

OPINION

SUBJECT COLLECTION: CILIA AND FLAGELLA

The cilium–centrosome axis in coupling cell cycle exit and cell fate

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ABSTRACT

The centrosome is an evolutionarily conserved, ancient organelle whose role in cell division was first described over a century ago. The structure and function of the centrosome as a microtubule-organizing center, and of its extracellular extension – the primary cilium – as a sensory antenna, have since been extensively studied, but the role of the cilium–centrosome axis in cell fate is still emerging. In this Opinion piece, we view cellular quiescence and tissue homeostasis from the vantage point of the cilium–centrosome axis. We focus on a less explored role in the choice between distinct forms of mitotic arrest – reversible quiescence and terminal differentiation, which play distinct roles in tissue homeostasis. We outline evidence implicating the centrosome–basal body switch in stem cell function, including how the cilium–centrosome complex regulates reversible versus irreversible arrest in adult skeletal muscle progenitors. We then highlight exciting new findings in other quiescent cell types that suggest signal-dependent coupling of nuclear and cytoplasmic events to the centrosome–basal body switch. Finally, we propose a framework for involvement of this axis in mitotically inactive cells and identify future avenues for understanding how the cilium–centrosome axis impacts central decisions in tissue homeostasis.

KEY WORDS: Centrosome, Primary cilium, Cell fate, Quiescence, Differentiation, Muscle stem cells, Cell cycle, RNA

Introduction

Tissue homeostasis in adult vertebrates requires episodic activation of dormant progenitor to replace and repair damaged cells. Most cells in adult vertebrate tissues are mitotically inactive, with the majority comprising terminally arrested differentiated cells performing specialized tissue functions and a minor subset of reversibly arrested quiescent stem cells. Resting stem cells break quiescence in response to tissue damage, and their progeny not only regenerate lost tissue but also replenish the stem cell pool (Dhawan and Rando, 2005). In adult stem cells (ASCs), regulation of cell fate differs from that in embryonic stem cells (ESCs) partly due to differences in proliferative rates. ESCs display distinct cell cycle kinetics with minimal gap phases, and consequently these cells proliferate very rapidly (Zaveri and Dhawan, 2018). Conditions that slow ESC proliferation lead to multi-lineage differentiation (Liu et al., 2019). However, in ASCs a third cell fate, called the quiescence program (or cellular dormancy), permits reversible arrest. Although its role in ASC biology has long been recognized (Cho et al., 2019; Collins et al.,

2005; Dhawan and Rando, 2005; Schultz et al., 1978), regulation of the quiescent state is still incompletely understood.

Cell cycle exit, like progression, involves integration of both intrinsic and extrinsic cues in the signal-responsive G1 phase (Blomen and Boonstra, 2007; Coller, 2019; Pardee, 1989). Notably, cell cycle exit in ASCs is coupled to cell fate decisions, leading to either reversible (quiescence) or irreversible (differentiation, senescence) arrest (Fig. 1A), which involve distinct mechanisms (Buttitta and Edgar, 2007; Pack et al., 2019). Although cellular senescence is an important feature of aging (Goodell and Rando, 2015; Sousa-Victor et al., 2015), in this article, we will focus on the divergent fates of quiescence and differentiation, where distinct roles for the cilium–centrosome axis are emerging. Terminal differentiation is appreciated to be a metabolically active state involving the induction and maintenance of complex programs of lineage and cell type-specific functions (Ruijtenberg and van den Heuvel, 2016; Ryall, 2013). By contrast, quiescence has been considered a dormant state with reduced biosynthetic functions, marked by the repression of both proliferation and differentiation (Rumman et al., 2015). Implementation of the quiescence program leads to a poised or primed state (van Velthoven and Rando, 2019) and preconfigures the dormant cell in anticipation of a return to proliferative activity (Gala et al., 2022; Puri et al., 2015). In this article, we use the terms ‘G0 phase’ and ‘quiescence’ to refer exclusively to reversible arrest and not the irreversible arrest associated with differentiation.

The centrosome is the major microtubule-organizing center (MTOC) of the cell, comprising a pair of age-asymmetric centrioles – the mother centriole (MC) and daughter centriole (DC) – surrounded by the pericentriolar material (PCM) and centriolar satellites (CSs) (Bornens, 2002; Gould and Borisy, 1977) (Box 1). The centrosome nucleates the spindle during mitosis (M) (Fig. 1A), but during interphase (G1) it transitions into the basal body (BB; an alternative form of the MTOC), which docks to the plasma membrane and elaborates the primary cilium, a filamentous membrane extension supported by hyper-stable microtubules (Kobayashi and Dynlach, 2011; Pitaval et al., 2017; Sorokin, 1962). For more detail on centrosome structure and the centrosome–BB–cilium axis, see Box 1. While the timing of primary cilium disassembly can vary, the switch between BB and centrosome function is a prerequisite for spindle formation (Ford et al., 2018; Rieder et al., 1979), suggesting that the cilium is a barrier to mitotic progression. These mutually exclusive roles couple the cilium–centrosome cycle not only to the cell cycle (Izawa et al., 2015) but also to tumor suppressive programs (Higgins et al., 2019; Mans et al., 2008) and implicate this organelle axis in controlling cell fate (Nigg and Stearns, 2011) (Fig. 1A).

In the following sections, we summarize our understanding of how the cilium–centrosome axis contributes to cell fate decisions. Although cilium–centrosome involvement in quiescence and differentiation has been identified in different cell types, the degree of conservation across cell types is unclear. Here, we focus on skeletal muscle, where proliferating, quiescent and

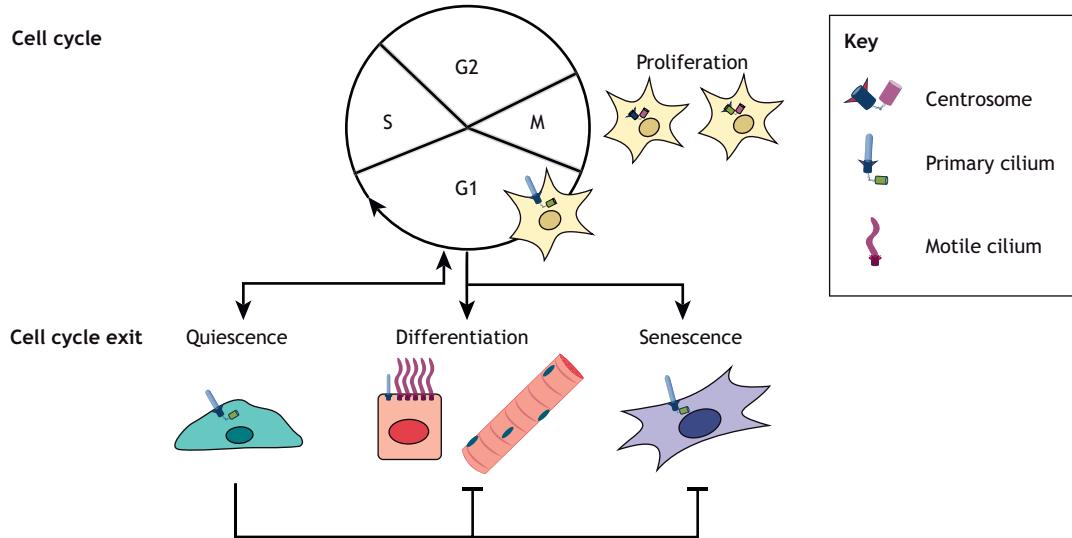
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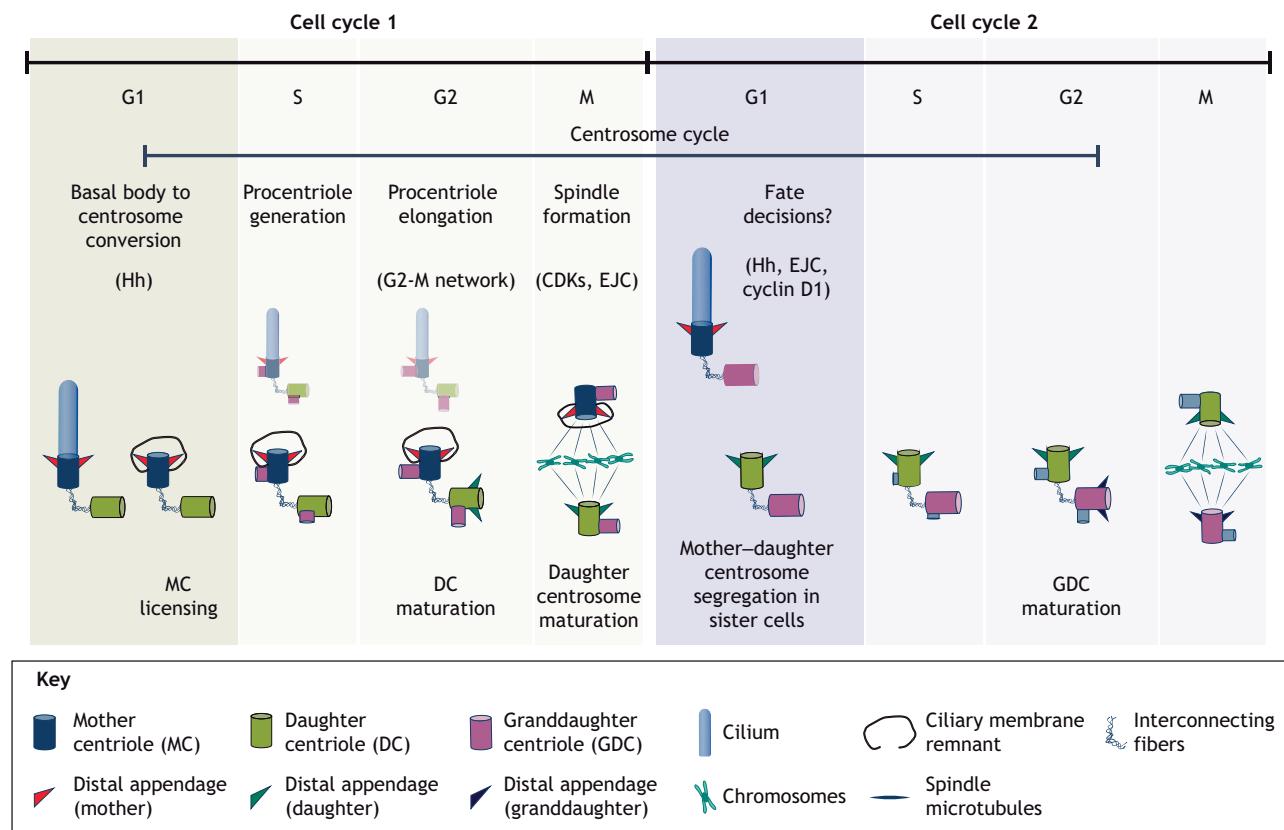


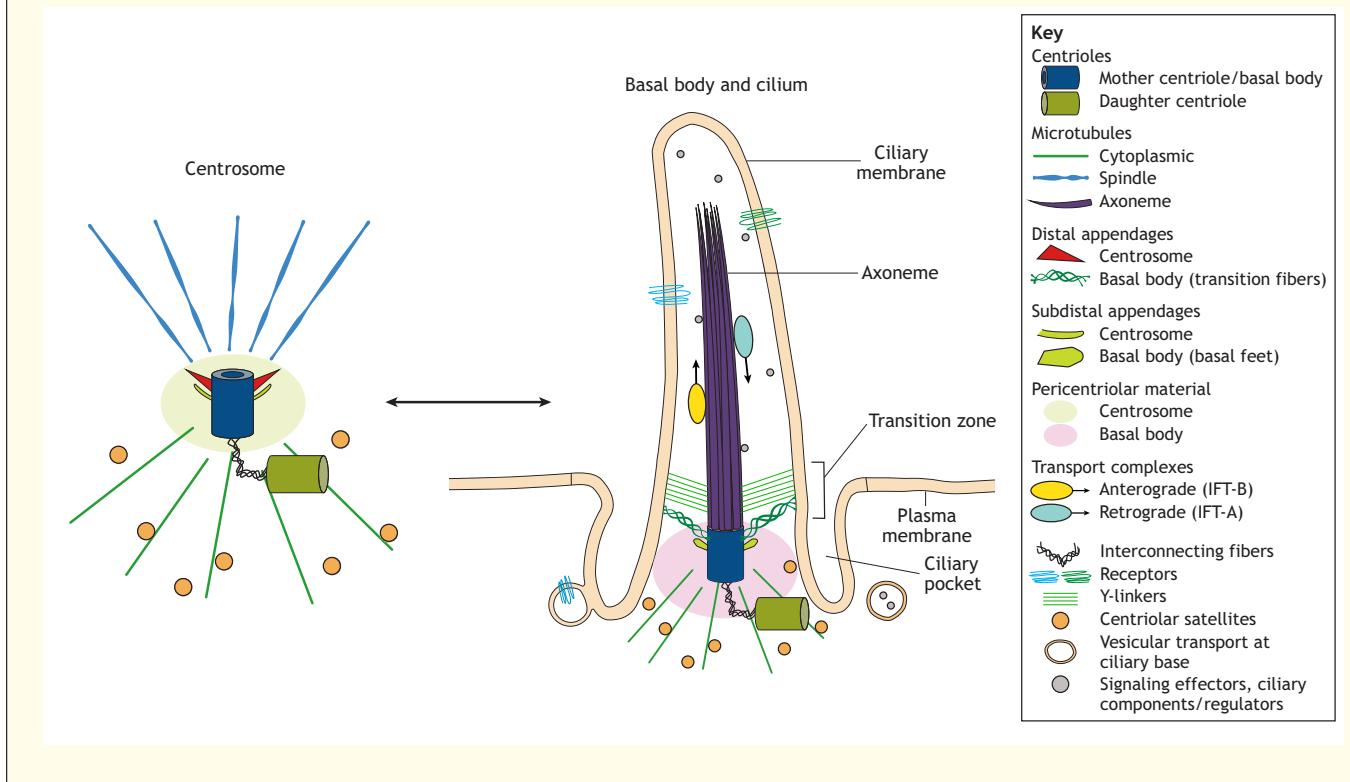
Fig. 1. Correlation between the cilium–centrosome and cell division cycles. (A) Classical view of the cell cycle, indicating the G1-phase decision point for cell fate. (B) The cilium–centrosome cycle is offset in reference to the cell division cycle. Cilium assembly occurs in G1 phase, but disassembly is cell type-dependent and can occur in S, G2 or M phases. Centrosome–BB interconversions are essential for ciliogenesis and spindle formation; cilium disassembly is a prerequisite for spindle formation. The MC and DC in a centrosome are asymmetric with respect to both age and function. The MC maintains PCM throughout the cycle and ciliary remnants at the M-G1 transition. Centriole licensing occurs in G1 phase: MC–DC disengagement and PCM expansion at the MC are essential for procentriole (granddaughter) formation and growth through S and G2 phases (duplication). In late G2 phase, DC maturation (characterized by acquisition of appendages) followed by daughter centrosome maturation (characterized by PCM expansion) facilitates bipolar spindle formation in M phase. The fate of sister cells in the subsequent cycle is linked to the asymmetric inheritance of the mother centrosome; the rate of cilium assembly (which is faster with ciliary remnants); and localization of mRNP complexes, the spliceosome, the EJC and translation factors. Carry-over of signaling effectors (Hh pathway) and cell cycle regulators (CDK2, CDK1 and cyclin D1 levels), and negative regulation of G2-M components by the cilium might influence fate decisions. Thus, some cell fate decisions are initiated in the previous cell cycle and are associated with the composition and nature of the inherited centrosome.

Box 1. The centrosome–basal body–cilium axis

The centrosome is a microtubule-based membraneless organelle composed of an MC and DC that differ structurally and functionally, with only the MC harboring appendages and PCM (Piel et al., 2000). Centrosomal composition, structure and function are dynamic and are regulated by the PCM and CSs. The PCM is a multi-functional docking platform consisting of proteins and RNA that is involved in centriole duplication, microtubule nucleation, spindle organization and signaling (Mennella et al., 2014; Woodruff et al., 2014). The CSs are membraneless granules orbiting the centrosome in a microtubule-dependent manner. They recruit, deliver and sequester cilium–centrosome proteins, and are crucial for duplication, ciliogenesis and PCM organization (Hori and Toda, 2016; Kubo et al., 1999).

Assembly of the primary cilium from the BB during interphase involves the intraflagellar transport complexes (IFT-A and IFT-B), which ferry cargo into and out of the shaft (axoneme) via microtubule-based motors (kinesin and dynein), with vesicular trafficking and the cytoskeleton supporting cilium extension and function (Hsiao et al., 2012; Ishikawa and Marshall, 2017; Malicki and Johnson, 2017; Mirvis et al., 2018).

The primary cilium–BB complex is a signaling center (Satir et al., 2010), not only because it facilitates extensive crosstalk in its small volume but also because it can maintain an environment different from that in the cytoplasm. The specialized ciliary membrane differs in composition from the bulk plasma membrane and is enriched in signaling receptors, with the ciliary pocket forming an important site for vesicular transport, and the transition zone serving as a diffusion barrier between the ciliary lumen and cytoplasm (Nachury and Mick, 2019). The composition, assembly–disassembly and reorganization of the cilium–centrosome in different cell types has been extensively reviewed (Avasthi and Marshall, 2012; Gönczy, 2012; Muroyama and Lechner, 2017; Vertii et al., 2016; Wang and Dynlach, 2018).



differentiated cells show clear distinctions in the status of the cilium–centrosome axis (Marican et al., 2016; Venugopal et al., 2020). We discuss how this axis controls cell cycle phases as well as signaling memory mechanisms across cell generations, in proliferating and quiescent states, but undergoes reorganization during differentiation.

Finally, we explore newly described mechanisms of RNA localization at the centrosome and/or BB (C/BB) that regulate cilium–centrosome functions. In light of these findings, we hypothesize links between C/BB-localized mRNAs, translation and the proliferation–quiescence switch.

Stem cell quiescence and cell fate decisions

Cell fate decisions are enforced during both transitions and maintenance of cell state. Induction of quiescence involves programmed alterations in chromatin organization, RNA biogenesis, RNA turnover, translation, metabolism and signaling

(Cheung and Rando, 2013; Marescal and Cheeseman, 2020). Whereas entry into and maintenance of differentiation involves active suppression of proliferation (Allen and Boxhorn, 1989; Olson, 1992; Rumman et al., 2015), reversible quiescence requires balanced suppression not only of proliferation, but also of alternative non-dividing programs (Fig. 1A) (Ancel et al., 2021; Bjornson et al., 2012; Mourikis et al., 2012; Sousa-Victor et al., 2015; Subramaniam et al., 2013). Irreversible arrest is coupled to induction of tissue-specific genes (Ruijtenberg and van den Heuvel, 2016), whereas reversible arrest involves suppression of lineage markers and activation of memory mechanisms to ensure their re-expression during cell cycle re-entry (Cheedipudi et al., 2015; Rumman et al., 2015; Sebastian et al., 2009). Quiescent cells also induce autophagy to optimize survival (Guan et al., 2013; Ho et al., 2017), repair pathways for genome maintenance and recycling metabolic pathways to conserve energy (Blanpain et al., 2011; Chandel et al., 2016; Esteves de Lima et al., 2021; Giordani and

Puri, 2013). Stem cell properties such as self-renewal are enhanced during quiescence *in vivo* (Ancel et al., 2021; Collins et al., 2005), and induction of quiescence in purified ASCs *ex vivo* promotes more efficient engraftment upon transplantation (Arjona et al., 2022; Asakura et al., 2007; Charville et al., 2015; Sacco et al., 2008; Shea et al., 2010; Zhang et al., 2015). Thus, reversible arrest is associated with enhanced cellular regenerative mechanisms.

Decisions during cell cycle exit involve integration of inputs from intrinsic and extrinsic pathways. Diverse extrinsic signals can trigger entry into reversible arrest, such as amino acid or nutrient starvation, mitogen limitation, loss of adhesion and mechanochemical cues (Cho et al., 2019; Coller et al., 2006; Dhawan and Laxman, 2015). Maintenance of quiescence requires a balance that opposes continuous activation signals (pro-proliferation and pro-differentiation) from the niche. Specific signals can contribute to quiescence in a cell type-dependent manner; for example, Hedgehog (Hh) signaling is suppressed in muscle stem cells (MuSCs) (Brun et al., 2022; Venugopal et al., 2020) but is required in lung mesenchymal cells (Peng et al., 2015). Similarly, activated Notch signaling maintains quiescence and suppresses differentiation in MuSCs (Bjornson et al., 2012) but promotes differentiation in keratinocytes (Ezratty et al., 2011). Whereas signaling is generally mediated by receptors on the plasma membrane, several types of receptors are enriched in the membrane encasing the non-motile primary cilium, highlighting its role as a sensory antenna (Anvarian et al., 2019) (Box 1).

Linking the cilium–centrosome axis and cell cycle to cell fate decisions – spindle versus cilium

The cilium–centrosome axis is involved in multiple processes, including cell division, migration and signaling (Arquint et al., 2014; Joukov and De Nicolo, 2019). Centriole duplication and maturation take 1 and 1.5 cell cycles, respectively, leading to the inheritance of centrosomes of different ages by sister cells derived from a single mitosis (Nigg and Holland, 2018) (Fig. 1B). Centriole and centrosome asymmetry influences MTOC functions; whereas age asymmetry is maintained through cell generations, functional asymmetry caters to spindle pole versus ciliation activities and changes in a cell cycle-dependent manner (Box 2; Fig. 1B).

Spatially, polarity establishment by the cilium–centrosome axis is evolutionarily conserved and contributes significantly to cell fate (Chen and Yamashita, 2021) (Box 2). Temporally, the dynamics of this axis are tightly coordinated with the cell cycle, with several intriguing features that have implications for cell fate (Box 2; Fig. 1A). The centrosome plays mutually exclusive roles in organizing a spindle during G2-M transition versus a BB elaborating a cilium in G1 phase (Box 2; Fig. 1B). Continuously proliferating cells transiently extend a cilium in G1 phase, which may persist as late as prophase but is lost before metaphase (Ford et al., 2018; Mytilis et al., 2022; Rieder et al., 1979; Tucker et al., 1979b). Cells that withdraw into G0 phase maintain a more stable cilium (Ho and Tucker, 1989; Tucker et al., 1979a). Ciliogenesis starts with MC to BB morphogenesis and involves recruitment of structural and regulatory proteins (Čajánek and Nigg, 2014; Graser et al., 2007; Mönnich et al., 2018; Tsang and Dynlacht, 2013). DC proteins regulate centriole duplication in proliferating cells (Mahjoub et al., 2010; Zou et al., 2005), but in quiescent and differentiated cells they promote ciliogenesis from the MC (Betleja et al., 2018; Gottardo et al., 2015; Ogungbenro et al., 2018). Thus, localization and asymmetric distribution of centriole proteins influences cell fate in an organism-, cell type- and cell state-dependent manner. Furthermore, after cytokinesis, the sister cell

Box 2. Asymmetric features of the cilium–centrosome axis

Centriole duplication and ciliation

The cilium–centrosome cycle is out-of-phase with, but closely follows, the cell cycle (Doxsey et al., 2005; Seeley and Nachury, 2010). In proliferating cells, the centrosome is as precisely duplicated as the genome (only once per cell cycle), using mechanisms that suggest coordinated regulation of the DNA replication and centrosome cycles (Knockleby and Lee, 2010; Nigg and Holland, 2018) (Fig. 1B). Duplication begins with generation of procentrioles (granddaughters) from the parental MC–DC centriole pair in late G1 and S phase, and is completed upon maturation and separation of mother and daughter centrosomes in late G2 phase and mitosis (Wang et al., 2011) (Fig. 1). Ciliogenesis is coordinated with centrosome duplication, with cilium assembly in G1 phase and disassembly between G1-S and G2-M phases depending on cell type (Ford et al., 2018; Rieder et al., 1979) (Fig. 1B).

Asymmetry of centrioles and centrosomes

Centrioles and centrosomes are asymmetric both by age and function (Chen and Yamashita, 2021; Piel et al., 2000; Reina and Gonzalez, 2014), regulating spindle and ciliation activities differently. Centrosome asymmetry functions within one cell cycle to influence the position, orientation, composition and microtubule nucleation of spindle poles (Gasic et al., 2015; Rusan and Peifer, 2007; Tang and Marshall, 2012). Asymmetry associated with ciliation spans cell generations, arising between the centrioles within each cell (only the MC elaborates a cilium) and between the centrosomes of sister cells (the older centrosome ciliates faster) (Anderson and Stearns, 2009) (Fig. 1B).

Polarity

The cilium–centrosome axis creates a polarity that nucleates signaling and structural asymmetries, leading to unequal segregation of lineage determinants between sister cells (Chen and Yamashita, 2021). Centrosome geometry and the cellular orientation of the MC–DC pair confer an inherent capacity for polarizing intracellular signals (Albrecht-Buehler and Bushnell, 1980; Regolini, 2019). This axis responds to external and internal factors, linking polarity factors and localization of fate determinants to the organization and function of the MTOC (Feldman and Priess, 2012; Higginbotham and Gleeson, 2007; Tang and Marshall, 2012). Control of spindle orientation during proliferation and reorganization of the MTOC during differentiation (Muroyama and Lechner, 2017; Tang and Marshall, 2012) appear to be evolutionarily conserved features that contribute to cell fate.

with the older MC undergoes ciliation more rapidly (Anderson and Stearns, 2009) (Fig. 1B), creating an intrinsic temporal heterogeneity in an otherwise homogeneous population, thereby impacting on the propensity of individual cells to proliferate and/or differentiate.

Thus, oscillation of the MTOC between BB and centrosome functions could be a defining property of the G1 phase that could serve as raw material for fate specification due to differential regulation of unique components. The asymmetry of centrioles and centrosomes, the association with polarity factors, and the centrosomal switch to form a BB and cilium are features that function across cell generations (Box 2; Fig. 1B). Importantly, the transient presence of the primary cilium during the switch in G1 phase suggests a fresh perspective from which to interrogate cell fate decisions. In this article, we focus on features of the cilium–centrosome axis in G1 phase, highlighting the decision between quiescence and differentiation that occurs prior to mitosis (Blomen and Boonstra, 2007), and we assess the evidence for a role of the cilium–centrosome axis in this divergence.

Expanding roles of the primary cilium in cell fate decisions

The primary cilium has been implicated in multiple signaling pathways that are important in development, adult tissue homeostasis

and disease, including the Hh (Ocbina and Anderson, 2008), Wnt (Wallingford and Mitchell, 2011), Notch (Ezratty et al., 2011), transforming growth factor (TGF) (Clement et al., 2013), platelet-derived growth factor (PDGF) (Schneider et al., 2005), insulin/insulin-like growth factor (IGF) (Zhu et al., 2009) and mammalian target of rapamycin (mTOR) (Boehlke et al., 2010) pathways, and these signaling roles of cilia have been reviewed in detail previously (Anvarian et al., 2019; Malicki and Johnson, 2017; Nachury, 2014; Satir et al., 2010; Wheway et al., 2018). These pathways coordinate with ciliary proteins to regulate cell cycle progression (Izawa et al., 2015). The signaling functions, prevention of inappropriate spindle assembly and regulation of centriole copy number were first thought to support a tumor suppressive role for the cilium (Mans et al., 2008) (Fig. 1). However, in some instances oncogenes may promote tumor formation via the cilium (Liu et al., 2018). Thus, proliferation control by the cilium–centrosome axis is context dependent, likely contributing to the variation seen across cell types.

In proliferating cells, transient ciliation in G1 phase might be associated with the restriction of mitogen receptivity to this phase (Tucker et al., 1979b). Of the major pathways, ciliary Hh signaling has been best delineated (Bangs and Anderson, 2017). Recent evidence suggests the existence of mitogenic memory between successive generations of continuously cycling cells, converging on cyclin-dependent kinase 2 (CDK2) and cyclin D1 (CCND1) levels (Spencer et al., 2013; Yang et al., 2017). Consistent with persistence of signal exposure between cell generations, ciliary Hh signaling has been shown to instruct mitogenic activation through signals received in the previous cell cycle (Ho et al., 2020), supporting the idea that signal-dependent cues can be inherited, extended or delayed in execution (Fig. 1B). While the mechanisms underlying signal information transfer from one cell generation to the next are unknown, it is tempting to speculate that the asymmetry and persistence of centrosomal proteins from one cell cycle to the next might play a role, with implications for mitogenic memory and fate determination. Interestingly, asymmetric segregation of ciliary membrane together with the MC into one sister cell promotes faster ciliation, increasing the propensity of that cell to differentiate or enter quiescence (self-renew) (Anderson and Stearns, 2009; Paridaen et al., 2013; Piotrowska-Nitsche and Caspary, 2012; Viol et al., 2020), indicating that ciliary remnants might also impact fate choice (Fig. 1B). Therefore, the differential retention of cilium and BB components might participate in distinguishing reversible from irreversible arrest.

Ciliary regulation of cell cycle progression is often studied by perturbation of ciliary assembly factors. In proliferating non-ciliated cells, knockdown of intraflagellar transport 88 (IFT88) has been used to show centrosomal association and cilia-independent control of the G1-S transition (Robert et al., 2007). However, in ciliated medulloblastoma cells, IFT88 knockdown has revealed a direct role of the cilium in regulating the length of each phase and the overall cell cycle (Youn et al., 2022), strengthening support for its role as a cell cycle checkpoint. Furthermore, compromising cilium extension in quiescent myoblasts derepresses a G2-M transcriptional network (Venugopal et al., 2020) by unknown mechanisms, and in medulloblastoma cells, the G2-M transition is negatively regulated by the cilium through control of CDK1 phosphorylation (Youn et al., 2022). Taken together, these findings suggest that signaling functions of the centrosome in the G2-M transition are inhibited by centrosome conversion to a BB (Fig. 1B). Since differentiation does not involve a return to the cell cycle, this regulatory role might be restricted to the proliferation–quiescence switch. However, whether modulation of

G2-M features by the centrosome–BB switch is universal remains to be established.

Cilium–centrosome dynamics in quiescence versus differentiation – a focus on skeletal muscle

Centrosomal MTOC activity changes depending on cell state in a cell type-specific manner. As outlined above, whereas quiescent ASCs exhibit stable ciliation until stimulated to re-enter the cell cycle, differentiated cells can harbor either transient or permanent primary cilia based on their cell type and context. For example, epithelial cells permanently exhibit both primary and motile cilia (Veland et al., 2009), whereas cardiac and skeletal myocytes transiently extend a primary cilium only during early differentiation (Clement et al., 2009; Fu et al., 2014; Koefoed et al., 2014; Rash et al., 1969; Venugopal et al., 2020) (Box 3; Fig. 2). During skeletal muscle regeneration, MuSCs transition between quiescence, proliferation and differentiation (Dhawan and Rando, 2005; Zammit, 2017), making this tissue an excellent model to analyze the spectrum of centrosomal and ciliary dynamics associated with these states (Box 3; Fig. 2).

Upon activation *in vivo*, quiescent MuSCs lose their primary cilium; only a small subset of activated MuSCs reassemble cilia and are capable of self-renewal, while the majority proliferate and differentiate (Marican et al., 2016). These observations suggest that the cilium functions to restrain both cell cycle re-entry and commitment to terminal differentiation (Fig. 2). In a culture model of MuSC quiescence and activation, cilia are observed to assemble during entry into G0 phase, rapidly disassemble during G0-G1 activation and re-extend in a subset of cells later in G1 phase. Blocking cilium extension by compromising intraflagellar transport leads to loss of hallmarks of quiescence, inducing both proliferative and myogenic transcriptional signatures, supporting the view that the cilium inhibits both these programs in G0 phase (Venugopal et al., 2020).

Interestingly, during differentiation, triggered myoblasts transiently assemble primary cilia, likely reflecting the coordination of commitment to differentiation with transit through G1 phase. However, cilia are disassembled prior to fusion of myoblasts into differentiated multi-nucleated myotubes, which is consistent with the finding that suppression of ciliation leads to upregulation of the myogenic program (Fu et al., 2014; Venugopal et al., 2020) (Fig. 2). Additionally, Hh signaling via the cilium is essential for the expression of muscle-specific transcription factors that drive myogenesis; aberrations in cilia assembly and Hh transduction prevent myotube formation and are implicated in rhabdomyosarcomas (skeletal muscle tumors) (Fu et al., 2014) and in aging (Palla et al., 2022). Taken together, these studies suggest that the cilium may function to fine-tune the decision between cell cycle progression and exit into either differentiation or quiescence (Fig. 2). However, the timing of ciliary assembly and retraction relative to differentiation is still unclear. In this context, the early distinction between transient and stable ciliation that accompanies the bifurcation of myogenic versus stem cell fate choices might be an important control point. Coupling of this MTOC-based function with transcriptional control by lineage-specific factors (discussed below) could provide a means of coordinating cellular cytoskeletal reorganization with cell cycle exit.

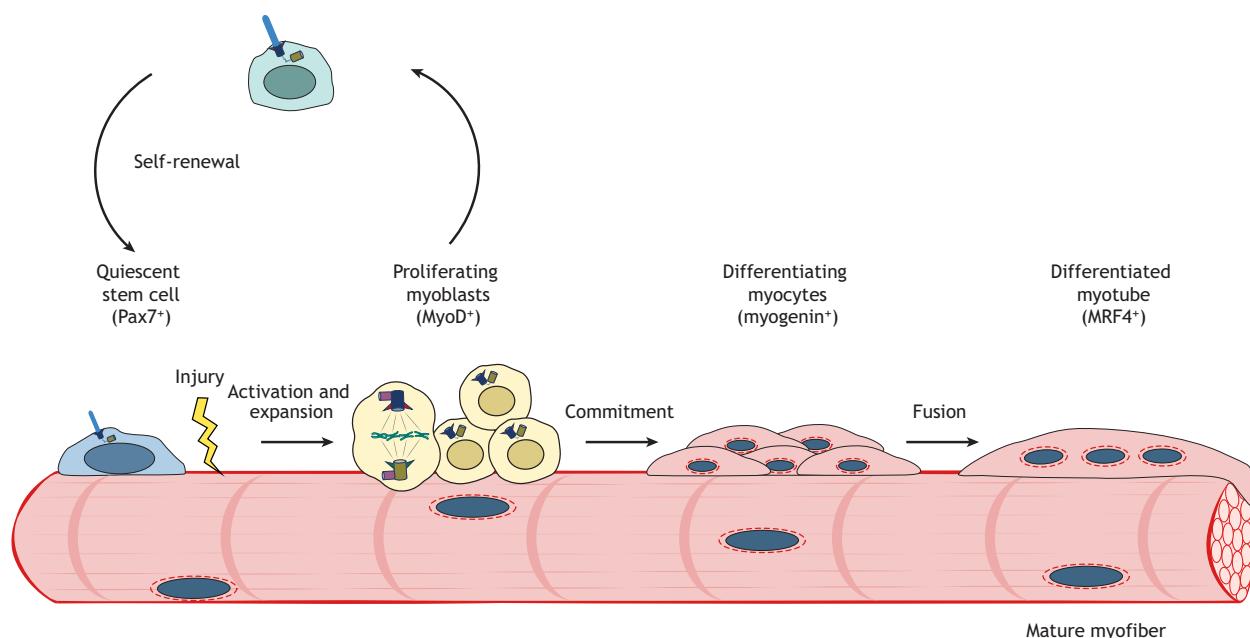
Centrosome reorganization in differentiation

MTOC function in differentiated cells is cell type dependent. Most postmitotic cells, including multi-ciliated epithelia, shift MTOC activity from centrosomal to non-centrosomal sites (Sanchez and

Box 3. Skeletal muscle and the cilium–centrosome axis

Skeletal muscle regeneration and homeostasis depend on the persistence of dormant MuSCs or satellite cells in adult tissue. In late embryonic development, MuSCs are distinguished by expression of the transcription factor Pax7 and take up residence in a niche between the muscle fiber membrane and basal lamina (Buckingham, 2007; Collins and Partridge, 2005; Mauro, 1961). Muscle determination and terminal differentiation involve hierarchical expression of the tissue-specific transcription factors MyoD, Myf5, myogenin and MRF4 (also known as MYF6), which form the core myogenic program. In adult muscle, Myf5 is expressed in quiescent Pax7⁺ MuSCs, but MyoD expression is induced during activation and oscillates during the cell cycle, marking phases for differentiation competence (Zammit, 2017). In response to extrinsic cues, MyoD induces expression of myogenin and cell cycle inhibitors, coupling arrest, fusion and differentiation. MRF4 is expressed in myonuclei only after fusion, sustaining the myogenic program initiated by MyoD and myogenin (Zammit, 2017). The multi-nucleated myofibers assemble a specialized muscle contractile cytoskeleton, and when mature, recreate the niche for the minor population of activated MuSCs that undergo self-renewal by re-entering quiescence, retaining competence for future repair. Thus, MuSCs regenerate both differentiated myofibers and stem cell compartments (Grounds, 1991).

Quiescent MuSCs harbor a primary cilium that is disassembled upon activation. During regeneration, cilium–centrosome dynamics differ in the two populations of activated MuSCs, coinciding with their divergent fates. The minor self-renewing subpopulation reassembles a stable cilium to enter quiescence. However, the major population transiently extends the cilium and commits to differentiation, along with altered MTOC organization. Centrosome reduction or inactivation (Muroyama and Lechner, 2017) results in handover of MTOC activity to non-centrosomal components at the nuclear periphery. During this process, centrosomal components and the microtubule cytoskeleton are reorganized from a polar–radial arrangement to a circumnuclear–parallel arrangement, which is essential for patterning the contractile actomyosin sarcomeric cytoskeleton (see Fig. 2). Thus, multi-nucleated muscle cells lose the ability to reassemble the centrosome, which might reinforce terminal differentiation by permanent loss of the capacity for spindle formation. The distinct spatial and temporal organization of the MTOC and primary cilium in quiescent versus differentiated muscle cells supports a role in cell fate decisions.



Feldman, 2017). However, in skeletal and cardiac muscle, early differentiation is accompanied by transient ciliation (Koefoed et al., 2014; Ng et al., 2021), the role of which is unclear. In skeletal muscle myotubes, MTOC reorganization (Box 3; Fig. 2) is critical for migration and alignment of nuclei, and formation of neuromuscular junctions (Tassin et al., 1985; Zaal et al., 2011). This involves the reorganization and relocation of the centrosome, Golgi and endoplasmic reticulum exit sites (Bugnard et al., 2005; Zaal et al., 2011). The muscle-specific transcription factors MyoD (also known as MYOD1) and myogenin control the expression of nuclear membrane proteins [nesprin 1 α (encoded by SYNE1) and A-kinase anchor protein 6 (AKAP6), respectively], which together with PCM and CS proteins nucleate a non-centrosomal MTOC at the nuclear envelope (Becker et al., 2021; Espigat-Georger et al., 2016; Gimpel et al., 2017; Srivastava et al., 2009; Steinfeldt et al., 2021) (Fig. 2). Similarly, in cardiac muscle, centrosome

reorganization promotes differentiation (Zebrowski et al., 2015). By contrast, in epithelia, reorganization of centrosomal components is initiated upon cell cycle exit independently of differentiation signaling pathways (Muroyama et al., 2016). Therefore, evidence for coordination of programs of terminal arrest with the loss of centrosomal MTOC functions is currently restricted to skeletal muscle. Nonetheless, the dispersal of centrosomal proteins in many differentiated cells has two implications: spindle functions of the centrosome critical to mitosis are abrogated, and extension of the cilium and reception of proliferative signals through this axis is also curtailed. Thus, centrosome reorganization supports and reinforces the terminal arrest.

Despite these recent advances, there are many unanswered questions about how the cilium–centrosome axis might influence the decision to either proliferate, enter quiescence or differentiate. The available evidence suggests that although quiescent cells

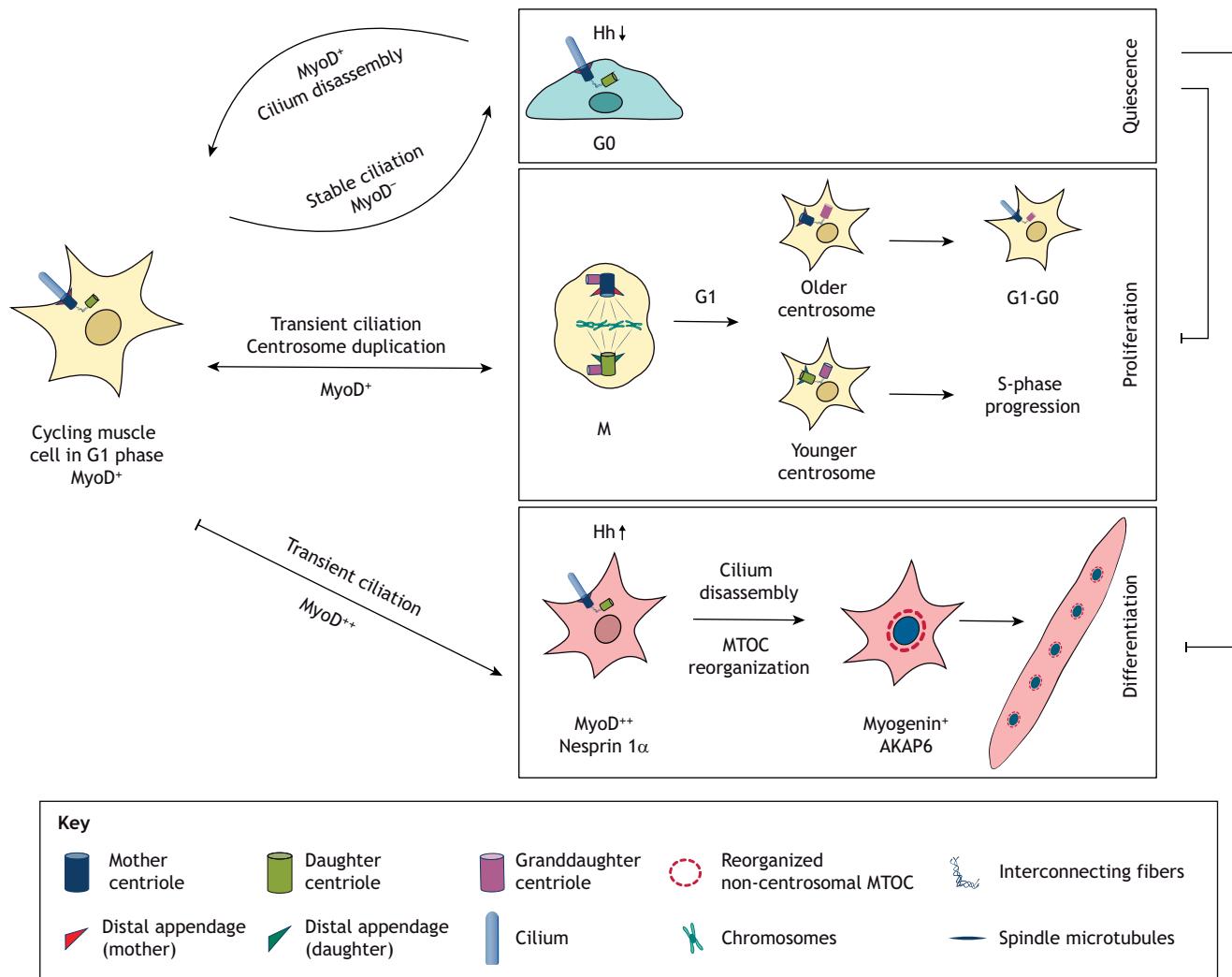


Fig. 2. The cilium–centrosome axis in skeletal muscle cell state transitions. Cycling muscle cells transiently ciliate in G1 phase, express the muscle determinant MyoD and duplicate their centrosomes. The sister cell receiving the older centrosome ciliates faster and can either exit the cell cycle or disassemble the cilium and continue to proliferate. Cells that assemble a stable cilium enter reversible quiescence and actively suppress expression of MyoD and myogenin, as well as terminal differentiation. By contrast, cells that sustain MyoD expression when they exit the cell cycle induce expression of myogenin and disassemble their cilium, thereby entering the muscle differentiation program. Here, synchronization and crosstalk between the core myogenic and centrosomal programs involve transcriptional control of nesprin 1 α by MyoD and of AKAP6 by myogenin. Concurrently, reorganization of the centrosome and loss of its MTOC function supports fusion, multi-nucleation and irreversible arrest of differentiating cells. Hh signaling is repressed in quiescence but is transiently active during early differentiation, indicating differential behavior between the two forms of arrest.

maintain a cilium that counteracts cell cycle progression and may transduce inhibitory signals, the small volume of the cilium and selectivity of the transition zone (Box 1) can support the selective enrichment of growth factor receptors in the ciliary membrane, which might facilitate poised mitogen responsiveness during the G0-G1 transition, but with a temporal delay. The transient presence of the cilium during G1 phase might influence the decision to exit G1 and differentiate rather than continue to the G1-S transition and proliferate, possibly depending on the extracellular signaling environment (Fig. 1A). Recent evidence suggesting that the cilium mediates signaling memory (Ho et al., 2020) warrants deeper investigation to decipher the role of this axis in G1 phase. The composition of the cilium and centrosome during stable versus transient ciliation is still incompletely documented. Below, we review recent discoveries of specific RNAs localizing to the centrosome that might shed new light.

Quiescence or differentiation – possible mechanisms of decisions involving the cilium–centrosome axis

The composition of the cilium–centrosome complex is dynamic (Doxsey et al., 2005; Nachury and Mick, 2019) and may well be central to distinctions between cell states. Although hundreds of proteins have been found to participate in forming this signaling axis (Arslanhan et al., 2020), accumulating evidence suggests a role not only for mRNA and regulation of localized translation at the centrosome (Chichinadze et al., 2012; Doxsey et al., 2005; O'Neill et al., 2022), but also for non-coding RNAs with diverse regulatory functions (Ito et al., 2020). Nuclear–centrosomal shuttling of proteins (Johnson and Malicki, 2019) couples their functions in both interphase and mitosis. While existing evidence suggests active roles for centrosome-localizing RNAs and RNA-binding proteins (RBPs) in the regulation of proliferation, we discuss three associated mechanisms that might contribute to fate decisions.

mTOR signaling

mTOR is the central nutrition, energy and stress sensor that integrates protein and lipid synthesis, autophagy and metabolism with the cell cycle (Laplante and Sabatini, 2012). mTOR is a key regulator of translation via two major downstream pathways: S6 kinase (S6K) pathway, which controls global translation, and 4E-binding protein (4E-BP) pathway, which specifically represses cap-dependent translation (Laplante and Sabatini, 2012). Global translation levels vary between cell states in a cell type-dependent manner. mTOR signaling regulates synthesis of ciliary components (Boehlke et al., 2010; Lai and Jiang, 2020) and is evolutionarily conserved (Yuan et al., 2012). In skeletal muscle, mTOR complex 1–S6K signaling regulates activation of quiescent MuSCs (Rodgers et al., 2014), whereas during differentiation, activity of 4E-BP1 (also known as EIF4E-BP1) is suppressed, facilitating myoblast fusion (Pollard et al., 2014). Presence of the cilium in quiescent myoblasts correlates with low mTOR activity, and compromising ciliation differentially affects the S6K and 4E-BP signaling pathway arms (Venugopal et al., 2020). Below, we summarize emerging evidence for functional associations of the translational machinery at the C/BB, suggesting an important role for localized protein synthesis, which might be cell state specific.

mRNA localization and translation

Localization of mRNA constitutes an efficient mechanism for spatial control of organelle proteomes and cellular functions (Buxbaum et al., 2015; Lécuyer et al., 2007; Ryder and Lerit, 2018). The C/BB has been known to harbor RNA molecules (Alliegro, 2011; Hoffman, 1965; Lerit, 2022; Marshall and Rosenbaum, 2000; Rieder, 1979) transported via the cytoskeleton (Das et al., 2021), but the identity and functions of these RNAs are not fully known. Although more evidence exists for the localization of mRNAs (Blower et al., 2007; Lambert and Nagy, 2002; Lécuyer et al., 2007; Zein-Sabatto and Lerit, 2021), RBPs, ribosomal proteins and translation factors (Müller et al., 2010; O'Neill et al., 2022) at the centrosome, proteomic surveys have revealed that the cilium–BB is also enriched for mRNAs and the protein synthesis machinery (Fingerhut and Yamashita, 2020; Hao et al., 2021; Jao et al., 2017; Kwon et al., 2021). Localization of mRNA to the C/BB depends both on structural features (such as untranslated regions, secondary structure and internal motifs) (Bergalet et al., 2020) as well as messenger ribonucleoprotein (mRNP) granule components, the dynamics and transport of which are microtubule dependent (Aizer et al., 2008). Together, they facilitate centrosome function (Ryder et al., 2020) and cilium formation (Moser et al., 2011).

Conversely, centrosomal proteins directly impact translation of specific mRNAs at the C/BB by controlling RBP-based mRNA localization and polysome-dependent recruitment through interactions with the core translational machinery (Iaconis et al., 2017). Recent studies have identified mechanisms that might facilitate cell-state specific functions of the cilium–centrosome axis (Fig. 3). High-throughput single-molecule fluorescence *in situ* hybridization (smFISH) screens in proliferating cells have revealed transcripts encoding centrosome components with spindle pole functions, further supporting C/BB-localized translation (Chouaib et al., 2020). Live tracking of polysomes containing centrosomal transcripts has indicated that mRNA localization can depend on the encoded nascent protein (Safieddine et al., 2021; Sepulveda et al., 2018), linking translation stalling with polysome transport to mitotic centrosomes. (Fig. 3). RBPs can also target mRNAs encoding proteins essential for ciliary assembly and function (Avolio et al., 2018), indicating that ciliogenesis might require

temporally controlled translation and delivery of components. Although these observations were made in proliferating cells, it is possible that specific localization assists the stable ciliation seen in quiescent cells.

Components of mRNA biogenesis and trafficking [including the spliceosome, pre-mRNA-processing factors (PRPFs), the exon junction complex (EJC) and the transcription-and-export complex (TREX)] not only enable mRNA surveillance mechanisms (Le Hir et al., 2016) but also localize to the C/BB (Busselez et al., 2023) to regulate cilium–centrosome function and coordinate fate choice (Haward et al., 2021; Michlewski et al., 2008; O'Neill et al., 2022; Stemm-Wolf et al., 2021). PRPFs also localize to the C/BB (Wheway et al., 2015) and differentially regulate C/BB composition in neural stem cells (NSCs) and neurons via localization of target mRNAs (O'Neill et al., 2022). Taken together, these findings suggest new mechanisms for regulation of C/BB composition and activity via differential localization of RNA. EJC components impact NSC numbers and neuronal differentiation via regulation of centrosome and spindle integrity (Silver et al., 2010), and also mediate the association of specific transcripts to the C/BB in quiescent mouse NSCs and RPE1 cells (Kwon et al., 2021). This EJC-dependent transcript enrichment is not seen in differentiated (ependymal) or proliferating (RPE1) cells, indicating cell state-specific effects, and suppression of EJC expression in G0 phase impairs cilium–centrosome organization (Kwon et al., 2021) (Fig. 3). Given that skeletal and cardiac muscle differentiation is accompanied by transient ciliation and subsequent reorganization of key centrosomal components, it would be interesting to investigate whether EJC-dependent mechanisms play a role in these cell types as well.

A recent study has reported that primary cilia regulate the RBP-dependent localized translation of β-catenin mRNA (*CTNNB1*) by preventing its sequestration into stress granules in medulloblastoma cells, facilitating tumorigenic growth (Youn et al., 2022) (Fig. 3). While the mechanisms of cilia-based control of localized translation need further investigation, one attractive hypothesis is that RBPs, the translation machinery and mRNP granule components are differentially used to regulate specific mRNA localization and translation in a cell state-dependent manner. Thus, the emerging focus on localized translation suggests tantalizing possibilities for the differences between centrosome and BB to serve as a substrate for fate determination.

Turnover of cilium–centrosome components

The turnover and trafficking of cilium–centrosome components are regulated by the ubiquitin–proteasomal system (UPS) and autophagy in a cell cycle-dependent manner (Boukhalfa et al., 2019; Goldsmith et al., 2020; Malicki and Johnson, 2017). Autophagy, a cellular degradation system that is important for regulation of cell state (Chang, 2020; Jang et al., 2016), also regulates centrosome integrity and protein homeostasis in a context- and cell type-dependent manner. For example, ciliary regulation of mTOR and Ih pathways feeds into the autophagic machinery, which in turn regulates centrosomal protein degradation and localization, affecting cilium assembly and length (Boukhalfa et al., 2019). Autophagy can therefore regulate selective mRNA translation and contribute to cell cycle progression via mechanisms involving sequestration or degradation of RBPs (Fig. 3). For example, oral-facial-digital syndrome type 1 protein (OFD1), a centriole and CS protein, undergoes autophagic degradation at CSs to promote ciliogenesis from the BB (Tang et al., 2013). OFD1 inhibits cap-dependent translation independent of mTOR by

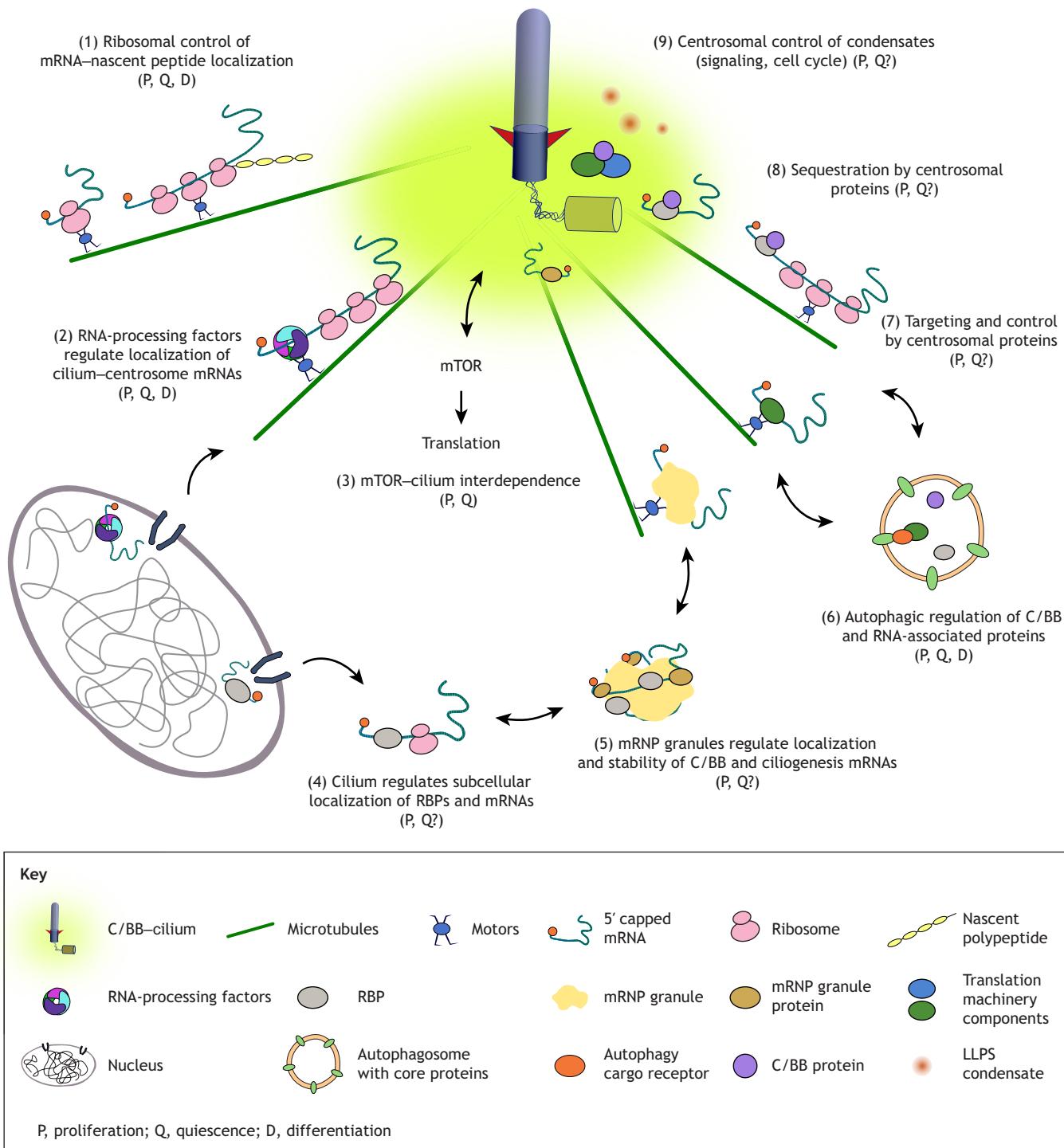


Fig. 3. Emerging mechanisms for regulation of cell fate decisions by the cilium–centrosome axis. Multiple mechanisms are implicated at the cilium–centrosome complex, including cytoskeleton-dependent recruitment of mRNAs, and an interplay with protein synthesis and degradation systems. (1) Co-translational targeting of polysome-bound mRNAs to the C/BB by nascent polypeptides occurs across different cell states. (2) Proteins involved in the RNA processing pathway (such as the spliceosome, PRPFs and the EJC) associate with cilium–centrosome-specific mRNAs, targeting them to the C/BB and regulating their composition and function. (3) mTOR signaling and the primary cilium reciprocally regulate each other to control translation in proliferating and quiescent cells. (4) The cilium promotes RBP-dependent subcellular localization and translation of mRNAs encoding signaling effectors in proliferating cells. (5) mRNP granules regulate and selectively target cilium–centrosome proteins to the C/BB in proliferating cells. (6) Autophagy regulates the maintenance and availability of cilium–centrosome proteins, RBPs and translation factors, influencing localization, transport and translation of select mRNAs across cell states. (7, 8) In proliferating cells, centrosome proteins facilitate transport of polysome-bound mRNAs to the C/BB, promoting localized translation. They also interact with and sequester components of translation initiation complexes and RBP-bound mRNAs to both repress translation and regulate the local concentration of the protein synthesis machinery. (9) In proliferating cells, centrosome–PCM peripheral zones nucleate LLPS condensates of signaling and centrosomal components, which can include mRNAs. Thus, localization mechanisms may involve microtubule-dependent transport, piggybacking on other centrosome-targeted components or diffusion from the initial location in the cell. Formation of condensates at the centrosome might sustain transient translation factories essential for the rapid changes required during cell state transitions.

sequestering translation factors and localization of specific mRNA–polysome complexes via RBPs at centrosomes (Iaconis et al., 2017) (Fig. 3). How the autophagic regulation of OFD1 synchronizes with its functions to modulate translation, and whether it differs across cell states, is yet to be determined.

The diverse modes of translational regulation therefore necessitate identification of subsets of mRNAs that localize to the cilium–centrosome complex, and/or are regulated by autophagy. Taken together, these studies provide unexpected new avenues for investigating the composition of the cilium–centrosome complex in different cellular states, and possible mechanisms by which centrosomal localization of proteins and RNA may impact cell fate.

Perspectives and conclusions

The cilium–centrosome axis is a regulatory hub controlling signaling, translation, metabolism and cell polarity. Multiple asymmetric features of the centrosome, such as age, composition, remnant ciliary components, and selective RNA and protein sequestration and/or enrichment confer the capacity to determine cell fate. While centriole duplication and reorganization and cilium assembly–disassembly have been extensively studied, many regulatory aspects of this axis in cell state maintenance and transitions remain unknown. Although there are tantalizing possibilities that ciliary composition and function influence the choice between different forms of arrest, molecular evidence is sparse. We propose that a more detailed analysis of the mechanisms that couple ciliogenesis to reversible arrest might illuminate this fate choice.

Two features stand out from the recent studies: involvement of spatially controlled posttranscriptional mechanisms, and temporal control across cell generations. Although selective protein localization is well documented (Devi et al., 2021; Gupta et al., 2015; Jakobsen et al., 2011), the newly described mechanisms of ciliary–centrosomal control of transcript localization and translation have amplified the regulatory arsenal of this axis. Ciliary regulation of β -catenin mRNA suggests that translation-dependent mechanisms fine-tune cellular signaling. Detecting such intertwined relationships requires multiplexing tools that permit monitoring of different types of molecules within and across cell generations. Although different transcripts within a single cell state appear to use different localization mechanisms, whether the same mRNA transporters and adaptors ferry the same or different sets of transcripts to the C/B β in other cell states is not known.

Regulatory control over the components or networks of a succeeding phase would facilitate seamless transitions between cell cycle phases, and identification of mechanisms that affect cell fate in successive cycles broadens the individual cycle-limited view. Increasing evidence suggests that crosstalk between the core cell cycle machinery and the cilium–centrosome axis occurs both spatially and temporally. The recently identified points of ciliary control from G1 to G2-M within a single cycle (Youn et al., 2022), from G1 of one cycle to the next (Ho et al., 2020), and from G0 to G2-M before re-entry into the cycle (Venugopal et al., 2020), suggest that this axis regulates cell fate networks. It is tempting to speculate, for a given combination of extrinsic and intrinsic signals, the existence of a cilium-based mechanism originating in G1 phase but spreading through cell cycle phases and across cell generations.

The biophysical nature of the centrosome has been enigmatic, and the recent suggestion that the PCM may form by liquid–liquid phase separation (LLPS) like other membraneless organelles is intriguing (Raff, 2019; Woodruff et al., 2017). In proliferating cells, condensate formation at the centrosome occurs not only for

centriole duplication (Park et al., 2019) but also for localized degradation of β -catenin by the destruction complex (Lach et al., 2022). The combination of optogenetics, microscopy and simulations is proving to be a powerful approach to understanding whether LLPS regulation of signaling plays a role during cell state transitions where rapid switching is required. Given that the cilium–centrosome axis also affects mRNA localization, and that RNA–protein complexes can form scaffolds for spindle assembly (Ito et al., 2020), it is possible that this axis integrates mRNA transport and localized translation with condensate nucleation of signaling components or cell cycle regulators to influence cell fate (Fig. 3). It would be interesting to investigate whether other cellular pathways are similarly regulated, and if such condensates also incorporate polyribosomal mRNA. In this regard, tools involving phase separation-prone domains, motor proteins, RNA reporters and chemically induced dimerization may be used to study condensate properties and the potential for selective recruitment and/or sequestration of mRNAs at the centrosome (Cochard et al., 2022 preprint).

Addressing these questions in the context of cell state transitions, such as quiescence versus differentiation, might help to delineate cell fate mechanisms that link nucleocytoplasmic mRNP transport with structural reorganization of the cilium–centrosome axis. In addition to providing fundamental information on an ancient organelle, such insights might eventually inform new therapeutic avenues for ciliopathies.

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Competing interests

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