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Fueled by Microtubules: Does Tubulin Dimer/Polymer Partitioning Regulate Intracellular Metabolism?

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Microtubules (MTs) or their subunits, tubulin dimers, interact with multiple components that contribute to intracellular metabolic pathways. MTs are required for insulin-dependent transport of glucose transporter 4 to the plasma membrane, they bind most glycolytic enzymes and are required for translation of the mRNA encoding hypoxia inducible factor-1α. Tubulin dimers bind the voltage-dependent anion channel of the mitochondrial outer membrane; this channel functions in metabolite transport in and out of mitochondria. We hypothesize that tubulin partitioning between dimer and polymer pools regulates multiple steps in metabolism, where metabolic output is greatest when both tubulin dimers and MT polymers are present and reduced by drug treatments that disrupt this normal balance. Experimental evidence from these drug-induced changes in tubulin dimer/polymer partitioning supports our model for several metabolic steps. Signal transduction pathways that stabilize or destabilize MTs can shift the normal ratio between unpolymerized and polymerized tubulin dimers, and one downstream consequence of this shift in tubulin partitioning could be a change in metabolic output. © 2012 Wiley Periodicals, Inc

Abbreviations used: 1,3-BPG, 1,3-bisphosphoglycerate; 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; DHAP, dihydroxyacetone phosphate; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; G6P, glucose-6-phosphate; GAP, glyceraldehyde-3-phosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT, glucose transporter; GSV, GLUT4 storage vesicle; HIF-1, hypoxia inducible factor; HK, hexokinase; MT, microtubule; PDK, pyruvate dehydrogenase kinase; PEP, phosphoenolpyruvae; PFK, phosphofructokinase; PGI, phosphoglucose isomerase; PGM, phosphoglycerate mutase; PI3K, phosphatidylinositol-3-kinase; PK, pyruvate kinase; PPP, pentose phosphate pathway; TPI, triosephosphate isomerase; VDAC, voltage-dependent anion channel; VHL, von Hippel-Lindau.

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Introduction

The microtubule (MT) cytoskeleton has well-characterized roles in cell polarity, motility, mitosis, and vesicle traffic. The dynamic turnover of MTs is critical to most of these processes, allowing the cell to reorganize an array of MTs rapidly for specific functions. Here we posit an additional role for MTs in regulating metabolic processes and propose that metabolism is greatest when both MTs and their tubulin subunits are present in cells. Disruption of this normal balance, to either inhibit or promote MT polymerization, is expected to decrease metabolic output. Our hypothesis predicts that chemotherapies that stabilize or destabilize MTs will significantly impact metabolic pathways and reduce ATP levels. Importantly, even a small decrease in ATP production can slow cell proliferation. For example, a 19% decrease in ATP production was sufficient to induce senescence in mouse embryo fibroblasts [Kondoh et al., 2005], indicating that even a relatively small contribution of the MT cytoskeleton to metabolic output could have a significant impact on cell fate.

The metabolic components and pathways we consider here include glucose transporters (GLUTs), glycolysis, the pentose phosphate pathway (PPP), and mitochondrial oxidative phosphorylation. Glucose enters cells through glucose transporters called GLUTs; the concentration of GLUTs at the plasma membrane can be regulated by insulin and requires trafficking of GLUT-containing vesicles along MTs to reach the plasma membrane. Within the cytoplasm, glucose is broken down to pyruvate during glycolysis, yielding ATP and NADH. Several intermediate products of glucose breakdown, including glucose-6-phosphate (G6P) and glyceraldehyde-3-phosphate (GAP), can also be shunted to anabolic pathways such as the PPP. Pyruvate enters the mitochondria, initially through the voltage-dependent anion channel (VDAC) of the outer

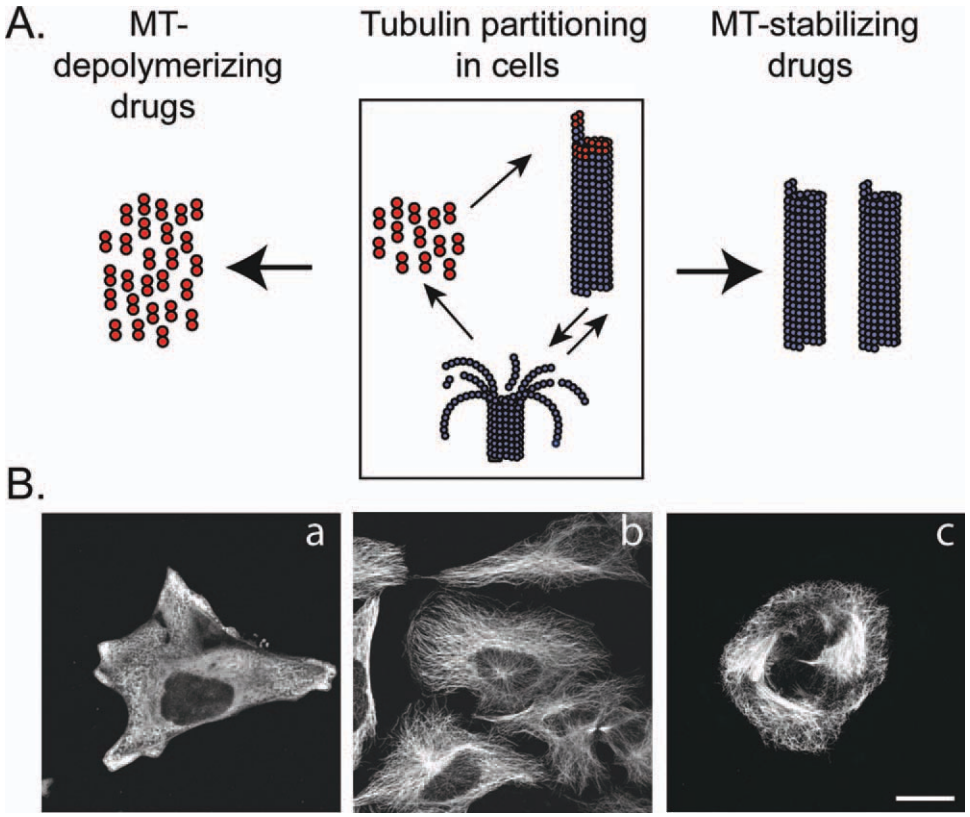

Fig. 1. Tubulin partitioning between dimers and polymers. (**A**) Diagrams represent (left to right) depolymerization of tubulins to individual dimers caused by MT-depolymerizing drugs (colchicine, nocodazole, or vinblastine); partitioning of tubulins between dimers and polymer (boxed section; both growing and shortening MTs are shown); hyperpolymerization of tubulins into MTs caused by MT-stabilizing drugs (paclitaxel and related molecules). GTP-tubulins are shown in red and for clarity are shown as larger spheres when present as dimers. GDP tubulins within MTs are shown in blue. Upon depolymerization, dimers release GDP and bind GTP to replenish the pool of GTP-dimers. (**B**) Images of antitubulin stained cells treated overnight prior to fixation with (a) 30 μM nocodazole, a MT-depolymerizing drug; (b) untreated; or (c) 1 μM paclitaxel. In the nocodazole-treated cell (a), the fluorescence represents soluble tubulins that were maintained during lysis and fixation in ice cold methanol.

mitochondrial membrane. Once within the mitochondrial matrix, pyruvate is converted to acetyl CoA and then further broken down in the citric acid cycle, yielding NADH and $FADH_2$. Oxidation of NADH and $FADH_2$ provides electrons to fuel the proton gradient and drive oxidative phosphorylation. Oxidative phosphorylation is significantly more efficient than glycolysis, generating 34–36 ATPs per glucose molecule, compared to only two ATPs from glycolysis. Most cancer cells show a greater reliance on glycolysis over oxidative phosphorylation, even under aerobic conditions, a phenomenon termed the Warburg effect [Buchakjian and Kornbluth, 2010; Maddocks and Vousden, 2011].

MTs are dynamic polymers composed of α/β tubulin heterodimers. These dynamic MTs continually turn over by dynamic instability, where at steady state MTs exist in one of two states (growing or shortening) with rare switches between states (catastrophe and rescue; Fig. 1). MT assembly consumes energy since tubulins bound to GTP are added to MT ends during polymerization. GTP is then hydrolyzed to GDP, and most of the MT, except its tip and possibly very small segments along the MT wall, is composed of tubulins bound to GDP [Howard and Hyman, 2003; Dimitrov et al., 2008; van der Vaart et al., 2009]. As a MT depolymerizes, the released tubulin dimers exchange their bound GDP for GTP. Dynamic instability in cells is regulated by a large number of regulatory proteins, allowing cells to tailor MT lengths and turnover rates to specific cell functions [van der Vaart et al., 2009]. Some of these MT assembly regulators also control the fraction of tubulin dimers present in soluble and polymer pools, referred to as "tubulin dimer partitioning" to reflect the distribution of tubulins between these two pools [Holmfeldt et al., 2009]. Cells can shift the percentage of tubulin in dimer and polymer pools. For example, cells transiently depolymerize most MTs at mitotic entry [Zhai and Borisy, 1994], and can change MT stability and polymer level in response to physiological signals [Gundersen and Cook, 1999].

Several successful chemotherapies target MT polymerization and block cells in mitosis; nocodazole, vinblastine, colchicine, and related molecules depolymerize MTs to

tubulin dimers, while paclitaxel and related taxanes promote MT assembly and shift most tubulins into the polymer form [Jordan and Kamath, 2007] (Fig. 1). Hyperpolymerization of MTs by incubation in paclitaxel can also disrupt the organization of MTs within cells (Fig. 1B).

Glucose Transport

Metabolism typically begins with facilitated glucose transport into cells by GLUTs on the plasma membrane. Different cell types express various members of the GLUT family allowing glucose uptake in response to signals or at differing extracellular glucose levels. In general, when glucose import is not required, GLUT levels on the cell surface are reduced by endocytosis and the transporters are stored in a membranous subcellular compartment. In contrast, when cells need to take up glucose, GLUTs in the intracellular storage pools are shuttled to the plasma membrane by exocytosis. Among the signals regulating GLUT level on the plasma membrane, insulin signaling to regulate GLUT4 level at the plasma membrane of skeletal muscle, cardiac muscle, and adipose tissue, is the best understood [James et al., 1988, 1989]. Insulin binding to its receptor activates the phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway and stimulates translocation (exocytosis) to the plasma membrane of a common recycling intracellular vesicle pool (endosomal pool) containing GLUT1, GLUT4, and the transferrin receptor [Holman and Kasuga, 1997]. Among the transporters, GLUT4 has a more acute response to insulin, partially due to the fact that GLUT4 is also found in a unique storage pool termed the "GLUT4 storage vesicle" (GSV). Up to 60% of insulin-responsive GLUT4 is present in these GSVs. In contrast, GLUT1 predominately resides at the plasma membrane to maintain basal levels of glucose uptake.

The MT network is required for GLUT4 translocation to plasma membrane in adipocytes [Fletcher et al., 2000; Guilherme et al., 2000; Emoto et al., 2001; Olson et al., 2001]. In response to insulin signaling, GLUT4 vesicles in 3T3-L1 adipocytes are transported along MTs from the perinuclear region to the plasma membrane [Fletcher et al., 2000]. GLUT4 transport is reduced by MT depolymerization [Fletcher et al., 2000; Emoto et al., 2001; Olson et al., 2001], while MTs are not required for insulin-dependent transport of the transferrin receptor, indicating that GSVs have a greater requirement for MT-based transport than do endosomes [Fletcher et al., 2000]. Dynein motors transport GLUT4-containing vesicles to a perinuclear localization, and this localization is required for a subsequent response to insulin [Guilherme et al., 2000]. Kinesin-1 (KIF5B), a MT-plus end directed kinesin, is responsible for insulin-dependent trafficking of GLUT4 vesicles along MTs to the plasma membrane [Semiz et al., 2003; see Lawrence et al., 2004 for standardized kinesin nomenclature]. An alternative role for MTs in GLUT4 translocation was proposed by Eyster et al. [2006], where MTs are required for formation of an insulin-signaling complex and stimulation of vesicle docking or fusion at the plasma membrane, rather than via motor-based transport. Although the mechanism responsible is a matter of debate, it is clear that MTs are required for GLUT4 translocation to the plasma membrane in response to insulin.

Depolymerizing MTs by treatment with nocodazole, colchicine, or vinblastine reduces insulin-dependent translocation of GLUT4 to the plasma membrane by 40–80% in the adipocyte cell line 3T3-L1 [Fletcher et al., 2000; Olson et al., 2001]. This reduction in GLUT4 level at the plasma membrane is sufficient to reduce insulin-induced glucose uptake by ~40% [Fletcher et al., 2000]. In contrast, basal levels of GLUT4 at the plasma membrane and constitutive glucose uptake are unaffected by MT disassembly [Fletcher et al., 2000]. MT depolymerization may reduce, but not eliminate, GLUT4 transport because MT depolymerization causes dispersal of the perinuclear vesicles containing GLUT4, which then allows actin/myosin-based transport at the cell cortex [Fletcher et al., 2000]. F-actin depolymerization by latrunculin B significantly inhibits insulin-dependent GLUT4 translocation to the plasma membrane, indicating that both MTs and F-actin contribute to GLUT4 translocation [Emoto et al., 2001]. Simultaneous depolymerization of both F-actin and MTs completely abolishes GLUT4 transport to the plasma membrane in response to insulin [Emoto et al., 2001]. A direct measure of glucose uptake was not reported by Emoto et al. [2001], but it is likely that depolymerization of both MTs and F-actin greatly reduces insulin-stimulated glucose uptake into adipocytes.

In contrast to adipocytes, insulin-stimulated glucose uptake in muscle cells does not show a convincing dependence on MTs. Treatment of rat skeletal muscle with colchicine, a MT depolymerizing drug, did not inhibit insulin-stimulated glucose transport [Ai et al., 2003]. Surprisingly, treatment of these same cells with nocodazole, another MT depolymerizing drug, inhibited insulin-stimulated glucose transport in a dose and time dependent manner [Ai et al., 2003]. Inconsistent results from treatment with two MT depolymerizing drugs raises concern that nocodazole may inhibit an additional target in these muscle cells. Based on this one study, it appears that MT-based transport of GLUT4-containing vesicles is less critical in muscle cells than in adipocytes.

The MT cytoskeleton's contribution to glucose uptake is restricted to adipocytes responding to insulin stimulation as discussed above. In these cells, MT depolymerization significantly reduces glucose uptake. It is not known whether paclitaxel treatment, to promote MT

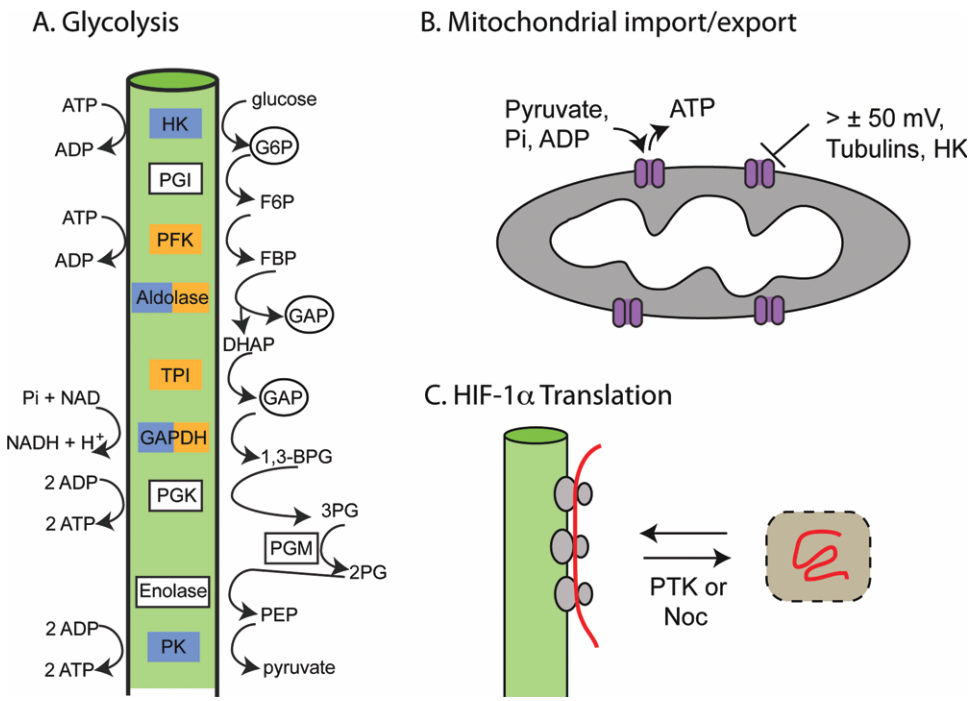

Fig. 2. Metabolic pathways regulated by MT assembly state. (**A**) Most glycolytic enzymes (shown in boxes) bind to MTs, shown in order for the glycolytic pathway. Some enzymes show greater activity when bound to MTs (blue boxes), others have decreased activity (yellow boxes). Blue/yellow boxes reflect inconsistent reports in the literature. Substrates and products are shown to their right of the MT. The circled products represent substrates that can be shunted to the PPP. To the left of the MT are ATP consumption and generation steps. Abbreviations for enzymes, substrates, and products are given in the text. (**B**) Metabolites cross the outer mitochondrial membrane through VDAC (purple channels). Pyruvate, ADP, and inorganic phosphate enter mitochondria through the channel, ATP exits mitochondria through the same channel. Several factors cause channel closing, including changes in outer mitochondrial membrane potential, tubulin dimers, and HK. The intermembrane space, between inner and outer mitochondrial membranes is shown in gray, the matrix is represented by the central white area. (**C**) The mRNA (red line) encoding HIF-1α is translated on MTs. For simplicity, ribosomes are shown binding to the MT, but the factor(s) binding HIF-1α to MTs is not known. HIF-1α mRNA is also present in P-bodies (brown box) where it is not translated. MT depolymerization (by nocodazole or colchicine) or hyperpolymerization (by paclitaxel) shifts the mRNA to P-bodies. PTX, paclitaxel; Noc, nocodazole. See text for citations for each process diagrammed here.

polymerization, disrupts GLUT4 trafficking. Paclitaxel treatment can change the normal MT organization (as shown in Fig. 1) and this disorganization of MTs is sufficient to inhibit directed virus translocation on MTs [Warren and Cassimeris, 2007]. Therefore, it is possible that either MT depolymerization or MT hyperpolymerization and/or MT reorganization will disrupt GLUT4 translocation to the plasma membrane. However, in most cells, MTs are expected to contribute little to glucose uptake. Instead, a more general role for MTs may be in regulating glycolysis and oxidative phosphorylation, as discussed next.

Most Glycolytic Enzymes Bind MTs

Once glucose enters the cell, it is available for glycolysis where it is broken down to pyruvate by a series of enzymatic reactions. Nearly all glycolytic enzymes have been reported to bind MTs to some degree, with the exception of phosphoglycerate mutase as outlined in Fig. 2. For phosphoglucose isomerase [Walsh et al., 1989], aldolase [Walsh et al., 1989; Volker et al., 1995], triosephosphate isomerase (TPI) [Orosz et al., 2000], glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [Walsh et al., 1989; Somers et al., 1990; Volker et al., 1995; Andrade et al., 2004; Chuong et al., 2004], phosphoglycerate kinase (PGK) [Walsh et al., 1989], and pyruvate kinase (PK) [Walsh et al., 1989; Volker et al., 1995; Kovacs et al., 2003], binding has been demonstrated by copelleting with MTs. In the case of TPI, binding of the native protein is relatively weak, but a mutant form found in a human disease shows much greater to binding to MTs [Orosz et al., 1999]. Others, including hexokinase (HK), phosphofructokinase (PFK), GAPDH, enolase, and PK, have been shown to colocalize with MTs in brain extracts (HK, Wagner et al. [2001]; PK, Kovacs et al. [2003]), myoblasts/muscle (enolase [Keller et al., 2007]), BHK cells (GAPDH [Andrade et al., 2004]) or melanoma cells (PFK [Glass-Marmor and Beitner, 1999]). Several enzymes bind both tubulin dimers and MT polymers, including HK [Wagner et al., 2001], enolase [Keller, 2007], and PK [Kovacs et al., 2003]. Most of the above observations

were reported in animal cells, but evidence for MT binding has also been presented in plants [e.g., Chuong et al., 2004; Romagnoli et al., 2010] and the yeast *Saccharomyces cerevisiae* [Gotz et al., 1999].

Binding of glycolytic enzymes to MTs has been shown to increase or decrease enzyme activity compared to that measured for the non-MT bound enzyme, as measured by the percentage of enzyme activity pelleting with MTs or remaining in solution. Figure 2A shows those enzymes whose activity is decreased (yellow) or increased (blue) by binding to MTs, although in some cases the evidence is contradictory (shown as both colors in Fig. 2A). The remaining enzymes have either not been tested (PGK) or their activities are unaltered by MT binding (enolase [Keller et al., 2007]). Enzymes showing reduced activity when bound to MTs are PFK (muscle isoform [Lehotzky et al., 1993; Vertessy et al., 1997]) and TPI [Orosz et al., 2000]. Enzymes with greater activity when bound to MTs include HK [Wagner et al., 2001] and PK [Walsh et al., 1989]. Enzymes reported to have either greater or lesser activity include GAPDH [Durrieu et al. 1987; Walsh et al., 1989] and aldolase [Walsh et al., 1989; Volker and Knull, 1993]. Some of the latter contradictory results could reflect differences in experimental methods or purity of enzyme components. Indeed, analysis of two sequential enzymes, PFK and aldolase, and their regulation by MT binding, demonstrates that a complex formed by the two enzymes no longer associates with MTs [Vertessy et al., 1997]. In some cases, e.g., aldolase [Vertessy et al., 1997], MT binding competes with substrate for enzyme binding and therefore MTs can act as a competitive inhibitor to reduce enzyme activity in these situations.

Binding of enzymes, which act in series, to a solid support (e.g., MTs) has been proposed to facilitate enzymatic reactions by providing a scaffold to organize enzymes sequentially [Srere, 1987]. For example, purine biosynthesis occurs in clusters bound to MTs, which increases the rate of purine synthesis [An et al., 2010]. In contrast, MTs do not appear to function as a simple scaffold to organize glycolytic enzymes in sequential order since individual enzymes vary in whether MT binding increases or decreases their activity. Instead, evidence from cells treated with MT-targeted drugs suggests that a balance between tubulin dimers and MT polymers is necessary for optimal glycolytic output as outlined below.

Although numerous studies have examined binding of individual glycolytic enzymes to MTs and how enzyme activity is modified by MT binding (as discussed above), fewer studies have examined whether drug-induced changes to the MT cytoskeleton impact glycolytic rate or ATP production in cells. Glass-Marmor and Beitner [1999] found that a mouse melanoma cell line treated with paclitaxel for 90 min caused PFK to release from MTs. This treatment also reduced the amount of an intermediate in glycolysis, fructose 1,6 bisphosphate, as well as total cellular ATP. Fructose 1,6 bisphosphate is produced by PFK in the rate-limiting step in glycolysis. Fructose 1,6 bisphosphate is an allosteric activator of PK; therefore, its reduction will also inhibit later steps in glycolysis. In this study, the paclitaxel-treated melanoma cells also showed reduced viability, correlated with reduced ATP levels, but whether cells would adapt to paclitaxel at longer incubation times was not addressed. In another study, vascular smooth muscle, isolated from pig cerebral microvessels, was treated with vinblastine or paclitaxel [Lloyd and Hardin, 1999]. Either treatment reduced labeled carbon incorporation into lactate, indicative of reduced glycolysis, while neither treatment reduced gluconeogenesis [Lloyd and Hardin, 1999]. These authors propose that binding of either tubulin dimers or MTs to various glycolytic enzymes may be responsible for the observed decline in glycolysis after either MT depolymerization (vinblastine) or hyperpolymerization (paclitaxel). Deletion of tubulin folding factor D (encoded by CIN1) from the yeast *S. cerevisiae* supports the idea that tubulin and/or MTs are necessary for efficient glycolysis, particularly in the yeast's ability to respond to a shift from growth on ethanol to glucose [Gotz et al., 1999]. Several reports have also modeled MT contributions to glycolytic rates [Aon and Cortassa, 2002; Ovadi et al., 2004], predicting that MTs generally inhibit glycolysis. Based on the results of drug-induced MT depolymerization or hyperpolymerization, it appears that a balance of tubulin dimers and MT polymers is necessary for optimal energy production via glycolysis, rather than the prediction of Aon and Cortassa [2002] that glycolysis will be favored by MT assembly and inhibited by MT depolymerization. We hypothesize that any shift in the normal balance of tubulin partitioning between dimer and polymer is likely to reduce glycolytic rate, while the normal distribution of tubulin between pools of dimer and polymer should allow maximal substrate flux through the glycolytic pathway.

In addition to the MT cytoskeleton, many glycolytic enzymes have been reported to bind actin filaments [e.g., Pagliaro and Taylor, 1988; Wang et al., 1997], particularly in human erythrocytes [Real-Hohn et al., 2010], a cell type that lacks a MT network. In *S. cerevisiae*, combined deletion of genes regulating the MT and actin cytoskeletons slowed the response of cells after a switch to glucose-containing medium [Gotz et al., 1999], indicating that both these cytoskeletal polymer systems are likely contributing to glycolytic regulation.

Two intermediates generated during glucose breakdown, G6P and GAP, can be shunted to the PPP and used in anabolic pathways. Disruption of the normal balance between unpolymerized and polymerized tubulin dimers should reduce the amount GAP available for the PPP since drug-induced changes in MT assembly reduce overall glycolytic rate (discussed above). We predict that the first reaction in glycolysis, catalyzed by HK, should yield

greater amounts of G6P when MT concentration is increased by paclitaxel. MTs will enhance HK activity and increase G6P concentration. G6P is the substrate for the first, and committed step, of the PPP, and an increase in G6P concentration could favor the PPP.

Tubulin Dimers Bind the Mitochondrial Outer Membrane VDAC

After glucose is broken down to pyruvate in the cytoplasm, subsequent metabolic processes, including the TCA cycle and oxidative phosphorylation, occur within the matrix and the inner membrane of mitochondria, respectively. These internal mitochondrial sites are inaccessible to either MTs or tubulin dimers, yet tubulin dimers can regulate these mitochondrial processes by controlling metabolite transport between cytosol and mitochondria. Transport across the outer mitochondrial membrane is mediated by the VDAC [Lemasters and Holmuhamedov, 2006; Shoshan-Barmatz et al., 2010] (Fig. 2B). Opening and closing of VDAC is controlled not only by membrane potential, but also by several cytosolic proteins, including tubulin dimers [Lemasters and Holmuhamedov, 2006; Rostovtseva and Berzrukov, 2008; Shoshan-Barmatz et al., 2010].

Higher eukaryotes express three VDAC isoforms: VDAC1, VDAC2, and VDAC3. VDAC1 is the most abundant in cells and is the best characterized of the VDAC isoforms [Messina et al., 2011; Shoshan-Barmatz and Ben-Hail, 2012]. Therefore, for the purposes of this review we will only consider VDAC1 and refer to the protein simply as VDAC.

When VDAC is associated with lipid membranes, VDAC exists in a stable, long-lived open state [Liu and Colombini, 1992]. In this open state, both metabolites and ions can travel through the channel, with anions slightly favored over cations [Lemasters and Holmuhamedov, 2006; Shoshan-Barmatz et al., 2010]. VDAC is open at low membrane potentials, but closes at either positive or negative membrane potentials (greater than $\pm$ 50 mV). In the "closed" state, the transport of anions and respiratory substrates (e.g., ATP, ADP, pyruvate) is blocked, yet VDAC remains permeable to cations such as Na^{2+}, Ca^{2+}, and K^{+} [Colombini et al., 1996; Lemasters and Holmuhamedov, 2006; Shoshan-Barmatz and Ben-Hail, 2012]. It is not yet known whether a change in potential across the outer mitochondrial membrane is a relevant physiological regulator of small molecule transport in and out of mitochondria. Binding of tubulin or HK to VDAC regulates channel open/closed states as discussed next.

Tubulin dimers bind to VDAC channels, both in artificial lipid bilayers and in isolated mitochondria [Carre et al., 2002; Rostovtseva et al., 2008]. When VDAC channels are incorporated into artificial lipid bilayers, nanomolar concentrations of tubulin cause VDAC closure at very low potentials ($\leq$10 mV) [Rostovtseva et al., 2008]. Addition of tubulin to isolated mitochondria also decreases respiration rate and raises the apparent Km for ADP by about 20-fold [Rostovtseva et al., 2008], indicating that tubulin binding to VDAC blocks metabolite exchange between cytoplasm and mitochondria, blocking ADP entry into mitochondria and ATP transport to the cytoplasm. The acidic C-terminus of tubulin is required to close VDAC and may insert into the lumen of the channel [Rostovtseva et al., 2008]. This C-terminal region of α and β tubulins is the site of many post-translational modifications, including detyrosination, polyglutamylation, and polyglycylation [Janke and Bulinksi, 2011], but whether these modifications prevent tubulin-mediated VDAC closure is not known.

Studies of artificial membranes or isolated mitochondria demonstrate that tubulin dimers can close VDAC, but is tubulin a physiological regulator of metabolite flux in and out of mitochondria within living cells? To date, this question has been addressed using a dye to measure mitochondrial inner membrane potential in cells treated with MT-targeting drugs to shift tubulin distribution into essentially all soluble dimers or all into polymer [Maldonado et al., 2010]. MT depolymerization with colchicine or nocodazole reduced mitochondrial inner membrane potential [Maldonado et al., 2010; Sheldon et al., 2011]. These data suggest that MT depolymerization and the resulting increase in free tubulin dimers will block mitochondrial respiration and deplete mitochondria of the respiratory substrates needed for ATP production. Increased soluble tubulin binding to VDAC channels should also prevent ATP from entering the cytoplasm. Conversely, treatment of cells with pacilitaxel, to drive most or all tubulins into MT polymer, caused mitochondrial inner membrane hyperpolarization [Maldonado et al., 2010], consistent with the idea that VDACs in the outer membrane remain in an open state longer, or that more channels are opened, when the soluble tubulin concentration is reduced. Curiously, this hyperpolarization was only observed in cancer derived cell lines and not in freshly isolated rat hepatocytes [Maldonado et al., 2010]. In separate studies, hyperpolarization of the inner mitochondrial membrane has been identified as an early step in apoptosis [Nagy et al., 2007]. When this hyperpolarization is prolonged, as observed in T cells isolated from systemic lupus erythematosus patients, ATP levels decline [Gergely et al., 2002]. Thus, paclitaxel treatment could reduce cytoplasmic ATP levels by opening VDAC and hyperpolarizing the mitochondrial inner membrane.

Cells may also regulate VDAC by phosphorylation and thus modify the extent of tubulin binding, and therefore VDAC open or closed states. Phosphorylation of VDAC by glycogen synthase kinase 3β or cAMP-dependent

protein kinase (PKA) increases tubulin binding to VDAC [Sheldon et al., 2011] and will therefore enhance the ability of tubulin dimers to modify transport in and out mitochondria.

HK also binds VDAC and this binding interaction is inhibited by G6P, the product generated by HK. When bound to VDAC, HK closes the channel, possibly blocking mitochondrial respiration [Lemasters and Holmuhamedov, 2006]. We assume that MTs and VDAC compete for HK binding, although this has not been tested directly. MT depolymerization should release MT-bound HK, making both tubulin (discussed above) and HK available to bind and close VDAC. MT polymerization driven by paclitaxel should reduce the pool of HK available to bind VDAC, opening more channels. The fraction of HK bound to MTs, VDAC, or free in the cytoplasm is unknown, making it difficult to know whether changes in MT polymerization state will significantly impact the amount of HK available to bind VDAC.

Hypoxia, HIF-1α, and MTs

The consideration of metabolic regulation by MTs discussed above is centered on normal environmental conditions, but MTs also play a role in metabolic regulation during hypoxia through regulation of hypoxia inducible factor (HIF)-1. Under conditions of low oxygen, cells trigger a number of responses leading to delayed cell cycle progression, production of prosurvival signals and alterations in cell metabolism to maintain ATP levels in reduced O_2. A key response to low O_2 is initiated by HIF-1, a heterodimeric complex of α and β subunits. As its name implies, HIF-1 is regulated by the presence of oxygen. In normoxia, O_2 molecules act as a cofactor for prolyl hydroxylation, which targets the HIF-1α subunit to the von Hippel-Lindau (VHL) ubiquitylation complex and subsequently to the proteasome for degradation. Under hypoxic conditions, this degradation mechanism is suppressed and HIF-1α associates with HIF-1β, enters the nucleus and activates transcription of genes containing hypoxia response elements [Jaakkola et al., 2001]. HIF-1 heterodimers induce the expression of a wide range of genes that regulate angiogenesis, erythropoiesis and life/death decisions, as well as promote a shift toward anaerobic metabolism by increasing expression of GLUTs, glycolytic enzymes and pyruvate dehydrogenase kinase (PDK). PDK prevents conversion of pyruvate to acetyl-CoA, further suppressing cellular consumption of oxygen. The activity of HIF-1 is controlled by the level of the HIF-1α subunit, which is controlled by both sequestration of its mRNA and protein degradation in the proteasome [Majmundar et al., 2010]. Interestingly, MTs are required for HIF-1α translation [Carbonarro et al., 2011], a prerequisite to activation of the HIF-1 transcription factor.

The amount of HIF-1α in the cell is regulated at the level of both proteolysis of the protein and by translation of HIF-1α mRNA. Translation of HIF-1α mRNA occurs on polysomes bound to MTs under conditions of either normoxia or hypoxia [Carbonarro et al., 2011] (Fig. 2C). Treatment of cells with MT depolymerizing drugs shifts the localization of HIF-1α mRNA from MTs to P-bodies, which are sites of mRNA storage and/or degradation present in the cytoplasm [Carbonarro et al., 2011] (Fig. 2C). This shift in HIF-1α mRNA localization from MTs to P-bodies occurs without a change in total HIF-1α mRNA level [Escuin et al., 2005]. The result of MT depolymerization is a reduction in HIF-1α protein level, even in hypoxia [Escuin et al., 2005; Carbonarro et al., 2011]. The accumulation of HIF-1α mRNA in P-bodies is reversible, demonstrated by washout of nocodazole to allow MT reassembly, which leads to reassociation of HIF-1α mRNA with MTs [Carbonarro et al., 2011]. HIF-1α mRNA binding to MTs is also disrupted by paclitaxel treatment, which also causes accumulation of HIF-1α mRNA at P-bodies and decreases the level of HIF-1α protein. It is possible that HIF-1α mRNA only binds to dynamic MTs [Carbonarro et al., 2011] or that the structure of MTs bound to paclitaxel is altered in a way that disrupts mRNA binding.

The MT requirement for HIF-1α expression suggests that MTs are necessary for transcription of those genes regulated by HIF-1 that allow cell survival in low O_2 environments. Such a relationship has been demonstrated by Teng et al. [2010] in rat cardiomyocytes. Under hypoxic conditions, MT depolymerization by colchicine treatment decreased HIF-1α protein level, expression of glycolytic enzymes, ATP content, and cell survival [Teng et al., 2010]. The response of cells to treatment with paclitaxel depended on the concentration of the drug. At concentrations <10 μM, the MT array appeared as a radial array, similar to cells in normoxia. At greater paclitaxel concentrations, MTs were present as short, aggregated bundles (see also Fig. 1B). At paclitaxel concentrations <10 μM, cardiomyocytes deprived of oxygen showed greater HIF-1α expression, increased levels of glycolytic enzymes and ATP, and greater survival compared to non-drug treated cells. At a concentration of paclitaxel (>10 μM) sufficient to disrupt MT organization, the presence of the drug did not increase cardiomyocyte survival in hypoxia [Teng et al., 2010]. It is not yet clear how to relate the paclitaxel-induced shift in HIF-1α mRNA to P-bodies [Carbonarro et al., 2011] with the doses of paclitaxel described by Teng et al. [2010]. It is possible that there is a paclitaxel concentration dependence, or MT organization requirement, to shift HIF-1α mRNA from MTs to P-bodies.

MT regulation of HIF-1α expression and downstream regulation of glycolysis may be important in conditions other than hypoxia. For example, B- and T-cell

differentiation requires increased HIF-1α levels to increase glycolytic enzyme expression [Kojima et al., 2010; Shi et al., 2011]. HIF1-α expression is also upregulated in many cancers, either due to the hypoxic environment within the tumor, or by mutations that function independent of hypoxia and result in increased HIF-α [Semenza, 2009, 2010]. For example, inactivating mutations in VHL or activating mutations in the PI3K pathway lead to increased HIF1-α expression [Semenza, 2010]. These data support a model where the MT cytoskeleton, by serving as a platform for HIF-1α translation, functions as a global regulator of cellular metabolism in many cancer cells and in several developmental processes.

Metabolic Enzymes and Regulators Also Contribute to Regulation of MT Assembly

If the partitioning of tubulins between dimer and polymer pools is important to regulation of metabolism, as outlined above, then we hypothesize that MT assembly state should be regulated by components of metabolic pathways. This feedback would allow cells to shift MT assembly levels, and in doing so, modify rates of metabolic pathways. In support of this hypothesis, several glycolytic enzymes are known to regulate MT assembly. For example, PK destabilizes MTs and impedes MT assembly [Vertessy et al., 1999; Kovacs et al., 2003]. PFK from *Dictystelium discoideum* is also an inhibitor of MT polymerization [Orosz et al., 1999]. VHL, a protein that contributes to HIF-1α degradation, is also a MT assembly regulator that stabilizes MTs without depleting the pool of tubulin dimers [Thoma et al., 2010]. Clinically identified VHL mutants show loss of different steps in regulation of MT dynamic instability [Thoma et al., 2010] and could increase or decrease the net amount of MT polymer over time. Thus, expression of some VHL mutants could lead to shifts in tubulin partitioning between dimer and polymer pools, which may then inhibit expression of glycolytic enzymes and GLUTs. Finally, glycogen, a storage form of glucose, is required for MT assembly in *Xenopus* meiotic egg extracts [Groen et al., 2011], again highlighting possible connections between metabolism and MT dynamics.

Disruption of metabolic pathways should be reflected in increased AMP and decreased ATP levels. This shift in nucleotide ratio between AMP and ATP will activate AMPK, a kinase that is responsible for sensing energy availability and serving as a master regulator of metabolic processes to maintain ATP levels. One target of AMPK is CLIP-170 [Nakano et al., 2010], a protein that binds specifically to the plus ends of growing MTs [Galjart, 2005]. Inhibition or siRNA depletion of AMPK increased CLIP-170 association with MT plus ends, decreased the rate of tubulin polymerization and resulted in greater amounts of nondynamic MTs, as marked by tubulin detyrosination [Nakano et al., 2010]. These data address the consequences of AMPK inhibition, but not AMPK activation at reduced ATP levels. Therefore, it is not known whether AMPK activation will shift the partitioning of tubulins between dimer and polymer pools. We suggest that active AMPK will only increase metabolic output if it also leaves unaffected the partitioning of tubulin between dimer and polymer pools unaffected.

Tuerk et al. [2007] suggested that reduced cytoskeletal dynamics signaled via AMPK functions to conserve energy by reducing the amount of GTP consumed by dynamic MTs. Such a scenario seems unlikely given the 1–10 mM pool of ATP normally present in a cell [Beis and Newsholme, 1975]. About half of the cell's 20 μM tubulin is in polymer form [Zhai and Borisy, 1994; Holmfeldt et al., 2009] and this 10 μM polymer turns over with a half-time of about 10 min during interphase [Saxton et al., 1984]. Therefore, ∼5 μM of tubulin dimers depolymerizes and repolymerizes every 10 min, consuming ∼5 μM of GTP at the same time. Dampening of MT dynamics by AMPK would, at best, conserve ∼30 μM of the cell's ATP pool per hour. Here, ATP is used to fuel the conversion of GDP to GTP by nucleotide diphosphate kinase, consuming 1 mole of ATP per mole of GTP produced.

Conclusions and Perspectives

The above summary demonstrates that MTs impact several steps in metabolism. Shifting the distribution of tubulins toward either all soluble dimers or all into polymers has been demonstrated to reduce output from several metabolic pathways and in other cases we hypothesize that such a decrease in metabolism will occur. Whether the normal partioning of tubulins between soluble and polymer forms (∼60–70% in polymer) favors maximal metabolic output is not known. It is possible that small shifts in partitioning between soluble and polymer pools, as occurs in response to several signals [Holmfeldt et al., 2009], could boost metabolic output, either by enhancing glucose uptake, glycolytic rate, transport across the outer mitochondrial membrane, or expression of HIF-1 inducible genes.

Much of the published work on glycolytic enzymes or mitochondrial VDAC binding to tubulin was done using purified components. An important test of tubulin/MT involvement in metabolic regulation will be to follow carbons within a cell, beginning with glucose uptake and continuing through glycolysis, the PPP and mitochondrial pathways. Such a system-wide approach would test whether drug-induced changes to MT assembly state impact the metabolic output of a cell. Within a pathway, such as glycolysis, it will be interesting to follow the localization of enzymes in living cells to see if sequentially

functioning enzymes are colocalized on MTs. It will also be interesting to measure the concentrations of glycolytic reactants and products after treatment of cells with drugs to shift tubulin into all dimers or all polymer forms. If correct, our hypothesis predicts that MT-targeted chemotherapies will be most effective when combined with metabolic inhibitors.

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