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Translation efficiencies of synonymous codons for arginine differ dramatically and are not correlated with codon usage in chloroplasts

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ABSTRACT

Codon usage in chloroplast mRNAs is different from that in prokaryotic and cytosolic mRNAs. We previously devised an *in vitro* assay for translation efficiencies using synthetic mRNAs, and measured translation efficiencies of five synonymous codon groups in tobacco chloroplasts. Using this assay, we here report our analysis of four additional synonymous codon groups in tobacco chloroplasts. We found that translation efficiencies of three arginine codons AGA, CGU and CGA differ dramatically, ca. 10-fold difference although the three arginine codons possess similar codon usage. Translation of AGA is very high, while CGA is translated extremely low. CGA is used frequently in chloroplasts but rare in *Escherichia coli*. The single tRNA species reads two histidine codons (CAU and CAC) and this is also the case for two glutamic acid codons (GAA and GAG) and two arginine codons (GCU and GCA). Their translation efficiencies, however, differ significantly. These observations suggest that individual codons posses their intrinsic efficiencies.

1. Introduction

The genetic code is degenerate, namely the common 20 amino acids except for methionine and tryptophan are coded for by two to six codons called synonymous codons. Synonymous codons are not used with equal frequency in protein-coding regions, and are used differently by different organisms. Codon usage has been generally calculated by simple summation of collected gene sequences. However, this method cannot be applied for plant mitochondria and chloroplasts because RNA editing causes codon conversion at the mRNA level (Yoshinaga et al., 1997; Giege and Brennicke, 1999; Sugiura, 2008). Observations in *Escherichia coli*, yeast and *Bacillus subtilis* showed that codon usage is positively correlated with tRNA content and tRNA gene copy number, especially for highly expressed genes (Ikemura, 1985; Kanaya et al., 2001). Attempts have been made to analyze the translation efficiency of synonymous codons in *E. coli*, and these experimental data indicated that different codons are translated at different rates and that there is generally a correlation between translation rate and codon usage or tRNA content (Robinson et al., 1984; Bonekamp and Jensen, 1988; Bonekamp et al., 1989; Sørensen and Pedersen, 1991).

The chloroplast genome in flowering plants contains about 80 protein-coding genes (Sugiura, 1992). These genes are believed to use the universal genetic code and contain all 64 possible codons. At least 32 tRNA species are required to read all codons according to the standard Wobble rule. However, the chloroplast genome lacks several tRNA genes (e.g. (Shinozaki et al., 1986)) and although there is no evidence for tRNA import into chloroplasts of photosynthetic organisms, it is very likely that chloroplasts of non-photosynthetic plants (e.g. parasitic plants such as *Epifagus*) have to import tRNAs. Many chloroplast tRNA genes are single copy while several genes are duplicated (Sugiura, 1992). The number of duplicated genes depends on the length of the large inverted repeat in the chloroplast genome (e.g. seven in tobacco while one in black pine, (Wakasugi et al., 1994)). Both *in vitro* and *in vivo* analyses suggested strongly that chloroplast-encoded tRNAs (30 in tobacco chloroplasts, (Shinozaki et al., 1986)) are sufficient to recognize all codons by the two-out of-three, U:N wobble and /or superwobble mechanisms (Pfitzinger et al., 1990; Rogalski et al., 2008). In addition, tRNA-Glu(UUC) is a cofactor for chlorophyll biosynthesis (Jahn et al., 1992). According to these unique features in chloroplasts, a question has arisen whether a relationship between codon usage and translation efficiency of synonymous codons in chloroplasts is different from that of eubacterial and cytoplasmic translation systems.

We previously developed an *in vitro* translation system from tobacco chloroplasts (Hirose and Sugiura, 1996) and recently improved extensively the system (Yukawa et al., 2007). Our improved *in vitro* translation system is highly active and allows us to measure relative translation rates of exogenous mRNAs without additional tRNAs. Therefore, tRNA populations in the *in vitro* system are

Abbreviations: mGFP, modified green fluorescent protein; Glu, glutamic acid; Arg, arginine; Asn, asparagine; Leu, leucine; His, histidine; Ile, isoleucine; kDa, kirodalton(s).

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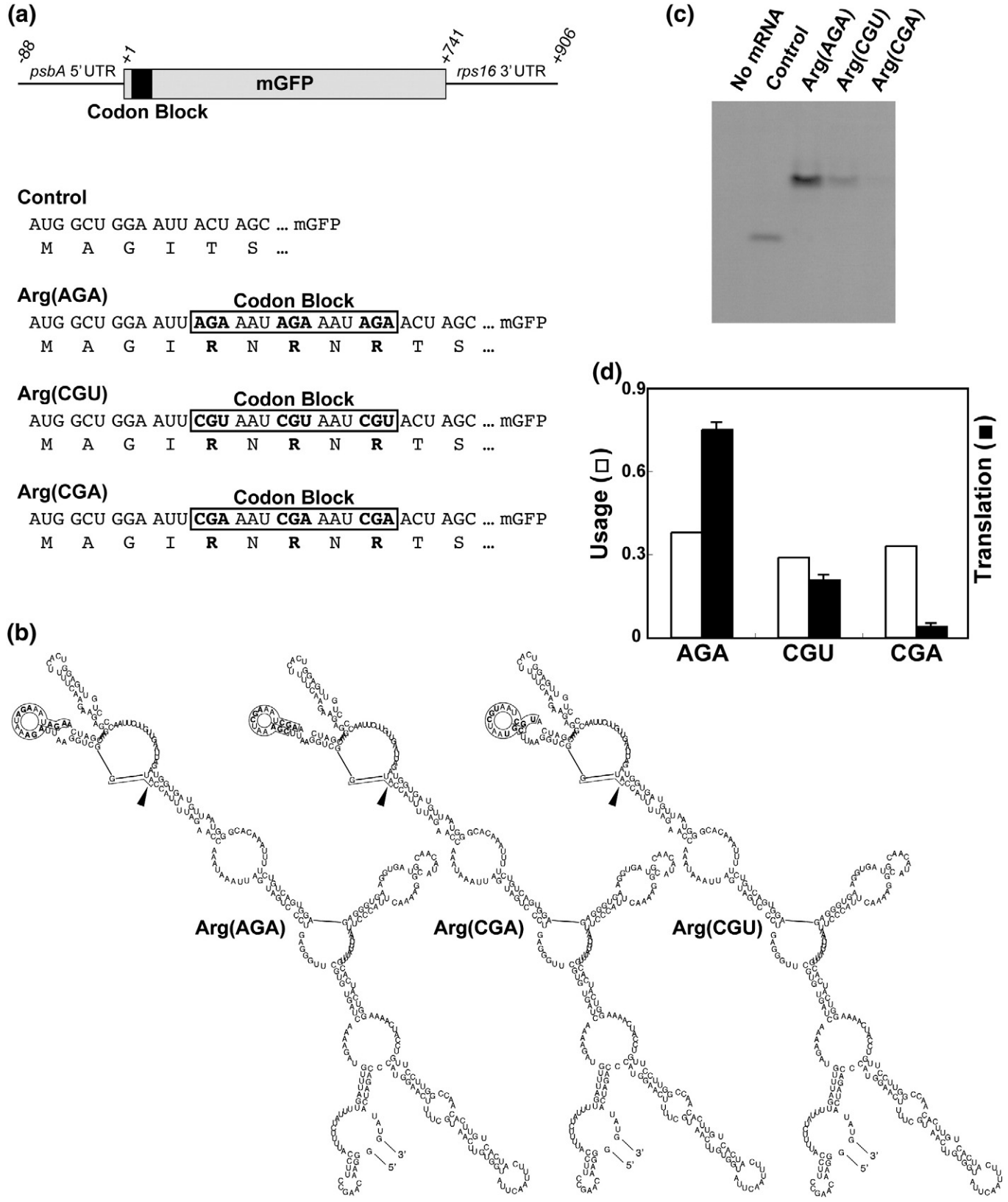

Fig. 1. Design of test mRNAs and translation analysis of arginine codons. (a) Schematic illustration of a test mRNA. Partial mRNA and amino acid sequences are shown below. Codon blocks are boxed in which Arg codons for assay are indicated by boldface. (b) Predicted secondary structures of the −88 to +256 regions (the A of the AUG codon as +1) in Arg (AGA), Arg(CGU) and Arg(CGA) mRNAs. Codon blocks are boxed in which Arg codons for assay are indicated by boldface. Arrowheads represent initiation codons. (c) Translation products on native PAGE. (d) Comparison of codon usage (open bar, the total number of three Arg codons as 1.0) and translation efficiency (solid bar, the sum of translation efficiencies of the three Arg codons as 1.0). Standard deviations were obtained from three experiments.