An obligatory role of protein glycosylation in the life cycle of yeast cells

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In the presence of 2–4 μg tunicamycin/ml, yeast cells stop growth after the cell number has increased 1.5–1.7-fold. The cells are arrested in G1: the rate of DNA synthesis greatly decreases and the budding index drops from 0.6 to 0.1. RNA synthesis is inhibited concomitantly, whereas protein synthesis is little affected.

It is postulated that for G1/S phase transition to occur one or more proteins have to be glycosylated.

Cell cycle *Tunicamycin* *G1 arrest* *Glycoprotein*

1. INTRODUCTION

The dolichol pathway for *N*-glycosylation has been shown to proceed in all eucaryotic cells [1–6]. Since the individual reactions of this pathway have been preserved during evolution in a very conservative way, protein glycosylation must be of fundamental importance for unicellular as well as for multicellular organisms. It is striking, therefore, that so little is known about the functions of carbohydrate sidechains on proteins. Evidence has been obtained that in some cases they are signals for endocytosis [7]. The suggestion that one of these signals, the mannosyl-6-phosphate residue, targets lysosomal proteins directly into their organelle seems questionable, however. Thus, this possibility has been ruled out for yeast cells [8] and is also doubtful at least for certain human tissues [9,10]. A role of glycoproteins in various cell–cell interactions has often been invoked. The evidence indicating that indeed the glyco-part is required for such interactions, is not abundant and in addition, for being a general principle, unicellular organisms would remain a problem.

As most glycosylated enzymes external invertase of *Saccharomyces cerevisiae* does not need its carbohydrate moiety to be active [11]. However, secretion of the protein is prevented somehow when glycosylation is inhibited by tunicamycin [12,13]. Since logarithmic growth of yeast cells is stopped after a while in tunicamycin, independent of the carbon source being sucrose or glucose [14], the antibiotic obviously effects a more general reaction essential for proliferation.

An analysis of *S. cerevisiae* growth inhibition by low concentrations of tunicamycin will be reported here. It will be shown that cells are specifically blocked in the cell cycle in the G1 stage. *N*-Glycosylation of protein(s), therefore, is postulated to be required for the G1/S transition.

2. MATERIALS AND METHODS

The haploid *S. cerevisiae* strain X2180–1A was used in this study. The cells were grown in a defined medium containing 2% glucose [15]. Tunicamycin has been obtained from Sigma. [2-^{14}C]Uracil and [^{14}C]phenylalanine plus [6-^{3}H]uracil were obtained from NEN (Dreieich) and from Amersham-Buchler (Braunschweig), respectively; α-factor from Bachem (Basel).

RNA and DNA synthesis was measured as in [16]; protein synthesis was followed by measuring [^{14}C]phenylalanine incorporation into trichloroacetic acid-insoluble material.

Cell number was determined in a Coulter counter, absorbance measured in an Eppendorf at 578 nm and the budding index (number of cells budding divided by number of total cells counted) was determined microscopically. The cells were sonified before counting for 30 s in an ultrasonic bath.

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3. RESULTS AND DISCUSSION

Tunicamycin prevents the first reaction of the dolichol pathway [17–19] and thus *N*-glycosylation of proteins. In the presence of tunicamycin a peculiar growth inhibition of logarithmically-growing yeast cells is observed (fig.1), which is typical for a cell cycle-specific growth stop [20]. The increase of cell number and absorbance is slightly inhibited for about 1 generation time (~2.5 h) and from then on a strong inhibition is observed; the increase in cell number even stops completely. After tunicamycin addition cell number generally stops after an increase of 1.5–1.7-fold. The increase in absorbance without a concomitant increase in cell number is due to an increase in cell size and indicates that protein synthesis is less affected in the presence of tunicamycin (see below). These results were obtained in the presence of 2 10 μg tunicamycin/ml at ~6 × 10^6 cells/ml.

The cell cycle in yeast cells can be easily followed since the emergence of the bud giving rise to the new daughter cell coincides closely with the beginning of the S phase; progress of the cycle from there on is reflected in the size of the bud [20]. The budding index of the culture after the addition of tunicamycin rapidly dropped from >0.6 to ~0.15 (fig.2). Tunicamycin obviously causes a first cycle stop in G1. Thus all cells which have either initiated S phase when tunicamycin is added or cells in G2 and M stage (altogether 60–70%) are able to finish the sequence of events until G1, but are no longer able to start a new S phase. This explains the increase in cell number by a factor of 1.5–1.7.

If the above interpretation were correct the rate of DNA synthesis should greatly decrease after a time interval required to finish S phase, i.e., after ~40–60 min. Since the test for DNA synthesis was not accurate enough to determine this time interval precisely the inhibitory effect of tunicamycin on DNA synthesis was compared with that caused by α factor. The α mating factor is known to prevent initiation of chromosomal DNA synthesis in haploid *S. cerevisiae* of mating type a [21]. Tunicamycin strongly inhibits DNA synthesis (fig.3A); time dependence of the effect is within experimental error not distinguishable from the inhibition by α factor; residual DNA synthesis in α factor-treated cells corresponds to mitochondrial DNA [22]. Tunicamycin also stops net RNA synthesis after about one generation time (fig.3B), which indicates that at least ribosomal RNA is not synthesized in G1 phase enforced by tunicamycin.

The increase in absorbance without change in cell number (fig.1) is due to a continued synthesis of protein. Tunicamycin does not inhibit the incorporation of [^{14}C]phenylalanine into protein within the first 3 h (fig.3C). In addition the rate stays constant thereafter for another 3 h. In the control the rate increases since the number of ribosomes and number of cells increase. Thus, tunicamycin prevents an increase in the rate of protein synthesis of the culture but does not seem to significantly inhibit ongoing protein synthesis. The latter phenomenon has generally been observed with tunicamycin [13,23,24].

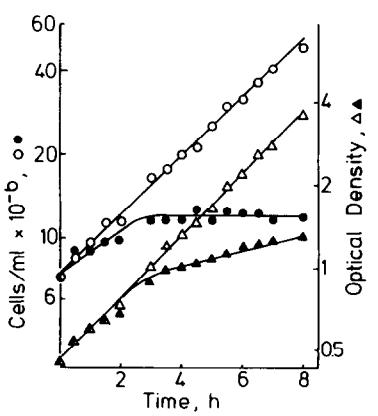

Fig.1. Growth of *S. cerevisiae* in the presence and absence of tunicamycin. Cells were grown at 30°C as in section 2. Tunicamycin (40 μg) was added at time 0 to one sample (10 ml).

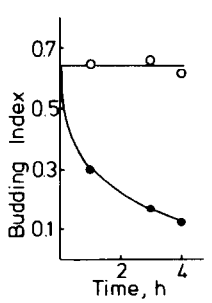

Fig.2. Decrease in the number of budding cells in the presence of tunicamycin (2 μg/ml). Budding index = budding cells/total cells.

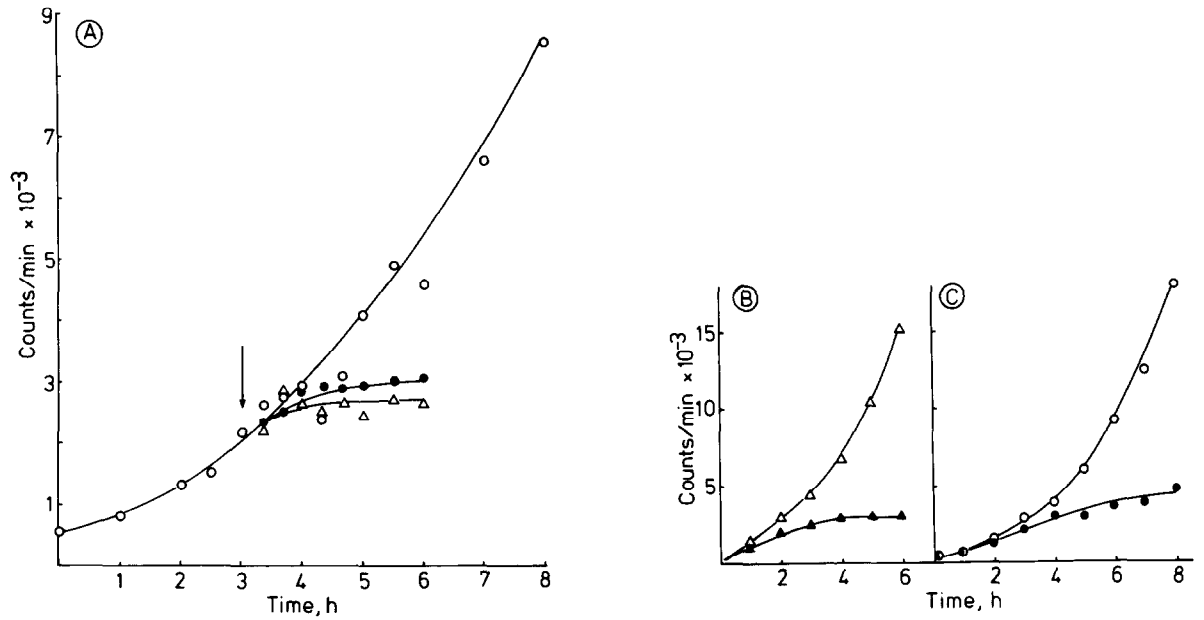

Fig.3. Effect of tunicamycin on DNA, RNA and protein synthesis. (A) DNA synthesis: 60 ml cells (5 × 10^6/ml) were incubated at 30°C with 225 μCi [6-^{3}H]uracil (62.5 Ci/mol). After 3 h, two aliquots were removed and tunicamycin 3 μg/ml (•) and α factor 10 μg/ml (△) were added, respectively; control without any additions (○). Aliquots of 1 ml were analyzed for radioactive DNA as in section 2. (B) RNA synthesis: 10 ml cells (3 × 10^6/ml) were incubated at 30°C (with 10 μCi [2-^{14}C]uracil (3.1 Ci/mol). Aliquots of 0.1 ml were analyzed for radioactive RNA as in section 2; control (△); tunicamycin 2 μg/ml (▲). (C) Protein synthesis: 10 ml cells (7 × 10^6/ml) were incubated with 2 μCi [^{14}C]phenylalanine (2 mCi/mol) at 30°C. Aliquots (0.5 ml) were analyzed for radioactive trichloroacetic acid-precipitable material.

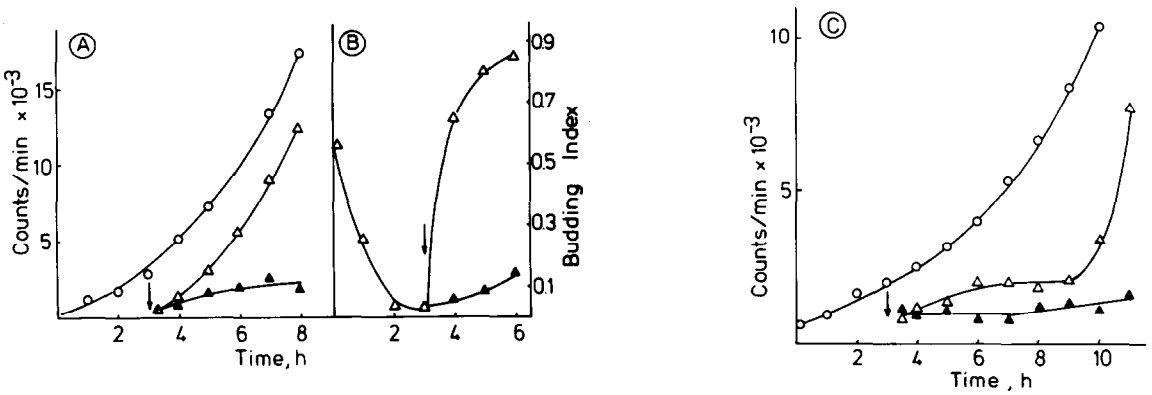

Fig.4. (A,B) Effect of tunicamycin on α factor-treated cells: (A) DNA synthesis: control without any α factor (○); α factor (10 μg/ml) present from 0–3 h (△); α factor after 3 h replaced by tunicamycin 3 μg/ml (▲). Other conditions as in fig.3 except that 30 μCi [6-^{3}H]uracil (30 Ci/mol) have been used in 10 ml total vol. (B) Budding index after addition and removal (arrow) of α factor (△); α factor replaced by tunicamycin (▲). (C) Effect of α factor on tunicamycin-treated cells. DNA synthesis: control without any tunicamycin (○); tunicamycin present from 0–3 h (△); tunicamycin after 3 h replaced by α factor (▲). Other conditions were as in (A) except that in the 2 samples after removal of tunicamycin 30 μCi [6-^{3}H]uracil (120 Ci/mol) have been used.

Since tunicamycin seems to prevent the G1/S phase transition stationary yeast cells which are in G1 [20] should not start growth in the presence of tunicamycin. This is indeed the case: when cells of an $A > 14$ were diluted and resuspended in fresh medium, they started growth after a lag time of 2–3 h. In the presence of 3 μg tunicamycin/ml neither an increase in budding index nor in cell number could be observed (not shown); solely the absorbance increased slightly. The same is true for cells treated with α factor and then transferred to new medium. In the presence of tunicamycin neither budding nor DNA synthesis starts (fig.4A,B). Therefore, the cell cycle block caused by tunicamycin either follows the α factor block or they both affect the cycle at the same site. To distinguish between the 2 possibilities cells treated for 3 h with tunicamycin were subsequently transferred to fresh medium with α factor. The tunicamycin inhibition is not easily reversible and it takes >5 h before DNA is synthesized again in the control (fig.4C). In the presence of α factor DNA synthesis, however, is not initiated at all. Therefore, α factor and tunicamycin seem to affect the cell cycle at the same site or at least sites very close to each other; however, their effects are not identical. Thus tunicamycin does not induce 'shmoos', the typical cell shape obtained with α factor, and α factor treatment, on the other hand, does not inhibit RNA synthesis [25].

The results obtained indicate that one or more proteins have to be glycosylated so that a yeast cell traverses the G1 phase and starts DNA and proceeds with rRNA synthesis. A very special feature of inhibition by tunicamycin therefore is that it synchronizes yeast cells in G1 of the cell cycle. Tunicamycin also synchronizes Burkitt lymphoma cells; their growth is stopped in G1, too [26]. These results were discussed as an inhibition of the formation of glycosylated receptors for growth factors. Growth factors and their interactions with corresponding receptors are required in mammalian cells for G1/S transition. However, these results make it likely that the phenomenon is a more general one; it might be related to the ubiquitous occurrence of protein *N*-glycosylation in all eucaryotic cells.

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REFERENCES

[1] Robbins, P.W., Hubbard, S.C., Turco, S.J. and Wirth, D.F. (1977) Cell 12, 893–900.
[2] Li, E., Tabas, I. and Kornfeld, S. (1978) J. Biol. Chem. 253, 7762–7770.
[3] Parodi, A.J. and Leloir, L.R. (1979) Biochim. Biophys. Acta 559, 1–37.
[4] Quesada-Allue, L.A. and Belocopitow, E. (1978) Eur. J. Biochem. 88, 529–541.
[5] Elbein, A.D. (1981) in: Plant Carbohydrates II, Encycl. Plant Physiol. new ser. vol. 13B (Tanner, W. and Loewus, F.A. eds) pp. 166–193, Springer-Verlag, Berlin, New York.
[6] Lehle, L. (1981) in: Plant Carbohydrates II, Encycl. Plant Physiol. new ser. vol. 13B (Tanner, W. and Loewus, F.A. eds) pp. 459–483, Springer-Verlag, Berlin, New York.
[7] Neufeld, E.F. and Ashwell, G. (1980) in: The Biochemistry of Glycoproteins and Proteoglycans (Lennarz, W.J. ed) pp. 241–266.
[8] Schwaiger, H., Hasilik, A., Von Figura, K., Wiemken, A. and Tanner, W. (1982) Biochem. Biophys. Res. Commun. 104, 950–956.
[9] Owada, M. and Neufeld, E.F. (1982) Biochem. Biophys. Res. Commun. 105, 814–820.
[10] Waheed, A., Pohlmann, R., Hasilik, A., Von Figura, K., Van Elsen, A. and Leroy, J.G. (1982) Biochem. Biophys. Res. Commun. 105, 1052–1058.
[11] Smith, W.L. and Ballou, C.E. (1974) Biochemistry 13, 355–361.
[12] Kuo, S.-C. and Lampen, J.O. (1974) Biochem. Biophys. Res. Commun. 58, 287–295.
[13] Hasilik, A. and Tanner, W. (1976) Antimicrob. Agents Chemoth. 10, 402–410.
[14] Tanner, W., Haselbeck, A., Schwaiger, H. and Lehle, L. (1982) Phil. Trans. Soc. Lond. B in press.
[15] Boller, T., Dürr, M. and Wiemken, A. (1975) Eur. J. Biochem. 54, 81–91.
[16] Hartwell, L.H. (1970) J. Bacteriol. 104, 1280–1285.
[17] Takatsuki, A., Kohno, K. and Tamura, G. (1975) Agr. Biol. Chem. 39, 2089–2091.
[18] Tkacz, J.S. and Lampen, J.O. (1975) Biochem. Biophys. Res. Commun. 65, 248–257.

[19] Lehle, L. and Tanner, W. (1976) FEBS Lett. 71, 167–170.
[20] Hartwell, L.H. (1974) Bacteriol. Rev. 38, 164–198.
[21] Bücking-Throm, E., Duntze, W., Hartwell, L.H. and Manney, T.R. (1973) Exp. Cell Res. 76, 99–110.
[22] Petes, T.D. and Fangman, W.L. (1973) Biochem. Biophys. Res. Commun. 55, 603–609.
[23] Struck, D.K., Sinta, P.B., Lane, M.D. and Lennarz, W. (1978) J. Biol. Chem. 253, 5332.
[24] Schwaiger, H. and Tanner, W. (1979) Eur. J. Biochem. 102, 375–381.
[25] Throm, E. and Duntze, W. (1970) J. Bacteriol. 104, 1388–1390.
[26] Nishikawa, Y., Yamamoto, Y., Kaji, K. and Mitsui, H. (1980) Biochem. Biophys. Res. Commun. 97, 1296–1303.