Mini-review

When replication travels on damaged templates: bumps and blocks in the road

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Abstract

Escherichia coli can accurately replicate their genome even when it contains hundreds of damaged bases. In this situation, processes such as DNA repair, translesion DNA synthesis, and recombination all contribute to the cell's ability to successfully complete this task. However, under conditions when these reactions go awry, these same processes can result in cell lethality, mutagenesis, or genetic instability. In order to understand the molecular events that can lead this normally faithful duplication of the genome to become less than perfect, it is essential to define the substrates and conditions when each of these processes are recruited to the replication fork.
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1. Introduction

Although chromosomal replication is extremely processive, DNA damage can prevent the replication machinery from accurately completing its task and can result in either mutagenesis or lethality for the cell in which it occurs. Considering the severe consequences that can result from the improper processing of damaged DNA templates, the molecular events that normally allow replication to accurately duplicate damaged templates have been intensely studied over the years. This has resulted in the identification of a large number of candidate genes in both prokaryotes and eukaryotes which, when mutated, are known to impair the accuracy and processivity of replication in the presence of DNA damage. A remaining challenge has been to determine the precise roles that these gene products play in the recovery process. This challenge has been confounded, in part, by the necessity to first identify the substrates and intermediates that are produced at the replication fork when it encounters DNA damage. Several recent advances, using both in vitro and in vivo approaches in the model system of *E. coli*, have helped to define these substrates and should facilitate the design of further experiments that will allow

us to characterize the enzymes which process these structures and maintain genomic stability in the presence of DNA damage.
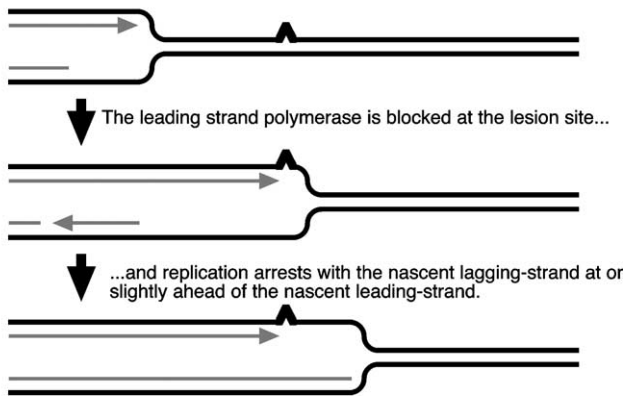
1.1. Replication fork structure

One interesting feature of the substrates generated by replicational encounters with DNA damage is that their structure varies depending upon which template strand contains the DNA lesion. The chromosome is duplicated by the coordinated replication of both the leading- and lagging-strand templates (reviewed in [41]). Since DNA polymerization on both strands occurs in a $5'$–$3'$ direction, the coordinated and simultaneous replication of both templates requires that unique enzymatic dynamics on each strand. Following a single priming event, the leading-strand template can be synthesized in a continuous, processive $5'$–$3'$ manner. However, the lagging strand template is synthesized in a direction opposite to the progress of the ongoing fork, and requires a primase activity that must constantly reprime the lagging strand template, resulting in discontinuous synthesis on the template (Okazaki fragments). These alternative mechanisms of synthesis on each template strand present different problems for the replication machinery when it encounters a DNA lesion. Higuchi and colleagues (2003) using a reconstituted system, examined how the replication holoenzyme behaves when it encounters a blocking

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A. Damaged Base in the Leading Strand Template

The leading strand polymerase is blocked at the lesion site...

...and replication arrests with the nascent lagging-strand at or slightly ahead of the nascent leading-strand.

B. Damaged Base in the Lagging Strand Template

The lagging strand polymerase is blocked at the lesion site...

...but the primase activity associated with the lagging strand allows synthesis to resume at the next downstream primer...

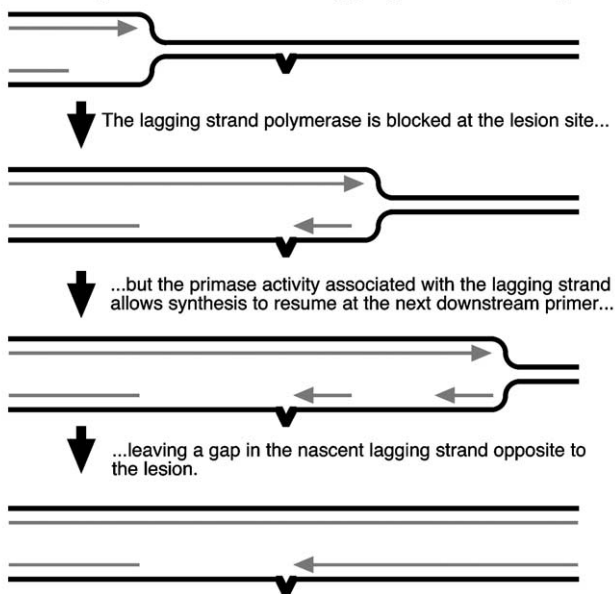*...leaving a gap in the nascent lagging strand opposite to the lesion.*

Fig. 1. Substrates generated when replication encounters a blocking DNA lesion in (A) the leading-strand template and (B) the lagging-strand template.

lesion, an AP-site, in either the leading- or lagging-strand template of a plasmid [25]. They observed that when the DNA lesion was found on the leading strand, the entire replication machinery was arrested. The substrate that resulted was a forked DNA structure that arrested with the nascent leading strand at the site of the lesion and the nascent lagging strand at, or slightly beyond, the lesion location (Fig. 1). Interestingly, when the lesion was placed in the lagging-strand, no disruption of replication was observed, although the polymerase that was blocked and failed to complete the Okazaki fragment in which the lesion was found. This resulted in the production of one intact daughter molecule and one gapped molecule containing the arresting DNA lesion. Nearly identical results have been obtained using a rolling-circle substrate (P. McInerney and M. O'Donnell, personal communication) and similar products were observed when plasmids containing a site-specific lesion in either the leading- or the lagging-strand template were trans-

formed into repair deficient cells [48]. These observations fit well with our understanding of the mechanics of how leading and lagging strands are coordinately synthesized. Blockage of the leading-strand polymerase might be expected to arrest replication due to the lack of any mechanism to prime and resume replication downstream of the lesion. By contrast, the primase activity associated with the lagging-strand polymerase allows replication to constantly reinitiate synthesis as new primers arise on the lagging-strand template, suggesting that when the lagging-strand polymerase is blocked at the DNA lesion, it may be able to simply reinitiate downstream when the next primer is synthesized, leaving the observed gap in the nascent lagging strand [25].

In vivo, it has long been observed that replication is transiently inhibited following DNA damage such as that induced by UV irradiation [58]. In addition, it was later shown that although replication is severely reduced, the limited DNA synthesis that does occur during this period of inhibition is in the form of short gapped fragments [54]. These observations would be consistent with the products that are observed in the in vitro assays described above. Following a moderate dose of UV-irradiation, DNA lesions would be randomly distributed between the leading and lagging strands. Thus, half of the replication forks would encounter lesions in the lagging strand template first, generating some gapped DNA substrates before all the replication forks are arrested at lesions in the leading-strand template. An important prediction from these observations, which remains to be tested in vivo, is that the gapped nascent DNA strands produced immediately after UV irradiation should be specific to the nascent lagging strand. The differences observed in vitro for leading- versus lagging-strand lesions implies that lesions are likely to require unique enzymatic processing events to repair and process the substrates produced in each situation. In addition, it also implies that lesions in either the leading or lagging strand may carry different biological consequences with respect to lethality and mutagenesis for the cell. For these reasons, it will be important to determine if the in vitro behavior of the replication machinery is similar to that which occurs in the cell following encounters with DNA lesions, so that the enzymatic pathways responsible for the accurate repair and replication of these templates can be determined.

2. Maintaining the replication fork

Several gene products are required to maintain and stabilize the replication fork upon encounters with DNA lesions. The most important of these is RecA, which binds and forms filaments on the single strand DNA regions generated when the replication fork encounters DNA lesions. RecA binding acts as the primary signal to upregulate the expression of more than forty "SOS" genes, many of whose products center on the task of repairing DNA damage, preventing premature cell division, and restoring processive replication [13,20]. In addition, the binding serves a more direct

role by maintaining the structural integrity of the replication fork itself when its progress is impeded. RecA performs this function by progressively pairing the single strand DNA regions with its replicated, sister duplex to create a RecA protein filament bound to a three strand DNA structure that is resistant to exonucleolytic degradation by cellular nucleases [10,14]. *recA* was originally identified and characterized as a gene that is essential for strand exchanges to occur during the sexual cell cycles of bacteria. During these recombinational processes, the strand-pairing activity of this protein plays a critical role in bringing different DNA molecules together to allow exchanges to occur. However, during the asexual reproductive cycle, this *same* biochemical activity of RecA also has nonrecombinational roles in maintaining the DNA replication fork [11,28]. Thus unlike the process of recombination, the strand-pairing activity of RecA may be required during replication to maintain, rather than rearrange, the strands of these arrested replication forks until after the offending lesion has been removed or bypassed, and replication can resume from these sites [8,11,14]. A role in maintaining the integrity of blocked replication forks until repair can occur is consistent with the observation that the survival promoted by *recA* following UV irradiation synergistically increases in the presence of nucleotide excision repair enzymes that can remove the UV-induced lesions [8,29].

In addition to RecA, RecF, RecO, and RecR are also required to maintain and allow the resumption of DNA synthesis following arrest by DNA damage [6,14]. Several lines of evidence, both in vivo and in vitro, suggest that these three gene products operate at a common step in promoting RecA's ability to maintain the blocked replication fork structure. Mutants lacking any one, or all three, of these gene products are equally sensitive to DNA damage, and delay the induction of SOS-regulated genes [24,27,36,40, 64,71], consistent with the idea that there is less activated RecA present at early times when RecF, RecO, or RecR is absent. In vitro, RecF, RecO, and RecR enhance the ability of RecA to bind DNA and prevent these filaments from disassembling at DNA ends [3,32,59,69,70], suggesting that these proteins play a role in stabilizing RecA filaments in their activated, bound form. In the absence of *recF, recO*, and *recR*, replication fails to recover following UV-induced DNA damage and the DNA at the replication fork is extensively degraded, although to a lesser extent than is observed in *recA* mutants, consistent with a role for these genes in facilitating the protection of the replication fork by RecA [6,14,53].

Other RecF pathway associated proteins, RecJ and RecQ, partially degrade and process the nascent lagging strand of the replication fork following arrest. RecQ is a $3'$–$5'$ helicase, and RecJ is a $5'$–$3'$ single-strand exonuclease [39,65]. Although the extent of degradation is limited by the presence of RecFOR and RecA, some nascent DNA processing is still detected in wild type cells. It is speculated that the processing may generate a much more

extensive substrate for RecA to bind and stabilize at the blocked replication fork, thereby ensuring that replication resumes from the same site at which disruption occurred. By analogy, *recQ* homologs in yeast, Drosophila, and humans play critical roles in maintaining processive replication and suppressing the frequency with which strand exchanges occur [15,19,23,33,44,57,61,67,68,73].

The degradation of nascent DNA and lack of further DNA synthesis suggests that RecFOR and RecA are essential for processing blocked replication fork substrates. However, it seems reasonable to assume that these proteins would also participate in protecting and processing gaps that are generated by nonarresting DNA lesions that are suspected to arise on the lagging strand template.

3. Dealing with the lesions

3.1. DNA repair

Following a moderate dose of UV irradiation, the replication machinery is transiently arrested, presumably upon encountering a lesion in the leading-strand template. One possible mechanism that may operate in this situation is that repair enzymes may come in and remove the blocking-DNA lesion (Fig 2A). In the case of UV irradiation (at 254 nm), two primary DNA lesions, the *cis*, *syn*-cyclobutane pyrimidine dimer (CPD) and the pyrimidine-6-4-pyrimidone photoproduct (6-4 PP), are formed in the DNA and block the DNA polymerases [4,42,43,58]. The *uvrA*, *uvrB*, and *uvrC* genes of *E. coli* are required to initiate nucleotide excision repair of UV-induced DNA lesions (reviewed in [56]). *E. coli* strains mutated in any one of these genes are unable to remove UV-induced lesions from DNA, exhibit elevated levels of recombination and lethality, and are associated with a severely impaired ability to recover replication [8,55]. Replication fails to resume in *uvr* mutants despite the protection of replication fork DNA and its structure [8]. These observations support the possibility that repair may be a prominent mechanism that acts when the progress of the replication apparatus is arrested by a DNA lesion.

It has also been speculated by our group that the recovery of replication may require a transient displacement of the nascent DNA (and potentially the replication machinery) so that repair enzymes can gain access to the offending lesion and effect repair. Consistent with this idea, the displacement of the nascent DNA at blocked replication forks can be observed on plasmids following UV-induced DNA damage in vivo [8,9]. In this case, the displacement of the nascent DNA at the blocked fork occurs spontaneously due to the supercoiling of the plasmid [49]. If fork reversal is required on the chromosome, it has been proposed that branch migration enzymes, such as RuvAB or RecG, may be involved in displacing the nascent strands of the replication fork, effectively pushing the junction point of the DNA fork backwards. Consistent with a potential role for RecG or
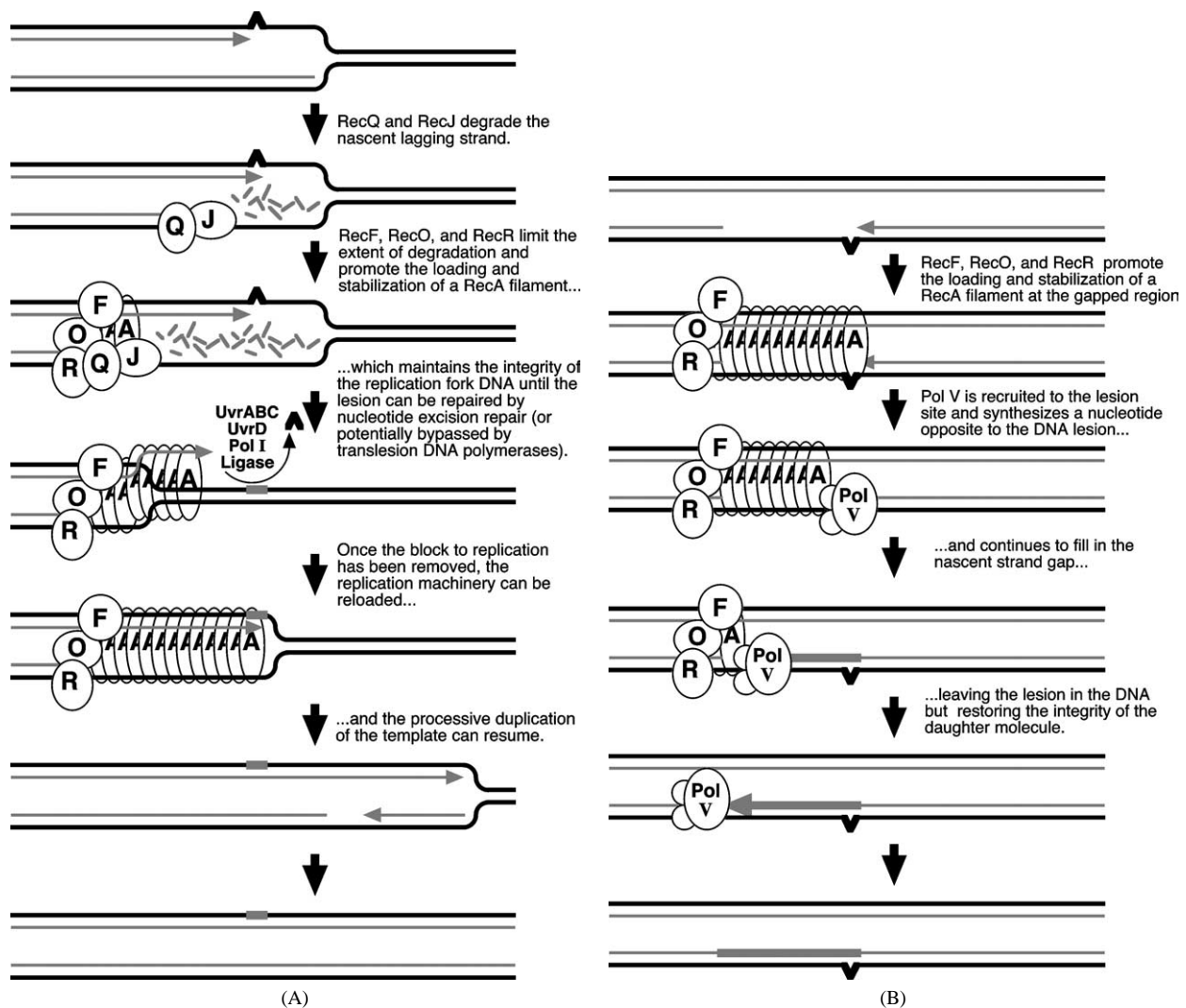
Fig. 2. Models for (A) the repair of a DNA lesion that arrests DNA replication and (B) the tolerance of a DNA lesion that does not arrest DNA replication.

RuvAB in catalyzing this event, both enzymes have been shown to be capable of catalyzing branch migration on synthetic replication-like structures in vitro [26,72] and mutants lacking either enzyme are moderately hypersensitive to UV irradiation [38,72]. However, other observations suggest that the UV hypersensitivity of *recG* or *ruvAB* mutants may be due to roles unrelated to the recovery at blocked replication forks. The absence of RecG or RuvAB does not affect the timing or rate that replication resumes following UV irradiation [17], implying that RuvAB- or RecG-catalyzed fork regression is not essential for DNA synthesis to resume following arrest at DNA damage. It remains possible that the displacement and degradation of the nascent DNA by RecJ and RecQ may be sufficient to restore the parental template to a form that is accessible to the repair enzymes (Fig. 2A). This leaves the question however, of what the essential role for RuvAB or RecG is that renders cells sensitive to DNA damage when they are absent. It remains possible that these enzymes may help to ensure that replication resets and resumes from the correct template, they may be required to

process nonreplication blocking DNA lesions, or they may act upon substrates that are not directly involved with the replication fork. The precise role that these enzymes play remains an interesting and important question to be addressed.

3.2. Translesion DNA synthesis

A second mechanism that functions following replicational encounters with DNA damage is translesion DNA synthesis. The *E. coli* genome encodes three damage-inducible DNA polymerases, *polB* (Pol II), *dinB* (Pol IV), and *umuDC* (Pol V), which are able to incorporate nucleotides opposite to DNA lesions with higher efficiency than the replicative polymerase, Pol III [1,2,5,31,34,35,47, 50,63,66]. Of these three polymerases, only mutants in Pol V, *umuDC*, render cells modestly hypersensitive to UV irradiation and reduce the level of mutagenesis following UV irradiation [1,34], indicating that Pol V is operating following UV-induced DNA damage but incorporates the incorrect nucleotide with elevated frequencies relative to the

replicative polymerase. In vitro, the translesion polymerase reaction also requires the activated form of RecA bound to the single-strand region, consistent with the structures and proteins that are present at replicated lesion-containing sites. In vivo, the absence of Pol V also modestly reduces the kinetics with which DNA synthesis resumes, and prolongs the persistence of gaps in the nascent DNA following UV [7]. The absence of the other polymerases does not render cells hypersensitive to UV irradiation and, in our hands, they do not affect the timing with which replication resumes [7]. However, other groups have observed a delay in the resumption of DNA synthesis following UV irradiation in Pol II mutants that were irradiated in very early log phase [51]. Napolitano and colleagues used different forms of DNA lesions, an *N*-2-acetylaminofluorene guanine adduct, a benzo(a)pyrene adduct, and a 6-4 PP, to demonstrate that the mutational spectrum that is produced depends upon which polymerases are present in the cell [46], suggesting that each polymerase may normally function at specific forms of DNA damage, perhaps similar to the specificity associated with DNA glycosylases for their respective structural lesions. Overall however, the observations that UV survival and the recovery of replication are not severely impaired in the absence of any or all of these inducible polymerases imply that these enzymes are not essential for replication to resume from lesions that block the progression of the replication fork. Yet it is also important to point out that these observations also cannot preclude the possibility that they do function at the arrested replication fork substrates.

Alternatively, several of the phenotypes associated with the translesion polymerases are consistent with the view that they may act on gapped substrates produced from nonreplication-blocking lesions. Lesions on the lagging strand template are not expected to inhibit the progression of replication, but instead produce gapped substrates. Therefore, mutants deficient in the processing of lagging strand gaps would not necessarily be expected to exhibit a severe reduction in the recovery of replication, perhaps similar to the phenotype exhibited by the *umuDC* mutants. In addition, *umuDC* mutants do exhibit a delay in the joining of short nascent DNA fragments that are produced following UV irradiation as measured in alkali sucrose gradients [7]. Interestingly, translesion DNA synthesis by UmuD$'_2$C in vitro requires a RecA-coated DNA template that has a DNA primer synthesized up to one base prior to the site of the lesion [52,60,62,63], conditions which are very similar to those predicted to be generated in vivo following replication through a lesion on the DNA template (Fig. 2B).

3.3. Recombination

A third process that occurs and may carry significant biological consequences during the asexual duplication of the cell is recombinational repair. This mode is most prominent in cells that are deficient in their ability to recover

replication normally following UV irradiation, such as nucleotide excision repair mutants. For this reason, most of the work characterizing this process has been done in *uvr* mutants. A large body of work using repair deficient mutants has documented that UV-induced DNA damage can lead to recombination events when replication encounters lesions that cannot be repaired [21,22,54,55]. These early studies revealed that the limited replication that occurs after UV irradiation in *uvr* mutants is fragmentary and accompanied by high frequencies of strand exchanges [21,22,54,55]. Furthermore, in the presence of RecA, these fragments are joined into larger fragments [21,22,54,55]. These observations were made shortly after *recA* mutants had first been isolated as a gene required for the formation of recombinant products during the sexual process of conjugation and led to the proposal that the primary function of RecA was to promote strand exchanges as a mechanism to reconstruct damaged genomes from the partially replicated sequences of undamaged regions [21,22,54,55].

Recombination can clearly occur during the asexual cell cycle and may in fact be essential for the repair of some forms of DNA damage, such as double-strand breaks. Under conditions where it does occur, genetic studies demonstrate that it can result in the production of some viable molecules. However, several observations also suggest that recombination may not necessarily be a primary or productive mechanism that operates when replication encounters DNA lesions in wild type cells. When cells that are dependant upon recombination for repair, such as *uvr* mutants, incur DNA damage to levels where strand exchanges can be observed, high levels of chromosome rearrangements, mutagenesis, and lethality are invariably observed as well [8, 54,55]. Furthermore, in mammalian organisms where strand exchanges can be observed more directly due to their large chromosomes, the frequency of recombination during replication correlates directly with genomic instability, cell death, and carcinogenic transformation [16,18,30,45]. Thus, although recombination clearly plays an essential role during sexual cell cycles and operates to promote some survival in dire situations during the asexual reproduction of the cell, it also carries several potentially detrimental repercussions for the genomic stability and viability of the cell in which it occurs. This should not exclude the notion that recombination is an active process operating in the cell, but we believe it is important to consider that recombination may not be the primary endpoint that is generated by RecA-catalyzed events, and that the genetic screens we often utilize to observe the products of these events may actually represent rare mistakes that occur when the recovery has gone awry.

4. Conclusions

While the models presented here for the recovery of replication following DNA damage are consistent with the current experimental observations, it is important to stress

that they are just models, and that the precise frequencies or conditions in which repair, translesion synthesis, or recombination occur remain important issues to address and require further characterization. In addition, the special roles that several less well-studied genes, many of which render cells hypersensitive or mutable to DNA damage (for reviews see [12,37]), remain to be identified. With the molecular tools available today and the identification of the substrates produced when replication encounters DNA damage, this is an exciting and appropriate time to reexamine old observations and models, develop new assays, and dissect these issues. The question of how the cell is able to accurately maintain and pass on its genetic information from generation to generation despite the constant barrage of chemicals and agents that react and damage it, is one in which we should expect to make significant progress in the near future.

Acknowledgements

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