Long W tracts are over-represented in the Escherichia coli and Haemophilus influenzae genomes

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ABSTRACT

The occurrence of DNA tracts of the three binary base combinations: R.Y, K.M and W;S has been mapped in the complete genomes of Haemophilus influenzae and Escherichia coli. A highly significant over-representation of W tracts is observed in both bacteria. The excess of W tracts is particularly striking in the 10% intercoding regions. Subdivision of intercoding regions into divergent (promoting), convergent (terminating) and sequential subregions shows that the excess of W tracts is most concentrated in the promoter regions. A particularly high excess of W tracts is observed in the first 200 bases upstream of coding start sites. The data suggest that W tracts have a role in promoter function. A function as unwinding centers, analogous to the role of R.Y tracts in eukaryotes, is proposed. R.Y and K.M tracts are only modestly over-represented in the two bacteria.

INTRODUCTION

Nucleotide base tracts consisting of only two bases (‘binary tracts’) can be found in just three combinations: (i) tracts made of purines on one strand and pyrimidines on the complementary one (‘R.Y tracts’); (ii) tracts made of G,T on one strand and A,C on the other (‘K.M tracts’); and (iii) the W;S pair which consists of either A,T or G,C tracts, each complementing itself. It has been known for some time that R,Y tracts are highly over-represented in higher eukaryotic DNA (1–6). More recently, we documented that R,Y tracts are not the only potential DUEs. We found that two S.cerevisiae promoters containing long R.Y tracts (CYC1 and DED1) are attacked by single strand-specific nucleases in the supercoiled but not in the linear state. These observations, supported by 2D topoiser analysis, indicate that in yeast promoters R.Y tracts have a similar tendency to assume an unwound (paranemic) state. It thus seems that, in yeast, both W tracts and R.Y tracts can serve as DUEs and support the notion that these binary tracts can readily form unwinding elements.

Escherichia coli is long known to be free of the excessive R.Y tracts present in the higher eukaryotes (1), but that its promoters are rich in A,T tracts (11–13). Studies by Blattner, Kornberg, Kowalski and their colleagues (14–16) indicated that unwinding elements may play a regulatory role in bacteria, and that these elements are A,T-rather than R.Y-rich. Classical DNA melting theory actually suggests that A,T-rich tracts are the first ones to unwind. Kowalski et al. proposed an algorithm to predict unwinding centers based on their A,T content (17,18). This approach has been refined to include the effect of super-helicity (19). The availability of the complete sequences of E.coli and Haemophilus influenzae makes it now possible to map the occurrence of the binary tracts in the entire genome of these prokaryotic organisms.

In this study we apply several unique DNA analysis programs (GENTRACTS, ANEX and DIVCON), in addition to TRACTS (5), to analyze the occurrence and distribution of long binary tracts in the E.coli and H.influenzae genomes. It is found that W tracts are in as large an excess in these two prokaryotes as are the R.Y tracts in eukaryotes. R.Y and K.M tracts are in only moderate excess. W tracts are thus the dominating excessive binary theme in both bacteria. It is further shown that the over-representation of W tracts, and to some extent also of R.Y and K.M tracts, is particularly high in promoter regions. This observation strengthens the proposition that in prokaryotes W tracts serve as the principal unwinding elements and may thus play a crucial role in prokaryotic gene regulation.

MATERIALS AND METHODS

The complete sequences of E.coli (20), GenBank entry U00096, and of H.influenzae (21), entry HIL42023, were analyzed.

The following programs were used:

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(i) Program GENTRACTS, written in python with an extension module in C (B.S.), reads multiple files from an external source (EMBL) and combines them into a logical annotated genome. It then computes the positions of the features selected, identifies binary or other tracts and determines the distance to the selected features (start or stop sites of the closest genes).

(ii) Program PLOTRACTS, written in python (B.S.) creates a graphical representation of the frequencies of various tracts generated by GENTRACTS versus their distances from the closest ORFs in a ‘pyramid’ form, as shown in Figure 1A and B. PLOTRACTS provides a clickable www interface which enables each section on the graph to be linked to vital information on the associated genes and their products.

(iii) Program ANEX, written in FORTRAN (G.Y.), parses the annotation data from a GenBank flat file and generates a file of gene start and stop sites. The file also lists the designation, length and a 50 letter description of each annotated gene.

(iv) Program TRACTS (formerly PUR) (5) calculates and lists the frequencies of tracts of each length, and lists all tracts above a certain length. Version 6.1 of TRACTS has been

Figure 1. The number of long W tracts in bins of 20 nt (from x to x + 20) plotted against the distance x from the first translated nucleotide (A when ATG). The length of the tracts is color coded as listed. (A) E.coli; (B) H.influenzae.
extended to calculate separate tract frequencies in coding and non-coding regions. This is performed by reading the output of ANEX and determining which bases are within ORFs and which are intercoding (mostly intergenic, but, as transcription start sites are presently mostly unavailable, 5' and 3' UTS are scored as intercoding). tRNA and tRNA regions are treated as genes.

(v) Program DIVCON (G.Y.) reads the lists of all tracts longer than a given length, l, generated by TRACTS, as well as the start and stop sites provided by ANEX, and assigns each intercoding region into one of four classes: divergent, convergent, or sequential of two kinds: ‘www’ when between two genes both coding on the GenBank listed strand, or ‘ccc’, when between two genes both coding on the complementary strand. DIVCON then calculates the number of tracts in each class and lists the cumulative number of bases in these tracts.

**Binary base frequencies expected in random DNA.** The number of tracts of length l and longer, n(l), expected in randomized DNA (with fraction of e.g. purines p, so that p + q = 1) are calculated as previously described (5), by:

\[
n(l) = L\left[(pq^l) + (qp^l)\right]
\]

where \( L \) is the number of bases in the input sequence (4 639 221 for *E.coli*). The number of bases in tracts \( \geq 1 \), \( N(\geq 1) \), is:

\[
N(\geq 1) = L \left[(p + q)p^l + (q + l)p^l\right]
\]

*Controls.* As control, two random DNA sequences of the length and composition of *H.influenzae* were generated, using IMSL routine GGUD. The average ratios of found over expected tracts were: for W:S: 0.98,1.05; for K:M: 0.96:0.97; for R.Y: 1.03,0.99 (the ratio expected for randomized DNA is of course unity).

These averages are for tracts from 10 nt to the longest consecutive tract found in the randomized genome (of 19,20 nt for R.Y and K.M, and of 25 nt for W:S); the randomized sequence had the same base composition as the studied sequence, e.g. 0.62 for *H.influenzae*.

**RESULTS**

**W tract distribution along *E.coli* and *H.influenzae* genomes**

The distribution of long W tracts between intercoding and coding regions of *E.coli* and *H.influenzae* is shown in Figure 1A and B, respectively. The number of tracts of each length, from 12 nt upwards, in bins of 20 nt along the sequence, is plotted against the distance of that bin from the first coding position (ATG) of the closest gene. The different colors represent tracts of increasing lengths, in steps of three, e.g. red represents tracts of 15–17 nt. A distinct peak is observed between positions –200 and –1, which makes it evident that a significant concentration of long W tracts (≥12 nt) is present in the first 200–250 bases upstream to the first ATG, in both *E.coli* and *H.influenzae*. This peak is relative to a background frequency averaging, for tracts ≥15 nt (red color), 4 nt for *E.coli* or 8 nt for *H.influenzae*. The excess of long W tracts over background is increasingly evident as tract size increases. However, statistics become less significant as tract size increases as discussed in greater detail below. A similar but much less significant excess was observed for R and Y tracts (not shown).

The high excess of W tracts in the first 200 bases upstream, is a strong indication of a possible role in promoter function. Since no systematic data on transcription start sites are available for either bacteria, part of the intercoding regions can be transcribed (UTS) and are not really intergenic. Also, many of the intercoding regions, especially the shorter ones, reside within operons, and therefore are probably not transcription promoters. Salgado *et al.* (22) list 292 operons in *E.coli*. If we assume that each operon contains on average two intercoding regions then 20–25% of the intercoding regions are within operons. UTS may contribute to the somewhat lesser concentration between positions –100 and –1 relative to –200 and –100 in *E.coli*. Altogether, one can expect most of the long W tracts to occur in promoter regions. This is a strong indication that the long tracts may have a role in promoting transcription.

**The extent of binary tract overrepresentation**

To obtain more quantiative information about the excessive W tracts, as well as of other binary tracts, program TRACTS was applied to the genomes of both *E.coli* (22) and *H.influenzae* (20). The results are shown in Table 1 and are plotted in Figure 2A and B. The number of bases in binary tracts of every length found in the genomes of *E.coli* and *H.influenzae* are listed in the tables. The length expected in randomized DNA of the same composition is also shown (Table 1, columns 3, 6 and 9). Also listed are the ratios between these two values (Table 1, columns 4, 7 and 10); these ratios give a direct measure of the
over-representations at each length, and are plotted in Figure 2 against the respective tract lengths. W tracts of every length up to 30 nt are found in both *E.coli* (Table 1A) and *H.influenzae* (Table 1B). It is seen that in both bacteria, stand alone W and S bases (*l* = 1) are under-represented (*r* = 0.89; 0.82), while W tracts of every length above four nt are over-represented to an increasing extent, up to enormous excesses for the longest tracts. Thus in *E.coli*, W tracts of *l* = 25 (4 tracts, 100 bases) are found at 54-fold excess over the average number expected in random DNA.

The most over-represented binary pair in *E.coli* is clearly W:S. A consideration of the full output of TRACTS shows, however, that only W tracts are involved; the longest S tract is a single 22 nt tract, while seven W tracts of that length are found. The longest W tract is of 30 nt, expected only 0.07/30 times in the entire *E.coli* genome of 4,693,221 nt, a 423-fold excess! The longest W tract expected in a random genome of that length is of 21 bases (24/21 = 1.14 tracts, see Table 1A).

The detailed outputs of TRACTS can be seen on the web site http://www.weizmann.ac.il/~lcyagil

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| %A,T = 0.492 | %A,G = 0.500 | %A,C = 0.500 |
(22-fold for the longest tract), but continuously from 5 nt up. In brief, a moderate excess of R.Y and K.M tracts is observed, much less pronounced than for the W tracts. W tracts are thus the dominant excessive binary motif in *E. coli*.

A similar situation is evident in *H. influenzae* (Fig. 2B). W tracts of every length up to 30 nt are found. The 30 nt W tract is only 6.8 times over-represented, due to the high (62%) A,T content of *H. influenzae*. In spite of this high A,T content, W tracts are continuously over-represented from 4 nt up. Up to 21 nt the over-representation of R.Y tracts is marginal. K.M tracts are in a continuous high excess, also up to 21 nt (Table 1B). Five extremely long K.M tracts, of 68–151 nt, are

Table 1. Continued

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</table>

%A,T = 0.62 %A,G = 0.50 %A,C = 0.50
found, as often encountered in mammalian genomes. The composition of all these long tracts is (AACC), on one strand and (GGTT), on the other. They are thus true microsatellites and require a special explanation. We should emphasize that the great majority of the tracts mapped by TRACTS have no particular repetitive or other symmetric feature, most of them are composed of just random mixtures of the two bases, as can be seen when all E.coli W tracts ≥25 nt are inspected (Table 2). A more detailed analysis is planned.

**Coding versus non-coding regions**

Is over-representation evenly distributed over the genome, or is there a difference between coding and non-coding regions? Coding regions compose 89% of the E.coli genome and 87% of the H.influenzae genome (Table 3). In Table 3 we see that W tracts ≥15 are somewhat less over-represented in the coding regions than in the total genome (see ratios). However, in the intercoding regions (11 and 13% of the genomes) W tracts are represented at a much higher degree than in the whole genomes: tracts 15 nt and longer (l ≥ 15) reach a 17.63-fold excess in E.coli over the value expected in uniform DNA (Table 3A). The over-representation in H.influenzae (6.38) is of a somewhat lesser magnitude, but still highly significant (Table 3B). The vast excess of long W tracts in intercoding regions is a further indication that W binary tracts may have a regulatory function in the bacterial genome. The excess of W tracts is evident whether one examines tracts ≥12 or ≥15 nt (there are 497 such tracts in E.coli; Table 3 lists the number of bases in these tracts). K.M and R.Y tracts also show a significant excess in the intercoding regions of both bacteria. The excess of K.M tracts in H.influenzae (7.84 for K.M ≥15; 794 tracts) is particularly notable.

**Over-representation is highest in promoting regions**

To determine whether the excess of long tracts is connected to promoting, the 4398 intercoding regions of E.coli, as well as the 1818 ones of H.influenzae, were dissected into four subclasses: (i) divergent intercoding subregions, which are promoting in both directions (on opposite strands); (ii) convergent subregions which are terminating in both directions, and consecutive subregions, comprising of (iii) ‘www’ regions, which are between two ORFs coded on the analyzed (GenBank listed) strand and (iv) ‘ccc’ regions which are between ORFs coded on the opposite strand. Consecutive regions have both terminator and promoter elements. The division was done with the program DIVCON, which parses the data in the gene list produced by ANEX. A similar dissection into subregions has recently been carried out in conjunction with termination signals (23). DIVCON assigns each tract to the proper class, and counts tracts, as well as bases within tracts, in each class in two ways as follows.

The first way is to consider the entire intercoding region as a potential promoter or terminator region. The data in Table 4A show that the E.coli genome has 645 divergent and 645 convergent intercoding regions. While 27% of the divergent regions contain at least one W tract ≥12 nt, only 9.5% of the convergent regions contain at least one of these longer tracts. Similarly low percentages (10.2; 9.9%) are observed for the www and ccc regions. Only 27% of all promoter regions include a long tract, but it should be borne in mind that many intercoding regions are quite short, often only a few bases, often within operons where no promoting features should be expected. If only tracts ≥15 are considered (next three rows), the excess in divergent regions becomes even more pronounced. However, the percentage of intercoding regions having long W tracts is now smaller, indicating that a tract of length 12 nt, or possibly shorter (or incomplete), may already fill the functional role, whatever it may be. It should be added that the number of tracts expected in random DNA is, in all subclasses, ~20% of those found, so that we are dealing with excessive, subclass-specific, tracts.

The second way to assess subclass distribution is to assume that promoting or terminating regions can extend into the preceding or following genes. To examine this possibility, tract frequencies 200 bases upstream from each ATG, whether extending into an upstream gene or not, as well as downstream
from each terminating codon, were counted. The results are also shown in Table 4 (rows 9–15 in each half table). In that case the percentage of convergent regions having tracts was somewhat increased (16%; 3.6% for \( /G_{02} \) 12 or \( /G_{02} \) 15 nt in \( E. coli \)), but was still significantly less than in the divergent regions.

As to \( H. influenzae \) (Table 4B), an even higher percentage of the diverging regions (56%) have at least one W tract \( /G_{02} \) 12 nt but the convergents also have a large amount of these tracts, so that the case in favor of promoters as a major unwinding sink is less strong than for \( E. coli \), but still significant; in particular when the \( \pm 200 \) nt range is considered. The data indicate, nevertheless, that long W tracts are present in terminator regions as well, often at the 3\(^{\prime} \) end of the RNA, or just beyond at the polyadenylation site. These regions are well known to contain W-rich elements which have been proposed to control polyadenylation and mRNA stability (19,24).

Concerning R.Y tracts, it was previously noted (5) that both the lac operon and pBR322, an \( E. coli \) derived plasmid, tend to have their few R.Y tracts concentrated in regulatory regions. Divergent intercoding regions contain three times as many R.Y tracts as convergent ones (Table 5). With K.M tracts divergent regions have nearly twice as many long tracts as convergent ones when examining all intercoding \( \pm 200 \) bases, but not beyond. It may be summarized that a certain amount of excessive R.Y and K.M tracts are present in \( E. coli \) promoters and also in terminators, but the significance is less obvious than with W tracts. \( H. influenzae \) also shows a certain excess of R.Y and K.M tracts, mainly in the divergent regions (not shown). Many promoters contain more than one binary tract, e.g. the ilv promoter (25) which has a W18 tract at \(-110\) and an R11 tract at \(-155\) from the first codon.

### DISCUSSION

The three main findings described are: (i) a very high over-representation of long W (A,T) tracts occurs in the \( E. coli \) and
Table 4. W tracts in different subclasses of intercoding regions

<table>
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<tr>
<th></th>
<th>Divergent</th>
<th>Convergent</th>
<th>www</th>
<th>cccc</th>
<th>Total</th>
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<tr>
<td></td>
<td>← →</td>
<td>→ ←</td>
<td>← →</td>
<td>← ←</td>
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A. *Escherichia coli*

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IC only (up to previous ORF)

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<td>156</td>
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Bases 200 nt upstream (even if entering previous ORF)

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B. *Haemophilus influenzae*

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IC only (up to previous ORF)

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<td>15.1</td>
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Bases ±200 nt upstream (even if entering previous ORF)

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<td>98000</td>
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<td>265000</td>
<td>726000</td>
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<td>125</td>
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<td>25.7</td>
<td>18.8</td>
<td>19.4</td>
<td>21.2</td>
</tr>
</tbody>
</table>

*Often partly overlapping.*

*This number is larger than the parallel number in Table 3A because negative ICs (when a gene starts within the preceding gene) are not subtracted here.*
H. influenzae genomes, as compared with random DNA; (ii) W tract over-representation is particularly high in promoter regions and, to a certain extent, in terminator and other inter-coding regions; (iii) a high fraction of all promoter regions contain one or several binary tracts.

The two bacteria studied here significantly differ from the eukaryotic genomes previously studied by us and others (3,4,9,10,26) in that W tracts are the most excessive binary theme. In the genomes of the higher eukaryotes, R.Y tracts were found to be dominating, while W and S tracts were at a marginal excess at most. Kowalski and co-workers (9,17,18) have demonstrated in yeast that A,T-rich regions tend to form DUEs in autonomous replication sequences (ARS) and in several yeast gene promoter regions. R.Y tracts were also shown to serve as unwinding centers, e.g. in the CYC1 and DED1 promoters (19). Yeast thus occupied an intermediate position, with all three binary motifs (except S) being in a large excess (7). As to archaea, the data for Methanococcus janaschii (unpublished) behave like eukaryotes rather than like the prokaryotes described here. Further organisms will have to be analyzed to verify the conclusion that an excess of W tracts characterizes prokaryotes in general.

What could the function of these W tracts be? If the excessive W tracts had no function, one would expect a more modest excess in compact genomes like the bacterial ones. Previous work on eukaryotic genomes, computational and experimental, has suggested that the binary tracts may serve as DNA unwinding centers in both transcription and replication control (27). The seminal study in this direction was by Larsen and Weintraub (28), who detected single-strand-specific DNA cleavage in active chick globin promoters. Many other susceptible promoter regions have been detected since, the theme common to most of them being the binary homopurine-homopyrimidine theme, although other binary themes do occur (summarized in ref. 27).

May the W tracts serve as unwinding centers as well? A,T-rich regions are well known to be the most readily melting form of DNA. Evidence in favor of melting of W tracts as a factor in gene activation in both E.coli and yeast exists. Susceptibility to cleavage by single strand-specific nucleases showed that A,T-rich regions in E.coli associated elements (phage lambda and pBR322) can serve as DUEs (14,29). Studies concerning the ori c replication origin of E.coli (15,16) led to the same conclusion. ori c unwinding occurs in preparation for replication, another major cellular process requiring a certain degree of DNA unwinding.

The propensity of W tracts to unwind in the two bacteria could thus be the parallel of the propensity of R.Y tracts to unwind in higher eukaryotes. Do R.Y tracts play any role in bacteria? As seen in Table 5, in E.coli the R.Y tracts are in a certain, yet small, excess, a situation paralleling the situation with W tracts in the higher eukaryotes. As for K.M tracts

### Table 5. R.Y and K.M tracts in E.coli intercoding subregions

<table>
<thead>
<tr>
<th></th>
<th>Divergent</th>
<th>Convergent</th>
<th>www</th>
<th>cccc</th>
<th>Total</th>
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<td>Number of ICs</td>
<td>645</td>
<td>645</td>
<td>1515</td>
<td>1593</td>
<td>4398</td>
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<tr>
<td>Bases within ICs</td>
<td>153 407</td>
<td>67 574</td>
<td>141 714</td>
<td>146 795</td>
<td>509 490</td>
</tr>
<tr>
<td>Bases in tracts within</td>
<td>1384</td>
<td>397</td>
<td>1471</td>
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<td>% having long IC tracts</td>
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<td>7.09</td>
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<tr>
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<td>258 000</td>
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<td>637 200</td>
<td>1 759 200</td>
</tr>
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<tr>
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<td>645</td>
<td>1515</td>
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<td>Bases within ICs</td>
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<td>64 574</td>
<td>141 714</td>
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<td>509 490</td>
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<tr>
<td>Bases in tracts within</td>
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<td>5.0</td>
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<td>258 000</td>
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<td>1 759 200</td>
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<td>12.6</td>
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(A,C,G,T), these show almost random ratios in E.coli (found over expected ratio = -1), but are systematically over-represented in H.influenzae. This supports the possibility that all DNA sequences made of only two bases have a propensity to unwind into a parameric state (6).

A general structural basis for a propensity of binary tracts to unwind is not available at present, but in the case of W tracts is in line with classical melting theory (30,31), which leads us to expect the W tracts to separate readily. Recent procedures to include the effect of supercoiling (19,32) strengthen that view and the presence of W-rich unwinding centers in certain bacterial promoters, such as the ilv promoter, has been experimentally documented (25). A structural basis for ready melting of R.Y tracts is less obvious and their melting under supercoiling tension deserves further investigation. It should be added that in eukaryotes other functions have been proposed for A,T-rich elements, including signals that control mRNA degradation or polyadenylation (at the 3’ end of the gene) (24), to serve as nuclear matrix attachment sites (MAR/SARs) (32) or even as preferred nucleosome attachment sites (4). These possibilities may explain some of the observed excessive tracts. The prefered nucleosome attachment sites (4). These possibilities may explain some of the observed excessive tracts. The prefered nucleosome attachment sites (4).

The linking deficiency can first be transformed into a duplex with the linking deficiency to the transcription/replication initiation site and permit unwinding when and where needed for entry of the copying machinery. Thus, in the lac operon, one W tract of 17 nt is found at the very end of the operon, i.e. at the termination of the lacI gene. This raises the possibility that a torsional sink may exist also at the end of a transcribing unit, remigrating to the initiation site by the supercoiling/decoupling mechanism just mentioned. All these inferences can be readily put to experimental examination. A regulatory role associated with unwinding events may open new alternatives for DNA expression control mechanisms.

ACKNOWLEDGEMENTS

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REFERENCES


NOTE ADDED IN PROOF

The identification in E.coli promoters of a UP element, containing a W11 tract consensus site, has been brought to our attention, thanks to Dr D. Charlier of Brussels. See Estrem,S.T. et al. (1999) Genes Dev., 13, 2134–2447.