

# The centrosome in cell cycle and signal transduction pathways

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Classically the centrosome is known as the microtubule organising centre in higher eukaryotic cells, coordinating microtubule-dependent functions. Although correct, this is only part of the story of what a centrosome does. The multitude of the microtubule-dependent functions in a cell (including intracellular transport, cell shape and polarity determination, motility, mitosis, cytokinesis) demands that the microtubule organising centre is both regulated by and regulating cell cycle and signal transduction events. In this sense the centrosome is "more" than a microtubule-organising centre, playing an active role also in cell cycle progression, stress response and checkpoint control, via interactions with numerous signal transduction molecules (Lange 2002). For example damaged or incompletely replicated DNA triggers centrosome destruction in *Drosophila* early embryos (Takada et al. 2003) suggesting an interconnection of the centrosome to DNA damage signalling pathways. In addition, following destruction of the centrosome in cultured cells by laser beam irradiation, a mitotic spindle is formed, chromosomes are separating, but the cells undergo abnormal cytokinesis and fail to exit the G1 phase of the cell cycle (Khodjakov & Rieder, 2001). This data suggests that the centrosome has a primary role in cytokinesis and cell cycle progression rather than in spindle formation. Indeed, there is not an absolute requirement for cells to possess a centrosome to form spindles. Spindles can be formed without centrosomes in naturally occurring systems such as in *Drosophila* oocytes (Theurkauf & Hawley, 1992) or *in vitro* systems (Heald et al. 1996) where motor proteins rather than centrosomes have been proposed to mediate spindle formation. This does not mean, however, that centrosomes are superfluous for spindle and microtubule organisation, because when centrosomes are present

they provide a dominant site for microtubule nucleation and play a role in positioning the spindle correctly in the cell (Rieder et al. 2001).

Why does this cell organelle respond to global cellular signalling cues after all? One answer might be that it is part of a complex cellular protection mechanism that brings the cell cycle to a stop when environmental conditions are harmful. At the moment we don't know how such signal transduction pathways are interconnected to the centrosome and which centrosomal proteins are substrates for signal transducer molecules. For sure, some of the answers to these questions lay in the molecular composition and organisation of the centrosome and thus reflecting its multiple functions. In this respect, good progress has been made in

the elucidation of the centrosome composition (Andersen et al. 2003; Bornens 2002; Doxsey 2002; Wigge et al. 1998) however, we still don't know the majority of components of the centrosome. If we really want to untangle the molecular spider web of signal transduction, cell cycle regulation and centrosome function then, no doubt, we have to tackle this problem. Luckily, we have now new technologies and knowledge at our hands and the next section will give an overview on how we are addressing this problem in our laboratory.

## A systematic screen of centrosome composition and function

In our work we are focusing on three fundamental questions: (1) How is the centrosome integrated in cell cycle and

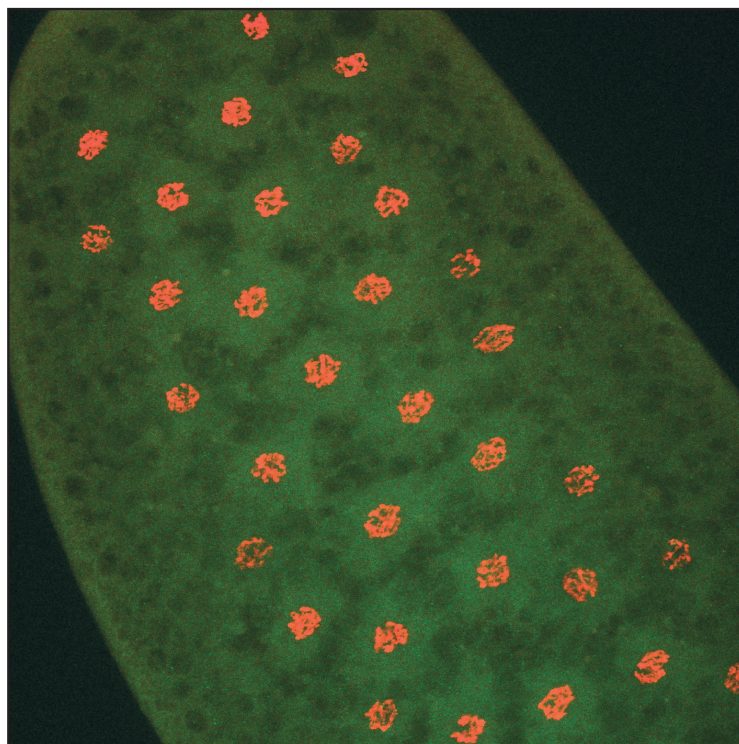
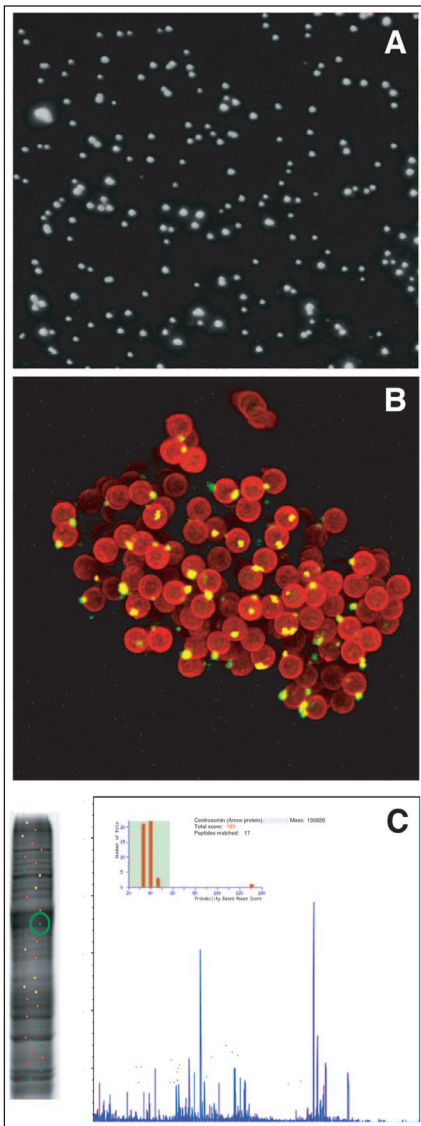


Fig. 1: Immunofluorescence microscopy image of the preblastoderm stage of a *Drosophila* embryo labeled with an antibody against phosphorylated histone H3. The condensing prophase chromosomes, shown in red, demonstrate the synchronous duplication and division cycles in the early embryo syncytium.



**Fig. 2:** (A, B) Immunofluorescence microscopy images of isolated *Drosophila* centrosomes labelled with an anti- $\gamma$ -tubulin antibody. (A) Centrosomes isolated by sucrose density centrifugation. (B) Centrosomes further purified by immuno-affinity isolation on magnetic beads coupled to antibodies. Beads are shown in red, centrosomes in yellow. (C) MALDI mass spectrometry fingerprinting of the centrosomal preparations isolated from coomassie-stained gels. The most abundant band identified (green circle) is the protein CNN. The diagram shows the hits of the peptide mass distribution that was used for the identification of the CNN protein, after tryptic digest (work in collaboration with Gustavsson & Gobom).

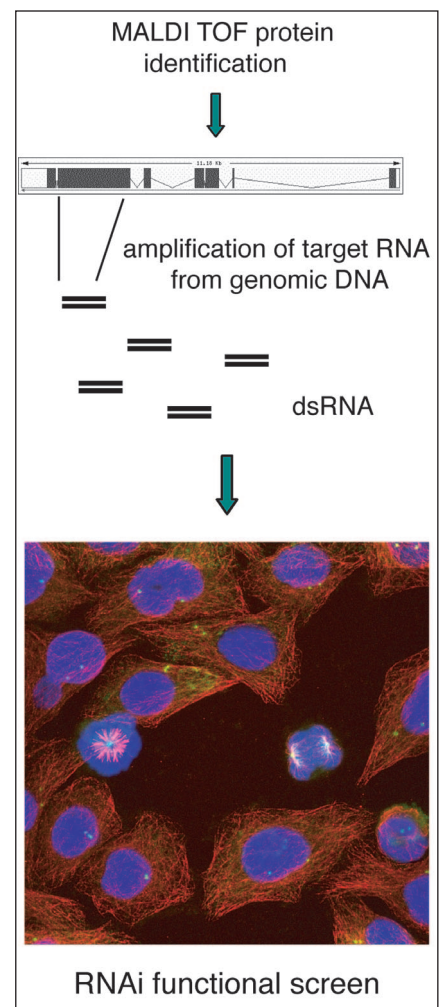
cell signalling pathways? (2) What holds the structure of the centrosome together and constitutes the scaffold for the assembly of functional protein complexes onto the centrosome? (3) Which molecular events mediate the change of microtubule nucleation capacity of the centrosome during the cell cycle?

In order to answer these questions we first need to have more complete information of the molecular make-up of the centrosome. The centrosome has so far escaped detailed biochemical and functional characterisation due to the small quantities of protein obtained through organelle isolation methods and due to lack of genetic approaches to study its function in higher eukaryotes. However, new and improved methods, such as better isolation techniques (Lange et al. 2000a; Müller, Lehmann & Lange unpublished), improved sensitivity of mass spectrometry (Yarmush & Jayaraman, 2002) and RNA interference (Clemens et al. 2000) are making a systematic biochemical and functional dissection of this organelle feasible.

To address these questions we initiated a systematic analysis of the molecular components of the centrosome using an affinity-purification isolation method in combination with mass spectrometry protein identification. We are using *Drosophila* as a model system for this work. Why *Drosophila*? First, *Drosophila* is an organism that is genetically manipulable and its genome has been sequenced. Therefore, we can study the function of individual molecules in the context of tissue and throughout development, providing functional clues that would be absent in a cell culture system. Secondly, the syncytial (cell membrane free) early embryonic stage of *Drosophila* is well suited for the isolation of centrosomes (Figure 1): these embryos are essentially tiny "bags", not constrained by cellular borders, full of cell organelles and native protein complexes that are easily amenable to biochemical isolation. Thirdly, growing large fly populations we can obtain per day 30–50 g of embryo starting material, required for the systematic mass isolation and characterisation of centrosomes.

To isolate centrosomes we developed an immuno-affinity approach using magnetic beads (Figure 2). This method has the advantage that most contaminants (which otherwise co-migrate with centrosomes in biochemical fractionation methods) are removed. In this way we have identified over 60 centrosomal protein candidates. We are screening those candidate molecules for their relevance by RNAi in *Drosophila* cultured cells. This is a fast, extremely efficient and

cost effective method to knock down protein expression from a gene of known sequence (Figure 3). The obtained phenotypes are classified according to their relevance for centrosome function and integration in signal transduction pathways. Subsequently, relevant proteins are cloned, their localisation is verified through GFP-tagged expression and characterised in flies. Furthermore, with the biochemical characterisation of these proteins we identify specific protein-protein interactions between novel or known centrosomal and signalling molecules. In summary, we developed a very efficient functional screen that integra-



**Fig. 3:** Schematic flow diagram of the RNAi functional screen: Once centrosomal proteins have been identified by MALDI mass spectrometry, dsRNA is generated by PCR amplification from genomic target DNA sequences. dsRNA is used for the protein "knock-down" assay in cultured *Drosophila* cells. Phenotypes are analysed by immunofluorescence microscopy: centrosomes are labelled with anti- $\gamma$ -tubulin antibodies (green), DNA is stained with DAPI (blue) and microtubules with anti- $\alpha$ -tubulin antibody (red).

tes the direct biochemical identification of novel centrosomal proteins with their functional characterisation. The following sections describe two examples of the application of this approach.

### Centrosome function depends on Hsp90

One of the centrosomal proteins identified, heat shock protein 90 (Hsp90) is a molecular chaperon maintaining the stability and activity of signalling molecules (Lange et al. 2000a). Hsp90 is present in the *Drosophila* centrosome in different developmental and cell cycle stages, in the early embryo as well as in larval spermatocytes. Moreover, Hsp90 localisation at the centrosome is conserved in all vertebrate cells from chicken to human. We demonstrated that Hsp90 is a core centrosomal protein necessary for the maintenance of centrosome function and integrity, required for the fidelity of chromosome segregation and cell cycle progression (Lange et al. 2000a). Abrogation of Hsp90 function in *Drosophila* or in mammalian cells results in abnormal centrosomal and mitotic phenotypes. In particular, centrosomal separation and maturation (i.e. the increase in pericentriolar material during the cell cycle; Lange et al. 2000b) are affected, resulting in cells with fragmented and abnormal in size centrosomes. Furthermore, lack of Hsp90 function triggers abnormal spindle formation with the subsequent chromosome segregation resulting in a high percentage of aneuploid and polyploid cells. In summary, Hsp90 has an essential role in centrosome function and mitosis in higher eukaryotic cells.

### Integration of microtubule organisation in the cell cycle machinery

We extended our functional assay to Cdc37, the kinase-targeting unit of Hsp90 that is involved in the functional maturation of protein kinases. Abrogating Cdc37 function (either by mutations or through "knock-down" RNAi experiments) in *Drosophila* demonstrated that this Hsp90 co-factor is essential for mitotic progression and cytokinesis (Lange et al. 2002). Moreover, our experiments showed that Cdc37 is essential for chromosome condensation, chromosome segregation and formation of a central spindle. Because Cdc37 is known to tar-

get kinases to Hsp90, it was likely that the observed phenotypes could be brought about by the inactivation of one or more kinases. Indeed, the inactivation of Hsp90 and Cdc37 lead to degradation of Aurora B kinase in mammalian cells and in *Drosophila* (Lange et al. 2002). Hence, the requirement of the Cdc37/Hsp90 complex for mitotic processes and microtubule organisation is evolutionary conserved.

### Outlook

Our approach integrates the direct biochemical identification of novel centrosomal proteins with their functional characterisation. This combination successfully exploits the advantages of the excellent biochemical and genetic system of *Drosophila*. So far, we have identified over 60 candidate centrosomal proteins which are currently being characterised. With this system at hand, the combination of cell biology approaches with the expertise and facilities for high-throughput analysis at the Max-Planck Institute for Molecular Genetics in Berlin provides the opportunity to obtain a genome wide view of protein complex structure and *in vivo* function. In addition, the structural characterisation of centrosomal protein complexes is ongoing, as part of the Ultra-Structural Network (USN) in Berlin/Berlin-Brandenburg, which is aiming at the high-throughput isolation and structural analysis of protein complexes via cryo-electron microscopy. The elucidation of centrosome structure and function will help us to understand the molecular events and the regulation of cell division and signal transduction in flies and humans.

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