(*26*), but not with the control peptide, reduced the number of parasite-associated IKK signalosomes containing P-IκBα by 83%. These data confirm a role for NEMO in parasite-associated IKK signalosome activation. Taken together, our observations strongly indicate that the parasite bypasses the common upstream adaptor proteins and kinases that link surface receptor stimulation to IKK activation. This mechanism is compatible with a model called "proximity-induced activation," in which the enforced oligomerization of IKK components or adaptor proteins suffices to induce NF-κB activation (*3*, *15*, *27–29*).

Despite extensive efforts, a direct association between parasite proteins and IKK could not be demonstrated. The focal pattern of IKK association with the parasite, together with the cell cycle–dependent appearance of activated IKK signalosomes, suggests that host cell molecules other than IKK may participate in the interaction.

Pathogen-derived products have been shown to interact directly with host cell signaling pathways at different levels. *Theileria* perverts the NF-κB pathway through an unusual mechanism involving the aggregation of critical pathway components at the surface of the transforming schizont stage. In contrast, the merozoite no longer requires host cell survival and ceases to activate the NF-κB pathway. Parasite-induced NF-κB activity is clearly crucial for the survival—and potentially also the proliferation—of *Theileria*-transformed cells (*7*). It can also be predicted, however, that NF-κB–dependent activation of inflammatory genes will contribute to the pathogenesis of theileriosis.

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RNA Polymerase II Transcription in Murine Cells Lacking the TATA Binding Protein

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Inactivation of the murine TATA binding protein (TBP) gene by homologous recombination leads to growth arrest and apoptosis at the embryonic blastocyst stage. However, after loss of TBP, RNA polymerase II (pol II) remains in a transcriptionally active phosphorylation state, and in situ run-on experiments showed high levels of pol II transcription comparable to those of wild-type cells. In contrast, pol I and pol III transcription was arrested. Our results show a differential dependency of the RNA polymerases on TBP and provide evidence for TBP-independent pol II transcriptional mechanisms that allow reinitiation and maintenance of gene transcription in vivo.

In vertebrates, TBP and the TBP-like factor (TLF, also called TBP-related factor 2 or TRF2) are two closely related proteins involved in RNA pol II transcription (*1*). Both proteins share an evolutionarily conserved saddle-like core domain responsible for DNA binding and interaction with the general transcription factors. TBP is a subunit of the pol II transcription factor TFIID and is thought to play a general role in pol II transcription as well as in pol I and pol III transcription (*2*).

In the African clawed toad *Xenopus laevis* and the zebrafish *Danio rerio*, both TBP and TLF are involved in gene expression at the onset of zygotic pol II transcription and during subsequent embryonic development (*3*, *4*). In contrast, murine TLF is neither expressed (fig. S1) nor required (*5*) during early embryogenesis, whereas murine TBP is expressed at all stages.

The gene encoding murine TBP was inactivated by homologous recombination after insertion of the hygromycin resistance gene into exon III (*6*) (fig. S2A). Embryonic stem cell clones bearing the inactivated allele were identified by Southern blot analysis, and $TBP^{+/-}$ mice (fig. S2B) were generated. $TBP^{+/-}$ mice were born at Mendelian frequency, were of normal size and weight, displayed no obvious abnormalities, and were fertile (*7*).

Crossing $TBP^{+/-}$ mice failed to generate viable newborn $TBP^{-/-}$ mice (fig. S2B). However, at day 3.5 postcoitum (E3.5), an approximately Mendelian ratio of $TBP^{-/-}$ blastocysts could be detected with a polymerase chain reaction (PCR) strategy (Fig. 1A). When examined by immunofluorescence for expression of the TBP protein, blastocysts were detected that were totally negative for TBP labeling (Fig. 1B, upper and middle panels). TBP was absent in explanted blastocysts grown for 1 day in vitro (Fig. 1C). Strongly reduced TBP levels were also detected at E2.5 in 8-cell-stage embryos (Fig. 1D), which indicates that the maternal TBP pool was significantly depleted at this stage and was undetectable by the blastocyst stage.

Blastocysts from $TBP^{+/-}$ crosses were explanted at E3.5 (day 0) and cultivated in vitro (*6*). Approximately 25% of the blastocysts

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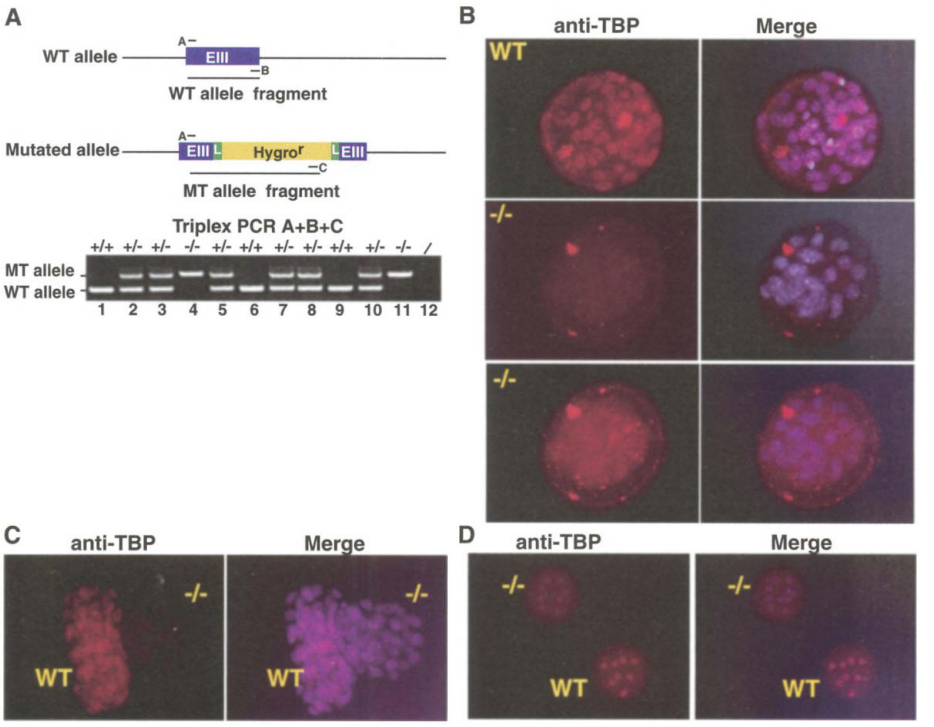

Fig. 1. Detection of TBP mutant blastocysts (40× magnification). **(A)** Schematic of a PCR strategy for genotyping blastocysts, indicating PCR products from the wild-type (WT) and mutant (MT) alleles and locations of the TBP exon III, the hygromycin resistance gene, and the oligonucleotide primers used. An example of the results of a genotyping experiment is also shown. **(B)** Immunodetection of TBP in E3.5 blastocysts derived from $TBP^{+/-}$ intercrosses. In the upper panel, TBP can be detected in the nuclei of all cells within the blastocyst with the 3G3 antibody to TBP. Because $TBP^{+/+}$ and $TBP^{+/-}$ blastocysts both stain positively for the presence of TBP, they are both designated as wild-type. The middle panel shows a blastocyst labeling negatively for TBP; the lower panel shows a red enhancement, in which only nonspecific background labeling can be observed, of the image in the middle panel. In each panel, the right-hand image shows the merge between the TBP fluorescence and Hoechst-stained DNA in blue. **(C)** Immunodetection of TBP in explanted blastocysts grown for 1 day in culture. Blastocysts labeling positively or negatively for TBP were found side by side. **(D)** Immunodetection of TBP in 8-cell-stage embryos as described in (B).

rapidly ceased growth and died, whereas the others hatched from the zona pellucida and continued to develop (Fig. 2A and fig. S3A). PCR genotyping detected wild-type and heterozygous blastocysts, but no $TBP^{-/-}$ embryos among those that grew (*6*). After 2 days, only rare apoptotic cells were observed in the proliferating wild-type blastocysts, whereas extensive apoptosis was observed in the growth-arrested $TBP^{-/-}$ embryos (Fig. 2B). Hence, $TBP^{+/+}$ or $TBP^{+/-}$ blastocysts grow normally, but $TBP^{-/-}$ blastocysts undergo growth arrest and apoptosis.

Embryos staining negatively for TBP were also recovered at E4.5 (fig. S3B). The $TBP^{-/-}$ E4.5 embryos typically comprise 30 to 40 cells, less than normally seen in wild-type E3.5 blastocysts, indicating that growth arrest occurs before E3.5, just as TBP levels become undetectable, and does not continue either in vitro or in vivo.

Treatment of wild-type blastocysts with α-amanitin indicated that continual pol II transcription was required for their short-term survival but did not induce apoptosis (fig. S4, A and B). The different phenotype of $TBP^{-/-}$ and α-amanitin–treated blastocysts suggests that loss of TBP does not induce a global arrest of pol II transcription. To address this question, blastocysts were labeled with antibody H5, which selectively recognizes the active elongating form of RNA pol II (*6*, *8*). After 1 or 2 days of culture in vitro, comparable H5 labeling of all wild-type and growth-arrested $TBP^{-/-}$ blastocysts was observed (Fig. 3, A and B). Hence, loss of TBP does not result in a significant reduction or disappearance of transcriptionally active pol

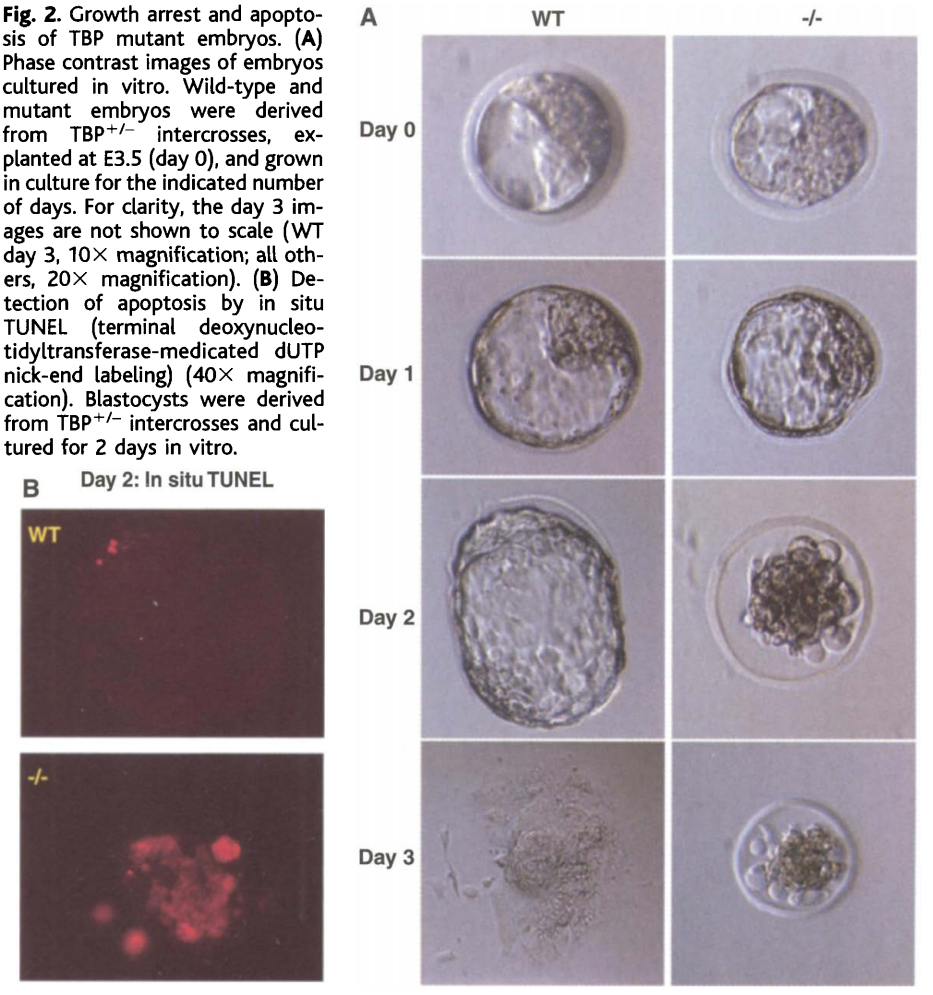

Fig. 2. Growth arrest and apoptosis of TBP mutant embryos. **(A)** Phase contrast images of embryos cultured in vitro. Wild-type and mutant embryos were derived from $TBP^{+/-}$ intercrosses, explanted at E3.5 (day 0), and grown in culture for the indicated number of days. For clarity, the day 3 images are not shown to scale (WT day 3, 10× magnification; all others, 20× magnification). **(B)** Detection of apoptosis by in situ TUNEL (terminal deoxynucleotidyltransferase-medicated dUTP nick-end labeling) (40× magnification). Blastocysts were derived from $TBP^{+/-}$ intercrosses and cultured for 2 days in vitro.

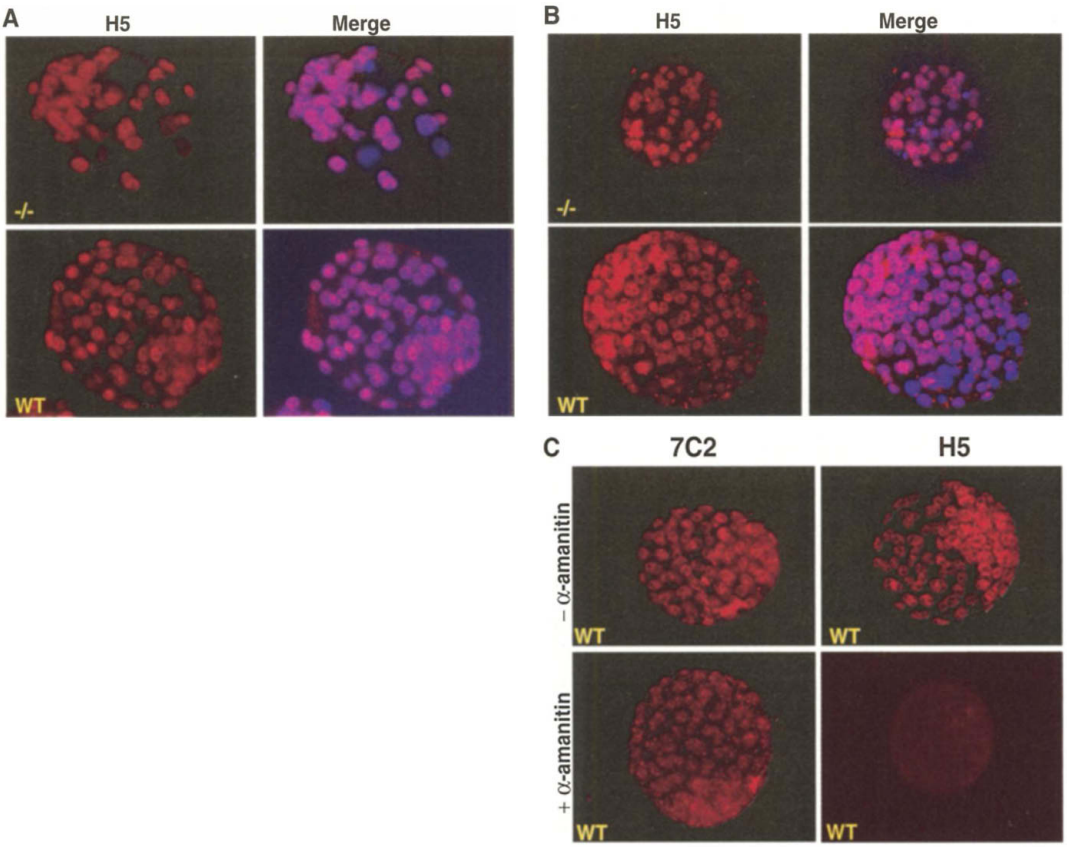

Fig. 3. Detection of actively transcribing RNA pol II in TBP mutant blastocysts (40× magnification). (**A** and **B**) Immunolabeling with monoclonal antibody H5 in blastocysts derived from $TBP^{+/-}$ intercrosses. Blastocysts were explanted at E3.5 and cultured for (A) 24 hours or (B) 48 hours before labeling. (**C**) Blastocysts derived from wild-type mice were cultured for 12 hours in vitro in the presence or absence of α-amanitin (24 μg/ml), then labeled with either H5 or 7C2 antibodies.

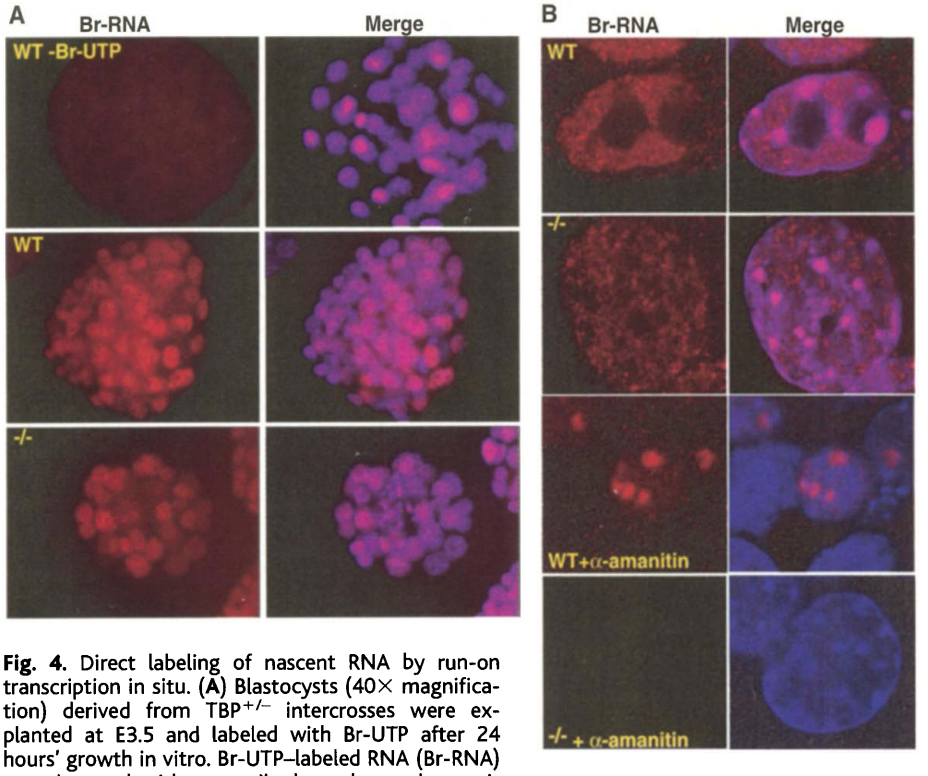

Fig. 4. Direct labeling of nascent RNA by run-on transcription in situ. (**A**) Blastocysts (40× magnification) derived from $TBP^{+/-}$ intercrosses were explanted at E3.5 and labeled with Br-UTP after 24 hours' growth in vitro. Br-UTP–labeled RNA (Br-RNA) was detected with an antibody to bromodeoxyuridine. The upper panels show a control labeling in the absence of Br-UTP. (**B**) Confocal sections (500× magnification) of wild-type or TBP mutant cells were generated from blastocysts labeled as described in (A). The presence or absence of α-amanitin (2 μg/ml) is indicated.

II. In contrast, H5 labeling was completely abolished in the presence of α-amanitin and pol II relocalized into discrete nuclear speckles (Fig. 3C) (*9*). Similar results were obtained with monoclonal antibody CC3 (*6*) (fig. S5).

To directly detect pol II transcription in $TBP^{-/-}$ blastocysts, nascent RNA chains were labeled in situ by incorporation of bromouridine 5′-triphosphate (Br-UTP) (*6*, *10*). After 24 hours in culture, strong nuclear labeling of both wild-type and $TBP^{-/-}$ blastocysts was observed, indicative of active transcription, whereas no labeling was observed in the absence of Br-UTP (Fig. 4A). Confocal microscopy showed comparable labeling in $TBP^{-/-}$ and wild-type cells (Fig. 4B). In $TBP^{-/-}$ cells, the nucleoplasmic labeling was abolished in the presence of low concentrations of α-amanitin, which shows that the labeling was due to pol II (Fig. 4B). These results are in agreement with the H5 labeling and show robust pol II transcription 24 and 48 hours after loss of TBP (fig. S6A).

In the presence of α-amanitin, nucleolar pol I transcription (*11*) is clearly evident in wild-type cells (Fig. 4B and fig. S5B). In contrast, no pol I transcription was observed in $TBP^{-/-}$ cells (Fig. 4B). The two to three large nucleoli in wild-type cells are disorganized in $TBP^{-/-}$ cells (fig. S6C), which is characteristic of arrested pol I transcription (*12*, *13*). Moreover, residual nucleoplasmic

labeling resistant to the low concentration of α-amanitin corresponding to pol III transcription can also be seen in wild-type but not $TBP^{-/-}$ nuclei (Fig. 4B and fig. S6B). Hence, loss of TBP leads to a shutdown of pol I and pol III transcription.

We show that depletion of TBP results in a concomitant and proportional loss of pol I and pol III but not pol II transcription. The contrasting effects of TBP depletion show that these polymerases have a differential dependency on TBP and are indicative of a TBP-independent mechanism for pol II transcription (*6*). This conclusion would appear to contradict previous experiments, in which inactivation of TBP in zebrafish embryos before the onset of zygotic transcription resulted in the absence of H5 labeling, indicating a major loss of zygotic transcription (*3*). However, in our experiments TBP is depleted only after zygotic transcription has begun. This observation, together with the observed growth arrest of $TBP^{-/-}$ cells and the growth and cell cycle defects of $TBP^{+/-}$ chicken DT40 cells (*14*), show that TBP is required for initiation of zygotic transcription and for cell proliferation, which also involves de novo initiation of gene expression during the cell cycle and after the mitotic division. In contrast, the high levels of pol II transcription seen in our experiments suggest that TBP is not required for transcription reinitiation and maintenance of gene expression in growth-arrested cells. Hence for many genes, there may be a distinct requirement for TBP at the de novo activation and reinitiation stages of transcription.

Distinct pathways for initiation and reinitiation have indeed been proposed based on in vitro studies where a subset of the general transcription factors remains at the promoter to form a scaffold facilitating reinitiation (*15*). Whereas TFIID is among the general factors that remain at the promoter in vitro, our results suggest that persistent transcription of many cellular genes can take place independently of TBP in vivo. The TFTC (TPB-free, TAF-containing) complex, containing a subset of TBP-associated factors but no TBP, has previously been shown to substitute for TFIID in vitro (*16*) and has recently been implicated in TBP-independent interferon-stimulated transcription in cultured cells (*17*). Here we provide genetic evidence for TBP-, TLF-independent pol II transcription mechanisms in vivo. It is possible that the TFTC complex or another transcription factor may play a role in the high levels of pol II transcription that we observe in the $TBP^{-/-}$ cells.

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Role of Histone H3 Lysine 27 Methylation in Polycomb-Group Silencing

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Polycomb group (PcG) proteins play important roles in maintaining the silent state of HOX genes. Recent studies have implicated histone methylation in long-term gene silencing. However, a connection between PcG-mediated gene silencing and histone methylation has not been established. Here we report the purification and characterization of an EED-EZH2 complex, the human counterpart of the *Drosophila* ESC-E(Z) complex. We demonstrate that the complex specifically methylates nucleosomal histone H3 at lysine 27 (H3-K27). Using chromatin immunoprecipitation assays, we show that H3-K27 methylation colocalizes with, and is dependent on, E(Z) binding at an *Ultrabithorax* (*Ubx*) Polycomb response element (PRE), and that this methylation correlates with *Ubx* repression. Methylation on H3-K27 facilitates binding of Polycomb (PC), a component of the PRC1 complex, to histone H3 amino-terminal tail. Thus, these studies establish a link between histone methylation and PcG-mediated gene silencing.

Maintenance of the spatially restricted expression pattern of HOX genes in both flies and vertebrates is controlled by PcG and trithorax group (trxG) proteins (*1*). Biochemical and genetic studies indicate that PcG proteins exist in at least two separate protein complexes, the Polycomb repressive complex 1 (PRC1) and the ESC-E(Z) complex. These two complexes function in a cooperative manner to maintain long-term gene silencing (*2*, *3*). Although components of both protein complexes are required to maintain the silenced state, the function of PRC1 appears to depend on the ESC-E(Z) complex (*4*). Recent studies on the covalent modifications of the histone NH_2-terminal tails have given rise to the "histone code" hypothesis (*5*). One of the covalent modifications, histone lysine methylation, has emerged as an important player in regulating gene expression and chromatin function (*6*). Histone lysine methylation occurs on lysines 4, 9, 27, 36, and 79 of H3 and on lysine 20 of H4. Biochemical and genetic studies indicate that methylation of different lysine residues, with the exception of H3-K79 (*7*–*9*), is catalyzed by different SET domain–containing proteins (*6*).

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