genic strains previously identified in the patient (*26*). The ability of type 1–piliated uropathogens to invade the urothelium suggests that recurrent UTIs may, in some cases, be a manifestation of a lingering chronic infection and not necessarily a reinoculation of the urinary tract.

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8. NU14 and recombinant bacteria were grown in static Luria-Bertani broth at 37°C for 48 hours to induce expression of type 1 pili (*2*). Expression was verified by mannose-sensitive agglutination of a 3% solution of guinea pig erythrocytes, by EM, and by immunogold labeling. Ten- to fifteen-week-old female C57BL/6 mice were anesthetized with methoxyfluorane and inoculated transurethrally with 50 μl of bacterial suspension (~1×10^8 colony-forming units) in phosphate-buffered saline (PBS) (*2*). Mice were killed, and their bladders were aseptically removed at the indicated time points.
9. NU14 type 1 pili were purified and characterized biochemically, genetically [S. J. Hultgren, J. L. Duncan, A. J. Schaeffer, S. K. Amundsen, *Mol. Microbiol.* **4**, 1311 (1990)], by immunogold EM, and by high-resolution EM.
10. Bladders were bisected, splayed, and pinned down lumenal sides up under NHC buffer [100 mM NaCl, 30 mM Hepes (pH 7.4), and 2 mM $CaCl_2$]. After rinsing gently, bladders were fixed in 2% glutaraldehyde in NHC for 1 to 2 hours at room temperature. For SEM, bladders were postfixed with 1% OsO_4/NHC, rinsed, dehydrated in ascending concentrations of ethyl alcohol, critical point dried from liquid CO_2, coated with ~150 Å of gold, and examined with a Hitachi S-4500 FEG Scanning Electron Microscope. Two or more bladders were examined for each type of sample.
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12. For high-resolution EM, fixed bladder samples were freeze-fractured, deep-etched, and rotary-replicated [J. E. Heuser, *J. Muscle Res. Cell Motil.* **8**, 303 (1987)]. Similar images of bacterial attachment through shortened pili were obtained whether bladders were infected in vivo for 2 hours or ex vivo (after removal from the mice) for 30 min.
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24. Mice were inoculated transurethrally with 50 μl of a 400 μM solution of BAF (Enzyme Systems Products) [M. Deshmukh *et al.*, *J. Cell Biol.* **135**, 1341 (1996)] in PBS 1 hour before inoculation with NU14 containing an additional dose of the drug. Control mice were inoculated with dimethyl sulfoxide in place of BAF. Bladders were recovered at 6 or 24 hours after infection for SEM (*10*). NU14 titers from bladders were determined at 12 hours after infection (*2*). BAF had no effect on the viability or growth rate of NU14 in control in vitro experiments.
25. Bladders were recovered and bisected at 2, 6, 12, 24, and 48 hours after infection (*8*). Each bladder half was splayed under warm Ringer solution [155 mM NaCl, 3 mM HCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 3 mM NaH_2PO_4, 10 mM glucose, and 5 mM Hepes (pH 7.4)] and gently rinsed. One-half of each bladder was incubated with, and one half without, Ringer solution supplemented with gentamicin (100 μg/ml) for 90 min at room temperature. This incubation with gentamicin was sufficient to kill any external bacteria. Bladder halves were washed with PBS, weighed, and homogenized in 1 ml of 0.025% Triton X-100/PBS, and surviving bacteria were plated (*2*).
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Requirement for p53 and p21 to Sustain G_2 Arrest After DNA Damage

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After DNA damage, many cells appear to enter a sustained arrest in the G_2 phase of the cell cycle. It is shown here that this arrest could be sustained only when p53 was present in the cell and capable of transcriptionally activating the cyclin-dependent kinase inhibitor p21. After disruption of either the p53 or the p21 gene, γ radiated cells progressed into mitosis and exhibited a G_2 DNA content only because of a failure of cytokinesis. Thus, p53 and p21 appear to be essential for maintaining the G_2 checkpoint in human cells.

DNA damaging agents in the form of γ radiation and chemotherapeutic drugs are the mainstays of most current cancer treatment regimens. This has stimulated much research to understand the cellular responses to DNA damage. After DNA damage, cells arrest at the transition from G_1 to S phase (G_1-S) or from G_2 to M phase (G_2-M) of the cell cycle, with DNA complements of $2n$ or $4n$, respectively (*1*). Arrest at these checkpoints prevents DNA replication and mitosis in the presence of unrepaired chromosomal alterations. The proportion of cells that arrest at G_1-S or G_2-M depends on cell type, growth conditions, and the checkpoint controls operative in the cell (*2*). The G_1-S arrest results, at least in part, from p53-regulated synthesis of the cell cycle inhibitor $p21^{WAF1/CIP1}$ (*3–5*), which leads to inhibition of the cyclin-cdk complexes required for the transition from G_1 to S phase. However, arrest in G_2 after DNA damage occurs in both murine and human cells in the absence of p21 or p53 (*3–6*). This arrest is thought to result from activation of a protein kinase, Chk1, that phosphorylates and inhibits the function of the protein phosphatase Cdc25C (*7*). Inhibition of Cdc25C prevents the removal of inhibitory phosphates from Cdc2, a protein kinase that complexes with mitotic cyclins and is required for mitotic entry (*8*).

The integrity of the Chk1-Cdc25C-Cdc2 pathway in p21- or p53-mutant cells would appear to explain the prolonged G_2-M arrest that occurs in such cells (*3–7*). However,

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there are some features of this arrest that have remained unexplained. Cells with disrupted p21 or p53 that are arrested in G_2 can undergo DNA synthesis, in some cases resulting in cells with DNA contents of 8*n* or higher (*9, 10*). Such rereplication also occurs in cells blocked in mitosis (*11*), but it is unclear how this could occur in cells arrested before mitosis, as it is thought that negatively acting factors that prevent DNA synthesis must be degraded during the mitotic phase (*12*) and that positively-acting "licensing factors" required for the next S phase cannot traverse the nuclear membrane and therefore can enter the nucleus only during mitosis (*13*).

We therefore investigated G_2-M arrest in p53-deficient cells in more detail. We initially used a panel of six human colorectal cancer cell lines, three with intact p53 genes and three with mutant genes. After irradiation, most of the cells in each culture were arrested with a DNA complement of 4*n* and >95% of the cells were in interphase (*9, 14*). To determine whether any of these cells entered M phase after irradiation, we treated them with nocodazole, a microtubule-disrupting agent that can trap cells in mitosis for several hours. In cells with wild-type p53, the mitotic index was very low after irradiation, as expected for cells truly blocked in G_2 (Fig. 1A). Unexpectedly, a large fraction of the p53-mutant cells entered mitosis after irradiation (Fig. 1B).

These experiments suggested that p53 controls a G_2 checkpoint that prevents entry into mitosis after DNA damage. However, this conclusion was tempered by the fact that the six lines, all derived from colorectal cancers, might differ in ways other than p53 status. We therefore disrupted the p53 gene by homologous recombination in one of the wild-type p53-containing cell lines. The HCT116 cell line was chosen because it has apparently intact DNA damage–dependent and spindle-dependent checkpoints, and it is suitable for targeted homologous recombination (*4, 9, 15*). Two promoterless targeting vectors, each containing a geneticin- or hygromycin-resistance gene in place of genomic p53 sequences, were used to sequentially disrupt the two p53 alleles in HCT116 cells (*16*). Cells with the desired genotypes (Fig. 2A) were used to test the response to DNA damage. After treatment with γ radiation, parental cells ($p53^{+/+}$) and those with one of the two p53 alleles disrupted (*14*) arrested in either G_1 or G_2, as expected for cells with intact checkpoints (Fig. 2B). The vast majority of cells with both p53 alleles disrupted ($p53^{-/-}$) appeared to arrest at G_2-M, with a DNA content of 4*n*, whereas a minor fraction had a DNA content of less than 2*n*, which is indicative of apoptosis (Fig. 2B). A substantial G_2 arrest was observed in cells of all genotypes from 24 to 72 hours after 12-gray (Gy) of γ radiation (Fig. 2B). However, morphologic examination revealed mitotic cells in the $p53^{-/-}$ cell population within 24 hours after irradiation, whereas cells with one or two intact copies of p53 remained mitotically inactive for the duration of the experiment (Fig. 2C). All cell types exhibited mitotic arrest in response to nocodazole treatment in the absence of irradiation (Fig. 2D). A nocodazole-trapping experiment, however, confirmed that only $p53^{-/-}$cells entered mitosis after irradiation (Fig. 2E). Thus, although G_2 arrest was initiated after irradiation in all cells tested, this arrest was not sustained in the absence of functional p53 (Figs. 1 and 2E).

Several potential mechanisms could account for these observations because p53 regulates the expression of many genes, including p21, that can affect the cell cycle (*3–5, 17, 18*). We examined the possible function of p21 in the maintenance of the G_2 arrest by using HCT116 cells in which the p21 genes were disrupted by homologous recombina-

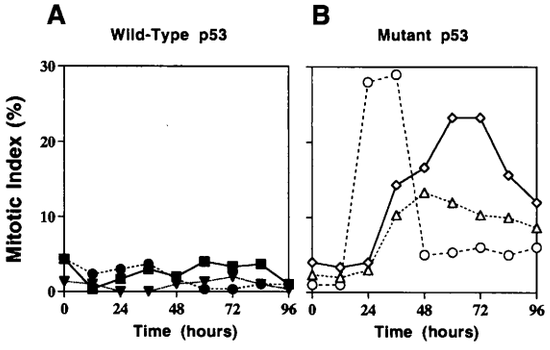
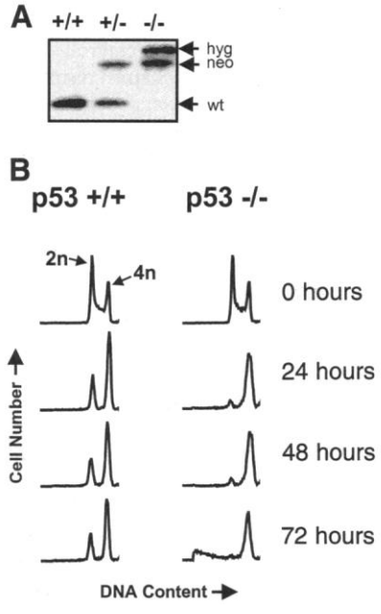


Fig. 1. Entry of colorectal cancer cell lines into mitosis after γ radiation. Cell lines with endogenous wild-type (**A**) or mutant (**B**) p53 genes were treated with nocodazole beginning 30 min after γ radiation (*32*). At the indicated times, cells were fixed, stained, and examined by fluorescence microscopy to determine the fraction of cells in mitosis (mitotic index). Cell lines were HCT116 (closed triangles), RKO (closed squares), SW48 (closed circles), DLD1 (open circles), HT29 (open diamonds), and Caco2 (open triangles).



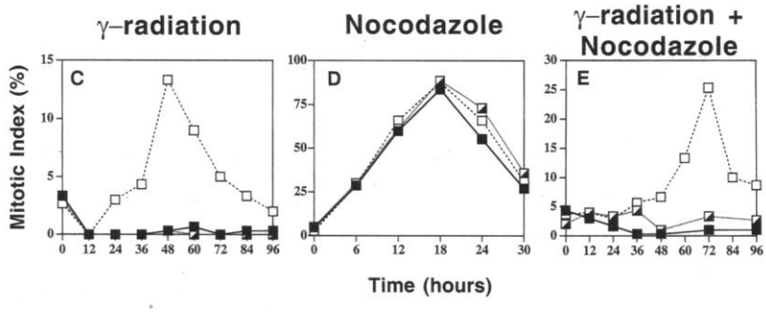


Fig. 2. Targeted deletion of p53 in a colorectal cell line. (**A**) Southern blot after Hind III digestion of genomic DNA of selected clones (*33*). Fragments corresponding to the wild-type allele (wt) (2.5 kb), and neomycin (neo) (3.5 kb) and hygromycin (hyg) (3.7 kb) homologous integrants are shown. (**B**) Flow cytometric analysis of parental HCT116 cells ($p53^{+/+}$) and HCT116 cells with targeted deletions of both ($p53^{-/-}$) p53 alleles at the indicated time points after 12-Gy γ radiation. Flow cytometry was done as described (*4*). Ten thousand cells were analyzed in each experiment; n represents haploid DNA content and results are plotted on a logarithmic horizontal axis. Mitotic indices of parental cells (closed squares) and cells with targeted deletions of one (half-closed squares) or both (open squares) alleles of p53 are shown at the indicated times after 12-Gy γ radiation alone (**C**), nocodazole treatment alone (**D**), or 12-Gy γ radiation followed by nocodazole treatment (**E**).

tion (*4*). The $p21^{-/-}$ HCT116 cells arrested with a DNA content of 4*n* after γ radiation (*4*), but they continued to enter mitosis (Fig. 3, A and C). A control HCT116 line with targeted disruption of both alleles of the Smad4 gene (*19*) behaved identically to the parental HCT116 cells (Fig. 3, A to C). The G_2 checkpoint was activated after exposure of cells to 2-, 6-, and 12-Gy doses of γ radiation in wild-type, p53-, and p21-deficient cells (Fig. 3, D to F). However, the p53- and p21-deficient cells escaped the G_2 arrest (Fig. 3, E and F), whereas the parental cells maintained it after exposure to 6 (Fig. 3D) or 12-Gy doses of γ radiation (Figs. 2E and 3C). The length of the G_2 arrest in the p21- or p53-deficient cells depended on the dose of γ radiation, with the higher doses delaying the appearance of mitotic nuclei proportionately (Fig. 3, D to F). Escape from G_2 arrest occurred earlier in p21- than in p53-deficient cells (Figs. 2E and 3C). This may be because some p53-independent p21 synthesis occurred after irradiation of $p53^{-/-}$cells (Fig. 4A). These results are consistent with the fact that p53 is a major, but not sole, transcriptional regulator of p21 in mammalian cells (*20*).

The most likely biochemical explanation for the entry into mitosis in the absence of p21 was lack of inhibition of the principal mitotic cyclin B1-cdc2 complex by p21 (*21*). The activity of this complex decreased within 12 hours after γ radiation in both cell types (Fig. 4B), which probably reflects activation of a checkpoint mechanism. This inhibition of cyclin B1-cdc2 kinase activity was not sustained in the absence of p21, as substantially increased activity was observed beginning 24 hours after DNA damage in p21-deficient cells (Fig. 4B).

To determine whether p21 and p53 are required to sustain the G_2 arrest in cells other than colorectal cancer or epithelial cells, we disrupted the p53 gene by homologous recombination in normal human fibroblasts (*22*). Nocodazole trapping was then used to monitor the escape from G_2 in parental fibroblasts and in a clone derived from the same fibroblasts in which the p21 genes had been disrupted by gene targeting (*5*). Again, the parental cells entered a sustained G_2 arrest while a substantial fraction of both p21- and p53-deficient fibroblasts escaped G_2 and entered mitosis (Fig. 3G).

Cells without p53 or p21 apparently proceed into mitosis after γ radiation but have a 4*n* DNA content (Fig. 2B) rather than the 2*n* DNA content expected for cells that had gone through mitosis. To investigate this contradictory result further, we stably transfected cells with a histone H2B–green fluorescent protein fusion vector to allow real-time visualization of the mitotic process (*23*). Time lapse experiments in the absence of nocodazole showed that 90 ± 6% of the $p21^{-/-}$ cells entered mitosis within 36 hours after 12-Gy γ radiation compared with less than 2% of the parental cells. The first stages of mitosis after irradiation of $p21^{-/-}$ (or $p53^{-/-}$) cells were indistinguishable from those in cells growing under normal conditions (Fig. 5A). After anaphase, however, the irradiated $p53^{-/-}$ and $p21^{-/-}$ cells never completed cytokinesis (Fig. 5B). These cells eventually flattened and the chromosomes decondensed, and >95% of the cells were found to contain abnormally shaped, multilobulated nuclei (Fig. 5, C, D, and E). A subset of these cells subsequently underwent programmed cell death. Staining with an antibody to the centrosome-specific γ-tubulin revealed that these cells always contained at least three centrosomes or pairs of centrosomes located in a cleft that likely was a remnant of the cleavage furrow associated with the failure of cytokinesis (Fig. 5, D and E). A large number of centrosomes, also observed in mouse cells that lack p53 (*24*), reflected the centrosome duplication that accompanies DNA synthesis (*25*) and was consistent with the fact that $p53^{-/-}$ and $p21^{-/-}$ cells often reenter S phase after irradiation, becoming tetraploid or octaploid (*9, 10*).

These results demonstrate that induced

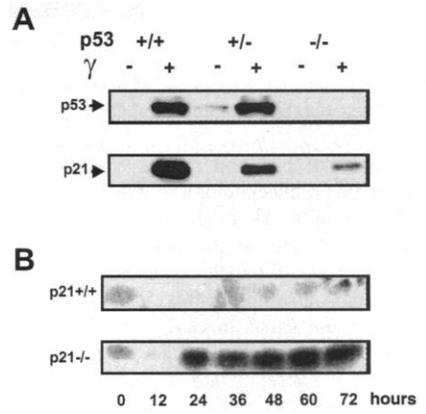


Fig. 4. Protein expression and kinase activity after γ radiation. (**A**) Expression of p53 and p21 in parental HCT116 cells (+/+) and HCT116 cells with targeted deletion of one (+/−) or both (−/−) p53 alleles before and 36 hours after 12-Gy γ radiation assessed by immunoblot analysis (*34*). (**B**) Analysis of in vitro cyclin B1–associated histone H1 kinase activity in parental HCT116 cells (+/+) and in HCT116 cells with targeted deletions of both p21 alleles (−/−) at the indicated times after γ radiation. Whole cell extracts were immunoprecipitated with antibodies to cyclin B1 and assayed for kinase activity as described (*35*).

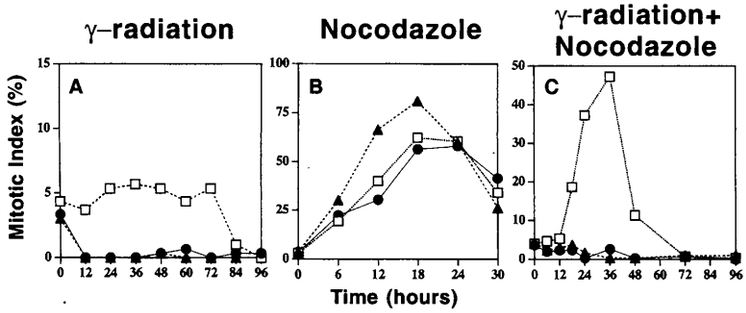
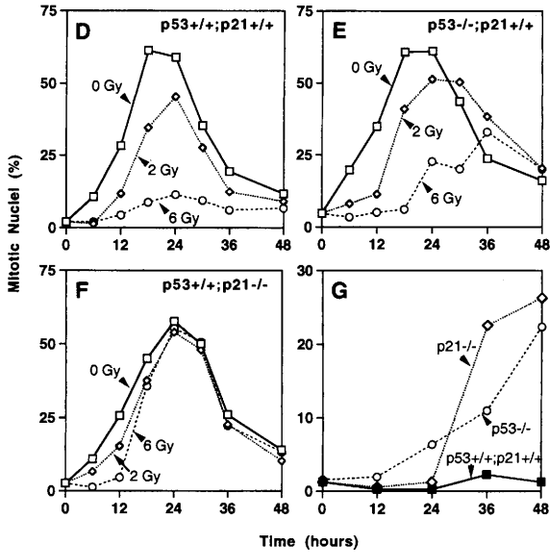


Fig. 3. Mitotic entry after γ radiation. Mitotic indices of parental HCT116 cells (closed circles), cells with targeted deletions of both p21 alleles (open squares), and cells with targeted deletions of both Smad4 alleles (closed triangles) after 12-Gy γ radiation (**A**), after nocodazole treatment alone (**B**), or after treatment with both 12-Gy γ radiation and nocodazole (**C**) (*32*). Response of wild-type HCT116 (**D**), p53-deficient (**E**), and p21-deficient derivatives (**F**) to lower doses of γ radiation in the presence of nocodazole. (**G**) Primary human fetal fibroblasts and their p21- and p53-deficient derivatives were treated with 12-Gy γ radiation plus nocodazole and were analyzed at the indicated times. Note that the scale on the *y* axis in (G) is different than in (D) to (F).



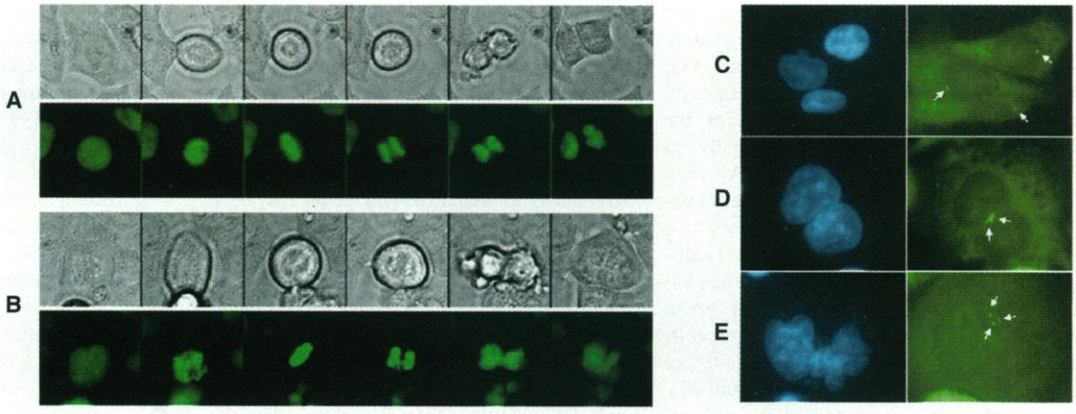


Fig. 5. Fluorescence microscopy of $p21^{-/-}$ cells. Clones stably expressing the histone H2B/GFP fusion protein were isolated and observed by time-lapse microscopy under bright-field (upper) and fluorescence (lower) illumination (*23*). (**A**) A $p21^{-/-}$ cell undergoing mitosis in the absence of radiation. The prometaphase-to-telophase transition time was 66 min. (**B**) An irradiated $p21^{-/-}$ cell. The prometaphase-to-telophase transition time was 114 min. Forty-eight hours after 12-Gy γ radiation, wild-type (**C**), $p21^{-/-}$ (**D**), and $p53^{-/-}$ (**E**) cells were fixed and stained with 4′,6-diamidino-2-phenylindole (left, blue) or immunostained with antibodies specific for the γ-tubulin component of centrosomes (*36*) (right, green). The nucleus in (D) was bilobed, with connections between the lobes visible in a different focal plane from the one shown.

expression of p21 and p53 is essential to sustain the G_2 checkpoint after DNA damage in human cells. Although most research on p53- and p21-regulated checkpoints has focused on the G_1-S transition, several previous observations are consistent with an important role for these genes in G_2-M (*26–29*). The p21 protein is synthesized in G_2 (*27, 28*), promotes a pause in late G_2 under normal growth conditions (*26, 28*), and, when expressed exogenously, causes cells to arrest in G_2 (*29*). In contrast, neither p53 nor p21 appears to play a major role in the spindle checkpoint because $p53^{-/-}$ and $p21^{-/-}$ cells respond normally to microtubule disruption (Figs. 2D and 3B). It is not yet clear whether cells with heavily damaged DNA fail to undergo cytokinesis because of a cytokinesis checkpoint (*18, 30*) or because of a simple mechanical problem.

Although p53 mutations provide cells with a selective growth advantage, such mutations burden them with a significant checkpoint deficit; they cannot respond normally to DNA-damaging agents and enter mitosis and subsequently replicate their genomes in the presence of DNA damage. Such checkpoint defects (*31*) may be exploited to treat the many cancers with abnormalities of p53 function.

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22. Lung-derived human fetal fibroblasts (cell strain LF-1) were electroporated with the neomycin-resistant p53 targeting vector (Fig. 2A) and grown under G418 selection (*5*). Southern blotting (DNA) analysis revealed that several resultant clones had undergone the desired recombination event. In three independent experiments, the efficiency of targeting was 5, 6, and 31%, respectively. Subclones from heterozygous cells were then serially cultured; 30% of them had an increased life span (an additional 30 population doublings before senescence). Southern blotting indicated that all such long-lived clones had sustained a loss of the remaining wild-type p53 gene. The p53-deficient clones used for nocodazole trapping experiments were in the early phases of life span extension.
23. Wild-type, $p21^{-/-}$, and $p53^{-/-}$ cells were transfected with a vector, pBOSH2BGFP-N1, that encodes H2B/GFP (T. Kanda, K. F. Sullivan, G. M. Wahl, *Curr. Biol.* **8**, 377 [1998]). Colonies with stable expression of this fusion protein were identified by fluorescence microscopy and expanded into cell lines. Normal mitoses were observed by time-lapse videomicroscopy and both fluorescent and bright-field images were acquired every 6 min with the MetaMorph software package (Universal Imaging).
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32. Cells were grown as monolayers in T-25 flasks and exposed to a ^{137}Cs source (GammaCell 40) for a total dose of 12 Gy delivered over 14.5 min. Where indicated, cells were exposed to lower doses for proportionally shorter time periods. It was important that the irradiation of cells occurred when they were ~70% confluent, as very sparse or completely confluent cells responded differently than subconfluent cells to γ radiation. Mitotic trapping experiments were done by adding nocodazole to the culture medium (0.2 μg/ml). Cells were collected by incubation with trypsin containing EDTA, centrifuged, and fixed in a solution containing 3.7% formaldehyde, 0.5% Nonidet P-40, and Hoescht 33258 (10 μg/ml) in phosphate-buffered saline. Nuclei were visualized by fluorescence microscopy. Nuclei with

condensed, evenly staining chromosomes were scored as mitotic. At least 300 cells were counted for each determination.
33. Genomic DNA was purified from cell lysates with the QiaAMP spin blood kit (Qiagen) and used as a substrate for polymerase chain reaction and Southern blot assessment of targeting vector integration.
34. Equal numbers of cells were collected, lysed in Laemmli sample buffer, and subjected to electrophoresis and protein immunoblotting. Filters were probed with antibodies to p53 (pAb 1801) and p21 {EA10 [W. S. El-Deiry *et al.*, *Cancer Res.* **55**, 2910 (1995)]}. Signals were visualized with enhanced chemiluminescence (Pierce).
35. Modified from K. Kaufmann *et al.*, *Cell Growth. Diff.* **8**, 1105 (1997). In brief, extracts for in vitro kinase assays were prepared by lysis of washed, centrifuged cells in 50 mM tris-HCl (pH 7.5), 0.5% Nonidet P-40, 10% (v/v) glycerol, 100 mM sodium chloride, 10 mM sodium orthophosphate, 5 mM β-glycerophosphate, 50 mM sodium fluoride, 0.3 mM sodium orthovanadate, 1 mM dithiothreitol, and 1× complete protease inhibitor cocktail (Boehringer Manheim) for 30 min at 4°C. Kinase complexes were immunoprecipitated by adding monoclonal antibody to cyclin B1 (150 ng, Santa Cruz) and protein A–Sepharose (Life Technologies). Immune complexes were washed with lysis buffer and incubated in 25 μl of a solution containing 20 mM tris-HCl (pH 7.5), 7.5 mM magnesium chloride, 1 mM dithiothreitol, 50 μM adenosine triphosphate (ATP), 20 μCi of [γ-^{32}P]ATP (6000 Ci/mmol), and 1 μg of histone H1 protein (Boehringer-Manheim) for 30 min at 30°C. After addition of 25 μl of 2× sample buffer and SDS–polyacrylamide gel electrophoresis, ^{32}P-labeled histone H1 was visualized by autoradiography.
36. Cells were fixed in methanol at −80°C and stained with antibody to γ-tubulin (Sigma) and a fluorescently labeled secondary antibody to mouse immunoglobulin G (Molecular Probes).
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A Rapidly Evolving Homeobox at the Site of a Hybrid Sterility Gene

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The homeodomain is a DNA binding motif that is usually conserved among diverse taxa. Rapidly evolving homeodomains are thus of interest because their divergence may be associated with speciation. The exact site of the Odysseus (*Ods*) locus of hybrid male sterility in *Drosophila* contains such a homeobox gene. In the past half million years, this homeodomain has experienced more amino acid substitutions than it did in the preceding 700 million years; during this period, it has also evolved faster than other parts of the protein or even the introns. Such rapid sequence divergence is driven by positive selection and may contribute to reproductive isolation.

The homeodomain is a stretch of 60 to 62 amino acids that was first discovered to be conserved between many homeotic genes in *Drosophila* (*1*). Proteins containing this DNA binding motif are usually transcription factors and have been found in most metazoans (*2*). Evolutionary conservation of homeoboxes has been well documented; for example, the homeodomains in the *Antp* gene of *Drosophila* and grasshoppers have identical sequences (*3*). Such a high degree of conservation in the protein sequences is often taken as evidence for conservation of the underlying functions. It is thus of great interest to find exceptions in such a highly conservative gene family. Are there homeobox genes that have evolved rapidly and, if there are, what are their functions? What are the selective forces that make them deviate from the norm for this class of genes? It is not inconceivable that their sequence divergence and evolution of the underlying functions may even play a role in differentiation among closely related species. In this report, we describe the cloning of a new homeobox gene that has experienced accelerated evolution in the *Drosophila melanogaster* clade. The acceleration is 100 to 1000 times greater than the rate experienced by its homologs in other taxa. The new homeobox gene was discovered in the search for a "speciation gene" that causes hybrid male sterility.

In a series of studies, several genetic elements responsible for reproductive isolation between *Drosophila simulans* and *Drosophila mauritiana* have been identified (*4*). One of them, mapped to the cytological interval 16D on the X chromosome, is named Odysseus (*Ods*) (*5, 6*). The introgression of an appropriate *Ods*-containing region of *D. mauritiana* into *D. simulans* renders males completely sterile. The allelic state of *Ods* is nearly fixed in both species (*7*). In other words, *Ods*-induced hybrid male sterility is observable between any pairwise combination of *D. mauritiana* and *D. simulans* lines.

To delineate the *Ods* locus precisely, we generated 190 new recombinants with progressively shortened introgressions (Fig. 1). With eight molecular markers (*8*), 63 of these introgressions are male fertile and the remaining 127 are male sterile. In agreement with (*6*), the distinction between fertile lines (>90% fertility penetrance for each line) and sterile lines (0%) is clear-cut. The two longest fertile introgressions and the two shortest sterile introgressions define the location of the *Ods* locus. Because the breakpoints of the four introgressions all fall within a genomic clone of 8.4 kb (U8 in Fig. 1), it is plausible that the *Ods* gene, or at least part of it, resides within this clone.

We first obtained the complete DNA sequence of the U8 clone from *D. melanogaster* and used DNA software programs to identify three putative exons. On the basis of the putative exon sequences, we designed polymerase chain reaction (PCR) primers to analyze transcripts in a series of experiments. By the reverse transcriptase–PCR (RT-PCR) procedure, we could detect transcripts of the predicted sizes spanning exons 2 and 3 in both larval and adult stages (*8*). Then, a near full-length cDNA was obtained from a testis cDNA library by PCR amplification with primers in the exons and in the cloning vector. Finally, the 3′ end is determined by the RACE (rapid amplification of cDNA ends) protocol (*8*). Translation of this cDNA sequence including exon 1, which is located distal to U8, is shown in Fig. 2. The putative protein is 349 amino acids long. Because of the presence of a homeobox in exons 2 and 3, we have named this new transcript *OdsH* (for *Ods*-site homeobox gene). The name implies the correspondence in position between the genetic and molecular data without stating their functional equivalence.

The best and highly significant matches with *OdsH* in the database are the *unc-4* gene of *Caenorhabditis elegans*, *uncx4.1* of mouse (and its rat homolog), and an unpublished sequence from planaria (*9*). These sequences comprise a homologous cluster belonging in the paired-type subfamily of homeobox genes. Some of these homologous genes from very divergent taxa—notably *Drosophila*, rodents, and planaria—also have significant matches beyond the homeodomain. For example, 13 of the 14 amino acids adjoining the COOH-terminus of the homeodomain are identical in mouse and *Drosophila*. A high level of conservation extends for 33 amino acids from the COOH-terminus. In this report, we focus on exons 2 and 3 because their products contain the conservative homeodomain, which would allow us to contrast long-term evolutionary stability (such as between mammals and *Drosophila*) with recent rapid changes (between sibling species). Homology with non-*Drosophila* species outside these two exons is too low to be informative.

In comparing homologous genes from dif-

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