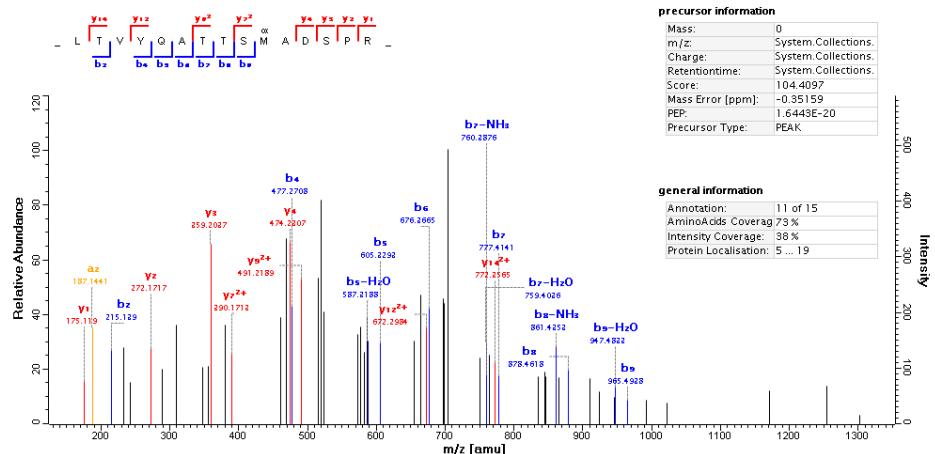


Supplementary Information

Re-annotation of the Mad1 start codon

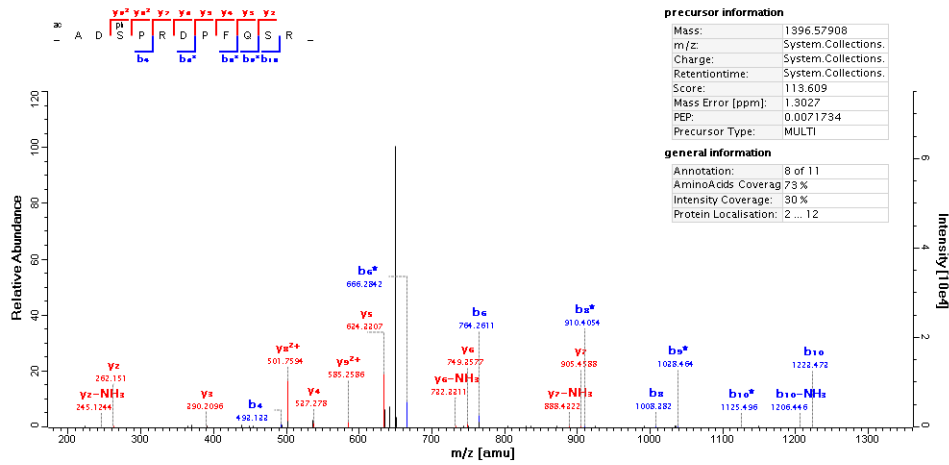
In the *Schizosaccharomyces pombe* genome database (www.pombase.org; Jan 8th, 2014), the *mad1+* coding sequence is annotated to start at position 1,277,098 on chromosome II. Alignment with the sequence of other *Schizosaccharomyces* species [5] indicates that the first 13 amino acids (MSSKLTVYQATTS) are not conserved and that it is likely that the start codon is amino acid 14, another Methionine. To corroborate this notion, we performed mass spectrometric analysis of immunopurified Mad1, digested with either Asp-N, Lys-C or Trypsin. We identified peptides supporting both the first as well as the second Methionine as start codon. In one experiment, where peptides for both termini were found (see below; LTVYQATTSM(ox)ADSPR (amino acid 5-19) supporting the first Methionine, and (ac)ADS(ph)PRDPFQSR (amino acid 15-25) supporting the second Methionine), the intensity of the second peptide was 170-times higher than of the first. Together with the phylogenetic evidence, this suggests that the second Methionine is the more prominent start codon, although the first one can be used. The annotation in the *Schizosaccharomyces pombe* genome database will be revised accordingly (Val Wood, personal communication).

Peptide 1 supporting translation from first Methionine:



a ion		b ion		y ion		y ²⁺ ion	
Δ dalton	mass	Δ dalton	mass	Δ dalton	mass	Δ dalton	mass
	86.09643	114.0913	1	L	14		
+0.107487	187.1441	-0.017483	215.139	2	T	13	1543.706
	286.2125		314.2074	3	V	12	1442.658
	449.2758	+0.147757	477.2708	4	Y	11	1343.59
	577.3344	+0.094794	605.3293	5	Q	10	1180.526
	648.3715	+0.061282	676.3665	6	A	9	1052.468
	749.4192	+0.023064	777.4141	7	T	8	981.4306
	850.4669	+0.046796	878.4618	8	T	7	880.3829
	937.4989	+0.083127	965.4938	9	S	6	779.3352
	1084.534		1112.529	10	M	5	692.3032
	1155.571		1183.566	11	A	4	545.2678
	1270.598		1298.593	12	D	3	474.2307
	1357.63		1385.625	13	S	2	359.2037
	1454.683		1482.678	14	P	1	272.1717
				15	R	0	175.119

Peptide 2 supporting translation from second Methionine:



b ion				γ ion			γ ²⁺ ion	
Δ dalton	mass	seq		Δ dalton	mass	Δ dalton	mass	
	114.05495494	1	A	10				
	229.08189797	2	D	9	1284.5368556	1284.5368556		
	396.08025679	3	S	8	1169.5099126	585.25859453	+0.0152092	
-0.1849616	493.13302064	4	P	7	1002.5115538	501.75941512	+0.0294032	
	649.23413167	5	R	6	905.45878993	905.45878993		
+0.0287813	764.2610747	6	D	5	749.3576789	749.3576789	+0.1810174	
	861.31383856	7	P	4	634.33073587	634.33073587	+0.0019668	
+0.0844223	1008.3822525	8	F	3	537.27797202	537.27797202	-0.1872127	
	1136.44083	9	Q	2	390.2095581	390.2095581	+0.1191772	
+0.1417656	1223.4728584	10	S	1	262.15098059	262.15098059	+0.0349935	
		11	R	0	175.11895218	175.11895218		

Supplementary References

5. Rhind N *et al* (2011) Comparative functional genomics of the fission yeasts. *Science* **332**: 930-936