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## The Bioenergetics of Neuronal Morphogenesis and Regeneration: Frontiers Beyond the Mitochondrion

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### Abstract

The formation of axons and dendrites during development, and their regeneration following injury, are energy intensive processes. The underlying assembly and dynamics of the cytoskeleton, axonal transport mechanisms and extensive signaling networks all rely on ATP and GTP consumption. Cellular ATP is generated through oxidative phosphorylation (OxP) in mitochondria, glycolysis and “regenerative” kinase systems. Recent investigations have focused on the role of the mitochondrion in axonal development and regeneration emphasizing the importance of this organelle and OxP in axon development and regeneration. In contrast, the understanding of alternative sources of ATP in neuronal morphogenesis and regeneration remains largely unexplored. This review focuses on the current state of the field of neuronal bioenergetics underlying morphogenesis and regeneration and considers the literature on the bioenergetics of non-neuronal cell motility to emphasize the potential contributions of non-mitochondrial energy sources.

### Keywords

mitochondrion; growth cone; glycolysis; creatine kinase; cytoskeleton

### INTRODUCTION

Neurons are characterized by complex morphologies. Following their terminal mitosis neuronal cell bodies migrate to their destination by generating leading edge processes that guide their migration (Jossin, 2020). As with other forms of cell migration, the nucleus follows in the direction of leading edge advance while the rear of the cell undergoes contraction allowing displacement of the cell as a whole. Upon arriving at their proper position neuronal cell bodies then generate multiple processes that develop into the axon and dendrites. The morphogenesis of neuronal processes is fundamental to the establishment of neuronal circuits and thus nervous system function. Depending on the neuron type, the axon can then extend up to meters in length in large animals. Dendrites tend to attain lengths from a few tens of microns to a few hundred depending on neuron type. Individual axons and

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dendrites form intricate patterns of branching. The branching of neuronal processes is crucial to the establishment of complex patterns of connectivity and in the case of axons allows the single axon to establish contacts with disparate targets within the nervous system. The final stage of morphogenesis involves the establishment of synaptic contacts and the refinement of neuronal projections (Riccomagno and Kolodkin, 2015). As a general rule, neurons develop more axon and dendrite branches than are present in the final configuration of the active circuit. The refinement process involves activity-dependent dynamic branch additions and retractions, even if in the absence of any further axon or dendrite elongation.

The formation of axons and dendrites involves the assembly and dynamics of the cytoskeleton (Dent and Gertler, 2003). The structure of axons and dendrites is supported by the underlying microtubule cytoskeleton. Depolymerization of microtubules results in the thinning and eventual fragmentation of axons and dendrites. The actin filament cytoskeleton is responsible for the surface features of axons and dendrites. All protrusive structures generated by axons and dendrites (e.g., filopodia, lamellipodia, synaptic structures) are dependent on actin filaments for their formation and maintenance. The tips of developing axons and dendrites are the sites of active elongation. In both cases, the tips develop structures termed growth cones.

Growth cones are highly dynamic subcellular domains that undergo complex changes in morphology and consist of a central (C) and peripheral (P) domain (Figure 1). Lamellipodia and filopodia are structures fully dependent of actin filaments for their formation and restructuring that characterize the P-domain of growth cones (Figure 1A,B) during the process of elongation and guidance to their targets. While actin filaments and thus lamellipodia and filopodia are not strictly required for axon elongation, in a context dependent manner (e.g., Abosch and Lagenaur, 1993; Selzer et al., 2006), they are required for the guidance of growth cones and are integral components of both the signaling and mechanical aspects of guidance (McCormick LE, Gupton, 2020). Myosin II is an ATPase molecular motor protein that generates contractile forces on actin filaments in an ATP hydrolysis dependent manner. Myosin II based contractility of filaments antagonizes the forward advance of axonal and dendritic growth cones and the regulation of actomyosin contractility is required for proper axon growth cone guidance (Turney and Bridgman, 2005; Loudon et al., 2006; Ketschek et al., 2007; Kollins et al., 2009; Turney et al., 2016). In growth cones the activity of myosin II at the interface of the P and C domains serves to pull the actin filaments in the P-domain toward to C-domain in a process termed actin retrograde flow (Figure 1C; Lin and Forscher, 1995; Brown and Bridgman, 2003; Kerstein et al., 2015). Microtubule plus tips are highly dynamic within the C-domain of growth cones (Figure 1D) and through cycles of polymerization and depolymerization, termed dynamic instability, probe the intracellular environment and allow the subcellular targeting and delivery of cargoes rides along them. Microtubule plus tip polymerization is required for the elongation of axons, although in some cases the transport of short microtubules within the axon to its tip may suffice. Microtubule dynamic instability is required for growth cone guidance. It is generally considered that interactions between actin filaments and the plus tips of microtubules are responsible for the directed turning of growth cones in response to extracellular signals. Myosin II-driven actin retrograde flow counters the ability of microtubules plus tips to advance into the P-domain (Ketschek et al., 2007; Schaefer et al.,

2008; Turney et al., 2016). Myosin II contractility at the region where the growth cone splay out from the main axon, often termed the “neck” of the growth cone, serves to maintain the growth cone polarized at the end of the axon and bundle microtubules into the axon as the growth cone advances (Burnette et al., 2008).

The formation of axon branches along the axon shaft, independent of the activity of the growth cone, also requires the dynamic reorganization of the axonal cytoskeleton (Armijo-Weingart L, Gallo, 2017; Menon and Gupton, 2018). Branches are initiated as filopodia that in turn emerge from transient localized “patches” of actin filaments. Axonal filopodia are transient and only a few mature into branches. The maturation requires the targeting and retention of a microtubule plus tip into the filopodium that subsequently loses its filopodial actin organization and develops a polarized accumulation of actin filaments at the tip of the nascent branch. As the branch matures further microtubules become stabilized to support continued elongation and the tip develops a growth cone, albeit usually smaller than that present at the tip of the main axon.

## BIOENERGETIC DEMANDS OF AXON AND DENDRITE DEVELOPMENT

Actin is an ATPase. The loading of an actin monomer with ATP greatly increases the polymerization competency of the monomer into a filament relative to its ADP bound state (Pollard, 2016). The importance of this aspect of actin biology is emphasized by the existence of actin regulatory proteins that regulate the loading of ATP onto actin (Goldschmidt-Clermont et al., 1992). Following polymerization into a filament the ATPase activity of actin hydrolyzes the ATP to ADP and following depolymerization from the filament that actin submit must be reloaded with ATP prior to reutilization (Figure 1A,B). Similarly, tubulin is a GTPase and has similar requirements for its polymerization into a microtubule as actin into a filament (Bowne-Anderson et al., 2015). Following polymerization into a microtubule the GTPase activity of tubulin hydrolyzes the bound GTP. The dynamic plus ends of microtubules thus contain a high content of GTP-bound tubulin relative to the rest of the microtubule lattice.

A difference between microtubules and actin filaments is that the latter are overall much more dynamic and undergo much faster turnover. While there are differences in the relative durations of filaments in different actin filament based structures (e.g., lamellipodia relative to stress fibers), overall actin filaments have life spans ranging from a few seconds to minutes. These high rates of filament turnover are particularly representative of filaments underlying protrusive structures, such as the P-domain of the growth cone. In contrast, in axons and dendrites microtubules have stable lattices that undergo relatively little tubulin exchange while their plus tips are the dynamic ends of the microtubules that exhibit dynamic instability and thus depend on GTP-loaded tubulin for their dynamics. The growth cone C-domain contains mostly the dynamic plus tips of axonal microtubules. Thus, the growth cone is a subcellular domain that is a sink for both ATP and GTP utilization by cytoskeletal turnover and assembly. Having said that, the axon shaft also contains dynamic microtubule plus tips and populations of dynamic actin filaments.

The bioenergetic drain (e.g., the relative utilization of ATP/GTP levels) of cellular processes in axons and dendrites is not well understood. In developing neurons undergoing process extension, the maintenance of membrane potential through the activity of ATP utilizing Na<sup>+</sup>/K<sup>+</sup> pump systems is estimated to account for approximately 50% of the bioenergetic drain for ATP (Bernstein and Bamberg, 2003; Engl and Attwell, 2015). Importantly, in the same developing neurons the drain of actin filament turnover is estimated to account for the remaining 50% of ATP utilization (Bernstein and Bamberg, 2003). A similar estimate for the bioenergetic drain of actin filament turnover was obtained for platelets (Daniel et al. 1986). Analysis of the relative contributions of actin turnover, through preventing filament polymerization, in slices of postnatal rat hippocampus arrived at estimates of oxygen utilization of approximately 25% (Engl et al., 2017), but this reflects the averaged bioenergetic drain of the various cell types present in slices and it should be noted that this experimental system is at post-developmental stages reflecting established circuitry and not developing axons and dendrites. The same study estimated a 22% utilization of oxygen by microtubule dependent processes as determined by treatment of the slices with high doses of the microtubule depolymerizing agent nocodazole, but this result cannot be attributed directly to the drain of microtubule dynamics as depolymerization of microtubules is expected to have occurred that would result in a variety of changes in cellular physiology including indirect alterations in the actin cytoskeleton. A specific analysis of the bioenergetic drain of microtubule dynamics under more controlled conditions is lacking, as is an analysis of the drain of axonal transport systems. Analysis of the bioenergetic drain of cytoskeletal dynamics in developing, and later stage, neurons under well controlled conditions is thus warranted. However, the current experimental evidence indicates that actin filament dynamics constitute a major bioenergetic drain in cells that exhibit high filament turnover rates such as developing neurons, and by inference specifically at the growth cone.

## MITOCHONDRIA AND OXIDATIVE PHOSPHORYLATION

Although a role for mitochondria in regulating cell migration in non-neuronal cells has been thoroughly investigated (Denisenko et al., 2019; Majumdar et al., 2019), the role of the mitochondrion in neuronal migration remains minimally considered. Intriguingly, the role of mitochondria in the migration of cortical neurons to their target positions is neuron type dependent (Lin-Hendel et al., 2016). Glutamatergic projection neurons (PN) are guided to their position by radial glial fibers that the neurons attach to and follow along a relatively linear trajectory. In contrast, interneuron GABAergic neurons (IN) take a more circuitous tangential route to reach their target positions. Mitochondria in IN neurons are dynamic and undergo changes in subcellular position and target to the leading edge, while in PN neurons they tend to reside perinuclearly. Pharmacological and genetic inhibition of OxP impaired the migration of IN neurons but not PN neurons. These observations pave the way for continued analysis of the role of mitochondria and OxP in the migration of the many other neuron types during both central and peripheral nervous system development.

In fully differentiated neurons, mitochondria undergo bidirectional transport along axons and dendrites. The molecular motor systems and aspects of the regulation of mitochondrial transport are well understood (Melkov and Abdu, 2018). Mitochondria undergo long distance transport on microtubules using kinesin and dynein motors and more local



promotion of mitochondria transport to the tips of regenerating axons increases the rate of regeneration in vitro and in vivo both in the peripheral and central nervous system (Zhou et al., 2016; Cartoni et al., 2016, 2017; Han et al., 2020). Mitochondrial calcium uptake after axon injury also promotes regenerative competency (Lee et al., 2019; Tang et al., 2020), possibly through the regulation of OxP. Chondroitin sulfate proteoglycans (CSPGs) are components of the extracellular matrix that inhibit axon extension during regenerative attempts (Tran et al., 2018) and stabilize axonal circuitry in the mature nervous system (Fawcett et al., 2019). On CSPGs mitochondria can target to growth cones but then exhibit increased rates of retrograde evacuation resulting in growth cones with lower mitochondria content than those on control growth permissive substrata (Sainath et al., 2017a). Myelin associated glycoprotein, an inhibitor of axon regeneration, and CSPGs impact the transport of mitochondria along axons in adult sensory neurons (Kalinski et al., 2019). CSPGs also induce depolarization of the mitochondrial membrane potential (Sainath et al., 2017a, 2017b; Kalinski et al., 2019) that is expected to result in decreased ATP production. In contrast, axon extension/regeneration promoting factors (e.g., neurotrophins) hyperpolarize the mitochondrial membrane potential (Huang et al., 2005; Verburg and Hollenbeck, 2008).

Axon branching is dependent on the positioning of stalled mitochondria along axons. The sites of the formation of axonal filopodia and in turn branching correlate with sites populated by stalled axonal mitochondria along both sensory and central nervous system axons (Courchet et al., 2013; Spillane et al., 2013; Tao et al., 2014). Experimentally decreasing the proportion of stalled mitochondria through suppression of syntaphilin, a microtubule based mitochondria anchoring molecule, suppresses branching of cortical axons in vitro and in vivo (Courchet et al., 2013). Inhibition of OxP prevents branching along sensory axons in response to nerve growth factor (NGF; Spillane et al., 2013). Although stalled mitochondria determine the sites of axons where branching may occur, following the initial maturation of a branchlet mitochondria undergoing transport then populate the branch as it develops further (Armijo-Weingart et al., 2019). Developing branches are preferentially populated by mitochondria representing the shorter subpopulation of axonal mitochondria (Armijo-Weingart et al., 2019). In the case of NGF induced sensory axon branching NGF drives a rapid bout of mitochondria fission and then maintains a new steady state of shorter mitochondria in axons that provide a greater number of ideally sized mitochondria for branch development (Armijo-Weingart et al., 2019). In contrast to axons, mitochondria aggregate in the proximal dendrites of cortical pyramidal neurons and locally negatively regulate branching (Kimura et al., 2014). The mechanism through which mitochondria suppress branching along proximal dendrites is not known but may involve the mitochondrion's role in calcium buffering (Konur and Ghosh, 2005).

The contribution of mitochondrial OxP to axonal actin dynamics is local. Axonal and dendritic filopodia arise from precursors axonal actin patches (Andresen et al., 2005; Gallo, 2013). Treatment of sensory axons NGF increases the proportion of patches that form in axon segments populated by stalled mitochondria (Ketschek and Gallo, 2010). Patches that form in axon segments populated by stalled mitochondria exhibit longer durations relative to those that form in segments lacking mitochondria (Sainath et al., 2017b; Armijo-Weingart et al., 2019). CSPGs depolarize axonal mitochondria (Sainath et al., 2017a, 2017b; Kalinski et al., 2019) and decrease the duration of patches formed in association with mitochondria

(Sainath et al., 2017b) but do not affect actin patches not associated with mitochondria. The effect of CSPGs on the duration of patches associated with mitochondria is reversed by pharmacologically promoting OxP (Sainath et al., 2017b). Furthermore, patches that form associated with mitochondria account for approximately 80% of sites of axonal filopodia formation from actin patches (Armijo-Weingart et al., 2019) and CSPGs suppress the formation of filopodia from patches (Sainath et al., 2017b), an effect partially reversed by pharmacologically promoting OxP. The induction of branches by NGF is dependent of the intra-axonal translation of mRNAs coding for actin regulatory proteins (Spillane et al., 2012). Stalled axonal mitochondria define hotspots of high intra-axonal translation of these mRNAs that is dependent on OxP (Spillane et al., 2013). A similar local role for mitochondria in defining sites of localized translation has also been described for dendrites (Rangaraju et al., 2019). CSPGs suppress axon branching and the intra-axonal translation of cortactin mRNA (Sainath et al., 2017b), one of the mRNAs whose mitochondria-dependent axonal translation contributes to branching. Pharmacological promotion of OxP on CSPGs restores cortactin translation along axons and the localized hotspots of translation along axons (Sainath et al., 2017b). Motile mitochondria can also contribute to axonal physiology. Mitochondria undergoing transport near a presynaptic site regulate synaptic vesicle release through local alterations in ATP levels (Sun et al., 2013).

## GLYCOLYSIS

Although glycolysis yield much less ATP than OxP per molecule of glucose, the rate of ATP production is greater through glycolysis than OxP and the two processes can generate equivalent amounts of ATP over equivalent time periods (Pfeiffer et al., 2001; Epstein et al., 2014; Shestov et al., 2014; Slavov et al., 2014) and glycolytic activity can be promoted to rapidly generate ATP (Epstein et al., 2014). Glycolysis is active in developing neurons. Glycolysis contributes proportionally more to ATP levels than OxP in embryonic neurons relative to later developmental stage neurons in both rat retinal ganglion cells (E20 relative to P5; Steketeer et al., 2012) and rat hippocampal neurons (E17/18 relative to P2/3; Surin et al., 2013). Glycolysis accounts for approximately 30% of cellular ATP during the early stages of cortical neuron differentiation in vitro (Agostini et al, 2016). In embryonic E12 chicken sympathetic neurons glycolysis accounts for the majority of cellular ATP (Wakade and Wakade, 1985; Wakade et al., 1985). In P1–2 rat superior cervical ganglion neurons glycolysis and OxP each contribute approximately one third and 2 thirds to ATP levels in the neurites of these neurons (Tolkovsky and Suidan, 1987). In unpublished work, using the ATP/ADP ratiometric sensor PercevalHR (Tarasov and Rutter, 2014), we find that OxP and glycolysis each account for approximately 50% of ATP in the distal axons of E7 chicken sensory neurons, respectively. These observations indicate that glycolysis may be a significant contributor to axonal bioenergetics, particularly during development. The full spectrum of the contribution of glycolysis in adult neurons remains to be determined. However, glycolysis has been involved in the regulation of synaptic physiology (reviewed in Ashrafi G, Ryan, 2017). Glycolytic enzymes associate with vesicles undergoing fast axonal transport and “on-board” glycolysis on the vesicle’s surface contributes the ATP to power its transport (Zala et al., 2013; Hinckelmann et al. 2016). In contrast, the axonal transport of mitochondria is powered by OxP independent of glycolysis (Zala et al., 2013; Spillane et al., 2013). Membrane addition is an important aspect of axon development (Winkle and Gupton,

2016). Glycolysis may thus contribute to neuronal morphogenesis through its role in fast axonal transport, but this remains to be determined.

Recent evidence has challenged the dogma that neurons rely on astrocytic lactate during activity (Díaz-García et al., 2017; Yellen, 2018; Díaz-García CM, Yellen G, 2019), further emphasizing the emerging significance of glycolysis in neuronal function. In vitro, cortical neurons initially form multiple “minor” processes (Stage II) and subsequently one of these processes differentiates into the axon (Stage III) and the remainder become dendrites. Inhibition of glycolysis using 2-deoxyglucose treatment starting at the time of culturing prevented cortical neurons from developing beyond stage II (Agostini et al., 2016), indicating a requirement for glycolysis to commence the differentiation of axons and dendrites from the undifferentiated precursor minor processes. However, the role of glycolysis in axon and dendrite development has not been specifically addressed. The observations that during in vivo development of cortical pyramidal cell dendrites there is a low density of mitochondria in distal dendritic segments, that mitochondria inhibit the branching of the more proximal apical dendrite and that suppression of mitochondria targeting into dendrites does not adversely impact the overall growth of the dendrites (Kimura et al., 2014) raise the question of whether glycolysis or other bioenergetic sources may contribute to the development of the more apical dendrite in this neuron type. GAPDH associates with GluA2- containing AMPA receptors (Lee et al., 2016). Treatment of cultured neurons with a peptide that blocks the association of GAPDH with AMPA receptors decreases dendritic growth in vitro, results in aggregation of the axonal marker TAU-1 and diminishes growth cone morphology (Lee et al., 2016). The effects of the inhibitory peptide on glycolytic activity have not been assessed and the effects have been ascribed to changes in the acetylation of p53 downstream of the GAPDH-AMPA receptor interaction (Lee et al., 2016). Thus, it is not clear whether these data are indicative of a role for GAPDH mediated glycolysis, and perhaps unlikely.

Although the migration of whole cells is not the same process as axon extension, growth cones share similarities to the leading edges of migratory cells. Some glycolytic enzymes (e.g., phosphofructokinase, aldolase, pyruvate kinase, lactate dehydrogenase, glyceraldehyde 3-phosphate dehydrogenase) have actin binding sequences and target to subsets of actin filaments in cells (Masters et al., 1987), ideally placing them for the regulation of actin based processes related to cell motility. Cancer cells have to contend with low oxygen environments and under these conditions rely on glycolysis for their bioenergetic needs (Dias et al., 2019; Yang et al., 2020) and cell types such as renal podocytes and astrocytes can survive without O<sub>2</sub> and rely on glycolysis (Supplie et al., 2017; Brinkkoetter et al., 2019; Yang et al., 2020). A growing literature demonstrates that the migration of cancer cells, crucial for their metastatic potential, is dependent on glycolysis (Beckner et al., 1990; Sottnik et al., 2011; Shiraishi et al., 2015; Verdone et al., 2015; Cantelmoet et al., 2016; Kathagen-Buhmann et al., 2016; Zhou et al., 2016; Yan et al., 2017). A role for glycolysis in mediating cell migration is not limited to cancer cells and has been shown in the regulation of lamellipodia, filopodia and cell migration has been shown in a variety of cell types (Nguyen et al., 2000; De Bock et al., 2013; Semba et al., 2016; Kishore et al., 2017; Guak et al., 2018; Liu et al., 2019; Qiao et al., 2019). Two salient examples are renal glomerular podocytes and endothelial cells. In renal glomerular podocytes mitochondria do not target to



the peripheral domains of the cell and also do not contribute to the morphology and migration of these cells which are instead under the control of glycolysis occurring the peripheral domains (Ozawa et al., 2015). Endothelial cells give rise to vasculature through their migration and tubulogenesis and derive approximately 80-90% of their ATP from glycolysis (De Bock et al., 2013). During migration endothelial cells form filopodia and lamellipodia. PFKFB3, an activator of glycolysis, and glycolytic enzymes target to the leading edge of migrating endothelial cells (De Bock et al., 2013). Suppression of glycolysis due to conditional knock out of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) in endothelial cells in vivo decreased their migration and formation of filopodia (De Bock et al., 2013). Similarly, in vitro the formation of filopodia and lamellae was also impaired.

Although it is known that glycolytic enzymes undergo axonal transport (Yuan et al., 1999; Hinckelmann et al., 2016) and most have axonal mRNAs that may undergo local translation (Gumy et al., 2011), our understanding of the distribution and subcellular targeting of glycolytic enzymes in developing or regenerating adult axons and dendrites is minimal. Hexokinase is the first enzyme in the glycolytic pathway. The activity and the targeting of hexokinase to mitochondria is required for axon extension in small and medium sized adult dorsal root ganglion neurons in vitro (Wang et al., 2008). It has been argued that reliance on glycolysis downstream of mitochondrial dysfunction contributes to the impairment of collateral sprouting and axon regeneration in diabetes (FERNYHOUGH, 2015). Glycolytic enzymes are subcellularly recruited to synapses in response to energy stresses (Jang et al., 2016), presumably representative of a metabolic compensatory response. Investigation of the role of glycolysis in neuronal morphogenesis and regeneration is expected to be a fruitful area of future investigation.

### **KINASE BASED ATP REGENERATION**

ATP regeneration refers to the phosphorylation of ADP to ATP by kinases. Of specific interest to the nervous system are creatine kinase and the arthropod expression specific arginine kinase. ATP regeneration systems are used by cells to rapidly replenish ATP in subcellular domains that have high bioenergetic drains. Creatine kinases are localized to ion pumps in the plasma membrane, actomyosin complexes and calcium pumps in muscle cell sarcoplasmic reticulum (Andres et al., 2008). Creatine kinase has two major forms, a mitochondrial and a cytoplasmic (CK-B) that is strongly expressed in brain tissue and neurons (Wallimann and Hemmer, 1994). CK-B localizes to the membrane of electrically active cells to provide a steady supply of ATP to the pump systems that control membrane potential (Wallimann and Hemmer, 1994) and also serves to provide ATP to the actomyosin system during muscular contraction (Wallimann et al., 1983, 1984; Hornemann et al., 2000, 2003). Creatine kinase is found throughout dendrites and axons of embryonic hippocampal neurons at all stages of in vitro morphogenesis (Kupier et al., 2008). Arginine kinase was shown to target to growth cones of embryonic grasshopper neurons, align with actin filaments and target to filopodia (Wang et al., 1998). The localization of CK-B to the plasma membrane places it in an ideal location to also provide ATP for actin filament polymerization as most polymerizing filament barbed ends are located just beneath the membrane (Figure 1A,B).

The functions of ATP regenerating systems in neuronal morphogenesis have been minimally investigated. Double CK knock out mice have behavioral deficits but the underlying circuitry has not been thoroughly investigated (Streijger et al., 2005). However, pharmacological inhibition of creatine kinase or shRNA-mediated depletion of either mitochondrial or CK-B impairs the growth of the dendrites of Purkinje cells (Fukumitsu et al., 2015). Whether CK contributes to axon development has not been addressed, but loss of CK-B increases the proportion of motile mitochondria without affecting APP vesicle movements (Kuiper et al., 2008a). In contrast, roles for CK-B in promoting actin filament protrusive structures and cell motility (Kuiper et al., 2009; Venter et al., 2015) and the regulation of actin filaments during phagocytosis (Kuiper et al., 2008b) have been established in non-neuronal cells. CK-B colocalizes with actin protrusive structures and targeting it to the membrane promotes protrusive activity.

Investigation of the roles of CK and arginine kinase in neuronal morphogenesis may yield interesting new observations. Considering that these ATP regeneration systems may be acting to provide energy under conditions of energy depletion or high levels of utilization, the roles of these systems may be in the context of specific signaling cues, environments or insults (e.g., hypoglycemia). The guidance of axons involves both attractant and repellent cues. Thrombin, a signal that causes cellular responses after vascular injury and extravasation into nervous tissue, elicits contractile responses resulting in growth cone collapse and axon retraction (Jalink et al., 1994; Fritsche et al., 1999). Similarly, developmentally relevant repellent cues cause growth cone collapse and subsequent contractile actomyosin dependent retraction of the axon tip (Gallo et al., 2002; Gallo, 2006; Brown and Bridgman, 2009; Brown et al., 2009). CK-B was found to associate with PAR-1, the receptor mediating the effects of thrombin, and was required for thrombin induced contractile responses (Mahajan et al., 2000). A role for CK-B in a contractile responses is consistent with its role in locally providing ATP regeneration for actomyosin contractility in muscle cell sarcomeres (Wallimann et al., 1983, 1984; Hornemann et al., 2000, 2003). It will be of interest to consider whether other extracellular signal receptor systems of relevance to neuronal morphogenesis may associate with ATP regenerating systems and the contributions thereof to the neuronal responses to these signals.

## FUTURE DIRECTIONS

This review emphasizes recent developments in the understanding of the role of mitochondria in neuronal morphogenesis and regeneration, but also highlights the relative void in the understanding of other bioenergetic sources. Figure 2 summarizes the aspects of neuronal morphogenesis to which specific ATP sources have been determined to contribute, and also notes the areas of investigation that remain unexplored/unreported (ND in Figure 2). While there is an extensive literature on the bioenergetics of the nervous system at the tissue level (for a recent and thorough review see Dienel, 2019), this knowledge base is devoid of information about the bioenergetics of specific cell types in the nervous system. As the nervous system has a high degree of cellular heterogeneity it will be important to address the issue of the bioenergetics of specific cell types during development, the response to injury and regeneration and normal activity. The availability of genetically encoded sensors for ATP, GTP and many signaling pathways and cellular processes dependent on

these energy sources in conjunction with modern imaging modalities will allow for revision and updating of our understanding of the bioenergetics of the nervous system. These new developments have already led to challenges to, and revision of, prior dogma regarding neuronal bioenergetics (Díaz-García et al., 2017; Yellen, 2018; Díaz-García CM, Yellen G, 2019).

The subcellular localization of bioenergetic sources is an important issue that promises to yield advances. Mitochondria distribution in cells is a controlled process and these organelles are recruited to subcellular sites where their functions are needed. This is also the case in developing and regenerating axons as mitochondria accumulate at growth cones, are recruited to actively growing branches of the same axon, and target to the ends of severed axons to promote their regeneration. Similarly, the localization of stalled mitochondria along axons allows these axon segments to give rise to branches. Mitochondria locally promote actin filament dynamics in axonal segments populated by mitochondria. Glycolysis is also spatio-temporally regulated. In neurons, populations of vesicles undergoing fast axonal transport associate with glycolytic enzymes and glycolysis occurs “on-board” and powers the transport of these vesicles. A number of glycolytic enzymes have actin binding domains and target to actin filaments in cells, indicating that glycolysis can be targeted to subcellular domains where actin filament dynamics are highest. Glycolytic enzymes can also be subcellularly recruited to specific sites such as synapses in response to energy stresses (Jang et al., 2016). The ATP regenerating systems are similarly targeted to subcellular domains where they are most needed. Creatine kinases associates with membranes where it is needed to power pump activity and the actomyosin system where it is required to power myosin II activity. How each one of the bioenergetic power sources available to the neuron is utilized and subcellularly regulated remains to be fully investigated and understood.

Whether bioenergetics are regulated during axon guidance has not been investigated. Although both attractant and repellent signals that can guide growth cones have been shown to alter mitochondrial membrane potential (e.g., neurotrophins, CSPGs, semaphorin 3A) whether this regulation occurs in the context of guidance remains to be directly addressed. It is possible that guidance signals may differentially impact bioenergetics across the growth cone. Single filopodial contacts with guidance cues elicit localized reorganization of the cytoskeleton and directed growth cone extension toward or away from the contact depending on the nature of the cue (Dent and Gertler, 2003). Similarly, when guidance cues are presented as soluble gradients across the growth cone they elicit graded intracellular responses. For example, some guidance signals use calcium fluxes and when presented as gradients across the growth cone establish intracellular spatio-temporal heterogeneities in calcium levels within the growth cone (Gasperini et al., 2017). Cytosolic calcium may then be taken up by mitochondria and in turn differentially control OxP in mitochondria across the growth cone. Mitochondria may also undergo regulated targeting within the growth cone during guidance. When growth cones encounter borders between a permissive substratum and CSPGs they turn and grow preferentially on the permissive substratum. Since when growth cones are growing on homogeneous CSPGs the mitochondria tend to be evacuated from growth cones, the same phenomenon may occur on a local scale within the portion of the growth cone making contact with the repellent signals (e.g., CSPGs). Alternatively, other bioenergetic sources such as glycolytic enzymes or creatine kinase may be differentially

targeted or activated across the growth cone during guidance. As at least some glycolytic enzymes associate with actin filaments, and actin filaments levels are increased and decreased respectively by attractant and repellent guidance cues, the subcellular localization of the glycolytic system may be regulated during guidance by changes in actin filament levels across the growth cone.

In addition to ATP, GTP is also an energy source used by the cell to regulate a variety of physiological processes of direct relevance to neuronal morphogenesis; microtubule plus tip dynamics, GTPases signaling mechanisms and protein synthesis. The understanding of the regulation of the level of GTP in developing neurons and its subcellular dynamics are an open frontier. The availability of GTP sensors for live imaging in cells should allow for rapid advance on this issue (Bianchi-Smiraglia et al., 2017). Furthermore, GTP biosynthetic enzymes are found at the leading edge of renal carcinoma cells (Wolfe et al., 2019), but their distribution in neurons is not known.

In conclusion, the understanding of the mitochondrion in neuronal morphogenesis and regeneration has advanced significantly in recent years. However, investigations into the contributions of other bioenergetic sources are lagging. The information reviewed in this manuscript indicates that glycolysis and ATP regenerating systems are likely to be of significance to neuronal morphogenesis and may also be of relevance to regeneration. As with other cellular systems, it is possible that in neurons different bioenergetic resources may be utilized in different contexts, such as different local environments, in response to different extracellular signals and at different developmental stages or following an injury. Continued analysis of neuronal bioenergetics can be expected to reveal a complex spatio-temporal and cell type specific landscape.

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## REFERENCES

- Abosch A, Lagenaur C. 1993 Sensitivity of neurite outgrowth to microfilament disruption varies with adhesion molecule substrate. *J Neurobiol.* 1993 3;24(3):344–55. [PubMed: 8492111]
- Agostini M, Romeo F, Inoue S, Niklison-Chirou MV, Elia AJ, Dinsdale D, Morone N, Knight RA, Mak TW, Melino G. 2016 Metabolic reprogramming during neuronal differentiation. *Cell Death Differ.* 23:1502–14. [PubMed: 27058317]
- Andersen R, Li Y, Resseguie M, Brenman JE. 2005 Calcium/calmodulin-dependent protein kinase II alters structural plasticity and cytoskeletal dynamics in *Drosophila*. *J Neurosci.* 25:8878–88. [PubMed: 16192377]
- Andres RH, Ducray AD, Schlattner U, Wallimann T, Widmer HR. 2008 Functions and effects of creatine in the central nervous system. *Brain Res Bull.* 76:329–43. [PubMed: 18502307]
- Armijo-Weingart L, Gallo G. 2017 It takes a village to raise a branch: Cellular mechanisms of the initiation of axon collateral branches. *Mol Cell Neurosci.* 84:36–47. [PubMed: 28359843]
- Armijo-Weingart L, Ketschek A, Sainath R, Pacheco A, Smith GM, Gallo G. 2019 Neurotrophins induce fission of mitochondria along embryonic sensory axons. *Elife.* 8:e49494. [PubMed: 31789589]
- Ashrafi G, Ryan TA. 2017 Glucose metabolism in nerve terminals. *Curr Opin Neurobiol.* 45:156–161. [PubMed: 28605677]

- Beckner ME, Stracke ML, Liotta LA, Schiffmann E. 1990 Glycolysis as primary energy source in tumor cell chemotaxis. *82*:1836–40.
- Bernstein BW, Bamburg JR. Actin-ATP hydrolysis is a major energy drain for neurons. *J Neurosci.* *23*:1–6. [PubMed: 12514193]
- Bianchi-Smiraglia A, Rana MS, Foley CE, Paul LM, Lipchick BC, Moparthy S, Moparthy K, Fink EE, Bagati A, Hurley E, Affronti HC, Bakin AV, Kandel ES, Smiraglia DJ, Feltri ML, Sousa R, Nikiforov MA. 2017 Internally ratiometric fluorescent sensors for evaluation of intracellular GTP levels and distribution. *Nat Methods.* *14*:1003–1009. [PubMed: 28869758]
- Bowne-Anderson H, Hibbel A, Howard J. 2015 Regulation of Microtubule Growth and Catastrophe: Unifying Theory and Experiment. *Trends Cell Biol.* *25*:769–779. [PubMed: 26616192]
- Brinkkoetter PT, Bork T, Salou S, Liang W, Mizi A, Özel C, Koehler S, Hagmann HH, Ising C, Kuczowski A, Schnyder S, Abed A, Schermer B, Benzinger T, Kretz O, Puellas VG, Lagies S, Schlimpert M, Kammerer B, Handschin C, Schell C, Huber TB. 2019 Anaerobic Glycolysis Maintains the Glomerular Filtration Barrier Independent of Mitochondrial Metabolism and Dynamics. *Cell Rep.* *27*:1551–1566 [PubMed: 31042480]
- Brown J, Bridgman PC. 2003 Role of myosin II in axon outgrowth. *J Histochem Cytochem.* *51*:421–8. [PubMed: 12642620]
- Brown JA, Bridgman PC. 2009 Disruption of the cytoskeleton during Semaphorin 3A induced growth cone collapse correlates with differences in actin organization and associated binding proteins. *Dev Neurobiol.* *69*:633–46. [PubMed: 19513995]
- Brown JA, Wysolmerski RB, Bridgman PC. 2009 Dorsal root ganglion neurons react to semaphorin 3A application through a biphasic response that requires multiple myosin II isoforms. *Mol Biol Cell.* *20*:1167–79. [PubMed: 19109430]
- Burnette DT, Ji L, Schaefer AW, Medeiros NA, Danuser G, Forscher P. 2008 Myosin II activity facilitates microtubule bundling in the neuronal growth cone neck. *Dev Cell.* *15*:163–9. [PubMed: 18606149]
- Cantelmo AR, Conradi LC, Brajic A, Goveia J, Kalucka J, Pircher A, Chaturvedi P, Hol J, Thienpont B, Teuwen LA, Schoors S, Boeckx B, Vriens J, Kuchnio A, Veys K, Cruys B, Finotto L, Treps L, Stav-Noraas TE, Bifari F, Stapor P, Decimo I, Kampen K, De Bock K, Haraldsen G, Schoonjans L, Rabelink T, Eelen G, Ghesquière B, Rehman J, Lambrechts D, Malik AB, Dewerchin M, Carmeliet P. 2016 Inhibition of the Glycolytic Activator PFKFB3 in Endothelium Induces Tumor Vessel Normalization, Impairs Metastasis, and Improves Chemotherapy. *Cancer Cell.* *30*:968–985. [PubMed: 27866851]
- Cartoni R, Norsworthy MW, Bei F, Wang C, Li S, Zhang Y, Gabel CV, Schwarz TL, He Z. 2016 The Mammalian-Specific Protein *Armcx1* Regulates Mitochondrial Transport during Axon Regeneration. *Neuron.* *92*:1294–1307. [PubMed: 28009275]
- Cartoni R, Pekkurnaz G, Wang C, Schwarz TL, He Z. 2017 A high mitochondrial transport rate characterizes CNS neurons with high axonal regeneration capacity. *PLoS One.* *12*:e0184672. [PubMed: 28926622]
- Cheng XT, Sheng ZH. 2020 Developmental regulation of microtubule-based trafficking and anchoring of axonal mitochondria in health and diseases. *Dev Neurobiol.* doi: 10.1002/dneu.22748.
- Courchet J, Lewis TL Jr, Lee S, Courchet V, Liou DY, Aizawa S, Polleux F. 2013 Terminal axon branching is regulated by the LKB1-NUAK1 kinase pathway via presynaptic mitochondrial capture. *Cell.* *2013* *153*:1510–25. [PubMed: 23791179]
- Daniel JL, Molish IR, Robkin L, Holmsen H. 1986 Nucleotide exchange between cytosolic ATP and F-actin-bound ADP may be a major energy-utilizing process in unstimulated platelets. *Eur J Biochem.* *156*:677–84. [PubMed: 3699030]
- De Bock K, Georgiadou M, Schoors S, Kuchnio A, Wong BW, Cantelmo AR, Quaegebeur A, Ghesquière B, Cauwenberghs S, Eelen G, Phng LK, Betz I, Tembuysen B, Brepoels K, Welti J, Gaudens I, Segura I, Cruys B, Bifari F, Decimo I, Blanco R, Wyns S, Vangindertael J, Rocha S, Collins RT, Munck S, Daelemans D, Imamura H, Devlieger R, Rider M, Van Veldhoven PP, Schuit F, Bartrons R, Hofkens J, Fraisl P, Telang S, Deberardinis RJ, Schoonjans L, Vinckier S, Chesney J, Gerhardt H, Dewerchin M, Carmeliet P. 2013 Role of PFKFB3-driven glycolysis in vessel sprouting. *154*:651–63.

- Denisenko TV, Gorbunova AS, Zhivotovsky B. 2019 Mitochondrial Involvement in Migration, Invasion and Metastasis. *Front Cell Dev Biol.* 7:355. [PubMed: 31921862]
- Dent EW, Gertler FB. 2003 Cytoskeletal dynamics and transport in growth cone motility and axon guidance. *Neuron.* 40:209–27. [PubMed: 14556705]
- Dias AS, Almeida CR, Helguero LA, Duarte IF. 2019 Metabolic crosstalk in the breast cancer microenvironment. *Eur J Cancer.* 121:154–171. [PubMed: 31581056]
- Díaz-García CM, Mongeon R, Lahmann C, Koveal D, Zucker H, Yellen G. 2017 Neuronal Stimulation Triggers Neuronal Glycolysis and Not Lactate Uptake. *Cell Metab.* 26:361–374. [PubMed: 28768175]
- Díaz-García CM, Yellen G. 2019 Neurons rely on glucose rather than astrocytic lactate during stimulation. *J Neurosci Res.* 97:883–889. [PubMed: 30575090]
- Dienel GA. 2019 Brain Glucose Metabolism: Integration of Energetics with Function. *Physiol Rev.* 99:949–1045. [PubMed: 30565508]
- Engl E, Attwell D. 2015 Non-signalling energy use in the brain. *J Physiol.* 593:3417–29. [PubMed: 25639777]
- Engl E, Jolivet R, Hall CN, Attwell D. 2017 Non-signalling energy use in the developing rat brain. *J Cereb Blood Flow Metab.* 37:951–966. [PubMed: 27170699]
- Epstein T, Xu L, Gillies RJ, Gatenby RA. 2014 Separation of metabolic supply and demand: aerobic glycolysis as a normal physiological response to fluctuating energetic demands in the membrane. *Cancer Metab.* 2:7. [PubMed: 24982758]
- Faits MC, Zhang C, Soto F, Kerschensteiner D. 2016 Dendritic mitochondria reach stable positions during circuit development. *Elife.* 5:e11583. [PubMed: 26742087]
- Fawcett JW, Oohashi T, Pizzorusso T. 2019 The roles of perineuronal nets and the perinodal extracellular matrix in neuronal function. *Nat Rev Neurosci.* 20:451–465. [PubMed: 31263252]
- Fernyhough P 2015 Mitochondrial dysfunction in diabetic neuropathy: a series of unfortunate metabolic events. *Curr Diab Rep.* 15:89. [PubMed: 26370700]
- Fritsche J, Reber BF, Schindelholz B, Bandtlow CE. 1999 Differential cytoskeletal changes during growth cone collapse in response to hSema III and thrombin. *Mol Cell Neurosci.* 14:398–418. [PubMed: 10588393]
- Fukumitsu K, Fujishima K, Yoshimura A, Wu YK, Heuser J, Kengaku M. 2015 Synergistic action of dendritic mitochondria and creatine kinase maintains ATP homeostasis and actin dynamics in growing neuronal dendrites. *J Neurosci.* 35:5707–23. [PubMed: 25855183]
- Gallo G, Letourneau PC. 1999 Different contributions of microtubule dynamics and transport to the growth of axons and collateral sprouts. *J Neurosci.* 19:3860–73. [PubMed: 10234018]
- Gallo G 2006 RhoA-kinase coordinates F-actin organization and myosin II activity during semaphorin-3A-induced axon retraction. *J Cell Sci.* 119:3413–23. [PubMed: 16899819]
- Gallo G 2013 Mechanisms underlying the initiation and dynamics of neuronal filopodia: from neurite formation to synaptogenesis. *Int Rev Cell Mol Biol.* 301:95–156. [PubMed: 23317818]
- Gallo G, Yee HF Jr, Letourneau PC. 2002 Actin turnover is required to prevent axon retraction driven by endogenous actomyosin contractility. *J Cell Biol.* 158:1219–28. [PubMed: 12356866]
- Gasperini RJ, Pavez M, Thompson AC, Mitchell CB, Hardy H, Young KM, Chilton JK, Foa L. 2017 How does calcium interact with the cytoskeleton to regulate growth cone motility during axon pathfinding? *Mol Cell Neurosci.* 84:29–35. [PubMed: 28765051]
- Goldschmidt-Clermont PJ, Furman MI, Wachsstock D, Safer D, Nachmias VT, Pollard TD. 1992 The control of actin nucleotide exchange by thymosin beta 4 and profilin. A potential regulatory mechanism for actin polymerization in cells. *Mol Biol Cell.* 3:1015–24. [PubMed: 1330091]
- Griffiths EJ, Rutter GA. 2009 Mitochondrial calcium as a key regulator of mitochondrial ATP production in mammalian cells. *Biochim Biophys Acta.* 1787(11):1324–33. [PubMed: 19366607]
- Guak H, Al Habyan S, Ma EH, Aldossary H, Al-Masri M, Won SY, Ying T, Fixman ED, Jones RG, McCaffrey LM, Krawczyk CM. 2018 Glycolytic metabolism is essential for CCR7 oligomerization and dendritic cell migration. *Nat Commun.* 9:2463. [PubMed: 29941886]

- Gumy LF, Yeo GS, Tung YC, Zivraj KH, Willis D, Coppola G, Lam BY, Twiss JL, Holt CE, Fawcett JW. 2011 Transcriptome analysis of embryonic and adult sensory axons reveals changes in mRNA repertoire localization. *RNA*. 17:85–98. [PubMed: 21098654]
- Han SM, Baig HS, Hammarlund M. 2016 Mitochondria Localize to Injured Axons to Support Regeneration. *Neuron*. 92:1308–1323. [PubMed: 28009276]
- Han Q, Xie Y, Ordaz JD, Huh AJ, Huang N, Wu W, Liu N, Chamberlain KA, Sheng ZH, Xu XM. 2020 Restoring Cellular Energetics Promotes Axonal Regeneration and Functional Recovery after Spinal Cord Injury. *Cell Metab*. 31:623–641. [PubMed: 32130884]
- Hinckelmann MV, Virlogeux A, Niehage C, Poujol C, Choquet D, Hoflack B, Zala D, Saudou F. 2016 Self-propelling vesicles define glycolysis as the minimal energy machinery for neuronal transport. *Nat Commun*. 7:13233. [PubMed: 27775035]
- Hornemann T, Stolz M, Wallimann T. 2000 Isoenzyme-specific interaction of muscle-type creatine kinase with the sarcomeric M-line is mediated by NH<sub>2</sub>-terminal lysine charge-clamps. *J Cell Biol*. 149:1225–1234. [PubMed: 10851020]
- Hornemann T, Kempa S, Himmel M, Hayess K, Furst DO, Wallimann T. 2003 Muscle-type creatine kinase interacts with central domains of the M-band proteins myomesin and M-protein. *J Mol Biol* 332:877–887 [PubMed: 12972258]
- Huang TJ, Verkhatsky A, Fernyhough P. 2005 Insulin enhances mitochondrial inner membrane potential and increases ATP levels through phosphoinositide 3-kinase in adult sensory neurons. *Mol Cell Neurosci*. 28:42–54. [PubMed: 15607940]
- Jalink K, van Corven EJ, Hengeveld T, Morii N, Narumiya S, Moolenaar WH. 1994 Inhibition of lysophosphatidate- and thrombin-induced neurite retraction and neuronal cell rounding by ADP ribosylation of the small GTP-binding protein Rho. *J Cell Biol*. 126:801–10. [PubMed: 8045941]
- Jang S, Nelson JC, Bend EG, Rodríguez-Laureano L, Tueros FG, Cartagena L, Underwood K, Jorgensen EM, Colón-Ramos DA. 2016 Glycolytic Enzymes Localize to Synapses under Energy Stress to Support Synaptic Function. *Neuron*. 90:278–91. [PubMed: 27068791]
- Jones SL, Selzer ME, Gallo G. 2006 Developmental regulation of sensory axon regeneration in the absence of growth cones. *J Neurobiol*. 66:1630–45. [PubMed: 17058187]
- Jossin Y 2020 Molecular mechanisms of cell polarity in a range of model systems and in migrating neurons. *Mol Cell Neurosci*. 106:103503. [PubMed: 32485296]
- Kalinski AL, Kar AN, Craver J, Tosolini AP, Sleigh JN, Lee SJ, Hawthorne A, Brito-Vargas P, Miller-Randolph S, Passino R, Shi L, Wong VSC, Picci C, Smith DS, Willis DE, Havton LA, Schiavo G, Giger RJ, Langley B, Twiss JL. 2019 Deacetylation of Miro1 by HDAC6 blocks mitochondrial transport and mediates axon growth inhibition. *J Cell Biol*. 218:1871–1890. [PubMed: 31068376]
- Kathagen-Buhmann A, Schulte A, Weller J, Holz M, Herold-Mende C, Glass R, Lamszus K. 2016 Glycolysis and the pentose phosphate pathway are differentially associated with the dichotomous regulation of glioblastoma cell migration versus proliferation. *Neuro Oncol*. 18:1219–29. [PubMed: 26917237]
- Kerstein PC, Nichol RH IV, Gomez TM. 2015 Mechanochemical regulation of growth cone motility. *Front Cell Neurosci*. 9:244. [PubMed: 26217175]
- Ketschek A, Gallo G. 2010 Nerve growth factor induces axonal filopodia through localized microdomains of phosphoinositide 3-kinase activity that drive the formation of cytoskeletal precursors to filopodia. *J Neurosci*. 30:12185–97. [PubMed: 20826681]
- Ketschek AR, Jones SL, Gallo G. 2007 Axon extension in the fast and slow lanes: substratum-dependent engagement of myosin II functions. *Dev Neurobiol*. 67:1305–20. [PubMed: 17638383]
- Kimura T, Murakami F. 2014 Evidence that dendritic mitochondria negatively regulate dendritic branching in pyramidal neurons in the neocortex. *J Neurosci*. 34:6938–51. [PubMed: 24828647]
- Kishore M, Cheung KCP, Fu H, Bonacina F, Wang G, Coe D, Ward EJ, Colamatteo A, Jangani M, Baragetti A, Matarese G, Smith DM, Haas R, Mauro C, Wraith DC, Okkenhaug K, Catapano AL, De Rosa V, Norata GD, Marelli-Berg FM. 2017 Regulatory T Cell Migration Is Dependent on Glucokinase-Mediated Glycolysis. *Immunity*. 47:875–889. [PubMed: 29166588]
- Kollins KM, Hu J, Bridgman PC, Huang YQ, Gallo G. 2009 Myosin-II negatively regulates minor process extension and the temporal development of neuronal polarity. *Dev Neurobiol*. 69:279–98. [PubMed: 19224562]

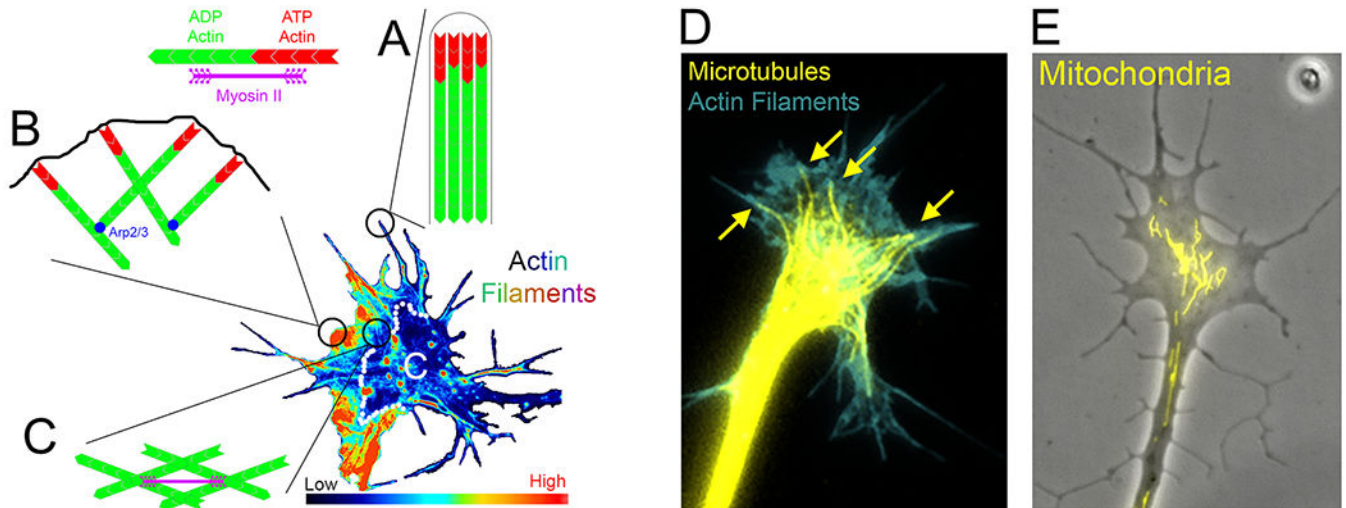
- Konur S, Ghosh A. 2005 Calcium signaling and the control of dendritic development. *Neuron*. 46:401–5. [PubMed: 15882639]
- Kuiper JW, Oerlemans FT, Fransen JA, Wieringa B. 2008a Creatine kinase B deficient neurons exhibit an increased fraction of motile mitochondria. *BMC Neurosci*. 9:73. [PubMed: 18662381]
- Kuiper JW, Pluk H, Oerlemans F, van Leeuwen FN, de Lange F, Fransen J, Wieringa B. 2008b Creatine kinase-mediated ATP supply fuels actin-based events in phagocytosis. *PLoS Biol*. 6:e51. [PubMed: 18336068]
- Kuiper JW, van Horssen R, Oerlemans F, Peters W, van Dommelen MM, te Lindert MM, ten Hagen TL, Janssen E, Fransen JA, Wieringa B. 2009 Local ATP generation by brain-type creatine kinase (CK-B) facilitates cell motility. *PLoS One*. 4:e5030. [PubMed: 19333390]
- Lee FH, Su P, Xie YF, Wang KE, Wan Q, Liu F. 2016 Disrupting GluA2-GAPDH Interaction Affects Axon and Dendrite Development. *Sci Rep*. 6:30458. [PubMed: 27461448]
- Lee S, Wang W, Hwang J, Namgung U, Min KT. 2019 Increased ER-mitochondria tethering promotes axon regeneration. *Proc Natl Acad Sci U S A*. 116:16074–16079. [PubMed: 31332012]
- Lin CH, Forscher P. 1995 Growth cone advance is inversely proportional to retrograde F-actin flow. *Neuron*. 14:763–71. [PubMed: 7536426]
- Lin-Hendel EG, McManus MJ, Wallace DC, Anderson SA, Golden JA. 2016 Differential Mitochondrial Requirements for Radially and Non-radially Migrating Cortical Neurons: Implications for Mitochondrial Disorders. *Cell Rep*. 15:229–37. [PubMed: 27050514]
- Liu J, Zhang X, Chen K, Cheng Y, Liu S, Xia M, Chen Y, Zhu H, Li Z, Cao X. 2019 CCR7 Chemokine Receptor-Inducible Inc-Dpf3 Restrains Dendritic Cell Migration by Inhibiting HIF-1 $\alpha$ -Mediated Glycolysis. *Immunity*. 50:600–615 [PubMed: 30824325]
- Masters CJ, Reid S, Don M. 1987 Glycolysis--new concepts in an old pathway. *Mol Cell Biochem*. 76:3–14. [PubMed: 3306346]
- Loudon RP, Silver LD, Yee HF Jr, Gallo G. 2006 RhoA-kinase and myosin II are required for the maintenance of growth cone polarity and guidance by nerve growth factor. *J Neurobiol*. 66:847–67. [PubMed: 16673385]
- Mahajan VB, Pai KS, Lau A, Cunningham DD. 2000 Creatine kinase, an ATP-generating enzyme, is required for thrombin receptor signaling to the cytoskeleton. *Proc Natl Acad Sci U S A*. 97:12062–7. [PubMed: 11050237]
- McCormick LE, Gupton SL. 2020 Mechanistic advances in axon pathfinding. *Curr Opin Cell Biol*. 63:11–19. [PubMed: 31927278]
- Melkov A, Abdu U. 2018 Regulation of long-distance transport of mitochondria along microtubules. *Cell Mol Life Sci*. 75:163–176. [PubMed: 28702760]
- Menon S, Gupton S. 2018 Recent advances in branching mechanisms underlying neuronal morphogenesis. *F1000Res*. 7:F1000 Faculty Rev-1779.
- Morris RL, Hollenbeck PJ. 1993 The regulation of bidirectional mitochondrial transport is coordinated with axonal outgrowth. *J Cell Sci*. 104:917–27. [PubMed: 8314882]
- Majumdar R, Steen K, Coulombe PA, Parent CA. 2019 Non-canonical processes that shape the cell migration landscape. *Curr Opin Cell Biol*. 57:123–134. [PubMed: 30852463]
- Nguyen TN, Wang HJ, Zalzal S, Nanci A, Nabi IR. 2000 Purification and characterization of beta-actin-rich tumor cell pseudopodia: role of glycolysis. *Exp Cell Res*. 258:171–83. [PubMed: 10912799]
- Ozawa S, Ueda S, Imamura H, Mori K, Asanuma K, Yanagita M, Nakagawa T. 2015 Glycolysis, but not Mitochondria, responsible for intracellular ATP distribution in cortical area of podocytes. *Sci Rep*. 5:18575. [PubMed: 26677804]
- Pfeiffer T, Schuster S, Bonhoeffer S. 2001 Cooperation and competition in the evolution of ATP-producing pathways. *Science* 292:504–507. [PubMed: 11283355]
- Pollard TD. 2016 Actin and Actin-Binding Proteins. *Cold Spring Harb Perspect Biol*. 8:a018226. [PubMed: 26988969]
- Qiao H, He X, Zhang Q, Yuan H, Wang D, Li L, Hui Y, Wu Z, Li W, Zhang N. 2019 Alpha-synuclein induces microglial migration via PKM2-dependent glycolysis. *Int J Biol Macromol*. 129:601–607. [PubMed: 30738168]



- Rangaraju V, Lauterbach M, Schuman EM. 2019 Spatially Stable Mitochondrial Compartments Fuel Local Translation during Plasticity. *Cell*. 176:73–84. [PubMed: 30612742]
- Riccomagno MM, Kolodkin AL. 2015 Sculpting neural circuits by axon and dendrite pruning. *Annu Rev Cell Dev Biol*. 31:779–805. [PubMed: 26436703]
- Ruthel G, Hollenbeck PJ. 2000 Growth cones are not required for initial establishment of polarity or differential axon branch growth in cultured hippocampal neurons. *J Neurosci*. 20:2266–74. [PubMed: 10704502]
- Ruthel G, Hollenbeck PJ. 2003 Response of mitochondrial traffic to axon determination and differential branch growth. *J Neurosci*. 23:8618–24. [PubMed: 13679431]
- Sainath R, Armijo-Weingart L, Ketschek A, Xu Z, Li S, Gallo G. 2017a Chondroitin sulfate proteoglycans negatively regulate the positioning of mitochondria and endoplasmic reticulum to distal axons. *Dev Neurobiol*. 77:1351–1370. [PubMed: 28901718]
- Sainath R, Ketschek A, Grandi L, Gallo G. 2017b CSPGs inhibit axon branching by impairing mitochondria-dependent regulation of actin dynamics and axonal translation. *Dev Neurobiol*. 77:454–473. [PubMed: 27429169]
- Schaefer AW, Schoonderwoert VT, Ji L, Mederios N, Danuser G, Forscher P. 2008 Coordination of actin filament and microtubule dynamics during neurite outgrowth. *Dev Cell*. 15:146–62. [PubMed: 18606148]
- Semba H, Takeda N, Isagawa T, Sugiura Y, Honda K, Wake M, Miyazawa H, Yamaguchi Y, Miura M, Jenkins DM, Choi H, Kim JW, Asagiri M, Cowburn AS, Abe H, Soma K, Koyama K, Katoh M, Sayama K, Goda N, Johnson RS, Manabe I, Nagai R, Komuro I. 2016 HIF-1 $\alpha$ -PDK1 axis-induced active glycolysis plays an essential role in macrophage migratory capacity. *Nat Commun*. 7:11635. [PubMed: 27189088]
- Shestov AA, Liu X, Ser Z, Cluntun AA, Hung YP, Huang L, Kim D, Le A, Yellen G, Albeck JG, Locasale JW. 2014 Quantitative determinants of aerobic glycolysis identify flux through the enzyme GAPDH as a limiting step. *Elife*. 3:e03342.
- Shiraishi T, Verdone JE, Huang J, Kahlert UD, Hernandez JR, Torga G, Zarif JC, Epstein T, Gatenby R, McCartney A, Elisseff JH, Mooney SM, An SS, Pienta KJ. 2015 Glycolysis is the primary bioenergetic pathway for cell motility and cytoskeletal remodeling in human prostate and breast cancer cells. *Oncotarget*. 6:130–43. [PubMed: 25426557]
- Slavov N, Budnik BA, Schwab D, Airoidi EM, van Oudenaarden A. 2014 Constant growth rate can be supported by decreasing energy flux and increasing aerobic glycolysis. *Cell Rep*. 7:705–14. [PubMed: 24767987]
- Smith GM, Gallo G. 2018 The role of mitochondria in axon development and regeneration. *Dev Neurobiol*. 78:221–237. [PubMed: 29030922]
- Sottnik JL, Lori JC, Rose BJ, Thamm DH. 2011 Glycolysis inhibition by 2-deoxy-D-glucose reverts the metastatic phenotype in vitro and in vivo. *Clin Exp Metastasis*. 28:865–75. [PubMed: 21842413]
- Spillane M, Ketschek A, Donnelly CJ, Pacheco A, Twiss JL, Gallo G. 2012 Nerve growth factor-induced formation of axonal filopodia and collateral branches involves the intra-axonal synthesis of regulators of the actin-nucleating Arp2/3 complex. *J Neurosci*. 32:17671–89. [PubMed: 23223289]
- Spillane M, Ketschek A, Merianda TT, Twiss JL, Gallo G. 2013 Mitochondria coordinate sites of axon branching through localized intra-axonal protein synthesis. *Cell Rep*. 5:1564–75. [PubMed: 24332852]
- Streijger F, Oerlemans F, Ellenbroek BA, Jost CR, Wieringa B, Van der Zee CE. 2005 Structural and behavioural consequences of double deficiency for creatine kinases BCK and UbCKmit. *Behav Brain Res*. 157:219–34. [PubMed: 15639173]
- Steketee MB, Moysidis SN, Weinstein JE, Kreymerman A, Silva JP, Iqbal S, Goldberg JL. 2012 Mitochondrial dynamics regulate growth cone motility, guidance, and neurite growth rate in perinatal retinal ganglion cells in vitro. *Invest Ophthalmol Vis Sci*. 53:7402–11. [PubMed: 23049086]
- Sun T, Qiao H, Pan PY, Chen Y, Sheng ZH. 2013 Motile axonal mitochondria contribute to the variability of presynaptic strength. *Cell Rep*. 4:413–419. [PubMed: 23891000]


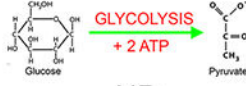

- Supplie LM, Düking T, Campbell G, Diaz F, Moraes CT, Götz M, Hamprecht B, Boretius S, Mahad D, Nave KA. 2017 Respiration-Deficient Astrocytes Survive As Glycolytic Cells In Vivo. *J Neurosci.* 37:4231–4242. [PubMed: 28314814]
- Surin AM, Khiroug S, Gorbacheva LR, Khodorov BI, Pinelis VG, Khiroug L. 2013 Comparative analysis of cytosolic and mitochondrial ATP synthesis in embryonic and postnatal hippocampal neuronal cultures. *Front Mol Neurosci.* 5:102. [PubMed: 23335879]
- Tang NH, Kim KW, Xu S, Blazie SM, Yee BA, Yeo GW, Jin Y, Chisholm AD. 2020 The mRNA Decay Factor CAR-1/LSM14 Regulates Axon Regeneration via Mitochondrial Calcium Dynamics. *Curr Biol.* 30(5):865–876. [PubMed: 31983639]
- Tao K, Matsuki N, Koyama R. 2014 AMP-activated protein kinase mediates activity-dependent axon branching by recruiting mitochondria to axon. *Dev Neurobiol.* 74:557–73. [PubMed: 24218086]
- Tarasov AI, Rutter GA. 2014 Use of genetically encoded sensors to monitor cytosolic ATP/ADP ratio in living cells. *Methods Enzymol.* 542:289–311. [PubMed: 24862272]
- Tolkovsky AM, Suidan HS. 1987 Adenosine 5'-triphosphate synthesis and metabolism localized in neurites of cultured sympathetic neurons. *Neuroscience* 23:1133–42. [PubMed: 3437992]
- Tran AP, Warren PM, Silver J. 2018 The Biology of Regeneration Failure and Success After Spinal Cord Injury. *Physiol Rev.* 98:881–917. [PubMed: 29513146]
- Turney SG, Bridgman PC. 2005 Laminin stimulates and guides axonal outgrowth via growth cone myosin II activity. *Nat Neurosci.* 8:717–9. [PubMed: 15880105]
- Turney SG, Ahmed M, Chandrasekar I, Wysolmerski RB, Goeckeler ZM, Rioux RM, Whitesides GM, Bridgman PC. 2016 Nerve growth factor stimulates axon outgrowth through negative regulation of growth cone actomyosin restraint of microtubule advance. *Mol Biol Cell.* 27:500–17. [PubMed: 26631553]
- Vaarmann A, Mandel M, Zeb A, Wareski P, Liiv J, Kuum M, Antsov E, Liiv M, Cagalinec M, Choubey V, Kaasik A. 2016 Mitochondrial biogenesis is required for axonal growth. *Development.* 143:1981–92. [PubMed: 27122166]
- van Spronsen M, Mikhaylova M, Lipka J, Schlager MA, van den Heuvel DJ, Kuijpers M, Wulf PS, Keijzer N, Demmers J, Kapitein LC, Jaarsma D, Gerritsen HC, Akhmanova A, Hoogenraad CC. 2013 TRAK/Milton motor-adaptor proteins steer mitochondrial trafficking to axons and dendrites. *Neuron.* 77:485–502. [PubMed: 23395375]
- Venter G, Polling S, Pluk H, Venselaar H, Wijers M, Willemse M, Fransen JA, Wieringa B. 2015 Submembranous recruitment of creatine kinase B supports formation of dynamic actin-based protrusions of macrophages and relies on its C-terminal flexible loop. *Eur J Cell Biol.* 94:114–27. [PubMed: 25538032]
- Verburg J, Hollenbeck PJ. 2008 Mitochondrial membrane potential in axons increases with local nerve growth factor or semaphorin signaling. *J Neurosci.* 28:8306–15. [PubMed: 18701693]
- Verdone JE, Zarif JC, Pienta KJ. 2015 Aerobic glycolysis, motility, and cytoskeletal remodeling *Cell Cycle.* 14:169–70. [PubMed: 25530323]
- Wakade AR, Prat JC, Wakade TD. 1985 Sympathetic neurons extend neurites in a culture medium containing cyanide and dinitrophenol but not iodoacetate. *FEBS Lett.* 190:95–8. [PubMed: 3899726]
- Wakade AR, Wakade TD. 1985 Sympathetic neurons grown in culture generate ATP by glycolysis: correlation between ATP content and [3H]norepinephrine uptake and storage. *Brain Res.* 359:397–401. [PubMed: 4075161]
- Wallimann T, Moser H, Eppenberger HM. 1983 Isozyme-specific localization of M-line bound creatine kinase in myogenic cells. *J. Muscle Res. Cell Motil* 4:429–441 [PubMed: 6355174]
- Wallimann T, Schlosser T, Eppenberger HM. 1984 Function of M-line-bound creatine kinase as intramyofibrillar ATP regenerator at the receiving end of the phosphorylcreatine shuttle in muscle. *J. Biol. Chem* 259:5238–5246. [PubMed: 6143755]
- Wallimann T, Hemmer W. 1994 Creatine kinase in non-muscle tissues and cells. *Mol Cell Biochem.* 133–134:193–220.
- Wang YE, Esbensen P, Bentley D. 1998 Arginine kinase expression and localization in growth cone migration. *J Neurosci.* 18:987–98. [PubMed: 9437020]

- Wang Z, Gardiner NJ, Fernyhough P. 2008 Blockade of hexokinase activity and binding to mitochondria inhibits neurite outgrowth in cultured adult rat sensory neurons. *Neurosci Lett.* 434:6–11. [PubMed: 18308470]
- Wang X, Zhang X, Wu D, Huang Z, Hou T, Jian C, Yu P, Lu F, Zhang R, Sun T, Li J, Qi W, Wang Y, Gao F, Cheng H. 2017 Mitochondrial flashes regulate ATP homeostasis in the heart. *Elife.* 6:e23908. [PubMed: 28692422]
- Winkle CC, Gupton SL. 2016 Membrane Trafficking in Neuronal Development: Ins and Outs of Neural Connectivity. *Int Rev Cell Mol Biol.* 322:247–80. [PubMed: 26940520]
- Wolfe K, Kofuji S, Yoshino H, Sasaki M, Okumura K, Sasaki AT. 2019 Dynamic compartmentalization of purine nucleotide metabolic enzymes at leading edge in highly motile renal cell carcinoma. *Biochem Biophys Res Commun.* 516(1):50–56. [PubMed: 31196624]
- Yan XL, Zhang XB, Ao R, Guan L. 2017 Effects of shRNA-Mediated Silencing of PKM2 Gene on Aerobic Glycolysis, Cell Migration, Cell Invasion, and Apoptosis in Colorectal Cancer Cells. *J Cell Biochem.* 118:4792–4803. [PubMed: 28543190]
- Yang J, Ren B, Yang G, Wang H, Chen G, You L, Zhang T, Zhao Y. 2020 The enhancement of glycolysis regulates pancreatic cancer metastasis. *Cell Mol Life Sci.* 77:305–321. [PubMed: 31432232]
- Yellen G 2018 Fueling thought: Management of glycolysis and oxidative phosphorylation in neuronal metabolism. *J Cell Biol.* 217:2235–2246. [PubMed: 29752396]
- Yuan A, Mills RG, Bamberg JR, Bray JJ. 1999 Cotransport of glyceraldehyde-3-phosphate dehydrogenase and actin in axons of chicken motoneurons. *Cell Mol Neurobiol.* 19:733–44. [PubMed: 10456234]
- Zala D, Hinckelmann MV, Yu H, Lyra da Cunha MM, Liot G, Cordelières FP, Marco S, Saudou F. 2013 Vesicular glycolysis provides on-board energy for fast axonal transport. *Cell.* 152:479–91. [PubMed: 23374344]
- Zhou B, Yu P, Lin MY, Sun T, Chen Y, Sheng ZH. 2016 Facilitation of axon regeneration by enhancing mitochondrial transport and rescuing energy deficits. *J Cell Biol.* 214:103–19. [PubMed: 27268498]
- Zhou L, Jiang S, Fu Q, Smith K, Tu K, Li H, Zhao Y. 2016b FASN, ErbB2-mediated glycolysis is required for breast cancer cell migration. *Oncol Rep.* 35:2715–22. [PubMed: 26936618]



**FIGURE 1. The growth cone cytoskeleton.**

(A-C) The actin filament cytoskeleton. In the center of the (A-C) is shown a growth cone stained with fluorescent phalloidin that specifically stains actin filaments. The signal is false colored to show the relative levels of actin filaments. The C-domain is denoted by a C and the border between the C and P domains is approximated by the line of white dots. **(A)** Shows the organization of actin filaments at the tip of a filopodium. Within filopodia actin filaments are bundled and form an aligned array. The polymerizing barbed ends, containing ATP-bound actin, are found beneath the distal end of the filopodium just beneath the membrane. **(B)** In lamellipodia actin filaments are organized as mesh works of varying geometries. The Arp2/3 filament nucleating system is responsible for the array of filament in lamellipodia that have a branched organization wherein filaments extend off the sides of other filaments. Many of the polymerizing barbed ends are found in close proximity to the leading edge of the lamellipodium. **(C)** Within the P-domain lamellipodial filaments and the filaments that form the base of filopodia intertwine into an interconnected network. At the border between the P and C domains, where the P-domain actin filament levels decrease sharply and referred to as the transition zone, the contractile motor protein myosin II pulls the P-domain network of filaments toward the C-domain resulting in actin retrograde flow. **(D)** Example of a growth cone stained with phalloidin and anti- $\alpha$ -tubulin using a preparation protocol that removes soluble proteins (e.g., soluble tubulin) and allows clear visualization of polymerized microtubules (Gallo and Letourneau, 1999). The microtubules are organized into a bundle within the axon shaft. Upon entering the growth cone the dynamic plus ends of microtubule splay out (yellow arrowheads). **(E)** Example of a sensory axon and its growth cone, visualized using phase contrast optics, with mitochondria fluorescently labeled using MitoTracker Green (as in Spillane et al., 2013).

			
Axon extension	YES	ND	ND
Axon regeneration	YES	ND	ND
Axon branching	YES	ND	ND
Dendrite extension	YES	ND	YES
Dendrite branching	YES	ND	ND
Synaptic activity	YES	YES	YES*
Other		Required for transport of some axonal vesicles	* inferred from behavioral deficits

**FIGURE 2.**

At a glance summary of investigations into the bioenergetics underlying aspects of neuronal development and regeneration. ND = not determined. YES = has been investigated and found to contribute, see main text for details.