The roles of the oncoprotein GOLPH3 in contractile ring assembly and membrane trafficking during cytokinesis

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Abstract

Cytokinesis is an intricate process that requires an intimate interplay between actomyosin ring constriction and plasma membrane remodelling at the cleavage furrow. However, the molecular mechanisms involved in coupling the cytoskeleton dynamics with vesicle trafficking during cytokinesis are poorly understood. The highly conserved Golgi phosphoprotein 3 (GOLPH3), functions as a phosphatidylinositol 4-phosphate (PI4P) effector at the Golgi. Recent studies have suggested that GOLPH3 is up-regulated in several cancers and is associated with poor prognosis and more aggressive tumours. In *Drosophila melanogaster*, GOLPH3 localizes at the cleavage furrow of dividing cells, is required for successful cytokinesis and acts as a key molecule in coupling phosphoinositide (PI) signalling with actomyosin ring dynamics. Because cytokinesis failures have been linked with pre-malignant disease and cancer, the novel connection between *GOLPH3* and cytokinesis imposes new fields of investigation in cancer biology and therapy.

Introduction

Cytokinesis is the conclusive act of cell division that separates the genomic material and the cytoplasm of the mother cell into two daughter cells [1]. In animal cells, constriction of a plasma membrane-anchored actomyosin ring leads to the formation of a cleavage furrow that ingresses until the two nascent daughter cells remain connected by a thin cytoplasmic bridge [1]. During the last step of cytokinesis, dubbed abscission, the intercellular bridge is ultimately severed, leading to complete separation of daughter cells. A network of scaffolding proteins, including septins and anillin, ensures the tightly anchoring of the actomyosin ring to the plasma membrane during furrow ingression and abscission [2,3]. In symmetrically dividing cells, the cleavage site is set up in a position that bisects the axis of chromosome separation, thus securing the equal distribution of genomic and cytoplasmic contents between the two daughter cells [1,4]. During anaphase, the mitotic spindle reorganizes to generate the central spindle (CS), an array of antiparallel, interdigitating microtubule (MT) bundles [4,5]. The CS MTs transmit the spatial information required for cleavage furrow formation by delivering regulators of the small Rho-GTPase to the equatorial cortex [6,7]. In turn, the accumulation of active Rho-GTPase at the equatorial cortex is the primary signalling event that sets up the site of cleavage furrow formation by controlling both profilin-mediated actin polymerization at the plasma membrane and myosin II activation [6,7]. The balance between the active state (GTP-bound) and inactive state (GDP-bound) of RhoA/Rho1 depends on the guanine-nucleotide-exchange factor (GEF) ECT2 (epithelial cell transforming sequence 2 oncogene)/Pebble and the GTPase-activating protein (GAP) MgcRacGAP/RacGAP50C. MgcRacGAP/RacGAP50C binds to the kinesin mitotic kinesin-like protein (MKLP1)/Pavarotti to form the centralspindlin complex, an evolutionary conserved hetero-tetramer required for CS formation [1,7]. Compelling data have demonstrated that the Rho GEF ECT2/Pebble associates with the MgcRacGAP/RacGAP50C component of centralspindlin to form a ternary complex leading to local activation of RhoA/Rho1 at the equatorial cortex [1,7]. Besides actomyosin ring constriction, animal cell cytokinesis involves vesicle transport from both the endocytic/recycling and the secretory pathways [8,9]. The requirement for membrane trafficking during cytokinesis has been associated with the increase in total surface area during furrowing and with the timely delivery of signalling factors that regulate this process [8]. Recent data have also demonstrated a role for specific lipids in the cleavage furrow and revealed changes in lipidome during cell division [10–12]. It has been suggested that a special lipid composition would facilitate the dynamic interplay between the plasma membrane and actomyosin apparatus and regulate vesicle targeting/fusion events at the cleavage furrow [8,9,11].

The highly conserved Golgi phosphoprotein 3 (GOLPH3) has been characterized as a phosphatidylinositol 4-phosphate

Key words: cancer, cytokinesis, *Drosophila*, Golgi, GOLPH3, vesicle trafficking.

Abbreviations: COG, conserved oligomeric Golgi-complex; CS, central spindle; ER, endoplasmic reticulum; fwd, four wheel drive; GEF, guanine-nucleotide-exchange factor; GOLPH3, Golgi phosphoprotein 3; MT, microtubule; MYO18A, myosin 18A; PI, phosphoinositide; PI4K, phosphoinositide 4-kinase; PIP, phosphatidylinositol phosphate; Vps74p, vacuolar protein sorting 74 protein.

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(PI4P) effector at the Golgi [13]. In addition *GOLPH3* functions as a potent oncogene and is amplified in several solid tumours [14]. Yet, the molecular mechanisms that link this protein to malignant transformation have not been clarified. In our recently published work [15], we provided the first evidence to date implicating GOLPH3 in cytokinesis. We demonstrated that the *Drosophila* homologue of GOLPH3 localizes at the cleavage site and controls both contractile ring formation and vesicle trafficking in dividing cells. Based on these data, GOLPH3 acts as a key molecule to coordinate membrane remodelling and cytoskeletal dynamics during cell cleavage.

GOLPH3 proteins localize to the Golgi through binding to PI4P and are required for Golgi architecture

Mapped in a chromosomal region that is frequently amplified in several solid tumours, human *GOLPH3* was validated as a new oncogene by combining integrative genomics with clinicopathological and functional analysis [14]. Since then, up-regulation of *GOLPH3* has been reported in several cancers including lung cancer, esophageal squamous-cell carcinoma, colorectal, prostate, breast cancer, melanoma, glyoma and connective tissue tumours [16–22]. Furthermore GOLPH3 overexpression has been linked with poor prognosis and more aggressive tumours [16,18–22]. Taken together these data have suggested that GOLPH3 might be used as a prognostic biomarker of tumour progression [16,18–22].

GOLPH3 family represents a group of Golgi proteins that are highly conserved across eukaryotes and serve an essential function for vesicle trafficking and Golgi structure [13,23–25]. Vertebrate species have two paralogues GOLPH3 (also referred to as GPP34, GMx33, MIDAS) and GOLPH3L (Golgi phosphoprotein 3-like; GPP34R/GMx33β), whereas lower organisms including *D. melanogaster* have a unique isoform [13,23,24]. The mammalian GOLPH3-proteins were firstly identified during a proteomic analysis of an isolated Golgi fraction and described as phosphorylated components of the Golgi matrix [23,24]. GOLPH3 was later demonstrated as a PI4P-binding protein through a high throughput proteomic screen based on the lipid-binding assay using *D. melanogaster* proteome [13]. To identify the minimal portion of GOLPH3 proteins that retains the ability to bind to PI4P, Dippold et al. [13] constructed a series of truncations of *Drosophil*a GOLPH3 that were tested by lipid blot assay. Based on this analysis, binding to PI4P requires amino acids 30–293 of *Drosophila* GOLPH3, which corresponds to the most evolutionary conserved region dubbed GPP34 domain by PFAM (Figure 1). The same series of truncations, when expressed in human embryonic kidney (HEK)-293 cells as GFP fusion proteins, revealed that Golgi localization requires the GPP34 domain of GOLPH3 [13]. Several studies, including our work, have led to demonstrate that GOLPH3 localizes to the Golgi membranes through binding to PI4P [13,15,26]. In budding yeast, mutations in the gene *PIK1,* which encodes the unique Golgi phosphoinositide 4-kinase (PI4K) [27] impaired recruitment of vacuolar protein sorting 74 protein (Vps74p) (the yeast orthologue of GOLPH3) to the Golgi [13,26]. Similarly, we showed that *Drosophila* GOLPH3 failed to concentrate at the Golgi in spermatocytes from males carrying in the gene *four wheel drive (fwd)*, which encodes the PI4K IIIβ [15]. In addition, the analysis of X-ray crystal structure of GOLPH3 and Vps74p revealed a conserved positively-charged pocket on the hydrophobic face of these proteins that might mediate PI4P binding [26]. Consistent with these data, mutant variants of GOLPH3/Vps74p, carrying amino acid substitutions in the putative PI4P-binding pocket, failed to localize at the Golgi when tested in either budding yeast, HeLa cells or *Drosophila* [13,15,26].

In human cells, PI4P and GOLPH3 protein are required to maintain the Golgi architecture [13]. Depletion of human GOLPH3 disrupts the Golgi morphology from an extended Golgi ribbon to a compacted structure at one end of the nucleus [13]. Remarkably, Dippold et al. [13] observed similar Golgi alterations after depletion of unconventional myosin 18A (MYO18A) or in cells treated with drugs that affect F-actin cytoskeleton. Furthermore, they found that GOLPH3 interacted with the unconventional MYO18A. These observations led them to propose a model whereby human GOLPH3 binds to PI4P-enriched *trans*-Golgi and MYO18A thus mediating a linkage with the F-actin cytoskeleton that facilitates the flattening of the Golgi stacks, as well as vesicle formation [13].

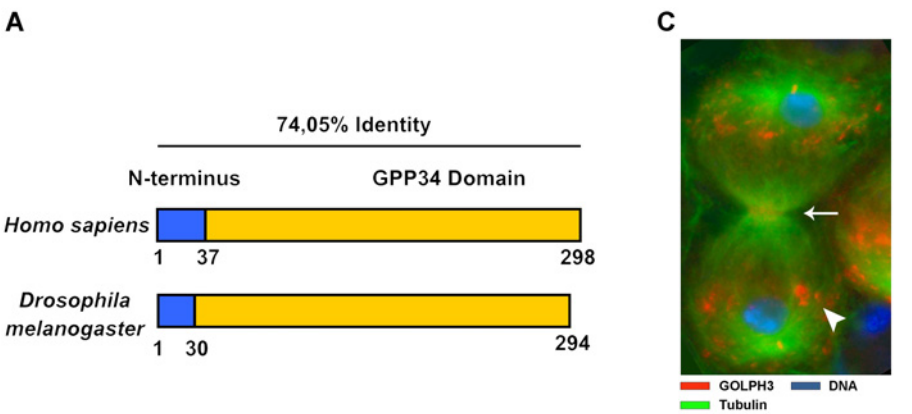
Our work demonstrated that *Drosophila* GOLPH3 too is required for normal Golgi structure [15]. Most *Drosophila* cells, including spermatocytes, lack a Golgi ribbon [28]. Each *Drosophila* Golgi has a paired structure consisting of two stacks held together through an actin-based mechanism [28]. Spermatocytes carrying mutations in *Drosophila GOLPH3*, exhibited a 1.9-fold increase in the number of Golgi bodies with the average size decreased by 50% indicating a role for GOLPH3 protein in maintaining the integrity of paired Golgi stacks [15]. It is then likely that GOLPH3 participates in a PI4P-dependent recruitment of actin-regulatory factors that contribute to regulate pairing of the Golgi stack structure.

GOLPH3 is required for contractile ring formation and membrane trafficking during cytokinesis

Drosophila male meiosis provides as a well-suited cell system for the analysis of membrane trafficking and membrane remodelling during cytokinesis [29]. Indeed mutant screens for mutants affecting male meiotic cytokinesis have allowed identifying a large number of vesicle-trafficking components and membrane remodelling factors required for this process [29]. Studies from our group and others revealed that spermatocyte cytokinesis requires the wild-type functions

Figure 1 | ***Drosophila melanogaster*** **GOLPH3 protein**

(**A**) Comparison between human and *Drosophila* GOLPH3 proteins. Amino acid identity for the entire protein is indicated. (**B**) Alignment of *Drosophila* GOLPH3 protein with human GOLPH3 (by ClustalW2). Green and red indicate mutations affecting binding of *Drosophila* GOLPH3 to PI4P, with green indicating substitution of lysine for glutamic acid (E273K), red indicating substitutions of alanine for lysine (K167A) and of alanine for arginine (R170A). Blue indicates the clathrin box sequence. (*) fully conserved residue; (**:**) conservation between groups of strongly similar properties; (.) conservation between groups of weakly similar properties. (**C**) In dividing spermatocytes, GOLPH3 protein is enriched in Golgi-derived vesicles (arrowhead) and at the cleavage furrow (arrow).



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H. sapiens              MTSLTQRSSGLVQRRTEASRNAADKERAAGG-GAGSSED--DA-QSRRDEQDD     49
D. melanogaster         MN----RSDGLV-RRSVKPR---ENGGAEGGLNANTPDDNQDALDNLKDQEDN     45
                        *.    **.*** **:  .*   ::  * ** .*.:.:*  ** :. :*::*:

H. sapiens              -DDKGDSKETRLTLMEEVLLLGLKDREGYTSFWNDCISSGLRGCMLIELALRG     101
D. melanogaster         IDD-GDSKETRLTLMEEVLLLGLKDKEGYTSFWNDCISSGLRGCILIELGLRG     97
                         ** ********************:******************:****.***

H. sapiens              RLQLEACGMRRKSLLTRKVICKSDAPTGDVLLDEALKHVKETQPPETVQNWIE     154
D. melanogaster         RVMIEKSGMRRRGLCTRKLILKSDQQTGDVLLDEALKHIKETDPPETVQSWIE     150
                        *: :* .****:.* ***:* ***  ************:***:******.***

H. sapiens              LLSGETWNPLKLHYQLRNVRERLAKNLVEKGVLTTEKQNFLLFDMTTHPLTNN     207
D. melanogaster         YLSGETWNPLKLRYQLKNVRERLAKNLVEKGVLTTEKQNFLLFDMTTHPLSDN     203
                         ***********:***:**********************************::*

H. sapiens              NIKQRLIKKVQEAVLDKWVNDPHRMDRRLLALIYLAHASDVLENAFAPLLDEQ     260
D. melanogaster         VVKCRLVKKIQDSVLSKWVNDPQRMDKRMLALIFLAHASDVIENAFAPLNDDD     256
                         :* **:**:*::**.******:***:*:****:*******:******* *::

H. sapiens              YDLATKRVRQLLDLDPEVECLKANTNEVLWAVVAAFTK                    298
D. melanogaster         YEVAMKRVRELLDLDFEAESAKPNANEILWAVFMAFTK                    294
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of several Golgi proteins including the conserved oligomeric Golgi-complex (COG) subunits Cog5 and Cog7 [30,31], the Golgi PI4K IIIβ Fwd [32], the endoplasmic reticulum (ER) to Golgi-vesicle docking protein syntaxin 5 [33] and Brunelleschi, the *Drosophila* orthologue of the yeast transport protein particle (TRAPP) II TRS120p subunit [34]. Another membrane-trafficking component required for male meiotic cytokinesis is Rab11. Implicated in both Golgi and recycling endocytic trafficking, this protein concentrates in vesicles that enrich at the cleavage furrow [35]. Phosphatidylinositol phosphates (PIPs) have emerged as important signalling molecules for cytokinesis in several organisms including *Drosophila* [11] and several proteins involved in the phosphoinositide (PI) cycle have been implicated in *Drosophila* cytokinesis. The *Drosophila* PI-transfer protein Giotto/Vibrator (Gio/Vib) concentrates at the ER membranes and at the cleavage furrow of dividing spermatocytes and is required for furrow ingression in both spermatocytes and larval neuroblasts [36,37]. Phenotypic analysis of loss-of-function *fwd* mutants also implicates the requirement for PI4P in spermatocyte cytokinesis [32]. Previously, work in Dr Brill's laboratory showed that Fwd protein binds Rab11 and co-localizes with both this protein and PI4P markers at the Golgi [38]. Mutations in *fwd* disrupt the synthesis of PI4P on Golgi membranes and impair the accumulation of Rab11- and PI4P-containing secretory

organelles at the cell equator [38]. However, Fwd protein does not accumulate at the cleavage furrow during cytokinesis [38]. Importantly, our recent study demonstrated that the PI4P-binding protein GOLPH3 accumulates at the cleavage furrow of *Drosophila* dividing spermatocytes and larval neuroblasts and is required for cytokinesis in both cell types [15]. We showed that GOLPH3 function in cytokinesis is intimately connected to its ability to bind PI4P. Mutations that abolish PI4P binding (Figure 1B), impair recruitment of GOLPH3 to both the Golgi and the cleavage furrow. Moreover, mutations that abolish GOLPH3–PI4P interaction also impair localization of PI4P-and Rab11-associated secretory organelles at the cleavage site [15]. Consistent with a role in targeting PI4P-and Rab11-secretory vesicles to the cleavage furrow, we found that GOLPH3 forms a complex with Rab11. Our biochemical studies also indicated a potential molecular interaction between GOLPH3 and clathrin which is further suggested by the presence of a putative clathrin-binding motif [39], 'LLDLD', in the GOLPH3 amino acid sequence (Figure 1B) [15].

GOLPH3 also interacts with components of the CS and the contractile ring and is required for maintenance of centralspindlin and Rho1 at cell equator and stabilization of myosin II and septin rings [15]. Several studies have shown that PI4P is the substrate for phosphatidylinositol 4-phosphate 5-kinase that generates the $PI(4,5)P_2$ lipid in the cleavage furrow where it regulates formation and stability of the cytokinetic structures [11]. Indeed, several cytokinesis proteins including Rho, the RhoGEF ECT2 and the centralspindlin subunit MgcRacGAP, contain protein domains that bind to $PI(4,5)P_2$ and/or PI4P and mediate plasma membrane interactions at the cleavage site [40–43]. Septins interact *in vitro* with PIPs and polymerization of these proteins into filaments is enhanced by association with lipid bilayers [11,44]. Finally, $PI(4,5)P_2$ is known to stimulate F-actin polymerization by modulating the activity of the actin-binding proteins profilin and cofilin [11,45]. Remarkably, visualization of the $PI(4,5)P_2$ in spermatocytes expressing phospholipase Cδ (PLCδ)–pleckstrin homology (PH)–GFP shows an enrichment of this lipid at the cleavage furrow membrane in wild-type but not in *GOLPH3* [15]. Based on these data, both PI4P–GOLPH3 and $PI(4,5)P_2$ are likely to regulate interaction of centralspindlin, septins and actomyosin with plasma membrane during cytokinesis. In the absence of GOLPH3, PI4P–GOLPH3 and $PI(4,5)P_2$ fail to concentrate at the cleavage furrow. As a result, localization of centralspindlin at the equatorial cortex is not maintained, centralspindlin-associated MTs fail to stably bundle and septin/myosin II rings are not stabilized.

Conclusions

Cytokinesis failures cause the formation of genetically-unstable tetraploid cells, thus promoting tumorigenesis [46,47]. Indeed, compelling data suggest that tetraploidy can lead to tumour initiation [46,47]. Tetraploid cells created from p53 null mouse mammary epithelial cells (MMECs) promote malignant cancer formation when transplanted into nude mice, in contrast with the diploid $p53^-$ null controls [48]. Similarly, APC (adenomatous polyposis coli) mutations found in human colorectal cancer impair cytokinesis and cause tetraploidy before the early steps of colorectal cancer development [49]. Importantly, recent data have suggested that *GOLPH3* might be a promising therapeutic target for cancer therapy [18,22]. However our finding that depletion of *GOLPH3* results in cytokinesis failures and tetraploidy raise new questions regarding the mechanisms of tumorigenesis associated with this oncogene.

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