Eppendorf Award for Young European Investigators

Assembling complex biological structures

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Introduction by Mónica Bettencourt-Dias

My work on the centrosome, which is the primary microtubule-organizing centre in animal cells, started when I was a post-doctoral researcher in the David Glover laboratory at the Cambridge University Department of Genetics. Subsequently, I started my laboratory at the Instituto Gulbenkian de Ciencia (IGC) in Oeiras, close to Lisbon, Portugal, in 2006 (http://sites.igc.gulbenkian.pt/ccr). Our research focuses on the regulation of cell-cycle progression in normal development and cancer. We are particularly interested in the roles of centrosomes and cilia, as little is known about their biogenesis or how it might go awry in human disease. In our research, we use an integrated approach, combining studies in the fruit fly (*Drosophila melanogaster*), and normal and cancerous human cells, along with bioinformatics and mathematical modelling. We predict that an understanding of the formation and function of centrosomes and cilia will generate new markers in cancer and ciliary diseases, and will provide novel therapeutic targets. I was very happy when I heard that I had received the Eppendorf Award for Young European Investigators, as it recognized our work and gave visibility to our science. This is crucial when starting a group in a small country, such as Portugal, where investment and productivity in science have come about relatively recently. The IGC is a recently renovated international research institute with excellent facilities, where truly multidisciplinary work is promoted. Perhaps the Eppendorf Award will motivate people throughout the world to consider Portugal, in particular the IGC, for their studies. In the following article, we describe some of our research in the context of developments in the field.

Of centrioles, basal bodies and centrosomes

What is the common thread linking the movement of spermatozoa, the sensing of light by our eyes and the cell-division apparatus of most of our cells? As implausible as it might seem, the structures that permit movement, light sensing and cell division are all made of microtubules, and are organized by the same organelle: the centriole or basal body (Fig. 1a). The presence of this nine-fold symmetrical structure in all branches of the eukaryotic 'tree of life' led to the suggestion of its existence in the last common eukaryotic ancestor. The basal body/centriole sets up the foundations for the axoneme, which forms the skeleton of cilia and flagella; it also participates in the formation of the centrosome[1] (Fig. 1a).

Although the details of these structures were revealed only recently with the advent of electron microscopy, their presence did not remain unnoticed by earlier cell biologists. Édouard Van Beneden and Theodor Boveri first described centrioles and centrosomes at the end of the nineteenth century using nematode eggs[2]. They suggested that these structures were important in setting up the cell-division apparatus (Fig. 1b, step 1(M)) and proposed that they were autonomous. Later, Boveri proposed that abnormal centrosome duplication would lead to an aberrant cell-division apparatus, which could result in cancer[3]. Since then, abnormalities of microtubule-organizing structures have indeed been observed in cancer and in a range of other human diseases, including cystic kidneys and retinal degeneration[1].

As centrioles and basal bodies are so important, their biogenesis should be highly regulated. Indeed, they duplicate only once every cell cycle (Fig. 1b, step 1), with one centriole (the 'daughter') forming close to an already existing centriole (the 'mother'; Fig. 1b, step 1(S)). However, numerous questions remain unanswered. How is centriole number controlled, what kick-starts their formation, how is their nine-fold symmetry defined, and how are their size and fate decided? What is their function? Because of their importance in cancer and other human diseases, there is an expectation that an understanding of the pathways involved in the regulation of the microtubule-organizing centre will help to generate new diagnostic and prognostic markers, and provide novel therapeutic targets.

SAK/polo-like kinase 4 (PLK4) is necessary for centriole biogenesis

In order to identify novel mechanisms involved in

Mónica Bettencourt-Dias is the thirteenth recipient of the Eppendorf Award for Young European Investigators, which recognizes talented young individuals working in the field of biomedical research in Europe. This is the first time that the Eppendorf Award has been presented to a researcher from the Iberian Peninsula. Mónica Bettencourt-Dias was born in Portugal in 1973. She entered the prestigious Gulbenkian Graduate Programme at the Instituto Gulbenkian de Ciência (IGC), Portugal, and did her Ph.D. at University College London in the UK under the supervision of Professor Jeremy Brockes. She then moved to the University of Cambridge in the UK, where she undertook postdoctoral research on cell-cycle regulation and centrosome function with Professor David Glover. Since 2006, Mónica Bettencourt-Dias has led an active research group at the IGC. The members of her laboratory use an integrated approach to study centrosome biogenesis and function in *Drosophila* and human cells. Here, Mónica Bettencourt-Dias describes, for a wider audience, the work that led to her receiving the Eppendorf Award, which she places into context within the broader research field.

The Eppendorf Award is presented in partnership with *Nature*. An independent jury of scientists under the Chairmanship of Kai Simons (Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany) selects the Eppendorf Award winner, and *Nature* and Eppendorf have no influence on the decision (http://www.eppendorf.com/awards).

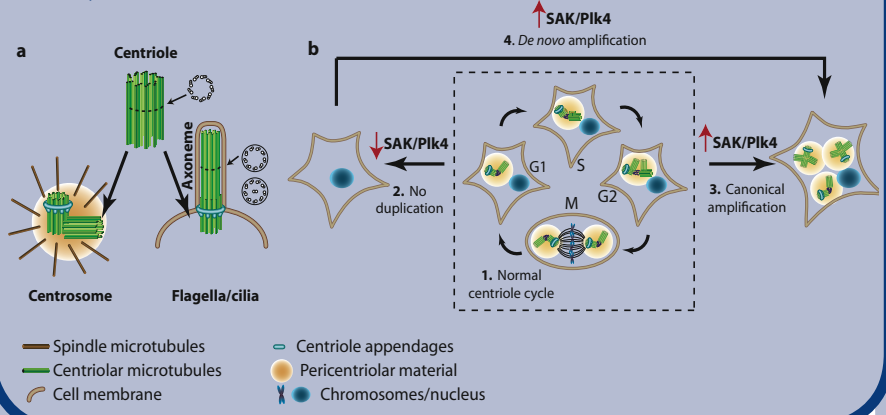

Figure 1. Centrioles, basal bodies and control of centriole number. **a**, Centrioles/basal bodies are composed of nine triplets of microtubules. These structures can function either as a centriole within a centrosome or, tethered at the membrane, as a basal body template for the formation of axonemes. The centrosome comprises two centrioles: a mother, with appendages (blue circle), and a daughter. It also has pericentriolar material (PCM; orange shading), which nucleates and anchors microtubules. **b**, Centrosomes undergo duplication once every cell cycle (step 1). A new procentriole is formed next to each existing centriole in S phase. In mitosis (M) each centrosome recruits PCM and forms one of the poles of the spindle. SAK/polo-like kinase 4 (PLK4) is an essential player in centrosome duplication (steps 1–4). Its absence leads to lack of duplication; if cells divide further, this leads to a dilution of centrosome number (step 2). Excessive amounts of SAK/PLK4 lead to centriole amplification[5,6,15–17] (step 3). In the absence of centrioles, these structures can form *de novo* (step 4)[13–15].

cell-cycle regulation, and centrosome function and biogenesis, we used RNA interference to screen quantitatively all of the fruit fly protein kinases for a cell-cycle function[4]. This was motivated by the high degree of evolutionary conservation of this protein family and its well-known role in signalling. We and others subsequently showed that one of those kinases, SAK/PLK4, which is a protein involved in hepatocellular carcinoma, is essential for centriole biogenesis in fruit flies and human cells[4–6] (Fig. 1b, step 2). In the absence of this kinase, fruit flies have few centrioles, and, hence, few centrosomes, cilia and flagella. We were surprised to see that fruit flies are able to develop under these conditions and that their somatic cell divisions are not disrupted, presumably because they use alternative pathways to make their cell-division apparatus[1,6,7]. However, they are sterile and highly uncoordinated, which are phenotypes associated with problems of flagella and sensory cilia[1,6,7]. Our results were further verified in other mutants that lack centrioles[7,8], suggesting that their main role is to template the axonemes of cilia and flagella[9]. As there is always an exception to the rule, some specialized cell divisions suffer as a result of the lack of centrioles, suggesting that tissue-specific constraints have selected for different contributions of centrosome-independent and centrosome-dependent mechanisms in cell division[8,10,11,12]. This heterogeneity should be taken into account when reaching an understanding of, and designing drugs that target, cell division.

Kick-starting centriole formation: self assembly plays a role

Boveri and Van Beneden originally proposed that centrioles must be capable of self-replication[2]. However, this assumption was questioned later on, as centrioles can form *de novo* in both naturally-induced and experimentally-induced acentriolar cells in the eggs of parthenogenic insects, *Chlamydomonas* (green algae) mutants and human tissue cultures[2,9,13,14]. Under such conditions, many centrioles can form at the same time, unlike in the controlled canonical pathway, where only one is formed close to each mother and just once per cell cycle (Fig. 1b, step 1). The realization that centrioles can form *de novo* demonstrated that these structures do not self replicate; indeed, a centriole template is not necessary for their formation. So, why do daughter centrioles appear next to their mothers? While observing embryos that overexpressed SAK/PLK4, we stumbled upon a notable phenotype. High levels of SAK/PLK4 were driving the formation of many more centrosomes than would normally be seen in a wild-type embryo at a similar stage. We consequently decided to look at unfertilized eggs. Centrioles are lost during oogenesis, so the egg does not have any of these structures. They are normally supplied by the spermatozoa upon fertilization, as there is a basal body/centriole at the base of the flagella. We were amazed to see that overexpression of SAK/PLK4 was sufficient to make centrioles *de novo* in unfertilized eggs[15,16] (Fig. 1b, step 4). We also showed that the same molecules are involved in canonical and *de novo* centriole biogenesis[15]. However, centrioles form faster next to a mother centriole than *de novo*[13–15]. This led us and others to suggest a model in which centriole biogenesis is a template-free self-assembly process that is locally triggered by SAK/PLK4 (refs 15,17). The role of the mother centriole is therefore not one of a *bona fide* 'template' that is replicated; rather, it acts as a platform where regulatory and structural centriole-assembly molecules meet, thereby catalysing and regulating daughter-centriole assembly. Recent data suggest that the ability of the mother centriole to recruit pericentriolar material (PCM) catalyses daughter-centriole formation close-by[18,19]. Perhaps that leads to microtubule-mediated recruitment of centriole precursors, thereby reducing their availability and consequent centriole formation elsewhere in the cell. This places PCM proteins high up in the molecular pathway leading to centriole formation. Indeed, early descriptions of centriole/basal body duplication indicated a role for an electron-dense material in centriole formation, which could correspond to the PCM. Moreover, γ-tubulin, which is a PCM and centriolar protein, has been implicated in centriole biogenesis in a range of species[1,17,19] (Fig. 2).

How centrioles are formed: defining the nine-fold symmetry

The structure of the centriole is amazingly conserved throughout the eukaryotic tree of life. How is its nine-fold symmetry defined? The first described intermediate in centriole assembly showing a nine-fold symmetry is the cartwheel, which consists of a central hub and nine spokes (Fig. 2c,d). In *Paramecium* and *Chlamydomonas*, this structure is reported to form in association with the electron-dense material (Fig. 2). However, the cartwheel can also self-organize *in vitro* in a solution with basal body components[20], suggesting that its constituent

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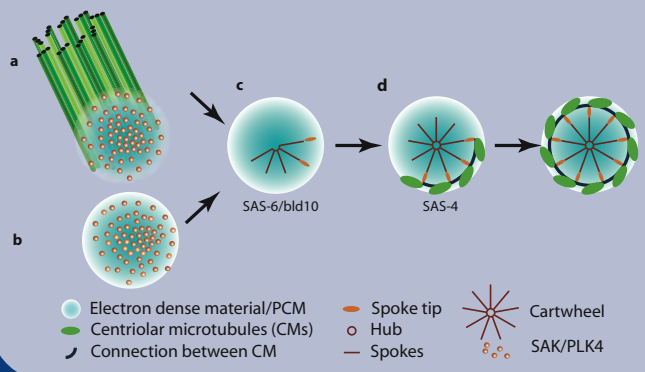
molecules have intrinsic properties that dictate its nine-fold symmetry. Only recently have components of this mysterious structure been identified, such as basal body-deficient protein 10 (Bld10) and spindle assembly-defective protein 6 (SAS-6; Fig. 2)[1,21–23]. We and others have observed that mutants in these molecules often fail to form centrioles in a range of species[1,8,10,11,16,17,22–24]. The conservation of these molecules is so great that, while presenting electron micrographs of centrioles from SAS-6 *Drosophila* mutants at a meeting, we discovered that they were similar to mutant centrioles/basal bodies of *Chlamydomonas*, which is evolutionarily separated from the fruit fly by 2 billion years; not surprisingly, this was the *Chlamydomonas* SAS-6 mutant. We observed abnormal centrioles, with no visible cartwheel, some of which lacked a few triplets, whereas others displayed an abnormal structure[8,22,23]. These results reinforce the importance of the cartwheel in establishing the nine-fold symmetry. The presence of some close-to-normal centrioles displaying no cartwheel suggests that other structures might also play a role in defining their architecture. Perhaps the PCM, the microtubule triplets and their connections have some self-assembly properties, and some structural constraints that normally act in coordination with the cartwheel, to enforce the nine-fold symmetry (Fig. 2).

Figure 2. Model for the assembly of a centriole/basal body. a,b, SAK/polo-like kinase 4 (PLK4) triggers centriole formation close to the mother centriole **(a)** or *de novo* **(b)** in the absence of this structure[15,16]. One of the first steps reported in centriole assembly is the formation of an electron-dense material (depicted in blue)[1], which might be the PCM, playing a role in the recruitment of centriole components. **c,** These molecules assemble the cartwheel, which defines the nine-fold centriolar symmetry. Spindle-assembly defective protein 6 (SAS-6) and basal body-deficient protein (bld10)[22] have a crucial role in the assembly of the cartwheel and centriole biogenesis[8,23,24]. **d,** Centriole microtubules are then tethered to this structure, a process that might be controlled by bld10 (ref. 22) and SAS-4 (refs. 19,24).



Future of centriole research

Centriole research started more than a century ago. The advent of electron microscopy led to a burst of research on this structure. Recently, the realization that they are misregulated in cancer and several other diseases, and the discovery of molecules involved in their formation, have led to a revival of this field. We have new tools with which to understand the architecture and the regulation of such complex structures, as well as how they might go awry in human disease. The use of functional genomics and proteomics has brought to light many new molecular players in the biogenesis and function of centrosomes and cilia. Comparative genomics within the framework of evolutionary analysis and better imaging technologies will help to improve our understanding of these structures and their role in human disease.

We thank the members of the Bettencourt-Dias laboratory, David Glover, Melanie Gatt, Adelaide Carpenter, Giuliano Callaini, Maria Riparbelli, Ryoko Kuriyama, François Balloux, Alex Dammerman and Polgen/Cyclacel for their collaboration and discussions of the work reported here. We also thank the Fly and Cilia clubs, and the Computational Genomics Laboratory at the IGC, for discussions. We apologize to all the authors whose work we could not cite due to space restrictions.


Kai Simons (Chairman of Eppendorf Award Jury) and Mónica Bettencourt-Dias

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