of these birds, like that of other species (*21, 22*), is adjusted specifically to the naturally occurring photoperiodic conditions.

In all experimental birds that had a complete molt, the molt started as the gonads began to regress, which is consistent with the situation in free-living conspecifics (*17*). This contrasts with findings in some other avian species, where the rhythms of gonadal size and molt tend to dissociate in the absence of environmental synchronizing agents (*16, 23, 24*). The rigidity of the temporal relation between the gonadal and molt cycles in the African stonechats suggests that circannual mechanisms in this species play an important role not only in the adjustment of seasonal activities to the environment but also in the maintenance of internal temporal order in an environment that lacks pronounced external seasonal information.

Although our results strongly suggest that the basic mechanism underlying annual periodicity in these tropical birds has a strong endogenous component, they do not exclude the participation of environmental timing cues. On the contrary, the fact that the period of rhythmicity tends to deviate from 1 year in constant conditions indicates that external stimuli are usually involved in synchronizing circannual rhythms with the calendar year. Factors related to the rainy or dry seasons are likely candidates (*1–4, 17*).

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18. Data obtained from other avian species suggest that full photoperiodic responses occur only at light intensities of 10 lux or more [G. A. Bartholomew, *Bull. Mus. Comp. Zool. Harv. Univ.* **101**, 433 (1949); M. Menaker and A. Eskin, *Science* **157**, 1182 (1967)]. Results from other species have indicated that the expression of circannual rhythms may depend on the appropriate duration of constant photoperiod (*21, 22*). It was therefore of interest to find out whether the circannual cycles of our equatorial stonechats would also require an appropriate simulation of constant photoperiod.
19. Five males and four females of experiment 1 (**A**, **B**, **E–K** in Fig. 1) and all birds of experiment 2 were collected in April and May near Nakuru, Kenya (0°15′S, 36°0′E), and transported to Andechs, West Germany, within 2 weeks. These birds were collected when they were 4 to 8 days old. Two males and six females (**C**, **D**, **L–Q** in Fig. 1) were bred from African birds caged in Andechs. The birds were handraised and subsequently fed as described [E. Gwinner, V. Neusser, D. Engl, D. Schmidl, L. Bals, *Gefiederte Welt* **5**, 118 (1987); *ibid.*, p. 145. They were kept in individual cages, housed in groups of six to nine in temperature-controlled environmental chambers (20° ± 3°C). Testicular width or follicular diameter were determined by laparotomy [E. Gwinner, *Tierpsychol.* **38**, 34 (1975).] We assessed molt by checking birds for the occurrence of growing body feathers (body molt) or flight feathers (flight-feather molt). We established the length of the circannual period of the gonadal cycles by determining the interval between times at which testicular width exceeded 1.2 mm and follicular width exceeded 0.5 mm during successive phases of gonadal growth.
20. Of the remaining two birds that exhibited circannual rhythms, one had a long period (about 18 months) during the first three cycles and a shorter one (about 14 months) during the last three cycles. The period of the other rhythmic bird was close to 12 months. In the two arrhythmic birds, gonadal size went through irregular cycles of growth and regression and molt occurred at variable intervals.
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Increased Life-Span of *age-1* Mutants in *Caenorhabditis elegans* and Lower Gompertz Rate of Aging

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A mutation in the *age-1* gene of the nematode *Caenorhabditis elegans* has been shown to result in a 65 percent increase in mean life-span and a 110 percent increase in maximum life-span at 25°C. One of the hallmarks of organismic aging and senescent processes is an exponential acceleration of age-specific mortality rate with chronological age. This exponential acceleration is under genetic control: *age-1* mutant hermaphrodites show a 50 percent slower rate of acceleration of mortality with chronological age than wild-type strains. Mutant males also show a lengthening of life and a slowing of the rate of acceleration of mortality, although *age-1* mutant males still have significantly shorter life-spans than do hermaphrodites of the same genotype. The slower rates of acceleration of mortality are recessive characteristics of the *age-1* mutant alleles examined.

A NOW CLASSIC OBSERVATION IN gerontology (*1*) is that human aging is associated with exponential accelerations of age-specific mortality rate, $m(t)$. Moreover, similar exponential relations between $m(t)$ and chronological age (t) are observed to result from mortality due to any one of many distinct causes, including both diseases and accidents (*2*). In a variety of other species, both vertebrate and invertebrate (*3, 4*), exponential accelerations of mortality rate with increasing chronological age also are observed; Sacher has termed this age-related acceleration the actuarial aging rate (*4*). These observations are consistent with the definition of senescence in evolutionary theory as "the tendency for the age-specific survival probabilities . . . to decline with increasing age . . ." (*5*, p. 214); however, theory makes no prediction about the form or nature of this decline. The nematode *Caenorhabditis elegans* shows exponential increases in age-specific mortality rate (*6*) with increasing chronological age that are specified in part by a polygenic system (*7*). Age-specific mortality rate can be accurately modeled as a function of chronological age with the use of only two parameters: A, initial mortality rate, which here is used as a measure of mortality rate at maturity; and α, the exponential Gompertz component, which describes the rate of acceleration of age-specific mortality with chronological age. These two parameters are related to $m(t)$ at age t by the Gompertz equation:

$$m(t) = Ae^{\alpha t}$$

Despite consistent findings of an expo-

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nential acceleration of age-specific mortality rate with chronological age, Gompertz analysis is not routinely performed. Here I show that an exponential acceleration of mortality rate also is observed in mutants of *age-1* [a gene of *C. elegans* in which recessive mutations that can increase maximum life-span more than twofold have been identified (*8–10*)] and that the rate of this exponential acceleration is 30 to 40% slower in *age-1* mutants than in wild-type strains.

Only two interventions, caloric restriction (*11*) and genetic manipulation (*8–10, 12–14*), have been shown to increase maximum life-span in any organism, and only in the analysis of caloric-restricted rodents (*11*) or of recombinant-inbred lines of *C. elegans* (*6*) has a slowing of the acceleration of mortality rate with chronological age been found. Both of these interventions extend life expectancy and, more importantly, maximum life-span. Recently, however, mutations (*15*) in the *age-1* gene of *C. elegans* that increased life expectancy and maximum life-span 60 and 110%, respectively, have been isolated (*8*) and mapped (*9*). Both of the mutant alleles (*hx542* and *hx546*) in this study are recessive (*10*) and are associated with a fourfold decrease in fertility (*9, 10*) but do not alter other life-history traits such as the length of the reproductive period or the rate of development (*9*). N2, the standard laboratory wild-type strain of *C. elegans*, carries the normal allele of *age-1* and has a life expectancy of about 19 days and a maximum life-span of about 37 days (*6–10, 12, 16, 17*). In the only previous assessment of $m(t)$ in *C. elegans* (*6*), an exponential Gompertz component of 0.053 (expressed in $\log_{10}$ units) was observed, leading to the doubling of mortality in 5.7 days; at this rate of doubling, 100% mortality was observed at 36 days of age. Because recombinant-inbred lines of *C. elegans* with a maximum life-span near 58 days (*6*) have a rate of acceleration that is significantly lower than that of N2 but show no change in initial mortality rate, it was of interest to know whether *age-1* mutants have a lower exponential Gompertz component as well. A priori, longer lived populations could result from a slowing in the rate of acceleration, from a lowering of the initial mortality rate, or from other changes including altered relations between age-specific mortality rate and chronological age.

Because the life-span of *C. elegans* is markedly sensitive to environmental conditions such as temperature and food concentration (*12, 16, 17*), the survival of the several genotypes was assayed under identical environmental conditions at the same time. Each genotype, in these experiments, consisted of four identical but independent populations of 50 nematodes (strain-genotype relations are given in Table 1), which were assayed for viability daily beginning at day 3 and continuing throughout life until all had

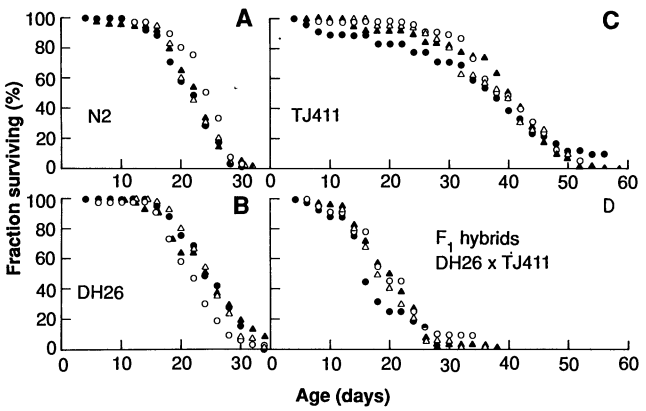

Fig. 1. Survival curves of two wild-type *age* strains, an *age* mutant strain, and a heterozygous strain of *C. elegans*. In each case, four identical populations of hermaphrodites (shown by different symbols) were separated at 3 days of age and monitored daily in separate mass cultures of 50 worms each until all worms had died. Survival analyses and statistical tests were performed as previously described (*7, 9, 12*) except that survivors were counted every 24 hours throughout life, and Bonferroni corrections were used where appropriate. (**A**) Survival curves for the wild-type, N2 (Bristol) hermaphrodites; life expectancies of the four identical subpopulations were 20.5, 23.0, 20.9, and 21.0 days; culture 2 was found to be significantly longer lived than the other three cultures ($P < 0.05$) and was not used in the further analyses described in Table 1; inclusion of this sample results in only minor modifications. (**B**) Survival curves for hermaphrodites of DH26, the parental strain in which the mutants were derived (*8*); life expectancies were 23.8, 20.7, 23.2, and 23.8 days and were not significantly different from each other. (**C**) Survival curves for TJ411 hermaphrodites (*10*); life expectancies were 34.3, 38.0, 37.6, and 36.6 days and were not significantly different from each other. (**D**) Survival curves for F_1 hermaphrodites from a cross of TJ411 hermaphrodites by DH26 males; life expectancies were 15.0, 18.7, 19.1, and 17.8 days and were not significantly different from each other.

Table 1. Weighted, age-specific mortality data.

Strain	Sex	Genotype	Life-span mean ± SE (days)	Total number of animals	Weighted Gompertz parameters* [$\log_{10}$ (probability of death/day)]		Initial mortality rate (probability of death/day)	Mortality rate doubling (days)
					A ± SE†	α ± SE		
				Experiment 1				
N2‡	Herm.	*age-1(+) fer-15(+)*	20.8 ± 0.4	123	−2.70 ± 0.07	0.087 ± 0.003	0.0036	3.5
DH26	Herm.	*age-1(+) fer-15(b26)*	22.9 ± 0.4	171	−2.48 ± 0.05	0.067 ± 0.002	0.0052	4.5
TJ411	Herm.	*age-1(hx542) fer-15(b26)*	36.6 ± 0.9	144	−2.70 ± 0.04	0.041 ± 0.001	0.0026	7.3
F_1	Herm.	*age-1(hx542)/age-1(+) fer-15(b26)*	17.8 ± 0.7	89	−2.06 ± 0.08	0.061 ± 0.004	0.0132	4.9
				Experiment 2				
DH26	Herm.	*age-1(+) fer-15(b26)*	25.5 ± 0.9	130	−1.76 ± 0.05	0.025 ± 0.002	0.0209	12.0
TJ401	Herm.	*age-1(hx546) fer-15(b26)*	56.2 ± 1.7	145	−2.54 ± 0.06	0.021 ± 0.001	0.0033	14.3
F_1	Herm.	*age-1(hx542)/age-1(+) fer-15(b26)*	24.3 ± 0.8	94	−1.78 ± 0.09	0.031 ± 0.003	0.0204	9.7
DH26	Male	*age-1(+) fer-15(b26)*	20.0 ± 0.4	173	−2.11 ± 0.05	0.057 ± 0.002	0.0115	5.3
TJ401	Male	*age-1(hx546) fer-15(b26)*	27.4 ± 0.6	185	−2.14 ± 0.04	0.038 ± 0.002	0.0093	7.9
F_1	Male	*age-1(hx542)/age-1(+) fer-15(b26)*	19.2 ± 0.4	170	−2.01 ± 0.05	0.054 ± 0.002	0.0141	5.6

*Mortality rate weighted by the number of animals dying within any time period. All standard errors provided in this table were calculated by the grouping of all worms after testing for heterogeneity as described in the legend to Fig. 1. †Values are mortality rate at 3 days of age; *C. elegans* is mature at this stage, and survival assays were initiated at this point. A and α are here represented as $\log_{10}$ rather than the natural logarithm. ‡Excluding one sample consisting of 39 worms (see legend to Fig. 1).

died. There is little evidence for heterogeneity among the four independent subsamples of each strain (Fig. 1); in only one strain (N2; Table 1, line 1) was there any statistically significant variation in survival among the subsamples (Fig. 1A). The three other strains that were similarly analyzed in experiment 1 (Fig. 1, B, C, and D) showed no signs of heterogeneity in survival, nor did any other strain described in Table 1. It is possible that the heterogeneity depicted in Fig. 1A resulted from stochastic effects, but the use of the Bonferroni inequality (*18*) failed to confirm this possibility. We have, therefore, excluded this one N2 subsample from these analyses, although the inclusion of that subsample does not affect any of these conclusions.

Because *C. elegans* is a self-fertilizing hermaphroditic species (*19*), all 200 nematodes of a given strain were genetically identical and homozygous. Nevertheless, as in all measures of life-span, significant variation in time of individual death is evident, even though nonaging causes of death, such as death from experimental error or inability to lay eggs prior to hatching, have been discounted (*9, 12*).

Simple linear regression of the age-specific mortality rate (after $\log_{10}$ transformation) on chronological age was used, and I derived standard errors for the regression coefficients by analyzing each of the four replicate subpopulations independently and calculating errors for these terms by averaging the four replicate subpopulations (Table 2). These empirically derived estimates of both the initial mortality rates and the rates of acceleration were not significantly different from those derived by an analysis of the entire population of 200 nematodes (Table 1). Thus, age-specific mortality rate can be modeled as an exponential function of chronological age, even in *age-1* mutant strains. This rules out several possible modes of action of the *age-1* mutant alleles (for example, that mutant alleles eliminate the acceleration of age-specific mortality rate with chronological age) and is consistent with the interpretation that the wild-type *age-1*(+) allele specifies an increase in the acceleration of age-specific mortality rate. Mathematical functions other than the Gompertz equation have been used to model the change in mortality rate with chronological age (*20*), and I have not attempted to exclude alternative mathematical models such as the Gamma or the Weibull as models for these data.

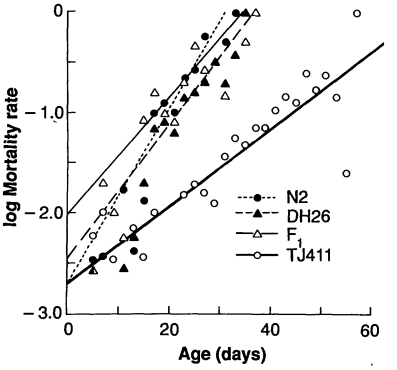

Fig. 2. Weighted, age-specific mortality rates calculated by the pooling of data shown in Fig. 1. Age-specific mortality rates were calculated as before (*6*) for each 2-day period throughout life; weighting is by number of worms dying within a 2-day interval. Lines are plotted on the basis of the least-squares regression estimates for initial mortality rate (A, age-specific mortality at day 3) and the exponential Gompertz component (α), which describes the age-related rate of acceleration of mortality rate. For DH26, both the initial mortality rate ($P < 0.01$) and the exponential Gompertz component ($P < 0.001$) differed significantly from the values for N2 (Table 1). The initial mortality rate for TJ411 was significantly lower ($P < 0.001$) than that of DH26 but not that of N2, whereas the exponential Gompertz component was significantly lower ($P < 0.001$) in all comparisons. The F_1 initial mortality rates were significantly higher in all comparisons, whereas exponential Gompertz components were lower for F_1 than for N2 ($P < 0.001$). In the statistical tests of difference in initial mortality rates and exponential Gompertz components, the t test for large unequal sample size was used.

Klass (*8*) had used the DH26 strain in the isolation of long-life mutants; this strain contains *fer-15(b26)*, a temperature-sensitive allele of *fer-15* that blocks sperm formation at 25°C (*21*), thus eliminating progeny production in his screens. Because we had not separated the *age-1* genetic locus from *fer-15* (*9, 10, 22*), we also examined the DH26 strain [*fer-15(b26)*] for its effects on mortality rate. In accord with earlier reports (*8–10, 16*), there was little difference in either life expectancy or maximum life-span of DH26 and N2 (Fig. 1; Table 1). However, there was a statistically significant 23% decrease in the age-related acceleration of mortality rate of DH26 as compared with N2, whereas the initial mortality rate of DH26 was significantly higher (Fig. 2 and Table 1, lines 1 and 2).

Consistent with previous observations (*8–10*), hermaphrodites in both TJ411 (Fig. 1C and Table 1, line 3) and TJ401 (Fig. 3A and Table 1, line 6), both of which carry an *age-1* mutant allele, lived much longer than either the wild-type N2 or DH26 strain. These *age-1* mutant strains averaged an increase in life expectancy for hermaphrodites of about 14 days in experiment 1 and more than 30 days in experiment 2, increases of 80 and 120%, respectively, over the control life expectancy. Maximum life-span also increased by 26 and 44 days, respectively (Figs. 1C and 3A).

The age-related acceleration of mortality rate was 20 to 60% slower in *age-1* mutant hermaphrodites than in the other genotypes examined and was statistically significant in all five of the five possible comparisons in Figs. 2 and 3C. The average doubling time of the age-specific mortality rate in TJ411 or TJ401 was 7.3 days in experiment 1 and 14.3 days in experiment 2 (Table 1, lines 3 and 6) as compared with doubling times averaging 4.3 days (Table 1, lines 1, 2, and

Table 2. Empirically derived age-specific mortality data. Initial mortality rate and the rate of acceleration of mortality here were estimated empirically by first deriving an estimate of each parameter for each of the four independent subpopulations used in each line of Table 1. These values were then used to derive the empirical values and errors shown in this table. Using these values, I observed statistically significant differences ($P < 0.05$) between the Gompertz exponential components of TJ411 and all other strains in experiment 1 and for TJ401 hermaphrodites in experiment 2 as compared with F_1. The initial mortality rate for TJ401 hermaphrodites in experiment 2 differed significantly from the rates for both of the other strains; males and hermaphrodites of both DH26 and TJ401 differed significantly in the estimates of the Gompertz exponential component. All other differences were not significant.

Strain	Sex	Empirical mortality parameters [$\log_{10}$ (probability of death/day)]	
		$A \pm$ SE	$\alpha \pm$ SE
		Experiment 1	
N2	Herm.	−2.79 ± 0.29	0.091 ± 0.011
DH26	Herm.	−2.44 ± 0.19	0.068 ± 0.007
TJ411	Herm.	−2.47 ± 0.14	0.039 ± 0.004
F_1	Herm.	−2.03 ± 0.08	0.065 ± 0.005
		Experiment 2	
DH26	Herm.	−1.78 ± 0.13	0.031 ± 0.002
TJ401	Herm.	−2.33 ± 0.09	0.021 ± 0.002
F_1	Herm.	−1.74 ± 0.11	0.036 ± 0.004
DH26	Male	−2.00 ± 0.17	0.056 ± 0.006
TJ401	Male	−2.11 ± 0.08	0.041 ± 0.002
F_1	Male	−1.97 ± 0.16	0.057 ± 0.007

4) and 10.8 days (Table 1, lines 5 and 7) for the strains carrying the wild-type allele of *age-1*. I conclude that the longer life-span observed in *age-1* mutants consistently involved a slower rate of acceleration of age-specific mortality with chronological age (*23*). Moreover, this slower rate of acceleration is recessive to the wild-type *age-1* allele (Table 1, lines 3, 4, 6, and 7); even in the empirically derived parameter estimates, values for TJ401 and TJ411 were significantly lower than for the other strains in four of five possible comparisons (Table 2).

Males of all three strains were shorter lived than hermaphrodites of the same genotype (Table 1, compare lines 5 and 8, 6 and 9, 7 and 10, values in the legend to Fig. 3). The acceleration of age-specific mortality for males was consistently about twofold faster than for hermaphrodites. In general, initial mortality rates of males were not significantly different from those of hermaphrodites. Although male-hermaphrodite comparisons reached statistical significance in a few cases (Fig. 3), statistical significance was not seen when empirically derived estimates of the parameters were used (Table 2). Survival parameters and life expectancy of DH26 and F_1 males were almost identical (Table 1, lines 8 and 10). As in hermaphrodites, TJ401 males have a large increase in life-span relative to DH26 and F_1 (increases in life expectancy of 7 days and increases in maximum life-span of 12 days, or about 35%) and a statistically significant 33% slower rate of acceleration of age-specific mortality, but no change in initial mortality rate (Fig. 3 and Table 1, lines 8 to 10).

In summary, *age-1* mutant hermaphrodites have longer life-spans than the other genotypes examined but still show exponential rates of increase of age-specific mortality with chronological age. The increase in life-span is primarily the result of a slower acceleration of age-specific mortality rate with increasing chronological age, although some effects on initial mortality rates are also possible. The slower accelerations of mortality are completely recessive to wild type, as previously reported for life-span (*8–10*). The mutational effects are also observed in the male.

These findings are consistent with a model in which the *age-1* mutation results in a reduction (hypomorph) or in the elimination (nullomorph) of the protein product specified by this gene. The normal allele of *age-1* would then specify a faster acceleration of age-specific mortality rate by some as yet unknown mechanism. These results, together with previously published studies of recombinant-inbred lines (*6, 7*), show clearly that species-specific life-span is genetically specified.

These results are likely to be relevant to two discussions currently under way within the gerontological research community. First, it was recently suggested that selective breeding for genetically longer lived lines should be initiated in mammals (*24*), following protocols that were successfully applied in *Drosophila* (*13, 14*). The results presented here show that single-gene, life-prolongation mutations in *C. elegans* have effects on mortality schedules that are analogous to those seen in the polygenic situation (*6*); single-gene mutant analysis may thus provide one alternative to such long-term selection studies (*24*). Second, Fries (*25*) has suggested that a "rectangularization of the survival curve" in human populations is currently being observed. Such a rectangularization leads to a "compression of morbidity" in the last years of life. If true, this observation has enormous fiscal and biomedical implications. However, because human populations show an exponential acceleration of mortality rate throughout life, as has been amply documented (*1–4, 26*), such a rectangularization of the survival curve may be inconsistent with the fundamental physiological constraints that are ultimately responsible for the shape of the survival curve. Instead, as has been suggested by Schneider and Brody (*27*) and others (*26*), both the size and relative morbidity of the elderly population may be unchanged or increasing. Further demographic analyses that take into account the nature of the age-related mortality function are in order.

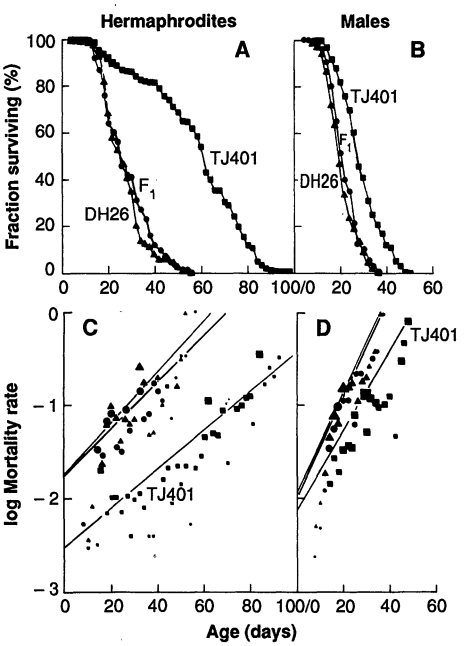

Fig. 3. (**A** and **B**) Survival data obtained as described in Fig. 1. (**C** and **D**) Plots of weighted age-specific mortality rate versus chronological age as in Fig. 1. Strains include DH26, TJ401, and their F_1 heterozygotes, both males and hermaphrodites. (A and C) Survival curves and age-specific mortality rates for hermaphrodites of the DH26 parent stock (▲), TJ401 (■), and F_1 hybrids from the TJ401 hermaphrodite X DH26 male cross (●). (B and D) Survival curves and age-specific mortality rates for males of the strains described in (A) and (C). The size of a point in (C) and (D) is proportional to the number of individuals dying within a given period, which was used as a weighting factor in these linear regressions (see Table 1 for quantitative values). Initial mortality rates were significantly lower ($P < 0.001$) in both comparisons of TJ401 hermaphrodites but not males. The exponential Gompertz component of TJ401 was lower in all comparisons for both males and hermaphrodites ($P < 0.001$, except for TJ401 with DH26 hermaphrodites, $P < 0.05$). Both males and hermaphrodites of F_1 and DH26 did not differ significantly from each other in either initial mortality rate or the exponential Gompertz component.

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Suppression of Human Colorectal Carcinoma Cell Growth by Wild-Type p53

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Mutations of the p53 gene occur commonly in colorectal carcinomas and the wild-type p53 allele is often concomitantly deleted. These findings suggest that the wild-type gene may act as a suppressor of colorectal carcinoma cell growth. To test this hypothesis, wild-type or mutant human p53 genes were transfected into human colorectal carcinoma cell lines. Cells transfected with the wild-type gene formed colonies five- to tenfold less efficiently than those transfected with a mutant p53 gene. In those colonies that did form after wild-type gene transfection, the p53 sequences were found to be deleted or rearranged, or both, and no exogenous p53 messenger RNA expression was observed. In contrast, transfection with the wild-type gene had no apparent effect on the growth of epithelial cells derived from a benign colorectal tumor that had only wild-type p53 alleles. Immunocytochemical techniques demonstrated that carcinoma cells expressing the wild-type gene did not progress through the cell cycle, as evidenced by their failure to incorporate thymidine into DNA. These studies show that the wild-type gene can specifically suppress the growth of human colorectal carcinoma cells in vitro and that an in vivo–derived mutation resulting in a single conservative amino acid substitution in the p53 gene product abrogates this suppressive ability.

ONE COPY OF THE SHORT ARM OF chromosome 17, which harbors the p53 gene, is lost in many human tumors, including those of the colon and rectum (*1–3*). In the majority of human colon carcinomas with allelic deletions of chromosome 17p, the remaining p53 allele contains a missense mutation (*3, 4*). In addition to colorectal carcinomas, p53 gene mutations have also been found in conjunction with chromosome 17p allelic deletions in tumors of the brain, breast, lung, and bone (*4–6*). These studies are consistent with the hypothesis that the normal (wild-type) p53 gene product may function as a suppressor of neoplastic growth, and that mutation or deletion, or both, of the wild-type gene inactivates this suppression. This hypothesis has been supported by studies in rodent cells. For example, p53 alleles are often rearranged or mutated as a result of viral integration events in Friend virus–induced mouse erythroleukemias (*7*). Additionally, in transfection studies, the wild-type murine p53 gene has been shown to inhibit the transforming ability of mutant p53 genes in rat embryo fibroblasts (*8*). Other studies, however, have suggested that expression of the wild-type p53 gene product is necessary (not inhibitory) for cell growth (*9, 10*). Thus, the effect of wild-type and mutant p53 genes on cell growth may depend on the cell type examined. We now show that expression of the wild-type p53 gene in human colorectal carcinoma cells dramatically inhibits their growth. Moreover, a p53 gene mutant cloned from a human colorectal carcinoma was biologically inactive in this respect, as it was incapable of inhibiting such growth.

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Table 1. Colony formation after transfection with wild-type and mutant p53 expression vectors. For each experiment, one or two 75-cm^2 flasks were transfected (*13*), and the total colonies counted after 3 to 4 weeks of selection in geneticin (0.8 mg/ml). Exp., experiment.

Cell line	Exp.	No. of geneticin-resistant colonies formed	
		pC53-SCX3 (mutant)	pC53-SN3 (wild-type)
SW837	1	754	66
	2	817	62
SW480	1	449	79
	2	364	26
RKO	1	1858	190
	2	1825	166
VACO 235	1	18	16
	2	26	28

The colorectal carcinoma lines SW480 and SW837, which are representative of 75% of colon carcinomas, have each lost one copy of chromosome 17p (including the p53 gene), and the remaining p53 allele is mutated (*3, 4*). The SW837 line contains an arginine to tryptophan mutation at codon 248 (*4*). The SW480 line contains two point mutations, arginine to histidine at codon 273 and proline to serine at codon 309 (*4*). The substitutions at codon 248 and 273 are typical of those observed in human tumors, occurring within two of the four mutation "hot spots" (*4*). For the transfection studies, we constructed a vector, pCMV-Neo-Bam, engineered to contain two independent transcription units (*11*). The first unit comprised a cytomegalovirus (CMV) promoter/enhancer upstream of a site for insertion of the cDNA sequences to be expressed, and splice and polyadenylation sites to ensure appropriate processing. The second transcription unit included a herpes simplex virus (HSV) thymidine kinase promoter/enhancer upstream of the neomycin resistance gene, allowing for selection of transfected cells in geneticin (*11*). A wild-type p53 cDNA was inserted into pCMV-Neo-Bam to produce pC53-SN3. Similarly, a vector, pC53-SCX3, expressing a mutant cDNA from human colorectal tumor CX3, was also constructed. The only difference between pC53-SN3 and pC53-SCX3 was a single nucleotide (C to T) resulting in a substitution of alanine for valine at p53 codon 143 in pC53-SCX3 (*12*).

The constructs were transfected into SW837 and SW480 cells (*13*), and geneticin-resistant colonies were counted 3 weeks later. Cells transfected with pC53-SN3 formed five- to tenfold fewer colonies than those transfected with pC53-SCX3 in both recipient cell types (Table 1). In both