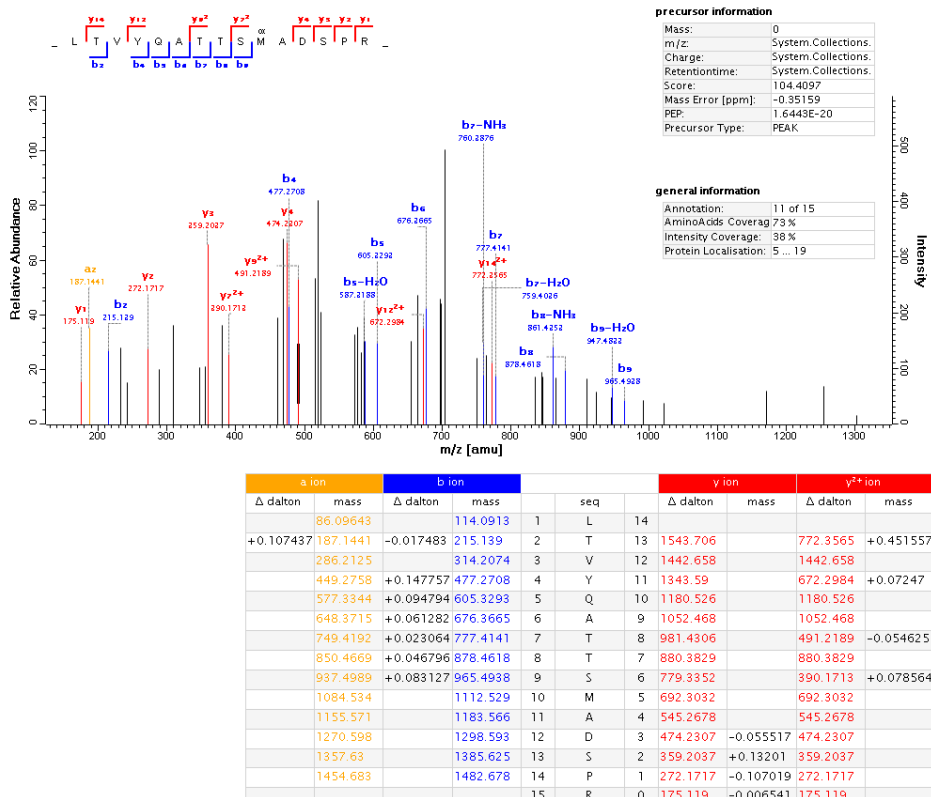


Supplementary Information

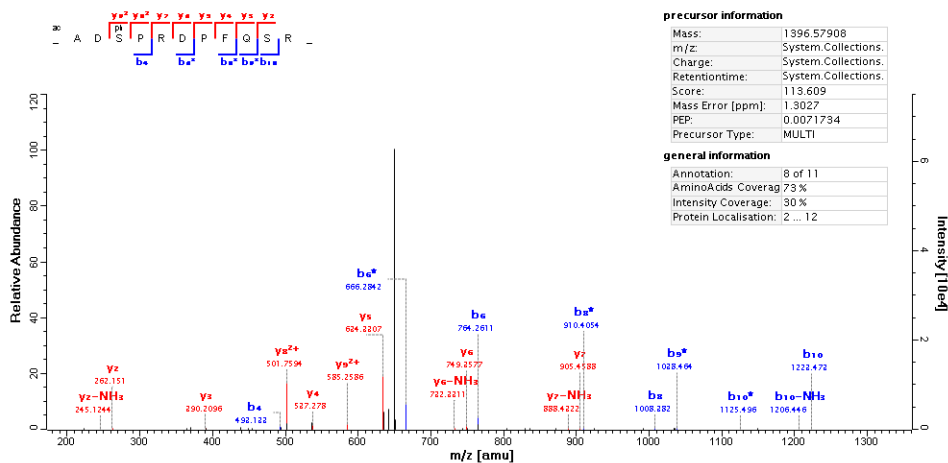
Re-annotation of the Mad1 start codon

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:



Peptide 2 supporting translation from second Methionine:



b ion				y ion			y 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	+0.1810174	749.3576789	
	861.31383856	7	P	4	634.33073587	+0.0019668	634.33073587	
+0.0844223	1008.3822525	8	F	3	537.27797202	-0.1872127	537.27797202	
	1136.44083	9	Q	2	390.2095581	+0.1191772	390.2095581	
+0.1417656	1223.4728584	10	S	1	262.15098059	+0.0349935	262.15098059	
		11	R	0	175.11895218		175.11895218	