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Conservation of the *Caenorhabditis elegans* timing gene *clk-1* from yeast to human: a gene required for ubiquinone biosynthesis with potential implications for aging

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Abstract. Mutations in the *Caenorhabditis elegans* gene *clk-1* have a major effect on slowing development and increasing life span. The *Saccharomyces cerevisiae* homolog COQ7 encodes a mitochondrial protein involved in ubiquinone biosynthesis and, hence, is required for respiration and gluconeogenesis. In this study, RT-PCR and 5′ RACE were used to isolate both human and mouse *clk-1*/COQ7 homologs. Human CLK-1 was mapped to Chr 16(p12–13.1) by Radiation Hybrid (RH) and fluorescence in situ hybridization (FISH) methods. The number and location of human CLK1 introns were determined, and the location of introns II and IV are the same as in *C. elegans.* Northern blot analysis showed that three different isoforms of CLK-1 mRNA are present in several tissues and that the isoforms differ in the amount of expression. The functional equivalence of human CLK-1 to the yeast COQ7 homolog was tested by introducing either a single or multicopy plasmid containing human CLK-1 cDNA into yeast *coq7* deletion strains and assaying for growth on a nonfermentable carbon source. The human CLK-1 gene was able to functionally complement yeast *coq7* deletion mutants. The protein similarities and the conservation of function of the CLK-1/*clk-1*/COQ7 gene products suggest a potential link between the production of ubiquinone and aging.

Introduction

A role for genetics in the aging process has long been proposed. In the nematode *Caenorhabditis elegans*, mutations in the timing gene clock-1 (*clk-1*) have been identified that increase longevity (Wong et al. 1995; Lakowski and Hekimi 1996; Ewbank et al. 1997). The phenotype of the *clk-1* mutants is pleiotropic, and the reported mutations slow the rate of embryonic and postembryonic development, cell cycle duration, and growth rate, and also lengthen the timing of adult behaviors including defecation, swimming, and pharyngeal pumping (Wong et al. 1995). From sequence comparisons, it has been proposed that *clk-1* is conserved in eukaryotes including yeast, rat, mouse, and human, although only a partial sequence was compared for the latter two species in the initial report (Ewbank et al. 1997).

In the yeast *Saccharomyces cerevisiae*, *COQ7* (Marbois and Clarke 1996), which is identical to the independently isolated *CAT5* gene (Proft et al. 1995), has been identified as the *clk-1* homolog (Ewbank et al. 1997). *COQ7* is required for the synthesis of ubiquinone (coenzyme Q, or Q) and influences respiration (Marbois and Clarke 1996). Q is a lipid soluble component of mitochondria that functions in respiration by transporting electrons in the inner mitochondrial membrane of eukaryotes (Brandt and Trumpower 1994). *COQ7* (*CAT5*) also functions in the derepression of gluconeogenic enzymes that accompanies the transfer of yeast from glucose to a nonfermentable carbon source such as glycerol or ethanol (Proft et al. 1995). Recently, it has been shown that the yeast Coq7p is a mitochondrial protein directly involved in Q synthesis, and its effect on gluconeogenesis is a secondary consequence of the defect in respiration (Jonassen et al. 1998). The *C. elegans clk-1* and rat *Coq7* genes rescue *coq7* deletion mutants of yeast by allowing growth on a nonfermentable carbon source, thus demonstrating a conserved biochemical function of *COQ7*/*clk-1*/*Coq7* in Q biosynthesis (Jonassen et al. 1996; Ewbank et al. 1997).

This report describes the full-length human and mouse *clk-1* cDNAs, demonstrates the functional equivalence of human CLK-1 to yeast *COQ7*, provides a physical map location and genomic organization for the human CLK-1 gene, and shows the levels of human CLK-1 mRNA in several tissues. The data show great nucleotide sequence conservation of human, mouse, and rat CLK-1/*Coq7* homologs. In addition, the functional complementation studies of yeast *coq7* deletion mutants by human, nematode, and rat CLK-1/*COQ7* shows conservation of function in Q biosynthesis across phylogenetic boundaries.

Materials and methods

Isolation and sequencing of human CLK-1 and mouse Coq7 *cDNA.* We used the reverse-transcription polymerase chain reaction (RT-PCR) and the 5′ and 3′ rapid amplification of cDNA ends (RACE) procedures to obtain the full-length human CLK-1 and mouse *Coq7* cDNA sequences. Total RNA from human heart (Clontech, Palo Alto, CA) or liver [isolated by standard methods (Chomczynski and Sacchi 1987)] was used to generate reverse transcribed cDNA for PCR amplification (RT-PCR). Mouse RNA was isolated from heart tissue by a standard method (Chomczynski and Sacchi 1987). RT-PCR was performed with either the Smart PCR cDNA synthesis kit (Clontech), the SuperScript Preamplification System kit (Gibco BRL, Rockville, MD), or the Genamp kit (Perkin Elmer, Foster City, CA). Oligonucleotide primers for amplifying human CLK-1 were designed based on rat *Coq7* (GenBank accession number U46149) and partial human CLK-1 (GenBank accession number U81276) cDNA sequences. Oligonucleotide primers for amplifying mouse *Coq7* were designed based on a partial sequence (GenBank accession number U81277). Kits for 5′ and 3′ RACE (Gibco, BRL) were used to amplify 5′ and 3′

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cDNA ends. Sequencing was performed on an ABI 373 instrument (Perkin Elmer). The sequences were deposited in the GenBank database, accession numbers AF032900 (human) and AF053770 (mouse). The primer sequences are available on request.

Nucleotide sequence and protein analysis. Sequences of human, mouse, rat, nematode, and yeast CLK-1/*Coq7*/*clk-1*/*COQ7* cDNA and encoded proteins (GenBank accession numbers AF032900, AF053770, U46149, U13642, and X82930) were aligned with CLUSTALW (Thompson et al. 1994). The proportion of identical nucleotides and amino acids was determined in pairwise comparisons with the MEGA program (Kumar et al. 1993). The predictions of protein secondary structure and of helical transmembrane segments were done with the PHDsec, PHDacc, and PHDhtm programs of PredictProtein (Rost and Sander 1993, 1994; Rost et al. 1994, 1995). The PredictProtein server is available at www.embl-heidelberg.de/predictprotein/predictprotein.html. The identification of protein motifs was performed by scanning the protein against the PROSITE database (Bairoch et al. 1997). The prediction of signal peptides was performed with SignalP (Nielsen et al. 1997).

Genomic organization of CLK-1. The intron and exon boundaries of CLK-1 were determined by sequencing PCR-amplified regions of human genomic DNA (Clontech) and amplified regions of a human BAC clone (Well 207P21, Genome Systems), and comparing the genomic and cDNA sequences. Sequencing was performed by the Nucleic Acid/Protein Research Core Facility at The Children's Hospital of Philadelphia. Primer sequences and PCR amplification conditions are available upon request.

Tissue-specific expression of CLK-1. Tissue-specific expression of CLK-1 mRNA was analyzed by probing a multiple tissue poly(A)+ RNA Northern blot (Clontech) with CLK-1 cDNA. The tissues represented on the blot were human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. G3PDH cDNA (Clontech) was used as a control. The −27 to 601 region of CLK-1 cDNA was cloned into the TA vector (Invitrogen, Carlsbad, CA). Two CLK-1 probes for hybridization were generated by PCR from the cDNA clone: (1) a 5′ 282-bp probe was generated with primers that begin (5′) at nucleotide positions 107 (forward) and 388 (reverse) (the positions are based on the cDNA sequence, with +1 beginning at the initiation codon) and (2) a 3′ 152-bp probe was generated with primers that begin at nucleotide positions 411 (forward) and 566 (reverse). The 282-bp PCR product was gel purified with the QIAquick gel extraction kit (Qiagen). The 152-bp probe was purified with the QIAquick PCR purification kit (Qiagen). With the CLK-1 probes, the blot was hybridized for 1 h at 68°C with ExpressHyb hybridization solution (Clontech) and washed two times at room temperature for 10 min in 2× SSC, 0.05% SDS, and two times at 50°C in 0.1× SSC, 0.1% SDS. The results with either CLK-1 probe were the same, and the hybridization with the 282-bp probe is presented (Fig. 2). The glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was hybridized overnight in 6× SSC, 5× Denhardt's solution, 0.5% SDS, and washed two times at room temperature for 10 min in 2× SSC, 0.05% SDS, and 2 times at 60°C in 0.1× SSC, 0.1% SDS. The blots were exposed to x-ray film at −70°C with two intensifying screens.

Mapping. The human CLK-1 gene was physically mapped by Radiation Hybrid (RH) mapping and by fluorescence in situ hybridization (FISH). The RH mapping was performed with a 167-bp PCR product from the 5′ region of the CLK-1 gene region and the Genebridge4 Radiation Hybrid screening panel (Research Genetics, Huntsville, AL). Oligonucleotide primers used for PCR were 5′ CCA GTG TCA GAT TTC GCA GTT CAG 3′ (forward) and 5′ TCT GAA TGA CTG GCC CGA CGC 3′ (reverse), which amplified a 167-bp fragment at the 5′ end of the CLK-1 gene region. PCR conditions are available upon request. Statistical analysis of the data was performed with RHMAPPER (Stein et al. 1995).

A human BAC clone carrying CLK-1 was identified and mapped by FISH. The Physical Mapping Core (NHGRI/NIH) screened Human BAC clones carrying the CLK-1 gene with oligonucleotide primers 5′ GGG GAC CGC CTT GCT CG 3′ (forward) and 5′ TGG TGC TCA AGC TCC TCA TC 3′ (reverse), which amplified a 1.6-kbp fragment. PCR conditions are available upon request. We chose one BAC clone (well 207P21, Genome Systems) for FISH, which was performed on the male control metaphase by the Cytogenetics Core Lab of National Human Genome Research Institute/NIH.

Rescue experiments. The functional equivalence of human CLK-1 to the yeast *COQ7* homolog was tested by introducing either a single or multicopy plasmid containing human CLK-1 cDNA into yeast *coq7* deletion strains and assaying for growth on a nonfermentable carbon source. Plasmid construction was as follows: the human CLK-1 cDNA was excised with *Eco*RI from the cloning vector pCR2.1 (TA Cloning Kit, Invitrogen) and blunt-end ligated into the *Hin*dIII site of pAH01 and the *Bam*HI site of pCH1. Both pAH01 and pCH1 were previously described (Hsu et al. 1996) and contain the yeast *CYC1* promoter. The generated constructs, pscQ7-1 and pmcQ7-1, allow the expression of the human CLK-1 cDNA behind the constitutive *CYC1* promoter in single- and multicopy, respectively. Yeast strains JM43ΔCOQ7 (α, *his4-580, leu2-3,112, trp1-289, ura3-52, coq7*Δ*-1::LEU2*) (Marbois and Clarke 1996) and CEN.MP3-1A (a, *his3-*Δ*1, leu2-3,112, trp1-289, ura3-52, MAL2-8^c, MAL3, SUC3, cat5::HIS3*) (Proft et al. 1995) were transformed with pscQ7-1, pmcQ7-1, pAH01, or pCH1 (Elble 1992). Transformants were selected for the presence of the *URA3* gene on SD-Ura plates (Adams et al. 1997). The Ura$^+$ colonies were subsequently replica-plated to YPG plate media (Adams et al. 1997) to test for growth on glycerol, a nonfermentable carbon source.

Results

The sequences were deposited in the GenBank database, accession numbers AF032900 (human) and AF053770 (mouse). Both sequences have an open reading frame of 537 nucleotides and are predicted to encode a 179-amino acid protein. In human CLK-1, multiple samples of cDNA revealed a T/C polymorphism at nucleotide position 194 (numbering from the start of the initiation codon, +1), which encodes a Met63Thr amino acid polymorphism (Fig. 1). The sequence reported here also shows a 1-bp mismatch with the previously published 140-bp partial CLK-1 sequence (GenBank accession number U81276) at nucleotide position 258, with G and C, respectively, although the sequences encode identical polypeptides. The mouse nucleotide sequence reported here shows 100% identity to a separate entry of a full-length mouse Coq7 cDNA (GenBank accession number AF080580).

An alignment and distance analysis of the nucleotide and encoded amino sequences of human CLK-1 and mouse, rat, nematode and yeast homologs shows that nucleotide similarity ranges from 52.0% (yeast and nematode) to 94.3% (mouse and rat), and amino acid similarity ranges from 41.4% (yeast and nematode) to 96.1% (mouse and rat) (Table 1). The amino acid alignment also shows that regions of the CLK-1/Coq7 proteins are highly conserved across species (Fig. 1). Overall, the CLK-1 protein is predicted to be mostly helical, with an α-helical membrane insertion region at residues 73-91 (Predict Protein). An α-helical membrane insertion region is also predicted in rat and nematode in the homologous regions (Fig. 1). Human CLK-1 contains one potential N-glycosylation site, one protein kinase C phosphorylation site, one casein kinase II phosphorylation site, and three N-myristolation sites (based on searching the PROSITE database). Except for the N-glycosylation site, these motifs are also found in the other CLK-1/Coq7 proteins, although usually not in homologous locations (not shown).

Tissue-specific expression of CLK-1 mRNA was analyzed by probing a Northern blot containing poly(A)+ RNA isolated from several human tissues with a CLK-1 cDNA. Two different cDNA probes were employed, one located at the 5′ end and the other at the 3′ end of the cDNA. With each probe, three bands were visible after hybridization followed by stringent washing conditions (see Materials and methods). The three RNAs may represent alternatively spliced CLK-1 transcripts, or RNA with high homology to CLK-1 given the stringent washing conditions. RT-PCR on human heart mRNA (Clontech) using primers outside of the coding region [primers at nucleotide positions −27 (forward) and 602 (reverse) relative to the initiation codon] yield a single PCR product. This suggests that exons II, III, and IV are not alternatively spliced, but does not rule out the possibility that exons I and IV (or exons 5′ or

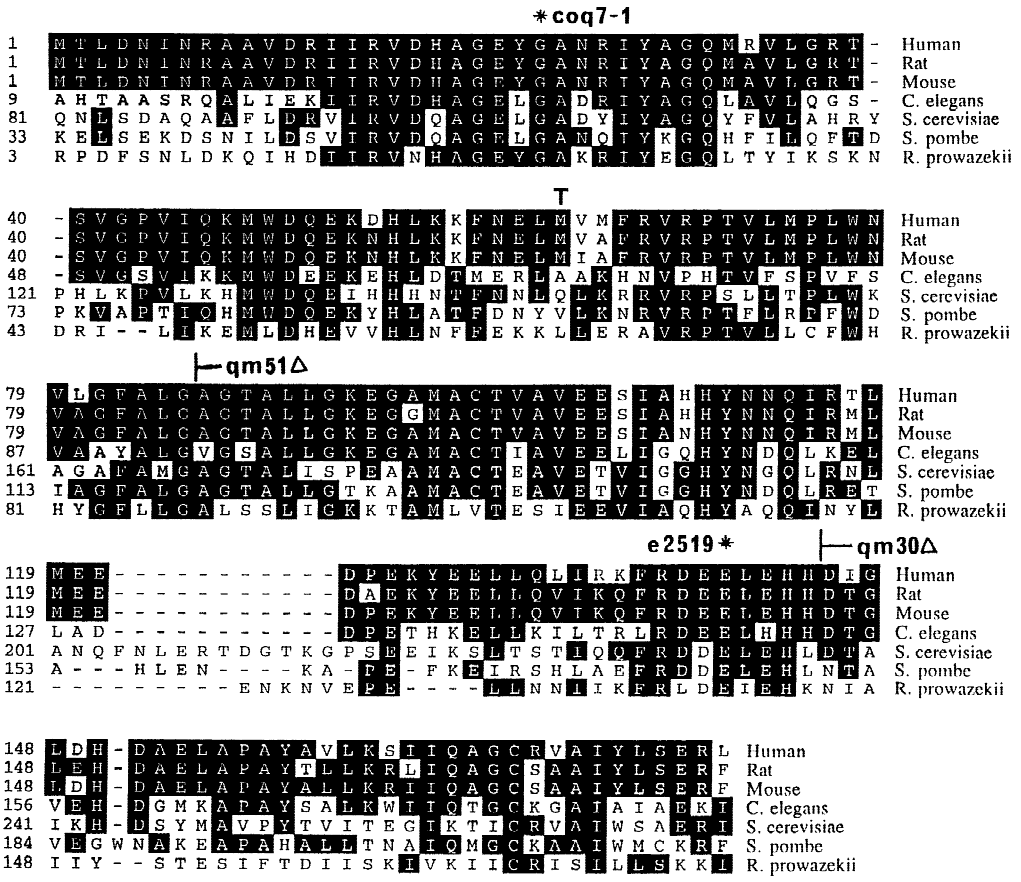

Fig. 1. Amino acid sequence comparison of CLK-1/Coq7 homologs. Alignment of the predicted COQ7 protein sequences of the *S. cerevisiae* (X82930, GenBank), *S. pombe* (CAA21285, GenBank) *R. prowazekii* (CAA14656, GenBank), *C. elegans* (U13642, GenBank), rat (U46149, GenBank), human (U81276, GenBank), and mouse (AF053770, GenBank) was done with the Clustal method in megalign of DNASTAR. Amino acid residues shared by all of the polypeptides are shaded; introduced gaps are designated with dashes. The Met_{63}/Thr polymorphism in human CLK-1 is noted by a T over the M at position 63. An α-helical transmembrane region is predicted for human CLK-1, residues 73–91, based on the PredictProtein program. An α-helical membrane insertion region is also predicted for each of the other homologs in the homologous region (based on the PredictProtein program). Also identified are the *coq7-1* allele (Marbois and Clarke 1996) and three *C. elegans clk-1* mutations (Ewbank et al. 1997).

Table 1. The proportion of identical nucleotides and amino acids in pairwise comparisons of full-length human, rat, mouse, nematode, and yeast CLK1/*Coq7*/*clk-1*/*COQ7* cDNA and encoded proteins using the MEGA program. Amino acid similarity is shown above the diagonal; nucleotide similarity is shown below the diagonal. The similarity is based on an alignment of human, mouse, rat, nematode, and yeast cDNA and amino acid sequences (GenBank accession numbers AF032900, AF053770, U46149, U13642, and X82930) with the program CLUSTALW.

	Human	Mouse	Rat	Nematode	Yeast
Human	—	91.6	89.9	54.2	48.6
Mouse	84.3	—	96.1	55.3	48.0
Rat	85.4	94.3	—	54.2	47.4
Nematode	57.3	55.8	56.6	—	41.7
Yeast	55.0	55.4	55.0	52.0	—

3′ to these) are involved in differential splicing. The different RNAs may also result from differential splicing of the 5′ or 3′ untranslated regions. Based on comparisons with G3PDH, the 1-kb RNA appears to be expressed more highly in heart than in the other represented tissues (Fig. 2), and the 3-kb band appears to be more predominant in skeletal muscle, kidney, and pancreas (Fig. 2).

Intron and exon boundaries of human CLK-1 were determined by sequencing genomic DNA or a BAC genomic DNA clone (Table 2). The number of introns (four, I–IV) and location of introns II and IV are identical to the *C. elegans clk-1* sequence (GenBank accession number U13642). Introns I and III are shifted downstream 26 bp and 44 bp respectively, relative to *C. elegans.*

By the radiation hybrid method, the human CLK-1 gene was mapped to Chr 16. The data vector (12000000121000000010011-0000011102010001010111011) mapped to Chr 16, 2.63 cR from AFMB354YF9 (lod >3.0) between D16S499 and D16S417 in the context of the Whitehead framework map (Stein et al. 1995). This

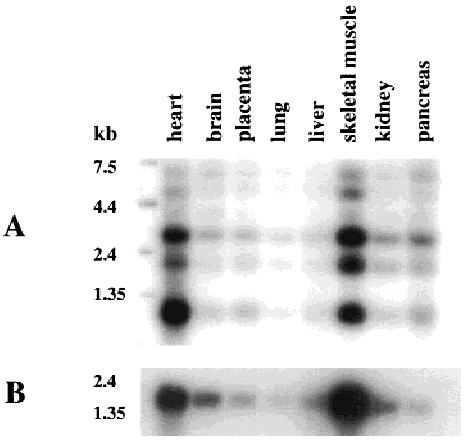

Fig. 2. Expression of CLK-1 in eight different human tissues. **(A)** A poly(A)+ RNA multiple tissue Northern blot was probed with a 283-bp region of CLK-1 cDNA. Three predominant mRNAs are detected, which are approximately 2.9 kbp, 1.9 kbp, and 1.0 kbp in size. The 1.0 kbp fragment is similar in length to the cDNA sequence (854 bp). **(B)** The same membrane was reprobed with G3PDH as a control for the poly(A)+ RNA in each lane.

location was confirmed by FISH, which mapped CLK-1 to Chr 16(p12–13.1).

The functional equivalence of human CLK-1 to the yeast *COQ7* homolog was tested by introducing either a single or multicopy plasmid containing human CLK-1 cDNA into yeast *coq7* deletion strains and assaying for growth on a nonfermentable carbon source. Introduction of the human construct in multicopy con-

Table 2. Nucleotide sequences of the intron/exon boundaries of human CLK-1. Invariant donor and acceptor splice site nucleotides are in bold. The intron location is noted by the amino acid residue at the intron splice site and is shown above the intron sequence. The size of the intron is noted, or an asterisk (*) indicates an approximate size.

Intron	5′ Donor site	3′ Acceptor site	Size (bp)
	Q_{46}↓	↓	
I	TCATTCAG **GT**GGGTGCTTCTTCATCTCCCTCA	CTTTATTTTTATGTTTCTTGC**AG** AAAATGTG	1826
	G_{85}		
II	TGCACTGG **GT**ACGTGTCTCTCTAGAAGTAGCT	TGGTCTGGGTTTAACAATCCC**AG** GGGCGGGG	1800*
	Q_{131}		
III	CTCTTCAG **GT**ATTTATCCGTGCTCTAGAACGG	TGGTGTCCTTTTTATTTAACC**AG** CTGATAAA	1500*
	L_{154}		
IV	CAGAATTG **GT**AGGGCCCTACTGTTACCTGTTC	TGTTTGCTTATTGTTTTTAAC**AG** GCTCCAGC	749

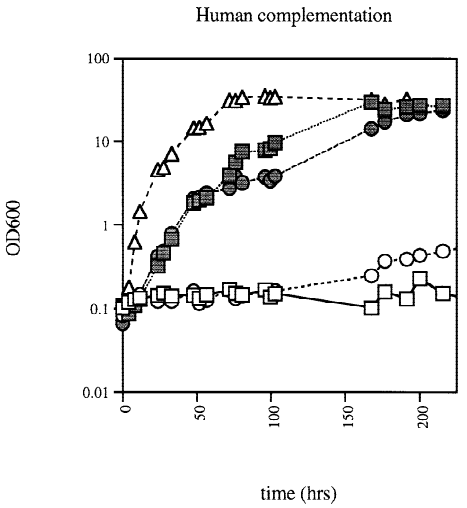

Fig. 3. Rescue of *coq7*Δ mutant yeast for growth on a nonfermentable carbon source by human CLK-1 cDNA. The following *coq7*Δ strains harbored the indicated plasmids and were monitored for growth on glycerol: JM43ΔCOQ7: pNMQ71 (yeast COQ7, △), JM43ΔCOQ7:pscQ7-1 (single-copy human CLK-1, ○), JM43ΔCOQ7:pmcQ7-1 (multicopy human CLK-1, ●), CEN.MP3-1A:pscQ7-1 (□), and CEN.MP3-1A:pmcQ7-1 (■). Samples were grown overnight in 5 ml of SD-Ura to stationary phase. The cultures were then diluted into 50 ml of YPG (OD_{600} = 0.1). Growth at 30°C was monitored by OD_{600} measurements.

ferred the ability of the yeast *coq7*Δ mutants to grow on glycerol (a nonfermentable carbon source) (Fig. 3).

Discussion

The CLK-1/*COQ7* gene is required for the synthesis of Q and respiration, and in *C. elegans*, several *clk-1* mutations have major effects on slowing development and increasing life span. As shown in Fig. 1, the CLK-1/Coq7 proteins show regions of high amino acid similarity across species. In general, CLK-1/Coq7 is absent from prokaryotic genomes (Ewbank et al. 1997), however, a CLK-1/Coq7 homolog is present in the recently determined sequence of *Rickettsia prowazekii*, an obligate intracellular parasitic bacterium whose genome is considered to be very closely related to mitochondria (Andersson et al. 1998). The *C. elegans* CLK-1 polypeptide has been localized to mitochondria (Felkai et al. 1999). In yeast, Coq7p has been localized to the inner mitochondrial membrane, and an α-helical membrane insertion region is predicted for residues 154–175 (Jonassen et al. 1998), corresponding to amino acid residues 73–91 in human CLK-1 (Fig. 1). The amino acid identities and shared structural features of the proteins suggest that the human CLK-1 protein is also an inner mitochondrial membrane protein. Similar to Coq7p in yeast, the amino terminus of the human CLK-1 protein lacks a typical mitochondrial import signal sequence (Nielsen et al. 1997). This is not unusual because it is known that numerous outer and inner membrane proteins, as well as proteins that reside in the intermembrane space, lack targeting sequences at the N-terminus, but have internal targeting signals (Neupert 1997).

The human CLK-1 gene was physically mapped to Chr 16(p12–13.1) by RH mapping and FISH. Interestingly, this location contains another gene required for respiration, the UQCRC2 gene encoding the core protein II subunit of the mitochondrial cytochrome bc_1 complex (Duncan et al. 1993).

Tissue-specific expression of CLK-1 mRNA was analyzed by probing a Northern blot of poly(A)$^+$ RNA isolated from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas with CLK-1 cDNA. It appears that different isoforms of CLK-1 are expressed in each of these tissues, although the three bands differ in the apparent relative amounts. The 1-kb RNA is the predominant RNA in heart, compared with G3PDH (Fig. 2). This correlates with amounts of Q that have been shown to be the highest in heart, kidney, and liver (Aberg et al. 1992). By contrast, the 3-kb band appears to be more predominant in skeletal muscle, kidney, and pancreas (Fig. 1).

Yeast *coq7* mutants lack Q and are unable to grow on nonfermentable carbon sources (Marbois and Clarke 1996). Introduction of the human constructs containing CLK-1 cDNA into yeast *coq7* deletion strains conferred the ability of the yeast *coq7*Δ mutants to grow on glycerol, a nonfermentable carbon source (Fig. 3). The ability to restore growth on glycerol demonstrates that human CLK-1 is required for the synthesis of ubiquinone and is the functional homolog of yeast *COQ7*. The ability of human CLK-1, *C. elegans clk-1*, and rat *Coq7* to complement yeast *coq7*Δ mutants, the considerable amino acid similarity among the CLK-1/Coq7/Coq7p proteins (Table 1), and the shared amino acid membrane insertion helical motifs (Fig. 1) indicate a similar function for the human polypeptide in the synthesis of Q and a similar location in the mitochondrial inner membrane.

On the basis of the conserved function of mammalian and yeast CLK-1/Coq7, it has been proposed that mutations in *clk-1* may influence the amount or distribution of Q (ubiquinone), which could have an effect on development and longevity (Jonassen et al. 1998). Although the cellular role(s) of *clk-1* in *C. elegans* is not clear, it has been proposed that *clk-1* mutations reduce metabolic rates, decrease energy requirements, and thus extend life span by leading to a slower accumulation of reactive oxygen species (Lakowski and Hekimi 1998). Reactive oxygen species have been proposed to exert a major influence on cellular damage and aging (Swartz and Mader 1995; Martin et al. 1996; Sohal and Weindruch 1996; Yu and Yang 1996; Miquel 1998). Thus, mutations in CLK-1, which may influence the amounts of Q, may impact the amounts of oxidants generated by mitochondrial respiration (Jonassen et al. 1998). According to this view, a reduction in Q biosynthesis could lead to slower metabolism and a concomitant decrease in the production of oxidants, which leads to molecular damage and aging.

The oxidative stress theory of aging has gained empirical support from studies showing that overexpression of the antioxidative enzymes copper-zinc superoxide dismutase (SOD1) and catalase extends the lifespan of transgenic *D. melanogaster* (Orr and Sohal 1994; Sohal et al. 1995). Recently, it has been shown that over-

expression of SOD1 in motor neurons of transgenic *Drosophila* extends normal lifespan up to 40% (Parkes et al. 1998). The extension of lifespan is attributed to elevated SOD1 activity and metabolism of reactive oxygen in this specific cell type, and not to an overall lower metabolism. The antioxidant role of Q in the aging process is less well characterized. Supplementation with Q in aged rats has been reported to improve age-associated arterial dysfunction (Lönnrot et al. 1998), stress tolerance, and baseline heart function (Rowland et al. 1998), and to provide neuroprotective effects that correlated with significant increases in Q content in brain mitochondria (Matthews et al. 1998). The cellular mechanism(s) by which Q exerts these effects is unknown. Perhaps the most compelling and direct evidence of the antioxidant role of Q/QH_2 is from in vivo studies in yeast, in which Q-deficient strains of yeast were shown to be hypersensitive to the autooxidation products of lipids (Do et al. 1996). The effects of *clk-1* mutations on levels of Q/QH_2 and its pro- and antioxidant activities, including its participation in the aging process, remain to be investigated.

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