Nucleotide-excision repair in mammalian cells can be separated into two steps[16]. First, the damaged DNA site is recognized and a fragment of 27–29 nucleotides containing the pyrimidine dimer is released by an incision/excision step, then PCNA-dependent repair synthesis closes the gap. In contrast, replicative DNA synthesis extends over much longer regions of DNA. It seemed plausible that the structural differences in DNA templates could lead to the differential effects of p21. Alternatively, a PCNA-dependent DNA polymerase other than polymerase δ, such as polymerase ε, could be responsible for repair synthesis and be more resistant to p21 inhibition. For example, polymerase ε requires less PCNA than polymerase δ for polymerase activity[17].

To test these models, we designed two DNA templates mimicking those involved in either DNA repair or replication. The first template contains two oligonucleotides annealed to a circular, single-stranded DNA, creating a 28-nucleotide gap (Fig. 4*a*). In this case, the upstream primer is labelled and the downstream primer serves as a 'terminator' that will retard progress of the DNA polymerase. The second template is a primed single-stranded DNA without the second oligonucleotide, thus resembling a template for DNA replication (Fig. 4*b*). Synthesis by polymerase δ or ε was carried out in the presence of replication protein A, replication factor C (a DNA-polymerase accessory factor) and PCNA (where indicated).

As shown previously[17–20], polymerase δ activity was dependent on the presence of PCNA (Fig. 4*c*, compare lanes 1 and 7 with 2 and 8). In the case of the gapped template, the polymerase paused at multiple sites near the 3′ end of the gap sequence (88 nucleotides); in addition, there was a considerable amount of strand displacement synthesis beyond the gap. Synthesis of long DNA products (for example, above the 564-nucleotide marker) on both templates was greatly diminished by increasing amounts of p21 (Fig. 4*c*, lanes 3–6, 9–12; the molar ratio of p21 to PCNA in lanes 6 and 12 was 20 to 1). Alkaline agarose gel electrophoresis confirmed that synthesis of larger DNA products (500–2,000 nucleotides) was greatly reduced by p21 (data not shown). In contrast, PCNA-dependent gap-filling DNA synthesis was relatively unaffected (Fig. 4*c*, lanes 3–6; 88 nucleotides). The effect of p21 on polymerase ε activity was assessed using conditions under which the polymerase activity was PCNA-dependent (Fig. 4*d*). Again, p21 blocked synthesis of long DNA fragments (Fig. 4*d*, lanes 9–12; around the 564-nucleotide marker), but not the short patch synthesis by polymerase ε (Fig. 4*d*, lanes 3–6; 88 nucleotides). Compared with polymerase δ, synthesis by polymerase ε on the gapped templates was more confined to the gap region (Fig. 4*d*, lanes 1–6), and its PCNA-dependent activity seemed to be more resistant to p21, suggesting that it was a better polymerase for DNA repair synthesis (for example, compare lanes 8 and 9 in Fig. 4*c* with those in Fig. 4*d*). Notably, both polymerases have been implicated in DNA repair[21–23]. Although our results do not address which polymerase plays a major role in nucleotide-excision repair *in vivo*, they show that short-gap synthesis by either polymerase is relatively insensitive to inhibition by p21.

PCNA has a dual function in SV40 DNA synthesis[13]. It recognizes a primer–template junction in cooperation with replication factor C and facilitates loading of polymerase δ; PCNA also enhances the processivity of polymerase δ during the elongation step. We suggest that the role of PCNA in processivity may be more sensitive to p21 than is its role in polymerase loading, and the PCNA–p21 interaction may arrest the polymerase and cause it to fall off the template. In repair synthesis, it is conceivable that the primary role of PCNA is to help the polymerase (δ or ε) localize to the junction of the incised DNA. Once bound to the template, the polymerase may be able to synthesize the short patch of DNA without the aid of PCNA. In addition, other repair-specific proteins[24,25] may further contribute to immunizing the repair machinery to p21.

DNA damage poses a tremendous challenge to the integrity of the genome. The post-damage events in normal cells ensure that any errors caused by the damage are corrected before propagation of the chromosomes. Based on our current understanding, elevated p21 levels block cell-cycle progression and DNA replication by coordinately inhibiting the functions of both CDKs and PCNA. The results presented here suggest a selectivity in p21 function which allows it to arrest DNA replication while permitting active DNA repair. p21 and its interaction with CDK, cyclin and PCNA may also be involved in normal proliferating cells. It is possible that p21 interaction with PCNA couples DNA repair to DNA replication during S phase and if the link between p21 and PCNA is disrupted, post-replicative repair could be abrogated, leading to genetic damage. We suggest that p21, PCNA and its partner, replication factor C might function, perhaps in cooperation with cyclin and CDK, to coordinate the replication and repair machineries with cell-cycle progression in both normal and damaged cells. □

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ERRATUM

Mice with targeted disruptions in the paralogous genes *hoxa-3* and *hoxd-3* reveal synergistic interactions

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FIGURES 1 and 2 in this Letter were accidentally transposed during the production process. The legends are correct as published. □