Molecular Biology of the Cell
Vol. 13, 3246–3256, September 2002

DNA Repair and Transcriptional Effects of Mutations in TFIIH in *Drosophila* Development

Carlos Merino, Enrique Reynaud, Martha Vázquez, and Mario Zurita*

Department of Genetics and Molecular Physiology, Institute of Biotechnology, Universidad Nacional Autónoma de México, Morelos 62250, México

Submitted February 14, 2002; Revised May 14, 2002; Accepted June 14, 2002
Monitoring Editor: Keith R. Yamamoto

Mutations in XPB and XPD TFIIH helicases have been related with three hereditary human disorders: xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy. The dual role of TFIIH in DNA repair and transcription makes it difficult to discern which of the mutant TFIIH phenotypes is due to defects in any of these different processes. We used *haywire* (*hay*), the *Drosophila XPB* homolog, to dissect this problem. Our results show that when *hay* dosage is affected, the fly shows defects in structures that require high levels of transcription. We found a genetic interaction between *hay* and *cdk7*, and we propose that some of these phenotypes are due to transcriptional deficiencies. We also found more apoptotic cells in imaginal discs and in the CNS of *hay* mutant flies than in wild-type flies. Because this abnormal level of apoptosis was not detected in *cdk7* flies, this phenotype could be related to defects in DNA repair. In addition the apoptosis induced by p53 *Drosophila* homolog (*Dmp53*) is suppressed in heterozygous *hay* flies.

INTRODUCTION

The TFIIH DNA repair/transcription factor provides an outstanding example of the complexity encountered in genotype–phenotype relationships. Mutations in some of the TFIIH components in humans may produce three hereditary disorders: xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD) (Lehmann, 1998, 2001). XP patients present sunlight hypersensitivity, abnormal skin pigmentation, and a high skin cancer predisposition (Cleaver, 2000; de Boer and Hoeijmakers, 2000; Rolig and McKinnon, 2000; Lehmann, 2001). CS individuals have slow postnatal growth and defects in nervous system development (Nance and Berry, 1992; Rolig and McKinnon, 2000). On the other hand, TTD patients share some of the neurological problems present in CS and have the particular phenotypes of brittle hair, fragile nails and ichthyosis (Itin and Pittelkow, 1990). In addition defects in TFIIH may have a role in the generation of cancer (Lehmann, 1998; Liu *et al.*, 2000).

TFIIH takes part in nucleotide excision repair (NER) (Buratowski, 1993; Feaver *et al.*, 1993; Schaeffer *et al.*, 1993; Drapkin and Reinberg, 1999; Conaway *et al.*, 2000; Le Page *et al.*, 2000). In eukaryotes, NER repairs many types of lesions that cause a distortion of the DNA helical structure, including pyrimidine dimers (Lehmann, 1987; Friedberg, 1996a; Wood, 1996). It has also been reported that TFIIH may participate in base excision repair (BER) when DNA suffers oxidative damage (Le Page *et al.*, 2000), increasing the number of roles that TFIIH plays in the eukaryotic genome maintenance.

TFIIH is formed by the DNA helicases XPB and XPD; the p62, p52, p44, and p34 polypeptides; and the complex known as cyclin-dependent kinase (Cdk)-activating kinase or CAK, which is formed by three proteins: Cdk7, CycH, and Mat1. Cdk7, CycH, and Mat1 are not involved in DNA repair, and there are no reported syndromes related to defects in these genes (Feaver *et al.*, 1994; Roy *et al.*, 1994; Serizawa *et al.*, 1995; Shiekhattar *et al.*, 1995; Rossignol *et al.*, 1997; Yankulov and Bentley, 1997). The Cdk7 kinase phosphorylates the C-terminal domain of RNA polymerase II. This phosphorylation is necessary for RNA polymerase II elongation (Gerber *et al.*, 1995). It has been suggested that the *Drosophila* Cdk7 homolog may also have a role in cell cycle control (Larochelle *et al.*, 1998). Therefore, the central role of TFIIH factor in transcription, DNA repair, and probably in cell cycle, explains the extremely pleiotropic phenotypes observed in XP, TTD, and CS disorders.

Functional analysis of TFIIH has been done using yeast, human cells, and in vitro transcription/DNA repair systems. More recently, the use of transgenic mice has allowed the generation of a TTD mouse model by introducing a mutation found in a human TTD patient into the mouse XPD gene (de Boer *et al.*, 1998). The mouse carrying the TTD allele has shown clearly TTD phenotypes. Unfortunately, this is the only example of a mammalian model that reproduces some manifestations found in humans affected in TFIIH (de Boer

Article published online ahead of print. Mol. Biol. Cell 10.1091/mbc.E02–02–0087. Article and publication date are at www.molbiolcell.org/cgi/doi/10.1091/mbc.E02–02–0087.

* Corresponding author. E-mail address: marioz@ibt.unam.mx.

et al., 1998; de Boer and Hoeijmakers, 1999; Rossi *et al.*, 2001). On the other hand, experiments in *Drosophila* have demonstrated that the fly is an excellent model for understanding and assaying the developmental function of genes that encode for the TFIIH complex components (Mounkes *et al.*, 1992; Larochelle *et al.*, 1998; Reynaud *et al.*, 1999; Leclerc *et al.*, 2000). In *Drosophila* the *XPB* homolog was identified as the *haywire* (*hay*) gene (Mounkes *et al.*, 1992). Alleles of *hay* mimic in the fly some of the defects found in XP and CS (Mounkes *et al.*, 1992). In this work we identified some phenotypes caused by defects either in transcription or by deficient DNA repair in *hay* flies, showing that the complex phenotype–genotype relationship of TFIIH genes in *Drosophila* can be genetically analyzed in detail. Our results show that when TFIIH is not functional during development, a high degree of apoptosis is induced. We also found that there is a genetic interaction between *Dmp53* and *hay* similar to the one found in human cells affected in XPB.

MATERIALS AND METHODS

Drosophila Strains

Wild-type strain in all experiments was OreR. *hay* alleles used in this work were hay^{nc2}, $hay^{nc2rv1-4}$, and hay^{nc2rv8} reported by Mounkes and Fuller (1999). Two new alleles, hay^{XPCS} and hay^{TTD}, were constructed. Deficiency *Df(3L)lxd6* uncovers the *hay* gene (Lindsley and Zimm, 1990). $cdk7^{P140S}$ is a transgenic conditional negative dominant allele. Therefore, all the crosses with this allele were performed at 29°C. The genotype of the *cdk7* stock is as follows: *w Df(1)JB254* Pw^{+} *[snf*$^{+}$*, dhd*$^{+}$*]/w Df(1)JB254* Pw^{+}*[snf*$^{+}$*, dhd*$^{+}$*]; +/+;* Pw^{+}*[Dmcdk7*^{P140S}*] Sb/TM3 Ser*; *Df(1)JB254* uncovers the 4F1-2 region of the X chromosome (Larochelle *et al.*, 1998). For p53 experiments the stocks used were *w118*; *Dmp53/CyO* (Brodsky *et al.*, 2000), *w1118*; *Dmp53R155H*; *Dmp53H159N*; *Dmp53K259H*; and *Dmp53C+* (Brodsky *et al.*, 2000; Ollman *et al.*, 2000) and *MS1096* as a *GAL4* wing imaginal disk driver located in the X chromosome (Capdevila and Guerrero, 1994).

Phenotypic Analyses of Wings, Cuticles, and Bristles

Wings were dehydrated and dissected in ethanol before being mounted in Permount (Fisher Scientific, Pittsburgh, PA) and then visualized with an optic microscope. The cuticle phenotypes were observed with a stereoscopic microscope; subsequently, abdominal regions of adult flies were dissected and prepared for electron microscopy as described previously (Stathakis *et al.*, 1999) and were examined in an EM900 transmission electron microscope (Carl Zeiss, Thornwood, NY). Bristle defects were visualized in a stereoscopic microscope. Flies were fixed in glutaraldehyde, postfixed in osmium tetroxide, dehydrated through a graded series of alcohol, and critical point dried before mounting on stubs and coated with carbon and gold. Samples were analyzed with a 5410 LV scanning electron microscope (JEOL, Tokyo, Japan).

Transgenic Flies

Mutations in the *hay* cDNA were introduced using a polymerase chain reaction-based method (Merino *et al.*, 1992). For the generation of the hay^{TTD} allele a base substitution (A-C) in position 385 on the *hay* cDNA (Koken *et al.*, 1992) was made using the oligonucleotide 5′-AGTACAAACTCCCCGCATACAGTTTATATG-3′. To generate a change in the reading frame hay^{XPCS} was made by introducing a guanine in position 2293. The oligonucleotide used was 5′-CCGACACGTCTCACCGATGCCGCCCG-3′. Mutant cDNAs were cloned by using the appropriate restriction enzymes in pCaSper *hsp83* vector, and the whole genes were sequenced to corroborate the mutations. Transgenic flies were constructed following a standard protocol (Spradling and Rubin, 1982). Two hay^{TTD} and four hay^{XPCS} independent lines in the second chromosome were isolated.

Staining of Imaginal Discs and CNS

Third instar imaginal discs and larval CNSs were dissected in 1× phosphate-buffered saline and stained with acridine orange vital dye (Sigma-Aldrich, St. Louis, MO) or Nile blue (Sigma-Aldrich) at a concentration of 5 and 100 μg/ml, respectively (Abrams *et al.*, 1993). Samples were analyzed with a conventional fluorescence microscope. For irradiated larvae, the imaginal discs were dissected and stained 24 h after irradiation. Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay was performed using the In Situ Cell Death Detection kit from Roche Applied Science (Indianapolis, IN) following the recommended protocol. The tissue was visualized on an MRC-600 confocal microscope (Bio-Rad, Hercules, CA).

Slot Blot Hybridization

Total RNA from adult flies was purified using the TRIzol kit (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. The integrity of the RNA was verified in a typical formamide-agarose gel. The RNA was loaded in Hybond-N^{+} membranes (Amersham Biosciences, Piscataway, NJ) by using a slot blot manifold (Hoeffer, San Francisco, CA). The blot was hybridized with labeled Pcp-1 and Actin probes (Roter *et al.*, 1985; Vigoreux and Tobin, 1987) in a 50% formamide hybridization solution. After hybridization, the membrane was washed at 65°C several times in 0.2× SSC, 0.1% SDS and exposed to XAR-5 film (Eastman Kodak, Rochester, NY). As control the membrane was hybridized with total rRNA labeled with [α-^{32}P]dCTP in a reverse transcriptase reaction. Signal quantification was performed by using the Scan Image system (Bio-Rad).

UV Irradiation

Third instar wild-type and hay^{nc2}/hay^{nc2} larvae were irradiated with the wild-type half LD by using 254-nm UV light with a germicide lamp (UVP), and the irradiation was measured using a UVX radiometer (UVP).

Antibody Generation and Western Blot

A Hay-Glutathione *S*-transferase recombinant protein was constructed using the first 70 residues from the Hay protein. The protein was purified as described previously (Smith, 1993) and used to elicit polyclonal antibodies in Wistar rats. The antibody obtained was used in Western blot experiments. In general, total protein soluble extracts were prepared from adult flies and standardized. Samples were loaded in 12% SDS-PAGE gels (Laemmli, 1970), blotted in nitrocellulose, and immunostained (Baurnette, 1981). Hay protein was visualized using a 1:1000 dilution of the anti-Hay antibody. Peroxidase-conjugated goat anti-rat was used as secondary antibody (Zymed Laboratories, South San Francisco, CA).

RESULTS

Multiple Cuticular Defects Are Present in haywire Mutant Flies

Several hypomorphic and antimorphic *hay* alleles have been analyzed at the genetic and molecular levels (Mounkes and Fuller, 1999). It has been reported that mutations in the *hay* gene produce an increase to UV light sensitivity, male sterility in combination with mutations in a particular tubulin gene, and some defects in the development of the nervous system (Mounkes *et al.*, 1992). These phenotypes resemble

some of the human manifestations produced in humans affected with XPB. However, except for the sensitivity phenotype caused by UV irradiation, which is attributed to defects in DNA repair, other phenotypes can be the consequence of DNA repair, transcriptional problems, or both. To understand the phenotypes produced by *hay* mutations in more detail, we reduced *hay* activity to its lowest viable level by making heteroallelic combinations of different antimorphic or hypomorphic alleles (Mounkes and Fuller, 1999) and two human-like alleles with the conditional hypomorphic hay^{nc2} mutation (Figure 1A). Progeny from these crosses had abdominal and wing defects, as well as deformations in the bristles (Table 1 and Figure 1). The abdominal abnormalities appeared as the loss of some cuticle portions. Electron microscopy showed that this phenotype is a consequence of a reduction of the deepest layers of the lamellate procuticle, whereas the superficial layers were not affected (Figure 1B). Both cuticular layers are derived from the same cell type (Fristrom and Liebrich, 1986), which suggest that this phenotype is due to a reduction of protein synthesis, rather than to the absence of these cells.

Compared with wild-type flies, bristles present in *hay* heteroallelic flies were very fragile and deformed in shape and texture (Figure 1C). This phenotype affects both the machrochaete and the microchaete. Some of the bristles have a fork-like structure at the tip and in general the macro- and microchaete in the thorax do not show the organization found in wild-type flies. Because of their similarities with the brittle hair phenotype present in TTD patients, we named this phenotype "brittle" bristles. Although the origin, function, and structure of the hair in mammals are basically different to the fly bristles, they do have molecular similarities in their development. The integrity of both hair and bristles requires high transcription levels of genes' structural coding for proteins that assemble these complex structures (de Boer *et al.*, 1998; Tilney *et al.*, 2000). Genetic evidence that the brittle bristle phenotype is due to transcriptional defects is presented below.

In addition to the cuticular and brittle bristle defects, wing defects were also observed in some of the *hay* heteroallelic combinations (Table 1 and Figure 1D). This phenotype, which we named wing phenotype A, is not as penetrant as are the brittle bristle and abdominal cuticular phenotypes. The hay^{nc2rv8} and hay^{nc2rv4} alleles as well as the *Df(3L)lxd6* with the hay^{nc2} allele do not show wing defects (Table 1). These results suggest that these wing defects were not due to the gene product dosage, but to allele-specific interactions.

Transgenic Flies Carrying Human-like Alleles with Mutations Reported in Human Patients Reproduce Defects Observed in hay Heteroallelic Flies

In addition to known EMS alleles (Mounkes and Fuller, 1999), we constructed two new *hay* alleles containing two mutations found in XPB patients. One of these mutations causes TTD, whereas the other produces both XP and CS manifestations (Weeda *et al.*, 1990, 1997; Riou *et al.*, 1999). These transgenes were named hay^{TTD} and hay^{XPCS}, respectively (Figure 1A). Although wild-type transgenes were able to rescue lethality of *hay* homozygous flies as well at the abdominal, bristle, and wing defects, mutant transgenes (hay^{TTD} and hay^{XPCS}) were not (our unpublished data). The presence of one copy of the hay^{TTD} or hay^{XPCS} transgenes in a hay^{nc2}/hay^{nc2} background decreases fly viability dramatically (Table 2). Transgenic flies carrying one copy of hay^{XPCS}, one copy of hay^{nc2} allele, and one copy of the wild-type gene reproduced defects observed in heteroallelic combinations of *hay*, but in a more severe manner. For instance, bristles were much more fragile and thinner and wings were practically amorphous (Figure 2A). In addition, locomotion impairments were observed. None of these defects were observed in transgenic flies carrying two wild-type *hay* alleles, demonstrating that, in individuals with this genotype, hay^{XPCS} is not dominant. Both transgenes overexpress the corresponding RNA (our unpublished data). A polyclonal rat antibody against the N-terminal domain of the wild-type Hay protein was produced (see MATERIALS AND METHODS). By using this antibody, we analyzed the Hay protein produced by the transgenic flies harboring the hay^{TTD} and hay^{XPCS} constructs. We found that both transgenic lines have a band corresponding to the expected size of wild-type and mutant proteins (~89 kDa) and a truncated product 30 kDa smaller than the wild type (Figure 2B). This result suggests that these mutant forms produce a nonstable protein that is processed.

cdk7 Genetically Interacts with Hay

The observed *hay* phenotypes could be due to problems either in DNA repair or in transcription. It has been demonstrated that Cdk7 participates in transcription as a component of TFIIH and is also involved in the control of the cell cycle, but it does not participate in DNA repair (Feaver *et al.*, 1994; Serizawa *et al.*, 1995; Svejstrup *et al.*, 1995). Thus, it is possible that *cdk7* mutants would present a subset of defects observed in *hay* flies affected in these processes. In an attempt to dissect the causes of different phenotypes observed in *hay* mutants, we performed crosses of *hay* flies with flies harboring different doses of *cdk7* wild-type gene, with or without the conditional dominant negative $cdk7^{P140S}$ (Larochelle *et al.*, 1998; see MATERIALS AND METHODS). If at least some of the defects observed in single *cdk7* or *hay* mutant flies are caused by defective transcription due to different TFIIH abnormal subunits, we should observe a genetic interaction in transheterozygous flies. The results are shown in Table 3. We found that when the only source of Cdk7 comes from the mutant allele $cdk7^{P140S}$, flies incubated at the restrictive temperature presented wing, cuticular, and bristle phenotypes. The last two were similar in appearance to the ones presented by single *hay* homozygous or heteroallelic mutants (Table 1, and Figure 1, D and E). Most of the *hay* alleles show a genetic interaction with $cdk7^{P140S}$ increasing the penetrance of bristle and cuticular phenotypes (Table 3). A stronger interaction was found between $cdk7^{P140S}$ and hay^{nc2rv8} alleles. Based on the appearance of the common bristle and cuticular phenotypes by single *hay* and *cdk7* mutants, and the enhancement of these phenotypes in transheterozygous *cdk7/hay* flies, we suggest that these two phenotypes were caused by transcriptional defects. These phenotypes were only observed at the restrictive temperature for $cdk7^{P140S}$. In addition to this result, no interaction was observed in *Df(1)JB254/+; hay/+* flies (Table 3), indicating that the bristle and cuticle defects are only due to the presence of the $cdk7^{P140S}$ mutant. Thus, the possibility of a second mutation that interacts with *hay* producing these phe-

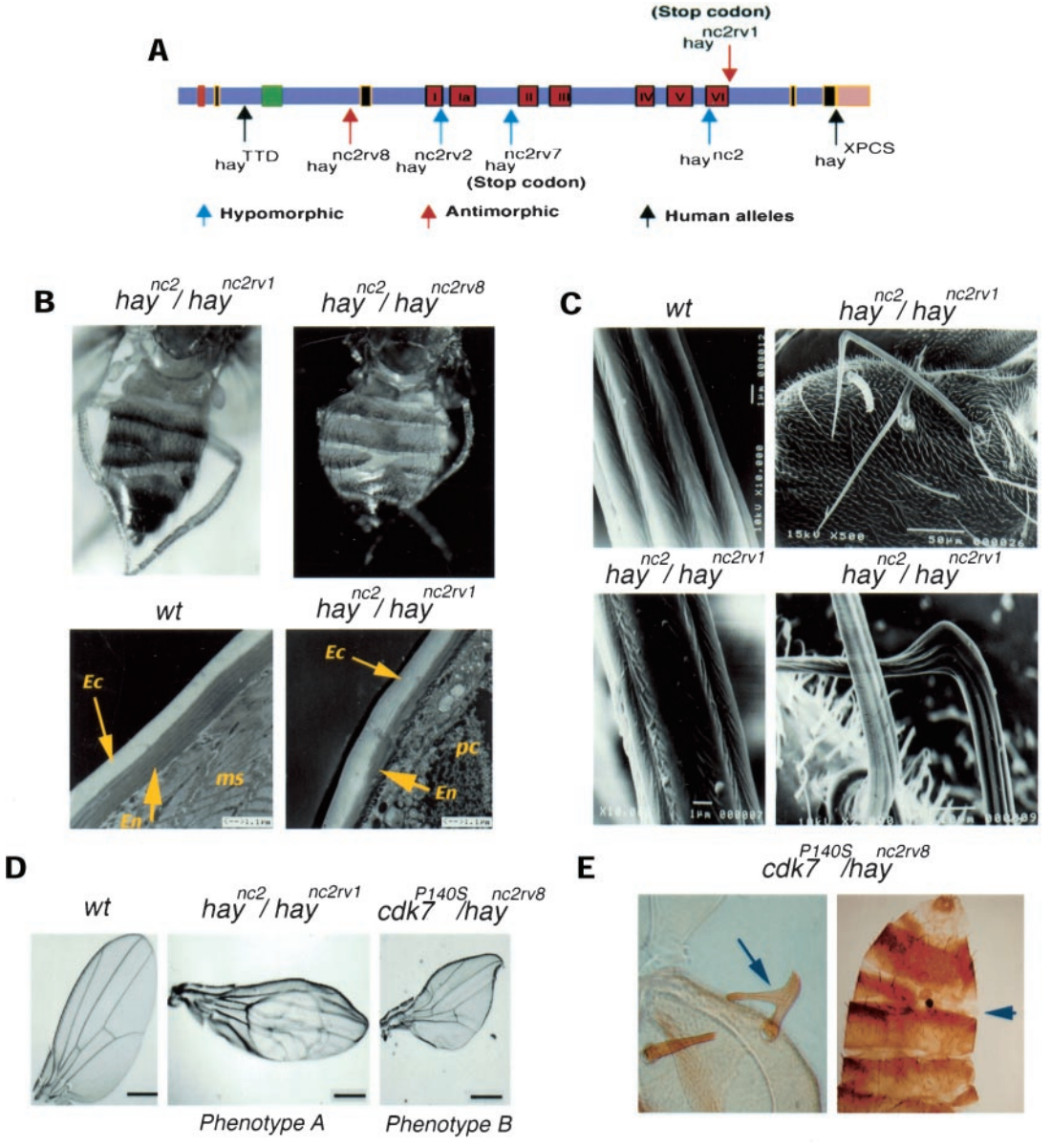

Figure 1. *hay* gene structure, and heteroallelic and transheterozygous phenotypes of *hay* and *cdk7* mutants. (A) Gene structure and localization of the mutations of the different *hay* alleles. The red boxes are the helicase domains, the black boxes are nuclear localization signals, the green box is the ATP-binding region, and the pink box is the COOH-terminal region affected in the XPCS human allele. The nucleotide sequence of the fly alleles has been reported previously (Mounkes and Fuller, 1999). The hay^{TTD} human-like allele has a change of Ser 119 for Pro (Weeda *et al.*, 1997), and the hay^{XPCS} human-like allele is an insertion of one base at the intron acceptor site that produces a phase change in the open reading frame at the COOH terminus, which is described in the human allele (Weeda *et al.*, 1990). Hypomorphic alleles are indicated with a blue arrow, antimorphic alleles with a red arrow, and the human alleles introduced in transgenic flies with a black arrow. The original hay^{nc2} allele was originally described as antimorphic in terms of its interactions with tubulin mutants (Mounkes eta al., 1992); however, in our genetic analysis it behaves as hypomorphic. (B) Dorsal view of *hay* heteroallelic flies with cuticle deformations in abdominal tergites (top panels) and electron microscopy pictures of cuticle preparations of wild-type and mutant flies (bottom panels). The arrows indicate the exocuticular (Ec) and endocuticular (En) layers, ms is muscle, and pc is parenchyma. (C) Brittle bristle phenotype. Scanning micrographs of wild-type and brittle bristles from the scutellum of *hay* heteroallelic flies. (D) Wing phenotypes A and B of hay^{nc2}/hay^{nc2rv1} and $cdk7^{P140S}/hay^{nc2rv8}$ flies. Note that both phenotypes are very different. The relevant genotypes are indicated. (E) Cuticle preparations of $cdk7^{P140S}/hay^{nc2rv8}$ flies showing the brittle bristle and abdominal cuticular phenotypes. Complete genotypes are given in MATERIALS AND METHODS.

Table 1. Viability and frequency of defects in heteroallelic *hay* flies
The data in table are percentages obtained from at least 150 individuals of each genotype. An example of each phenotype is shown in Figure 1.

Genotype	Viability	Brittle bristles[a]	Abdominal cuticular defects[b]	Phenotype A wing[c]
$hay^{nc2}/+$	100	0	0	0
hay^{nc2}/hay^{nc2}	46.3	14	13	9
hay^{nc2}/hay^{nc2rv1}	47.9	19.9	21.5	14.3
hay^{nc2}/hay^{nc2rv2}	45.6	49.1	12.0	9.6
hay^{nc2}/hay^{nc2rv4}	12.4	47.9	16.6	0
hay^{nc2}/hay^{nc2rv7}	38.8	46.1	16.7	1.0
hay^{nc2}/hay^{nc2rv8}	49.7	11.1	15.3	0
$hay^{nc2}/Df(3L)lxd6$	50.0	26.6	26.0	0

[a] Brittle bristles phenotype consists of deformed and fragile macro and microchaete.

[b] Abdominal cuticular defects consist of a reduction of the endocuticular layer.

[c] Abnormal wing phenotype A consists in the lost of marginal regions and blistered wings.

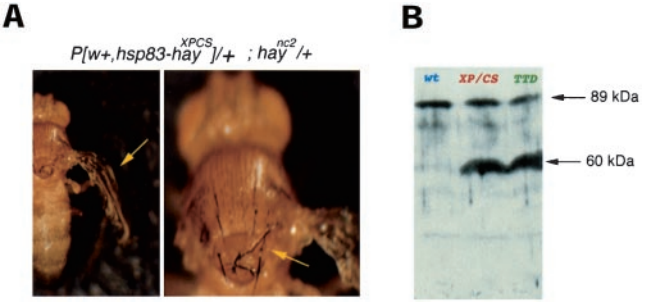

Figure 2. hay^{XPCS} allele enhances the *hay* phenotypes. (A) Phenotype of a hay^{nc2} heterozygous fly carrying one copy of the hay^{XPCS} allele expressed from the fly *hsp83* promoter. Similar phenotypes are presented with the hay^{TTD} allele. $hay^{nc2}/+$ flies have a wild-type phenotype. (B) Western analysis of soluble proteins from wild-type and transgenic third instar larvae expressing either the Hay^{TTD} and Hay^{XPCS} proteins. After Western transfer Hay proteins were visualized using a polyclonal antibody against a glutathione *S*-transferase fusion protein harboring the N terminus of Hay. The genotypes of the larvae are wild-type: *OreR*; *XPCS:* w^{118}, hay^{XPCS}/CyO; *TTD:* w^{118}, hay^{TTD}/CyO. The wild-type protein is indicated by a red arrow and the blue arrow indicates a truncated form which is observed in both mutants.

notypes could be discarded. On the other hand, bristle and cuticle phenotypes emerge in homozygous *Df(1)JB254* flies in the presence of one copy of the $cdk7^{P140S}$ allele (Table 3), reflecting that these phenotypes were due to the loss of function of *cdk7*.

In contrast, wing defects observed in $cdk7^{P140S}$ flies were clearly different from the ones presented by *hay* mutant flies, thus we named it wing phenotype B (Figure 1D). This phenotype was present even when there is a wild-type *cdk7* copy, which is not the case for bristle and cuticular phenotypes. $cdk7^{P140S}$ flies present small and deformed wings (phenotype B), whereas wings of heteroallelic *hay* flies show loss of marginal regions and the presence of blisters (Figure 1D, phenotype A). All transheterozygous *hay/cdk7* combinations showed the $cdk7^{P140S}$ wing phenotype B. Unexpectedly, the wing phenotype of $cdk7^{P140S}$ is not enhanced by the addition of a defective *hay* allele with the exception of the hay^{nc2rv8} allele, showing that the interaction between $cdk7^{P140S}$ and hay^{nc2rv8} is allele specific (Table 3, in bold). Lack of enhancement of $cdk7^{P140S}$ wing phenotype with different *hay* alleles, with the exception of hay^{nc2rv8} (see DISCUSSION), supports that the different wing defects in *hay* and *cdk7* mutants (phenotype A and B) are caused by failure in different mechanisms. Thus, because Cdk7 is only required for transcription and for the progression of cell cycle, the *hay* wing phenotype is probably caused by a deficiency in DNA repair rather than by transcription.

To complement the genetic interaction data between *cdk7* and *hay*, mRNA levels of cuticular protein Pcp-1 and Actin (Rother *et al*., 1985; Vigoreaux and Tobin, 1987), which

Table 2. Fly viability in heteroallelic and homozygous hay^{nc2} flies in the presence of the hay^{TTD} and hay^{XPCS} alleles
Data are presented as the percentage of expected individuals of each genotype and are an average of at least 500 embryos of all genotypes laid in each cross.

Genotype	*wt*	hay^{nc2}/hay^{nc2rv1}	hay^{nc2}/hay^{nc2}	$hay^{nc2}/+$
	100	47.9	46.9	100
P[w, hay+]		100	nd	nd
$P[w, hay^{TTD}]$	100	2.1	0	92
$P[w, hay^{XPCS}]$	100	0.4	0.6	70

Table 3. Wing and bristles phenotypes in *cdk7* flies and genetic interaction with *hay*

Genotype	Wing phenotype B	Brittle bristles	Cuticle
Df/+	0	0	0
$Df/+; +/hay^{nc2}$	0	0	0
$Df/+; cdk7^{P140S}/+$	20.9	0	0
$Df/Df; cdk7^{P140S}/TM3$	36.3	16.3	5
$Df/+; cdk7^{P140S}+/hay^{nc2}$	17.2	10.4	4.03
$Df/+; cdk7^{P140S}+/hay^{nc2rv2}$	17.5	14.4	2.12
$Df/+; cdk7^{P140S}+/hay^{nc2rv7}$	18.0	6.7	n.d.
$Df/+; cdk7^{P140S}+/hay^{nc2rv8}$	**49.3**	**30.0**	**5**

Data represent percentages. Because *cdk7* is located in the X chromosome, the flies used in these crosses were *w Df(1)JB254* Pw^+ *[*snf^+*,* dhd^+*]/ w Df(1)JB254* Pw^+ *[*snf^+*,* dhd^+*]; +/+;* Pw^+ *[*$Dmcdk7^{P140S}$*] Sb/ TM3 Ser. Df(1)JB254* is deficient for the *cdk7*, gene and for the *snf* and *dhd* genes. The *snf* and *dhd* genes are complemented by wild-type alleles carried in a P element inserted in the same X chromosome (Larochelle *et al*., 1998). In the table this genotype is named *Df*. The genetic interaction between $cdk7^{P140S}$ and hay^{nc2rv2} is indicated in bold. The wing phenotype B consists in shape deformations and size reduction (phenotype B; see Figure 1). The percentages of flies presenting the three phenotypes with combinations of $cdk7^{P140S}$ and *hay* alleles are significant ($P < 0.0005$).

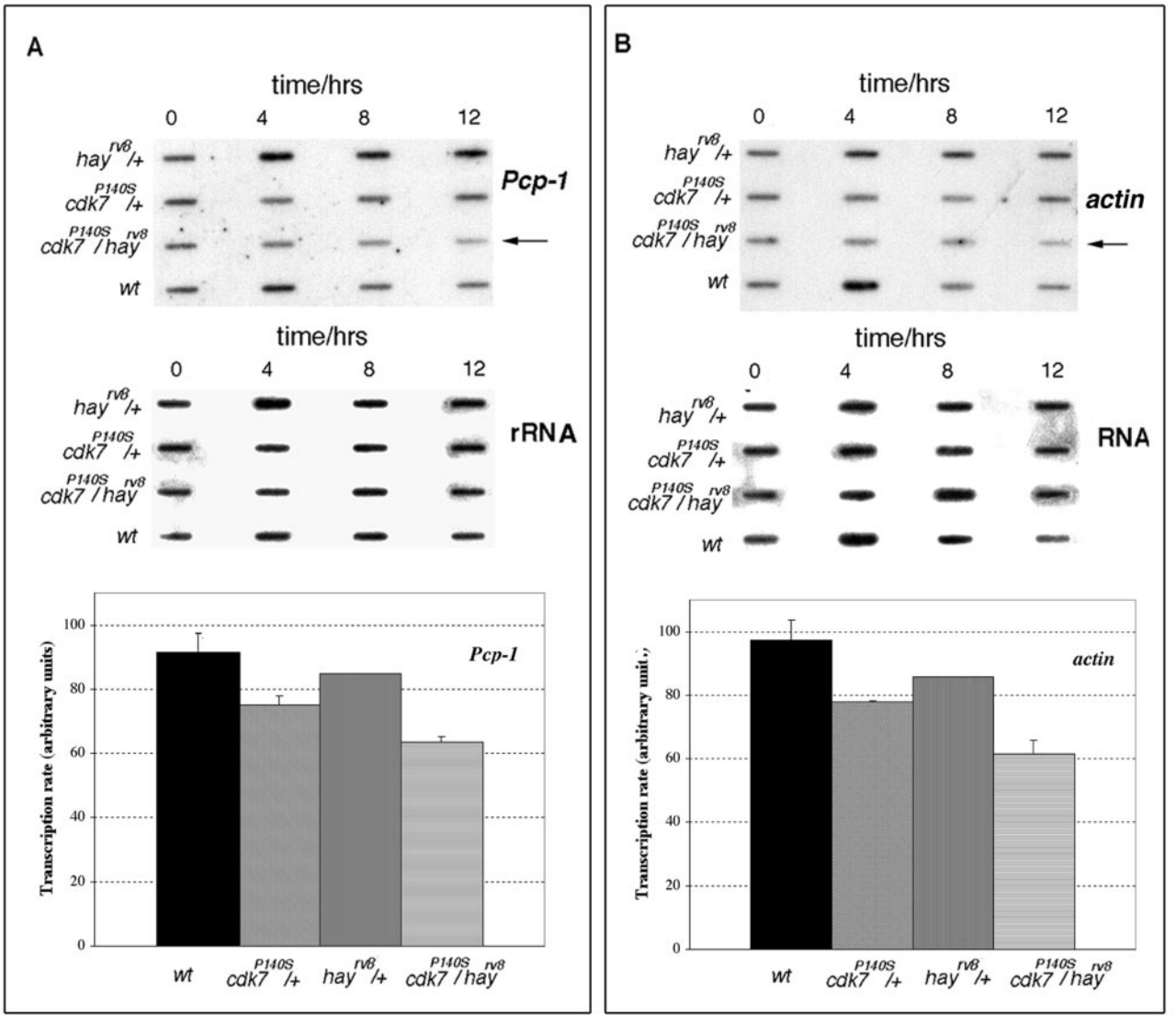

Figure 3. RNA levels of the Pcp-1 cuticular and Actin proteins in *cdk7*^{P140S} and *hay* double mutant flies. Slot blot hybridization of the Pcp-1 (A) and Actin probes (B) against total RNA purified at different times from adult flies incubated at 29°C. The quality of the RNA was confirmed in typical formamide-agarose gels. Independent membranes were hybridized for each probe. The different genotypes are indicated in the figure. The same blots were washed and hybridized against rRNA as loading control. Note that in the *cdk7*^{P140S}/*hay*^{rv8} flies (indicated with an arrow) the *Pcp-1* and *Actin* transcript levels are reduced with the incubation time. The graphs show for each genotype, the average (n = 3) percentage of reduction in specific transcript levels after 12 h of incubation at the restrictive temperature (29°C) compared with rRNA levels.

should be related to the observed phenotypes, were analyzed in *hay*^{nc2rv8}/*cdk7*^{P140S} flies and compared with heterozygous and wild-type flies. *hay*^{nc2rv8} flies were crossed with *cdk7*^{P140S} organisms and the obtained transheterozygous adults were incubated at the restrictive temperature (29°C) at different times (0, 4, 8, and 12 h). Then total RNA from three different experiments was purified, and similar amounts of for each sample were loaded in a slot blot and hybridized against a *Pcp-1* and *Actin* cDNA probes. Because mutations in the TFIIH components should affect a large number of genes transcribed by RNA polymerase II we used as control the levels of rRNA. Figure 3 shows that RNA levels of *Pcp-1* and *Actin* were reduced in the *hay*^{nc2rv8}/*cdk7*^{P140S} flies incubated at 29°C compared with *hay* and *cdk7* heterozygous flies incubated at a similar temperature. Quantification of the *Pcp-1* and *actin* mRNA levels in *hay*^{nc2rv8}/*cdk7*^{P140S} flies, of three independent blots, indicates that the reduction was of ~35 and 40%, respectively, if compared with the wild-type at 12 h of incubation at the restrictive temperature (Figure 3, A and B). The reduction of both mRNA levels occurs at 29°C, confirming that this is due to the *cdk7*^{P140S} conditional mutant. These results support that

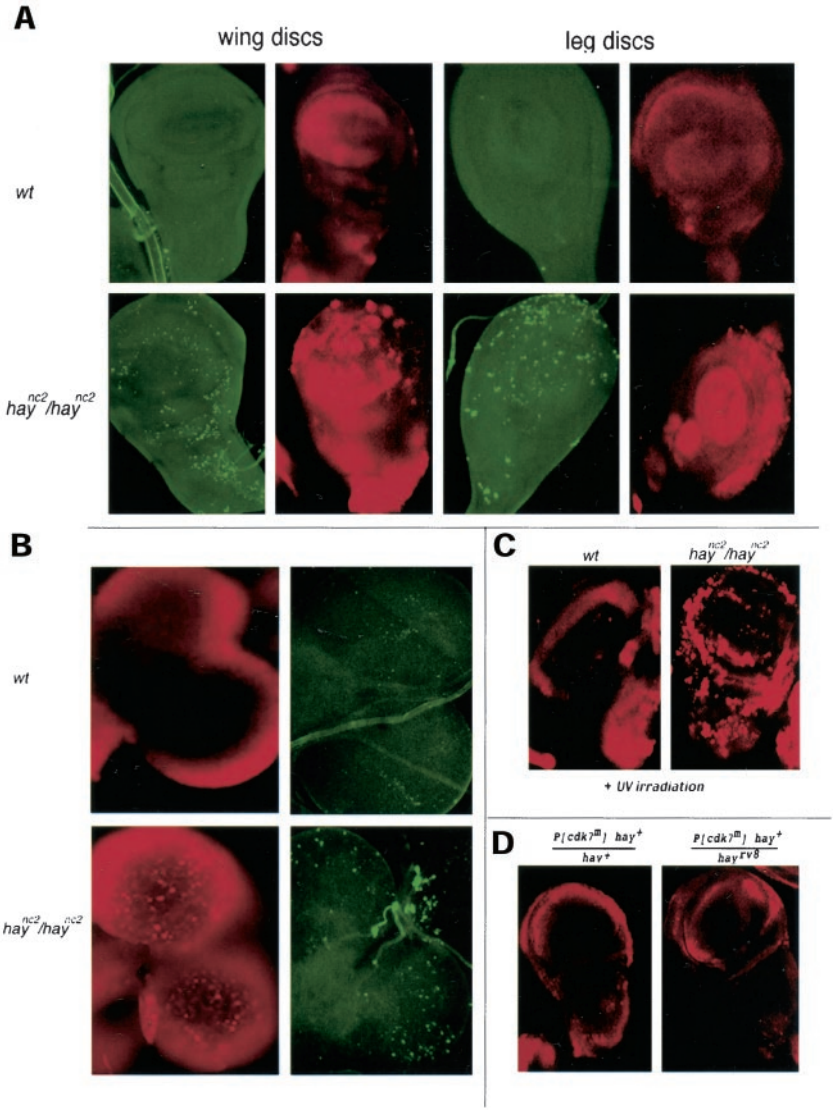

Figure 4. Apoptosis in *hay* imaginal discs and brains. (A) Wing and leg disc preparations from wild-type and hay^{nc2}/hay^{nc2} flies stained with acridine orange (red) and TUNEL assay (green) for the presence of apoptotic cells. (B) CNS preparations from wild-type and hay^{nc2}/hay^{nc2} flies stained with acridine orange (red) and TUNEL (green). Note the high number of apoptotic bodies in the *hay* larval tissues. (C) Wild-type and hay^{nc2}/hay^{nc2} wing discs stained for apoptosis 24 h after UV irradiation (half LD for the wild-type, 164 J/m^2). (D) $cdk7^{P140S}$ hay^{+}/hay^{+} and $cdk7^{P140S}$ hay^{+}/hay^{nc2rv8} discs. Note that only background is detected as in the wild-type discs. In all cases cell death is also detected with Nile blue (our unpublished data). Relevant genotypes of these flies are as in Figure 1 and MATERIALS AND METHODS. Acridine orange-treated samples were observed under a fluorescence microscope. The TUNEL assay was visualized using confocal microscopy. For each tissue sample maximum projections of the whole set of images are shown.

the interaction between *cdk7* and *hay* mutants has an effect on transcription that may result in the observed phenotypes.

Flies Affected in Hay Have a High Rate of Apoptosis in Imaginal Discs and in CNS

It has recently been shown that human cells with defects in XPB are not able to repair oxidative DNA damage by BER during transcription (transcription-coupled repair or TCR) (Le Page *et al.*, 2000). These defects in TCR by XPB have been related to neurodegenerative problems found in CS patients, suggesting that deficient TCR may induce apoptosis (Hanawalt, 2000; Le Page *et al.*, 2000; Vermeulen and Hoeijmakers, 2000). To know whether apoptosis was increased in *hay* flies, we analyzed the presence of apoptotic bodies in larval CNS and in the imaginal discs. Interestingly, an increase of apoptotic bodies in both kinds of tissues in *hay* larvae was found (Figure 4, A and B). The amount of apoptotic cells is increased in *hay* discs after UV irradiation compared with irradiated wild-type discs (Figure 4C). In contrast, we could not detect abnormal apoptotic bodies in $cdk7^{P140S}$ imaginal discs and in the CNS, even in $cdk7^{P140S}/hay^{nc2rv8}$ transheterozygous flies (Figure 4D). Because *cdk7* only participates in transcription and in cell cycle control, but not in DNA repair, we suggest that apoptosis in hay^{nc2} mutants is due to DNA repair defects. These results also show that DNA repair by TFIIH is required during development and constitutes the first in vivo evidence that defects in the *hay* gene may induce apoptosis. $cdk7^{P140S}$ and *hay* flies present different wing phenotypes. For *cdk7*, the allele-specific interaction with hay^{nc2rv8} suggests that wing phenotype is related to deficient transcription, although a deficiency in cell cycle cannot be ruled out. With the evidence presented herein, we propose that *hay* wing phenotype is probably a consequence of an abnormal high apoptosis found in wing

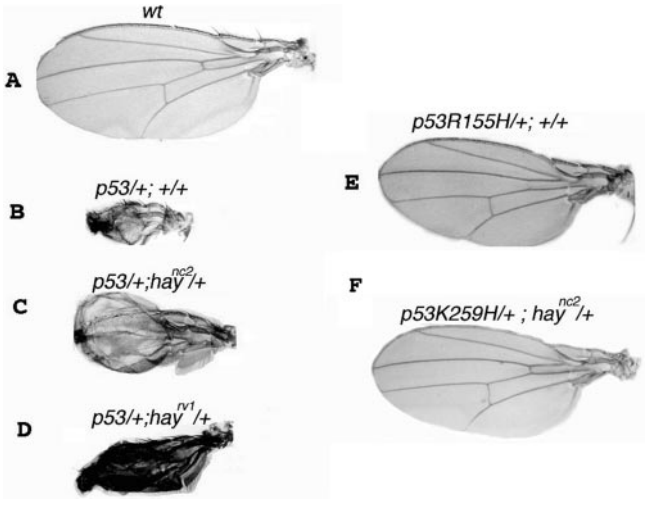

Figure 5. *hay* and *Dmp53* genetic interaction. Wild-type *Dmp53* and a dominant negative *Dmp53R155H*, *Dmp53H159N*, *Dmp53K259H*, and *Dmp53C+* alleles were overexpressed in the wing disc by using the *MS1096 GAL4* driver in the presence or the absence of *hay* alleles. (A) Wild-type OreR wing. (B) Overexpression of the wild-type *Dmp53* produces aberrant wings. (C and D) Wing overexpression of the wild-type *Dmp53* in hay^{nc2} and hay^{nc2rv1} heterozygous backgrounds. Note that the apoptotic phenotype is reduced. (E) Overexpression of the dominant negative *Dmp53R155H* allele does not produces defects in the wing. (F) Example that shows that no genetic interaction occurs between *Dmp53* negative dominant mutants and *hay* alleles.

imaginal discs of mutant *hay* flies, caused by a deficient DNA repair during development.

Dmp53/hay Genetic Interaction

In mammalian cells, apoptosis induced by DNA damage is mediated by p53 (Ford and Hanawalt, 1995). A physical interaction between p53 and TFIIH has been demonstrated (Wang *et al.*, 1995), and p53-mediated apoptosis seems to be defective in XPB and XPD mutant cells (Wang *et al.*, 1996). The *Drosophila* p53 homolog gene (*Dmp53*) can activate apoptosis in response to DNA damage by γ-irradiation (Brodsky *et al.*, 2000; Ollman *et al.*, 2000). Wild-type *Dmp53* overexpression in the eye and wing discs induces apoptosis and severe deformations in both adult organs (Brodsky *et al.*, 2000; Ollman *et al.*, 2000; Figure 5B). Wing deformations are characterized by abnormal shape and a dramatic reduction of the wing blade as a consequence of massive cell death (Figure 5B). To know whether there is an interaction between *Dmp53* and TFIIH in *Drosophila*, we used transgenic flies with either wild-type *p53* (*Dmp53*) gene or with several dominant negative alleles affected in the DNA binding domain (*Dmp53R155H*, *Dmp53H159N*, *Dmp53K259H*, and *Dmp53C*) (Brodsky *et al.*, 2000; Ollman *et al.*, 2000). Both wild-type and mutant p53 alleles were overexpressed under *GAL4-UAS* system in the third instar larval wing discs of *hay* mutant individuals, by using the *GAL4* wing driver *MS1069* (see MATERIALS AND METHODS).

We found that wing defects produced by the *Dmp53* overexpression were suppressed in the presence of a *hay* mutant allele. Although this suppression was partial, the penetrance was 100%, even in the presence of a single *hay* mutant allele (hay^{nc2}/+ and hay^{nc2rv1}/+ flies; Table 4 and Figure 5, C and D). These results showed that *Dmp53* needs an intact TFIIH to induce apoptosis because it occurs in human cells derived from patients affected in XPB and XPD (Wang *et al.*, 1996).

Table 4. Genetic interaction between *Dmp53* and *hay*

Data represent percentages from at least 50 individuals of each genotype. *MS1096* is a wing imaginal disc GAL4-driver. *Dmp53R155H* is a p53 negative dominant mutation that is not able to induce apoptosis (Ollman *et al.*, 2000).

Genotype	Apoptotic phenotype[a]	Phenotype suppression[b]
MS1096	0	-
Dmp53	0	-
MS1096; Dmp53	100	-
MS1096; Dmp53; hay^{nc2}	-	**100**
MS1096; Dmp53; hay^{nc2rv1}	-	**100**
MS1096; Dmp53R155H	0	-
MS1096; Dmp53R155H; hay^{nc2}	0	-

[a] Apoptotic wing phenotype is identically to phenotype presented in Figure 4 in all flies with the same genetic background.

[b] The suppressed wing phenotype is identical to the phenotype presented in the figure with the same genetic background in each case. The suppression is fully penetrant.

DISCUSSION

Phenotypic defects produced by some of the components of TFIIH have been difficult to characterize in mammalian systems. Analyzing combinations of mutant alleles of genes encoding two TFIIH subunits (*hay* and *cdk7*), we have been able to dissect phenotypes associated with transcription and with DNA repair.

Brittle Bristles and Cuticular Phenotypes Are Associated with TFIIH Transcriptional Deficiencies

Reduction of the Hay activity in different heteroallelic combinations produces analogous defects to the ones observed in humans affected in the XPB gene. Besides the increase in sensitivity to UV irradiation and sterility that were described by Mounkes *et al.* (1992), the most obvious defects in adult *hay* heteroallelic flies are severe cuticular deformations in the abdomen, brittle bristles, and aberrant wings. These defects can be rescued by overexpression of the Hay wild-type form in all the heteroallelic combinations. From the data reported herein, we propose that both the cuticular abdominal deformations and the brittle bristles are associated with deficiencies in transcription caused by defective TFIIH. For the adult cuticles, electron microscopy studies showed that this phenotype is due to a reduction of the deeper lamellate procuticle layers. As in the cuticle, construction of hair-like structures of bristle requires the abundant transcription of genes that encode bristle structural components (Frinstrom and Frinstrom, 1993). Bristles in *hay* flies are very fragile and they have severe deformations in their structure and texture. These defects resemble defects

observed in TTD patients' hair caused by reduced transcription of genes encoding structural components such as the sulfur-rich proteins of the hair (Lehmann, 2001). This phenotype is also caused by a mutation in *cdk7* producing a defective TFIIH. We found a clear genetic interaction between *hay* and *cdk7* for the brittle bristle and the abdominal cuticular phenotypes. Because these two TFIIH subunits are involved in transcription, but Cdk7 is not involved in DNA repair, we concluded that lower levels of transcription cause brittle bristles and the abdominal cuticular phenotypes. This conclusion was supported by the fact the double *hay/cdk7* mutants can affect transcript levels of genes related to the cuticular and bristle defects (Figure 3). In addition, we have observed similar defects by the genetic interaction of *hay* and mutant alleles of other basal transcription factors genes (our unpublished data).

The general transcription machinery is affected in *hay* mutants used in this work. Nevertheless, some *hay* individuals, although defective in many senses, are able to have a proper cell differentiation and to develop until adults. However, we believe that in *hay* cells that require the overexpression of specific genes, the TFIIH machinery is probably exhausted before the transcriptional program is completed, resulting in defects such as cuticle and bristles phenotypes. These defects could be the result of a partial reduction in the transcriptional rate of highly expressed genes. This could be similar to the proposed explanations for the brittle hair and ichthyosis manifestations observed in TTD patients (Lehmann, 2001).

The penetrance of the defects associated with *hay* is enhanced in the presence of hay^{XPCS} and the hay^{nc2} alleles (Figure 2). The XPCS patient carrying the mutation similar to the hay^{XPCS} allele is heterozygous, and it has been speculated that this mutation could be a dominant allele (Weeda *et al.*, 1990). However, the presence of a second mutation in the control region of the other *XPB* allele (paternal) or in other gene has not yet been ruled out, in particular, because it was observed that the transcript of the paternal allele is not detectable in the patient (Weeda *et al.*, 1990). Our results show that in *Drosophila,* hay^{XPCS} allele is not dominant, and it only produces a *hay* phenotype when it is in combination with other *hay* mutant alleles. It is important to note that, although the nature of the two mutations (TTD and XP/CS) is different, in *Drosophila* they both produce a truncated Hay form that could affect the function of TFIIH. Interestingly, Mounkes and Fuller (1999) reported that hay^{nc2} mutant also accumulates a truncated product of similar size to the one found in this work, suggesting that in the fly some *hay* mutations produce a nonstable product. It has not been tested whether this truncated protein retains some function or whether it could be assembled into TFIIH complexes.

Wing Defects in hay Flies Can Be Associated with Defects in DNA Repair during Development

Both $cdk7^{P140S}$ and *hay* flies have aberrant wings, but these defects are different in each case. Results presented herein show that the wing phenotype present in $cdk7^{P140S}$ (phenotype B) is not enhanced by most of the *hay* alleles, with the exception of hay^{nc2rv8} allele (see below). This suggests that wing defects observed in both mutants are due to deficiencies in different processes. Wing defects in *cdk7* mutant can be due to a deficiency in the cell cycle control and/or in transcription (Larochelle *et al.*, 1998; Leclerc *et al.*, 2000). However, an allele-specific interaction with hay^{nc2rv8} is observed also for wing defects. hay^{nc2rv8} allele has a modest effect in the bristles in combination with hay^{nc2}, but it does not cause any wing defects in this situation (Table 1). In contrast, a single copy of this allele in the presence of a wild-type *hay* gene strongly enhances $cdk7^{P140S}$ wing phenotype (Table 3, in bold). We propose that the nature of these two mutations produces an interaction that has a strong effect on TFIIH activity, most likely affecting transcription. Conversely, aberrant wings observed in *hay* heteroallelic combinations (phenotype A) seem not to be related to transcription. This hypothesis is supported by the fact that most of the *hay* alleles do not increase wing phenotype B observed in $cdk7^{P140S}$ flies. Taking these results plus the fact that apoptosis is not detected in $cdk7^{P140S}/hay^{nc2rv8}$ flies as it occurs in the hay^{nc2}/hay^{nc2} background, we suggest that aberrant wings observed in *hay* mutant flies are not due to transcription but to DNA repair.

Defects in hay Increase Apoptosis during Fly Development: Interaction with Dmp53

A higher rate of apoptotic bodies is detected in *hay* heteroallelic combinations and in hay^{nc2} homozygous flies. Apoptosis is also dramatically increased in *hay* imaginal discs after UV irradiation but not in wild-type discs. As stated above, apoptosis is not observed in *cdk7* mutant flies, suggesting that the presence of a larger number of apoptotic cells in *hay* flies is due to abnormal DNA repair. These results suggest that DNA repair function of TFIIH is required during development even without the challenge of external physical or chemical agents that damage DNA. This is the first in vivo evidence that defective *hay*, an *XPB* homolog, may induce cell death during development. The TFIIH function in DNA repair could be either in NER or in BER. TFIIH is required for TCR of 8-oxo-guanine and thymine glycol caused by oxidative damage of DNA (Le Page *et al.*, 2000). It has been proposed that in CS patients the accumulation of 8-oxo-guanine and/or thymine glycol caused by defective TCR may induce apoptosis during development of the nervous system (Hanawalt, 2000). We also propose that apoptosis observed in *hay* imaginal discs and in CNS is mostly due to an accumulation of oxidative damage that normally occurs during development. This accumulation of oxidative damage may be caused by defective TFIIH.

Interestingly, the presence of a *hay* mutant allele suppresses the penetrance of the wing defects due to the overexpression of *Dmp53* in wing disc. It is remarkable that this suppression is fully penetrant (Table 4), showing a clear genetic interaction between *Dmp53* and *hay*. This information is of particular relevance because it has been reported that primary cultured fibroblast derived from individuals with xeroderma pigmentosum, which are deficient in DNA repair by mutations in XPD or XPB, are not able to undergo p53-induced apoptosis (Wang *et al.*, 1996). This deficiency can be rescued by transferring the wild-type XPD or XPB genes in the corresponding mutant cells, suggesting that XPB and XPD are components of the p53-mediated apoptosis pathway (Wang *et al.*, 1996). The results presented in this work are in agreement with this information and confirm that the fly is an excellent animal model to study the role of TFIIH during development. However, a paradox emerges from these results. Mutations in *hay* induce apoptosis during development, but the same mutations sup-

press the apoptotic effect of the *Dmp53* overexpression. *Dmp53* apparently needs an intact TFIIH to induce apoptosis as in cultured human cells from XP patients. Then how is the apoptosis produced by *hay* mutations activated? It is possible that the apoptosis seen in *hay* mutants could be triggered by a *Dmp53*-independent mechanism. Alternatively, it cannot be ruled out that *Dmp53* may still respond to other molecules that may activate it as a response to DNA damage produced by a defect in TFIIH during development. TFIIH is able to phosphorylate p53 in vitro, and the phosphorylated p53 is able to bind more efficiently to its target DNA sequences (Lu *et al.*, 1997). There is evidence that p53 can modulate the TFIIH-associated nucleotide excision repair activity (Wang *et al.*, 1995). Thus, there is an intricate cross talk between p53 and TFIIH.

In conclusion, mutations in TFIIH components produce as diverse phenotypes in *Drosophila* as they do in humans. Some of these defects are caused by faulty transcription, such as the bristle and cuticular phenotypes. Other defects, such as aberrant wings in *hay* flies are related to problems in DNA repair, which could increase cell death during development. A genetic interaction between *hay* and the fly homolog of p53 seems to be similar in some aspects to what has been found with human alleles in the same genes, confirming the value of the fly as a model for human diseases produced by mutations in TFIIH. Studies of humans affected in TFIIH have proposed that some mutant alleles of *XPB* and *XPD* genes may impair only DNA repair or transcription and others may affect both. Most of the *hay* (*XPB* homolog) alleles analyzed in this work seem to affect both mechanisms. This work shows that by using *Drosophila* genetics, the developmental role of the different components of the TFIIH complex can be analyzed in detail and that this information is relevant for the analysis of the syndrome manifestations in humans.

ACKNOWLEDGMENTS

We thank Dr. M.A. West and J. Sepúlveda for scanning microscopy; Biol. L. López for histological sample preparation and electron microscope analysis; Dr. Patricia León, Dr. Veronica Narvaez, M.C. Miranda Gonzalez, and Dr. Diana Escalante for commenting on the manuscript; V. Barajas for technical support; Dr. Margaret Fuller for providing the different *hay* alleles used in this work; and the Bloomington stock center. We also thank Drs. Gerald Rubin and Casey Kopczynski for providing the different *Dmp53* stocks and Dr. Juan Riesgo-Escovar for the *GAL-4* driver. This work was supported by the Consejo Nacional de Ciencia y Tecnologia Grant 31786, DGPA Grant IN-200799, and the Howard Hughes Medical Institute Grant 55003712.

REFERENCES

Abrams, J.M., White, K., Fessler, L.I., and Steller, H. (1993). Programmed cell death during *Drosophila* embryogenesis. Development *117*, 29–43.

Brodsky, M.H., Nordstrom, W., Tsang, G., Kwan, E., Rubin, G.M., and Abrams, J.M. (2000). *Drosophila* p53 binds a damage response element at the *reaper* locus. Cell *101*, 103–113.

Buratowski, S. (1993). DNA repair and transcription: the helicase connection. Science *260*, 37–38.

Burnette, W. (1981). Electrophoretic transfer of proteins from SDS-polyacrilamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein. Anal. Biochem. *112*, 195–203.

Capdevila, J., and Guerrero, I. (1994). Target expression of the signaling molecule decapentaplegic induces pattern duplications and growth alterations in *Drosophila* wings. EMBO J. *13*, 4459–4468.

Chen, X., Ko, L.J., Jayaraman, L., and Prives, C. (1996). p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells. Genes Dev. *10*, 2438–3451.

Cleaver, J.E. (2000). Common pathways for ultraviolet skin carcinogenesis in the repair and replication defective groups xeroderma pigmentosum. J. Dermatol. Sci. *23*, 1–11.

Conaway, J.W., and Conaway, R.C. (1999). Transcription elongation and human disease. Annu. Rev. Biochem. *68*, 301–319.

de Boer, J., Donker, I., de Wit, J., Hoeijmakers, J.H.J., and Weeda, G. (1998a). A mouse model for the basal transcription/DNA repair syndrome trichothiodystrophy. Mol. Cell *1*, 981–990.

de Boer, J., Donker, I., de Wit, J., Hoeijmakers, J.H.J., and Weeda, G. (1998b). Disruption of the mouse xeroderma pigmentosum group D DNA repair/basal transcription gene results in preimplantation lethality. Cancer Res. *58*, 89–94.

de Boer, J., and Hoeijmakers, J.H.J. (1999). Cancer from outside, aging from the inside: mouse models to study the consequences of defective nucleotide excision repair. Biochimie *81*, 127–137.

de Boer, J., and Hoeijmakers, J.H.J. (2000). Nucleotide excision repair and human syndromes. Carcinogenesis *21*, 453–460.

Feaver, W.J., Svejstrup, J.Q., Henry, N.L., and Kornberg, R.D. (1994). Relationship of CDK-activating kinase and RNA polymerase II CTD kinase TFIIH/TFIIK. Cell *79*, 1103–1109.

Friedberg, E.R. (1992). Xeroderma pigmentosum, Cockayne's syndrome, helicases, and DNA repair: what's the relationship? Cell *71*, 887–889.

Friedberg, E.R. (1996). Relations between DNA-repair and transcription. Annu. Rev. Biochem. *65*, 15–42.

Frinstrom, D., and Frinstrom, J.W. (1993). The metamorphic development of the adult epidermis. In: The Development of *Drosophila melanogaster*, vol. II, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 843–898.

Frinstrom, D., and Liebrich, W. (1986). The hormonal coordination of cuticulin deposition and morphogenesis in *Drosophila* imaginal disc *in vivo* and *in vitro*. Dev. Biol. *114*, 1–11.

Ford, J.M., and Hanawalt, P.C. (1995). Li-Fraumeni syndrome fibroblasts homozygous for p53 mutations are deficient in global DNA repair but exhibit normal transcription-couple repair and enhance UV resistance. Proc. Natl. Acad. Sci. USA *92*, 8876–8880.

Gerber, H., Hagmann, M., Seipel, K., Georgiev, O., West, M.A.L., Litingtung, Y., Schaffner, W., and Corden, J.L. (1995). RNA polymerase II C-terminal domain required for enhancer-driven transcription. Nature *374*, 660–662.

Hanawalt, P.C. (2000). The bases for Cockayne's syndrome. Nature *405*, 415–416.

Itin, P.H., and Pittelkow, M.R. (1990). Tricothiodystrophy: review of sulfur-deficient brittle hair syndromes and association with the ectodermal dysplasias. J. Am. Acad. Dermatol. *20*, 705–717.

Koken, M.H.M., Vreeken, C., Bol, S.A.M., Cheng, N.C., Jaspers-Dekker, I., Hoeijmakers, H.J.H., Eeken, J.C.J., Weeda, G., and Pastink, A. (1992). Cloning and characterization of the *Drosophila* homologue of the xeroderma pigmentosum complementation-group B correcting gene, *ERCC3*. Nucleic Acids Res. *20*, 5541–5548.

Larochelle, S., Pandur, J., Fisher, R.P., Salz, H.K., and Suter, B. (1998). Cdk7 is essential for mitosis and for in vivo Cdk-activating kinase activity. Genes Dev. *12*, 370–381.

Leclerc, V., Raisin, S., and Léopold, P. (2000). Dominant-negative mutants reveal a role for the Cdk7 kinase at the mid-blastula transition in *Drosophila* embryos. EMBO J. *19*, 1567–1575.

Lehmann, A.R. (1998). Dual functions of DNA repair genes: molecular, cellular and clinical implications. BioEssays *20*, 146–155.

Lehmann, A.R. (2001). The xeroderma pigmentosum group D (XPD) gene: one gene, two functions, tree diseases. Genes Dev. *15*, 15–23.

Le Page, F., Kwoh, E., Avrutskaya, A., Gentil, A., Leadon, S., Sarasin, A., and Cooper P. (2000). Transcription-coupled repair of 8-oxoGuanine: requirement for XPG, TFIIH, and CSB and implications for Cockayne syndrome. Cell *101*, 159–171.

Lindsley, D.L., and Zimm, G.G. (1990). The genome of *Drosophila melanogaster*, San Diego: Academic Press.

Ljungman, M., and Fenfen, Z. (1996). Blockage of RNA polymerase as possible trigger for UV light-induced apoptosis. Oncogene *13*, 823–831.

Lu, H., Fisher, R.P., Bailey, P., and Levine A.J. (1997). The CDK7-CycH-p36 complex of transcription factor IIH phosphorylates p53, enhancing its sequence-specific DNA binding activity *in vitro*. Mol. Cell. Biol. *17*, 5923–5934.

Merino, E., Osuna, J., Bolívar, F., and Soberón, X. (1992). A general, PCR-based method for single or combinatorial oligonucleotide-directed mutagenesis on pUC/M13 vectors. Biotechniques *12*, 508–510.

Mounkes, L.C., Jones, R.S., Bee-Choo, L., Gelbart, W., and Fuller, M.T. (1992). A *Drosophila* model for xeroderma pigmentosum and Cockayne's syndrome: *haywire* encodes the fly homologue of *ERCC3*, a human excision repair gene. Cell *71*, 925–937.

Mounkes, L.C., and Fuller, M.T. (1999). Molecular characterization of mutant alleles of the DNA repair/basal transcription factor *haywire/ERCC3* in *Drosophila*. Genetics *152*, 291–297.

Nance, M.A., and Berry, S.A. (1992). Cockayne Syndrome: review of 140 cases. Am. J. Med. Genet. *42*, 68–84.

Ollman, M., *et al*. (2000). *Drosophila* p53 is a structural and functional homologue of the tumor suppressor p53. Cell *101*, 91–101.

Reynaud, E., Lomelí, H., Vázquez, M., and Zurita, M. (1999). The *Drosophila melanogaster* homologue of the xeroderma pigmentosum D gene product is located in euchromatic regions and has a dynamic response to UV light-induced lesions in polytene chromosomes. Mol. Biol. Cell *10*, 1191–1203.

Riou, L., Zeng, L., Chevallier-Lagente, O., Stary, A., Nikaido, O, Taieb, A., Weeda, G., Mezzina, M., and Sarasin, A. (1999). The relative expression of mutated XPB genes results in xeroderma pigmentosum/Cockayne's syndrome or trichothiodystrophy cellular phenotypes. Hum. Mol. Genet. *8*, 1125–1133.

Robles, A.I., Wang, X.W., and Harris, C.C. (1999). Drug-induced apoptosis is delayed and reduced in XPD lymphoblastoid cell lines: possible role of TFIIH in p53-mediated apoptotic cell death. Oncogene *19*, 4681–4688.

Rolig, R.L., and McKinnon, P.J. (2000). Linking DNA damage and neurodegeneration. T. Neurobiol. Sci. *23*, 417–423.

Rossi, D.J., Londesborough, A., Korsisaari, N., Pihlak, A., Lehtonen, E., Henkemeyer, M., and Mäkelä, T.P. (2001). Inability to enter S phase and defective RNA polymerase II CTD phosphorylation in mice lacking Mat1. EMBO J. *20*, 2844–2856.

Rossignol, M., Kolb-Cheynel, I., and Egly, J.M. (1997). Substrate specificity of the cdk-activating kinase (CAK) is altered upon association with TFIIH. EMBO J. *16*, 1628–1637.

Roter, A.H., Spofford, J.B., and Swiff, S. (1985). Synthesis of the major adult cuticle proteins of *Drosophila melanogaste*r during hypoderm differentiation. Dev. Biol. *107*, 420–431.

Roy, R., Adamczewski, J.P., Seroz, T., Vermeulen, W., Tassan, J.-P., Schaeffer, L., Nigg, E.A., Hoeijmakers, J.H.J., and Egly, J.M. (1994). The MO15 cell cycle kinase is associated with the TFIIH transcription-DNA repair factor. Cell *79*, 1093–1101.

Schaeffer, L., Roy, R., Humbert, S., Moncolin, V., Vermeulen, W., Hoeijmakers, J.H.J., Chambon, P., and Egly, J.M. (1993). DNA repair helicase: a component of BTF2 (TFIIH) basic transcription factor. Science *260*, 58–63.

Serizawa, H., Makela, T.P., Conaway, J.W., Conaway, R.C., Weinberg, R.A., and Young, R.A. (1995). Association of Cdk-activation kinase subunits with transcription factor TFIIH. Nature *374*, 280–282.

Shiekhattar, R., Mermelstein, F., Fisher, R.P., Dynlacht, B., Wesslilng, H.C., Morgan, D.O., and Reinberg, D. (1995). Cdk-activating kinase complex is a component in human transcription factor TFIIH. Nature *374*, 283–287.

Smith, D.B. (1993). Purification of glutathione-S-transferase fusion proteins. Methods Mol. Cell. Biol. *4*, 220–229.

Spradling, A.C., and Rubin, G.M. (1982). Transposition of cloned P-elements into *Drosophila* germline chromosomes. Science *218*, 341–347.

Stathakis, D.G., Burton, D.Y., McIvor, W.E., Krishnakumar, S., Wright, T.R., and O'Donell J.M. (1999). The catecholamines up (Catsup) protein of *Drosophila melanogaster* functions as a negative regulator of tyrosine hydroxylase activity. Genetics *153*, 361–382.

Svejstrup, J.Q., Wang, Z., Feaver, W.J., Wu, X., Bushnell, D.A., Donahue T.F., Friedberg, E.C., and Kornberg, R.D. (1995). Different forms of TFIIH for transcription and DNA repair: holo-TFIIH and a nucleotide excision repairosome. Cell *80*, 21–28.

Taylor, M.E., Bernard, C.B., Botta, E., Stefanini, M., Sarasin, A., Jaspers, N.G.J., Fawcett, H., A., Colin, F.A., and Lehmann, A.R. (1997). Xeroderma pigmentosum and trichothiodystrophy are associated with different mutations in the XPD (ERCC2) repair/transcription gene. Proc. Natl. Acad. Sci. USA *94*, 8658–8663.

Tilney, L.G., Connelly, P.S., Vranich, K.A., Shaw, M.K., and Guild, G.M. (2000). Regulation of actin filament cross-linking and bundle shape in *Drosophila* bristles. J. Cell Biol. *148*, 87–99.

Vermeulen, C.E., and Hoeijmakers, J.H. (2000). Transcriptional healing. Cell *101*, 447–450.

Vigoreaux J., O., and Tobin, S.L. (1987). Stage-specific selection of alternative transcriptional initiation sites from the 5C actin gene of *Drosophila melanogaster*. Genes Dev. *10*, 1161–1171,.

Wang, X.W., *et al*. (1996). The XPB and XPD helicases are components of the p53-mediated apoptosis pathway. Genes Dev. *10*, 1219–1232.

Wang, X.W., Yeh, H., Schaefer, L., Roy, R., Moncollin, V., Egly, J.M., Wang, Z., Friedberger, E.C., Evans, M.K., and Taffe, B.G. (1995). p53 modulation of TFIIH-associated nucleotide excision-repair activity. Nat. Genet. *10*, 188–195.

Weeda, G., Eveno, E., Donker, I., Vermeulen, W., Chevallier-Lagente, O., Taieb, A., Stary, A., Hoeijmakers, J.H.J., Mezzina, M., and Sarasin, A. (1997). A mutation in the *XPB/ERCC3* DNA repair transcription gene, associated with trichothiodystrophy. Am. J. Hum. Genet. *60*, 320–329.

Weeda, G., van Ham, R.C.A., Vermeulen, W., Bootsma, D., van der Eb, A.J., and Hoeijmakers, J.H.J. (1990). A presumed DNA helicase encoded by ERCC-3 is involved in repair disorders xeroderma pigmentosum and Cockayne's syndrome. Cell *62*, 777–791.

Wood, R.D. (1996). DNA repair in eukaryotes. Annu. Rev. Biochem. *65*, 135–167.

Yankulov, Y.K., and Bentley, D.L. (1997). Regulation of CDK7 substrate specificity by MAT1 and TFIIH. EMBO J. *16*, 1638–1646.