicken erythrocyte chromatin, we demonstrate that this feature is not due to inability of *Physarum* histone H1 to aggregate chromatin. We show that the abnormal behavior of *Physarum* chromatin can be explained by its association with charged contaminant.

Journal of General Microbiology (1987), 133, 3175–3182.

Polymorphism and Uniparental Inheritance of Mitochondrial DNA in *Physarum polycephalum*

By S. KAWANO,[1]* R. W. ANDERSON,[2] T. NANBA[1] AND T. KUROIWA[1]

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[2] *Department of Genetics, University of Sheffield, Sheffield S10 2TN, UK*

Restriction endonuclease analysis was done on mitochondrial DNA (mtDNA) from 19 plasmodial strains of *Physarum polycephalum*. The extent of mtDNA variation among these strains was high in comparison with other organisms, and provides a useful source of cytoplasmic genetic markers. The strains were classified into seven groups according to their mtDNA types. Although plasmodia of *P. polycephalum* are diploid, formed by fusion of amoebal isogametes, each of the 19 plasmodia possessed mtDNA of only a single type. The transmission pattern of mtDNA during plasmodium formation was studied by mating pairs of amoebal strains that contained mtDNA of different types. Transmission was uniparental; the plasmodia that were formed carried mtDNA with the restriction pattern of only one of the two parental types. Since diploid zygotes develop into plasmodia by repeated mitotic cycles in the absence of cell division, it is clear that this uniparental transmission of mtDNA does not depend upon random partitioning either of mitochondria or of mtDNA molecules during cell division.

THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 263, No. 1, Issue of January 5, pp. 305–313, 1988

Amino Acid Sequence of the Calcium-binding Light Chain of Myosin from the Lower Eukaryote, *Physarum polycephalum**

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We have established a new method for preparing *Physarum* myosin whose actin-activated ATPase activity is inhibited by micromolar levels of Ca^{2+}. This Ca^{2+}-inhibition is mediated by the Ca^{2+} binding to the myosin rather than by the Ca^{2+}-dependent modification of the phosphorylated state of the myosin (Kohama, K., and Kendrick-Jones, J. (1986) *J. Biochem. (Tokyo)* 99, 1433–1446). Ca^{2+}-binding light chain (CaLC) has been suggested to be of primary importance in this Ca^{2+} inhibition (Kohama, K., Takano-Ohmuro, H., Tanaka, T., Yamaguchi, T., and Kohama, T. (1986) *J. Biol. Chem.* 261, 8022–8027).

The amino acid sequence of CaLC was determined; it was composed of 147 amino acid residues and the N terminus was acetylated. The molecular weight was calculated to be 16,131. The homology of CaLC in the amino acid sequence with 5,5′-dithiobis-(2-nitrobenzoic acid) light chain and alkali light chain of skeletal muscle myosin were rather low, *i.e.*, 25% and 30%, respectively. Interestingly, however, the CaLC sequence was 40% homologous with brain calmodulin.

This amino acid sequence was confirmed by sequencing the cloned phage DNA accommodating cDNA coding CaLC. Northern and Southern blot analysis indicated that 0.8-kilobase pair mRNA was transcribed from a single CaLC gene.

This is the first report on the amino acid sequence of myosin light chain of lower eukaryotes and nucleotide sequence of its mRNA.

Development, Growth and Differentiation, Vol. 29, No. 4, 1987, p. 394

cDNA of Ca-binding light chain of *Physarum* myosins.

K. Kohama*, M. Kawaguchi, and Y. Hamada. Dept. of Pharmacology, Fac. of Medicine, Univ. of Tokyo, Tokyo and National Inst. for Basic Biology, Okazaki.

Amoebal and plasmodial stages in *Physarum* life cycle have Ca-sentitive, stage specific myosins. However characterization on the basis of protein analysis revealed that Ca-binding light chain (CaLc) are common to both myosins, [Kohama et al. J. Biol. Chem. 261, 8022, (1986)]

cDNA bank with a phage vector λgt 11 was constructed from poly(A)$^+$RNA prepared from the plasmodia. cDNA coding CaLc (λCaLc1) was screened by a probe of oligonucleotide synthesized according to C-terminal portion of amino acid sequence of CaLc (Kobayashi et al. Seikagaku 28, 972, Abstr.)

Although λCaLc1 lacked 5' untranslated sequence and the sequence coding the N-terminal amino acid (Thr), it comprised of nucleotides coding Res.2-Res. 147 of amino acid sequence followed by 3' untranslated sequence. Northern blots analysis showed that the λCaLc1 hybridized at 0.8Kb with both amoebal and plasmidial poly(A)$^+$RNA. Southern bolts of the restricted genomic DNA of *Physarum* hybridized with λCaLc1 at one bands for each restriction enzymes. We conclude that mRNA of CaLc is transcribed from a single copy gene in both amoebal and plasmidial cells. Supported by grants from Uehara Memorial Foundation and from the Ministry of Education Science and Culture, Japan.

J. Biochem. 102, 947–956 (1987)

A 250K-Molecular-Weight Actin-Binding Protein from Actin-Based Gels Formed in Sea Urchin Egg Cytoplasmic Extract[1]

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The actin-based gel formed at 35°C in the cytoplasmic extract from eggs of a sea urchin, *Tripneustes gratilla*, contains several high-molecular-weight proteins. Among them, the 250K-molecular-weight protein was isolated and characterized. This protein migrated slightly more slowly than filamin from chicken gizzard upon polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. It reacted only very weakly with antibodies against chicken gizzard filamin or against a high-molecular-weight actin-binding protein from *Physarum* plasmodia. It did not react with antibodies against chicken erythrocyte alpha-spectrin nor against the 220K protein from the same egg. A chemical crosslinking experiment revealed the presence of dimers in the purified 250K protein preparation. A rotary shadowed specimen of such a preparation showed wavy single-stranded molecules 120–170 nm long, having five to six globular domains, which may represent dimers. The appearance was different from that of spectrin or actin-binding protein from macrophage or chicken gizzard filamin. This protein increased the viscosity of F-actin solution. It bound to F-actin preferably at low KCl concentrations such as 20 mM. The binding ability was not influenced by pH between 6.0 and 7.5, although it was somewhat reduced above pH 8.0. The binding was insensitive to low Ca ion concentrations. Electron microscopy using the negative staining technique supported the idea that this protein crosslinks actin filaments. In addition, a second protein from egg gels, with a reported molecular weight of about 220K (Kane, R.E., *J. Cell Biol.* 66, 305–315 (1975)), comigrated with human erythrocyte alpha-spectrin on an SDS-gel and reacted with antibodies against chicken erythrocyte alpha-spectrin. This suggests that this protein is a sea urchin egg spectrin. The role of these proteins in the cytoskeleton formation in the sea urchin egg is discussed.

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Towards an understanding of the biological function of histone acetylation

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A model is presented which explains the biological function of posttranslational acetylation of core histones in chromatin. Along the lines of this model histone acetylation serves as a general mechanism to destabilize nucleosome core particles during various processes occurring in chromatin. Acetylation acts as a signal that modulates histone-protein and histone-DNA interactions and finally leads to the displacement of particular histones from nucleosome cores. The high specificity of the acetylation signal for different processes (DNA replication, transcription, differentiation-specific histone replacement) is achieved by site specificity and asymmetry of acetylation in nucleosomes. The essential features of this model are in accord with the more recent results on histone acetylation.

Biology of the Cell, Vol. 60 No. 2. 1987. Pgs. 87-96.

Ultrastructural investigation of the effects of supraoptimal temperature on late interphase nuclei of *Physarum polycephalum* plasmodia

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When *Physarum polycephalum* plasmodia in the G2 period were subjected to a temperature shift from 25° to 33.8° C, they continued to grow for about 70 hr without entering mitosis. During this period the nuclei became approximately twice as large as before. After about 17 hr, the nucleoplasm took on a coarser texture and 2 major types of nucleoli were observed. Some nucleoli exhibited vacuoles of various sizes consisting mostly of granules that, from labelling experiments, were shown to contain RNA. Certain of these vacuoles also showed patches of dense material that reacted with the DNase-gold complex and, therefore, contained DNA. In the course of this temperature shift the fibrillar regions migrated to the outer portion of the nucleolus and interchromatin-like granules appeared within both this organelle and the nucleoplasm. A number of these morphologic changes closely mimicked, but on a smaller scale, lesions induced by heat shocks as well as by various other stress conditions.

Curr Genet (1988) 13:71–74

Transient expression of a chloramphenicol acetyltransferase gene following transfection of *Physarum polycephalum* myxamoebae

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Summary. A plasmid was constructed containing a replication origin sequence from the *Physarum* ribosomal DNA molecule, and a bacterial chloramphenicol acetyltransferase (CAT) gene linked to a putative promoter of the long terminal repeat (LTR) of the *Physarum* "HpaII-repeat" element. The plasmid was transfected into *Physarum* myxamoebae either by electroporation or $CaCl_2$ treatment. In both cases significant transient levels of CAT gene expression were detected. Results were compared with those obtained with plasmids in which CAT gene expression was driven by eukaryotic virus promoters.

Biology of the Cell, Vol. 60 (1987)

CARACTERISATION BIOCHIMIQUE ET IMMUNOLOGIQUE D'UNE PROTEINE CYTOSQUELETTIQUE DE 45 KD CHEZ LE DINOFLAGELLE NOCTILU[illegible] SCINTILLANS : COMPARAISON AVEC L'ACTINE MUSCULAIRE ET L'ACTINE NON MUSCULAIRE (PHYSARUM).

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L'analyse électrophorétique unidimensionnelle des protéines du cytosquelette de N. scintillans préparées en pré[illegible] détergent non ionique (0,1 % Triton X-100) révèle l'existence de 3 bandes majeures de poids moléculaire : 220, 95 et 4[illegible] L'observation de ces cytosquelettes en M.E. montre qu'ils sont formés d'un épiplasme fibrillo-granulaire périphérique e[illegible] filaments organisés en myonèmes striés (1).
Du fait de sa similitude de poids moléculaire avec l'actine, nous avons isolé cette protéine de 45 KD par électropho[illegible] préparative en condition dénaturante, en présence d'urée 7M selon la méthode de Huiatt et al. (2) et l'avons comparée à[illegible] l'actine musculaire et non musculaire (Physarum) (3). En électrophorèse bidimensionnelle, la protéine 45 KD se présente sous f[illegible] d'une protéine acide composée de trois isoformes (β, γ, δ) de PI : 5.2-5.5. Ses caractéristiques électrophorétiques (PM et PI)[illegible] sont avérées identiques à celles des actines musculaire et non musculaire, lorsque ces deux types d'actine ont été ajoutés co[illegible] marqueurs internes dans les électrophorèses bidimensionnelles.
Après digestion enzymatique par la protéase V8 de S. aureus, la comparaison entre la protéine 45 KD et les deux actines montre [illegible] faible analogie de sites de coupures induisant uniquement la formation d'un polypeptide de 16 KD en commun, ce qui ne permet [illegible] de conclure que la protéine de 45 KD soit de type actine musculaire ou de type actine de Physarum.
Ces résultats ont été confirmés par les tests biochimiques et immunologiques suivants : - Absence d'inhibition de l'activité D[illegible] I par la protéine 45 KD dans les extraits cellulaires de N. scintillans, - Réaction négative aux tests croisés réalisés/[illegible] immunoblotting avec les anticorps préparés contre la protéine de 45 KD de N. scintillans et les anticorps anti-actine musculai[illegible] - Absence de décoration avec la HMM des cellules de N. scintillans traitées au glycérol ou des cytosquelettes isolés, - Enf[illegible] l'anticorps anti 45 KD ne reconnait pas l'actine de Physarum.
La préparation d'anticorps polyclonaux contre la protéine 45 KD nous a permis de la localiser par immunoélectromarquage ap[illegible] couplage à l'or colloïdal au niveau de l'épiplasme périphérique de la noctiluque. Réfs.: Métivier C. et al. 1987, soumis (2) Huiatt T.W. et al., J. Biol. Chem. 255, 6981-6989, 1980 (3) Pahlic M., Eur. J. Cell Biol. 36, 169-177, 1985.

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Review of Biological Research in Aging 3:41-50 (1987)

Aging of Fungi

Kenneth D. Munkres

INTRODUCTION

This review summarizes recent advances in research on aging of fungi: the Myxomycete slime molds and the Ascomycetes *Podospora* and *Neurospora*.

In recent years, biogerontologists have attempted to integrate various theories about the mechanisms of aging. Aging may be understood within the broad concepts of differentiation or "dysdifferentiation." Such an approach can reconcile seemingly disparate stochastic "wear and tear" and genetic program theories. Accordingly, unlike previous reviews in this series, this one includes selected, recent advances in studies of fungal differentiation that appear to be especially relevant to fungal aging.

The Journal of Biological Chemistry

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Expression of Poriferasterol Monoglucoside Associated with Differentiation of *Physarum polycephalum**

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A glycolipid which was expressed during a differentiation from haploid myxoamoebae to diploid plasmodia of a true slime mold, *Physarum polycephalum*, has been examined. In the amoeboid stage, cells did not contain this glycolipid, but after conjugation of the haploid cells, this substance appeared and increased in its amount.

From structural studies of the purified glycolipid, it has been identified as poriferasterol monoglucoside.

Superoxide Dismutase Activity and Glutathione Concentration During the Calcium-Induced Differentiation of *Physarum polycephalum* Microplasmodia

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

Cytologia 52: 599–614, 1987

Light and Electron Microscopic Observations of Mitochondrial Fusion in Plasmodia Induced Sporulation in *Physarum polycephalum*

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The growing plasmodium of *Physarum polycephalum* provides a system for the investigation of mitochondrial division. In *Physarum polycephalum* mitochondrial division consists of two main processes, mitochondrial kinesis and mitochondrial nuclear division (Kuroiwa *et al.* 1977). Mitochondrial division is semi-synchronized in the plasmodium (Kuroiwa *et al.* 1978) Light and thin-sectioned electron microscopic observations have shown the presence of a mitochondrial nucleus (mt-nucleus) situated in the central portion of the inner matrix of *P. polycepahlum* mitochondria throughout the mitochondrial division cycle (Guttes *et al.* 1967, Kuroiwa 1973, Kuroiwa *et al.* 1977). The mt-nucleus elongates longitudinally with the growth of mitochondria. Just before mitochondrial division, the mt-nucleus divides by constriction of the limiting membrane of the dividing mitochondria and is distributed almost equally to the daughter mitochondria. The mt-DNA content per mitochondrial nucleus is estimated to be about 32 molecules, which are linear and have molecular weights of 45×10^6 D per mitochondrion in spherical mitochondria (Kawano *et al.* 1982). This is contrast to the finding that during spherulation in *P. polycephalum* mitochondria and mitochondrial nuclei become smaller (Kawano *et al.* 1983). Diminutive mitochondria contain very small particle-shaped mitochondrial nuclei and the amount of DNA, RNA and protein per mitochondrion decreases remarkably with spherulation (Kawano *et al.* 1983). Thus, in *P. polycephalum* mitochondrial differentiation to inactive state (spherulation) was known to be characterized by a decrease not only in dimension but also in DNA, RNA and protein content.

Very little information was available, however, concerning the behavior of mitochondria and mt-nuclei during sporulation. The present paper described the behavior of cell nuclei, mitochondria and mt-nuclei during sporulation, during which starved plasmodia differentiate into sporangia.

Volume 16 Number 3 1988 Nucleic Acids Research

Primary and hypothetical secondary structure of *Drosophila melanogaster* U6 RNA

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Submitted December 29, 1987

Drosophila melanogaster flies were used to isolate and sequence the U6 small nuclear RNA (1,2,3). The hypothetical secondary structure was determined by computer folding (4) and multiple sequence alignments (5).

Primary structure:

```
                 1          10         20         30
             5' NpppGUUCUUGCU UCGGCAGAAC AUAUACUAAA
      40               50          60          70          80
 AYUGGAACGA YAC m6AGAGAmAG AUUAmGmCAUGG CmCCmCAGCGCmA AGGAUGACmAC
      90         100        108
 GCAAAAYCGU GAAGCGUUCC ACAUUUUU(OH) 3'
```

Hypothetical secondary structure:

A comparison of the nucleotide sequence of Drosophila U6 RNA with that of rat (6) reveals a homology of 90.7%. Of the 10 differences, 4 represent sugar and base modifications (positions 64, 69, 71, 73) while 6 are nucleotide replacements (positions 4, 6,18,65,86,102). Multiple alignements show that the nucleotide sequences of broad bean (7) and Physarum polycephalum U6 snRNA (8) have much weaker homologies than those described above. Interestingly, in domains where the U6 sequences show poor homology, the secondary structures seem to be highly conserved. Detailed computer studies are available from the authors or from Ch. Nager, Bio-Informatics, Friedrich Miescher Institut, 4002 Basel, Switzerland.

Journal of Cell Biology, Vol. 105, No. 4 Part 2, 1987, p. 69A

Construction and Screening of a Genomic Library for U6 Small RNA Genes in the Myxomycete *Physarum polycephalum*. H.B. Skinner and D.S. Adams, Worcester Polytechnic Institute, Worcester, Massachusetts 01609.

Our laboratory previously identified, characterized and sequenced U6 small RNA from the vegetative plasmodia of the myxomycete *Physarum polycephalum* (Skinner and Adams, 1987, Nucl. Acids Res. 15: 371). Here, we report the construction of a partial genomic library in the lambda phage gt10 from *EcoRI* digested genomic DNA. 2 x 10^6 plaques were obtained. We estimate that 2.1 x 10^4 plaques represent 1 genomic equivalent. Approximately 80,000 plaques were screened by *in situ* hybridization, using a reverse transcribed oligo primed U6 probe. 16 hybridization signals were detected from the primary screening, and 13 plaques were confirmed by rescreening. Southern analysis of *EcoRI* digested *Physarum* genomic DNA indicated two major U6 gene families, sized at approximately 6.7 kb and 10 kb. Since lambda gt10 can accomodate inserts up to 7 kb, the 10 kb family may be excluded. Hybridization analysis indicated a copy number of approximately 350 genes, for the U6 6.7 kb family, per haploid genome. One plaque (designated SPL2-B4) contained a 1 kb insert which may represent either a low or single copy number U6 family, or a truncated representitive of the 6.7 kb U6 gene family. Our laboratory is currently sequencing the screened clones.

Age, Vol. 10, No. 3, 1987, p. 112

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ROLE OF OXIDATIVE STRESS IN CELLULAR DIFFERENTIATION AND CELLULAR SENESCENCE. *R.S. Sohal*, Dept. of Biology, Southern Methodist University, Dallas, TX 75275, USA.

There are several lines of evidence which indicate that active oxygen species play a causal role in the induction of cellular differentiation and senescence. Our studies on the slime mold *Physarum polycephalum* and the housefly *Musca domestica* indicate that undifferentiated and rapidly dividing cells exhibit low levels of cyanide-insensitive respiration and superoxide dismutase activity, and high concentrations of reduced glutathione (GSH). During cellular differentiation rate of cyanide-insensitive respiration, SOD activity and peroxide concentration increase whereas GSH concentration decreases. Cellular differentiation in the slime mold can be induced or delayed by manipulations of oxidative stress. During cellular senescence, activity of antioxidant enzymes and GSH concentrations decline while products of oxygen-free radical reactions such as H_2O_2, GSSG, TBA-reactants, n-pentane exhalation, and lipofuscin increase in their levels. In addition, rate of antimycin A-insensitive respiration increases in old organisms. Overall, results of these studies suggest that cellular differentiation and senescence represent a continuum of progressive oxidative stress. It is postulated that effects of oxidative stress are expressed through changes in redox state and ion distribution which then affect the chromatin structure and pattern of gene expression.

BIOCHEMICAL SOCIETY TRANSACTIONS

Vol. 15, No. 5, 1987, pp. 899-900

Analysis of pre-rRNAs in total RNA from *Physarum*

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The plasmodial phase of the slime mould *Physarum polycephalum* exhibits natural mitotic synchrony, allowing the synthesis of macromolecules to be studied in relation to the nuclear division cycle (Turnock, 1979). The net production of rRNA rises smoothly during the cycle, increasing 5–6-fold between early S phase (there is no G1) and late G2 phase (Hall & Turnock, 1976). This increase in rate of synthesis could be indicative of control of the initiation of transcription from the transcription units for rRNAs. However, we have shown that rRNA transcripts constitute a constant fraction (about 40%) of total pulse-labelled RNA throughout the cycle (Hunter & Turnock, 1985). As one strategy for determining whether the post-transcriptional processing reactions are also subject to regulation, a method has been developed for measuring the relative proportions of the major pre-rRNAs.

A 233 bp DNA fragment from the spacer region (sequenced by Otsuka *et al.*, 1983) between the 5.8 S and 26 S rRNA genes was cloned into bacteriophage M13 (mp93). The recombinant was designated SII-6, and it has been used for the synthesis of radioactive DNA for detection of pre-rRNAs that retain the spacer region by RNA–DNA hybridization.

Total RNA (Hall & Turnock, 1976) was fractionated by electrophoresis in 1% (w/v) agarose gels containing formaldehyde (Maniatis *et al.*, 1982) and blotted onto Hybond-N (Amersham International). Radioactive SII-6 probe, labelled from [α-^{32}P]dCTP, was synthesized by the method of Farrell *et al.* (1983) and hybridized to the Northern blots in 6 × SSC and 50% (w/v) formamide at 42°C (Sweeney *et al.*, 1987). After autoradiography, tracks corresponding to individual RNA samples were cut into 3 mm transverse sections and the distribution of ^{32}P measured quantitatively by scintillation spectrometry.

Fig. 1 shows a profile of hybridization relative to the positions of the 19 S and 26 S rRNAs, detected by staining adjacent tracks of the gel with Methylene Blue. The hybrid peaks P1, P2 and P4 are estimated to correspond to the classes of pre-rRNAs described by Gubler *et al.* (1980). A faint band (P3?) between P2 and P4 could be seen on autoradiographs. P4, the immediate precursor to 26 S rRNA, is clearly the major species. According to Gubler *et al.* (1980), it still retains the 5.8 S rRNA and at least part of the sequence between the 19 S and 5.8 S rRNAs.

Peaks A, B and C must represent combinations of the spacer plus 19 S rRNA or subsections of the 26 S molecule. They could originate from molecules in which the sequence of endonucleolytic cleavages occurs in a different order to the predominant pattern, and which are made evident by the denaturing conditions used for the analysis. Peak A, for example is the size expected for a molecule of 19 S plus the sequence to the boundary of the 26 S rRNA. B and C could be combinations of the 5.8–26 S spacer and subsections of 26 S transiently available during removal of the two introns, while D is probably the spacer ± 5.8 S after cleavage from the 26 S molecule.

Preliminary measurements of the pre-rRNAs that hybridize to SII-6 in RNA prepared from plasmodia at different times during the mitotic cycle have shown an increase in P4, together with A, B, C and D, relative to P1 and P2 in the later part of the cycle by comparison with S phase. This might indicate a role for regulation of the conversion of P2 (P3) to P4 in the synthesis of mature rRNAs. The endonucleolytic cleavage 1.7 kb from the 5′ end of the primary transcript is unlikely to be rate limiting (Blum *et al.*, 1986), and Fig. 1 shows that the molecules that collectively make up P1 (Gulber *et al.*, 1980) constitute only a small fraction of the sum of the different precursors.

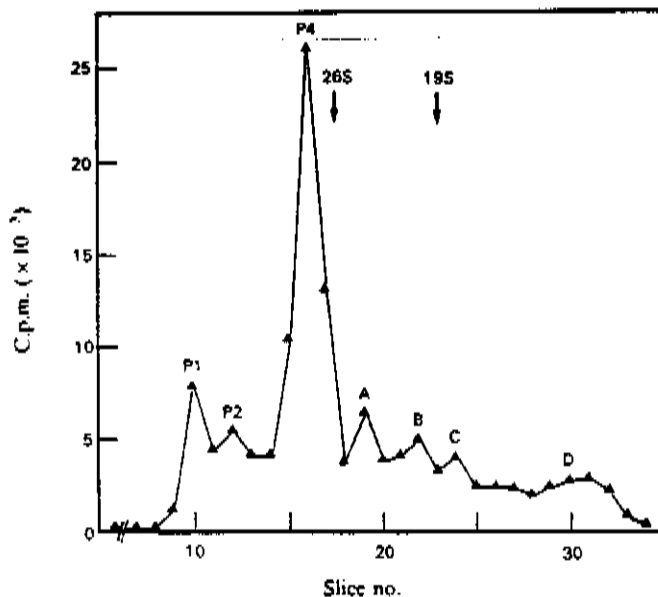

Fig. 1. *Hybridization of SII-6 DNA to total plasmodial RNA*

RNA was fractionated by electrophoresis in agarose in the presence of formaldehyde, blotted on to Hybond-N and hybridized to ^{32}P-labelled SII-6 DNA. Slices, 3 mm thick, along one track of the membrane were assayed for ^{32}P in a scintillation spectrometer. The positions of 19 S and 26 S rRNA are indicated.

Abbreviations used: bp, base pair; rRNA, ribosomal RNA; pre-rRNA, precursor to ribosomal RNA; SSC, 0.15 M-NaCl, 0.015 M-Na citrate, pH 7.0.

Protoplasma (1987) 141: 83–94

The Plasma Membrane of *Physarum* Cell Fragments: a Morphological and Electrophysiological Study

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Summary

Small, spherical cell fragments derived from macroplasmodia of the acellular slime mold *Physarum polycephalum* by incubation in a 15 mM caffeine solution were investigated with morphological and electrophysiological techniques. Analysis of cell surface composition with the fluorescence microscope and different RITC-conjugated lectins revealed strong binding of ConA and RCA-I, weak binding of PEA, DBA and WGA and no binding of UEA-I. In addition, binding sites for external calcium ions were detected by chlorotetracycline-fluorescence. Electron microscopical staining with ruthenium red, iron or lanthanum delivered evidence for localization of lectin and calcium binding sites in a thin mucous layer on the cell surface.

Electrical recordings by means of intracellular microelectrodes yielded an average membrane potential (MP) of −113 mV. Spontaneous depolarizations of the MP, with amplitudes between 10 and 80 mV and a duration of 20–30 s, failed to show a correlation with contractile activity. The ionic nature of MP was studied by varying the composition of the perfusing medium. The MP was not much affected by changes in external [Ca^{2+}], [K^+], or [Na^+] but was sensitive to changes in [Cl^-] or [H^+], with a linear dependence on pH_o in the range between 7 and 5. Metabolic inhibition by potassium cyanide or low temperature (11 °C) as well as application of the protonophore CCCP caused a depolarization of the MP. The results strongly support the hypothesis that the MP in *Physarum* cell fragments is mainly generated by an electrogenic H^+-extrusion mechanism.

Photochemistry and Photobiology Vol. 47, No. 2, pp. 271–275, 1988

CHANGES IN cAMP AND cGMP CONCENTRATION, BIREFRINGENT FIBRILS AND CONTRACTILE ACTIVITY ACCOMPANYING UV AND BLUE LIGHT PHOTOAVOIDANCE IN PLASMODIA OF AN ALBINO STRAIN OF *Physarum polycephalum*

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Abstract—Photoavoidance by plasmodia of an albino strain of *Physarum polycephalum* was studied. When the organism was irradiated locally, the protoplasm moved away from the irradiated region. The action spectrum for this avoidance showed three peaks at about 260, 370 and 460 nm. The organism was about one hundred times as sensitive to far UV as to near UV and blue light, and high intensity far-UV caused the gelation of the protoplasm. Irradiation with UV or blue light increased the mean level or the amplitude of oscillation in intracellular cAMP and cGMP concentrations. Upon UV irradiation, birefringent fibrils, presumably microfilaments of F-actin, became thick and numerous, and the plasmodial strand generated a strong tensile force. It is postulated that UV or blue light brings about an increased concentration of cyclic nucleotides which leads to an enhanced local development of contractile fibrils which squeeze protoplasmic sol from the area, resulting in photoavoidance.

Protoplasma (1987) 140: 190–192

ATP-induced Relative Movement Between Microfilaments and Microtubules in Myxomycete Flagellates

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Summary

Permeabilized flagellates of the myxomycete *Physarum polycephalum* were treated with ATP, and changes in the cytoskeletal organization were examined by fluorescence microscopy. The backbone structure in permeabilized flagellates consisted of a coaligned bundle of microfilaments and microtubules. Treatment of such permeabilized flagellates with ATP caused relative movement between the microtubules and the bundle of microfilaments, so that the microtubules and the microfilaments apparently slid apart. Similar movement was observed using the isolated backbone structures.

The Coleopterists Bulletin, 41(4):395-403, 1987.

A NEW SPECIES OF *AGATHIDIUM* ASSOCIATED WITH AN "EPIMYCETIC" SLIME MOLD PLASMODIUM ON *PLEUROTUS* FUNGI (COLEOPTERA: LEIODIDAE—MYXOMYCETES: PHYSARALES—BASIDIOMYCETES: TRICHOLOMATACEAE)

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ABSTRACT

Agathidium aristerium, new species (Coleoptera: Leiodidae), is described from upstate New York and reported to feed on the plasmodial stage of the slime mold *Physarum polycephalum* Schweinitz (Myxomycetes: Physarales) which in turn grows over the surface of the fleshy oyster mushroom *Pleurotus ostreatus* Fries (Basidiomycetes: Tricholomataceae). Coleoptera associated with *Physarum* are briefly reviewed, but this is only the second report of a predator of a plasmodium associated with a higher basidiomycete. Possible significance of this beetle/slime mold/fungus association is discussed.

Journal of Cell Biology, Vol. 4, No. 4 Part 2, 1987, p. 153A

Sequence and expression of an α-tubulin isotype from Physarum polycephalum that contains a coded C-terminal methionine.

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The complete nucleotide sequence of a second α-tubulin gene isolated from Physarum polycephalum has been determined. The gene forms part of the complex altB locus. The coding sequence is interrupted by 7 introns and encodes an α-tubulin polypeptide with a C-terminal methionine. S1 nuclease analysis shows that the gene is subject to a developmental switch since it is expressed in the plasmodial but not the amoebal phase of the life cycle. The gene product (E α-tubulin) was identified by raising anti-peptide antibodies. The same approach was used to identify the gene product (N α-tubulin) of another complete α-tubulin gene sequence (Monteiro & Cox, 1987). Both E α-tubulin and N α-tubulin are present in plasmodial lysates but absent from amoebal lysates. Separation of plasmodial tubulin isotypes by IEF-SDS 2D gel electrophoresis revealed that E α-tubulin co-localises with the α2 isotype whereas N α-tubulin co-localises with the α1 isotype. Both E α-tubulin and N α-tubulin are components of the plasmodial mitotic spindle.

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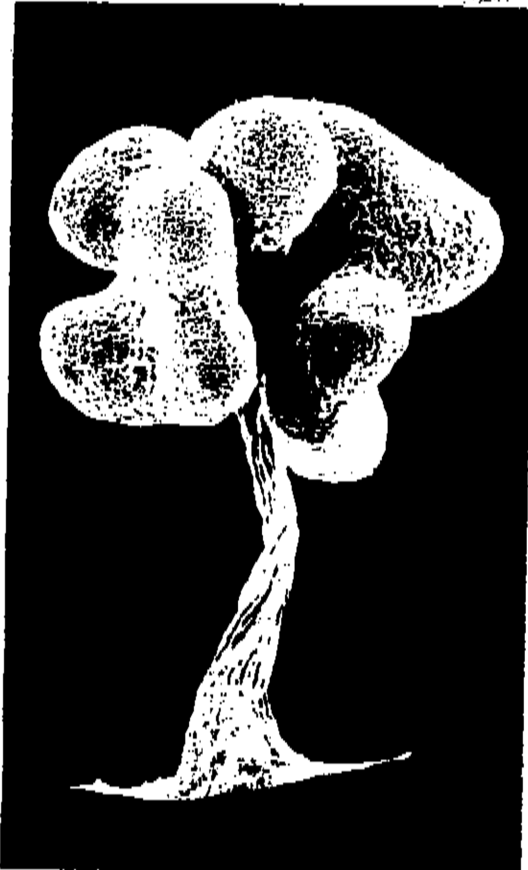

ABSTRACTS NOT AVAILABLE

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