# Chapter I

## Introduction

### 1.1 Glial cells in central nervous system (CNS)

Nervous system of vertebrates is categorised into central nervous system (CNS) and peripheral nervous system (PNS). The CNS consists of brain and spinal cord. Till recently, the neurons were considered to be the major cell type in brain functions due to their excitability, but the more recent evidences indicate equal numbers of glial cells as neurons and more complexity of glia throughout the evolution towards mammals (Azevedo et al. 2009, Molofsky et al. 2012). Thus complexity of brain in higher vertebrates is considered to be attributed to the glial cells (Azevedo et al. 2009). There are four major types of glial cells known in brain: (i) Astrocytes (ii) Oligodendrocytes (iii) Ependymal cells (iv) Microglia.

Among all the glial cells, astrocytes are more explored due to their abundance, complexity and wide spectrum of functions in mammals (Molofsky et al. 2012, Wang and Bordey 2008).

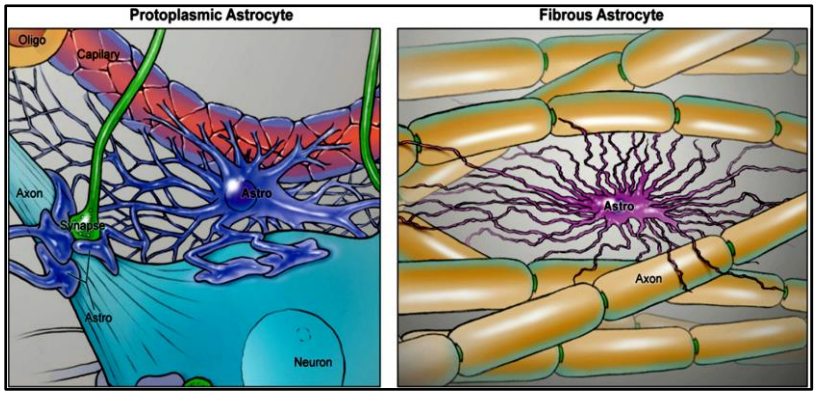
### 1.2 Morphology, physiology, development and functions of astrocytes

#### 1.2.1 Morphology & physiology

Astrocytes, or astroglia, are named with the Greek root word ‘astro’, which means star. This nomenclature is because of the star shaped appearance after Golgi staining of these cells. The earlier neuroscientists- Camillo Golgi and Ramon y Cajal had already noticed that even though astrocytes appear star-shaped, the morphology is diverse (Zhang and Barres 2010). With the advancement in electrophysiological, molecular and genetics methods, it is now well evident that astrocytes represent a heterogenous population of cells in CNS (Kimelberg 2004). They are broadly classified into sub-types: (i) Protoplasmic astrocytes (ii) fibrous astrocytes, in cerebrum and bergmann glia in cerebellum (Raff et al. 1983, Yong et al. 1990, Bailey and Shipley 1993, Wang and Bordey 2008, Kimelberg 2004). Protoplasmic astrocytes are located in the gray matter and possess many branching processes, which envelop synapses and whose end feet cover blood vessels whereas; fibrous astrocytes possess long, thin, unbranched processes whose end feet envelop nodes of Ranvier (Miller and Raff 1984, Privat and Rataboul 2007, Bushong et al. 2002) (Fig.1.1). Bergmann glia is located in cerebellum with cell bodies in the Purkinje cell layer and

processes that extend into the granule cell layer and terminate at the pial surface. The Bergmann glia also enwraps synapses and modulates synaptic functions similar to protoplasmic astrocytes (Reichenbach, Derouiche, and Kirchhoff 2010). Morphological changes have also been associated with pathological condition of brain; astrocytes with hypertrophy and increased GFAP expression are named 'reactive' astrocytes (Liddelow and Barres 2017). By translating ribosome affinity purification (TRAP) method, the translated mRNAs in cortical astrocytes, cerebellar astrocytes, and cerebellar Bergman glia have been evaluated and substantial differences in gene expression between astrocytes from different brain regions are observed (Doyle et al. 2008). The microarray analysis also revealed major gene expression differences in astrocytes isolated from four different brain regions (Yeh et al. 2009). Other than these genome wide gene expression studies, the various other *in vitro* and *in vivo* studies have shown differential expression of genes in different subsets of astrocytes (Zhang and Barres 2010). *These observations suggest that astrocytes are not only morphologically different but also varies with respect to their gene expression, which could be responsible for functional heterogeneity.*

**Fig.1.1 Astrocytes are morphologically heterogeneous** A protoplasmic astrocyte is shown in close connection with a neuron and a capillary, constituting the so-called ''neurovascular unit'' and highlighting the roles of astrocytes in developmental synaptogenesis and in modulating the BBB. (Right) A fibrous astrocyte is shown in a white matter tract, where it may interact with oligodendrocytes to promote myelination (Source: (Molofsky et al. 2012)).




Major markers specific for astrocytes are GFAP, GLT-1, GLAST, S100β, and BLBP (Molofsky et al. 2012, Wang and Bordey 2008) (Table 1.1).

**Table 1.1 Markers of astrocytes and their progenitors** (Source:(Molofsky et al. 2012))

| Marker | Expression onset | Advantages | Caveats |
|---|---|---|---|
| Glast | mE11.5, cE5 | Early expression | Also expressed in some oligodendrocyte precursors |
| NFI A/B | mE11.5, cE5–cE6 | Early expression | Also expressed in oligodendrocyte precursors and mature motor neurons |
| FABP7/BLBP | mE8–mE9, cE2 | Early expression | Also expressed during neurogenic stages in radial glia |
| FGFR3 | mE9.5, cE3 | Early expression | Also expressed during neurogenic stages in radial glia and ligodendrocyte precursors |
| Sox9 | mE9.5–E10, cE3–cE4 | Early expression | As above |
| Reelin/slit | cE4 | Early expression; subtype specificity | Also expressed by neurons; uncharacterized outside spinal cord |
| AldhlL1 | mE9.5 | Early expression that persists in mature astrocytes; labels both fibrous and protoplasmic astrocytes | Incompletely characterized; expressed early in radial glia, (unclear whether these are fated to become astrocytes only) |
| Id3 | mE14.5 | Early expression | Also expressed in some oligodendrocyte precursors |
| Aldolase C | mE15 | Antibody detects fibrous and protoplasmic astrocytes | Also expressed in pial and purkinje cells |
| GFAP | mE17.5–mE18.5 | Robust, well-characterized marker of mature fibrous astrocytes and reactive astrocytes | Poorly labels protoplasmic astrocytes; turned on relatively late in development |
| S100β | mE15.5–mE16.5 | Robust, good antibodies available | Also labels oligodendrocytes; turned on late |
| Aquaporin-4 | mE18 | Subtype specific? (preferentially labels endfeet near blood vessels and pia) | Also in ependymal cells; white matter-restricted Expressed in fibrous and protoplasmic subtypes |
| CD44 | E18.5–P0, cE10 | Cell surface marker potentially useful for flow cytometry | Incompletely characterized |
| Glutamine synthetase | Embryonic to early postnatal | Expressed in fibrous and protoplasmic subtypes | Incompletely characterized |

Astrocytes are non-excitable brain cells as they do not generate action potential in response to voltage-gated channel activation (Verkhratsky, Rodríguez, and Parpura 2012). However, they can respond to several external stimuli such as mechanical activation, transmitter application, and synaptic activity with increase in intracellular $Ca^{2+}$. Astrocytic calcium oscillations are independent of neuronal excitability (Pirttimaki and Parri 2013). The resting membrane potential of astrocytes ranges from about −85 to −25 mV in acute hippocampal slices (McKhann, D'Ambrosio, and Janigro 1997) and isolated optic nerves (Bolton et al. 2006). This variation in resting membrane potential also suggests existence of distinct types of astrocytes (McKhann, D'Ambrosio, and Janigro 1997). Current profiles also have been observed to be distinct in astrocytes from different brain regions (Guatteo, Stanness, and Janigro 1996) and also in astrocytes within the same brain region (Matthias et al. 2003, Steinhäser, Jabs, and Kettenmann 1994, Wallraff et al. 2004). Astrocytes are arranged not in layers but they are distributed fairly, even where a single astrocyte ensheaths number of proximal synapses, connects with soma and dendrites of different neurons (Pirttimaki and Parri 2013). Astrocytes contact not only neurons but are interconnected by gap junctions and also through gliotransmitters with adjacent astrocytes, thus confers synchronized actions/ activity of the brain (Verkhratsky, Rodríguez, and Parpura 2012). However, the extents of coupled actions of astrocytes, owing to their connection through gap junctions, differ widely in cells from different brain regions (Zhang and Barres 2010, Lee et al. 1994, Blomstrand et al. 1999).

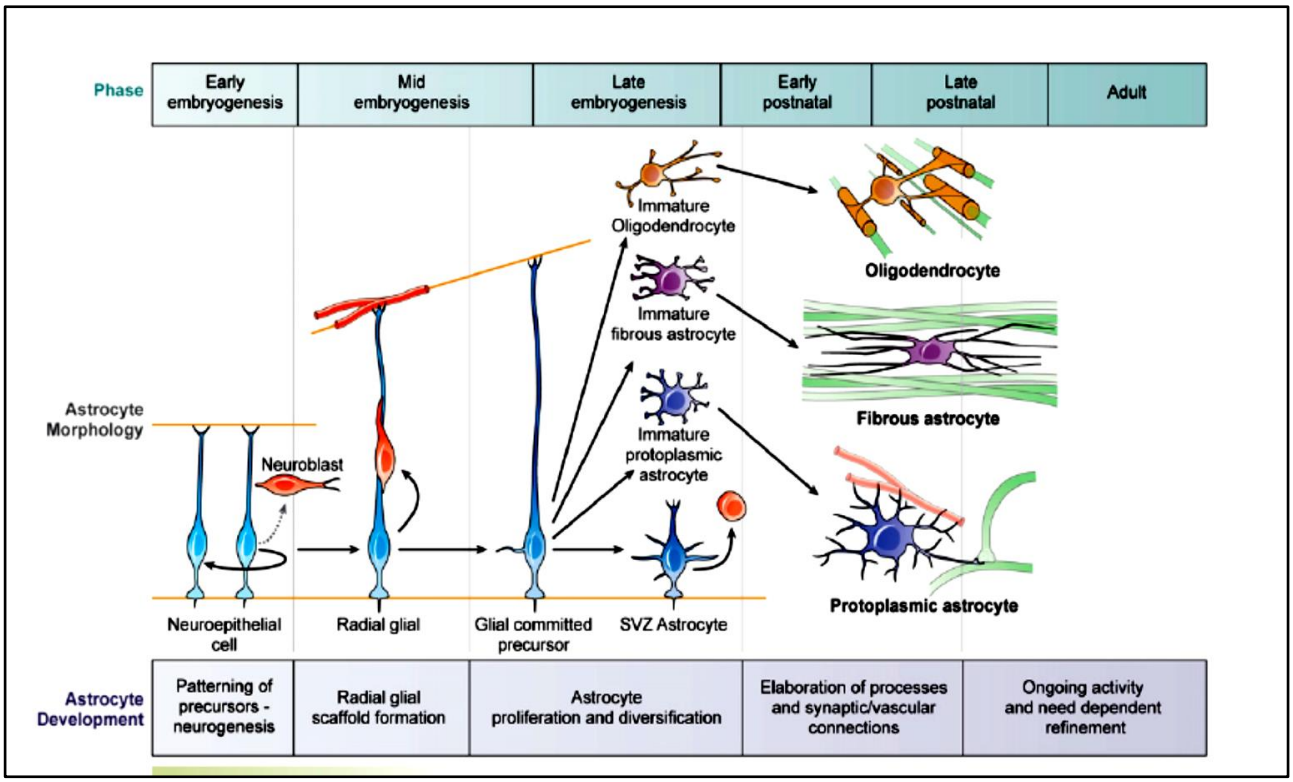
### 1.2.2 Astrocyte development/ lineages

Brain development is a spatiotemporal process involving neurogenesis followed by gliogenesis (the generation of astrocytes and oligodendrocytes) in most regions (Noctor et al. 2001). The gliogenesis begins late in embryonic development and continues during the neonatal and postnatal period in mammals (Fig. 1.2). The cerebral cortex is one of the best-studied regions for gliogenesis (Wang and Bordey 2008). In cerebral cortex, the astrocytes derive from four different sources: (i) radial glia (RG) (ii) subventricular zone (SVZ) progenitors (iii) locally proliferating glia (iv) NG2 glia (Ge and Jia 2016). During embryonic development, the neuroepithelial cells derived RG cells located at the ventricular zone (VZ) produce neurons and astrocytes (Malatesta, Hartfuss, and Gotz 2000, Noctor et al. 2001). Astrocytes first appear transformed from RG cells at around E16 in cortical region of mice. However, major gliogenesis occurs at the first month of post natal period (Bayraktar et al. 2015). These RG cells either directly transforms into mature astrocytes or astrocyte progenitors (intermediate progenitor cells)(Ge and Jia 2016, Noctor et al. 2004, Schmechel and Rakic 1979). Astrocyte progenitors generated by RG cells in VZ region

migrate to adjacent SVZ region, proliferates further and migrate to cortex where they differentiate (Noctor et al. 2004, Ganat et al. 2006, Levison and Goldman 1993). More recent studies suggest that neonatal SVZ contains multipotent, bipotential progenitors and astrocyte-restricted progenitors which give rise to astrocytes (Levison and Goldman 1997). RG cells can also transform into specialized astrocytes such as Bergmann glia in the cerebellum(Rakic 2003). Postnatally generated astrocytes arise mainly in SVZ region (Ge and Jia 2016). Dividing astrocytes locally has also been observed to be contributing to astrocytes population (Ge and Jia 2016). NG-2 glia are the major contributors of oligodendrocytes, but minor quantity of astrocytes is observed to be derived from oligodendroglial lineage cells in the postnatal rodent brain (Ge and Jia 2016, Aguirre and Gallo 2004, Chittajallu, Aguirre, and Gallo 2004). The different developmental astrocyte lineages provide an explanation for astrocyte diversity in the same brain region. The protoplasmic astrocytes, present in gray mater, are generated from embryonic radial glia and, to a lesser extent, from intermediate progenitors migrating from the neonatal SVZ. The fibrous astrocytes, present in white matter, are predominantly generated from neonatal SVZ progenitors. Thus different waves or lineages of astrocytic development and regional differences in radial glia fate could be responsible for different patterns of gene expression and functions of astrocytes (Malatesta et al. 2003, Cai et al. 2007, Hochstim et al. 2008).

The patterning by differential expression of transcription factors and activation of intracellular signalling pathways influences gliogenesis. Local chemical cues and cell-cell interactions also influence astrocytogenesis which could be responsible for astrocytic heterogeneity (Zhang and Barres 2010). The major transcription involved in astrocyte lineage determination are revealed using iPSC technology by conversion of fibroblasts into functional astrocytes (Caiazzo et al. 2015). Many recent studies show the transcription factors responsible for specification of astrocyte lineage (Muroyama et al. 2005, Hochstim et al. 2008).

**Fig.1.2 Development of astrocytes** Neuroepithelial cells give rise to radial glia, which generate first neurons, and then become glial-committed, giving rise to precursors that proliferate and diversify into fibrous and protoplasmic astrocytes, which then go through a protracted stage of postnatal maturation (source: (Molofsky et al. 2012))




### 1.2.3 Functions of astrocytes

Several functions of astrocytes are known sharing part of the workload of CNS. They support not only the structure and function of neurons but also synchronize with other glial functions and development. There are interesting reviews focusing on diverse functions of astrocytes (Zhang and Barres 2010, Wang and Bordey 2008, Molofsky et al. 2012, Gorshkov et al. 2018, Kimelberg and Nedergaard 2010).

In addition to abundance in CNS, the diverse functions of astrocytes make this glial cell essential to know for their contribution in CNS diseases. Over the last decade, the experimental evidences have suggested that astrocytes play a key role in acute, chronic and also neurodevelopmental CNS diseases. Astrocyte dysfunction has been documented in schizophrenia, autism, drug abuse, major depression disease and neurodegenerative disorders such as Alexander Disease (AxD), Amyotrophic Lateral Sclerosis (ALS), and Alzheimer's Disease (AD) (Molofsky et al. 2012, Schitine et al. 2015, Gorshkov et al. 2018, Hirase and Koizumi 2018, Jha et al. 2017).

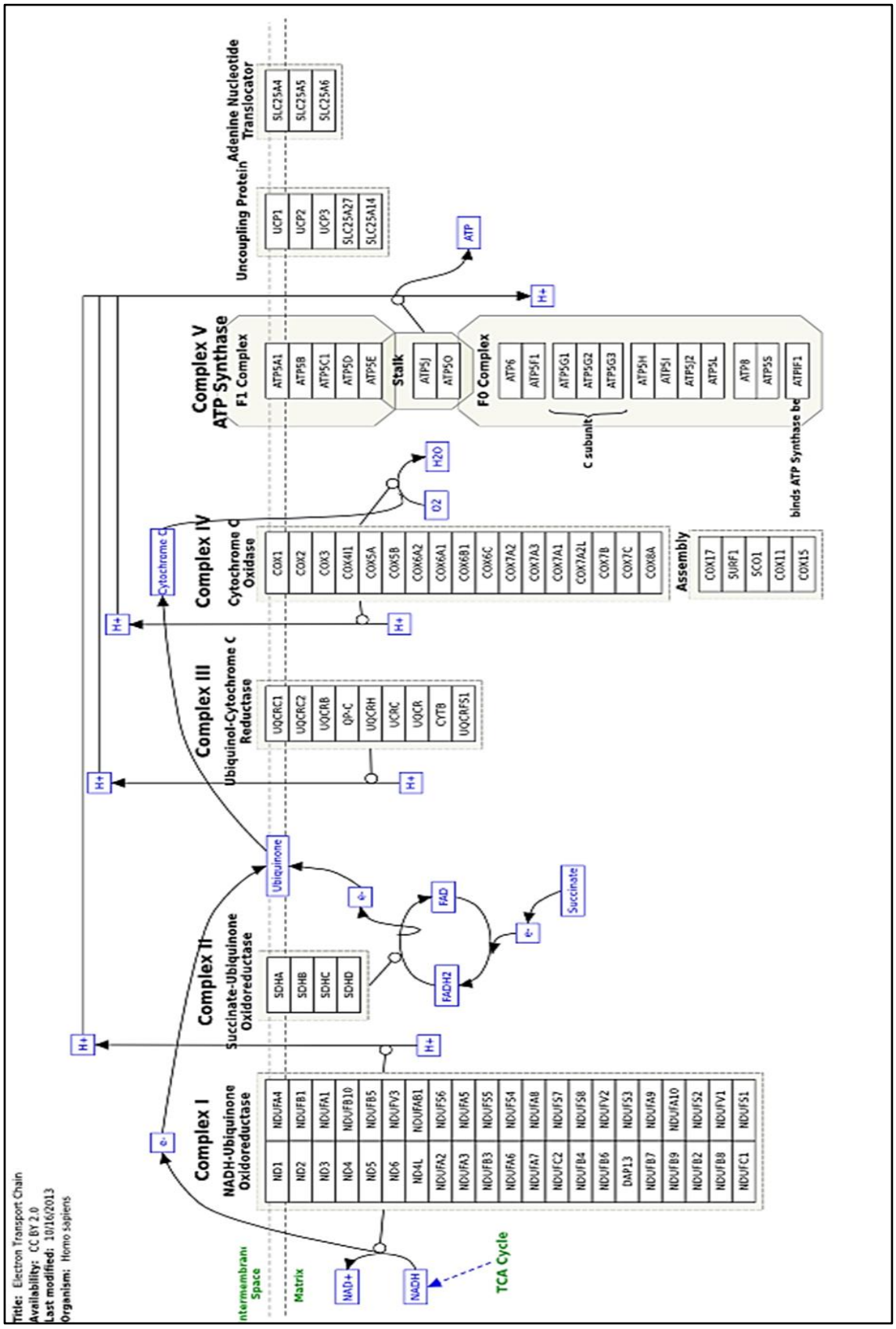
## 1.3 Mitochondrial functions and associated central nervous system diseases

Mitochondrion is called the *powerhouse* of the cell as it is mainly involved in generating energy in the form of adenosine triphosphate (ATP) (Hatefi 1994). It is involved in metabolic pathways (Scheffler 2002) and also programmed cell death- apoptosis (Green and Reed 1998).The cellular

energy (ATP) production pathway i.e. oxidative phosphorylation (electron transport chain) is present in inner mitochondrial membrane and consists of a series of five enzyme complexes: (i) Complex I is NADH dehydrogenase, or NADH:ubiquinoneoxidoreductase (ii) complex II is succinate dehydrogenase (SDH), or succinate:ubiquinoneoxidoreductase; (iii) complex III is the bcl complex, or ubiquinone:cytochrome c oxidoreductase (iv) complex IV is cytochrome c oxidase (COX),or reduced cytochrome c:oxygen oxidoreductase; and (v) complex V is ATP synthase or proton-translocating ATP synthase (Kühlbrandt 2015).

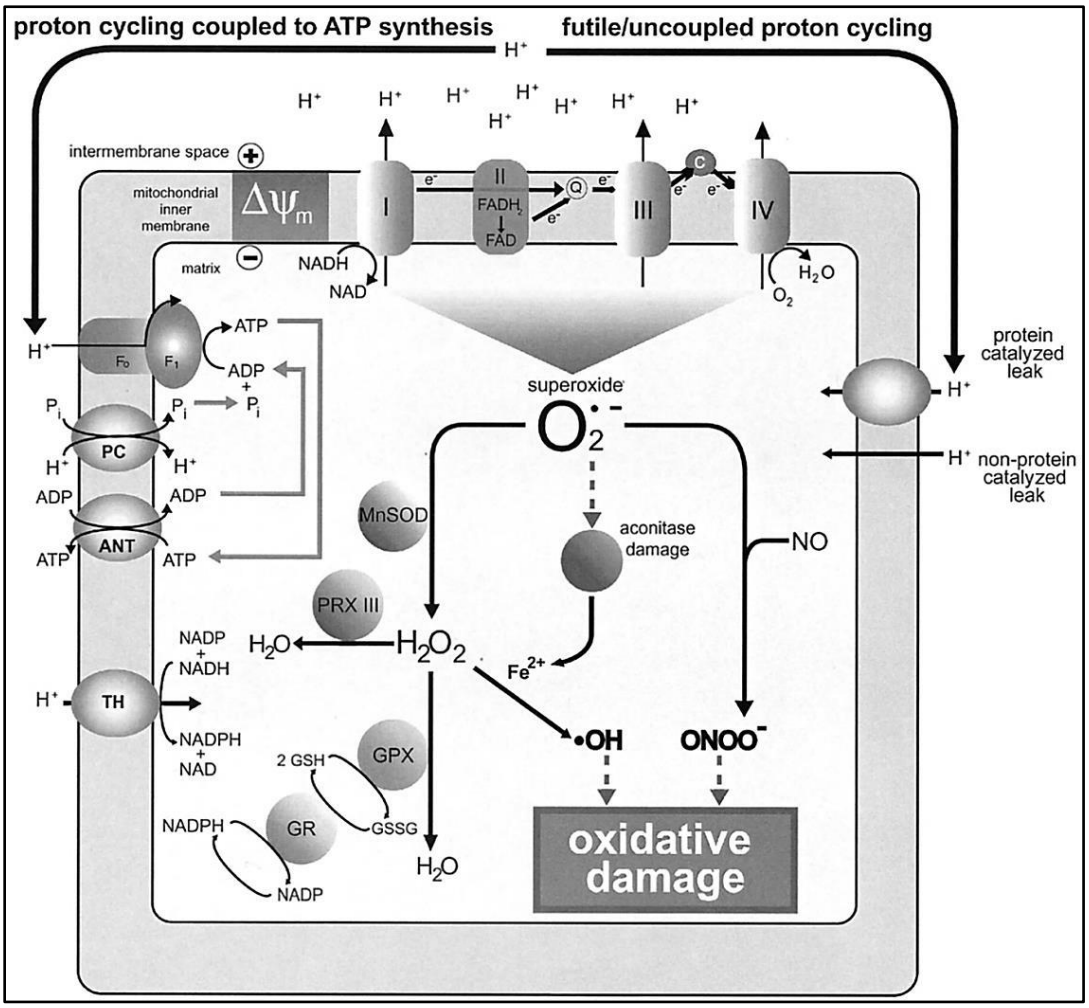
Mitochondrial function is under the dual genetic regulation i.e. mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). The 13 subunits of complexes I, III, IV and V are encoded by 37 mtDNA genes whereas; the other subunits of the ETC complexes are coded by more than 850 nDNA genes (Cotter et al. 2004, Dhar, Ongwijitwat, and Wong-Riley 2008) (Fig. 1.3). The expression, replication and maintenance of mtDNA is also under the control of nuclear-encoded genes (Taanman 1999, Gleyzer, Vercauteren, and Scarpulla 2005, Sirey and Ponting 2016, Scarpulla 2008, Dinkova-Kostova and Abramov 2015, Holmström, Kostov, and Dinkova-Kostova 2016).

**Fig.1.3 Mitochondrial electron transport chain** Image includes nuclear encoded and mitochondrial encoded genes involved in forming super complexes I- V (Source: Wikipedia:Electron_transport_chain)



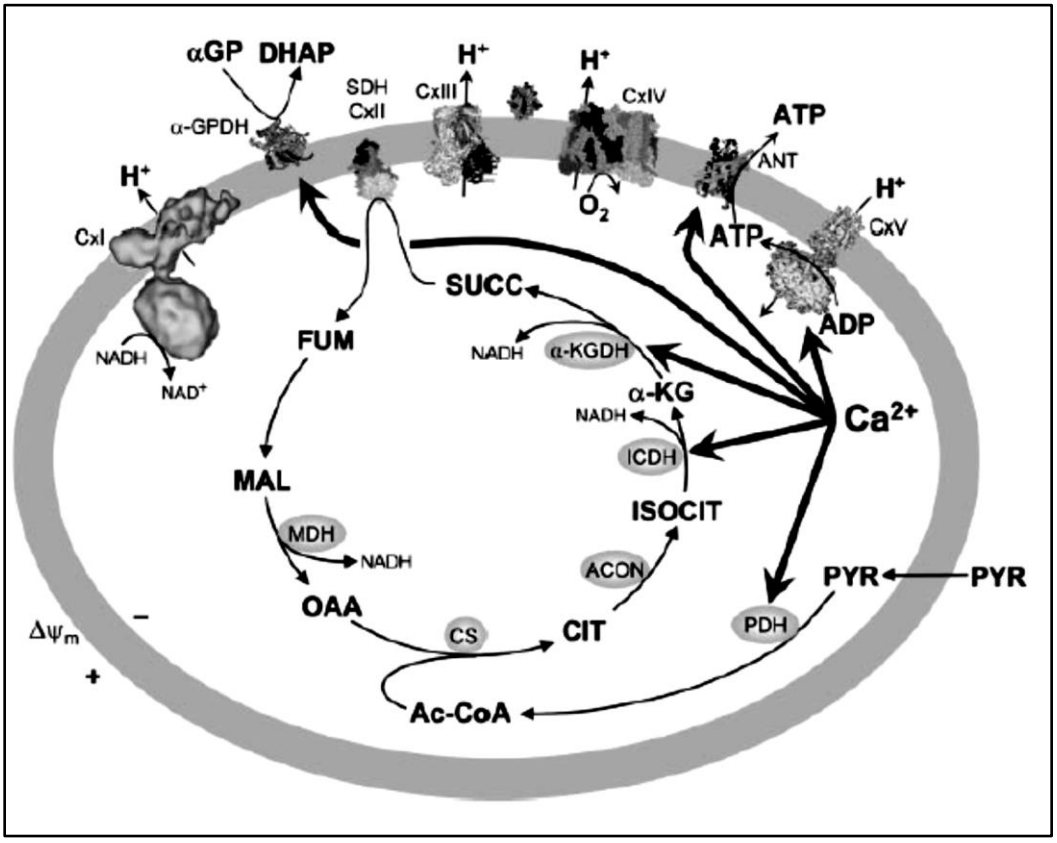
NADH and FADH generated by the tricarboxylic acid cycle (TCA cycle) operated in mitochondrial matrix carry electrons to the electron transport chain complex-I & II, which flow through the series of ETC complexes down the electrochemical gradient pumping $H^+$ ions from matrix to inter-membrane space. In the process, the consumed oxygen gets converted into the superoxide ($O_2-$) radical which then transforms into hydrogen peroxide ($H_2O_2$) and then to water($H_2O$) by superoxide dismutase (SOD) and glutathione peroxidase (GPX) respectively. The inability of these enzymes to cope up the superoxide ($O_2-$) radical conversion to water ($H_2O$) molecules causes accumulation of superoxides in the mitochondria. Additionally, mitochondrial nitric oxide synthase (mtNOS) produces nitric oxide in mitochondria which reacts with $O_2-$ radical and forms another radical, peroxynitrite ($ONOO^-$). These free radicals ultimately lead to **oxidative stress/damag**e (Fig. 1.4). The oxidative stress includes damage to mitochondria lipids, proteins, oxidative phosphorylation enzymes, and mtDNA and the cumulative effects finally compromise mitochondrial functions (Pieczenik and Neustadt 2007).

**Fig.1.4 Mitochondrial oxidative damage**. The mitochondrial respiratory chain (top) passes electrons from the electron carriers NADH and $FADH_2$ through the respiratory chain to oxygen. This leads to the pumping of protons across the mitochondrial inner membrane to establish a proton electrochemical potential gradient ($\Delta\mu_{H+}$), negative inside: only the membrane potential ($\Delta\psi_m$) component of $\Delta\mu_{H+}$ is shown. The $\Delta\mu_{H+}$ is used to drive ATP synthesis by the $F_0F_1$ATP synthase. The exchange of ATP and ADP across the inner membrane is catalyzed by the adenine nucleotide transporter (ANT) and the movement of inorganic phosphate ($P_i$) is catalyzed by the phosphate carrier (PC) (top left). There are also proton leak pathways that dissipate $\Delta\mu_{H+}$ without formation of ATP (top right). The respiratory chain also produces superoxide ($O_2\cdot^-$), which can react with and damage iron sulfur proteins such as aconitase, thereby ejecting ferrous iron. Superoxide also reacts with nitric oxide (NO) to form peroxynitrite ($ONOO^-$). In the presence of ferrous iron, hydrogen peroxide forms the very reactive hydroxyl radical (·OH). Both peroxynitrite and hydroxyl radical can cause extensive oxidative damage (bottom right). The defenses against oxidative damage (bottom left) include MnSOD, and the hydrogen peroxide it produces is degraded by glutathione peroxidase (GPX) and peroxiredoxin III (PRX III). Glutathione (GSH) is regenerated from glutathione disulfide (GSSG) by the action of glutathione reductase (GR), and the NADPH for this is in part supplied by a transhydrogenase (TH). (Source: Green, Brand, and Murphy 2004)




Another crucial role of electrochemical gradient created by electron transport chain is buffering the signal ion $Ca^{2+}$. Mitochondrion possesses calcium transporters and channels and is also one of the calcium stores of the cell (Nicholls 2005, Celsi et al. 2009). Mitochondrial calcium is also involved in regulating oxidative phosphorylation and ATP synthesis (Brookes et al. 2004) (Fig.1.5). It is known that oxidative stress causes $Ca^{2+}$ influx into the cytoplasm from extracellular environment through plasma membrane channels or from the endoplasmic reticulum(ER) through ER channels (Contreras et al. 2010).This rise in cytosolic $Ca^{2+}$ concentration further leads to $Ca^{2+}$ influx into mitochondria and nuclei, which can cause disrupted mitochondrial metabolic pathways and modulated gene transcription in nucleus by regulating calcium dependent kinases (Contreras et al. 2010, Ermak and Davies 2002).Thus, it is established fact that mitochondria is involved in maintaining cellular calcium homeostasis too and impaired mitochondrial enzymes can imbalance mitochondrial calcium flux and vice versa(Contreras et al. 2010).

**Fig.1.5 $Ca^{2+}$ activation of the TCA cycle and oxidative phosphorylation.** Thin arrows represent metabolic pathways/ reactions; thick arrows represent actions of $Ca^{2+}$. The outer membrane is omitted for clarity. Where possible, known 3D structures obtained from the Protein Data Bank are shown. For α-glycerophosphate (α-GP) dehydrogenase (α-GPDH), the cytosolic isoform structure is shown. Succ, succinate; α-KG, α-ketoglutarate; Isocit, isocitrate; Cit, citrate; OAA, oxaloacetate; Mal, malate; Fum, fumarate; Ac-CoA, acetyl coenzyme A; Pyr, pyruvate; PDH, pyruvate dehydrogenase; Acon, aconitase; CS, citrate synthase; MDH, malate dehydrogenase; ICDH, isocitrate dehydrogenase; α-KGDH, α-ketoglutarate dehydrogenase; DHAP, dihydroxyacetone phosphate; CxI–V, complexes I–V;SDH, succinate dehydrogenase (source: (Brookes et al. 2004))




Thus, Mitochondrial dysfunction through glutathione depletion, glutamate excitotoxicity, altered gene expression of electron transport chain complexes, abnormal $Ca^{+2}$ elevation, oxidative stress, decreased ATP levels and mutation in mtDNA or nDNA, deficiency of micronutrients are possible underlying mechanisms (Pieczenik and Neustadt 2007, Streck et al. 2014).

Many of the diseases have mitochondrial dysfunction as an underpinning culprit including some of them associated with nervous system such as Alzheimer's disease, Parkinson's disease, Bipolar syndrome, Schizophrenia, Multiple sclerosis, Amyotrophic lateral sclerosis and Anxiety disorders

(Pieczenik and Neustadt 2007, Keane et al. 2011, Contreras et al. 2010, Celsi et al. 2009, Chauhan, Gu, and Chauhan 2012). Interestingly, astrocytes play the crucial role in above CNS diseases. Recently it has also been reported that astrocytes donate healthy mitochondria to neurons after stroke (Hayakawa et al. 2016) thus mitochondrial dysfunction in astrocytes makes neurons also vulnerable to cell death (Ouyang et al. 2007).

### 1.4 Role of MeCP2 in mitochondrial dysfunction

Epigenetic regulations such as DNA methylation, histone modifications, and microRNAs (miRNAs) regulation are the major regulatory mechanisms responsible for the control of metabolic pathways at the transcriptional or post-transcriptional level (Xu et al. 2016).

MeCP2, a global transcription factor, binds to methylated, nonmethylated CpG islands present in promoter region as well as intergenic regions within imprinted loci (Georgel et al. 2003, Harikrishnan et al. 2010, Yasui et al. 2007); and acts as activator as well repressor for gene transcription (Ben-Shachar et al. 2009, Chahrour et al. 2008). It's highly explored methyl-CpG-binding protein due to its contribution in neurodevelopmental disorder- Rett syndrome (Amir et al. 1999) that has symptoms like mental retardation, loss of acquired skills such as speech and purposeful hand use, stereotypical movements, acquired microcephaly, seizures, autistic features, and respiratory abnormalities (Hagberg et al. 1983). MeCP2 functions in pleiotropic ways by binding to various transcription partner proteins (Chahrour et al. 2008, Forlani et al. 2010, Kokura et al. 2001, Nan et al. 1998, Young et al. 2005) and with multiple types of post-translational modifications (Bellini et al. 2014, Parikh, Tripathi, and Pillai 2017) in stimulus-dependent manner that makes it multifaceted and this very complexity also worsens diseases associated with MeCP2 functions.

Mitochondrial dysfunction has been observed in brain of MeCP2 null mouse and RTT syndrome patients reviewed in detail by (Shulyakova et al. 2017). There are several reports showing altered mitochondrial respiratory chain complexes genes, proteins and enzyme activities, ROS generation, impaired calcium homeostasis, reduced glutathione level and altered ROS stabilizing enzymes such as superoxide dismutase, catalase, Glutathione S-transferase, glutamate excitotoxicity, and reduced ATP level in RTT condition (Kriaucionis et al. 2006, Pecorelli et al. 2013, Gold et al. 2014, Valenti et al. 2017, De Filippis et al. 2015, Li et al. 2013, Saywell et al. 2006, Gibson et al. 2010, Jin et al. 2015, Janc and Müller 2014, Formichi et al. 1998, Großer et al. 2012, Mironov et al. 2009, Marchetto et al. 2010, Dong et al. 2018).

### 1.5 Neuroinflammation and associated central nervous system diseases

Inflammation is a first line defensive mechanism against injury or infection. It involves release of pro-inflammatory and anti-inflammatory cytokines (Rothwell and Luheshi 2000, Tansey and Wyss-Coray 2008). This cytokines release further regulates activation of immune cells and their immune functions (Hohlfeld, Kerschensteiner, and Meinl 2007, Allan and Rothwell 2003a). During inflammation, production of eicosanoids (prostaglandins, thromboxane and leukotrienes) derived from arachidonic acid by cyclooxygenase (COX) and lipoxygenase (LOX) pathways also increase (Khanapure et al. 2007, Robinson 1989). Along with its beneficial effects, detrimental effects have also been documented in many of the diseases including various acute, chronic and neurodevelomental disorders (Allan and Rothwell 2003a, Schmidt et al. 2004, Myers, Campana, and Shubayev 2006, Allan and Rothwell 2001, Iadecola et al. 2001, Nagayama et al. 1999, Araki et al. 2001, Candelario-Jalil et al. 2007, Candelario-Jalil and Fiebich 2008, Griffin and Mrak 2002, Van Everbroeck et al. 2002, Pardo, Vargas, and Zimmerman 2005, Harry , Gruol and Nelson 1997, Gadient and Otten 1997, Lucas, Rothwell, and Gibson 2006).

The inflammation in central nervous system disorders is not attributed to only microglia, a myeloid lineage (immune) cells resident into CNS but also astrocytes play a critical role in elevating inflammation and inflammation mediated damages (Maragakis and Rothstein 2006b, Li et al. 2011, Malmeström et al. 2006, Selmaj et al. 1990, Penkowa et al. 1999, Colombo and Farina 2016, Hansson et al. 2016, Rossi 2015, Hsieh and Yang 2016, Sofroniew 2015). Several studies have found astrocytes acting as a bridge between nervous and immune system by expressing receptors involved in innate immunity including toll like receptors (TLR-2,TLR-4), components of complement system, and MHC-II, B7, CD-40 receptors which are critical in T-cell activation (Carpentier et al. 2005, Dong and Benveniste 2001, Marinelli et al. 2015). Moreover, they produce pro-inflammatory and anti-inflammatory cytokines, chemokines and eicosanoids that act as immune mediators in cooperation with microglia and provoke adaptive immune system (Dong and Benveniste 2001, Font-Nieves et al. 2012, Gruol and Nelson 1997). In LPS activated murine primary astrocytes, study on pharmacological inhibitors of signalling molecules show that TNF-α is regulated by activation of ERK1/2, JNK, p38, AP-1 and NF-κB pathways.IL-1β and IL-6 are regulated by ERK1/2, JNK, p38 and AP-1 but not NF-κB whereas, MCP-1 and iNOS in LPS activated astrocytes (Gorina et al. 2011, Font-Nieves et al. 2012, Lu et al. 2010). Astrocytes also undergo reactive astrogliosis, a hallmark of many neurodegenerative diseases which involves proliferation, morphological changes and enhanced glial fibrillary acidic protein (GFAP) expression. On the one hand astrogliosis is beneficial due to the increased production of growth

factors and neurotrophins by astrocytes as that supports neuronal survival and promotes neuronal growth, but on the other hand it forms glial scars ultimately which is detrimental for neuronal function (Hatten et al. 1991, Sofroniew and Vinters 2010).

### 1.6 Structure, metabolism and properties of Quercetin- a plant flavonol

Quercetin (3,3′,4′,5,7-pentahydroxyflavone), a most abundant plant flavonol, is majorly present in fruits and vegetables such as apples, berries, onions and capers (Costa et al. 2016). It is mostly present in the form incorporated to sugar moieties such as quercetinglucoside, quercetingalactoside, and quercetinarabinoside(Costa et al. 2016). These glycosides are aglycosylated by lactase phlorizin hydrolase (a beta-glucosidase) and/or gut microbiota-derived beta glucosidase into aglyconic form of quercetin (Costa et al. 2016). Quercetin (aglycone) modifies into methylated, sulfated and glucuronidated metabolites in enterocytes (Costa et al. 2016).

Quercetin can traverse through blood brain barrier (BBB) owing to P-glycoprotein transporters and lipophilicity (Faria et al. 2010, Youdim et al. 2004) (Fig.1.6). Although the evidences confirm quercetin entry through blood brain barrier, the quantity of quercetin accumulated in brain is low relative to other tissues and retention plateau occurs within one week of administration in rat brain (Ishisaka et al. 2011). In *in vivo,* highest level of quercetin aglycone has been observed in lungs whereas, lowest level was observed in brain, spleen and adipose tissue in rats after 11 weeks and highest level in liver and kidney while lowest levels in brain, heart and spleen in pigs after 3 days of feeding quercetin-rich diet (de Boer et al. 2005).

Several reports suggest role of quercetin as anti-oxidant (Çevik et al. 2013, Song et al. 2013), anti-inflammatory (Al-Fayez et al. 2006, Bhaskar, Shalini, and Helen 2011, Cho et al. 2003, Jackson et al. 2006, Shen et al. 2002, Zhang et al. 2011, Hämäläinen et al. 2007), cell protective (Cao et al. 2007, Schültke et al. 2010, Silva et al. 2008, Yousef et al. 2010, Wang et al. 2011), anti-proliferative or apoptotic (Spencer, Rice-Evans, and Williams 2003), proliferative and regenerative (Dihal et al. 2006, Wang et al. 2011, Wu et al. 2014) compound in *in vitro* and *in vivo*. There are many other beneficial effects of quercetin and quercetin-rich diet reported in *in vitro* and *in vivo* studies (Henagan et al. 2015, Seo et al. 2015, Moon et al. 2013). Clinical trials using quercetin (Hamdy and Ibrahem 2010, Akhlaghi et al. 2018, Hughes et al. 2008, Pfeuffer et al. 2013, Edwards et al. 2007, Rezvan et al. 2017, Hassan, Sharrad, and Sheri 2018)or plant extracts containing high amount of quercetin (Shrivastava and John 2006, Bondonno et al. 2018, Choi et al. 2015) in various disease conditions also show beneficial effects of quercetin.

### 1.7 Effect of quercetin on CNS cells

Quercetin has been shown to attenuate neuroinflammation in the CNS by down-regulating pro-inflammatory cytokines expression through modulating Wnt5a signalling pathway in rodent model (Yang et al. 2018). Several other studies also show neuroprotective role of quercetin in CNS cells by targeting different pathways and molecules (Suganthy et al. 2016, Rinwa and Kumar 2013, Testa et al. 2014, Mehta, Parashar, and Udayabanu 2017, Bournival et al. 2012, Kao et al. 2010, Alugoju, Swamy, and Periyasamy 2018, Carvalho et al. 2018, Ding et al. 2018, Fereidounni and Dhawan 2018, Gargouri et al. 2018, Gupta et al. 2018, Jabir, Khan, and Tabrez 2018, Li et al. 2018, Naeimi et al. 2018, Sarchielli et al. 2018, Vargas-Restrepo, Sabogal-Guáqueta, and Cardona-Gómez 2018b, a, Wang, Zhao, et al. 2018, Wang, Li, et al. 2018).

### 1.8 Structure, metabolism and properties of Docosahexaenoic acid- an omega-3 polyunsaturated fatty acid

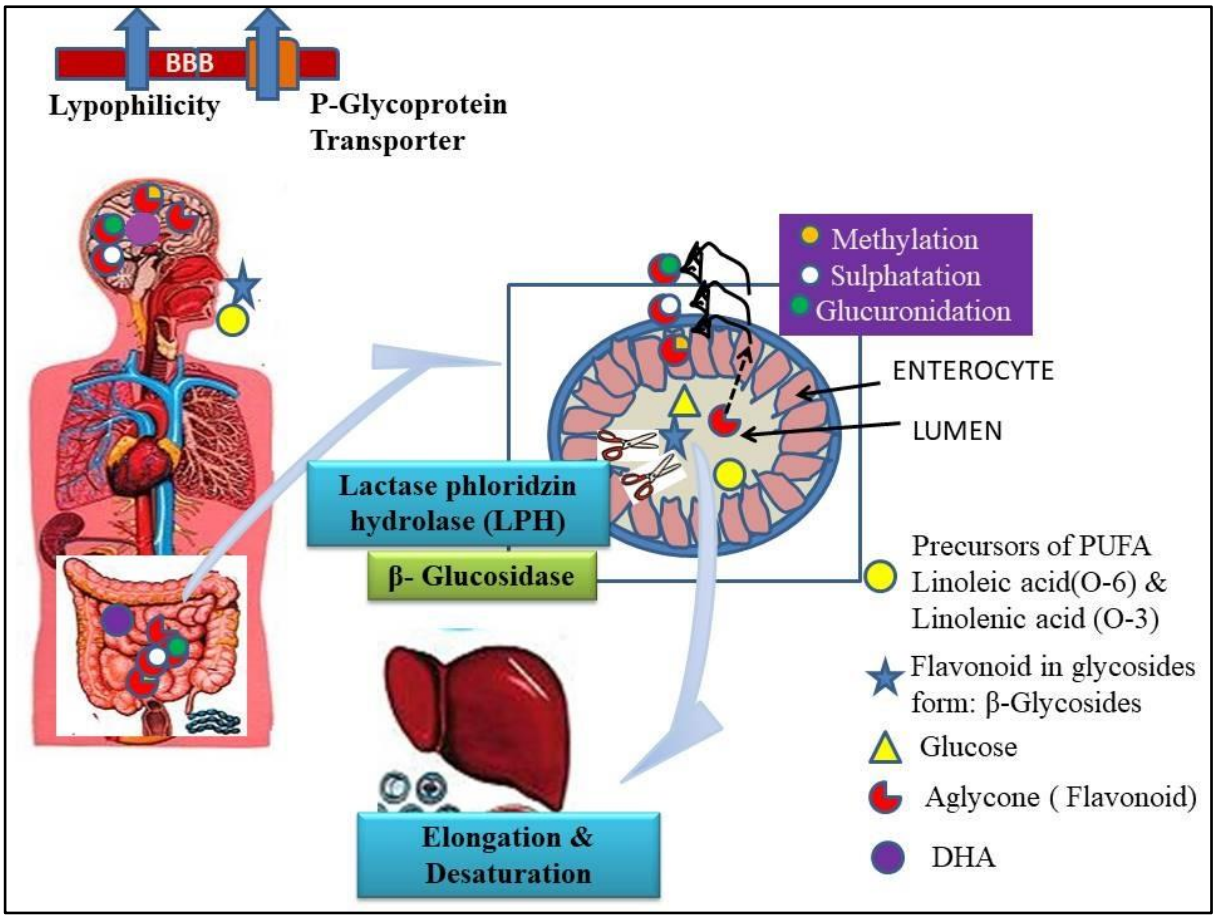
Docosahexaenoic acid (DHA; 22:6n-3) is an omega-3 polyunsaturated fatty acid derived from linoleic acid (18:2n-6, LA) and α-linolenic acid (18:3n-3; ALA) (Essential fatty acids) which cannot be synthesised by mammals. Although LA and ALA cannot be synthesised by humans they can be metabolised to other fatty acids. Both Arachidonic acid (AA) and Eicosapentanoic acid (EPA) can be further metabolised into Docosahexaenoic acid (DHA) in liver (Burdge 2018). DHA has been also shown to synthesis by cerebral endothelial cells and astrocytes as reviewed by (Horrocks and Farooqui 2004). However, synthesis of DHA in astrocytes is lesser than DHA supplied to brain tissue from plasma. DHA can metabolize into new class of lipid mediators called D-Resolvins and neuroprotectin D1(Dyall 2015). LA is abundant in many vegetable oils, including corn, sunflower and soybean oils, and in products made from such oils, such as margarines whereas; ALA is found in green plant tissues, in some common vegetable oils, including soybean and rapeseed oils, in some nuts, and in flaxseed (also known as linseed) and flaxseed oil. EPA, Docosapentaenoic acid and DHA are found in fish, especially so-called ‘oily’ fish (tuna, salmon, mackerel, herring, sardine). The commercial products known as fish oils also contain these long chain n-3 PUFAs (Calder 2008).DHA accumulates in second highest amount in brain tissue after adipose tissue (Horrocks and Farooqui 2004).

DHA, being the unsaturated fatty acid, can traverse through blood brain barrier owing to its lipophilicity (Jump 2002a) (Fig.1.6). The fluidity of membrane due to incorporated DHA affects the signal processing properties of cells but the excess dietary PUFAs reduce membrane-bound cholesterol that can cause membrane rigidity (Horrocks and Farooqui 2004). DHA has also been

reported to modulate gene expression of many enzymes associated with signal transduction processes. DHA modulates ion channels and neurotransmitters and their receptors but its effects on ion channels are quite complex (Horrocks and Farooqui 2004). Thus, it has been found to have pleiotropic effects which suggest its multiple target sites for the action.

The consumption of DHA has been shown with many health benefits including beneficial effects on brain (Horrocks and Farooqui 2004, Dyall 2015). DHA and its metabolites have been known to have anti-inflammatory (Calder 2008), anti-apoptotic (Mukherjee et al. 2004, Bryner et al. 2012) and cell protective (Blondeau et al. 2002, Adkins and Kelley 2010), anti-oxidant (Kusunoki et al. 2013, Wu, Ying, and Gomez-Pinilla 2004), anti-cancerous (Narayanan, Narayanan, and Reddy 2001, Moloudizargari et al. 2018) effects in different conditions *in vitro* and *in vivo*. Clinical trials in unhealthy individuals show positive role of DHA at some extent (Mann et al. 2018, Torquato et al. 2018, AbuMweis et al. 2018, Alfaddagh et al. 2018, Chang et al. 2018, Smith et al. 2018, Allaire et al. 2018).

**Fig.1.6 Metabolism of quercetin and DHA and availability to brain** Quercetinis present in glycoside form in nature which gets metabolized by lactate phloridzin hydrolase and β-glucosidase enzymes into lumen of intestine. Quercetinaglycones then get modified by sulphatation, glucuronidation or methylation processes in enterocytes. These products and unmodified quercetinaglycone can traverse through blood brain barrier(BBB) due to their lipophilic nature and through P-glycoprotein transporter. Linoleic acid (18:2n-6, LA) and α-linolenic acid (18:3n-3; ALA) (Essential fatty acids) undergo elongation and desaturation in liver and produce DHA. DHA also can traverse through BBB due to lipophilicity.




### 1.8 Effect of DHA on CNS cells

Docosahexaenoic acid (DHA; C22: 6n-3) is one the major omega-3 polyunsaturated fatty acids in the brain. It has been shown to be important for neurological development (Green and Yavin 1998, Kawakita, Hashimoto, and Shido 2006, Calderon and Kim 2004), learning and memory (Wu, Ying, and Gomez-Pinilla 2008, Kotani et al. 2006, Wu, Ying, and Gomez-Pinilla 2004), synaptic plasticity (Wu, Ying, and Gomez-Pinilla 2008, McGahon et al. 1999), regulating certain genes expression and intracellular signalling pathways(Jump 2002b, Salem et al. 2001, Duplus, Glorian, and Forest 2000, Kitajka et al. 2002, Kitajka et al. 2004, Barcelo-Coblijn et al. 2003, de Urquiza et al. 2000, Vaidyanathan, Rao, and Sastry 1994). In CNS disorders also rescuing effects of DHA has been observed (Hashimoto et al. 2002, Salem et al. 2001, Lim et al. 2005, Calon et al. 2004, Wu, Ying, and Gomez-Pinilla 2011).

**Rationale of the study:**

Mitochondrial impairment caused due to MeCP2 mutation in Rett syndrome and neuroinflammation in central nervous system diseases are well documented problems. There are reports suggesting the compounds that can rescue these disease conditions partially but there is no promising drug available to treat these diseases completely. Several studies have shown health benefits of quercetin and DHA in CNS cells and in *in vivo* CNS disease models. Astrocytes are the key regulators of CNS metabolism and neuroinflammation and hence the current study was aimed to find out if there is any beneficial role of quercetin and DHA in above mentioned conditions.

**Major Objectives:**

1. To determine the effects of quercetin on mitochondrial dysfunction mediated by MeCP2 deficiency in astrocytes.
2. To determine the effects of DHA on mitochondrial dysfunction mediated by MeCP2 deficiency in astrocytes.
3. To evaluate anti-inflammatory role of quercetin and DHA on LPS activated astrocytes.