during the 0.5 m-NaCl extraction, as compared with a 22-fold purification and a 146% yield of the aqueous method used before (Tsopanakis et al., 1976). This result may be due to the presence of ethylene glycol in the solutions.

Ethylene glycol seems to play a ‘protective role’ for the enzyme, independent of the temperature effect it may have at −20 °C.

It is noteworthy that the most purified fraction still shows apparent enzyme activity in the absence of added protein acceptors. This may be due either to self modification of the enzyme molecules or of the added histone H1, or it may be due to the synthesis of long acid-insoluble free polymers.

This work was supported by the Science Research Council, the Medical Research Council and the Cancer Research Campaign.


Study of the Translational Stability of Messenger Ribonucleic Acids for Lytechinus pictus and HeLa-Cell Histones Injected into Xenopus Oocytes

G. HUEZ,* G. MARBAIX,* E. WEINBERG,† D. GALLWITZ,‡ E. HUBERT* and Y. CLEUTER*

*Département de Biologie Moléculaire, Université Libre de Bruxelles, 1640-Rhode St-Genèse, Belgique, †Mergenthaler Laboratory for Biology, The Johns Hopkins University, Baltimore, MD 21218, U.S.A., and ‡Physiologisch-Chemisches Institut I, Philipps-Universität, Marburg, 355, Marburg, Lahn, West Germany

We have previously shown that the removal of the poly(A) segment from globin mRNA markedly decreases its stability when injected into frog oocytes (Nudel et al., 1976). To strengthen further the idea that the poly(A) is required to ensure the stability of eukaryotic mRNA, we decided to study the stability of an mRNA in oocytes which naturally lacks poly(A). Histone mRNA was chosen (Adesnik & Darnell, 1972).

RNA fractions enriched in histone mRNA were prepared from either early embryos of Lytechinus pictus (Weinberg et al., 1972) or synchronized HeLa cells in S-phase (Gallwitz & Breindl, 1972).

Oocytes (100) from a single Xenopus laevis female were injected with 50 nl each of aqueous...
solutions of histone mRNA (750 μg/ml). At different times after injection, batches containing 10 oocytes were incubated in 100 μl of Barth medium containing 1 mCi of [3H]alanine or [3H]lysine/ml at 19°C. [For details of micro-injection and culture of oocytes, see Gurdon et al. (1971).]

At the end of the incubations, protein samples were prepared from oocytes and analysed by sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis. Reference [14C]histones were used as a control. At the end of the electrophoresis, the gels were dried and submitted to fluorography (Laskey & Mills, 1975).

Synthesis of histones H3, H2 and H4 is clearly detected in oocytes injected with histone mRNA preparations from either L. pictus or HeLa cells. The synthesis rate of histones H3, H2 and H4 (HeLa cells) and of histones H2a and H4 (L. pictus) is maximal 5–10 h after injection, then decreases rapidly and becomes almost undetectable after 20 h.

From these experiments, we conclude that a naturally poly(A)-lacking mRNA behaves, as far as its translation is concerned, in a very similar way to globin mRNA from which the poly(A) segment has been artificially removed.

Although we cannot definitely conclude that the poly(A)-lacking histone mRNA is really physically unstable, these results nevertheless suggest that the addition of a poly(A) segment at the 3'-end of the molecule might be a general mechanism by which the stability of eukaryotic mRNA is regulated.

This study was made possible through the financial support of the Caisse Générale d'Epargne et de Retraite. G. H. and G. M. are Chercheurs qualifiés of the Fonds National de la Recherche Scientifique.


Mammalian Cells Recognize Growth Factors in the G1-Interval, and Organize a Series of Signals and Events Necessary for Deoxyribonucleic Acid Replication and Cell Division

LUIS JIMÉNEZ DE ASUA, MINNIE O'FARRELL, DOROTHIE CLINGAN and PHILIP S. RUDLAND

Department of Cell Regulation, Imperial Cancer Research Fund Laboratories, Lincoln’s Inn Fields, London WC2A 3PX, U.K.

The replication of mammalian cells both in culture and in animals is a highly ordered process, and its rate changes in response to alterations in the external environment, mainly by expanding or contracting the average time of the G1-phase of the cell cycle. Here we examine the hormonal requirements for replication of cultured mouse 3T3 and 3T6 fibroblastic cells. The effect of three growth factors, prostaglandin F2α, fibroblastic growth factor and epidermal growth factor on the replication of 3T3 fibroblastic cells can be modified by cortisol or insulin (Rudland et al., 1974, 1977; Jiménez de Asua et al., 1975, 1977a). The three growth factors can synergize with insulin to the same extent and can initiate DNA synthesis in many quiescent cultured fibroblastic cells within 24 h (M. O’Farrell, D. Clingan, P. S. Rudland & L. Jiménez de Asua, unpublished work). We have investigated the kinetics of stimulation of DNA synthesis and cell division by the purified growth factors.

Swiss-mouse 3T3 fibroblastic cells were allowed to grow and become quiescent in a...