

Research Article

The 3' terminal sequence of the inosine monophosphate dehydrogenase gene encodes an active domain in the yeast *Schizosaccharomyces* pombe

Semian Karaer¹, Aysegül Topal Sarikaya^{1,2}, Nazli Arda^{1,2} and Güler Temizkan^{1,2}

Abstract

The gua1 gene encoding inosine monophosphate dehydrogenase (IMPDH), which catalyses the first step in $de\ novo$ biosynthesis of guanosine monophosphate (GMP), was cloned in the yeast $Schizosaccharomyces\ pombe$ by functional complementation of a gua1ura4-D18 mutant strain from a $S.\ pombe$ DNA genomic library. Complementation analysis revealed a 1.2 kb fragment which segregation analysis confirmed did not code for a suppressor gene. Only 446 nucleotides of the gua1 gene encoding the IMPDH C-terminal residues were found within this 1.2 kb sequence (GenBank, AJ293460). The comparison of this wild-type fragment with the same fragment from the gua1ura4-D18 mutant revealed that there was a point mutation at position 1261 (guanine \rightarrow adenine) from the 5' end, corresponding to the amino acid residue 421 (glycine \rightarrow serine) of the enzyme. Dot and Northern analyses showed that the gua1 gene was expressed in transformants as well as in the wild-type and the gua1ura4-D18 mutant, but enzyme activity was only detected in wild-type and transformant cells. It seems likely that a 446 bp fragment from the 3' end of the gua1 gene abolished the point mutation in the mutant strain, suggesting that this fragment participates in the sequences encoding the active domain of IMPDH in $S.\ pombe$.

Key words: Schizosaccharomyces pombe, inosine monophosphate dehydrogenase, gua1 gene, purine nucleotide pathway. Received: July 4, 2005; Accepted: December 16, 2005.

Introduction

The de novo biosynthesis of purine nucleotides is essentially the same in all groups of organisms studied so far (Henderson and Paterson, 1973; Michal, 1999) and inosine monophosphate dehydrogenase (IMPDH; E.C.1.1.1.205) is one of the key enzymes for the regulation of this pathway. This enzyme catalyses the NAD-dependent conversion of inosine monophosphate (IMP), which serves as a branch point between the adenine and guanine specific branches, to xanthosine monophosphate (XMP) which is the rate-limiting step in de novo guanine nucleotide biosynthesis (Hedstrom, 1999). Inhibition of IMPDH causes a reduction in the guanine nucleotide pool with subsequent interruption of DNA and RNA synthesis which results in cytotoxicity. The reduction in guanine nucleotides also compromises the ability of G-proteins to function as transducers of intracellular signals (Manzoli et al., 1995). In-

Send correspondence to Semian Karaer. Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, 34118 Vezneciler, Istanbul, Turkey. E-mail: semka@istanbul. edu. tr.

creased IMPDH activity and consequently GMP synthesis has been shown in a variety of cancer cell lines and it appears that IMPDH may be a target for cancer chemotherapy and the development of immunosuppressive drugs (Weber, 1983). The structural and functional properties of IMPDHs from different organisms and its mode of action and inhibition have been well documented (Hedstrom, 1999) and IMPDH sequences from at least 163 organisms from bacteria to plants have been reported to GenBank (http://www.ncbi.nlm.nih.gov/sutils/blink.cgi?pid=39959).

The yeast Schizosaccharomyces pombe is an attractive model system for eukaryotic cell and molecular biology studies. This yeast is known to have 12 loci (ade1 to ade10 plus gua1 and gua2) involved in the de novo biosynthesis of purine nucleotides (Heslot, 1972). The chromosomal location of all these genes have been determined and the ade1, ade2, ade4, ade6 and ade10 genes have been cloned and sequenced (McKenzie et al., 1987; Szankasi et al., 1988; Speiser et al., 1992; Ludin et al., 1994; Liedtke et al., 1998). Pourquié (1974) conducted the first study of the genes belonging to the S. pombe guanine nucleotide biosynthesis pathway and identified two types of genetically

¹Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, Vezneciler, Istanbul, Turkey.

²Research and Application Center for Biotechnology and Genetic Engineering, Istanbul University, Vezneciler, Istanbul, Turkey.

552 Karaer et al.

unlinked auxotrophic mutants designated *gua1* and *gua2*, the *gua1* mutant having no IMPDH activity. Haploidization and tetrad analyses showed that the *gua1* gene was located at the centromeric region of chromosome II (Oraler *et al.*, 1990).

During the study described in this paper we used complementation techniques to clone a partial *gua1* gene from a *S. pombe* genomic library and, interestingly, found that the transformant containing only a 446 bp long fragment from the 3' end of the gene was able to produce an active enzyme.

Material and Methods

Strains, plasmids and growth conditions

The Schizosaccharomyces pombe wild-type strains 972h and 975h plus the IMPDH-negative gual mutant and the ura4-D18 mutant containing a full deletion of the ura4 gene (Grimm et al., 1988) were obtained from Istanbul University, Molecular Biology Laboratory collection (Address Above). All the S. pombe strains were grown using minimal media (MM) or enriched media (EM) broth or agar and sporulated in synthetic sporulation agar (SPA) as previously described by Gutz et al. (1974). The media were supplemented with guanine and uracil (50 mg/L) as required.

Two types of plasmid were used, the pUR19 yeast shuttle cloning vector and the pUC18 bacterial cloning vector. The *Escherichia coli* DH5 α was used for plasmid amplification. DNA manipulations, including plasmid preparation, subcloning, restriction mapping, agarose gel electrophoresis, and transformation and *E. coli* growth techniques were performed according to standard protocols (Sambrook *et al.*, 1989). All enzymes for restriction mapping and subcloning were obtained from MBI Fermentas (Lithuania).

Gene isolation and subcloning

The *gua1* gene was isolated by complementation using an *S. pombe* genomic library established in pUR19 (provided by Dr. Clive Price, University of Sheffield, Department of Molecular Biology and Biotechnology). To obtain the *gua1ura4*-D18 double-mutant, strains were crossed on SPA and the double-mutant selected from tetrads according to its guanine and uracil requirements. The mating type of the double-mutant was determined as described by Leupold (1970) and was transformed using 3.5 µg of DNA for each experiment (Warshawsky and Miller, 1994). Plasmids from transformants were rescued according to the protocol of Topal *et al.* (1997). To determine the smallest fragment containing the *gua1* gene, subcloning was performed in pUR19 and plasmids were transformed into the *gua1ura4*-D18 mutant strain.

Suppressor gene analysis, sequencing and RNA isolation and analysis

Randomly selected *S. pombe* transformants carrying the insert in their genome were crossed with the wild-type 975h⁺ and genotypes of the spores were determined on selective media by tetrad analysis (McKenzie *et al.*, 1987).

For sequencing, the smallest DNA fragment carrying the *gua1* gene (determined by complementation) was cloned to pUC18. Sequencing reactions of this fragment and the PCR product of the mutant allele from the *gua1* strain were performed using a Pharmacia Fluorescence Kit and a Perkin Elmer model 377 automatic DNA sequencer with a universal M13 reverse primer followed by primers, corresponding to the internal sequences of the insert. Sequence analysis of the insert was evaluated using the UWGCG (University of Wisconsin Genetic Computer Group) programs. A search of the GenBank database was made using the NCBI BLASTP 2.2.5 program (Altschul *et al.*, 1997).

Total RNA was isolated as described by Burke *et al.* (2000) and dot and Northern hybridizations performed using the DNA Labeling and Detection (DIG) Kit according to the manufacturer's instructions (Boehringer Mannheim). The RNA samples (\sim 10 μ g) were blotted on a nylon membrane (Schleicher & Schuell) for dot hybridization and the RNAs separated on 1.2 % (w/v) agarose gel containing 0.66 M formaldehyde and 0.5 μ g/mL ethidium bromide and transferred to the membrane using a capillary system. A DIG-11-dUTP labeled DNA fragment carrying the *gua1* gene was used as a probe for hybridizations.

Inosine monophosphate dehydrogenase (IMPDH) assay

We prepared S. pombe lysates according to the method of Pourquié (1974), with a slight modification. Cells were grown in EM broth to the late log phase in a rotary shaker (30 °C, 150 rpm), harvested by centrifugation for 10 min at 0 °C and 4000 x g, washed twice in distilled water, re-centrifuged. The pellet was resuspended in 1.5 mL of breakage buffer (1 M Tris-HCl, pH 8.4; 0.1 M KCl) per gram of cell wet-weight and 0.45-0.50 mm ∅ glass beads were added to just below the meniscus of the suspension and the mixture homogenized in a cell Braun homogenizer chilled with CO2 at 15 s intervals for three minutes. After cell disruption, the homogenate was clarified by ultra-centrifugation for 90 min at 4 °C and 90 000 x g and the resultant supernatant (crude extract) was used for the enzyme assay. Protein concentration of the crude extract was measured by the method of Lowry et al. (1951) and IMPDH activity was determined spectrophotometrically by monitoring the formation of NADH at 340 nm (Carr et al., 1993) by adding 0.9 mL of crude extract to 4.1 mL of reaction mixture (100 mM Tris-HCl, pH 8.0, 100 mM KCl, 3 mM EDTA, 200 µM inosine mono-

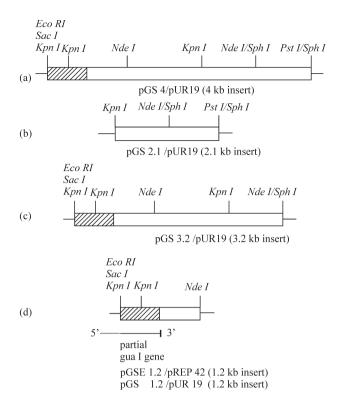


Figure 1 - The cloned 4kb *Schizosaccharomyces pombe* fragment in plasmid pUR19 containing a partial *gua1* gene (a) and three subclones (b-d). Subclone pGS1.2 contained the shortest (1.2 kb) DNA fragment that complements the *gua1* mutant. The position and orientation of the partial *gua1* gene is represented by the hatched box and arrow.

phosphate (IMP) and 400 μ M NAD) and a second reaction mixture supplemented with 0.12 mM allopurinol, a potent inhibitor of IMPDH (O'Gara *et al.*, 1997). After incubation at 37 °C for 15 min, the optical density was read at $\lambda = 340$ nm (OD₃₄₀) against a blank consisting of breakage buffer minus crude extract. Enzyme activity was expressed as OD₃₄₀ per mg per mL of protein in the crude extract.

Results

Cloning of the qua1 gene

The *S. pombe gualura4*-D18 strain was transformed with a pUR19/Sau3A genomic library and cells exhibiting gua⁺ura⁺ phenotype were selected. Five positive colonies were obtained from approximately 3.6 x 10⁴ transformants. The plasmids from these transformants were isolated and amplified in *E. coli*, and their sizes were determined by restriction analysis. These plasmids were named according to the size of insert as (in sequence) pGS9.3, pGS7.5, pGS4.8 (two plasmids of similar size) and pGS4. The plasmid used for further studies was pGS4. To determine the location of the gual gene within the 4 kb insert, the gualura4-D18 double-mutant was transformed with several subcloned DNA fragments of variable lengths. The smallest fragment complementing the mutation was a 1.2 kb *KpnI/NdeI* fragment (Figure 1). Then, this fragment was cloned into

pUR19. New construct was designated as pGS1.2, and the positive *S. pombe* transformants designated as SG1.

Suppressor gene analysis

Suppressor gene analysis was performed to determine whether the 1.2 kb *KpnI/NdeI* fragment contained the *gua1* gene itself or an extragenic suppressor. The segregation of the *gua1* gene localized on chromosome II (Oraler *et al.*, 1990) with the *ura4* gene localized on chromosome III (Gygax and Thuriaux, 1984) was investigated. To achieve this the wild-type *S. pombe* 975h⁺ strain was crossed with the stable SG1 *S. pombe* transformant, which carries the *gua1* gene integrated into its genome. We analyzed 556 spores from 139 tetrads for guanine and uracil auxotrophy and found that 43 spores were *ura* and all of them were *gua*⁺. This indicated that the insert had integrated precisely at the *gua1* locus and that the complementation did not originate from a suppressor gene, but instead represented a cloned functional *gua1* gene.

Sequence analysis

We cloned a 1.2 kb KpnI/NdeI DNA fragment carrying the gual gene into pUC18 and the new construct (pGSC1.2) was transformed in E. coli DH5 α as described above. The DNA sequence of the fragment was analyzed and compared with the sequence present in cosmid c2F12 which is assumed to be carrying the putative gua1 gene and which is available at the S. pombe genome project in the Sanger Center (http://srs6.ebi.ac.uk/srsbin/cgi-bin/wgetz? -id+3HhqU1NNOGG+-e+[EMBL:`SPBC2F12']+-qnum+ 1+-enum+12). We were surprised to find that the whole gual gene did not exist within the cloned 1.2 kb fragment but only a 446 bp long region from the 3' terminus of the gual gene was located in this insert, the remaining approximately 750 bp belonged to the gene encoding a kinesinelike protein. This partial sequence of the gual gene has been deposited in the National Center for Biotechnology Information (NCBI) data bank under the Accession Number AJ293460. Sequence identity comparisons showed that this partial gene fragment was correlated with the C-terminal residues of the enzyme, the partial sequence revealing an open reading frame of 446 nucleotides encoding a polypeptide of 148 amino acids (Figure 2).

The size of the IMPDH gene PCR products from wild and mutant strains was also similar, leading us to determine the mutation type of the gua1 strain. The results of the sequence analysis of the wild-type and mutant strain proved that the defect within the gua1 gene was a point mutation at position 1261 ($ggt \rightarrow agt$), resulting a substitution at residue 421 (glycine \rightarrow serine) in *S. pombe* IMPDH. Thus a 446 bp from the 3' terminus of the gene complemented with this mutation whereas PCR product of the remaining longer 1129 bp part did not (Figure 2).

A search of the GenBank database using NCBI BLASTP 2.2.5 program (Altschul *et al.*, 1997) showed that

554 Karaer et al.

1					16					31					811					826					841				
ata	tot	acc	+++	220		tat	act	ass.	act	ttg	022	ata	ctt	220		act	aat	++=	asc		att	gtc	ato	asc		tat	cad	aat	aat
M	S	A	F	K	P			E	A	L	E	V	L	K	E			L		A		V		D	S	S	O	G	N
2000	5	A	r	K		1	1	- E	A	7	E.	V	П	N		A	G	П	D		V	V	1	D		8	Q	G	14
46					61					16					856					871					886				
										gac				cgt		tgc						att			att				
K	Y	E	K	K	D	G	L	S	I	D	D	L	I	R	S	C	F	Q	I	E	M	I	K	W	I	K	K	T	Y
91					106					121					901					916					931				
cac	aat	ttt	caa	gat	gga	tta	acc	ttc	aat	gat	ttc	tta	atc	tta	cct	aaa	att	gat	att	att	act	ggt	aat	att	ata	act	cac	gag	caa
H	N	F	0	Ğ	G	L	T	F	N	D	F	L	I	L	P	K	I	D	v	I		G	N	v	v	T	R	E	0
136		-	~	-	151	-			**	166	-	-	-	_	946	**	-	-		961	**	-	**	*	976	•		-	×
	1						1222	27272	222							120,202			T.L.			United and	1202121	20292			212		
										gtc												ggt							ggt
P	G	Y	I	D		V	P	N	N	V	S	L	E	T	T	A	S	L	I		A	G	A	D	G	L	R	V	G
181					196					211					991					1006	5				1021				
cgt	att	tct	cgt	aat	att	gtt	ctt	aag	act	ccc	ttt	atg	agt	tct	atg	ggt	agt	ggt	agt	gca	tgt	atc	act	caa	gag	gtt	atg	gca	tgt
R	I	S	R	N	I	V	L	K	T	P	F	M	S	S	M	G	S	G	S	A	C	I	T	0	E	v	M	A	C
226	150	177.66	45.76	17740	241		1000	100	250	256	-650			65000	1036			2.50	15.00	105		0.770	- 57.4	-	1066		2.5	170	
	ata	ant	ant	att		~~~	ant	022	2+0		2++	+20	2+0	ant			cat	020	ant			att	acc	000			000	+++	act
	acy									gct			aty																gcc
P	M	D	T	V	T	E	D	Q	M	A	I	Y	M	A	G		P	Q	A	T	A	I	A	Q	V		E	F	A
271					286					301					108					109	_				111	7			
ctt	ttg	ggt	ggc	att	ggt	gta	att	cat	cac	aat	tgc	act	cct	gag	agt	caa	ttt	ggt	att	ggt	gtc	att	gct	gac	ggt	ggt	att	cag	aac
L	L	G	G	I	G	V	I	H	H	N	C	T	P	E	S	Q	F	G	I	G	V	I	A	D	G	G	I	Q	N
316					331					346					112	6				114	1				115	5		10(000)	
	can	act	act	ata		cac	aad	atc	222	aaa	tac	gaa	aat	aaa	att	aat	cat	atq	atc	aag	agt	ctc	age	tta	gat	act	act	act	att
E	cag		A	M	V	R	K	V	K	K	Y	E	M	999	V		Н	M	V	K	S	L	S	L	G		T	J.	Arr.
	V	A	A	1-1		K	K		IV.		1	E	14	G	100		n	1-1	V			-	3	ы		0.00	-	A	V
361					376		2.00	55.55		391					117			_	_	118		_		_	120			_	_
		ttg								caa			gtt	ggt								ggt							gaa
F	I	L	D	P	V	V	F	S	P	0	H	T	V	G	M	M	G	G	L	L	A	G	T	T	E	S	P	G	E
406					421					436					121	6				123	1				124	5			
	ata	tta	aaq	att		gaa	acc	aaa	aat		agt	aat	att	ccc	-		atc	cat	gag			cat	tac	aaa			cat	aat	ato
gat					aaa					ttc					tac	tat				gga	caa	cgt			tca	tac			atg
gat D	gtg V	ttg L	aag K	att I	aaa K		acc T	aaa K	ggt G	ttc F	agt S	ggt G	att I	ccc P	tac	tat	gtc V		gag E	gga G	caa	cgt R	tac Y	aaa K	tca	tac	cgt R	ggt G	atg M
gat D 451	V	L	K	I	aaa K 466	E	T	K	G	ttc F 481	S	G	I	P	tac Y 126	tat Y	V	R	Е	gga G 127	caa Q 6	R	Y	K	tca S 129	tac Y	R	G	М
gat D 451 att	V	L gaa	K aat	I gga	aaa K 466 aaa	E	T cgt	K gga	G aag	ttc F 481 ttg	s gtt	G ggt	I att	P gtt	tac Y 126 g gt	tat Y 1 tcc	V	R	gca	gga G 127 atg	caa Q 6 gaa	R	Y act	K	tca S 129 gtt	tac Y 1 aat	R	Gaat	М
gat D 451 att I	V	L	K	I	aaa K 466 aaa K	E	T	K gga	G	ttc F 481 ttg L	S	G	I	P	tac Y 126 ggt G	tat Y 1 tcc S	V	R	Е	gga G 127 atg M	caa Q 6 gaa E	R	Y	K	s 129 gtt V	tac Y 1 aat N	R	G	М
gat D 451 att I 496	V act T	gaa E	K aat N	I gga G	aaa K 466 aaa K 511	E ctt L	T cgt R	K gga G	G aag K	F 481 ttg L 526	gtt V	ggt G	I att I	p gtt V	tac Y 126 ggt G 130	tat Y 1 tcc S	V atc I	gct A	gca A	gga G 127 atg M 132	caa Q 6 gaa E	R ggt G	act T	ggt G	s 129 gtt V 133	tac Y 1 aat N	R aag K	aat N	M gct A
gat D 451 att I 496	V act T	gaa E	K aat N	I gga G	aaa K 466 aaa K 511	E ctt L	T cgt R	K gga G	G aag K	ttc F 481 ttg L	gtt V	ggt G	I att I	p gtt V	tac Y 126 ggt G 130	tat Y 1 tcc S	V atc I	gct A	gca A	gga G 127 atg M 132	caa Q 6 gaa E	R	act T	ggt G	s 129 gtt V 133	tac Y 1 aat N	R aag K	aat N	M gct A
gat D 451 att I 496	V act T	gaa E	K aat N	I gga G	aaa K 466 aaa K 511	E ctt L	T cgt R	K gga G	G aag K	F 481 ttg L 526	gtt V	ggt G	I att I	p gtt V	tac Y 126 ggt G 130	tat Y 1 tcc S	V atc I	gct A	gca A	gga G 127 atg M 132 ttc	caa Q 6 gaa E	R ggt G	act T	ggt G	s 129 gtt V 133	tac Y 1 aat N	R aag K	aat N	M gct A
gat D 451 att I 496 act	V act T tct	gaa E cgt	K aat N gat	I gga G gtt	aaa K 466 aaa K 511 caa Q	E ctt L ttc	T cgt R cac	gga G aaa	aag K gac	ttc F 481 ttg L 526 acc	gtt V aat	ggt G act	att I cct	gtt V gtc	tac Y 126 ggt G 130 tct S	tat Y 1 tcc S 6 act	V atc I	gct A	gca A tat	gga G 127 atg M 132 ttc	caa Q 6 gaa E 1 tct	R ggt G	y act T	ggt G	tca S 129 gtt V 133 gct A	tac Y 1 aat N 6 gtc V	R aag K cgt	G aat N gtt	M gct A
gat D 451 att I 496 act T 541	V act T tct S	gaa E cgt R	aat N gat D	gga G gtt V	aaa K 466 aaa K 511 caa Q 556	E ctt L ttc F	cgt R cac H	gga G aaa K	aag K gac D	ttc F 481 ttg L 526 acc T 571	gtt V aat N	ggt G act T	att I cct P	gtt V gtc V	tac Y 126 ggt G 130 tct S	tat Y 1 tcc S 6 act T	V atc I ggc G	gct A cgc	gca A tat Y	gga G 127 atg M 132 ttc F 136	caa Q 6 gaa E 1 tct S	ggt G gaa E	act T aac	ggt G gat D	tca S 129 gtt V 133 gct A 138	tac Y 1 aat N 6 gtc V	aag K cgt R	g aat N gtt V	M gct A gcc A
gat D 451 att I 496 act T 541 act	V act T tct S	gaa E cgt R	K aat N gat D atg	gga G gtt V acc	aaa K 466 aaa K 511 caa Q 556 cct	E ctt L ttc F cgt	cgt R cac H	gga G aaa K gaa	aag K gac D	ttc F 481 ttg L 526 acc T 571 atc	gtt V aat N	ggt G act T acc	att I cct P gcc	gtt V gtc V	tac Y 126 ggt G 130 tct S 135 caa	tat Y 1 tcc S 6 act T 1 ggt	V atc I ggc G	R gct A cgc R	gca A tat Y	gga G 127 atg M 132 ttc F 136 ttg	caa Q 6 gaa E 1 tct S 6	ggt G gaa E gtc	Y act T aac N	ggt G gat D	tca S 129: gtt V 133: gct A 138: ggc	tac Y 1 aat N 6 gtc V 1 tcg	R aag K cgt R	aat N gtt V	M gct A
gat D 451 att I 496 act T 541 act	V act T tct S	gaa E cgt R	aat N gat D	gga G gtt V	aaa K 466 aaa K 511 caa Q 556 cct P	E ctt L ttc F	cgt R cac H	gga G aaa K	aag K gac D	ttc F 481 ttg L 526 acc T 571 atc	gtt V aat N	ggt G act T	att I cct P	gtt V gtc V	tac Y 126 ggt G 130 tct S 135 caa Q	tat Y 1 tcc S 6 act T 1 ggt G	V atc I ggc G	gct A cgc	gca A tat Y	gga G 127 atg M 132 ttc F 136 ttg L	caa Q 6 gaa E 1 tct S 6 gtc	ggt G gaa E	act T aac	ggt G gat D	tca S 129 gtt V 133 gct A 138 ggc	tac Y 1 aat N 6 gtc V 1 tcg S	aag K cgt R	g aat N gtt V	M gct A gcc A
gat D 451 att I 496 act T 541 act T 586	V act T tct S gaa E	gaa E cgt R gtt V	K aat N gat D atg	gga G gtt V acc T	aaa K 466 aaa K 511 caa Q 556 cct P 601	E ctt L ttc F cgt R	cgt R cac H gag E	gga G aaa K gaa E	gac D ttg	ttc F 481 ttg L 526 acc T 571 atc I 616	gtt V aat N act	ggt G act T acc	att I cct P gcc A	gtt V gtc V gag E	tac Y 126 ggt G 130 tct S 135 caa Q	tat Y 1 tcc S 6 act T 1 ggt G	V atc I ggc G V	R gct A cgc R tct S	gca A tat Y ggt G	gga G 127 atg M 132 ttc F 136 ttg L	caa Q 6 gaa E 1 tct S 6 gtc V	ggt G gaa E gtc V	act T aac N gac	ggt G gat D aag	tca S 129: gtt V 133: gct A 138: ggc G	tac Y 1 aat N 6 gtc V 1 tcg S	R aag K cgt R ctt L	aat N gtt V ctt L	M gct A gcc A cgc R
gat D 451 att I 496 act T 541 act T 586 gga	V act T tct S gaa E atc	gaa E cgt R gtt V	K aat N gat D atg M ttg	gga G gtt V acc T	aaa K 466 aaa K 511 caa Q 556 cct P 601 cgt	E ctt L ttc F cgt R gcc	T cgt R cac H gag E aac	gga G aaa K gaa E	gac D ttg L atg	ttc F 481 ttg L 526 acc T 571 atc I 616 ttg	gtt V aat N act T	ggt G act T acc T	I att I cct P gcc A tcc	P gtt V gtc V gag E aaa	tac Y 126 ggt G 130 tct S 135 caa Q 139	tat Y 1 tcc S 6 act T 1 ggt G 6 tta	V atc I ggc G V cct	R gct A cgc R tct S tat	gca A tat Y ggt G	gga G 127 atg M 132 ttc F 136 ttg L 141 tac	caa Q 6 gaa E 1 tct S 6 gtc V 1 act	ggt G gaa E gtc V	y act T aac N gac D ttg	ggt G gat D aag K	tca S 1299 gtt V 1339 gct A 1389 ggc G 1420 cac	tac Y 1 aat N gtc V 1 tcg S	R aag K cgt R ctt L	aat N gtt V ctt L	M gct A gcc A cgc R
gat D 451 att I 496 act T 541 act T 586	V act T tct S gaa E	gaa E cgt R gtt V	K aat N gat D atg	gga G gtt V acc T	aaa K 466 aaa K 511 caa Q 556 cct P 601 cgt	E ctt L ttc F cgt R	cgt R cac H gag E	gga G aaa K gaa E	gac D ttg	ttc F 481 ttg L 526 acc T 571 atc I 616	gtt V aat N act	ggt G act T acc	att I cct P gcc A	gtt V gtc V gag E	tac Y 126 ggt G 130 tct S 135 caa Q 139	tat Y 1 tcc S 6 act T 1 ggt G	V atc I ggc G V	R gct A cgc R tct S	gca A tat Y ggt G	gga G 127 atg M 132 ttc F 136 ttg L 141 tac Y	caa Q 6 gaa E 1 tct S 6 gtc V 1 act	ggt G gaa E gtc V	act T aac N gac	ggt G gat D aag K	tca S 129: gtt V 133: gct A 138: ggc G	tac Y 1 aat N 6 gtc V 1 tcg S	R aag K cgt R ctt L	aat N gtt V ctt L	M gct A gcc A cgc R
gat D 451 att I 496 act T 541 act T 586 gga	V act T tct S gaa E atc	gaa E cgt R gtt V	K aat N gat D atg M ttg	gga G gtt V acc T	aaa K 466 aaa K 511 caa Q 556 cct P 601 cgt	E ctt L ttc F cgt R gcc	T cgt R cac H gag E aac	gga G aaa K gaa E	gac D ttg L atg	ttc F 481 ttg L 526 acc T 571 atc I 616 ttg	gtt V aat N act T	ggt G act T acc T	I att I cct P gcc A tcc	P gtt V gtc V gag E aaa	tac Y 126 ggt G 130 tct S 135 caa Q 139	tat Y 1 tcc S 6 act T 1 ggt G tta L	V atc I ggc G V cct	R gct A cgc R tct S tat	gca A tat Y ggt G	gga G 127 atg M 132 ttc F 136 ttg L 141 tac	caa Q 6 gaa E 1 tct S 6 gtc V 1 act	ggt G gaa E gtc V	y act T aac N gac D ttg	ggt G gat D aag K	tca S 1299 gtt V 1339 gct A 1389 ggc G 1420 cac	tac Y 1 aat N 6 gtc V 1 tcg S 6 gct A	R aag K cgt R ctt L	aat N gtt V ctt L	M gct A gcc A cgc R
gat D 451 att I 496 act T 541 act T 586 gga G 631	V act T tct S gaa E atc I	gaa E cgt R gtt V agt	K aat N gat D atg M ttg L	gga G gtt V acc T gag E	aaa K 466 aaa K 511 caa Q 556 cct P 601 cgt R 646	E ctt L ttc F cgt R gcc A	cgt R cac H gag E aac N	gga G aaa K gaa E gaa E	G aag K gac D ttg L atg M	ttc F 481 ttg L 526 acc T 571 atc I 616 ttg L	gtt V aat N act T cgc R	ggt G act T acc T aaa K	I att I cct P gcc A tcc S	P gtt V gtc V gag E aaa K	tac Y 126 ggt G 130 tct S 135 caa Q 139 ttt F	tat Y 1 tcc S 6 act T 1 ggt G tta L	V atc I ggc G gtc V cct P	R gct A cgc R tct S tat Y	gca A tat Y ggt G ctc L	gga G 127 atg M 132 ttc F 136 ttg L 141 tac Y 145	caa Q 6 gaa E 1 tet S 6 gtc V 1 act T	ggt G gaa E gtc V	Y act T aac N gac D ttg L	ggt G gat D aag K caa Q	tca S 1299 gtt V 1339 gct A 1389 ggc G 1420 cac H 1471	tac Y 1 aat N 6 gtc V 1 tcg S 6 gct A	R aag K cgt R ctt L	G aat N gtt V ctt L caa Q	M gct A gcc A cgc R gat D
gat D 451 att 1496 act 541 act 586 g G 631 aag	V act T tct S gaa E atc I gga	gaa E cgt R gtt V agt S aag	K aat N gat D atg M ttg L ctt	gga G gtt V acc T gag E	aaa K 466 aaa K 511 caa Q 556 cct P 601 cgt R 646 gtt	E ctt L ttc F cgt R gcc A gtt	T cgt R cac H gag E aac N gac	gga G aaa K gaa E gaa E	G aag K gac D ttg L atg M gat	ttc F 481 ttg L 526 acc T 571 atc I 616 ttg L 661 gac	gtt V aat N act T cgc R	ggt Gact Tacc Taaa Kctg	I att I cct P gcc A tcc S	P gtt V gtc V gag E aaa K gct	tac Y 126 ggt G 130 tct S 135 caa Q 139 ttr F	tat Y 1 tcc S 6 act T 1 ggt G 6 tta L 1	V atc I ggc G gtc V cct P	R gct A cgc R tct S tat Y	gca A tat Y ggt G ctc L	gga G 127 atg M 132 ttc F 136 ttg L 141 tac Y 145 ctt	caa Q 6 gaa E 1 tct S 6 gtc V 1 act T 6 gat	R ggt G gaa E gtc V ggt G	y act T aac N gac D ttg L ctt	ggt G gat D aag K caa Q cat	tca S 129 gtt V 133 gct A 138 ggc G 142 cac H 147 gaa	tac Y 1 aat N 6 gtc V 1 tcg S 6 gct A 1	R aag K cgt R ctt L ctt L	G aat N gtt V ctt L caa Q gac	M gct A gcc A cgc R gat D aag
gat D 451 att I 496 act T 541 act T 586 g G 631 aag K	V act T tct S gaa E atc I	gaa E cgt R gtt V agt	K aat N gat D atg M ttg L	gga G gtt V acc T gag E	aaa K 466 aaa K 511 caa Q 556 cct P 601 cgt R 646 gtt	E ctt L ttc F cgt R gcc A	cgt R cac H gag E aac N	gga G aaa K gaa E gaa E	G aag K gac D ttg L atg M	ttc F 481 ttg L 526 acc T 571 atc I 661 gac D	gtt V aat N act T cgc R	ggt G act T acc T aaa K	I att I cct P gcc A tcc S gtt	P gtt V gtc V gag E aaa K	tac Y 126 G G 130 tct S 135 caa Q 139 ttt F 144 att	tat Y 1 tcc S 6 act T 1 ggt G tta L 1 ggt G	V atc I ggc G gtc V cct P	R gct A cgc R tct S tat Y	gca A tat Y ggt G ctc L	gga G 127 atg M 132 ttc F 136 ttg L 141 tac Y 145 ctt	caa Q 6 gaa E 1 tct S 6 gtc V 1 act T 6 gat	ggt G gaa E gtc V ggt G	Y act T aac N gac D ttg L	ggt G gat D aag K caa Q	tca S 129: gtt V 133: gct A 138: ggc G 142: cac H 147: gaa E	tac Y 1 aat N 6 gtc V 1 tcg S 6 gct A 1 gct A	R aag K cgt R ctt L	G aat N gtt V ctt L caa Q	M gct A gcc A cgc R gat D
gat D 451 att I 496 act T 586 gga G 631 ack 676	V act T tct S gaa E atc I gga G	gaa E cgt R gtt V agt S aag	K aat N gat D atg M ttg L ctt L	gga G gtt V acc T gag E	aaa K 466 aaa K 511 caa Q 556 cct P 601 cgt 646 gtt V 691	E ctt L ttc F cgt R gcc A gtt V	cac H gag E aac N gac D	gga G aaa K gaa E gaa E	gac D ttg L atg M gat D	ttc F 481 ttg L 526 acc T 571 atc I 616 ttg Gac D 706	gtt V aat N act T cgc R aac	ggt G act T acc T aaa K ctg L	att I cct P gcc A tcc S gtt V	P gtt V gtc V gag E aaa K gct A	tac Y 126 ggt G 130 tct S 135 caa Q 139 ttt F 144 att	tat Y 1 tcc S 6 act T 1 ggt G 6 tta L 1 ggt G 6	V atc I ggc G gtc V cct P acc T	R gct A cgc R tct S tat Y aaa K	gca A tat Y ggt G ctc L tct	gga G 127 atg M 132 ttc F 136 ttg L 141 tac Y 145 ctt L 150	caa Q 6 gaa E 1 tct S 6 gtc V 1 act T 6 gat D	ggt G gaa E gtc V ggt G gag E	y act T aac N gac D ttg L ctt L	ggt G gat D aag K caa Q cat H	tca S 129: gtt V 133: gct A 138: ggc G 142: cac H 147: gaa E 151:	tac Y 1 aat N 6 gtc V 1 tcg S 6 gct A 1	R aag K cgt R ctt L ctt L y	G aat N gtt V ctt L caa Q gac D	M gct A gcc A cgc R gat D aag K
gat D 451 496 act 541 act 586 gga 631 g 676 ctt	V act T tct S gaa E atc I gga G ttg	gaa E cgt R gtt V agt S aag K tct	K aat N gat D atg M ttg L ctt L tta	I gga G gtt V acc T gag E cct P	aaa K 466 aaa K 511 caa Q 556 cct P 601 cgt 646 gtt V 691 gac	E ctt L ttc F cgt R gcc A gtt V ttg	T cgt R cac H gag E aac N gac D atg	K gga G aaa K gaa E gaa E aag K	G aag K gac D ttg Atg M gat D aac	ttc F 481 ttg L 526 acc 571 atc I 616 ttg D 706 ttg	gtt V aat N act T cgc R aac	ggt Gact Tacc Taaa Kctg Lttc	att I cct P gcc A tcc S gtt V cct	P gtt V gtc V gag E aaa K gct A ctt	tac Y 126 ggt G 130 tct S 135 caa Q 139 tt F 144 att I 148 cat	tat Y 1 tcc S 6 act T 1 ggt G 6 tta L 1 ggt G 6 gaa	V atc I ggc G gtc V cct P acc T gtt	R gct A cgc R tct S tat Y aaa K cgc	gca A tat Y ggt G ctc L tct S	gga G 127 atg M 132 ttc F 136 ttg L 141 tac Y 145 ctt L 150 gag	caa Q 6 gaa E 1 tct 6 gtc V 1 act T 6 gat D 1 tta	R ggt G gaa E gtc V ggt G gag E cgt	y act T aac N gac D ttg L ctt L	ggt G gat D aag K caa Q cat H	tca S 129 gtt V 133 gct A 138 ggc G 142 cac H 147 gaa E 151 gct	tac Y 1 aat N 6 gtc V 1 tcg S 6 gct A 1 gct A 6	R aag K cgt R ctt L ctt L ctt V atc	G aat N gtt V ctt L caa Q gac D	M gct A gcc A cgc R gat D aag K
gat 451 451 496 496 541 542 586 631 676 611 L	V act T tct S gaa E atc I gga G	gaa E cgt R gtt V agt S aag	K aat N gat D atg M ttg L ctt L	gga G gtt V acc T gag E	aaa K 466 aaa K 511 caa Q 556 cct P 601 cgt R 646 gtt 691 gac D	E ctt L ttc F cgt R gcc A gtt V ttg	T cgt R cac H gag E aac N gac D atg	gga G aaa K gaa E gaa E	gac D ttg L atg M gat D	ttc F 481 ttg L 526 acc 571 atc I 661 g D 706 ttg L L	gtt V aat N act T cgc R aac	ggt G act T acc T aaa K ctg L	att I cct P gcc A tcc S gtt V	P gtt V gtc V gag E aaa K gct A	tac Y 1266 ggt G 130 tct S 135 caa Q Q 139 ttt F 144 att I 148 cat H	tat Y 1 tcc S 6 act T 1 ggt G 6 tta L 1 ggt G 6 gaa E	V atc I ggc G gtc V cct P acc T	R gct A cgc R tct S tat Y aaa K	gca A tat Y ggt G ctc L tct	gga G 127 atg M 132 ttc F 136 ttg L 141 tac Y 145 ctt L 150 gag E	caa Q 6 gaa E 1 tct 6 gtc V 1 act T 6 gat D 1 tta L	ggt Ggaa Egtc Vggt Ggag E	y act T aac N gac D ttg L ctt L	ggt G gat D aag K caa Q cat H	tca S 129 gtt V 133 gct A 138 ggc G 142 cac H 147 gaa E 151 gct A	tac Y 1 aat N 6 gtc V 1 tcg S 6 gct A 1 gct A 6 gcc A	R aag K cgt R ctt L ctt L y	G aat N gtt V ctt L caa Q gac D	M gct A gcc A cgc R gat D aag K
gat D 451 att 196 act T 541 586 G 631 a K 676 c L 721	V act T tct S gaa E atc I gga G ttg L	gaa E cgt R gtt V agt S aag K tct S	K aat N gat D atg M ttg L ctt L tta L	gga Ggtt Vacc Tgag Ect Pact	aaa K 466 aaa K 5111 caa C Q C CCt P 6011 cgt R 6466 gtt V G 91 G G D 736	E ctt L ttc F cgt R gcc A gtt V ttg L	cac H gag E aac N gac D atg M	gga Gaa K gaa E gaa E aag K	gac D ttg L atg M gat D aac N	ttc F 481 ttg L 526c T 571 atc I 661 gac D 706g ttg 751	gtt V aat N act T cgc R aac N	G ggt G act T acc T aaa K ctg L ttc F	att I cct P gcc A tcc S gtt V cct P	gtt V gtc V gag E aaa K gct A	tac Y 1266 ggt G 130 tct S 135 caa Q 139 ttt F 144 att I 148 cat H	tat Y 1 tcc S 6 act T 1 ggt G 6 tta L 1 ggt G 6 ggaa E 1	V atc I ggc G gtc V cct P acc T gtt V	R gct A cgc R tct S tat Y aaa K cgc R	gca A tat Y ggt G ctc L tct S	gga G 127 atg M 1322 ttc F 136 ttg L 141 tac Y 145 ctt L 150 gag E 154	caa Q 6 gaa E 1 tct S 6 gtc V 1 act T 6 gat D 1 tta L	ggt Ggaa Egtc Vggt Ggag Ecgt R	act T acc N gac D ttg L ctt L tcg S	ggt G gat D aag K caa Q cat H agc S	tca S 129 gtt V 133 gct A 138 ggc G 142 cac H 147 gaa E 151 gct A	tac Y 1 aat N 6 gtc V 1 tcg S 6 gct A 1 gct A 6	R aag K cgt R ctt L ctt L gtc V atc I	gac D cgt	M gct A gcc A cgc R gat D aag K gaa E
gat D 451 496 act T 541 act 586 gga 676 ctt L 721 gcc	V act T tct S gaa E atc I gga G ttg L agc	gaa E cgt R gtt V agt S aag K tct S aaa	K aat N gat D atg M ttg L ctt L tta L aca	gga G gtt V acc T gag E cct P act T tcg	aaa K 466 aaa K 5111 caa Q 556 cct P 6011 cgt V 691 gab 736 gat	E ctt L ttc F cgt R gcc A gtt V ttg L act	T cgt R cac H gag E aac N gac D atg M aag	gga G aaa K gaa E gaa E aag K aag K caa	gac D ttg L atg M gat D aac N ctt	ttc F 481 ttg L 526 acc T 571 atc I 661 gac D 706 ttg L 575 atg L 575 atg L 575 atg	gtt V aat N act T cgc R aac N cac H gtt	G ggt G act T acc T aaa K ctg L ttc F	I att I cct P gcc A tcc S gtt V cct P gct	gtt V gtc V gag E aaa K gct A	tac Y 1266 ggt G 130 tct S 135 caa Q 139 ttt F 144 att I 148 cat H	tat Y 1 tcc S 6 act T 1 ggt G 6 tta L 1 ggt G 6 ggaa E 1	V atc I ggc G gtc V cct P acc T gtt V	R gct A cgc R tct S tat Y aaa K cgc R	gca A tat Y ggt G ctc L tct S	gga G 127 atg M 1322 ttc F 136 ttg L 141 tacc Y 145 ctt L 150 gag E 154 ttt	caa Q 6 gaa E 1 tct S 6 gtc V 1 act T 6 gat D 1 tta L	R ggt G gaa E gtc V ggt G gag E cgt R aca	y act T aac N gac D ttg L ctt L tcg S tac	K ggt G gat D aag K caa Q cat H agc S gaa	tca S 129 gtt V 1333 gct A 138 ggc G 142 cac cac E 151 get A	tac Y 1 aat N 6 gtc V 1 tcg S 6 gct A 1 gct A 6	R aag K cgt R ctt L ctt L gtc V atc I	gac D cgt	M gct A gcc A cgc R gat D aag K gaa E
gat D 451 att 196 act T 541 586 G 631 a K 676 c L 721	V act T tct S gaa E atc I gga G ttg L	gaa E cgt R gtt V agt S aag K tct S	K aat N gat D atg M ttg L ctt L tta L	I gga G gtt V acc T gag E cct P	aaa K 466 aaa K 5111 caa C Q C CCt P 6011 cgt R 6466 gtt V G 91 G G D 736	E ctt L ttc F cgt R gcc A gtt V ttg L	cac H gag E aac N gac D atg M	gga G aaa K gaa E gaa E aag K aag	gac D ttg L atg M gat D aac N	ttc F 481 ttg L 526c T 571 atc I 661 gac D 706g ttg 751	gtt V aat N act T cgc R aac N cac H gtt	G ggt G act T acc T aaa K ctg L ttc F	att I cct P gcc A tcc S gtt V cct P	gtt V gtc V gag E aaa K gct A	tac Y 1266 ggt G 130 tct S 135 caa Q 139 ttt F 144 att I 148 cat H	tat Y 1 tcc S 6 act T 1 ggt G 6 tta L 1 ggt G 6 ggaa E 1	V atc I ggc G gtc V cct P acc T gtt V	R gct A cgc R tct S tat Y aaa K cgc R	gca A tat Y ggt G ctc L tct S	gga G 127 atg M 1322 ttc F 136 ttg L 141 tac Y 145 ctt L 150 gag E 154	caa Q 6 gaa E 1 tct S 6 gtc V 1 act T 6 gat D 1 tta L	ggt Ggaa Egtc Vggt Ggag Ecgt R	act T acc N gac D ttg L ctt L tcg S	ggt G gat D aag K caa Q cat H agc S	tca S 129 gtt V 133 gct A 138 ggc G 142 cac H 147 gaa E 151 gct A	tac Y 1 aat N 6 gtc V 1 tcg S 6 gct A 1 gct A 6	R aag K cgt R ctt L ctt L gtc V atc I	gac D cgt	M gct A gcc A cgc R gat D aag K gaa E
gat D 451 496 act T 541 act 586 gga 676 ctt L 721 gcc	V act T tct S gaa E atc I gga G ttg L agc	gaa E cgt R gtt V agt S aag K tct S aaa	K aat N gat D atg M ttg L ctt L tta L aca	gga G gtt V acc T gag E cct P act T tcg	aaa K 466 aaa K 5111 caa Q 556 cct P 6011 cgt V 691 gab 736 gat	E ctt L ttc F cgt R gcc A gtt V ttg L act	T cgt R cac H gag E aac N gac D atg M aag	gga G aaa K gaa E gaa E aag K aag K caa	gac D ttg L atg M gat D aac N ctt	ttc F 481 ttg L 526 acc T 571 atc I 661 gac D 706 ttg L 575 atg L 575 atg L 575 atg	gtt V aat N act T cgc R aac N cac H gtt	G ggt G act T acc T aaaa K ctg L ttc F gcc	I att I cct P gcc A tcc S gtt V cct P gct	P gtt V gtc V gag E aaa K gct A ctt L gct	tac Y 1266 ggt G 130 tct S 135 caa Q 139 ttt F F 144 att 148 cat H 153 ggt	tat Y 1 tcc S 6 act T 1 ggt G 6 tta L 1 ggt G 6 tta L 1 ggt G 6 gaa E 1 gat	V atc I ggc G G C V cct P acc T gtt V atc	R gct A cgc R tct S tat Y aaa K cgc R	gca A tat Y ggt G ctc L tct S ttc F ggt	gga G 127 atg M 1322 ttc F 136 ttg L 141 tacc Y 145 ctt L 150 gag E 154 ttt	caa Q 6 gaa E 1 tct S 6 gtc V 1 act T 6 gat D 1 tta L 6 gct	R ggt G gaa E gtc V ggt G gag E cgt R aca	y act T aac N gac D ttg L ctt L tcg S tac	K ggt G gat D aag K caa Q cat H agc S gaa	tca S 129 gtt V 1333 gct A 138 ggc G 142 cac cac E 151 get A	tac Y 1 aat N 6 gtc V 1 tcg S 6 gct A 1 gct A 1 gct A 1 gct A	R aag K cgt R ctt L ctt L gtc V atc I ctt	G aat N gtt V ctt L caa Q gac D cgt R tac	M gct A gcc A cgc R gat D aag K gaa E
gat D 4511 att I 4966 act T 5866 G31 aag K 6766 Ctt L 721c A 766	V act T tct S gaa E atc I gga G ttg L agc S	L gaa E cgt R gtt V agt S aaag K tct S aaaa K	K aat N gat D atg M ttg L tta L aca T	I gga G G V acc T gag E cct P act T tcg S	aaa K 466 aaa K 511 caa Q 5566 cct P 601 cgt R 646 gt V 691 gac D 7366 gat D 781	E ctt L ttc F cgt R gcc A gtt V ttg L act T	T cgt R cac H gag E aac N gac D atg M aag K	gga G aaaa K gaa E gaa E aaag K aaag K Caaa Q	G aag K gac D ttg L atg M gat D aac N ctt L	ttc F 481 ttg L 526 acc T 571 atc L 661 ttg D 706 ttg L T 751 atg M	gtt V aat N act T cgc R aacc N cac H gtt V	ggt G act T acc T ttc F gcc A	att I cct P gcc A tcc S gtt V cct P gct A	P gtt V gtc V gag E aaa K gct A ctt L gct A	tac Y 1266 ggt G 130 tct S 135 caa Q 139 ttt F F 144 att 148 cat H 153 ggt	tat Y 1 tcc S 6 act T 1 ggt G 6 tta L 1 ggt G 6 tta L 1 ggt G 6 gaa E 1 gat	V atc I ggc G G C V cct P acc T gtt V atc	R gct A cgc R tct S tat Y aaa K cgc R	gca A tat Y ggt G ctc L tct S ttc F ggt	gga G 127 atg M 1322 ttc F 136 ttg L 141 tacc Y 145 ctt L 150 gag E 154 ttt	caa Q 6 gaa E 1 tct S 6 gtc V 1 act T 6 gat D 1 tta L 6 gct	R ggt G gaa E gtc V ggt G gag E cgt R aca	y act T aac N gac D ttg L ctt L tcg S tac	K ggt G gat D aag K caa Q cat H agc S gaa	tca S 129 gtt V 1333 gct A 138 ggc G 142 cac cac E 151 get A	tac Y 1 aat N 6 gtc V 1 tcg S 6 gct A 1 gct A 1 gct A 1 gct A	R aag K cgt R ctt L ctt L gtc V atc I ctt	G aat N gtt V ctt L caa Q gac D cgt R tac	M gct A gcc A cgc R gat D aag K gaa E

Figure 2 - The sequence of the *Schizosaccharomyces pombe gua1* gene showing the predicted inosine monophosphate dehydrogenase (IMPDH) amino acid residues. The cloned and complemented partial *gua1* gene discovered in this study is shadowed. The location of the point mutation is shown in bold-faced type. Altering the guanine (g) at the 1261 nucleotide position to adenine (a) caused the IMPDH 421_{Gly} residue to be substituted by a 421_{Ser} residue.

the partial IMPDH amino acid sequence had homologies with IMPDH from *Candida albicans* (64%), *Saccharomyces cerevisiae* (62%), *Drosophila melanogaster* (53%), human type I (53%), *Mus musculus* type I (52%), human type II (52%), *M. musculus* type II (52%) *Arabidopsis thaliana* (45%) and *E. coli* (40%) (Figure 3).

Transcription analysis

To demonstrate expression of the *gua1* gene we isolated RNAs from three different types of the cells, wild-type, double-mutant (*gua1ura4*-D18) and the transformant (SG1). Dot and Northern hybridizations were carried out with a 1.2 kb fragment as a probe containing partial gene fragments of a kinesine-like protein and IMPDH. Labeling was detected with the samples tested by dot hybridization (data not shown) and two bands were observed for all samples in the Northern blot hybridizations (Figure 4). One of these bands was consistent with the size of a full-length *S. pombe* IMPDH transcript of 1575 nucleotides, indicating that expression of this gene occurred at the transcriptional level in the double mutant. The size of the other band, cor-

responding to the kinesine-like protein transcript was about 2 kb.

Enzyme assay

The IMPDH activities of the crude extracts from the 972h wild-type, *gualura4*-D18 double-mutant and the SG1 transformant were determined in the reaction mixture with or without the IMPDH inhibitor allopurinol. Significant IMPDH activity existed in wild-type and the SG1 transformant but almost none in the *gualura4*-D18 mutant and there was also a clear difference between the activities detected for each sample in the presence and absence of allopurinol. These results confirmed that allopurinol had an inhibitory effect on IMPDH *in vitro* (Weber, 1983) and decreased the enzyme activity (Table 1).

Discussion

We found that the *S. pombe* DNA fragment cloned in this study is part of the IMPDH gene as indicated by complementation with the *gualura4*-D18 double- mutant as well as comparison of the sequence of the fragment with

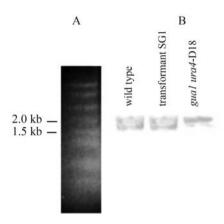


Figure 3 - Northern blot analysis of inosine monophosphate dehydrogenase (IMPDH) transcripts in the *Schizosaccharomyces pombe* wild-type, mutant and SG1 transformant. Total RNA (~10 μg) was separated using denaturing formaldehyde/agarose gel electrophoresis. RNA markers separated on agarose gel (a); Northern blot hybridization of IMPDH mRNA with a DIG-11-dUTP labeled DNA fragment carrying the gua1 gene as a probe.

the putative *gua1* gene cloned to chromosome II cosmid c2F12.

The BLASTP search of the GenBank database showed that the partial IMPDH amino acid sequence had 40 to 64% homology with IMPDH from, in increasing order of size, *E. coli*, *A. thaliana*, *M. musculus* type II, human type II, *Mus musculus* type I, *Drosophila melanogaster*, *S. cerevisiae* and *C. albicans* (Figure 3), indicating that this sequence seems to be conserved between unrelated species.

The observation of similar-sized IMPDH mRNAs in both the wild-type and the mutant *S. pombe* strain indicated that the defect of the *gua1* gene was due to a point mutation. Furthermore, sequence analyses showed that the point mu-

Table 1 - Inosine monophosphate dehydrogenase (IMPDH) activity (OD_{340} per mg per ml of protein) in crude extracts of *Schizosaccharomyces pombe*. Activity measurements were carried out using two different reaction mixtures, one with allopurinol (O'Gara *et al.*, 1997) and the other without allopurinol (Carr *et al.*, 1993). Values represent the average \pm the standard deviation for three independent determinations.

	IMPDH activity							
Cell type	With allopurinol	Without allopurinol						
Wild type (972h ⁻)	0.465 ± 0.057	0.759 ± 0.015						
Double mutant (gualura4-D18)	0.061 ± 0.008	0.045 ± 0.003						
Transformant (SG1)	0.408 ± 0.009	0.549 ± 0.014						

tation was exactly at position 1261 ($ggt \rightarrow agt$), the first nucleotide of codon 421 which encodes a glycine residue in IMPDH (Figure 2), and that this mutation changed this residue to a serine residue.

There was significant enzyme activities in both the wild-type strain and the SG1 transformant but the enzyme activity in the mutant was so low that it could be disregarded. These results show that the SG1 transformant containing the partial *gua1* gene showed nearly as much catalytic activity as wild-type *S. pombe*. In addition, when we cloned the rest of the gene (1129 bp) into the *gua1ura4*-D18 double-mutant, no complementation was observed. Having shown that the enzyme activity obviously originated from one of the IMPDH C-terminal residues encoded by a small portion of *gua1* gene, complementing the mutation raised the interesting question of how only a partial gene sequence could give rise to a level of IMPDH expression similar to that of the wild-type *S. pombe*.

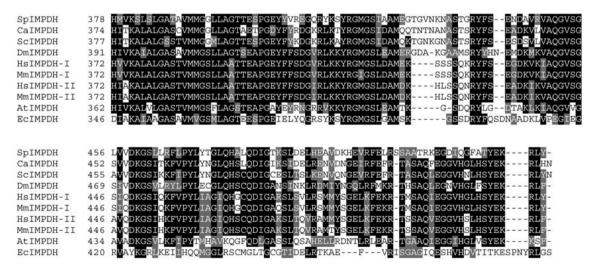


Figure 4 - Partial amino acid alignment of *Schizosaccharomyces pombe* inosine monophosphate dehydrogenase (IMPDH) (GenBank CAB97003) homologues with *Candida albicans* (O00086), *Saccharomyces cerevisiae* (P50094), *Drosophila melanogaster* (Q07152) Human type I (P20839), *Mus musculus* (P50096), Human type II (P12268), *M. musculus* type II (P24547), *Arabidopsis thaliana* (Q9SA34) and *Escherichia coli* (P06981). Abbreviations for the species are: Sp = *S. pombe*; Ca = *C. albicans*; Sc = *S. cerevisiae*; Dm = *D. melanogaster*; Hs = *Homo sapiens*; Mm = *M. musculus*; At = *A. thaliana*; Ec = *E. coli*. Identical amino acids are shown with a black background, similar amino acids with a gray background. Dashes (gaps) have been introduced for optimal alignment. The alignments revealed that the regions of putative catalytic domain amino acids (378-524) were highly conserved.

556 Karaer *et al.*

The IMPDH enzyme is a tetramer formed by monomers consisting of two domains, an α/β barrel core domain (catalytic domain) and a cystathione-B-synthase (CBS) subdomain (Carr et al., 1993; Huete-Perez et al., 1995; Zhou et al., 1997; Colby et al., 1999; Zhang et al., 1999). Zhang et al. (1999) showed that site specific mutations in the CBS subdomain of Streptococcus pyrogenes did not result in loss of IMPDH activity but the construction of a point mutation in the active site by changing Arg₄₀₆ to alanine resulted in complete loss of IMPDH activity. However it had been previously reported (Zhou et al., 1997) that IMPDH from Borrelia burgdorferi did not contain a CBS subdomain and yet maintained enzymatic activity. Nimmesgern et al. (1999) demonstrated the expression of the core domain and the CBS subdomain of human IMPDH separately in E. coli and determined that the core domain was enzymatically active while the CBS subdomain was inactive. Futer et al. (2002) reported that the mutations of three active site residues to alanine in the IMP binding pocket reduced IMPDH activity to less than 0.1 % of that found in human wild-type IMPDH.

All these findings suggest that the region close to the C terminus of the core domain, rather than the CBS subdomain, is responsible for IMPDH activity. Our results were also consistent with this conclusion because the SG1 transformant contained a 446 bp fragment of the gene encoding the IMPDH C-terminal residues and was capable of producing active enzyme.

However, IMPDH CBS subdomains from different species vary considerably in size and the subdomain sequences are much less conserved than the core domain sequences (Nimmesgern *et al.*, 1999). Moreover, mutation studies on the gene fragment encoding the CBS domain in *Methanococcus janaschii* (Archeae) suggest that the CBS domain is responsible for the regulation of cystathione-β-synthase activity (Bateman, 1997). Thus it can be speculated that the function of the *S. pombe* IMPDH CBS domain may also be related to regulation of IMPDH expression. However, the presence of this domain in the alignment of all but one (from *B. burgdorferi*) of the 56 IMPDHs studied by Nimmesgern *et al.* (1999) still raised questions with regarding its functional role.

In our study, complementation in the SG1 transformant carrying the 446 bp part of the *gua1* gene in the plasmid pGS1.2 suggested that the insert integrated into the exact region containing the mutation in the genome by homologous recombination. Hence, it can be concluded that a 446 bp from the 3' end of *gua1* gene participates in the sequences encoding the catalytic domain of *S. pombe* IMPDH.

Acknowledgments

We appreciate the critical comments on the manuscript by D.E. Kelly. We are grateful to Gökhan Akman for

help with the alignment. This work was supported by the Research Fund of The University of Istanbul (Project numbers T-404/270697, B-280/200899 and B-1016/07062001) and by the Research and Application Center for Biotechnology and Genetic Engineering (Project number BIYOGEM-98/01).

References

- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zang Z, Miller W and Lipman DJ (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res 25:3389-3402.
- Bateman A (1997) The structure of a domain common to archaebacteria and the homocystinuria disease protein. Trends Biochem Sci 22:12-13.
- Burke D, Dawson D and Stearns T (2000) Methods in Yeast Genetics. Cold Spring Harbor Laboratory Press, New York, 205 pp.
- Carr SF, Papp E, Wu JC and Natsumeda Y (1993) Characterization of human type I and type II IMP dehydrogenases. J Biol Chem 268:27286-27290.
- Colby TD, Vanderveen K, Strickler MD, Markham GD and Goldstein BM (1999) Crystal structure of human type II inosine monophosphate dehydrogenase: Implications for ligand binding and drug design. Proc Natl Acad Sci USA 96:3531-3536.
- Futer O, Sintchak MD, Caron PR, Nimmesgern E, DeCenzo MT, Livingston DJ and Raybuck SA (2002) A mutational analysis of the active site of human type II inosine 5'-monophosphate dehydrogenase. Biochim Biophys Acta 1594:27-39.
- Grimm C, Kohli J, Murray J and Maundrell K (1988) Genetic engineering of *Schizosaccharomyces pombe*: A system for gene disruption and replacement using the *ura4* gene as a selectable marker. Mol Gen Genet 215:81-86.
- Gutz H, Heslot H, Leupold U and Loprieno N (1974) Schizosaccharomyces pombe. In: King RC (ed) Handbook of Genetics. Plenum Press, New York, pp 395-446.
- Gygax A and Thuriaux P (1984) A revised chromosome map of the fission yeast *Schizosaccharomyces pombe*. Curr Genet 8:85-92.
- Hedstrom L (1999) IMP dehydrogenase: Mechanism of action and inhibition. Current Med Chemistry 6:545-560.
- Henderson JF and Paterson ARP (1973) Nucleotide Metabolism. Academic Press, New York, pp 207-263.
- Heslot H (1972) Genetic control of the purine nucleotide pathway in *Schizosaccharomyces pombe*. Proc IV IFS/Ferment Technol Today 867-876.
- Huete-Perez JA, Wu JC, Whitby FG and Wang CC (1995) Identification of the IMP binding site in the IMP dehydrogenase from *Tritrichomonas foetus*. Biochem 34:13889-13894.
- Leupold U (1970) Genetical methods for *Schizosaccharomyces pombe*. In: Prescott DM (ed) Methods in Cell Physiology, v. 4. Academic Press, New York, pp 169-177.
- Liedtke C and Schmidt H (1998) Molecular cloning and sequence analysis of the *Schizosaccharomyces pombe ade10*⁺ gene. Yeast 14:1307-1310.
- Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951) Protein measurement with the Folin-phenol reagent. J Biol Chem 193:265-275.

- Ludin KM, Hilti N and Schweingruber ME (1994) The ade4 gene of Schizosaccharomyces pombe: Cloning, sequence and regulation. Curr Genet 25:465-468.
- Manzoli L, Billi AM, Gilmour RS, Martelli AM, Matteucci A, Rubbini S, Weber G and cocco L (1995) Phosphoinositide signaling in nuclei of friend-cells-tiazofurin down-regulates phospholipase-C beta (1). Cancer Res 55:2978-2980.
- McKenzie R, Schuchert P and Kilbey B (1987) Sequence of the bifunctional *ade1* gene in the purine biosynthetic pathway of the fission yeast *Schizosaccharomyces pombe*. Curr Genet 12:591-597.
- Michal G (1999) Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology. John Wiley & Sons Inc, New York, pp 99-102
- Nimmesgern E, Black J, Futer O, Fulghum JR, Chambers SP, Brummel CL, Raybuck SA and Sintchak MD (1999) Biochemical analysis of the modular enzyme inosine 5'-monophosphate dehydrogenase. Protein Expr Purif 17:282-289.
- O'Gara MJ, Lee C-H, Weinberg GA, Nott JM and Queener SF (1997) IMP dehydrogenase from *Pneumocystis carinii* as a potential drug target. Antimicrob Agents Chemother 41:40-48.
- Oraler G, Olgun A and Karaer S (1990) An addition to the chromosome map of *Schizosaccharomyces pombe*: The localization of *gua* genes. Curr Genet 17:543-545.
- Pourquié MJ (1974) Controle génétique du métabolisme purique chez la levure *Schizosaccharomyces pombe*. Ph.D. Thesis L'Université Paris VI, Paris.

- Sambrook J, Fritsch EF and Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.
- Speiser DM, Ortiz DF, Kreppel L, Scheel G, McDonald G and Ow DW (1992) Purine biosynthetic genes are required for cadmium tolerance in *Schizosaccharomyces pombe*. Mol Cell Biol 12:5301-5310.
- Szankasi P, Heyer WD, Schuchert P and Kohli J (1988) DNA sequence analysis of the *ade6* gene of *Schizosaccharomyces pombe*. Wild-type and mutant alleles including the recombination host spot allele *ade6*-M26. J Mol Biol 204:917-925.
- Warshawsky D and Miller L (1994) Improved method for rapid transformation of intact Schizosaccharomyces pombe cells. Biotechniques 16:798-800.
- Weber G (1983) Biochemical strategy of cancer cells and the design of chemotherapy: G.H.A. Clowes memorial lecture. Cancer Res 43:3466-3492.
- Zhang R-G, Evans G, Rotella FJ, Westbrook EM, Beno D, Huberman E, Joachimiak A and Collart FR (1999) Characteristics and crystal structure of bacterial inosine-5'-monophosphate dehydrogenase. Biochemistry 38:4691-4700.
- Zhou X, Cahoon M, Rosa P and Hedstrom L (1997) Expression, purification, and characterization of inosine-5'-monophosphate dehydrogenase from *Borrelia burgdorferi*. J Biol Chem 272:21977-21981.

Internet Resources

Topal A, Karaer S and Temizkan G (1997) A simple method for rescuing autonomous plasmid from fission yeast. Technical Tips Elsevier Trends Journals. http://www.elsevier.com//locate/tt0.T01070.

Associate Editor: Sérgio Olavo Pinto da Costa