

Review

Role of magnesium in genomic stability

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Abstract

In cellular systems, magnesium is the second most abundant element and is involved in basically all metabolic pathways. At physiologically relevant concentrations, magnesium itself is not genotoxic, but is highly required to maintain genomic stability. Besides its stabilizing effect on DNA and chromatin structure, magnesium is an essential cofactor in almost all enzymatic systems involved in DNA processing. Most obvious in studies on DNA replication, its function is not only charge-related, but very specific with respect to the high fidelity of DNA synthesis. Furthermore, as essential cofactor in nucleotide excision repair, base excision repair and mismatch repair magnesium is required for the removal of DNA damage generated by environmental mutagens, endogenous processes, and DNA replication. Intracellular magnesium concentrations are highly regulated and magnesium acts as an intracellular regulator of cell cycle control and apoptosis. As evident from animal experiments and epidemiological studies, magnesium deficiency may decrease membrane integrity and membrane function and increase the susceptibility to oxidative stress, cardiovascular heart diseases as well as accelerated aging. The relationship to tumor formation is more complex; magnesium appears to be protective at early stages but promotes the growth of existing tumors. With respect to the magnesium status in humans, the daily intake in most industrialized countries does not reach the current recommended daily dietary allowances (RDA) values, and thus marginal magnesium deficiencies are very common. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Magnesium is the second most abundant element in cellular systems. It exerts a large variety of biological functions, ranging from structural roles by complexing negatively charged groups, i.e. phosphates in nucleic acids, catalytic roles in enzyme activation or inhibition, and regulatory roles by modulating cell prolifer-

ation, cell cycle progression and differentiation. Even though less understood as compared to calcium homeostasis, the intracellular magnesium content appears to be regulated by Mg^{2+} uptake, efflux, and intracellular compartmentization, also in response to external stimuli. With respect to genomic stability, several aspects are of major importance. They include the role of magnesium in DNA replication and protein synthesis, its function as cofactor in DNA repair proteins, its role in maintaining the anti-oxidative status of the cell and finally its effect on cell cycle regulation and apoptosis. Magnesium deficiency or the displacement of Mg^{2+}

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by other toxic divalent metal ions leads to an increased genomic instability, as evident by inhibited DNA repair, oxidative stress, aging, and carcinogenicity.

1.1. Magnesium homeostasis and effects on cellular functions related to genomic stability

1.1.1. Magnesium homeostasis at the cellular level

While much attention has been given to elucidate the homeostasis of Ca^{2+} , H^+ , K^+ , or Na^+ , the knowledge about the intracellular magnesium distribution is still incomplete, mainly because of technical limitations measuring Mg^{2+} inside cells. The total magnesium content in various cell types ranges from 5 to 30 mM, while the free Mg^{2+} content has been determined mostly between 0.4 and 0.6 mM. Magnesium is transported across the plasma membrane in both directions, most likely via Na^+/Mg^+ exchange. Whether influx and efflux of Mg^{2+} are mediated by the same mechanism operating in opposite directions or whether there are distinct pathways is still not known. Both influx and efflux are hormonally controlled and regulated by modifications in intracellular cAMP levels and in protein kinase C activity. Concerning the intracellular distribution of magnesium, major intracellular compartments are mitochondria, the nucleus, and the endoplasmic reticulum (for reviews see [1–3]). As discussed in more detail below, bound magnesium can be mobilized from these compartments to increase the concentration of free intracellular Mg^{2+} if required, for example for cell cycle progression or apoptosis. Thus, the distribution of magnesium within cells is highly regulated, and many enzymes in different biochemical pathways are activated or inhibited by changes in free Mg^{2+} , some of which are relevant for maintaining genomic stability.

1.2. Effect of magnesium on the fidelity of DNA replication

One important prerequisite for maintaining the genomic integrity is the faithful transmission of genetic information during DNA replication. DNA templates are copied with remarkably high fidelity, checking for correct base-pair formation both at the nucleotide insertion and at subsequent DNA extension steps. Back in 1976, Loeb and coworkers have shown that magnesium ions are essential for the fidelity of

DNA replication. Even though Co^{2+} , Mn^{2+} , and Ni^{2+} could substitute for Mg^{2+} in DNA polymerases from different origins, the replacement resulted in a marked decrease of replication fidelity [4,5]. Only recently, crystal structures of different DNA polymerases complexed with DNA substrates and incoming nucleotides revealed the exact role of divalent metals, usually magnesium ions, in this process. Very similar in all different polymerases investigated, they position the nucleotide and promote phosphoryl transfer. Metal ion A interacts with the 3'-hydroxyl group of the primer strand and has been proposed to lower its $\text{p}K_a$, thereby facilitating its attack on the α -phosphate of the incoming dNTP. Metal ion B binds to and facilitates the leaving of the β - and γ -phosphates. Both metal ions together are also thought to stabilize the structure and charge of the pentavalent transition state occurring during this reaction [6–8].

1.3. Role of magnesium in DNA repair processes

DNA is continuously damaged by environmental mutagens and by endogenous processes. To keep mutation frequencies low, prokaryotic and eukaryotic cells have evolved different types of DNA repair systems. Nucleotide excision repair (NER) is mainly involved in the removal of DNA damage induced by environmental mutagens, such as UV radiation, polycyclic aromatic hydrocarbons, and heterocyclic aromatic amines. The repair process is mediated by the coordinated action of more than 20 different proteins, most of them involved in damage recognition and incision at both sides of the lesion, including those defect in xeroderma pigmentosum. Magnesium is an essential cofactor in basically all steps of NER. Thus, when applying an *in vitro* incision reaction, optimal magnesium concentrations were found to be 4.5–7 mM, whereas the incision was inhibited completely in the absence of Mg^{2+} as well as at very high concentrations (18 mM) [9]. Examples for incision factors with a reported requirement for magnesium are the DNA-damage recognition protein UV-DDBP [10], the helicase XPD [11], and the nuclease XPG [12]. Magnesium-dependent post-incision events are both polymerization as described above and ligation [13,14].

Perhaps most important among endogenous processes causing DNA damage, reactive oxygen species

(ROS) are generated as a consequence of oxygen metabolism, leading to DNA strand breaks, DNA–protein cross-links and a broad spectrum of oxidative DNA base modifications [15]. Further DNA damaging reactions are hydrolytic cleavages of N–glycosidic bonds causing apurinic/apyrimidinic sites, hydrolytic desaminations as well as methylations of oxygen and nitrogen atoms in DNA bases [16]. Endogenous DNA damage is mainly repaired by base excision repair (BER). According to current models, BER is started by removal of the modified base by a specific N–glycosylase, generating an AP site, which is subsequently incised at its 5' side by an AP endonuclease. After excision of the 5' terminal dRP, the single nucleotide gap is filled by DNA polymerase β and sealed by ligase. Alternatively, AP sites are further processed involving proliferating cell nuclear antigen and DNA polymerase δ for the excision and DNA synthesis reaction (for recent reviews see [17]). While DNA glycosylases usually do not require magnesium, the opposite is true for enzymes involved in later steps of BER. Thus, hydrolytic nucleases usually depend on the participation of metal ions, mainly magnesium, at active sites, which are involved either in substrate interactions or directly in the cleavage of the phosphate–oxygen bond. During the last few years, the exact role of magnesium ions has been elucidated in some cases. For example, in the major human AP site-specific DNA repair endonuclease HAP 1 (also called APE or Ref-1), a single magnesium ion binds to a defined Glu residue in the active center and aids the attack on the P–O3' bond by polarization of the P–O bond, and perhaps by correctly orientating the phosphate group rather than directly participating in the nucleolysis reaction. Even though the enzyme can be activated in principle by manganese and nickel ions as well, its activity is greatly reduced (50 and 90%, respectively) as compared to magnesium [18]. Other examples of magnesium-dependent endonucleases in BER are the mammalian apurinic/apyrimidinic endonuclease activity associated with the 5-hydroxymethyluracil-DNA glycosylase [19] as well as the flap-endonuclease-1 (FEN-1), a structure-specific endonuclease involved in DNA replication and DNA repair [20,21]. Finally, not only the DNA synthesis mediated by pol β , but also its DNA deoxyribophosphodiesterase (dRPase) activity acts in a Mg^{2+} -dependent manner [22].

The third DNA repair system which has attracted special attention during the last few years is the mismatch repair pathway (MMR). MMR contributes greatly to genomic stability by correcting replication errors; defects in MMR have been associated with increased susceptibility to hereditary nonpolyposis colon cancer (HNPCC). In prokaryotes, mismatches are recognized by MutS and initiation as well as subsequent steps in methyl-directed repair depend on MutL, which interacts with the sequence-dependent endonuclease MutH and UvrD helicase [23]. Analysis of the crystal structure of MutL revealed an ATPase activity which is highly conserved also in eucaryotic homologs [24]. This activity has been proposed to play a role in switching from a mismatch-repair initiation to a processing complex. It appears to be essential for the entire repair function, since the majority of mutations in human MLH1 found in HNPCC patients and almost all of the mutations in *Escherichia coli* MutL causing the mutator phenotype are located in or around the ATP-binding site. Recent studies show that MutL has an absolute requirement for Mg^{2+} ; in the absence of magnesium, the MutL–ATP association is abolished completely [25]. In addition to being involved in MMR, the eucaryotic homologs MLH and PMS have been shown to play a role in gene conversion and chromosome segregation during and after meiosis [26,27].

Finally, magnesium is also required for double-strand break repair arising for example after ionizing radiation and during meiosis. In this context, the hPOMp75 protein isolated from HeLa nuclei has been shown to catalyze DNA annealing and D-loop formation during homologous recombination in an ATP-independent but Mg^{2+} -dependent reaction. Supporting its role in the maintenance of genomic integrity, this protein has recently been shown to be identical to the human proto-oncoprotein TLS commonly mutated in liposarcomas [28].

1.4. Effect of magnesium on chromosome structure and segregation

Under physiological conditions of pH and intracellular K^+ and Mg^{2+} concentrations, approximately 0.2 mol Mg^{2+} is bound per mole phosphate in polynucleotides, and in case of DNA, stabilizes the double helix (reviewed in [29]). Moreover, magnesium retains

the chromatin structure, and critical levels of Mg^{2+} are required for the maintenance of the compact state of heterochromatin [30]. Besides these electrostatic interactions, magnesium is also involved in chromosome function. For example, both free Mg^{2+} as well as GTP-Mg play an essential role in the extent of tubulin polymerization and thus in chromosome segregation during mitosis [31].

1.5. Role of magnesium in cell cycle progression, differentiation, and apoptosis

Accumulating evidence supports the involvement of magnesium in the regulation of cell cycle, proliferation, apoptosis, and differentiation [32]. Back in 1975, Rubin proposed magnesium as a key factor in the coordinate control of metabolism and growth in animal cells [33]. Later on, Maguire [34] summarized evidence for magnesium to be a regulated and regulatory cation, mainly based on data concerning the regulation of intracellular magnesium concentrations, the hormonal regulation of Mg^{2+} transport, and the intracellular compartmentation of Mg^{2+} . Even though at that time no specific physiological event was known which was regulated by magnesium, he concluded that Mg^{2+} may be a chronic regulatory agent as opposed to Ca^{2+} , which mediates acute “on-off” signals. Nevertheless, these early studies were limited by the lack of simple and direct methods to study intracellular free Mg^{2+} in living cells. In the meantime, the role of magnesium in the regulation of distinct cellular processes has been elucidated in more detail. Thus, levels of free intracellular magnesium increase in cells undergoing apoptosis. This increase is an early event in apoptosis, preceding DNA fragmentation and externalization of phosphatidylserine, and is likely due to a mobilization of magnesium from mitochondria. Therefore, the raise in intracellular free magnesium appears to serve as a “second messenger” for downstream events in apoptosis [35]. On the protein level, Ca^{2+} - and Mg^{2+} -dependent endonucleases have been implicated in DNA fragmentation during apoptosis [36]. With respect to proliferation and differentiation, it has been shown that magnesium restriction causes differentiation in human leukemic HL-60 cells. Again, a shift in intracellular magnesium was observed on these conditions: while the total magnesium content markedly decreased, no change occurred

in the concentration of free intracellular magnesium. As possible reason for cell cycle arrest and differentiation, a marked increase in the expression level of the CDK inhibitor p27^{kip1}, a negative regulator of cell cycle progression, has been observed [37]. Taken together, current evidence suggests that besides being a cofactor of many enzymatic reactions involved in basically all cellular processes, magnesium is involved in the regulation of cell cycle control and apoptosis, mediated mainly by the mobilization of magnesium from intracellular pools. Nevertheless, much remains to be done on this field to further clarify this aspect.

2. Genotoxicity and anti-genotoxicity

From studies conducted either in bacteria or in mammalian cells in culture, there is no evidence for genotoxic effects of magnesium salts at physiologically relevant doses. Thus, at concentrations up to 20 mM magnesium sulfate, no increased mutation frequency was observed after treatment of diverse strains of *Salmonella typhimurium* and no enhancement of chromosomal aberrations was seen in Chinese hamster lung cells [38]. Similarly, no enhanced frequency of sister-chromatid exchanges was observed in Chinese hamster ovary (CHO) cells [39]. Only at 8 mg/ml (40 mM) $MgCl_2$, an increase in chromatid gaps and chromatid breaks in Chinese hamster lung fibroblasts was detected, most likely due to ionic imbalance [40]. In contrast, magnesium exerts pronounced protective effects in combination with certain transition metals. For example, raising the extracellular concentration of $MgCl_2$ inhibited nickel-induced DNA strand breaks, DNA-protein cross-links, sister-chromatid exchanges, chromosomal aberrations, and cell transformation [39]. Furthermore, the addition of magnesium prevented nickel- and cobalt-induced inhibition of nucleotide excision repair [41,42]. The reason for these anti-genotoxic effects may include not only altered metal transport at the cellular level, but also competitions at critical binding sites. Thus, excess magnesium given to nuclear extracts of nickel-treated human HeLa cells reversed the nickel-induced disturbance of DNA-protein interactions involved in DNA-damage recognition during nucleotide excision repair, indicating the displacement of Mg^{2+} by Ni^{2+} and vice versa. Nevertheless, these

protective effects are metal-specific, since in the same test system no reversal was seen in case of nuclear extracts derived from cadmium-treated cells [43].

3. Carcinogenicity, anti-carcinogenicity, and aging as related to nutrition in animals and humans

The relationship between magnesium and cancer is rather complex, and several aspects have to be considered separately, including (i) the potential impact of magnesium deficiency on tumor incidences, (ii) disturbed magnesium homeostasis frequently observed in tumor cells, and (iii) the effect of either magnesium deficiency or supplementation on the progression of existing tumors.

3.1. Animal studies

In a long-term feeding study in mice, magnesium chloride at dose levels up to 2% in the diet was shown to be not carcinogenic [44]. On the other hand, there is convincing evidence from animal studies that magnesium-deficient diets increase the incidences of thymic tumors and leukemias [45,46]. Furthermore, magnesium-deficient diets increased chromosomal aberrations in rats in maternal and fetal tissues [47]. However, as emphasized by Durlach et al. [48], carcinogenic effects of magnesium deficiency were restricted to rats and not observed in other species.

In combination with other carcinogens, magnesium has been shown to increase or decrease their tumorigenic potency, depending on the compound investigated and on the experimental conditions. This issue has been extensively reviewed [47,49] and the main findings can be summarized as follows. With respect to toxic metal compounds, magnesium prevented the formation of pulmonary tumors after i.p. injections of nickel or lead in mice [50]. Furthermore, when magnesium carbonate was administered together with the strong carcinogen Ni_3S_2 i.m. in male Fischer rats, it strongly inhibited the formation of local tumors as well as kidney tumors after simultaneous injection into the renal cortex; however, no protection towards Ni_3S_2 was seen after dietary treatment with magnesium carbonate. On the other hand, dietary magnesium did protect from 3-methyl cholantrene-induced fibrosarcomas [51]. Enhanced tumor incidences in

the respiratory tract were observed when magnesium oxide was used as a carrier dust for intra-tracheal instillation of benzo(a)pyrene [52].

Nevertheless, it is important to emphasize that the predominantly protecting effects of magnesium exist only at the very early stage of tumorigenesis. Thus, magnesium deficiency antagonizes tumor implantation and inhibits growth of induced or spontaneous tumors in the rat (e.g. [53]), while magnesium intake stimulates tumor development. The reason appears to be a disturbance in magnesium homeostasis in carcinogenesis, leading to magnesium accumulation in tumors with magnesium depletion in non-neoplastic tissues. These alterations in magnesium distribution may play an important role on the neoplastic development at the membrane, cytosol, or chromatin level [48].

One other aspect repeatedly discussed in literature is the relationship between magnesium deficiency and oxidative stress. Thus, magnesium-deficient animals show an increased susceptibility to an in vivo oxidative stress, and their tissues are more susceptible to in vitro peroxidation. Consequences are increased oxidative damage of cellular lipids, proteins, and nucleic acids, which may lead to altered membrane functions, perturbations of intracellular calcium metabolism, cardiovascular diseases, accelerated aging, and carcinogenesis (for reviews see [54]). This is also supported experimentally in isolated bovine endothelial cells: when growing in magnesium-deficient medium, they exerted higher levels of lipid peroxidation and intracellular oxidative injury after exposure to oxygen radicals produced by dihydroxyfumarate and Fe^{3+} -ADP as compared to control cells [55].

Finally, experimental magnesium deficiency leads to alterations in the immune response. Thus, for example, rats on magnesium-deficient diet showed accelerated thymus involution accompanied by enhanced apoptosis [56].

3.2. Humans

The average dietary intake of magnesium is approximately 300 mg per day; main sources are green vegetables, grains, meat, and — depending on its hardness — drinking water. Magnesium balance is mediated by intestinal absorption and renal excretion. Thus, the amount of magnesium absorbed in the small intestine from a low magnesium diet may be as high as

75%, from a high magnesium diet as low as 25% [57]. In most industrialized countries, magnesium intake is marginal in the entire population. Recommended daily dietary allowances (RDA) values for the US have been increased recently to 320 mg Mg per day for adult women and to 420 mg Mg per day for adult men [58]. In Germany, Switzerland, and Austria, the reference values are 300 for women and 350 for men of 25 years and older [59]. However, the actual magnesium intake has been found to be 67–77% of RDA in West Germany, England, and some regions of North America. Even lower magnesium intakes of barely 50% RDA were observed in Newfoundland and Japan [60]. These low magnesium intakes were confirmed in a recent study conducted in Germany, where magnesium intake and balance were determined in a control and in a magnesium-supplemented group. Additionally, the authors showed that a full balance required at least 375 mg Mg per day for women and men, while positive balances were observed between 950 and 1020 mg Mg per day [61]. Whether the current RDA values are sufficient in stress situations and to prevent cardiovascular diseases was also questioned by Seelig [62], emphasizing inter-individual genetic differences and interactions with other dietary constituents in magnesium-dependent reactions. Reasons

for magnesium deficiency can be manifold. They include inadequate dietary intake, malabsorption in the gastrointestinal tract, and renal losses, for example due to a drug therapy with diuretics. Alterations in magnesium metabolism are being increasingly implicated as significant factor in the pathogenesis of ischemic heart disease (IDH); hypomagnesemia has been shown to cause an increased incidence of arrhythmias, coronary vasospasm, and hypertension, and may predispose to the pathogenesis of atherosclerosis [63]. On the other hand, oral magnesium supplementation of magnesium-deficient persons can lower blood pressure, improve brain performance, concentration, and stress tolerance [60]. As emphasized by Durlach et al. [64], magnesium deficiency may be particularly pronounced in elderly persons, since magnesium absorption decreases with age, and in some cases, urinary magnesium leakage may be increased, leading to a reduction of magnesium-exchangeable pools. Due to altered membrane functions and increased susceptibility to oxidative stress described above, magnesium-deficiency may constitute an important factor in accelerated aging processes. With respect to carcinogenicity, early epidemiological studies pointed towards higher incidences of neoplasms and lymphoproliferative disorders in region with low magnesium

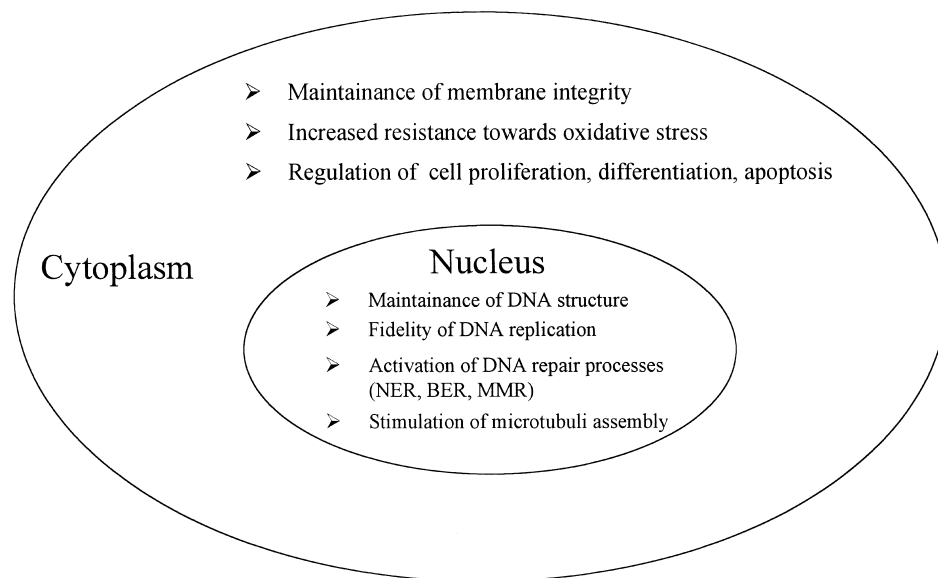


Fig. 1. Effect of magnesium on cellular processes related to genomic stability.

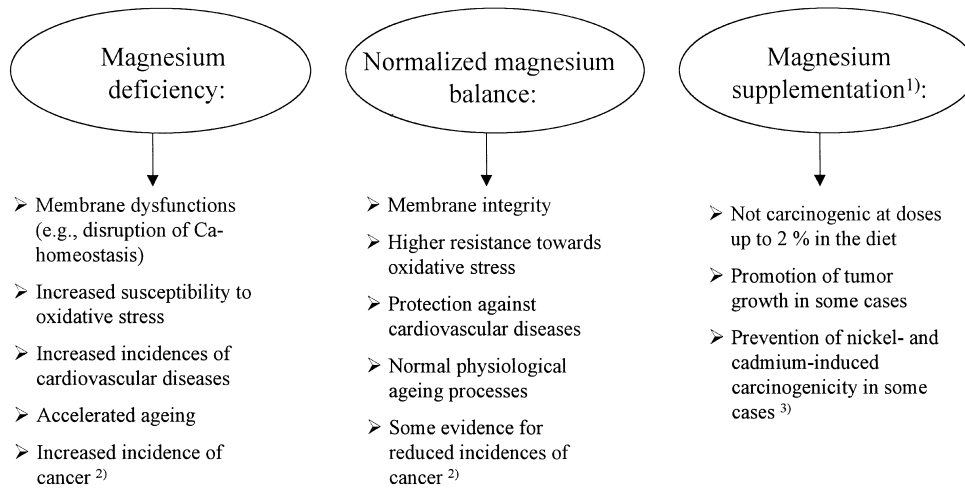


Fig. 2. Proposed effects of magnesium status on genomic stability in experimental animals and humans: (1) results from animal studies; (2) protective effects are restricted to very early stages of tumor development; (3) requires simultaneous administration via the same route; dietary supplementation usually ineffective. For references and further details see text.

contents in soil and drinking water (reviewed in [65]). This is supported by a recent case-control study conducted in Taiwan, where high levels of magnesium in drinking water exerted protective effects against gastric cancer [66].

4. Conclusions and perspectives

As evident from the multiple functions of magnesium in basically all cellular processes, magnesium is highly required to maintain genomic stability (summarized in Fig. 1). Being not genotoxic at physiologically relevant concentrations itself, it keeps mutation frequencies low by facilitating high replication fidelity and by supporting basically all DNA repair processes as well as chromosome segregation during mitosis. In some cases, it exerts protective effects in combination with other genotoxic and/or carcinogenic agents such as certain toxic metal ions, most likely by competition at critical binding sites. Thus, it is not surprising that intracellular magnesium is compartmentalized, and changes in free Mg^{2+} concentrations serve as signals for cell cycle regulation and apoptosis. Animal experiments and epidemiological studies show inverse correlations between magnesium status and cardiovascular diseases, and magnesium deficiency appears

to be one susceptibility factor for accelerated aging. Nevertheless, the relationship between magnesium status and tumorigenesis is more complex: while magnesium deficiency tends to increase tumor incidences in animals and humans, magnesium promotes the growth of pre-existing tumors due to profound changes of magnesium homeostasis in tumor cells; thus, protecting effects are restricted to early steps in tumor development (summarized in Fig. 2). With respect to magnesium status in humans, magnesium intake in many countries is considerably lower compared to current RDA values and marginal magnesium deficiency is very common. Thus, it is highly recommended to increase actual magnesium intake to maintain genomic stability and to protect from age-related diseases.

References

- [1] A. Romani, A. Scarpa, Regulation of cell magnesium, *Arch. Biochem. Biophys.* 298 (1992) 1–12.
- [2] R.D. Grubbs, Hormonal regulation of magnesium homeostasis in cultured mammalian cells, in: H. Sigel, A. Sigel (Eds.), *Metal Ions in Biological Systems*, Marcel Dekker, New York, 1990, pp. 177–192.
- [3] T. Guenther, Membrane transport of magnesium, in: H. Sigel, A. Sigel (Eds.), *Metal Ions in Biological Systems*, Marcel Dekker, New York, 1990, pp. 215–225.
- [4] M.A. Sirover, L.A. Loeb, Metal activation of DNA synthesis, *Biochem. Biophys. Res. Commun.* 70 (1976) 812–817.

- [5] M.A. Sirover, L.A. Loeb, On the fidelity of DNA replication, *J. Biol. Chem.* 252 (1977) 3605–3610.
- [6] S. Doublié, S. Tabor, A.M. Long, C.C. Richardson, T. Ellenberger, Crystal structure of a bacteriophage T7 DNA replication complex at 2.2 Å resolution, *Nature* 391 (1998) 251–258.
- [7] J.R. Kiefer, C. Mao, J.C. Braman, L.S. Beese, Visualization DNA replication in a catalytically active *Bacillus* DNA polymerase crystal, *Nature* 391 (1998) 304–307.
- [8] T.A. Steitz, DNA polymerases: structural diversity and common mechanisms, *J. Biol. Chem.* 274 (1999) 17395–17398.
- [9] P. Calsou, B. Salles, Properties of damage-dependent DNA incision by nucleotide excision repair in human cell-free extracts, *Nucleic Acids Res.* 22 (1994) 4937–4942.
- [10] S. Keeney, G.J. Chang, S. Linn, Characterization of a human DNA damage binding protein implicated in xeroderma pigmentosum E, *J. Biol. Chem.* 268 (1993) 21293–21300.
- [11] P. Sung, V. Bailly, C. Weber, L.H. Thompson, L. Prakash, S. Prakash, Human xeroderma pigmentosum group D gene encodes a DNA helicase, *Nature* 365 (1993) 852–855.
- [12] A. O'Donovan, R.D. Wood, Identical defects in DNA repair in xeroderma pigmentosum group G and rodent ERCC group 5, *Nature* 363 (1993) 185–188.
- [13] H. Teraoka, K. Tsukada, Eucaryotic DNA ligase. Purification and properties of the enzyme from bovine thymus, and immunochemical studies of the enzyme from animal tissues, *J. Biol. Chem.* 257 (1982) 4758–4763.
- [14] S.W. Yang, J.Y.H. Chan, Analysis of the formation of AMP-DNA intermediate and the successive reaction by human DNA ligases I and II, *J. Biol. Chem.* 267 (1992) 8117–8122.
- [15] B. Halliwell, O.I. Aruoma, DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian cells, *FEBS Lett.* 281 (1991) 9–19.
- [16] T. Lindahl, Instability and decay of the primary structure of DNA, *Nature* 362 (1993) 709–715.
- [17] V.A. Bohr, G.L. Dianov, Oxidative DNA damage processing in nuclear and mitochondrial DNA, *Biochimie* 81 (1999) 155–160.
- [18] G. Barzilay, C.D. Mol, C.N. Robson, L.J. Walker, R.P. Cunningham, J.A. Tainer, I.D. Hickson, Identification of critical active-site residues in the multifunctional human DNA repair enzyme HAP1, *Nat. Struct. Biol.* 2 (1995) 561–568.
- [19] S. Cannon-Carlson, H. Gokhale, G.W. Teebor, Purification and characterization of 5-hydroxymethyluracil-DNA glycosylase from calf thymus, *J. Biol. Chem.* 264 (1989) 13306–13312.
- [20] B. Shen, J.P. Nolan, L.A. Sklar, M. Park, Essential amino acids for substrate binding and catalysis of human flap endonuclease 1, *J. Biol. Chem.* 271 (1996) 9173–9176.
- [21] G. Frank, J. Qiu, M. Somsouk, Y. Wenig, L. Somsouk, J.P. Nolan, B. Shen, Partial functional deficiency of E160D flap endonuclease-1 mutant in vitro and in vivo is due to defective cleavage of DNA substrates, *J. Biol. Chem.* 273 (1998) 33064–33072.
- [22] Y. Matsumoto, K. Kim, Excision of deoxyribose phosphate residues by DNA polymerase β during DNA repair, *Science* 269 (1995) 699–702.
- [23] P. Modrich, R. Lahue, Mismatch repair in replication fidelity, genetic recombination, and cancer biology, *Annu. Rev. Biochem.* 65 (1996) 101–133.
- [24] C. Ban, W. Yang, Crystal structure and ATPase activity of MutL: implications for DNA repair and mutagenesis, *Cell* 95 (1998) 541–552.
- [25] C. Ban, M. Junop, W. Yang, Transformation of MutL by ATP binding and hydrolysis: a switch in DNA mismatch repair, *Cell* 97 (1999) 85–97.
- [26] M.S. Williamson, J.C. Garne, S. Fogel, Meiotic gene conversion mutants in *Saccharomyces cerevisiae*. I. Isolation and characterization of pms1-1 and pms1-2, *Genetics* 110 (1985) 609–646.
- [27] N. Hunter, R.H. Borts, Mlh1 is unique among mismatch repair proteins in its ability to promote crossing-over during meiosis, *Genes Dev.* 11 (1997) 1433–1442.
- [28] H. Baechtold, M. Kuroda, J. Sok, D. Ron, B.S. Lopez, A. Akhmedov, Human 75 kDa DNA pairing protein is identical to the pro-oncoprotein TLS/FUS and is able to promote D-loop formation, *J. Biol. Chem.* 274 (1999) 34337–34342.
- [29] G.L. Eichhorn, The effect of metal ions on the structure and function of nucleic acids, in: G.L. Eichhorn, L.G. Marzilli (Eds.), *Metal Ions in Genetic Information Transfer*, Elsevier, New York, 1981, pp. 1–46.
- [30] A. Weith, Mg²⁺-dependent compactness of heterochromatic chromosome segments, *Exp. Cell Res.* 146 (1983) 199–203.
- [31] S. Grover, E. Hamel, The magnesium-GTP interaction in microtubule assembly, *Eur. J. Biochem.* 222 (1994) 163–172.
- [32] F.I. Wolf, A. Cittadini, Magnesium in cell proliferation and differentiation, *Frontiers Biosci.* 4 (1999) 1–11.
- [33] H. Rubin, Central role for magnesium in coordinate control of metabolism and growth in animal cell, *Proc. Natl. Acad. Sci. U.S.A.* 72 (1975) 3551–3555.
- [34] M.E. Maguire, Magnesium: a regulated and regulatory cation, in: H. Sigel, A. Sigel (Eds.), *Metal Ions in Biological Systems*, Marcel Dekker, New York, 1990, pp. 135–153.
- [35] M.M. Chien, K.E. Zahradka, M.K. Newell, J.H. Freed, Fas-induced B cell apoptosis requires an increase in free cytosolic magnesium as an early event, *J. Biol. Chem.* 274 (1999) 7059–7066.
- [36] C. Giannakis, I.J. Forbes, P.D. Zalewski, Calcium/magnesium dependent nuclease: tissue distribution, relationship to inter-nucleosomal DNA fragmentation and inhibition by zinc, *Biochem. Biophys. Res. Commun.* 181 (1991) 915–920.
- [37] V. Covacci, N. Bruzzese, A. Sgambato, A. Di Francesco, M.A. Russo, F.I. Wolf, A. Cittadini, Magnesium restriction induces granulocytic differentiation and expression of P27^{Kip1} in human leukemic HL-60 cells, *J. Cell. Biochem.* 70 (1998) 313–322.
- [38] Y. Oguma, F. Yokota, K. Inoue, K. Shimamura, Mutagenicity studies of magnesium sulfate — reverse mutation test with bacteria and chromosomal aberration test with mammalian cells in culture, *J. Toxicol. Sci.* 23 (Suppl. 1) (1998) 81–90.
- [39] K. Conway, X.-W. Wang, L. Xu, M. Costa, Effect of magnesium on nickel-induced genotoxicity and cell transformation, *Carcinogenesis* 8 (1987) 1115–1121.

- [40] J. Ashby, M. Ishidate Jr., Clastogenicity in vitro of the Na, K, Ca and Mg salts of saccharin, and of magnesium chloride, consideration of significance, *Mutat. Res.* 163 (1986) 63–73.
- [41] A. Hartwig, L.H.F. Mullenders, R. Schlepegrell, U. Kasten, D. Beyersmann, Nickel(II) interferes with the incision step in nucleotide excision repair, *Cancer Res.* 54 (1994) 4045–4051.
- [42] U. Kasten, L.H.F. Mullenders, A. Hartwig, Cobalt(II) inhibits the incision and the polymerization step of nucleotide excision repair in human fibroblasts, *Mutat. Res.* 383 (1997) 81–89.
- [43] M. Hartmann, A. Hartwig, Disturbance of DNA damage recognition after UV-irradiation by nickel(II) and cadmium(II) in mammalian cells, *Carcinogenesis* 19 (1998) 617–621.
- [44] Y. Kurata, S. Tamano, M.A. Shibata, A. Hagiwara, S. Fukushima, N. Ito, Lack of carcinogenicity of magnesium chloride in a long-term feeding study in B6C3F1 mice, *Food Chem. Toxicol.* 27 (1989) 559–563.
- [45] P. Bois, Tumour of the thymus in magnesium-deficient rats, *Nature* 204 (1964) 1316.
- [46] K.S. Kasprzak, M.P. Waalkes, The role of calcium, magnesium, and zinc in carcinogenesis, in: L.A. Poirier, P.M. Newbame, M.W. Pariza (Eds.), *Essential Nutrients in Carcinogenesis*, Plenum Press, New York, 1986, pp. 497–515.
- [47] L.T. Bell, M. Branstrator, C. Roux, L.S. Hurley, Chromosomal abnormalities in maternal and fetal tissues of magnesium- or zinc-deficient rats, *Teratology* 12 (1975) 221–226.
- [48] J. Durlach, M. Bara, A. Guet-Bara, Magnesium and its relationship to oncology, in: H. Sigel, A. Sigel (Eds.), *Metal Ions in Biological Systems*, Marcel Dekker, New York, 1990, pp. 549–578.
- [49] K.S. Kasprzak, Effects of calcium, magnesium, zinc, and iron on nickel carcinogenesis: inhibition versus enhancement, in: N.D. Hadjilias (Ed.), *Cytotoxic, Mutagenic and Carcinogenic Potential of Heavy Metals Related to Human Environment*, Kluwer Academic Publishers, Dordrecht, 1997, pp. 93–106.
- [50] L.A. Poirier, J.C. Theiss, L.J. Arnold, M.B. Shimkin, Inhibition by magnesium and calcium acetates of lead subacetate- and nickel acetate-induced lung tumors in strain A mice, *Cancer Res.* 44 (1984) 1520–1522.
- [51] T. Patiroglu, G. Sahin, O. Kontas, K. Üzümlü, R. Raraymen, Protective effect of magnesium supplementation on experimental 3-methyl cholantrene-induced fibrosarcoma and changes in tissue magnesium distribution during carcinogenesis in rats, *Biol. Trace Element Res.* 56 (1997) 179–185.
- [52] F. Stenback, A. Sellakumar, P. Shubik, Magnesium oxide as carrier dust in benzo[a]pyrene-induced lung carcinogenesis in Syrian hamsters, *J. Natl. Cancer Inst.* 54 (1975) 861–867.
- [53] B.J. Mills, R.D. Lindemann, C.A. Lang, Magnesium deficiency inhibits biosynthesis of blood glutathione and tumor growth in the rat, *Proc. Soc. Exp. Biol. Med.* 181 (1986) 326–332.
- [54] Y. Rayssiguier, J. Durlach, E. Gueux, E. Rock, A. Mazur, Magnesium and ageing. I. Experimental data: importance of oxidative damage, *Magnesium Res.* 6 (1993) 369–378.
- [55] B.F. Dickens, W.B. Weglicki, Y.-S. Li, I.T. Mak, Magnesium deficiency in vitro enhances free radical-induced intracellular oxidation and cytotoxicity in endothelial cells, *FEBS Lett.* 311 (1992) 187–191.
- [56] C. Malpuech-Brugere, W. Wojciech, E. Gueux, J. Kuryszko, E. Rock, Y. Rayssiguier, A. Mazur, Accelerated thymus involution in magnesium-deficient rats is related to enhanced apoptosis and sensitivity to oxidative stress, *Br. J. Nutr.* 81 (1999) 405–411.
- [57] R.J. Elin, The assessment of magnesium status in humans, in: H. Sigel, A. Sigel (Eds.), *Metal Ions in Biological Systems*, Marcel Dekker, New York, 1990, pp. 579–596.
- [58] Food and Nutrition Board, *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*, National Academy Press, Washington, DC, 1999.
- [59] Deutsche Gesellschaft für Ernährung (DGE), Österreichische Gesellschaft für Ernährung (ÖGE), Schweizerische Gesellschaft für Ernährung (SGE), Schweizerische Vereinigung für Ernährung (SVE), *Referenzwerte für die Nährstoffzufuhr*, Umschau Braus GmbH, Frankfurt am Main, 2000.
- [60] J.R. Marier, Dietary magnesium and drinking water, in: H. Sigel, A. Sigel (Eds.), *Metal Ions in Biological Systems*, Marcel Dekker, New York, 1990, pp. 85–104.
- [61] M. Wangemann, A. Selzer, C. Leitzmann, S. Golf, V. Graef, N. Katz, *Empfehlungen zur Magnesium-Zufuhr*, *Magnesium Bull.* 17 (1995) 79–85.
- [62] M.S. Seelig, Nutritional status and requirements of magnesium, *Magnesium Bull.* 8 (1986) 170–185.
- [63] N. Brautbar, A.T. Roy, P. Hom, D.B.N. Lee, Hypomagnesia and hypermagnesia, in: H. Sigel, A. Sigel (Eds.), *Metal Ions in Biological Systems*, Marcel Dekker, New York, 1990, pp. 285–320.
- [64] J. Durlach, P. Bac, V. Durlach, Y. Rayssiguier, M. Bara, A. Guet-Bara, Magnesium status and ageing: an update, *Magnesium Res.* 11 (1997) 25–42.
- [65] J.M. Blondell, The anticarcinogenic effect of magnesium, *Med. Hypotheses* 6 (1980) 863–871.
- [66] C.-Y. Yang, M.-F. Cheng, S.-S. Tsai, Y.-L. Hsieh, Calcium, magnesium, and nitrate in drinking water and gastric cancer, *Jpn. J. Cancer Res.* 89 (1998) 124–130.