

Nucleotide sequence of a second glutamine tRNA gene in wheat mitochondrial DNA

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We present here the sequence of a wheat mitochondrial (mt) tRNA^{Gln} gene (Q2, boxed in Fig. 1) and flanking regions. We show an 857 bp *Sau3AI* subclone (sequenced on both strands) of a >15 kbp *HindIII* fragment, H-K1/Q2, which also contains a tRNA^{Lys} gene (K1) [1]. K1 and Q2 are in opposite orientation on H-K1/Q2 and separated by ~1 kbp (based on restriction data). The protocols used to isolate and sequence tRNA gene-containing clones have been described [2].

Q2 is the second tRNA^{Gln} gene we have isolated from wheat mtDNA. Differences between Q2 and Q1 [3] and their flanking regions are shown in Fig. 1, with vertical arrows demarking the extent of obvious sequence similarity and lower case letters denoting the positions at which the sequence of H-Q1 [3] differs from that of H-K1/Q2. A single base substitution at position 39 of the coding sequence changes a G-T bp in the anticodon stem of Q1 to a G-C bp in Q2 (Fig. 2). This is the first report of a sequence difference in duplicate copies of the same plant mt tRNA gene. In contrast, we found no differences within the coding region of duplicate tRNA^{Pro} genes [4].

Using criteria outlined in [2], there are no inverted repeats downstream (within 100 bp) of Q2 and only one major direct repeat (overlined, Fig. 1). There are three pairs of direct repeats (overlined, Fig. 1) and two pairs of inverted repeats (underlined, Fig. 1) upstream of Q2 (within 150 bp). A purine-rich sequence, AAGAAARR, found upstream of other wheat mt tRNA genes [1,2,3] is also found here (dotted underline, Fig. 1).

When H-Q1 was used as a hybridization probe, a signal corresponding to H-K1/Q2 was visible only after lengthy exposure. The low sequence similarity between H-Q1 and H-K1/Q2 in flanking regions (59.3% in the 54 bp 5' to the gene and none 3'), as well as the single base change within the coding region, presumably accounts for this result. When wheat mtDNA was probed with ³²P-labeled 4S RNA, H-Q1 and H-K1/Q2 were seen at equal intensity, suggesting that these fragments are present in similar amounts in the wheat mt genome.

From preliminary sequence analysis of tRNA^{Gln} isolated from wheat mitochondria, it is not clear whether both of these genes are transcribed. Enzymatic sequencing suggests a C at position 39, but without further analyses we cannot exclude the possibility that there is a modified U at this position.

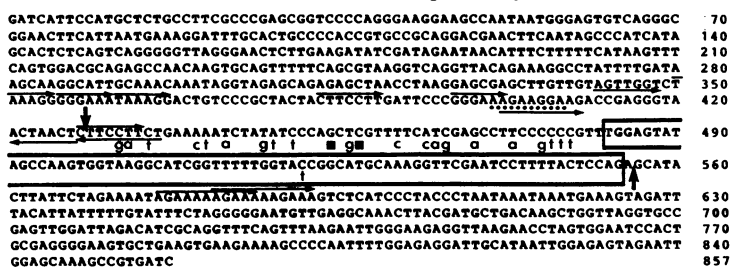


Figure 1

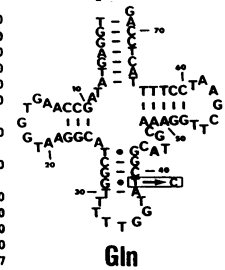


Figure 2

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