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Polo-like Kinase 4 Shapes Up

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Polo-like kinase 4 (Plk4) is a master regulator of centriole duplication and targets to centrioles through the association of its cryptic polo box domain with centriole receptors. In this issue of *Structure*, Shimanovskaya and colleagues unveil a new dimeric architecture of Plk4's cryptic polo box that reveals a conserved mechanism for centriole targeting of the kinase.

Centrioles are cylindrical, microtubule-based structures that generate the basal bodies required for the formation of cilia and are the core of the centrosome, an organelle that plays an important role in organizing the microtubule cytoskeleton (reviewed in Gönczy, 2012). At the beginning of the cell cycle, cells contain a single centrosome comprised of a pair of centrioles surrounded by a proteinaceous pericentriolar material (PCM). Exactly one new "daughter" centriole is created adjacent to each pre-existing "mother" centriole in each cell cycle. This tightly coordinated process ensures that the centrosome reproduces only once prior to mitosis. The two centrosomes instruct the formation of the bipolar spindle apparatus and segregate equally into the daughter cells during division. Defects in proper centriole biogenesis can result in chromosome segregation errors and developmental disorders and have been linked with tumorigenesis. Despite the key role that centrioles play in cellular homeostasis, the molecular mechanisms controlling centriole biogenesis remain poorly understood.

The serine/threonine kinase, Polo-like kinase 4 (Plk4), has emerged as a critical regulator responsible for coupling centriole duplication with cell cycle progression. Polo-like kinases (PLKs) are marked by the presence of an N-terminal kinase domain followed by two or more C-terminal polo box domains (PBDs; reviewed in Zitouni et al., 2014). Plk4 is the most divergent member of the PLK family and possesses a C-terminal PBD and a central domain known as the cryptic polo box (CPB) (Figure 1A). The CPB dimerizes and is responsible for binding to the partner proteins Asterless/Cep152 and SPD-2/Cep192 to allow centriolar targeting of Plk4 (Cizmecioglu et al., 2010; Delattre et al., 2006; Dzhindzhev et al., 2010; Hatch et al., 2010; Kim et al., 2013; Pelletier et al., 2006; Slevin et al., 2012; Sonnen et al., 2013). Dissecting how the CPB functions to coordinate Plk4 targeting is an essential step for our understanding of centriole copy number control.

A crystal structure of the CPB of *Drosophila* Plk4 revealed a previously unidentified pair of polo boxes known as PB1 and PB2 (Slevin et al., 2012). The CPB is a stable homodimer in solution, and several intermolecular interfaces were observed in the crystal structure. One interface involved PB1 and PB2 interacting along their length in a side-by-side pseudo-symmetric homodimer (Figures 1B and 1C) (Slevin et al., 2012). The side-by-side arrangement was proposed to represent the biologically relevant dimeric conformation of the CPB; however, this configuration did not explain how the CPB mediates Plk4 recruitment to the centrioles.

In this issue of *Structure*, Shimanovskaya et al. (2014) determine the structure of the CPB of *C.elegans* ZYG-1/Plk4 at 2.3 Å resolution and generate a new crystal form of the *Drosophila* Plk4 CPB. Crystal analysis coupled with biophysical characterization suggests that the CPB of Plk4 exists as a dimer in solution that broadly resembles an X shape. In this conformation, the PB2 domains associate end-to-end to generate a 12-stranded, intermolecular β sheet. The two PB1 domains do not directly interact with each other, because they are positioned on opposite sides of the intermolecular β sheet (Figures 1D and 1E) (Shimanovskaya et al., 2014). The X-shaped dimer is distinct from the side-by-side arrangement of the CPB that was originally proposed to represent the native conformation. Importantly, the X-shaped dimer is the only intermolecular interface present in all of the CPB crystal structures (Park et al., 2014; Shimanovskaya et al., 2014; Slevin et al., 2012).

Plk4 is present in animals and fungi, but a clear functional ortholog of Plk4 is notably absent from the genome of the roundworm, *Caenorhabditis elegans*. *C. elegans* possesses a kinase known as ZYG-1 that controls centriole duplication and functions in a manner analogous to Plk4 (O'Connell et al., 2001). Nevertheless, ZYG-1 and Plk4 are highly divergent in their amino acid sequences, leading some to speculate that these kinases may have convergently evolved to play similar cellular functions. The work by Shimanovskaya et al. (2014) shows that the CPB of *C. elegans* ZYG-1 and *Drosophila* Plk4 contain a similar configuration of two polo boxes arranged as an X-shaped end-to-end dimer. This provides compelling evidence that ZYG-1 is a bona fide (if highly divergent) Plk4 ortholog with analogous functional domains (Shimanovskaya et al., 2014). This central regulator of centriole duplication is therefore conserved from worms to humans.

In vertebrates, recruitment of Plk4 to the centriole relies on the interaction of the CPB with an acidic region at the N terminus of the centriolar proteins Asterless/Cep152 and SPD-2/Cep192 (Cizmecioglu et al., 2010; Dzhindzhev et al., 2010; Hatch et al., 2010; Kim et al., 2013; Sonnen et al., 2013). Although both of these molecules control Plk4 recruitment in vertebrates, only one of these interactions is employed in *Drosophila* and *C. elegans*. In *Drosophila,* SPD-2/Cep192 lacks the acidic N-terminal region required for Plk4 binding, and Asterless/Cep152 alone controls Plk4 recruitment (Dzhindzhev et al., 2010). On the other hand, *C.elegans* lacks an Asterless/Cep152 homolog and recruitment of ZYG-1/Plk4 is controlled by SPD-2/Cep192 (Figure 1F) (Delattre et al., 2006; Pelletier et al., 2006).

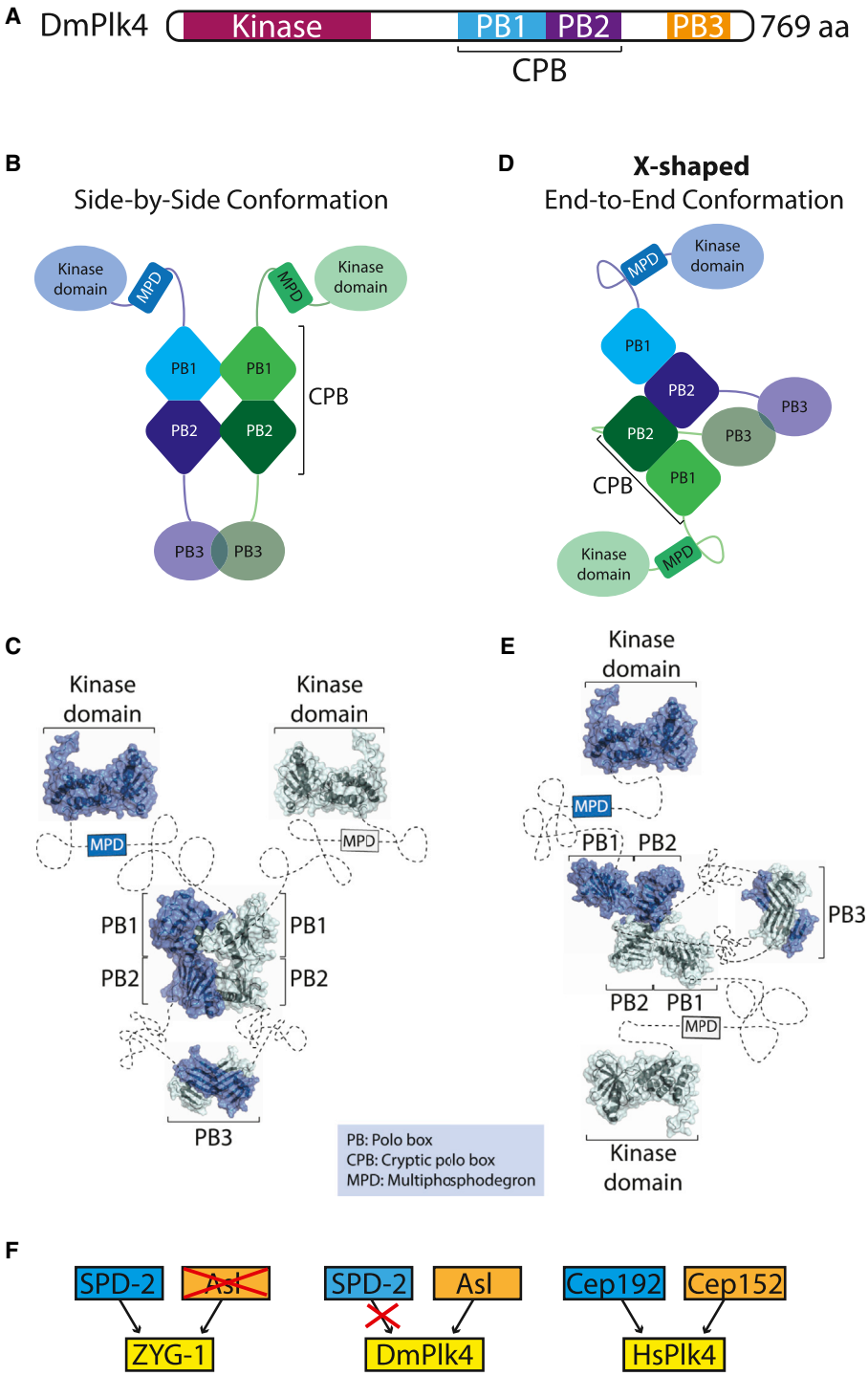

Figure 1. Two Structural Models of the Serine/Threonine Kinase, Plk4

(A) Schematic showing the structural domains of *Drosophila* Plk4.

(B and C) Model representation (B) and crystal structure (C; surface plot and ribbon) of the CPB of DmPlk4, published by Slevin et al. (2012). The authors show that the CPB is comprised of two polo boxes, PB1 and PB2. They propose that the PBs form an intermolecular homodimer arranged in a side-by-side manner.

(D and E) Model representation (D) and crystal structure (E) of the DmPlk4 CPB published in this issue by Shimanovskaya et al. (2014) showing the two polo boxes in the CPB orienting end-to-end to form an X-shaped dimer, with the two PB2 domains interacting with each other. Protein Data Bank IDs: 3COK (kinase domain), 4G7N (CPB domain), and 1MBY (PB3).

(F) Schematic presentation of the differential binding of ZYG-1/Plk4 to its receptors, SPD-2/Cep192 and Asterless(Asl)/Cep152. *C.elegans* lacks an Asterless/Cep152 homolog, and SPD-2/Cep192 is responsible for binding and recruitment of ZYG-1/Plk4. In *Drosophila*, SPD-2/Cep192 lacks the acidic N-terminal region required for Plk4 binding, and Asterless/Cep152 alone controls Plk4 recruitment. In humans, Cep152 and Cep192 associate with Plk4 in a mutually exclusive manner to control centriole recruitment.

While the proteins responsible for recruiting Plk4 to the centriole have been identified, the molecular mechanism underlying this interaction has not been established. The structure revealed by Shimanovskaya et al. (2014) offers new information about these interactions. The X-shaped dimeric arrangement of the Plk4 CPB contains a conserved basic patch running across one side of the CPB homodimer. This indicates that Plk4 is recruited to the centriole by electrostatic interactions between residues of the CPB basic patch and those of the acidic N-terminal region of SPD-2/Cep192 and Asterless/Cep152. Consistent with this arrangement, each Plk4 CPB dimer binds to the acidic region of one centriole receptor. In addition, the authors used charged swapped point mutations to identify specific amino acids in the ZYG-1/Plk4 CPB and the acidic region of Asterless/Cep152 and/or SPD-2/Cep192, which are required for binding. This revealed distinct binding footprints for the acidic region of Asterless/Cep152 and SPD-2/Cep192 on the surface of the CPB (Shimanovskaya et al., 2014). Using elegant functional replacement in *C. elegans*, Shimanovskaya et al. (2014) dissected the contribution of the electrostatic interactions of SPD-2/Cep192 and ZYG-1/Plk4 in centriole assembly. *C. elegans* SPD-2/Cep192 is required for both centriole duplication and recruitment of the surrounding pericentriolar material. Consistent with this dual role, SPD-2/Cep192 mutants defective in ZYG-1/Plk4 binding are able to recruit PCM but fail to rescue ZYG-1/Plk4 recruitment and centriole duplication. In addition, CPB mutants of ZYG-1/Plk4 that are defective in SPD-2/Cep192 binding fail to localize to the centriole and cannot support centriole assembly (Shimanovskaya et al., 2014). Together with in vitro biochemical analysis, these data support the view that the binding of the ZYG-1/Plk4 CPB to SPD-2/Cep192 is mediated by key charged residues on the surfaces of both proteins.

Concurrent with the publication of the manuscript by Shimanovskaya et al. (2014), another manuscript reported the structure of the human Plk4 CPB bound to the acidic peptides of Asterless/Cep152 and SPD-2/Cep192 (Park et al., 2014). This report supports the X-shaped dimeric structure of the Plk4 CPB. Importantly, human CPB Plk4 dimers were found to interact in a mutually exclusive manner with the acidic N terminus of either Asterless/Cep152 or SPD-2/Cep192. Both Shimanovskaya et al. (2014) and Park et al. (2014) concluded that each Plk4 CPB dimer associates with a single Asterless/Cep152 molecule. However, an important disparity between the two studies is the stoichiometry with which the Plk4 CPB binds to SPD-2/Cep192. Shimanovskaya et al. (2014) report that the ZYG-1/Plk4 CPB dimer associates with a single 147 amino acid (aa) SPD-2/Cep192 molecule, while Park et al. (2014) identify two 58 aa SPD-2/Cep192 peptides associated with each CPB dimer. It is possible that a larger SPD-2/Cep192 fragment extends across both subunits of the CPB dimer and occludes the second binding site. We postulate that in vivo one Asterless/Cep152 or SPD-2/Cep192 molecule will associate with one Plk4 dimer.

Cellular Plk4 levels are carefully managed as excessive Plk4 triggers the formation of supernumerary centrioles, whereas Plk4 depletion results in a failure of centriole duplication. Endogenous Plk4 controls its own stability through self-phosphorylation, *in trans*, of a multisite phosphodegron (MPD; reviewed in (Zitouni et al., 2014). This autocatalyzed destruction is required to limit centriole duplication to once per cycle. The X-shaped arrangement of the Plk4 cryptic polo box implies that the two-kinase domains in the Plk4 dimer would emerge on opposite sides of the CPB dimerization interface (Figures 1D and 1E). This raises the question of how Plk4's MPD is positioned for *trans* autophosphorylation. The lengthy linker sequence between the kinase domain and PB1 may be flexible enough to accommodate bridging the two-kinase domains to promote self-phosphorylation.

Elucidating the dimeric arrangement of ZYG-1/Plk4's CPB domain leaves us with several tantalizing questions. Is monomeric Plk4 kinase active but unable to self regulate? What is the role of the third PB domain of Plk4 that is not required for centriole recruitment? How is the binding of Plk4 to Asterless/Cep152 and SPD-2/Cep192 controlled to allow for dynamic changes in the localization of Plk4? Do Asterless/Cep152 and/or SPD-2/Cep192 homodimerize or oligomerize, and if so, does it aid in bringing together Plk4 dimers? Lastly, how can Plk4 be protected from self-destruction when it is concentrated at the site of daughter centriole assembly? Further studies will be necessary to elucidate the complex series of events allowing spatiotemporal control of centriole biogenesis.

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REFERENCES

Cizmecioglu, O., Arnold, M., Bahtz, R., Settele, F., Ehret, L., Haselmann-Weiss, U., Antony, C., and Hoffmann, I. (2010). J. Cell Biol. *191*, 731–739.

Delattre, M., Canard, C., and Gonczy, P. (2006). Curr. Biol. *16*, 1844–1849.

Dzhindzhev, N.S., Yu, Q.D., Weiskopf, K., Tzolovsky, G., Cunha-Ferreira, I., Riparbelli, M., Rodrigues-Martins, A., Bettencourt-Dias, M., Callaini, G., and Glover, D.M. (2010). Nature *467*, 714–718.

Gönczy, P. (2012). Nat. Rev. Mol. Cell Biol. *13*, 425–435.

Hatch, E.M., Kulukian, A., Holland, A.J., Cleveland, D.W., and Stearns, T. (2010). J. Cell Biol. *191*, 721–729.

Kim, T.S., Park, J.E., Shukla, A., Choi, S., Murugan, R.N., Lee, J.H., Ahn, M., Rhee, K., Bang, J.K., Kim, B.Y., et al. (2013). Proc. Natl. Acad. Sci. USA *110*, E4849–E4857.

O'Connell, K.F., Caron, C., Kopish, K.R., Hurd, D.D., Kemphues, K.J., Li, Y., and White, J.G. (2001). Cell *105*, 547–558.

Park, S.Y., Park, J.E., Kim, T.S., Kim, J.H., Kwak, M.J., Ku, B., Tian, L., Murugan, R.N., Ahn, M., Komiya, S., et al. (2014). Nat. Struct. Mol. Biol. Published online June 29, 2014. http://dx.doi.org/10.1038/nsmb.2846.

Pelletier, L., O'Toole, E., Schwager, A., Hyman, A.A., and Müller-Reichert, T. (2006). Nature *444*, 619–623.

Shimanovskaya, E., Viscardi, V., Lesigang, J., Lettman, M.M., Qiao, R., Svergun, D.I., Round, A., Oegema, K., and Dong, G. (2014). Structure *22*, this issue, 1090–1104.

Slevin, L.K., Nye, J., Pinkerton, D.C., Buster, D.W., Rogers, G.C., and Slep, K.C. (2012). Structure *20*, 1905–1917.

Sonnen, K.F., Gabryjonczyk, A.M., Anselm, E., Stierhof, Y.D., and Nigg, E.A. (2013). J. Cell Sci. *126*, 3223–3233.

Zitouni, S., Nabais, C., Jana, S.C., Guerrero, A., and Bettencourt-Dias, M. (2014). Nat. Rev. Mol. Cell Biol. *15*, 433–452.