RESEARCH

Identification of the Complete Coding Sequence and Genomic Organization of the Treacher Collins Syndrome Gene

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Treacher Collins syndrome (TCS) is an autosomal dominant disorder of craniofacial development, the features of which include conductive hearing loss and cleft palate. Recently, the demonstration of a series of 10 mutations within a partial-length cDNA clone have indicated that the TCS gene (*TCOF1*) has been positionally cloned. Although it has been shown that the gene is expressed in a wide variety of fetal and adult tissues, database sequence comparisons have failed to provide significant information on the function of the gene. In the current investigation, a combination of cDNA library screening and rapid amplification of cDNA ends has permitted the isolation of the complete coding sequence of *TCOF1*, which is encoded by 26 exons and predicts a low complexity, serine/alanine-rich protein of ~144 kD. The use of a variety of bioinformatics tools has resulted in the identification of repeated units within the gene, each of which maps onto an individual exon. The predicted protein *Treacle* contains numerous potential phosphorylation sites, a number of which map to similar positions within the repeated units, and shows weak but significant homology to the nucleolar phosphoproteins. Although the precise function of *Treacle* remains unknown, these observations suggest that phosphorylation may be important for its role in early embryonic development and that it may play a role in nucleolar–cytoplasmic shuttling. The information presented in this study will allow continued mutation analysis in families with a history of TCS and should facilitate continued experimentation to shed further light on the function of the gene/protein during development of the craniofacial complex.

[The sequence data described in this paper have been submitted to the GenBank data library under accession nos. U40847 and U79645–U79660.]

Treacher Collins syndrome (TCS) is an autosomal dominant disorder of facial development, which was probably first reported by Thompson (1846), but is named after E. Treacher Collins, who described the essential features of the syndrome in 1900 (Treacher Collins 1900). The incidence of TCS is thought to be ~1/50,000 live births (Fazen et al. 1967; Gorlin et al. 1990), with 60% of cases appearing to arise as the result of a de novo mutation (Jones et al. 1975). TCS displays high penetrance, with only one reported case of nonpenetrance (Dixon et al. 1994a) and a high degree of both inter- and intrafamilial phenotypic variability. The clinical manifestations include the following. (1) Abnormalities of the external ears, which are frequently

associated with narrowing of the ear canals and abnormalities of the middle-ear ossicles. Bilateral conductive hearing loss is therefore a common feature of this disorder (Phelps et al. 1981). (2) Hypoplasia of the facial bones, particularly the mandible and zygomatic complex. (3) Downward slanting of the palpebral fissures with colobomas (notching) of the lower eyelids and a lack of eyelashes medial to the defect. (4) Cleft palate. These features are usually bilaterally symmetrical (Kay and Kay 1989); however, because of the variability in expression, it can be extremely difficult to reach a clinical diagnosis and to provide accurate genetic counselling.

The Treacher Collins syndrome locus (*TCOF1*) was initially linked to polymorphic markers from chromosome 5 at 5q31–34 (Dixon et al. 1991). This localization was subsequently confirmed by Jabs et al. (1991), and more recent studies have resulted in

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the creation of a combined genetic and radiation hybrid map around the *TCOF1* locus (Dixon et al. 1993; Loftus et al. 1993). This map allowed a yeast artificial chromosome (YAC) contig of the region to be constructed (Jabs et al. 1993; Dixon et al. 1994b). Transcription mapping of the *TCOF1* candidate region eventually led to the identification of a cDNA clone with an open reading frame (ORF) of 4142 bp that did not contain a translation initiation signal, a polyadenylation signal, or a poly(A) tail (Loftus et al. 1996; Treacher Collins Syndrome Collaborative Group 1996). Investigation of this cDNA led to the identification of different mutations in 10 unrelated families, all of which resulted in the introduction of a premature termination codon into the predicted protein product, *Treacle* (Gladwin et al. 1996; Treacher Collins Syndrome Collaborative Group 1996).

As the structures affected in TCS arise from the first and second pharyngeal arches, which in turn have a significant contribution from the neural crest, it has been proposed that the disorder may be the result of a defect in neural crest cell migration or improper cellular differentiation during development (Poswillo 1975; Wiley et al. 1983). The identification of the *TCOF1* gene has, however, failed to elucidate the biochemical nature of the disorder as initial database comparisons have indicated that it has no strong homologies with previously identified genes, gene families, or protein motifs of classic importance. In this study we report the complete sequence of *TCOF1* and its genomic organization, which has allowed us to identify repeated units within the gene that map onto individual exons. Analysis of *TCOF1/Treacle* using a number of bioinformatics tools has suggested further that protein phosphorylation may be important for its function.

RESULTS

Isolation of the Entire Coding Sequence of *TCOF1*

Screening of a number of cDNA libraries with portions of the original cDNA clone (Treacher Collins Syndrome Collaborative Group 1996) failed to identify any clones that extended the sequence previously presented in a 3' direction. Extension of the sequence of the *TCOF1* gene in this direction was therefore achieved using rapid amplification of cDNA ends (RACE). Sequence analysis of a 3' RACE product of ~600 bp isolated using this methodology revealed that it extended the cDNA sequence presented previously by 565 bp, including an additional 40 bp of coding sequence prior to the first

in-frame termination codon. Initial screening of cDNA libraries also failed to identify a clone that extended the sequence in a 5' direction. 5' RACE produced two PCR products, the smaller of which did not extend the previously reported cDNA sequence. Sequencing of the larger product resulted in the identification of an additional 69 bp of sequence, including a strong Kozak consensus initiation sequence (Kozak 1987a,b). Screening of a human fetal brain cDNA library with the larger of the 5' RACE products identified three cDNA clones, all of which provided additional sequence information. The longest of these clones contained the start codon and an in-frame termination codon (TAA) 75 bp upstream. RT-PCR analysis of the 5' end of the gene flanking the initiation codon using RNA extracted from skeletal muscle and a lymphoblastoid cell line yielded a single PCR product of the predicted size, the sequence of which was in perfect agreement with that of the cDNA clone. The combined cDNA cloning and RACE strategies have therefore resulted in the identification of the complete coding sequence of *TCOF1*, which consists of an ORF of 4233 bp, followed by a termination codon and a 3' untranslated region (UTR) of 507 bp, which contains a single polyadenylation signal. A 5' UTR of 93 bp has also been identified (Fig. 1). This sequence predicts a 144-kD protein of 1411 amino acids (Fig. 1). The predicted protein is of low complexity with 5 amino acids, alanine (14.86%), serine (13.59%), lysine (11.18%), glutamic acid (9.13%), and proline (9.06%), accounting for the majority of residues.

Genomic Organization of the Gene

Experiments aimed at determining the genomic organization of *TCOF1* indicated that the gene is encoded by 26 exons, ranging in size from 49 to 561 bp (Table 1). Exon 1 contains the translation initiation signal, preceded by the 5' UTR, which contains a large number of rare-cutter restriction sites, including two *Bss*HII, two *Fse*I, and two *Eag*I sites, within 93 bp. Exon 25 contains the last 24 bp of the coding sequence, the termination codon, and the first 22 bp of the 3' UTR. The remainder of the 3' UTR is encoded by exon 26. The intron/exon boundary sequences conform to the published consensus sequences (Table 1) (Breathnach and Chambon 1981), with the exception of exon 14, the splice donor site of which displays the sequence GC rather than the more usual GT. The intron/exon boundaries are of type 0 (splicing occurring between codons) for introns 1, 4, 6–16, 18, 19, 21, and 24;

CHARACTERIZATION OF *TCOF1*

		AGTGGGGCGCGCGA																					
		GGTCTAAGGGCGCGAGGGGAAGTGGCGGGCGGGGACTAAGGCGGGGCGCTGCAGGTAGCCGGCCGGCCGGGGGGTTCGCGGGT																					
1	1	ATG	GCC	GAG	GCC	AGG	AAG	CGG	CGG	GAG	CTA	CTT	CCC	CTG	ATC	TAC	CAC	CAT	CTG	CTG	CGG	60	
		M	A	E	A	R	K	R	R	E	L	L	P	L	I	Y	H	H	L	L	R	20	
61	21	GCT	GGC	TAT	GTG	CGT	GCG	GCG	CGG	GAA	GTG	AAG	GAG	CAG	AGC	GGC	CAG	AAG	TGT	TTC	CTG	120	
		A	G	Y	V	R	A	A	R	E	V	K	E	Q	S	G	Q	K	C	F	L	40	
121	41	GCT	CAG	CCC	GTA	ACC	CTT	CTG	GAC	ATC	TAT	ACA	CAC	TGG	CAA	CAA	ACC	TCA	GAG	CTT	GGT	180	
		A	Q	P	V	T	L	L	D	I	Y	T	H	W	Q	Q	T	S	E	L	G	60	
181	61	CGG	AAG	CGG	AAG	GCA	GAG	GAA	GAT	GCG	GCA	CTG	CAA	GCT	AAG	AAA	ACC	CGT	GTG	TCA	GAC	240	
		R	K	R	K	A	E	E	D	A	A	L	Q	A	K	K	T	R	V	S	D	80	
241	81	CCC	ATC	AGC	ACC	TCG	GAG	AGC	TCG	GAA	GAG	GAG	GAA	GAA	GCA	GAA	GCC	GAA	ACC	GCC	AAA	300	
		P	I	S	T	S	E	S	S	E	E	E	E	E	A	E	A	E	T	A	K	100	
301	101	GCC	ACC	CCA	AGA	CTA	GCA	TCT	ACC	AAC	TCC	TCA	GTC	CTG	GGG	GCG	GAC	TTG	CCA	TCA	AGC	360	
		A	T	P	R	L	A	S	T	N	S	S	V	L	G	A	D	L	P	S	S	120	
361	121	ATG	AAA	GAA	AAA	GCC	AAG	GCA	GAG	ACA	GAG	AAA	GCT	GGC	AAG	ACT	GGG	AAT	TCC	ATG	CCA	420	
		M	K	E	K	A	K	A	E	T	E	K	A	G	K	T	G	N	S	M	P	140	
421	141	CAC	CCT	GCC	ACT	GGG	AAG	ACG	GTG	GCC	AAC	CTT	CTT	TCT	GGG	AAG	TCT	CCC	AGG	AAG	TCA	480	
		H	P	A	T	G	K	T	V	A	N	L	L	S	G	K	S	P	R	K	S	160	
481	161	GCA	GAG	CCC	TCA	GCA	AAT	ACT	ACG	TTG	GTC	TCA	GAA	ACT	GAG	GAG	GAG	GGC	AGC	GTC	CCG	540	
		A	E	P	S	A	N	T	T	L	V	S	E	T	E	E	E	G	S	V	P	180	
541	181	GCC	TTT	GGA	GCT	GCT	GCC	AAG	CCT	GGG	ATG	GTG	TCA	GCG	GGC	CAG	GCC	GAC	AGC	TCC	AGC	600	
		A	F	G	A	A	A	K	P	G	M	V	S	A	G	Q	A	D	S	S	S	200	
601	201	GAG	GAC	ACC	TCC	AGC	TCC	AGT	GAT	GAG	ACA	GAC	GTG	GAG	GTA	AAG	GCC	TCT	GAA	AAA	ATT	660	
		E	D	T	S	S	S	S	D	E	T	D	V	E	V	K	A	S	E	K	I	220	
661	221	CTC	CAG	GTC	AGA	GCT	GCC	TCA	GCC	CCT	GCC	AAG	GGG	ACC	CCT	GGG	AAA	GGG	GCT	ACC	CCA	720	
		L	Q	V	R	A	A	S	A	P	A	K	G	T	P	G	K	G	A	T	P	240	
721	241	GCA	CCC	CCT	GGG	AAG	GCA	GGG	GCT	GTA	GCC	TCC	CAG	ACC	AAG	GCA	GGG	AAG	CCA	GAG	GAG	780	
		A	P	P	G	K	A	G	A	V	A	S	Q	T	K	A	G	K	P	E	E	260	
781	261	GAC	TCA	GAG	AGC	AGC	AGC	GAG	GAG	TCA	TCT	GAC	AGT	GAG	GAG	GAG	ACG	CCA	GCT	GCC	AAG	840	
		D	S	E	S	S	S	E	E	S	S	D	S	E	E	E	T	P	A	A	K	280	
841	281	GCC	CTG	CTT	CAG	GCG	AAG	GCC	TCA	GGA	AAA	ACC	TCT	CAG	GTC	GGA	GCT	GCC	TCA	GCC	CCT	900	
		A	L	L	Q	A	K	A	S	G	K	T	S	Q	V	G	A	A	S	A	P	300	
901	301	GCC	AAG	GAG	TCC	CCC	AGG	AAA	GGA	GCT	GCC	CCA	GCG	CCC	CCT	GGG	AAG	ACA	GGG	CCT	GCA	960	
		A	K	E	S	P	R	K	G	A	A	P	A	P	P	G	K	T	G	P	A	320	
961	321	GTT	GCC	AAG	GCC	CAG	GCG	GGG	AAG	CGG	GAG	GAG	GAC	TCG	CAG	AGC	AGC	AGC	GAG	GAA	TCG	1020	
		V	A	K	A	Q	A	G	K	R	E	E	D	S	Q	S	S	S	E	E	S	340	
1021	341	GAC	AGT	GAG	GAG	GAG	GCG	CCT	GCT	CAG	GCG	AAG	CCT	TCA	GGG	AAG	GCC	CCC	CAG	GTC	AGA	1080	
		D	S	E	E	E	A	P	A	Q	A	K	P	S	G	K	A	P	Q	V	R	360	
1081	361	GCC	GCC	TCG	GCC	CCT	GCC	AAG	GAG	TCC	CCC	AGG	AAA	GGG	GCT	GCC	CCA	GCA	CCT	CCT	AGG	1140	
		A	A	S	A	P	A	K	E	S	P	R	K	G	A	A	P	A	P	P	R	380	
1141	381	AAA	ACA	GGG	CCT	GCA	GCC	GCC	CAG	GTC	CAG	GTG	GGG	AAG	CAG	GAG	GAG	GAC	TCA	AGA	AGC	1200	
		K	T	G	P	A	A	A	Q	V	Q	V	G	K	Q	E	E	D	S	R	S	400	
1201	401	AGC	AGC	GAG	GAG	TCA	GAC	AGT	GAC	AGA	GAA	GCA	CTG	GCA	GCC	ATG	AAT	GCA	GCT	CAG	GTG	1260	
		S	S	E	E	S	D	S	D	R	E	A	L	A	A	M	N	A	A	Q	V	420	
1261	421	AAG	CCC	TTG	GGG	AAA	AGC	CCC	CAG	GTG	AAA	CCT	GCC	TCT	ACC	ATG	GGC	ATG	GGG	CCC	TTG	1320	
		K	P	L	G	K	S	P	Q	V	K	P	A	S	T	M	G	M	G	P	L	440	
1321	441	GGG	AAA	GGC	GCC	GGC	CCA	GTG	CCA	CCT	GGG	AAG	GTG	GGG	CCT	GCA	ACC	CCC	TCA	GCC	CAG	1380	
		G	K	G	A	G	P	V	P	P	G	K	V	G	P	A	T	P	S	A	Q	460	
1381	461	GTG	GGG	AAG	TGG	GAG	GAG	GAC	TCA	GAG	AGC	AGT	AGT	GAG	GAG	TCA	TCA	GAC	AGC	AGT	GAT	1440	
		V	G	K	W	E	E	D	S	E	S	S	S	E	E	S	S	D	S	S	D	480	
1441	481	GGA	GAG	GTG	CCC	ACA	GCT	GTG	GCC	CCG	GCT	CAG	GAA	AAG	TCC	TTG	GGG	AAC	ATC	CTC	CAG	1500	
		G	E	V	P	T	A	V	A	P	A	Q	E	K	S	L	G	N	I	L	Q	500	
1501	501	GCC	AAA	CCC	ACC	TCC	AGT	CCT	GCC	AAG	GGG	CCC	CCT	CAG	AAG	GCA	GGG	CCT	GTA	GCC	GTC	1560	
		A	K	P	T	S	S	P	A	K	G	P	P	Q	K	A	G	P	V	A	V	520	
1561	521	CAG	GTC	AAG	GCT	GAA	AAG	CCC	ATG	GAC	AAC	TCG	GAG	AGC	AGC	GAG	GAG	TCG	TCG	GAC	AGT	1620	
		Q	V	K	A	E	K	P	M	D	N	S	E	S	S	E	E	S	S	D	S	540	
1621	541	GCG	GAC	AGT	GAG	GAG	GCA	CCA	GCA	GCC	ATG	ACT	GCA	GCT	CAG	GCA	AAA	CCA	GCT	CTG	AAA	1680	
		A	D	S	E	E	A	P	A	A	M	T	A	A	Q	A	K	P	A	L	K	560	

Figure 1 (See p. 227 for legend.)

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1681	ATT	CCT	CAG	ACC	AAG	GCC	TGC	CCA	AAG	AAA	ACC	AAT	ACC	ACT	GCA	TCT	GCC	AAG	GTC	GCC	1740
561	I	P	Q	T	K	A	C	P	K	K	T	N	T	T	A	S	A	K	V	A	580
1741	CCT	GTG	CGA	GTG	GGC	ACC	CAA	CCC	CCC	CGG	AAA	GCA	GGA	ACT	GCG	ACT	TCT	CCA	GCA	GGC	1800
581	P	V	R	V	G	T	Q	P	P	R	K	A	G	T	A	T	S	P	A	G	600
1801	TCA	TCC	CCA	GCT	GTG	GCT	GGG	GGC	ACC	CAG	AGA	CCA	GCA	GAG	GAT	TCT	TCA	AGC	AGT	GAG	1860
601	S	S	P	A	V	A	G	G	T	Q	R	P	A	E	D	S	S	S	S	E	620
1861	GAA	TCA	GAT	AGT	GAG	GAA	GAG	AAG	ACA	GGT	CTT	GCA	GTA	ACC	GTG	GGA	CAG	GCA	AAG	TCT	1920
621	E	S	D	S	E	E	E	K	T	G	L	A	V	T	V	G	Q	A	K	S	640
1921	GTG	GGG	AAA	GGC	CTC	CAG	GTG	AAA	GCA	GCC	TCA	GTG	CCT	GTC	AAG	GGG	TCC	TTG	GGG	CAA	1980
641	V	G	K	G	L	Q	V	K	A	A	S	V	P	V	K	G	S	L	G	Q	660
1981	GGG	ACT	GCT	CCA	GTA	CTC	CCT	GGG	AAG	ACG	GGG	CCT	ACA	GTC	ACC	CAG	GTG	AAA	GCT	GAA	2040
661	G	T	A	P	V	L	P	G	K	T	G	P	T	V	T	Q	V	K	A	E	680
2041	AAG	CAG	GAA	GAC	TCT	GAG	AGC	AGT	GAG	GAG	GAA	TCA	GAC	AGT	GAG	GAA	GCA	GCT	GCA	TCT	2100
681	K	Q	E	D	S	E	S	S	E	E	E	S	D	S	E	E	A	A	A	S	700
2101	CCA	GCA	CAG	GTG	AAA	ACC	TCA	GTA	AAG	AAA	ACC	CAG	GCC	AAA	GCC	AAC	CCA	GCT	GCC	GCC	2160
701	P	A	Q	V	K	T	S	V	K	K	T	Q	A	K	A	N	P	A	A	A	720
2161	AGA	GCA	CCT	TCA	GCA	AAA	GGG	ACA	ATT	TCA	GCC	CCT	GGA	AAA	GTT	GTC	ACT	GCA	GCT	GCT	2220
721	R	A	P	S	A	K	G	T	I	S	A	P	G	K	V	V	T	A	A	A	740
2221	CAA	GCC	AAG	CAG	AGG	TCT	CCA	TCC	AAG	GTG	AAG	CCA	CCA	GTG	AGA	AAC	CCC	CAG	AAC	AGT	2280
741	Q	A	K	R	S	P	S	K	V	K	P	P	V	R	N	P	Q	N	S		760
2281	ACC	GTC	TTG	GCG	AGG	GGC	CCA	GCA	TCT	GTG	CCA	TCT	GTG	GGG	AAG	GCC	GTG	GCT	ACA	GCA	2340
761	T	V	L	A	R	G	P	A	S	V	P	S	V	G	K	A	V	A	T	A	780
2341	GCT	CAG	GCC	CAG	ACA	GGG	CCA	GAG	GAG	GAC	TCA	GGG	AGC	AGT	GAG	GAG	GAG	TCA	GAC	AGT	2400
781	A	Q	A	Q	T	G	P	E	E	D	S	G	S	S	E	E	E	S	D	S	800
2401	GAG	GAG	GAG	GCG	GAG	ACG	CTG	GCT	CAG	GCG	AAG	CCT	TCA	GGG	AAG	ACC	CAC	CAG	ATC	AGA	2460
801	E	E	E	A	E	T	L	A	Q	A	K	P	S	G	K	T	H	Q	I	R	820
2461	GCT	GCC	TTG	GCT	CCT	GCC	AAG	GAG	TCC	CCC	AGG	AAA	GGG	GCT	GCC	CCA	ACA	CCT	CCT	GGG	2520
821	A	A	L	A	P	A	K	E	S	P	R	K	G	A	A	P	T	P	P	G	840
2521	AAG	ACA	GGG	CCT	TCG	GCT	GCC	CAG	GCA	GGG	AAG	CAG	GAT	GAC	TCA	GGG	AGC	AGC	AGC	GAG	2580
841	K	T	G	P	S	A	A	Q	A	G	K	Q	D	D	S	G	S	S	S	E	860
2581	GAA	TCA	GAC	AGT	GAT	GGG	GAG	GCA	CCG	GCA	GCT	GTG	ACC	TCT	GCC	CAG	GTG	ATT	AAA	CCC	2640
861	E	S	D	S	D	G	E	A	P	A	A	V	T	S	A	Q	V	I	K	P	880
2641	CCC	CTG	ATT	TTT	GTC	GAC	CCT	AAT	CGT	AGT	CCA	GCT	GGC	CCA	GCT	GCT	ACA	CCC	GCA	CAA	2700
881	P	L	I	F	D	P	N	R	S	P	A	G	P	A	A	T	P	A	Q		900
2701	GCC	CAG	GCT	GCA	AGC	ACC	CCG	AGG	AAG	GCC	CGA	GCC	TCG	GAG	AGC	ACA	GCC	AGG	AGC	TCC	2760
901	A	Q	A	A	S	T	P	R	K	A	R	A	S	E	S	T	A	R	S	S	920
2761	TCC	TCC	GAG	AGC	GAG	GAT	GAG	GAC	GTG	ATC	CCC	GCT	ACA	CAA	TGC	TTG	ACT	CCT	GGC	ATC	2820
921	S	S	E	S	E	D	E	D	V	I	P	A	T	Q	C	L	T	P	G	I	940
2821	AGA	ACC	AAT	GTG	GTG	ACC	ATG	CCC	ACT	GCC	CAC	CCA	AGA	ATA	GCC	CCC	AAA	GCC	AGC	ATG	2880
941	R	T	N	V	V	T	M	P	T	A	H	P	R	I	A	P	K	A	S	M	960
2881	GCT	GGG	GCC	AGC	AGC	AGC	AAG	GAG	TCC	AGT	CGG	ATA	TCA	GAT	GGC	AAG	AAA	CAG	GAG	GGA	2940
961	A	G	A	S	S	S	K	E	S	S	R	I	S	D	G	K	K	Q	E	G	980
2941	CCA	GCC	ACT	CAG	GTG	TCA	AAG	AAG	AAC	CCA	GCT	TCC	CTC	CCA	CTG	ACC	CAG	GCT	GCC	CTG	3000
981	P	A	T	Q	V	S	K	K	N	P	A	S	L	P	L	T	Q	A	A	L	1000
3001	AAG	GTC	CTC	GCC	CAG	AAA	GCC	AGT	GAG	GCT	CAG	CCT	CCT	GTT	GCC	AGG	ACC	CAG	CCT	TCA	3060
1001	K	V	L	A	Q	K	A	S	E	A	Q	P	P	V	A	R	T	Q	P	S	1020
3061	AGT	GGG	GTT	GAC	AGT	GCT	GTG	GGA	ACA	CTC	CCT	GCA	ACA	AGT	CCC	CAG	AGC	ACC	TCC	GTC	3120
1021	S	G	V	D	S	A	V	G	T	L	P	A	T	S	P	Q	S	T	S	V	1040
3121	CAG	GCC	AAA	GGG	ACC	AAC	AAG	CTC	AGA	AAA	CCT	AAG	CTT	CCT	GAG	GTC	CAG	CAG	GCC	ACC	3180
1041	Q	A	K	G	T	N	K	L	R	K	P	K	L	P	E	V	Q	Q	A	T	1060
3181	AAA	GCC	CCT	GAG	AGC	TCA	GAT	GAC	AGT	GAG	GAC	AGC	AGC	GAC	AGT	TCT	TCA	GGG	AGT	GAG	3240
1061	K	A	P	E	S	S	D	D	S	E	D	S	S	D	S	S	S	G	S	E	1080
3241	GAA	GAT	GGT	GAA	GGG	CCC	CAG	GGG	GCC	AAG	TCA	GCC	CAC	ACG	CTG	GGT	CCC	ACC	CCC	TCC	3300
1081	E	D	G	E	G	P	Q	G	A	K	S	A	H	T	L	G	P	T	P	S	1100
3301	AGG	ACA	GAG	ACC	CTG	GTG	GAG	GAG	ACC	GCA	GCA	GAG	TCC	AGC	GAG	GAT	GAT	GTG	GTG	GCG	3360
1101	R	T	E	T	L	V	E	E	T	A	A	E	S	S	E	D	D	V	V	A	1120
3361	CCA	TCC	CAG	TCT	CTC	CTC	TCA	GGT	TAT	ATG	ACC	CCT	GGA	CTA	ACC	CCA	GCC	AAT	TCC	CAG	3420
1121	P	S	Q	S	L	L	S	G	Y	M	T	P	G	L	T	P	A	N	S	O	1140

Figure 1 (Continued)

CHARACTERIZATION OF *TCOF1*

type 1 (splicing occurring after the first base of the codon) for introns 3, 5, 17, 20, 22, and 23; and type 2 (splicing occurring after the second base of the codon) for intron 2. In many cases, the introns were sequenced in their entirety, which permitted their sizes to be determined accurately, whereas the sizes of other introns were estimated by PCR (Table 1). Interestingly, sequence analysis of the 3' UTR indicated that it was not single exonic, an intron of ~730 bp, as determined by the PCR, being present between exons 25 and 26. Introns 1, 3, 6, 13, and 16 consistently could not be amplified using PCR, and thus these introns were considered to be out of the range of PCR amplification under the conditions used in this study. The entire sequence data generated in this study have been submitted to GenBank under the following accession numbers: U79645 (exon 1); U79646 (exon 2); U79647 (exon 3);

U79648 (exon 4); U79649 (exon 5); U79650 (exon 6); U79651 (exon 7 to exon 13); U79652 (exon 14 to exon 16); U79653 (exon 17); U79654 (exon 18); U79655 (exons 19 and 20); U79656 (exon 21); U79657 (exon 22); U79658 (exon 23); U79659 (exons 24 and 25); and U79660 (exon 26). All available intronic sequence has been submitted under the appropriate accession number.

Bioinformatics Analysis

As initial database sequence comparisons failed to show any strong homologies between *TCOF1* and previously identified genes, gene families, or motifs of classic importance, a number of bioinformatics programs were employed in the current study. The use of dot plots allowed the identification of repeated units within the gene, each of which was

3421 1141	GCC A	TCA S	AAA K	GCC A	ACT T	CCC P	AAG K	CTA L	GAT D	TCC S	AGC S	CCC S	TCA V	GTT S	TCC S	TCT S	ACT T	CTG L	GCC A	GCC A	3480 1160
3481 1161	AAA K	GAT D	GAC D	CCA P	GAT D	GGC G	AAG K	CAG Q	GAG E	GCA A	AAG K	CCC P	CAA Q	CAG Q	GCA A	GCA A	GGC G	ATG M	TTG L	TCC S	3540 1180
3541 1181	CCT P	AAA K	ACA T	GGT G	GGA G	AAA K	GAG E	GCT A	GCT A	TCA S	GGC G	ACC T	ACA T	CCT P	CAG Q	AAG K	TCC S	CGG R	AAG K	CCC P	3600 1200
3601 1201	AAG K	AAA K	GGG G	GCT A	GGG G	AAC N	CCC P	CAA Q	GCC A	TCA S	ACC T	CTG L	GCG A	CTG L	CAA Q	AGC S	AAC N	ATC I	ACC T	CAG Q	3660 1220
3661 1221	TGC C	CTC L	CTG L	GGC G	CAA Q	CCC P	TGG W	CCC P	CTG L	AAT N	GAG E	GCC A	CAG Q	GTG V	CAG Q	GCC A	TCA S	GTG V	GTG V	AAG K	3720 1240
3721 1241	GTC V	CTG L	ACT T	GAG E	CTG L	CTG L	GAA E	CAG E	GAA E	AGA R	AAG K	AAG K	GTG V	GTG V	GAC D	ACC T	ACC T	AAG K	GAG E	AGC S	3780 1260
3781 1261	AGC S	AGG R	AAG K	GGC G	TGG W	GAG E	AGC S	CGC R	AAG K	CGG R	AAG K	CTA L	TCG S	GGA G	GAC D	CAG Q	CCA P	GCT A	GCC A	AGG R	3840 1280
3841 1281	ACC T	CCC P	AGG R	AGC K	AAG K	AAG K	AAG K	AAG K	CTG L	GGG G	GCC A	GGG G	GAA E	GGT G	GGG G	GAG E	GCC A	TCT S	GTT V		3900 1300
3901 1301	TCC S	CCA P	GAA K	AAG K	ACC T	TCC S	ACG T	ACT T	TCC S	AAG K	GGG G	AAA K	GCA A	AAG K	AGA D	GAC K	AAA A	GCA A	AGT S	GGT G	3960 1320
3961 1321	GAT D	GTC V	AAG K	GAG E	AAG K	AAA K	GGG G	AAG G	GGG G	TCT S	CTT L	GGC G	TCC S	CAA Q	GGG G	GCC A	AAG K	GAC D	GAG E	CCA P	4020 1340
4021 1341	GAA E	GAG E	GAG E	CTT L	CAG Q	AAG K	GGG G	ATG M	GGG G	ACG T	GTT E	GAA G	GGT G	GGA D	GAT Q	CAA S	AGC S	AAC N	CCA P	AAG K	4080 1360
4081 1361	AGC S	AAG K	AAG K	GAG E	AAG K	AAG K	AAA K	TCC D	GAC A	AAG R	AGA K	AAA K	AAA K	GAC D	AAA K	GAA E	AAA K	AAA K	GAA E	AAG K	4140 1380
4141 1381	AAG K	AAG K	AAA K	GCA A	AAA K	AAG K	GCC A	TCA S	ACC T	AAA K	GAT D	TCT S	GAG E	TCA S	CCG P	TCC S	CAG Q	AAG K	AAA K	AAG K	4200 1400
4201 1401	AAG K	AAA K	AAG K	AAG K	AAG K	ACA T	GCA A	GAG E	CAG A	GAT T	GTA V	TGA *									4236 1412
4237	CGAGCACCAGCACCAGGCACAGGGATTTCCTAGCCGAGCAGTGCCCATCCCCATGCCCTCTGACCTCCACCGACCTCTGC																				4316
4317	CCACCATGGGTTGGAACCTAACTGTTACCTTCCCTTCGCTCCACAGAGAAGACAGCCAGCTTCAGGGGTCCCTGTGCTG																				4395
4396	GCCAAGCCAGTGAGCCTGCGGGGAGGCTGGTCCAAGGAGAAAGTGACAGCTCCCATGACCTCACCCCACTCCCCAA																				4474
4475	CACAGGACGCTTCATATAGATGTGTACAGTATATGATTTTTTAAAGTGACCTCCTCTCTCTCCACAGACCCACATGC																				4553
4556	CCAAAGGCCTCGGGACTTCCCAACCACTTGTCTCCACAGATCCAGCTAGGCCTGACCTGTGCCTCATCCCGTGCCGCTCG																				4632
4633	GTCTCTGGCTGATCCCGAGGCTTTGTCTTCTCTCTCTCGTCAGTTCTTTTGGTTGTGTTTTTGTGTTTTTTTAAATAACTCA																				4711
4712	AAAAAAAAATAAAGACTTGGAGGAAGGGTGAAAAAAAAAAAAAAAAAAAA																				4760

Figure 1 Complete nucleotide sequence of *TCOF1* (U40847) and the derived amino acid sequence of the protein Treacle. Position 1 is taken as the first base of the initiation codon. The single polyadenylation signal is underlined. The new sequence data (nucleotide and amino acid) are in boldface type.

Table 1. Genomic Organization of the *TCOF1* Locus

Exon	Size (bp)	Splice Donor	Splice Acceptor	Nucleotide Position	Intron Size
1	-	AGC GGC CAG gtaagcgttc	ctctctgcag AAG TGT TTC	5' UTR - 108	-
2	56	TGG CAA CA gtaagtgggtg	tgtcctgcag A ACC TCA	109-164	2.7kb (P)
3	140	AAA GCC A gtaagagcct	tttcttgcag CC CCA AGA	165-304	-
4	74	AAA GCC AAG gtgagtggga	ttctctgtag GCA GAG ACA	305-378	860bp (P)
5	187	AAG CCT G gtaagaagtc	cgatcctcag GG ATG GTG	379-565	600bp (P)
6	74	GAC GTG GAG gtaattgcca	ttttaccag GTA AAG GCC	566-639	-
7	213	CTG CTT CAG gtgaggcctg	gtttctccag GCG AAG GCC	640-852	230bp (S)
8	195	CCT GCT CAG gtgaggcaga	ctcactccag GCG AAG CCT	853-1047	142bp (S)
9	210	GCA GCT CAG gtgaggctgg	tgtctcccag GTG AAG CCC	1048-1257	175bp (S)
10	216	CCG GCT CAG gtgaggcccc	ctcactccag GAA AAG TCC	1258-1473	166bp (S)
11	189	GCA GCT CAG gtgaggcctg	gtccctcag GCA AAA CCA	1474-1662	171bp (S)
12	249	GTG GGA CAG gtgaggcctg	gtcatcccag GCA AAG TCT	1663-1911	93bp (S)
13	198	CCA GCA CAG gtgaggccta	ctccactcag GTG AAA ACC	1912-2109	-
14	138	CCA TCC AAG gcaagtgggg	tgcaattcag GTG AAG CCA	2110-2247	185bp (S)
15	180	CTG GCT CAG gtgaggggga	ctccctccag GCG AAG CCT	2248-2427	118bp (S)
16	201	TCT GCC CAG gtaagacttg	gtttttcaag GTG ATT AAA	2428-2628	-
17	187	ACT CCT G gtgagcgacc	tccatttcag GC ATC AGA	2629-2815	2kb (P)
18	137	GCC ACT CAG gtacctggtg	ccaccacag GTG TCA AAG	2816-2952	1.6kb (P)
19	114	TCA AGT GGG gtgagcttec	accgaattag GTT GAC AGT	2953-3066	298bp (S)
20	220	ACG CTG G gtgagggtgc	tctccagtag GT CCC ACC	3067-3286	540bp (P)
21	83	CCA TCC CAG gtaactgcaa	gcttcttcag TCT CTC CTC	3287-3369	570bp (P)
22	181	AAA ACA G gtaagttaag	ctctccatag GT GGA AAA	3370-3550	2.5kb (P)
23	561	GAC AAG A gtgagtgacc	cttcccttag GA AAA AAA	3551-4111	1.6kb (P)
24	98	AAG AAA AAG gtagagagtt	ctcctcacag AAG AAG ACA	4112-4209	570bp (S)
25	49	GGCACAG gtacgttcc	cttcccctag GGATTTC	4210-4258	730bp (P)
26		3' untranslated region		4259-4740	

The cDNA position of the intron/exon boundaries is defined relative to the sequence presented in this study with position 1 being the first base of the initiation codon. The intronic sequence is indicated in lowercase; the exonic sequence in uppercase. The codon usage is also indicated. The intron sizes were either determined by sequencing (S) or by PCR analysis (P). The genomic sequence information has been submitted to GenBank under accession nos. U79645–U79660, inclusive.

shown to map onto an individual exon. With the exception of exon 14, the repeated units extended from exon 7 to 16. In this region all of the intron/exon boundaries are of type 0 and the splice donor sites are all of the consensus sequence /gtgagg, with the exception of the atypical intron 14 (Table 1).

Moreover, all of the introns in this region, with the exception of intron 13, which precedes the atypical exon 14, have been sequenced and shown to be small, ranging in size from 93 to 230 bp (Table 1). The repeat units were multiply aligned and shown to be highly conserved (Fig. 2). Exons 7, 8, 9, 10, 13,

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8 - AKASGKTSQV GAASAPAKES PRKGAAPA... PPGKTGP AVAKAQ... AGKREEDS QSSSEE.SD. SSEEA.... PAQ
9 - AKPSGKAPQV RAASAPAKES PRKGAAPA... PPRKTGP AAAQVQ... VGKQEDS RSSSEE.SD. SDREALAAMN AAQ
16 - AKPSGKTHQI RAALAPAKES PRKGAAPT... PPGKTGP SAAQAG... KQ.DDS GSSSEE.SD. SDGEAPAAVT SAQ
7 - VKASEKILQV RAASAPAKGT PGKGATPA... PPGKAGA VASQTK... AGKPEEDS ESSSEESSD. SEETPAKA LLQ
10 - VKPLGKSPQV KPASTMGMP LGKGAGPV... PPGKVGP ATPSAQ... VGKWEEDS ESSSEESSDS SDGEVPTAVA PAQ
13 - AKSVGKGLQV KAASVPVKG LGQGTAPV... LPGKTGP TVTQVK... AEK.QEDS ESSEESSDSE EAAAS.... PAQ
11 - EKSLGNILQA KPTSSPAKG. .... PPQKAGP VAVQVK... AEKPMDS ESSEESSDSA DSEAPAAMT AAQ
15 - VKPPVRNPQN STVLARG... PASVP... SVGKAVA TAAQAQ... TG.PEEDS GSSEESSDSE EEAET.... LAQ
12 - AKPALKIPQT KACPKNNTT ASAKVAPRV GTQPPRKAGT ATSPAGSSPA VAGGTQRPAE DSSSEESSDS SEETGLAVT VGQ

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Figure 2 Alignment of the most closely related repeated units identified within *Treacle*. The exon onto which each motif maps is indicated at left; the area of multiple potential sites for CKII phosphorylation is in boldface type.

and 16 were particularly homologous, with the most closely related being exons 8 and 16 (77.41% identity, 87.10% similarity) and the least closely related being exons 7 and 13 (50% identity, 59.09% similarity) (Fig. 2). The protein sequence was also compared against pattern databases in an attempt to identify functional motifs within the sequence. These comparisons resulted in the identification of multiple motifs for casein kinase II phosphorylation (CKII) and protein kinase C phosphorylation. However, as these elements tend to be overpredicted by the programs, their significance is uncertain. It is, however, striking that multiple sites for CKII phosphorylation were observed in an identical position within each repeated unit (Fig. 2). Weak similarity to *Xenopus laevis* nucleolar phosphoprotein (S57757; $P = 4.2 \times 10^{-9}$) (Cairns and McStay 1995) and nucleolar phosphoprotein 140 (M94288; $P = 2.6 \times 10^{-8}$) (Meier and Blobel 1992) were demonstrated using the BLAST programs (Altschul et al. 1990). These highly phosphorylated proteins have a role in protein transport between the cytoplasm and the nucleus (Meier and Blobel 1992). Alignment of *Treacle* with these proteins indicated that this was predominantly a result of homology in the regions of the CKII sites within the repeated units of *Treacle* (Fig. 3). As is the case for the nucleolar phosphoproteins, the CKII phosphorylation sites occur within clusters of acidic amino acids, which are separated by stretches of residues that are relatively rich in lysine, proline, and alanine, with few acidic residues and are, therefore, basic in nature (Fig. 2). In addition, both *Treacle* and the nucleolar phosphoproteins display a number of motifs of the type K-K/R-X-R/K, which represents the minimal nuclear localization signal consensus sequence, toward the 3' end of the coding sequence.

DISCUSSION

In this study we report the entire coding sequence of the *TCOF1* gene and its genomic organization. The gene has an ORF of 4233 bp encoded by 25

exons. Exons 4, 5, 6, 15, 18, and 25 have also been recovered by exon amplification strategies (Treacher Collins Syndrome Collaborative Group 1996; Gladwin et al. 1996; this study). All of the splice junctions of the gene conform to the published consensus sequences (Breathnach and Chambon 1981), with the exception of the splice donor site of exon 14, which has GC in place of the expected GT. A number of other genes exhibiting this unusual variation have been published, including the fibrillin gene (Pereira et al. 1993). Although the significance underlying this splice variant is unclear, it seems to be prone to mis-splicing events resulting from apparently minor nucleotide changes in the extended splice consensus sequence (Gladwin et al. 1996). A second unusual variation in the genomic organization of the gene has been observed in the current study in that the 3' UTR of *TCOF1* is not single exonic as has been reported for the vast majority of genes (Wilcox et al. 1991). Exceptions to this rule include the p58^{cl^k-1} protein kinase gene (Eipers et al. 1992) and the genes encoding the β 1-subunit of the voltage-dependent sodium channel in mouse, rat, and human (Dib-Hajj and Waxman 1995). These genes do not, however, appear to be related. The presence of the intron in the 3' UTR is validated by the fact that exon 25 was successfully "rescued" by exon amplification using the pSPL3 vector system, which does not rescue the 3' terminal exon. The sequence and splicing of the exon-amplified product corresponds exactly to the sequence of exon 25.

The nucleotide sequence of *TCOF1* predicts a protein, *Treacle*, of 1411 amino acids, that is relatively rich in alanine and serine residues. Although *Treacle* does not exhibit strong homology to any known proteins, the use of a number of bioinformatics tools has highlighted several interesting features of the gene. There is a series of repeated units of unknown function within the gene that map onto individual exons. The splicing phase of all of the exons in this region is the same (type-0 junctions); therefore, it is possible that this region of the

(export) or ribosomal proteins (import) (Meier and Blobel 1992). The role of phosphorylation may be to increase the net negative charge in the acidic domain, thus increasing the affinity of the protein for oppositely charged species such as basic ribosomal proteins (Cairns and McStay 1995). Interestingly, recent data suggest that the complex events of epithelial–mesenchymal transformation in the neural crest cell system can be triggered by epigenetic events involving differential protein phosphorylation (Newgreen and Minichiello 1995). Nevertheless, although these interesting similarities provide potential insight into the function of the protein, additional experiments are required to confirm its role during facial development.

On the basis of the TCS phenotype, the gene must play a fundamental role in early embryonic development, possibly via an effect on neural crest cell migration and/or differentiation (Poswillo 1975; Wiley et al. 1983). In this regard, determination of the precise spatiotemporal expression patterns of the gene, and its protein product *Treacle* during development will be important in the investigation of the function of the gene. Here, the use of animal models, where accurately staged embryos from a wide variety of developmental stages are readily obtainable, will be extremely valuable. Furthermore, the isolation of the complete coding sequence of *TCOF1* and the determination of its genomic organization reported here will prove to be helpful in the isolation and characterization of its murine homolog, which is essential in the creation of a mouse model of TCS by gene targeting.

As the vast majority of the mutations that have been reported in *TCOF1* to date are unique to the family in which they were identified and are spread throughout the gene (Gladwin et al. 1996; Treacher Collins Syndrome Collaborative Group 1996; M.J. Dixon and S.J. Edwards, unpubl.), elucidation of the complete genomic organization of the gene will facilitate continued mutation screening, which may provide further information on functionally important domains within the gene and confirm the hypothesis that TCS results from haploinsufficiency. With the exception of exon 23 of *TCOF1*, all of the exons are <250 bp in size and are therefore of an appropriate size to be analyzed as a single fragment by single-stranded conformation polymorphism

(SSCP) analysis (Orita et al. 1989). Although this technique is not sufficiently sensitive to identify all potential mutations, it does provide an excellent balance between simplicity and sensitivity of detection. In addition to providing information on the mutational spectrum underlying TCS, continued mutation analysis will also prove to be important for postnatal diagnosis of TCS, particularly in cases where there is some doubt as to diagnosis of either parent of a child with obvious clinical signs of TCS. Molecular diagnosis will also be important in providing prenatal diagnostic predictions for “at-risk” families (Edwards et al. 1996) and in assessing whether patients with conditions in which the facial gestalt has some similarities to the TCS facies, such as Nager and Miller syndrome (Gorlin et al. 1990), are also attributable to mutations in *TCOF1*.

METHODS

5' and 3' RACE

First-strand cDNA synthesis was performed on 500 ng of poly(A)⁺ RNA isolated from skeletal muscle (Clontech) and a lymphoblastoid cell line using the 5' or 3' RACE kit (BRL) according to the manufacturer's instructions. In the case of 5' RACE, cDNA synthesis was initiated from the gene-specific primer 5'-TTCCCAGTCTTGCCAG-3'. The original mRNA template was then removed by treatment with RNase H. In the case of 5' RACE products, a homopolymeric tail was added to the 3' end of the cDNA using terminal deoxynucleotide transferase (Tdt) and dCTP. PCR amplification of the target cDNA was performed using the universal amplification primer and a 5' RACE gene-specific primer, 5'-TTTCGGCTTCTGCTTCTTCC-3', or a 3' RACE gene-specific primer, 5'-GGCCTCAACCAAAGATTC-3'. After electrophoretic separation on a 1% agarose gel, the PCR product was diluted and subject to a second round of PCR using the abridged universal amplification primer and a nested 5' RACE gene-specific primer, 5'-CTGAATTCAGATGTCCAGAAGGGT-TACG-3' or a nested 3' RACE gene-specific primer, 5'-AAGAATTCTGAGTCACCGTCCCAG-3'. The resulting PCR products were gel-purified, digested with *EcoRI/Sall*, cloned into M13mp18/19, and sequenced via the dideoxy chain termination method (Sanger et al. 1977) using the Sequenase version 2.0 kit (U.S. Biochemical Corp.).

RT-PCR

One microgram of RNA extracted from skeletal muscle or a lymphoblastoid cell line was incubated with 100 ng of random primer at 70°C for 10 min. The samples were chilled on ice, and Moloney murine leukemia virus (MMLV) reverse

Figure 3 Comparison of the predicted amino acid sequences of the human *Treacle* protein and the rat nucleolar phosphoprotein using BESTFIT alignment. The *top* line represents the single-letter amino acid sequence of human *Treacle*; and the *bottom* line is the rat nucleolar phosphoprotein sequence. The sequences display 32.43% identity and 46.86% similarity. The regions containing multiple potential sites for CKII phosphorylation are in boldface type.

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transcriptase buffer, 10 mM DTT, 1 mM dNTPs (all BRL), and 0.5 units of RNasin (Promega) were added. The reactions were equilibrated at 37°C for 2 min, 100 units of MMLV reverse transcriptase was added, and the samples incubated at 37°C for 1 hr. The samples were then heated to 95°C for 5 min, and 3 μ l of cDNA was used in the PCR with the primers 5'-TTGGATCCAAGTGGGGCGCGCGAGGT-3' and 5'-TCGAATTCTGGTAGATCAGGGGAAGTAG-3'. Control reactions included those performed in the absence of RNA or in the absence of reverse transcriptase.

PCR Conditions

PCR assays were performed in 25- μ l volumes containing 50 pmoles of each primer; 200 μ M each of dCTP, dGTP, dTTP, and dATP; 10 mM Tris-HCl at pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, and 0.01% gelatin. The samples were overlaid with mineral oil, heated to 96°C for 10 min and cooled to 55°C. After addition of 0.75 units of *Taq* DNA polymerase, the samples were processed through 35 amplification cycles of 92°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec using a Hybaid thermal cycler. The final extension step was lengthened to 10 min. Positive and negative controls were established for all reactions. The PCR products were analyzed on 2%–3% agarose gels.

Screening of cDNA Libraries

Bacteriophage from muscle, placental (Stratagene Cloning Systems), and fetal brain (Clontech) cDNA libraries were plated at 5×10^4 PFU/140-mm petri dish. Approximately 5×10^5 plaques were screened with restriction fragments of the original *TCOF1* cDNA (Treacher Collins Syndrome Collaborative Group 1996) or RACE products using standard procedures. Positive primary clones were purified by two additional rounds of screening and subcloned into pBluescript. The resulting plasmids were restriction mapped, and suitable restriction fragments were subcloned into M13mp18/19 and sequenced.

Exon Amplification

Genomic DNA from cosmids 17-1 and 18-3 were digested to completion with either *Pst*I, or double-digested with *Bam*HI and *Bgl*II. The restriction fragments were ligated into corresponding sites of the pSPL3 vector. The exon amplification protocol of Church et al. (1994) was followed with modifications reported previously (Treacher Collins Syndrome Collaborative Group 1996). Exon amplification clones were sequenced as above.

Determination of Intron/Exon Boundaries

Cosmids 17-1 and 18-3 were digested with *Sau*3A1, *Alu*I, *Pst*I, and *Sst*I and shotgun-cloned into M13. Recombinant plaques were screened with restriction fragments of the *TCOF1* cDNA or RACE products. Sequence data generated from the positive clones were compared with the cDNA sequence, and intron-exon boundaries were identified by comparison with the published consensus sequences (Breathnach and Chambon 1981).

Bioinformatics Analysis

DNA and derived protein sequences were used to query the GenBank, NBRF, Swissprot, and TREMBL databases using the BLAST suite of programs; BLASTP was used to compare *Treacle* to the OWL protein database (Altschul et al. 1990; Bleasby et al. 1994). A dot plot of *Treacle* versus *Treacle* was created using the GCG programs COMPARE (window size, 30; stringency, 18) and DotPlot (Devereux et al. 1984). Multiple alignments of the repeated units of *Treacle* were produced using GCG PILEUP and edited using LINEUP. PROSITE, PRINTS, and BLOCKS databases were searched to identify protein motifs within *Treacle* (Bairoch 1991; Attwood et al. 1994; Henikoff and Henikoff 1994). The above programs were accessed using either SEQNET or the Human Genome Mapping Resource Centre (Hinxton, UK).

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