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REVIEW

SUBJECT COLLECTION: CYTOSKELETON

Fueling the cytoskeleton – links between cell metabolism and actin remodeling

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ABSTRACT

Attention has long focused on the actin cytoskeleton as a unit capable of organizing into ensembles that control cell shape, polarity, migration and the establishment of intercellular contacts that support tissue architecture. However, these investigations do not consider observations made over 40 years ago that the actin cytoskeleton directly binds metabolic enzymes, or emerging evidence suggesting that the rearrangement and assembly of the actin cytoskeleton is a major energetic drain. This Review examines recent studies probing how cells adjust their metabolism to provide the energy necessary for cytoskeletal remodeling that occurs during cell migration, epithelial to mesenchymal transitions, and the cellular response to external forces. These studies have revealed that mechanotransduction, cell migration, and epithelial to mesenchymal transitions are accompanied by alterations in glycolysis and oxidative phosphorylation. These metabolic changes provide energy to support the actin cytoskeletal rearrangements necessary to allow cells to assemble the branched actin networks required for cell movement and epithelial to mesenchymal transitions and the large actin bundles necessary for cells to withstand forces. In this Review, we discuss the emerging evidence suggesting that the regulation of these events is highly complex with metabolism affecting the actin cytoskeleton and vice versa.

KEY WORDS: Actin, Cytoskeleton, Force, Mechanotransduction

Introduction

Actin, the most abundant protein in eukaryotic cells, can organize into a diverse collection of cellular architectures, including branched or crosslinked networks, parallel bundles and antiparallel structures (Svitkina, 2018). The type of actin architecture formed depends on the regulators or proteins that bind to actin (Pollard and Earnshaw, 2002). The actin structure and its associated actin-binding proteins is collectively known as the actin cytoskeleton. Dynamic rearrangements of the actin cytoskeleton are critical for eukaryotic cell migration, mechanical integrity, cell shape, polarity and the regulation of transcription (Blanchoin et al., 2014).

Actin exists as a globular monomer (G-actin) bound to ADP or ATP that can polymerize into a filamentous structure known as F-actin (Pollard and Earnshaw, 2002). After the polymerization process, the ATP bound to actin is slowly hydrolyzed. The hydrolysis of ATP is not required for the formation of actin filaments. Rather, the bound ATP accelerates polymerization and dramatically affects other aspects of filament formation and integrity. For instance, ATP can affect the affinity for actin-binding partners, which ultimately impacts actin dynamics by controlling the polymerization and branching of actin filaments (Blanchoin and Pollard, 1999; Cai et al., 2007a,b; Pollard and Earnshaw, 2002; Pollard and Borisy, 2003; Suarez et al., 2011). Additionally, ATP affects the structural integrity of actin filaments with ATP-bound filaments being more rigid than the ADP-bound protein (Janmey et al., 1990). Thus, ATP is an important determinant of actin filament dynamics.

The amount of ATP needed to support the actin cytoskeleton has been the subject of intense scrutiny. Studies of unstimulated platelets revealed ∼50% of the total ATP consumption is used to support the actin cytoskeleton (Daniel et al., 1986). It is tempting to speculate that the large energy expenditure required of platelets is because of their extraordinarily dynamic nature. However, that does not seem to be the case as neurons require similar amounts of energy (Bernstein and Bamburg, 2003). In neurons, when actin turnover is inhibited by either blocking actin disassembly or assembly, ATP depletion is reduced by 50% (Bernstein and Bamburg, 2003). These studies collectively indicate that about half of the ATP in a cell is needed to support actin cytoskeletal rearrangements. Understanding how this energy is derived is an area of active investigation and is the focus of this Review. Here, we present a brief introduction of cell metabolism and a historical perspective about what is known with regards to metabolic enzymes binding to the actin cytoskeleton. We then consider more recent work uncovering links between the metabolic machinery and the actin cytoskeletal rearrangements that occur during mechanotransduction, migration, and the transition from an epithelial to mesenchymal phenotype. Finally, we present emerging mechanisms for how metabolism is altered by the cytoskeleton.

Cell metabolism and the actin cytoskeleton

ATP provides the energy for supporting key cellular functions. ATP can be formed by glycolysis and oxidative phosphorylation. Glucose does not diffuse into cells, rather it is actively transported into cells via a series of glucose transporters. Inside the cell, glucose is oxidized in a series of ten steps (Lehninger et al., 2013; Mulukutla et al., 2016). In the first step, glucose is phosphorylated by hexokinase (Fig. 1). The addition of the polar phosphate group effectively traps glucose in the cell. The phosphorylated form of glucose is isomerized to fructose-6-phosphate which is phosphorylated by phosphofructokinase-1 (PFK-1) to form fructose-1,6-bisphosphate. This step is the rate-limiting and commitment step for glycolysis and thus is highly regulated. Fructose-1,6-bisphosphate is cleaved by aldolase to form glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. The latter is isomerized in the next step of glycolysis to form further glyceraldehyde-3-phosphate. In the final five steps of glycolysis, glyceraldehyde-3-phosphate is oxidized (Fig. 1). The first oxidative product is 1,3-bisphosphoglycerate, which in most cells is rapidly phosphorylated to form 3-phosphoglycerate, which is then converted into 2-phosphoglycerate. In the next step, the dehydration of 2-

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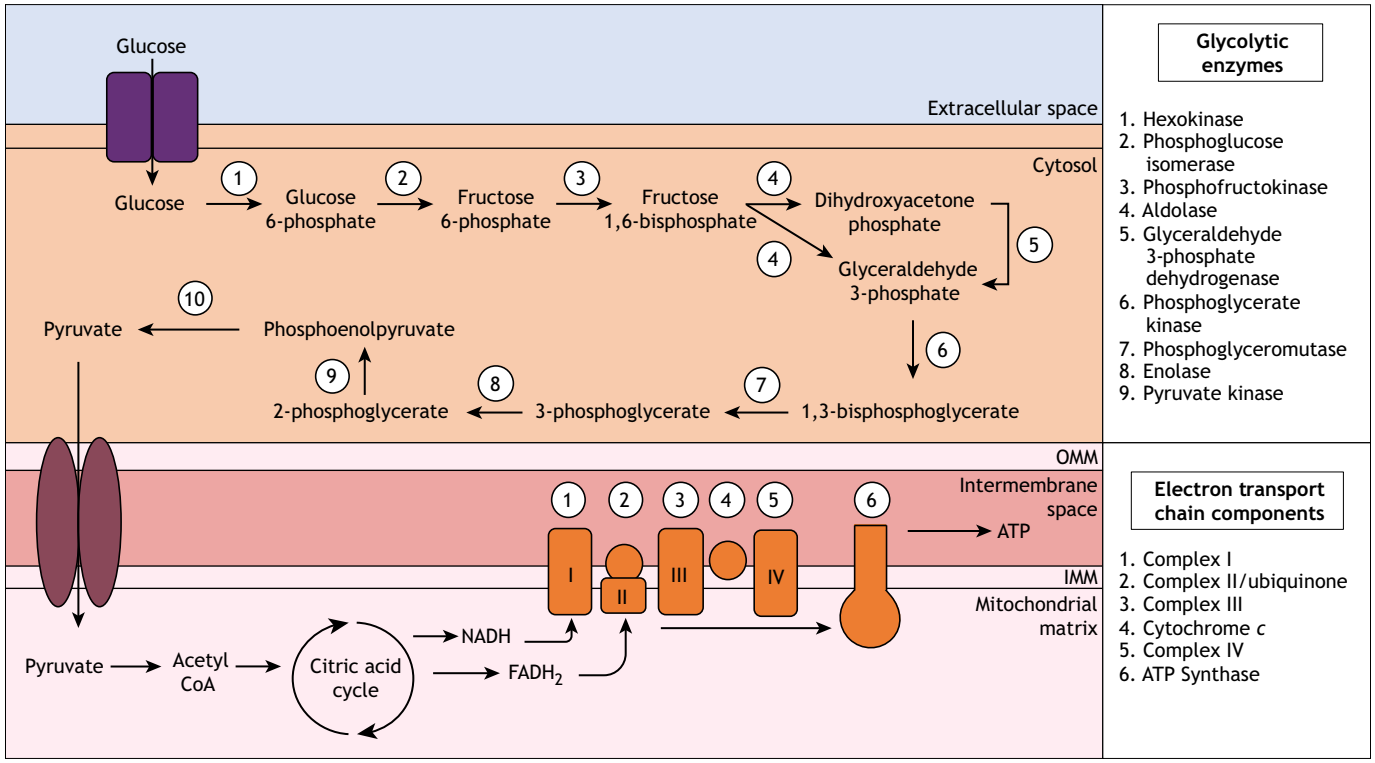

Fig. 1. Oxidative glucose metabolism. Once glucose enters the cell, it must be phosphorylated to remain in the cytosol. After phosphorylation, the 6-carbon glucose molecule goes through a serious of nine enzymatic reactions breaking it down to two molecules of pyruvate. Pyruvate is then shuttled into the mitochondrial matrix, where it is converted into acetyl CoA, which can be further oxidized in the citric acid cycle. Two electron carriers, NADH and $FADH_2$ are produced from the citric acid cycle and are components of a series of redox reactions in a process called the electron transport chain. The flow of electrons through the electron transport chain produces an electrochemical proton gradient that drives the synthesis of ATP via ATP synthase. IMM, inner mitrochondrial membrane; OMM, outer mitochondrial membrane.

phosphoglycerate is catalyzed by enolase, forming phosphoenolpyruvate. In the final step of glycolysis, pyruvate kinase catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate to ADP, thereby forming ATP.

The pyruvate formed can funnel into the citric acid cycle to be fully metabolized to CO_2, generating NADH and $FADH_2$ (Urry et al., 2017). As indicated in Fig. 1, in the mitochondria, these reduced coenzymes can donate electrons to protein complexes that act as electron carriers, denoted complex I to IV (Urry et al., 2017). As electrons pass through these electron carriers, they lose their free energy. This energy is captured and is used to phosphorylate ADP yielding ATP – a process known as oxidative phosphorylation.

Glycolysis and oxidative phosphorylation are regulated by many of the same molecular effectors. Consequently, they typically do not operate independently. Instead, the two events are highly coupled in cells and are regulated by the amount of mitochondria, as well as the microenvironment and the availability of oxygen (Balaban, 1990; Yellen, 2018). For example, in endothelial cells, which have a low mitochondria content, glycolysis predominates (Fitzgerald et al., 2018). However, as endothelial cells age (Walker et al., 2010) or when they proliferate (Coutelle et al., 2014), they switch to oxidative phosphorylation. Conversely, in muscle cells, oxidative phosphorylation provides the ATP when oxygen is available (Greenhaff and Timmons, 1998). Only when exercise exceeds the oxidative capacity of the muscle does the muscle turn to anaerobic glycolysis for ATP generation (Greenhaff and Timmons, 1998). An alteration in the preference of a cell for metabolites is known as metabolic reprogramming. Accumulating evidence suggests that metabolic reprogramming is critical for fueling the actin cytoskeletal rearrangements necessary for a cell to withstand mechanical forces or for it to undergo epithelial-to-mesenchymal transition (EMT) (Bays et al., 2017; Shiraishi et al., 2015).

Glycolytic enzymes binding to actin

The earliest indications that metabolism provides fuel for the actin cytoskeleton arose from older studies reporting direct interactions between enzymes that catalyze glycolysis and actin itself. For the most part, these studies have focused on one of three enzymes: PFK-1, aldolase or glyceraldhyde-3-phosphate dehydrogenase (GAPDH), and they provide evidence that binding of these enzymes to actin not only allows for spatial, but also functional regulation.

There are several determinants of PFK-1 binding to actin. PFK-1 binds to actin through electrostatic interactions (Roberts and Somero, 1989). *In vitro* studies revealed that PFK-1 binding to actin is inhibited by low concentrations of ATP and ADP, but is not affected by the concentration of its substrate, fructose-6-phosphate, suggesting that the binding site for actin overlaps with the binding site for the ADP and ATP (Roberts and Somero, 1989). Several pieces of evidence suggest that the enzymatically active form of PFK-1 binds to the actin cytoskeleton. First, PFK-1 phosphorylation increases binding to F-actin (Luther and Lee, 1986). Second, PFK-1 binding to actin is thought to stabilize PFK-1 in its tetrameric conformation and the tetrameric conformation of the enzyme is thought to be its fully active form (Roberts and Somero, 1987). Third, PFK-1 binding to actin lowers the K_m and renders the enzymes less susceptible to allosteric inhibition by ATP, citrate, and lactate (Luther and Lee, 1986; Masters, 1984; Poglazov and Livanova, 1986; Sola-Penna et al., 2010). Finally, in insulin-stimulated muscle and erythrocyte cells, more PFK-1

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associates with F-actin (Real-Hohn et al., 2010; Silva et al., 2004). Thus, all the available evidence to date suggests that the enzymatically active form of PFK-1 binds to the actin cytoskeleton (Fig. 2A).

Aldolase, the enzyme that catalyzes the fourth step of glycolysis, binds preferentially to F-actin over G-actin (Ouporov et al., 1999). Early *in vitro* studies indicated that binding is mediated by electrostatic interactions with a basic patch on aldolase and an acidic patch on actin, and that aldolase binds actin in an inactive state (Ouporov et al., 1999). In support of this notion, binding of substrate forces aldolase to adopt a conformation that is unfavorable for actin binding and thus promotes dissociation (Schindler et al., 2001; Wang et al., 1996). Furthermore, the release of aldolase from the cytoskeleton can be triggered by insulin. This release is important for actin remodeling and increasing glycolytic flux in response to insulin (Fig. 2B) (Hu et al., 2016). Thus, aldolase, unlike PFK-1, is held in an enzymatically inactive form by binding to the actin cytoskeleton (Fig. 2A).

GAPDH, the enzyme that catalyzes the sixth step of glycolysis also binds directly to F-actin (Fig. 2A). *In vitro* studies and Brownian dynamic simulations suggest that GAPDH is inactive when bound to F-actin (Forlemu et al., 2011; Poglazov and Livanova, 1986). In support of this notion, when metabolism is low, such as in non-proliferating, confluent or serum-starved cells, GAPDH is primarily in the insoluble fraction and bound to actin (Cao et al., 1999; Schmitz and Bereiter-Hahn, 2002). Conversely, in proliferating cells where metabolism is presumably high, GAPDH is in the soluble cytoplasmic fraction (Schmitz and Bereiter-Hahn, 2002). Thus, GAPDH is likely sequestered when bound to F-actin and released from the actin cytoskeleton when active. Collectively, these findings indicate that the actin cytoskeleton not only consumes energy via glycolysis but also controls the localization, activity, and stabilization of these key glycolytic enzymes.

It remains unclear why binding to F-actin serves to activate some of the glycolytic enzymes whereas others are inhibited. One possibility is that when PFK-1 receives a stimulus, it is activated and binds to the actin cytoskeleton, initiating new glycolysis locally where it is needed. As the cytoskeleton changes, the release of the active forms of the other glycolytic enzymes would allow the signal propagated by PFK-1 in the form of fructose-1,6-bisphosphate to diffuse locally and be converted ultimately into ATP along the growing cytoskeleton as the need for ATP arises. An alternative possibility that should be considered, given that the early experiments were performed prior to the advent of affinity tag purification, is that the early PFK-1 preparations lacked integrity. Thus, more work is needed to understand the consequences actin binding has on the enzymatic activity of the glycolytic enzymes. Emerging work investigating how different actin-mediated processes, such as migration and response of cells to external forces, as well as EMT, affect energy consumption and production are beginning to shed light on this phenomenon.

Links between actin cytoskeletal rearrangements and energy consumption

Many cellular processes require a dynamic rearrangement and assembly/disassembly of the actin cytoskeleton. This phenomenon is best described in migrating cells, which assemble a branched actin network at the leading edge to allow for protrusion, extension, and cell guidance, while actin bundles near the trailing edge provide the forces to retract the cell rear and move the cell body forward (DeMali and Burridge, 2003). Similarly, large rearrangements and increased actin assembly occur to allow cells to reinforce their actin cytoskeletons to withstand external forces. Finally, when cells undergo EMT necessary for cells to metastasize, changes in cell morphology are accompanied by reorganization of actin cytoskeleton. (Lamouille et al., 2014). In this section, we discuss links between these biological processes and cell metabolism.

Mechanotransduction

All cells experience force, and cells respond to external forces by reinforcing their actin cytoskeleton. In cultured cells, the number

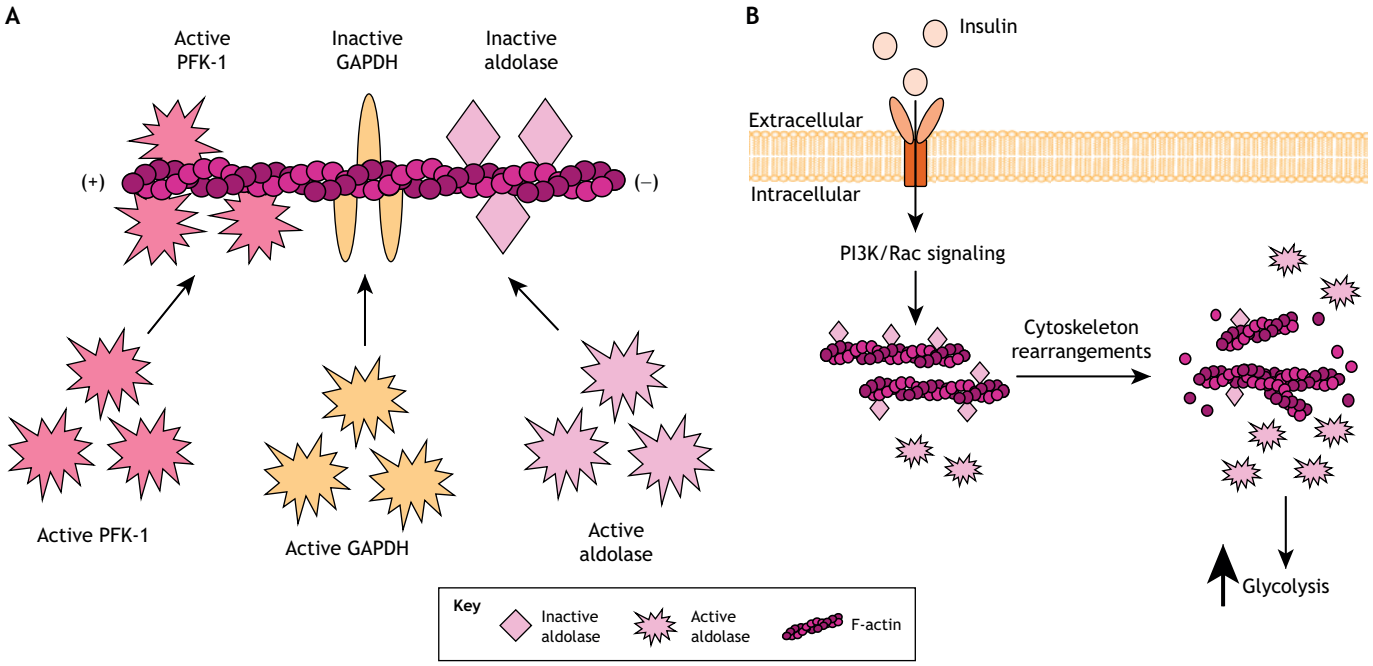

Fig. 2. Glycolytic enzymes bind to filamentous actin. (A) PFK-1, aldolase and GAPDH bind to F-actin with unique properties. (B) Insulin stimulates PI3K and Rac signaling, which triggers cytoskeleton rearrangements, causing F-actin-bound aldolase to be released. This release event leads to an increase in total aldolase activity, which aids in driving glycolytic flux.

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and thickness of actin stress fibers increases in response to force (Borghi et al., 2012; Liu et al., 2010) to allow the cell to withstand the force. In epithelial cells, this response to force requires recruitment and phosphorylation of the adapter protein vinculin at Y822 (Fig. 3; Bays et al., 2014). Vinculin links the actin cytoskeleton to force transmission receptors. Recruitment of vinculin leads to actin polymerization and the activation of the Rho GTPase pathway, which regulates the myosin light chain (MLC) of non-muscle myosin II, causing an increase in actomyosin contractility. Considering that 50% of the ATP in a resting cell may be necessary to support the actin cytoskeleton (Bernstein and Bamburg, 2003; Daniel et al., 1986), the amount of ATP required to support the actin cytoskeleton in cells under force is expected to be far greater. Several recent studies have examined how cells under force derive the energy needed to support actin remodeling. Application of shear stress to epithelial cells or tensional forces directly to E-cadherin, the major force-sensing receptor in epithelial tissues, stimulate glucose uptake and ATP production (Fig. 3; Bays et al., 2017). Importantly, Bays et al. discovered that this increase in ATP production was sensitive to oligomycin A inhibition (indicating a role for glycolysis and oxidative phosphorylation) and was necessary to support force-induced cytoskeletal rearrangements in order to reinforce cell–cell contacts and form an epithelial barrier (Bays et al., 2017).

A recent study further expands this paradigm between cell mechanics and metabolism by providing a novel mechanism of how cells tune their energy production in response to changes in the mechanical properties of cells (Park et al., 2020). Switching cells from a soft to a stiff matrix induces integrin engagement and actin-based morphological changes with the appearance of stress fibers (Fig. 4). This substrate switch triggers an increase in the glycolytic machinery and flux (Park et al., 2020). In contrast, others have shown that in certain cancer cell lines, glycolysis is downregulated when cells are grown on higher density matrices (Morris et al., 2016; Velez et al., 2019). This suggests that upregulation of glycolysis in response to stiff matrices may be cell-type specific. Indeed, it has been demonstrated that in more invasive type breast cancers, there is a shift towards glycolysis when collagen density is increased. However, here, less invasive breast cancer cells showed no changes regardless of substrate (Mah et al., 2018). Collectively, these data suggest that certain cell types increase glycolysis to provide the energy to remodel the actin cytoskeleton and allow them to withstand mechanical forces imparted by stiffer matrices, while other type of cells rely on other energy-producing mechanisms (Mah et al., 2018; Morris et al., 2016; Park et al., 2020; Velez et al., 2019).

Cell migration

Another energetically costly function in cells is the process of cell migration. Cell migration is required for processes such as embryonic development, immune responses and wound repair (Trepat et al., 2012). In addition to these normal physiological processes, cell migration is also crucial for tumor cell metastasis. Both the leading edge and trailing edge of migrating cells comprise actin-rich structures, which require energy to reorganize. This section examines the requirements for energy in single and collective cell migration and will show that the requirements are different (Fig. 5).

Many different types of cells require glycolysis to migrate (Alon, 2017; Marelli-Berg and Jangani, 2018; Shiraishi et al., 2015). Studies of T cells are providing important clues into how this phenomenon occurs. Regulatory T cells rely heavily upon oxidative metabolism for some of their functions, such as proliferation (Kishore et al., 2017, 2018). However, upon receiving a cue to migrate, a regulatory T cell upregulates glycolysis (Kishore et al., 2017, 2018). Key to this metabolic reprogramming is induction of glucokinase, a hexokinase isoform that transfers a phosphate from ATP to glucose to generate glucose-6-phosphate for the first step of glycolysis (Kishore et al., 2017, 2018). While glucokinase has a much lower affinity than hexokinase for glucose, it is less susceptible to inhibition by glycolytic metabolites. This advantage allows the T cells to maintain the glycolytic flux in the metabolic soup at sites of inflammation and infection. This is important because between day five and seven after infection, the inflammatory environment changes in most sites of infection, from a pro-inflammatory, infection-fighting mode, to a pro-suppressive, wound-healing mode (Kishore et al., 2017, 2018). Thus, the glycolytic changes in this instance confer a unique ability of the cell to withstand its environment. Interestingly, in these studies, glucokinase was reported to bind directly to the actin cytoskeleton, suggesting that it could localize ATP production to sites where the cytoskeleton is actively being rearranged (Kishore et al., 2018).

Similarly, dendritic cells, which present antigens to T cells, also exhibit metabolic reprogramming when activated (Guak et al., 2018). Activated dendritic cells switch from mitochondrial metabolism to glycolysis, and this switch is required to support cell shape and motility (Guak et al., 2018). More recent studies

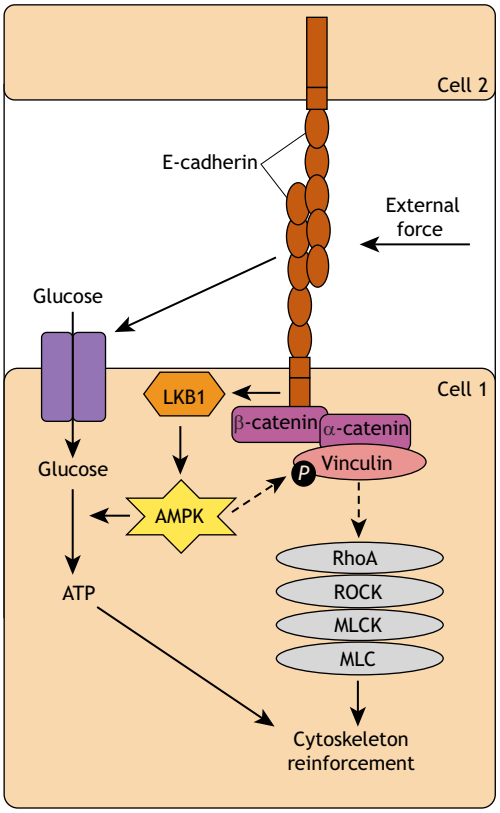

Fig. 3. Force-induced E-cadherin signaling for increased metabolism. In response to external forces, E-cadherin stimulates AMPK via its upstream activator, LKB1, both of which are activated and recruited to the E-cadherin adhesion complex. Increased AMPK signaling has two effects. It acts on a series of kinases to increase phosphorylation of vinculin at Y822; this triggers activation of the RhoA–ROCK–MLCK–MLC pathway, culminating in reinforcement of the actin cytoskeleton. AMPK also signals for increased glucose uptake and its conversion into ATP. The ATP produced provides the energy to polymerize new actin filaments in order to reinforce the actin cytoskeleton. ROCK, Rho-associated protein kinase; MLCK, myosin light chain kinase; MLC, myosin light chain.

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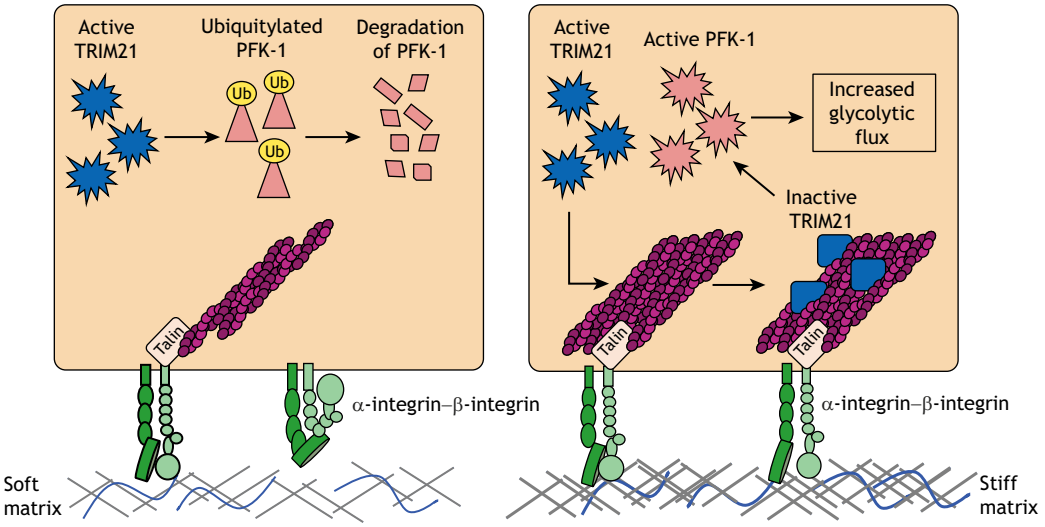

Fig. 4. Integrins respond to changes in matrix stiffness. On soft extracellular matrices, active E3 ubiquitin ligase TRIM21 binds and ubiquitylates PFK-1 in the cytosol, leading to the targeted degradation of PFK-1, which keeps glycolysis relatively low. When cells are transferred to a stiff extracellular matrix, contractility is elevated and F-actin binding to talin-bound integrins is increased, thereby stimulating actin bundling and TRIM21 sequestration and inactivation. As a result, PFK-1 accumulates in the cytosol and increases glycolysis. Ub, ubiquitin.

support this notion and suggest that the glycolytic reprogramming is prolonged and involves an upregulation of gene transcription (Liu et al., 2019). Thus, in response to migratory cues, immune cells tune their metabolism towards glycolysis to support cell migration.

The studies described above focus on how single cells tune their metabolism to provide the energy necessary to support cell migration. Studies of groups of two or more migrating cells reveal that the energy requirements are different. Collectively migrating cells consist of leader cells at the front of the migrating group that control the directionality of movement (Mayor and Etienne-Manneville, 2016). Follower cells trail the leaders and rely on them for directionality (Mayor and Etienne-Manneville, 2016). Emerging work indicates that the leader cells take up more glucose, have higher ATP:ADP ratios and are more contractile than follower cells (DeCamp et al., 2020; Zhang et al., 2019). Leader MDA-MB-231 cells need energy levels above a certain threshold in order to remain a leader cell. Once a leader cell has depleted its energy and drops below the threshold, a follower cell with the adequate energy stores takes its place (Zhang et al., 2019). The dependency of the leader cells on glycolysis may be cell-type dependent as in other cell types (H1299), the leader cells depend on mitochondrial respiration for energy and the follower cells rely on glycolysis (Commander et al., 2020). Nonetheless, these data suggest that migrating cells have mechanisms in place to tune their metabolism to meet the metabolic demands of cell migration. While groups of migrating cells do not all employ the same metabolic pathways, the energy states of the leader and follower cells are highly coordinated. The factors that control whether some leader cells employ glycolysis and others are heavily reliant on oxidative phosphorylation remain an area of active investigation. Certainly, one possibility is that the

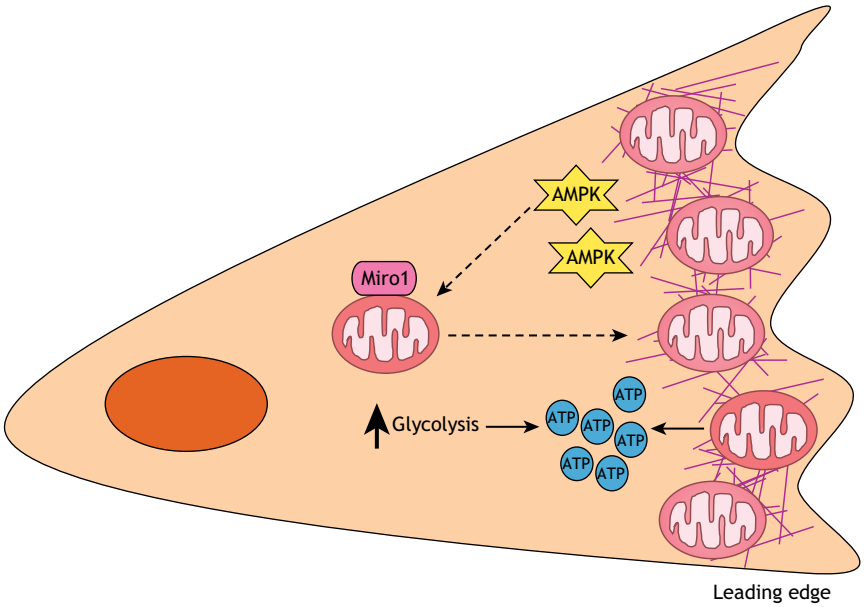

Fig. 5. Migrating cells increase energy production at the leading edge. Localized activation of AMPK at the leading edge of a migrating cell causes the recruitment of mitochondria to this location where increased actin cytoskeleton polymerization takes place. Miro1 mediates mitochondria trafficking from the cell body to the leading edge. Activated AMPK at the leading edge causes increased mitochondrial flux, ATP levels and membrane ruffling in cell protrusions. This localized synthesis of ATP contributes to the actin cytoskeletal dynamics required at the leading edge of a migrating cell. In addition to localized energy production at sites of highly dynamic actin polymerization, migrating cells upregulate glycolysis to contribute to the ATP levels in the cell to provide energy to migrate.

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energy demands put upon the cell by migrating through dense versus sparse microenvironments may be one determinant of the metabolic pathway utilized.

EMT

Epithelial cells line most of the major organs and cavities of the body. These cells are highly polarized and form extensive cell–cell adhesions, including adherens junctions and tight junctions, with neighboring cells. Cells transition from an epithelial to a mesenchymal phenotype during development, repair processes, such as wound healing and tissue regeneration, and the initiation of cancer cell metastasis.

EMTs are initiated by the activation of transcriptional factors, which decrease epithelial cell-to-cell adhesion, stimulate a loss of apical-basal polarity and promote the acquisition of migratory and invasive properties. During these events, the thin bundles of actin filaments that align the cortex of epithelial cells are transformed into thick, parallel, contractile bundles in mesenchymal cells – the thick actin bundles are necessary to generate traction forces so cells can migrate and adhere to the underlying basement membrane. Without this actin cytoskeleton remodeling, EMT does not occur, cells revert to an epithelial state and are unable to adopt an invasive phenotype (Peng et al., 2018; Sousa-Squiavinato et al., 2019).

How the energy is derived to support this cytoskeletal remodeling is emerging (summarized in Fig. 6). There is increased recognition that metabolic alterations drive EMT. Epithelial cells undergoing a transition to a mesenchymal phenotype generally have increased glucose uptake and flux. This is best characterized in neural crest cells, which delaminate from the neural tube and undergo EMT before giving rise to peripheral neurons; in these cells, a transient increase in glycolytic flux was observed prior to EMT and, importantly, shown to be a prerequisite to undergoing EMT (Bhattacharya et al., 2020). Increased glycolysis in normal cells undergoing EMT is likely to support increased actin dynamics that are required for a cell to adopt a mesenchymal phenotype. While it is possible that the elevations in glycolysis during EMTs that occur as cells progress to a cancerous phenotype also support actin reorganization, all the data to date support the idea that increased glycolysis supports the uncontrolled proliferation of tumor cells (Liberti and Locasale, 2016; Vander Heiden et al., 2009).

Accompanying changes in glycolysis in cells undergoing EMT is a downregulation of mitochondrial-associated gene expression and, hence, protein levels. Recently, an investigation into the link between the downregulation of mitochondrial-associated proteins and EMT was examined in 20 different cancer types (Gaude and Frezza, 2016). This analysis revealed that in cancers where genes associated with oxidative phosphorylation were downregulated, the most upregulated function in those cells were EMT-associated genes (Gaude and Frezza, 2016). Similarly, an examination of gene expression profiles in primary versus metastatic cancer samples revealed that oxidative phosphorylation was significantly downregulated and EMT-associated genes were significantly upregulated in metastatic cancers when compared to primary cancers (Gaude and Frezza, 2016).

However, the significance of decreased mitochondrial function during EMT is not well understood. Mitochondrial dysfunction has been associated with increased invasiveness, metastatic potential and drug resistance of cancer cells (Chen, 2012; Guerra et al., 2017; Moro et al., 2008, 2009; Porporato et al., 2014).

How are rapid metabolic changes necessary for cytoskeletal rearrangements achieved?

Above, we discussed how cells adjust their metabolism to meet the energetic demands imposed by the actin cytoskeleton. In this section, we will discuss emerging mechanisms for providing the energy necessary to fuel actin remodeling.

Changes in signal transduction pathways that govern cell metabolism

Cell metabolism is tightly regulated through the actions of AMP-activated protein kinase (AMPK), an energy sensor in all living organisms. AMPK is activated when cells are deficient in nutrients. Consequently, AMPK stimulates glucose uptake and lipid oxidation, and turns off energy-consuming processes (Herzig and Shaw, 2018; Ke et al., 2018; Lin and Hardie, 2018; Wang et al., 2018). Emerging data indicates that, in response to eternally applied forces, cells activate AMPK, allowing cells to provide energy for actin cytoskeletal rearrangements (Bays et al., 2017; Campbell et al., 2019; Guo et al., 2020; Steele et al., 2019). For example, in epithelial cells, application of shear stress or tensional force directly to E-cadherin, a major force-transducing receptor, stimulates activation of AMPK. Active AMPK is recruited to the cadherin adhesion complex via liver kinase B1 (LKB1; also known as STK11) and signals for the increased uptake of glucose and the production of ATP (Bays et al., 2017). The ATP produced then provides the energy to allow for cortical actin remodeling (Bays et al., 2017). In further support of a role for AMPK in providing the energy necessary to remodel the actin cytoskeleton, AMPK is locally activated in the leading edge of migrating cells (Cunniff et al., 2016), sites of active actin polymerization, which generate a protrusive force that pushes the membrane forward. This localized

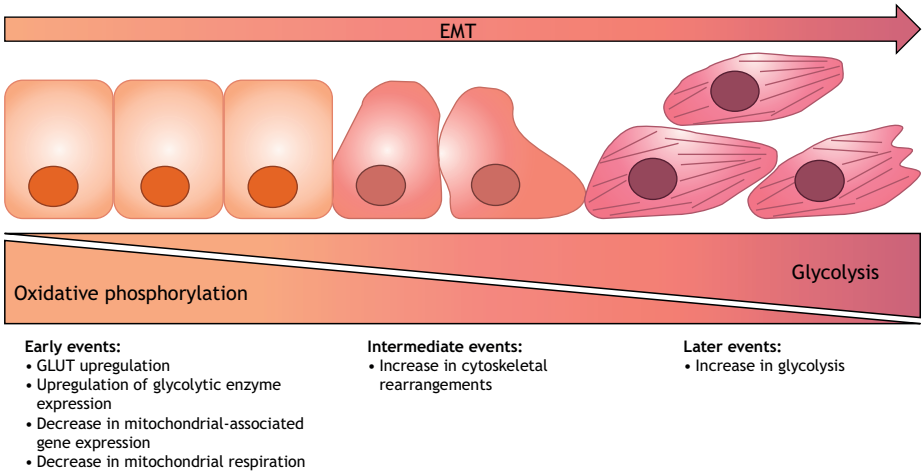

Fig. 6. Epithelial to mesenchymal transitions alter cell metabolism. Epithelial cells that undergo EMT generally upregulate glycolysis and downregulate mitochondrial oxidative respiration. Cells can increase their glycolytic rate by upregulating the expression of glucose transporters (GLUTs) and various glycolytic enzymes. Concurrently, cells downregulate mitochondrial-associated genes, resulting in a decrease in oxidative phosphorylation. These metabolic and genetic changes coincide with increased actin cytoskeletal rearrangements that fuel the transition from an epithelial to a mesenchymal phenotype.

activation of AMPK increases ATP, mitochondrial flux and cytoskeletal dynamics (Cunniff et al., 2016), and the resulting energy may fuel actin cytoskeletal reorganization.

Liberation and activation of metabolic enzymes

Another mechanism for stimulating metabolism is to liberate sequestered metabolic enzymes. As discussed previously, several glycolytic enzymes are bound to the actin cytoskeleton. Recent studies revealed that stimulation of epithelial cells with insulin leads to phosphoinositide-3-kinase (PI3K) and Rac activation, culminating in cytoskeletal remodeling (Hu et al., 2016). In response, aldolase A is released from actin causing an increase in aldolase A activity, which contributes to stimulating glycolytic flux (Hu et al., 2016). These data provide evidence that energy for actin remodeling can be coordinated with glycolysis by modulating the availability of metabolic enzymes.

Controlling the amount of key metabolic enzymes via proteasomal degradation

An additional mechanism for regulating the availability of metabolic enzymes is to coordinate their degradation. It has been recently examined how cells control their energy production in response to changes in stiffness of their microenvironment (Fig. 4) (Park et al., 2020). When human bronchial epithelial cells are transferred from a stiff to soft matrix, F-actin bundling decreases and the E3 ubiquitin ligase tripartite motif (TRIM)-containing protein 21 (TRIM21) is released from the actin cytoskeleton. Active TRIM21 stimulates the degradation of phosphofructokinase-1, resulting in decreased glycolysis. Interestingly, when similar experiments are performed on non-small cell lung cancer cells, the switch from stiff to soft matrices has no effect, and the cells retain their actin bundles. This study thus suggests that loss of contractility promotes the disassembly of the actin cytoskeleton and controls metabolism by modulating the proteasomal degradation of the rate-limiting enzyme of glycolysis (Park et al., 2020).

Increased mobilization of energy-producing organelles to sites of actin remodeling

Another mechanism for coordinating actin remodeling and metabolism is to mobilize the metabolic machinery to sites of actin assembly. Numerous studies in normal and transformed cells demonstrate that mitochondria are enriched in the leading edge of cells, an area rich in actin polymerization (Cunniff et al., 2016; Daniel et al., 2019 preprint; Prudent et al., 2016; Schuler et al., 2017; Zhao et al., 2013). Not only are mitochondria present in the leading edge but they also locally increase ATP concentrations and are required for efficient cell migration (Cunniff et al., 2016).

Recent studies reveal mechanistic insight into how mitochondria are trafficked and immobilized at the leading edge of migrating cells. The mitochondrial Rho-GTPase Miro1, a mediator of microtubule-based mitochondrial motility, has emerged as a candidate for this localization (Schuler et al., 2017). In support of this notion, mouse embryonic fibroblasts deficient in Miro1 have mitochondria that do not translocate to the cell periphery, thus dramatically reducing not only the ATP:ADP ratio at the cell cortex but also actin cytoskeletal dynamics (Schuler et al., 2017). Studies aimed at understanding how mitochondria are immobilized suggest that at least two possibilities exist. Evidence from budding yeast and cultured tobacco cells indicates that the actin cytoskeleton itself immobilizes mitochondria (Boldogh and Pon, 2006). Another possibility is that mitochondria are tethered to focal adhesions, sites where integrins bind to the extracellular matrix and the actin cytoskeleton. (Daniel et al., 2019 preprint). Interestingly, when mitochondrial activity is inhibited, the size of focal adhesions and migratory speed is decreased. These phenotypes could be explained by a loss of actin cytoskeleton in the vicinity or directly associated with the focal adhesions (Daniel et al., 2019 preprint). Thus, mobilizing and retaining mitochondria at sites of actin remodeling is another way that cells can rapidly adjust metabolism to account for the energetic demands of reorganizing the actin cytoskeleton.

Changes in protein and gene expression of the glycolytic components

The mechanisms described thus far provide fast-acting ways to mobilize the metabolic machinery into action. In addition to these, other more time-consuming mechanisms exist. The vast majority of these involve the transcriptional regulation of genes encoding metabolic enzymes or the metabolic machinery. One class of metabolic proteins frequently targeted are the glucose transporters, which traffic glucose into cells. In response to force on E-cadherin, glucose uptake is stimulated by increasing the membrane retention of GLUT1 (also known as SLC2A1) (A.M.S., J. L. Bays, S. R. Mackin, R. M. Mege, and K.A.D, unpublished data). Inducing EMT triggers an increase in expression of glucose transporter 3 (GLUT3) in some cell types and GLUT1 and GLUT3 (SLC2A3) in others (Masin et al., 2014). ZEB1, a transcription factor promoting changes associated with EMT, has been shown to directly activate transcription of *GLUT3* (Masin et al., 2014). Other mechanisms for regulating the level of mRNAs exist. Degrading hyaluronic acid, a glycosaminoglycan that impedes cell migration by encapsulating the cell in a dense matrix, produces a robust increase in glycolysis (Sullivan et al., 2018). The increase in glycolysis occurs through a degradation of mRNA transcripts for thioredoxin-interacting protein (TNIP), a protein that controls internalization of the GLUT1 (Sullivan et al., 2018). The loss of TNIP enriches GLUT1 at the plasma membrane and consequently increases cell migration (Sullivan et al., 2018). Taken together, these data indicate that the levels of glucose transporters can be modulated at the level of increased synthesis of mRNA transcripts or increased degradation of a negative regulator. Regardless of the exact mechanism, increased expression of glucose transporters is a prerequisite for processes such as migration and EMT, which require extensive actin remodeling.

Another group of proteins transcriptionally regulated during processes involving extensive actin remodeling are the glycolytic enzymes themselves. Extensive evidence show hexokinase, PFK-1 and pyruvate kinase, the three enzymes that catalyze the irreversible steps of glycolysis, are subject to transcriptional regulation. In the case of hexokinase, evidence suggests that its expression is increased during EMT and elevates glycolytic rate (Chen et al., 2018). Preventing the increase in hexokinase expression suppresses glycolysis and decreases not only EMT but also cell migration (Chen et al., 2018).

PFK-1 is also transcriptionally regulated. Its expression is suppressed by Snail (SNAI1) – a well-known transcriptional repressor of E-cadherin in EMT – and the suppression of PFK-1 switches glycolytic flux towards the pentose phosphate pathway, which maintains an adequate level of NADPH that is critical for surviving oxidative stress (Kim et al., 2017). A comparison of normal mammary epithelial and breast cancer cell lines revealed that normal mammary cells express the platelet and muscle isoforms of PFK-1; indeed, breast cancer cells that have undergone EMT and are more migratory, have elevated levels of a different isoform, namely the liver isoform (Zancan et al., 2010).

Like hexokinase and PFK-1, the expression of pyruvate kinase, the enzyme that catalyzes the final irreversible step of glycolysis, is transcriptionally regulated (Hamabe et al., 2014). Gene expression of pyruvate kinase and its splice variant pyruvate kinase M2 is elevated upon induction of EMT. Interestingly, once cells begin to undergo EMT, a fraction of the cytosolic pyruvate kinase M2 enters the nucleus and associates with transcription factors, culminating in suppression of E-cadherin (Hamabe et al., 2014). This study demonstrates that the initiation of EMT precedes alterations in gene expression. Thus, it is EMT that calls for increased metabolism, as opposed to the opposite. Collectively, these studies indicate that modulation of gene expression is an effective means of regulating the glycolytic machinery in response to events such as EMT, which occurs on a slower timescale than other processes that call for a much more immediate increase in metabolism.

Conclusions and perspectives

The actin remodeling that occurs during the response of cells to external forces, EMT and cell migration is a major metabolic drain on cells. Emerging data suggest that energy for these events is provided by reprogramming of the cell metabolism. Many mechanisms have been identified for increasing metabolism. Some of these, such as activating signal transduction cascades that regulate the availability, activity and levels of metabolic enzymes, or mobilization of the metabolic machinery, can provide rapid adjustments to cell metabolism. Others, such as changes in gene expression, are slower acting but are exquisitely coordinated with actin cytoskeletal changes.

Despite the many advances in understanding how metabolism is coupled to the actin cytoskeleton, several major unanswered questions remain. First, do changes in cell metabolism stimulate actin cytoskeletal remodeling or vice versa? Our work shows that application of force on E-cadherin stimulates AMPK activity, suggesting that force calls for changes in metabolism (Bays et al., 2017). However, blocking actin cytoskeletal remodeling while leaving AMPK activity intact does not stimulate glycolytic flux, suggesting that the increase in ADP:ATP ratios, not AMPK activation directly, signals for increased metabolism (Bays et al., 2017). It will be interesting to determine whether activation of AMPK or an increase in ADP:ATP ratios signals for more energy in other systems. Second, there is very little information about how elevations in cell metabolism affect the actin cytoskeleton. The events that signal for increased metabolism are accompanied by increased actin dynamics. For example, we and others have found that actin stress fibers grow and increase in number when cells are under force (Bays et al., 2017; Borghi et al., 2012; Liu et al., 2010). Increases in actin dynamics are highly dependent upon ATP. Indeed, as new filaments grow, G-actin bound to ATP adds to the barbed ends of new and existing actin filaments (Pollard and Earnshaw, 2002; Suarez et al., 2011). The hydrolysis of the bound ATP and release of inorganic phosphate is known to modulate the physical properties of actin filaments and the binding affinity of various actin-binding proteins (Bugyi and Carlier, 2010). Thus, it is tempting to speculate that elevated levels of ATP in cells would allow for increased recruitment of binding partners that bundle and stabilize actin filaments. Third, the metabolic heterogeneity of collectively migrating cells needs closer examination, as there are contradictory ideas about the energy consumption between leader and follower cells (Commander et al., 2020; Zhang et al., 2019). Additionally, what is the mechanism for follower cells to reach an energetic threshold that allows them to take the place of and position itself as a leader cell and subsequently change its actin dynamics to that of a leader cell? These questions and others will need further examination to fully understand the coordination between the actin cytoskeleton and cell metabolism.

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Competing interests

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