We have previously demonstrated that sequential activation of the bacterial ilvIH-leuO-leuABCD gene cluster involves a promoter-relay mechanism. In the current study, we show that the final activation of the leuABCD operon is through a transcriptional derepression mechanism. The leuABCD operon is transcriptionally repressed by the presence of a 318-base pair AT-rich upstream element. LeuO is required for derepressing the repressed leuABCD operon. Deletion analysis of the repressive effect of the 318-bp element has led to the identification of a 72-bp AT-rich (78% A+T) DNA sequence element, AT4, which is capable of silencing a number of unrelated promoters in addition to the leuABCD promoter. AT4-mediated gene silencing is orientation-independent and occurs within a distance of 300 base pairs. Furthermore, an increased gene-silencing effect was observed with a tandemly repeated AT4 dimer. The possible mechanism of AT4-mediated gene silencing in bacteria is discussed.

The leu-500 mutation is an A to G transition in the -10 region of the promoter of the Salmonella typhimurium leuABCD operon (1). The transcriptional activity of the mutant promoter is DNA supercoiling-dependent (2). The mechanism whereby the leu-500 promoter (pleu-500) is activated in the topA mutants is intriguing (3–7). Previous studies using a plasmid system have demonstrated that activation of plasmid-born pleu-500 in topA mutants requires an upstream transcriptional activity transcribing away from pleu-500 (8–11). This notion has been confirmed in a recent study using the chromosomal setting (12). Transcriptional activation of the ilvIH promoter (pilvIH) located 1.9 kilobases upstream of pleu-500 was shown to be responsible for pleu-500 activation (5). Transcription-driven DNA supercoiling (13) has been suggested to play a role in this long-range promoter-promoter interaction.

The intervening promoter that relays the distant interaction between pilvIH and pleu-500 is the leuO promoter (pleuO). In addition to transcriptional activity from pleuO, the leuO gene product, LeuO, is also required to provide a trans-acting function for activation of pleu-500 (6). It appears that the functional pleuO (or other replaced promoter) and LeuO are coupled in activating pleu-500. The molecular basis for pleu-500 activation by the combined action of pleuO and LeuO is still a mystery.

There is a stretch of 434 base pairs (bp) that is AT-rich DNA flanked by the divergently arrayed leuO and leuABCD (14). Besides the promoter sequences of the flanking genes, the function of the remaining 318-bp AT-rich (69% A+T) DNA is unknown (illustrated in Fig. 1). By monitoring pleu-500 activation, we found that the 318-bp AT-rich intervening DNA appears to repress the short-range interaction (11) between the two flanking promoters. Interestingly, LeuO relieves the repression. The repressive effect of the AT-rich intervening DNA on the short-range promoter-promoter interaction (pleuO and pleu-500) could potentially be due to anchoring of the AT-rich DNA to a large mass, which restricts DNA rotation and thereby abolishes short-range promoter-promoter interaction via DNA supercoiling. However, detailed analysis to search for DNA rotation blockage has ruled out this anchorage possibility.

The repressive activity of the 318-bp AT-rich intervening DNA has been narrowed down to a 72-bp AT-rich (78% A+T) DNA, referred to as AT4 in this work. AT4 is located at the pleuO end of the 318-bp AT-rich DNA. AT4 can repress promoter activity within a 300-bp distance. This repression is independent of the orientation of AT4. AT4-mediated repression of the promoter activity appears to be promoter nonspecific, because all promoters tested are repressed by AT4. These results support a role for AT4 as a gene silencer in bacteria.

**EXPERIMENTAL PROCEDURES**

**Plasmids and Bacterial Strains—**pWU802T, pEV101, pS01000, pAO, pW270, and pBR322 have been previously described (6, 15–18). pWU912T was derived from pWU802T. To construct pWU812T, a 320-bp promoterless non-AT-rich (47% A+T) DNA was generated by polymerase chain reaction (PCR) from the coding region of human cathepsin B gene (19), with the primers introducing HinII and BstXI sites at the ends. The digested HinII-BstXI fragment was then used to replace the 318-bp HinII-BstXI segment containing the native AT-rich sequence (69% A+T) between the divergently transcribing tac and pleu-500 in pWU802T. The native 318-bp AT-rich intervening DNA was also PCR-amplified with primers containing AatII restriction sites. The AatII-digested AT DNA fragment was inserted into the unique AatII site on pAO to yield pAO-AT and pAO-ATR. The plasmid carrying an AT DNA insert, with the leuO end of the DNA orientated proximal to pbla, was designated pAO-AT. The plasmid carrying the DNA insert in the opposite orientation was designated as pAO-ATR. Similarly, the 72-bp DNA located near the pleuO end of the 318-bp AT-rich DNA was PCR-amplified with primers so that the 72-bp AT4 DNA was flanked by AatII restriction sites on both ends. The AatII-digested AT4 DNA was inserted at the unique AatII site on pAO to yield pAO-AT4 (the leuO end of the DNA insert was proximal to pbla) and pAO-AT4R (the opposite orientation). The AT4 DNA sequence was 5’-CACAATTCAATACCGAACAA-

1 The abbreviations used are: bp, base pair(s); PCR, polymerase chain reaction; IPTG, isopropyl-1-thio-β-D-galactopyranoside; H-NS, histone-like nucleoid structuring protein.
GTGAATGATCATTTAAGTTTCAATTAAATGTTTAATATTAATAGTCAAAAGTT-3'. The nucleotide sequence of the rest of the intervening DNA between the divergently arranged leuO and leuABCD genes can be obtained from the GenBank database (accession number AF108556). Other testing plasmids were all derived from the above-described plasmids and were individually described in the corresponding experiments. The following 72-bp DNA sequence from the lacZ coding region was used to replace the AT4 DNA on pWU802T as described in Fig. 6. 5\'-AACCACCGAAGTGACCAGGATATCCGTTCGTTAGACGGAT-3'. The following four synthetic DNA oligomers, consisting of nucleotide sequence of the lacZ coding region, were used to sequentially extend the distance between the AT4 insert and pbla in pAO-AT4 as described in Fig. 8: 5\'-GCCGATCTTCTTCTTAGGCCGATCTGCTGTTCCCGCTCAACTCGGCGGATC-3'; 5\'-GATCCACCGGTTACGTCGGCGCTCCATACCAACCCCTTACCTCCATGATCC-3'; 5\'-AATTCATAGCGGCGTGGTTCCACCGGAGATCGGGTTGTATCTGTACG-3'; and 5\'-CTTTAATGTTGTGAAGAAGGCTGCTACAGGCAA-GGCGAGATTTACAC-3'.

**RESULTS**

The 318-bp AT-rich Intervening DNA Represses Short-range Promoter-promoter Interaction—The 318-bp AT-rich DNA is located at the 1.5-ml sample was analyzed by agarose gel electrophoresis for quantification is the average of at least two experiments. The signals of primer extension DNA product was visualized and quantified using a DNA sequence ladder was prepared using each individual primer for verifying the initiation site at a DNA sequence level. The radioactivity of primer extension DNA was visualized.

**Chromosomal Targeting of leuO**—As described (11). Several DNA oligomers were used as primers in the reactions. Two primers were mixed in the primer extension reactions and 50 μl ampicillin or 12.5 μl tetracycline was added as needed.

**Primer Extension**—Primer extension was carried out as previously described (11). Several DNA oligomers were used as primers in the study. 5\'-CTGTGTTGAGAGCACAAACAGGGGGG-3' was used for detecting pbla-mediated transcripts; 5\'-AGAATTCCTCTAGGGTATACACG-3' was used for detecting pAO-AT4-mediated transcripts; 5\'-CACTAGAATTTAATAGGCGTATCACGAGGCCCT-3' was used for detecting pAO-AT4-mediated transcripts.

**Immunoblotting Detection of Overexpressed LeuO in a leuO Strain**—A LeuO-specific antisera was raised by injecting the purified overexpressed S. typhimurium His-tagged LeuO into a rabbit. The affinity-purified IgG (1.4 mg/ml) from the antisera was used at a dilution factor of 1.5000 as the primary antibody to detect the cellular LeuO protein. The secondary antibody was anti-rabbit IgG conjugated to alkaline phosphatase. The blot was developed by ECL in a modification of a previous procedure (24) using an ECF Western blotting kit (Amersham Pharmacia Biotech). The chemiluminescence signal was detected and quantified by the Storm 840 imaging system (Molecular Dynamics).
which contained the 318-bp AT-rich DNA instead of the lac operator, no accumulation of the positively supercoiled DNA topoisomers was observed (Fig. 2C). In fact, the topoisomer distribution of pAO was almost identical to that of pAO-AT (Fig. 2, compare A and C), suggesting that the 318-bp AT-rich DNA insert did not significantly restrict DNA helix rotation and therefore could not block DNA supercoils generated by transcription of the bla gene. In addition, IPTG induction (the production of LeuO protein from pEV101) did not affect the topoisomer distribution of either pAO or pAO-AT (Fig. 2, B and D). These results suggest that the 318-bp AT-rich DNA-mediated repression is unlikely to be due to restriction of DNA helix rotation.

The 318-bp AT-rich DNA Represses Transcription of Adjacent Promoters—The 318-bp AT-rich DNA could repress transcription from one of the flanking promoters (either tac or plev-500) and thereby abolishes short-range promoter-promoter interaction. To test this possibility, we examined the transcription activity of tac in pWU802T and pWU812T. Primer extension results indicated that the tac activity (Fig. 3) strikingly correlated with the plev-500 activity (Fig. 1). The tac functioned normally if the 318-bp AT-rich DNA was replaced with a neutral DNA sequence of similar size as in pWU812T (lanes 6 and 8 in Fig. 3). In the presence of the native 318-bp AT-rich DNA, the tac activity on pWU802T was severely impaired (Fig. 3, lane 4). On the same plasmid, the tac activity was partially restored if LeuO was provided in trans (Fig. 3, lane 2). This result strongly supports the notion that the 318-bp AT-rich intervening DNA is a negative regulatory element for transcription.

A 72-bp DNA Element Within the 318-bp AT-rich DNA Silences Adjacent Genes—To test whether or not the repressive effect of the 318-bp AT-rich DNA element on transcription can be observed with other promoters, the 318-bp DNA was inserted at the unique AatII site located 99 bp upstream of the bla promoter (pbla) in pAO. In either orientation (pAO-AT and pAO-ATR), the 318-bp AT-rich DNA insert caused an ~45% reduction of the pbla activity (Fig. 4A, compare lanes 2 and 3 with lane 1). Deletion analysis using pAO-AT had located a predominant gene-silencing effect (more than 80% reduction on pbla activity) in AT4, a 72-bp AT-rich DNA located near the plevO end of the 318-bp DNA (lane 6 in Fig. 4A). Those DNA inserts containing the 72-bp AT4 plus all or part of the rest of 318-bp AT-rich DNA (AT, ATR, and AT2) exerted lesser gene-silencing effects (lanes 2, 3, and 4 in Fig. 4A). Furthermore, the AT1 DNA segment, which represents the 146-bp plev-500 end of the 318-bp AT-rich DNA, enhanced the pbla activity (lane 5 in Fig. 4A). These results indicate that, although the 72-bp AT4 exhibits a clear gene-silencing effect, a complex transcriptional effect is present in the rest of the 318-bp AT-rich DNA. The stronger silencing effect that is associated with AT4 may be due to elimination of other complex and opposing effects within the 318-bp AT-rich intervening DNA.

AT4-mediated Gene Silencing Is Additive, Orientation-independent, and topA Genetic Background-independent—Inversion of the AT4 DNA insert did not significantly affect gene silencing (compare lanes 8 and 10 in Fig. 4A). The reduction of pbla activity was ~80% with either orientation. Furthermore, the gene-silencing effect was additive. In either orientation, an ~95% reduction was achieved when the AT4 DNA insert was tandemly repeated (lanes 9 and 11 in Fig. 4A). Thus far, characterization of the 72-bp gene silencer had been carried out in the S. typhimurium topA- strain, CH582, where plev-500 activation was originally studied. The topA- genetic background has been shown to enhance short-range promoter-promoter interaction such as activation of a plasmid-borne plev-500 (11). To examine whether AT4-mediated gene-silencing effect was dependent on the topA- genetic background, pAO-AT was tested in an S. typhimurium topA+ strain, CH601, which is the parental strain of CH582. The same degree of gene silencing (~80% reduction of the pbla activity) was observed in both the topA+ and the topA- strains (Fig. 4B).

LeuO Protein Negates AT4-mediated Gene Silencing—The trans-acting LeuO protein was shown to relieve 318-bp AT-rich DNA-mediated repression of the short-range pleuO (tac)-pleu-500 interaction in pWU802T (Fig. 1). To test whether or not
LeuO can also suppress AT4-mediated gene silencing, the effect of LeuO on AT4-mediated silencing of pbla was examined in an E. coli leuO strain, MF1 (Fig. 5). A slightly stronger gene-silencing effect (~88% reduction of the pbla activity) was found in the LeuO-free strain (Fig. 5A, compare lanes 1 and 2). When LeuO was provided in trans in MF1 from a coexisting expression vector, pEV101, AT4-mediated gene silencing was nearly abolished even without IPTG induction (Fig. 5A, lane 3). This was probably due to the leakage of LeuO from the expression vector, pEV101. Such a leakage was evidenced from immunoblotting analysis (Fig. 5C, lane 2; 8.6 ng of LeuO was detected in the 100 µg of total protein loaded). Upon further increase of cellular LeuO due to IPTG induction (Fig. 5C, lanes 3–6), AT4-mediated gene silencing was completely eliminated (Fig. 5A, lanes 4–7). The pbla activity was fully restored with 50 µM IPTG treatment (Fig. 5A, compare lanes 5 and 1). In a control experiment using the parental plasmid pAO, the pbla activity was unaffected by IPTG treatment (data not shown). These results indicate that LeuO negates AT4-mediated gene silencing.

However, the effect of LeuO on transcription could be non-specific. To test whether or not the effect of LeuO is specific for AT4, a 72-bp DNA consisting of a DNA sequence from the lacZ coding region was synthesized and used to replace the 72-bp AT4 DNA in pWU802T (illustrated in Fig. 6). In the absence of LeuO (i.e. the absence of pEV101), this replacement resulted in a LeuO-independent plevu-500 activation on the mutant plasmid (Fig. 6B, lane 2). A significant ptc-mediated transcription activity was also detectable in the mutant plasmid (Fig. 6A, lane 2). With the native 72-bp AT4 in place, a significantly reduced ptc activity was detected in pWU802T (Fig. 6A, lane 1). Apparently, the reduced ptc activity was too weak to activate plevu-500 at this distance (Fig. 6B, lane 1). Our study, thus far, has clearly indicated that LeuO relieves 318-bp intervening DNA-mediated repression (Fig. 1) by specifically negating AT4-mediated gene silencing.

The Effect of Adjacent Transcriptional Activity on AT4-mediated Gene Silencing—The 72-bp gene silencer, AT4, is located at the 5′-ends of the divergently arrayed leuO and leuABCD genes. Such a chromosomal organization may not be a coincidence, because transcription-generated negative DNA supercoiling is known to accumulate in such a topological domain (13, 21). To test whether or not AT4 functions most effectively when placed between divergently arrayed promoters, we inserted AT4 at the unique Aatt site in pBR322 DNA so that AT4 was flanked by the divergently arrayed bla and tac genes (illustrated in Fig. 7). As expected, AT4 caused reductions in both pbla and ptac activities (Fig. 7, A and B). However, the gene-silencing effect of AT4 in pBR322 (Fig. 7A, lanes 2) was lower than that in pAO-AT4 (Fig. 4). The anti-tet transcription activity (16) that read through the AT4 insert in pBR322 could be the reason for this reduction. Despite the reduced effect, AT4-mediated gene silencing on pBR322 was still additive in the presence of a tandemly repeated AT4 dimer (Fig. 7, A and B, lanes 3). Due to the simultaneous gene-silencing effects on both flanking promoters, it remained unclear whether or not the bacterial gene silencer was affected by an adjacent transcription activity.

To test the effect of adjacent transcription on AT4-mediated gene silencing, pJW270-based plasmid constructs were used. pJW270-based plasmids are essentially the same as pBR322, except that the tac promoter is replaced by an IPTG-inducible lacUV5 promoter and that an iq promoter-controlled lacI gene was inserted at the 5′-end of the lacUV5 promoter (Refs. 17 and 21, and plasmid maps in Fig. 7). The opposite orientation of the lac gene in pJW270 and pJW270II was designed to test the effect of an adjacent transcription activity on AT4-mediated gene silencing. As expected, AT4-mediated gene silencing on placa was observed in pJW270II-AT4 and pJW270II-AT4R when lacI was transcribing away from the AT4 DNA inserts (Fig. 7C, lanes 4–6). Strikingly, the gene-silencing effect was abolished when lacI was inverted in pJW270-AT4 and pJW270-AT4R (Fig. 7C, lanes 1–3). With a location at the 3′-end of an adjacent transcription unit, AT4 not only exerted no gene-silencing effect on the pbla, but the transcription activity of

![Graph](image-url)

**Fig. 2.** The 318-bp AT-rich DNA does not restrict the rotational motion of DNA helix. pAO-AT is identical to pAO except that it carries the 318-bp AT-rich DNA insert. AS19 harboring either pAO (A and B) or pAO-AT4 (C and D) with (B and D) or without (A and C) the coexisting pEV101 was treated with 100 µM novobiocin 30 min prior to cell harvest. To provide LeuO in trans, cells harboring pEV101 were grown in MF1 from a coexisting pEV101. Such a leakage was evidenced from immunoblotting analysis (Fig. 5C, lane 2; 8.6 ng of LeuO was detected in the 100 µg of total protein loaded). Upon further increase of cellular LeuO due to IPTG induction (Fig. 5C, lanes 3–6), AT4-mediated gene silencing was completely eliminated (Fig. 5A, lanes 4–7). The pbla activity was fully restored with 50 µM IPTG treatment (Fig. 5A, compare lanes 5 and 1). In a control experiment using the parental plasmid pAO, the pbla activity was unaffected by IPTG treatment (data not shown). These results indicate that LeuO negates AT4-mediated gene silencing.

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p bla was actually increased (Fig. 7C, lanes 2 and 3). This result demonstrates that a parallel (convergently) arrayed adjacent transcription unit abolished AT4-mediated gene silencing. Transcription-driven positive DNA supercoiling at the 3' end of a transcription unit could be the reason why AT4 function was abolished at such a location. Another interpretation is that AT4 is functional only when it is located at the 5' end of a transcription unit. For maximum gene-silencing activity, adjacent transcription activities should be transcribing away from the gene silencer. Read-through transcription activity and a 3'-end location of a transcription unit will either weaken or impair AT4-mediated gene silencing.

AT4-mediated Gene Silencing Is Effective Up to a 300-bp Distance—The chromosomal location of AT4 was centered at the −283 position of leuO and at the −2351 position of the divergently arrayed leuABCD. In pBR322-AT4, AT4 was located at the unique AatII site located at the −99 position of bla in pAO. Plasmids were assayed in CH582. Primer extension results were quantified and shown. All quantified results were compared with the p bla activity on pAO (A, lanes 1 or 7), which was arbitrarily set as 1. The identified AT4-mediated gene-silencing effect was tested in CH601(B). Again, p bla activity on pAO in either CH601 (lane 1) or CH582 (lane 3) was quantified and served as the standard for comparison with the p bla activity in pAO-AT4 in either CH601 (lane 2) or CH582 (lane 4), respectively.
AT-rich DNA-mediated Gene Silencing

**Fig. 6.** AT4 represses the short-range interaction between \( \text{ptac} \) and \( \text{pleu-500} \). \( \text{pWU802T} \) was derived from \( \text{pWU802T} \) by replacing the 72-bp AT4 DNA element with a promoterless neutral DNA of same size derived from the \( \text{lacz} \) coding region. \( \text{pWU802M} \) was derived from \( \text{pWU802T} \) by deleting the 66-bp \( \text{ptac} \). Plasmids were assayed in CH582, in which \( \text{ptac} \) activity is not required IPTG induction due to the lack of \( \text{lac} \) repressor in \( \text{S. typhimurium} \). The transcription activities of both \( \text{ptac} \) and \( \text{pleu-500} \) were simultaneously detected using primer extension (A and B).

**Fig. 7.** An adjacent transcription activity affects AT4-mediated gene silencing. AT4-mediated gene silencing was analyzed by placing the AT4 DNA insert at the unique AatII site on \( \text{pBR322} \), \( \text{pJW270} \), and \( \text{pJW270I} \), respectively. Both flanking \( \text{pbla} \) and \( \text{ptetA} \) activities on various \( \text{pBR322} \) vectors in CH582 were detected using primer extension and shown in A and B, respectively. The constructs were: lane 1, \( \text{pBR322} \) vector with no insert; lane 2, \( \text{pBR322} \) vector with AT4 insert; lane 3, \( \text{pBR322} \) with a tandemly repeated AT4 dimer. The \( \text{pbla} \) activity of \( \text{pJW270} \) or \( \text{pJW270I} \) vector in CH582 with or without the AT4 insert in either orientation was detected by primer extension and shown in C.
looping-mediated transcriptional activation in nitrogen (ntr) regulation (28) and DNA looping-mediated transcriptional repression of the araBAD promoter (29). However, if DNA looping due to protein-protein interaction is important for AT4-mediated gene silencing, the optimal distance for gene silencing should be about 500 bp (30). Using the lac operator as a model system, it has been experimentally demonstrated that, starting from a distance of ~150 bp, DNA looping mediated by repressor binding to the operator increased dramatically when the size of the intervening DNA increased. This effect peaked at a distance of 500 bp (30). In contrast, AT4 retained its transcriptional repressive effect up to a distance of 300 bp. The repressive effect was abolished at a distance longer than 350 bp (Fig. 8). Starting from a distance of 188 bp up to the 300-bp limit, the repressive effect was slightly reduced rather than dramatically enhanced as one might expect for the above-discussed protein-protein interaction at a distance scenario. This clear difference argues against the possibility that AT4 functions as a binding site for a repressor “acting at a distance,” which represses transcription via a direct contact with the RNA polymerase complex at a distance.

What is the mechanism whereby AT4 silences an adjacent transcription activity at a distance of 300 bp? LeuO-mediated reversal effect (Fig. 5) has provided a possible clue. Overexpression or underexpression of LeuO has been linked to a number of hns−-associated phenotypes (23, 31, 32). Mizuno’s group has shown that LeuO relieves bgl silencing in E. coli (23). Both H-NS and AT-rich DNA flanking the bgl promoter have been shown to be responsible for bgl silencing (33–35). In addition, using the pfluv-500 activity as a reporter, we have shown genetically that H-NS plays a repressive role in the transcriptional regulation. Together, these results suggest a possible involvement of H-NS in AT4-mediated gene silencing.

H-NS has been known to bind preferentially to curved DNA (36, 37). Once H-NS is recruited to the local site (the AT-rich DNA sequence element, AT4), the binding cooperativity of H-NS may cause a cis-spreading (oligomerization) of H-NS to the promoter region. The H-NS oligomer may physically block RNA polymerase complex from accessing the promoter (~35 and ~10 sequences). The binding cooperativity of H-NS may determine the size of the H-NS oligomer and hence the 300-bp distance limit of the AT4-mediated gene-silencing effect. The proposed mechanism is similar but distinct from the nucleoprotein filament model for the bacterial centromere site-mediated transcriptional silencing, which affects genes within several kilobases (38). Our model is also different from the DNA sequestration-mediated gene silencing model (39).

A similar direct transcriptional repressor role of H-NS was proposed by Ueguchi and Mizuno (40). They have shown in vitro that H-NS inhibits proV (proU)-mediated transcription by directly binding to the promoter region. The repressive H-NS complex is strikingly local and highly specific to the DNA sequence in the proV promoter, because H-NS does not affect transcription from pTAC on the same DNA molecule (40). One or more cis-elements in the proV promoter must be responsible for H-NS recruitment to the local site. The 72-bp AT4 DNA may contain one or more similar elements that trigger H-NS localization in the proV promoter. Because DNA structure rather than the specific DNA sequence is known to be important for H-NS localization (36, 37 and reviewed in Ref. 41), no DNA sequence homology is expected between the promoters that utilize such a mechanism in their transcriptional silencing.

The idea that DNA structural elements could serve as signals for the formation of a transcriptionally repressive nucleoprotein structure may also be applicable to explain the well known eukaryotic heterochromatin gene-silencing mechanism, because high A+T composition and repetitiveness are the two common features for DNA structural elements involved in heterochromatin formation. The LINE-1 element in X chromosome inactivation (42 and reviewed in Ref. 43), the satellite DNAs in the centromeric- or telomeric-heterochromatin (reviewed in Ref. 44), and the silencers I and E in the yeast MAT loci (reviewed in Ref. 45) are all AT-rich. Combinations of any pair of the yeast silencers, HMR-E, HML-R, and HML-I, can result in inactivation of any gene activity flanked by the AT-rich DNA elements (46). However, the mechanistic link between DNA structural elements in the eukaryotic silencers and the formation of heterochromatin has been unclear. Detailed studies of the 72-bp AT-rich DNA-mediated gene silencing in bacteria could shed light on the mechanism of gene silencing in both prokaryotes and eukaryotes.

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AT-rich DNA-mediated Gene Silencing

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