Mg²⁺ and K⁺ content in Ehrlich Ascites tumor cells during cell cycle

By *T. Günther, J. Vormann, E. Liss* *

Institute of Molecular Biology and Biochemistry, Free University of Berlin
* Institute of Clinical Chemistry and Clinical Biochemistry, Free University of Berlin

Zusammenfassung

Von in vitro asynchron wachsenden Ehrlich-Ascites-Tumorzellen wurden Zellen in der Gl-Phase mittels Zentrifugal-Elutriation abgetrennt. Die Gl-Zellen wurden in Wachstumsmedium reinkubiert. Nach verschiedenen Zeiten wurde der Gehalt an Protein, Mg²⁺ und K⁺ bestimmt. Der Mg²⁺- und K⁺-Gehalt, bezogen auf mg Protein, blieb während des Zellzyklus konstant. Daraus ergibt sich, daß Wachstum und Aufnahme von Mg²⁺ und K⁺ koordiniert sind.

Summary

Gl-phase cells were separated from in vitro asynchronously growing Ehrlich ascites tumor cells by centrifugal elutriation. The cells were reincubated in culture medium. Protein, Mg²⁺ and K⁺ contents were measured at various times. The Mg²⁺ and K⁺ content per mg protein remained constant during the cell cycle, indicating that cell growth and uptake of Mg²⁺ and K⁺ are coordinated processes.

Résumé

Des cellules en phase Gl ont été séparées par élutriation centrifuge de cellules ascitiques tumorales d'Ehrlich croissant de façon asynchrone in vitro. Les cellules ont alors été reincubées dans le milieu de culture. Les taux de protéine, de Mg²⁺ et de K⁺ ont été mesurés à intervalles variables. Les taux de Mg²⁺ et de K⁺ par mg de protéine demeurent constants durant le cycle cellulaire, indiquant que la croissance cellulaire et la captation de Mg²⁺ et de K⁺ représentent des processus coordonnés.

Introduction

Walker and *Duffus* [21] suggested that the intracellular concentration of free Mg²⁺ regulates cell division. According to this hypothesis no Mg²⁺ is taken up during cell growth and due to the cell volume expansion, the concentration of intracellular free Mg²⁺ drops to a level which permits tubulin polymerization and subsequent spindle formation. A rapid influx of Mg²⁺ then serves to trigger chromosome condensation and eventually spindle breakdown, enabling nuclear and cell division to proceed.

This hypothesis has various weak points. Total Mg²⁺ content was measured during the cell cycle of Schizosaccharomyces pombe. However, yeast cells contain a vacuole, in which Mg²⁺ is highly concentrated. According to *Okorokov* et al. [17], the concentration of free Mg²⁺ in the vacuole amounts to 20 mM, whereas the concentration of free Mg²⁺ in the cytosol amounts to 1 mM.

Furthermore, yeast cells contain a high quantity of polyphosphates [11] that bind Mg²⁺ with high affinity [14]. The behaviour of the vacuole and polyphosphates during the cell cycle was not considered in "the magnesium cell cycle regulator model" [21]. During the cell cycle, the vacuole of yeasts multiplies by fission into small vacuoles. Part of them enter into the bud and fuse [13]. Total volume and functional properties of the vacuoles during this process are unknown. The content of polyphosphates changes during the cell cycle [6, 9].

On the other hand, the "sawtooth" pattern of total Mg²⁺ in yeast [21] was not found in E.coli cells which have no vacuole and only a low polyphosphate content [8]. Contrarily to yeast cells, in synchronized E.coli cells, the Mg²⁺ content rose proportionally to cell mass [4, 10].

The magnesium cell cycle regulator model [21] should be applicable to other eucaryotic cells. Therefore, we studied the Mg²⁺ content during the cell cycle in synchronized Ehrlich ascites tumor cells, because animal cells contain no vacuole and only negligible amounts of polyphosphate located in the nucleus [2, 16].

Materials and Methods

Ehrlich ascites tumor cells were grown for 44 hours at 37° C in sterile RPMI 1640 medium with addition of 10 % fetal calf serum (Seromed, München, FRG), 25 mM Hepes, pH 7.4, 15 mM NaHCO₃, 0.1 g/l streptomycin and 0.06 g/l penicillin. Under these conditions, the cells could reach a concentration of 5×10^5 cells/ml with a constant growth rate. Separation of Gl-phase cells was performed with 2.4×10^8 cells in a Beckman centrifuge J2−21 with the elutriator rotor JE-6 according to *Meistrich* et al. [15]. The rotor speed was maintained at 1900 ± 10 rpm. The cells were introduced into the chamber at 20 °C at a flow rate of 10 ml/min. Thereafter, growth medium was introduced into the chamber and the flow rate was gradually increased by about 0.5−1 ml/min, resulting in various cell fractions. The first cell fractions amounting to a total of about 10^7 cells, containing dead cells and cell debris, were discarded. The next fraction of 10^7 cells, sampled at a flow rate of 13−15 ml/min, according to the

various experiments, contained Gl-phase cells.

The cell cycle phase and purity of the separated cells were characterized by pulse cytophotometry. 10^5 cells, sedimented at $1200\ g \times 10$ min, were resuspended in 1 ml 0.15 M NaCl and fixed by adding 10 ml methanol. After centrifugation, the pellet was incubated with 1 ml 0.5 % pepsin in 50 mM HCl for 5 min. Then 10 ml 0.001 % ethidium bromide in 0.1 M Tris buffer, pH 7.5, and 0.1 ml ribonuclease (0.1 %) were added and 20 min later measurements were performed with a pulse cytophotometer ICP 11 (Phywe, Göttingen, FRG). The DNA histograms of the cells were plotted with a printer plotter (Wang, model 2281 P). The doubling time was estimated by cell counting in a Coulter counter (model DN).

The separated Gl-cells were reincubated in the same RPMI 1640 medium, as described above, at 37 °C by shaking. After 0.5, 2.5, 5 and 7.5 hours, aliquots were centrifuged at 1000 g for 10 min and washed twice in 0.15 M NaCl. The washed pellets were extracted with 3 ml 10 % TCA containing 0.1 % La^{3+}. The supernatants were taken for the determination of Mg^{2+} by atomic absorption spectrophotometry (Perkin-Elmer, model 303) and for the determination of K^+ by flame photometry (Beckman). The pellets were taken for protein measurements according to *Lowry* et al. [12].

Results and Discussion

Characterization of cells and cell cycle phase

The doubling time of the cells in culture was 10.5 ± 0.5 hours. The Gl-, S-, G2- and M-phase amounted to 1.5, 6, 1.5 and 1 h, respectively. As shown in Figure 1, the asynchronous cell population (Fig. 1a) can be separated to almost uniformly Gl-phase cells (Fig. 1b). Therefore,
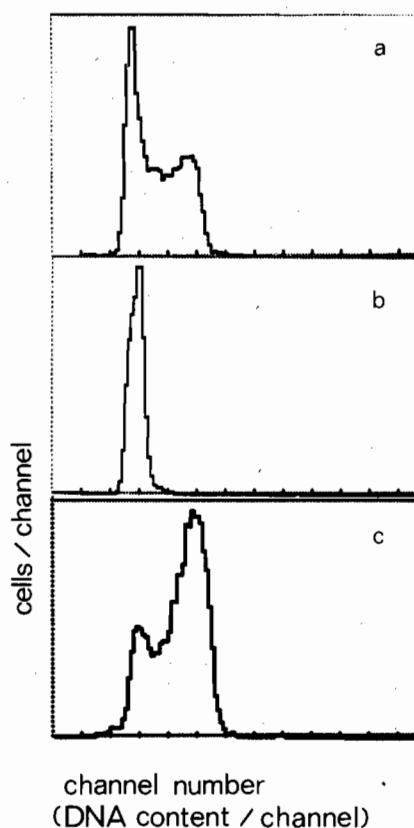

cells / channel

channel number
(DNA content / channel)

Figure 1:   DNA histograms of Ehrlich ascites tumor cells. Printer plot of pulse cytophotometry:
a) asynchronous cell population
b) isolated G1-phase cells
c) G1-phase cells 7.5 hours after reincubation in growth medium

after 0.5 h incubation the cells are in the Gl-phase, after 2.5 h incubation at the beginning of the S-phase, after 5 h in the middle of the S-phase and after 7.5 h about one third of the cells have entered the G2-phase. However, as can be seen from Figure 1c, a small portion of the cells has not entered the cell cycle.

Mg^{2+} and K^+ content during cell cycle

When Ehrlich ascites tumor cells, being in the Gl-phase, were reincubated in sterile RPMI 1640 medium with 10 % fetal calf serum, they continuously synthesized protein (Fig. 2), as it is typical for other neoplastic cells [22]. The increase in protein within an incubation time of 7 hours amounted to 50 %. This value is in agreement with the doubling time of these cells and with the fact that a small part of the cells did not enter the cell cycle.

The Mg^{2+} and K^+ contents of the tumor cells increased in the same manner as protein. Thus, the Mg^{2+} and K^+ content related to protein remained constant throughout the cell cycle (Fig. 2), its value being the same as in un-
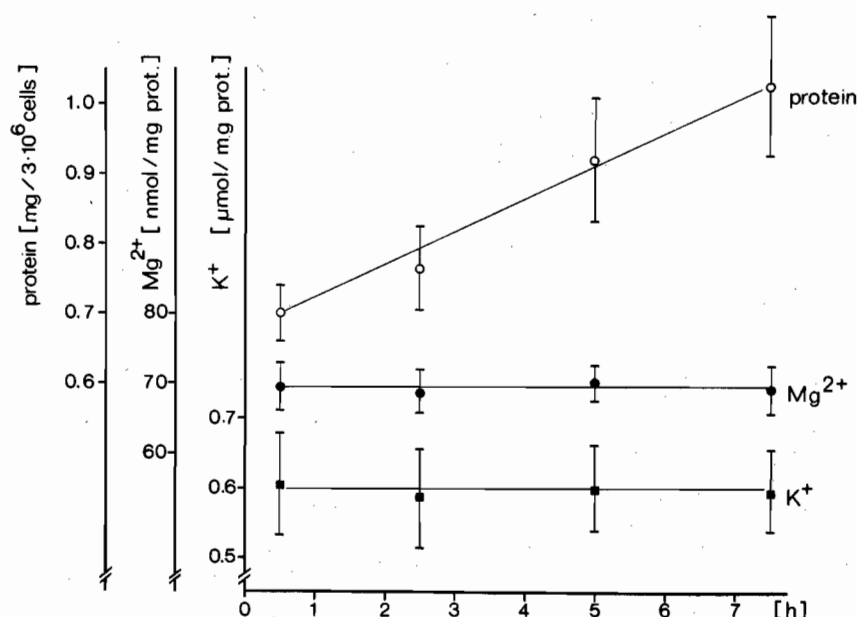

Figure 2:   Contents of protein, Mg^{2+}, and K^+ in synchronized Ehrlich ascites tumor cells after reincubation of G1-phase cells.
Mean ± SEM from 5 experiments.

synchronized cells (not shown). This behaviour is identical with the Mg^{2+} and the K^+ content in synchronized E. coli during the cell cycle [4, 10]. Therefore, the "sawtooth" pattern of the intracellular Mg^{2+} content, as found in Schizosaccharomyces pombe during the cell cycle [21], may be caused by the vacuole and the polyphosphates.

The behaviour of intracellular free Mg^{2+} during cell cycle is not defined because there are no satisfying methods. With different methods different values were measured in the same cell type [3]. Moreover, free Mg^{2+} is not uniformly distributed within the cell, Mg^{2+} ions being enriched at charged surfaces, like membranes.

Theoretically, the concentration of intracellular free Mg^{2+} may be changed at constant total Mg^{2+} content when the concentration of Mg^{2+} binding ligands is changed during cell cycle. In synchronized fibroblasts, the ATP content was increased by 40 % when Gl-phase cells proceeded to the S-phase [19]. Thus, the concentration of intracellular free Mg^{2+} may be decreased. At the same time, however, Mg^{2+}, bound to other ligands with lower Mg^{2+}-binding constants than ATP, would be liberated. These Mg^{2+}-binding sites are part of the intracellular Mg^{2+} buffer.

During the cell cycle, the intracellular content of polyamines is changed, particularly the concentration of putrescine increased [7]. Polyamines, having similar binding properties as Mg^{2+} [20], compete with Mg^{2+} for the same binding sites. Thus, an increase in polyamines can increase the concentration of free Mg^{2+} by liberation of bound Mg^{2+}.

Moreover, intracellular free Mg^{2+} can be regulated by Mg^{2+} efflux [5], and by Mg^{2+} uptake (in preparation). Thus, the intracellular pMg may be held constant, indicating that Mg^{2+} plays a permissive and not a regulatory role in cellular metabolism [3].

For the regulation of cell cycle, other mechanisms, involving cAMP, Ca^{2+} and calmodulin may be responsible [1, 18, 18a].

Our results indicate that Mg^{2+} is taken up continuously in growing cells in a constant relation to the protein content which remains in parallel to cell volume [22]. From this result, it can be concluded that a coordinated mechanism exists between cell growth or biosyntheses and the net uptake of Mg^{2+}, K^+ and probably other substances. The mechanisms regulating and coordinating these processes are still unknown.

Literature

[1] *Boynton, A. L., Whitfield, J. F.:* The role of cyclic AMP in cell proliferation: A critical asessment of the evidence. Adv. Cyclic Nucl. Res. **15** (1983) 193 – 294.

[2] *Griffin, J. B., Davidian, N. M., Penniell, R.:* Studies of phosphorus metabolism by isolated nuclei. VII. Identification of polyphosphate as a product. J. Biol. Chem. **240** (1965) 4427 – 4434.

[3] *Günther, T.:* Biochemistry and pathobiochemistry of magnesium. Magnesium-Bull. **3** (1a) (1981) 91 – 101.

[4] *Günther, T., Hoffmann, C. F.:* Zum Magnesium-Stoffwechsel von E. coli. Z. klin. Chem. klin. Biochem. **11** (1973) 237 – 242.

[5] *Günther, T., Vormann, J., Förster, R.:* Regulation of intracellular magnesium by Mg^{2+} efflux. Biochem. Biophys. Res. Commun. **119** (1984) 124 – 131.

[6] *Harold, F. M.:* Inorganic polyphosphates in biology: Structure, metabolism, and function. Bacteriol. Rev. **30** (1966) 772 – 794.

[7] *Inouye, M., Pardee, A. B.:* Requirement of polyamines for bacterial division. J. Bacteriol. **101** (1970) 770 – 776.

[8] *Kornberg, S. R.:* Adenosine triphosphate synthesis from polyphosphate by an enzyme from Escherichia coli. Biochim. Biophys. Acta **26** (1956) 294 – 300.

[9] *Kuhl, A.:* Die Biologie der kondensierten anorganischen Phosphate. Ergeb. Biol. **23** (1960) 144 – 186.

[10] *Kung, F. C., Raymond, J., Glaser, D. A.:* Metal ion content of Escherichia coli versus cell age. J. Bacteriol. **126** (1976) 1089 – 1095.

[11] *Liss, E., Langen, P.:* Versuche zur Polyphosphat-Überkompensation in Hefezellen nach Phosphatverarmung. Arch. Mikrobiol. **41** (1962) 383 – 392.

[12] *Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J.:* Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193** (1951) 265 – 275.

[13] *Matile, P. H., Moor, H., Robinow, C. F.* in: *A. H. Rose, J. S. Harrison* (eds): The Yeasts, AP, London/New York 1969, pp. 218 – 302.

[14] *Martell, A. E., Calvin, E.:* Chemistry of the Metal Chelate Compounds. Prentice Hall, Inc. Englewood Cliffs, N. J., 1952, p. 545.

[15] *Meistrich, M. L., Meyn, R. E., Barlogie, B.:* Synchronization of mouse L – P 59 cells by centrifugal elutriation separation. Exptl. Cell Res. **105** (1977) 169 – 177.

[16] *Offenbacher, S., Kline, E. S.:* Evidence for polyphosphate in phosphorylated nonhistone nuclear proteins. Arch. Biochem. Biophys. **231** (1984) 114 – 123.

[17] *Okorokov, L. A., Lichko, L. P., Kulaev, I. S.:* Vacuoles: Main compartments of potassium, magnesium, and phosphate ions in Saccharomyces carlsbergensis cells. J. Bacteriol. **144** (1980) 661 – 665.

[18] *Poenie, M., Alderton, J., Steinhardt, R., Tsien, R.:* Calcium activity correlates with the activation state and specific events in the cell cycle. J. Cell Biol. **99** (1984) 429a.

[18a] *Ralph, R. K.:* Cyclic AMP, calcium and control of cell growth. FEBS Lett. **161** (1983) 1 – 8.

[19] *Rapoport, E., Garcia-Blanco, M. A., Zamecnik, P. C.:* Regulation of DNA replication in S phase nuclei by ATP and ADP pools. Proc. Natl. Acad. Sci. USA **76** (1979) 1643 – 1647.

[20] *Tabor, H., Tabor, C. W.:* Biosynthesis and metabolism of 1.4-diaminobutan, spermidine, spermine and related amines. Adv. Enzymol. **36** (1972) 203 – 268.

[21] *Walker, G. M., Duffus, J. H.:* Magnesium as the fundamental regulator of the cell cycle. Magnesium Exptl. Clin. Res. **2** (1983) 1 – 16.

[22] *Warmsley, A. M. H., Pasternak, C. A.:* The use of conventional and zonal centrifugation to study the life cycle of mammalian cells. Biochem. J. **119** (1970) 493 – 499.

For the authors: Prof. Dr. T. Günther, Institut für Molekularbiologie und Biochemie, Freie Universität Berlin, Arnimallee 22, D-1000 Berlin 33