



Centrosome Biology

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The work in our laboratory focuses on the centrosome, an organelle best known for its role as a major microtubule organising centre. Emerging evidence suggests that the centrosome also acts as a communication hub that spatially concentrates diverse signalling pathways. While centrosome number, structure and function are carefully regulated within healthy cells, tumours display a multitude of centrosomal abnormalities. Such anomalies can disrupt microtubule organisation as well as impede essential signalling cascades. The question remains, however, as to whether centrosome dysfunction is a cause or a consequence of tumourigenesis. Our primary objectives are to probe the function of the centrosome in genomic instability, cell polarity and in tumour formation.

Multipolar spindles and genomic instability

The centrosome determines the temporal and spatial distribution of microtubule networks in most animal cells. In proliferating cells the duplication, separation and microtubule nucleation capacity of the centrosome are tightly linked to the cell cycle. Centrosome duplication takes place simultaneously with DNA replication, ensuring that at the time of mitotic entry, cells have two centrosomes available to form the poles

of the bipolar spindle. Excess numbers of centrosomes and perturbed microtubule dynamics are both important causes of multipolar spindle formation, a common feature of cancer cells (Gergely et al. *Genes Dev.* 2003; 17:336; Gergely and Basto *Genes Dev.* 2008; 22:2291). When a diploid cell divides in a multipolar manner, most resulting daughter cells die because they inherit an incomplete genome. Therefore, introducing multipolarity could be an efficient way to kill tumour cells and could be exploited in future therapies. It is important to remember, however, that multipolar divisions of polyploid cells could result in aneuploid albeit viable cells. Therefore, at the same time as being a lethal weapon against diploid cells, multipolar cell divisions of polyploid cells have the potential to initiate genomic instability, hence contributing to tumourigenesis as well as to the development of drug resistance.

Ch-Tog guards against multipolarity

The colonic hepatic tumour overexpressed gene (ch-Tog) is the human homologue of a highly conserved family of microtubule-stabilising proteins. Most members of this family localise along microtubules as well as at the centrosome, but their role at the centrosome is unclear.

To address the function of ch-Tog in human cells, we used small interfering RNA duplexes to alter its expression levels (Gergely et al. *Genes Dev.* 2003; 17:336; Barr and Gergely *Mol. Cell Biol.* 2008; 28:7199). Cells depleted of ch-Tog spend up to 4–14 hours in mitosis trying to align chromosomes and stabilise a metaphase plate. After failing to do so, they exit catastrophically and give rise to multinucleated cells. Moreover, in the absence of ch-Tog, the majority of mitotic cells contain multipolar spindle arrangements. We have recently demonstrated that despite their appearance, these cells contain two intact centrosomes (Barr and Gergely *Mol. Cell Biol.* 2008; 28:7199). Our studies also revealed that cells lacking ch-Tog are defective in establishing dynamic centrosomal microtubule arrays. In particular, microtubules within ch-Tog-depleted spindles are fewer, shorter and more

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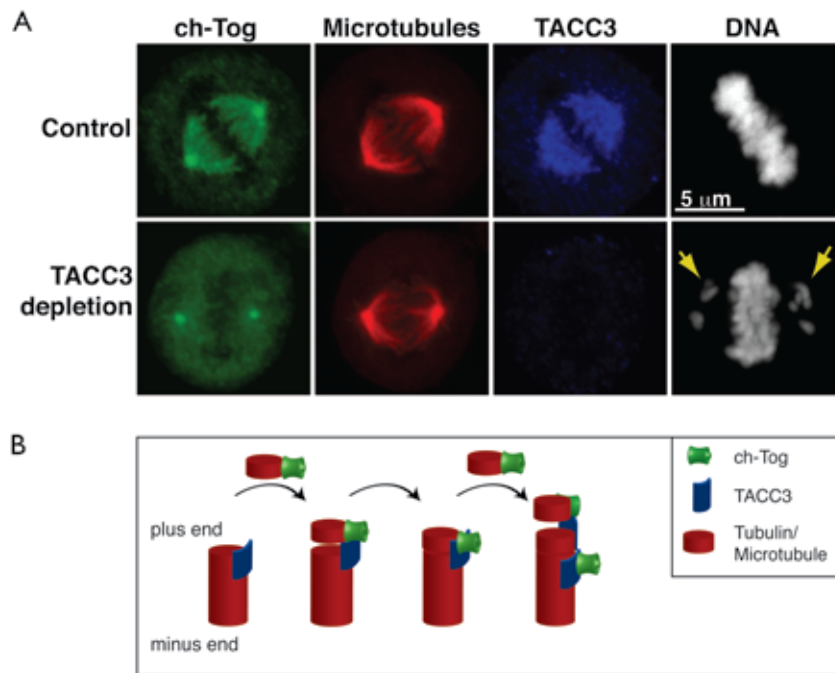


Figure 1. (a) Ch-Tog (in green) localises to the spindle poles and to spindle microtubules in a control cell (top panels). Depleting TACC3 protein displaces ch-Tog from spindle microtubules, but it does not perturb its localisation to the spindle poles (bottom panels). Unaligned chromosomes (arrows), a sign of abnormal mitotic spindle microtubules, are visible in the TACC3-depleted cell. (b) Schematic model of how ch-Tog and TACC3 promote the polymerisation of microtubules (red). Ch-Tog (green) binds to tubulin dimers in the proximity of microtubules. TACC3 (blue) facilitates the recruitment of ch-Tog to microtubule plus ends. In addition, the interaction between TACC3 and ch-Tog could also serve to maintain ch-Tog in the proximity of growing microtubule plus ends.

long-lived than normal. Furthermore, these microtubules do not seem to exert tension across kinetochore pairs of sister chromatids, which explains why chromosomes fail to align on the metaphase plate in the absence of ch-Tog. Our observations suggest an essential role for ch-Tog in providing a dynamic mitotic spindle, a prerequisite for faithful chromosome segregation and successful cell division.

TACC3 protein regulates the activity of ch-Tog

Similarly to ch-Tog, its binding partner, the transforming acidic coiled-coil containing protein 3 (TACC3), is also upregulated in tumours. Unlike ch-Tog, which is centrosomal throughout the cell cycle, TACC3 is only expressed during mitosis, hence raising the possibility that TACC3 regulates ch-Tog in the mitotic spindle poles. Our most recent data indicate that TACC3 function is important to promote efficient microtubule elongation in early mitosis (Barr and Gergely *Mol. Cell. Biol.* 2008; 28:7199). When TACC3 is absent, ch-Tog levels are much reduced on centrosomal microtubules and chromosomes do not align efficiently on the metaphase plate (Figure 1a). We propose that TACC3 plays a particularly important part in loading and maintaining ch-Tog in the proximity of microtubule ends, hence facilitating their growth away from the centrosome (Figure 1b). It is clear that TACC3 function is intricately linked with that of ch-Tog. However, ch-Tog also interacts with two additional members of the TACC family of centrosomal proteins, TACC1 and TACC2. As the levels of *tacc1* and *tacc2* genes are dysregulated in many cancers, it is essential to find out if TACC1 and TACC2 are also involved in mitotic spindle assembly.

To address this point we have used the chicken B-cell line,

DT40, which allows the creation of isogenic knockout cell lines via homologous gene targeting. We have disrupted the *tacc1* and *tacc2* genes alone and in combination. Our preliminary results suggest that while TACC1 and TACC2 play only minor roles in mitotic spindle assembly, they seem to affect the distribution of ch-Tog and TACC3 on mitotic spindle microtubules. Experiments are underway to assess the nature and purpose of the molecular interactions between the TACC proteins and ch-Tog. An attractive hypothesis is that TACC1 and TACC2 negatively control the binding of TACC3 to ch-Tog. If this is true, a delicate balance could exist in the expression of these proteins that maintains effective and timely spindle assembly in normal cells, a process that might break down during malignant transformation.

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