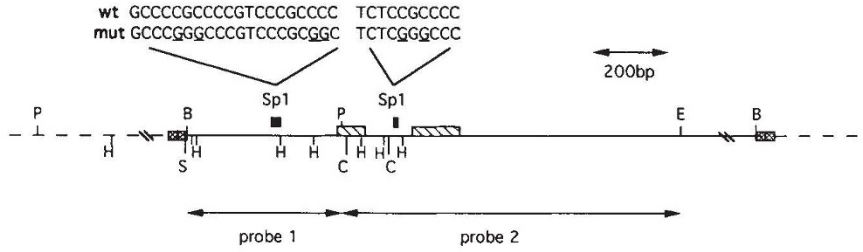

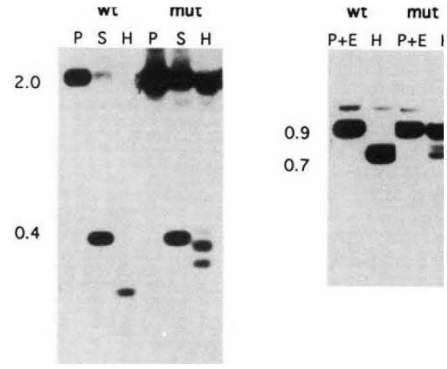

FIG. 4 Sp1 elements protect the APRT CpG island from *de novo* methylation *in vivo*. The diagram shows a map of the APRT transgene in the head-to-tail configuration. Restriction sites for *Hpa*II (H), *Cfo*I (C), *Pvu*II (P), *Bam*HI (B), *Eco*RI (E) and *Sal*I (S) are indicated. The locations and sequences of the Sp1 sequences that were mutated in this experiment are also shown. The larger hatched boxes mark the positions of the APRT exons and the smaller boxes show the polylinker of the pUC19 vector. wt, Wild type; mut, mutant.

METHODS. Using a pUC19 construct containing the 3.8-kb *aprt Bam*HI genomic fragment[24], the three Sp1 elements at positions 229, 239 and 558 were mutated by modifying two bases at each site using the Clonetech kit[26]. All these wild-type sequences were able to compete for Spl binding in a gel-retardation assay. In contrast, a potential Spl site at position 626 showed only weak competition (data not shown). The wild-type and mutant *Bam*HI fragments were then used to generate transgenic animals[27]. DNA from total founder embryos (14 days post-coitum) was extracted and subjected to Southern blot analysis to detect the samples containing the transgene and analyse their methylation patterns. The gene construct was found to be integrated in 2 of 12 control and 4 of 16 mutant embryos. To assay the 3′ portion of the CpG island, DNA was restricted by *Pvu*II/*Eco*RI either alone or together with *Hpa*II and hybridized with the *Pvu*II/*Eco*RI probe (2). To study the 5′ end of the CpG island, DNA was digested with *Pvu*II either alone or together with *Sal*I or *Hpa*II and hybridized to the *Bam*HI/*Pvu*II probe (1). In this latter strategy, it is possible to visualize the head-to-tail recombinations of the construct allowing measurement of the degree of methylation of the most 5′ CpG island sites. Note that for both the 5′ and 3′ regions of the island, multiple modifications are required to obtain the full-length fragment, suggesting that each individual site is highly methylated. The single *Sal*I site located in the pUC19 polylinker immediately adjacentto the APRT island also underwent methylation in the mutant construct. The *Hpa*II locus in the 3′ non-island end of the APRT *Bam*HI fragment apparently underwent *de novo* methylation in both constructs. CpG island *de novo* methylation was also observed in two other mutant embryos. In contrast, this island was unmodified in two control embryos from this experiment and was previously shown to remain unmethylated in three founder mice carrying this same APRT transgene.

tutively unmethylated *in vivo* although transcriptionally inactive in most cell types[1]. The mechanism of CpG island demethylation may be analogous to that involved in the demodification of tissue-specific genes that occurs in individual cell types during organ differentiation. B-cell-specific demethylation of κ-chain sequences, for example, is driven by κ-enhancer-associated *cis*-acting elements in a reaction that occurs independently of transcription and operates regionally over 3–4 kilobase (kb) distances on either side of the inducing sequence[22]. It thus appears that well known *cis*-acting transcriptional regulatory elements are involved in both stage- and tissue-specific demethylation processes.

Note added in proof: It was recently found that Spl elements play a similar role in the mouse APRT gene[28]. □

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CORRECTION

The mitotic feedback control gene *MAD2* encodes the α-subunit of a prenyltransferase

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WE previously reported that the product of budding yeast spindle assembly checkpoint gene *MAD2* was a component of a geranylgeranyl transferase. Further analysis by our colleague Rey-Huei Chen has shown that the prenyltransferase component is encoded by the gene adjacent to the *MAD2* gene. Thus our conclusion that a prenyltransferase played a role in the spindle assembly checkpoint was in error, although the conclusions that the gene that we had previously identified as *MAD2* encodes the α-subunit of an essential geranylgeranyl transferase, and that this enzyme modifies Ypt1 and Sec4, remain valid. The prenyltransferase-encoding gene has been renamed *BET4* as one of the other subunits of the geranylgeranyl transferase is the product of the previously identified *BET2* gene. The bona fide *MAD2* gene encodes a 196-amino-acid open reading frame (GenBank accession number U14132), which lacks homology to any known genes. □