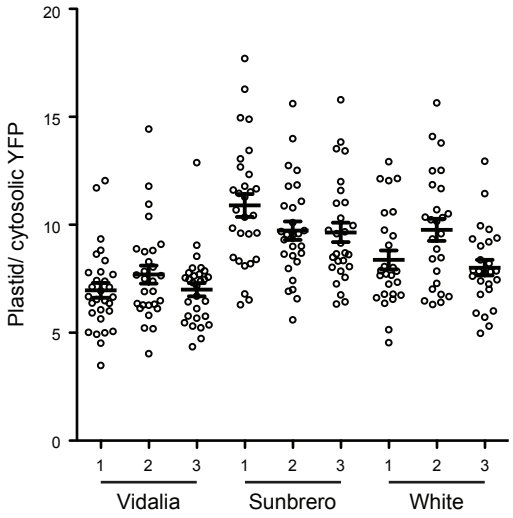
Supplemental Information

Non-native, N-terminal Hsp70 Molecular Motor-recognition Elements in Transit Peptides Support Plastid Protein Translocation

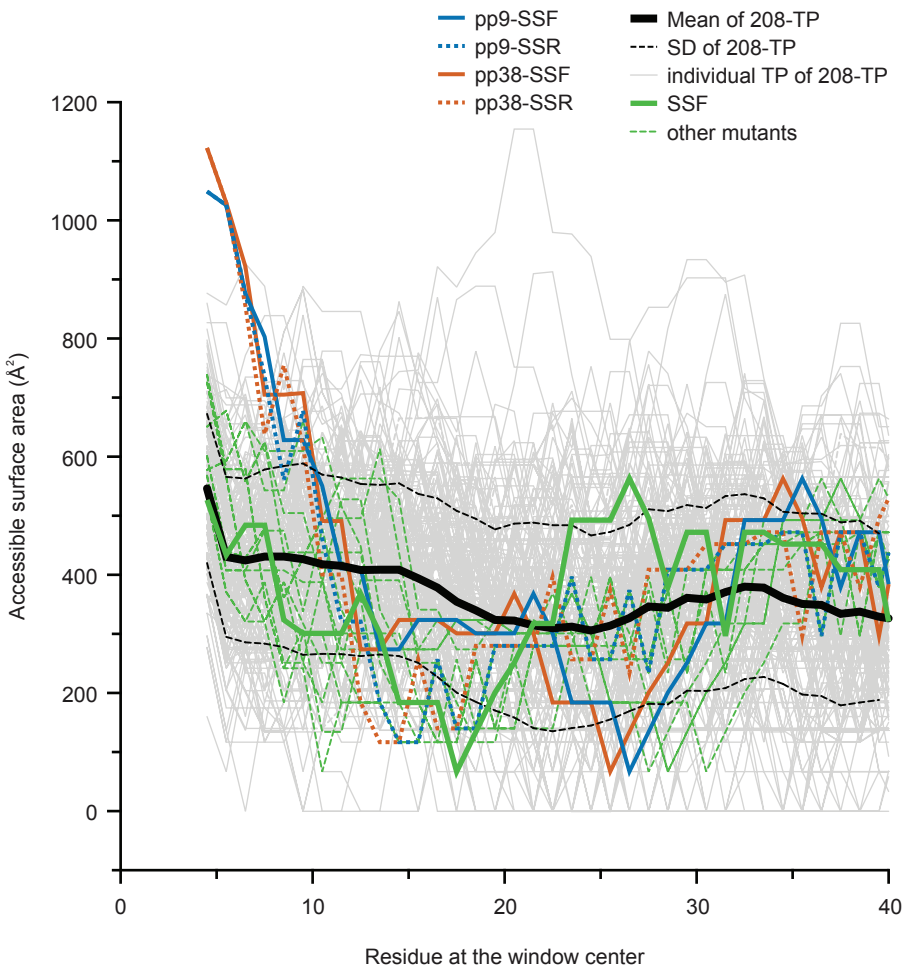
Prakitchai Chotewutmontri[1] and Barry D. Bruce [2§]

[1]The Graduate School of Genome Science and Technology, [2]the Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN 37996

[§]To whom correspondence should be addressed: Barry D. Bruce, Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN 37996, Tel.: (865) 974-4082; Fax: (865) 974-0978; E-mail: bbruce@utk.edu



Supplemental Figure S1. Effect of onion cultivar on the targeting of fluorescent proteins into the plastids. The plastid targeting ratios of tobacco SStp-20-YFP were determined using 3 cultivars of onion: Vidalia, Sunbrero and White Onion. The ratios were determined from 3 independent assays (labeled 1,2 and 3) for each cultivar. Tukey's test showed that the average ratios between experiments within the same cultivar are not different ($p>0.05$). However, when the data from the same cultivar were combined, the average ratios between cultivars were significantly different ($p<0.001$). Means ± SE are shown. $26 \leq n \leq 30$.



Supplemental Figure S2. HASA of the N-ter mutants. The combined HASA within an 8-aa window are plotted along the length of the aa sequences. The averaged HASA calculated from the 208-TP data set is shown as a reference (black line) with 95% CI (black dashed lines).

Supplemental Table S1. Sequence of oligonucleotides used in cloning and mutagenesis.

Generated construct	Oligonucleotide name	Sequence [a]
pBS-pp38-MtoA-SSF-YFP	pp38-MtoA-SSF-F	GGTGGTGCTAGCATGTTCTGGGGTCTCTGGCCTTGGGCGGCTTCTAGC
pBS-pp9-MtoA-SSF-YFP	pp9-MtoA-SSF-F	GGTGGTGCTAGCATGTGGATCTTCCCTTGGATTCAACTTGCGGCTTCTAGC
pBS-PepG-MtoA-SSF-YFP	PepG-MtoA-SSF-F	GGTGGTGCTAGCATGGGTTGGTATGGTTTCCGTCATCAGAACTGCGCGGCTTCTAGC
pBS-V10-MtoA-SSF-YFP	V10-MtoA-SSF-F	GGTGGTGCTAGCATGTTCTACCAACTTGCTAAGACCTGTCCAGTTGCGGCTTCTAGC
pBS-DRC8-MtoA-SSF-YFP	DRC8-MtoA-SSF-F1	GGACCAAGAGGTCATTTCTACGATGCAGCGGCTTCTAGC
	DRC8-MtoA-SSF-F2	GGTGGTGCTAGCATGTACCTTGTTGGACCAAGAGGTCATTTCTACG
pBS-A6R-MtoA-SSF-YFP	A6R-MtoA-SSF-F	GGTGGTGCTAGCATGGCCAGCCATCTGGGTCTGGCCCGTGCGGCTTCTAGC
pBS-HbS-MtoA-SSF-YFP	HbS-MtoA-SSF-F	GGTGGTGCTAGCATGGTGCATCTGACCCCGGTGGAAAAAGCGGCTTCTAGC
pBS-np09-MtoA-SSF-YFP	np09-MtoA-SSF-F	GGTGGTGCTAGCATGCGTGTTGATCCGGTTGTTGCTTTCGCGGCTTCTAGC
pBS-HA-MtoA-SSF-YFP	HA-MtoA-SSF-F	GGTGGTGCTAGCATGTACCCGTACGATGTTCCGGACTACGCAGCGGCTTCTAGC
pBS-pp38-MtoA-SSR-YFP	pp38-MtoA-SSR-F	GGTGGTGCTAGCATGTTCTGGGGTCTCTGGCCTTGGGCGTGTAAGGTACG
pBS-pp9-MtoA-SSR-YFP	pp9-MtoA-SSR-F	GGTGGTGCTAGCATGTGGATCTTCCCTTGGATTCAACTTGCGTGTAAGGTACG
pBS-PepG-MtoA-SSR-YFP	PepG-MtoA-SSR-F	GGTGGTGCTAGCATGGGTTGGTATGGTTTCCGTCATCAGAACTGCGCGTGTAAGGTACG
pBS-V10-MtoA-SSR-YFP	V10-MtoA-SSR-F	GGTGGTGCTAGCATGTTCTACCAACTTGCTAAGACCTGTCCAGTTGCGTGTAAGGTACG
pBS-DRC8-MtoA-SSR-YFP	DRC8-MtoA-SSR-F1	GGACCAAGAGGTCATTTCTACGATGCAGCGTGTAAGGTACGTGG
	DRC8-MtoA-SSR-F2	GGTGGTGCTAGCATGTACCTTGTTGGACCAAGAGGTCATTTCTACG
pBS-A6R-MtoA-SSR-YFP	A6R-MtoA-SSR-F	GGTGGTGCTAGCATGGCCAGCCATCTGGGTCTGGCCCGTGCGTGTAAGGTACGTGG
pBS-HbS-MtoA-SSR-YFP	HbS-MtoA-SSR-F	GGTGGTGCTAGCATGGTGCATCTGACCCCGGTGGAAAAAGCGTGTAAGGTACGTGG
pBS-np09-MtoA-SSR-YFP	np09-MtoA-SSR-F	GGTGGTGCTAGCATGAGAGTTGATCCAGTTGTGGCTTTCGCGTGTAAGGTACGTGG
pBS-HA-MtoA-SSR-YFP	HA-MtoA-SSR-F	GGTGGTGCTAGCATGTACCCATACGATGTTCCTGACTACGCAGCGTGTAAGGTACGTGG
pBS-FlipN10SSF-YFP	Flip-SSF-F	GGTGGTGCTAGCATGGTTGCTTCCTCCAGCATCATGAGCGCAACCACTGTTTCCCGTGC
pBS-FlipN10FDF-YFP	Flip-FDF-F	GGTGGTGCTAGCATGGTTTCTCTGACTTCCCTGACCTCTGCCTCTGCTTCTCTGCTGC
pBS-ScrambleN10SSF-YFP	Scramble-SSF-F	GGTGGTGCTAGCATGGTTTCTTCAGCAATCAGCGCATCCACCACTGTTTCCCG
pBS-ScrambleN10FDF-YFP	Scramble-FDF-F	GGTGGTGCTAGCATGTCTTCGCTGACCGTGACCCTGGCATCTTCTGCTTCTCTGCTGC
All of pBS construct	nos-R	CTTAACGTAATTCAACAGAA
pET-NheI-YFP	XbaI-RBS-NcoI-F	CTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATGCTAGCC
	XbaI-RBS-NcoI-R	CATGGGCTAGCATCTCCTTCTTAAAGTTAAACAAAATTATTT
pET-m20-YFP	m20-NdeI-F	GGTGGTCATATGCAGGTGTGGCCACC
	T7ter	GCTAGTTATTGCTCAGCGG
pET-m20-YFP6xHis	6xHis-F	GTACAAGGGCAGCCATCACCATCACCATCACTAAC
	6xHis-R	TCGAGTTAGTGATGGTGATGGTGATGGCTGCCCTT
pET-30a-prSSU	NheI-prSSU-Nt-F	GGTGGTGCTAGCATGGCTTCCTCAGTTCTTTCC
	BsrGI-prSSU-Nt-R	GGTGGTTGTACAGGTAGCCTTCTGGCTTGTAGG
pET-30a-prSSU-3xHAH	T7pro	TAATACGACTCACTATAGGGG
	1xHA-6xHis-R	GGTGGTCTCGAGGTAATCTGGAACATCGTATGGGTAGCCGGAGTAGCCTTCTGGCTTGTA
	2xHA-6xHis-R	GGTGGTCTCGAGATAGTCCGGGACGTCATACGGATAGCCAGCGTAATCTGGAACATCGTA
	3xHA-6xHis-R	GGTGGTCTCGAGGTAGTCTGGAACATCGTACGGGTAGGAACCTGCATAGTCCGGGACGTC
pET-His-mCSS1	NdeI-His-mCSS1-F	GGTGGTCATATGCACCATCATCATCATCATTCTTCTGGTGTCAGTGAGAAAGTCGTTG
	NotI-mCSS1-R	GGTGGTGCGGCCGCTCATTTAGAGTCTGTAAAATC
pET-His-mCSS1-mut	L167-F	CGGCAACGTTAAACTGGATTGTCCTGCTATCG
	L270-F	CTTTTGATGTCTCAGTGCTGGAGGTTGGTGACGGAG
	L373-F	GAAGAGCTGTGTTCAGATCTGCTTGACAGACTTAGGACAC
	L652-F	CCTTGGCTGCCCTGAACCAGGAAGTTATGCAG

[a] The restriction sites are underlined.

Supplemental Table S2. H70BS clustering and TargetP prediction of the 208-TP dataset.

Hsp70 cluster group	SWISS-PROT ID	Lee el at. (2008) Group	TargetP prediction		
			Localization	Reliability class	TP length
1	Q9SUI7	None	Chloroplast	3	44
1	Q8W0Y8	None	Chloroplast	2	49
1	Q949Y5	None	Chloroplast	3	18
1	Q9XI84	DnaJ-J8	Chloroplast	1	57
1	P27521	PORA	Chloroplast	2	49
1	P52032	None	Chloroplast	1	72
1	Q9LYU9	DnaJ-J8	Chloroplast	3	80
1	Q9SAG8	DnaJ-J8	Chloroplast	1	46
1	Q41932	None	Chloroplast	3	48
1	Q39195	DnaJ-J8	Chloroplast	1	69
1	P93009	RbcS	Chloroplast	3	70
1	P25856	PORA	Chloroplast	2	45
1	P10797	RbcS	Chloroplast	3	54
1	Q96242	BCCP	Chloroplast	2	32
1	P25697	DnaJ-J8	Chloroplast	3	54
1	O22170	None	Other	2	-
1	Q9C5W3	None	Chloroplast	2	61
1	O49347	Cab	Chloroplast	2	50
1	O22886	None	Chloroplast	2	35
2	O24621	BCCP	Chloroplast	1	32
2	Q9STE8	DnaJ-J8	Chloroplast	1	79
2	Q9SCY0	DnaJ-J8	Chloroplast	5	52
2	Q9FKP0	None	Secretory Pathway	5	16
2	O22056	GLU2	Chloroplast	3	39
2	Q93WC9	None	Other	4	-
2	P82538	None	Chloroplast	1	74
2	Q949X0	TOCC	Chloroplast	2	53
2	Q9SI53	TOCC	Mitochondrion	5	76
2	Q0WNZ5	None	Chloroplast	5	33
2	Q8L493	RbcS	Chloroplast	2	57
2	Q9ZR03	None	Chloroplast	3	50
2	Q9C642	None	Chloroplast	2	48
2	P25702	None	Chloroplast	4	51
2	P25701	DnaJ-J8	Chloroplast	2	51
2	Q8LPN3	BCCP	Chloroplast	1	45
2	Q9S7N7	None	Chloroplast	1	59
2	Q9SR43	DnaJ-J8	Chloroplast	2	45
2	Q9SYI0	TOCC	Chloroplast	4	61
2	Q9FYC2	RbcS	Chloroplast	1	49
3	Q96291	BCCP	Chloroplast	1	83
3	O24600	None	Chloroplast	1	95
3	P04777	None	Chloroplast	3	23
3	Q8LCA1	GLU2	Chloroplast	1	45
3	Q9LS03	None	Chloroplast	1	78
3	Q93ZB2	None	Secretory Pathway	1	16
3	Q8S9K3	Cab	Chloroplast	4	33
3	P10796	RbcS	Chloroplast	3	54
3	P10798	RbcS	Chloroplast	4	54
3	P10795	RbcS	Chloroplast	3	54
3	Q9SRL5	RbcS	Chloroplast	2	44
3	Q9FYL3	PORA	Chloroplast	3	45
3	P50318	RbcS	Chloroplast	1	95
3	Q9SMW0	None	Chloroplast	3	49
3	Q93ZC5	DnaJ-J8	Chloroplast	1	52
3	Q93W77	RbcS	Chloroplast	1	69
3	Q39101	RbcS	Chloroplast	2	47
3	O24629	None	Other	5	-
3	Q9SUI5	DnaJ-J8	Chloroplast	4	32
3	Q9SKP6	DnaJ-J8	Chloroplast	2	58
3	Q9SUI4	RbcS	Chloroplast	2	50
3	Q39194	RbcS	Chloroplast	3	44
3	Q9LQK7	BCCP	Chloroplast	2	36
3	Q02166	None	Chloroplast	2	63
3	O78310	None	Chloroplast	1	61
3	P04778	None	Chloroplast	3	23
3	Q9FUZ2	RbcS	Chloroplast	5	56
3	Q3EAJ6	Cab	Chloroplast	4	35
4	Q9LDH1	None	Chloroplast	3	54
4	Q9LTF4	None	Other	5	-
4	Q9ZS97	None	Chloroplast	1	63
4	Q9M7I7	None	Chloroplast	4	47
4	P57720	RbcS	Chloroplast	2	39
4	P46248	RbcS	Chloroplast	4	41
4	Q9SQK3	PORA	Chloroplast	1	39
4	P82658	None	Chloroplast	4	32
4	Q8LBP4	None	Chloroplast	2	55
4	P25851	RbcS	Chloroplast	1	57
4	Q9FGS4	DnaJ-J8	Chloroplast	1	69

Supplemental Table S2. (continue)

Hsp70 cluster group	SWISS-PROT ID	Lee el at. (2008) Group	TargetP prediction		
			Localization	Reliability class	TP length
4	Q9LER7	Cab	Chloroplast	1	64
4	P46644	None	Chloroplast	4	64
4	P21238	None	Chloroplast	2	45
4	Q05753	RbcS	Chloroplast	4	36
4	Q9LS01	GLU2	Chloroplast	1	56
4	Q9SA14	DnaJ-J8	Mitochondrion	5	35
4	Q944G9	None	Chloroplast	2	46
4	Q9C5U8	RbcS	Chloroplast	4	29
4	O48782	None	Chloroplast	1	54
4	Q39199	DnaJ-J8	Chloroplast	2	51
4	Q39002	None	Chloroplast	5	21
4	O80575	None	Chloroplast	2	68
4	Q9M5K4	None	Chloroplast	1	23
4	O82499	BCCP	Mitochondrion	2	46
4	Q94FY7	TOCC	Chloroplast	1	98
4	Q9XFH8	BCCP	Chloroplast	2	57
4	Q8W033	BCCP	Other	5	-
4	Q9LYN2	RbcS	Chloroplast	1	48
4	Q9S720	None	Chloroplast	2	26
5	Q9XF89	None	Chloroplast	2	48
5	Q9S714	None	Chloroplast	1	46
5	Q3E9T1	None	Chloroplast	2	33
5	Q94AY1	None	Chloroplast	5	74
5	Q9SRN1	None	Other	4	-
5	Q3EA16	RbcS	Other	4	-
5	P81760	GLU2	Chloroplast	4	33
5	Q9S831	None	Chloroplast	2	44
5	Q42029	None	Chloroplast	2	31
5	Q9ZNZ7	GLU2	Chloroplast	2	62
5	Q42536	PORA	Chloroplast	1	53
5	Q9ZU32	Cab	Mitochondrion	3	26
5	Q42588	Cab	Other	2	-
5	Q948R9	None	Chloroplast	2	47
5	Q00218	RbcS	Chloroplast	4	47
5	Q8LEB5	None	Other	1	-
5	O82730	None	Other	3	-
5	Q9S841	None	Chloroplast	3	28
5	Q01908	None	Chloroplast	1	42
5	P21218	PORA	Chloroplast	1	43
5	P10896	DnaJ-J8	Chloroplast	1	58
5	Q5XET4	PORA	Chloroplast	2	60
5	Q84JH7	None	Chloroplast	2	46
5	Q9XIK3	None	Chloroplast	1	55
5	Q9XF91	DnaJ-J8	Chloroplast	5	59
5	Q39161	None	Chloroplast	3	25
5	O04921	None	Chloroplast	1	49
5	Q9T0P4	GLU2	Chloroplast	2	72
5	P69834	DnaJ-J8	Mitochondrion	5	92
5	O64903	None	Chloroplast	1	62
5	O22160	None	Chloroplast	1	34
6	O04130	None	Chloroplast	3	49
6	O48741	PORA	Chloroplast	3	66
6	Q9LD95	None	Chloroplast	1	55
6	Q9SW33	None	Chloroplast	4	21
6	O22773	None	Chloroplast	1	38
6	Q9M401	PORA	Chloroplast	2	60
6	Q9CAK8	RbcS	Chloroplast	1	52
6	P92935	None	Mitochondrion	5	80
6	Q84W65	None	Chloroplast	2	66
6	P46310	DnaJ-J8	Chloroplast	2	81
6	Q9LW57	GLU2	Chloroplast	1	72
6	Q9MBA1	None	Chloroplast	2	36
6	Q9XF88	None	Chloroplast	2	39
6	Q9SAK2	BCCP	Chloroplast	3	29
6	Q9SX22	None	Chloroplast	2	56
6	O49292	None	Chloroplast	5	52
6	O49196	None	Chloroplast	4	59
6	Q9M439	PORA	Mitochondrion	4	22
6	Q9S756	None	Chloroplast	1	52
6	Q9LR75	RbcS	Chloroplast	2	48
6	Q43316	RbcS	Chloroplast	1	86
6	Q9SI93	TOCC	Mitochondrion	5	10
6	Q9SZ52	None	Chloroplast	5	62
7	P37271	None	Chloroplast	1	70
7	Q9SEU8	None	Chloroplast	1	72
7	Q07473	None	Chloroplast	3	40
7	Q94IC9	TOCC	Mitochondrion	4	58
7	Q38802	BCCP	Chloroplast	1	60
7	Q9S7W1	None	Chloroplast	3	26
7	P42699	BCCP	Chloroplast	1	52
7	Q9SKT0	RbcS	Chloroplast	1	67
7	Q43307	BCCP	Chloroplast	2	62
7	Q42533	BCCP	Chloroplast	1	82
7	Q38933	None	Other	2	-
7	P49077	PORA	Chloroplast	2	68
7	Q66GR6	None	Chloroplast	3	75

Supplemental Table S2. (continue)

Hsp70 cluster group	SWISS-PROT ID	Lee el at. (2008) Group	TargetP prediction		
			Localization	Reliability class	TP length
7	Q8RWW6	None	Chloroplast	1	70
7	P49107	RbcS	Chloroplast	5	81
7	Q8LD49	None	Chloroplast	1	67
7	P27202	RbcS	Chloroplast	3	40
7	Q93VA3	None	Chloroplast	4	18
7	P11490	DnaJ-J8	Chloroplast	1	66
7	Q9SEU7	None	Chloroplast	1	67
7	Q9M591	Cab	Chloroplast	1	36
7	P42732	RbcS	Chloroplast	2	46
7	Q9FMT1	GLU2	Other	5	-
7	Q43349	None	Chloroplast	1	65
7	P16127	RbcS	Chloroplast	1	60
8	Q84RQ7	None	Chloroplast	2	99
8	Q93WX6	DnaJ-J8	Chloroplast	2	35
8	Q9LUD9	None	Chloroplast	3	39
8	Q9LS02	None	Chloroplast	1	77
8	Q8GXU8	None	Chloroplast	4	56
8	Q9SJE1	None	Chloroplast	2	49
8	Q9S7H1	None	Chloroplast	2	44
8	Q9LFV0	BCCP	Chloroplast	1	45
8	Q9SA56	None	Chloroplast	1	43
8	Q38854	None	Chloroplast	1	58
8	Q9S7D1	None	Chloroplast	2	58
8	Q9CA35	DnaJ-J8	Chloroplast	1	71
8	Q8W105	Cab	Chloroplast	2	90
8	Q38885	RbcS	Chloroplast	1	67
8	O23403	None	Chloroplast	1	75
8	Q9SUI6	None	Chloroplast	4	44
8	O81439	DnaJ-J8	Chloroplast	2	55
9	Q39089	None	Chloroplast	2	22
9	O50039	RbcS	Chloroplast	1	53
9	Q9XFT3	None	Chloroplast	2	44
9	O22265	RbcS	Chloroplast	1	56
9	P42762	None	Chloroplast	1	89
9	Q9SCX9	None	Chloroplast	3	32
9	O82796	GLU2	Chloroplast	4	15
9	P32068	RbcS	Chloroplast	5	60
9	Q96255	PORA	Chloroplast	1	63
9	Q9CAP8	None	Chloroplast	5	30
9	P21240	None	Chloroplast	1	53
9	Q93W20	None	Chloroplast	1	16
9	Q9SJU4	None	Chloroplast	3	10
9	P16972	None	Chloroplast	1	52
9	O04090	PORA	Chloroplast	2	69