



Organisation of microtubules during cell division and differentiation

Microtubules are a component of the cytoskeleton and are required for many essential cellular processes. To carry out their functions, microtubules are assembled into highly ordered arrays. Defects in the structure and function

of the microtubule network are frequently observed in cancer cells and are implicated in certain developmental disorders. Cells contain sites that nucleate the polymerisation of new microtubules and control where and when these structures are made. These nucleation sites provide a unique molecular environment, not only for the control of microtubule nucleation but also for regulating microtubule behaviour, and are thus central to our understanding of microtubule organisation. Microtubule nucleation sites are formed by interaction of the γ -tubulin ring complex, a microtubule nucleator, with microtubule organising centres such as the centrosome. However, the exact molecular composition of microtubule nucleation sites and the spatio-temporal regulation of their assembly are poorly understood. Our long-term goal is to achieve a molecular understanding of the function of centrosomal and non-centrosomal microtubule nucleation pathways in distinct cell types and how defects are linked to disease.

Centrosome maturation

The centrosome, the major microtubule organising centre (MTOC), contributes to the assembly of the mitotic spindle by organising the spindle poles and by nucleating microtubule (MT) polymerisation (Lüders and Stearns, 2007). Centrosomal de-

fects, such as aberrant size, integrity and MT nucleation activity can impair proper spindle assembly and function, and result in genomic instability.

The centrosome is composed of a pair of barrel-shaped centrioles surrounded by a dense proteinaceous matrix, the pericentriolar material (PCM). The nucleation of MT polymerisation occurs within the PCM and requires recruitment of γ -tubulin ring complexes (γ TuRCs) from the cytoplasm. These complexes contain γ -tubulin, a tubulin family member that is not incorporated into the MT polymer, and additional proteins. We have previously shown that the interaction of γ TuRCs with centrosomes is mediated by the γ TuRC subunit GCP-WD (also known as NEDD1) (Lüders *et al*, 2006).

In late G2 phase of the cell cycle, cells prepare for mitosis by increasing the size and MT nucleating activity of the centrosomes. This process, also termed centrosome maturation, depends on the activity of mitotic kinases such as Polo-like kinase 1 (Plk1), but the molecular details of this pathway are largely unknown.

We have discovered that Plk1 associates with GCP-WD, the γ -tubulin targeting factor, and that Plk1 activity contributes to mitotic phosphorylation of GCP-WD (Haren *et al*, 2009; Figure 1).

Plk1 depletion or inhibition revealed that accumulation of γ -tubulin at centrosomes is regulated by controlling the levels of centrosomal GCP-WD. Surprisingly, GCP-WD mutants that are defective in Plk1 binding and phosphorylation still accumulate at mitotic centrosomes and recruit γ -tubulin. Our studies further

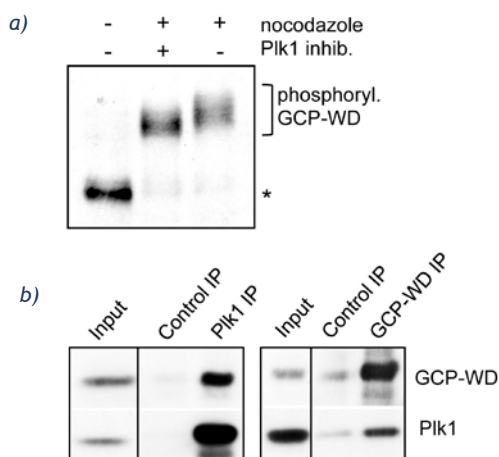


Figure 1. *Plk1 interacts with GCP-WD in mitosis and contributes to its mitotic phosphorylation. (a) HeLa cell lysates immunoblotted for GCP-WD. Cells were synchronised in mitosis by nocodazole treatment and treated with the Plk1 inhibitor BI2536, as indicated. The asterisk shows the position of unphosphorylated GCP-WD. (b) Endogenous GCP-WD and Plk1 were immunoprecipitated from mitotic cell extracts and probed with antibodies against the indicated proteins after Western blotting.*

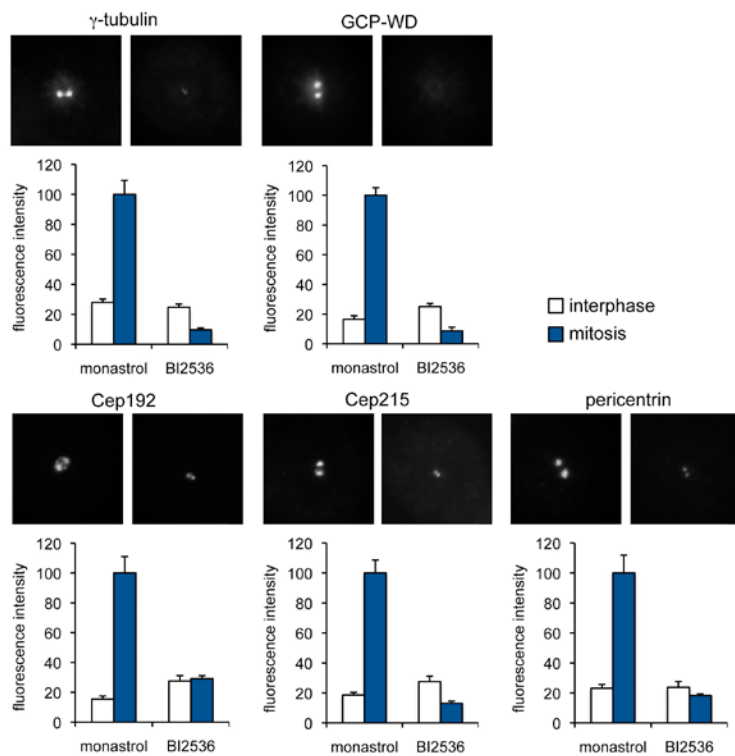


Figure 2. *Plk1 inhibition interferes with the recruitment of Cep192, Cep215 and pericentrin to mitotic centrosomes. HeLa cells were treated with monastrol or the Plk1 inhibitor BI2536. They were then fixed and immunostained for GCP-WD, γ -tubulin, Cep192, Cep215 or pericentrin. Fluorescence intensities at the centrosomes were quantified in interphase and in mitosis and plotted as a percentage of the intensities measured in mitotic cells treated with monastrol.*

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show that Plk1 also controls the recruitment of other PCM proteins implicated in centrosomal γ -tubulin attachment (Cep192/hSPD2, pericentrin, Cep215/Cdk5Rap2; Figure 2).

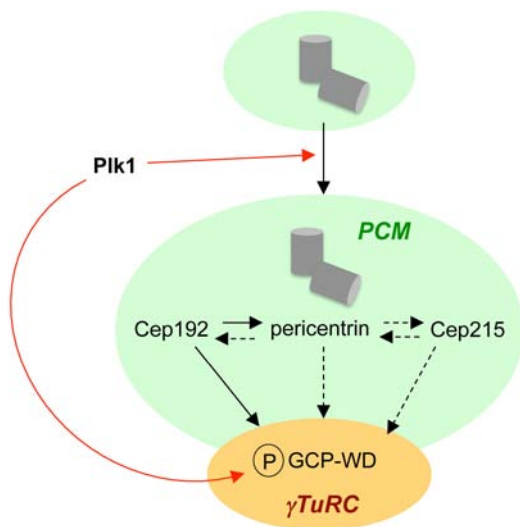


Figure 3. Interdependencies of components involved in the recruitment of γ -tubulin complexes to mitotic centrosomes. During centrosome maturation, Plk1 phosphorylates PCM components, which results in the accumulation of PCM, including γ TuRC recruitment factors, at mitotic centrosomes. Plk1-dependent phosphorylation of GCP-WD does not affect centrosome recruitment of γ TuRCs, which is regulated upstream of GCP-WD. Upstream regulators and potential Plk1 substrates include Cep192, pericentrin, and Cep215. Pericentrin and Cep215 contribute to each other's centrosome localisation and to γ TuRC binding. Pericentrin is also important for centrosome localisation of Cep192, which has the strongest impact on centrosome recruitment of GCP-WD/ γ TuRC (Gomez-Ferreria et al., 2007; Zhu et al., 2008). Solid black arrows indicate an essential role in the recruitment of the component they are pointing at, dashed black arrows indicate a partial role. Red arrows indicate Plk1-dependent regulation.

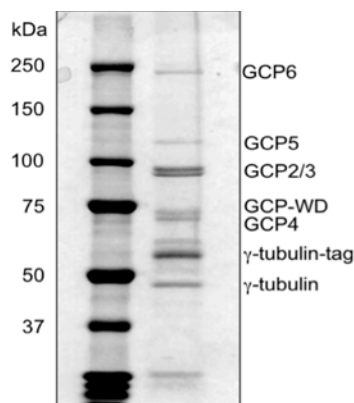


Figure 4. Purified human γ TuRC analysed by PAGE and coomassie staining. The positions of the γ TuRC subunits are indicated on the right.

Systematic testing of interdependencies between Cep215, pericentrin and GCP-WD for their localisation to mitotic centrosomes using RNAi demonstrated that pericentrin and Cep215 strongly depend on each other for their localisation to mitotic centrosomes and that each protein only partially contributes to γ -tubulin recruitment. In contrast, GCP-WD is an absolute requirement for γ -tubulin recruitment and seems to function more proximal to the γ TuRC in the recruitment pathway, most likely downstream of Cep215/pericentrin.

On the basis of our results, we propose that recruitment of γ -tubulin to mitotic centrosomes is regulated upstream of GCP-WD and involves multiple PCM proteins and potentially multiple Plk1 substrates (Haren et al., 2009; Figure 3).

Composition and regulation of the γ TuRC, a nucleator of MT polymerisation

The γ TuRC is a key component required for MT nucleation at MTOCs. γ TuRCs are composed of γ -tubulin and additional subunits named gamma-tubulin complex proteins (GCPs) in humans. Assembly of the γ TuRC involves subcomplexes composed of two molecules of γ -tubulin and one of GCP2 and GCP3, respectively (gamma-tubulin small complex, γ TuSC). According to a current model, the additional GCPs 4-6 promote assembly of ~13 γ TuSCs into the higher order ring-shaped γ TuRC by forming a stabilising cap on one side of the ring. However, in flies only γ -tubulin and the homologues of GCP2 and 3 are essential and in the absence of the homologues of GCPs 4-6 γ -tubulin is still recruited to centrosomes and supports MT nucleation. Similar results were recently obtained in *Aspergillus*. It is likely that in these cases, as well as in budding yeast, which naturally lacks homologues of GCP4-6, assembly of the γ TuSC into a higher order ring structure occurs only upon interaction with MTOCs. In addition to promoting the assembly of cytoplasmic γ TuRCs, GCPs 4-6 might have other regulatory functions, for example in controlling MT length and dynamics, either directly or by interaction with other proteins.

It is currently unclear how γ TuRC-dependent MT nucleation is regulated in space and time and how cooperation with other components involved in MT assembly and organisation occurs.

To address this issue, we are collaborating with Carme Caelles and Joan Roig (Molecular Medicine Programme, IRB Barcelona). We have developed a new method to purify γ TuRC from human cells, which greatly improves yield and purity (Figure 4). By combining the purification of γ TuRC with mass spectrometry, we will gain insight into cell cycle-dependent interactions as well as post-translational modifications such as phosphorylation. Our studies are the first to systematically analyse the cell cycle-dependent regulation of the γ TuRC and will provide new insight into the spatio-temporal control of MT assembly during spindle formation.

Identification and characterisation of novel centrosome and spindle-associated proteins

The proteomes of specific structures of the MT cytoskeleton have been described, but many proteins still await functional characterisation. Assigning functions to these proteins and analysing

their interplay is crucial for our understanding of the architecture and regulation of complex MT arrays such as the mitotic spindle.

We screened uncharacterised centrosome proteins by expression as GFP fusions in human cells for their localisation to specific cytoskeletal structures. Using this approach, we identified several proteins that localised to centrioles throughout the cell cycle and to mitotic spindle MTs in mitosis. This unique localisation pattern prompted us to study these proteins in more detail. Three of the proteins identified were recently described by other labs as subunits of the augmin complex (Lawo *et al*, 2009; Uehara *et al*, 2009). This complex associates with a subset of spindle

MTs in mitosis and recruits γ TuRC to promote proper spindle assembly and mitotic progression. According to a current model, augmin recruits γ TuRC via its targeting factor GCP-WD, to induce non-centrosomal MT nucleation from the sides of existing MTs within the spindle. Our results indicate that spindle targeting of the γ TuRC is regulated by mitotic phosphorylation of GCP-WD (Lüders *et al*, 2006). We are currently studying the molecular details of this novel pathway. In addition to augmin subunits, our expression screen also identified another uncharacterised protein. Experiments are underway to characterise this novel protein in human cells and to test whether it also plays a role in the augmin pathway.

Scientific output

Publications

Haren L, Stearns T and Lüders J. Plk1-dependent recruitment of gamma-tubulin complexes to mitotic centrosomes involves multiple PCM components. *PLoS One*, 4(6), e5976 (2009)

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Research networks and grants

Microtubule organizing centers and microtubule nucleation in mitosis

European Commission, PEOPLE-2007-4-3-IRG (2008-2012)
Principal investigator: Jens Lüders

Organización molecular de los sitios de nucleación de microtubulos centrosómicos y no centrosómicos

Spanish Ministry of Science and Innovation, BFU2009-08522 (2009-2012)
Principal investigator: Jens Lüders

Collaborations

Composition and regulation of the human γ TuRC

Carne Caelles and Joan Roig, Molecular Medicine Programme, IRB Barcelona (Barcelona, Spain)

Recruitment of γ -tubulin complexes to mitotic centrosomes

Andreas Merdes and Laurence Haren, Institut de Sciences et Technologies du Médicament de Toulouse, Centre National de la Recherche Scientifique/Pierre Fabre (Toulouse, France)