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Supplemental Information

Conditional Switch between Frameshifting Regimes upon Translation of *dnaX* mRNA

Neva Caliskan, Ingo Wohlgemuth, Natalia Korniy, Michael Pearson, Frank Peske, and Marina V. Rodnina

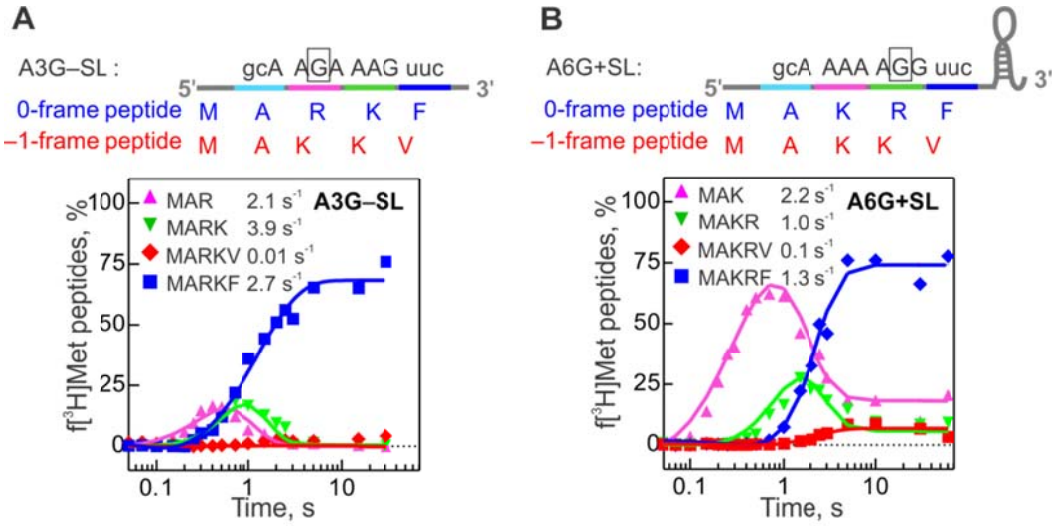


Figure S1. Kinetics in the presence of the cognate Arg-tRNA^{Arg}. Related to Figures 2 and 3.

(A) A3G–SL mRNA.
(B) A6G+SL mRNA. MAR (pink), MARK (green), MARKF(blue) and MARKV (red) peptides are monitored.

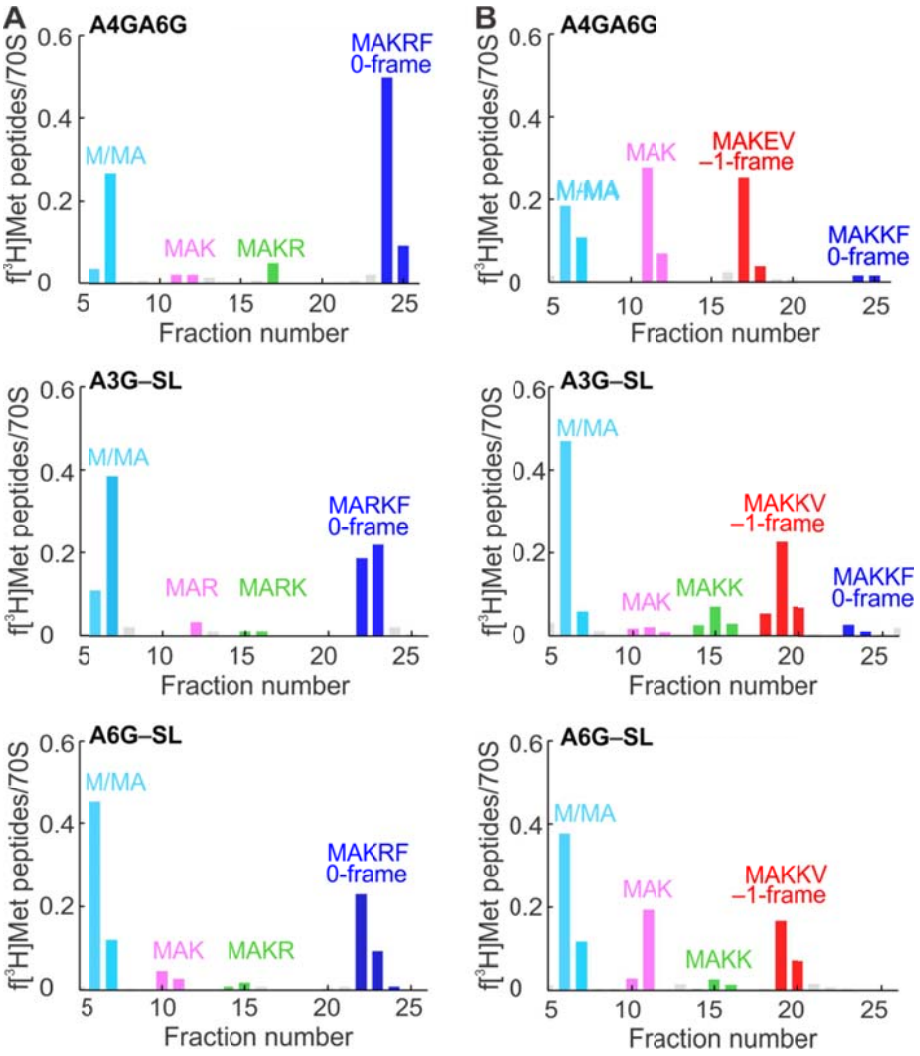
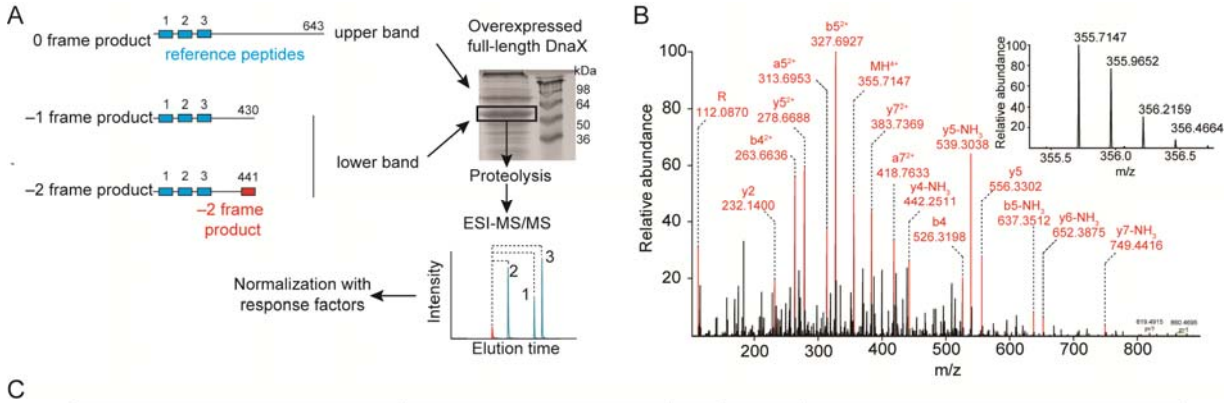


Figure S2. Analysis of translation peptides. Related to Figures 2, 3 and 4.

(A) Example chromatograms for peptide synthesis on the A4GA6G (top), A3G−SL (middle panel) and A6G−SL mRNA variants in the presence of Arg-tRNA^{Arg}. Monitored peptides are M-MA (light blue), MAK or MAR (pink), MAKR or MARK (green), and MAKRF or MARKF (blue).

(B) Same as in **(A)**, but without Arg-tRNA^{Arg}. Monitored peptides are M-MA (light blue), MAK (pink), MAKK (green), MAKEV-MAKKV (red) and MAKKF (blue).



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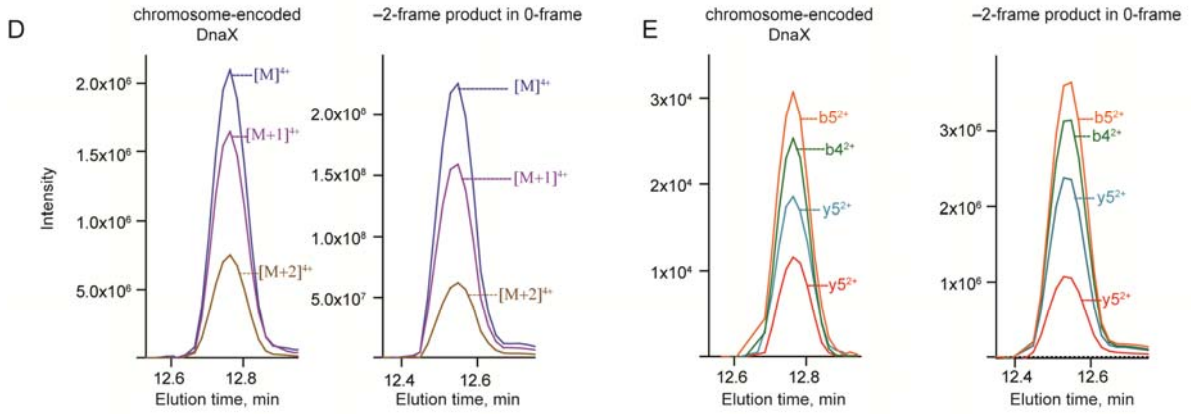
Peptide	Sequence	z	m/z	Extracted ions
Reference peptide 1	VGK.TSIARLLAK.GLN (51, 59)	2	486.814	$[M]^{2+}$, $[M+1]^{2+}$, $[M+2]^{2+}$, y7, y6, b7
Reference peptide 2	LLK.TLEEPPEHVK.FLL (141, 150)	3	393.540	$[M]^{3+}$, $[M+1]^{3+}$, $[M+2]^{3+}$, y6, y5, $y6^{2+}$
Reference peptide 3	HVK.FLLATTDPQK.LPV (151, 160)	2	567.314	$[M]^{2+}$, $[M+1]^{2+}$, $[M+1]^{2+}$, y8, y7, y3, b2
−2-frame peptide	AKK.RVNRQPLPARGR (430, 441)	4	355.716	$[M]^{4+}$, $[M+1]^{4+}$, $[M+2]^{4+}$, $y7^{2+}$, $y5^{2+}$, $b4^{2+}$,$b5^{2+}$



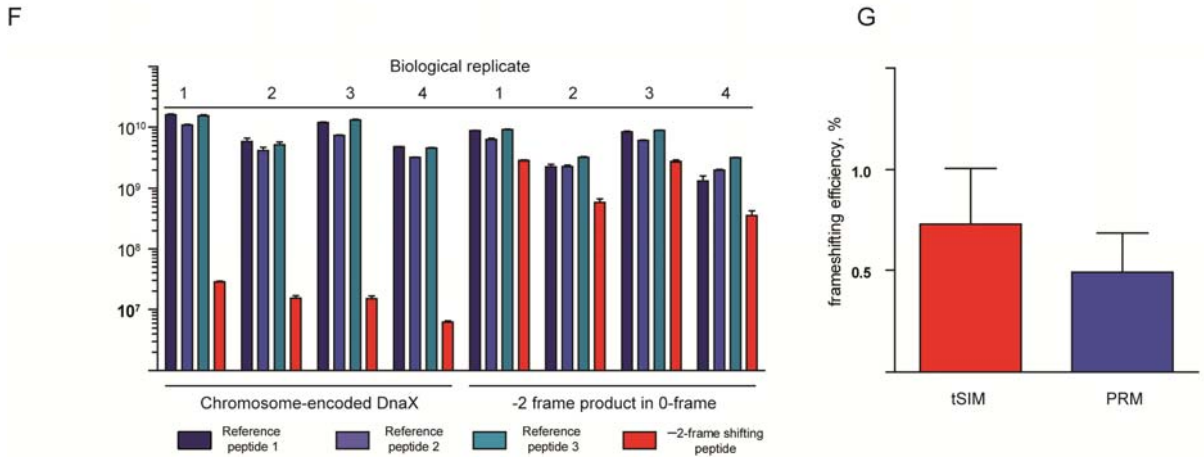


Figure S3. Quantification of –2-frame product of *dnaX* translation *in vivo* using mass spectrometry. Related to Figure 5. See also Table S3.
(A) Quantification work flow. The –2-frame product was quantified relative to the –1-frame product. The two products have a similar size and co-migrate on SDS PAGE. The protein band that comprised both isoforms was excised from the gel and subjected to proteolysis with LysC. The amount of –2-frame relative to –1-frame product was quantified by label-free targeted mass spectrometry monitoring the elution of precursor- (targeted selected ion monitoring; tSIM) and fragment- (Parallel Reaction Monitoring; PRM) ions over time.

(B) The –2-frame peptide identified by high resolution MS (inset, isotope dot product 0.99) and MS/MS spectra as well as by co-elution and co-fragmentation with an isotope-labeled internal standard peptide (AQUA, ratio dot product 0.99)**.**

(C) Sequence and extracted ions of the quantified peptides.

(D) Representative elution profiles of the –2-frame peptide precursors ions (tSIM).

(E) Representative elution profiles of the –2-frame peptide fragment ions (PRM).

(F) Integrated areas for the –2-frame peptide and the three reference peptides (shown for PRM) (**Table S3**). For both samples four biological replicates were analyzed. Error bars show the standard deviation of three technical replicates.

(G) Efficiency of –2- relative to –1-frameshifting. The frameshifting efficiency was independently quantified on MS1 (0.7%; red tSIM) and MS2 (0.5%; red; PRM) level. Error bars represent the SD of four biological replicates with three technical replicates each.

Table S1. Rate constants of translation steps upon –1PRF on *dnaX*. Related to Figure 1.

mRNA variant	Rates, s^{-1}				–1FS, %	
	LysI[a]	LysII[a]	Val[a]	Phe[a]	QF	IVT
SS / SL	1.7±0.2	2.0±0.2	0.46±0.03	0.13±0.02	78±15	72±4
– / SL	2.1±0.3	1.3±0.1	0.03±0.01	0.3±0.0	9±3	4±1
SS / –	2.3±0.5	2.2±0.1	0.7±0.03	2.3±0.1	23±2	16±5
– / –	2.3±0.5	2.2±0.5	0.03±0.01	2.9±0.6	1±0	0

[a] Rate constants of amino acid incorporation were determined by global fitting of the data shown in Figures 1E-1H; error bars are s.e.m. of the fit. IVT, *in-vitro* translation. The frameshifting efficiency (–1FS) was calculated from the end points of IVT experiments shown in Figures 1C and 1D; the values are mean ± s.d. (n=3 independent experiments).

Table S2. Rate constants of translation steps upon NHF on various *dnaX* constructs. Related to Figures 2, 3 and 4.

mRNA variant	Rates, s^{-1}		–1FS, %
	Lys[a]	Val[a]	IVT
A3G+SL	n.d.	0.06±0.01	80±4
A3G–SL	n.d.	0.04±0.02	61±4
A6G+SL	2.3±0.1	0.03±0.00	52±8
A6G–SL	2.4±0.1	0.02±0.00	44±4
A4GA6G	1.4±0.1	0.014±0.002	36±4

[a] Rates constants of amino acid incorporation were determined by global fitting of the data shown in Figures 2F, 2G, 3F, 3G and 4D; error bars are s.e.m. of the fit. The frameshifting efficiency (–1FS) was calculated from the end points of IVT experiments shown in Figures 2D, 2E, 3D, 3E, and 4C; the values are mean ± s.d. (n=3 independent experiments).

Table S3. Determination of the –2 frameshifting efficiency by mass spectrometry *in vivo*. Related to Figure 5 and S3.

This table is a separate file.

Table S4. mRNA constructs used in this study. Related to Star Methods.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
SS / SL mRNA: GCGUGCAGGGAGCAACCAUGGCAAAAAAGUUC UAACCGGCAGCCGCUACCCGCGCGCGGCCGGU GAA	This paper	N/A
− / SL mRNA: GCGUGCAGGGAGCAACCAUGGCGAAGAAGUUC UAACCGGCAGCCGCUACCCGCGCGCGGCCGGU GAA	This paper	N/A
SS / − mRNA: GCGUGCAGGGAGCAACCAUGGCAAAAAAGUUC UAG	This paper	N/A
− / − mRNA: GCGUGCAGGGAGCAACCAUGGCGAAGAAGUUC UAG	This paper	N/A
A3G–SL mRNA: GCGUGCAGGGAGCAACCAUGGCAAGAAAGUUC UAG	This paper	N/A
A3G+SL mRNA: GCGUGCAGGGAGCAACCAUGGCAAGAAAGUUC UAACCGGCAGCCGCUACCCGCGCGCGGCCGGU GAA	This paper	N/A
A6G–SL mRNA: GCGUGCAGGGAGCAACCAUGGCAAAAAGGUUC UAG	This paper	N/A
A6G+SL mRNA: GCGUGCAGGGAGCAACCAUGGCAAAAAGGUUC UAACCGGCAGCCGCUACCCGCGCGCGGCCGGU GAA-	This paper	N/A
A4GA6G mRNA: GCGUGCAGGGAGCAACCAUGGCAAAGAGGUUC UAACCGGCAGCCGCUACCCGCGCGCGGCCGGU GAA	This paper	N/A