Structural and Functional Conservation of the *Caenorhabditis elegans* Timing Gene *clk-1*

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Mutations in the *Caenorhabditis elegans* gene *clk-1* affect biological timing and extend longevity. The gene *clk-1* was identified, and the cloned gene complemented the *clk-1* phenotypes and restored normal longevity. The CLK-1 protein was found to be conserved among eukaryotes, including humans, and structurally similar to the yeast metabolic regulator Cat5p (also called Coq7p). These proteins contain a tandem duplication of a core 82-residue domain. *clk-1* complemented the phenotype of *cat5/coq7* null mutants, demonstrating that *clk-1* and *CAT5/COQ7* share biochemical function and that *clk-1* acts at the level of cellular physiology.

The activity of *clk-1* in the nematode *Caenorhabditis elegans* controls the rate of the worms' development, the pace of their behaviors, and when they die (*1*). In *clk-1* mutants, the timing of a wide range of physiological processes is deregulated (*1*). This leads to an average lengthening of such diverse processes as the worms' early cell cycles, their embryonic and postembryonic development, and the period of rhythmic adult behaviors, such as swimming, pharyngeal pumping, and defecation. *clk-1* mutants also have an extended life-span. This pleiotropic alteration of developmental and behavioral timing is also exhibited by worms with mutations in any one of the genes *clk-2*, *clk-3*, or *gro-1* (*1*, *2*). Mutations in these four genes interact genetically to affect developmental rate and longevity (*3*).

Mutations of *clk-1* exhibit a maternal effect: homozygous mutant (*clk-1*/*clk-1*) progeny from a heterozygous hermaphrodite (*clk-1*/+) are phenotypically wild type; only homozygous mutants from a homozygous mother exhibit a Clock (Clk) phenotype. The maternal rescue not only influences early events, such as embryonic development, but it extends to adult phenotypes, such as defecation and longevity (*1*). This and other evidence [(*1*), reviewed in (*4*)] suggests the existence of a pervasive timing mechanism whose intrinsic rate can be set early in development and that influences diverse timed processes throughout the worm's life.

The *clk-1* gene lies on linkage group III, between *dpy-17* and *lon-1* (Fig. 1). Overlapping cosmids from the candidate region were assayed for their ability to rescue the mutant phenotype by microinjection into *clk-1* mutants. The cosmid ZC400 rescued the mutant phenotype of both strong (*qm30*) and weaker (*e2519*) *clk-1* alleles and fully recapitulated the maternal effect. Over a number of generations, ZC400-containing extrachromosomal arrays lost their ability to rescue fully the slow-growth phenotype of *clk-1* mutants, but the rescue of slow defecation persisted. This loss of rescue of the developmental phenotype probably reflects a ubiquitous phenomenon in *C. elegans*, in which transgenic arrays undergo transcriptional silencing in the germ line and in early embryos, possibly because of their complex repeated structure (*5*). If this is so, it implies that later zygotic expression of *clk-1*(+) is sufficient to rescue adult behavioral defects, but maternal or early zygotic expression is needed to rescue slow development.

Injection of ZC400 subclones localized *clk-1* to a 1.9-kb Eco RI fragment. Extrachromosomal arrays containing this fragment restored developmental and behavioral rates to wild-type speed for at least one generation. Again, stable rescue over many generations was only obtained for the behavioral phenotypes such as defecation (*6*). This fragment is predicted to contain a single gene, ZC395.2 (*7*), which is altered in three *clk-1* alleles, thereby confirming the identity of this gene as *clk-1* (Fig. 1). The weaker *clk-1* allele is a missense mutation; the stronger ones involve the disruption of entire exons.

We previously demonstrated that all phenotypes of *clk-1* mutants can be fully maternally rescued, that all alleles exhibit the same pattern of phenotypes, and that all alleles fail to complement each other for all phenotypes (*1*, *2*). Given this, the molecular evidence presented here unequivocally establishes that mutations in the *clk-1* gene are responsible for all of the phenotypes seen in *clk-1* mutant worms.

The *clk-1* gene lies just downstream of the predicted gene, ZC395.3 (Fig. 1). Using reverse transcription polymerase chain reaction (RT-PCR) (*8*), we established the 5′ and 3′ ends of both genes and their splicing patterns. We found that *clk-1* is exclusively trans-spliced to the splice leader SL2 at its 5′ end, and the upstream gene is trans-spliced to SL1, in both cases immediately upstream of the initiator AUG

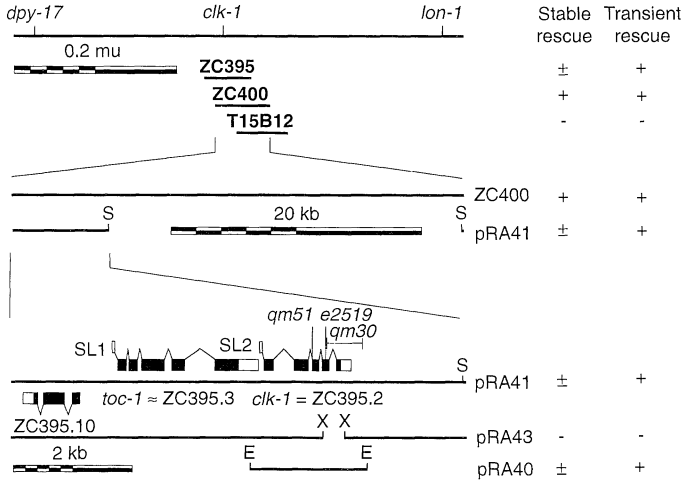

Fig. 1. Cloning of *clk-1*. The top line shows the genetic map in the *clk-1* region (*7*). *clk-1* had been previously mapped to this region (*1*, *2*), but we have refined its location (*25*). Some cosmids tested for rescuing activity (*5*) are shown. Stable rescue that persisted for many generations was obtained only with ZC400. A plus sign indicates rescue of all phenotypes; a plus-minus sign indicates rescue of just the defecation phenotype; and a minus sign indicates no rescue. pRA41 is a derivative of ZC400 with an internal Sac I deletion. The insert in pRA41 contains three predicted genes, ZC395.10, ZC395.3, and ZC395.2 (*7*). A deletion in ZC395.2 eliminates *clk-1* rescuing activity, but pRA40 rescues *clk-1*, indicating that ZC395.2 is the *clk-1* gene. S, Sac I; X, Xba I; E, Eco RI. The nematode expressed sequence tag CEESX93F (*7*) matches the 3′ end of *toc-1*. We resequenced *clk-1* from the mutant alleles (*26*). In allele *e2519*, a G→A transition introduces a new Hind III site and causes a Glu→Lys (E→K) missense alteration in an absolutely conserved residue (Fig. 3). Allele *qm30* results in a 590–base pair deletion, starting 12 nucleotides 3′ of the lesion in *e2519* and encompassing the entire last exon. Allele *qm51* alters the absolutely conserved terminal G in the intron 2 splice acceptor.

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codon. The 3′ end of the upstream gene lies 105 base pairs from the 5′ end of *clk-1*. The pattern of trans-splicing and the intergenic distance are typical of genes organized into operons in *C. elegans* (9), suggesting that the two genes share a promoter 5′ of the upstream gene. The introns in *clk-1* were correctly predicted, but the real product of the upstream gene (Fig. 1) lacks the first predicted exon. This gene potentially encodes a protein that has similarity to a family of divalent metal ion transporters, so we have named it *toc-1*, for transporter-like protein in an operon with *clk-1*. There is no obvious functional relation between *clk-1* (see below) and *toc-1*.

To demonstrate that the lesion in *clk-1(e2519)* was responsible for the mutant worms' extended longevity, rather than it resulting from a difference in genetic background (*10*), we assayed the longevity of fully rescued *e2519* mutant worms carrying the ZC400-containing extrachromosomal array *qmEx109*, as well as those carrying *qmEx96*, an extrachromosomal array containing the *clk-1* 1.9-kb Eco RI fragment. As adults, *e2519; qmEx96* worms showed full rescue of their defecation cycle, even though their development was slow. We found that the presence of either *qmEx96* or *qmEx109* restored a wild-type life-span to *e2519* mutant worms. The life-spans of both *e2519; qmEx96* and *e2519; qmEx109* worms were indistinguishable from that of N2 worms (Fig. 2).

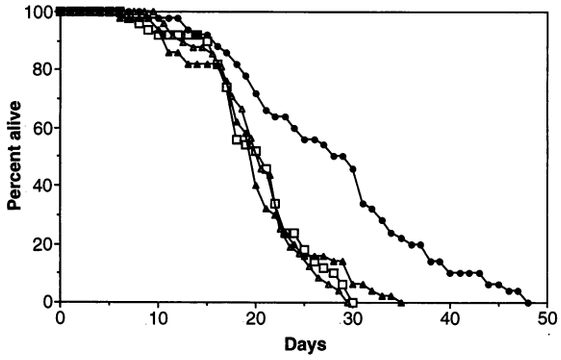

Fig. 2. The extension of life-span exhibited by *clk-1(e2519)* is rescued by the presence of extrachromosomal arrays containing the wild-type *clk-1* gene. The graph shows the percentage of worms alive on a given day after being laid as eggs during a 2.5-hour period on day 0 for N2 (□), *clk-1(e2519)* (●), and *e2519; qmEx109* (▲). For *e2519; qmEx96* (△) a 6-hour limited hatching was used. The mean life-spans, with standard errors, are 20.4 ± 0.8, 28.1 ± 1.4, 20.2 ± 0.9, and 20.4 ± 0.7 days, respectively. The worms were maintained at 18°C throughout and their longevity scored as described (*3*). The sample size was 50 worms of each genotype, except for *e2519; qmEx109* which was 48.

The *clk-1* gene is predicted to encode a 187-residue protein (CLK-1) that is similar to the product of the *Saccharomyces cerevisiae* gene *CAT5/COQ7* (Cat5p/Coq7p) (*11*, *12*) (Fig. 3A). A rat homolog of Cat5p/Coq7p has also been described (*13*). Using the sequence of the rat gene, we identified and partially sequenced murine and human homologs of *clk-1* (*14*). The three full-length proteins are 33% identical over 177 residues, although their NH_2-termini show no similarity, either in length or composition (Fig. 3A). Over the available predicted sequences of 43 and 126 amino acids, the human and mouse proteins are 93 and 97% identical to the rat protein, respectively (Fig. 3). These five proteins are unrelated to any other known sequence, and there are few indications as to their biochemical function (*15*, *16*). The protein sequences can each be split and aligned to reveal the presence of an 82-residue tandemly repeated core domain, which we call the TRC domain, for tandemly repeated in CLK-1 (or Cat5p/Coq7p or rat COQ7) (Fig. 3B). For all the repeats, residues are absolutely conserved at eight positions, and at an additional 12 positions all the residues are similar. For each protein, its two TRC domains are juxtaposed without any linking

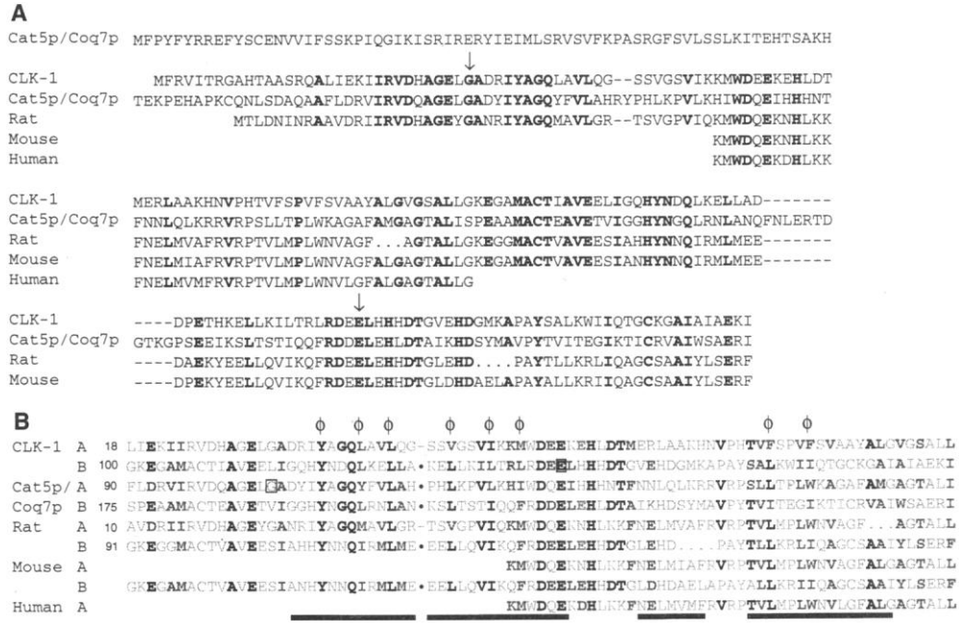

Fig. 3. CLK-1 is highly conserved between nematodes, yeast, rodents, and humans. (**A**) Alignment of the nematode CLK-1, rat COQ7, and yeast Cat5p/Coq7p sequences, together with the partial sequence of murine and human CLK-1 homologs (*14*). Over the length of the rat protein, the identity between CLK-1 and its yeast and rat homologs is 42 and 53%, respectively. Introduced gaps are marked by dashes. Reduction-of-function alleles are indicated by arrows in (A) and are boxed in (B); G→D in *coq7-1* (*12*) and E→K in *e2519*. The rat sequence (GenBank accession number U46149) appears to contain sequencing errors in the vicinity of residues 82 through 84 and 151 through 154 (marked by dots). It is presumably a coincidence that the tripeptide motif AGE (residues 28 through 30 in CLK-1) is present in all three proteins. (**B**) Duplication within the CLK-1 sequence and its homologs. Each of the sequences shown in (A) can be split and aligned to reveal the presence of a tandemly repeated TRC domain. There is a single site of insertions for both NH_2- and COOH-terminal domains; these have been removed for this alignment, as marked by the small black dots. Those residues identical in more than half of the domains are shown in black lettering; those that are similar in more than half are shown in dark gray. A bar is drawn under those residues predicted to be in a helical conformation [(*16*), with input being the alignment shown here]. Within the extended helical regions, positions where there is absolute conservation of a hydrophobic residue are marked by the symbol ϕ. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

sequence. For each domain, there appears to be only a single point at which insertions are tolerated, flanked by regions predicted to be helical (Fig. 3B). Within these helical regions (residues 34 through 56 and 116 through 144 for CLK-1), the spacing of conserved hydrophobic residues is suggestive of an interface for protein-protein interaction, such as a surface for dimerization (*17*) (Fig. 3B). The proteins' two-domain primary structure likely reflects two equivalent domains at the level of tertiary structure (*17*). Given the functional complementation of yeast *cat5/coq7* mutants by the rat homolog (*13*), the level of identity between rat and human sequences strongly supports the notion of functional conservation of *clk-1* from yeast to human.

CAT5/COQ7 is required for the derepression of *PCK1*, which encodes the gluconeogenic enzyme phosphoenolpyruvate carboxykinase, that accompanies the transfer of yeast from glucose to a nonfermentable carbon source such as glycerol or ethanol (*11*). This derepression is mediated by transcriptional activation (*18*), and *CAT5/COQ7* is required for the formation of a specific transcriptional activation complex (*11*). *CAT5/COQ7* also appears to be involved in the control of expression of other enzymes of gluconeogenesis and those of respiration and the glyoxylate cycle (*11*). But its role in all these processes appears to be indirect, because Cat5p/Coq7p is not part of the transcriptional complex (*11*). In addition, the expression of *CAT5/COQ7* appears to be highly regulated, by glucose and by itself (*11*).

cat5/coq7 mutants do not synthesize ubiquinone (coenzyme Q), a lipid-soluble two-electron carrier, which is essential for respiration and consequently for nonfermentative growth (*12*). *CAT5/COQ7* appears to control ubiquinone biosynthesis at two or more steps, although its mode of action is obscure (*11, 12*). The pleiotropic effects of mutation of *CAT5/COQ7* have led to the proposal that there is a co-regulation of respiratory chain components, the biogenesis of mitochondria, and gluconeogenesis, with *CAT5/COQ7* being a likely link connecting glucose derepression with respiration (*11*). Thus, *CAT5/COQ7* appears to be important in the regulation of multiple parallel processes of metabolism, consistent with our view that *clk-1* regulates many disparate physiological and metabolic processes in *C. elegans*.

To test whether the structural similarity extends to functional equivalence, we constructed a *cat5/coq7* deletion yeast strain (Δ*cat5/coq7*), which, as expected (*11, 12*), did not grow on glycerol (*19*). Introduction of a multicopy plasmid containing the *C. elegans clk-1* coding sequence, within an expression cassette with the constitutive promoter and 3′ sequence of the *ADH1* gene, conferred to the Δ*cat5/coq7* strain the ability to grow on glycerol (*20*). This functional complementation of the Δ*cat5/coq7* null mutation by *clk-1* is consistent with the functional complementation of the yeast mutant by the rat homolog (*13*) and is indicative of a common biochemical function for these three genes. In spite of their common biochemical function and roles in regulatory mechanisms, one must be cautious in attempting to understand the physiological defects seen in *clk-1* worms in terms of the phenotypic defects of *cat5/coq7* mutant strains because yeast have some highly specialized systems of metabolic regulation not seen in most other eukaryotes (*21*). Nevertheless, the interspecific functional complementation suggests that a central mechanism of metabolic coordination, which regulates many distinct downstream regulators, is conserved in all eukaryotes, including humans.

The action of *clk-1* is highly pleiotropic; it acts at the level of single-celled eggs and subsequently apparently affects all tissues of the worm at all stages of life (*1*). The *clk-1* homolog *CAT5/COQ7* affects cellular physiology in yeast. These observations suggest that *clk-1* increases animal longevity by slowing down general cellular aging. It remains possible, however, that the longer life of *clk-1* mutants reflects a decreased rate of deterioration of the cells of a particular organ critically required for life, rather than a reflection of the health of the whole of the animal's cells.

In conclusion, *clk-1* is structurally and functionally homologous to a yeast central metabolic regulator. This is consistent with our previous speculation that the long life of *clk-1* mutants might be a consequence of slower cellular metabolism, with an attendant reduction in the rate of production of detrimental by-products (*3*). Our findings also lend support to the idea that multicellular organisms age because their cells age (*22*).

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6. Defecation was scored as previously described (*1*). Five worms of each genotype were scored for five cycles at 20°C. The means of individual animals' mean defecation period, ± standard deviation, in seconds, were as follows: N2, 44.1 ± 2.1; *e2519*, 55.4 ± 5.4; *e2519; qmEx81*[ZC400, pRF4], 46.2 ± 1.6; and *e2519; qmEx96*[pRA40, pRF4], 43.6 ± 1.5. The defecation period of *clk-1*(*e2519*) worms is both longer and more irregular than that of wild-type worms (*1*). Both aspects of the phenotype are rescued in the transgenic animals.
7. Genetic and physical map data for *C. elegans* were obtained by anonymous FTP from the public repository at ftp://nlm.nih.gov/repository/acedb/celegans and viewed by using the program ACeDB [F. H. Eeckman and R. Durbin, *Methods Cell Biol.* **48**, 583 (1995)], obtained from the same source. The sequence of cosmid ZC395, with predicted genes, made available by the *C. elegans* genome sequencing consortium [J. Sulston *et al.*, *Nature* **356**, 37 (1992); R. Wilson *et al.*, *ibid.* **368**, 32 (1994)] can also be obtained from GenBank (accession number U13642).
8. Mixed-stage nematode RNA was prepared [P. Chomczynski and N. Sacchi, *Anal. Biochem.* **162**, 156 (1987)] and used to generate reverse-transcribed libraries for cDNA amplification [M. A. Frohman, M. K. Dush, G. R. Martin, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8998 (1988)]. For amplification of the 3′ end of *clk-1* mRNA, we used the nested primer pairs SHP4 and R_o and then SHP4 and R_i on single-stranded cDNA that had been synthesized by priming with R_t. For the 3′ end of *toc-1* (see text), the same single-stranded template was used with the nested primer pairs SHP64 and R_o and then SHP64 and R_i. The genes produce mRNAs with 3′ untranslated regions of 367 and 176 nucleotides for *toc-1* and *clk-1*, respectively. For amplification of the 5′ end of the *clk-1* message we used the nested primer pairs SL and SHP12 and then SL and SHP10 {where both trans-spliced leaders SL1 and SL2 were tested [D. A. Zorio, N. N. Cheng, T. Blumenthal, J. Spieth, *Nature* **372**, 270 (1994); J. Spieth, G. Brooke, S. Kuersten, K. Lea, T. Blumenthal, *Cell* **73**, 521 (1993)]} on cDNA that had been synthesized by priming with SHP12. For amplification of the 5′ end of *toc-1*, SHP15 and SHP14 replaced SHP12 and SHP10, respectively. Methods were essentially those given in (*23*). The sequences of primers are available on request.
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10. Such differences have been shown to be a confounding factor in the reported extended longevity of *spe-26* mutant worms [D. Gems and D. L. Riddle, *Nature* **379**, 723 (1996)].
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14. RT-PCR was performed, with mouse embryonic RNA and human placental RNA exactly as for nematode RNA (*8*). An internal product from the mouse and human cDNA was amplified with the nested primer pairs CCC3 and CCC4 and then CCC5 and CCC6, and the 3′ end of the mouse cDNA was amplified by the nested primer pairs CCC3 and R_o and then CCC5 and R_i. Both mouse and human internal products were entirely sequenced, and the longer mouse 3′ amplification product was partially sequenced. The human and mouse sequences have been deposited into GenBank with accession numbers U81276 and U81277, respectively. A mouse expressed sequence tag (GenBank accession number AA030846) corresponds to the gene amplified here, and in the region of sequence overlap there were no discrepancies. The mouse sequence in Fig. 3 is a fusion of our sequence and the expressed sequence tag. The sequences of primers are available on request.
15. The CLK-1 sequence contains one potential protein kinase C phosphorylation site, one casein kinase II phosphorylation site, and three *N*-myristoylation sites [A. Bairoch, P. Bucher, K. Hofmann, *Nucleic Acids Res.* **24**, 189 (1995); http://expasy.hcuge.ch/sprot/prosite.html]. All of these are common among proteins and none of them is also present in both the yeast and rat homologs, suggesting that they may not be functional. Two cysteine residues are conserved between the three species, suggesting that they could have a structural or functional role (*17*).

16. The protein is predicted to be largely helical [B. Rost and C. Sander, *Proteins* **19**, 55 (1994)], but prediction-based threading [B. Rost, in *Protein Folds, A Distance-Based Approach*, H. Bohr and S. Brunak, Eds. (CRC Press, Boca Raton, FL, 1995), pp. 132–151; B. Rost, in *The Third International Conference on Intelligent Systems for Molecular Biology (ISMB)*, C. Rawlings *et al.*, Eds. (AAAI Press, Menlo Park, CA, 1995), pp. 314–321] fails to identify any other proteins of similar structure; http://www.embl-heidelberg.de/predictprotein/predictprotein.html.
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19. All yeast manipulations were in the SEY6210 background (*MAT***a**, *leu2-3, ura3-52, his3-Δ200, lys2-801, trp1-Δ901, suc2-Δ9*). The *CAT5/COQ7* locus was disrupted with a PCR-mediated approach [A. Baudin, O. Ozier-Kalogeropoulos, A. Denouel, F. Lacroute, C. Cullin, *Nucleic Acids Res.* **21**, 3329 (1993); the primers used were SHP84 and SHP85]. The *CAT5/COQ7* gene was entirely replaced with a DNA fragment containing a disruption module encoding the green fluorescent protein and the *HIS3* gene [R. K. Niedenthal, L. Riles, M. Johnston, J. H. Hehemann, *Yeast* **12**, 773 (1996)]. Haploid cells were transformed [R. D. Gietz, R. H. Schiestl, A. R. Willems, R. A. Woods, *ibid.* **11**, 355 (1995)] with the PCR product, and *HIS3* integrants were selected on minimal medium lacking histidine. Gene disruptions were confirmed by PCR analysis with primers SHP82, SHP83, and ML138. The Δ*cat5/coq7* strain failed to grow (*24*) on YEPG or $YEPE_3$, which contains ethanol (*11*). The sequences of the primers are available on request.
20. The *CAT5/COQ7* locus was directly amplified from yeast genomic DNA by PCR with *Pfu* polymerase (Stratagene) and primers SHP69 and SHP70. A cDNA corresponding to the entire *clk-1* coding sequence was obtained by PCR amplification, also with *Pfu* polymerase, and nested primer pairs SHP57 and SHP59 and then SHP57 and SHP58 on single-stranded cDNA that had been synthesized by priming with SHP59. The respective yeast and nematode PCR products were digested with Hind III and ligated to Hind III–cut and dephosphorylated pVT102-U [T. Vernet, D. Dignard, D. Y. Thomas, *Gene* **52**, 225 (1987)]. As well as restoring growth on glycerol, both the *CAT5/COQ7*- and *clk-1*–containing plasmids restored the ability of the Δ*cat5/coq7* strain to grow on ethanol [$YEPE_3$ medium (*24*)]. The Δ*cat5/coq7* strain transformed with the *CAT5/COQ7*-containing plasmid did not grow as well as the wild-type yeast strain on nonfermentable carbon sources. When the yeast gene was reintroduced in the context of its own promoter on a centromeric vector, full restoration of wild-type growth was obtained (*24*). *CAT5/COQ7* is known to be involved in the regulation of its own expression (*11*); presumably, the presence of excess Cat5p/Coq7p perturbs the normal metabolic balance of yeast. The sequences of the primers are available on request.
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25. By picking Sma non-Dpy recombinant progeny of *dpy-17(e164) sma-4(e729)/unc-79(e1030) clk-1(e2519) lon-1(e185)* hermaphrodites, we were able to position *clk-1* more precisely: *dpy-17* 25/79 *clk-1* 27/79 *lon-1* 27/79 *sma-4*. To interpolate the physical position of *clk-1*, we estimated that the separation between *ced-4* and *dpy-17* is ~0.2 centimorgans on the basis of data in the database ACeDB (*7*). By using linked double mutants, we also directly determined (*24*) the two point distances between *dpy-17* and *sma-4* (0.85 cM), *dpy-17* and *lon-1* (0.5 cM), and *lon-1* and *sma-4* (0.35 cM). Details of the mapping data can be found in ACeDB (*7*).
26. The sequencing of allele *qm11*, which has a phenotype essentially identical to *e2519* (*1*), revealed an identical lesion. The low probability of independently obtaining the same mutation twice suggests that the original allele was lost. Sequencing of *qm47* failed to reveal a mutation. Subsequent reexamination of the phenotype of *qm47* homozygotes and new complementation tests suggest that *qm47* is not a *clk-1* allele.
27. We thank A. Coulson for cosmids; K. Kemphues for strains; J.-C. Labbé, A Kothari, and J. Mes-Mason for nematode, mouse, and human RNA, respectively; and A. Wong, A.-M. Sdicu, and R. Durbin. Some nematode strains used in this work were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources. Supported by a Royal Society–National Science and Engineering Research Council of Canada exchange fellowship and a Medical Research Council of Canada fellowship to J.J.E., a Medical Research Council of Canada grant to S.H., a Canadian Genome Analysis and Technology grant to H.B., and by fellowships to B.L. from the J. W. McConnell Foundation and Fonds pour la Formation de Chercheurs et l'Aide à la Recherche Québec.

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Structure of Bcl-x_L–Bak Peptide Complex: Recognition Between Regulators of Apoptosis

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Heterodimerization between members of the Bcl-2 family of proteins is a key event in the regulation of programmed cell death. The molecular basis for heterodimer formation was investigated by determination of the solution structure of a complex between the survival protein Bcl-x_L and the death-promoting region of the Bcl-2–related protein Bak. The structure and binding affinities of mutant Bak peptides indicate that the Bak peptide adopts an amphipathic α helix that interacts with Bcl-x_L through hydrophobic and electrostatic interactions. Mutations in full-length Bak that disrupt either type of interaction inhibit the ability of Bak to heterodimerize with Bcl-x_L.

Programmed cell death (apoptosis) occurs during the course of several physiological processes, and when dysregulated contributes to many diseases, including cancer, autoimmunity, and neurodegenerative disorders (*1*). The Bcl-2 family of proteins plays a central role in the regulation of apoptotic cell death induced by a wide variety of stimuli (*2*). Some proteins within this family, including Bcl-2 and Bcl-x_L, inhibit programmed cell death, and others, such as Bax and Bak, can promote apoptosis. Interactions between these two groups of proteins antagonize their different functions and modulate the sensitivity of a cell to apoptosis (*3*, *4*). Several regions of the death-inhibiting proteins participate in their antiapoptotic activity and heterodimerization with the death-promoting proteins, including the Bcl-2 homology 1 (BH1) and BH2 regions (*3*, *5*, *6*). In contrast, only a relatively small portion of the death-promoting proteins encompassing the BH3 region is critical for the ability to promote apoptosis (*7*– *10*). For example, small, truncated forms of Bak are necessary and sufficient both for promoting cell death and binding to Bcl-x_L (*7*).

The three-dimensional (3D) structure of the cell survival protein Bcl-x_L consists of two central hydrophobic α helices surrounded by five amphipathic helices (*11*). To understand how Bak interacts with Bcl-x_L and inhibits the ability of Bcl-x_L to promote cell survival, we determined the solution structure of Bcl-x_L complexed with a 16-residue peptide derived from the BH3 region of Bak. We also measured the binding affinities of Bcl-x_L to alanine mutant Bak peptides and to peptides corresponding to the BH3 regions of other Bcl-2 family members (*12*, *13*).

The minimal region of Bak required to bind to Bcl-x_L was examined in a fluorescence-based assay (*14*). A 16–amino acid peptide derived from the BH3 region of Bak (residues 72 to 87) bound tightly to Bcl-x_L (Table 1). In contrast, smaller peptides from this region, such as an 11–amino acid peptide corresponding to residues 77 to 87, did not bind (Table 1). The 16–amino acid peptide of Bak corresponds precisely to the

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