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Insights into Anaphase Promoting Complex TPR subdomain assembly from a CDC26–APC6 structure

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Abstract

The multi-subunit Anaphase Promoting Complex (APC) is an essential cell cycle regulator. Although CDC26 is known to play a role in APC assembly, its molecular function has remained unclear. Biophysical, structural, and genetic studies presented here reveal that CDC26 stabilizes the structure of APC6, a core TPR protein required for APC integrity. Interestingly, CDC26–APC6 association involves an intermolecular TPR mimic composed of one helix from each protein.

The Anaphase Promoting Complex (APC) controls cell division by promoting ubiquitin-mediated proteolysis of key cell cycle regulatory proteins. The importance of the folding and assembly of APC subunits is underscored by the fact that many of their genes were identified in classic screens for cell division cycle (Cdc) mutants in *S. cerevisiae* and *S. pombe*. The ~13 APC subunits can be broadly divided into subcomplexes, one of which contains the small heat shock protein CDC26 and multiple proteins containing tetratricopeptide (TPR) motifs1–6. The TPR motif forms a ~34-residue helix-turn-helix structure. Multiple TPRs pack into a superhelical protein-protein interaction domain, with a hydrophobic central peptide-binding groove7. Although it has been proposed that peptide binding can stabilize TPR domains, this notion is controversial because several TPR domains can fold in the absence of their ligands8.

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Author contributions

JW designed, performed, and analyzed biochemical, biophysical, and crystallography experiments and wrote the manuscript; BTD designed, performed, and analyzed biochemical and yeast genetic experiments and wrote the manuscript; KRR and IK performed and analyzed crystallography experiments; BAS advised on all aspects of the project and wrote the manuscript.

Accession codes Coordinates and structure factors for $CDC26^{N}$–$APC6^{TPR}$ have been deposited in the RCSB with accession code 3HYM.

Genetic and biochemical studies have revealed a link between CDC26 and the assembly of the TPR protein APC6 into the APC. The fission yeast CDC26 (Hcn1) was isolated as a high-copy suppressor of mutations in APC6 (Cut9)9, and interacts with Cut9 in a 2-hybrid assay10. In budding yeast, CDC26 is lost from APC complexes also having lost APC6 (Cdc16)6, and zebrafish APC6 and CDC26 were co-identified in a screen for genes controlling post-embryonic eye growth11. CDC26 is essential in fission yeast10, and loss of its function in budding yeast interferes with APC assembly and leads to temperature-sensitive growth12–14. Despite its critical role, the CDC26 sequence lacks motifs to indicate either its structure or function.

To confirm direct CDC26–APC6 interaction, we co-purified human CDC26 and APC6 co-expressed in insect cells and further analyzed the structural effects of this interaction using CD spectroscopy (Fig. 1a). As with other TPR proteins, APC6 does show the potential to independently adopt a helical structure. However, relative to either protein alone, the CDC26–APC6 complex displayed increased helical content, cooperativity of unfolding, and thermal stability. Furthermore, CDC26 decreases the propensity of APC6 to aggregate (Supplementary Fig. 1). Thus, CDC26 appears to stabilize APC6 structure.

To further characterize the complex, we subjected CDC26–APC6 to limited trypsinization. A proteolytically-stable complex containing the N-terminus of CDC26 (residues 1–29, hereafter called $CDC26^{N}$) and all 8 predicted tandem APC6 TPRs (residues 212–539, $APC6^{TPR}$) was subsequently purified by gel filtration (Supplementary Fig. 2). This represents a structurally stable core because it is resistant to trypsin despite having numerous potential cleavage sites (Lys and Arg residues) distributed throughout the $CDC26^{N}$ and $APC6^{TPR}$ sequences (7 and 27, respectively). $CDC26^{N}$–$APC6^{TPR}$ maintains biophysical characteristics observed in the full-length CDC26–APC6 complex as compared to each protein alone, including (1) increased helix content, (2) cooperative unfolding, (3) increased thermal stability, and (4) decreased aggregation (Fig. 1a, b, Supplementary Fig. 1). Notably, $CDC26^{N}$ is sufficient to fulfill these functions in complex with full-length APC6 (Fig. 1c, Supplementary Fig. 1, 2).

To gain detailed insights into CDC26–APC6 interactions, we determined the $CDC26^{N}$–$APC6^{TPR}$ crystal structure at 2.8 Å resolution (Fig. 1d, Supplementary Table 1, Supplementary Fig. 3). $CDC26^{N}$ forms a rod-like structure, with residues 1–12 adopting an extended conformation, and residues 13–25 forming a helix. In agreement with TPR prediction analyses, $APC6^{TPR}$ contains 8 full TPRs that together adopt a solenoid-like structure wrapping around the length of $CDC26^{N}$. $CDC26^{N}$ and $APC6^{TPR}$ are configured with their N- and C-termini aligned, and the TPR motifs of APC6 pack in a manner that resembles other TPR-containing proteins15.

The structure explains the core biophysical properties of CDC26–APC6. First, CDC26 alone is unfolded (Fig. 1a), which might be expected for a protein that interacts with its binding partner in an extended conformation. Second, the $CDC26^{N}$ "rod" supports the helical architecture of $APC6^{TPR}$, consistent with the increased structural stability observed for the complex (Fig. 1, Supplementary Fig. 1). Third, the $CDC26^{N}$–$APC6^{TPR}$ structure rationalizes

their proteolytic resistance, as potential cleavage sites are protected upon forming a stable complex.

The CDC26^{N}–APC6^{TPR} interface buries 3320 Å^{2}, with extensive hydrophobic and electrostatic interactions (Fig 1d). CDC26 residues 1–4 are almost completely buried by APC6 TPRs 1–4, such that CDC26 Leu2 is encircled by APC6 Tyr242, Cys307, Ile338, Ala339, His342, and Leu369 (Fig. 2a). Consistent with this, inclusion of an N-terminal tag on CDC26 severely impairs its binding to APC6 (data not shown). CDC26 residues 5–12 interact with APC6 TPRs 5–8. Key interactions here are mediated by CDC26 Thr7, which is proximal to 5 different residues of APC6 (Leu376, His406, Glu407, Val410, and Asn450) and by CDC26 Leu9, which approaches 4 APC6 residues (Phe413, Asn449, Asn450, and His453) (Fig. 2d).

Interestingly, the CDC26^{N} helix interacts with the non-TPR helix 9A of APC6^{TPR} such that their geometry mimics two helices in a TPR motif (Supplementary Fig. 4). Although this helix pair adopts a parallel orientation, it otherwise aligns well with the individual TPRs of APC6^{TPR}. This intermolecular TPR mimic continues the sinuous form of the overall structure and packs against the 8th TPR of APC6 to form a 4-helix bundle (Fig. 1d, 2c).

Several lines of data suggest that key features of the CDC26^{N}–APC6^{TPR} structure are maintained throughout evolution. First, CDC26^{N} and APC6^{TPR} encompass highly conserved regions of sequence overall, with strong homology for their interacting residues (Supplementary Fig. 5). Second, human CDC26^{N} can substitute for some functions of full-length CDC26 from other species (Supplementary Fig. 2c,d). Third, the physiological importance of the CDC26^{N} interaction is demonstrated by the sufficiency of a highly homologous N-terminal fragment of budding yeast CDC26 (residues 1–31) to rescue the temperature-sensitive growth of a *cdc26*Δ yeast strain (Fig. 2d). Fourth, this rescue is sensitive to mutation at positions analogous to those making structurally important contacts in the complex (Fig. 2).

The CDC26^{N}–APC6^{TPR} structure provides a basis for understanding the deleterious effects of several previously identified mutations (Supplementary Fig. 6). Residues whose mutation confer temperature-sensitive growth defects in budding yeast Cdc2612 and budding and fission yeast Cdc16/Cut916,17 correspond to key interacting residues or positions within the hydrophobic core, mutation of which would be expected to result in temperature-sensitive misfolding. Additionally, APC6 mutations found in human cancer cell lines18 would disrupt the integrity of TPRs 3 and 7.

Together, our data provide a rationale for the role of CDC26 in orchestrating assembly of the APC TPR subcomplex. CDC26^{N} serves as a lynchpin providing critical support for the APC6^{TPR} superhelix. Given that other APC subunits also have TPRs, it is tempting to speculate that their superhelical grooves bind similarly to extended and/or helical portions of their partners19,20. Future studies will be required to reveal the bases of these interactions and to investigate other potential functions of CDC26 and APC6. Since both CDC26 and APC6 are required for the stable incorporation of other TPR proteins into the APC6,13, the

stable CDC26–APC6 complex may itself serve as a platform for assembling a higher-order multi-TPR complex required for APC function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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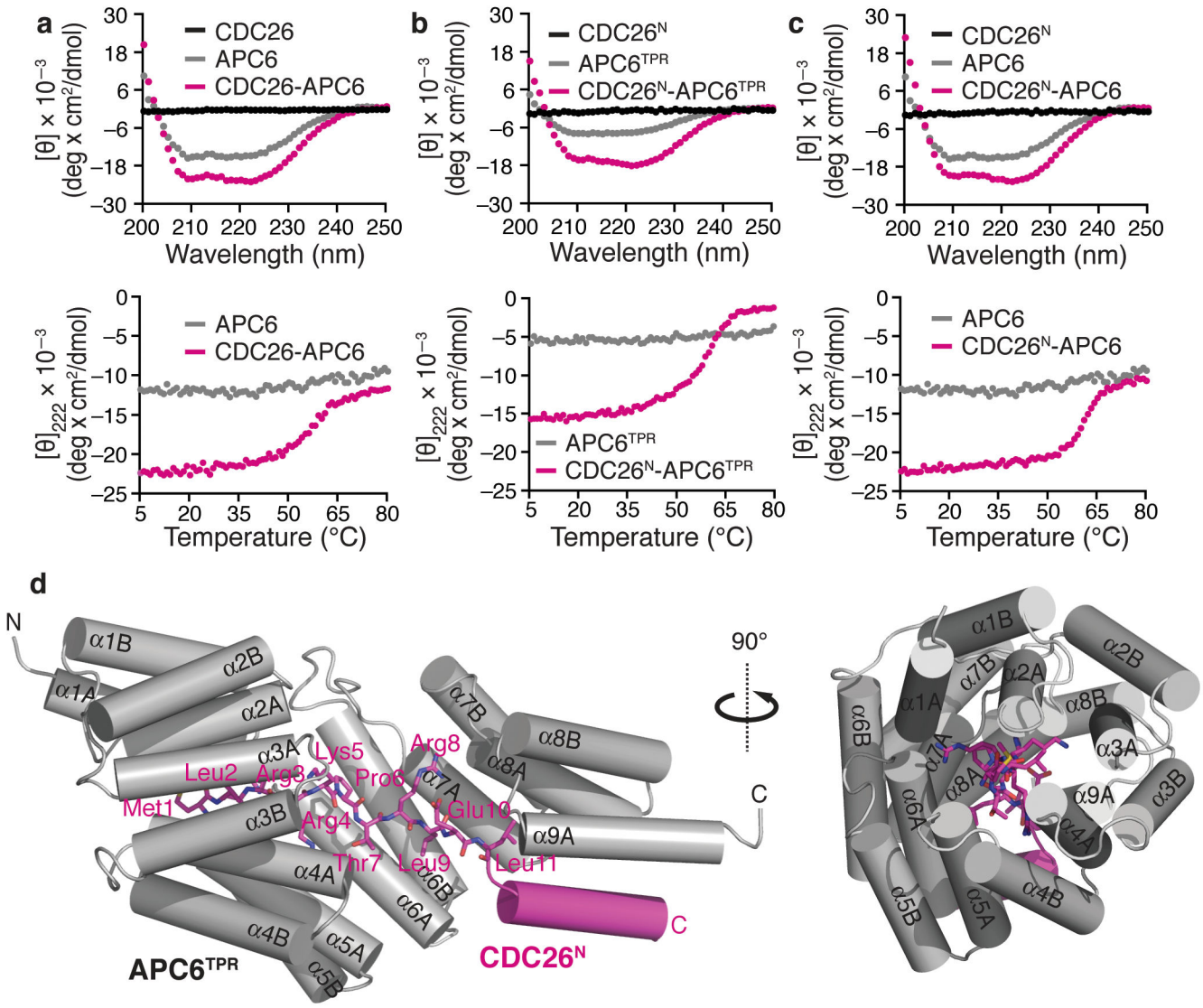

Figure 1. CDC26 stabilizes APC6 structure

(**a**) Far-UV circular dichroism wavelength spectra (top) or thermal-denaturation profiles (bottom) for full-length CDC26 alone (black), APC6 alone (grey), or the CDC26–APC6 complex (magenta). (**b**,**c**) Experiments as in **a**, except with the proteolytically-defined $CDC26^{N}$ and/or $APC6^{TPR}$, as indicated. (**d**) Two views of $CDC26^{N}$ (magenta)–$APC6^{TPR}$ (grey) crystal structure, rotated 90° about the vertical axis.

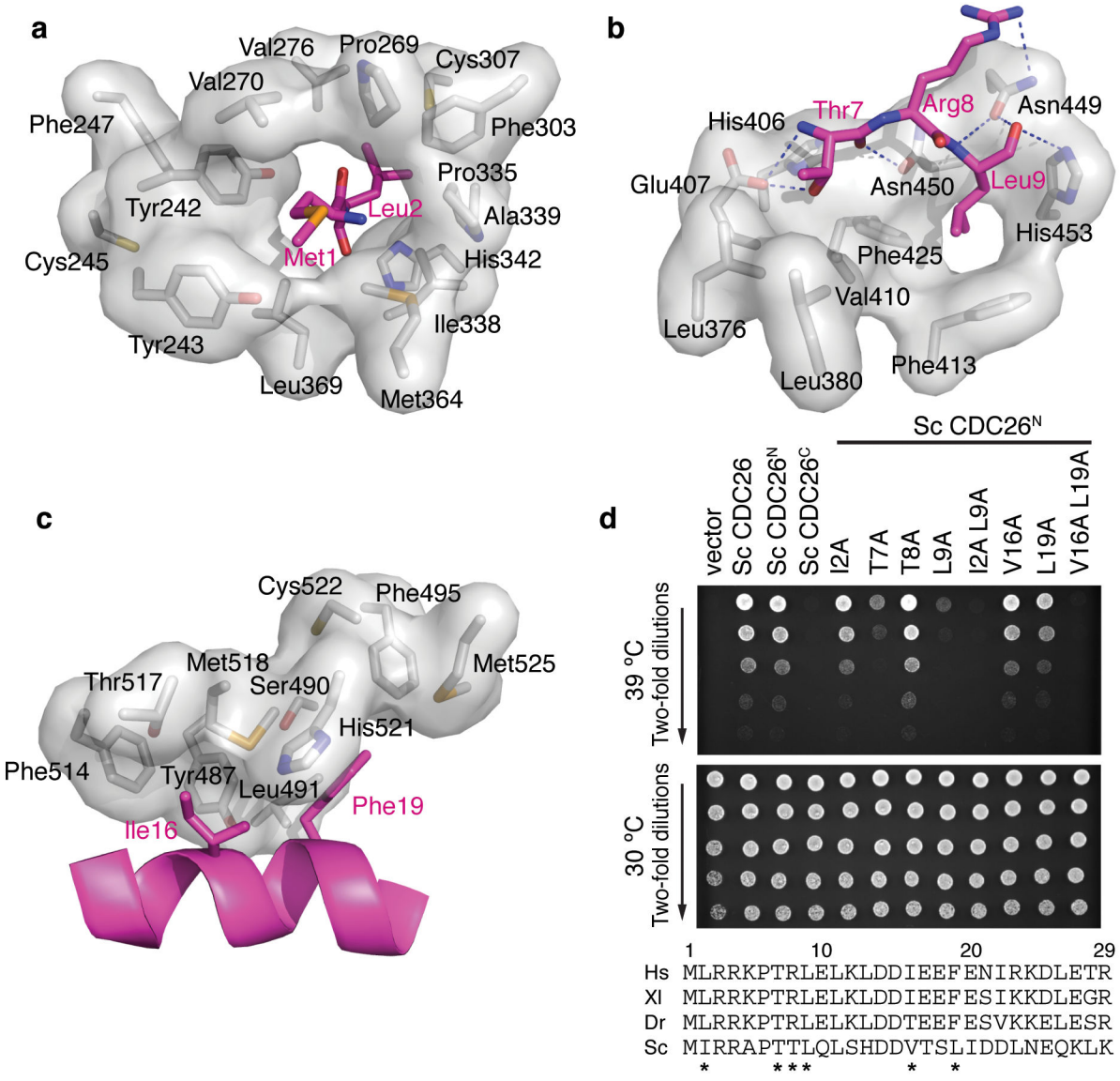

Figure 2. CDC26^{N}–APC6^{TPR} interactions and yeast complementation

(**a–c**) Closeup views of key interactions between CDC26^{N} (magenta sticks) and APC6^{TPR} (grey, surface and sticks). Dotted lines mark electrostatic interactions. (**d**) Top, complementation of temperature-sensitive *cdc26*Δ *S. cerevisiae* growth by expression of the indicated proteins. Bottom, alignment of human (Hs) CDC26^{N} with sequences from frog (Xl), zebrafish (Dr), and budding yeast (Sc). Asterisks mark residues mutated in complementation experiments.