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Title: Roles of mitochondria in neuronal development

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ABSTRACT

      Mitochondria are ubiquitous and multi-functional organelles involved in diverse metabolic processes, namely energy production and biomolecule synthesis. The intracellular mitochondrial morphology and distribution change dynamically, which reflect the metabolic state of a given cell type. A dramatic change of the mitochondrial dynamics has been observed in early development that led to further investigations on the relationship between

mitochondria and the process of development. A significant developmental process to focus on, in this review, is a differentiation of neural progenitor cells into neurons. Information on how mitochondria-regulated cellular energetics is linked to neuronal development will be discussed, followed by functions of mitochondria and associated diseases in neuronal development. Lastly, the potential use of mitochondrial features in analyzing various neurodevelopmental diseases will be addressed.

INTRODUCTION

All cells undergo cellular respiration, whether it uses oxygen or not, to produce energy for survival. The process using oxygen to respire is called aerobic respiration and all aerobically respiring mammalian cells contain and utilize mitochondria for energy production (1). Dependency on mitochondria for energy production varies on different cell types. High energy-demanding cells rely on mitochondria for adenosine triphosphate (ATP) production because mitochondria; a major powerhouse of cells, generate ATP in the most efficient way via oxidative phosphorylation (OXPHOS) (2). OXPHOS creates a proton gradient that induces mitochondrial membrane potential (MMP), then uses oxygen for the synthesis of ATP (3). On the other hand, certain cell types prefer utilizing cytoplasmic metabolic pathways of glycolysis and pentose phosphate pathway (PPP) for cellular metabolism even though mitochondria are found in these cells (4,5). Glycolysis and PPP use glucose then generate pyruvate and NADPH in addition to ATP (Fig. 1A). Pyruvate and NADPH are essential molecules for the synthesis of amino acids and nucleotides required for highly proliferative cells to divide.

Cells in the developmental process change metabolic states to support any newly acquired structural and functional properties (6,7). Although ATP production is more efficient in OXPHOS, glycolysis is enhanced in actively proliferating cells since diverse metabolic

substances (not limited to ATP) are required (8,9). Cells need a lot of energy to sustain homeostasis and support specialized functions they have acquired from cellular differentiation. Therefore, energy metabolism shifts from glycolysis to OXPHOS and mitochondrial maturation occurs during cellular differentiation (9).

Mutations on genes necessary for mitochondrial maturation are associated with a failure in metabolic transition that can result in developmental defects (10). Since mitochondria is present everywhere, they can have an impact on all types of tissues with no limits. Studies linking mitochondrial dysfunction and developmental diseases are beginning to re-emerge. Knowing how mitochondria behave in a given condition and which genes regulate mitochondrial dynamics will facilitate an understanding of many developmental disease-etiologies. This review will focus on distinct features of mitochondria in neuronal development and diseases. We will address the roles of mitochondria along with the process of neurodevelopment.

Genes regulating mitochondria

Mitochondria are thought to have been engulfed by an ancestral cell during evolution, via a process named endosymbiosis, for more efficient cell survival (11–13). Mitochondria, therefore, are double-membraned: including a permeable outer membrane that is structurally similar to the plasma membrane and an inner membrane forming cristae that divides the mitochondrial matrix and the intermembrane space. Mitochondrion has its own genome and machinery for its gene expression. Mitochondrial genome encodes 37 genes and only makes 13 polypeptides that belong to the OXPHOS complexes (Fig. 1B) (14).

OXPHOS components excluding all 13 polypeptides synthesized in mitochondria are translated in cytoplasm from the nuclear genome transcripts (10,15–17). Nuclear genome-derived mitochondrial proteins are transported through TOM (translocase of outer

mitochondrial membrane), TIM (translocase of inner mitochondrial membrane), OXA (oxidase assembly machinery), etc. (11,18–22) The five OXPHOS complexes in the inner mitochondrial membrane (IMM) comprise of NADH dehydrogenase (Complex I), succinate dehydrogenase (Complex II), cytochrome c reductase (Complex III), cytochrome c oxidase (Complex IV), and ATP synthase (Complex V).

Mitochondria mainly facilitate energy production through OXPHOS, consisting of an electron transport chain (ETC) and an ATP synthase (17). The ETC carries electrons step-by-step that triggers proton gradient across the IMM. Keeping a constant MMP and cellular respiration cycle are significant in operating the OXPHOS, since the ATP producing complex, ATP synthase, needs a proton gradient to convert adenosine diphosphates (ADPs) to ATPs. Mitochondria participate in other cellular processes like calcium signaling, trafficking and apoptosis by interacting with many other intracellular organelles such as endoplasmic reticulums, lysosomes, peroxisomes, etc. Proteins necessary for additional mitochondrial functions are originated from the nuclear genome and transported into mitochondria. Exploration of mitochondrial protein composition, localization, and topology are in progress to fully investigate the role of mitochondria (23–28).

Mitochondria change their morphology and localization in cells under given conditions to function properly. Mitochondria are regulated dynamically with a balanced and continuous cycle of fusion and fission (Fig. 1C) (29). Fusion allows mitochondria to exchange membranous materials including various metabolites and rescue damaged mitochondria. On the other hand, fission can segregate and degrade mitochondria with impaired mitochondrial DNA (mtDNA), only leaving healthy mitochondria inside the cell. Proteins helping the fusion: MFN1/2 and OPA1, and the fission: DRP1 and FIS1, have been identified and studies are ongoing to regulate mitochondrial dynamics (30–32). Mitochondria move to a specific region of a cell where high-energy consumption is required (33). Rho

GTPases of mitochondria that affect mitochondrial motility, mitochondrial transport, etc. in mammals have been identified: MIRO-1 and MIRO-2 (34). These are proteins of the outer mitochondrial membrane (OMM) interacting with motor proteins such as KINESIN and transporting mitochondria along microtubules.

Databases demonstrating the localization of mitochondrial proteins in representative tissues have been developed. Databases such as MitoCarta, MitoMiner including Integrated Mitochondrial Protein Index (IMPI), MitoP2, MitoProteome, etc. include protein information mostly obtained by an approach to isolate mitochondria from cells (35–38). Biochemical isolation of mitochondria from cells removes the OMM. This approach identifies proteins of mitochondrial matrix and IMM. Recently, proteins localized in OMM facing the cytosol can be analyzed by an enzymatic method called engineered ascorbate peroxidase (APEX) that labels proximal and interacting proteins (28). The latest version of MitoCarta, named MitoCarta2.0, includes additional proteomic data reported in literatures and discovered via APEX (35). However, most of above databases are limited to a number of tissues and only focused on certain complexes. An extensive research that uncovers the entire protein composition of mitochondria, linked to each protein's physiological role, is necessary.

Developmental process of neurons

Mammalian neurogenesis begins at the prenatal stage and continues to the postnatal stage even for adult brains. During neonatal development, neural stem cells (NSCs) appear by the end of gastrulation and majority of the brain structure is formed by the end of embryogenesis (39). In early fetal development, NSCs called radial glial cells (RGCs) reside in the ventricular zone and produce neurons that assemble the neocortex. Newborn neurons migrate inside-out to the neocortex and form the cortical layer; younger neurons are at the outer layer of the cortex. Following neurogenesis, NSCs produce glial cells as well. In the

postnatal brain, certain population of the fetal NSCs are retained in two restricted regions of the brain and maintained as adult NSCs (40,41). While adult NSCs are surrounded by glial cells, adult NSCs keep their multi-potency and produce neurons. One of the neurogenic niches in the adult brain is the dentate gyrus (DG) of the hippocampus. Radial glia-like (RGL) NSCs reside in the subgranular zone and add newborn neurons to the granule cell layer (GCL) of DG with an outside-in pattern. Younger neurons are at the inner GCL. The occurrence of adult hippocampal neurogenesis in human brains is like other mammalian brains except for cetaceans such as whales and dolphins. Although debated recently, this phenomenon has been widely accepted for two decades (42–44).

Fetal and adult NSCs share common features in the process of neuronal development even though the environment of neurogenic niche and the layering patterns of newborn neurons are different from each other. RGCs and RGL NSCs displaying a bipolar structure produce intermediate neural progenitor cells (NPCs, also referred to as IPCs) with a non-polar structure. NPCs continue to retain stem cell markers such as SOX2 and actively proliferate. Neuronal cell fate becomes more apparent at the NPC stage. Neurons differentiated from NPCs undergo morphological changes via axonal and dendritic arborization, resulting in a change in cell polarity. Then, newborn neurons migrate to their destination and make connections with pre-existing neurons by forming synapses and integrating into an established neuronal circuit.

Neurogenesis is modulated by diverse molecular mechanisms. A representative mechanism that regulates neurogenesis is the transcriptional gene regulation (45,46). Transcription factors drive a change in the transcriptome profile of cells during neuronal development. Extrinsic factors such as signaling molecules also affect many steps of neurogenesis. For example, the WNT protein family affects proliferation of NPCs, morphogenesis of newborn neurons, migration of newborn neurons, etc. (47,48). Recently,

interests on studying the mechanisms of neurogenesis have expanded to lipid metabolism (49,50). In adult hippocampal neurogenesis, fatty acid oxidation is required for maintenance and proliferation of NPCs and lipogenesis is critical for neuronal differentiation (49). These processes aid the metabolic shift during neuronal development. Lipid can be used as an alternative energy source in addition to glucose in (an anaerobic) glucose metabolism. In this regard, significance of the mitochondrial role in neuronal development is now recognized and receiving more attention.

Mitochondrial dynamics during neuronal development and its potential association with developmental brain diseases

The significance of mitochondrial dynamics in neuronal development has been described in the animal brain. Ablating some genes involved in mitochondrial fission and fusion resulted in defects of brain development, although fusion-and-fission dynamics during neuronal development under physiological conditions is unknown (51–53). Recent studies have reported on morphological changes of mitochondria when NSCs are differentiated in the developing brain and the adult brain (54–56). In the developing brain, mitochondria shape themselves with an elongated morphology in NSCs and a fragmented morphology in NPCs (54). On the other hand, in the adult hippocampus, mitochondria form a mixture of globular and tubular structures in NSCs and a thin and more elongated morphology in NPCs (56). However, divergent observations in fetal and adult brains come to an agreement for neurons. Mitochondria of the differentiated neurons reveal an elongated morphology in the developing brain and a wider and highly elongated morphology in the adult hippocampus (54,56). Morphological changes of mitochondria during neurogenesis illustrate maturation of mitochondria and reflect metabolic shift of cells from glycolysis to OXPHOS for an increase in bioenergetics (Fig.2) (57).

Adding on to morphological changes of mitochondria during neurogenesis, RNA expression profiles of single cells during neuronal differentiation demonstrate a metabolic shift from glycolysis to OXPHOS (58,59). In adult hippocampus, it clearly demonstrates that RGL NSCs highly express glycolytic genes and lose expression of those genes with differentiation (58). Corresponding to this, increased expression of OXPHOS genes are characterized in post-mitotic neurons. Particularly, genes of Complex V in OXPHOS are dramatically increased in their expression level upon neuronal differentiation. Expression levels of ETC genes, referring to other OXPHOS complexes: Complex I - IV, are quite consistent during neuronal development. When cells enter the post-mitotic stage in the developing brain, expression patterns of the metabolic genes also change dramatically (59). For example, expression levels of glycolytic genes such as *ALDOC* (Aldolase C) and *HK2* (Hexokinase 2) decreased once NSCs started to differentiate into neurons. Although changes in the level of some metabolism-related transcripts have been reported, a deeper analysis on the expression level changes of mitochondria- and metabolism-related genes in neurogenesis will augment mechanism studies of neuronal development. Further investigations to elucidate which mitochondrial genes and proteins contribute to mitochondrial maturation and functions at each stage of neurogenesis will be necessary.

Mitochondrial dynamics and a metabolic shift have also been investigated in human neurogenesis by utilizing NPCs, derived from human pluripotent stem cells (60,61). Expression patterns of metabolic genes were analyzed at different stages of neuronal differentiation. The expression level of MFN2, a key player of mitochondrial fusion, increase along with the differentiation of NPCs (60). Depletion of MFN2 in NPCs delays neuronal development when the overexpression promotes neuronal development, indicating significance of mitochondrial dynamics in human neurogenesis. Expression of key glycolytic genes*, HK2* and *LDHA,* are decreased while transcript levels of most OXPHOS genes do not

change when NPCs are differentiated into neurons that correspond to the results from the study of adult hippocampal neurogenesis (61).

The gene expression pattern indicates that human NPCs undergo a bioenergetic shift from glycolysis to OXPHOS. Increased mass of mitochondria in the process of NPC differentiation also support that NPCs rely on the mitochondrial function with differentiation (62,63). However, there is a caveat to understanding mitochondrial function based solely on the RNA transcript level. The level of transcripts does not always correlate to the level of proteins (64,65). Thus, additional layers of analysis on the translational and post-translational gene regulation should also be considered in order to interpret the functions of mitochondria.

Developing neurons extend neurites and generate axons required for migration of mitochondria from the soma toward axon terminals. Mitochondria supply ATPs to modulate actin filaments at the axon terminal. Regarding the transport system in mammals, two Rho GTPases of mitochondria: MIRO-1 and MIRO-2, have effects on the mitochondrial motility and transport (66). MIROs have calcium binding domains and are proteins of the OMM (67). They interact with calcium and regulate cellular motor proteins, mainly KINESIN-1. MIRO-1 is also known to mediate mitochondrial fusion and fission depending on the level of calcium in human cell lines (68). In fully differentiated neurons, mitochondria are concentrated at the pre-synapse and the post-synapse: location where a lot of energy is necessary (33,69,70). Mitochondria at synaptic terminals provide regional ATPs to neurons and modulate cytosolic calcium levels. Thus, if mitochondria are unable to reach the signal exchanging center during development and even after development, neuronal function will be impaired (71).

Mitochondrial dysfunctions due to mutations may affect the proliferation rate of NPCs and also change the efficiency of differentiation into neurons, resulting in delayed or paused neurogenesis (72). Dysfunctional mitochondria are associated with neurological diseases such as Leigh syndrome, Rett syndrome, Angelman syndrome, Autism Spectrum

Disorder (ASD), Schizophrenia, and Bipolar disorder. Leigh syndrome is a representative mitochondrial disease of dysfunctional Complex I or IV and also occurring due to a mutation in *MT-ATP6* gene belonging to Complex V (73–78). Defective Complex I and IV prevents the proton gradient from being maintained, but dysfunctional Complex V will not produce ATP even when sufficient proton gradient is generated. Rett syndrome caused by a mutation in *MECP2* gene on X-chromosome is a neurodevelopmental disease (79–81). A mutation in *MECP2* can alter the epigenetic status of the nuclear genome (82,83). As most OXPHOS subunit proteins are encoded in the nuclear genome, transcription of these genes can be affected. Deficiency in Complex IV activity is observed in animal models of Rett syndrome (84). Angelman syndrome is caused by UBE3A loss-of-function (85). Reduced activity of Complex III and change in mitochondrial morphology are observed in the Angelman syndrome. ASD is not always associated with mitochondrial dysfunctions (86). However, it is reported to be more severe with mitochondrial dysfunctions and correlated with decreased level of an antioxidant defense mechanism and an elevated level of ROS and lactate (87–91). Schizophrenia can be caused due to defective Complex I, III, and/or IV that result in decreased ATP production, higher anaerobic metabolism of glucose, and increased lactate level (91–93). Bipolar disorder is also affected by oxidative stresses similar to that of schizophrenia: higher lactate level and decreased number of protons in the mitochondrial matrix (89,94,95). Hence, studies to reveal the hidden molecular mechanisms of the neurological disease-causing mitochondrial dysfunctions will be necessary.

Perspectives

Several studies have linked mitochondria to neurological diseases by observing stage-dependent and metabolism-related changes of neurogenesis. This has opened an era of more in-depth investigations on neurometabolic diseases. Here, diverse aspects of metabolism as

main factors associated with neurodevelopmental diseases examined by many research groups have been introduced. Although a correlation between mitochondria and neuronal differentiation has been demonstrated by many groups, most have not demonstrated the underlying mechanisms in connecting mitochondria and various neurological diseases. Especially, functional implications of mitochondria on neurological diseases are lacking scientific findings that may be applied to clinical settings. However, specific features of mitochondria have been identified and are used as biomarkers or in treatments for some diseases, leaving hope for such application in neurodevelopmental diseases (96–98). Studies using human NSCs and unbiased identifications of functional proteins in mitochondria will bring in novel insights and thought-provoking discoveries to the field. The significance and function of mitochondria in neurodevelopmental diseases should not be underestimated.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FIGURE LEGENDS

Figure 1. Mitochondrial proteins, functions and dynamics

(A) Major function of mitochondria is energy production through OXPHOS. Glycolysis occurring in the cytosol produce pyruvate, which is necessary to fuel the tricarboxylic acid

(TCA) cycle. The pentose phosphate pathway (PPP) is a shunt for glycolysis. Through the PPP, cells acquire required components for other cellular processes including nucleotide synthesis. In mitochondria, beta-oxidation occurs as the other mechanism of converting lipid to generate energy. (B) Most proteins localized in the mitochondria are produced from the nuclear genome (nDNA) and transported into mitochondria. Mitochondria contain its own genome (mitochondrial DNA, mtDNA) and produce 13 proteins comprising oxidative phosphorylation (OXPHOS) complex. (C) Dynamically changing morphology of mitochondria through continuous cycle of fusion and fission.

Figure 2. Mitochondrial features and bioenergetics during neuronal development.

Neural stem cells (NSCs) and intermediate neural progenitor cells (NPCs, also referred to as IPCs) have self-renewing capacities. NSCs are differentiated into NPCs, which are then differentiated into neurons. The changes in mitochondrial morphology during neuronal development should be noted. In corticogenesis in developing brains (A), the mitochondrial morphology change from elongated structure to fragmented, then elongated again, followed by more complex structure due to further elongation. In adult hippocampal neurogenesis (B), the mitochondrial morphology changes from mixed globular and tubular structures to thin and elongated, then elongated more, followed by a wider and more complex structure due to further elongation. Level of glycolysis is decreased in both (A) and (B) when level of OXPHOS is increased along with neuronal differentiation.

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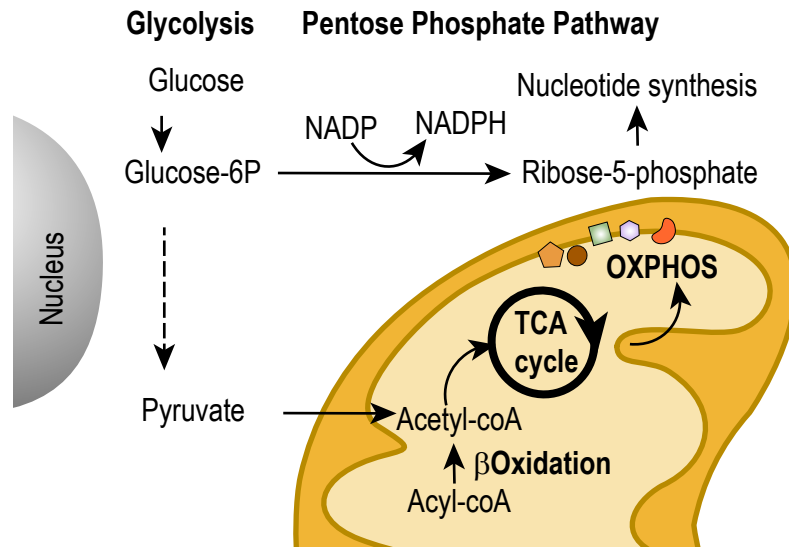
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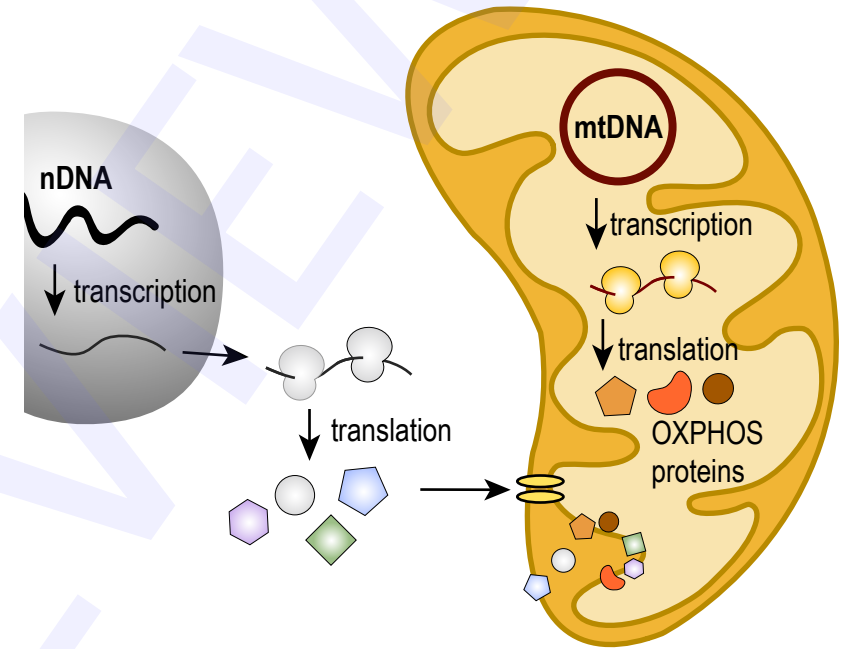
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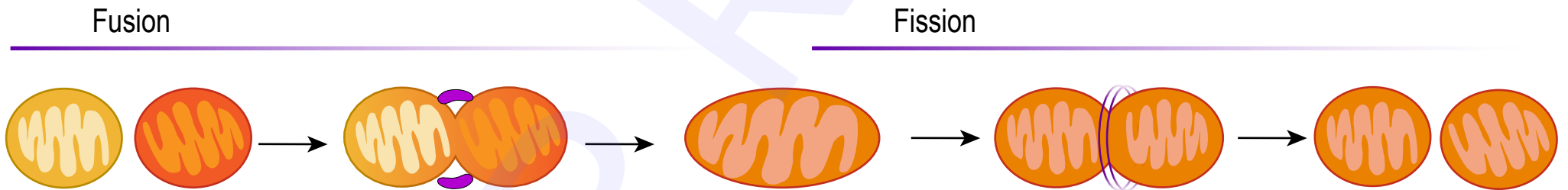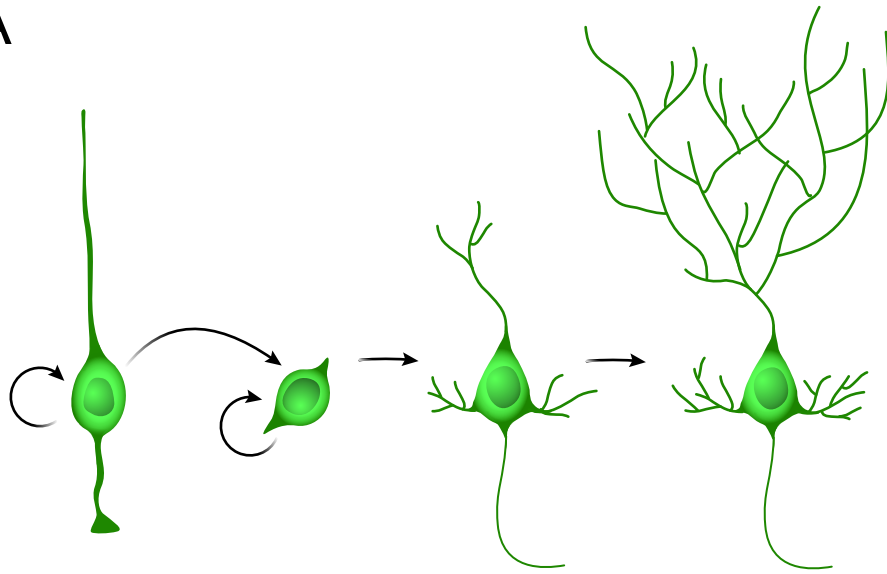
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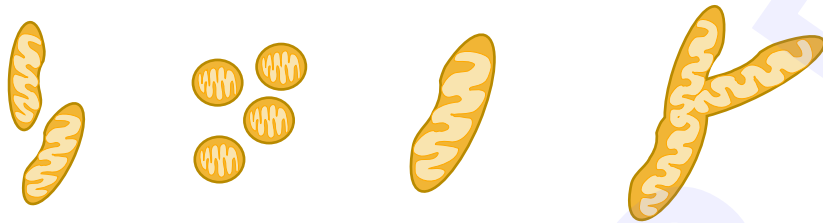**Glycolysis**   **Pentose Phosphate Pathway**

Nucleus

Glucose

Nucleotide synthesis

Glucose-6P   NADP → NADPH   Ribose-5-phosphate

OXPHOS

TCA cycle

Pyruvate → Acetyl-coA

βOxidation

Acyl-coA

B

nDNA

transcription

translation

mtDNA

transcription

translation

OXPHOS proteins
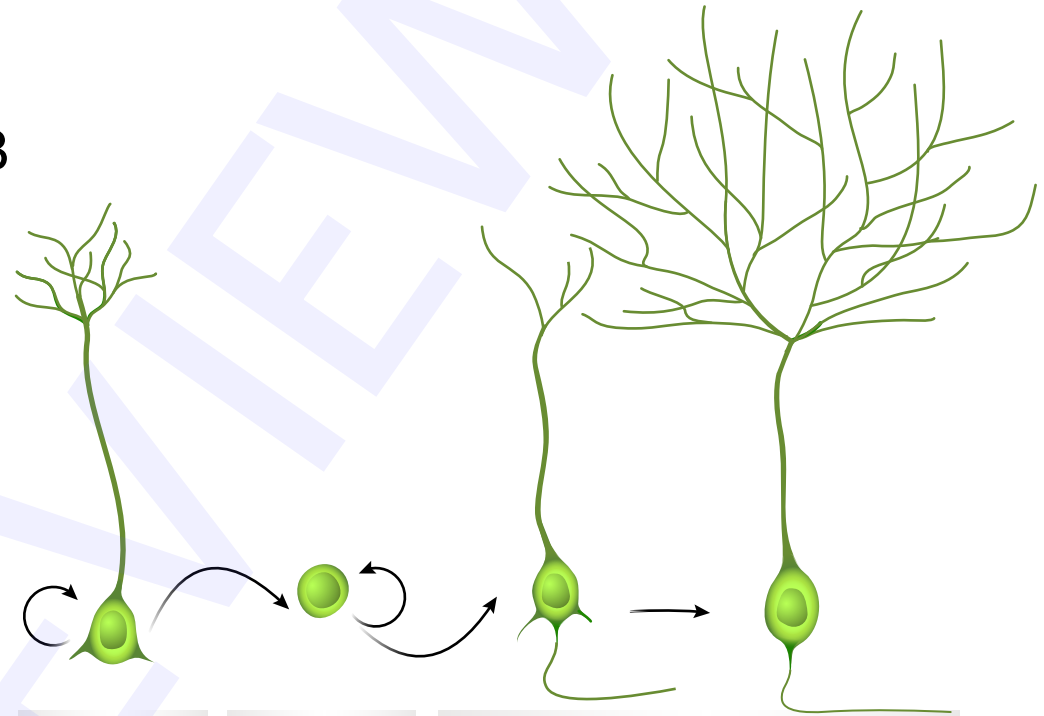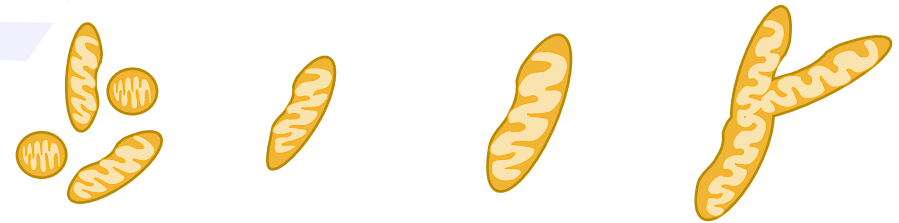
C

Fusion

Fission

A

RGC NSCs | NPCs | Neurons

Glycolysis

OXPHOS

B

RGL NSCs | NPCs | Neurons

Glycolysis

OXPHOS