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Extracellular Mg^{2+} regulates intracellular Mg^{2+} and its subcellular compartmentation in fission yeast, *Schizosaccharomyces pombe*

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Abstract. Effects of extracellular magnesium ions ($[Mg^{2+}]_o$) on intracellular free Mg^{2+} ($[Mg^{2+}]_i$) and its subcellular distribution in single fission yeast cells, *Schizosaccharomyces pombe*, were studied with digital-imaging microscopy and an Mg^{2+} fluorescent probe (mag-fura-2). Using 0.44 mM $[Mg^{2+}]_o$, $[Mg^{2+}]_i$ in yeast cells was 0.91 ± 0.08 mM. Elevation of $[Mg^{2+}]_o$ to 1.97 mM induced rapid (within 5 min) increments in $[Mg^{2+}]_i$ (2.18 ± 0.11 mM). Lowering $[Mg^{2+}]_o$ to 0.06 mM, however, exerted no significant effects on $[Mg^{2+}]_i$ (0.93 ± 0.14 mM), at least for periods of up to 30 min. Irrespective of the $[Mg^{2+}]_o$ used, the subcellular distribution of $[Mg^{2+}]_i$ remained heterogeneous, i.e. where the sub-plasma membrane region > cytoplasm > nucleus. $[Mg^{2+}]$ in all three subcellular compartments increased significantly, two- to threefold, concomitant with $[Mg^{2+}]_i$ when placed in 1.97 mM $[Mg^{2+}]_o$. We conclude that $[Mg^{2+}]_i$ in fission yeast is maintained at a physiologic level when $[Mg^{2+}]_o$ is low, but intracellular free Mg^{2+} rapidly rises when $[Mg^{2+}]_o$ is elevated. Like most eukaryotic cells, yeast may have a Mg^{2+} transport system(s) which functions to maintain gradients of Mg^{2+} from the outside to inside the cell and among its subcellular compartments.
Key words. Magnesium; *Schizosaccharomyces pombe*; mag-fura-2.

The fission yeasts, *Schizosaccharomyces pombe* (*S. pombe*), are small, relatively simple unicellular eukaryotes which share many of the same fundamental cellular properties of larger, multicellular organisms [1, 2]. Such unique characteristics provide a valid model system for studying eukaryotic genetics and its physiologic properties in yeast. Understanding of the cytology, intermediary metabolism and molecular genetics of this organism has advanced substantially over the past several years [1–3]. Although important roles for magnesium (Mg) in enzymatic reactions, DNA synthesis and the cell cycle have been studied in detail [4–6], the ionic aspects of magnesium and its regulation in fission yeast have received little experimental attention.

Magnesium is widely distributed in nature and is the second most abundant intracellular cation next to potassium [7, 8]. While most of the internal magnesium is bound to nucleotides, proteins or sequestered into intracellular organelles, only a small fraction is present in its free, divalent ionized form, Mg^{2+}, which is thought to be responsible for many of the biological actions of magnesium [8, 9]. Measurement of intracellular free Mg^{2+} concentrations ($[Mg^{2+}]_i$) would be desirable to understand the physiologic role of Mg^{2+} in various intracellular processes. Here, we report for the first time the values of $[Mg^{2+}]_i$ and its subcellular distribution in single *S. pombe* cells, using digital imaging analysis and the Mg^{2+}-sensitive fluorescent dye mag-fura-2. We show that the cellular distribution of $[Mg^{2+}]_i$ is not homogeneous and increases as a consequence of increments in extracellular Mg^{2+} concentration ($[Mg^{2+}]_o$).

Materials and methods

Strain and culture conditions. *S. pombe* used in our studies was a wild-type strain SP66 (h^{90} leu 1-32 ade 6-216) which was derived from 972 h^{-s} isolates of *S. pombe* originally introduced by Leupold [10]. *S. pombe* cells were grown in minimal media [3] at 30 °C with shaking.

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Determination of $[Mg^{2+}]_i$. A 1-ml aliquot of cells from mid-log phase cultures ($0.5-1 \times 10^7$ cells/ml) was taken into a microtube and spun for 10 s. The supernatant was removed, and the pellet was washed twice with 1 ml of Tris-HCl buffer solution, which contained 1.2 M sorbitol, 135 mM NaCl and 10 mM Tris-HCl, pH 7.6. The cells were loaded with mag-fura-2 (Molecular Probes, Eugene, OR) by incubating them with 5 μM mag-fura-2/Acetoxymethylester in Tris-HCl buffer solution at 30 °C for 60 min. The loading solution was then removed, and the *S. pombe* cells were divided into three microtubes and washed three times with Tris-HCl buffer solutions which contained 0.06, 0.44 and 1.97 mM Mg^{2+}, respectively. The ionic activities of Mg^{2+} in Tris-HCl buffer solutions were monitored by ion-selective electrodes (NOVA Biomedical Corp., Waltham, MA) [11] and adjusted with $MgSO_4$. The cells in each microtube were plated onto a microscopic slide with a thin film of Tris-HCl buffer. The preparations were then covered with cover glasses and placed in a chamber on a thermostatically regulated stage (30 °C) of a Nikon fluorescence microscope. Measurement of $[Mg^{2+}]_i$ was performed using a TN8500 FluorPlex III Image Analyser (Tracor Northern, Madison, WN) [12]. Images of mag-fura-2 fluorescence at 510 nm emission were obtained with 335- and 370-nm excitation wavelengths
using a silicon intensified target (SIT) camera. Background fluorescence for both excitation wavelengths was acquired from blanks for each experiment and subtracted from each pair of images separately before ratioing. Fluorescence ratios ($R_{335/370}$) were obtained by dividing the 335-nm image by the 370-nm image [12].
To obtain absolute values of $[Mg^{2+}]_i$ in single cells, a final concentration of 5 μM mag-fura-2 pentapotassium salt containing either 10.0 mM (max) or 0 mM $MgSO_4$ (min) was used for an in vitro calibration [12]. The calibration solutions also contained (in mM): KCl 115, NaCl 20 and HEPES 5, buffered with NaOH to pH 7.1 under air, at 37 °C. From these Mg-standard solutions, the maximum and minimum intensities of fluorescence were obtained at the 335-nm and 370-nm wavelengths, and a ratio of ($R_{335/370}$) was generated. $[Mg^{2+}]_i$ was calculated according to the following equation [13]:

$$[Mg^{2+}]_i = K_d \times B \times (R - R_{min})/(R_{max} - R)$$

and a K_d of 1.5 mM [13] was used for the mag-fura-2/Mg^{2+} complex. B is the ratio of fluorescence intensity of free mag-fura-2 to Mg- bound mag-fura-2 at 370 nm.
Localization of cellular nuclei. To map and determine intracellular compartmentation of $[Mg^{2+}]_i$, nuclei of *S. pombe* were visualized using DAPI (4,6-diamidino-2-phenylindole) [3]. After measurement of $[Mg^{2+}]_i$, the cells were stained by adding 2 μl of DAPI onto thin-cell films from the edge of the coverslips and then examined using fluorescent microscopy, as above, with an ultraviolet (UV) filter. According to images of nuclei identified with DAPI staining, each yeast cell was arbitrarily divided into three compartments, e.g. nucleus, sub-plasma membrane (peripheral region) and cytoplasm (area between nucleus and sub-plasma membrane).
Where appropriate, means ± SEM were calculated and compared for statistical significance using Student's t-test and analysis of variance (ANOVA).

Results and discussion

When *S. pombe* cells were incubated in 0.44 mM $[Mg^{2+}]_o$, $[Mg^{2+}]_i$ in these yeast cells was 0.91 ± 0.08 mM (mean ± SEM) (table 1), which was about 2.1-fold higher than $[Mg^{2+}]_o$. These values of $[Mg^{2+}]_i$ that we have observed in yeast appear to be within the range of the resting levels found in many mammalian cells [9, 12, 13]. In many yeast cells there was some variations of $[Mg^{2+}]_i$, ranging between values of 0.47 mM and 2 mM, among the cells tested. The reason for this observed variation in $[Mg^{2+}]_i$ between individual cells is not known, but may be due to the fact that the *S. Pombe* cells used in the present study were not synchronized; cells at different stages of the cell cycle are thought to contain different amounts of magnesium [14]. Since $[Mg^{2+}]_i$ is a known cofactor of about 300 enzymes [8], the variation of $[Mg^{2+}]_i$ we observed could be of significant physiological relevance. Further studies are needed to discern whether or not such fluctuations of $[Mg^{2+}]_i$ and its subcellular distributions in *S. pombe* cells are, indeed, related to different stages of the cell cycle. Using 1.97 mM $[Mg^{2+}]_o$ solutions, the mean value of $[Mg^{2+}]_i$ in *S. Pombe* rose to 2.18 ± 0.11 mM, i.e. about 2.4-fold higher when compared with 0.44 mM $[Mg^{2+}]_o$ and about equivalent to the elevated $[Mg^{2+}]_o$ (table 1). Consistent with 0.44 mM $[Mg^{2+}]_o$, variation of $[Mg^{2+}]_i$ among 1.97 mM $[Mg^{2+}]_o$-treated cells was also observed, with a range between 1.70 mM and 3.03 mM. Such $[Mg^{2+}]_i$ levels were reached within 5 min, which is similar to findings in some mammalian cells [12]. Since no significant dye (mag-fura-2) leakages were noted during the experiments, changes of membrane integrity or permeability could not account for such elevation of $[Mg^{2+}]_i$. Our data clearly indicate that the cell wall and the plasma membrane of *S. pombe* cells are permeable to external Mg^{2+} ions, and transport of

Table 1. Effects of alteration of $[Mg^{2+}]_o$ on $[Mg^{2+}]_i$ in single fission yeast cells, *Schizosaccharomyces pombe*.

$[Mg^{2+}]_o$ (mM)	n[a]	$[Mg^{2+}]_i$[b] (mM)
0.06	13	0.93 ± 0.14
0.44	12	0.91 ± 0.08
1.97	12	2.18 ± 0.11*

[a] n = number of cells; [b] mean ± SEM.
* Significantly different from other values ($P < 0.001$).

Table 2. Effects of $[Mg^{2+}]_o$ on the subcellular compartmentation of $[Mg^{2+}]_i$ in single fission yeast, *Schizosaccharomyces pombe*.

Cellular compartment	$[Mg^{2+}]_i$ (mM)[a]		
	0.06 mM $[Mg^{2+}]_o$ (n = 13)[b]	0.44 mM $[Mg^{2+}]_o$ (n = 12)	1.97 mM $[Mg^{2+}]_o$ (n = 12)
Sub-plasma membrane	1.29 ± 0.29*,**	1.14 ± 0.08*,**	3.11 ± 0.20**
Cytoplasm	0.84 ± 0.12*,**	0.95 ± 0.14*,**	1.78 ± 0.12**
Nucleus	0.38 ± 0.04*	0.43 ± 0.03*	0.91 ± 0.05

[a] Values are means ± SEM.
[b] n = number of cells.
* Significantly different from 1.97 mM $[Mg^{2+}]_o$ ($P < 0.01$).
** Significantly different from nucleus ($P < 0.01$).

Mg^{2+} probably occurs much faster than thought heretofore [7, 8].

At first glance, it might appear that higher $[Mg^{2+}]_i$ over $[Mg^{2+}]_o$ could result from active accumulation of Mg^{2+} against a transmembrane gradient across the cell membrane. However, considering the cell membrane of *S. pombe*, which has a voltage of about −53 mV [15], $[Mg^{2+}]_i$ was far away from its electrochemical equilibrium, irrespective of the $[Mg^{2+}]_o$ tested (either 0.44 mM or 1.98 mM). Therefore, yeast cells must possess a transporter(s) which extrudes Mg^{2+} from the cell and/or sequesters Mg^{2+} into intracellular organelles or physiologic binding components, such as a Mg^{2+}/Ca^{2+} exchanger or a Na^+/Mg^{2+} exchanger, as proposed for mammalian cells [7, 9]. Studies of Mg^{2+} transport in unicellular eukaryotes have been rather limited [16]. In *S. pombe*, it is known that Ca uptake can be inhibited by $[Mg^{2+}]_o$ [17] and that mutation in the gene *sep1*$^+$ shows increased sensitivity to the toxic effects of high concentrations of $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ [18]. A precise role of cation exchangers in regulation of $[Mg^{2+}]_i$ in yeast cells remains to be determined.

Interestingly, removal of most extracellular Mg^{2+} (0.06 mM) did not affect the level of $[Mg^{2+}]_i$ (0.93 ± 0.14 mM) (table 1), for at least 30-min observation periods. These unexpected findings suggest that in yeast cells $[Mg^{2+}]_i$ may be regulated by a unique mechanism(s). At present, little is known about Mg^{2+} transport system(s) in yeast. Unlike some mammalian cells [19], the fission yeast cells seem to be relatively resistant to depletion of $[Mg^{2+}]_i$, when environmental $[Mg^{2+}]$ is low. The fission yeast cells may thus maintain $[Mg^{2+}]_i$ either by preventing Mg^{2+} loss from the plasma membrane and the cell wall or by releasing Mg^{2+} from internal binding sites. Such characteristics may account, in part, for why fission yeast cells can survive after placing them into Mg^{2+}-deficient media for prolonged periods [14].

Divalent cations may be compartmented in yeast cells, probably sequestered in cytoplasm granules, as suggested previously by using differential extraction or energy-dispersive X-ray microanalysis [16, 20]. But such studies do not give any indication of the form of magnesium, i.e. bound, complexed or ionized in the cells. Using digital-imaging analysis and a Mg^{2+} fluorescence probe, we found that, irrespective of the $[Mg^{2+}]_o$ used, the subcellular distribution of $[Mg^{2+}]_i$ appeared heterogeneous in *S. pombe* cells. With DAPI staining, we further defined $[Mg^{2+}]$ in three areas, i.e. nucleus, cytoplasm and sub-plasma membrane region, among each single yeast cell. Differences in $[Mg^{2+}]$ gradients from the periphery to the nuclei are clearly evident among these regions in these cells and show a relative order of concentration, where the sub-plasma membrane region > cytoplasm > nucleus (table 2). It is also noteworthy that in all three subcellular compartments $[Mg^{2+}]_i$ increased significantly, two- to threefold, concomitant with $[Mg^{2+}]_i$ when $[Mg^{2+}]_o$ was elevated to 1.97 mM (table 2). A spatial heterogeneity of $[Mg^{2+}]_i$ in *S. pombe* demonstrated that, in addition to the plasma membrane, the nuclear membrane and discrete localized processes within the cell may regulate Mg^{2+} activity at the subcellular level. The high $[Mg^{2+}]_i$ in the sub-plasma membrane area could represent Mg^{2+} release from magnesium binding sites and uptake elements there (probably the vacuoles) which limit Mg^{2+} diffusion. Further interpretation of our data is made difficult by our lack of knowledge of how Mg^{2+} is transported and the unavailability of confocal microscopy in the present studies. However, although specific molecular regulatory processes are not known, the $[Mg^{2+}]_i$ homeostasis revealed here could be critical, since the Michaelis constant values for Mg^{2+} activation and inhibition of many enzymes fall within the $[Mg^{2+}]i$ range found here.

Last, the demonstrated similarities to mammalian cells, as well as the ability of yeast cells to actively transport Mg^{2+} and maintain a fairly constant level of $[Mg^{2+}]_i$ in low $[Mg^{2+}]_o$, suggest that $[Mg^{2+}]_i$ could serve as a fundamental physiological regulator of cellular processes involved in metabolism and growth in eukaryotic cells.

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1 Zhao Y. and Lieberman H. B. (1995) *Schizosaccharomyces pombe*: a model for molecular studies of eukaryotic genes. DNA Cell Biol. **14:** 359–371
2 Allshire R. C. (1995) Elements of chromosome structure and function in fission yeast. Seminars Cell Biol. **6:** 55–64
3 Moreno S., Klar A. and Nurse P. (1991) Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. Methods Enzymol. **194:** 795–823
4 Walker G. M. (1986) Magnesium and cell cycle control: an update. Magnesium **5:** 9–23
5 Wu W. I. and Carman G. M. (1994) Regulation of phosphatidate phosphatase activity from the yeast *Saccharomyces cerevisiae* by nucleotides. J. Biol. Chem. **269:** 294–295
6 Bhargava P. and Mayalagu S. (1990) Magnesium induced activation of endogenous nuclease from isolated yeast nuclei: liberation of chromatin fragments of mono- and oligo-nucleosome size. Biochim. Internat. **20:** 1041–1048
7 Flatman P. W. (1984) Magnesium transport across cell membranes. J. Membr. Biol. **80:** 1–14
8 Grubbs R. D. and Maguire M. E. (1987) Magnesium as a regulatory cation: criteria and evaluation. Magnesium **6:** 113–127
9 Altura B. M., Zhang A. and Altura B. T. (1993) Magnesium, hypertension vascular diseases, atherogenesis, subcellular compartmentation of Ca^{2+} and Mg^{2+} and vascular contractility. Mineral Electr. Metab. **19:** 323–336
10 Leupold U. (1950) Die Verebung von homothallic and heterothallie bei *Schizosaccharomyces pombe*. Ctes rend Lab Carsberg, Ser Physiol. **24:** 381–480
11 Altura B. T., Shirey T. L., Young C. C., Dell'Orfano K., Hiti J., Welsh R. et al. (1994) Characterization of a new ion selective electrode for ionized magnesium in whole blood, plasma, serum, and aqueous samples. Scand. J. Clin. Lab. Invest. **54:** (Suppl. 217) 21–36
12 Zhang A., Cheng T. P.-O., Altura B. T. and Altura B. M. (1992) Extracellular magnesium regulates intracellular free Mg^{2+} in vascular smooth muscle cells. Eur. J. Physiol. **421:** 467–469
13 Raju R., Murphy E., Levy L. A., Hall R. D. and London R. E. (1989) A fluorescent indicator for measuring cytosolic free magnesium. Am. J. Physiol. **256:** C540–C548
14 Walker G. M. and Duffus J. H. J. (1980) Magnesium ions and the control of the cell cycle in yeast. Cell Sci. **42:** 329–356
15 Kotyk A. and Georghiou G. (1993) Effects of the physiology state of five yeast species on H^+-ATPase-related processes. Folia Microbiol. **38:** 467–472
16 Borst-Pauwels G. W. F. H. (1981) Ion transport in yeast. Biochim. Biophys. Acta **650:** 88–127
17 Boutry M., Foury F., and Goffeau A. (1977) Energy-dependent uptake of calcium by the yeast *Schizosaccharomyces pombe*. Biochim. Biophys. Acta **464:** 602–612
18 Sipiczki M. and Miklos I. (1993) Mycelial and syncytial growth in *Schizosaccharomyces pombe* induced by novel septation mutations. J. Cell Sci. **104:** 485–493
19 Altura B. M., Zhang A., Cheng T. P.-O. and Altura B. T. (1993) Cocaine induces rapid loss of intracellular free Mg^{2+} in cerebral vascular smooth muscle cells. Eur. J. Pharmacol. **246:** 299–301
20 Okorokov L. A., Lichko L. P., Kholodenko V. P., Kadomtseva V. M., Petrikevich S. B., Zaichkin E. I. et al. (1975) Free and bound magnesium in fungi and yeasts. Folia Microbiol. **20:** 460–466