JCB Journal of Cell Biology

SPOTLIGHT

Fast and furious . . . or not, Plk4 dictates the pace

Simon Gemble and Renata Basto

In each duplication cycle, daughter centrioles grow to the same length as their mothers. Which mechanisms regulate this fidelity to maintain centriole length is not known. In this issue, Aydogan et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201801014) report a novel role for Polo-like kinase 4 (Plk4). They found that Plk4 functions in a homeostatic manner to balance growth rate and growth period to set the final centriole size.

Centrioles are highly conserved and structured microtubule-based organelles. Centrioles function as platforms for multiple functions: (A) During both mitosis and interphase, centrioles recruit and organize microtubule-nucleating factors and their adapters, the pericentriolar material, to form the centrosome, the major site for microtubule nucleation in animal cells. In mitosis, the centrosome facilitates the rapid generation of a bipolar microtubule array to segregate chromosomes in two daughter cells. Furthermore, the centrosome also contributes to mitotic spindle positioning, which is essential during tissue morphogenesis. (B) Centrioles behave as basal bodies to nucleate cilia or flagella with essential roles in cell signaling, polarization, and locomotion (Conduit et al., 2015).

The bipolar status of the mitotic spindle ensuring equal segregation of the chromosome set into two daughter cells requires that cells enter mitosis with only two centrosomes. This is achieved through control of centriole duplication along the cell cycle. During interphase, the master centriole regulator Polo-like kinase 4 (Plk4) is recruited to the two preexisting centrioles, also called mother centrioles. Superresolution microscopy research has revealed that Plk4 is first recruited in a ring configuration surrounding the mother centriole. Subsequently, Plk4 relocalizes in a dot-like structure, which most likely seeds the site for daughter centriole nucleation (Kim et al., 2013). Procentriole assembly is initiated at right angles of the preexisting mother centrioles thanks to the recruitment of Ana2/STIL and Sas-6 to form the first detectable structure: the cartwheel (Dzhindzhev et al., 2017). The cartwheel is located at the proximal site and is composed of a centrally located hub and nine radial spokes that dictate its ninefold symmetry. The spokes contact centriolar microtubules that are extended at the final steps of procentriole elongation (Conduit et al., 2015).

Interestingly, overexpression of any one of the core components of the Plk4-STIL–Sas-6 module is sufficient to promote the formation of more than one centriole per mother centriole (Arquint and Nigg, 2016), resulting in the generation of supernumerary centrosomes, a condition also known as centrosome amplification. Centrosome amplification is a hallmark of cancer cells and has been shown to be a tumor-initiating event in many different mode systems (Nigg and Holland, 2018). Overall, these findings highlight the importance of controlling centriole number as well as the levels of proteins involved in centriole duplication.

Although great progress has been made in the past 15 years in understanding the regulation of centriole number, less is known about centriole length control. Procentrioles normally grow to the same size as their mothers, maintaining stable centriole length over many generations. Importantly, abnormally long centrioles have been shown to be unstable structures that can provide additional microtubule-nucleating sites and promote genetic instability, illustrating the importance of maintaining correct centriole size at each division (Kohlmaier et al., 2009). However, the mechanism by which daughter centrioles grow to achieve the correct size was not known. To tackle this question, Aydogan et al. developed elegant tools to follow procentriole assembly and growth by live imaging in the early *Drosophila melanogaster* embryo. They report in this issue a novel function for Plk4 in controlling centriole length. Surprisingly, they provide evidence that procentriole lengthening can occur in two distinct ways: (1) fast procentriole growth occurs in a short period of time as high Plk4 activity results in rapid kinase inactivation, and (2) slow procentriole growth takes place for a longer period of time as Plk4 levels are more stably maintained. Overall, this study shows for the first time that Plk4 can function in a homeostatic manner to balance growth rate and growth period, determining final centriole size.

Aydogan et al. (2018) analyzed Sas-6 dynamics using 3D structured illumination microscopy–FRAP in GFP-tagged Sas-6–expressing embryos (Sas-6-GFP). These experiments rely on the detection of FRAP to ascertain the dynamic properties

Institut Curie, Paris Sciences et Lettres Research University, Centre National de la Recherche Scientifique, UMR144, Paris, France.

Correspondence to Renata Basto: renata.basto@curie.fr.

https://doi.org/10.1083/jcb.201802084

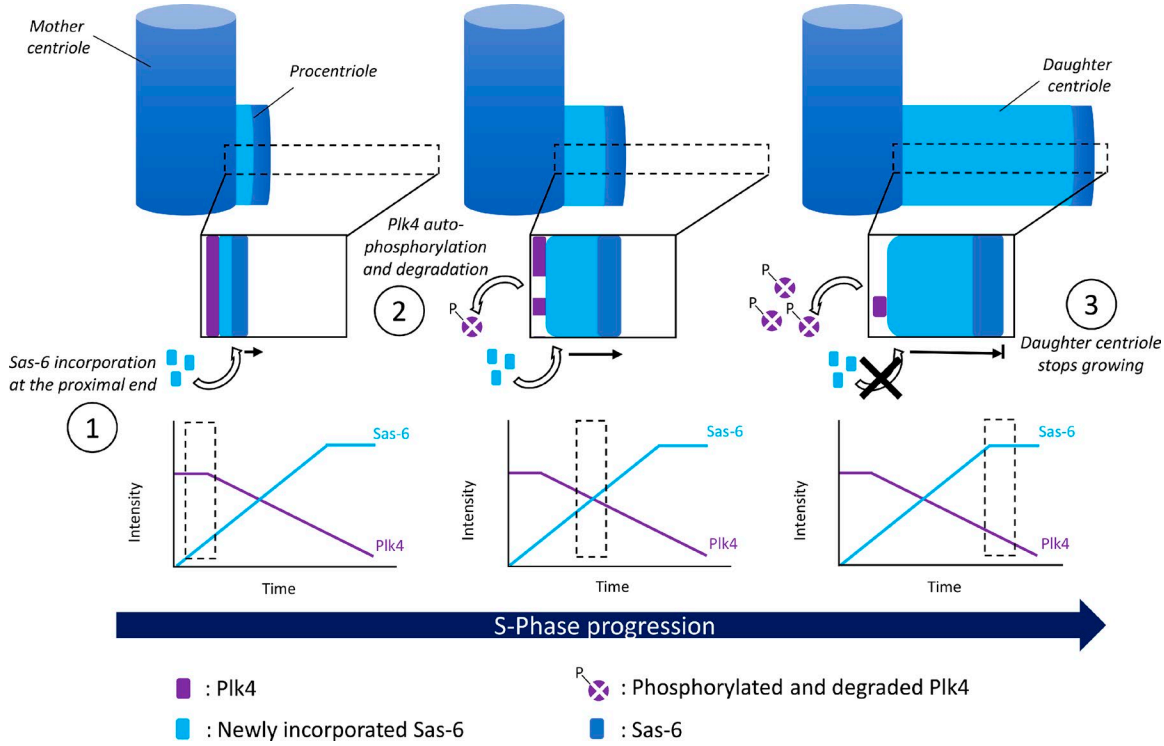

Figure 1. **Plk4 controls centriole size by regulating Sas-6 incorporation.** (1) Centriole growth relies on Sas-6 incorporation at the proximal end. (2) Throughout S phase, centriolar Plk4 levels decrease as a result of its autophosphorylation associated with its degradation. As Plk4 levels decrease, Sas-6 incorporation increases, which is associated with centriole growth. (3) However, when centriolar Plk4 levels decrease below a certain threshold, Sas-6 incorporation ceases.

of fluorescent molecules in living cells. Complete Sas-6–GFP photobleaching allowed Aydogan et al. (2018) to monitor de novo Sas-6–GFP recruitment (newly incorporated Sas-6) by fluorescence recovery and to conclude that Sas-6 is incorporated exclusively and irreversibly into the growing procentriole. This behavior established Sas-6 incorporation as a readout of procentriole growth kinetics. Analysis of hundreds of events of centriole duplication revealed that Sas-6–GFP is incorporated in the growing procentriole in a linear manner during S phase. Just before mitotic entry, however, Sas-6–GFP reaches a plateau that appears to correlate with the final growth period. Interestingly, Sas-6 also follows a period of linear growth before reaching a plateau in the early worm embryo (Dammermann et al., 2008), suggesting a conserved mechanism of Sas-6 incorporation in the growing procentriole. Importantly, these findings raise the obvious question of which signal leads to growth arrest at the end of S phase.

With the purpose of identifying this signal, Aydogan et al. (2018) first investigated whether S phase duration could influence centriole growth. During early *Drosophila* embryogenesis, S phase length gradually increases from one cycle to the next, allowing the comparison of procentriole growth parameters in S phases of increasing durations. Unexpectedly, they found that the growth rate (defined as the speed of Sas-6–GFP incorporation) decreased as S phase lengthens. This inverse correlation always generated a daughter centriole of equivalent size (measured by Sas-6–GFP fluorescence intensity at the end of the growing period). This inverse relationship between growth rate and growth period puts forward the hypothesis of the existence of a homeostatic mechanism regulating these two parameters in order to finally generate daughter centrioles of the right size at the end of S phase. Next, Aydogan et al. (2018) tested whether S phase length by itself can control daughter centriole growth parameters. However, using astute genetic tools to either extend or shorten S phase duration, they observed a lack of correlation between daughter centriole growth rate or growth period and S phase duration.

Because Plk4 plays major roles in centriole duplication, Aydogan et al. (2018) tested whether this kinase was also involved in centriole growth control. They generated flies that contained either reduced or increased Plk4 levels. Furthermore, they also included in their study flies that exhibited reduced kinase activity. Consistent with the inverse relationship between growth rate and growth period, they found that embryos containing reduced Plk4 levels still assembled centrioles of the correct size. The decrease in growth rate was compensated by an increase in growth period. Interestingly, in embryos with increased Plk4 levels or in embryos with reduced kinase activity, centriole size was affected. Indeed, in any of these conditions, Aydogan et al. (2018) found reduced centriole size. Importantly, this decrease did not seem to have the same origin. Although Plk4 overexpression did not compromise growth rate, it restricted growth period. In contrast, reduced kinase activity resulted in altered growth rate without influencing growth period. These results are coherent with a model whereby Plk4 activity can independently contribute to influence procentriole growth rate and growth period.

To gain insight into Plk4 activity and spatial distribution, Aydogan et al. (2018) generated flies expressing GFP-tagged Plk4 under the control of its own promoter expressed in the Plk4 mutant background. This strategy was essential to allow Plk4 detection without causing defects in centriole duplication. The lowest GFP-Plk4 levels were noticed at metaphase. During late mitosis and early S phase, Plk4 levels increased to reach a maximum before undergoing a gradual decrease as S phase proceeded. Interestingly, overlap of Plk4 kinetics seemed to contrast with Sas-6 behavior. Indeed, Sas-6 incorporation reached the plateau period when centriolar Plk4 levels were at their lowest. These observations put forward the hypothesis that the number of Plk4 molecules at the centriole functions as the mark for growth arrest. Plk4 has been described as a suicide kinase because it triggers its own degradation through autophosphorylation (Cunha-Ferreira et al., 2009; Rogers et al., 2009; Holland et al., 2010). Aydogan et al. (2018) proposed that the levels of Plk4 at the centriole are crucial to regulate the consequent rate of Plk4 recruitment and then its loss from the centriole. If the centriole contains higher Plk4 levels at the beginning of S phase, this will impact the Plk4 recruitment rate and thus the rate by which Plk4 is subsequently lost from the centriole. On the contrary, reduced Plk4 levels at the beginning of S phase will result in lower recruitment and loss rates. Thus, these data suggest that the initial recruitment of Plk4 could be sufficient to establish the centriolar Plk4 rate controlling Sas-6 incorporation period and centriole size.

To further understand how Plk4 regulates centriole growth, Aydogan et al. (2018) investigated the directionality of cartwheel assembly. Using RFP Asterless (Asl-RFP-Asl-), which is the *Drosophila* homologue of human Cep152, to label mother centrioles, they observed that SAS-6–GFP was incorporated closer to the mother centriole. These unexpected findings suggest that growth occurs preferentially at the proximal procentriole end, which, from a spatial configuration point of view, appears more difficult to achieve.

To conclude, the study by Aydogan et al. (2018) identified a novel mechanism responsible for setting daughter centriole size, which is governed by Plk4 (Fig. 1). In this model, Plk4 functions like a builder (or a group of builders) constructing a house. The more builders show up for work early in the morning, the faster the house will be completed. With less builders, it will take longer for the work to be done. Surprisingly, they appear to follow an unusual building plan, as though they were assembling the roof first and then the floors one by one, pushing the roof away or upwards in doing so. Indeed, the building site for Sas-6 incorporation stays close to the proximal site, and each subsequent Sas-6 building block therefore pushes the previous one away as it is assembled.

The exciting and surprising findings of Aydogan et al. (2018) open several new questions worth being considered: How are Sas-6 molecules incorporated at the proximal site? What is the identity of Plk4 substrates involved in centriole growth? Is there a crosstalk between the rate of Sas-6 incorporation and centriolar microtubule growth? Is the same mechanism present in cells that contain shorter cartwheels that do not extend throughout the entire centriole length?

Finally, it is worth mentioning that this study represents an excellent example of how in vivo studies performed in robust and reproducible model organisms can contribute to decipher the basic and essential mechanisms of outstanding biological questions like organelle size control.

Acknowledgments

Work in our laboratory is supported by a European Research Council consolidator grant (ChromoNumber - LS3, ERC-2016-COG), Institut Curie, and the Centre National de la Recherche Scientifique. Our laboratory is a member of the Labex CelTisPhyBio.

The authors declare no competing financial interests.

References

Arquint, C., and E.A. Nigg. 2016. The PLK4-STIL-SAS-6 module at the core of centriole duplication. *Biochem. Soc. Trans.* 44:1253–1263. https://doi.org/10.1042/BST20160116

Aydogan, M.G., A. Wainman, S. Saurya, T.L. Steinacker, A. Caballe, Z.A. Novak, J. Baumbach, N. Muschalik, and J.W. Raff. 2018. A homeostatic clock sets daughter centriole size in flies. *J. Cell Biol.* https://doi.org/10.1083/jcb.201801014

Conduit, P.T., A. Wainman, and J.W. Raff. 2015. Centrosome function and assembly in animal cells. *Nat. Rev. Mol. Cell Biol.* 16:611–624. https://doi.org/10.1038/nrm4062

Cunha-Ferreira, I., A. Rodrigues-Martins, I. Bento, M. Riparbelli, W. Zhang, E. Laue, G. Callaini, D.M. Glover, and M. Bettencourt-Dias. 2009. The SCF/Slimb ubiquitin ligase limits centrosome amplification through degradation of SAK/PLK4. *Curr. Biol.* 19:43–49. https://doi.org/10.1016/j.cub.2008.11.037

Dammermann, A., P.S. Maddox, A. Desai, and K. Oegema. 2008. SAS-4 is recruited to a dynamic structure in newly forming centrioles that is stabilized by the gamma-tubulin-mediated addition of centriolar microtubules. *J. Cell Biol.* 180:771–785. https://doi.org/10.1083/jcb.200709102

Dzhindzhev, N.S., G. Tzolovsky, Z. Lipinszki, M. Abdelaziz, J. Debski, M. Dadlez, and D.M. Glover. 2017. Two-step phosphorylation of Ana2 by Plk4 is required for the sequential loading of Ana2 and Sas6 to initiate procentriole formation. *Open Biol.* 7:170247. https://doi.org/10.1098/rsob.170247

Holland, A.J., W. Lan, S. Niessen, H. Hoover, and D.W. Cleveland. 2010. Polo-like kinase 4 kinase activity limits centrosome overduplication by autoregulating its own stability. *J. Cell Biol.* 188:191–198. https://doi.org/10.1083/jcb.200911102

Kim, T.S., J.E. Park, A. Shukla, S. Choi, R.N. Murugan, J.H. Lee, M. Ahn, K. Rhee, J.K. Bang, B.Y. Kim, et al. 2013. Hierarchical recruitment of Plk4 and regulation of centriole biogenesis by two centrosomal scaffolds, Cep192 and Cep152. *Proc. Natl. Acad. Sci. USA.* 110:E4849–E4857. https://doi.org/10.1073/pnas.1319656110

Kohlmaier, G., J. Loncarek, X. Meng, B.F. McEwen, M.M. Mogensen, A. Spektor, B.D. Dynlacht, A. Khodjakov, and P. Gönczy. 2009. Overly long centrioles and defective cell division upon excess of the SAS-4-related protein CPAP. *Curr. Biol.* 19:1012–1018. https://doi.org/10.1016/j.cub.2009.05.018

Nigg, E.A., and A.J. Holland. 2018. Once and only once: mechanisms of centriole duplication and their deregulation in disease. *Nat. Rev. Mol. Cell Biol.* https://doi.org/10.1038/nrm.2017.127

Rogers, G.C., N.M. Rusan, D.M. Roberts, M. Peifer, and S.L. Rogers. 2009. The SCF Slimb ubiquitin ligase regulates Plk4/Sak levels to block centriole reduplication. *J. Cell Biol.* 184:225–239. https://doi.org/10.1083/jcb.200808049