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The Intrinsic Topological Information of the Wild-type and of Up-promoter Mutations of the *Saccharomyces cerevisiae* Alcohol Dehydrogenase II Regulatory Region*

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A 569-base pair fragment encompassing the upstream regulatory region, the RNA initiation sites, and the initial part of the coding region of the *Saccharomyces cerevisiae* alcohol dehydrogenase II gene has been analyzed for the presence of sites which undergo conformational modification under torsional stress. Fine mapping of P1 and S1 endonuclease-sensitive sites was obtained on *single* topoisomers produced by *in vitro* ligation. It was shown that the upstream activator sequence, the TATA sequence, a region directly upstream to the RNA initiation sites, and several positions in the first segment of the transcribed region change conformation as a function of the applied torsional stress in a precisely *coordinate* fashion. The superhelical density optima for this coordinate modifications have been determined. Analysis of the conformational changes of the promoter sequence in several naturally occurring (Young, E. T., Williamson, V. M., Taguchi, A., Smith, M., Sledziewski, L., Russel, D., Osterman, J., Denis, C., Cox, D., and Beier, D., (1982) in *Genetic Engineering of Microorganisms for Chemicals* (Hollander, A., De Moss, R. D., Kaplan, S., Konisky, J., Savage, D., and Wolle, R. S., eds) pp. 335–361, Plenum Publishing Corp., New York) up-promoter constitutive mutants was performed.

This analysis has shown that the conformation of functionally relevant sites changes as a function of sequence mutations that have taken place elsewhere; this shows that the conformational behavior of the whole promoter region is linked and suggests transmission *in cis* of topological effects in RNA polymerase II promoters.

Regulation of transcription of RNA polymerase II-served genes requires the ordered positioning of *trans*-acting protein factors along *cis*-acting regulatory DNA elements. This interaction ultimately leads to the productive binding of RNA polymerase on RNA initiation site(s). A commonly accepted interpretative model of the complex interplay among *trans*-acting regulatory elements and RNA polymerase relies on protein-protein interaction, the role of the DNA of the regulatory region being limited to that of a semi-inert scaffold, necessary only for the correct positioning of regulatory and enzymatic proteins. In these types of models, the problem set by the distance of the interacting sites is solved by applying to the marked bending ability of DNA (2).

Knowledge of the *in vitro* properties of unusual DNA structures (3–6) supports the concept of DNA conformational microheterogeneity (6–8). A key role of DNA heterogeneity in genetic processes, including transcription, has been proposed (8). A class of models of promoter function, alternative to the scaffold model mentioned above, is derived from this proposal and essentially relies on the "conformational information" (3, 7, 8) contained in the secondary structure of DNA in promoter regions. This information is considered a basic feature of the regulatory process of transcription (8–10). Alteration of DNA structure may result from covalent modification, interaction with proteins, and/or torsional stress. We explore in this study the possibility that the relevant regulatory sequences of an RNA polymerase II promoter alter their conformation as a function of torsional stress.

We define as *intrinsic* the information expressed by the double helix upon the sole variation of topological or environmental (ionic and thermal) parameters as opposed to the *extrinsic* information, activated upon interaction with *trans*-acting macromolecular factors (*i.e.* the modifications induced on DNA by regulatory proteins (11–13).

The model system that we have analyzed consists of a DNA segment containing 569 base pairs of the *Saccharomyces cerevisiae* alcohol dehydrogenase II gene and encompassing the upstream sequences identified as relevant for its *in vivo* transcription and regulation, the multiple RNA initiation sites, and the initial part of the coding sequence (251 bp[1] downstream of the ATG) (1, 14, 15).

The topological information that can be expressed by this promoter was analyzed converting the linear DNA into a closed circular domain; programmed torsional stress was obtained by variation of the linking number of the domain. The resulting alterations of the DNA conformation have been localized.

Different linking numbers were obtained by ligation of DNA fragments in the presence of defined concentrations of the intercalating agent EtdBr. The modifications of the DNA conformation induced as a function of the torsional stress were monitored by isolation of *single* topoisomers and analysis of the appearance of sites hypersensitive to the endonucleases P1 and S1. As for the enzymatic or chemical probes, this assay has a *relative* character: it reveals a *variation* in the conformation of the double strand as a function of the adopted variable (variation of the linking number) but does not in principle provide information on the nature of the resulting

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[1] The abbreviations used are: bp, base pair(s); EtdBr, ethidium bromide; PHS, P1 hypersensitive site(s); RIS, RNA initiation site(s); UAS, upstream activator sequence.

structure. The precise localization of the induced hypersensitivities along the nucleotide sequence has allowed understanding of the supercoiling-induced structures in several instances (reviewed in Refs. 3 and 16–18).

In topologically closed domains, the torsional stress induced by linking deficiency is in principle a global property. The free energy of supercoiling is partitioned between writhing and local modifications of twisting. We show that modifications occur on DNA sites which correspond to the sequences identified in the wild type alcohol dehydrogenase II promoter as relevant for its genetic function (the UAS, the TATA sequence, the RNA initiation region) and in few other, well defined locations. Several naturally occurring constitutive mutants of the alcohol dehydrogenase II promoter were analyzed, revealing that mutant promoters have a completely altered topology.

EXPERIMENTAL PROCEDURES

Materials—P1 and S1 endonucleases were obtained from Pharmacia LKB Biotechnology Inc. and Boehringer Mannheim, respectively. Restriction endonucleases and T4 ligase were purchased from New England Biolabs and Boehringer Mannheim: EtdBr was purchased from Sigma, radiochemicals from Du Pont New England Nuclear. Topoisomerase I was purified from frozen erythrocytes according to Ref. 19.

The recombinant plasmid ADRII.BS.pBR322 (1, 14, 20) contains a *Bam*HI-*Sau*3A 2.2-kilobase pair fragment of *S. cerevisiae* DNA cloned in the *Bam*HI site of pBR322. This fragment encompasses 1 kilobase pair of the upstream region and the complete coding sequence of the alcohol dehydrogenase II gene; the sequence is reported in Ref. 20. A 569-bp *Sau*3A-*Sau*3A DNA fragment that contains 318 bp of the region upstream of the ATG and the initial part of the coding sequence was used in the present study. A schematic map is reported in Fig. 1.

DNA of constitutive mutants was derived 1) from the recombinant plasmid ADR3-5c-yRp7 for the constitutive mutation caused by the increase in length of the dA stretch (from 20 to 55 dA residues) (located in the wild-type at positions −223 to −242 upstream of the ATG (1, 14) and 2) from the recombinants ADR3-6c-yRp7 and ADR3-8c-yRp7, which contain a 13-kilobase pair fragment derived from *S. cerevisiae* mutants made constitutive for the alcohol dehydrogenase II expression by the Ty insertion occurring, respectively, at positions −161 and −124 from the alcohol dehydrogenase II ATG (1, 21, 22).

Circularization of DNA Fragments—The procedure detailed in Ref. 23 was followed with minor modifications (24–26). Circularization was performed at 4 °C at the appropriate concentration of EtdBr in 500 μl. The complete range of topoisomers was obtained by ligations performed in EtdBr concentrations ranging from 0 to 1.5 μg/ml. Lk4 was obtained with 0.2, Lk5, 6, and 7 with 0.7, Lk8 with 1.2 μg/ml EtdBr. Single uncontaminated topoisomers were prepared (as described in Refs. 24 and 26). by recovery of cut-out bands from preparative gel electrophoresis. The restriction sites used for terminal labeling and ligation were the *Sau*3A sites at positions +251 and −318 relative to the ATG in the wild type alcohol dehydrogenase II, at positions +251 and −353 in the 5c constitutive mutant , at position +251 and at a position in the Ty's δ element, 153 nucleotides upstream of the insertion site for the Ty-induced constitutive mutations (1, 21, 22). Schematic maps are shown in Figs. 1, 5, and 6.

Mapping of the P1 and S1 Hypersensitive Sites—After elution and purification, the isolated topoisomers were treated with P1 or S1 as detailed (24, 25), phenol-extracted, alcohol-precipitated, and treated with a secondary restriction endonuclease to allow directional mapping of the P1 or S1 cutting sites. The secondary restriction used was *Nci*I (position −297) or *Hpa*II (position +209): the resulting linear DNA fragments were denatured and analyzed on thin polyacrylamide gels containing 8 M urea. The lengths of the single-stranded DNA fragments, produced by P1 on one side and by restriction on the other, were identified by comparison with a reference ladder composed by a complete *Bsp*RI digest of pUR250. The ambiguity resulting from this procedure is of plus or minus two nucleotides depending on whether the P1 cut is on the upper or on the lower strand. Scanning densitometries were made with an LKB UltroScan XL.

Topoisomerase I Assay—0.5 units of enzyme (27, 28) were reacted in 50 μl of 0.1 M NaCl, 10 mM Tris-HCl, pH 7.8, and 1 mM EDTA at 35 °C for the time specified with ~50 ng of supercoiled DNA.

RESULTS

Analysis of the Alterations of the Secondary Structures of the Alcohol Dehydrogenase II Promoter as a Function of Increasing Linking Deficiency

Localization of Conformational Alterations—The 569-bp DNA fragment encompassing the alcohol dehydrogenase II promoter was ligated in the presence of concentrations of EtdBr ranging from 0.0 to 1.5 μg/ml. The topoisomers (from Lk1 to Lk8) were prepared as described and analyzed for the presence of the P1 hypersensitive sites (PHS). Fig. 1 (*panel A1*) shows the PHS on Lk4, 6, 7, and 8, alongside the sequence of the UAS (localized in the inverted repeat, from position −292 to −271 (15)) and of the 52 adjacent nucleotides. No PHS were detected in Lk1, 2, and 3; Lk4 always behaved as Lk5. The major sites are observed on Lk8, centered on the UAS (at the center of the inverted repeat, position −281 ±2), at position −263 ±2 (at the center of a complex region characterized by several conformational possibilities: directed repeats, inverted repeats, $d(GA)_n \cdot d(CT)$ motifs), and on a group of sites between positions −27 and +38 (first part of the transcribed region, around the ATG, detailed below). Overexposure (10 ×) of the autoradiogram shows two additional sites: −157 (center of the TATA box) (Fig. 1, *panel A2*) and a larger region of sensitivity located immediately upstream of the RNA initiation sites (RIS) (Fig. 1, *panel 2*). The sensitive sites are spread along a region characterized by several CTT repetitions and, more in general, by a quasi-homogeneous pyrimidine sequence (31 pyrimidine/36 between positions −71 and −106). *Panel 2A* also details the mapping of the cluster of PHS distributed between positions −27 and +38 (exposure = ⅓ ×), in a region that, besides the indicated direct repeats, shows no obvious reasons for deformation under torsional stress. Fig. 1*B* reports the densitometries of the whole UAS-to-ATG region for exposures 1 × and details the TATAAAT and RNA initiation sequences (exposure 10 ×). Fig. 1*C* quantitatively describes the superhelical density optima for selected positions representative of the UAS, of the TATA sequence, of the RNA initiation region, and of one site inside the first transcribed segment. Each pattern is normalized to the maximal intensity (=100) observed for the reported position.

This analysis has revealed that (*a*) the conformation of the UAS, of the TATA sequence, of a region proximal to the multiple RNA initiation sites, and of a region encompassing the first part of the transcribed region varies markedly after a threshold of $-\sigma = 0.073$. (*b*) Several other minor positions change conformation along the promoter region. See Fig. 5, map on the *left*, for a localization. (*c*) These variations are *coordinate*. By this term we mean that the intensity of the alterations not only increases, but also decreases as a function of the *increasing* superhelical density, according to a precisely defined although complex pattern. The pattern shows the existence of different optima for the free energy requirement for the nucleation and maintainance of alternate DNA conformations as predicted by statistical mechanics (see "Discussion").

Additional evidences for this coordinate behavior was obtained by an alternative method, *decreasing* the torsional stress of a highly supercoiled topoisomer (Lk8) in the P1 sensitivity assay; Fig. 2 reports the scanning densitometries, relative to selected PHS positions, representative of the relevant sites. Decreasing stress was obtained by increasing the EtdBr concentration in the endonucleolytic assay. The difference of the $-\sigma$ optimal for each sensitive position are again evident. This method yields $-\sigma$ optima slightly different (*cf.*

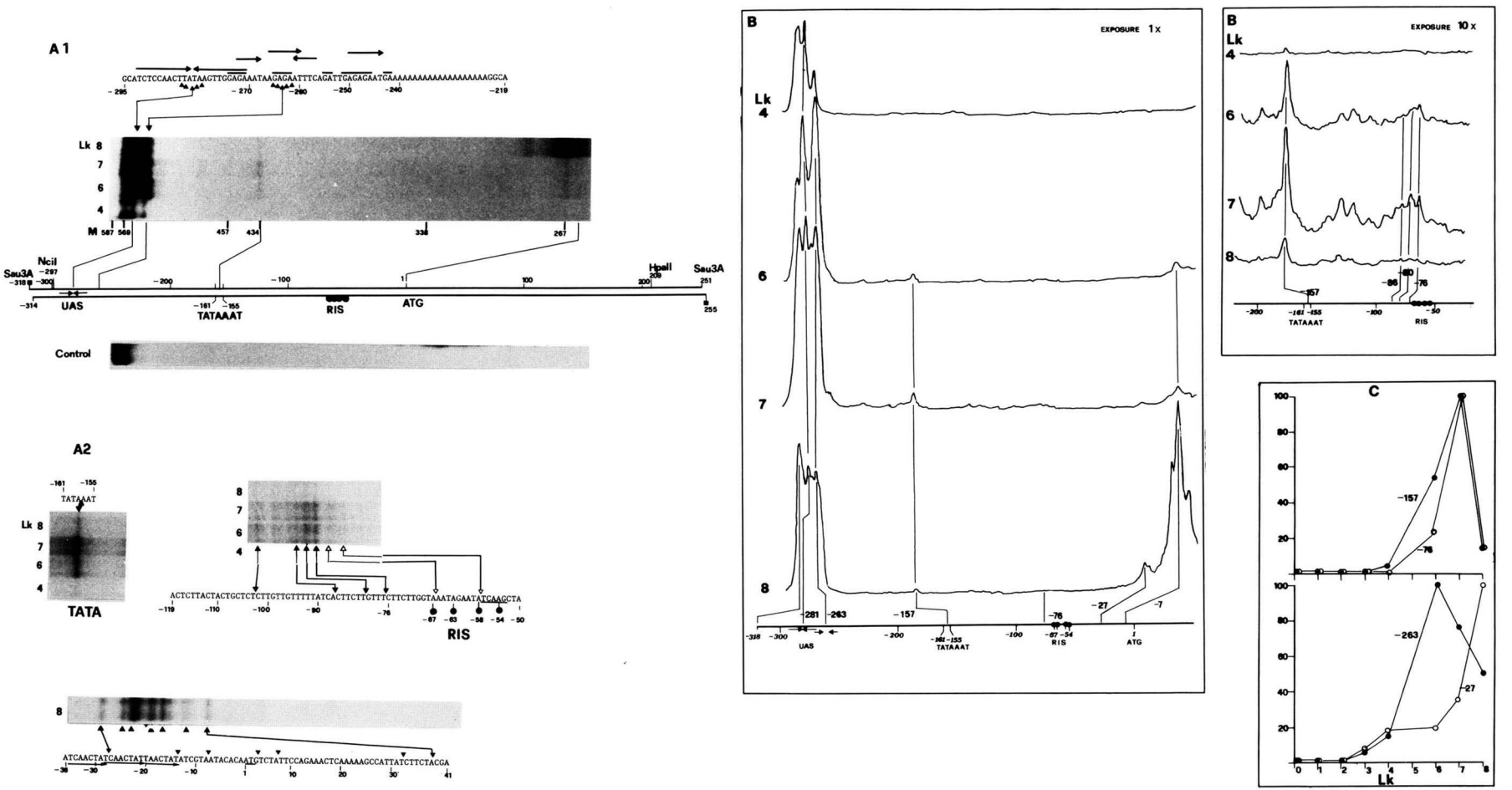

FIG. 1. **Localization of the PHS as a function of the increasing linking deficiency.** *Panel A*, the linear map of the whole fragment is shown in the *center*. The *Sau*3A extremities used for terminal labeling and ligation and the secondary restriction sites used for orientation (*Nci*I and *Hpa*II) are indicated. References for the DNA sequences and the localization of the RNA initiation sites are under "Materials."

The photo shows the mapping of the PHS (0.08 units of P1, see "Experimental Procedures") on topoisomers Lk4, 6, 7, and 8. *M*, markers. A *control* lane obtained in the absence of any endonuclease treatment is also reported (exposure 5 ×). The *upper* sequence refers to the UAS (the long inverted repeats) and to the 52 downstream nucleotides. The *arrows* indicate other inverted and direct repeats. GA motifs are *overlined*. 5000 cpm were loaded for each sample (~30 ng of DNA). Exposure 20 h (1 ×). *Panel A2, upper photo left*, the PHS on the TATA sequence (exposure 10 ×); *upper photo right*, the PHS on the RNA initiation region (sequence is shown, the dots indicate the four RNA initiation sites; −58 and −54 are the two major sites (1). *Lower photo*, the sequence and the PHS in the first part of the transcribed region (between −30 and +40 relative to the ATG) (exposure ⅓ ×). *B*, scanning densitometries of the whole UAS-to-ATG region for the indicated topoisomers. The *smaller panel* details the TATA-to-RIS region, blown up and overexposed (10 ×). *C*, superhelical density optima for selected positions (see *A*). Each profile is reported normalized to the maximal intensity (=100) observed for each reported position.

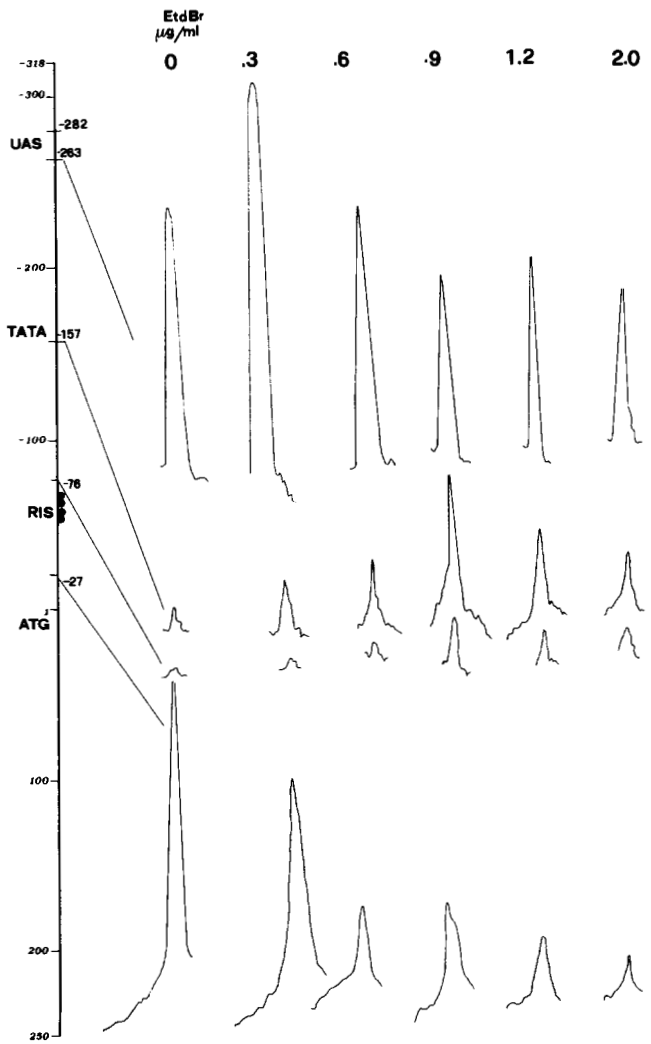

FIG. 2. **The intensity of PHS changes as a function of the variation of torsional stress.** LK8 was analyzed for the presence of P1 hypersensitive sites (as described) in the absence or in the presence of the indicted (*top line*) increasing concentrations of EtdBr (to decrease the torsional stress) (*horizontal* variable). The densitometries of several selected positions (maps) are reported (*vertical* variable). This technique has two advantages relative to the analysis of purified topoisomers: simplicity (the tedious production and recovery of single topoisomers is avoided) and possibility of a detailed analysis (the analytical limitation set by the small integer number of topoisomers that can be obtained for short DNA segments is circumvented). For the possibility of defining a precise correlation between superhelical density and concentration of EtdBr in the assay see Refs. 46 and 47. Position 263 = ⅓ ×, relative to the others.

TATA *versus* UAS) from those obtained with isolated topoisomers.

Fig. 3 shows the partial similarity of sensitivity to S1 and to P1. Similarity is evident for several positions, although the relative intensities and $-\sigma$ dependencies vary (see the comparison of the scanning densitometries, Fig. 3*B*).

The Major PHS Are Present Contemporaneously on the Same Molecules—Evidence is shown in Fig. 4. Lk8 was treated with P1, *not* followed by restriction. The appearance of a labeled fragment whose size accomodates the distance between the UAS and the ATG sites shows that the two sites were present (and cut) together on the same circular molecule (see legend to Fig. 4 for details).

Alterations of the Secondary Structure of the Constitutive Up-promoters Mutations

Two types of naturally occurring up-promoter constitutive mutations for the alcohol dehydrogenase II have been described (1, 20–22), one caused by the expansion of a normal 20-base pair dA-dT sequence to a 54- or 55-base pair homopolymer stretch, the other by the insertion of a Ty1 element in one of several positions between the dA stretch and the RNA initiation sites. We have analyzed the distribution of PHS in one dA- and two Ty-caused mutations. Fig. 5 shows the analysis of the PHS on the 5c alcohol dehydrogenase II promoter (characterized by the 20–55 dA expansion) as a function of the decrease of torsional stress (*lanes 6–10*), compared to the wild-type promoter (*lanes 1–5*).

The pattern of conformational alterations in the wild type is completely different from that of the mutant promoter: 1) the dA stretch is P1-insensitive in the wild type and highly sensitive in the mutant. 2) The TATA box of the constitutive promoter is much less sensitive. 3) The region proximal to the RNA initiation sites is less sensitive in the mutant, although a new site (position −105) appears. 4) In the constitutive promoter, the first part of the transcribed region (between positions −30 and +10, around the ATG) shows a less marked series of deformations. Underexposures showed no difference in the sensitivities of the UAS region (not shown). No additional sites were observed downstream at position +50 (not shown).

Fig. 6 shows the same type of analysis performed on circularized promoters made constitutive by Ty insertion at position −161 (just before the TATA box sequence, 6c) or at −124 (between TATA and RIS, 8c).

Both 6c and 8c *Sau*3A-*Sau*3A circularized molecules contain an identical 153-bp fragment of the Ty element (from the inner *Sau*3A site to the right-most extremity of the δ segment, sequence reported at the bottom of the figure), the 5-bp duplication due to Ty insertion (29) plus, respectively, 412 or 374 bp, from the δ insertion point to the *Sau*3A site used for circularization located inside the coding region. In these circular molecules, UAS and poly(dA) are replaced by a portion of the δ element.

Lanes 1 and *6* show the PHS pattern of highly supercoiled mutant promoters; the effect of the decrease of the linking deficiency (obtained, as for Fig. 5, with increasing concentration of EtdBr) is also reported (*lanes 2–5* and *7–10*).

A major sensitive site is in the δ, at positions −221 and −223 for 6c. The site is also present in 8c, in the corresponding positions (−184 and −186). See the sequence at the *bottom* of Fig. 6, which shows that this site maps at one side of the direct repeat. A secondary site maps at position −183 for 6c (−146 for 8c, 22 positions from the right extremity of the δ, see sequence at the *bottom* of Fig. 6).

The major finding of this analysis is the complete variation of the pattern of the PHS in the alcohol dehydrogenase II sequences: both for 6c and 8c the RNA initiation region is P1-sensitive (around the position −58 for 6c and between −53 and −82 for 8c); this is not the case for the wild type (see Fig. 1). The major deformation present in the wild type, between the −30 and +10 region, is absent in the mutants, and a new series of sites appears inside the gene (see Fig. 6: positions −152, −98, and −91 for 6c and −98 for 8c).

DISCUSSION

We have shown that the DNA sequences encompassing the alcohol dehydrogenase II regulatory region of *S. cerevisiae* respond to torsional stress by undergoing conformational changes at sites corresponding to regulatory sequences: the

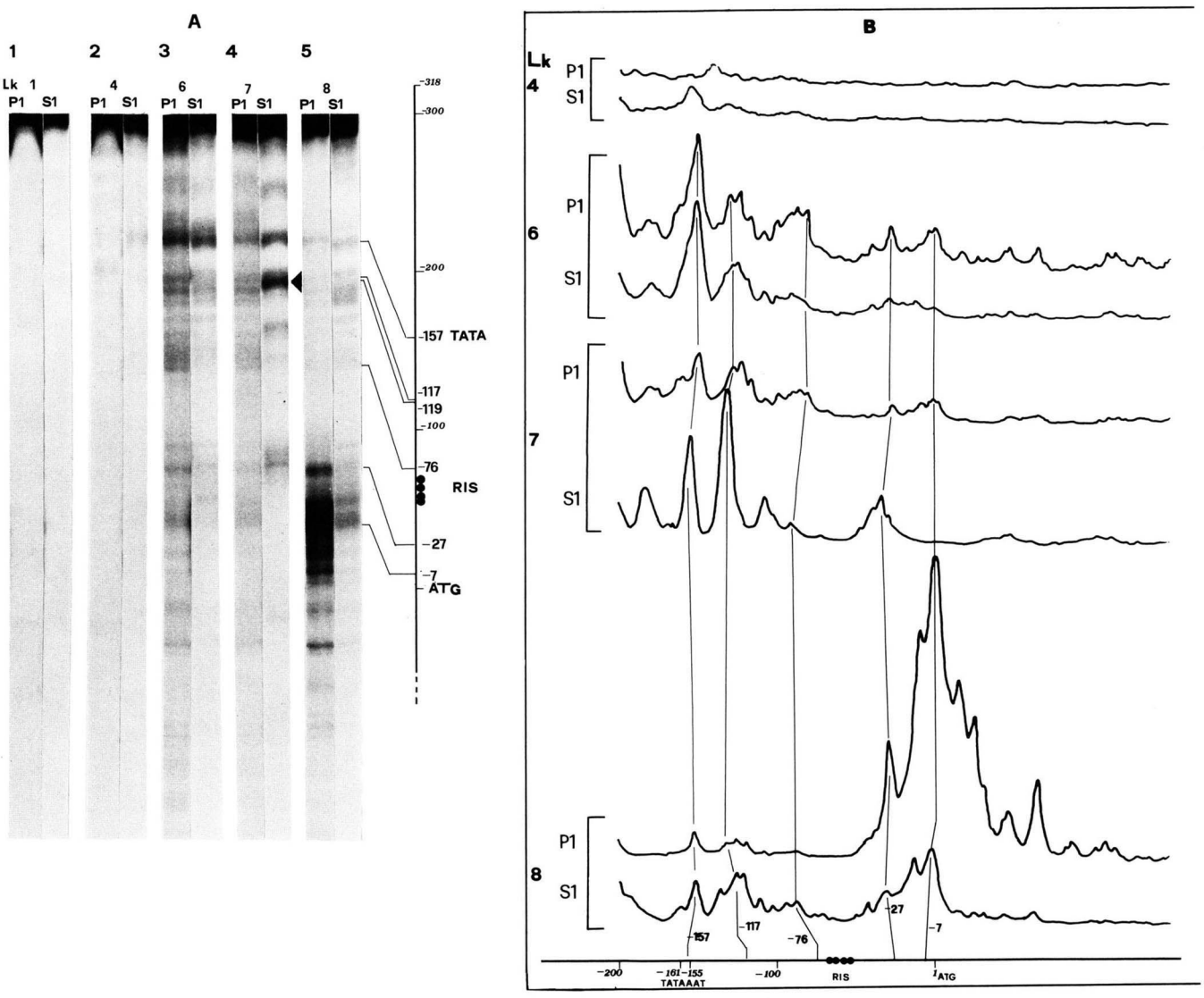

FIG. 3. **Comparison of the P1 and S1 hypersensitive sites.** Analysis for P1 (0.08 units) and S1 (100 units; as in Ref. 47) as reported. The topoisomers studied are indicated on *top* (Lk1, 4, 6, 7, 8). *A*, autoradiogram. *B*, scanning densitometries.

UAS, the TATA box, the region around the multiple RNA initiation sites. The same sequences in naturally occurring up-promoter constitutive mutants respond to torsional stress in a completely different manner.

In particular we observe that the localization of the sites which undergo conformational modification has revealed that several major deformations occur in the functionally relevant sites starting at Lk4 ($-\tau = 2$, $-\sigma = 0.037$) and Lk5 ($-\tau = 3$, $-\sigma = 0.053$) (these are intermediate superhelical density values, comparable with those of bacterial plasmids or animal viruses) and are more evident at high stress (Lk8, $-\tau = 6$, $-\sigma = 0.109$). The presence of a region of major conformational variation inside the transcribed region cannot be directly correlated with part of the promoter function. It maps in and around a segment characterized by direct repeats (see Fig. 1, *panel 2B*). The presence of the ATG signal in this region is probably fortuitous.

These modifications are *coordinate*: increase and decrease in their frequency appear as a function of the variations of the linking deficiency, and each is characterized by a specific superhelical density optimum. This is especially evident for the PHS in the TATA sequence and for those proximal to the RNA initiation sites (*i.e.* at Lk6, $-\tau = 4$, $-\sigma = 0.073$) (Fig. 1). A similar coordinate behavior has been observed for the *S. cerevisiae* GAL1-GAL10 divergent promoters subjected to the same topological analysis (25) and has been theoretically predicted by statistical mechanics of the competition for free energy in the formation of alternative secondary structures in DNA under limiting stress (30, 31).

Topological Domains—From these and from parallel studies (24–26), the view of the RNA polymerase II promoters as *topological domains* emerges. With this definition we refer here to the behavior of a DNA domain in which (i) torsional stress elicits a defined response and which (ii) changes the pattern of its alternate conformations as a function of the variations of torsional stress (as predicted, Refs. 30, 31) and (iii) modifies its response to stress as a function of modifications of its sequence composition. According to this definition, the results that we report here show that the alcohol dehydrogenase II promoter behaves as a topological domain.

It has been postulated that the difference between active and inactive conformation relies on the topological behavior of the components of the system (the "conformational information" model "7, 8)).

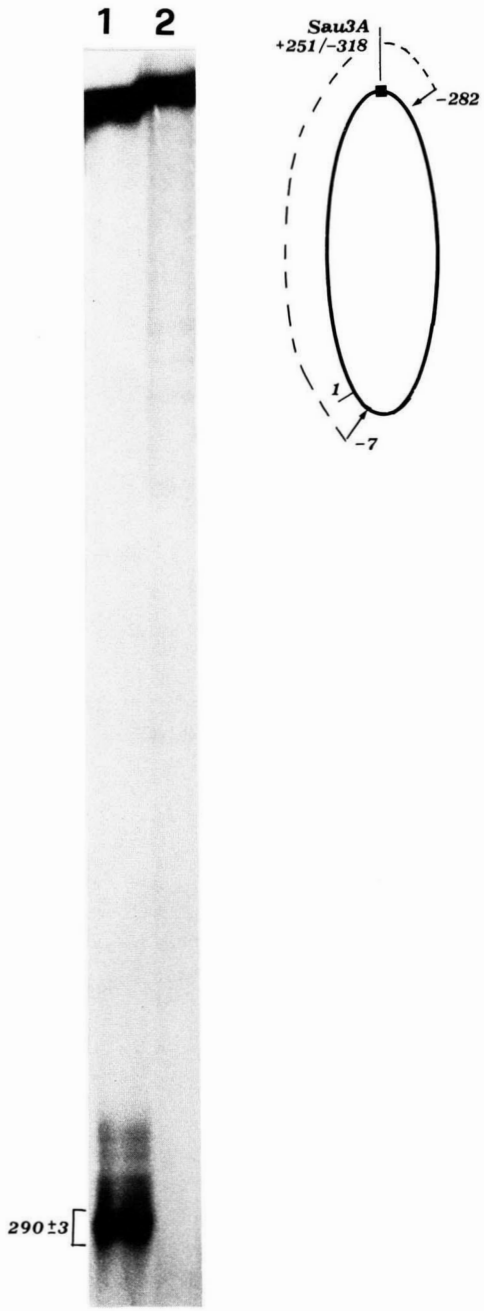

FIG. 4. **Alternate conformations are present contemporaneously on the same DNA molecules.** Lk 8 was treated with P1 and analyzed on gel without further restriction (*lane 1*). *Lane 2* is a P1-untreated sample. The two major PHS are, respectively, on the first part of the transcribed region (centered at position −7) on the UAS (a doublet of sensitive sites centered at positions −263 and −282) (see Fig. 1). If the two sites are cut on the same circular molecule, two fragments will be generated, one of about 290 bp which encompasses the *in vitro* ligated labeled position and another, unlabeled, will be about 280 bp long. A labeled 290-bp fragment is actually generated and is shown in Fig. 1. The schematic drawing indicates the cut position relative to the labeled ligated site; for simplicity the scheme is drawn as a relaxed molecule, not as the supercoiled (Lk8) form.

Alternate DNA Conformations—The major problem facing the verification of the conformational information model and the evaluation of the physiological meaning of the topological variations found in the circularized promoter *in vitro* has been so far the inability to directly analyze the *in vivo* topology. Recent reports have completely reversed the situation and have defined methods to study the occurrence of non-B DNA *in vivo*. These methods have been applied to the analysis of the induction of left-handed DNA (32) and to the formation of cruciforms (33). A photofootprinting technique has also been described (48), which *in vivo* detects transcription-dependent changes in yeast TATA boxes. The altered sensitivity to photomodification in the TATA control elements is believed (48, 49) to result from a DNA structural change mediated by the binding of a specific protein. The major changes in photofootprinting as a function of the transcriptional state of the cell occur on the same DNA sites, UAS and TATA, which we have described, for the same gene, as sites of conformational modification under torsional stress *in vitro* (24, 25).

Both the existence of alternate non-B DNA conformations *in vivo* (32) and the structural changes induced *in vivo* in *S. cerevisiae* by variation of the transcriptional state (48) lend support to the hypothesis that the changes in conformation that we observe as a response to stress in the functional elements of the circularized promoter are related to their function.

Additional support is provided by the analysis of the topological organization of naturally occurring mutant promoters of alcohol dehydrogenase II. This analysis links a defined behavior *in vivo* (constitutive up-promoter) with an alteration of the topological response to torsional stress (Figs. 5 and 6). The pattern of hypersensitive sites in the mutants is completely different from that of the wild type: this fact reinforces the view of the RNA polymerase II promoter as a topological domain (see above), in which an alteration of the sequence in one location alters the possibility of conformational modification elsewhere. The analysis of the hypersensitivity patterns reveals that both types of mutations (both poly(dA) expansion and δ insertion) cause a generalized conformational modification (as described in Figs. 5 and 6).

The fact that the sites in which the modified response to torsional stress takes place are away from the mutated sequences is only explained by the existence of mechanisms of transmission of a conformational information.

Telestability (50, 51), transmission of allostery in DNA (34), induction of conformational modifications at a distance by homopyrimidine stretches (16, 18) and by alternating sequences (17) are examples of possibly related phenomena. In these last instances, it was shown that torsional stress is relieved by the formation of alternate structures at sites which (despite their S1 sensitivity) are regions of duplex DNA. Sites of structural alteration are often detected in positions several residues away from the sequences which are the primary cause of the stress-induced deformation (6 and 8 residues away from the cloned $d(TC)_n$ sequence in (17); up to more than 30 residues away from the cloned poly(dG)·poly(dC) sequences in (18)). In agreement with these observations, nuclease hypersensitivity has been noted in proximity to the naturally occurring dC stretch in the promoter region of the chicken β-globin (23). Additional evidence for asymmetrical DNA deformation has been provided recently (35) in the analysis of intrinsic bending induced by repetitions of A.T tracts; Reynolds *et al.* (36) have furthermore described the asymmetrical cleavage at the 3′ end of A tracts and in the TATA box by the antitumor antibiotic CC-1065.

The example we report here is the first instance of major conformational alterations induced at a distance and caused by natural mutations.

The existence of the up-promoter constitutive mutants, due to expansion of the poly(dA-dT) sequences (14, 20) is explained by the observation that naturally occurring poly(dA-

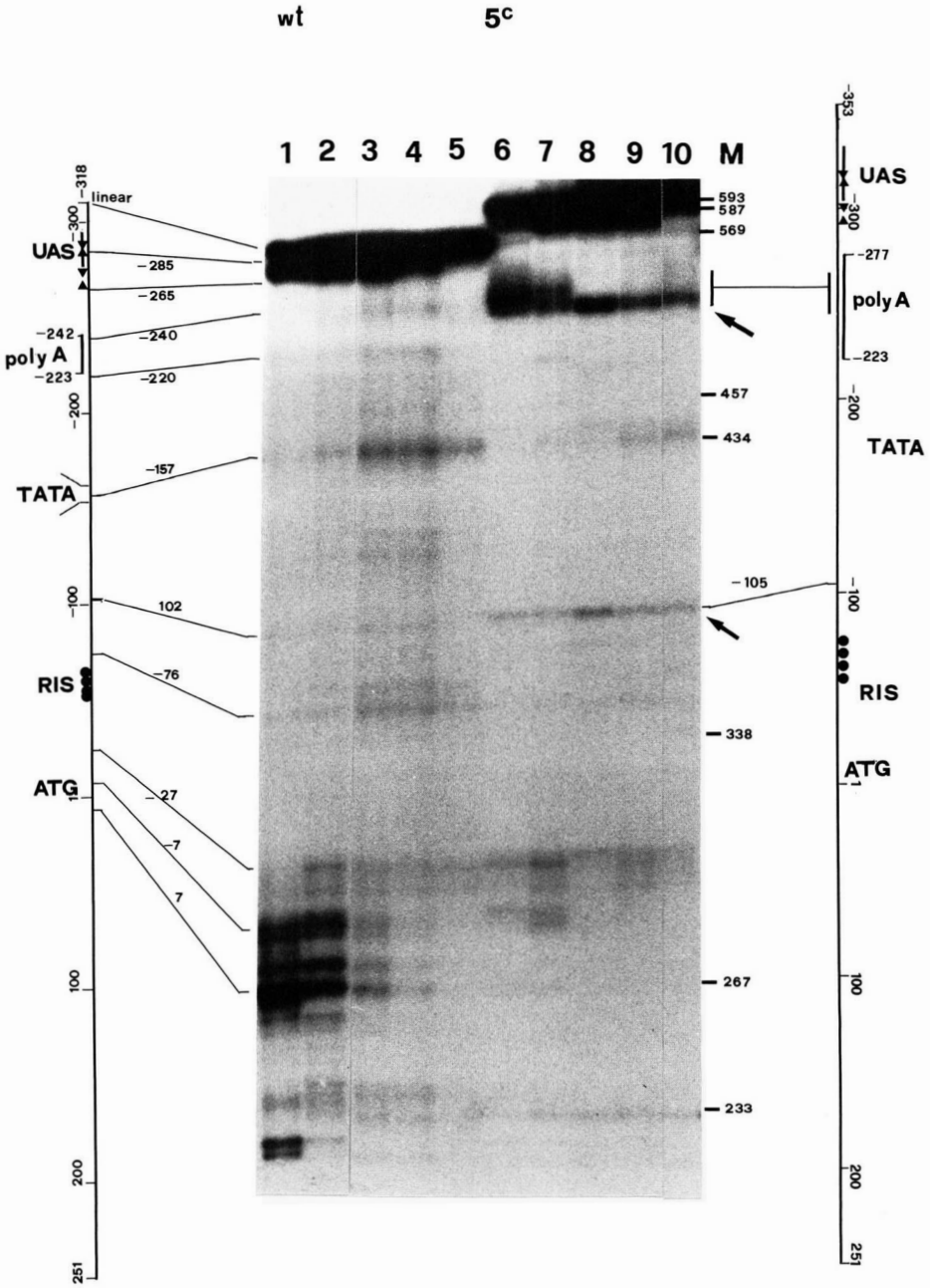

FIG. 5. **The pattern of PHS changes in the 5c constitutive alcohol dehydrogenase II promoter.** The 569-base pair alcohol dehydrogenase II promoter was circularized at 1.5 μg/ml EtdBr and analyzed for the presence of PHS as a function of the decrease of torsional stress (obtained with the presence of EtdBr in the P1 assay). *Lanes 1–5*, 0, 0.3, 0.6, 0.9, 1.2 μg/ml EtdBr, respectively. *Lanes 6* to *10*: as above, for the same *Sau*3A-*Sau*3A fragment from the ADR3-5c-yRp7 plasmid carrying the up-promoter constitutive mutation caused by the expansion on the dA tract, see "Materials"). The *upper arrow* (on the *right*) points to the changed sensitivity in the dAs, the *lower arrow* points to the new site at −105. *wt*, wild type.

dT) sequences are upstream promoter elements for constitutive transcription in yeast (37). The fact that the poly(dA-dT) tract becomes a site of major conformational deformation in the mutant (where it is the only mutated site) but not in the wild type, suggests a correlation between its deformation and its function. The correspondence of other sites of conformational alteration with functional sites (UAS, TATA, RIS, which are all established protein binding sites) stresses the potential general importance of local polymorphism of DNA structure (reviewed in Ref. 3) and suggests that the intrinsic topological information is actually used in *in vivo* DNA-protein interaction.

Altered DNA conformations observed in chromatin, because of their hypersensitivity to S1 nuclease, often correspond to DNase I-hypersensitive regions, associated with the regulatory regions of active genes (39–42). S1-sensitive sites have been detected in positions known to be factor(s) binding sites in the upstream regulatory region of the chick β-globin (43–45).

Evidence for a cause-effect relationship between topology of closed DNA domains and *in vitro* activation of transcription has been obtained (26, 38).

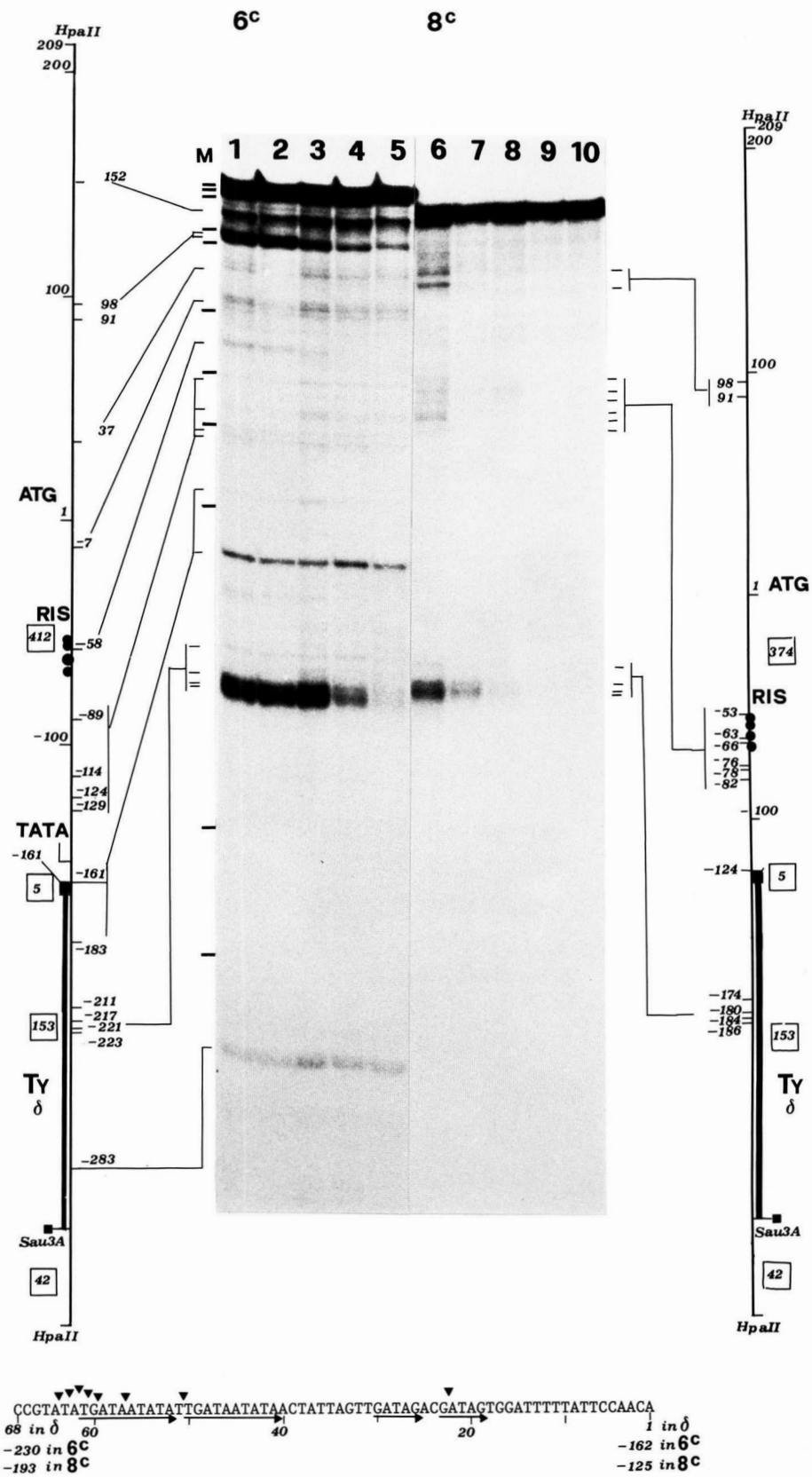

FIG. 6. **The pattern of PHS in two up-promoter mutations caused by Ty insertion.** PHS on the *Sau*3A-*Sau*3A circularized DNA obtained from the ADR3-6c-yRp7 (*left*) or ADR3-8c-yRp7 (*right*). The upstream *Sau*3A used for ligation is the one of Ty's δ sequence (153 base pairs upstream from the δ border, the downstream one is the same used in the wild type gene (position +251)). Secondary restriction: *Hpa*II (position +209). The *numbers* in *squares* indicate the length of the various subcomponents of the DNA fragment. Insertion of the Ty is at position −161 for the 6c and at −124 for 8c. The sequence at the *bottom* is the right border of the δ, reported to show the positions of PHS (from Ref. 29; the minor modifications of the sequences observed in this type of mutants are discussed in Ref. 22). Numbering of the δ sequence starts from 1 = the rightmost nucleotide of the Ty element. To allow mapping of the PHS in the different mutants, which have the same sequence inserted in different locations, the numbering of the first and last position in the 6c and 8c mutants is reported (*bottom*). Imprecision of mapping in this experiment is ±3 nucleotides in the *upper part* of the gel (*above* the *RIS*), ±2 in the *lower part*. P1 analysis as reported, in the presence of 0, 0.3, 0.6, 0.9, and 1.2 μg/ml EtdBr (*lanes 1–5* for 6c, *lanes 6–10* for 8c).

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