Interphase differential sensitivity to protein synthesis inhibition

G. Garcia-Herdugo

Instituto de Biología Celular, C. S. I. C., Velázquez 144, Madrid-6 (Spain), 21 March 1977

Summary. The effect of protein synthesis inhibition during interphase of *Allium cepa* L. root meristem cells was studied. Anisomycin and cycloheximide were used in intermittent treatments during interphase of a synchronous cell subpopulation labelled as binucleate by caffeine, and the delays in reaching prophase were recorded. High sensitivity to protein synthesis inhibition was detected in G_1 and in the S/G_2 boundary, while protein synthesis in most of the S and G_2 phase was not required for the normal timing of next prophase.

The role of protein synthesis in the different stages of the cell cycle has been studied by many methods and in many biological materials during the last years. Effects on mitotic prophase[1,2], on nucleologenesis[3,4] and on replication[5] have been recorded in cells of plant meristems. The aim of this work is to study the differential sensitivity of late mitosis and of the different interphase periods to inhibition of protein synthesis as reflected in a lengthening of the interphase which is measured by a delay of mitosis.

Material and methods. The material used was the root meristem of *Allium cepa* L. The onion bulbs were grown in the dark at a constant temperature of 15 °C ± 0.5 °C in cylindrical receptacles of 70 ml capacity, with tap water which was renewed every 24 h and aerated continuously by bubbling at 10–20 ml air/min. For the cytological analysis, the roots were fixed in a 3:1 mixture of ethanol-acetic acid, and the specimens were prepared by staining the roots with acetic orcein according to the technique of Tjio and Levan[6].

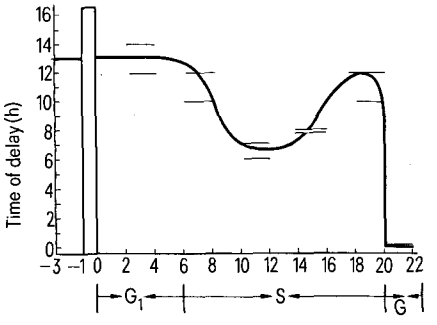

Fig. 1. Delays in reaching prophase induced by cycloheximide treatment at different of the cell cycle. Horizontal lines represent the 2-h-length of the treatments with inhibitor. The vertical column represents the 1-h-caffeine labelling period during which 'spontaneously synchronous' cells traversing telophase become binucleate.

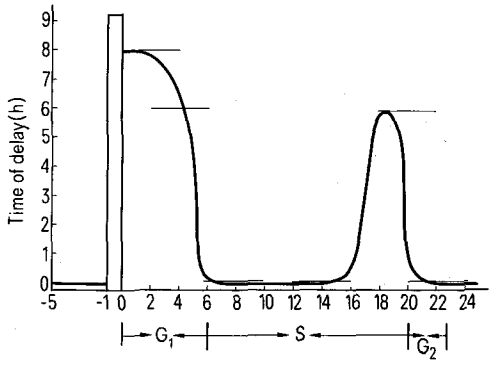

Fig. 2. Delays induced by 4-h-anisomycin treatment during mitosis and different segments of interphase.

To evaluate inhibitor efficacy, different bulbs were incubated with 0.02 μCi/ml, 10^{-4} M ^{14}C-leucine (with a specific activity of 34.4 μCi/mM). They were considered control bulbs, while others were incubated after labelling in the same way in solutions containing inhibitors[5].

Results and discussion. To study the sensitivity of cells to protein synthesis inhibition during different periods of the cell cycle, we have used a synchronous cell population labelled in telophase as binucleates by 1 h treatment with 0.1% caffeine[7]. We have confirmed that in our experimental conditions (15 °C), the binucleate cells reach prophase 22 h after the caffeine treatment.

Different inhibitors of protein synthesis (cycloheximide and anisomycin) which have been previously evaluated in our material[5] were applied for short periods at various stages of the cell cycle. Both of them inhibit the protein synthesis by more than 90% at a 1 μg/ml concentration. The possible effect on the whole interphase should be detected as a delay in the time when the first binucleate cells reach their prophase.

Figure 1 shows the delays in reaching mitosis which are produced by 2-h treatments with cycloheximide, applied at different phases during interphase as well as immediately before interphase initiation (expressed as h −3 to −1 before caffeine labelling in figure 1). There is a general though nonuniform sensitivity to cycloheximide during interphase. A slightly greater sensitivity seems to exist to treatments during G_1 than in those during early and mid S, while there is a high sensitivity zone in late S. Finally, the G_2 appears not to be sensitive to cycloheximide.

Figure 2 shows the effects of the different 3-h anysomicin treatments. This figure essentially shows the same zones of high sensitivity. However, there are striking differences with respect of the sharpness of the response. When using anisomycin prior to the initiation of interphase (from 5 h to 1 h before the end of the caffeine treatment), there is no prolongation of the next interphase while cycloheximide induced a large delay (13 h). Since both drugs inhibit protein synthesis, we can interpret these results in the sense that protein synthesis by late mitosis is not necessary for normal development of the next interphase, and that cycloheximide either shows additional side effects or it has a strong residual effects, that is to say, its effect is reverted at a much slower rate.

1 G. Garcia-Herdugo, M. E. Fernandez-Gomez, J. Hidalgo and J. F. Lopez-Saez, Expl Cell Res. *89*, 336 (1974).
2 G. Garcia-Herdugo and M. E. Fernandez-Gomez, Experientia *33*, 37 (1977).
3 M. E. Fernandez-Gomez, C. De La Torre and G. Gimenez-Martin, Cytobiologie *5*, 117 (1972).
4 G. Gimenez-Martin, C. De La Torre, M. E. Fernandez-Gomez and A. Gonzalez-Fernandez, J. Cell Biol. *60*, 502 (1974).
5 G. Garcia-Herdugo, A. Gonzalez-Fernandez and J. F. Lopez-Saez, Expl Cell Res. *104*, 1 (1976).
6 J. H. Tjio and A. Levan, Ann. Est. Exp. Aula Dei *2*, 21 (1950).
7 G. Gimenez-Martin, A. Gonzalez-Fernandez and J. F. Lopez-Saez, J. Cell Biol. *26*, 305 (1965).

The sensitivity of G_1 to protein synthesis inhibition agrees with previous results[8] which showed that the initiation of the S period depends on previous protein synthesis. The lack of sensitivity of the early and middle S period to protein synthesis inhibition was not expected; although completion of DNA replication does not require concurrent protein synthesis, it was logical to assign a role in mitosis to protein(s) synthesized during this period. The present results suggest that proteins synthesized during this period are not strictly required to reach the next division. On the other hand, some protein(s) must be synthesized at the S/G_2 transition in order to trigger the next prophase, as shown by the great delay recorded after such treatments.

In conclusion, G_1 and S/G_2 boundaries appear as phases highly sensitive to protein synthesis inhibition, while most of S and G_2 do not require protein synthesis for the normal timing in reaching the next prophase. Lastly, this technique is proposed as a simple method to analyze the cycle by specific metabolic inhibitors.

8 A. Gonzalez-Fernandez, G. Gimenez-Martin, M. E. Fernandez-Gomez and C. De La Torre, Expl Cell Res. *88*, 163 (1974).

The dependence of heart rate and locomotor activity on water temperature in the carp (*Cyprinus carpio* L.)

R. Siegmund and J. Vogel[1]

Sektion Biologie der Humboldt-Universität, Bereich Verhaltenswissenschaften, Invalidenstrasse 43, DDR-104 Berlin (German Democratic Republic), 28 February 1977

Summary. Long-term measurements of heart rate and locomotor activity in relatively free moving carps demonstrated dependence on water temperature. Under these conditions, the heart rate has a circadian and circannual rhythm.

As fish are poikilo-thermal animals, their body temperature reponds to changes in the temperature of the environment, although little is known of the actual nature of their body temperature maintenance. The environmental temperature and all organic functions of poikilo-thermal animals show positive correlation[2-4]. Rueth[5] found a high positive correlation of heart-rate and body temperature with environmental temperature, in amphibians. Up to now these relations have hardly been investigated in the case of carps. Iriki et al.[6] carried out thermal stimulation of the spinal cord and thus heart-rate in some cyprinid fish. Albrecht[7] was able to show that temperature has an influence on certain physiological changes in inner organs. Little behavioural analysis of temperature-dependence has been carried out, although such analysis could clarify various ecological questions and problems in fishery research[8-10]. Field observations,

1 The authors express their thanks to Prof. Dr G. Tembrock for this encouragement and support and for his review of the manuscript. We gratefully acknowledge the assistance given by Frau E. Schmidt.

2 J. J. Cech, D. W. Bridges, D. M. Rowell and P. J. Balzer, Can. J. Zool. *54*, 1383 (1976).

3 E. D. Stevens and A. M. Sutterlin, J. exp. Biol. *65*, 131 (1976).

4 J. R. Burns, Phys. Zool. *48*, 142 (1975).

5 E. Rüth, Zool. Jb. Physiol. *78*, 513 (1974).

6 M. Iriki, S. Murata, M. Nagai and K. Tsuchiya, Comp. Biochem. Physiol. *53A*, 61 (1975).

7 M. L. Albrecht, Z. Binnenfisch. DDR *21*, 103 (1974).

8 C. Meske, Aquakultur von Warmwasser-Nutzfischen. Stuttgart 1973.

9 T. L. Beitinger, Biol. Bull. *149*, 96 (1975).

10 B. L. Olla and A. L. Studholme, Proc. Eur. mar. biol. Symp. 75 (1975).

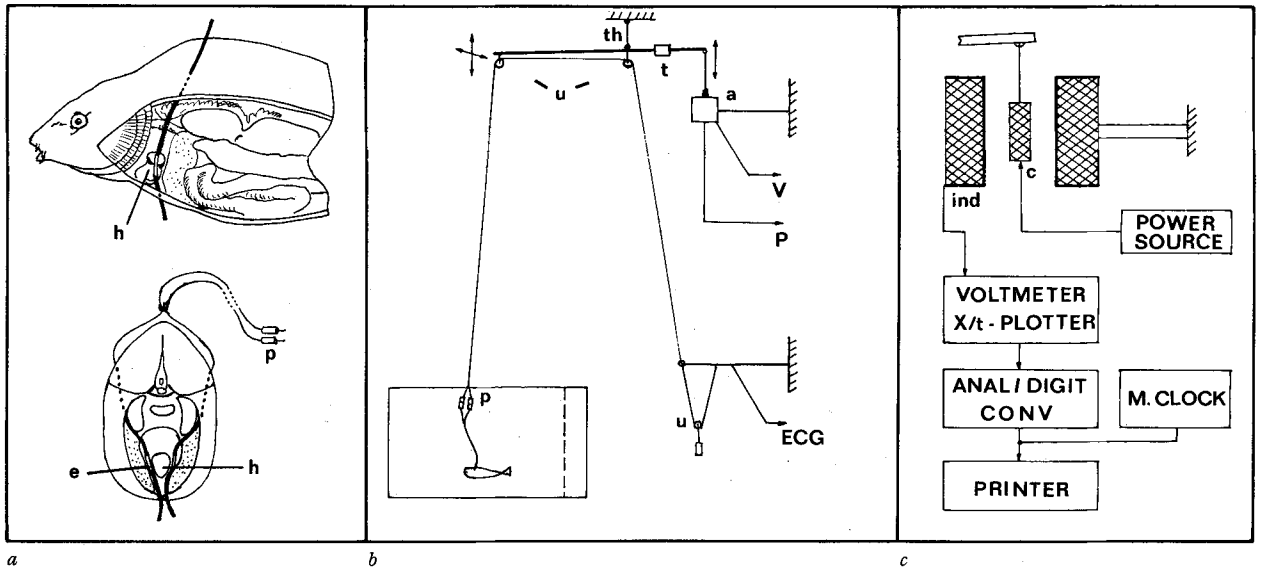

Fig. 1. *a* Arrangement of the electrodes in the fish: h, heart; e, electrodes. *b* Recording system: u, turn back rollers; a, activity receiver; th, theread suspension; l, lever; p, plug connection; v, voltmeter; P, power source. *c* Measuring and processing device for the locomotor activity (schematic); c, current coil; ind, induction coil.