PERIODIC SYNTHESIS OF ABUNDANT PROTEINS IN THE CELL CYCLE OF *SACCHAROMYCES CEREVISIAE**

by

KAY GULLØV and JØRGEN FRIIS

Department of Molecular Biology, University of Odense,
Campusvej 55, DK-5230 Odense M., Denmark

Keywords: Cell cycle, periodicity, two-dimensional electrophoresis, density variation, ribosome idling

Centrifugation in isotonic self-generated Percoll gradients was used to separate exponentially growing cells of *Saccharomyces cerevisiae* according to a cell cycle-related density variation. Two-dimensionally polyacrylamide electrophoresis revealed that the rate of synthesis of most of the cellular proteins varied in the cell cycle-related manner as formerly described for bulk cellular protein (6).

1. INTRODUCTION

In a previous report (6) we measured the net rate of synthesis of bulk protein in yeast cells fractionated according to their position in the cell cycle and thereby verified a marked variation which reflects a changing degree of ribosomal idling during the cell cycle. Irrespective of the growth rate, a high rate of protein synthesis was observed during the G2 period. In this report we discuss whether the cell cycle-related variation of the net rate of protein synthesis per presynthesized equivalent of protein, $dP/dtxP^1$, as measured on bulk protein synthesis may be applied to the synthesis of individual proteins. Alternatively, a more complex pattern of synthesis of individual proteins leads to the established synthesis of bulk protein.

2. MATERIALS AND METHODS

A diploid prototrophic strain X951 was grown in a synthetic medium containing 2% w/v glu-

cose as carbon source. Spheroplasts were isolated as described earlier (4). Cell cycle fractionations in Percoll and sucrose gradients were performed according to usual procedures (6). Prior to electrophoresis cellular proteins were extracted with 62 mM-Tris, HCl (pH 6.8); 5% SDS; 10% glycerol; 5% mercaptoethanol; 100 mM-EDTA at 95 °C for 4 min. Following a low speed centrifugation the supernatant was diluted with lysis buffer A (9) to a final concentration of 0.25% SDS. O'FARRELL electrophoresis was performed according to standard procedures. Fluorography and radioactivity assays were performed as previously described (5). Each sample was counted until a counting error of a least $\pm 5\%$ was recorded in the ^{14}C channel.

3. RESULTS AND DISCUSSION

To reveal a changing rate of synthesis of individual proteins during the cell cycle, cells were pulse labelled and fractionated according

*On the occasion of his 80th birthday we dedicate this paper to HEINZ HOLTER who introduced one of us (J.F.) to the science of cell biology

Figure 1. Two-dimensional polyacrylamide gel electrophoresis of cellular proteins. The isoelectric focusing gel of the first dimension was in the pH range of 10, left side of the picture, to 3.5, including an expansion zone in the pH range of 5-7.

to their density in Percoll gradients. From an exponentially growing culture with a generation time of 125 min, 2×10 ml subcultures were withdrawn when a density of 10^6 cells/ml was attained. One culture was labelled with L-[U- 14 C]-leucine (4 μ C/ml; 4 μ g/ml) for 3 hrs and chased with 50 μ g/ml L-leucine. Coincidentally with the chase of the first culture, the second one was pulse labelled with L-[4,5- 3 H]-leucine (0.1 mC/ml; 0.23 μ g/ml) for 6 min. Following chase of this culture, the subcultures were combined and subjected to Percoll centrifugation. Eluted fractions were scored for cell type morphology by hemocytometer countings, and the gradient was subdivided into four sections which clearly alternated with respect to cell type frequencies. The first section, Percoll fractions 3-6, contained 46% unbudded cells, 50% budded cells with small buds and 4% budded cells with the bud $> 1/3$ of mother cell. The fourth section, section, fractions 18-24, contained 22% unbudded cells, 8% budded cells with small buds and 70% budded cells with large buds. For details of separation principles, see Figure 1 of (6). Following addition of carrier cells, cells from each section were treated with glusulase for 30 min, proteins were extracted and prepared for electro-

phoresis (Figure 1). The $^1\text{H}/^{14}\text{C}$ ratio of approximately sixty different proteins was registered within the four sections. The results for a number of typical proteins are given in Table I. The greater number of the most abundant cellular proteins exhibit the typical continuous, sigmoid increase of the ratio towards the top of the Percoll gradient as seen for bulk cellular protein. When the ratio between the two outermost sections, fractions (3-6) and (18-24), of all registered proteins were compared, a mean figure of 1.62 ± 0.21 (13%) was calculated. This value is compatible with the ratio 1.56 calculated for bulk cellular protein. Rather few proteins exhibit an atypical pattern. In Nos. 22 and 33, the incorporation rate during the pulse is seemingly low. Less than 10% of the registered proteins exhibit this pattern. Likely candidates within this group are the mitochondrial proteins which are known to be processed following cytoplasmic synthesis (1). Protein No. 23 exhibits the opposite pattern, a seemingly high rate of incorporation of the ^3H -leucine pulse. This observation might be attributed to a significant instability of the protein. Disregarding the aberrant incorporation rates these proteins exhibit the generally observed variation of the incorporation rate in Percoll fractionated cells as seen for bulk protein.

Our results indicate that most of the analysed abundant cellular proteins show an increase in the rate of synthesis during the G2 phase of the cell cycle in yeast, including the putative microtubule proteins (5) (Nos. K6 and K10/11 of Figure 1). A similar periodic synthesis of these proteins was observed in *Physarum polycephalum* (7) and in HeLa cells (2). The same pattern of protein synthesis has also been reported for the fission yeast *Schizosaccharomyces pombe* (3), using three different labelled amino acids including leucine. As seen from Table I this periodic synthesis of proteins is not the only pattern to be found in the yeast cell as a few proteins Nos. 11, 46 and 54, indicated with arrows in Figure 1, seem to manifest a rather constant ratio throughout the Percoll gradient. A pattern which is compatible with an exponential increase of the proteins during the cell cycle. Furthermore, some major proteins have been reported to be synthesized in G1 and the early

Table I.

 $^3\text{H}/^{14}\text{C}$ ratios of individual proteins from Percoll separated cells

Protein No.	Sections (fractions Nos.)			
	(3-6)	(7-10)	(11-15)	(18-24)
Bulk protein	0.77	0.80	1.10	1.20
4	0.58	0.70	0.78	0.97
10	0.68	0.79	0.94	1.08
13	0.56	0.63	0.73	0.83
14	0.70	0.77	0.91	1.03
15	0.61	0.69	0.79	1.03
16	0.65	0.63	0.76	0.99
24	0.70	0.78	0.93	1.15
25	0.73	0.87	0.98	1.18
26	0.75	0.75	0.97	1.07
40	0.58	0.66	0.83	0.91
47	0.67	0.69	0.86	1.04
53	0.67	0.74	0.85	1.03
58	0.59	0.61	0.76	0.85
K6	0.45	0.47	0.57	0.72
K8	0.60	0.66	0.86	1.05
K10, 11	0.61	0.57	0.71	0.86
11	0.80	0.84	0.95	0.90
22	0.43	0.50	0.66	0.89
23	0.82	1.19	1.96	2.14
33	0.44	0.42	0.59	0.82
46	0.92	0.85	0.84	0.90
54	0.59	0.65	0.65	0.65

Prefix K denotes proteins located in the nuclear compartment.

S-phase (8) using a semiautomatic procedure for quantitating gel electropherogram-autoradiographs.

ACKNOWLEDGEMENTS

The authors wish to thank I. HOLMBLAD and S. ANDERSEN for their technical assistance. The financial support received by the Carlsberg Foundation is likewise acknowledged.

REFERENCES

1. BORST, P.: Control of mitochondrial biogenesis in yeast. In: Molecular genetics in yeast. Eds. D. von Wettstein, J. Friis, M. Kielland-Brandt & A. Stenderup, Alfred Benzon Symp. 16, Munksgaard, Copenhagen, pp. 211-223 (1981)
2. BRAVO, R. & J.E. CELIS: A search for differential polypeptide synthesis throughout the cell cycle of HeLa cells. *J. Cell Biol.* 84, 795-802 (1980)
3. CREANOR, J. & J.M. MITCHISON: Patterns of protein synthesis during the cell cycle of the fission yeast *Schizosaccharomyces pombe*. With a statistical appendix by D.A. Williams. *J. Cell Sci.* 58, 263-285 (1982)
4. GULLØV, K. & J. FRIIS: Isolation of chromatin from *Saccharomyces cerevisiae*: The metabolic state in relation to the protein content of chromatin and its susceptibility to micrococcal nuclease. *Exp. Mycol.* 2, 161-172 (1978)
5. GULLØV, K. & J. FRIIS: Chromosomal proteins in *Saccharomyces cerevisiae* I. Number and properties of individual proteins. *Current Genet.* 2, 69-74 (1980)
6. GULLØV, K., J. FRIIS & B. BONVEN: Rates of protein synthesis through the cell cycle of *Saccharomyces cerevisiae*. *Exp. Cell Res.* 136, 295-304 (1981)

7. LAFFLER, T.G., M.T. CHANG & W.F. DOVE: Periodic synthesis of microtubular proteins in the cell cycle of *Physarum*. Proc. Natl. Acad. Sci. USA 78, 5000-5004 (1981)
8. LÖRINCZ, A.T., M.J. MILLER, N.-H. XUONG & E.P. GEIDUSCHEK: Identification of proteins whose synthesis is modulated during the cell cycle of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 2, 1532-1549 (1982)
9. O'FARRELL, P.H.: High resolution two-dimensional gel electrophoresis of proteins. J. Biol. Chem. 250, 4007-4021 (1975)