Proteolysis of Sxa2, a carboxypeptidase involved in pheromone adaptation in yeast.

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The fission yeast Schizosaccharomyces pombe exists in one of two mating types (Plus or Minus) and conjugation is controlled by the reciprocal action of diffusible mating pheromones (for review, see 1). Exposure to pheromone causes changes that prepare the cell for mating but persistent stimulation does not induce a continuous response as the cells recover and adapt to the presence of the stimulus (2). Part of the recovery process in M-cells involves the P-factor-dependent production of Sxa2. Sequence analysis suggests that Sxa2 is a carboxypeptidase that is secreted into the medium (3) and we have shown that inactivation of P-factor by removal of the C-terminal leucine residue occurs in an Sxa2-dependent manner (4). It seems likely that Sxa2 is the enzyme that inactivates P-factor but other explanations are possible and we have sought more direct evidence. One approach involves placing the sxa2 gene under the control of the thiamine regulatable nmt1 promoter and introducing the construct into P-cells (these do not normally produce Sxa2) (5). Growing these cells in the absence of thiamine (to allow expression of the nmt1 promoter) led to the release of a carboxypeptidase that was able to degrade P-factor. We now report the analysis of this medium by gel electrophoresis (Figure 1) and present evidence that Sxa2 undergoes proteolysis during its release from the cell (Figure 2).

The most significant difference between the samples is the strong band at ~25 kDa in medium from cells expressing Sxa2 (this is also seen at a lower level when the same cells are cultured in the presence of thiamine). This protein appears to mirror the presence of the carboxypeptidase activity but it is much smaller than we would expect for Sxa2. Sxa2 contains 507 residues and, after removal of the signal sequence, would be expected to be ~54 kDa in size. This would be further increased by N-glycosylation at any of the five potential acceptor sites. Indeed, we have previously found that translation of sxa2 mRNA in a coupled translation-translocation system that supports both signal sequence cleavage and core glycosylation, generates a product of ~64 kDa (Ladds and Davey, unpublished). To resolve this issue we prepared sufficient material to allow sequencing of the ~25 kDa product and found that it is the C-terminal fragment that results from cleavage after the arginine residue at position 310 in Sxa2 (Figure 2). The enzyme most likely to be responsible for this cleavage is Krp1, an endopeptidase that cleaves secretory proteins following a pair of basic residues (6).

Cycle 1 5 10 15

-25 kDa EALDGEDIGNVFNSI

Sxa2 FSTSTSLRKR BaldGEDIGNVFNSISGC

Figure 2 Sxa2 is cleaved during secretion.

The ~25 kDa protein from Figure 1 was subjected to 15 rounds of cycle sequencing (Alta Bioscience, University of Birmingham) and compared to the sequence of Sxa2. Krp1 is a member of the kexin family of processing enzymes that are responsible for the proteolytic maturation of a variety of pro-proteins and its apparent cleavage of Sxa2 could have important implications for the maturation of this enzyme. It is possible, for example, that Sxa2 is synthesised as a precursor that is activated by cleavage following Arg310. Alternatively, the cleavage we observe has no role in the maturation of Sxa2 and represents a non-productive fate for the enzyme. Whatever the outcome, our results raise questions that need to be resolved. What, for example, happens to the N-terminal portion of Sxa2? This must be produced in equivalent amounts to the C-terminal fragment and yet we do not detect any product in the medium (we would expect a product of ~40 kDa) and can only assume that it is retained within the cell. A similar retention of the full length Sxa2 would also explain its apparent absence from the medium. Perhaps the most important question is whether the C-terminal fragment is active and it is this problem that we are now seeking to resolve.

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