Mitotic repression of the transcriptional machinery

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Nuclear RNA transcription is silenced when eukaryotic cells enter mitosis. Until recently, this repression was thought to derive solely from the condensation of interphase chromatin into mitotic chromosomes. Recent studies, however, have shown that changes in chromatin structure and occupancy of promoter elements by both general and gene-specific transcription factors also play a role in transcriptional silencing. In addition, studies with simplified systems reveal that reversible phosphorylation of the basal transcriptional machinery represses transcription at mitosis.

TRANSCRIPTION of the eukaryotic genome, which is highly active in interphase, is abruptly silenced when cells enter mitosis. Experiments carried out over 30 years ago with cells in culture demonstrated that the incorporation of RNA precursors ceases in late prophase and only resumes in telophase, when cells exit from mitosis (see Refs 1, 2 and lit. cit. therein). Indeed, mitotic repression of transcription has been observed *in vivo* for genes transcribed by all three nuclear RNA polymerases[1,3–5]. Furthermore, autoradiographic studies clearly show a lack of incorporation of labeled RNA precursors in mitotic chromosomes, suggesting that essentially all transcription is suppressed at mitosis[1].

Transcription is also repressed throughout the early cleavage stages of both *Xenopus* and *Drosophila* embryos. Here, the cell cycle rapidly alternates between mitosis and S phase every 10–30 minutes, with no intervening G1 or G2 phases[6–8]. The repression of transcription observed in early development can be prevented by arresting the embryo in interphase using either the protein synthesis inhibitor cycloheximide or a DNA synthesis inhibitor[9]. Thus, when embryonic cells are prevented from entering mitosis, transcription can take place. These observations are consistent with the idea that the early embryonic repression of transcription and the repression of transcription observed at mitosis in all cells are linked.

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Mechanisms of transcriptional repression at mitosis

Over the years, numerous hypotheses have been put forward as molecular explanations for the mitotic repression of transcription (Fig. 1). The possible mechanisms are listed in Table I and include the following: (1) The most obvious candidate mechanism is condensation of the interphase chromatin into mitotic chromosomes[4]. Chromatin condensation would naturally limit the accessibility of the DNA template to transcription factors and RNA polymerase. One possible chromosomal protein that might be responsible for condensation leading to repression of transcription at mitosis is DNA topoisomerase II, an enzyme that is required for condensation both *in vivo*[10] and *in vitro*[11]. Modification of the linker histone H1 and the core histone H3 at mitosis might also be key components of a general mechanism for repression of gene expression at mitosis. These histone proteins become phosphorylated at mitosis and their phosphorylation has been linked to some level of chromatin condensation[12].

(2) The production or activation of general repressor proteins or of other proteins involved in chromosome structure at mitosis could be responsible for transcriptional repression. Recent genetic and biochemical studies have uncovered a class of chromosomal proteins that are required for mitotic chromosome assembly and silencing of transcription at specific loci[13–15]. These include the *Caenorhabditis elegans* gene *DPY-27*, essential for dosage compensation[13], *Xenopus* chromosome associated polypeptides-C and -E (XCAP-C and -E), required for the assembly and structural maintenance of chromosomes[14] and yeast SMC1 (stability of mini-chromosomes), required for the meiotic segregation of yeast chromosomes[15]. Each of these proteins are involved in chromosome structure and condensation, and are related to one another in sequence. Members of this protein family might thus be involved in the general silencing of the genome at mitosis.

(3) Alternatively, an efficient and global repression of transcription could be accomplished by inhibitory phosphorylation of basal transcription factors and/or RNA polymerases at mitosis. Gene-specific transcription factors could also be directly phosphorylated at mitosis and/or be displaced from mitotic chromosomes[16–19].

(4) Evidence has been obtained for premature termination of transcription at mitosis in the early stages of *Drosophila* embryogenesis[8]. Such termination is a potential, albeit probably not global, way of silencing transcription, especially in the case of large genes.

Ultimately, the mechanism(s) of transcriptional repression must be set in motion by the master mitotic kinase, *cdc2*–cyclin B, which is known to initiate mitosis. In each of the models above, the proposed DNA-binding proteins, chromosomal proteins, transcription factors or components of the basal transcription machinery (Table I) would be the phosphorylation targets of the *cdc2*–cyclin B kinase, or of a secondary kinase, which would be activated by this master mitotic kinase[20].

Table I. Repression of transcription at mitosis

Possible mechanisms	Refs
Chromosome condensation mediated by:	
DNA topoisomerase II	10, 11
Histone phosphorylation	12
Nucleosomal changes	16, 17, 41–43
General repressor proteins	
SMC-like chromosomal proteins	13–15
Inactivation/displacement of transcriptional machinery	
Basal transcription factors	18, 23, 24, 26
RNA polymerase	2, 32
Gene specific factors	16, 19, 36–39
Premature termination	8, 40, 48

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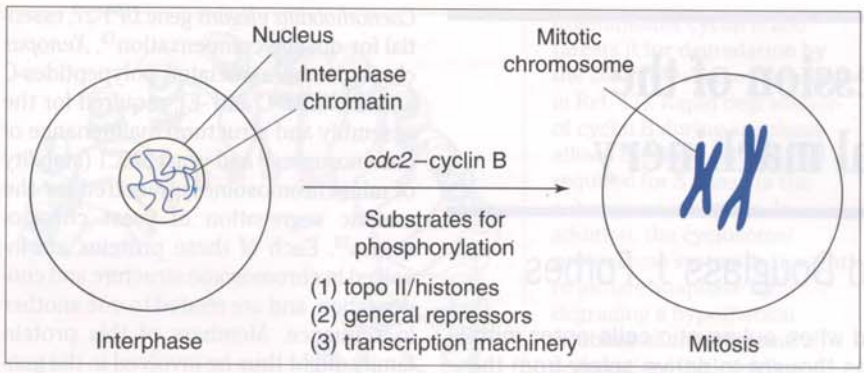


Figure 1

Mitotic repression of transcription is set in motion by the master mitotic kinase, *cdc2*–cyclin B. Either this kinase, or a kinase that is activated by the cdc2 kinase, is responsible for the phosphorylation of proteins involved in chromosome condensation and repression of transcription.

Mitotic repression of RNA polymerase III transcription *in vitro*

With respect to distinguishing between the models for mitotic repression, the most progress has been made *in vitro* using RNA polymerase III (pol III) systems. Consequently, this area will be discussed at some length here. Substantial progress has also been made recently in the *in vitro* analysis of mitotic repression of pol II transcription (see below).

In a first step towards dissecting mechanisms of mitotic transcriptional repression, Hartl *et al.* established that mitotic repression of transcription could be reproduced *in vitro*[20]. Extracts prepared from *Xenopus* eggs can easily be shifted between phases of the cell cycle: an interphase extract can be shifted to a mitotic state by the addition of protease-resistant cyclin B to generate active *cdc2*–cyclin B mitotic kinase[21,22]. (The resulting mitotic extract will convert the chromatin of added nuclei into mitotic chromosomes[20].) When template DNA is added to an interphase extract, it is highly active in the transcription of pol III genes, including 5S RNA and tRNA genes; however, Hartl *et al.*[20] found that, upon conversion to a mitotic state, the extract was severely inhibited for transcription by pol III. This mitotic repression of transcription could be blocked by the kinase inhibitor 6-dimethylaminopurine (DMAP), demonstrating that one or more mitotic phosphorylation events were responsible for the transcriptional repression. It was possible to show that repression in the *Xenopus* system did not require chromosome condensation, nucleosome assembly or any general DNA-binding repressor protein[20]. Instead, it was found that full mitotic repression of transcription *in vitro* was accomplished by the mitotic phosphorylation of the pol III transcription machinery itself. Indeed, simple combination of the *cdc2*–cyclin B kinase and a partially purified pol III transcription system was found to lead to a complete shut off of transcription[23].

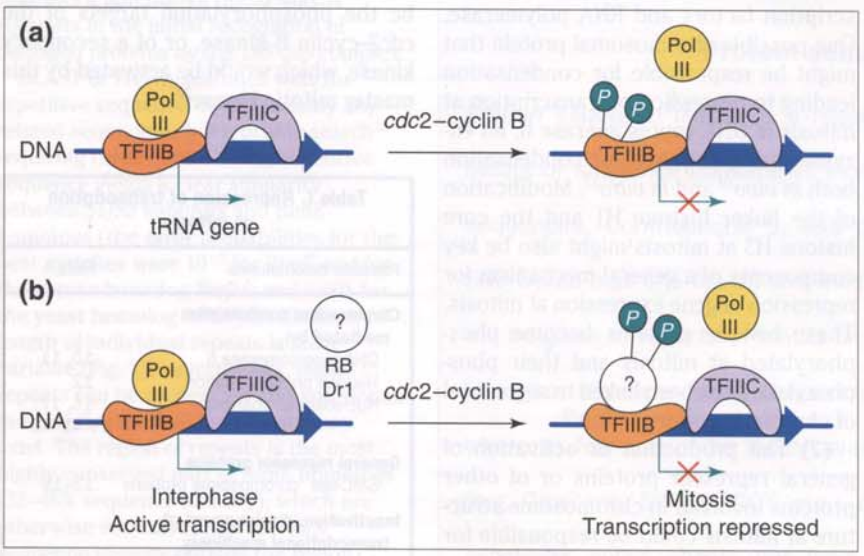


Figure 2

Models for mitotic repression of RNA polymerase III (pol III) transcription. **(a)** The target of phosphorylation is a component of the general pol III transcription factor, TFIIIB. A tRNA gene bound with the factors TFIIIB and TFIIIC is shown. **(b)** Another protein, such as the retinoblastoma protein (RB) or Dr1, is phosphorylated at mitosis and binds and inactivates TFIIIB, thereby repressing pol III transcription.

A search for the target of the mitotic kinase mediating pol III repression

As *cdc2*–cyclin B kinase represses the transcription system itself at mitosis, the next puzzle was identification of the target(s) of the kinase. To determine this for the pol III system, the transcription system was first repressed with mitotic kinases immobilized on agarose beads. After removal of the beads, individual unmodified transcription factors were added back to see if a single fraction could reverse mitotic repression. Pol III transcription is known to require the general transcription factors TFIIIB and TFIIIC, and for 5S RNA genes, the gene-specific factor TFIIIA. Only TFIIIB could restore transcription, indicating that the target of the mitotic kinase in the pol III system is a component of TFIIIB[23].

Recently, White and co-workers[24] observed mitotic repression of pol III transcription *in vitro* with extracts prepared from synchronized mitotic HeLa cells. Unsynchronized HeLa cell extracts were found to be active in pol III transcription, but mitotic cell extracts were repressed for transcription. Similar to the results in the *Xenopus* system, transcriptional activity could be restored to the mitotic extract with interphase TFIIIB, again indicating that a protein in the TFIIIB fraction is the target of mitotic phosphorylation and repression.

Intense study by numerous labs has revealed that TFIIIB comprises the TATA-binding protein (TBP) and a number of pol III-specific TBP-associated factors, or TAFs (reviewed in Ref. 25). Interestingly, while highly purified TFIIIB can restore activity in both the *Xenopus* and HeLa systems, TBP cannot do so[23,24], suggesting that a pol III TAF is the likely target of mitotic repression (Fig. 2a). Recent studies indicate that TBP and a 90 kDa TFIIIB-associated polypeptide are phosphorylated in the *Xenopus* system by mitotic kinases[26]. A human TFIIIB TAF of 90 kDa has recently been isolated and a cDNA encoding this TAF cloned[27]. Interesting future experiments will address whether the

target 90 kDa phosphoprotein observed in the *Xenopus* system corresponds to this TAF and whether it is this phosphorylation event that mediates the mitotic repression of pol III transcription.

Another plausible model (Fig. 2b) for repression of pol III transcription at mitosis would involve an inhibitory phosphoprotein(s) that interacts with TFIIIB and thereby represses transcription. Several repressors of pol III transcription have been identified, such as the retinoblastoma susceptibility gene product RB[28,29] and the TBP-binding protein Dr1 (Ref. 30). These proteins are known to be phosphorylated and, indeed, RB can be phosphorylated *in vitro* by the cdc2 kinase[29]. As these proteins repress transcription even in their non-phosphorylated forms, one would have to hypothesize that mitotic phosphorylation is necessary to release them from a partner protein so that they can gain access to the gene targets they repress at mitosis.

Reactivation of transcription upon exit from mitosis

In a normal somatic cell cycle, one would predict that dephosphorylation of the transcription apparatus must occur at the exit from mitosis to reactivate transcription. Indeed, experiments with the *Xenopus* system indicate that a phosphatase present in the extract can reverse mitotic repression of transcription[20]. Interestingly, White *et al.*[31] have recently monitored the reactivation of pol III transcription upon exit from mitosis and found that reactivation is a late event during the G1 phase of the cell cycle. Taken together, the data are consistent with an *in vivo* scenario where, at mitosis, phosphorylation would inactivate the basal transcription machinery (TFIIIB for pol III). Upon exit from mitosis, dephosphorylation would then reactivate both free transcriptional machinery and DNA-bound transcription complexes. In support of this scenario, *in vitro* mitotic repression of pol III transcription could be induced before transcription complexes form, but could also be observed on pre-formed transcription complexes[20].

Mitotic repression of RNA polymerase II transcription

Many lines of evidence now indicate that mitotic repression of pol II transcription is at the level of the transcriptional machinery itself, including modification of the basal transcription machinery, modification of certain gene-specific factors, promoter occupancy by basal and gene-specific factors, and control of the elongation/termination phase of transcription (Fig. 3a–e):

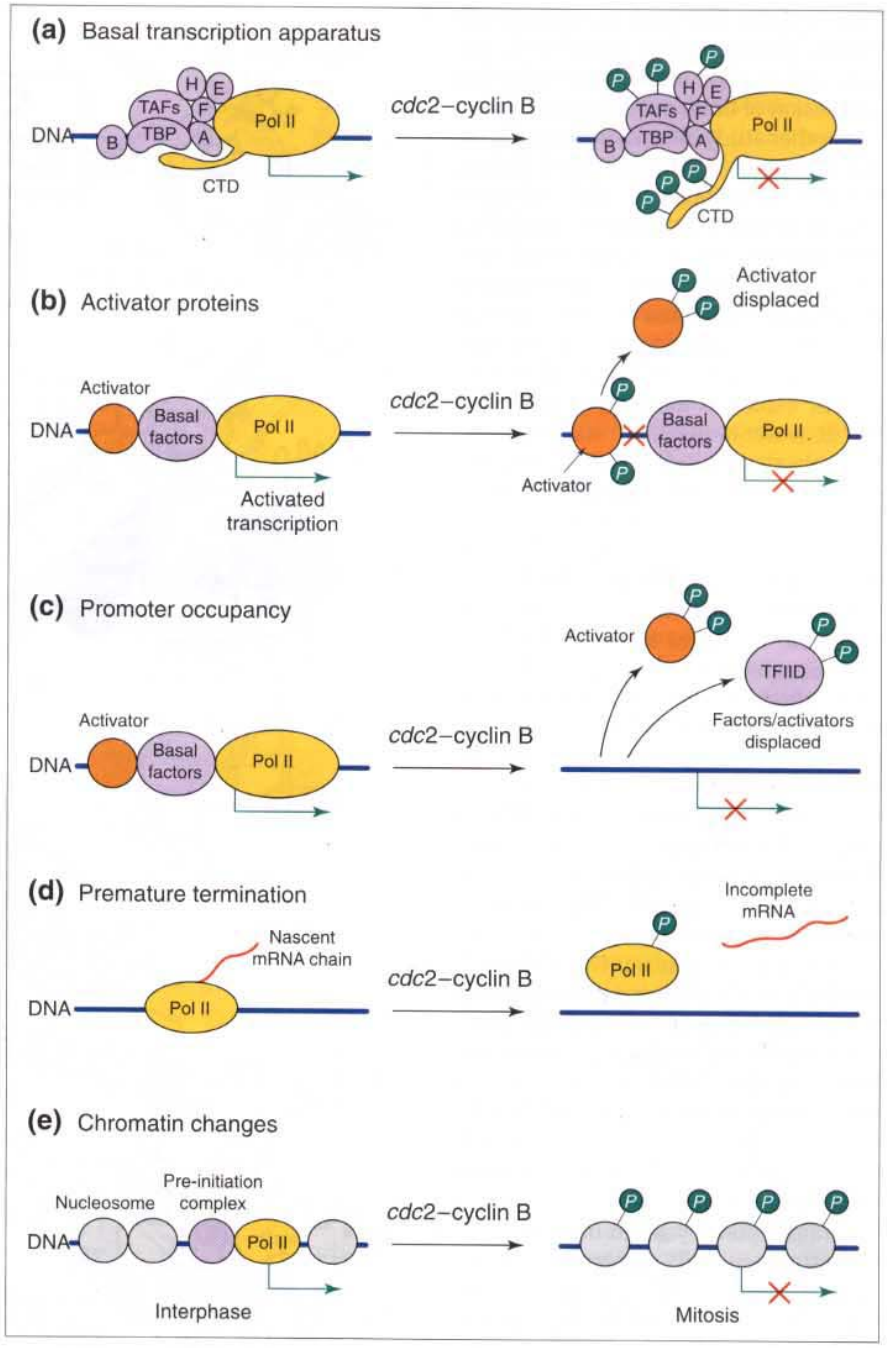


Figure 3

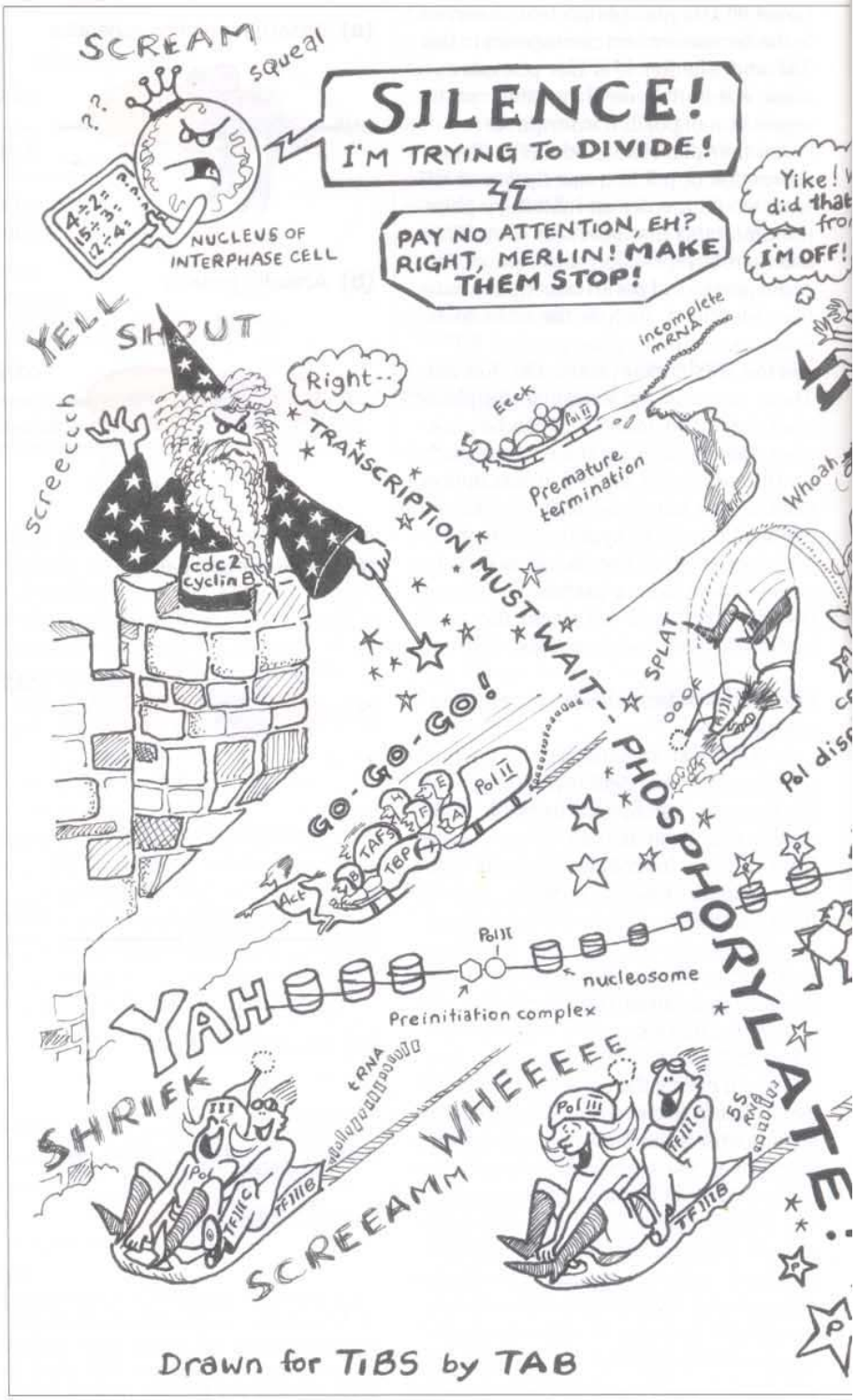
Models for repression of RNA polymerase II (pol II) transcription at mitosis. **(a)** Components of the basal transcription machinery for pol II are the targets of phosphorylation at mitosis. Potential targets include the TATA-binding protein (TBP)-associated factors of TFIID (TAFs), the carboxy-terminal domain (CTD) of pol II and other components of the basal machinery, such as TFIIH. **(b)** Activator proteins that bind enhancer or upstream elements are phosphorylated and inactivated and/or displaced at mitosis. **(c)** Activator proteins and components of the basal machinery, such as TFIID, are displaced from the template at mitosis (promoter occupancy). **(d)** Transcription is prematurely terminated at mitosis and incomplete transcripts are released. **(e)** Changes in the nucleosome composition or position at mitosis mask promoter elements leading to repression of transcription.

Alteration of the basal pol II machinery. Basal transcription of pol II genes is known to require pol II, TFIIA, -B, -D, -E, -F and -H [see *TiBS* September (1996) Special Issue on Transcription]. Like pol III transcription, pol II transcription *in vitro* can be inhibited by mitotic *cdc2*–cyclin B kinase and inhibition can be prevented with a protein kinase inhibitor[26]. Transcription in this study was of the basal type, using a template containing

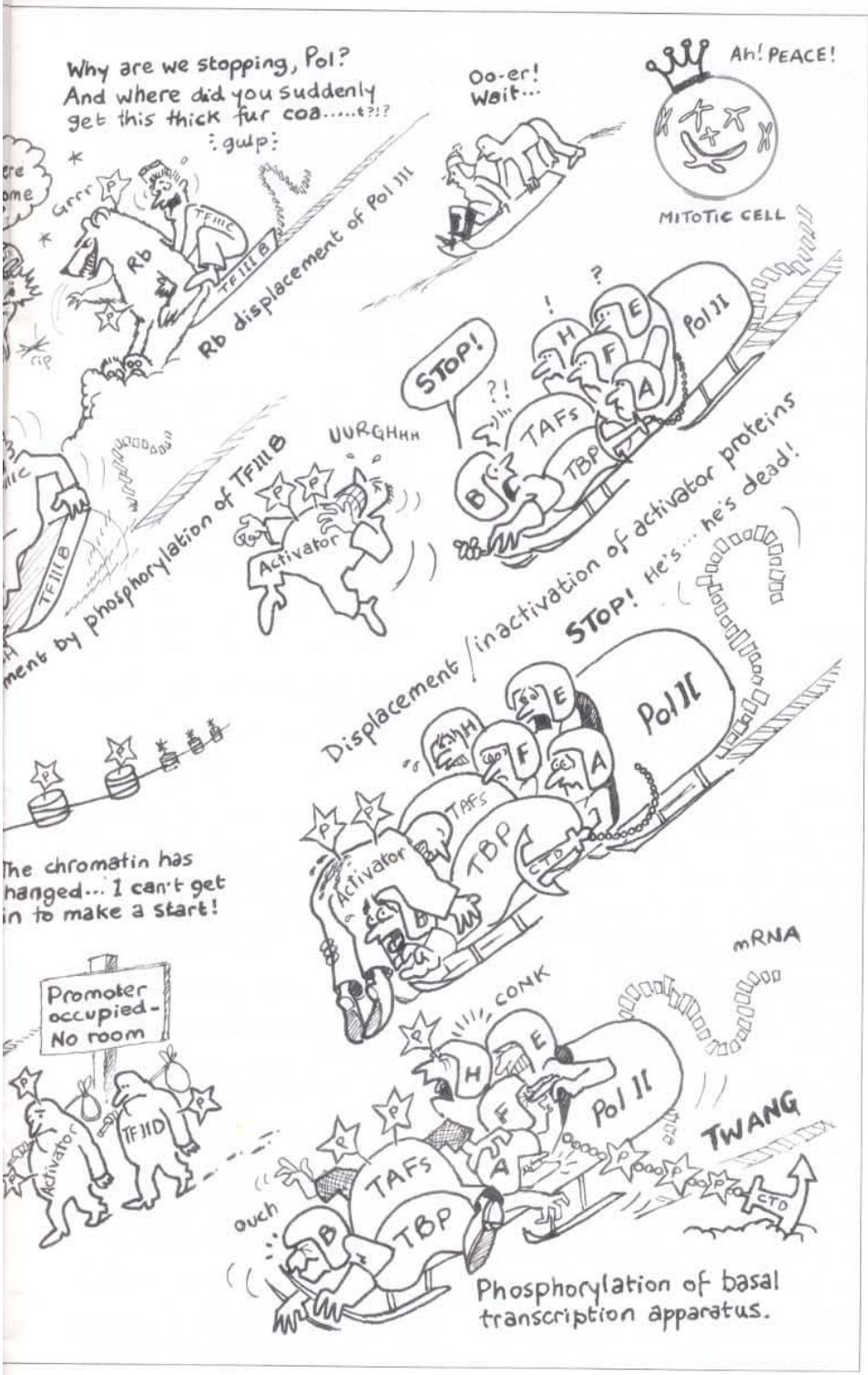
only a TATA box and an initiator element. Future studies will identify the target(s) of phosphorylation mediating repression of basal pol II transcription.

Another study focused specifically on the activity of TFIID at mitosis[18], finding that several subunits of TFIID are hyperphosphorylated at mitosis, including TBP and three TAF_{II}s. Coincident with this phosphorylation, immunocytochemistry experiments indicated that the majority of TFIID was released from the chromatin at mitosis. The TFIID isolated from mitotic cells was found to be defective in activator-dependent transcription, but only slightly defective in basal transcription. As basal transcription does not require the TAFs of TFIID, Segil *et al.*[18] suggest that phosphorylation of one or more TAFs is responsible for the mitotic repression of activator-dependent transcription. It should be noted that the only component in this study derived from mitotic cells was TFIID, thus future studies will be needed to determine the targets mediating full repression of basal transcription at mitosis.

Several studies suggest that pol II itself may be a target of regulation at mitosis. Sequence analysis indicates that the largest subunit of pol II has multiple potential phosphorylation sites in its carboxy-terminal domain (CTD)[32]. Indeed, pol II can be phosphorylated by a *cdc2*-related kinase *in vitro*[33], and the phosphorylated form of pol II interacts less well with the TBP component of the basal transcription apparatus than non-phosphorylated pol II (Ref. 34). The role of CTD phosphorylation in the transition from the initiation to elongation phases of the transcription cycle has been reviewed recently [see *TiBS* September (1996) Special Issue]. CTD phosphorylation may also affect other protein–protein interactions within the initiation complex. Thus, mitotic phosphorylation of the CTD may be an additional mechanism for general repression of pol II transcription. Future experiments will be needed to assess this possibility or the possibility that other components may exist that mediate pol II repression.



Alteration of specific pol II transcription factors. Numerous studies have documented that phosphorylation in general can alter the activity of gene- and tissue-specific transcription factors involved in pol II transcription[35,36]. Phosphorylation has been shown to affect, either positively or negatively, the DNA-binding activity of certain factors, the transcriptional activation activity of others, and the nuclear/cytoplasmic localization of still others. In several cases, specific inhibitory phosphorylations have been linked to the cell-cycle control of transcription. For example, Oct-1, a transcription factor used by a subset of genes transcribed by pol II and pol III, is hyperphosphorylated as cells enter mitosis and dephosphorylated as cells exit mitosis[18]. Moreover, phosphorylation of the Oct-1 homeodomain protein negatively regulates its DNA-binding activity[37]. These results indicate that the inactivation of Oct-1 may play a role in the mitotic repression of the subset of genes regulated by Oct-1. Another member of the POU family of homeodomain proteins (found in the Pit 1, Oct-1 and Unc-86 gene products), GHF-1, is also subject to mitotic phosphorylation and this event inhibits the DNA-binding activity of GHF-1 (Ref. 38). Interestingly, the mitotic phosphorylation sites in Oct-1 and GHF-1 are at analogous amino acid positions within these proteins. As these sites are



conserved in all members of the POU family of transcription factors, all may be repressed in a similar manner at mitosis. Lastly, the general pol II factor Sp1 and the oncoproteins Myc and Myb are also hyperphosphorylated at mitosis and exhibit reduced binding activity in mitotic cell extracts[16,19]. Thus, mitotic phosphorylation of individual specific transcription factors may well be an important component in the mitotic repression of RNA pol II transcription.

Promoter occupancy. Recent studies have found evidence that the transcription factor occupancy of the promoter or enhancer regions of certain pol II-transcribed genes is altered upon mitotic chromosome formation. Hershkovitz and Riggs[17] found that the promoter region upstream from the human *PGK1* gene contains proteins in interphase chromatin, but is clearly protein-free in mitotic chromosomes[17]. These data suggest that the transcription complex on the *PGK1* gene is erased at mitosis and must be re-established following each mitosis. A similar study by Martínez-Balbás *et al.*[16] of the stress-inducible human *hsp70* gene showed that transcription is repressed in HeLa cells at mitosis. Genomic footprinting indicated that the sequence-specific transcription factors Sp1, C/EBF and HSF1 had all been displaced from the *hsp70* promoter in the mitotic chromosomes. Consistent with this, immunocytochemical localization studies demonstrated that these factors and several other transcription factors were dispersed from bulk chromatin at mitosis[16].

In addition to these DNA-binding transcription factors, a recent study has shown that two components of the human SWI–SNF protein complex are phosphorylated at mitosis and are excluded from mitotic chromosomes[39]. The SWI–SNF multiprotein complex is involved in transcriptional activation of many genes through its ability to alter chromatin structure and thus allow transcription factors to access promoter elements (reviewed in Ref. 39). Thus, phosphorylation of SWI–SNF subunits at mitosis and concomitant exclusion from chromatin may contribute to mitotic repression of pol II transcription.

By contrast to these findings, mitotic dispersal was not observed for AP-2 or for the serum response factor p67SRF[16,40]. Similarly, the GAGA transcription factor does not appear to be displaced from GA/CT-rich regions on the mitotic chromosomes of early *Drosophila* embryos[41]. However, this study did not determine whether the GAGA factor remained bound to particular gene promoters rather than to non-transcribed GA/CT-rich repetitive DNA elements.

The data above argue that, although certain specific pol II factors are retained on mitotic chromosomes, in many cases, specific pol II transcription factors are displaced from the chromosomes at mitosis. How might displacement from the genome occur at mitosis? Of the *trans*-acting factors required for *hsp70* expression, only Sp1 DNA-binding activity was reduced in mitotic cell extracts. Martínez-Balbás *et al.*[16] have discussed a possible general mechanism for displacement, which invokes chromatin condensation during mitosis and the displacement of transcription factors. Factors that might conceivably cause chromatin condensation and concurrent factor displacement include the charge neutralization of DNA by: deacetylation of the core histones in mitotic chromosomes,

the binding of polycations such as spermine and spermidine to mitotic chromatin, and the mitotic phosphorylation of histones H1 and H3 (see Ref. 16 and lit. cit. therein). At present, however, it is unclear what mechanism causes transcription factor displacement.

Premature termination of pol II transcripts at mitosis. For the transcripts of very large genes, premature termination of pol II transcription has been observed at mitosis. Using *Drosophila* embryos, Shermoen and O'Farrell[8] report that nascent transcripts of the *Ubx* gene, which can be visualized by *in situ* hybridization, are seen to disappear at mitosis and reappear during the next cell cycle. The data are most consistent with the model that all RNA polymerase molecules may prematurely terminate and dissociate from the Ubx template during mitosis. As the *Ubx* transcription unit is 77 kb in length, and requires ~55 mins to transcribe, complete synthesis of the transcript requires a longer period than that of the early embryonic *Drosophila* cell cycle (≤ 10–11 min), effectively limiting the synthesis of the Ubx protein until the cell cycle lengthens later in development. Additional studies in *Drosophila* embryos indicate that the length of the mitotic cycle can indeed provide a barrier to transcript length and thus can play an important regulatory role in development[42].

Chromatin structural changes. Several studies have compared the chromatin structure of specific genes in interphase chromatin to that of mitotic chromosomes[16,43–45]. Both the general nuclease sensitivity of active genes and the presence of DNaseI-hypersensitive sites, which are located primarily in promoter or enhancer regions, appear to be preserved in mitotic chromosomes. This suggests that the nucleosome level of chromatin organization is not grossly affected at mitosis. Interestingly, however, the precise location of the hypersensitive sites upstream of some specific genes appears to differ between interphase and mitotic chromatin[43], suggesting that there is a rearrangement or displacement of some of the protein factors controlling these genes at mitosis, as discussed above.

Mitotic repression of pol I. Transcription by pol I is also repressed during mitosis *in vivo*[2]. This inhibition of ribosomal RNA transcription is not due to displacement of either pol I molecules[46–48] or the pol I-specific transcription factor UBF (upstream binding factor) from the DNA template[47–49]. Furthermore, it has been found that TBP and the TAF_I63 and TAF_I110 subunits of the pol I factor SL1 remain associated with the ribosomal RNA genes at mitosis[47,48]. These results are in contrast to the finding that the majority of pol II-specific TBP is dispersed from the chromatin at mitosis. Weisenberger and Scheer[50] suggest that repression of the rRNA genes at mitosis is regulated at the level of transcript elongation rather than at initiation, because most transcripts were found to be released from the ribosomal genes at mitosis. As for pol III transcription, the RB protein has been shown to repress pol I transcription[29,51] and, thus, phosphorylation of RB at mitosis might be involved in mitotic repression of pol I transcription. Biochemical studies are in progress to assess the mechanisms responsible for repression of rRNA synthesis at mitosis.

Summary and future directions

Although repression of transcription at mitosis has been known for over three decades, the biochemical events responsible for this global repression of transcription have yet to be fully elucidated. With the advent of *in vitro* systems that reproduce mitotic repression, it will now be possible to determine the mechanisms of repression, the protein kinases responsible for repression and the targets of such mitotic phosphorylation. Simplified systems have now been established that mimic mitotic repression of transcription. Although modification of the transcription machinery accounts for the majority (≥ 90%) of the repression observed *in vitro*, at least in the case of pol III (Ref. 20), it is likely that multiple levels of control exist *in vivo*. Full repression of transcription at mitosis could result from inactivation of the basal transcription machinery, loss of transcription factors from promoters or enhancers, modification of the chromatin template, and condensation of mitotic chromosomes (Fig. 3). The latter two mechanisms could provide an even tighter repression of transcription during the crucial time of genome segregation.

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