Molecular Characterization of Mutant Alleles of the DNA Repair/ 
Basal Transcription Factor haywire/ERCC3 in Drosophila

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ABSTRACT

The haywire gene of Drosophila encodes a putative helicase essential for transcription and nucleotide excision repair. A haywire allele encoding a dominant acting poison product, lethal alleles, and viable but UV-sensitive alleles isolated as revertants of the dominant acting poison allele were molecularly characterized. Sequence analysis of lethal haywire alleles revealed the importance of the nucleotide-binding domain, suggesting an essential role for ATPase activity. The viable hay⁻² allele, which encodes a poison product, has a single amino acid change in conserved helicase domain VI. This mutation results in accumulation of a 68-kD polypeptide that is much more abundant than the wild-type haywire protein.

The haywire locus of Drosophila encodes the fly homolog of ERCC3, a human gene associated with the DNA repair deficiency disease xeroderma pigmentosum group B (XP-B) (Mounkes et al. 1992). Mutations in the Saccharomyces cerevisiae homolog SSL2/RAD25 are defective in overall nucleotide excision and transcription-coupled repair (Swedner and Hanawalt 1994). In addition, these proteins appear to play a role in transcription. The human ERCC3 (Schaeffer et al. 1993) and S. cerevisiae SSL2/RAD25 (Feaver et al. 1993) gene products are found to be associated with the basal transcription factor TFIIH. Purified TFIIH complements the excision repair defect of lysates from cells lacking ERCC3 function (Drapkin et al. 1994). Consistent with a function in transcription, SSL2/RAD25 is an essential gene (Park et al. 1992), and a temperature-sensitive allele of SSL2/RAD25 inhibits transcription at the restrictive temperature (Qui et al. 1993). Different alleles of SSL2/RAD25 show defects that can separate the roles of the protein in excision repair and transcription (Guzder et al. 1994). As for SSL2/RAD25 of yeast, extreme alleles of haywire are lethal (Regan and Fuller 1990), whereas viable mutant alleles are UV sensitive (Mounkes et al. 1992). Only a few cases of XP-B are known to date, suggesting that ERCC3 is also essential in humans, and that only patients carrying special weak mutant alleles of ERCC3 survive to birth (Weeda et al. 1990; Vermeulen et al. 1994). A viable point mutation in ERCC3 now defines a new trichothiodystrophy complementation group which, however, does not cause XP-B (Weeda et al. 1997).

The fly, yeast, and human ERCC3 homologs show hallmark conserved domains characteristic of the superfamily of DNA/RNA helicases (Gorbalenya et al. 1989; Linder et al. 1989). Two of these, the predicted nucleotide- and magnesium-binding domains, are responsible for the essential ATPase activity of the SSL2/RAD25 (Park et al. 1992) and ERCC3 (Ma et al. 1994a) products. Helicase activity is associated with the TFIIH transcription complex (Schaeffer et al. 1993; Drapkin et al. 1994), and helicase activity of the ERCC3 protein has been demonstrated in vitro (Ma et al. 1994b). Helicase activity of ERCC3 is required for the remaining conserved helicase domains, which have been shown by mutational analysis to function in excision repair of UV-sensitive cell cultures (Ma et al. 1994a).

The Drosophila ERCC3 homolog haywire was originally identified through an unusual mutant allele, hay⁻², that acted as a dominant enhancer of mutations in the testis-specific β-tubulin B2t. Males heterozygous for either hay⁻² or B2tnull and wild type are fertile at 25°C. However, flies transheterozygous for one copy of hay⁻² and one copy of B2tnull are male sterile at 25°C. The genetic interaction between hay⁻² and B2tnull suggested the hay⁻² allele encoded a poison product because flies transheterozygous for B2t mutations and a deficiency of haywire were male fertile (Regan and Fuller 1988). Revertant alleles of hay⁻² abolish the genetic interaction between hay⁻² and B2tnull, but still result in novel abnormal phenotypes with respect to male fertility (Regan and Fuller 1990), suggesting that a poison product encoded by the hay⁻² allele is responsible for the failure to complement tubulin mutations.

In this article, we report the molecular characterization of mutations that identify regions of haywire that are important either for viability or DNA repair. In addition, we show data indicating that the hay⁻² allele causes production of a 68-kD polypeptide that accumulates to much higher levels than wild-type haywire protein. Mutant haywire alleles that revert the genetic interaction between hay⁻² and B2tnull abrogate production of the
stable, truncated protein, suggesting that the same defect causes accumulation of the 68-kd polyepitide and the poison product activity.

**MATERIALS AND METHODS**

**Fly strains and culture:** Fly stocks and crosses were maintained using standard fly media at 25°. Balancer chromosomes, visible markers, Df(1)w, B2thd, and Df(3L)E(z)7e1 (67E1-4:67F1-3) are described in Lindley and Zimm (1992). The following haywire alleles were used: haync2, a recessive male sterile mutation that fails to complement mutations in B2t (Regan and Fuller 1988), and six alleles recovered as revertants of the failure of haync2 to complement B2thd (Regan and Fuller 1990). Fertility of mutant combinations of alleles was determined by testis dissections, as described (Mounkes et al. 1992).

**Sequencing of haywire alleles:** The coding region of haywire was divided into halves by two sets of PCR primers, each amplifying ~1.5-kb fragments from haywire. Primers were used to amplify the 5’ half of haywire were 5’-GGAGAGCTTGCCATACGCT GTGGTAC-3’, and 5’-CCAAGGGCCATACGCTGATGCCGTC CCC-3’. Primers used to amplify the 3’ half of haywire were 5’-GGGCCTGTTGAATTTGTTGACACCATAC-3’ and 5’-GGCTGTTGGGTTACTGACGTGGTAAAG-3’. For the viable alleles haync2 and haync2rv1,2,3,7,8, templates for the PCR reactions were genomic DNA made from flies heterozygous for the viable haywire allele and a deficiency for haywire, Df(3L)E(z)7e1. For lethal alleles, template DNA for the PCR reactions was made from flies heterozygous for the lethal revertant allele and the parent chromosome, haync2. PCR products were purified and sequenced as described (Sanger et al. 1977; Biggin et al. 1983; Kretz et al. 1989). One strain of the revertant alleles was sequenced, except in areas where there were changes. Changes were verified by sequencing the complementary strand.

**Northern analysis:** Total RNA was isolated by RNAzol extraction according to Cinna Scientific. RNA (30 μg) was run on 0.1 m sodium phosphate gel, blotted onto a Hybond membrane (Amersham, Arlington Heights, IL), and hybridized as described (Sambrook et al. 1989). Blots were probed with a haywire riboprobe made from a HindIII-linearized cDNA construct (Mounkes et al. 1992) and a random-primed-labeled 1.5-kb EcoRI-Sall fragment from the plasmid containing p49 (O’Connell and Rosbash 1984).

**Western analysis:** Protein samples were made by grinding 20 adult flies in an Eppendorf tube in 0.15 ml of solution A (0.1 m Tris, pH 9.0, 0.1 m EDTA, 1% SDS, 0.5% DEPC) plus 1 mm PMSF. Samples were spun in the microforge for 30 sec to pellet large body parts. Supernatants were transferred to a new tube, 10 μl each were brought to 1× sample buffer (Laemmli 1970), and proteins were separated by 10% SDS-PAGE. Gels were blotted to nitrocellulose (Schleicher & Schuell, Keene, NH) as described (Towbin et al. 1979). Haywire was visualized by enhanced chemiluminescence (ECL) staining as described by the manufacturer (Amersham). Affinity-purified primary antibody was used at 1:1500. Horseradish peroxidase-conjugated donkey anti-rabbit secondary antibody (Amersham) was used at 1:4000.

**Germline transformations:** PCR mutagenesis of a 380-nucleotide BglII-Sad fragment of the 3’ end of the haywire cDNA was performed to introduce the same C-to-T transition found according to Cinna Scientific. RNA (30 μg) was run on 0.1 m sodium phosphate gel, blotted onto a Hybond membrane (Amersham, Arlington Heights, IL), and hybridized as described (Sambrook et al. 1989). Blots were probed with a haywire riboprobe made from a HindIII-linearized cDNA construct (Mounkes et al. 1992) and a random-primed-labeled 1.5-kb EcoRI-Sall fragment from the plasmid containing p49 (O’Connell and Rosbash 1984).

**TABLE 1**

Alterations in lethal and viable haywire alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>Type a</th>
<th>Nucleic acid change</th>
<th>Amino acid change</th>
<th>Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>haync2</td>
<td>L</td>
<td>CAG → TAG</td>
<td>Gln → STOP</td>
<td>657</td>
</tr>
<tr>
<td>haync2v</td>
<td>L</td>
<td>TGC → TAC</td>
<td>Cys → Tyr</td>
<td>377</td>
</tr>
<tr>
<td>haync3</td>
<td>L</td>
<td>CGA → TGA</td>
<td>Arg → STOP</td>
<td>380</td>
</tr>
<tr>
<td>haync7</td>
<td>L</td>
<td>TGG → TGA</td>
<td>Trp → STOP</td>
<td>441</td>
</tr>
<tr>
<td>haync4</td>
<td>V, uv</td>
<td>GAG → AAG</td>
<td>Glu → Lys</td>
<td>278</td>
</tr>
<tr>
<td>haync12</td>
<td>L</td>
<td>Rearranged</td>
<td>Deletes GAGKS</td>
<td>265-269</td>
</tr>
<tr>
<td>haync4d</td>
<td>V, uv</td>
<td>CGT → TGT</td>
<td>Arg → Cys</td>
<td>652</td>
</tr>
</tbody>
</table>

a haywire alleles are either lethal (L) or viable (V) and UV sensitive (uv).

b Single nucleotide changes in haync2rv1,2,3,7,8 and haync4 are consistent with the mode of induction of these alleles by EMS mutagenesis (Regan and Fuller 1990), a potent inducer of point mutations (Ashburner 1989) that favors GC-to-AT transitions (Williams and Shaw 1987).

c The haync2rv1,2 mutation was induced by diepoxybutane (L. C. Mounkes and K. Schuske, unpublished results), a known inducer of deficiencies (e.g., Olsen and Green 1982).
MGPPKSKDRSGGDKFKGGEAGDFQPVDNDSLPDSESQGAASKAETNDDQTINTQMEGKYSQMQPQHPGHPVWWAPPNGIMELFESQPYXKANDHPDLIAEYPEVRPEHIEYKLTAISYLANDVGLQTHIDIVEYLAKRKSCTSPEGILFIRLCILSYGKVLVHKNYEFIESPHPELVKLDKQPIQKCRISGEDEOFQRTLQLDKPAIQTQFGTKLP

WT

P

XXX

K

T

P

XXX

K

KIEVIQKRIEIEHPILAEYDPDNTPNDPINDKPLPAVLPQLYQKSLKMWFGNRA

rv1

rv2

rv3

---Nucleotide Binding (Dom. I)---

rv7

$S$

SEAKDKPMGCGLVLTYSMTTHQKSWAEQTRMNWLEQENGCIWYDEVHEATMPAMFR

---My2 bind. (Dom. III)---

RVLTVQH5CKLGLTALTLLREDKDIADNLFLIGKLEYANKLQKGGYIAVRGCAEVW

---Dom. III------

CPMSPEPYRELTKTSSKLLYVNPSSKFRSCQLTKHYEQGDQKITDVSDNFVKFITY

ALKNMFFITGYPTSGONERIQILONQFKNXKIVTIVSNADTSFDPLEANWQLTISSHG

-----Dom. IV-------Dom. V-------

nc2

rv1

WT

C

S

H

GSRQEAQLRQGRILRAKKGGAIEAEYNAFFYWTLSQDTMEMYSISRKRQLFVNGQSYKV

-----Dom. VI------

ITHLKGGMDSLSMLGQTESEQQLVLQVLASDLDCEDEKLPGEYPGSNGSGYRRV

GG5LSM53GDOAAYHEHKNKNGSIVPFLKFRG

Figure 1.—Alterations in lethal and viable haywire alleles. Amino acid changes are indicated above the appropriate residues. X indicates sequences deleted in the e1 wild-type strain; $ indicates a mutation to a stop codon. The conserved helicase domains are defined by dashed lines under the sequences. The asterisk indicates the conserved lysine within the P loop of the nucleotide-binding domain. WT, wild type; rv2, haync2rv2; rv3, haync2rv3; rv6, haync2rv6; rv12, haync2rv12; rv7, haync2rv7; rv1, haync2rv1. Alleles in boldface type are homozygous viable. All other mutant haywire alleles are lethal.

excised with HindIII and cloned into the pCaSpeR transformation vector (Pirotta et al. 1988) previously linearized with HindIII and treated with calf intestinal phosphatase (CIP). All cloning junctions within haywire and the mutated site were sequenced to verify sequence integrity at the cloning junctions and introduction of the mutation. Restriction enzymes, CIP, and Tα DNA polymerase were purchased from Boehringer Mannheim (Indianapolis). DNA (200 μg/ml) twice purified by cesium chloride banding was injected by standard methods (Rubin and Spradling 1982) into Df(1)w embryos with p[yw, Δ2–3] (Robertson et al. 1988) at 200 μg/ml as a source of transposase. One transformant linked to the X chromosome was isolated.

RESULTS

Molecular lesion in the haync2 allele responsible for the dominant genetic interaction with B2t: The original haync2 mutation caused replacement of arginine 652 with a cysteine (Table 1) in conserved helicase domain VI (Figure 1). This missense mutation was present in all the revertant alleles of haync2, as expected. As haync2 homozygotes are viable, arginine 652 is not absolutely required for haywire function in basal transcription, but it may be important in DNA repair since the haync2 allele is UV sensitive (Mounkes et al. 1992).

The haync2 mutation resulted in accumulation of a polypeptide that migrated on SDS polyacrylamide gels as 68 kD, cross-reacted with anti-haywire antiserum, and was present in all the lethal alleles of haywire. WT in first lanes (A and B) indicates homogenates from the wild-type background strain, red e, on which the haync2 mutation and subsequent revertant alleles were made. Positions of molecular weight markers are indicated on the left in kilodaltons. (A) Mutant haywire alleles over a wild-type balancer. (B) Comparison of mutant haywire alleles in combination with a deficiency (Δf) or wild-type balancer chromosome (+).

Figure 2.—The haync2 mutation results in accumulation of a 68 kD polypeptide (nc2) not seen in the wild type. Western analysis of whole-fly homogenates from haywire alleles. WT in first lanes (A and B) indicates homogenates from the wild-type background strain, red e, on which the haync2 mutation and subsequent revertant alleles were made. Positions of molecular weight markers are indicated on the left in kilodaltons. (A) Mutant haywire alleles over a wild-type balancer. (B) Comparison of mutant haywire alleles in combination with a deficiency (Δf) or wild-type balancer chromosome (+).
Table 2

| Transgenic animals made by introduction of the Arg652-to-Cys change of hay^B2tnull into a genomic rescue fragment show a genetic interaction with the B2t^null allele |
|---|---|---|
| 1<sup>1</sup> | + B2t^null | hay<sup>nc2</sup> + | Sterile<sup>4</sup> |
| 2<sup>1</sup> | B2t^null/+ | hay<sup>nc2</sup>/+ | 35 | 0 |
| 3<sup>1</sup> | hay<sup>nc2</sup>/+ | B2t^null/+ | 27 | 0 |
| 4 | + B2t^null | Df(hay) + | 32 | 0 |
| 5<sup>1</sup> | P[A3, w<sup>1+</sup>] >; + B2t^null | Df(hay) + | 1 | 51 |
| 6 | P[A3, w<sup>1+</sup>] >; + Df(hay) + | 42 | 0 |
| 7 | P[A3, w<sup>1+</sup>] >; + + B2t^null | 23 | 8 |
| 8 | P[A3, w<sup>1+</sup>] >; + + | 33 | 0 |

Lines 1–3, Sibling progeny from the cross hay<sup>nc2</sup>/TM3 × B2t<sup>null</sup>/TM3.

Line 4, Df(hay) indicates Df(3L)E(z)7e11, a deletion that removes haywire. Test class flies were derived from crossing Df(1)w; B2t<sup>null</sup>/TM3 and Df(1)w; E(z)7e1/TM3 flies. A deletion of hay complements B2t<sup>null</sup>, while the original hay<sup>nc2</sup> allele fails to complement B2t<sup>null</sup>.

Lines 5–7, sibling progeny from the cross P[A3, w<sup>1+</sup>]/P[A3, w<sup>1+</sup>]; Df(3L)E(z)7e1/TM3 virgin females × Df(1)w; riB2t<sup>null</sup>/TM6B males. The hay<sup>nc2</sup> transgene caused failure to complement B2t<sup>null</sup> in the presence of one endogenous wild-type copy of haywire partially alleviated male sterility (line 7), suggesting that the poison product encoded by the hay<sup>nc2</sup> allele on the transgene acts as an antimorph and competes with wild-type haywire protein.

Line 8, The transgene insert did not cause male sterility on its own.

1 Fertility was scored by visual inspection of dissected testes.
2 The original genetic interaction between hay<sup>nc2</sup> and B2t<sup>null</sup>.
3 Control class siblings.
4 P[A3, w<sup>1+</sup>] indicates an insertion on the X chromosome of the mutant copy of haywire, introducing the same Arg652-to-Cys change found in the hay<sup>nc2</sup> allele.

Although these flies were not as sterile as hay<sup>nc2</sup>+/+. Thus, the genetic interaction appeared to be sensitive to the ratio between mutant and wild-type haywire (Table 2, compare lines 5 and 7). Similar behavior was observed in the failure of a missense mutation in α-tubulin to complement B2t<sup>null</sup> (Hays et al. 1989), and it is consistent with hay<sup>nc2</sup> encoding an antimorphic poison that can compete with the wild-type protein. The ArGen52-to-Cys altered transgene caused both accumulation of the 68-kD polypeptide and failure to complement B2t mutations, demonstrating that the single mutation was responsible for both phenotypes associated with hay<sup>nc2</sup>.
A missense mutation (hay<sup>rv2</sup>) and small rearrangement (hay<sup>rv2</sup>) indicate that the nucleotide-binding domain of haywire is essential for viability. The hay<sup>rv2</sup> mutation replaced cysteine 377 with a tyrosine residue (Table 1). This cysteine, which lies eight amino acids downstream of the P loop (Walker et al. 1982) in the nucleotide-binding domain (Figure 1), is conserved among the Drosophila, human, mouse, and yeast homologs. The hay<sup>rv2</sup> lesion was a small chromosomal rearrangement located at the ‘GAGKS’ P loop of the nucleotide-binding site (brackets in Figure 1). All these revertants of the genetic interaction of hay<sup>rd2</sup> also reverted the defect that leads to accumulation of the 68-kD polypeptide (Figure 2).

A viable revertant of hay<sup>rd2</sup>: The hay<sup>rd2v1</sup> viable mutation changed glutamic acid 278 to lysine, introducing a charge change and possibly affecting protein structure or stability. hay<sup>rd2v1</sup> homozygotes are viable, indicating that glutamic acid 278 is not essential for basal transcription. Glu278 could be important for the repair of UV damage, since this allele also showed UV sensitivity (Mounkes et al. 1992). Alternatively, the UV sensitivity could be caused by the still-present hay<sup>rd2</sup> lesion, while the hay<sup>rd2v1</sup> reversion merely alleviated the poison nature of the hay<sup>rd2</sup> product. Again, the hay<sup>rd2v1</sup> missense mutation also reverted the defect leading to accumulation of the 68-kD polypeptide characteristic of hay<sup>rd2</sup>.

Altered amino acids encoded by wild-type haywire alleles: The sequences of three wild-type alleles from different genetic backgrounds revealed regions of haywire/ERCC3 that are not essential (Figure 1). A wild-type cDNA from a dp cn bn strain and two different wild-type genomic sequences (red e and e<sup>1</sup>) differed at several positions within the amino acid coding region. Alanine residues at positions 238 and 244 in the dp cn bn and red e backgrounds were changed to threonine and proline, respectively, in wild-type haywire from the e<sup>1</sup> chromosome. Alanine acid 663 was a methionine in the e<sup>1</sup> strain but a leucine in both the red e and dp cn bn strains. In addition, the e<sup>1</sup> wild-type allele of haywire had a four-amino-acid deficiency at positions 253–256 compared with haywireon both the red eand dp cn bn chromosomes. The four consecutive amino acids missing in the e<sup>1</sup> background are valine/valine/alanine/alanine. Most of the wild-type polymorphisms occur in regions that are poorly conserved between the Drosophila, yeast, and mammalian homologs, and may identify residues of haywire not essential for function.

**DISCUSSION**

The single nucleotide mutation in the hay<sup>rd2</sup> allele that causes an arginine-to-cysteine change is responsible for both the production of a shortened polypeptide associated with the hay<sup>rd2</sup> allele and the genetic interaction observed between hay<sup>rd2</sup> and B2t mutations. Several molecular mechanisms of reverting the genetic interac-
tion between hay^\text{nd} and B2^\text{null} are suggested by sequence analysis of the haywire alleles obtained as revertants of hay^\text{nd}. Destruction of the P loop of the nucleotide-binding domain reverted the genetic interaction between hay^\text{y2} and B2^\text{null} (hay^\text{nd}, Table 1) possibly by destroying the ATP-binding or helicase function of the hay^\text{nd} primary product. Two other revertant alleles, hay^\text{y2v3} and hay^\text{y2v8}, caused missense mutations that could alter protein conformation or stability. hay^\text{nd2}, which caused a change from a cysteine to tyrosine, altered a potential disulfide bond partner. hay^\text{y2v8} which caused a change from a glutamic acid to lysine, caused a change from a negatively charged residue to a positively charged residue. Finally, nonsense mutations hay^\text{ndv1}, hay^\text{y2v3}, and hay^\text{y2v7} reverted the genetic interaction between hay^\text{nd} and B2^\text{null}. The finding that at least four out of six revertants of hay^\text{nd} (the three stop codons and the P-loop mutation) are mutations that should knock out haywire function supports the idea that the original hay^\text{nd} allele encodes a poison product.

The shortened polypeptide that accumulates in hay^\text{nd} flies is likely to be either responsible for or a direct result of the poison nature of the hay^\text{nd} allele, as the 68-kD polypeptide no longer accumulated in six out of six mutants that reverted the dominant enhancer effect of hay^\text{y2} (Figure 2). The 68-kD polypeptide that accumulates in hay^\text{y2} flies is not likely to be caused by a simple truncation of the haywire protein near the site of the nd mutation. The stop codon mutation in hay^\text{y2v1} (Table 1) suggests that a simple truncation event near amino acid 652 results in neither production of a poison product nor accumulation of the 68-kD polypeptide (Figure 2). Furthermore, the hay^\text{ndv1} allele is lethal, perhaps because of truncation of the protein within the last conserved helicase domain, whereas the hay^\text{y2} allele is viable (Table 1).

The hay^\text{nd} allele encodes a product that must contain at least some wild-type function, as hay^\text{nd}/Df(hay) flies were fully viable. As the mutation responsible for causing accumulation of the truncated product is not a stop codon, full-length haywire protein could be initially expressed from the hay^\text{nd} allele. It is possible that this full-length but mutant haywire protein could provide sufficient haywire function for viability when initially expressed. However, defects in functioning or processing of the defective protein because of the Arg652-to-Cys mutation could alter or disrupt normally occurring processing of the full-length haywire protein, resulting in accumulation of a shortened form of the protein in hay^\text{y2}. The observation that the 68-kD shortened polypeptide accumulated to much higher levels than the wild-type haywire product also suggests the possibility of turnover of haywire/ERCC3 as a normal part of its function. We would like to raise the possibility that the haywire protein might normally be degraded as part of the functional cycle of TFIIH in transcription initiation. It is also possible that the 68-kD polypeptide that accumulates in hay^\text{y2} flies is the product of some other gene that is upregulated in this particular mutant background. If so, however, the product of this hypothetical upregulated gene would have to cross-react with the affinity-purified, anti-haywire antiserum. In addition, upregulation of the other hypothetical gene appears to depend strictly on the poison product effect of the hay^\text{nd} allele, as the polypeptide was not detected in any of the six revertants of hay^\text{nd}.

The hay^\text{nd} allele genetically interacts with mutations in the B2 locus (Regan and Fuller 1988), which encodes a testis-specific $\beta$-tubulin isoform (Kempf et al. 1979). Levels of $\beta$-tubulin are critical for spermatogenesis in flies. Flies carrying a deficiency for B2 are already close to a threshold requirement of $\beta$-tubulin, since B2^\text{null} heterozygotes are fertile at 25° but sterile at 18° (Fuller et al. 1989). Further decreasing the level of transcription by altering the function of a basal transcription factor might bring the level of expressed $\beta$-tubulin below the critical threshold required for the many microtubule-mediated tasks necessary for spermatogenesis. It is feasible that either full-length haywire protein with the Arg652-to-Cys mutation or accumulation of a 68-kD fragment of the haywire protein in hay^\text{y2} flies could cause such a deleterious effect on transcription, causing the hay^\text{y2} mutation to act as a dominant enhancer of B2 mutations.

We thank Barbara Robertson and Kim Schuske, who conducted genetic screens to identify new revertant alleles of hay^\text{nd}, and Cricket Wood for technical assistance. We thank Stig Hansen and Robert Tjian for help and advice in producing affinity-purified antibodies to the haywire protein. We acknowledge the protein and nucleic acid (PAN) facility, Stanford University, for synthesizing oligonucleotides used in this work. This work was supported by National Institutes of Health grants HD-18127 and HD-29194 to M.T.F.

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