PERSPECTIVES: TRANSCRIPTION

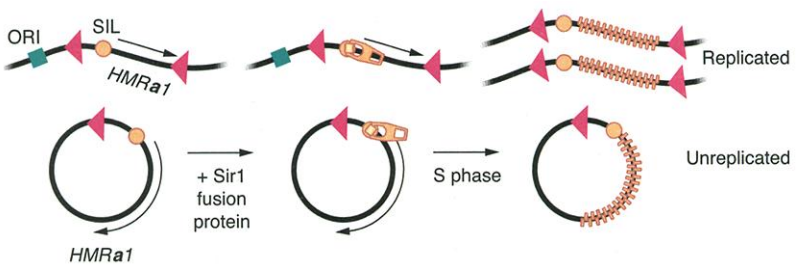
Is S Phase Important for Transcriptional Silencing?

Jeffrey S. Smith and Jef D. Boeke

Transcriptional silencing is a mysterious phenomenon that renders large sections of chromosomal DNA inert. This process alters the structure of chromatin, thus preventing the transcriptional machinery from reaching genes, which consequently remain switched off. Well-known examples of silencing include the inactivation of one X chromosome during development of the female human embryo, and position effect variegation in the fruit fly. The common denominator of these forms of silencing and many others (including silencing of the mating type loci *HMRa* and *HMLα* in the yeast *Saccharomyces cerevisiae*) is the formation and maintenance of a repressive chromatin structure called heterochromatin. For more than 15 years, researchers have known that silencing of chromatin depends on progression of cells through S phase and they have assumed that DNA replication must be the key event required before silencing can commence.

Heterochromatin is replicated during the last portion of S phase, whereas the rest of the DNA is replicated much earlier, suggesting that the way in which heterochromatin is replicated may be quite different. Classic experiments by Miller and Nasmyth in the early 1980s (*1*) suggested that cells must pass through S phase of the cell cycle before silencing can be established (that is, before unsilenced chromatin can be converted to the silenced state). Moreover, several genes involved in DNA replication also participate in silencing (see the table, next page). Using clever genetic tricks in yeast, Kirchmaier and Rine (page 646) and Li *et al.* (page 650) now challenge the assumption that DNA replication during S phase is a prerequisite for chromatin silencing (*2*, *3*).

Silencing of yeast mating type–specificity genes in the *HMRa* and *HMLα* loci depends on sequences called silencers that flank these genes. These silencer sequences contain binding sites for proteins such as Rap1p, Abf1p, and proteins of the origin recognition complex (ORC) (*4*). Together with the Sir (silent information regulator) proteins, silencers and the proteins that bind them establish a heterochromatin-like structure in yeast. This structure is characterized by H3 and H4 histone proteins with fewer acetyl groups in their amino termini and by alterations in the topology of the DNA that is wound around the histones (*5*–*7*). Silencing of genes in the *HMRa* and *HMLα* loci is required for sexual competence in yeast.



Zipping up unreplicated chromatin. Yeast cells arrested in the pre–S-phase state were engineered to contain the *HMRa1* gene of the *HMRa* mating-type locus on an excised ring lacking a replication origin (bottom panel). The *HMRa1* gene in its normal chromosomal location is shown in the upper panel. Triangles represent recombination sites in the excised ring; the arrow represents the unsilenced *HMRa1* transcript. Expression of a Sir1 fusion protein (zipper tab), which binds to *HMRa* at the E silencer (SIL, orange dot) sequence, established silencing of the *HMRa1* gene after release of the cells into S phase. The *HMRa1* DNA in its native chromosomal location is replicated through the action of an adjacent origin of replication (ORI, blue square), whereas the excised DNA circle cannot replicate because it does not have an ORI. Subsequently, silenced chromatin (closed zipper) was established on both the replicated DNA and unreplicated ring, resulting in equivalent amounts of *HMRa1* gene silencing in both native and ring DNA.

Besides initiating DNA replication by binding to replication origins in the DNA, the ORC recruits Sir1p to the silencer sequence, which in turn recruits other Sir proteins (*8*). Artificially targeting a Sir1 fusion protein to a silencer engineered to lack an ORC binding site has been shown to result in the establishing of chromatin silencing (*9*). In the new studies, the two groups deleted the ORC binding site from the *HMR-E* silencer of the yeast *HMRa* locus and replaced it with either LexA or Gal4 DNA binding sites. This elegant genetic trick rendered the modified *HMRa* locus incapable of DNA replication. In a remarkable convergence of strategy, both groups then used the same genetic approach for their next trick—they flanked *HMRa* with recombination sites for FLP (or the related R) recombinase. This allowed the controlled excision of *HMRa* from the genome as a nonreplicating DNA ring (see the figure). After excision of the ring, the investigators expressed the Sir1 fusion protein (composed of Sir 1 and either the Gal4 or LexA DNA binding domain) and examined whether silencing had been established. Both groups went to heroic lengths to detect very small amounts of ring replication, yet they saw none. Efficacy of silencing was then evaluated by RNA blot analysis of the *HMRa1* gene. In both studies, silencing was established as efficiently on the excised, nonreplicating *HMRa* ring as on the replicated chromosome. Thus, DNA that had not been replicated during the previous S phase could still be silenced. Importantly, the S-phase requirement for establishing silencing was still retained in this somewhat contrived system, even though DNA replication per se was not required.

It could be argued that the silenced chromatin established in these experiments was not authentic because silencing was ORC independent and required artificial recruitment of a Sir1 fusion protein to a single modified silencer. This argument, however, is largely refuted by the experiments of Li *et al.* (*3*), who found that *HMRa* rings had physical characteristics indistinguishable from those of native silent chromatin. These characteristics include hypoacetylation of histones H3 and H4, excessive negative supercoiling, and dependence on the *SIR2*, *SIR3*, and *SIR4* genes. The silent chromatin observed appears, therefore, to be authentic. It remains formally possible that some aspect of the ORC-Sir1p interaction might be affected by replication, given that the interaction was by necessity not evaluated in these experiments.

It is important to emphasize that establishment, maintenance, and inheritance of silencing are three different processes. The current reports do not address the possible importance of DNA replication in the maintenance or heritability of silent chromatin. There is still compelling evidence that DNA replication could be involved in the maintenance or heritability of silencing at the *HMRa* and *HMLα* loci and probably at other loci, too; it may also be required for silencing genes in telomeres and in ribosomal DNA that lack clearly defined silencer elements. Mutations in subunits of yeast chromatin assembly factor–I (CAF-I)

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PROTEINS INVOLVED IN BOTH DNA REPLICATION AND SILENCING

Element	Replication	Silencing
HMR ARS	Binds ORC	Binds ORC
ORC	Scaffold for preinitiation complex	Recruits Sir1p
CAF	Nucleosome assembly during replication	Assembly of silent chromatin?
DNA polymerase α	Initiate nascent DNA chains	?
DNA polymerase ε	Elongation; repair	?
PCNA	Sliding clamp	?
RF-C	Clamp loader; elongation processivity	?

A variety of DNA sequences and proteins are important for transcriptional silencing. The ORC is involved in both DNA replication and silencing, and different portions of ORC proteins carry out its separate functions (*16*). The ORC recruits Sir1p, which is required for silencing to be established (*9*). In contrast, other components of the ORC involved in replication seem to be important for movement of the replication fork along the DNA; any part that they may play in silencing remains obscure. Although some ORC components are required for efficient silencing, others inhibit silencing; differential effects of these proteins are seen depending on whether silencing is examined in yeast mating-type loci or in ribosomal DNA (*12, 13, 17*). ARS, autonomously replicating sequence (the site of ORC binding); RF-C, replication factor C.

reduce the stable inheritance of silencing at telomeres and impair the maintenance of silencing at *HMRa*, *HMLα*, and other loci (*10–12*). The connection with DNA replication is that CAF-I deposits newly synthesized histones onto newly replicated DNA. Furthermore, mutations in proliferating cell nuclear antigen (PCNA) that disrupt the association of this replication protein with CAF-I also impair the inheritance of silencing (*13*). The implication is that DNA replication may well prove crucial for "persistence" of the silent state.

This returns us to the original question: What makes S phase important for transcriptional silencing? Strictly speaking, the phase of the cell cycle required for silencing to be established is somewhere between early S phase (the point where hydroxyurea blocks cell cycle progression) and mitosis (where nocodazole has its inhibitory effect) (*1*). This suggests that the S-phase requirement for silencing may in reality be a point somewhere in late S phase, in G_2, or possibly even in early mitosis.

The cell's DNA replication machinery is fully capable of replicating conventional chromatin or previously silenced chromatin. However, it may be challenged by the drastic alterations in chromatin structure that accompany the establishment of silencing, during which the chromatin changes from the relatively open unsilenced state to the closed heterochromatic state. To prevent such molecular conflicts, the silencing machinery may be activated by an intracellular signal sent when DNA replication has been completed. In fact, Sir proteins can move to new locations inside the cell in response to various forms of DNA damage (*14, 15*), indicating that they can respond to signals sent by changes in DNA state. Needless to say, there are many other possible events that could control the establishment of silencing through the generation of specific cell cycle signals or changes in nuclear structure. But what was once considered the most likely event—replication of the DNA prior to silencing—does not appear to be among them.

References

1. A. M. Miller, K. A. Nasmyth, *Nature* **312**, 247 (1984).
2. A. L. Kirchmaier, J. Rine, *Science* **291**, 646 (2001).
3. Y.-C. Li, T.-H. Cheng, M. R. Gartenberg, *Science* **291**, 650 (2001).
4. S. Loo, J. Rine, *Annu. Rev. Cell Dev. Biol.* **11**, 519 (1995).
5. M. Braunstein *et al.*, *Mol. Cell. Biol.* **16**, 4349 (1996).
6. X. Bi, J. R. Broach, *Mol. Cell. Biol.* **17**, 7077 (1997).
7. T. H. Cheng *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 5521 (1998).
8. T. Triolo, R. Sternglanz, *Nature* **381**, 251 (1996).
9. C. A. Fox *et al.*, *Science* **276**, 1547 (1997).
10. S. Enomoto, J. Berman, *Genes Dev.* **12**, 219 (1998).
11. E. K. Monson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13081 (1997).
12. J. S. Smith *et al.*, *Mol. Cell. Biol.* **19**, 3184 (1999).
13. Z. Zhang *et al.*, *Nature* **408**, 221 (2000).
14. S. G. Martin *et al.*, *Cell* **97**, 621 (1999).
15. K. D. Mills *et al.*, *Cell* **97**, 609 (1999).
16. A. Dillin, J. Rine, *Genetics* **147**, 1053 (1997).
17. A. E. Ehrenhofer-Murray *et al.*, *Genetics* **153**, 1171 (1999).

PERSPECTIVES: ARCHAEOLOGY

What Drives Societal Collapse?

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The archaeological and historical record is replete with evidence for prehistoric, ancient, and premodern societal collapse. These collapses occurred quite suddenly and frequently involved regional abandonment, replacement of one subsistence base by another (such as agriculture by pastoralism), or conversion to a lower energy sociopolitical organization (such as local state from interregional empire). Each of these collapse episodes has been discussed intensively within the archaeological community, commonly leading to the conclusion that combinations of social, political, and economic factors were their root causes.

That perspective is now changing with the accumulation of high-resolution paleoclimatic data that provide an independent measure of the timing, amplitude, and duration of past climate events. These climatic events were abrupt, involved new conditions that were unfamiliar to the inhabitants of the time, and persisted for decades to centuries. They were therefore highly disruptive, leading to societal collapse—an adaptive response to otherwise insurmountable stresses (*1*).

In the Old World, the earliest well-documented example of societal collapse is that of the hunting and gathering Natufian communities in southwest Asia. About 12,000 years ago, the Natufians abandoned seasonally nomadic hunting and gathering activities that required relatively low inputs of labor to sustain low population densities and replaced these with new labor-intensive subsistence strategies of plant cultivation and animal husbandry. The consequences of this agricultural revolution, which was key to the emergence of civilization, included orders of magnitude increases in population growth and full-time craft specialization and class formation, each the result of the ability to generate and deploy agricultural surpluses.

What made the Natufians change their lifestyle so drastically? Thanks to better dating control and improved paleoclimatic interpretations, it is now clear that this transition coincided with the Younger Dryas climate episode about 12,900 to 11,600 years ago. Following the end of the last glacial period, when southwest Asia was dominated by arid steppe vegetation, a shift to increased seasonality (warm, wet winters and hot, dry summers) led to the development of an open oak-terebinth parkland of woods and wild cereals across the interior Levant and northern Mesopotamia. This was the environment exploited initially by the hunting and gathering Natufian communities. When cooler and drier conditions abruptly returned during the Younger Dryas, the harvests of wild resources dwindled, and foraging for these resources

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