

Early Replicating DNA of *Physarum* is Denser than Late Replicating DNA

The myxomycete *Physarum polycephalum* can be grown as plasmodia, in which most nuclei go through naturally synchronous mitoses¹. The main nuclear DNA is replicated during a 3 h period immediately following mitosis; under the conditions used here the whole intermitotic time is about 9 h. It has been shown previously that DNA molecules that replicate in a subfraction of one S-period again replicate in the corresponding subfraction of the following S-period². This shows that DNA molecules are replicated in a specific time sequence, at least under standard growth conditions. The following experiment shows that DNA replicating at different times of the S-period has a somewhat different density.

Surface cultures of *Physarum* were grown by routine methods and the times of mitosis determined by phase contrast microscopy. Three cultures were prelabeled from inoculation of surface cultures until 3 h after mitosis 2

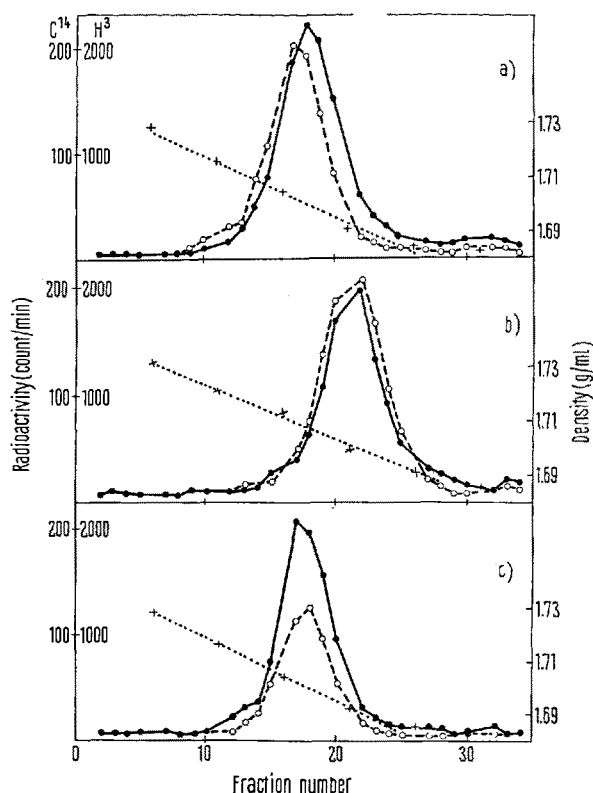
with H³-thymidine (0.5 μ C/ml) and then transferred to an unlabeled medium. After mitosis 3, cultures were pulse labeled at different times of the S-period with C¹⁴-thymidine (2 μ C/ml). Immediately following the labeling, nuclei were isolated, DNA extracted and cesium chloride gradients prepared by methods described previously³. It can be seen from the Figure that H³ and C¹⁴ do not band at the same position at all times. DNA replicated early in the S-period is denser than bulk DNA (Figure a) and DNA replicated late in the S-period is somewhat less dense than bulk DNA (Figure c). Although nuclear satellite DNA (density 1.714 g/ml) does not replicate together with the bulk nuclear DNA (density 1.702 g/ml), it cannot have any influence on the outcome of these experiments⁴. The nuclear satellite DNA represents only 2% of the total DNA and it replicates from the middle of the S-period to the end of the G-2-period⁴.

For mammalian cells in culture very similar results have been obtained: DNA replicated early in the S-phase has a higher G + C content than DNA replicated late in the S-phase⁵⁻⁷. It follows that in very different eukaryotic organisms DNA replication proceeds in a well defined time sequence at least under standard growth conditions. Whether this sequence is also maintained during differentiation remains to be established. It is not clear what functional significance this fixed time sequence of replication has, nor by what mechanism it is controlled: conceivably activating proteins may induce the replication of one segment after another⁸.

Résumé. Le DNA du myxomycete *Physarum polycephalum* a été marqué pendant des temps de 10 min à différentes étapes de la phase S. L'analyse sur gradients de densité préparatifs de chlorure de césium a montré qu'une partie de ce DNA, reproduit tôt dans la phase S est plus dense que le reste du DNA, celui-ci étant lui-même plus dense que le DNA reproduit tardivement.

R. BRAUN and HELEN RÜEDI-WILI

Department of Cell Biology,
Swiss Institute for Experimental Cancer Research,
CH-1011 Lausanne (Switzerland), 4. August 1971.



Cesium chloride gradients of pulse labeled DNA. Cultures were pre-labeled with H³-thymidine, then allowed to continue growth in unlabeled medium and pulse-labeled for 10 min with C¹⁴-thymidine at the following times during the S-period: a) from 10 min after mitosis 3 to 20 min after mitosis 3; b) from 50 min after mitosis 3 to 60 min after mitosis 3; c) from 90 min after mitosis 3 to 100 min after mitosis 3. ●—●, H³-radioactivity; ○—○, C¹⁴-radioactivity; × ... ×, density. Bottom of gradients at the left.

¹ H.P. RUSCH, in *Advances in Cell Biology* (Ed. D.M. PRESCOTT, L. GOLDSTEIN and E. MCCONKEY; Appleton-Century-Crofts, New York 1970), vol. 1, p. 297.

² R. BRAUN and H. WILI, *Biochim. biophys. Acta* 174, 246 (1969).

³ A. ZELLWEGER, U. RYSER and R. BRAUN, *J. molec. Biol.*, in press.

⁴ R. BRAUN and T.E. EVANS, *Biochim. biophys. Acta* 182, 511 (1969).

⁵ A.M. TOBIA, C.L. SCHILDKRAUT and J.J. MAIO, *J. molec. Biol.* 54, 499 (1970).

⁶ W.G. FLAMM, J.N. BERNHEIM and P.E. BRUBACKER, *Expl. Cell Res.* 64, 97 (1971).

⁷ C.J. BOSTOCK and D.M. PRESCOTT, *Expl. Cell Res.* 64, 267 (1971).

⁸ J.E. CUMMINS, in *The Cell Cycle, Gene-Enzyme Interactions* (Ed. G.M. PADILLA, G.L. WHITSON and I.L. CAMERON, Academic Press, New York 1969) p. 141.

⁹ Supported by grant No. 3.183.69 SR of the Swiss National Foundation for Scientific Research.

The Effect of Pressure Changes on Esterification of Cholesterol and Hydrolysis of Cholesterol Esters in Rat Aorta and Serum

Enzyme activity in the tissues may be influenced by various factors. It is known that there is adaptation of enzymes on alterations in the diet¹, after the administration of hormones², drugs³, etc. It was found previously

that also the activity of enzymes esterifying and hydrolyzing cholesterol esters in vascular wall and blood serum is affected by diet changes⁴. When studying other factors which could control the activity of these enzymes in the