Macromolecular Transport in Oligodendrocytes and Structure of the MBP Gene and Function of Its Gene Products

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Macromolecular Transport in Oligodendrocytes

Lots of things are happening in the oligodendrocyte as it prepares itself for myelination, many more than one appreciated even 5 or 6 years ago. Part of the interest of our group is in how the synthesis of the myelin membrane is regulated, both during myelination and prior to it, as the cells of the oligodendrocyte lineage differentiate. As the previous speakers have so eloquently expressed it, both proteins and lipids are required to synthesize myelin, and a whole host of genes must be turned on for myelin synthesis to occur. It has become apparent in recent years that targeting of a macromolecules, i.e., proteins, in cells can occur by two mechanisms. One that we know a lot about involves targeting of the protein itself by means of signal sequences, etc. A second mechanism involves targeting and transport of the mRNA molecule itself and its transcription in the vicinity of the site where the protein is supposed to be. This second type of transport is probably important in the brain, the most clear-cut example being MBP, which is translated and inserted into the myelin sheath at some distance from the cell body. This has been shown in mouse brain by an immunohistochemical technique using antibodies to the hapten digoxigenin. PLP is synthesized on membrane-bound ribosomes and is located primarily within the cell body in oligodendrocytes. The same is true of cAMPase, another major myelin protein. If you look at MBP early on during differentiation of the oligodendrocyte, the gene for MBP is turned on but the mRNA remains localized in the cell body. This is the situation in a 6-day-old mouse brain. At later stages the mRNA for MBP moves out of the cell body and you can actually locate it within the fibers of the myelin sheath. Note that we are looking at MBP mRNA (not protein), which is detected by using an appropriate RNA probe coupled to digoxigenin and anti-(digoxigenin) coupled to a fluorescent probe. So the mRNA is actually moving through cytoplasmic channels and infiltrating the myelin sheath. This process can also be seen in vitro using mixed glial cultures; you can watch the message move with time from the cell body to processes.

We noticed that if you remove other cell types from the oligodendrocytes by shaking the cultures, MBP mRNA very readily moves out of they cell body into the processes. Dr. Sashiyama in my lab has discovered that if you plate oligodendrocytes onto astrocytes, the MBP message tends to be present primarily in the cell body of the oligodendrocytes. Thus this process of translocation is, in a sense, plastic and is dependent on interaction between the oligodendrocyte and other cell types. Dr. Sashiyama’s most recent finding is that addition of neuronal conditioned medium reverses the effect of cell–cell interaction and mRNA comes out of the cell body again.
These results suggest that also an interplay between oligodendrocytes, astrocytes, and neurons may be important for the regulation of myelin synthesis. On the other hand, this could, of course, be an in vitro artifact.

**Structure of the MBP Gene and Function of Its Gene Products**

The MBP gene comprises about 35 kb and contains 7 exons. Alternative splicing leads to formation of a variety of mRNA species. Some types of transcripts produced from the MBP gene could not be accounted for by the known structure of the gene. What we found is that, in the mouse, and as we now know, also in man, the MBP gene is localized in a much larger transcription unit that we call the GOLLI-mbp gene. This gene has three transcription start sites, in exons 1, 4 and 5b, for the production of mRNA species that encode MBP and MBP-related proteins.

We have now worked out the structure of this gene complex in both the mouse and in man. They have essentially the same structure except that in the mouse the gene is 105 kb long whereas in man it is 179 kb. If we ignore the MBP transcription start sites (or the MBP transcription unit within the larger unit), there are three major products that have in common that they use exons 1, 2, and 3 of the Golli transcription unit. Each of these products appears to have a different developmental pattern of expression. In the mouse, we localized these products by in situ hybridization to cells of the oligodendrocyte lineage. The use of probes that are GOLLI specific but do not react with MBP leads to lighting up of oligodendrocytes (or a subset of oligodendrocytes) in corpus callosum.

The products of the GOLLI gene are expressed not only in the nervous system but also in thymus, macrophage cell lines, and B cell lines. Thus the GOLLI-MBP gene complex is expressed not only in the brain but also in the immune system. Now, for people interested in experimental allergic encephalomyelitis (EAE), this will represent a very surprising finding, since it has always been thought that the blood/brain barrier essentially protects the immune system from contact with epitopes of MBP. Our suggestion is that these gene products are made in the spleen and in the thymus and that we must revise our ideas about the pathogenesis of EAE.

We can readily translate the messages derived from the GOLLI-MBP gene complex in the rabbit reticulocyte system, in bacteria, and by transfection of CHO cells and oligodendrocyte cell lines. At present we are purifying these proteins, which we can make in large amounts, and are generating antibodies to specific portions of the GOLLI gene products.

The GOLLI-MBP gene produces two sets of products and they are developmentally regulated. The products are made in human embryonic neurons as well as at specific stages in the differentiation of neurons in the cortex. As already mentioned the gene is expressed in a number of different cell types both in the nervous system and in the immune system.