Global activity modulations can result in positively biased correlations, but correlations around the mean activity imposed by these modulations can still be extremely small (Fig. 4, D and E; and figs. S5 and S8). Similarly weak correlations have been reported in visually driven neural populations in area V1 of awake behaving monkeys (29). However, as the constellation of inputs driving a cortical circuit is, in general, unknown to the experimenter, positive correlations may persist even after all experimentally controlled variables are accounted for (II, 12). Whether “residual” correlations of this nature will have a strong impact on coding will depend on the extent to which downstream networks are able to disambiguate modulations in activity due to different sources. In either case, we suggest that cortical circuitry does not itself constitute an irreducible source of “noise.”

References and Notes

15. The correlation between two quantities, each given by sum of N variables correlated by an amount \( r_{ij} \), out of which \( N_p \) are common, is equal to \( \rho + r_{ij}(N - |1|) \), which is approximately equal to \( \rho + r_{ij}N \), when \( r_{ij}N < 1 \).
16. When the firing statistics of the \( E \) and \( I \) populations are identical, the leading order effect of positive firing correlations on \( c \) can only be \( \geq 0 \). If excitation and inhibition are precisely balanced, the equality is realized and the fraction of shared input sets the population-averaged firing correlation (9).
17. Details of the theory are available in the Supporting Online Material on Science Online.
21. Materials and methods are available as supporting material on Science Online.
24. M. Steriade, Electroencephalography (Williams & Wilkins, Baltimore, MD, ed. 4, 1999).
30. We thank A. Amarsingh, N. Brunel, G. Buzsaki, D. Boiron, A. Ecker, S. Fisjawa, A. Heimel, A. Kohn, D. Robbe, S. Sakata, E. Stark, and A. Tolas for comments on an earlier version of this manuscript; C. van Vreeswijk for discussions; and A. Compte, S. Ardid, and J. M. Marrie for sharing their codes. This study was supported by NIH grants MH073245 and DC009947; NSF grant SBE-0542013 to the Temporal Dynamics of Learning Center, an NSF Science of Learning Center; a National Institute on Deafness and Other Communication Disorders, NIH, grant DC-005787-01A1; and a Spanish grant FIS 2006-09294. K.D.H. is an Alfred P. Sloan fellow. We would like to dedicate this work to the memory of D. J. Amit.

Supporting Online Material

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SOM Text
Figs. S1 to S11
References
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Direct Restart of a Replication Fork Stalled by a Head-On RNA Polymerase

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In vivo studies suggest that replication forks are arrested by encounters with head-on transcription complexes. Yet, the fate of the replisome and RNA polymerase (RNP) after a head-on collision is unknown. We found that the Escherichia coli replisome stalls upon collision with a head-on transcription complex, but instead of collapsing, the replication fork remains highly stable and eventually resumes elongation after displacing the RNP from DNA. We also found that the transcription-repair coupling factor Mfd promotes direct restart of the fork after the collision by facilitating displacement of the RNP. These findings demonstrate the intrinsic stability of the replication apparatus and a previously unknown role for the transcription-coupled repair pathway in promoting replication past a RNP block.

In vivo studies suggest that replication forks are arrested by head-on transcription complexes, but are unaffected by codirectional transcription complexes (1) [supporting online material (SOM) Text S1]. Mechanisms that resolve head-on collisions in favor of the replisome are therefore necessary for chromosome duplication and may preserve genomic integrity by preventing fork collapse. In vivo data indicate that head-on replisome-RNA polymerase (RNP) collisions cause chromosomal deletions, which suggests dissociation of the replisome (2). Genetic studies implicate recombinational repair in resolving conflicts between replication and transcription, which also suggests the possibility of fork collapse (3, 4). Similarly, in vitro data imply that the replisome dissociates after encountering a lac repressor, which arrests the fork (5). In contrast, several in vivo studies indicate that although replication forks stall at protein barriers, the replisome remains stable and resumes elongation after removal of the block (6). Thus, replisome stalling may not necessitate fork collapse (7). We investigated the stability of the Escherichia coli replisome after it encounters a head-on RNP in vitro.

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The E. coli replisome is a multiprotein complex that copies DNA with high speed (~630 nucleotides (nt) s⁻¹) and processivity (~50 kb) (8). A solid-phase assay was used to study a replisome-RNAP head-on collision (Fig. 1A). An RNAP-halted elongation complex was assembled on linear DNA, immobilized to streptavidin beads, then washed with a high concentration of salt to remove unstable RNAP-DNA complexes (fig. S1) (9). Next, the replisome was assembled in two steps: First, the replicative DnaB helicase that encircles the lagging strand was added; second, DNA polymerase III (Pol III), the clamp loader, and the β clamp were added along with ATP (adenosine 5′-triphosphate), [³²P]-α-dCTP (2′-deoxyctydine 5′-triphosphate), and [³²P]-α-dGTP (2′-deoxyguanosine 5′-triphosphate). Fork movement was initiated by adding [³²P]-α-dATP and [³²P]-α-dTTP with single-strand DNA binding protein (SSB). We observed a 2.5-kb product equal to the distance from the fork to the promoter, indicating that the replisome was impeded by the RNAP (Fig. 1B, lane 2). Full-length DNA (3.6 kb) was also produced, suggesting incomplete promoter occupancy by RNAP or replisome read-through of the transcription complex (Fig. 1B, lane 2). Omitting the promoter specificity factor, σ²⁰, resulted in only full-length DNA (Fig. 1B, lane 1). An average of 50% (n = 8) of the replisomes produced full-length DNA in the presence of a head-on RNAP (Fig. 1B, right), which exceeded the number of templates that lacked RNAP (24%; fig. S2). This suggested that ~26% of the replisomes passed a head-on RNAP during the 10-min time course. We determined whether the replisome reads-through RNAP directly by performing a pulse-chase experiment in which cold dNTPs (deoxynucleoside triphosphates) were added after 5 min, and extension of the 2.5-kb product was monitored (Fig. 1C). A steady increase in the ratio of full-length to intermediate-length product was observed, indicating that the replisome passes the RNAP, albeit after pausing for a considerable duration (Fig. 1C). The relatively large amount of full-length product (39%) observed after 5 min suggests that some replisomes might have passed the RNAP with high efficiency. Replisome read-through of RNAP on a different template ruled out any sequence-specific effects (fig. S3). The stalled replisome remained active for 60 min after the collision without the need for primosomal proteins, such as PriA/C, that are necessary to reload DnaB onto SSB-coated single-strand DNA (Fig. 1C) (10, 11). DnaB therefore stayed bound to the stalled fork. Consistent with previous studies, we demonstrated that DnaB was required for replication and that SSB prevented DnaB loading in the absence of primosomal proteins (fig. S4). Thus, although the replication fork stalls upon encountering a head-on RNAP, the replisome remains intact and resumes elongation, presumably after displacing the transcription complex.

We determined whether the replisome displaces the RNAP from DNA by Xho I digestion...
of the promoter-proximal sequence, which is protected by RNAP (Fig. 2A). RNAP occupancy of the promoter blocks digestion, whereas RNAP displacement allows digestion. Removing ribonucleotides (NTPs) by washing prevents reassembly of the halted RNAP. A significant increase in digestion was observed only when replication was performed (Fig. 2A, right; compare blue and red bars). Little RNAP displacement was detected at 20 min, which is likely due to replication of only 8.5% of the DNA, probably as a result of RNAP binding to the primer-template (12) (fig. S5). Nevertheless, the large increase in digestion due to replication indicates that the replisome displaces the RNAP. Similar results were observed in the presence of GreB and limiting NTPs, which inhibit RNAP backtracking (fig. S6). We further investigated RNAP displacement by monitoring transcription of a challenge template in the presence of ε250 and limiting NTPs. Transcription of the challenge template was only observed after replication, which indicates that the replisome displaced the RNAP (Fig. 2B, compare lanes 2 and 3). These results are consistent with the ability of DnaB to displace a protein block during DNA unwinding (13).

Genetic data implicate RNAP modulators such as Mfd in resolving conflicts between replication and transcription (3). Mfd displaces a halted RNAP from DNA and recruits the nucleotide excision repair machinery to the site, which results in preferential repair of the transcribed strand when RNAP stalls at lesions—referred to as transcription-coupled repair (TCR) (14–18). TCR has been postulated to promote fork progression by displacing RNAP blocks from DNA (16). We examined whether Mfd facilitates restart of the fork after a head-on collision. After providing 5 min to allow the replisome to collide with RNAP, we divided the reaction and treated it with either Mfd or buffer for a further 5 min. Nearly full extension of the 2.5-kb product was observed upon addition of Mfd (Fig. 3A, compare lanes 1 and 2), which removed the RNAP block (fig. S7). We determined whether Mfd-mediated replication restart requires a supply of DnaB in solution (Fig. 3B). For this experiment, the beads were washed after the collision then resuspended in buffer containing dNTPs and all the replication proteins except DnaB either in the presence or absence of Mfd. The addition of Mfd again resulted in extension of the 2.5-kb product, providing further evidence that DnaB stays bound to the stalled fork. Next, the experiment of Fig. 3A was repeated with Mfd K634N, which is defective in ATP hydrolysis and can no longer dislodge RNAP (17). Mfd K634N failed to promote extension of the 2.5-kb product (Fig. 3C, compare lanes 1 and 2). These results indicate that Mfd promotes fork progression after the collision by using the energy of ATP, and hence translocase activity, to dissociate the transcription complex ahead of the stalled fork. Finally, we demonstrated that the ability of Mfd to promote replication past a head-on RNAP was unaffected by the addition of all four NTPs and GreB, which promote transcription elongation (fig. S8).

In conclusion, we find that although the replication fork stalls upon collision with a head-on transcription complex, the replisome remains stable and resumes elongation after displacing the RNAP (fig. S9, left). It is conceivable that the collision may induce RNAP backtracking. However, the lack of stimulation of RNAP endonuclease activity after the collision suggests that this is not the case (fig. S10). Moreover, the addition of GreB and NTPs, which inhibit backtracking, have no effect on our assays (figs. S6, S8, and S11). A previous study of the T4 replisome reported that a head-on RNAP remains bound to the DNA, but the RNAP and transcript switch strands as the replication fork passes (19). This result is difficult to reconcile with the current view of transcription. We find that Mfd promotes direct restart of the fork following the collision by facilitating displacement of the RNAP (fig. S9, right). Codirectional collisions are resolved without auxiliary factors; the replisome uses mRNA as a primer to reinitiate leading-strand synthesis after displacing a co-directional RNAP from DNA (9). Pol III extension of the RNA was not observed in our study, probably due to displacement of the transcript. Genetic data suggest that recombinational repair and other RNAP modulators help resolve replisome-RNAP conflicts, thus explaining the normal growth rate of cells lacking Mfd (3). mfd cells, however, demonstrate a greater lapse in replication and cell growth following ultraviolet irradiation, which supports a role for Mfd in facilitating replication through transcription complexes arrested by lesions in vivo (20).

Our data demonstrate a new role for TCR in promoting replication past an RNAP block and may have implications for human disorders that result from deficient TCR, such as Cockayne syndrome (15, 16).

References and Notes
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