CHAPTER II

Review Literatures

Iron

The essential element iron plays a wide variety of physiological and biochemical roles for all living organisms. It is an essential component of, or cofactor for, hundreds of proteins and enzymes (Fairbanks, 1999; Beard et al., 1996). For example, iron is used in oxygen transport (Brody, 1999), as a cofactor of many enzymes involved in electron transport, energy metabolism (Yip and Dallman, 1996), the synthesis of DNA (Fairbanks, 1999; Beard et al., 1996), neurotransmitters including dopamine, serotonin and GABA (Beard, 2001), and lipid–rich myelin (Larkin and Rao, 1990). Iron is also required for a number of vital functions including growth, reproduction, healing and immune function. For these reasons, iron is indispensable for cellular metabolism of almost all living organisms. All growing cells contain surface TfR that avidly bind Tf, after which the receptor-bound Tf is subjected to endocytosis. After iron is taken into the cell, it distributes to utilization and the excess of iron will be stored in ferritin (Crichton et al., 2002).

Iron uptake

At present, there are two known systems for iron uptake. The first system, shown in Figure 1 and known as transferrin bound iron (TBI) uptake, involves several important steps including the binding of iron to Tf, endocytosis of iron-bound Tf by the TfR found on the cell surface, acidification, dissociation, and translocation of the iron across the endosomal membrane into the cytoplasm, which is mediated by divalent metal transporter 1 (DMT1) (Fleming et al., 1997; Gunshin et al., 1997). After that, intracellular iron transport molecule shuttles iron to different cellular compartment for utilization and the excess iron will be stored in ferritin protein (Crichton et al., 2002). The TfR/iron-free Tf complex is then recycled back to the cell surface (Karin and Mintz, 1981; Klausner et al., 1983; Iacopetta and Morgan, 1983). Cells can also bring

iron across plasma membrane into the cell through a non-transferrin bound iron (NTBI) uptake mechanism, which is mediated by low molecular weight organic anions, such as ascorbate and citrate (Telfer and Brock, 2004).

While TfR-mediated endocytosis is relatively well characterized and is believed to be the major route of cellular iron sequestration (Ke and Ming Qian, 2003), potential carriers for NTBI have only recently been identified (Gutierrez et al., 1997; Gunshin et al., 1997). NTBI uptake process is associated with p97, Nramp1 and divalent metal transporter-1 (DMT1, also known as DCT1 and Nramp2 protein). NTBI uptake appears to require energy (Gutierrez et al., 1998; Qian and Morgan, 1991; Quail and Morgan, 1994), but exactly how this is coupled to trans-bilayer movement of iron is unclear. However, there is limited evidence suggesting that NTBI transport is regulated in response to cellular iron loading.

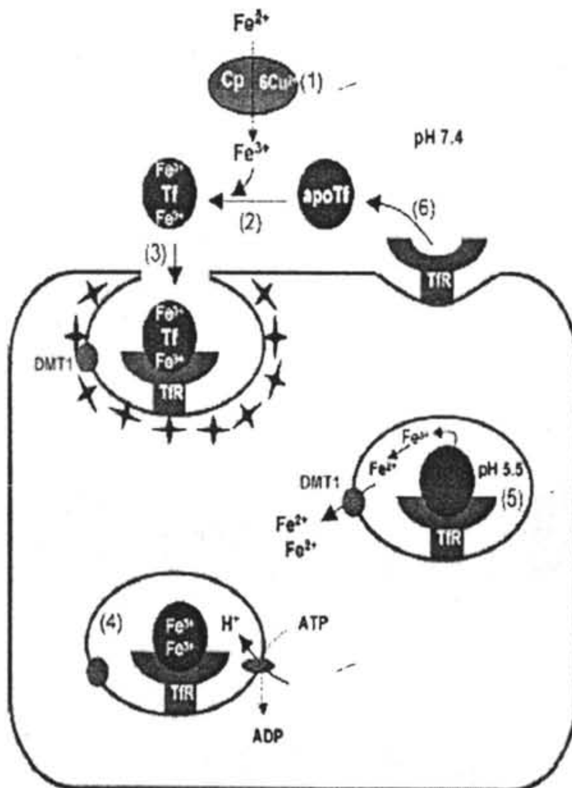

Figure 1 ***Regulation of cellular iron uptake.*** *(1) Ferrous iron (Fe2+) is converted to ferric iron (Fe3+) by the ferroxidase activity of ceruloplasmin (Cp). (2) Transferrin (Tf) binds two atoms of ferric iron. (3) Tf binds to the transferrin receptor (TfR) on the cell membrane and is internalized along with the divalent metal transporter (DMT1) by receptor mediated endocytosis. (4) Endocytic vesicles develop ATPases that pump protons resulting in an acidified compartment. (5) The reduction in pH reduces the affinity of Tf for iron. Following reduction, the iron is delivered to the cytosol via DMT1. (6) Endosomes are recycled to the plasma membrane. At neutral pH the affinity of TfR for apo-Tf is low, permitting recycling of both Tf and TfR (Papanikolaoua and Pantopoulos, 2005).*

Iron storage

Living organisms have evolved an elegant mechanism to tightly control an excess of "free" iron, this is because redox active iron catalyses the generation of highly reactive oxygen species (Halliwell and Gutteridge, 1999; Welch et al., 2002). Such mechanisms are involved iron-storage protein ferritin and the presence of labile iron pool (LIP). Ferritin is a multimeric protein with a great capacity for storage up to 4500 atoms of iron, therefore representing cellular iron storage site (Vaisman et al., 2000). Ferritin composes of 24 heavy and light subunits (Figure 2). The two type subunits appear to have different functions in iron metabolism. H-ferritin has been linked to iron utilization (Arosio et al., 1991), while L-ferritin is more closely associated with cellular iron storage (Bomford et al., 1981). This distinction is important because the ratio of H: L is cell and tissue specific and determines the pattern of iron storage and utilization (Blissman et al., 1996). For example, neurons contain predominantly H-ferritin, indicating that neurons have a high iron requirement with little capacity to store iron, whereas microglia contains predominantly L-ferritin, consistent with their role as scavenger cells. Interestingly, the only cells to contain a mixture of H- and L-ferritin are oligodendrocytes, indicating both a relatively high iron storage capacity and high utilization rate for iron (Connor and Menzies, 1995). Since cellular uptake and storage of iron is carried out by different proteins as reviewed above, it is necessary to have a pool of accessible iron, called LIP. LIP constitutes a crossroad of metabolic pathways of iron-containing proteins and also midway between the cellular need for iron and the hazard of excessive generation of hydroxyl radical, as it has been proposed that LIP is a cellular source of iron available for Fenton reaction (Breuer et al., 1997).

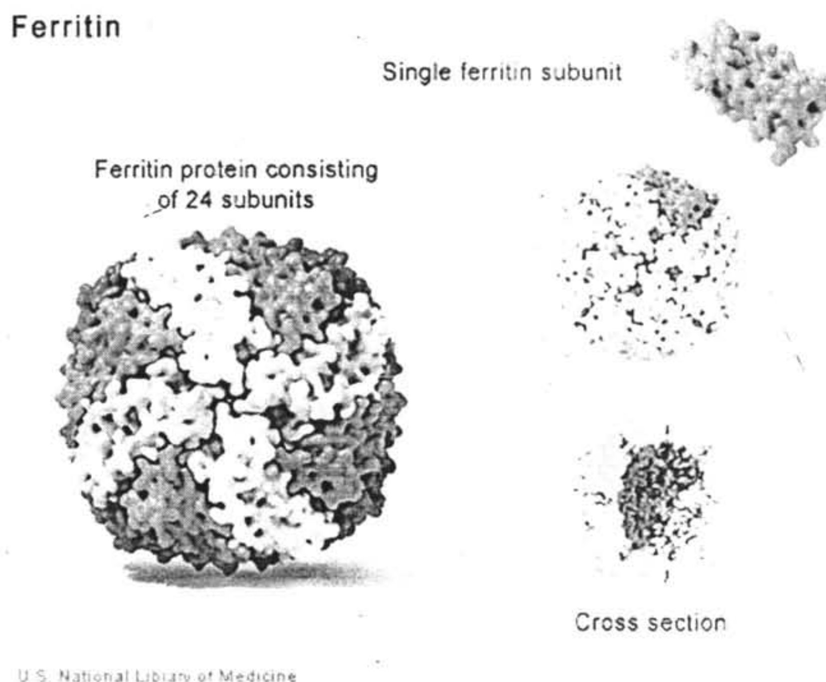

Figure 2 *Ferritin, a protein made up of 24 identical subunits, is involved in iron storage.*

Iron regulation

As shown in Figure 3, alternation of iron pools of an organism is the result of a complex network of events, acting at the transcriptional and translational level to change the expression of proteins involved in transport, cellular iron uptake, utilization, and storage of iron. TfR and ferritin are crucial proteins for the control of cellular iron homeostasis (Hentze et al., 2004). The mRNAs encoding TfR and ferritin contain iron responsive elements (IREs) in their 3′ or 5′ untranslated regions (UTRs), respectively (Johansson and Theil, 2002). The IRE motifs provide a binding site for two iron regulatory proteins, IRP1 and IRP2, which are activated in iron-starved cells to bind with high affinity to cognate IREs. The IRE–IRP interactions stabilize the otherwise unstable TfR mRNAs, which contain five IRE copies in its long and complex 3′ UTR and is the only thus far identified mRNA with multiple IREs. As a result, TfR expression levels increase and the iron-starved cells acquire more iron from Tf. On the other hand, the IRE–IRP interaction in the 5′UTR of ferritin mRNAs specifically

inhibits their translation and the iron-starved cells do not synthesize ferritin, which is under these conditions obsolete. Conversely, in iron-replete cells, IRP1 and IRP2 get inactivated, thereby permitting TfR mRNA degradation and ferritin mRNA translation. This response inhibits further iron uptake from Tf and promotes the storage (and detoxification) of excess intracellular iron.

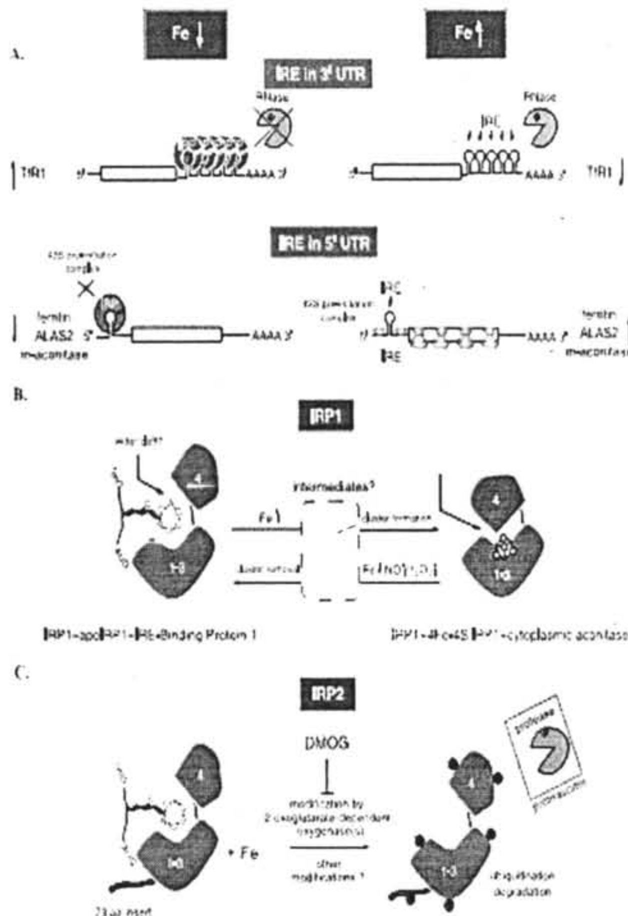

Figure 3 ***(A) Homeostatic responses to iron supply mediated by IRE–IRP interactions.*** *Decreased iron supply activates binding of IRPs to IREs, resulting in stabilization of TfR mRNA and translational inhibition of the mRNAs encoding ferritin (H- and L-chains), erythroid-specific ALAS2, and mitochondrial aconitase. Conversely, IRPs do not bind to cognate IREs in iron-replete cells, permitting degradation of TfR mRNA and translation of ferritin, ALAS2, and mitochondrial aconitase mRNAs.* ***(B) Posttranslational regulation of bifunctional IRP1 in response to iron, NO, and H_2O_2 via an iron–sulfur cluster switch. (C) Iron-dependent degradation of IRP2 by a mechanism involving 2-oxoglutarate-dependent oxygenases*** *(Papanikolaoua and Pantopoulos, 2005).*

Iron and CNS myelination

In the central nervous system, evidence has provided that iron is an absolute requirement for oligodendrocytes myelination (Connor and Menzies,1996 ; Dickinson and Connor, 1994). Iron is required for cholesterol and lipid synthesis, both of which are abundant in and key components of myelin (Larkin and Rao, 1990) Furthermore, cholesterol and lipid synthesis occurs at a higher rate in oligodendrocytes, CNS myelin making cells than other cell type in brain (Cammer, 1984). Indeed, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the initial step in cholesterol biosynthesis is considered a marker for oligodendrocytes (Pleasure et al., 1977). Iron-requiring enzymes leading to lipid synthesis (fatty acid desaturase) and degradation (lipid dehydrogenase) are enriched in oligodendrocytes (Bourre, 1984; Cammer, 1984; Tansey and Cammer, 1988). During the initial stages of their development, oligodendrocyte precursors proliferate and migrate to the white matter, before undergoing dramatic morphological changes as they differentiate to mature, myelinating cells. As determined by immunohistological method, immature oligodendrocytes in the white matter express TfRs before the expression of any myelin-associated proteins (Espinosa de los monteros et al., 1988; Lin and Connor, 1989). A close relationship of iron to oligodendrocyte myelination was further demonstrated by alteration in iron acquisition pattern during postnatal development induced by prenatal ethanol exposure leading to a delayed onset of myelination and, subsequently hypomyelination of the white matter tracts (Miller et al., 1995). This can be simply explained by the fact that iron is a cofactor for enzymes involved in cholesterol and lipid synthesis, which occurs at a higher rate in oligodendrocytes than any other cell types in the brain (Cammer, 1984). Therefore, the relationship of iron to oligodendrocytes and myelination is well documented. On the other hand, our knowledge of iron on Schwann cells, PNS myelin making cells myelination is still infancy.

PNS myelination

The major glial cells which play a vital role in the peripheral nervous system (PNS) are Schwann cells. They are flattened cells with only a few mitochondria and a small Golgi complex. Schwann cells are derived from neural crest cells like melanocytes. The counterparts of Schwann cells in CNS are oligodendrocytes which they are derived from ectoderm. Schwann cells synthesized the insulating and protective lipoprotein myelin, but they are capable of producing far less than oligodendrocytes. This is reflected by the fact that oligodendrocytes are able to myelinate more than 60 internodes of neuronal axons in CNS, whereas Schwann cells can only myelinate one internode of axon in PNS.

Development of Schwann cells

As shown in Figure 4, the development of Schwann cells can be divided into 4 stages. There are neural crest cells, Schwann cell precursors, immature Schwann cells and mature Schwann cells. During the development of the PNS, Schwann cell precursors arise from the neural crest, migrate out and contact developing peripheral axons (Harrison, 1924; Le Douarin and Dupin, 1993). These immature Schwann cells then invade and ensheath bundles of developing axons, in which they further, differentiate into myelinating or non-myelinating Schwann cells (Webster, 1993). During this stage of development, some Schwann cells establish a one-to-one association with an axon, the so called promyelinating stage, a step necessary for myelination to proceed (Webster, 1993). These cells then initiate a program of myelin-specific gene expression, turning on a set of genes encoding the major myelin proteins and turning off a subset of genes previously expressed (Scherer, 1997). At this early stage, MAG appears first, followed closely by P0 and MBP (Martini and Schnachner, 1986; Owens and Bunge, 1989). In contrast, immature Schwann cells that do not establish a one-to-one relationship with an axon do not activate the program of myelin gene expression and become non-myelinating Schwann cells (Webster et al, 1983; Mirsky and Jessen, 1990). The differentiation of non-myelinating Schwann cells,

however, also depends on continuing interactions with axons, which provide, at least in part, the signal (or signals) necessary to initiate this process (Jessen et al, 1987).

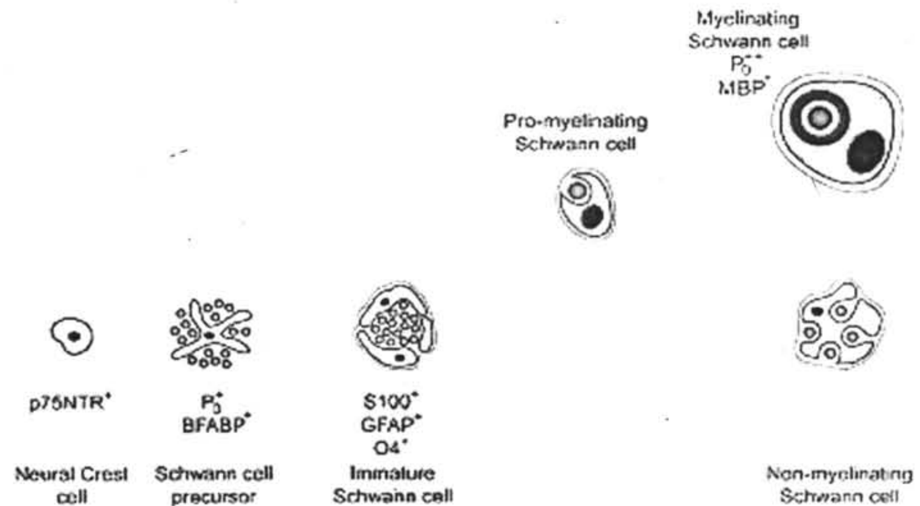

Figure 4 ***Schwann cell development and some of the molecular markers that can be used to differentiate each stage from the preceding one.*** *The lineages arise from multipotent cells in steps that are broadly comparable. Note that only the Schwann cell lineage generates two distinct cell types and includes a fate decision point, the immature Schwann cell stage. Also, fully differentiated Schwann cells retain an unusual plasticity throughout life and can readily de-differentiate to form cells similar to immature Schwann cells (open arrows). Basal lamina (gray line) is associated with Schwann cells but not with Schwann cell precursors .Blue: axons. Red: myelin. MBP: myelin basic protein.p75NTR: Low affinity p75 neurotrophin receptor. P0: protein zero. BFABP: brain fatty acid binding protein. GFAP: glial fibrillary acidic protein (Jessen, 2004).*

There are several Schwann cell molecules that are useful for identifying Schwann cells, as well as, useful for identifying Schwann cells phenotype (premyelin, myelinating or non myelinating Schwann cells) (Table 1). These molecules or Schwann cell markers are often used in immunocytochemical analysis following nerve injury because the relationship between the axon and Schwann cell altered and

identification of Schwann cell by morphology alone becomes difficult (Gould et al., 1992).

Markers	Precursors	Nonmyelin producing cell	Myelinating cell
S100	-	+	+
P0	-	-	+
MAG	-	-	+
GFAP	-	+	-
NCAM	+	+	-

Table 1 Expression of Schwann cell markers in different Schwann cell phenotypes (Gould et al., 1992).

In addition to produce and maintain PNS myelin, Schwann cells were also proliferation, releasing growth factor and capable phagocytosis, when the peripheral nerve injury. If a peripheral nerve is cut, severing the axon and its Schwann cells from the neuronal cell body, axons degenerate and demyelination occurs; initiating the process of Wallerian degeneration. During Wallerian degeneration myelinating Schwann cells change their pattern of gene expression, turning off the set of gene encoding the major myelin proteins and turning on the same set of gene expressed prior to myelination as immature Schwann cells (Scherer and Salzer, 1996). If the nerve is crushed, however, allowing regeneration to occur after Wallerian degeneration, Schwann cell differentiation and myelination can be reinitiated as the axons regenerate through the crushed segment, recontacting denervated Schwann cells (Aguayo et al., 1976a, b; Weinberg and Spencer, 1976). In addition, regenerating unmyelinated axons are ensheathed but not remyelinated, even if the Schwann cells previously synthesized

a myelin sheath (Aguayo et al., 1976b). Both the initiation and maintenance of myelinating Schwann cell phenotype thus depend on continuing Schwann cell-axonal interactions.

PNS myelin components

Myelin has a water content of about 40%. The dry mass of PNS myelin is composed of a high proportion of lipid (70-85%) and, consequently, a low proportion of protein (15-30%).

Peripheral myelin contains some unique proteins and some shared with CNS myelin. The comparison between the percentage of myelin protein components in CNS and PNS was shown in Table 2.

Proteins	CNS (% of total)	PNS (% of total)
1. PLP	50	<0.01
2. MBP	30	18
3. CNPase	4	0.4
4. MOG	0.05	<0.01
5. MAG	1	0.1
6. P0	<0.01	>50
7. PMP22	<0.01	5-10

Table 2 The comparison between the percentage of myelin protein components in CNS and PNS

PNS myelin proteins

A number of different proteins are associated specifically with myelin in the nervous system. They are vital for normal myelination, and their dysfunction either

through malformation due to genetic mutations or attack through autoimmune disease is responsible for the pathology in many disorders of myelination (Matenson, 1992).

Protein Zero (P0). The major PNS myelin protein present is P0. It does abundance comprise more than 50% of PNS myelin proteins and found throughout compacted myelin. P0 is similar to MAG in that it is a member of the immunoglobuin superfamily; it has only a single transmembrane segment. P0 has a glycosylated extracellular domain that functions as an adhesion protein. During myelin spiraling and compaction, the extracellular domain of one P0 molecule interacts with the same domain on another P0 molecule on the surface of the opposed membrane, adhering the two membranes to each other. At least 60 mutations of the P0 gene (on chromosome 1) have been identified, most of which are point mutations resulting in production of aberrant protein; these are implicated in several hereditary motor sensory neuropathies including a severe autosomal recessive form of Charcot-Marie Tooth disease known as Dejerine-Sottas disease (CMT III), and an autosomal dominant form of Charcot-Marie Tooth disease (CMT 1B) (Schwartz, 1991).

Myelin basic Protein (MBP). In PNS, MBP content varies from approximately 5-18% of total protein. It is a cytoplasmic membrane protein (unlike most of the others, which are integral membrane proteins). MBP has an important role in myelin compaction and in maintaining the basic structure of the myelin sheath. A single MBP gene produces at least seven related proteins via alternative RNA splicing. MBP is crucial in the initiation of myelination, possibly by inducing plasmalemma growth and compaction. This protein appears to be the major autoimmune target in Multiple Sclerosis in CNS, in contrast to the PNS, which has almost normal myelin amount and structure but lacks MBP (Schwartz, 1991).

Peripheral myelin protein (PMP22). Another important PNS myelin protein is PMP22, present in compact myelin. Also a member of the immunoglobulin superfamily, the 4-transmembrane-segment protein is implicated in growth and formation of the myelin sheath. The gene, on chromosome 22, is significantly altered

by a number of different point mutations leading to reductions in the mutant proteins, resulting in a number of different hereditary demyelinating neuropathies. In contrast, a gene duplication mutation of PMP22 results in increased production of the PMP22 protein, which results in growth arrest and apoptosis (programmed cell death) and causes the most common form of Charcot-Marie-Tooth disease (type 1A).

Myelin-associated glycoprotein (MAG). A single transmembrane-segment protein with a glycosylated extracellular domain, MAG protein is a member of the immunoglobulin superfamily and a cell adhesion molecule with many similarities to a number of other proteins involved in cell-cell recognition, including neural cell adhesion molecule (NCAM). MAG is present in both CNS and PNS; its functions include stabilization of contact between the axon and glial processes in early myelination and in myelin compaction. In the Schwann cells, MAG is expressed early during peripheral myelination and is situated at the margin of the mature myelin sheath just adjacent to the axon, suggesting its importance in the initiation of myelination. MAG is also found in the compacted myelin of the adult and is responsible for maintenance of the size and compaction of myelin through adulthood. The only well known disorder associated with MAG protein is an autoimmune disease, Anti-MAG Syndrome, that occurs in adulthood; no mutations leading to disease in humans have yet been found (Matenson, 1992).

Proteolipid Protein (PLP). The majority of the total protein in CNS myelin is PLP, a four-transmembrane-segment molecule which, like other proteolipids, is soluble only in organic solvents due to the long chains of fatty acids linked to amino acid residues throughout the molecule. PLP is an important protein in the formation of the multilamellar structure of myelin; it is also thought to be involved in myelin compaction (Schwartz, 1991).

2′3′-Cyclic nucleotide-3′-phosphodiesterase (CNPase). Myelin contains enzymes that function in metabolism and possibly ion transport. A few enzymes, including CNPase, are believed to be fairly myelin-specific, although they are probably

present very low in peripheral nerve and PNS myelin. CNPase is not a major component of compact myelin but is concentrated in specific regions of the myelin sheaths associated with cytoplasm. Previously, it has been reported CNPase plays a role in events involving the cytoskeletal network of myelin. Besides, CNPase was involved iron. *In vivo* study has been reported that CNPase activity was decreased when iron deficiency in rat brain. This report suggested that iron associated with CNPase activity (Beard et al., 2003).

Iron and PNS myelination

At present, there are little known about the relationship between iron to Schwann cell and myelination. However, previous study was shown that axotomy led to a massive but transient increase in Tf binding at the site of the injury and in the distal part of the crushed or resected sciatic nerve, shortly preceding the time course of Schwann cell proliferation (Raivich et al, 1991). Immunocytochemistry revealed strong and simultaneous expression of the transferrin receptor on two different cell types: invading macrophages and Schwann cells reacting to the injury (Raivich et al., 1991). Using Schwann cell culture system, it was demonstrated that treatment of cultured Schwann cells with iron saturated-Tf, but not with apo-Tf, prevented dedifferentiation of Schwann cells (Salis et al., 2002). Therefore, available data have lead us to hypothesize that activation of Schwann cell iron metabolism could be essential for the process of nerve regeneration and could recapitulate the relationship of iron to oligodendrocytes and myelination.