Trans-splicing as a Novel Mechanism to Explain Interallelic Complementation in Drosophila

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ABSTRACT

Two mutant alleles of the same gene, each located in one of the two homologous chromosomes, may in some instances restore the wild-type function of the gene. This is the case with certain combinations of mutant alleles in the *mod(mdg4)* gene. This gene encodes several different proteins, including Mod(mdg4)2.2, a component of the *gypsy* insulator. This protein is encoded by two separate transcription units that can be combined in a *trans*-splicing reaction to form the mature Mod(mdg4)2.2-encoding RNA. Molecular characterization of complementing alleles shows that they affect the two different transcription units. Flies homozygous for each allele are missing the Mod(mdg4)2.2 protein, whereas wild-type *trans*-heterozygotes are able to synthesize almost normal levels of the Mod(mdg4)2.2 product. This protein is functional as judged by its ability to form a functional insulator complex. The results suggest that the interallelic complementation in the *mod(mdg4)* gene is a consequence of *trans*-splicing between two different mutant transcripts. A conclusion from this observation is that the *trans*-splicing reaction that takes place between transcripts produced on two different mutant chromosomes ensures wild-type levels of functional protein.

INTERALLELIC complementation refers to the restoration of the wild-type function of a gene by the combination of two different mutant alleles. Such complementation patterns, which may seem anomalous in the context of classical complementation analyses, have been the subject of very detailed studies. Fundamentally, two classes of mechanisms can account for the observations. One class explains complementation at loci whose alleles retain the ability to encode a mutant protein. Taken individually, mutant proteins do not possess the complete wild-type function of the gene, but retain part of it. The combination of two different mutant proteins can restore the wild function, each protein ensuring a part of it. A classical example is the yeast *HIS4* locus, first thought to encode three different enzymes involved in histidine biosynthesis (Fink 1966) and later shown to actually encode a single polypeptide carrying three separately mutable enzymatic activities (Keesey *et al.* 1979). α-Complementation of β-galactosidase is a second example that also falls into this category (Ullman *et al.* 1967; Jacobson *et al.* 1994; Juers *et al.* 2000). A variation on this theme is defined by mutations affecting the ability of a protein to engage in a homomultimeric functional complex. For example, an essential interaction at the dimerization interface may be lost in a mutant protein, but compensated for by another mutation in another copy of the gene. A classical example is given by the *Escherichia coli* alkaline phosphatase gene (Garen and Garen 1963). Transvection is the basis for the second, radically different, class of explanatory mechanisms. This term, coined by Ed Lewis (Lewis 1954), refers to phenomena that cause gene expression to be sensitive to pairing of homologous chromosomes (for reviews, see Henikoff and Comai 1998; Pirrotta 1999; Wu and Morris 1999). Complementation by transvection is typically observed when an allele carrying a mutation in the coding region is combined with an allele mutant in its regulatory region. The wild-type regulatory region is able to control transcription of the homologous locus *in trans*. Well-studied examples of this phenomenon include the Drosophila *yellow* (Geyer *et al.* 1990), *decapentaplegic* (Gelbart 1982), *white* (Davison *et al.* 1985; Zachar *et al.* 1985), and *bithorax* complex (Martinez-Laborda *et al.* 1992; Muller *et al.* 1999) loci.

Here we report another mechanism that accounts for interallelic complementation in the Drosophila *modifier of mdg4* [*mod(mdg4)*] gene. This gene has coding potential for >25 different mRNAs (Gerasimova *et al.* 1995; Buchner *et al.* 2000). The encoded proteins share four common exons spanning the N-terminal domain and differ by one or two different additional exon(s). These proteins are believed to be involved in the control of nuclear functions through the regulation of chromatin structure. Mutations in the gene show enhancement of position-effect variegation and homeotic transformations and affect viability and fertility of the fly (Dorn *et al.* 1993; Gerasimova *et al.* 1995). *mod(mdg4)* interacts

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genetically with *trithorax* group genes in the transcriptional regulation of homeotic genes (Gerasimova and Corces 1998). The exact function of most *mod(mdg4)*-encoded proteins remains to be determined, and only the Mod(mdg4)2.2 product has been studied in depth using genetic and biochemical approaches (Gerasimova *et al.* 1995). The Mod(mdg4)2.2 protein interacts with Suppressor of Hairy-wing [Su(Hw)], a zinc-finger DNA-binding protein, to form the *gypsy* insulator protein complex (Gerasimova *et al.* 1995; Ghosh *et al.* 2001). This complex, when bound to the insulator DNA sequence, is able to block the action of enhancers on their target promoters when placed between them (for recent reviews on insulators, see Bell *et al.* 2001; Gerasimova and Corces 2001). The *gypsy* insulator is also able to shield a transgene against position effects (Roseman *et al.* 1993). The complex genomic structure of *mod(mdg4)2.2* has been recently described (Labrador *et al.* 2001). The coding regions of some *mod(mdg4)*-encoded transcripts, including Mod(mdg4)2.2, originate from the two strands of the DNA. In addition to the four exons common to all variants, the Mod(mdg4)2.2 mRNA is encoded by two exons lying in the opposite DNA strand. This organization requires the combination of two independent transcripts to produce the final mRNA. The molecular nature of the combination reaction is unknown, but a *trans*-splicing event is the most likely (Dorn *et al.* 2001; Labrador *et al.* 2001). In such a reaction, the splice donor and the splice acceptor sites are present on a separate premessenger RNA. In this article, we show that some *mod(mdg4)* alleles can complement each other by providing part of the information required to encode a final functional Mod(mdg4)2.2 mRNA. In this new type of complementation mechanism the effective complementation is achieved at the RNA level, rather than at the protein or transcription level. This provides a new paradigm with which to interpret interallelic complementation events. It also raises questions regarding genome complexity and evolution and may have an impact on the way we search for new genes in the genomic databases now available.

MATERIALS AND METHODS

Fly crosses: All crosses were performed at 25° on yeast, cornmeal, molasses, and agar medium containing tegosept and propionic acid as mold inhibitors. Two lethal mutations, $mod(mdg4)^{L3101}$ and $mod(mdg4)^{03852}$, were obtained from the Bloomington Stock Center, and a third one, $mod(mdg4)^{E(var)3\text{-}93D}$, was described earlier (Dorn *et al.* 1993). These mutations are caused by *P*-element insertions in the third exon at nucleotide 153,269, in the third intron at nucleotide 153,049, and in the third intron at nucleotide 153,036, respectively (numbers given correspond to the genomic sequence, accession no. AE003734). All three alleles show homozygous larval polyphasic lethality. The $mod(mdg4)^{u1}$ allele is caused by an insertion of the Stalker transposable element in the sixth exon, at nucleotide 137,206; $mod(mdg4)^{T6}$ is a viable EMS-induced point mutation at nucleotide 137,511 (Gdula *et al.* 1996; T. Gerasimova and V. Corces, unpublished results). To select for larvae homozygous for lethal *mod(mdg4)* alleles, stocks were balanced with a *TM6B, P{Ubi-GFP.S65T}* balancer, and nonfluorescent live larvae were picked up. Such larvae die around the second instar stage.

To analyze the effect of lethal *mod(mdg4)* mutations on the activity of the *gypsy* insulator, $y^2\ w\ ct^6;\ mod(mdg4)^{u1}$ and $y^2 w\ ct^6;\ mod(mdg4)^{T6}$ virgin females were crossed with $mod(mdg4)^{E(var)3\text{-}93D}/TM6B$, $mod(mdg4)^{03852}/TM3$, and $mod(mdg4)^{L3101}/TM3$ males, respectively. The $y^2\ w\ ct^6;\ mod(mdg4)^{u1}/mod(mdg4)^{E(var)3\text{-}93D}$, $y^2\ w\ ct^6;\ mod(mdg4)^{T6}/mod(mdg4)^{E(var)3\text{-}93D}$, $y^2\ w\ ct^6;\ mod(mdg4)^{u1}/mod(mdg4)^{083852}$, $y^2\ w\ ct^6;\ mod(mdg4)^{T6}/mod(mdg4)^{083852}$, $y^2\ w\ ct^6;\ mod(mdg4)^{u1}/mod(mdg4)^{L3101}$, and $y^2\ w\ ct^6;\ mod(mdg4)^{T6}/mod(mdg4)^{L3101}$ male offspring were examined for the y^2 and ct^6 phenotypes and compared with the balanced *mod(mdg4)*/TM3 and *mod(mdg4)*/TM6B siblings.

Ectopic expression of the Mod(mdg4)2.2 mRNA: A UAS-Mod(mdg4) 2.2-kb cDNA was constructed by inserting the full-length 2.2-kb cDNA (accession no. U30905) into the cloning site of the pUASp vector (Rorth 1998). The UAS-Mod (mdg4)2.2 plasmid was microinjected into *y w*; P{Δ2-3}/TM6 embryos, and flies carrying the transgene in the second chromosome were selected by standard procedures (Robertson *et al.* 1988). Transcription of this transgene was driven by an Act5C-GAL4 construct (Ito *et al.* 1997). Flies of the genotype $y^2\ w\ ct^6$; *UAS-Mod(mdg4)2.2/act5C-GAL4*; $mod(mdg4)^{u1}/mod(mdg4)^{u1}$ were obtained and the y^2 and ct^6 phenotypes were compared with siblings of the genotype $y^2 w\ ct^6$; *act5C-GAL4/Cyo*; $mod(mdg4)^{u1}/mod(mdg4)^{u1}$.

Protein immunolocalization and Western analysis: Exons 5 and 6 of the sequence encoding Mod(mdg4)2.2 were cloned into pET30 (Novagen, Madison, WI) and the protein was expressed in *E. coli* as a 6× His tag fusion protein and purified on a nickel-agarose column (QIAGEN, Chatsworth, CA) under denaturing conditions. Antibodies were prepared in rats by standard procedures (Pocono Rabbit Farm & Laboratory, Canadensis, PA). Preparation of antibodies against Su(Hw) has been reported previously (Gerasimova and Corces 1998). Western analysis was carried out by standard procedures using the enhanced chemiluminescence kit from Amersham Pharmacia Biotech (Piscataway, NJ) for detection. Protein extracts were prepared from equal amounts of larvae of different genotypes and loaded in the gels. After immunodetection, membranes were stained with Coomassie blue to further control for equal loading of the samples. Immunolocalization of proteins on polytene chromosomes was as previously described (Harrison *et al.* 1993). Proteins were visualized using FITC- or Texas red-conjugated secondary antibodies; chromosomes were examined using a Zeiss Axiophot microscope and a Photometrics cooled CCD camera (Roper Scientific, Trenton, NJ).

RESULTS

Interallelic complementation in the *mod(mdg4)* locus: Figure 1 depicts the genomic organization of the *mod(mdg4)* locus. The molecular lesions associated with mutations used in this study are described in materials and methods and summarized in Figure 1. To dissect the genetic properties of the *mod(mdg4)* locus, a complementation analysis of different mutants affecting the Mod(mdg4)2.2 protein was carried out. The functionality of the protein was assessed by its effect on the *gypsy* insulator using two different phenotypic markers, *cut-6* (ct^6) and *yellow-2* (y^2). Both mutations are caused by the presence of the *gypsy* insulator between the enhancer and promoter sequences of the *cut* and *yellow* genes. In

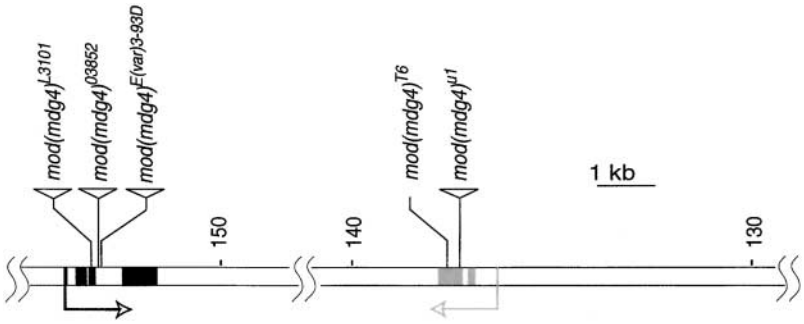

FIGURE 1.—Genomic structure of the *mod (mdg4)2.2* coding sequences. Exons 1–4 are represented by solid boxes, and exons 5 and 6 by shaded boxes. Arrows mark the promoter of each transcription unit and indicate the direction of transcription. Position of the promoter for exon 5 and 6 is inferred at nucleotide 136,626 from sequence analysis using Genescan (BURGE and KARLIN 1997). The locations of different *mod(mdg4)* mutations are indicated above the gene. Numbering in kilobases are with reference to the genomic sequence with accession no. AE003734.

the presence of wild-type Mod(mdg4)2.2 protein, the insulator disrupts the activation of the promoter by the enhancers, resulting in the formation of severe cuts in the wing margins and the loss of abdominal pigmentation (Figure 2, a and b). The *mod(mdg4)^{u1}* and *mod(mdg4)^{T6}* mutations are viable and affect the sequences encoding the carboxy-terminal end of the Mod(mdg4)2.2 protein (Figure 1). When homozygous, these alleles result in the loss of insulator activity and reversion of the *ct^6* and *y^2* phenotypes: the wing margins show only a few notches and the abdomen pigmentation is partially restored (Figure 2, c and d). The three *P*-element-induced mutants of the *mod(mdg4)* gene are lethal during larval stages and, therefore, their effect on the *gypsy* insulator and the *y^2* and *ct^6* phenotypes cannot be assessed in homozygous adult mutant individuals. To determine the effect of these mutations on the functionality of the *gypsy* insulator, we tested their ability to modify the *y^2* and *ct^6* phenotypes in heterozygous combinations with *mod (mdg4)^{u1}* and *mod(mdg4)^{T6}*. Surprisingly, flies of the genotype *mod(mdg4)$^{Evar3\text{-}93D}$/ mod(mdg4)^{u1}* display normal *y^2* and *ct^6* phenotypes, suggesting that the *gypsy* insulator is functionally normal in the background of this combination of *mod(mdg4)* alleles (Figure 2, e and f). The same is true for all other possible combinations between lethal and viable alleles (Figure 2, g and h). These results suggest that mutations affecting the Mod(mdg4)2.2 protein fall into two complementation groups. Group I comprises *mod(mdg4)^{L3101}*, *mod(mdg4)^{03852}*, and *mod(mdg4)$^{E(var)3\text{-}93D}$* whereas group II consists of *mod(mdg4)^{u1}* and *mod(mdg4)^{T6}*. The interallelic complementation observed when crossing group I to group II *mod(mdg4)* alleles is demonstrated by the restoration of strong *ct^6* and *y^2* phenotypes, suggesting the restoration of wild-type insulator function.

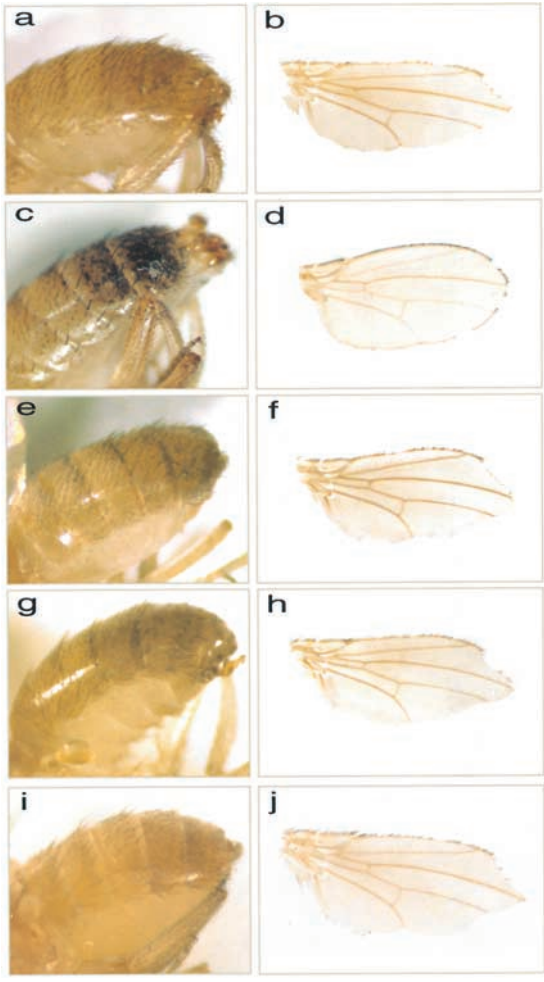

FIGURE 2.—Body cuticle and wing phenotypes of flies with different combinations of *mod(mdg4)* alleles. (a and b) *y^2 ct^6*; *mod(mdg4)^{u1}/+*. (c and d) *y^2 ct^6*;*mod(mdg4)^{u1}/ mod(mdg4)^{u1}*. (e and f) *y^2 ct^6*; *mod(mdg4)^{u1}/ mod(mdg4)$^{E(var)3\text{-}93D}$*. (g and h) *y^2 ct^6*; *mod (mdg4)^{u1}/ mod(mdg4)^{L3101}*. (i and j) Transgenic fly with genotype *y^2 w ct^6; UAS-mod2.2/act5C-GAL4; mod(mdg4)^{u1}/ mod(mdg4)^{u1}*.

The Mod(mdg4)2.2 protein rescues the *mod(mdg4)^{u1}* phenotype: Group I mutations affect the amino-terminal region of all *mod(mdg4)*-encoded proteins, whereas group II mutations affect the carboxy-terminal region of the Mod(mdg4)2.2 protein. Since group II mutations are the only ones that can be shown to have an effect on the *gypsy* insulator, it is possible that only the Mod (mdg4)2.2 protein is involved in *gypsy* insulator function. To demonstrate that the Mod(mdg4)2.2 protein is not only necessary but also sufficient for insulator function we tested whether it can rescue the activity of the *gypsy* insulator in a *mod(mdg4)^{u1}* background. We established transgenic flies carrying the full-length Mod(mdg4)2.2 cDNA under the control of the GAL4 transcriptional activator. These flies also express GAL4 under the control of an actin gene promoter that can drive transcription in all tissues of the fly. This binary

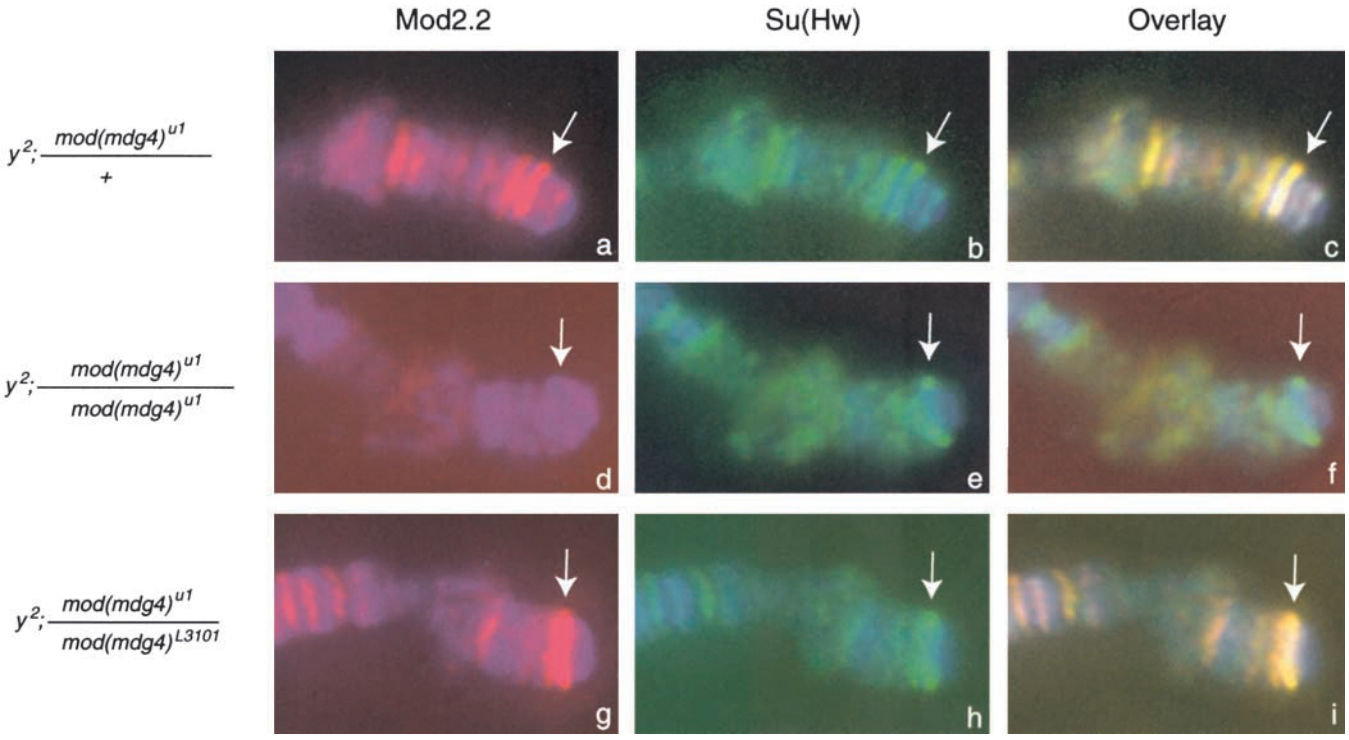

FIGURE 3.—Immunolocalization of Su(Hw) and Mod(mdg4)2.2 proteins carrying the *gypsy*-induced y^2 allele and different combinations of *mod(mdg4)* mutations. Arrows point to the *yellow* locus. The location of Mod(mdg4)2.2 protein is depicted in red and that of Su(Hw) in green. DNA was counterstained with 4′,6-diamidino-2-phenylindole (blue).

system provides constitutive expression of the transgene. Transgenic individuals with a y^2 ct^6; $mod(mdg4)^{u1}$ genotype that express the Mod(mdg4)2.2 protein show y^2 and ct^6 phenotypes, suggesting that the *gypsy* insulator is functional (Figure 2, i and j). This result clearly indicates that the Mod(mdg4)2.2 protein is sufficient to rescue the *mod(mdg4)* function in a $mod(mdg4)^{u1}$ mutant. This construct does not rescue lethality of the group I alleles, indicating that additional products of the *mod (mdg4)* locus are required for viability.

Immunolocalization of the Mod(mdg4)2.2 protein upon interallelic complementation: Combination of class I and class II complementing alleles restores normal insulator function. Since the Mod(mdg4)2.2 protein appears to be necessary and sufficient for the activity of the *gypsy* insulator, we tested whether Mod(mdg4)2.2 protein is present at insulator sites in polytene chromosomes from third instar larvae carrying two complementing alleles. To this purpose, an antibody specific to exons 5 and 6 of Mod(mdg4)2.2 was produced. It has been previously shown that a functional insulator complex requires the Su(Hw) protein to bind to the insulator sequence and recruit Mod(mdg4)2.2 (GERASIMOVA *et al.* 1995). In larvae carrying a wild-type copy of the *mod(mdg4)* gene, both proteins are detected, as expected, in the *gypsy* insulator present in the *yellow* locus y^2 allele (Figure 3, a–c). In a $mod(mdg4)^{u1}$ mutant, no Mod(mdg4)2.2 protein can be detected at the y^2 locus, and only Su(Hw) is present (Figure 3, d–f). In larvae of the genotype y^2 *w* ct^6; $mod(mdg4)^{u1}/mod(mdg4)^{L3101}$, normal levels of the Su(Hw) and Mod(mdg4)2.2 are observed at the *yellow* locus, indicating that the Mod(mdg4)2.2 protein is produced in these flies and is able to interact with Su(Hw) at insulator sites on larval polytene chromosomes (Figure 3, g–i).

Expression of a normal Mod(mdg4)2.2 protein upon interallelic complementation: To further identify the mechanism leading to the observed interallelic complementation and to verify that the Mod(mdg4)2.2 protein synthesized in flies carrying a combination of complementing alleles is the same as in wild-type flies, Western analyses were performed using antibodies specific for this protein. Wild-type pupae produce an abundant protein of 115 kD and a minor protein of 86 kD; a second minor protein of 150 kD recognized by the antibody might not be encoded by *mod(mdg4)*, since it is not affected by mutations in this gene (Figure 4a). Wild-type larvae produce the same abundant 115-kD protein but the 86-kD product is synthesized at higher levels (Figure 4b). Mutations in $mod(mdg4)^{u1}$ affect the accumulation of the 115- and 86-kD proteins in both larvae and pupae (Figure 4, a and b). Although the three *P*-element mutants behave similarly in the complementation analysis, $mod(mdg4)^{03852}$ and $mod(mdg4)^{Evar3\text{-}93D}$ carry *P*-element insertions in an intron and might produce low levels of the wild-type protein by splicing of *P*-element sequences. Consequently, we chose to analyze the $mod(mdg4)^{L3101}$ mutant that carries the *P* element in the third exon for its effect on the expression of the Mod (mdg4)2.2 protein. Because this mutation is lethal, only

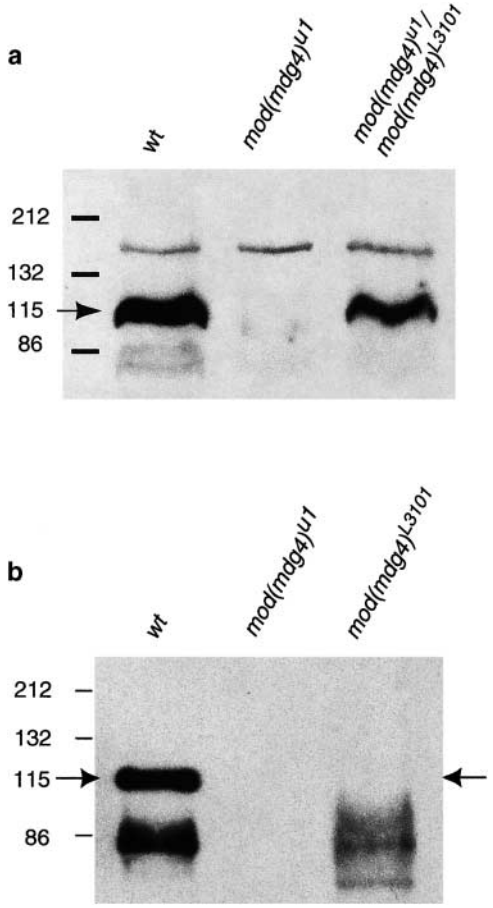

FIGURE 4.—Western analysis of the Mod(mdg4)2.2 protein in wild-type and *mod(mdg4)* mutant tissues. Arrows point to the Mod(mdg4)2.2 band. (a) Pupae, (b) first instar larvae.

early larvae could be analyzed. As expected, the Mod(mdg4)2.2 protein is absent from $mod(mdg4)^{L3101}$ homozygous first instar mutant larvae (Figure 4b). A weak smear is detected instead of the sharp band normally found in the wild-type larvae. This residual signal may arise from either degraded maternally provided proteins or from aberrant, low-abundance products of the mutant gene. A striking result was observed when analyzing the protein content of pupae of the genotype $mod(mdg4)^{u1}/mod(mdg4)^{L3101}$, a stage during which the *yellow* and *cut* genes are expressed. The Mod(mdg4)2.2 protein, absent from mutant individuals homozygous for either allele, is present at almost normal levels in pupae (Figure 4a). These results suggest that interallellic complementation between the two mutant alleles is accomplished by synthesizing the normal Mod(mdg4)2.2 protein.

DISCUSSION

It has been previously shown that different exons of the Mod(mdg4)2.2 mRNA can be encoded by sequences present in opposite strands of the gene (DORN *et al.* 2001; LABRADOR *et al.* 2001). Results described here go one step further to show that different domains of a single Mod(mdg4)2.2 protein can be encoded by DNA sequences residing in the two homologous chromosomes and that this characteristic is the molecular basis for the interallelic complementation observed between some alleles of the *mod(mdg4)* gene. This principle is illustrated in Figure 5. Two independent RNAs, transcribed from the *mod(mdg4)* gene and present in two different chromosomes, provide the information required to encode a normal functional Mod(mdg4)2.2 protein. Western analysis and immunolocalization on third instar larvae polytene chromosomes confirmed the absence of the protein from each of the homozygous mutants. When one homolog carries a mutation in one of the two transcription units and the other carries a mutation in the second transcription unit, the final, functional messenger may still be produced by combining the information contained in the wild-type transcription units of each homolog. Thus, in a complementation analysis, this combination of alleles restores the phenotypes associated with wild-type *mod(mdg4)* functions, and the phenotypes of the y^2 and ct^6 *gypsy*-induced mutations are reversed. At the protein level, the complementation is readily seen by the production of nearly normal amounts of the wild-type Mod(mdg4)2.2 protein, which is required and sufficient to confer a wild-type phenotype to a $mod(mdg4)^{u1}$ mutant.

The interallelic complementation mechanism we report here is different from those previously described. Interallelic complementation between mutations affecting the coding region of the gene has been observed when each allele is affected in only one of two separate functional domains of a multifunctional protein. Two alleles, each deficient in a different domain, may complement each other. Such a complementation mechanism requires the production of abnormal proteins by the mutant loci. This is not the case for *mod(mdg4)*, as assessed by Western blots and *in situ* immunodetection. The complementation observed here has its molecular origin in the cell's ability to produce a wild-type RNA by combining information present in two mutant transcripts. A *trans*-splicing event is most probably involved in the production of the final Mod(mdg4)2.2 mRNA. How *trans*-splicing is integrated with transcription and pre-mRNA processing reactions remains to be addressed. Abundant evidence suggests that transcription and processing of the mRNA are coordinated nuclear events. For example, splicing factors are recruited to the sites of transcription by RNA polymerase II. Similarly, mRNA capping and polyadenylation seem to occur right at the transcription site (for recent reviews, see HIROSE and MANLEY 2000; COLE 2001). Finally, even the packaging of the mature mRNA into heterogeneous ribonucleoparticles prior to cytoplasmic export could be coupled to other pre-mRNA processes (LEI *et al.* 2001). One may therefore argue that to enter a *trans*-splicing reaction, two premessenger RNAs need to be in physical proximity also during their transcription, before they engage

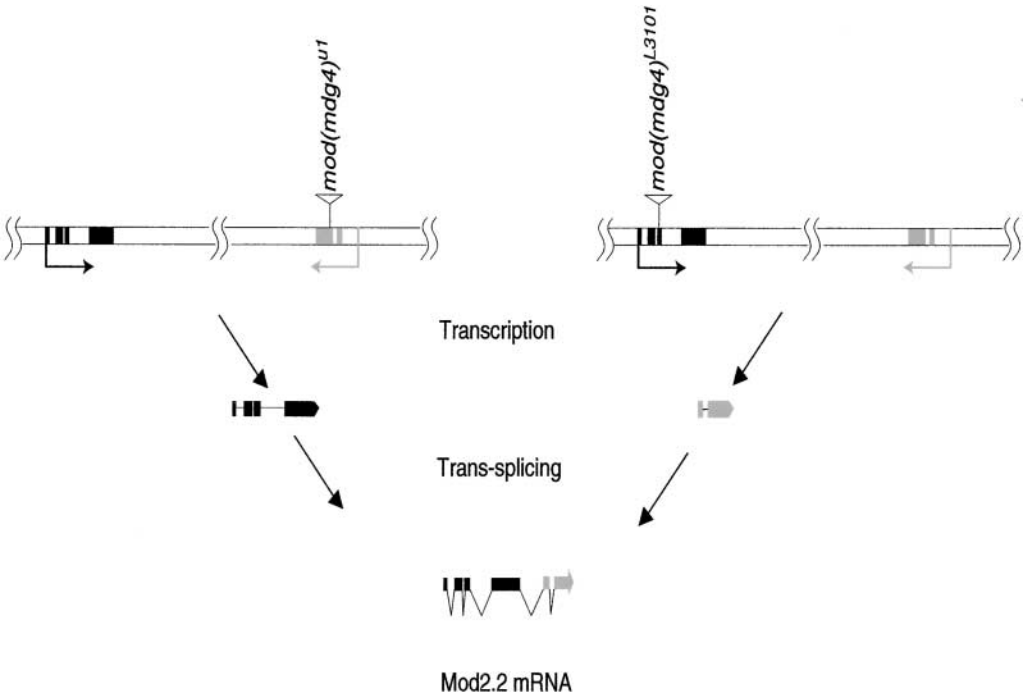

FIGURE 5.—Model depicting the *trans*-splicing-induced interallelic complementation. The two *mod(mdg4)* loci of a *trans*-heterozygous animal are depicted. One locus (left) carries the *mod(mdg4)*^{u1} allele, which affects the transcription unit encoding exons 5 and 6, but is still able to encode a wild-type transcript for exons 1–4. The second locus (right) carries the *mod(mdg4)*^{L3101} mutation and can still encode a wild-type transcript for exons 5 and 6. The combination of the two transcripts in a *trans*-splicing reaction creates a functional wild-type full-length mRNA.

in *cis*-splicing and other processing events. In the case of a wild-type *mod(mdg4)* locus, this condition is always met, thanks to the configuration of the gene: the two transcription units are in the same locus. In the case of flies undergoing interallelic complementation, this proximity condition may be met because of the extensive somatic pairing that exists between homologous chromosomes in both polytene (METZ 1916) and diploid cells (HIRAOKA *et al.* 1993) throughout the cell cycle (CSINK and HENIKOFF 1998).

It remains an open question whether a physiologically significant number of *trans*-splicing events are possible when the transcripts involved are produced at distant nuclear locations. It has been recently shown that two transcripts, one produced by the normal *mod(mdg4)* gene and a second one by a transgene inserted elsewhere in the genome, may be combined, presumably by *trans*-splicing (DORN *et al.* 2001). This latter study used a nonquantitative RT-PCR-based assay to detect the *trans*-spliced mRNA. It is therefore difficult to assess whether the increased distance between sites of transcription of both pre-mRNAs diminishes the efficiency of *trans*-splicing. If the level of *trans*-splicing is low when the mutant alleles are physically far away in the genome, the levels of protein synthesized might not be sufficient to restore the wild-type function of the *mod(mdg4)* gene. If this is the case, interallelic complementation at the *mod(mdg4)* locus will exhibit properties similar to transvection, in which phenotypic complementation is sensitive to the pairing of the two complementing alleles. The combined analysis of *trans*-splicing and somatic pairing of homologous chromosomes may constitute a powerful tool to study the intricate succession of events involved in the transcription and processing of the RNA in higher eukaryotes.

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