also function as inhibitors of programmed cell death. A large fraction of cells are lost through apoptosis during in vitro myogenesis. Mitotic cells committed to terminal differentiation (myogenin-positive and p21-negative) and noncommitted cells are susceptible to apoptosis. However, the subsequent induction of $p21^{CIP1}$ is correlated with the acquisition of the apoptosis-resistant phenotype, and the ectopic expression of $p21^{CIP1}$ protects differentiating myocytes from apoptosis. Similarly, ectopic expression of $p16^{INK4A}$ also protects differentiating myocytes from death. Because $p16^{INK4A}$ is specific for the CDK4 and CDK6 Rb kinases (9), these data also suggest that Rb may mediate the survival effects of the Cdk inhibitors. Consistent with this hypothesis is the observation that differentiated $Rb^{-/-}$ myotubes are highly susceptible to apoptotic cell death (7). Though the specific links between cell cycle control and apoptosis are currently unknown, the data presented here are consistent with observations that apoptosis in other cell types is associated with deregulated Cdk activity or can be inhibited by Rb overexpression (10). Cdk inhibitor expression may also influence myocyte survival in embryos, in which it is observed that cells in somites show patterns of death that depend on their location and stage of development (11). Because many nonmyocyte cell lines also induce Cdk inhibitors as they differentiate (12), the regulation of these molecules during development may be a general mechanism that influences whether a cell dies or continues with its differentiation program.

Note added in proof: Recently, antisense oligonucleotides to $p21^{CIP1}$ were shown to enhance cell death in differentiating neuroblastoma cells (13).

REFERENCES AND NOTES

1. F. E. Stockdale and M. Holtzer, *Exp. Cell Res.* **24**, 508 (1961); B. Nadal-Ginard, *Cell* **15**, 855 (1978).
2. K. Guo, J. Wang, V. Andres, R. Smith, K. Walsh, *Mol. Cell. Biol.* **15**, 3823 (1995); O. Halevy *et al.*, *Science* **267**, 1018 (1995); S. B. Parker *et al.*, *ibid.*, p. 1024.
3. W. Gu *et al.*, *Cell* **72**, 309 (1993).
4. M. Collins and A. Lopez Rivas, *Trends Biochem. Sci.* **18**, 307 (1993); G. Williams and C. Smith, *Cell* **74**, 777 (1993); G. Evan *et al.*, *ibid.* **69**, 119 (1992); L. D. Tomei, J. P. Shapiro, F. O. Cope, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 853 (1993).
5. V. Andrés and K. Walsh, *J. Cell Biol.* **132**, 657 (1996).
6. J. Chen, P. K. Jackson, M. W. Kirschner, A. Dutta, *Nature* **374**, 386 (1995).
7. J. Wang and K. Walsh, unpublished data.
8. Flow cytometry analysis of apoptosis was performed as described [L. Zhu *et al.*, *Genes Dev.* **7**, 1111 (1993)]. Subconfluent cultures of murine C2C12 myocytes were transfected with 1 μg of pCMV-CD20 DNA plus 20 μg of pCDNA-p21 by the Lipofectamine method (BRL-Life Technologies). Thirty-six hours after transfection, the growth medium was changed to medium containing 0.5% horse serum for 16 hours and both floating and adhering cells were collected. Cells were subsequently stained with mouse antibody to CD20 and fluorescein isothiocyanate–conjugated antibody to mouse immunoglobulins (Dako). Cells were then fixed in 70% ethanol, stained with propidium iodide, and analyzed on a Becton-Dickinson FACScan. A gate was set to select CD20-positive cells that were more than 20 times as bright as the negative untransfected cells. Propidium iodide staining was simultaneously recorded on the CD20-positive and CD20-negative subpopulations.
9. M. Serrano, G. I. Hannon, D. Beach, *Nature* **366**, 704 (1993); M. Serrano, E. Gomez-Lahoz, R. A. DePinho, D. Beach, D. Bar-Sagi, *Science* **267**, 249 (1995).
10. D. A. Haas-Kogan *et al.*, *EMBO J.* **14**, 461 (1995); Y. Haupt, S. Rowan, M. Oren, *Oncogene* **10**, 1563 (1995); L. Shi *et al.*, *Science* **263**, 1143 (1994); C. J. Li, D. J. Friedman, C. Wang, V. Metelev, A. B. Pardee, *ibid.* **268**, 429 (1995).
11. A. Glücksmann, *Biol. Rev.* **26**, 59 (1951); P. Jeffs and M. Osmond, *Anat. Embryol.* **185**, 589 (1992).
12. H. P. Jiang *et al.*, *Oncogene* **9**, 3397 (1995); R. A. Steinman *et al.*, *ibid.*, p. 3389; K. F. Macleod *et al.*, *Genes Dev.* **9**, 935 (1995); A. F. Lois, L. T. Cooper, Y. Geng, T. Nobori, D. Carson, *Cancer Res.* **55**, 4010 (1995).
13. W. Poluha *et al.*, *Mol. Cell. Biol.* **16**, 1335 (1996).
14. C2C12 myocytes were maintained in Dulbecco's modified eagle's medium (DMEM) supplemented with fetal bovine serum (20%, growth medium), and myogenic differentiation was initiated by shifting subconfluent cultures into differentiation medium [DMEM supplemented with horse serum (2%)]. For immunofluorescence microscopy, cells grown on sterile glass cover slips were fixed for 10 min with 2% paraformaldehyde (in phosphate-buffered saline) and permeabilized with 0.5% NP-40. After they were blocked for 5 min with bovine serum albumin (2%), cells were stained with a monoclonal antibody to skeletal MHC proteins (MF20) and rhodamine-conjugated antibody to mouse immunoglobulin G. The cells were then incubated with digoxigenin-dUTP terminal dioxynucleotide transferase mixture and subsequently stained with fluorescein-conjugated antibody to digoxigenin (ApopTag, Oncor), counterstained with Hoechst 33258, and mounted. Specimens were examined and photographed with a Nikon Diaphot fluorescence microscope.
15. Rabbit polyclonal antibody to p21 was from Santa Cruz Biotech. This antibody raised to the COOH-terminal peptide of $p21^{CIP1}$ recognizes both full-length p21 and p21 proteins truncated at the COOH-terminus. Monoclonal antibody to myogenin antibody was a gift of W. Wright [W. E. Wright, M. Binder, W. Funk, *Mol. Cell. Biol.* **11**, 4104 (1991)].

16. We thank E. Cosgrove for the CC42 myocytes, A. Dutta for pCDNA3-p21 and pCDNA3-p21C, G. Hannon for pCMV-$p16^{INK4A}$, L. Zhu for pCMV-CD20, and W. Wright for antibody to myogenin. Supported by NIH grants HL50692 and AR40197.

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Interaction Between a Putative Mechanosensory Membrane Channel and a Collagen

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The degenerin family of proteins in *Caenorhabditis elegans* is homologous to subunits of the mammalian amiloride-sensitive epithelial sodium channels. Mutations in nematode degenerins cause cell death, probably because of defects in channel function. Genetic evidence was obtained that the *unc-105* gene product represents a degenerin homolog affecting *C. elegans* muscles and that this putative channel interacts with type IV collagen in the extracellular matrix underlying the muscle cell. This interaction may serve as a mechanism of stretch-activated muscle contraction, and this system could provide a molecular model for the activation of mechanosensitive ion channels.

Studies of muscles in the nematode *C. elegans* have identified many components that are conserved in higher organisms (1). Mutations in many of the muscle genes cause abnormal animal motility and behavior. Among these, semidominant mutations in the *unc-105* gene cause muscle disorganization and hypercontraction, with resultant severe paralysis of the animal (2). The hypercontracted muscle in *unc-105* mutants can be relieved by mutations in many genes affecting the myofilament lattice, which results in animals displaying the phenotypes of these secondary mutations (3, 4). However, rare mutations in a suppressor gene, previously known as *sup-20*, behave as the only true suppressors, restoring the wild-type motility in *unc-105* animals (3). Genetic analyses have shown that the suppression of *unc-105* defects by *sup-20* mutations occurs in muscle cells (3).

We cloned the *unc-105* gene by Tc1 transposon tagging and sequenced both genomic and cDNA clones (5) (Fig. 1). A homology search with the predicted amino acid sequence revealed that the UNC-105 protein is similar to MEC-4, MEC-10, and DEG-1, members of the degenerin protein family in *C. elegans* (6–8). MEC-4 and MEC-10 are postulated to be involved in the mechanical signal transduction in the touch reception system (6, 7). As with degenerins, UNC-105 also showed extensive similarity with the α-, β-, and γ-subunits of the mammalian amiloride-sensitive epithelial sodium channel (ENaC) (9–11). Recently, mutations in these subunits that increase their apical cell-surface expression in renal epithelia were shown to be the cause of

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Liddle syndrome, an inherited form of hypertension (*12*). The similarity between UNC-105 and ENaC extends throughout the protein and includes all of the conserved domains of ENaC (Fig. 1). However, the predicted UNC-105 protein contains approximately 150 amino acids at the COOH-terminus that are not represented in other C. *elegans* degenerin proteins.

We identified the mutational changes in three dominant alleles of *unc-105*: *n490*, *n506*, and *n1274* (*3*, *5*). All three mutations occur in the predicted extracellular loop (Fig. 1), with the two stronger alleles (*n490* and *n1274*) near the first membrane-spanning region and one weaker allele (*n506*) near the second membrane-spanning region. Both *n490* and *n1274* change the proline residue at position 134 (to serine and threonine, respectively), and *n506* substitutes glutamic acid for lysine at residue 677 (E677K). The E677K change is close to but not in the predicted β-hairpin region where degeneration-causing mutations are found in MEC-4 (*6*), MEC-10 (*7*), and DEG-1 (*8*).

Three independent alleles of *sup-20* were isolated that suppressed all three semidominant alleles of *unc-105* (*3*). Although these suppressor mutations had no phenotype in an otherwise wild-type background, loss-of-function alleles of this gene were lethal and caused an embryonic arrest phenotype that is similar to, but distinct from, that of the *pat* genes (*13*–*15*). We mapped *n821*, one of the suppressor alleles, to a small interval on the right arm of linkage group X, using chromosomal deficiencies and duplications and using three-factor crosses. This region contains a previously identified gene *let-2*, which encodes the α2 chain of type IV collagen found in the basement membrane between muscle cells and the hypodermis (*14*, *15*).

Because strong mutations in *let-2* caused embryonic arrest similar to that observed for the lethal allele of *sup-20*, we examined through a complementation test whether *n821* is an allele of the *let-2* gene. Because the suppression by *n821* was recessive to the wild-type function and dominant over a putative null, we first examined the effect of *n821* over a loss-of-function allele of *let-2(g30ts)*. As shown in Table 1, *n821* was able to suppress in the presence of *let-2(g30ts)*, which suggests that *let-2(g30ts)* eliminated the wild-type activity of the suppressor gene. Thus, *let-2(g30ts)* failed to complement *n821*, and both affected the *let-2* gene. In a second test, we found that the *let-2* gene contained the wild-type function of the suppressor gene. Independent transformation rescue experiments with the use of either cosmid C12F7, which contains *let-2*, or a subclone in which *let-2* is the only known C. *elegans* gene showed that *let-2(+)* in extrachromosomal arrays eliminated the *n821* suppression effect (Table 1 and Fig. 2A), which indicates that *n821* was a mutation in *let-2* (*16*).

We identified the mutational change in three independently derived suppressor alleles: *let-2(n821)*, *let-2(n1168)*, and *let-2(n1169)* (*17*). The identical base substitution was found in all three alleles. The change resulted in a substitution of lysine for arginine at residue 1783 (R1783K), near the end of the COOH-terminal NC1 domain of the LET-2 protein (Fig. 2B). This arginine residue is conserved in all known type IV collagens (Fig. 2C), which suggests that it is a critical residue. Because the suppressor mutation had no phenotype in an otherwise wild-type background, this conservative change apparently does not disrupt most functions of the LET-2 collagen. By contrast, all other available mutations in *let-2* occur in the triple helical region of the collagen and cause embryonic arrest (*13*).

To confirm that the R1783K change conferred the suppression activity, we introduced the change into a wild-type *let-2* plasmid and established transformed lines in both *let-2(g30ts)* and *unc-105(n490); let-2(n821)* backgrounds. Although this plasmid rescued *let-2(g30ts)*, it did not convert the

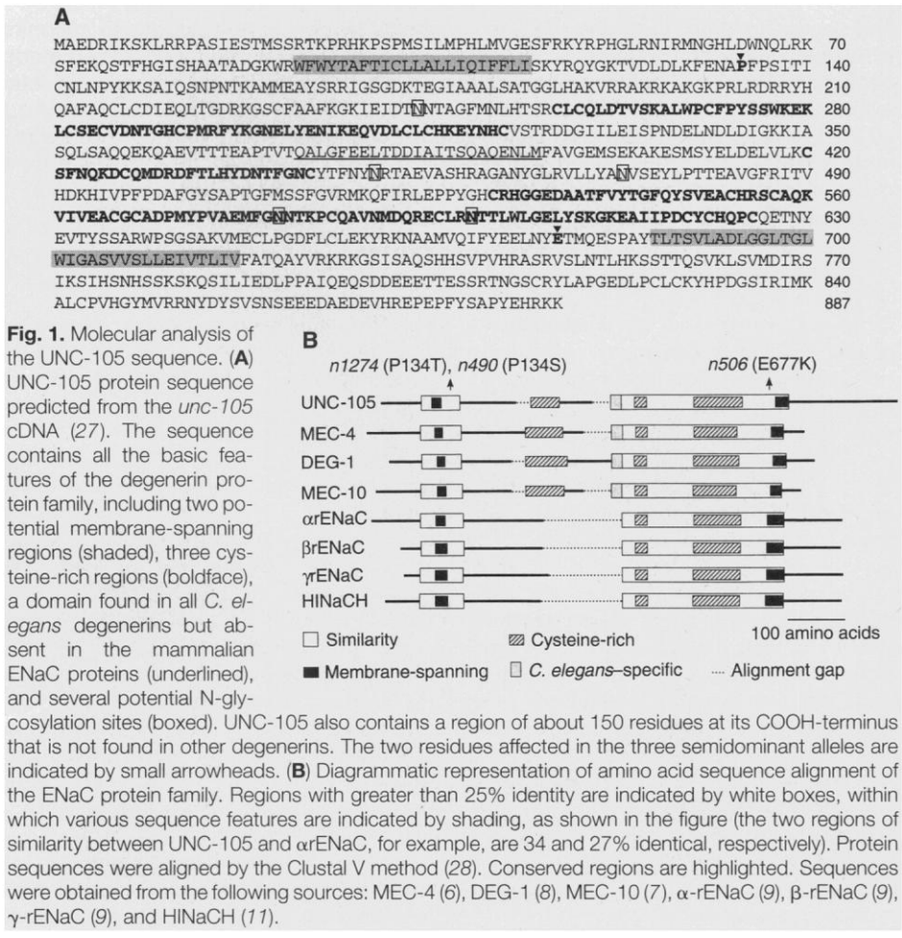

Fig. 1. Molecular analysis of the UNC-105 sequence. (**A**) UNC-105 protein sequence predicted from the *unc-105* cDNA (*27*). The sequence contains all the basic features of the degenerin protein family, including two potential membrane-spanning regions (shaded), three cysteine-rich regions (boldface), a domain found in all C. *elegans* degenerins but absent in the mammalian ENaC proteins (underlined), and several potential N-glycosylation sites (boxed). UNC-105 also contains a region of about 150 residues at its COOH-terminus that is not found in other degenerins. The two residues affected in the three semidominant alleles are indicated by small arrowheads. (**B**) Diagrammatic representation of amino acid sequence alignment of the ENaC protein family. Regions with greater than 25% identity are indicated by white boxes, within which various sequence features are indicated by shading, as shown in the figure (the two regions of similarity between UNC-105 and αrENaC, for example, are 34 and 27% identical, respectively). Protein sequences were aligned by the Clustal V method (*28*). Conserved regions are highlighted. Sequences were obtained from the following sources: MEC-4 (*6*), DEG-1 (*8*), MEC-10 (*7*), α-rENaC (*9*), β-rENaC (*9*), γ-rENaC (*9*), and HINaCH (*11*).

Table 1. Noncomplementation between *n821* and *let-2(g30ts)* in an *unc-105* background (*29*). The suppressor allele *n821* suppressed *unc-105(n490)* recessively—that is, the presence of the wild-type function eliminated the suppression (row 3). However, a previously identified putative null allele of the *n821* gene had no effect on the suppression by *n821* [(*3*), row 4]. In the test strain (row 5), *let-2(g30ts)* showed no effect on the suppression, thus behaving as a null allele of the suppressor gene. By contrast, a wild-type *let-2* clone in an extrachromosomal array eliminated the suppression activity, which demonstrated that it carried the wild-type function of the suppressor gene (row 6) (also see Fig. 2A). Furthermore, the same clone modified to contain the *n821* mutation was able to rescue *let-2(g30ts)* and suppress *unc-105(n490)* animals (row 7).

Genotype	Phenotype
+/+	Unc
n821/n821	Wild type
n821/+	Unc
n821/null	Wild type
n821/let-2(g30)	Wild type
n821/n821; stEx17 [let-2(+)]	Unc
n821/n821; stEx36 [let-2(R1783K)]	Wild type

phenotypically wild-type *unc-105(n490); let-2(n821)* animals to an Unc phenotype, and it behaved as the suppressor allele.

Thus, UNC-105 appears to be a new member of the degenerin channel family that affects muscle function, and defects in this putative channel can be corrected by a change in a basement membrane collagen. The nature of the functional link, implied by the suppression effect, between UNC-105 and the LET-2 collagen is currently unknown. Because the suppression seems to be caused by specific changes in the collagen, these two proteins may interact either directly or through other intermediate molecules (*18*). Because the degenerins MEC-4 and MEC-10 are likely to mediate the mechanosensory transduction in the touch-reception system, we propose that UNC-105 is also a mechanosensitive ion channel in muscle and that gating of this channel involves interaction with components in the basement membrane underlying muscle cells. An interaction between the extracellular loop of the putative UNC-105 channel and the collagen network could provide an anchor for the channel, and stretch-induced distortion of this network could activate the channel, leading to muscle contraction.

To explain our observations in terms of such a model, we suggest that *unc-105* dominant mutations produce a prolonged or exaggerated response of the channel to distortion of the collagen network, which in turn leads to sustained muscle contraction. The suppressor mutation in the collagen disrupts the linkage but not the collagen network itself, so that the UNC-105 channel is not activated. Control of muscle contraction in *unc-105(n490); let-2(n821)* animals is carried out by the redundant functions or other mechanisms that also must act in *unc-105* null mutants. These redundant functions might represent other subunits of the channel that can function in the absence of UNC-105.

Other available data on these genes are also consistent with this model. First, the muscle hypercontraction in *unc-105* mutants and their relief by mutations in other muscle genes suggest that the UNC-105 protein is produced in muscle cells. We found that the *unc-105* promoter is active in muscle cells using an *unc-105*-green fluorescent protein (gfp) promoter fusion construct (*gfp*) (*19*). Moreover, the *let-2* gene products are also produced in muscle (*20*). These expression patterns are consistent with the early observation that *n821* suppressor activity resides in muscle (*3*). Second, localization of the *unc-105* mutations in the extracellular domain suggests that this region may be involved in regulating the function of the channel. Similarly, analyses of mutations in MEC-4 and DEG-1 also suggested a regulatory role of the extracellular domain for gating these channels (*8*). Third, the R1783K change in the NC1 domain of the collagen is unique in both its location and functional consequences. Because the NC1 domain is involved in intermolecular interactions with collagens and other basement membrane components (*21*), it is conceivable that this region may also interact with molecules in the plasma membrane, such as UNC-105. Finally, this model is also consistent with the existence of a stretch receptor that conducts myogenic, stretch-activated muscle contraction as proposed previously (*22*). Gating of the UNC-105 channel by basement membrane collagen molecules could fulfill the role of such a receptor.

Mechanical signaling through interactions between degenerin channels and the extracellular matrix might also occur with the touch receptors in *C. elegans*. One of the genes involved in touch reception was recently found to encode a novel type of collagen (*23*), and a mutation in β-laminin, a basement membrane component, appears to influence degenerin-induced touch cell degeneration (*24*). The putative connection between the extracellular domain of UNC-105 and the α2 subunit of type IV collagen is similar in concept to the mechanical signal transduction in the auditory system, where extracellular tip links physically transduce force to the mechanosensitive channels in the hair cell (*25*). Our model extends this concept by identifying the specific molecules involved.

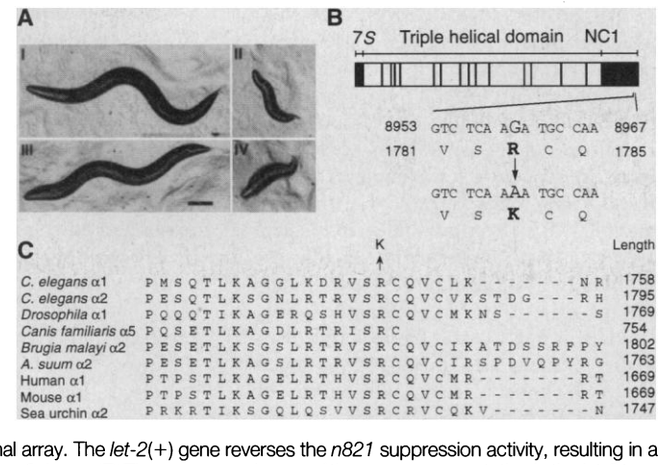

Fig. 2. The *n821* mutation is in *let-2*, a type IV collagen gene. (**A**) Phenotypes of *unc-105(n490)* and its suppressor *n821*. I, wild-type N2. II, *unc-105(n490)* mutant. This animal is hypercontracted and paralyzed. III, *unc-105(n490); let-2(n821)*. *n821* suppresses *unc-105(n490)*, restoring wild-type motility and morphology. IV, *unc-105(n490); let-2(n821); stEx17[let-2(+)]*. This mutant contains the same chromosomal genotype as III but carries a *let-2(+)* gene in an extrachromosomal array. The *let-2(+)* gene reverses the *n821* suppression activity, resulting in a hypercontracted animal. Bar = 0.1 mm. (**B**) The sequence change of the suppressor mutation in the *let-2* type IV collagen gene (*27*). The NH_2-terminal *7S* domain, the COOH-terminal NC1 domain, and many nonhelical interruptions in the triple-helical region are indicated. A single nucleotide change from G to A was found near the end of the NC1 domain, resulting in an amino acid substitution of lysine for arginine at residue 1783. (**C**) Conservation of the NC1 domain and the arginine residue altered in *n821*. Sequences of several type IV collagens are aligned with regions of identical residues highlighted by shading. The arrow points to Arg^{1783} in LET-2. Although not all available type IV collagen sequences are included in this alignment, Arg^{1783} is conserved among all type IV collagen sequences in GenBank. The sequences of the type IV collagen molecules were aligned with the Clustal V method (*28*). The sources of the sequences are as follows: *C. elegans* α2 (*14*) (GenBank accession number U22327), *C. elegans* α1 (X56979), *Drosophila melanogaster* α1 (M23704), *Canis familiaris* α5 (U07888), *Ascaris suum* α2 (M67507), *Brugia malayi* α2 (U07224), *Strongylocentrotus purpuratus* (X76730), human α1 (Y00706), and mouse α1 (J04694).

REFERENCES AND NOTES

1. R. H. Waterston, in *The Nematode* Caenorhabditis elegans, W. B. Wood, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988), pp. 281–335.
2. E.-C. Park and H. R. Horvitz, *Genetics* **113**, 821 (1986).
3. ———, *ibid.*, p. 853.
4. D. Eide and P. Anderson, *ibid.* **109**, 67 (1985).
5. To clone the *unc-105* gene, we first localized *unc-105* to a small physical interval on chromosome II by aligning the genetic map and physical map, using known markers present on both maps. We then identified three intragenic revertants of *unc-105(n490)* in a Tc1 active background RW7000 [D. Moerman, G. M. Benian, R. H. Waterston, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2579 (1986)] and demonstrated the presence of Tc1-induced restriction fragment length polymorphisms, using as probes C30D12 and C41C1, two overlapping cosmids in the *unc-105* region. These three Tc1 insertions were localized to a 2.3-kb Hind III fragment. A 7-kb Sac I fragment from C30D12 that includes the Hind III fragment was subcloned and sequenced by the dideoxy method with the use of a shotgun approach and was found to contain the genomic sequence of *unc-105* that included all the cDNA sequences identified later. C41C4 was later sequenced by the *C. elegans* genome sequencing project (*26*) (GenBank accession number Z48045). Several candidate cDNA clones were isolated from a mixed-stage cDNA library [R. J. Barstead and R. H. Waterston, *J. Biol. Chem.* **264**, 10177 (1989)], with the use of the 2.3-kb Hind III fragment as probe. The longest cDNA clone was found to be 3179 base pairs (bp) by sequencing and was used to derive the amino acid sequence of UNC-105 (887 amino acid residues) and to determine exon-intron boundaries in the genomic sequence. We found 12 exons and 11 introns, which were all correctly predicted by the gene prediction program GeneFinder (*26*). A BLASTP search was then conducted with the use of the UNC-105 sequence as a query [F. S. Altschul, W. Gish, W. Miller,

E. W. Myers, D. Lipman, *J. Mol. Biol.* **215**, 403 (1990)]. We identified mutations in *unc-105* mutants by sequencing all regions of the *unc-105* genomic DNA obtained by polymerase chain reaction amplification of overlapping genomic fragments from mutant animals [B. D. Williams, B. Schrank, C. Huynh, R. Shownkeen, R. H. Waterston, *Genetics* **131**, 609 (1992)].
6. M. Driscoll and M. Chalfie, *Nature* **349**, 588 (1991); K. Hong and M. Driscoll, *ibid.* **367**, 470 (1994); C.-C. Lai *et al.*, *J. Cell Biol.*, **133**, 1071 (1996).
7. M. Huang and M. Chalfie, *Nature* **367**, 467 (1994).
8. M. Chalfie and E. Wolinsky, *ibid.* **345**, 410 (1990); J. Garcia-Anoveros, C. Ma, M. Chalfie, *Curr. Biol.* **5**, 441 (1995); W. Shreffler, T. Margardin, C. Shekdar, W. Wolinsky, *Genetics* **139**, 1261 (1995).
9. C. M. Canessa, J.-D. Horisberger, B. C. Rossier, *Nature* **361**, 467 (1993); C. M. Canessa *et al.*, *ibid.* **367**, 463 (1994).
10. E. Lingueglia, N. Voilley, R. Waldmann, M. Lazdunski, P. Barbry, *FEBS Lett.* **318**, 95 (1993).
11. F. J. McDonald, P. M. Snyder, P. B. McCray Jr., M. J. Welsh, *Am. J. Physiol.* **266**, L728 (1994).
12. J. H. Hansson *et al.*, *Nature Genet.* **11**, 76 (1995); P. M. Snyder *et al.*, *Cell* **83**, 969 (1995).
13. B. D. Williams and R. H. Waterston, *J. Cell Biol.* **124**, 475 (1994).
14. M. H. Sibley, J. J. Johnson, C. C. Mello, J. M. Kramer, *ibid.* **123**, 255 (1993); J. M. Kramer, *Annu. Rev. Genet.* **28**, 95 (1994).
15. M. H. Sibley, P. L. Graham, N. von Mende, J. M. Kramer, *EMBO J.* **13**, 3278 (1994).
16. Gonadal injections were performed essentially as described [C. C. Mello, J. M. Kramer, D. Stinchcomb, V. Ambros, *ibid.* **10**, 3959 (1992)]. Carrier DNA (pRF4) and test DNA were mixed and injected at a ratio of 20:1, at a concentration of 200 mg/ml of total DNA. The rescue of *n821* by *let-2*(+) was demonstrated in two transformation experiments. First, a *let-2*(+) extrachromosomal array was first established in a *let-2(g30ts)* background with cosmid C12F7 and then brought into an *unc-105(n490); let-2(n821)* background by crossing. The cross progeny were found to have an Unc phenotype. Second, UNC-105–like F_1 transformed progeny were also found when pJJ353, a subclone of C12F7 containing only *let-2* (*19*), was injected directly into phenotypically wild-type strain MT1679 [*unc-105(n490); lon-2(e678) let-2(n821)*].
17. To sequence the mutations in *n821*, *n1168*, and *n1169*, we amplified genomic DNA fragments from the *let-2* gene in the three mutants by polymerase chain reactions, using KlenTaq LA polymerase [W. M. Barnes, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2216 (1994)]; their sequences were then determined. To confirm the mutation identified by DNA sequencing, we used a 797-bp Bst XI–Nde I fragment (which spanned the mutation from the mutant DNA) to replace the same region in the wild-type clone and introduced the resulting plasmid into an *unc-105(n490); let-2(n821)* strain. Transformed animals identified through the Roller phenotype induced by the pRF4 carrier DNA were otherwise normal in motility, which indicates that the plasmid contains the suppressor allele. A similar result was obtained when a rescuing line was first established in a *let-2(g30ts)* background and then crossed into *unc-105(n490); let-2(n821)*.
18. It seems unlikely that the suppression is caused by general decreases in the collagen function, as we did not observe suppression of *unc-105(n490)* by *let-2(g30ts)* even at intermediate temperatures.
19. We constructed an *unc-105::gfp* promoter fusion by fusing the 5.1-kb genomic fragment upstream of the start codon of *unc-105* with the *gfp* expression vector pPD95.73 (A. Fire, J. Ahnn, G. Seydoux, S. Xu, personal communication). When this construct was introduced into wild-type N2 animals, we observed nuclear-localized fluorescent signals in body-wall muscle cells and did not detect expression in hypodermal or motor neuron cells.
20. J. M. Kramer, personal communication.
21. E. C. Tsilibary *et al.*, *J. Cell Biol.* **111**, 1583 (1990).
22. H. D. Crofton, *Nematodes* (Hutchinson University Library, London, 1966); J. G. White *et al.*, *Philos. Trans. R. Soc. London Ser. B* **275**, 326 (1976).
23. H. Du, G. Gu, C. M. William, M. Chalfie, *Neuron* **16**, 183 (1996).
24. G. Kao, M. Driscoll, W. Wadsworth, personal communication.
25. J. O. Pickles and D. Corey, *Trends Neurosci.* **15**, 254 (1992); A. J. Hudspeth and P. G. Gillespie, *Neuron* **12**, 1 (1994).
26. R. Wilson *et al.*, *Nature* **368**, 32 (1994).
27. 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.
28. D. G. Higgins and P. M. Sharp, *Comput. Appl. Biosci.* **5**, 151 (1989).
29. In a cross between MT1679 [*unc-105(n490); lon-2(e678) let-2(n821)*] and GG30 [*let-2(g30ts)*] males, F_1 cross progeny, identified as non-Lon animals, were found to have the wild-type phenotype at 25°C and 20°C and the Unc phenotype at 15°C. In comparison, a similar cross between MT1679 and N2 males produced F_1 progeny with an Unc phenotype.
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Oxygenic Photoautotrophic Growth Without Photosystem I

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Contrary to the prediction of the Z-scheme model of photosynthesis, experiments demonstrated that mutants of *Chlamydomonas* containing photosystem II (PSII) but lacking photosystem I (PSI) can grow photoautotrophically with O_2 evolution, using atmospheric CO_2 as the sole carbon source. Autotrophic photosynthesis by PSI-deficient mutants was stable both under anaerobic conditions and in air (21 percent O_2) at an actinic intensity of 200 microeinsteins per square meter per second. This PSII photosynthesis, which was sufficient to support cell development and mobility, may also occur in wild-type green algae and higher plants. The mutants can survive under 2000 microeinsteins per square meter per second with air, although they have less resistance to photoinhibition.

In the Z scheme, first proposed by Hill and Bendall (*1*), PSII can split water but is not thought to be able to perform one of PSI's assigned functions: the reduction of ferredoxin (Fd)/nicotinamide adenine dinucleotide phosphate ($NADP^+$), which is essential for CO_2 assimilation. The Z scheme therefore requires that both PSII and PSI work in sequence for complete photosynthesis, using water as the source of electrons and CO_2 as the terminal electron acceptor. Despite some disagreement (*2–5*), the Z scheme has become the textbook model of photosynthesis (*6*, *7*). Sustained photoassimilation of CO_2 and evolution of H_2 and O_2 in minimal medium can be achieved by the PSII light reaction without involvement of PSI in a PSI-deficient mutant of *Chlamydomonas* grown photoheterotrophically with the use of an organic nutrient (acetate) (*8*). Here we report that PSI-deficient mutants of *Chlamydomonas* were capable of growing photoautotrophically. Because the Z scheme requires both PSI and PSII working in series, it predicts that PSI-deficient mutants of green algae will not grow photoautotrophically. The discovery of photoautotrophic growth of PSI-deficient green algae without any organic nutrients, therefore, suggests the existence of PSII photosynthesis that is an alternative to the Z scheme. Our discovery may provide an explanation for many reports of anomalous quantum requirements that cannot be explained by the Z scheme.

Photoautotrophic growth of several PSI-deficient mutants of *Chlamydomonas*, such as F8 and B4, was observed when photoheterotrophically grown aliquots were inoculated into 75 ml of minimal medium (*9*) in sterilized Erlenmeyer flasks and incubated under continuous actinic illumination [photosynthetically active radiation (PAR) of 20 microeinsteins (μE) m^{-2} s^{-1}] provided by daylight fluorescent lamps (an einstein is the energy of 1 mol of photons). The minimal medium contained only water and mineral elements. The flasks that contained the liquid culture were capped with phenolic screw caps that allowed air (CO_2) exchange. Constant shaking of the culture flasks with a gyratory shaker at a speed of 140 rpm facilitated air exchange for CO_2 supply. Under these conditions, the algal cultures grew for

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