trough containing chocolate (followed by the rat consuming the chocolate), *or* by the rat traveling down the arm to encounter chocolate (on its own) and consuming it [1]. Thus, familiarity of having received chocolate (semantic memory) was identical in both instances. But the source (episodic memory) of the chocolate ('directed' placement by the experimenter, or 'discovered' by the rat) was different.

In Experiment 1, the place where the rat discovered chocolate on its own was replenished with chocolate on the test, but not the place where the rat was placed in front of the trough containing chocolate. Rats accurately remembered this source rule. In another experiment (Experiment 4), the rule was reversed and the rats accurately remembered this opposite source rule — showing that handling (the rats) was not an artifact that produced a negative result (disrupted memory). In Experiment 2, rats were shown to accurately transfer the appropriate source rule to a second, different maze in which they had no previous 'source' training — thereby demonstrating that overlearned cues from a particular maze were not an artifact that produced a positive result (good memory).

Additionally, the rats' source memory was shown to be special (Experiment 3) — as it should be if it really was episodic memory — by lasting much longer (seven days or more) than 'run of the mill' memory (one day) for regular rat chow. And lastly, the rats' source memory was shown (Experiment 5) to be disrupted by temporarily disabling (with lidocaine) a brain area (CA3 region of the hippocampus) thought to be crucial for accurate human source memory. This last result adds important converging evidence that this procedure really is testing something very close to source memory.

This study [1] sets the stage for exploring and better understanding the neural basis of source memory (and episodic memory), not possible with humans even with high-resolution imaging. In addition to the CA3 region of the hippocampus investigated in this work, the role of other brain areas could be tested in future experiments with this procedure. Studies have shown the importance of other medial temporal lobe structures in related memory tasks (for example, the CA1 region of the hippocampus, dentate gyrus, parahippocampal and entorhinal cortecies) [6]. But such memories are not 'stored' in the medial temporal lobes. Memories are distributed (neural circuits). Often (maybe always) remembering reactivates sensory association areas (for example, parietal lobe, located dorsal to the temporal lobe) and even primary sensory areas (for example, occipital lobe located caudal to the parietal lobe) that produced those memories in the first place [7].

Often (maybe always) what controls reactivation of a memory comes from a very different brain region — the prefrontal cortex of the frontal lobe [7]. So, reactivation of memories coupled with reactivation along paths of the original activation form loops of memory activity. With a rat model of source memory like that shown by Crystal *et al.* [1], neural firing in several brain regions could be recorded in real time as the rat makes correct (and incorrect) source judgments. These techniques along with others (e.g., molecular, genetic) may someday be able to specify pathways and mechanisms of how episodic memory works, perhaps leading to approaches for repairing memory when it begins to fail.

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Chromosome Biology: Conflict Management for Replication and Transcription

A recent study has uncovered a new mechanism that attenuates DNA replication during periods of heightened gene expression to avoid collisions between replication and transcription.

James M. Dewar and Johannes C. Walter

In all organisms, chromosomes host two essential metabolic process, gene transcription and DNA replication, which would appear to conflict with one another. DNA replication copies the genetic information in preparation for cell division and is initiated at sites called origins [1]. At each origin, two replisomes are established that consist of a replicative DNA helicase, leading and lagging strand DNA polymerases, and many accessory factors. The two replisomes travel away from the origin in opposite directions, copying both strands of the duplex as they go. While prokaryotes generally replicate their genomes from a single origin, eukaryotic cells employ up to hundreds of thousands of origins in every S phase. Like DNA replication, transcription involves copying the information encoded in the genome, in this case by an RNA polymerase that

transcribes one DNA strand into RNA. When a replication and transcription complex collide, the consequences are potentially disastrous. In a recent paper, Duch *et al.* [2] report an elegant new mechanism that prevents collisions between replication and transcription during a heightened transcriptional response.

Cells are known to use a variety of strategies to resolve the conflict between replication and transcription [3,4]. The most dangerous situation involves a head-on collision between RNA polymerase and the replication fork. Here, RNA polymerase runs headlong into the lagging strand DNA polymerase (Figure 1, lower panel). The fork might be able to displace or circumvent a single RNA polymerase. However, if it encounters an array of RNA polymerases on a highly transcribed gene, the replication fork can stall, and eventually collapse, leading to genome rearrangements. In *Eschericia coli*, where the directionality of replication throughout the genome is well-defined due to the use of a single origin, highly transcribed genes are oriented so as to be co-directional with replication. Another strategy is the use of replication fork barriers. In the heavily transcribed rDNA locus in eukaryotes, a unidirectional replication fork barrier residing at the 3' end of the rDNA gene prevents head-on collisions of RNA polymerase and the replisome.

A problematic situation arises when there is a sudden and dramatic increase in the expression of many genes, as seen during osmotic stress. In yeast, ~600 genes are upregulated in response to 'osmostress' by the stress-activated protein kinase (SAPK) Hog1 [5,6]. Hog1 activates gene expression by phosphorylating transcription factors such as Smp1 and Sko1, and by recruiting RNA polymerase and chromatin remodelers [7]. Importantly, Hog1 is activated in response to osmotic stress in all stages of the cell cycle, including S phase [8]. How cells manage the conflict between replication and many highly induced genes has been unclear.

Duch *et al.* [2] have tackled this problem. In a proteomic screen, they identified the replication factor Mrc1 as a direct target of Hog1 phosphorylation. Mrc1 is required for optimal replication fork progression [9–11], possibly due to a physical interaction with DNA polymerase

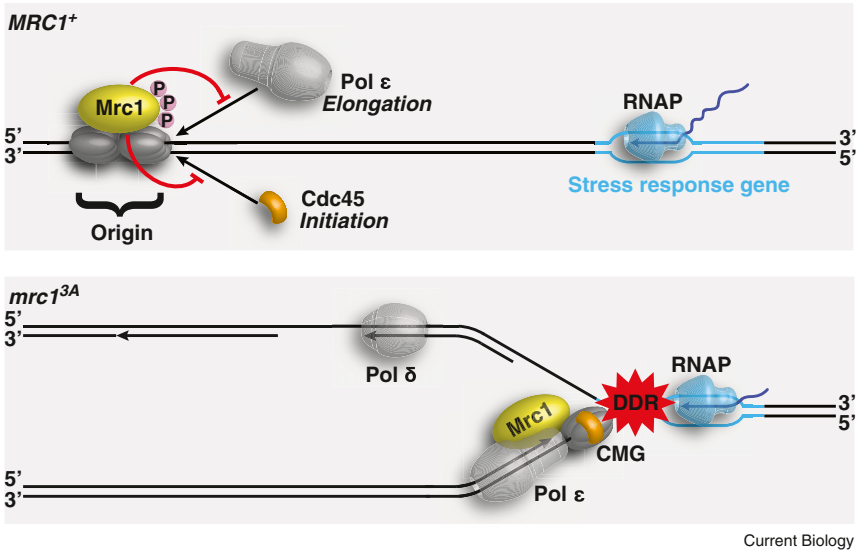

Figure 1. Model for how Mrc1 prevents transcription–replication collisions.

In response to osmotic stress, many genes are upregulated by the protein kinase Hog1, potentially causing collisions between the transcription and replication machineries. In wild-type cells, Hog1 phosphorylates Mrc1, which in turn inhibits origin firing (initiation) and replisome progression (elongation), reducing the probability of replisome and transcription complex collisions. In *mrc1*^{3A} cells, Mrc1 does not inhibit replication during osmostress, and a DNA damage response (DDR) results from transcription–replication collisions.

epsilon (Pol ε) [12]. Duch *et al.* show that Hog1 phosphorylates Mrc1 on three MAPK consensus sites. When yeast cells experience osmostress, S phase progression is severely delayed. This effect does not require signaling by the Rad53 or Mec1 checkpoint kinases. Instead, it depends critically on Hog1-dependent Mrc1 phosphorylation as no S phase slowing is seen with an *MRC1* allele (*mrc1*^{3A}) lacking the three Hog1 phosphorylation sites. Therefore, Hog1-dependent phosphorylation of Mrc1 is required to slow S phase progression in response to osmostress.

How does Mrc1 phosphorylation delay S phase? Chromatin immunoprecipitation (ChIP), 2-D gel electrophoresis, and DNA-combing assays suggest that the rate of replication fork progression is significantly diminished in response to osmostress, perhaps due to a reduced interaction between Mrc1 and Pol ε (Figure 1, upper panel). In addition, firing of both early and late origins was inhibited due to defective loading of the helicase co-factor Cdc45. The effects of osmostress on initiation and elongation were abrogated in the *mrc1*^{3A} mutant (Figure 1, lower panel), consistent with the lack of a delayed S phase in these cells and with both effects being mediated through inhibition of mrc1 function.

Duch *et al.* next addressed the consequences of disrupting the osmoregulation of Mrc1. They found that unlike wild-type (WT) cells, *mrc1*^{3A} cells exhibited a dramatic increase in chromosomal instability after osmotic shock. They suspected that the observed chromosomal instability might be due to conflicts between DNA replication and transcription. To test this, they used a plasmid in which an osmostress-driven reporter gene consisting of tandem repeats is located in two orientations with respect to an origin of replication. In the presence of fork collapse, the tandem repeats recombine, yielding a functional reporter gene. As such, the system reads out transcription-associated recombination (TAR). Strikingly, *mrc1*^{3A} but not WT cells exhibited a massive increase in TAR after osmostress, but only when the promoter and origin were oriented such as to yield head-on collisions of the replication and transcription machines. No TAR was observed with co-directional promoters. Although *mrc1*^{3A} cells had normal viability during osmostress, when they also lacked Rad53, viability was reduced. This suggests that when the Hog1–Mrc1 pathway is disrupted, the damage

caused by fork collisions can be dealt with by the traditional DNA damage checkpoint. In summary, the data provide compelling evidence that cells attenuate DNA replication during periods of heightened transcription to avoid genomic catastrophes.

Mrc1 is not only required for replication fork progression but also for amplification of checkpoint signaling during replicative stress, for example when deoxyribonucleotides are depleted by hydroxyurea (HU) [9,13]. Specifically, Mrc1 serves to amplify checkpoint signaling by the Rad53 kinase, which involves Mrc1 phosphorylation by Rad53. Importantly, the Rad53 and Hog1 phosphorylation sites in Mrc1 are distinct, and the *mrc1*^{3A} allele supports normal checkpoint signaling and cell survival during replication stress. Thus, Mrc1 participates in distinct osmostress and replication stress pathways, governed by Hog1 and Rad53, respectively.

The study raises numerous interesting questions. One concerns the mechanism of how Mrc1 phosphorylation prevents replication initiation. Previous results suggest that assembly of the Cdc45, Mcm2-7, GINS (CMG) replicative helicase complex is required for recruitment of Mrc1 and Pol ε to the replisome [14,15], whereas Mrc1 is not normally required for recruitment of Cdc45 [16]. In this light, it is surprising that Hog1-phosphorylated Mrc1 binds origins and delays loading of Cdc45 and Pol ε [2]. The data imply that osmostress converts Mrc1 into a dominant negative inhibitor that binds pre-RCs and prevents loading of Cdc45 and Pol ε. A possible precedent for Mrc1 functioning as an inhibitor is seen in fission yeast, where deletion of Mrc1 enhances replication initiation efficiency at some origins and where Mrc1 can bind origins independently of Cdc45 [17]. In the future, it will be interesting to explore the mechanistic basis of how Mrc1 inhibits origin firing under different conditions. An interesting point is that slowing down replication forks should increase rather than decrease the probability that an RNA polymerase encounters a replication fork. Perhaps Mrc1 phosphorylation not only slows, but also stabilizes the fork in the event of collision with RNA polymerase.

Another pressing question is whether the conclusions of this study apply to metazoans. In support of this notion, high osmolarity and other stresses promote phosphorylation of the MCM2-7 loading factor Cdt1 by the mammalian SAPKs p38 and JNK, thereby inhibiting origin licensing [18,19]. Whether post-licensing events of origin firing and fork progression are also inhibited, and whether this avoids clashes with transcription, remains to be tested. Based on the findings by Duch *et al.*, it seems likely that cells will use many creative strategies to manage the conflict between replication and transcription.

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Causality: Perceiving the Causes of Visual Events

Adapting to visual collisions increases the tendency to see the colliding objects as sliding over one another, rather than as one 'launching' another, but only in the adapted retinal location. This demonstrates a low-level perceptual component to the interpretation of the causes of visual events.

Alan Johnston

Imagine a billiard ball rolling directly towards another: it makes contact, stops and then the other ball rolls forwards. Naturally, we see a collision and have the impression that the first ball caused the second to move.