Tracking of centriole inheritance in *C. elegans*
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**Figure 1:** Centrosomes can be successfully tracked within *C. elegans* applying photo-conversion to Dendra2::SAS-4 tagged centrioles. (A) Schematic representation of the UV photo-conversion experiment to analyze the inheritance of Dendra2::SAS-4 tagged centrioles in the ABprpppa cell lineage. Vertical lines indicate the progression of time; horizontal lines indicate cell division events. In this study, grandmother cell refers to: ABprpppa; mother cell: ABprppppaa or ABprppppap; daughter cell: ABprppppaaa, ABprppppapa, or ABprppppapp. (B) Schematic representation of the sas-4p::dendra2::sas-4::sas-4 construct coding for the Dendra2::SAS-4 fusion protein. The *dendra2* coding sequence was fused to the endogenous sas-4 coding region, flanked by the endogenous sas-4 regulatory regions. (C) DIC image of a *C. elegans* embryo shortly after the ABprpppa cell division took place (left panel). The right panel shows DIC and fluorescence images of the anterior daughter cell (ABprpppaaa), which inherited a centrosome comprising a younger...
unconverted (cyan, white arrow head) and an older converted (magenta, filled white arrow head) centriole. In the merge channel, the young and old centrioles are seen right next to each other as expected for a centrosome before the onset of a new round of centrosome duplication. Scale bar: 5µm, 3µm in the insets. (D) Fluorescence micrographs of a strain expressing Dendra2::SAS-4 and GFP::PH. The expression of GFP::PH allows for clear detection of the cell boundaries and reliable scoring of the segregation of photo-converted centrioles. Images show a photo-converted centriole being inherited by the anterior daughter cell born from the ABprpppaa division. A magenta centriole is present only in the ABprppppaaa cell, but not in the ABprppppap cell. Only recordings that clearly showed a converted centriole in one daughter cell, but not its sister, were used for the analysis. Scale bars: 5µm. (E) Analysis of age-dependent centriole segregation in the ABprpppaa and ABprppppap cell divisions.

Description

The nematode *C. elegans* possesses a relatively small subset of centrosome proteins and hence, has emerged as an important model system in elucidating mechanisms of centrosome biogenesis and dynamics. The most basic factors of the centrosome assembly pathway were discovered and characterized in the worm. The centrosome consists of a pair of centrioles, surrounded by the pericentriolar material, and its duplication is strictly coupled to the cell cycle. In worms, the centriole duplication pathway comprises the protein SPD-2Cep192, which recruits the kinase ZYG-1PLK4 to centrioles to initiate centriole assembly (O’Connell et al., 2001; Kemp et al., 2004; Pelletier et al., 2004). ZYG-1PLK4 in turn, recruits SAS-6sAS6, which in complex with SAS-5STIL, triggers the formation of the central tube (Dammermann et al., 2004; Delattre et al., 2004; Leidel et al., 2005; Kitagawa et al., 2009; Qiao et al. 2012; Hilbert et al., 2013; Lettman et al., 2013; Rogala et al., 2015). Subsequently, the coiled-coil protein SAS-4CPAP is stably incorporated into the centriole wall and assembles singlet microtubules around the forming centriole (Kirkham et al., 2003; Leidel and Gönczy, 2003, Balestra et al., 2015). SAS-7Cep29S is required for paddlewheel structure formation and recruits SPD-2Cep192 for a new round of centriole formation in the following cell cycle (Chang et al., 2016; Saurya et al., 2016; SugioKA et al., 2017). Due to the nature of the duplication of centrioles, there is always one older and one younger centriole present in a centriolar pair. Hence, in the subsequent cell division, daughter cells will inherit centrosomes carrying mother centrioles of different ages. Studies in *Drosophila melanogaster* and mammals have shown that mother centrosome and daughter centrosome can be segregated in a non-random manner during stem cell divisions and that this segregation pattern correlates with the fates of the daughter cells (Yamashita et al., 2007; Wang et al., 2009; Conduit et al., 2010; Januschké et al., 2011). Due to the invariant cell lineage of *C. elegans* and the possibility to follow individual cell fates, tracking centriole inheritance in regard to their age in worms could provide valuable information about the impact of centriole age on differentiation (Sulston and Schierenberg, 1983). However, factors that localize to one of the two centrioles in an age-dependent manner have not been identified in *C. elegans* to date, making it impossible to distinguish older from younger centrosomes in worms. To overcome the limitation of following age-related centrosome inheritance in *C. elegans*, we generated a strain in which centrioles are labeled with a photo-switchable marker. Once centrioles are photo-converted, they can be tracked over several cell cycles, and centrosome age can be distinguished after the second round of duplication (Figure 1A). The photo-switchable fluorescent protein Dendra originates from the octocoral *Dendronephthya sp.* The protein can be irreversibly converted from a green-to-red fluorescence state by exposure to visible blue or ultraviolet light. In this study, we made use of the bright and fast-maturing Dendra2 version of the protein, which was fused to the centriolar protein SAS-4CPAP and expressed under endogenous regulatory sequences (Figure 1B, Gurskaya et al., 2006, Ihara et al., 2011). SAS-4CPAP is stably incorporated into centrioles and shows no cytoplasmic exchange once centrioles are formed (Dammermann et al., 2004, Balestra et al., 2015). We did not notice any significant difference in embryonic lethality of the strain at 25°C. On average we found 1.4% embryonic lethality for *sas-4p::dendra2::sas-4* (n=1322 embryos); in comparison to 1.1% for a wild-type control strain (n=1692 embryos). In this study, we show that the older and younger centrosome can be successfully distinguished within cells of different lineages in *C. elegans* (Figure 1C).

L4 larvae were grown at 25°C overnight to adulthood. The next day, worms were dissected to collect embryos shortly after fertilization in H2O on a coverslip (Carl Roth GmbH; 18 x 18 mm, #1 thickness; Cat. no. 0657.2). Embryos were reversely mounted on a 4 % agarose pad on a microscope slide and sealed with petroleum jelly. The development of embryos starting at 4-cell stage was followed under a 4D-microscope at 25°C. The imaging of the cells of interest was performed simultaneously while recording. Images were taken using a Zeiss Axioskop 2 microscope equipped with epifluorescence and the Time to Live software from Caenotec. Differential interference contrast (DIC) micrograph Z-stacks were taken every 35 sec at 25°C. Fluorescent scans were taken as required. The Simi BioCell software was used for the lineage analysis (Simi Reality Motion Systems GmbH; http://www.simij.com) as previously described (Schnabel et al., 1997). Embryos were allowed to develop until shortly after the onset of the ABprpp cell division. Subsequently, embryos were exposed to UV light on a Zeiss Axioscop 2 microscope for photo-conversion of the Dendra2 fluorophore (conversion time: 15-17 sec; whole embryos were exposed to UV light, and all centrosomes present at this stage were photo-converted). All centrioles formed prior to photo-conversion display red fluorescence (magenta) after the exposure to UV light,
whereas centrioles formed after conversion display green fluorescence (cyan; Figure 1 A). Lineaging of the embryos was continued on the 4D microscope after photo-conversion.

As proof of principle, we analyzed the age-dependent inheritance pattern of centrioles in a strain expressing Dendra2::SAS-4, as well as GFP::PH, to reliably mark the outlines of individual cells. The cell lineage chosen was still easy to track but close to the final differentiation state. Centrioles were photoconverted in the grandmother cell ABprpppaa (Figure 1 A). The divisions of the daughter cells ABprpppaa and ABprppppp were followed to determine the segregation of the centrioles (Figure 1 D and E). Fluorescence images were taken after the divisions of the sister cells. The ABprpppaa daughter cell divides into equally sized granddaughter cells. Here, in 57 % of the divisions, the older converted centriole is inherited by the anterior cell, and in 43 % of the divisions by the posterior cell (n=7, Figure 1 E). The granddaughter cells deriving from the ABprppppp cell division are unequal in size, with the posterior granddaughter being smaller and fated to die. In 80 % of the divisions, the older converted centriole segregates into the anterior cell. In the remaining 20 % of the cases, the posterior cell inherits the converted centriole (n=5, Figure 1 E). Thus, despite what fate the granddaughter cells adapt, centrioles are segregated randomly in both lineages. Taken together, our approach allows the successful tracking of centrosome inheritance in the invariant divisions of the \textit{C. elegans} lineage.

\textbf{Methods}

\textbf{Request a detailed protocol}

We applied standard methods for DNA amplification, analysis, and manipulation. For PCR amplification, the Phusion® High-Fidelity DNA Polymerase (New England Biolabs) was used according to the manufacturer’s protocol. The \textit{sas-4} sequence and regulatory regions were amplified from \textit{C. elegans} genomic DNA and pAD154. The \textit{dendra2} nucleotide sequence was introduced upstream of the \textit{sas-4} coding sequence in the MosSCI vector pCF3350 to generate the TMD29 [\textit{sas-4p::dendra2::sas-4::sas-4}] plasmid (Figure 1 B). Cloning was performed via the sequence and ligation independent cloning (SLIC) method (Jeong \textit{et al.}, 2012) using the T4 DNA polymerase (New England Biolabs) and NEBuffer 2.1 (New England Biolabs). Sanger sequencing was applied to analyze DNA sequences. The TMD29 plasmid was integrated into the second chromosome of the \textit{C. elegans} genome by the universal MosSCI single-copy integration method (Frøkjær-Jensen \textit{et al.}, 2014, integration strain: EG6699 [\textit{ttTi5605; unc-119(ed3)}]) to generate the \textit{mikSi1} allele. Germline microinjection was performed as described by Mello \textit{et al.}. (1991) (Mello and Kramer, 1991). Image analyses were performed with Fiji/ImageJ 2.0.0 (Schindelin \textit{et al.}, 2012, \url{https://fiji.sc/}). To test for embryonic lethality, singled L4 worms were allowed to lay eggs at 25°C overnight. Total embryos laid and hatched worms were scored in three independent experiments. The N2 Bristol strain was used as control.

\textbf{Reagents}

\textbf{Nematode strains:}

N2 \textit{C. elegans} wild isolate

TMD42 \textit{mikSi1[sas-4p::dendra2::sas-4]II; unc-119(ed3)III}

TMD57 \textit{mikSi1[sas-4p::dendra2::sas-4]II; bcIs57[pie-1p::gfp::plcδph]}

Nematode strains were maintained at 15 °C under standard conditions (Brenner, 1974).

TMD57 will be made available on CGC.

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\textbf{References}


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