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Chromatin structure and transcriptional activity of MAG gene

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Abstract. Myelin associated glycoprotein (MAG) is an essential component of the periaxonal architecture of the myelin sheath. Because of its potent neurite growth repressive activity, MAG is also likely to play an important role in axonal guidance during the CNS development, and to be responsible for abortive neuronal regeneration in adult CNS. The MAG gene chromatin from approximately -1.6 to +0.6 kb features MNase hypersensitivity that maydelineate the gene control region. The proximal upstream region of the gene is organized into an array of five nucleosomes with hypersensitive linkers. The core promoter is located within the first upstream linker that becomes highly hypersensitive in the course of oligodendrocyte differentiation. The adjacent upstream region contains positive and negative enhancers that are likely to streamline oligodendrocyte specific expression of the gene. The TATA-less core promoter contains novel, as yet uncharacterized initiator elements that direct the assembly of transcriptional complexes. The promoter appears to be controlled by both, the addition of activating trans-factors and the removal of inhibitory trans-factors as progenitor cells differentiate into mature oligodendrocyte. The developmental activation of the gene is also concomitant with profound DNA demethylation that may provide auxiliary regulatory mechanisms. Hence, the upregulation of the MAG gene in differentiating oligodendrocytes entails chromatin remodeling as well as changes in the assortment of nuclear trans-factors.

Key words: myelin associated glycoprotein gene, oligodendrocytes, trascription, chromatin

Myelin associated glycoprotein (MAG) is a highly glycosylated, transmembrane protein expressed exclusively by myelin-forming cells, i.e., oligodendrocytes in the CNS, and Schwann cells in the PNS. MAG is a member of the immunoglobulin superfamily (its large extracellular portion contains five Ig-like domains), and shares structural similarity to such potent cell recognition molecules of the nervous system as N-CAM, L1, TAG-1, contactin, faciclin and neuroglian (Yoshihara et al. 1991). MAG is also functionally coupled to Fyn tyrosine kinase, and acts as a transmembrane signal transducer across the periaxonal membrane (Umemori et al. 1994). MAG has been postulated to mediate the cell-cell recognition events during initial stages of myelinogenesis (Quarles et al. 1990), and to be essential for the formation and stabilization of the periaxonal architecture of the myelin sheath (Umemori et al. 1994, Fruttiger et al. 1995). Recent demonstration that MAG is the dominant component of myelin that suppresses neurite growth (McKerracker et al. 1994, Mukhopadhyay et al. 1994) extends the biological functions of MAG beyond its role in myelinogenesis into putative involvement in axonal guidance during the CNS development, and later on in the stabilization of neuronal network by preventing axonal sprouting. The inhibitory effect on neurite growth, however, may play a pivotal role in the suppression of neuronal regeneration in the adult CNS.

The MAG gene features temporal and tissue--specific expression in myelin-forming cells, however, the mechanisms of this developmental regulation are poorly understood. The MAG gene is also expressed in rat glioma C6 cells (Bong et al. 1991, Kanoh et al. 1991), and hence, this cell line provides a convenient model system to study transcriptional mechanisms of the gene. For example, the gene expression can be experimentally manipulated by a variety of agents and culture conditions (Bong et al. 1991, Kanoh et al. 1991, 1992, Laszkiewicz et al. 1992, Zhu et al. 1992, 1994, Ye et al. 1992, 1994). The most profound upregulation of the gene can be induced by cAMP potentiators (Ye et al. 1992, 1994), while retinoic acid abrogates the gene expression in C6 cells (Zhu et al. 1992, 1994). Furthermore, the kinetics and extent of the MAG gene response to various treatments usually differs from the response of the PLP gene that encodes major structural protein of CNS myelin. Also, the expression of the MAG gene in quaking mutant brain is significantly upregulated, whereas the expression of other myelin-specific genes is down-regulated (Konat et al. 1988). These findings indicate that the MAG gene is controlled by unique transcriptional mechanisms.

The rat MAG gene encompasses approximately 16 kb, contains 13 exons, and expresses a multiplicity of mRNAs through alternative splicing and polyadenylation (Lai et al. 1987). Primary structure of 4.4 kb of the 5'end of rat MAG gene encompassing approximately 2.8 kb of upstream and 1.6 kb of intragenic sequences has been determined (Montag 1992). Chromatin analysis (Grubinska et al. 1994)) revealed a broad region of nuclease hypersensitivity stretching from approximately -1.6 to +0.6 kb that may delineate the gene control region. Out of nine distinguishable hypersensitive sites (HS), two are located within the transcription unit, namely, in intron 1 and at exon 2/intron 2 junction. The first upstream HS site comaps with the core promoter of the gene (*vide infra*). High resolution HS mapping showed that the region directly upstream from the structural gene is organized into an array of nucleosomes with hypersensitive linkers (Konat et al. 1995, Laszkiewicz et al. 1995). These linkers represent the initial five upstream HS sites.

Genomic DNA of the MAG gene showed a highly heterogenous methylation pattern among cells and tissues as assessed by digestion with methylation sensitive restriction enzymes (Grubinska et al. 1994). For example, liver DNA (non-expressing cells) was the most heavily methylated. In contrast, most of the testable CpG palindromes were partly demethylated in the brain, and in cultured oligodendrocyte lineage cells. Two sites located at approximately -1.9 and at -0.1 kb were significantly less methylated in mature oligodendrocytes than in O-2A progenitor cells, indicating progressive demethylation concomitant with oligodendrocyte dif-

ferentiation. However, certain CpG dinucleotides remained heavily methylated even in the fully active gene in mature oligodendrocytes indicating that they may be essential for maintaining proper chromatin structure.

Transfection analysis using deletion mutants of the upstream region of MAG gene showed that the core promoter of the gene is located within the initial 152 bp as this region was sufficient to drive the CAT gene transcription in permissive C6 cells (Ye et al. 1994, for mouse promoter; Konat and Ye, unpublished results for rat promoter). The promoter is also responsive to enhancers as demonstrated by a 36 fold increase in the CAT expression induced by the addition of the ubiquitously active SV40 enhancer to the constructs (Ye 1994). The upstream region proximal to the promoter contains strong activators, while the region from approximately -0.5 to -0.8 kb harbors potent inhibitory cis-acting elements. It can be envisaged that the repressive elements are crucial for cell-specific expression of the gene by inhibiting its transcription in cells other than myelin-producing oligodendrocytes and Schwann cells.

Primer extension analysis using RNA from mature oligodendrocytes revealed several transcription initiation sites (Laszkiewicz et al. 1996). There is no canonical TATA box within this region, and hence the promoter belongs to the family of TATA-less promoters. The TATA-less promoters are found mainly in housekeeping genes, and in several tissue-specific genes. These promoters contain special cis-elements, called initiators (Inr) that encompass the transcription start site, and direct the assembly of transcription complexes (Weis and Reinberg 1992). Although, Inr alone are sufficient to direct basal levels of transcription, additional cis-elements that bind other transcription trans-factors, e.g., Sp1 exert strong regulatory effects (Weis and Reinber 1992). No sequences homologous to the known Inr motifs can be identified within the MAG gene core promoter. Furthermore, only two sequences reminiscent of common cis-elements, i.e., AP1, TCE are present. Hence, the promoter probably contains novel, as yet uncharacterized Inr elements that govern the formation of transcription complexes.

The upregulation of the MAG gene in differentiating oligodendrocyte lineage cells is accompanied by profound changes in the assortment of nuclear trans-factors that bind to the core promoter region of the gene as revealed by electrophoretic mobility shift analysis (Laszkiewicz et al. 1996). Thus, the trans-factors present in O-2A nuclei are absent in oligodendrocyte nuclei, while oligodendrocyte nuclei contain a specific set of trans-factors that are not present in O-2A nuclei. The results strongly suggest that the promoter is controlled by both positive and negative mechanisms in the course of oligodendrocytic differentiation, i.e., the removal of O-2A-specific inhibitory trans-factors, and concurrent addition of oligodendrocyte-specific activating trans-factors. Furthermore, these trans-factors are present at very low concentrations in the nuclear extracts indicating that they represent a restricted pool of unique regulators that undoubtedly include specific Inr-binding proteins (IBP), and probably other auxiliary trans-factors. Hence, the oligodendrocyte-specific expression of the MAG gene may be mediated by the formation of stereospecific nucleoprotein complexes between unique cis-elements within the core promoter and their cognate trans-factors that appear timely in the nuclei of differentiating oligodendrocytes. Such promoter-driven cell specificity has been demonstrated for some TATA-less genes including a neurone-specific gene (Salomon et al. 1993, Faraonio et al. 1994).

Limited MNase digestion analysis indicated that the 5' end of core promoter is delimited by a positioned nucleosome (Laszkiewicz et al. 1995, 1996). Both, the downstream linker that harbors the core promoter, and the upstream linker of the nucleosome becomes highly hypersensitive in the process of gene activation as O-2A progenitors differentiate into mature oligodendrocytes. Hence, the upstream linker region is likely to contain important regulatory cis-information, and the nucleosomal core particle may provide chromatin conformation conducive to the interaction of regulatory nucleo-

protein complexes formed within the upstream linker with transcriptional complexes formed within the core promoter.

In summary, the upregulation of the MAG gene in differentiating oligodendrocytes entails both chromatin remodeling, and alteration in the assortment of unique trans-factors. The TATA-less gene promoter itself may control the cell-specific gene expression. Also, the DNA methylation seems to play a regulatory role in the gene activity. The understanding of transcriptional mechanisms of the MAG gene in oligodendrocytes will provide for experimental control of the gene status, and consequently may facilitate the development of gene therapies aimed at promoting neuronal regeneration in the CNS.

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