DNA damage, particularly oxidative lesions derived from normal metabolism, is thought to contribute to aging, but the mechanisms involved remain obscure (1–4). To counteract the effects of DNA damage, an intricate network of DNA repair pathways has evolved (5, 6). One important pathway is nucleotide excision repair (NER), which removes helix-distorting damage including major ultraviolet (UV)-induced lesions, bulky chemical adducts, and some forms of oxidative damage (7). Xeroderma pigmentosum (XP) patients show the consequences of inherited defects in NER: sun (UV) hypersensitivity, cancer predisposition, accelerated aging of the skin, and, frequently, neurodegeneration (8).

Of the seven XP genes (XPA–G), XPB and XPD are exceptional because different mutations in these genes also cause Cockayne syndrome (CS) and a photosensitive form of the brittle hair disorder trichothiodystrophy (TTD) (8–11). TTD and CS are characterized by postnatal growth failure, progressive neurological dysfunction, impaired sexual development, skeletal abnormalities, and a strongly reduced life expectancy, but not cancer predisposition (8, 12). A clue to the intriguing clinical heterogeneity linked with XPB and XPD mutations came with the discovery that these genes encode DNA helicase subunits of the transcription factor IIH (TFIIH) complex (13, 14), which have dual functions: local opening of the DNA around a lesion during NER (15) and opening of the promoter DNA during transcription initiation (16). Thus, XPB and XPD mutations may not only compromise NER, causing photosensitivity, but may also affect transcription (17). To obtain insight into the complex pathophysiology of TTD, we generated mice carrying an XPD point mutation [Arg122→Trp (R122W)] found in TTD patients. TTD mice displayed many features of the human disease and partial defects in transcription and repair (18, 19). Here, we report that TTD mice develop premature aging features caused by DNA damage.

Premature aging phenotype. Through regular observation of a large group of TTD and wild-type (wt) littermates (20), we noticed that TTD mice acquired an “aged” appearance beginning at ∼3 months of age (Fig. 1). This, together with a shortened life-span (average <12 months, compared with >2 years for wt littermates, P < 0.0001), early cessation of development (see 18), and cachectic dwarfism, prompted us to conduct a more systematic analysis of parameters indicative of premature aging.

TTD mice have brittle hair, the hallmark of TTD (18), that is normally pigmented (Fig. 2A) and appears depigmented by 12 months of age in TTD but not wt mice (Fig. 2B). Follicular dilation and sebaceous gland hyperplasia (asterisk) in TTD mice (Fig. 2C and D) are not seen in wt littermates. In addition, TTD mice show a range of systemic symptoms of premature aging, detailed below.

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**Fig. 1.** TTD mice develop normally, then show a premature aging phenotype. Shown are wt (A and C) and TTD (B and D) mice at age ∼3 months (A) and (B) and 15 to 16 months (C) and (D). Progeroid symptoms (cachexia and kyphosis) start to develop in TTD mice at age 3 to 4 months onward and become increasingly severe.

**Fig. 2.** Cutaneous symptoms of aging in TTD mice. (A) Typical example of early depigmentation in the fur of a 13-month-old black TTD mouse. No depigmentation was observed in age-matched wt mice (see table 1 in (21)]. (B) Follicular dilation and sebaceous gland hyperplasia (asterisk) in TTD compared to wt skin. Note the hyperkeratotic TTD epidermis (indicated by an arrow). The bar is 100 μm.
However, they showed patchy depigmentation (Fig. 2A) earlier and more frequently than did wt littersmates [table 1 in (21)]. Melanocytes were absent from grey skin patches, with foci of melanin granules in macrophages as found in normal greying (22). Young TTD mice also developed greasy hair and showed (benign) hyperplasia of the sebaceous gland (Fig. 2B), as observed in human aging (23).

The sexual behavior of most young female TTD mice appeared unimpaired (indicative of a normal hormonal status) and occasionally led to full-term pregnancy. TTD females were also fertile until at least 7 months of age [table 1 in (21)]; thus, initial sexual development per se is unimpaired. However, TTD females appeared to lose fertility over time, and to lose it early, because they never produced more than one litter and never after 6 months of age. TTD females (n = 8, age ~16 months) displayed ovarian dysfunction ranging from complete anovulation to sporadic, seemingly normal, ovulation (Fig. 3).

There was no correlation between the severity of cachexia and the degree of anovulation, which suggests that the fertility defects were not due to nutritional problems. Rather, they resembled the fertility defects seen in aging rodents (24) and menopausal women.

Although 2- to 4-month-old TTD mice showed no detectable skeletal abnormalities, radiographs of 14-month-old TTD mice revealed prominent kyphosis (curvature of the spinal column) (Fig. 4, A to D) and a generalized reduction in radiodensity of the skeleton, except for the skull. The mineral density of TTD vertebrae was 56% that of wt mice (P < 0.01), and the density of the TTD skull was 119% that of wt (P < 0.05, Fig. 4E). Osteosclerosis of the cranium and characteristic birdlike facies have been reported in TTD patients (25). The osteoporosis and concomitant kyphosis exhibited by TTD mice are hallmarks of aging in humans.

The most life-threatening symptom of TTD patients is failure to thrive, which leads to cachexia and, in turn, to a susceptibility to infections, which is a frequent cause of death (12). Cachexia in TTD mice was progressive, heterogeneous in onset and severity, and followed by premature death. At 6 months of age, TTD mice showed mild normochromic anemia [table 2 in (21)] and significantly decreased serum levels of the branched-chain amino acids (valine, leucine, and isoleucine) [table 3 in (21)], which is indicative of starvation (26). Anatomical, histological, and biochemical analysis indicated that starvation was not due to aberrant food uptake or malabsorption (22). The TTD mice did not show histological abnormalities in other vital organs such as the liver, kidney, or heart, except for an enlarged spleen, which might be related to the mild anemia.

A complete NER defect dramatically enhances the severity of the TTD phenotype. In view of the dual function of XPD, the accelerated aging features could be a result of impaired transcription, impaired NER, or a combination of the two. A DNA repair defect alone seemed unlikely because TTD patients and mice have considerable residual NER activity (19), whereas XP-A patients and mice, who have a complete NER defect, do not display premature aging (8, 27, 28).

To examine whether TTD aging was due to a defect in transcription, independent of NER status, we crossed TTD mice with mice carrying an XPA null allele (27). Combined homozygosity for XPA and TTD was found to be compatible with normal embryogenesis but was associated with increased neonatal lethality [table 4 in (21)], which suggests that the combined mutations reduced the tolerance of mice to stress. The surviving double-mutant mice exhibited a retarded but steady growth in the first 1.5 weeks, but failed to gain further weight after 2 to 3 weeks, and developed dramatically runted growth and extreme cachexia resulting in a severely shortened life-span of only 22 days (n = 10).
TTD double mutants escaped juvenile death and lived to 4 and 12 months of age. Pathological analysis did not reveal defects in any organ, except for complete absence of body fat, including subcutaneous fat (Fig. 5C). The cachexia in the XPA/TTD mice resembled the progressive pathology of the TTD mutants in manifestation and as a cause of death, but it developed at a vastly accelerated rate. Surprisingly, other typical TTD characteristics were also much more pronounced in XPA/TTD mutants: excessive epidermal hyperkeratosis and severe dilation of hair follicles (Fig. 5C). This suggests that the complete absence of NER enhances transcriptional insufficiency thought to be responsible for the cutaneous abnormalities (18).

**TTD/XPA double-mutant cells are hypersensitive to oxidative stress.** These findings suggest that unrepaired DNA lesions of endogenous origin aggravate the TTD symptoms in double-mutant mice. Although oxidative DNA damage is primarily (but not exclusively) repaired by the base-excision repair pathway rather than by NER (6), we focused on this type of endogenous damage, because it has already been implicated in aging. As expected, experiments measuring survival, DNA repair synthesis, and the recovery of RNA synthesis after UV exposure all showed that the partial repair deficiency of TTD was converted to the total NER defect of XPA (Fig. 6, A to C). Sensitivity to oxidative injury was determined by exposure of cells to a continuous low dose of paraquat for 3 days (21, 29, 30). Although XPA and TTD single-mutant cells showed a survival curve similar to that of wt cells, XPA/TTD double mutants were clearly more sensitive (Fig. 6D), showing a survival curve similar to that of Cockayne Syndrome group B (CSB)–deficient fibroblasts, which are completely deficient in repair of transcription-blocking lesions (7). The TTD/XPA and CSB cells were also hypersensitive to a fractionated dose of x-irradiation (22). The synergistic effect of the XPA/TTD double mutant both at the organismal level and in terms of sensitivity to oxidative damage provides evidence for a causal link between DNA damage and the dramatically enhanced aging features.

**Discussion.** The fact that TTD mice develop normally until adulthood indicates that the phenotype we describe here is not the result of aberrant development but rather reflects bona fide aging. Because TTD is associated with several features of normal aging, it can be considered a segmental progeroid disorder (31). In comparison to other progeroid disorders, such as Werner, Cockayne, and Bloom syndromes (32, 33), TTD is associated with much faster aging. Patients with the XPD R722W mutation mimicked in the mouse did not live longer than 5 years (34). This interpretation is reinforced by the recent description of two TTD sisters who were less than 5 years old but were described as looking prematurely aged (35). Moreover, many TTD symptoms overlap with those seen in CS, a well-characterized progeroid condition (8, 17).

Several observations suggest that DNA is...
Telomere shortening has been implicated in the aging phenotype of highly proliferative tissues (36–39). Cells from patients with Werner, Cockayne, and Bloom syndromes display genome instability that is caused by defects in DNA helicases (40–42) similar to the XPB and XPD helicases affected in TTD. Our work on TTD mice, and particularly on XPA/TTD double-mutant mice, highlights the role of DNA damage, repair, and transcription in the onset of premature aging. Interestingly, CSB and Cockayne Syndrome Group A mice, which have a defect in the repair of transcription-blocking lesions (transcription-coupled repair) but normal global genome NER (43), exhibit the same dramatic TTD/XPA double-mutant phenotype when crossed with XPA mutants (22). However, when the NER defect is incomplete—as in the cases of TTD/CSB and TTD/XPC double mutants (which have some residual repair)—the enhancement of the TTD features is less pronounced (22, 36). Finally, mice defective in the duo- 

molecular basis of aging.

section-coupled repair II complex may persist longer in TTD, in turn preventing repair (46, 51, 52). Conceivably, this would cause gene inactivation and trigger apoptosis (53–55), leading to functional decline and depletion of cell renewal capacity. Both cell death and impaired cell functioning may underlie the aging phenotype in TTD. In support of this model is recent work showing that mice expressing a hyperactive p53 mutant also exhibit accelerated aging (56) that is likely to be due to increased apoptosis. Obviously, any events causing gene inactivation or cell death such as telomere attrition, chromosomal instability, and increased levels of oxidative damage might accelerate aging.

In conclusion, our data strongly support the DNA damage theory of aging and suggest a significant role of transcription decay and subsequent cell death in its pathophysiology. The TTD mice may also prove to be a useful experimental model for further dissecting the molecular basis of aging.

References and Notes
20. The generation of TTD mice by gene targeting of the ERCC1/XPF complex (22). Finally, mice defective in the dou-

ble-strand break-repair protein Ku86 exhibit features of early aging (50). Together, these observations indicate that DNA damage–induced genome dysfunction underlies the aging process.

What is the molecular mechanism underlying the premature aging in TTD mice? We propose that DNA damage persists longer and triggers TTD mice because the XPD mutation impairs not only global genome NER but also transcription-coupled repair of any lesions that stall elongating RNA polymerase II. Because XPD is also thought to function in removal of the blocked polymerase (5–7, 46), the stalled RNA polymerase II complex may persist longer in TTD, in turn preventing repair (46, 51, 52). Conceivably, this would cause gene inactivation and trigger apoptosis (53–55), leading to functional decline and depletion of cell renewal capacity. Both cell death and impaired cell functioning may underlie the aging phenotype in TTD. In support of this model is recent work showing that mice expressing a hyperactive p53 mutant also exhibit accelerated aging (56) that is likely to be due to increased apoptosis. Obviously, any events causing gene inactivation or cell death such as telomere attrition, chromosomal instability, and increased levels of oxidative damage might accelerate aging.

In conclusion, our data strongly support the DNA damage theory of aging and suggest a significant role of transcription decay and subsequent cell death in its pathophysiology. The TTD mice may also prove to be a useful experimental model for further dissecting the molecular basis of aging.