**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 Ciência (IGC) in Oeiras, close to Lisbon, Portugal, in 2006 (http://sites.igc.gulbenkian.pt/erc). 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 spermatocytes, 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 organic entity: 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

(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. Edouard Van Beneden and Theodor Boveri first described centrioles and centrosomes at the end of the nineteenth century using nematode eggs. 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. 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.

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 makers, and provide novel therapeutic targets.

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

In order to identify novel mechanisms involved in the regulation of centrosome biogenesis, we studied centriole duplication in the fruit fly, Drosophila melanogaster, and normal and cancerous human cells. In normal cells, centriole duplication is required for the normal cell-division apparatus (Fig. 1b, step 1(M)).

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 Brookes. 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).*
Kick-starting centriole formation: self assembly plays a role

Boveri and Van Beneden originally proposed that centrioles must be capable of self-replication. 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. 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. At the origin of centriole/flagella, it 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 bound ‘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. 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 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 (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, suggesting that its constituent...
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). 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\cite{11,12,22,23}. 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 concert with the cartwheel, to enforce the nine-fold symmetry (Fig. 2).

**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, has revived interest in 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.

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