Supplemental Information:

Title: Selective charging of tRNA isoacceptors induced by amino acid starvation.

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1. E. coli tRNA probes and theoretically predicted charged levels.

Amino Acid	tRNA Probe	Anticodon	Predicted charged level upon starvation [a]	Operons
Ala	Ala-1	UGC	Intermediate	V,U,T
	Ala-2	GGC	Low	X,W
Arg	Arg-2	ACG	Low	Q,Z,Y,V
	Arg-3	CCG	High	X
	Arg-4	UCU	High	U
	Arg-5	CCU	High	W
Asn	Asn	GUU	-	T,W,U,V
Asp	Asp	GUC	-	U,V,T
Cys	Cys	GCA	-	T
Gln	Gln-1	UUG	Low	W,U
	Gln-2	CUG	Low	X,V
Glu	Glu	UUC	-	W,U,T,V
Gly	Gly-1	CCC	High	T
	Gly-2	UCC	High	U
	Gly-3	GCC	Low	W,V,X,Y
His	His	GUG	-	R
Ile	Ile-1	GAU	Low	V,U,T
	Ile-2	CAU	High	Y,X
Leu	Leu-1	CAG	Low	T,V,P,Q
	Leu-2	GAG	Low	U
	Leu-3	UAG	Low	W
	Leu-4	CAA	High	X
	Leu-5	UAA	Intermediate	Z
Lys	Lys	UUU	-	T,W,Y,Z,Q,V
Met	Met	CAU	-	U,T
	fMet	CAU	-	Z,W,V,Y
Phe	Phe	GAA	-	V,U
Pro	Pro-1	CGG	Low	K
	Pro-2	GGG	High	L
	Pro-3	UGG	Low	M
Sec	Sec	UCA	-	C
Ser	Ser-1	UGA	Low	T
	Ser-2	CGA	Intermediate	U
	Ser-3	GCU	Intermediate	V
	Ser-5	GGA	Low	W,X
Thr	Thr-1/3	GGU	Low	V,T
	Thr-2	CGU	High	W
	Thr-4	UGU	Intermediate	U
Trp	Trp	CCA	-	T
	Tyr	GUA	-	V,T,U
Val	Val-1	UAC	Low	T,Z,U,X,Y
	Val-2A	GAC	Low	V
	Val-2B	GAC	Low	W

a. Charged level: high: 0.50 – 1.00; intermediate: 0.10 – 0.50; low: < 0.10.

2. Determination of charged levels of a single sample by microarray (Fig. 2):

The Cy3 and Cy5 succinimidyl ester derivatives often exhibit differential reactivity for different RNA molecules. In addition, many *E. coli* tRNAs have post-transcriptional modifications (Bjork, 1995; Rozenski et al., 1999) that contain either an aliphatic primary amine (lysidine at position 34, ^{acp3}U at position 47) or a secondary amine (Q, ^{mnm5}U, ^{mnm5s2}U and ^{cmnm5}U, all at position 34). The Cy3 and Cy5 succinimidyl ester reacts preferentially with 5-aminoallyl group in the tagging oligonucleotide to the 3' end of tRNA (data not shown). However, the amine groups derived from internal tRNA modifications can also react with the dye and affect the calculation of charged levels.

To accurately measure the charged levels for a particular sample (Fig. 2), four array experiments were performed:

Array #	X in the tagging oligonucleotide	Periodate-treated sample label	mock-treated sample label
1	5NU	Cy5	Cy3
2	5NU	Cy3	Cy5
3	dT	Cy5	Cy3
4	dT	Cy3	Cy5

The third and fourth arrays measure the contribution of internal tRNA modifications to the observed fluorescent values in the first and second arrays. tRNAs containing internal modifications made of primary or secondary amines are more reactive than those without in the third and fourth arrays (data not shown). The data for Arg-5, Ser-2 and Sec-1 are less certain due to the very low fluorescent intensities of the corresponding tRNAs.

A correction factor is used in the calculation of charged levels for each tRNA to correct for the fluorescent signals derived from internal modifications:

$$\text{Charged level I} = (Cy5/Cy3)^{Array1} * (1 - Cy5^{Array3}/Cy5^{Array1})/(1 - Cy3^{Array3}/Cy3^{Array1}) \quad [1]$$

$$\text{Charged level II} = (Cy3/Cy5)^{Array2} * (1 - Cy3^{Array4}/Cy3^{Array2})/(1 - Cy5^{Array4}/Cy3^{Array2}) \quad [2]$$

Both $(Cy5/Cy3)^{Array1}$ and $(Cy3/Cy5)^{Array2}$ for each tRNA have been normalization to the added yeast $tRNA^{Phe}$ control with a known charged level measured by thin-layer chromatography (0.64 - 0.68, (Wolfson and Uhlenbeck, 2002)). In the absence of dye bias, both measurements should produce the same charged levels for each tRNA regardless of which sample is labeled with which dye (Fig. 2).

3. Determination of relative charged levels for the starvation series (Fig. 4 and Table 1):

In order to determine the relative charged levels, the Cy5/Cy3 ratios for samples before and after starvation need to be corrected for signals derived from internal amine modifications. Sine these internal tRNA modifications are not expected to change during the period of starvation, we used the following formula to calculate the relative charged levels:

[3a] $F5(t) = Cy5(t) - [Cy3(t)/Cy3(0)]*Cy5^{Array3}$

[3b] $F3(t) = Cy3(t) - [Cy3(t)/Cy3(0)]*Cy3^{Array3}$

Relative charged level = $\{F5(t)/F3(t)\} / \{F5(0)/F3(0)\}$ [3]

Where Cy5(t) and Cy3(t) are the Cy5 and Cy3 fluorescence signals of the samples from varying time points after starvation and Cy5(0) and Cy3(0) are the fluorescence signals of the sample before starvation. The factor Cy3(t)/Cy3(0) is used to correct for the relative amount of total tRNA in each array experiment.

4. Remarks on microarray measurements:

(i) Cross-hybridization: Our array contains probes for tRNA from three organisms, *E. coli*, *Bacillus subtilis* and *Sacchromyces cerevisiae*. As in previous work (Dittmar et al., 2004), the inclusion of tRNA from different organisms is useful as a specificity control. There is little detectable cross-hybridization between *S. cerevisiae* and *E. coli* tRNA. *E. coli* samples cross-hybridize to several probes designated for *B. subtilis* tRNA. Sequence similarities between tRNAs from *E. coli* and *B. subtilis* can explain some cross-hybridizations (Ala-1, Ala-2, Asp, Gly-3, Ile-1, Val-1), but not others (Leu-1, fMet).

(ii) Hybridization to Arginine probes: The absolute fluorescence intensity before starvation of the four $tRNA^{Arg}$ isoacceptors has the same rank order to those determined by two-dimensional gel electrophoresis (Dong et al., 1996), suggesting that there is no significant cross-hybridization to these probes from other *E. coli* tRNAs. Furthermore, we looked at all *E. coli* tRNA sequences and cannot find an obvious candidate for cross-hybridization to the $tRNA^{Arg2}$ probe.

$tRNA^{Arg}$	Relative Cy3-value from t=0 sample (Fig. 2A)	2-D gel, at doubling times of 0.7-1.1/h
2	1.0	1.0
3	0.16	0.13 ± 0.04
4	0.55	0.16 ± 0.02
5	0.07	0.10 ± 0.01

The actual differences may be derived from variations in (a) labeling and hybridization efficiencies of these isoacceptors, (b) *E. coli* strains used in these experiments, (c) growth conditions leading to differences in tRNA concentrations.

(iii) Dilution series: $tRNA^{Arg2}$ is present at the highest level among all $tRNA^{Arg}$ isoacceptors. The observed high charged level of this tRNA after arginine starvation may be due to saturation of the Arg-2 probe under standard condition for the array experiment which uses 0.9 μg each of Cy5 and Cy3 labeled total RNA. To ensure that this probe was not saturated under the above condition, another array experiment was performed using 0.3 μg each of Cy5 and Cy3 labeled total RNA after 45 minutes of arginine starvation. Comparing the same total RNA sample at 0.9 and 0.3 μg used in hybridization, the relative charged ratios for $tRNA^{Arg2, 3, 4}$ isoacceptors remain constant (1.09; 1.10; 0.95, respectively. A high variation for $tRNA^{Arg5}$ was observed, likely due to its very low signal intensity, Fig. 2A). This result demonstrates that the observed high charged level for $tRNA^{Arg2}$ does not result from probe saturation.

(iv) Arginine starvation series: The microarray experiment differentiates tRNAs having protected 3' ends against periodate oxidation from those that are not protected. Such protecting groups include covalently attached amino acids, peptides released from ribosomes, as well as unidentified cryptic compounds (Yegian and Stent, 1969a; Yegian and Stent, 1969b). However, these cryptic charged forms of $tRNA^{Arg2}$ during arginine starvation were not detected by the Northern blot analysis (Sorensen, 2001).

The primary protecting group against periodate oxidation is the amino acid under non-starving conditions (Jakubowski and Goldman, 1984; Morris and DeMoss, 1966). Under starvation conditions, however, the other two protecting groups may make more significant contributions to the Cy5/Cy3 ratio, depending on the tRNA isoacceptor. It is possible that non-amino acids contribute significantly against periodate oxidation of $tRNA^{Arg2}$ during arginine starvation. The microarray results on $tRNA^{Arg2}$ after arginine starvation are similar to a previous

result in which the charged level was measured by periodate oxidation followed by tRNA-synthetase charging with radioactive arginine (Morris and DeMoss, 1965).

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