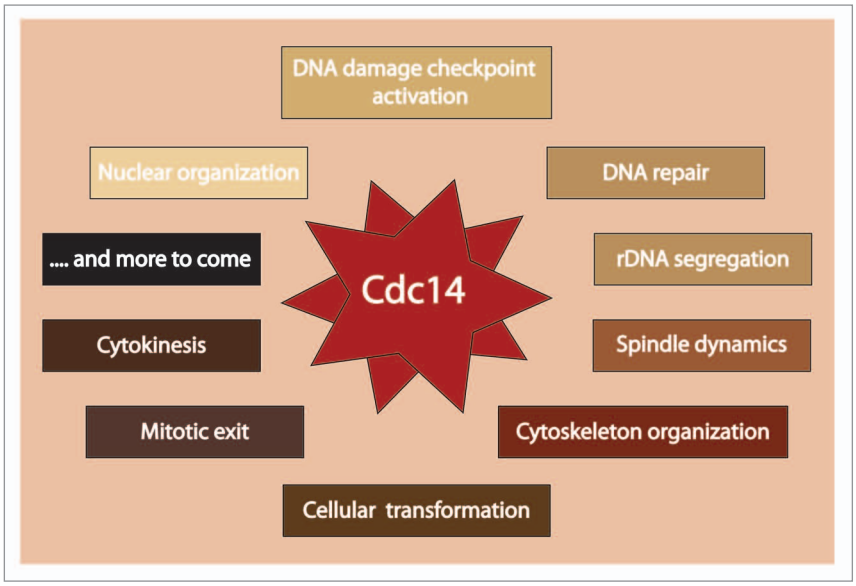

Figure 1. The multiple functions of Cdc14 phosphatases. Work from different species has linked Cdc14 to a wide variety of cellular processes portraying a somewhat confusing picture of the Cdc14 phosphatase family and its functions. Likely in the years to come additional roles will be unveiled explaining how nature has recycled and successfully adapted a single phosphatase to satisfy multiple purposes.

protein H-RasV12. These included anchorage-independent growth, loss of contact inhibition and changes in cell shape.[8] To explore the link between Cdc14B and H-RasV12, the authors investigated and compared the cellular transcription profiles caused by overexpression of the two oncoproteins in stable NIH-3T3 clones. Similar deregulations in gene expression were found. These data combined with the finding that co-expressing Cdc14B and H-RasV12 did not give an exacerbated phenotype led the authors to conclude that Cdc14B and H-RasV12 may act in a common pathway. Additional analyses by Chiesa et al. suggested that, among the many signaling pathways activated by Ras,[9] the Ras-Raf-Mek pathway may be mainly responsible for mediating the oncogenic effects of Cdc14B. Although the details of the mechanism by which Cdc14B elicits cellular transformation remain to be determined, the new findings are intriguing, as they suggest that mammalian phosphatases, besides acting as tumor suppressors (e.g., PP2A; PTEN), may also function as oncogenes (e.g., CDC14B).

Forty years after its discovery, the Cdc14 phosphatase continues to surprise. Functional clues coming from different species suggest that the phosphatase is used in various pathways aimed at fine tuning growth and cell division. Future identification of CDC14B substrates and interactors as well as the characterization of the spatio-temporal control of its activity promise to reveal which events CDC14B coordinates in higher eukaryotes. What is now seen as difficult to comprehend or even controversial may soon make sense and explain how a generally good kid can turn bad.

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It's all about flaps: Dna2 and checkpoint activation

Comment on: Budd ME, et al. Cell Cycle 2011; 10:1690–8

Peter M. Burgers; Washington University School of Medicine; St. Louis, MO USA; Email: burgers@biochem.wustl.edu; DOI: 10.4161/cc.10.15.15851

DNA flaps function as intermediates in DNA metabolism. 3′-Flaps are generated as intermediates for homologous recombination. 5′-flaps, on the other hand, arise as necessary but dangerous DNA structures during DNA replication and DNA damage response. 5′-Flaps are generated either through strand displacement synthesis by a DNA polymerase or through the action of a DNA helicase, and generally, their degradation by 5′-nucleases is tightly coupled to their formation (**Fig. 1A**). Members of the family of 5′-flap endonucleases, as exemplified by FEN1, cut such 5′-flaps. Recently, however, another type of 5′-endonuclease, Dna2, has gained attention because of its involvement in multiple pathways involving the degradation of 5′-flaps. In the May 15th issue of *Cell Cycle*, Budd et al. showed that deletion of *S. cerevisiae DNA2* leads to a permanent checkpoint arrest, resulting in a lethality that can be alleviated by the inactivation of key cell cycle checkpoint factors.[1] In fact, it had been known for over a decade that the temperature sensitivity of *dna2* hypomorphs could be suppressed by deletion of the checkpoint mediator RAD9.[2] However, at that time, the mechanistic significance of this result was unclear, since the role of Dna2 nuclease in DNA metabolism had not yet been delineated.

Dna2 was originally identified as a DNA helicase with an associated nuclease activity, but subsequent studies showed that its nuclease activity performs the primary function in the pathways studied and is essential for viability.[3,4] The function of the helicase appears to be more ancillary in character. During Okazaki fragment maturation, 5′-flaps are generated through strand displacement synthesis by DNA polymerase δ.[5] Most flaps remain small and undergo concomitant cleavage by FEN1, but some flaps escape cleavage and grow to a length of >25 nt, which allows coating by the single-stranded binding protein RPA. These RPA-coated flaps are refractory to FEN1 cutting, but they become substrates for Dna2. Since Dna2 cutting is not precise but leaves a small 5′-flap of 2-6 nucleotides, further trimming by FEN1 or another

flap endonuclease is still necessary (**Fig. 1B**). Similarly, during long-patch base excision repair in the mitochondria of mammalian cells, flaps generated by DNA polymerase γ are cut by FEN1 when they are small and by Dna2 when they are longer.[6,7] Most recently, resection of the 5′-strand at a double-stranded break has been shown to be mediated by the coupled action of Sgs1 helicase and the Dna2 nuclease.[8,9] This degradation process is unique in that FEN1 is apparently not involved. None of these pathways show a substantial dependence on the helicase function of Dna2.

Okazaki fragment maturation proceeds primarily through the generation of short flaps that are cut by FEN1, with the Dna2-dependent long flap pathway being a rare backup mechanism.[10] Biochemical and genetic studies have shown that several factors can influence the balance of short versus long flaps during Okazaki fragment maturation (**Fig. 1B**).[5] This balance is an important consideration, because there is good evidence that the checkpoint-mediated lethality of a *DNA2* deletion is caused specifically by the accumulation of long flaps during Okazaki fragment maturation. Lethality of *dna2-Δ* is suppressed by mutations and conditions that limit the accumulation of long flaps on the lagging strand of the replication fork; for instance, by the reduction in efficiency with which Pol δ carries out strand displacement synthesis (in *pol32-Δ,* that deletes its third subunit), or by deletion of *PIF1*, a helicase which actually promotes the generation of longer flaps.[11] In addition, overexpression of *RAD27* (encoding FEN1) increases the utilization of short flaps, thereby also limiting the accumulation of long flaps and thus suppressesing the lethality of *dna2* hypomorphs. In contrast, the phenotypic defects caused by an increase in the generation of long flaps, either in a *rad27-Δ* mutant or in a proofreading-defective mutant of Pol δ, which results in increased strand displacement synthesis, can be rescued by overexpression of *DNA2*. Finally, the balance of short versus long flaps can also be affected by post-translational modification of FEN1 and Dna2.[12]

Given the high importance assigned to the FEN1 pathway, one may wonder why a *rad27-Δ* mutant is viable but *dna2-Δ* is not. Budd et al. show that the answer lays in checkpoint activation.[1] Rad27 deficiency is partially compensated by the action of another flap exonuclease, Exo1, and partially by Dna2 cutting of the longer flaps, which are generated more frequently in the *rad27-Δ* mutant. However, the *rad27-Δ* defect also leads to the generation of double-stranded breaks which require homologous recombination for repair, hence the lethality of *rad27-Δ rad52-Δ* double mutants. On the other hand, Dna2 deficiency leads to the persistent accumulation of long 5′-flaps, and these RPA-coated flaps likely serve as recruitment sites for the principal checkpoint kinase Mec1, initiating a continual checkpoint response with subsequent lethal consequences in G_2/M. Remarkably, both the DNA damage checkpoint, which proceeds via the Rad9 mediator, and the replication checkpoint, which proceeds via the Mrc1 mediator, contribute to the terminal arrest of a *DNA2* deletion, since maximal growth is recovered when the checkpoint functions of both mediators are eliminated. The latter observation is an indication that Okazaki fragment maturation is tightly coupled to its maturation by FEN1, Dna2 and DNA ligase, since failure in maturation leads to replication-fork stalling with associated activation of the Mrc1-dependent checkpoint pathway.

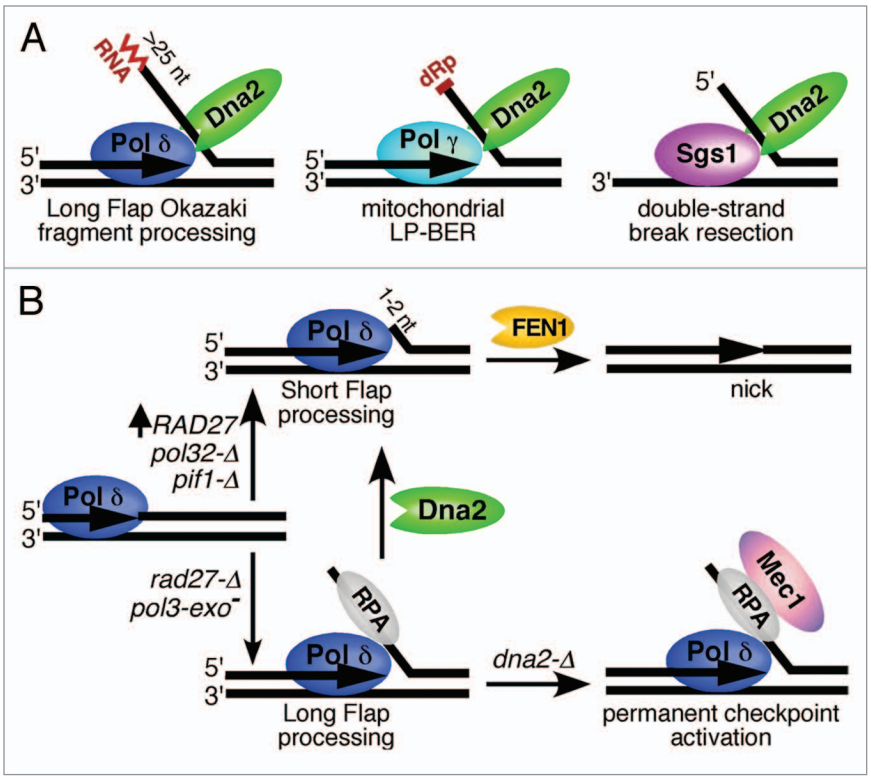

Figure 1. Dna2 nuclease in DNA metabolism. (A) Pathways in which Dna2 nuclease participates. (B) Balance of short versus long flap distribution during Okazaki fragment maturation is affected by various mutations. RAD27 = FEN1. See text for details.

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