G-quadruplexes: the beginning and end of UTRs

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ABSTRACT

Molecular mechanisms that regulate gene expression can occur either before or after transcription. The information for post-transcriptional regulation can lie within the sequence or structure of the RNA transcript and it has been proposed that G-quadruplex nucleic acid sequence motifs may regulate translation as well as transcription. Here, we have explored the incidence of G-quadruplex motifs in and around the untranslated regions (UTRs) of mRNA. We observed a significant strand asymmetry, consistent with a general depletion of G-quadruplex-forming RNA. We also observed a positional bias in two distinct regions, each suggestive of a specific function. We observed an excess of G-quadruplex motifs towards the 5'-ends of 5'-UTRs, supportive of a hypothesis linking 5'-UTR RNA G-quadruplexes to translational control. We then analysed the vicinity of 3'-UTRs and observed an over-representation of G-quadruplex motifs immediately after the 3'-end of genes, especially in those cases where another gene is in close proximity, suggesting that G-quadruplexes may be involved in the termination of gene transcription.

INTRODUCTION

There are numerous mechanisms that regulate gene expression either at the DNA level or at the RNA level. Mechanisms for post-transcriptional regulation include the control of mRNA processing, nucleocytoplasmic transport, cellular and subcellular localization, translation efficiency and stability. Several studies have demonstrated that the genetic information required for post-transcriptional control is located mainly in the 5'- and 3'-untranslated regions (UTRs) of mRNA, and may involve both the primary sequence and secondary structure of non-protein-coding elements (1). Control via primary sequence recognition is exemplified by the action of ~22 nt single-stranded trans-acting regulatory elements, called micro-RNAs, that target mRNA sites, generally in their 3'-UTR, leading to translation repression (2). Secondary structures formed in 5'- and 3'-UTRs can also serve as regulatory elements by acting as target sites for RNA-binding factors such as proteins (3) or small molecule metabolites (4), or by interacting directly with the translation machinery (5–7).

There is scope for non-canonical nucleic acid structures to form in RNA transcripts. Certain guanine-rich nucleic acid sequences are predisposed to adopting four-stranded structures known as G-quadruplexes (8) that comprise stacks of hydrogen bonded G-tetrads, each containing four guanines. There is evidence that G-quadruplexes can form in the DNA at telomeres (10) under the control of telomere-binding proteins (9,10). Owing to the functional relationship between telomere maintenance, cell proliferation, and cancer, the telomeric DNA G-quadruplex is under consideration as a potential molecular target for anticancer therapeutics (11). It has also been proposed that DNA G-quadruplex motifs found within gene promoters may be involved in controlling gene expression at the transcriptional level (12). There is some in vitro experimental evidence for the promoter-quadruplex hypothesis from chemical biology studies on proto-oncogenes that including c-myc (13), k-ras (14) and c-kit (15). Furthermore, genome-wide computational analysis has revealed that putative quadruplex-forming sequences (putative quadruplex sequences, PQS) are prevalent in the human genome (16,17), and that there is a significant enrichment in gene promoter regions relative to the rest of the genome, with almost half of all protein-coding genes found to have putative quadruplex-forming motifs in their promoters (18). This has also been found to be the case for a variety of warm-blooded animals and the presence of putative G-quadruplex motifs in the first intron of genes has recently been studied (19).

While significant attention has hitherto been focused on DNA G-quadruplexes and their potential role in biology, certain G-rich RNA sequences can also fold into quadruplexes (20,21). Indeed, a few cases have been reported where intramolecular G-quadruplex formation within mRNAs has been proposed to be associated with function. For example, G-quadruplex formation in the 3'-UTR of insulin-like growth factor IGF-II mRNA was shown to

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occur downstream of an endonucleolytic cleavage site (22), the fragile X mental retardation protein (FMRP) has been shown to bind a G-quadruplex within the coding region of the corresponding mRNA (23) and an intramolecular RNA G-quadruplex motif has been found within the fibroblast growth factor (FGF-2) internal ribosome entry site (24). A cytoplasmic exonuclease, mXRN1p, has been shown to exhibit a substrate preference for G-quadruplex RNA (25). We recently discovered a conserved, intramolecular G-quadruplex motif within the 5′-UTR of the gene transcript of the human NRAS proto-oncogene, and we have demonstrated that this RNA G-quadruplex inhibits translation (26).

### METHODS

Sequence data and gene descriptions were extracted from Ensembl with biomart, using build 36 of the human genome sequence throughout. Sequences analysed were known transcripts of known protein-coding genes, using the Ensembl definitions and flags throughout. We have considered every transcript individually, unless they had identical UTR sequences; thus there are in some cases more than one UTR per gene. We investigated the key conclusions of this work using only genes with precisely one transcript; the results were broadly the same, and are shown in supplementary material. Where gene-level analyses are reported, a gene was considered to have a UTR PQS if any of its transcripts did so. PQS were identified using the program quadparser (16), which is available online at http://www.quadruplex.org/?view=quadparser. Briefly, we used the default parameters for this program, which searches for sequences of the form G\(_{5+n}\)N\(_{1-7}\)G\(_{3+n}\)N\(_{1-7}\)G\(_{3+n}\) on either strand of the sequence given. Other analyses were performed using custom-written perl scripts. Statistical analyses were performed using chi-squared tests or as otherwise appropriate. Full details are available in Supplementary material.

### RESULTS AND DISCUSSION

Computational approaches can provide insights into the potential roles of sequence motifs that cluster in specific structural regions of the genome. We have previously used computational analysis of genomic data to suggest a functional role for G-quadruplex sequence motifs within gene promoters (18). Here, we have mapped the location of G-quadruplex motifs with high resolution in and around the 5′- and 3′-UTRs of human protein-coding genes.

#### Incidence of G-quadruplex motifs in 5′-UTRs and in 3′-UTRs

Using build 36 of the human genome sequence, we extracted unique 5′- and 3′-UTRs corresponding to all known protein-coding transcripts post-splicing. This yielded 21 658 unique genes, with a total of 32 985 annotated 5′-UTRs, and 32 818 3′-UTRs. The 5′-UTRs were in general significantly shorter than the 3′-UTRs, with a mean length of 243 bases, compared with 899 bases for the 3′-UTRs. The median lengths were 120 and 494 bases, respectively, showing that the distribution of UTR lengths comprised many relatively short UTRs and a tail of long UTRs, with the longest 5′-UTR being 24 kb, and the longest 3′-UTR 14 kb. From these data, using our search program quadparser (16), we investigated whether these UTRs contain PQS, on either of the two strands present in the cDNA sequence (Table 1). To distinguish between the two strands, we have referred to motifs identified in the coding strand as G-PQS (as the transcribed RNA sequence would contain a G-rich sequence capable of forming a G-quadruplex), and motifs identified in the template strand as C-PQS (as the transcribed RNA sequence would be C-rich and not form a G-quadruplex) (Figure 1).

#### Table 1. Summary of results for the association of 5′- and 3′-UTRs with PQS

|                  | 5′ UTRs | 3′ UTRs |
|------------------|---------|---------|
| No. UTRs         | 32,985  | 32,818  |
| Average length   | 243 bases | 899 bases |
| No. UTRs with PQS (%) | 4141 (12.6%) | 5041 (15.3%) |
| With G-PQS       | 2034 (6.2%) | 2740 (8.3%) |
| With C-PQS       | 2525 (7.7%) | 3252 (9.9%) |
| Ratio C/G        | 1.24    | 1.19    |
| No. G-PQS        | 2334    | 3530    |
| No. C-PQS        | 3070    | 4526    |
| G-PQS density    | 0.291/kb | 0.120/kb |
| C-PQS density    | 0.382/kb | 0.153/kb |
| Ratio C/G        | 1.32    | 1.28    |

The number of UTRs with G-PQS and the number with C-PQS do not sum to the total number with PQS, as some UTRs contain both a G-PQS and a C-PQS.

![Figure 1. Schematic of DNA and transcribed RNA. Where the DNA sequence in the coding strand (blue) is G-rich (shown as GGG) a DNA G-quadruplex could form in that strand, and is here referred to as a G-PQS. A C-rich region in the coding strand is shown equivalently as CCC, and would allow a G-quadruplex to form on the template strand (red) and is here referred to as a C-PQS. After transcription, G-PQS, but not C-PQS, also results in the formation of a G-quadruplex in the mRNA (green).](https://example.com/f1.png)
Of the 32,985 5′-UTRs, 4,141 (12.6%) exhibited one or more PQS on one of the two strands. However, the two strands were not equivalent, with only 2034 (6.2%) having a G-PQS, whereas 2525 (7.7%) were associated with a C-PQS. We also calculated the overall densities of PQS in 5′-UTRs to be 0.291 G-PQS/kb and 0.382 C-PQS/kb. This shows a significantly greater proportion of C-PQS than G-PQS by a factor of 1.31 ($P = 3 \times 10^{-29}$). Of the 32,818 3′-UTRs, 5041 (15.3%) exhibited one or more PQS on one of the two strands. The proportion of 3′-UTR with PQS is therefore higher, but this could be easily explained (indeed, overcompensated for) by the observation that 3′-UTRs are in general much larger that 5′-UTRs, by a factor of about 4. On considering the two strands separately, we found that in the 3′-UTR, 2740 (8.3%) were associated with G-PQS, and 3252 (9.9%) with C-PQS. However, the densities of the two motifs are significantly lower ($P = 1 \times 10^{-53}$) in the 3′-UTR as compared with the 5′-UTR, at 0.120 G-PQS/kb and 0.153 C-PQS/kb. This gives a strand asymmetry ratio of 1.28 ($P = 2 \times 10^{-24}$) for 3′-UTR PQS, broadly the same as for PQS in the 5′-UTRs. These results are summarized in Table 1.

The occurrence of G-quadruplex motifs is strongly affected by base composition. Therefore, we investigated whether the asymmetry observed could be accounted for by this factor. In the 5′-UTR, there are more G bases than C bases (29.8% against 28.8%), which would suggest that more G-PQS would be expected to be present than C-PQS, in contradiction to the observed result. In the 3′-UTR, both bases are present in almost exactly equal proportions, at 21.7 and 21.6%, respectively. This could account for the difference in PQS density between the 5′- and 3′-UTRs, but not the excess presence of C-PQS.

To further study this effect, we generated simulated UTRs. To make these simulated, we studied every UTR, and counted the frequency with which each base was found at every position from the 5′-end. For each position, we counted the number of times the real UTRs terminated at that position. We then generated simulated UTRs in which the base frequencies at each position reflected the natural base frequencies at that position, and with UTR termination occurring at each position depending on the natural termination probability. One hundred replicates each consisting of 100,000 5′- and 3′-UTRs were generated, and searched for G-quadruplex motifs as above. As expected from the base frequencies, G-PQS were more frequently observed than C-PQS. For the 5′-UTRs, the simulates gave a ratio of C-PQS/G-PQS of 0.67 ± 0.09, compared to an observed ratio of 1.31 (significant, $P = 5 \times 10^{-14}$ based on the normal distribution curve). For the 3′-UTRs the simulated asymmetry was lower, at 0.96 ± 0.10, compared to the observed ratio of 1.28 (significant, $P = 4 \times 10^{-4}$ based on the normal distribution curve). Thus, base composition alone cannot account for the observed effects.

For comparison, we also examined the G-quadruplex densities of the entire transcriptome (defined as the total transcript of known human genes, including introns, according to Ensembl) as well as the whole genome [as described previously (16)]. Both of these showed virtually no strand asymmetry. In the transcriptome as a whole (1.1 Gb) we found 86,472 G-PQS and 86,038 C-PQS. This gives a transcriptome PQS density of 0.077 PQS/kb for each strand, for a total of 0.153 PQS/kb overall, slightly higher than that for the whole genome, which had an overall density of 0.115 PQS/kb, also split equally between the two strands.

Next, we analysed the Gene Ontology (GO) codes associated with all of these genes, to see if they corresponded to any over- or under-represented categories of genes (Tables 2 and 3). We used the same methodology reported in our previous analysis of gene promoters (18), which compared the number of genes in a particular GO category with G-PQS in a region to the total number of genes in that GO category, which varies significantly. We found that for the 5′-UTR G-PQS, 22 GO categories were significant at our required threshold of $P < 3.9 \times 10^{-6}$ (using the conservative Bonferroni correction to a $P$-value of 0.05, considering two tests on each of 6447 GO codes). For the 3′-UTR G-PQS, 28 GO categories were significant at the same level. All of these categories, together with the associated $P$-values, are listed in Tables 2 and 3. Twelve of these GO categories were the same as for the 5′-end, again showing a relationship between the two UTRs. Interestingly, some of the GO categories shown to be unlikely to have promoter PQS (18) were also found to be very unlikely to have G-PQS in either their 5′- or 3′-UTRs, such as the genes involved in olfaction and immune response.

Association of G-quadruplex motifs in both 3′- and 5′-UTRs

We considered whether genes with G-quadruplexes in their 5′-UTRs were also more likely to have them in their 3′-UTRs, or whether these were independent properties. Here, we have restricted our analysis to only include G-PQS sequences, as there is some evidence to support the hypothesis that these motifs may be associated with function in RNA, although the C-PQS could potentially form DNA G-quadruplexes on the template strand or a C-rich RNA secondary structure called the i-motif (27,28).

We found that 314 genes had G-PQS motifs in both the 5′- and 3′-UTRs, out of a total of 1,665 genes with G-PQS in the 5′-UTR and 2,154 with G-PQS in the 3′-UTR, from an overall total of 21,658 genes (Figure 2). The proportion of genes with 5′-UTR G-PQS that also have 3′-UTR PQS was 18.9% (314/1,665) as compared to the proportion of all genes with 3′-UTR PQS, which was 9.9% (2,154/21,658). If these two sets of motifs were entirely independent, the two proportions would necessarily be identical. Thus, there is clearly a significant association ($P = 5 \times 10^{-34}$) between the 5′- and 3′-UTRs and the presence of G-PQS in the two ends is not independent. This may be suggestive of a functional long-range interaction. Indeed, such long-range interactions between sequences in the 5′- and 3′-UTRs have been proposed to enhance translation efficiency (6,29). We tested whether this correlation was simply due to a correlation in UTR lengths, and found no significant correlation between the length of a 5′-UTR and the corresponding 3′-UTR (Pearson $r = 0.02 \pm 0.04$).
We also confirmed that the same result was found using only non-redundant UTRs (see Table S3).

**Table 3. Gene families with significantly rare G-PQS in either the 5′- or 3′-UTRs**

| GO code   | Description                                      | 5′ UTR −log p | 3′ UTR −log p |
|-----------|--------------------------------------------------|---------------|---------------|
| GO:0000786| Nucleosome                                       | 6.5           | –             |
| GO:0003735| Structural constituent of ribosome               | –             | 10.2          |
| GO:0004872| Receptor activity                                | 9.0           | –             |
| GO:0005757| Extracellular region                             | 22.3          | 26.6          |
| GO:0005739| Mitochondrion                                    | 10.2          | –             |
| GO:0005840| Ribosome                                         | –             | 8.3           |
| GO:0006334| Nucleosome assembly                               | 7.5           | 6.2           |
| GO:0006412| Translation                                       | 6.3           | 8.2           |
| GO:0009555| Immune response                                  | 9.7           | 7.2           |
| GO:0007186| G-protein coupled receptor protein signaling pathway | 18.1          | 12.0          |
| GO:0007508| Sensory perception of smell                      | 20.0          | 27.7          |
| GO:0008152| Metabolic process                                | –             | 8.5           |
| GO:0002612| MHC class I protein complex                      | 5.9           | –             |
| GO:0005896| Response to stimulus                             | 18.3          | 14.8          |

Where significant under-representation was found, the P-value associated with the test is presented as a negative logarithm. Where the test was not significant for one of the UTRs, this is marked as ‘–’.

We also confirmed that the same result was found using only non-redundant UTRs (see Table S3).

**Positional bias and strand asymmetry of PQS in the 5′-UTR**

We then considered whether PQS within the 5′-UTR showed any positional bias. The data presented in Table 4 shows strong clustering of G-PQS in the first ~50 bases, with a gradually declining frequency upon moving further away from the 5′-end. It was noteworthy that there was a strand bias to this positional effect, where C-PQS showed significantly less clustering than G-PQS (significant, \( P = 2 \times 10^{-9} \) within 50 bases). We then performed a high-resolution mapping of all PQS in the UTRs, together with the upstream (promoter) region for comparison, studying the frequency with which every base position was occupied by a PQS (Figure 3a).

Examination of the 5′-UTR region at this higher resolution (Figure 3a) confirms the results described earlier (Table 4), with clear strand asymmetry and the highest density of G-PQS at the 5′-end of the 5′-UTR, decreasing approximately linearly along the 5′-UTR. In contrast, the C-PQS on the complementary strand are relatively depleted in the first 50 bases at the 5′-end of the 5′-UTR, and then become...
Table 4. Frequency of PQS motifs at the 5′-end of the 5′-UTR and the 3′-end of the 3′-UTR

| Start                  | 5′-end of 5′-UTR | 3′-end of 3′-UTR |
|------------------------|------------------|------------------|
|                        | G-PQS (%)        | C-PQS (%)        | G-PQS (%)        | C-PQS (%)        |
| Within first 10 bases  | 193 (8.3)        | 135 (4.4)        | 13 (0.4)         | 0 (0)            |
| Within first 20 bases  | 316 (13.5)       | 241 (7.9)        | 18 (0.5)         | 0 (0)            |
| Within first 50 bases  | 653 (28.0)       | 569 (18.5)       | 106 (3.0)        | 34 (0.8)         |
| Within first 100 bases | 1091 (46.7)      | 1113 (36.3)      | 356 (10.1)       | 266 (5.9)        |
| Within first 1/20th    | 253 (10.8)       | 201 (6.5)        | 102 (2.9)        | 57 (1.3)         |
| Within first 1/10th    | 437 (18.7)       | 356 (11.6)       | 287 (8.1)        | 210 (4.7)        |

Figure 3. (a) High-resolution map of PQS at the TSS junction. Distances are shown in units of bases in the 5′ direction (−ve) or 3′ direction (+ve). The frequencies represent the number of times a PQS was observed to include each individual base position, normalized for the number of times each position was observed. The equivalent frequencies across the whole genome and transcriptome are also shown for comparison. The upstream region is taken from whole genomic data, the 5′-UTR data is taken from the mature mRNA post-splicing, with a gap to highlight the junction. (b) Analysis of the excess of G-PQS over C-PQS for 5′-UTRs in the human genome, calculating the excess of G-PQS over C-PQS as Excess = (G-PQS − C-PQS). (G-PQS + C-PQS) at every position along the 5′-UTR, counting in bases from the TSS. (c) A simple model to predict over-/under-representation of G-PQS motifs compared to C-PQS motifs suggests an overall profile given by the red line, in good agreement with the observed data.
increasingly common over the next 50 bases, before decreasing linearly. Over most of the 5′-UTR, and with the striking exception of the first 50 bases at the 5′-end, C-PQS are 30% more common than G-PQS, despite the fact that there are more G than C bases. This strand asymmetry, which is not shared by the PQS in the promoter vicinity of the transcription end site (TES) junction. First, the 5′-UTRs may be involved in tran-lation regulation (Figure 4a), and hence that there may be an evolutionary pressure in favour of the positional bias of G-quadruplex motifs towards this initial region.

**Positional bias and strand asymmetry of PQS in and around the 3′-UTR**

We have also carried out a high-resolution mapping of the 3′-UTR and the region immediately downstream of the gene (Figure 5), in analogous fashion to that performed for the 5′-UTRs. The 3′-UTRs of genes have a lower density of PQS than the 5′-UTRs, but there is still a strand bias within the 3′-UTR, with an excess of C-PQS. Two noteworthy features were observed in the immediate vicinity of the transcription end site (TES) junction. First, PQSs are extremely rare, compared to all other areas studied, in a region stretching from 20 bases within the 3′-end of the 3′-UTR to 10 bases downstream from the TES. Second, just 3′ of this, there is a very sharp peak in G-PQS density that is not accompanied by an equivalent C-PQS effect (i.e. the strand bias is strong), and is not reflected by an increase in the proportion of G bases (see Supplementary material). These results are suggestive of RNA PQS function localized proximal to the junction between the 3′-UTR and the 3′-downstream region.

In considering the possible functional implications of a G-quadruplex immediately downstream of the TES, we were drawn to observations and suggestions made by Proudfoot and co-workers (32,33) that structures formed by G-rich sequences at the 3′-end of a gene may help to demarcate the end of transcription, especially in cases where there is another gene shortly after the TES. Therefore, given our observation of a peak in G-PQS density in the 100 bases immediately 3′ of the TES, we were prompted to investigate whether known protein-coding genes with G-PQS in this 100 bases region were particularly likely also to have nearby genes. We identified 562 genes with G-PQS in this region, giving a total of 859 such G-PQS. In each case, we measured the distance from these genes to the next known gene in the 3′ direction relative to the gene. In one case, there was no next gene before the end of the chromosome, and that gene was not considered further. The results are shown in Table 5. Of the remaining 561 genes, 50 (8.9%) overlapped with other known genes. Of the remainder, the mean distance to the next gene was 47.3 kb. Of these genes, 97 (17.3%) had another gene within a kilobase. These results were compared to a control set where all known protein-coding genes were considered. Among this dataset of 21 679 genes, 36 did not have a next gene, and 1589 (7.3%) were overlapping. The mean distance to the next gene among the remainder was 93.4 kb, considerably larger than that found for the set with TES-associated G-PQS. Of the entire set of genes, 1633 (7.5%) had another within a kilobase, significantly less than the proportion found for
genes with TES-associated G-PQS (17.3%), by a factor of 2.3 (significant, \( P = 2 \times 10^{-16} \)).

A requirement for 3′-end processing of mRNAs is efficient transcription termination (36,37). This appears to be especially important in the cases of closely spaced genes (33). It has been shown that downstream G-rich sequences promote efficient transcription termination. Proudfoot and co-workers (34,35) have previously shown that the sequence (GGGGAGGGGG)₄, a tetramer of four MAZ-binding sites, strongly activates transcription termination in vitro when positioned downstream of a synthetic poly(A) site, in a manner that does not require the expression of the MAZ protein. We have noted with interest that this particular G-rich sequence is predicted by quadparser to form a stable G-quadruplex (16). Proudfoot and co-workers have further shown that mutation of the sequence to (GGTGAAAGGTG)₄, which quadparser (16) does not predict to form a G-quadruplex, does not efficiently terminate transcription. Specifically, it was shown in vivo that the parent, but not the mutated sequence, promotes efficient transcription termination of a heterologous β-globin construct, and that a naturally occurring G-rich sequence, located 100 nt downstream of the poly (A) site in the human β-actin gene, is essential for transcription termination. Equally, it has been previously noted that some G-rich RNA sequences are involved in

Table 5. Distance from the 3′-end of genes to the next gene either for all known protein-coding genes with a gene to their 3′-end, or only those with a predicted G-quadruplex immediately to the 3′-end of the gene

| All known protein-coding genes | Class | Genes with G-PQS in 100 bases 3′ of TES | Enrichment |
|--------------------------------|-------|----------------------------------------|------------|
| No. (%)                        |       | No. (%)                                |            |
| 21643 (100)                    | All with next gene                        | 561 (100)  |            |
| 1589 (7.3)                     | Overlapping \( (d \leq 0) \)              | 50 (8.9)   | 1.21       |
| 20054 (92.7)                   | Non-overlapping \( (d > 0) \)             | 511 (91.1) | 0.98       |
| 1633 (7.5)                     | Near \( (0 < d \leq 1000) \)              | 97 (17.3)  | 2.29       |
| 3376 (15.6)                    | Medium \( (1000 < d \leq 5000) \)        | 114 (20.3) | 1.30       |
| 15045 (69.5)                   | Far \( (5000 < d) \)                      | 300 (53.5) | 0.77       |
| 3317 (15.3)                    | Very far \( (100000 < d) \)              | 51 (9.1)   | 0.59       |

The data is binned into categories where the next gene overlaps the previous one, is within 1 kb (‘near’, shown in bold), from 1 to 5 kb away (‘medium’) or >5 kb away (‘far’). The subset where the next gene is >100 kb away (‘very far’) is also shown. Genes where there was no subsequent gene (because they were near a chromosome end) are not shown. Enrichment is calculated as percentage with G-PQS/% of all genes for each class.

Figure 5. High-resolution map of PQS at the TES junction. Distances are shown in units of bases in the 5′ direction (−ve) or 3′ direction (+ ve). The frequencies represent the number of times a PQS was observed to include each individual base position, normalized for the number of times each position was observed. The equivalent frequencies across the whole genome and transcriptome are also shown for comparison. The downstream region is taken from whole genomic data, the 3′-UTR data is taken from the mature mRNA post-splicing, with a gap to highlight the junction.
regulating 3'-end processing of alternatively processed mammalian pre-mRNAs by interaction with hnRNP H protein subfamily members (36,37). Our observation that G-PQS cluster immediately after the end of the 3'-mammalian pre-mRNAs by interaction with hnRNP H immediately after the 3'-end of the mRNA, particularly in cases of genes that have a proximal gene in the 3'-UTR supportive of function relating to transcriptional termination, cleavage and polyadenylation (Figure 4b).

CONCLUSIONS

From a comprehensive survey of G-quadruplex motifs in and around human genomic UTRs, we have shown that their incidence shows significant strand asymmetry and positional bias, suggestive of functional roles in RNA. In 5'-UTRs, G-quadruplex motifs tend to exist towards the 5'-end of the 5'-UTR supportive of function relating to transcription initiation, as depicted in Figure 4a, and for which there is now some experimental support (26). With respect to 3'-UTRs, G-quadruplex motifs tend to cluster immediately after the 3'-end of the mRNA, particularly in cases of genes that have a proximal gene in the 3' direction. In such cases, failure to terminate transcription could lead to problems associated with the additional transcription of the adjacent gene. We propose that G-quadruplex motifs may serve as pause elements that promote transcriptional termination, cleavage and polyadenylation (Figure 4b).

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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