The zebrafish genome contains two distinct selenocysteine tRNA^{[Ser]Sec} genes

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Abstract The zebrafish is widely used as a model system for studying mammalian developmental genetics and more recently, as a model system for carcinogenesis. Since there is mounting evidence that selenium can prevent cancer in mammals, including humans, we characterized the selenocysteine tRNA [Ser]Sec gene and its product in zebrafish. Two genes for this tRNA were isolated and sequenced and were found to map at different loci within the zebrafish genome. The encoding sequences of both are identical and their flanking sequences are highly homologous for several hundred bases in both directions. The two genes likely arose from gene duplication which is a common phenomenon among many genes in this species. In addition, zebrafish tRNA[Ser]Sec was isolated from the total tRNA population and shown to decode UGA in a ribosomal binding assay.

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Key words: Zebrafish; Selenocysteine; tRNA; Gene; Selenium

1. Introduction

The zebrafish is becoming a prominent model organism for studying mammalian genetics and development [1] and more recently, as a model for studying oncogenes and carcinogenesis (see [1-4] and references therein). Selenium suppresses cancer in rodents, and possibly in humans, and selenoproteins are the most likely candidates responsible for the chemopreventive effect of this element (see [5,6] and references therein). As selenocysteine (Sec) tRNA^{[Ser]Sec} is the central component in the selenoprotein biosynthesis [7], we examined this critical molecule as the initial step in determining if zebrafish may also serve as a model organism for studying selenium as a chemopreventive agent in cancer. We found that zebrafish, unlike any other known animal in which the tRNA [Ser] Sec gene has been sequenced, encodes two copies of this gene. All other higher and lower animals, including several mammals, chickens, frogs, fruit flies and worms, in which the tRNA[Ser]Sec gene has been sequenced, contain this gene only in a single copy (reviewed in [8]). Both zebrafish genes have identical encoding sequences, contain highly homologous sequences for several hundred bases upstream and downstream of the gene and map at different loci. The two genes likely result from gene duplication which is a common occurrence with many genes of the zebrafish lineage [9,10] and they may

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have resulted from genome duplication in an early ancestor

2. Materials and methods

2.1. Materials

The zebrafish genomic library cloned in λ (EMBL 3 SP6/T7) was purchased from Clontech (Palo Alto, CA, USA) and all reagents were commercial products of the highest grade available. Adult zebrafish were quickly frozen in liquid nitrogen and stored at -80° C until ready for use.

2.2. Library screening, gene isolation and sequencing

All molecular cloning procedures including plaque lifting, Southern blotting, subcloning, restriction enzyme digestions and gel electrophoresis followed standard techniques [12] or the manufacturer's protocol unless otherwise mentioned. The zebrafish genomic library (18 plates with approximately 2×10⁴ phages/plate) was screened with a 193 bp fragment encoding the human tRNA[Ser]Sec gene [13] labelled with [α-3²P]dCTP as probe. Following hybridization for 2 h, membranes were washed twice for 10 min each in SSC (1×SSC was used during library screening and 0.2×SSC in all subsequent hybridizations) and 0.5% SDS at room temperature and exposed to a phosphur screen for 0.5–2 h or to X-ray films for 2–10 h. Bacteriophages giving positive signals were amplified and phage DNA was isolated using Qiagen columns. Fragments encoding the Sec tRNA[Ser]Sec gene obtained by restriction endonuclease digestion were subcloned into pUC 19 for sequencing. GCG-Lite+Clustalw in the NIH Network was used for sequence alignment and analysis.

2.3. Genomic mapping

Genomic mapping of the two ZStR genes was performed using a zebrafish-hamster radiation hybrid panel (Research Genetics). PCR analyses were carried out in a final volume of 10 μl containing $1\times PCR$ buffer (Perkin Elmer), 2 μM of each primer, 200 μM of each dNTP, 1 μl of template DNA (25 ng) and 0.5 U of AmpliTaq Gold DNA polymerase (Perkin Elmer). After an initial activation of polymerase at 95°C for 10 min, 36 cycles at 95°C for 15 s, 58°C for 15 s and 72°C for 40 s were carried out on a GeneAmp PCR System 9600 (Perkin Elmer). PCR products were separated on a 2.5% agarose gel (Gibco BRL). Analysis of the radiation hybrid panel data was performed by Dr Robert Geisler of the Max-Planck Institute.

2.4. Isolation, fractionation and codon recognition studies of $tRNA^{\{Ser\}Sec}$

Total tRNA was isolated from 45 g of frozen adult zebrafish as described [13]. 315 A_{260} U of total tRNA were applied to a RPC-5 column in 0.45 M NaCl, 0.01 M Mg(OAc)₂, 0.01 M NaOAc and 0.001 M EDTA, pH 4.5, and the attached tRNA eluted in a linear 0.50 M–0.70 M NaCl gradient as described [14]. Sec tRNA^{[Ser]Sec} was identified in fractionated tRNA by dot blotting 5 μ l of every other eluted fraction onto nitrocellulose filters and hybridizing with a labelled 193 bp human DNA fragment encoding the Sec tRNA^{[Ser]Sec} gene as probe. Fractions containing tRNA^{[Ser]Sec} were pooled, amino-acylated with [3 H]serine [13], the resulting [3 H]seryl-tRNA^{[Ser]Sec} was fractionated on a RPC-5 column and individual peaks of [3 H]seryl-tRNA were isolated, prepared for encoding studies and encoding

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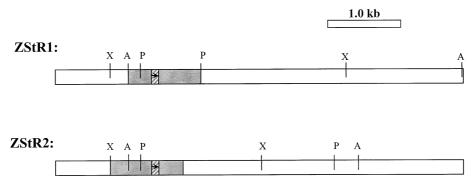


Fig. 1. Restriction map of zebrafish ZStR1 and ZStR2. The shaded regions were sequenced including the hatched areas that correspond to the encoding sequences of the tRNA^{[Ser]Sec} genes as shown in Fig. 2. Arrows in the hatched boxes indicate the transcription orientation. X designates XbaI, P, PstI and A, AccI. The PstI fragment of ZStR1 was subcloned and sequenced. The sequence of an additional 171 bp upstream of the PstI site within ZStR1 was obtained by subcloning a 1 kb AccI fragment of recombinant DNA that was cloned in λ near the 3'-terminus of the tRNA^{[Ser]Sec} gene and contained an AccI site inside the vector DNA. The 2.1 kb XbaI fragment of ZStR2 was subcloned and 1031 bp sequenced.

studies were carried out by the procedure of Nirenberg and Leder [15] as described [14].

3. Results

3.1. Restriction analysis of recombinant and genomic DNAs

The genomic library of zebrafish was screened and six positive clones were isolated. Digestion of each with endonucleases showed that they fell into two classes, designated ZStR1 and ZStR2 (see restriction map in Fig. 1). ZStR1, ZStR2 and zebrafish genomic DNA were digested with *XbaI* and with *PstI* and the resulting fragments analyzed by Southern blotting (see Fig. 2). Genomic DNA yielded two fragments in both digests as shown in lanes 4 and 5. One fragment in each digest corresponded to those generated from ZStR1 (lanes 2 and 6), while the other corresponded to those generated from ZStR2 (lanes 3 and 7). Thus, ZStR1 and ZStR2 are located at different loci within the zebrafish genome.

3.2. Sequencing of recombinant DNAs

A total of 1031 bp in ZStR1 and 1006 bp in ZStR2 were sequenced and aligned with the corresponding tRNA^{[Ser]Sec} gene and flanking regions in *Xenopus* as shown in Fig. 3. The encoding sequences of the two zebrafish genes are identical and differ by 5 bp from the tRNA^{[Ser]Sec} gene in *Xenopus*. The flanking sequences of both zebrafish genes are highly homologous (97.5% in the 5'-flank and 93.6% in the 3'-flank). The upstream sequence encodes three regulatory regions, a TATA box at -30, a proximal sequence element (PSE) at -66 and an activator element (AE) at -205. These regulatory elements correspond to similar elements in *Xenopus* (see Fig. 3) that govern expression of the tRNA^{[Ser]Sec} gene [8].

3.3. Genomic mapping of the zebrafish Sec tRNA genes

3.4. Identification and codon recognition properties of the gene products

Total tRNA from zebrafish was fractionated on a RPC-5

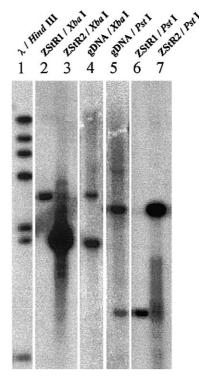


Fig. 2. Hybridization of zebrafish genomic and recombinant DNAs. Genomic DNA or recombinant DNA was digested, electrophoresed on an agarose gel, transblotted to a filter and hybridized with a 193 bp fragment of human DNA encoding the tRNA^{[Ser]Sec} gene (see Section 2). Lanes 2 and 6 contain ZStR1, lanes 3 and 7 contain ZStR2 and lanes 4 and 5 contain genomic DNA (designated gDNA) digested with *XbaI* and *PstI*, respectively. Lane 1 contains labelled DNA markers digested with *HindIII*.

	-600									-501
ZStR1										
	AAAGTATTTA	AAGTCTTCTT	GTATAATACT	TGTTGTATCA	GATATTTTAC	TTAATAACAA	TTCAGCTTCT	TTTTCATTGT	AAGGGTCTGA	
XTRSP		• • • • • • • • • • • • • • • • • • • •								
	-500									-401
ZStR1								COMMUNICACION OF		
	TTCTTATAAA									
2111101				0000010111						
20121	-400					Cm	CTA CTTA CAA	Unive	rsal FW Prin	ner -301
ZStRI ZStR2	GTTTTTCTAA	ATAAAAAAAA	ATATTTAGTA	TTTTTTTCTC	CTTGTTCAAT	CCACTTTTGT	CTACTTACAA	ATGCACCTTT	AGAAGTCTTG	AAGATGTCAT
	GTCACCTCCT									
	200									-201
ZStR1	-300 TAATTAACAA	ACAACGAA	CAATGACATA	AAAACTCCAC	CCAAGTCTCA	ACCAATAGCA	AACA	.ATTAATCTG	CATGCGGT.G	
ZStR2	TCATTAACAA	ACAACGAA	CAATGACATG	AAAACTCCAC	CCAAGTCTCA	ACCAATAGCA	AACA	.ATTAATCTG	CATGCGGT.G	CAGCGCTGTG
XTRSP	CCACTAACAA	ACAGCAACCA	CACGCCCCTC	CTCCCTCTTC	CCGTTTTTTC	ATTGAAAATA	AACCGAAGCG	TATTGTTATG		CATGCCTCGC AE
	-200								F	-101
ZStR1	TTTAATGCAG								GATGATGATA	AGATGATGAT
ZStR2									GATGATGATA	
XTRSP	GCGCGTGTAT	GCTATGCGAT	CTTGTTTGAT	TTCCCTCGAT	TTCACGTTAG	ACTAGTCGGG	GTATGTAAGC	GGCGATACGT	T.TAACTAGA	AAAGGAAI
	-100									-1
ZStR1	ATGCTGCCAA	AT.ATAGTTT	TAGAAATATC	CCCTCACCAC	AGATGTAAAG	CACATCACTG	CCACGACCTG	TATATAAGGG	TGGTTTCCGC	TGCTTCGAAT
ZStRZ	ATGCTGCCAA AGTCAGATTT	AT.ATAGTTT	TAGAAATTTC	TTGTCACCAC	AGATGTAAAG A.ATATATAA	TATAATGGTG	GGAGGGGGTA	TAAAAGGAAA	TGGGTTTCAGC	TGAGGTATTT
MINDI	1101011011111	110/11/100/10	THEORETTE	1101010000	PSE			TATA		
	+1					0.000.000.00	or omcommor	2 mm 0 0 2 0 0 mm		+100
ZStR1									TCGGGCGACT	
ZStR2 XTRSP	000000000								TCGGGCGAGG	300
MINDL			*	11000110111	00101110010		0110110011011			
		* *	*			* ^				
	+101		Specific FV		War e Wamar r		CDD2 CDD200	7.7.M. 7.0M. M. M	cióm ca milima a	+200
ZStR1	+101 CATCACTACA	AAAA.TA TTT	Specific FV	ACAAAACAGA	CGATACTGAA AGATGCTGAA	AATAATAAAC	GTTACTTATA	AAATACTTTG AAATACTTTG	CGTCATGTGC	CGCTTTATAA
ZStR2	+101	AAAA .TA TTT AAAAATA CTT	Specific FV AAGAAATTAC AAGAAATTTC	ACAAAACAGA ACTAAACAGC	A GATGCTGAA	AATAATAAAC	GTTACTTCTA	AAATACTTTG	CATCATTTGC	CGCTTTATAA CGCTTTATAA
ZStR2	+101 CATCACTACA CATTACTACA TTTCATCACT	AAAA .TA TTT AAAAATA CTT	Specific FV AAGAAATTAC AAGAAATTTC	ACAAAACAGA ACTAAACAGC	A GATGCTGAA	AATAATAAAC	GTTACTTCTA TTAGCA	AAATACTTTG ATATTCTTTA	CATCATTIGC CCGAAAC	CGCTTTATAA CGCTTTATAA GGCTTTCTAT
ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201	AAAA.TA TTT AAAAATA CTT TCTATCCTCT	Specific FV AAGAAATTAC AAGAAATTTC GATAAATAGC	ACAAAACAGA ACTAAACAGC TCATCTCAGT	AGATGCTGAA GGCTA	AATAATAAAC TATAATGCGC	GTTACTTCTA TTAGCA Univers	AAATACTTTG ATATTCTTTA sal RV Prime	CATCATTTGC CCGAAAC	CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300
ZStR2 XTRSP ZStR1 ZStR2	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA	AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG	Specific FV AAGAAATTC AAGAAATTC GATAAATAGC .CAGCCACGC .CAGCCACGC	ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC	AATAATAAAC TATAAACACT ATTAAACACT	GTTACTTCTA TTAGCA Univers CAACTGGACC CAACTGGAAC	AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC	CATCATTTGC CCGAAAC er ACAGCGCTCA ACAGCGCTCA	CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTGAAGTGTC
ZStR2 XTRSP ZStR1 ZStR2	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA	AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG	Specific FV AAGAAATTC AAGAAATTC GATAAATAGC .CAGCCACGC .CAGCCACGC	ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC	AATAATAAAC TATAAACACT ATTAAACACT	GTTACTTCTA TTAGCA Univers CAACTGGACC CAACTGGAAC	AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC	CATCATTTGC CCGAAAC er ACAGCGCTCA ACAGCGCTCA	CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTGAAGTGTC
ZStR2 XTRSP ZStR1 ZStR2	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTACATTCA	AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG	Specific FV AAGAAATTC AAGAAATTC GATAAATAGC .CAGCCACGC .CAGCCACGC	ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC	AATAATAAC TATAATGCGC ATTAAACACT ATTAAACACT ATTATGTTTG	GTTACTTCTA TTAGCA Univers CAACTGGACC CAACTGGAAC TCATTTCTGA	AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT	CATCATTTGC CCGAAAC er ACAGCGCTCA ACAGCGCTCA	CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTGAAGTGTC
ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATAGGA	AAAA. TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC	Specific FT AAGAAATTC AAGAAATTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG	ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG GTTAGCTTCA	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTT TGCA.TGCTG	AATAATAAC TATAAACACT ATTAAACACT ATTATGTTTG Spe GATTGAGAGG	GTTACTTCTA TTAGCA Univers CAACTGGACC CAACTGGAAC TCATTTCTGA ecific RV Pr CTAGCA.GGC	AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT cimers TAACATTTAT	CATCATTTGC CCGAAAC er ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA	CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA
ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATACGA TGTCATACGA	AAAA. TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC	Specific FT AAGAATTAC AAGAATTTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG AGTGCCCTTG	ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG GTTAGCTTCA GTTAGCTTCA	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTTT TGCA.TGCTG TGCA.TGCTG	AATAATAAC TATAAACACT ATTAAACACT ATTATGTTTG Spg GATTGAGAGG GATTGAAAAG	GTTACTTCTA TTAGCA Univers CAACTGGACC CAACTGGAAC TCATTTCTGA ecific RV Pr CTAGCA.GGC CTAACAAGGC	AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT rimers TAACATTTAT TAACATATAT	CATCATTIGC CCGAAAC PI ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA CCTGTA.AAA	CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA CAATTTATAA
ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATAGGA	AAAA. TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC	Specific FT AAGAATTAC AAGAATTTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG AGTGCCCTTG	ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG GTTAGCTTCA GTTAGCTTCA	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTTT TGCA.TGCTG TGCA.TGCTG	AATAATAAC TATAAACACT ATTAAACACT ATTAAACACT ATTATGTTTG Spg GATTGAGAGG GATTGAAAAG	GTTACTTCTA TTAGCA Univers CAACTGGACC CAACTGGAAC TCATTTCTGA ecific RV Pr CTAGCA.GGC CTAACAAGGC	AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT rimers TAACATTTAT TAACATATAT	CATCATTIGC CCGAAAC PI ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA CCTGTA.AAA	CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA CAATTTATAA
ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATAGGA TGTCATAGGA GCCAGTCTA +401	AAAA. TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA	Specific FT AAGAAATTAC AAGAAATTTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG AGTGCCCTTG GCTGCTCCCT	ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG GTTAGCTTCA GTTAGCTTCA TATATCTTGC	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC	AATAATAAC TATAAACACT ATTAAACACT ATTAAACACT ATTATGTTTG Spi GATTGAGAGG GATTGAAAAG TTATATATCA	GTTACTTCTA TTAGCA Univers CAACTGGACC CAACTGGAAC TCATTTCTGA ecific RV Pr CTAGCA.GGC CTAACAAGGC GTAACAAGGTTA	AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT rimers TAACATTTAT TAACATATAT ATACAGTTAA	CATCATTTGC CCGAAAC er ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA CCTGTA.AAA AAGGGATAAA	CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA CAATTTATAA AAAAACATAT +500
ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATAGGA TGTCATACGA .GCCAGTCTA +401CATTTCC	AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC	Specific FT AAGAATTAC AAGAATTTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG AGTGCCCTTG GCTGCTCCCT	ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG GTTAGCTTCA GTTAGCTTCA TATATCTTGC AAGAGTGCTG	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC	AATAATAAC TATAAACACT ATTAAACACT ATTAAACACT ATTATGTTTG GATTGAGAGG GATTGAAAAG TTATATATCA TAATATTAGT	GTTACTTCTA TTAGCA Univers CAACTGGACC CAACTGGACC TCATTTCTGA ecific RV Pr CTAGCA.GGC CTAACAAGGC GTAACAGTTA ATATCGATAT	AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT rimers TAACATTTAT TAACATATAT ATACAGTTAA TATACAGTTAA	CATCATTTGC CCGAAAC PT ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA CGTGTA.AAA AAGGGATAAA GGATCAGATG	CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTGAAGTGTC CTCCAG.GTG -400 CAATTTATAA CAATTTATAA AAAAACATAT +500 ATTTCATGTG
ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTTCATAAA TTTCATACAT +301 TGTCATACGA TGTCATACGA .GCCAGTCTA +401CATTTCCCATTTCC	AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCG	Specific FT AAGAAATTC AAGAAATTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG AGTGCCCTTG GCTGCTCCCT	ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCCA GTTAGCTTCA GTTAGCTTCA TATATCTTGC AAGAGTGCTG	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC	AATAATAAC TATAAACACT ATTAAACACT ATTAAACACT ATTATGTTTG GATTGAAAG GATTGAAAAG TTATATATCA TAATATTAGT	GTTACTTCTA TTAGCA Univers CAACTGGACC CAACTGGAAC TCATTTCTGA ecific RV PP CTAGCA.GGC CTAACAAGGC GTAACAGTTA ATATCGATAT	AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT cimers TAACATTTAT TAACATTTAT ATACAGTTAA TATAGTTGTA	CATCATTTGC CCGAAAC PT ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA ACGTGTA.AAA AAGGGATAAA GGATCAGATG	CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA CAATTTATAA AAAACATAT +500 ATTTCATGTG
ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATAGGA TGTCATAGGA GCCAGTCTA +401CATTTCC GTGCAGTACA	AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCG	Specific FT AAGAAATTC AAGAAATTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG AGTGCCCTTG GCTGCTCCCT	ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCCA GTTAGCTTCA GTTAGCTTCA TATATCTTGC AAGAGTGCTG	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC	AATAATAAC TATAAACACT ATTAAACACT ATTAAACACT ATTATGTTTG GATTGAAAG GATTGAAAAG TTATATATCA TAATATTAGT	GTTACTTCTA TTAGCA Univers CAACTGGACC CAACTGGAAC TCATTTCTGA ecific RV PP CTAGCA.GGC CTAACAAGGC GTAACAGTTA ATATCGATAT	AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT cimers TAACATTTAT TAACATTTAT ATACAGTTAA TATAGTTGTA	CATCATTTGC CCGAAAC PT ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA ACGTGTA.AAA AAGGGATAAA GGATCAGATG	CGCTTTATAA CGCTTTATAA GGCTTTCTAT +300 CTGAAGTGTC CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA CAATTTATAA AAAAACATAT +500 ATTTCATGTG ATTGTGCCTTG
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ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATAGA TGTCATAGA GCCAGTCTA +401CATTTCC GTGCAGTACA +501 ACATAAAG TATTACAGGT	AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCG TGATCACAGT AGTAGAGCTG	Specific FT AAGAATTTC AAGAATTTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG AGTGCCCTTG GCTGCTCCCT AATCCCCGGT	ACAAACCAA ACTAAACACCA GAACAACCCA GAACAACCCA GTAACAACCCA GTTAGCTTCA GTTAGCTTCA TATATCTTGC AAGAGTGCTG GAGTGTAAGA AGGTTTAAAC	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC .TTCTTTTCTG	AATAATAAC TATAAACACT ATTAAACACT ATTAAACACT ATTATGTTTG GATTGAGAGG GATTGAAAG TTATATATCA TAATATTAGT GAAGTTGCAT AATGACAATA	GTTACTTCTA TTAGCA Univer: CAACTGGACC CAACTGGAAC TCATTTCTGA ecific RV Proceed CTAACAGCC CTAACAAGCC GTAACAAGTTA ATATCGATAT TTTCCTTTAG TGCTGAGTAA	AAATACTTTG ATATTCTTTA sal RV Prime AATC AATC TATCTTTAGT rimers TAACATTTAT TAACATTTAT ATACAGTTAA TATAGTTGTA TTTAATTCTA AACATGGTGG	CATCATTTGC CCGAAAC PT ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA ACGTGTA.AAA AAGGGATAAA GGATCAGATG TTATGGACTA TTATAGGTG	CGCTTTATAA CGCTTTATAA CGCTTTATA +300 CTGAAGTGTC CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA AAAACATAT +500 ATTTCATGTG
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ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATAGA TGTCATAGA GCCAGTCTA +401CATTTCC GTGCAGTACA +501 ACATAAAG TATTACAGGT +601 TATAAAGCAA TTTTCAAAAAA +701	AAAA.TATT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCG TGATCACAGT AGTAGAGCTG AGTAGAGCTG ATGGGATCCG AGTATAATTA TGATTCCTT	Specific FT AAGAATTAC AAGAATTTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG AGTGCCCTTG GCTGCTCCCT AATCCCCGGT TTACTTAT.T CTGAAGATTC TTAACTGGAA TTTAATCTTA TTTCTCTGTA	ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG GTTAGCTTCA GTTAGCTTCA TATATCTTGC AAGAGTGCTG	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTTT TGCA.TGCTG TCGA.TGCTG TCAAGTGAAC AATTGTTTGC .TTCTTTTCTG CACCGGATAA	AATAATAAC TATAAACACT ATTAAACACT ATTAAACACT ATTATGTTTG GATTGAAAG TTATATATCA TAATATTACA TAATATTAGT	GTTACTTCTA TTAGCA Univers CAACTGGACC CAACTGGAAC TCATTTCTGA ecific RV Pr CTAGCA.GGC CTAACAAGGC GTAACAGGTTA ATATCGATAT TTTCCTTTAG TGCTGAGTAA AGCCCTTTTC TTTAAATGGT	AAATACTTTG ATATTCTTTA sal RV Prime AATC TATCTTTAGT rimers TAACATTTAT TAACATATAT ATACAGTTAA TATAGTTGTA TATAGTTGTA CTATAGTTGTA CTATAGTTGTA CTATAGTTGTA CTATAGTTGTA CTATAGTTCTC CG.CTTTTAA	CATCATTTGC CCGAAAC PT ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA ACGTGTA.AAA AAGGGATAAA GGATCAGATG TTATGGACTA TTATAGGTG CATGTATCTA TATGTCAGAA TATGTCAGAA	CGCTTTATAA CGCTTTATAA CGCTTTATATA 4300 CTGAAGTGTC CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA CAATTATAA AAAAACATAT +500 ATTTCATGTG
ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATAGGA TGTCATAGGA .GCCAGTCTA +401CATTTCC GTGCAGTACA +501 ACATAAAG TATTACAGGT +601 TATAAAAGCAA TTTTCAAAAAA +701 GCGTTCGACA	AAAA.TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC CGATCGCGTC CGATCG TGATCACAGT AGTAGAGCTC AGTAGAGCTC AGTATAATTA TGATTTCCTT	Specific FT AAGAATTAC AAGAATTTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG AGTGCCCTTG GCTGCTCCCT AATCCCCGGT .TATCTTAT.T CTGAAGATTC .TTAACTGGAA TTTAATCTTA .TTTCTCTGTA	ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA GATAGCTTCA GTTAGCTTCA GTTAGCTTCA TATATCTTGC AAGAGTGCTG AAGAGTGCTG AAGATGTAAAC	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC GTTCCATTT TGCA.TGCTG TGCA.TGCTG TCAAGTGAAC AATTGTTTGC TCTTTTCTG CACCGGATAA CAGAAGCTCA TGTTTCATGG AGTACTTTGG	AATAATAAAC TATAAACACT ATTAAACACT ATTAAACACT ATTATGTTTG GATTGAAAG TTATATATCA TAATATTACA TAATATTACA AATGACAATA GAATTACGGA AAATGTGATC ACTTTATCTT +760	GTTACTTCTA TTAGCA Univers CAACTGGACC CAACTGGAAC TCATTTCTGA ecific RV Pr CTAGCA.GGC CTAACAAGGC GTAACAGGTTA ATATCGATAT TTTCCTTTAG TGCTGAGTAA AGCCCTTTTC TTTAAATGGT	AAATACTTTG ATATTCTTTA sal RV Prime AATC TATCTTTAGT rimers TAACATTTAT TAACATATAT ATACAGTTAA TATAGTTGTA TATAGTTGTA CTATAGTTGTA CTATAGTTGTA CTATAGTTGTA CTATAGTTGTA CTATAGTTCTC CG.CTTTTAA	CATCATTTGC CCGAAAC PT ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA ACGTGTA.AAA AAGGGATAAA GGATCAGATG TTATGGACTA TTATAGGTG CATGTATCTA TATGTCAGAA TATGTCAGAA	CGCTTTATAA CGCTTTATAA CGCTTTATATA 4300 CTGAAGTGTC CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA CAATTATAA AAAAACATAT +500 ATTTCATGTG
ZStR2 XTRSP ZStR1 ZStR2 XTRSP ZStR1 ZStR2 XTRSP	+101 CATCACTACA CATTACTACA TTTCATCACT +201 TTTTCATAAA TTTTCATAAA TTTTCATAAA TTTACATTCA +301 TGTCATAGGA TGTCATAGGA .GCCAGTCTA +401CATTTCC GTGCAGTACA +501 ACATAAAG TATTACAGGT +601 TATAAAAGCAA TTTTCAAAAAA +701 GCGTTCGACA	AAAA. TATTT AAAAATACTT TCTATCCTCT CCCGGAAGCG CCCGGAAGCG GTCACAGTAT AATGACGTAC AATGACGTAC TCAGTCATAA CGATCGCGTC CGATCG TGATCACAGT ATGGGATCCG AGTATAATTA TGATTTCCTT TGAAGCTCAG	Specific FT AAGAATTAC AAGAATTTC GATAAATAGC .CAGCCACGC .CAGCCACGC ACAGTATCCA AGTGCCCTTG AGTGCCCTTG GCTGCTCCCT AATCCCCGGT TATCTTAT.T CTGAAGATTC TTAACTGGAA TTTAATCTTA .TTTCTCTGTA CTGCAG	ACAAACAGA ACTAAACAGC TCATCTCAGT GAACAACCCA GAACAACCCA TAACAACCAG GTTAGCTTCA GTTAGCTTCA GTTAGCTTCA TATATCTTGC AAGAGTGCTG	AGATGCTGAA GGCTA ATCAGCGC ATCAGCGC ATCAGCGC GTTCCATTT TGCA.TGCTG TCAAGTGAAC AATTGTTTGC TTCTTTTCTG CACCGGATAA CAGAAGCTCA TGTTTCATGG AGTACTTTGG	AATAATAAC TATAAACACT ATTAAACACT ATTAAACACT ATTAAACACT ATTATGTTTG GATTGAAAG TTATATATCA TAATATTAGT GAAGTTGCAT AATGACAATA AATGACAATA AATGACAATA AAATGTGATC ACTTTATCTT +760	GTTACTTCTA TTAGCA Univers CAACTGGACC CAACTGGAAC TCATTTCTGA ecific RV Pr CTAGCA.GGC CTAACAAGGC GTAACAGGTTA ATATCGATAT TTTCCTTTAG TGCTGAGTAA AGCCCTTTTC TTTAAATGGT	AAATACTTTG ATATTCTTTA sal RV Prime AATC TATCTTTAGT rimers TAACATTTAT TAACATATAT ATACAGTTAA TATAGTTGTA TATAGTTGTA CTATAGTTGTA CTATAGTTGTA CTATAGTTGTA CTATAGTTGTA CTATAGTTCTC CG.CTTTTAA	CATCATTTGC CCGAAAC PT ACAGCGCTCA ACAGCGCTCA ACATGGGTAA CATGTA.AAA ACGTGTA.AAA AAGGGATAAA GGATCAGATG TTATGGACTA TTATAGGTG CATGTATCTA TATGTCAGAA TATGTCAGAA	CGCTTTATAA CGCTTTATAA CGCTTTATATA 4300 CTGAAGTGTC CTGAAGTGTC CTCCAG.GTG +400 CAATTTATAA CAATTATAA AAAAACATAT +500 ATTTCATGTG

Fig. 3. Alignment of zebrafish recombinant DNA sequences and a *Xenopus* DNA sequence encoding the tRNA^{[Ser]Sec} gene. The encoding sequences of the tRNA^{[Ser]Sec} genes of zebrafish and *Xenopus* (boxed region) and the flanking sequences of ZStR1, ZStR2 and *Xenopus* are shown. The sequences from *Xenopus* were taken from Lee et al. [19]. Base differences within the encoding sequences are indicated with an asterisk. TATA boxes at -30 in ZStR1 and ZStR2 and at -31 in XtRSP, the PSE at -66 in ZStR1 and ZStR2 and at -64 in XStR and the AE at -205 in ZStR1 and ZStR2 and at -209 in XStR are underlined. Base differences in the flanking sequences of ZStR1 and ZStR2 are shaded. To maximize homology between sequences, dots were used to indicate gaps with the exception of the 3'- and 5'-ends of sequences where dots indicate unidentified bases. Specific and universal primers used for PCR analysis of the radiation hybrid panel are shown in bold. GenBank accession numbers for ZStR1 and ZStR2 are AF135236 and AF135237, respectively.

column and Sec tRNA^{[Ser]Sec} identified in the eluted fractions by Northern hybridization (see Fig. 4A). Two peaks were observed that were pooled as shown in the figure, amino-acylated with [³H]serine and refractionated over the column (Fig. 4B and C, respectively). Most of the serine tRNA^{Ser} eluted

with the earlier eluting peak of $tRNA^{[Ser]Sec}$. Hence, the amount of $[^3H]$ seryl- $tRNA^{[Ser]Sec}$ relative to $[^3H]$ seryl- $tRNA^{Ser}$ is much less in the earlier eluting Sec $tRNA^{[Ser]Sec}$ isoacceptor than the latter one. Both seryl- $tRNA^{[Ser]Sec}$ isoacceptors decode UGA in a ribosomal binding assay.

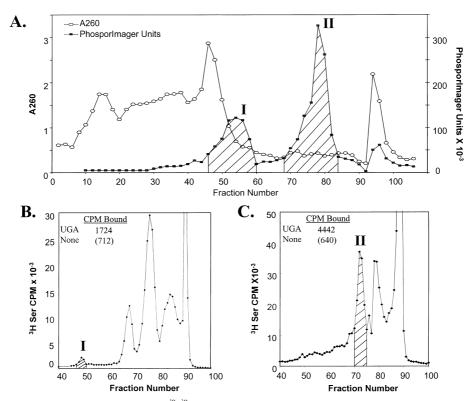


Fig. 4. Fractionation and coding responses of Sec tRNA^{[Ser]Sec}. In A, total tRNA was loaded onto a RPC-5 column, the column was washed and tRNA fractionated in a linear 0.50–0.70 M NaCl gradient, aliquots of every other fraction were blotted on a filter and the filter was hybridized with probe (see Section 2). Fractions were pooled as shown by the hatched areas in A, amino-acylated with [³H]serine and the resulting [³H]seryl-tRNAs were fractionated on the column in a linear 0.6–0.825 M NaCl gradient without Mg²+ (see Section 2). In B, peak I from graph A is shown and in C, peak II from graph A is shown. The hatched peak in B and that in C were pooled, prepared for encoding studies and encoding studies were carried out as given in Section 2. The total number of cpm added to assays shown in B was 4500 and that shown in C was 8430. None designates the amount of binding of [³H]seryl-tRNA^{[Ser]Sec} to ribosomes in the absence of trinucleoside diphosphate. Codon UGA was a gift of M.W. Nirenberg.

4. Discussion

Zebrafish contain two distinct tRNA^{[Ser]Sec} genes. Both have a TATA box, a PSE and an AE in the 5'-flanking region which are the three regulatory elements [16] that govern expression of all other eukaryotic Sec tRNA^{[Ser]Sec} genes examined to date [8]. Both genes are transcribed following their microinjection into *Xenopus* oocytes, but the level of transcription was quite low (data not shown) and comparable to that observed for the chicken tRNA^{[Ser]Sec} gene in this transcription system [17]. The genes map at different loci within the zebrafish genome, but at present, we cannot determine whether they are localized on the same or different chromosomes

Two Sec tRNAs^{[Ser]Sec} were found in the total tRNA population. The two isoacceptors most certainly differ by base modification as the primary transcripts of both genes would have identical sequences. The isoacceptor that elutes first from the RPC-5 column may likely lack a N^6 -isopentenyladenosine (i^6A) modification at position 37. This modification is characteristic for Sec tRNAs^{[Ser]Sec} isolated from other animals [8] and the absence of i^6A causes tRNA^{[Ser]Sec} to elute much earlier from the RPC-5 column [18] as was observed in the elution of the initial peak of zebrafish tRNA^{[Ser]Sec} (see Fig. 4A).

The present studies demonstrate for the first time that the Sec tRNA^{[Ser]Sec} gene occurs in two copies in the genome of an animal even though gene duplication appears to be a frequent occurrence in zebrafish [9–11]. The present study provides an

initial characterization of the machinery involved in the selenoprotein biosynthesis in zebrafish and provides a foundation on which to expand the use of this organism as a model for exploring mechanisms of selenium-mediated chemoprevention in cancer.

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