A Nuclear Matrix/Scaffold Attachment Region Co-localizes with the Gypsy Retrotransposon Insulator Sequence*

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The 5′-untranslated region of the Drosophila gypsy retrotransposon contains an “insulator,” which disrupts the interactions between enhancer and promoter elements located apart. The insulator effect is dependent on the suppressor of Hairy-wing (su(Hw)) protein, which binds to reiterated sites within the 350 base pairs of the gypsy insulator, whereby it additionally acts as a transcriptional activator of gypsy. Here, we show that the 350-base pair su(Hw) binding site-containing gypsy insulator behaves in addition as a matrix/scaffold attachment region (MAR/SAR), involved in interactions with the nuclear matrix. In vitro experiments using nuclear matrices from Drosophila, murine, and human cells demonstrate specific binding of the gypsy insulator, not observed with any other sequence within the retrotransposon. Moreover, we show that the gypsy insulator, like previously characterized MAR/SARs, specifically interacts with topoisomerase II and histone H1, i.e. with two essential components of the nuclear matrix. Finally, experiments within cells in culture demonstrate differential effects of the gypsy MAR sequence on reporter genes, namely no effect under conditions of transient transfection and a repressing effect in stable transgenic genes, namely no effect under conditions of transient transfection and a repressing effect in stable transgenic experiments. In vitro experiments presented in this report disclose the attachment of chromosomes to the nuclear matrix.

Insulator elements play a fundamental role in genome organization. They isolate independent transcriptional units from cross-reaction with the neighboring regulatory elements (reviewed in Refs. 1–4). Within complex genetic loci, insulators participate in refined modulations of internal enhancer-promoter interactions (e.g. the bithorax complex in Drosophila; see Refs. 5 and 6).

One of the most extensively analyzed insulator element in Drosophila is part of a retrotransposon, the gypsy retrotransposon, where it occupies the 5′-untranslated region (5′-UTR). The 5′-untranslated region of the Drosophila gypsy retrotransposon contains an “insulator,” which disrupts the interactions between enhancer and promoter elements located apart. The insulator effect is dependent on the suppressor of Hairy-wing (su(Hw)) protein, which binds to reiterated sites within the 350 base pairs of the gypsy insulator, whereby it additionally acts as a transcriptional activator of gypsy. Here, we show that the 350-base pair su(Hw) binding site-containing gypsy insulator behaves in addition as a matrix/scaffold attachment region (MAR/SAR), involved in interactions with the nuclear matrix. In vitro experiments using nuclear matrices from Drosophila, murine, and human cells demonstrate specific binding of the gypsy insulator, not observed with any other sequence within the retrotransposon. Moreover, we show that the gypsy insulator, like previously characterized MAR/SARs, specifically interacts with topoisomerase II and histone H1, i.e. with two essential components of the nuclear matrix. Finally, experiments within cells in culture demonstrate differential effects of the gypsy MAR sequence on reporter genes, namely no effect under conditions of transient transfection and a repressing effect in stable transgenic experiments. In vitro experiments presented in this report disclose the attachment of chromosomes to the nuclear matrix.

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‡ The abbreviations used are: UTR, untranslated region; bp, base pair(s); MAR/SAR, matrix/scaffold attachment region; CAT, chloramphenicol acetyltransferase; LIS, lithium diiodosalicylate; LTR, long terminal repeat; topo, topoisomerase.

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as a transcriptional activator (8, 34), therefore also interacts with components of the nuclear matrix. The complex in vivo regulatory effects of the gypsy element are discussed in relation with the characteristic features of this insulator.

**EXPERIMENTAL PROCEDURES**

**Plasmids and DNA**—The gypsy DNA was from plasmid cDm111 (35), re-cloned as BglII-EcoRI plus EcoRI-BamHI fragments into the pSK (Stratagene) polycloner; a plasmid containing the gypsy LTR plus 5'-LTR was obtained by cloning the Klenow-treated HpaI-NcoI fragment (positions 2–1201 in Ref. 36) from the above plasmid into the EcoRV site of the pSK polycloner; plasmids with fragments I to IV (see Fig. 1A) were obtained by inserting the corresponding DNA fragments, obtained either by restriction from the plasmids above or after polymerase chain reaction amplification, into the pSK polycloner.

For the assays with histone H1, a plasmid containing one copy of the gypsy insulator as a XmnI-Sau3AI fragment (positions 670–1029 in Ref. 36) cloned into the BamHI site of pSK after Klenow treatment of both vector and insert was used, as well as a plasmid containing three copies of the same fragment cloned into the Klenow-treated SpeI, XhoI, and BamHI sites of the pSK polycloner.

For the transfection experiments, we used the previously described p6 plasmid (37), which contains the chloramphenicol acetyltransferase (CAT) gene under control of the EcoRV site of the pSK polycloner (for sequence analysis, see below) followed by the SV 40 polyadenylation sequence. The XmnI-Sau3AI gypsy insulator fragment (positions 670–1029) with all 12 copies of the binding site for the su(Hw) protein was inserted both upstream and downstream to the reporter gene into the BamHI and XhoI sites, respectively. As a reference, we used plasmid p8 (37), derived from plasmid p6 upon addition on both sides of the reporter gene of the 960-bp intergenic scaffold-associated regions from the Drosophila melanogaster hsp 70 gene.

**In Vitro MAR/SAR Binding Experiments**—High salt extraction of nuclei from Drosophila Schneider II, murine L, or human HeLa cells (2 M NaCl extraction after Dnase I digestion) and in vitro MAR binding assays were performed as in Refs. 29 and 38. In vitro SAR binding assays with LIS-extracted nuclei (25 mM lithium diiodosalicylate) digested with a series of restriction nucleases (XhoI, BstXI, and XmnI) were performed as in Refs. 39 and 40. The gypsy fragments, radiolabeled either by Klenow treatment after restriction with appropriate enzymes or upon polymerase chain reaction amplification using [32P] labeled primers, were incubated 2 h with nuclear matrices prepared from 5 A590 units of purified nuclei, in the presence of competitor DNA (0.5 mg/ml sonicated salmon sperm DNA). Pellet (P) and supernatant (S) fractions were separated by centrifugation (1 min at 4 °C, 10,000 0 g), treated, in parallel, with polycrylamide or agarose gels (and transferred onto Hybond N membranes in the latter case); dried polyacrylamide gels or membranes were then autoradiographed. For competition experiments, the labeled fragment corresponding to the gypsy insulator (XmnI-Sau3AI) was first preincubated with the antibiotics distamycin (Sigma) or chloromycin (Boehringer Mannheim) as in Refs. 41 and 42, and then assayed as above.

**Precipitation and Cleavage Assay with Topoisomerase II**—For the precipitation assay, radiolabeled fragments of the gypsy retrotransposon were incubated 30 min at 30 °C with increasing amounts of highly purified yeast and human topoisomerase II (topo II; gift from Dr. A. Larsen) in TEN buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 150 mM NaCl, 0.1% digitonin) in the presence of competitor DNA (sonicated salmon sperm DNA) as in Refs. 41 and 42. Pellet and supernatant fractions, separated by centrifugation (10 min at 4 °C, 12,000 0 g), were then recovered and treated with 0.5% SDS and 0.5 mg/ml protease K (final concentrations), were analyzed on polyacrylamide gels, which were dried and autoradiographed. For the topo II cleavage assay, a fragment containing the 5' part of gypsy (positions 1–1201 in Ref. 36) plus 1.5 kilobase pairs of the pSK vector was incubated with increasing amounts of topo II in buffer B (20 mM Tris-HCl, pH 7.4, 20 mM KCl, 10 mM MgCl2, 70 mM NaCl, 0.05 mM spermine, 0.125 mM spermidine, 0.1% digitonin, 1 mM phenylmethylsulfonyl fluoride, 25 μM/ml bovine serum albumin, 1 mM dithiothreitol, and 1 mM ATP), supplemented with the anti-tumor drug VM26 (final concentration 50 μM; gift from Dr. A. Larsen) as in Ref. 42. After electrophoresis and blotting, the cleavage pattern was analyzed by hybridization with the indicated probe (indirect end-labeling).

**Precipitation and Protection Assay with Histone HI**—Precipitation assay and protection from digestion by Dnase I (Sigma) were performed as in Refs. 43 and 44, with the gypsy insulator fragment XmnI-Sau3AI either as a single copy or as three directly repeated copies subcloned into the pSK plasmid. Plasmids were digested with restriction enzymes in the pSK polycloner, and fragments were labeled by Klenow treatment. DNA (2–5 ng of labeled fragments with 1 μg of sonicated salmon sperm DNA) was then incubated with histone H1 (Boehringer Mannheim) for 3 h at 37 °C. For the protection assay, pellet was recovered by centrifugation (15 min at 4 °C, 12,000 0 g) and analyzed as above.

For the DNase I protection assay, DNA with histone H1 was digested with Dnase I (50 ng; Sigma) for 3 min at room temperature in the presence of 1 mM CaCl2, purified by phenol/chloroform extractions and analyzed by gel electrophoresis (44).

**Cells, Transfections, and CAT Assays**—Drosophila SII cells were grown in Schneider medium (Life Technologies, Inc.) with 10% fetal calf serum (Life Technologies, Inc.), and murine L and human HeLa cells in Dulbecco’s modified Eagle’s medium with the same serum. Transfection of cells (about 5 × 105 cells) were performed by the standard calcium phosphate precipitation procedure, with 10 μg of DNA. Stable transformants were obtained upon co-transfection with the plasmid pSV2neo conferring Genetin resistance (45), followed by selection with G-418 (0.7 mg/ml; Life Technologies, Inc.). For transient assays, cells were co-transfected with a plasmid containing the luciferase gene under control of the Rous sarcoma virus LTR to normalize transfection efficiency. Protein extracts were prepared as in Ref. 37 for CAT activity as in Ref. 46 using appropriate dilutions to obtain less than 50% conversion of [14C]chloramphenicol to acetylated forms. Reaction products were quantitated by a Bio-Imaging analyzer (BAS model 1000, Fuji, Tokyo, Japan). CAT activity was expressed as the percentage of [14C]chloramphenicol acetylated in 30 min/mg of protein at 37 °C. Reporter plasmid copy number in the stable transformants was measured by Southern blot analysis of the genomic DNAs restricted with HindIII and EcoRI, using a CAT gene fragment from the p6 plasmid as a probe. DNA isolation and Southern blot hybridization were as in Ref. 47.

**RESULTS**

**Sequence Analysis of the gypsy Insulator**—The gypsy insulator is a short sequence (350 bp) with a complex organization (Fig. 1, A and B). It contains reiterated binding sites for the suppressor of Hairy-wing (su(Hw)) protein, which both regulate transcriptional efficiency and acts as a transcriptional enhancer (see Introduction). These sites are part of a sequence characterized by 12 copies of a core 12-bp sequence, 5'-PyPuTTGCATACCPy-3', with homology to the mammalian octamer motif (see Ref. 30). These reiterated binding sites are interspersed among an AT-rich domain (>70%), which is composed of short homopolymeric runs of dA-dT base pairs and AT dimer repeats (Fig. 1B), and is involved in the refined modulation of su(Hw) binding (30). Although there is no definite consensus sequence for MAR/SARs, analysis of previously characterized sequences revealed ATC regions with G residues only on one strand of the DNA duplex, which were demonstrated to contain binding sites for nuclear matrix specific proteins and which in a multimerized form actually bind to the nuclear matrix (48–50). Close examination of the gypsy insulator actually discloses ATC regions, with, for instance, four almost perfect (T/C)TTT-TAATAAA(T/A)A(T/C)ATT repeats (Fig. 1B), organized as the ATC sequences in the MAR of the mouse IgH gene, which harbors binding sites for the nuclear matrix-specific SATB1 and Bright proteins (ATC stretches with A-rich core, flanked by AT-dimer repeats, see reference above). Finally, MAR/SARs usually contain sequences related to the in vitro topoisomerase II cleavage core consensus (AT/A/C/T)ATT (51, 52); such sequences can be found in the present sequence (see Fig. 1B). The possibility that the gypsy insulator co-localizes with a MAR/SAR was therefore tested, using classical assays for the identification of such elements.

**Binding of the gypsy Insulator to the Nuclear Matrix**—In vitro binding assay of gypsy sequences with high salt-extracted nuclei was performed as described in Refs. 29 and 38, using either Drosophila (SII), murine (L), or human (HeLa) cells. Insoluble material (i.e. associated with the nuclear matrix) was recovered as a pellet after centrifugation and treated, in par-
allel with the supernatant, with proteinase K, purified and analyzed on agarose or polyacrylamide gels. As illustrated in Fig. 1C using end-labeled fragments from the gypsy retrotransposon, a fragment is specifically recovered in the insoluble fraction, which coincides with the 5′-UTR of gypsy; the other gypsy fragments are not associated with the nuclear matrix. A more refined analysis of the 5′-UTR region using for the binding assay four labeled fragments (I–IV, see Fig. 1A) including the gypsy LTR, the most 5′ part of the untranslated region (also AT-rich), the gypsy insulator (10, 12) and the region corresponding to the N-terminal part of ORF1, further showed that the MAR coincides with the insulator domain (Fig. 1C). Identical results were obtained with nuclear scaffold (SAR assay, see Fig. 1D) isolated after lithium diiodosalicylate (LIS) extraction of nuclei from the SII cells as described in Refs. 39 and 40. In typical MAR/SAR assays, about 50% of total gypsy insulator DNA was recovered in the pellet, as also observed (data not shown) with the previously characterized histone MAR/SAR from Drosophila (37). Finally, binding assays performed with nuclear matrices from cells of other species revealed a conservation of the MAR/SAR activity of the gypsy insulator (see Fig. 1C for murine and human cells), as observed for other such domains (48, 53).

Drug interference experiments as devised in Refs. 41, 42, and 52 for MAR/SARs, further demonstrated that the oligo(dA)-oligo(dT) tracks within the gypsy insulator should be the main structural determinants of the nuclear matrix binding activity. As illustrated in Fig. 1E, binding of the gypsy insulator to the...
nuclear matrix was specifically inhibited by the peptidic distamycin antibiotic, and was not affected by chromomycin, which selectively interact with the minor groove of AT- and GC-rich DNA regions, respectively.

Interaction of Topoisomerase II with the gypsy Insulator—All MAR/SARs so far described have been shown to be functional targets of topo II, one of the main protein components of the nuclear matrix (54, 55). Topo II is a key protein mediating interconversions between different topological states of the DNA, through transient double-strand breaks and rejoining. This enzyme is also required for chromatin assembly and packaging, and it plays a significant role in the establishment of chromatin loops (29, 56, 57). Assays for both the aggregation of the gypsy MAR/SAR and for cleavage were therefore performed, using highly purified yeast or human topoisomerase II. Topo II-mediated aggregation was monitored as in Ref. 42 by centrifugation using end-labeled gypsy fragments and analysis of the pellet after centrifugation were analyzed on acrylamide gels; the specifically precipitated 5′-UTR and insulator fragment III are indicated by arrows. B, cleavage assay. A linearized fragment containing the gypsy 5′ end and plasmidic DNA (see structure on the right) was incubated with yeast topo II in the appropriate cleavage buffer (see “Experimental Procedures”); reaction products were analyzed after electrophoresis on an agarose gel and blotting, by an indirect end-labeling procedure upon hybridization of the membrane with the probe indicated in the figure (heavy bar).

![Fig. 2. Topoisomerase II preferentially interacts with the gypsy insulator. A, precipitation assay. Labeled gypsy fragments from the full-length gypsy restricted with a series of enzymes (XhoI, BstXI, EcoRV and HpaII; upper part) or fragments II and III from the gypsy 5′-UTR (see Fig. 1A; lower part) were incubated with increasing amounts of highly purified yeast topo II; total DNA (input DNA) and pellet after centrifugation were analyzed on acrylamide gels; the specifically precipitated 5′-UTR and insulator fragment III are indicated by arrows. B, cleavage assay. A linearized fragment containing the gypsy 5′ end and plasmidic DNA (see structure on the right) was incubated with yeast topo II in the appropriate cleavage buffer (see “Experimental Procedures”); reaction products were analyzed after electrophoresis on an agarose gel and blotting, by an indirect end-labeling procedure upon hybridization of the membrane with the probe indicated in the figure (heavy bar).](image1)

![Fig. 3. The gypsy insulator DNA specifically nucleates histone H1. A, precipitation assay. Labeled fragments corresponding to the pSK plasmid vector and a three direct repeat of the gypsy insulator were incubated with the indicated amount of histone H1 (expressed as percent in weight of histone H1 to DNA); total DNA (input DNA) and DNA in the pellet after centrifugation were analyzed as in Fig. 1C; positions of the pSK plasmid vector and of the gypsy insulator are indicated with arrows. B, DNase I protection assay. The histone H1 binding reaction was as in A, and then DNase I digestion was allowed (+) or not (−) to proceed for 3 min at room temperature. DNA was then purified, electrophoresed, blotted, and analyzed by autoradiography.](image2)
centrations of histone H1. After centrifugation, DNA in the insoluble fraction recovered as a pellet was extracted and analyzed on agarose gels. As illustrated in Fig. 3A, histone H1 at a low concentration preferentially aggregates the insulator-containing DNA (10-fold ratio over control plasmid DNA), whereas higher histone H1 concentrations finally resulted in a general aggregation of both DNA fragments, as expected (43). Similar results were obtained using a single copy of the gypsy insulator (instead of 3-mer), but with a reduced specificity (data not shown). To analyze further the binding of histone H1, a second series of experiments was performed as in Ref. 43, to measure the possible protection of the insulator DNA domain from digestion by DNase I (Fig. 3B). The insulator DNA, together with the vector plasmid as a control, were incubated with purified histone H1 as above, then DNase I was added for 3 min and DNA was recovered and analyzed. As illustrated in Fig. 3B, the addition of histone H1 results in the protection of the insulator DNA, whereas complete degradation of the vector plasmid DNA takes place. This two series of experiments therefore strongly suggest that histone H1 actually binds in a specific manner to the gypsy insulator, resulting in a DNase I-resistant structure.

Differential Effect of gypsy MAR/SAR-containing Reporter Genes in Transient and Stable Transfection Assays—Common to the studies on MAR/SAR is the observation that MAR/SARs flanking a test gene have either a repressive or stimulatory effect on the level of its expression (Refs. 21 and 60–62; see the “chromatin switch model” in Refs. 22, 44, and 59). Moreover, in contrast to transcriptional enhancer elements, MAR/SARs exert this regulatory effect only if the reporter gene is stably integrated into the genome but have no effect in transient transfection assays (Refs. 20, 63, and 64; reviewed in Ref. 22). The gypsy MAR/SAR was therefore assayed as in Ref. 37 upon transfection of cells in culture under both transient and stable conditions. The SV-CAT reporter gene in Ref. 37 was inserted between the gypsy MAR/SAR, using as a negative control a vector without any bordering sequences and, as a positive control, a reporter with the previously characterized MAR/SAR from the hsp70 gene. These reporter plasmids were then introduced into mouse L cells by transfection and were either assayed 2 days after transfection or stably integrated upon further selection in G418 medium (the cells being co-transfected with a neo resistance-encoding plasmid). As illustrated in Fig. 4A, no effect of either the hsp or gypsy sequence can be detected in the transient assay, as expected for MAR/SAR sequences. However, both sequences had a significant effect in the stable transformants (Fig. 4B); the hsp MAR/SAR resulted, as reported previously for the same construction (37), in a 10–20-fold increase in CAT activity, whereas the gypsy sequence resulted in a 3–5-fold decrease. Identical results were similarly obtained using human HeLa cells (data not shown), with no effect in the transient assay and, again, a repressing effect (versus an increase for the hsp MAR/SAR) in stable transformants.

DISCUSSION

The present investigation reveals an as yet unidentified characteristic feature of the insulator domain of the gypsy retrotransposon, i.e. a MAR/SAR activity. This 350-bp domain, located in the 5′-untranslated region of this Drosophila retrotransposon, therefore combines three functions, namely insulation, transcriptional enhancement, and nuclear matrix attachment. This co-localization might be the consequence of the pressure for compactness of retroviral genomes and could be responsible for the complex “phenomenologic” behavior of the gypsy insulator and the often divergent models that have been proposed to account for the gypsy effects (12–14, 65).

A Multifunctional Domain within 350 bp of the gypsy Retrotransposon: an Enhancer, an Insulator, and a MAR/SAR—The 350-bp gypsy sequence has been the subject of extensive analyses, which had already revealed two characteristic features of this domain. Actually, it had been demonstrated by several groups that this sequence acts as an insulator, therefore inhibiting in a directional manner the effect of enhancer sequences located distally from the promoter (10–13). Refined in vitro analyses have unambiguously demonstrated that this blockade is not due to a repression of the enhancer per se but to an inhibition of the effect of the enhancer on the insulated promoter, as promoters proximal to the enhancer remained fully regulated (12, 13). Most importantly, this insulating activity was shown to require the presence of the suppressor of Hairwing protein, which binds to the insulator sequence via well-identified sites (reviewed in Refs. 2 and 4). Actually, a second protein is required for insulation, the mod(mdg4) protein, which most probably binds to the su(Hw) protein to form a complex active for insulation (14, 65).

A second function associated with the gypsy insulator is an enhancer activity which, although less extensively documented, also requires the su(Hw) protein (8, 34). Actually, in vitro analyses of the expression of a transgene containing the gypsy promoter plus 5′-UTR region driving the expression of a lacZ reporter gene (34) have revealed an enhancer activity of the gypsy insulator containing sequence, which was su(Hw)-dependent. This enhancer activity is also suggested by in vivo analyses of the expression of a mini-white gene flanked by the gypsy sequence, which have revealed transgenic flies with increased eye pigmentation, whose intensity decreased in a
su(Hw) mutant background.²

The third function that was unwarered in the present work is associated with the presence of a MAR/SAR within gypsy, which precisely co-localizes with the insulator domain. Indeed, we have shown that the gypsy insulator possesses all the in vitro characteristic features of previously characterized MAR/SARs, including the occurrence of functional binding sites for topoisomerase II and histone H1 ("switch model," see Refs. 22, 41, 44, and 59). Ex vivo analyses of reporter genes flanked by the gypsy MAR/SAR sequence have further shown, as expected, differential effects of this sequence on reporter genes depending on whether the assay was under transient or stable conditions, a characteristic feature of chromatin structure-dependent effects (see references under "Results"). Actually, the presence of a MAR/SAR within gypsy is not totally unexpected, as "boundary" elements are in general regions which contains not only enhancer and insulating elements, but also matrix attachment domains (reviewed in Ref. 2). The rather original feature of the gypsy sequence is that all three domains, which in general are sufficiently "dispersed" so as to allow isolation of "pure" enhancers, MAR/SAR, or insulators (reviewed in Refs. 2 and 22; see also Refs. 19 and 21), are in the present case "gathered" within a single and relatively short (350 bp) sequence. This rather uncommon situation might in fact be relevant to the pressure for compactness within retroviral sequences, as it is known that retroviruses can only package a limited amount of genetic information (66).

A Tentative Model for gypsy Insulation—A consequence of compaction is that the gypsy insulator and its associated components are most probably interacting, in vivo, with elements of the nuclear matrix. Accordingly, proteins of the nuclear matrix might play a role in the insulation process, and conversely the su(Hw) protein (which is essential for insulation) might interact with proteins of the matrix. Such interactions could actually account for the data on gypsy insulation and fit with previously proposed models for the gypsy effects. A first series of data strongly suggests that the gypsy insulator, as all previously characterized insulators, essentially prevents interactions between distal enhancer and promoter, without any direct repressing effect on the enhancer itself (12, 13). This directional effect can most easily be accounted for by the "looping model" involving generation of structural domains isolated one from the other by attachment of boundary sequences (MAR/SAR) to the nuclear matrix (see Ref. 22 and Introduction). Alternatively, a series of data on gypsy insulation (essentially in mod/mdg4 mutants) discloses bidirectional repressing effects (14, 65), which can be accounted for by a model involving heterochromatinization (65). The present data (showing that the gypsy insulator behaves as a MAR/SAR) are clearly in agreement with the structural looping model, but also support the heterochromatinization model. Indeed, the gypsy MAR/SAR DNA per se, in the absence of su(Hw) protein, is involved in histone H1 nucleation (as shown in this paper), and it has been demonstrated that histone H1 nucleation is associated with both DNA compaction and transcriptional silencing (reviewed in Ref. 67); in addition, Laemmli and co-workers have found that histone H1 could be removed from MAR/SAR domains by distamycin and distamycin-like proteins (D-like proteins, such as the high mobility group proteins), leading to the proposal that MAR/SARs could activate or repress transcription of adjacent genes depending on the nucleation/depletion of histone H1 ("switch model," see Refs. 22, 41, 44, and 59). The gypsy MAR/SAR could then be responsible for the repressing effect observed in the mod/mdg4 mutants (65), as well as in the present assay within heterologous cells (assuming further that appropriate D-like proteins are absent in these cells). Taking into account, in addition, that mutations in the mod/mdg4 or the su(Hw) genes modify position-effect variegation (65), it could be further hypothesized that the su(Hw)/mod/mdg4 complex acts as the D-like proteins and makes nucleation processes to switch from a repressing to an active state. Accordingly, a model in which the su(Hw) binding sites and the associated su(Hw)/mod/mdg4 complex modulate the effects of the MAR/SAR DNA sequence would rather simply account for the biological effects of the gypsy insulator in both the wild type and su(Hw)/mod/mdg4 mutants. The proposed model would then reconcile the two previous models for gypsy insulation, i.e. the heterochromatinization and the looping models.

In conclusion, we have shown that components of the nuclear matrix interact specifically with the gypsy insulator, and accordingly could be involved in establishing chromatin boundaries. The next important question, along these lines, concerns the role of the su(Hw) protein in this interaction. The su(Hw) protein could (i) be directly involved, as a possible component of the nuclear matrix itself, (ii) be involved as a "bridge" or intermediate protein, or even (iii) be dispensable (although necessary for insulation) if interaction with the nuclear matrix is mediated solely by the DNA sequences per se, possibly by the AT-rich interdomains within the insulator. Experiments to answer this question and delineate the role of the MAR/SAR for insulation in vivo are now in progress.

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