Human Mob1 proteins are required for cytokinesis by controlling microtubule stability.
Claudia Florindo, Joana Perdigão, Didier Fesquet, Elmar Schiebel, Jonathon Pines, Alvaro A Tavares

To cite this version:
Claudia Florindo, Joana Perdigão, Didier Fesquet, Elmar Schiebel, Jonathon Pines, et al.. Human Mob1 proteins are required for cytokinesis by controlling microtubule stability.. Journal of Cell Science, Company of Biologists, 2012, pp.3085-3090. 10.1242/jcs.097147. hal-00715099

HAL Id: hal-00715099
https://hal.archives-ouvertes.fr/hal-00715099
Submitted on 1 Jun 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Human Mob1 proteins are required for cytokinesis by controlling microtubule stability

Claudia Florindo1,2,3, Joana Perdigão1, Didier Fesquet4, Elmar Schiebel5, Jonathon Pines6 and Álvaro A. Tavares1,2,3,*

1Instituto Gulbenkian de Ciência, P-2780-156 Oeiras, Portugal
2Regenerative Medicine Program, Departamento de Ciências Biomédicas e Medicina, Universidade do Algarve, 8005 Faro, Portugal
3Centre for Molecular and Structural Biomedicine, CBM/IBB, University of Algarve, Faro 8005-139, Portugal
4Université Montpellier, Sud de France, CRBM, 34293 Montpellier, France
5ZMBH, Universität Heidelberg, 69120, Germany
6The Gurdon Institute, Cambridge CB2 1QN, UK

*Author for correspondence (astavares@ualg.pt)

Summary
The completion of cytokinesis requires abscission of the midbody, a microtubule-rich cytoplasmic bridge that connects the daughter cells before their final separation. Although it has been established that both the midbody structure and membrane fusion are essential for abscission, the biochemical machinery and the cellular processes of abscission remain ill-defined. Here we report that human Mob1A and Mob1B proteins are involved in the regulation of abscission of the intercellular bridge. The Mob family is a group of highly conserved proteins in eukaryotes, described as binding partners as well as co-activators of protein kinases of the Ndr family, and as members of the Hippo pathway. We show that depletion of Mob1A and Mob1B by RNAi causes abscission failure as a consequence of hyper-stabilization of microtubules in the midbody region. Interestingly, depleting Mob1 also increases cell motility after cytokinesis, and induces prolonged centriole separation in G1 phase. In contrast, centrosomes fail to split when either Mob1A or Mob1B is overexpressed. Our findings indicate that human Mob1 proteins are involved in the regulation of microtubule stability at the midbody. We conclude that Mob1A and Mob1B are needed for cell abscission and centriole re-joining after telophase and cytokinesis.

Key words: Cytokinesis, Mob1, Microtubule stability

Introduction
At the end of cytokinesis cells have to execute abscission, which results in the physical separation of two daughter cells, and return to interphase. It is generally accepted that a particular sequence of events are required for abscission to occur: cortical anchorage of the ingressed furrow, splitting the plasma membrane between the sister cells, and the disassembly of midbody-associated microtubules (Barr and Gruneberg, 2007; Guizetti and Gerlich, 2010). Recent work has demonstrated that endosome fusion and microtubule reorganization are essential for abscission. It was shown that disassembly of microtubules can be mediated by the microtubule-severing protein spastin (Connell et al., 2009; Guizetti et al., 2011; Schiel et al., 2011) and several proteins of the endosomal sorting complex are required for transport (ESCRT)-III localize to the midbody and are required for cytokinesis (Morita et al., 2007; Wollert et al., 2009; Guizetti et al., 2011; Schiel et al., 2011).

Mob1 is a conserved protein required for cytokinesis and for the regulation of mitotic exit (McCollum and Gould, 2001; Hergovich, 2011). Mob1 proteins are binding partners and co-activators, of protein kinases of the Ndr and Lats families (reviewed in Hergovich et al., 2006; Trammell et al., 2008). In fly and human cells, Mob1 participates in the control of cell proliferation and apoptosis as part of the Hippo pathway (Lai et al., 2005; Wei et al., 2007; Praskova et al., 2008). There is also a genetic interaction between the Drosophila Mob-like genes and tricornered (trc), another Ndr protein kinase required for normal morphogenesis of a variety of polarized outgrowths (He et al., 2005). In humans, Lats1 interacts with Mob1 and this complex appears to be functionally required for cytokinesis (Bothos et al., 2005).

Here we have investigated the function of the human Mob1A and Mob1B proteins to further explore their role during cytokinesis. We show that Mob1 proteins are required for abscission. Furthermore, we show that upon Mob1 downregulation, centrioles fail to rejoin at the end of cytokinesis, cells acquire increased motility, and microtubule stability is increased. Finally, we also show that depolymerization of microtubules is a prerequisite for abscission, and that Mob1 proteins are involved in the regulation of this process.

Results and Discussion
Human Mob1A and Mob1B proteins are essential for centriole cohesion
We first examined the distribution of human Mob1A and Mob1B proteins. In HeLa cells expressing GFP-centrin, where the two centrioles were clearly distinguishable, we detected a broad single Mob1 dot in the center of the centrosome by indirect immunofluorescence microscopy (Fig. 1A). This staining persisted until the end of mitosis (Fig. 1B). However, in late telophase cells with two separated centrioles, Mob1 was detected just in the stronger GFP-centrin signal, often the one closer to the midbody.

Mob1 was also observed at the kinetochores (supplementary material Fig. S2) and in the central spindle and midbody regions during late anaphase and telophase (Fig. 1). The specificity of the Mob1 centrosomal staining was confirmed by time-lapse video microscopy of HeLa cells expressing either GFP-Mob1A or GFP-Mob1B (Fig. 1C).

The high degree of identity between the two Mob1 proteins indicated a possible functional redundancy (supplementary material Fig. S1) (Vitulo et al., 2007). Therefore, we sought to disrupt the expression of both Mob1s by simultaneous co-transfection of siRNA directed against each Mob1. We used three different siRNA duplexes for each type of Mob1, and found that Mob1 protein levels were substantially repressed (>95%) by 48 h post-transfection (Fig. 2A; supplementary material Fig. S1C). Identical results were obtained using any pair of different siRNAs against either Mob1s, indicating that siRNA treatment effectively and specifically reduced the abundance of Mob1 proteins. We will refer to cells in which neither Mob1A nor Mob1B were detected by immunoblotting as ‘Mob1(A+B)-depleted’.

Although Mob1(A+B)-depleted cells formed apparently normal interphase arrays of microtubules and mitotic spindles, downregulation of Mob1 had a striking effect upon centriole behavior. Two distinct, widely separated GFP-centrin positive structures were observed in >28% of interphase Mob1(A+B)-depleted cells, compared with 5% in control cells (Fig. 2B). The distance between separated centrioles varied considerably (between 2 and 30 μm, Fig. 2B), compared to control cells (1.4 ± 0.2 μm). This effect on centrioles was observed with all Mob1-siRNAs, but not with the control siRNAs, and was also observed in U2OS and RPE1 cell lines (supplementary material Fig. S3A,B), indicating that is not cell type specific, nor linked to the transformed state of Hela cells.

The abrogation of Mob1 function seemed to cause centrioles to split during G1 phase, firstly because mitotic spindle poles

**Fig. 1.** Mob1 localizes to the centrosome and to the midbody. (A) Cells were stained for GFP-centrin (green), DAPI (blue) and anti-Mob1 (red). Mob1 accumulates on the centrosome but does not completely overlap with the centrioles (two centrin dots). This pattern remains while the centrioles duplicate and centrosomes separate (A1 to A4). (B) HeLa GFP-centrin cells immunostained for α-tubulin (blue) and Mob1 (red). At the end of telophase (3), Mob1 is detected in the midbody and in just one centriole. (C) Time lapse analysis of a stable cell line expressing GFP-Mob1A. GFP-Mob1A is visible on the centrosomes from the start of mitosis until anaphase (arrows), and in the central spindle and midbody (arrowheads) at the end of mitosis. GFP-Mob1B protein has an identical behavior.

**Fig. 2.** Depletion of Mob1 results in centriole separation and cytokinesis failure. Unsynchronized HeLa-GFPcentrin cells were analyzed after RNAi for Mob1(A+B) two days post-transfection. (A) Western blots using α-Mob1, α-Lamin A/C and anti-γ-tubulin antibodies. (B) HeLa-GFPcentrin cells with centriole/centrosome separation induced by Mob1(A+B) depletion. Cell a shows normal centriole separation; cell b has centrosome separation of ~3 μm; cell c: this distance between centrosomes is rarely seen in control cells; cell d has centrioles separated more than 30 μm, never detected in control cells. Scale bar: 10 μm. (C) Percentage of cells with centriole/centrosome separation greater than 3 μm in asynchronous cultures, and in synchronized cells analyzed in S/G2 phase. (D) DIC + fluorescence images from Mob1(A+B)-depleted cells showing delayed/failed cytokinesis (1) and three connected cells (2) with the midbodies in the intercellular bridges (arrows) (DNA: blue, γ-tub: red). (E) For the quantification of linked cells after Mob1(A+B) depletion only cells with a visible midbody, as in D1, were considered.
always had two centrioles each (even after 5 days of siRNA treatment); secondly, because we never observed cells with four separated centrioles (or with one pair plus two separated centrioles) indicating that no splitting occurred after centriole duplication. To confirm this hypothesis, we analyzed cells 3.5 h after release from a S-phase block and we found that 32% of the Mob1(A+B)-depleted cells had two separated pairs of centrioles compared to ∼7% in control cells (Fig. 2C), indicating that a large proportion of Mob1-depleted cells initiated centriole duplication with already separated centrioles.

Centrioles often split at the end telophase and re-join after abscission (Chevrier et al., 2002; Meraldi and Nigg, 2002). In contrast to the effect of depletion, overexpression of either Mob1A or Mob1B drastically reduced this number to 15% and 4%, respectively (three independent experiments, n=∼100 dividing cells per exp.). Therefore, an excess of Mob1 prevents centriole separation at the end of cell division, and Mob1 is important for the re-association of centrioles after mitosis (supplementary material Fig. S7).

Mob1 downregulation results in abscission failure and increased cell motility
We found that Mob1(A+B)-depleted cells remained connected at the end of cytokinesis by thin cytoplasmic bridges containing the midbody (Fig. 2D; Fig. 3A; supplementary material Fig. S3C,D). Chains of 3 or 4 connected cells were also frequently observed (Fig. 2D2). Time-lapse imaging revealed that Mob1(A+B)-depleted cells proceeded through mitosis with similar kinetics to control cells but took longer to execute abscission (Fig. 3). Most control cells took up to 4 h (80%, n=60) from constriction to complete separation of daughter cells. In contrast, abscission was completed by 4 h in only 30% Mob1(A+B)-depleted cells (Fig. 3B), and 38% took longer than 6 h (n=70). No DAPI-staining or anti-lamin staining was detected at the midbody region after Mob1(A+B)-depletion (data not shown), indicating that chromosome bridges or lagging chromosomes were unlikely to be the major cause of abscission failure (Steigemann et al., 2009). In addition, treatment of the cells with ZM1 (a specific Aurora B inhibitor) did not accelerated the timing of abscission, indicating that the delay was not caused by activation of the Aurora B-dependent checkpoint (supplementary material Fig. S4).

Remarkably, while control cells moved slightly apart after cytokinesis before adhering again and flattening onto the dish (supplementary material Movie 1), Mob1-depleted cells became highly mobile immediately after telophase/cytokinesis and remained connected by long intercellular bridges (Fig. 3; supplementary material Movies 2, 3). Cells moved continuously for several hours and with frequent changes of direction (Fig. 3C–D) even after resolution of the intercellular bridge, indicating that movement was not dependent on abscission failure.

Fig. 3. Depletion of Mob1 increases cell motility. (A) Mob1(A+B) depleted cells are frequently connected by very long intercellular bridges, indicating that cells have moved away from each other (scale bar: 10 μm). (B) The time from nuclear envelope breakdown (NEB) to the start of anaphase and from anaphase until abscission was measured by time-lapse imaging. 65 control and 70 Mob1(A+B)-depleted cells were analyzed. (C) Representation of the movement of all dividing cells in an optical field over 23 h. Each pair of cells is represented in a different color. 30 control and 36 Mob1(A+B)-depleted cells divided in each movie respectively. (D) Time-lapse of Mob1-depleted cells (supplementary material Movie 2). The blue and red arrows indicate the path followed by daughter cells A and B, respectively. The black arrow indicates the position of the midbody, between the two connected cells. After Mob1(A+B) down regulation daughter cells fail abscission, do not adhere strongly to the dish, and continue moving for hours.
Mob1 depletion reduces dynamic instability of microtubules

We found that Mob1(A+B)-depletion did not affect the midbody localization of Aurora B, MKPL1, PLK1 or Cep55, in agreement with (Wilmeth et al., 2010). Both endobrevin and syntaxin 2 also localized correctly, indicating that abscission failure was not caused by deficient recruitment of exocyst components to the midbody region (not shown).

Because over-stabilization of microtubules can prevent or delay cytokinesis (Hong et al., 2007; Manabe et al., 2002), we wondered whether hyper-stabilization of microtubules prevented abscission in Mob1-depleted cells. We found that Mob1-depleted cells had increased levels of acetylated tubulin (a marker of stabilised microtubules) (Piperno et al., 1987), and of microtubules resistant to both nocodazole and cold treatments (Fig. 4; supplementary material Fig. S6). In dividing cells, acetylated microtubules were easily detected in the midbody region (Fig. 4A). But after 30min incubation with nocodazole and cold this staining was lost in 46% of control cells, while the majority of Mob1(A+B)-depleted cells (>80%) retained strong acetylated tubulin staining on the intercellular bridge (Fig. 4). Therefore down regulation of Mob1A+B resulted in increased microtubule stability. If hyper-stabilization of the microtubules did prevent abscission, then treatments that reduced microtubule stability should decrease the number of unsuccessful cell divisions. In agreement with this, we found that Mob1(A+B)-depleted cells could execute abscission if microtubules were artificially destabilized by nocodazole treatment (Fig. 4C).

In summary, we have shown that Mob1(A+B) depletion inhibits the removal of midbody-localized microtubules that is required to allow membrane fusion and abscission (Guizetti and Gerlich, 2010). Although poorly explored, microtubule dynamics do have an important role in cytokinesis (D’Avino et al., 2005; Glotzer, 2009). Cells lacking Spastin, a microtubule-severing protein, fail abscission because they retain midbody microtubules (Yang et al., 2008; Connell et al., 2009; Guizetti et al., 2011) making spastin a potential candidate for regulation by Mob1. Also, it is tempting to think that there may be a causal connection between the unusual centriole behavior in Mob1-depleted cells and the contribution of the mother centriole to signal abscission observed by (Piel et al., 2001).

Populations of microtubules with different stabilities are involved in several aspects of cytokinesis (Foe and von Dassow, 2008; Shen et al., 2009; Connell et al., 2009). Microtubule dynamics and reorganization are also crucial for cell motility (Gundersen and Bulinski, 1988; Pegtel et al., 2007; Drabek et al., 2006) and, interestingly, altered microtubule dynamics can induce centriele separation (Jean et al., 1999). At present it is unclear whether the centriele splitting, abscission failure and hypermotility of Mob1(A+B)-depleted cells are all consequences of altered microtubule stability or reflect different functions of Mob1A and Mob1B (supplementary material Fig. S7).

The only Mob-like protein in Tetrahymena (TtMob1) is a cell polarity marker and is essential for division plane placement and cytokinesis (Tavares et al., 2012), therefore, since directed cell movement is dependent on cell polarization, the effect on cytoskeletal organization may to be the ancestral function of Mob-like proteins. The underlying molecular mechanism of Mob1 activity likely involves binding and activating Dbf2-like
Mob1 regulates microtubule stability

kinases, since both Lats1/2 and NDR kinases contribute to the regulation of the cytoskeleton (Justice et al., 1995; Yang et al., 2004; Hergovich et al., 2006). Consistent with this idea, components of the Hippo pathway cooperate with the Nek2 kinase to regulate centrosome disjunction (Mardin et al., 2010). Although other centrosome components are required to prevent premature centriole/centrosome splitting (Bahlamanyar et al., 2008; Wang et al., 2008; Nakamura et al., 2009), Mob1 is unique in the sense that it affects centriole re-joining.

In conclusion, we have shown that membrane recruitment to the midbody area is not in itself enough, and that microtubules have to be reorganised before membrane vesicles can fuse. Our results open the door to explore the regulation of microtubule dynamics by the Mob1-dependant kinase(s). Moreover, they indicate the exciting possibility that the Hippo pathway, which has a key role in regulating cell proliferation and apoptosis (reviewed in Pan, 2010), may influence microtubule dynamics in response to extracellular and intracellular signals and thereby influence cell polarity.

Materials and Methods

Antibodies and siRNAs

The α-Mob1 serum (Ponchon et al., 2004), recognized both Mob1A and Mob1B in HeLa extracts. The signal could be competed with either Mob1A or Mob1B expressed bacterially, and was suppressed upon Mob1A+Mob1B RNAi depletion (supplementary material Fig. S1). Primary antibodies: γ-tubulin (GTU–88, Sigma), α-tubulin (DM1a, Sigma), acetylated tubulin (C3B9). Three different siRNAs were used per gene with identical results: 5′-AACACGACGAA-GCCACACUU-3′ and Ambion’s #s30511 and #s30510 for Mob1A, oligos 5′-AACACACUU-3′ and Ambion’s #s40077 and #s40978 for Mob1B. As controls Lamin A/C oligo (Elbashir et al., 2001) and Dharmacharn Scramble II oligo were used. GenBank sequences: Human Mob1A, AJ577473; Human Mob1B, AJ577474.

Cell culture and synchronization

HeLa and U2OS cells were cultured in DMEM supplemented with 10% heat-inactivated fetal calf serum (FCS) and 2 mM glutamine. HeLa-GFP-centrin cells were seeded 131 331 Nat. Rev. Mol. Cell Biol. 65 331 Curr. Biol. 9 331 (2005). Control of cell proliferation and apoptosis by mob as tumor suppressor, mat. Cell 183, 651-670.

References


References

Mob1 regulates microtubule stability 3089

Supplementary material available online at http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.097147/-/DC1

Acknowledgements

We would like to thank Michel Bornens, Thomas Meyer, Thomas Weimbs, Isabel Martinez Garay and Iain Cheeseman for reagents.

Funding

C. F. and J. P. were recipients of FCT [grant numbers PRAXIS XXI/DB/19823/99 to C. F. and SFRH/BPD/20698/2004 to J. Perdigao]. Work in the Tavares lab was financed by the following FCT programs [grant numbers 331 331 POCTI/BIC/34405/09, POCTI/CQB/39099/2001, POCTI/BIA/PRO/60337/2004, PDTA/SQAD/105234-2008 and PesOE/EQEB/LA/0023/2011], and in the Fesquet lab by Contract ARC N° 5479.

Journal of Cell Science


