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inates contamination by microbes, which is an important criterion for maintaining food industry standards. Fermentative growth of naturally heterotrophic microalgae has resulted in dry biomass accumulation to 100 g/liter (3, 27), which is 10 to 50 times the yields obtained by using light-dependent culture systems. Fermentation-based systems can reduce production costs of microalgae by an order of magnitude relative to those incurred by photosynthesis-based production; cost reduction analyses factor in expenses for both fixed-carbon supplementation and equipment operation (28). Commercial benefits of fermentation-based systems result from increased biomass, productivity, harvesting efficiency, and reduced losses from contamination. The ability to grow microalgae heterotrophically increases the feasibility for developing a large range of new algal products.

Marine ecosystems also depend on diatoms, which contribute substantially to the reduction of inorganic carbon in marine habitats. Such a contribution may increase substantially as the ecology of oceanic environments is altered (29–32). The exploitation of diatoms that can be genetically manipulated and that can grow heterotrophically will facilitate the use of mutants to augment our understanding of both photosynthesis and other metabolic pathways that are essential for competing in marine ecosystems.

References and Notes
15. After 4 weeks in the dark, the transformants that have become too short (the Hayflick limit). This growth arrest is mediated by a DNA damage response to telomeres (Telomere Position Effect, TPE) can result in the reversible silencing of a gene near a telomere by a mechanism that depends on TPE on telomere length and on the distance to the gene (4–6). Because telomeres in most human cells shorten with age, TPE would provide a mechanism to incrementally alter phenotype with increasing cellular age (7). However, previous efforts to identify TPE in mammalian cells have not been successful (8–10). We demonstrate here the presence of TPE in human cells and that the strength of the silencing effect is dependent on telomere length.

Most normal human cells lack the enzyme telomerase, which maintains telomeres, and as a consequence, telomeres shorten with each division until the cells reach replicative senescence (the Hayflick limit). This growth arrest is mediated by p53 and has been suggested to be the result of a DNA damage response to telomeres that have become too short (1–3). No mechanism has been demonstrated in vertebrates that can account for differences between young and old (but not yet senescent) cells. In Saccharomyces cerevisiae, telomere position effect (TPE) can result in the reversible silencing of a gene near a telomere by a mechanism that depends on TPE on telomere length and on the distance to the gene (4–6). Because telomeres in most human cells shorten with age, TPE would provide a mechanism to incrementally alter phenotype with increasing cellular age (7). However, previous efforts to identify TPE in mammalian cells have not been successful (8–10). We demonstrate here the presence of TPE in human cells and that the strength of the silencing effect is dependent on telomere length.

Telomere Position Effect in Human Cells

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In yeast, telomere position effect (TPE) results in the reversible silencing of genes near telomeres. Here we demonstrate the presence of TPE in human cells. HeLa clones containing a luciferase reporter adjacent to a newly formed telomere express 10 times less luciferase than do control clones generated by random integration. Luciferase expression is restored by trichostatin A, a histone deacetylase inhibitor. Overexpression of a human telomerase reverse transcriptase complementary DNA results in telomere elongation and an additional 2- to 10-fold decrease in expression in telomeric clones but not control clones. The dependence of TPE on telomere length provides a mechanism for the modification of gene expression throughout the replicative life-span of human cells.

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wise identical linearized construct that lacked telomere repeats. As expected with plasmid transfections, there was a high degree of variation within each group. The clones with a telomeric reporter nonetheless expressed luciferase at a 10 times lower average level than did the clones with an internal integration site (Fig. 2A).

We next sought to demonstrate that the lower expression levels in telomeric clones were the result of heterochromatin formation rather than of damage to the transgene or the presence of a mixed population of clones. Heterochromatin in mammalian cells is normally dependent on histone deacetylation. We therefore investigated whether treatment with trichostatin A (TSA), a highly specific inhibitor of histone deacetylases (13), could eliminate the telomeric silencing effect we had observed. Telomeric and internal clones were treated with TSA. After treatment, both sets of clones expressed the reporter at an enhanced level, representing a 2.6 ± 0.4-fold increase for the internal clones and a 51 ± 37-fold increase for the telomeric clones (Fig. 2B). The initial difference in the level of luciferase expression is thus histone deacetylase–dependent. Enhancement of transgene expression by histone deacetylase inhibitors has been noted previously (14). Luciferase expression returned to pre-experiment levels within 72 hours after withdrawal of the TSA (15). Although the TSA dose used in these experiments is somewhat cytotoxic, the toxicity did not play a role in increasing luciferase expression, because nonspecific treatment with toxic doses of hygromycin led to a moderate decrease in luciferase activity (15).

We next extended telomerases in order to establish the length dependence of the observed silencing effect. Increasing the telomerase activity of HeLa cells by infection with a human telomerase reverse transcriptase (hTERT)–encoding retrovirus causes them to elongate their telomeres (Fig. 3A), as has been observed in several other cell lines (16). We observed an additional 2- to 10-fold decrease in luciferase activity after telomeric clones were infected with a telomerase-containing retrovirus, as compared to control, vector-only infections (Fig. 3B). This change was not observed in clones with an internal luciferase reporter. These results demonstrate that this effect shares some similarities with yeast TPE and provides a mechanism by which the expression of subtelomeric human genes could increase with replicative age.

The strongest evidence against the existence of mammalian TPE comes from a comparison of mRNA levels for a telomeric neo gene in subclones of SV40-transformed human fibroblasts with varying telomere lengths (8). This cell line uses the ALT (alternative lengthening of telomeres) pathway to maintain its telomeres, a phenotype that involves altered telomere biology and a substantial increase in total telomere biology and a substantial increase in total gene expression by histone deacetylase inhibitor TSA. Three telomeric and three internal clones were treated with TSA (200 ng/ml) (Sigma, St. Louis, Missouri) for 24 hours. The medium was replaced, and the cells were incubated for an additional 24 hours before collection for luciferase assays. Note the switch to a logarithmic scale. rlu, relative light units.
telomeric DNA (17). It is possible that the extra telomeric sequences in ALT cells are titrating out factors essential for TPE, as has been observed in yeast (18), so that ALT cells might not exhibit TPE. Another report may have failed to identify TPE, because the healed telomere appears to have been extremely short and/or because it was located >50 kb from the nearest gene that could be examined (9). In at least one case, data consistent with a very mild physiology of aging or cancer. It will be important to identify endogenous genes whose expression is influenced by telomere length in order to predict expression that might affect both cell and division, leading to altered patterns of gene expression that might affect both cell and organ function. It will be important to identify endogenous genes whose expression is influenced by telomere length in order to determine whether TPE actually influences the physiology of aging or cancer.

A number of proteins have been reported to change in expression level as a function of the replicative age of the cell (21, 22). The existence of TPE in mammalian cells raises the possibility that some presenescent changes could be “programmed” by the progressive shortening of telomeres with ongoing cell division, leading to altered patterns of gene expression that might affect both cell and organ function. It will be important to identify endogenous genes whose expression is influenced by telomere length in order to determine whether TPE actually influences the physiology of aging or cancer.

Epigenetic silenced alleles of the Arabidopsis SUPERMAN locus (the clark kent alleles) are associated with dense hypermethylation at noncanonical cytosines (CpXPg and asymmetric sites, where X = A, T, C, or G). A genetic screen for suppressors of a hypermethylated clark kent mutant identified nine loss-of-function alleles of CHROMOMETHYLASE3 (CMT3), a novel cytosine methyltransferase homolog. These cmt3 mutants display a wild-type morphology but express 2 to 10 times lower levels of luciferase activity as compared to control, vector-only infections. Internal clones having comparable initial values retain full expression of the luciferase reporter after infection with hTERT.

Cytosine methylation plays a major role in determining the epigenetic expression state of eukaryotic genes. This methylation is most often found at the symmetrical dinucleotide CG (or CpG sites). CpG methylation is maintained by the well-studied DNMT1 subfamily of methyltransferases, which includes Arabidopsis MET1 (1–3). Methylation at sites other than CpG is also found in many organisms (4), but the mechanism by which this methylation is maintained is poorly understood. Arabidopsis can tolerate major disruptions in DNA methylation (2, 3, 5), making it useful for genetic analysis of methylation pattern-