CORE
Metadata, citation and similar papers at core.ac.uk
Provided by epublications@Marquette

Marquette University
e-Publications@Marquette

Biological Sciences Faculty Research and Publications

Biological Sciences, Department of

10-1-2002

ASI1, a Gene Encoding a Novel Leucine Zipper Protein, Is Induced during Development of the Macronucleus in *Tetrahymena*

Rupa A. Udani
Marquette University

Kathleen M. Karrer
Marquette University, kathleen.karrer@marquette.edu

Accepted version. *Mechanisms of Development*, Vol. 118, No. 1-2 (October 2002): 215-218. DOI.
Published under Creative Commons License Attribution-Noncommercial-NoDerivs 4.0.
NOTICE: this is the author's version of a work that was accepted for publication in *Mechanisms of Development*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *Mechanisms of Development*, VOL 118, ISSUE 2, October 2002, DOI.

ASI1, a gene encoding a novel leucine zipper protein, is induced during development of the macronucleus in *Tetrahymena*

Authors: Rupa A. Udani, Kathleen M. Karrer*

Abstract: *Sexual reproduction in the ciliate Tetrahymena follows a complex developmental program involving the sequential regulation of dozens of genes. Genes that are up-regulated during post-zygotic development in Tetrahymena were isolated by subtractive hybridization. Anlagen stage induced gene 1 (ASI1) encodes a 2.8 kb transcript that contains a single intron and is induced during macronuclear development. ASI1 is a single copy gene in both the micronucleus and the macronucleus. It encodes a 95 kDa conceptual protein with a leucine zipper near the amino terminus.*

1. Results and discussion

The ciliated protozoan *Tetrahymena* is a unicellular eukaryote that contains two nuclei, a germ line micronucleus and a somatic macronucleus. The sexual phase of the *Tetrahymena* life cycle follows a complex developmental program that occurs over a period of ~24 h. At about 12 h after mixing of two complementary mating types, the macronucleus is degraded and a new one develops from a mitotic product of the zygotic micronucleus. Macronuclear development involves massive genome reorganization including elimination, amplification, and/or methylation of specific DNA sequences (reviewed in Karrer et al., 1999).

A subtracted cDNA library was constructed to clone genes that are up-regulated during development of the new macronucleus (macronuclear anlagen). The method complements the successful approach of isolating genes based on the abundance of the protein products (Madireddi et al., 1996; Nikiforov et al., 2000; Nikiforov et al., 1999). The products of genes that produce less abundant messages are likely to encode a different class of proteins with qualitatively different roles in development.

Since *Tetrahymena* are starved to induce mating, a cDNA library from mating cells at 12 h post-mixing was subtracted with mRNA from starved cells. After several rounds of screening, 14 partial cDNA clones were isolated that were up regulated during macronuclear anlagen development. One of these clones, anlagen stage induced, gene 1 (*ASI1*) hybridized to a transcript of ~2.8 kb, whose abundance was dramatically increased in RNA from mating cells.

Genomic clones of the *Hin*dIII and *Cla*I fragments that cover the *ASI1* gene (Fig. 1A) were obtained in two inverse PCR experiments using primers P1 and P2 to amplify the *Hin*dIII fragment

and primers P2 and P3 to amplify the *Cla*I fragment. Southern analysis (Fig. 1B) indicated that *ASI1* is a single copy gene in both the micronucleus and the macronucleus. Since there are no differences in the sizes of fragments from the two nuclei, the data suggest there are no elements that undergo developmentally regulated deletions within ~1.2 kb 5′ and ~2.5 kb 3′ of *ASI1*.

Open reading frame analysis of the genomic DNA (Accession #AF435076) suggested that *ASI1* contained at least one intron. A reverse transcriptase-polymerase chain reaction (RT-PCR) experiment was performed using mRNA from mating cells at 12 h post-mixing as template and primer P4 (Fig. 1A, Table 1) for the synthesis of the first strand cDNA. Double stranded cDNA was obtained using the first strand cDNA as template and primers P5 and P6 (Table 1) for the PCR. A RT-PCR product of ~1.3 kb was obtained (Fig. 1A). The product was ~300 bp smaller than the PCR product obtained in a control experiment using macronuclear DNA as the template, suggesting that an intron of ~300 bp was removed from the primary transcript. The ~1.3 kb cDNA was cloned and sequenced in both directions. Comparison of the cDNA sequence to that of the genomic clone indicated there was an intron of 337 bp with the consensus nucleotides AG and GA at the 5′ and 3′ splice sites. The intron had a low GC content (17%), as expected for non-coding regions of *Tetrahymena* genome (Wuitschick and Karrer, 1999). In order to determine whether there were any additional introns in *ASI1*, RT-PCR was performed using primers P7 and P8 (Fig. 1A, Table 1) to obtain a 1.6 kb cDNA covering the 5′ end of the *ASI1 gene*. The presence of the intron was confirmed. No additional introns were found.

The open reading frame (ORF) of the spliced *ASI1* message is 2.53 kb. The size is in good agreement with the size of the ~2.8 kb transcript observed on Northern blots. Three additional lines of evidence supported the assignment of the proposed ORF. First, the transcription start of *ASI1* was determined, by an RNase protection experiment, to be ~160 bp upstream of the first ATG (data not shown). Second, there is an in frame TGA stop codon at position -21 from the putative initiator ATG. Third, the putative 5′ NTS is only 18% GC, as expected for non-coding DNA in *Tetrahymena*. The poly(A) addition site at the 3′ end of the mRNA is known from the structure of the original cDNA clone.

Northern blot analysis demonstrated that *ASI1* is developmentally induced (Fig. 1C). Aliquots were removed from a population of mating cells at 6, 9, and 12 h post-mixing of the two complementary mating types for isolation of poly(A)$^+$ RNA. RNA was also isolated from starved and vegetatively growing cells. Northern blots of the RNAs hybridized with a probe for *ASI1* hybridized to a single transcript of ~2.8 kb in lanes with mRNA from 6, 9, and 12 h post-mating cells. The strongest hybridization was observed with mRNA from cells at 9 and 12 h post-mixing.

The result was repeatable: *ASI1* RNA also showed maximal abundance at 9 and 12 h after pairing in cells from a different mating (data not shown). Thus *ASI1* transcripts were up-regulated in mating cells, and the RNA abundance is highest early in the development of the macronuclear anlagen.

The integrity of the mRNA in each lane was demonstrated by hybridization with control probes (Fig. 1C). The 1.4 kb transcript of the cysteine protease gene *CYP1* was detected predominantly in RNA from starved cells. Histone *H4* gene transcripts of 0.9 and 0.6 kb were found in cells engaged in DNA replication.

ASI1 encodes a novel deduced polypeptide of 95 kDa (Fig. 2). The putative gene product of 805 amino acids, Asi1p, contains a leucine zipper motif (aa 243–265). Leucine zippers are known to function in protein–protein interactions (Hurst, 1995). Thus it is likely that the Asi1p functions as a homodimer or a heterodimer during anlagen development. Many, but not all, leucine zipper proteins are transcription factors.

Asi1p does not have significant homology to any known proteins. Blast P analysis showed a low degree of homology ($6e^{-04}$) between Asi1p and a motif of unknown function in prokaryotic methyl-accepting chemotaxis proteins (Hanlon and Ordal, 1994).

Upregulation of the *ASI1* message coincides with macronuclear anlagen development involving chromosome fragmentation, DNA elimination, telomere synthesis and DNA amplification (Blackburn, 1991; Yao et al., 2002). It is reasonable to expect that Asi1p may have an enzymatic or regulatory role in one or more of these processes.

2. Materials and methods

Tetrahymena thermophila strains CU428 and CU427 were obtained from Dr Peter Bruns, Cornell University, Ithaca, New York. The strains were mated as described previously (Hamilton and Orias, 1999).

For developmental Northern blot analysis, poly(A)$^+$ RNA was isolated from aliquots of cells removed from a mass mating at various time points after mixing of the two complementary mating types. The amount of RNA loaded in the various lanes was determined by measuring the A_{260} of each sample, and checked by running an aliquot of the sample on a separate gel stained with ethidium bromide.

Acknowledgements

The authors wish to thank Dr Daniel Wexler for providing the subtracted cDNA library, and

Michael Schlappi and G. Thomas Hayman for helpful comments on the manuscript. The research was supported by grant MCB 9974885 from National Science Foundation. R.A.U. was funded in part by Marquette University Graduate Fellowship and a Richard W. Jobling Research Fellowship.

References

Blackburn, E.H., 1991. Structure and function of telomeres. Nature 350, 569–573.

Hamilton, E.P., Orias, E., 1999. Genetic crosses: setting up crosses, testing progeny, and isolating phenotypic assortants. *Tetrahymena thermophila*, Asai, D.J., Forney, J.D. (Eds.), Methods in Cell Biology, vol. 62. Academic Press, San Diego, CA, pp. 219–228.

Hanlon, D.W., Ordal, G.W., 1994. Cloning and characterization of genes encoding methyl-accepting chemotaxis proteins in Bacillus subtilis. J. Bio1. Chem. 269, 14038–14046.

Hurst, H.C., 1995. Transcription factors 1: bZip proteins. Protein Profile 2, 101–168.

Karrer, K.M., 1999. *Tetrahymena* genetics: two nuclei are better than one. *Tetrahymena thermophila,* Asai, D.J., Forney, J.D. (Eds.), Methods in Cell Biology, vol. 62. Academic Press, San Diego, CA, pp. 127–186.

Madireddi, M.T., Coyne, R.S., Smothers, J.F., Mickey, K.M., Yao, M.-C., Allis, C.D., 1996. Pdd1p, a novel chromodomain-containing protein, links heterochromatin assembly and DNA elimination in *Tetrahymena*. Cell 87, 75–84.

Nikiforov, M.A., Gorovsky, M.A., Allis, C.D., 2000. A novel chromodomain protein, Pdd3p, associates with internal eliminated sequences during macronuclear development in *Tetrahymena thermophila*. Mol. Cell. Biol. 20, 4128–4134.

Nikiforov, M.A., Smothers, J.F., Gorovsky, M.A., Allis, C.D., 1999. Excision of micronuclear-specific DNA requires parental expression of Pdd2p and occurs independently from DNA replication in *Tetrahymena thermophila*. Genes Dev. 13, 2852–2862.

Wuitschick, J.D., Karrer, K.M., 1999. Analysis of genomic G + C content, codon usage, initiator codon context and translation termination sites in *Tetrahymena thermophila*. J. Eukaryot. Microbiol. 46, 239–247.

Yao, M.-C., Duharcourt, S., Chalker, D.L., 2002. Genome-wide rearrangements of DNA in ciliates. In: Craig, N., Craigie, R., Gellert, M., Lambowitz, A.M. (Eds.). Mobile DNA II, ASM Press, Washington, DC, pp. 730–758.

Notes

- Department of Biological Sciences, Marquette University, Milwaukee, WI 53201-1881, USA
- *Corresponding author. Tel.: 11-414-288-1474; fax: 11-414-288-7357. E-mail address: kathleen.karrer@marquette.edu (K.M. Karrer).

Appendix

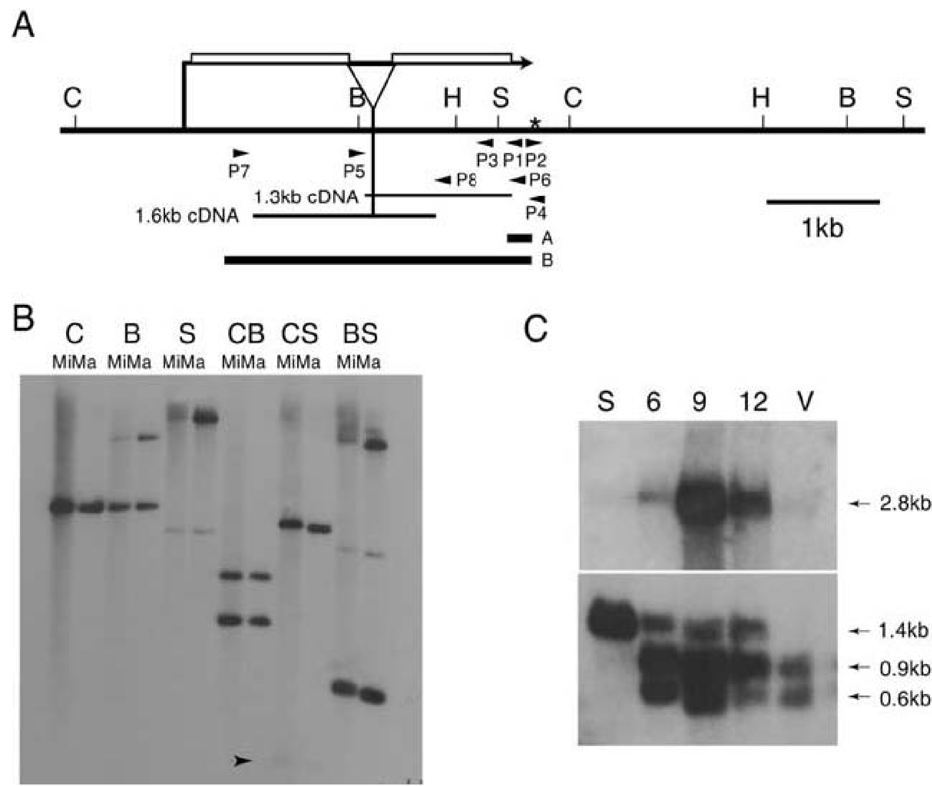
Table 1

***ASI1* PCR primers**[a]

Primer	Sequence	Location
P1	TATTGTTCGATGTGGTTCATGAAC	2883R
P2	CTTCCCAATTTCTAAATGCCC	2885F
P3	ATCCATAATCTACCAGCTGTTC	2666R
P4	ACAAACCCTTTAAATATGTCTC	2928R
P5	TGCTCTCAGAGGGAAAGACAAG	1344F
P6	GGGCATTTAGAAATTGGGAAG	2905R
P7	TCTCTTGAATCCAGGATTAAAGG	203F
P8	AGCAGAACCAGCAACTTTAAAT	2170R

[a] F, forward primers; R, reverse primers.

Figure 1



(A) Genomic restriction map of the *ASI1* gene. Angled arrowhead depicts the orientation of the *ASI1* transcript. Open boxes represent the open reading frames separated by a 337 bp intron. The line extending from the inverted triangle represents the site of joining of the two exons. Small arrowheads indicate the location of the primers P1–P8 employed for inverse PCR and RT-PCR. Filled boxes A and B denote hybridization probes for the Southern blot in B. The asterisk represents the site of poly(A) tail addition to the *ASI1* mRNA. C, *Cla*I; B, *Bgl*II; H, *Hin*dIII; S, *Sac*I. (B) Southern analysis of micronuclear and macronuclear DNA. The arrow indicates a small *Cla*I-*Sac*I fragment whose hybridization signal is more intense in longer exposures. C, *Cla*I; B, *Bgl*II; S, *Sac*I. Mi and Ma indicate lanes containing micronuclear and macronuclear DNA, respectively. (C) Northern analysis of *ASI1* transcripts. The upper panel shows hybridization of the 1.6 kb PCR product (Fig. 1A) to a developmentally regulated transcript of ~2.8 kb. The source of the poly(A)+ RNA in each lane was: S, starved cells; 6, 9 and 12, mating cells at the corresponding number of hours after mixing of two complementary mating types; V, vegetatively growing cells. The lower panel shows control Northern blots. The cysteine protease gene *CYP*1 probe hybridized to a message of ~1.4 kb that is induced in starved cells. The hybridization in lanes containing mRNA from mating cells is likely to be due to the presence of starved non-maters in the mating population. There was no hybridization of this probe to mRNA from vegetatively growing cells. A histone H4 probe hybridized to transcripts of the *H4I* and *H4II* genes at ~0.9 and 0.6 kb. Histone H4 messages are present only in cells undergoing DNA replication. Thus there was no hybridization in lanes containing mRNA from starved cells. The blots were exposed for 24 h at -80°C with one intensifying screen.

Figure 2

The genomic sequence of *ASI1*

```
TAGCAAATTTTAATTATTGTTACAAGATTTTTACTATTTAATCTTAAAAAAATTAATAGATAGGCAATCAAATTACCAAATTTTTTAAGA   90
GATACAAACTAAGAATTAAGCTTATTCTATTATAATTTAATCTGTTGAAGTAACAGAAATTTAATAATGCAAGATACCTTAGATGCTAAA  180
                                *                              M  Q  D  T  L  D  A  K

AACAGCTATCTTAATTTACTTTCTCTGAATCCAGGATTAAAGGAATTTCAAATATAAAAAATGCTAATCTGCTTAAAAATAATTAAGCT  270
 N  S  Y  L  N  F  T  F  S  E  S  R  I  K  G  I  S  N  I  K  N  A  N  L  L  K  N  N  Q  A

ACAAGTATTATGGAATTAGATCAAACACTTCAAGCATTCCATAAAGCGTCACATGGTAATTTGAAGAATATAATCAAGAATGCTGAAATA  360
 T  S  I  M  E  L  D  Q  T  L  Q  A  F  H  K  A  S  H  G  N  L  K  N  I  I  K  N  A  E  I

TGCGCATTAGATCAATATAGTATCCAAAACAGATAATAAAATTTTGAAACTATAACATTAAATAAAAACAAATCTTAAAGTAGAGTTAGC  450
 C  A  L  D  Q  Y  S  I  Q  N  R  Q  Q  N  F  E  T  I  T  L  N  K  N  K  S  Q  S  R  V  S

AAAACTGAAATTAGGAGTCTAAATCCACTTTCATGCAATGAATCTGAGAAAACAAAGTACTTTGAAAATGAATAGGATAATTTATGTAAT  540
 K  T  E  I  R  S  L  N  P  L  S  C  N  E  S  E  K  T  K  Y  F  E  N  E  Q  D  N  L  C  N

TTAGAAGATGAATACGAAAATGAGAGTAATTATCATAAAAATAATTTAGAGTCATAGTAAAGTAGCACACAGCTATTTGCAAATATTAGG  630
 L  E  D  E  Y  E  N  E  S  N  Y  H  K  N  N  L  E  S  Q  Q  S  S  T  Q  L  F  A  N  I  R

CAAACTAAGATATTTTAATAAATGAGAGACTATGTACGAACTCTAAAGAAGAATAAAATTATCTAAAAATTTTGGTTTTGTTAAATAAAT  720
 Q  T  K  I  F  Q  Q  M  R  D  Y  V  R  T  L  K  K  N  K  I  I  Q  K  F  W  F  C  Q  I  N

AGCAGATGGACTCGATAAAAGTAGCTTAATGTTTCATTCTTAATGATGAGTATTTGTATAATTAGTATTTTATTGATTTGCTGGGTTCTA  810
 S  R  W  T  R  Q  K  Q  L  N  V  S  F  L  M  M  S  I  C  I  I  S  I  L  L  I  C  W  V  L

CTTTTATTCTTTATGTATCGTATTTTTGATGACACTTACTATCCAAATTACTCTGAAAGTGAAAAAAATTAACTAATTTATACCTTATCC  900
 L  L  F  F  M  Y  R  I  F  D  D  T  Y  Y  P  N  Y  S  E  S  E  K  N  Q  L  I  Y  T  L  S

TCTTTACAAGAATCATTAGTTAGTCTCTTAAGAAGAACTGACCATCTTCACCAAGTCGCCATTAGCATTTATTAATCACAATAAAAAGGT  990
 S  L  Q  E  S  L  V  S  L  L  R  R  T  D  H  L  H  Q  V  A  I  S  I  Y  Q  S  Q  Q  K  G

CCCAAAAACCCTTTTTACGATATTAATTTTGATTCTCATATATTAAAAGGAAAACAAAATTACATTTCAATAAACTAAGATGTGTCACCT 1080
 P  K  N  P  F  Y  D  I  N  F  D  S  H  I  L  K  G  K  Q  N  Y  I  S  I  N  Q  D  V  S  P

CAATTTAATGATACTTTAGGCTTTTACTTTTAAAATGTTGATACTCCAATTACATATGATTTTTTTAATGGGCTACGTTTATAGACCAA 1170
 Q  F  N  D  T  L  G  F  Y  F  Q  N  V  D  T  P  I  T  Y  D  F  F  Q  W  A  T  F  I  D  Q

AGAAATTACACTCATATTACACCAGAATCTTAGAAGATTCTTGATGACTTTGATATACTTAGTTATTTCATAAAAAACTTAATTGCTAAT 1260
 R  N  Y  T  H  I  T  P  E  S  Q  K  I  L  D  D  F  D  I  L  S  Y  F  I  K  N  L  I  A  N

GCAAAATTTTCATCTTCTTACTCTTATTATTGTGGATTTGAAACAGATGAATTAGTTGTCTATTATCCAGCTGGTTTATAAAATGCTCTC 1350
 A  K  F  S  S  S  Y  S  Y  Y  C  G  F  E  T  D  E  L  V  V  Y  Y  P  A  G  L  Q  N  A  L

AGAGGGAAAGACAAGGTACCTGATTTTAGTTTTCACCCAAATTAGAGGGATTGGTACTCCTAAATAATTAAAAATAACCTATAGTATACA 1440
 R  G  K  D  K  V  P  D  F  S  F  H  P  N  Q  R  D  W  Y  S  Q  I  I  K  N  N  L  Q  Y  T

AATCAAACTTACTTTACTCTCCCCTATAAAGATTAAATAACTTAGCAATATATAATGACCATGTCTGTTGCTCTTCGTGATTAGgtaaaa 1530
 N  Q  T  Y  F  T  L  P  Y  K  D  Q  I  T  Q  Q  Y  I  M  T  M  S  V  A  L  R  D  Q

gatctatttaatattttttatttttttgtcaatatttttttgtaaaaagatatttcacctgaatcaggttaaaaaccttctaataaata 1620
tcctgcagaaacaataaatttaattattaaatcaaattctaaattctgaattgatttttctatcaatcagaatcaaaaattatttattac 1710
caaatttcaaaacaaaaataataaattaattaatttactagtgtaacattgaacaatgagttatttatttatttattgatttaatgaatg 1800
ataaaaaacaaaatattcttaagcttttcccttaagacaattattatttaattttaaaaagGATGGAAAATTTTAAGGTGTTTGTGCTAC 1890
                                                              D  G  K  F  Q  G  V  C  A  T

TGACTATTACTTAGATAAATTTGACAAATATATAAATTCAATAGGCATAAATGAGTATTCTATTATGGCTCTGATAGATGCCAGTAACGA 1980
 D  Y  Y  L  D  K  F  D  K  Y  I  N  S  I  G  I  N  E  Y  S  I  M  A  L  I  D  A  S  N  D

TAATAGTATTATTCTAACATAAAACAGTAGTATTATTAACTTTACTCCATAACAGTTCTTACAATTTTCTCCCTCTTAATGGACAGATAT 2070
 N  S  I  I  L  T  Q  N  S  S  I  I  N  F  T  P  Q  Q  F  L  Q  F  S  P  S  Q  W  T  D  M

GAAATAGCAAATACTTTAAACATTTAACAATAATACTAACAGCTTAAATTCTTATAGCTATTTTTAAGACATTTAAGGATTTAAAGTTGC 2160
 L  K  Q  Q  Q  T  F  N  N  N  T  I  N  S  L  N  S  Y  S  Y  F  Q  D  I  Q  G  F  K  V  A

TGGTTCTGCTTTTAAATCCAAAAATTCAAATGATCAAACCTTTATTTTATTAGTGAAGGAATCTACAAATTAGTTCGAAAGACTTTACGA 2250
 G  S  A  F  K  S  K  N  S  N  D  Q  T  F  I  L  L  V  K  E  S  T  N  Q  F  E  R  L  Y  D

TACAATAATTTAAAGAGTTTAAGATAAATATATTAAACCGAATATTAACTTTATAATCGCATTTCTAGTGATAAGCTTTGCTCACATTAT 2340
 T  I  I  Q  R  V  Q  D  K  Y  I  K  P  N  I  N  F  I  I  A  F  L  V  I  S  F  A  H  I  I

TTATTGCTTAATAATGAGTTATTATGTTACTCAACCTTATAGAAAAATAAAGTAGATATCTAACTAAATTACATAGAATTTAAAGGCATC 2430
 Y  C  L  I  M  S  Y  Y  V  T  Q  P  Y  R  K  I  K  Q  I  S  N  Q  I  T  Q  N  L  K  A  S

AAATTAAATAGGATCACTTGATTCATACTTAAACACTTTAGATTTTAAAAGTGATAGAAATTTATATATTCTAGTCAAGTCTGCAAAAAA 2520
 N  Q  I  G  S  L  D  S  Y  L  N  T  L  D  F  K  S  D  R  N  L  Y  I  L  V  K  S  A  K  K

AATGGTTTTAAAAATATAAAAAGCCAGTACTAAGAAAATAATCGAAAATTAAAAATATTATCATGCTTATCATTATGAGTAAAATGAGTA 2610
 M  V  L  K  I  Q  K  A  S  T  K  K  I  I  E  N  Q  K  Y  Y  H  A  Y  H  Y  E  Q  N  E  Y

TTTTGATAGTCAAATGGAGTGGAGAGATGAAATAGAACAGCTGGTAGATTATGGATTATCAAAAAACTTTAATTCACTCCCTACAAACCT 2700
 F  D  S  Q  M  E  W  R  D  E  I  E  Q  L  V  D  Y  G  L  S  K  N  F  N  S  L  P  T  N  L

AGAATTCTTCAACGATTGTGAATCAAAAATCACTGAGCTCAGAGATAACTCTCCCTTTGCTATTGTCAACAATAAATAAGAATCGCTAAA 2790
 E  F  F  N  D  C  E  S  K  I  T  E  L  R  D  N  S  P  F  A  I  V  N  N  K  Q  E  S  L  K

AATTAAATAAAAAATACTATCTGAAGATTCATTTTGCAAATCTGTCTCTGTTCCATACATTGACTAAAAGTTCATGAACCACATCGAACA 2880
 I  K  Q  K  I  L  S  E  D  S  F  C  K  S  V  S  V  P  Y  I  D  Q  K  F  M  N  H  I  E  Q

ATATCTTCCCAATTTCTAAATGCCCTGAGACATATTTAAAGGGTTTGTAAATAAATAAATTTATTTTATTAAAAATATAATCAATCAATA 2970
 Y  L  P  N  F  Q  M  P  *

TAATTAAATTATTCAAACAAGCACTAATTAATATATAGAATCTTTAAAAATATGTATATTATGTTAAATTAAAAATTTTTATAATTTATG 3060
AAAGGCAAAATAA
```

The nucleotide at position +1 represents the approximate start of the *ASI1* transcript. The asterisk at position +137 indicates a stop codon in frame with the putative initiator ATG. The leucine zipper motif is highlighted in gray with each seventh leucine residue double underlined. Lower case letters represent the 337 bp intron. The asterisk at the end of the sequence denotes the stop codon. The double underlined 37 nucleotides near the end of the sequences indicates a consensus poly(A) addition sequence. The underlined amino acids represent the region of Asi1p similar to the methyl-accepting chemotaxis proteins.