A *trans*-Acting Regulatory Gene That Inversely Affects the Expression of the *white*, *brown* and *scarlet* Loci in Drosophila

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ABSTRACT

A *trans*-acting regulatory gene, *Inr-a*, that alters the level of expression of the *white* eye color locus as an inverse function of the number of its functional copies is described. Several independent lines of evidence demonstrate that this regulatory gene interacts with *white* via the promoter sequences. Among these are the observations that the inverse regulatory effect is conferred to the *Adh* gene when fused to the *white* promoter and that *cis*-regulatory mutants of *white* fail to respond. The phenotypic response to *Inr-a* is found in all tissues in which *white* is expressed, and mutants of the regulator exhibit a recessive lethality during larval periods. Increased *white* messenger RNA levels in pupal stages are found in *Inr-a*/+ individuals versus +/+ and a coordinate response is observed for mRNA levels from the *brown* and *scarlet* loci. All are structurally related and participate in pigment deposition. These experiments demonstrate that a single regulatory gene can exert an inverse effect on a target structural locus, a situation postulated from segmental aneuploid studies of gene expression and dosage compensation.

IN a variety of multicellular organisms (Drosophila, maize, mouse, human), variation in the dosage of particular chromosomal segments inversely affects the expression of individual genes encoded elsewhere in the genome (BIRCHLER 1979, 1981; BIRCHLER and NEWTON 1981; DEVLIN, HOLM and GRIGLIATTI 1988; KLOSE and PUTZ 1983; WHATLEY *et al.* 1984; REICHERT 1986; BIRCHLER, HIEBERT and KREITZMAN 1989; BIRCHLER, HIEBERT and RABINOW 1989; BIRCHLER, HIEBERT and PAIGEN 1990). That is, when a given chromosomal segment is present at one half the normal diploid level, the expression of specific unlinked genes is increased to a twofold maximum. When the same segment is trisomic, the expression of the affected genes is reduced to two-thirds of the diploid value. Any one structural gene is inversely affected by multiple chromosomal segments, but the available data suggest they are not cumulative beyond the inverse level in most cases (DEVLIN, HOLM and GRIGLIATTI 1988). This response is mediated on the messenger RNA level (DEVLIN, GRIGLIATTI and HOLM 1984; BIRCHLER, HIEBERT and PAIGEN 1990) and is conferred upon a reporter gene when tested with a transformed promoter fusion construct (BIRCHLER, HIEBERT and PAIGEN 1990).

An examination of segmental aneuploid studies for the localization of structural genes for many enzymes reveals that virtually any region of the Drosophila genome, averaging about 3% of the total, will inversely affect one or more gene products when present as a trisomic (DETWILER and MACINTYRE 1978; HALL and KANKEL 1976; HODGETTS 1975; MOORE and SULLIVAN 1978; O'BRIEN and GETHMAN 1973; OLIVER, HUBER and WILLIAMSON 1978; PIPKIN, CHAKRABARTTY and BREMMER 1977; RAWLS and LUCCHESI 1974). Six of seven segmental dosage series examined in maize inversely or directly affected the expression of proteins expressed in the tissue studied (BIRCHLER and NEWTON 1981). This suggests that there are scores of regulatory genes in any one species that exert such a dosage effect on the expression of subsets of structural genes. The extensive studies of DEVLIN, HOLM and GRIGLIATTI (1988) on whole arm trisomy in Drosophila indicate that the phenomenon is operative upon all of the sixteen loci examined.

This response, referred to as the inverse effect, is sufficiently pervasive and of the proper magnitude to account for the dosage basis of both *X* and autosomal dosage compensation (BIRCHLER 1979, 1981; BIRCHLER, HIEBERT and KRIETZMAN 1989; BIRCHLER, HIEBERT and PAIGEN 1990), although an additional process that discriminates between the *X* and the autosomes must be invoked for the former. Autosomal dosage compensation refers to the observation that genes contained in long segmental aneuploids usually do not exhibit a dosage effect (BIRCHLER 1979, 1981; DEVLIN, HOLM and GRIGLIATTI 1982; 1985a,b; DEVLIN, GRIGLIATTI and HOLM 1984; BIRCHLER, HIEBERT and PAIGEN 1990). In contrast, shorter segmental three dose aneuploids surrounding an affected structural gene exhibit elevated product levels (*e.g.*, GRELL 1962). In the example of alcohol dehydrogenase, an inverse effect included in a longer chromosomal segment cancels the structural gene dosage

effect to produce the compensation (BIRCHLER, HIEBERT and PAIGEN 1990).

While there are multiple chromosomal segments that inversely affect the expression of any one locus, a change of whole genomic ploidy results in a directly proportional increase in gene product per cell (LUCCHESI and RAWLS 1973; BIRCHLER 1981; BIRCHLER, HIEBERT and PAIGEN 1990). When considered along with the widespread phylogenetic occurrence of the inverse effect and segmental dosage compensation, a balance or stoichiometric relationship among the responsible genes is suggested. This type of balance phenomenon parallels phenotypic observations involving aneuploidy known from early genetic work in Datura (BLAKESLEE, BELLING and FARNHAM 1920; BLAKESLEE 1921; 1934) and Drosophila (BRIDGES 1921a,b, 1925; PATTERSON, STONE and BEDICHEK 1935, 1937; PATTERSON, BROWN and STONE 1940) and basically all other higher eukaryotic organisms (for reviews see BIRCHLER 1983; EPSTEIN 1988). We believe that this balance phenomenon is a reflection of a regulatory system central to gene expression in eukaryotes that manifests itself as the inverse effect.

The present study was initiated to test whether the inverse effect could be reduced to the action of specific genes. Mutations exhibiting properties predicted for lesions in genes producing an inverse effect, which we will refer to as inverse regulators, would allow study of this phenomenon without the complicating factor of varying many loci in studies using segmental aneuploids.

We have chosen the *white* locus of Drosophila for these studies. *white* has been studied extensively both genetically and molecularly and many aspects of its *cis*-acting regulatory features are known. *Cis*-acting mutants have been characterized that exhibit a variety of regulatory phenotypes (ZACHAR and BINGHAM 1982; O'HARE, LEVIS and RUBIN 1983; PIRROTTA and BROCKL 1984) and an analysis of constructs with progressively truncated regulatory sequences has been conducted via *P* element mediated transformation (HAZELRIGG, LEVIS and RUBIN 1984; LEVIS, HAZELRIGG and RUBIN 1985; PIRROTTA, STELLAR and BOZZETTI 1985). Numerous leaky or hypomorphic alleles at *white* facilitate the visual identification of *trans*-acting mutants that modulate its expression. An advantage of such a system is that it can reveal regulatory factors that act either directly or indirectly.

Because a heterozygous chromosomal deficiency or segmental monosomic for a particular region elevates the expression of a monitored gene approximately twofold above the diploid level, a mutation in an "inverse regulator," when present as a heterozygote, would be expected to be dominant, and double the expression of the monitored gene. In this paper, the initial characterization of the first such locus is described. It was found that this regulator not only affects *white*, but also has a specific coordinate effect upon the accumulation of RNA from the *brown* and *scarlet* loci, all of which are structurally related and are thought to function together in pigment deposition.

MATERIALS AND METHODS

Fly culture: Flies were grown on Instant Drosophila Medium (Carolina Biological Supply Co.) at 25°.

Hybrid dysgenesis: Harwich P strain males were crossed to w^a M strain females. The F_1 w^a dysgenic males were backcrossed to w^a females. This generates a screened progeny that is homozygous for w^a and heterozygous for mutagenized chromosomes. Because the desired mutants were expected to be dominant and potentially recessive lethal, they were selected as heterozygotes.

Chemical mutagenesis: Males of the respective genotype were fed ethyl methansulfonate (EMS) by the method of LEWIS and BACHER (1968). To produce specific mutations in *Inr-a*, males of a second chromosome marker stock carrying *aristaless* (*al*), *black* (*b*), *curved* (*c*) and *speck*^{2} (*sp*^{2}) were treated with EMS. The males were mated to +; *b Tft*/*Cy0*^{DTS519}. This *Cy0* balancer chromosome carries a dominant temperature sensitive lethal in addition to the Curly wing marker. Individual F_1 males of genotype +; *Cy0*^{DTS519}/*al b c sp*^{2} were mated to w^a; *Inr-a*^{hd1}/*SM6a, Cy* and the progeny reared at 29°. The flies bearing the DTS chromosome are killed at this temperature. The eye color of females is wild type and that of males, apricot. If no mutant at *Inr-a* is generated, Curly and non-Cy flies will be present. If, however, a mutant is present that fails to complement *Inr-a*^{hd1}, only balanced *SM6a* flies survive. The protocol allows the immediate observation of the new mutant effect on w^a in the males and the presence of wild-type females serves as a check on virginity of the tester flies. Non-virgins would also give all *Cy* progeny but the females would be w^a. The marked second chromosome carried *al* and *sp*^{2} as does *SM6a*; therefore, it serves as a control to discern newly induced mutants. A total of 5750 pair matings were scored.

Mutants induced on *SM1* were recovered as previously described (BIRCHLER and HIEBERT 1989).

Gamma irradiation: Gamma irradiation (4000 rad) was applied to w^a (or w^{b1}) males, which were allowed to mate to w^a (or w^{b1}) virgin females for 2 days after which time they were removed. Mated females were transferred to new food daily for two weeks. Exceptionally light or dark-eyed progeny were mated to w^a (or w^{b1}) to perpetuate the mutant. Heritable examples were mated to w^a; *In(2LR) Gla,Gla*/*SM6a,Cy* and w^a; *TM3, Ser/MKRS, Sb* to localize to chromosome and to produce balanced stocks.

Pigment determination: For pigment quantification, flies were separated into appropriate genotypes and frozen on dry ice. The frozen flies were vortexed to remove their heads. When this material is placed on a U.S. sieve no. 35 (500 umeter) opening, the bodies are retained but the heads are not. Fifty heads per genotype were collected, homogenized in one ml of methanol, acidified with 0.1% HCl (EPHRUSSI and HEROLD 1944) and centrifuged. The supernatant was used to measure absorbance at 480 nm.

Genetic mapping: To map *Inr-a*^{hd1} on the second chromosome, males of the constitution, w^a; *Inr-a*^{hd1}/*SM6a*, were crossed to a w^a; *al b c sp*^{2} stock. These markers are described further in LINDSLEY and GRELL (1968). The heterozygous w^a/w^a; *al b c sp*^{2}/*Inr-a*^{hd1} females were backcrossed to w^a/*Y*; *al b c sp*^{2} males and the progeny scored. For mapping to

straw (*stw*) and *bloated* (*blo*), a w^a; *b Tft Inr-a^{hd1}/SM6a* stock was crossed by the respective mutants. The non-Cy females were backcrossed to males from the respective mutant stock. Males that were w^a were scored to determine the recombination frequency between each mutant and *Inr-a*. All presumptive recombinants were progeny tested against w^a to confirm the *Inr-a* constitution. To map *Inr-a^{hd1}* relative to the *vestigial* inversions, females of w^a; *Inr-a^{hd1}/SM6a* were mated to the balanced *vestigial* mutants. The F_1 *Inr-a/vg^U* or *vg^W* were selected and crossed by w^a males. The w^a progeny were scored to determine the frequency of recombination. Presumptive recombinants were testcrossed against w^a to confirm their constitution. An example of an *Inr-a^{hd1} vg^U* and an *Inr-a^{hd1} vg^W* recombinant was balanced by *In(2LR)Gla, Gla*. The *Inr-a^{hd1} vg^W* chromosome was used to map *Inr-a* relative to *cinnabar*. This chromosome was made heterozygous with *pr cn* and backcrossed to the same. From 133 males scored, two were ++++. Progeny tests to w^a females confirmed this genotype. Two flies were thought to be w^a; *pr cn Inr-a vg^W*. Progeny tests confirmed the presence of *Inr-a*.

To map *Inr-a* relative to the deficiency *Df(2R)eve$^{1.27}$* (NUSSLEIN-VOLHARD, WIESHAUS and KLUDING 1984), the w^a; *b Tft Inr-a^{hd1}/SM6a* females were crossed by +; *cn Df(2R)eve$^{1.27}$/Cy0* males. The heterozygous non-Curly females were backcrossed to +; *cn Df(2R)eve$^{1.27}$/Cy0* males. Only the w^a non-Curly males were scored. None of these flies can be homozygous for the Df and therefore the vast majority will be *Inr-a*. If the order is *Inr-a-Df(2R)eve$^{1.27}$*, then non-*Inr-a* flies will be + *cn* ++. However, if the order is *Df(2R)eve$^{1.27}$-Inr-a*, then non-*Inr-a* chromosomes will be *Tft* +++. One such fly of the latter type was recovered and confirmed to carry neither *Inr-a* or *Df(2R)eve$^{1.27}$* in progeny tests. This is consistent with the order *Df(2R)eve$^{1.27}$-Inr-a*.

Deficiency and complementation tests: Balanced stocks of deficiencies in proximal 2R were crossed as males to a w^a; *Inr-a^{hd1}/SM6a* stock. The class with no balancer indicates complementation between the deficiency and the recessive lethality of *Inr-a*. At least 50 progeny were scored in each case. Tests of complementation with various visible markers in 2R were performed by crossing males to the w^a; *Inr-a^{hd1}/SM6a* stock. *Inr-a^{hd1}* complements the following loci in proximal 2R: *puff* (*puf*), *bloated* (*blo*), *intersex* (*ix*), *chaetelle* (chl), *withered* (*whd*), *faint* (*fai*), *lines* (*lin*), *faint sausage* (*fas*), *arrow* (*arr*), *brown head* (*brh*), *filzig* (*flz*), and *ghost* (*gho*) (LINDSLEY and GRELL 1968; NUSSLEIN-VOLHARD, WEISHAUS and KLUDING 1984; LINDSLEY and ZIMM 1987).

Test of trisomic effect of *T(Y:2)G44* on white alleles and transformed constructs: To test the response of segmental trisomy for the *Inr-a* region on various *white* alleles and transformed constructs, the *T(Y:2)G44* insertional translocation was used. This aberration has an insert from polytene section 44C to 50C in a y^+ marked *Y* chromosome (LINDSLEY *et al.* 1972; L. CRAYMER, personal communication). The stock has an inverted attached *XY*, *In(1)EN, y*, that is marked by a recessive *yellow* allele. The deficiency corresponding to the *Y* insertion is balanced by *SM1, Cy*. The females in the balanced stock carry an attached *X*. When outcrossed as males to free *X* stocks, there are two classes of viable males. One class has the *Y* insertion and the corresponding deficiency. These flies are euploid. The other class has the *Y* insertion but inherit the *SM1* chromosome instead of the deficiency. These males are Curly (from *SM1*) and have three copies of the region 44C-50B. Females from the appropriate *white* allele stocks to be tested were crossed by G44 males. The male progeny will all be hemizygous for the *white* allele. A comparison of Curly and non-Cy brothers is made to determine any trisomic effect. For autosomal transformed constructs, the same type of cross is made. These transformants are carried in the presence of a *white* deficiency, w^{1118}. Therefore, all the males exhibit the construct phenotype despite their location on the autosomes.

In experiments that combined *Inr-a* with the G44 trisomic, the original G44 stock was crossed to females from a *In(1)EN, y; Sco/Cy^t* stock. The F_1 *Sco* males were selected and crossed to w^a; *Inr-a^{hd1}/SM6a* females. This extra cross substitutes the dominant marker, *Scutoid* (*Sco*), on the normal chromosome two.

Segmental trisomy: To examine the effect on *white* of segmental trisomics in the region of *Inr-a*, a series of *T(Y:2)*'s were selected from the collection of LINDSLEY *et al.* (1972). These are listed in Table 4. Examples were chosen that have lost the *Bar-Stone* (B^S) marker but retained y^+. These translocations were crossed as males to the attached *X* stock *C(1)RM, y^2 sc w^a ec*. The F_1 y^+ females carry the attached *X* and the respective y^+ marked translocation. When crossed to the appropriate adjacently broken translocation stock as males, the trisomic and euploid females can be distinguished by the presence or absence of the y^+ and male balancer marker (*Cy*) (LINDSLEY *et al.* 1972).

Enzyme activity, protein and DNA measurements: Activity measurements for glucose-6-phosphate, 6 phosphogluconate, β-hydroxy acid and isocitrate dehydrogenases as well as fluorescent estimates of DNA were as described by BIRCHLER, HIEBERT and KRIETZMAN (1989). Alcohol dehydrogenase activity from the *w* promoter-*Adh* reporter strains was assayed as follows. Nine replicas each of ten pupae from each of the three genotypes examined were homogenized in 250 μl of extraction buffer. One hundred microliters of the extraction was added to 900 μl of assay solution and incubated at 30° for 30 min. After the reactions were stopped, absorbance was measured at 340 nm in a Beckman DU-50 spectrophotometer. Other details of the reaction protocol are given in BIRCHLER, HIEBERT and PAIGEN (1990). Total protein measurement used 5 μl of the extraction in a final one ml volume of Bradford reagent (Biorad) and absorbance measured at 595 nm as described (BIRCHLER, HIEBERT and PAIGEN 1990).

RNA extraction, Northern transfer, probe preparation and hybridization: RNA was extracted from frozen flies by guanidine-HCl extractions followed by repeated ethanol precipitations as described by COX (1968) and modified by BIRCHLER, HIEBERT and KREITZMAN (1989) and BIRCHLER, HIEBERT and RABINOW (1989).

Procedures for northern transfer were conducted as previously described (RABINOW and BIRCHLER 1989). Single stranded RNA probes were generated with T7 polymerase from a plasmid vector with a T7 promoter driving an antisense cDNA of *white* extending from the 3′ end to within the third exon.

Anti-sense *rudimentary* (SEAGRAVES *et al.* 1984), *brown* (DREESEN, JOHNSON and HENIKOFF 1988), *scarlet* (TEARLE *et al.* 1989) *alcohol dehydrogenase* (BIRCHLER, HIEBERT and PAIGEN 1990; GOLDBERG 1980) and *vermilion* (SEARLES and VOELKER 1986) probes were prepared by the same method using either T7 or SP6 RNA polymerase. T7 generated antisense *rp49* (KONGSUWAN *et al.* 1985) was used as a loading control. RNA levels were estimated by laser densitometry of autoradiographs using an LKB Ultrascan. The level of the respective RNAs was estimated relative to the loading control in each lane.

Construction of the *white* promoter-*Adh* reporter fusion: The *white* promoter was isolated by digestion of pm 11.5 (LEVIS, BINGHAM and RUBIN 1982a) with *Hph*I to yield a 2.5-kb fragment. This fragment contains all of the cis regulatory sequences thought to be required for *white* func-

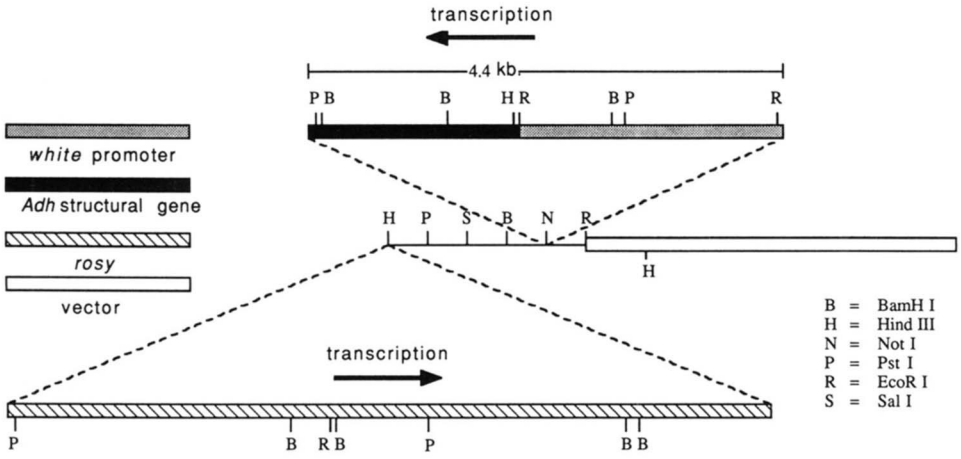

FIGURE 1.—Construction and restriction map of *pDM30-w-Adh*. The orientation of the *white* promoter, *Adh* structural gene, *rosy* transformation marker and vector sequences are shown by the noted shadings.

tion (HAZELRIGG, LEVIS and RUBIN 1984). The 3′ cut is within the 5′ nontranslated leader of the *white* mRNA encoding sequences. The fragment was blunt ended with T4 DNA polymerase (New England Biolabs), ligated to *Eco*RI linkers, and cloned into the *Eco*RI site of pBlueScript SK+ (Stratagene, Inc.). This fragment was then transferred into an *Eco*RI site preceding the *Adh* structural gene in a Bluescript derived plasmid. To construct this latter plasmid, Bluescript was digested with *Eco*RI, blunt ended with T4 DNA polymerase to destroy the site in the vector and religated. The *Adh* gene was removed from pDM28 (D. MISMER and G. M. RUBIN, personal communication) in a 1.9-kb *Not*I digest and ligated into the modified Bluescript. This plasmid has a unique *Eco*RI site preceding the *Adh* structural gene for acceptance of the *white* promoter fragment to yield p*BlueScript-w-Adh*. Digestion with *Bam*HI gave diagnostic fragment sizes to select bacterial transformants with the *white* promoter in the same orientation as *Adh* (GOLDBERG 1980). The *white* promoter-*Adh* gene ensemble was isolated from the plasmid with a *Not*I digest and cloned into the unique *Not*I site in pDM30 (MISMER and RUBIN 1987), a *P* element vector carrying the ry^+ gene marker. Restriction analysis with six enzymes (*Eco*RI, *Hin*dIII, *Pst*I, *Sal*I, *Not*I, and *Bam*HI) was performed to confirm the structure of the transformation vector. The construction and final map of the construct is given in Figure 1.

Germline transformation: Microinjection of preblastoderm embryos and other aspects of the transformation procedure were performed as described (RUBIN and SPRADLING 1982; SPRADLING and RUBIN 1982) with the following modifications. The pDM30-*w-Adh* plasmid and the helper plasmid pII2.57wc (KARESS and RUBIN 1984) were injected at a concentration ratio of 0.8 μg/μl into the recipient strain Adh^{fn6} *cn*; ry^{506}. Emerging flies were mated to Adh^{fn6} *cn*; ry^{506} and the progeny screened for ry^+ transformants. Isolates on the *X* and on the third chromosome were recovered. The third chromosome isolate, designated 9g12, was made homozygous as Adh^{fn6} *cn*; P[*w-Adh*; ry^+] ry^{506}. This strain was used for analysis with *Inr-a*.

Genetic crosses involving the *w* promoter-Adh reporter fusion strain: To test for an effect of *Inr-a* on the *white* promoter-*Adh* reporter fusion construct, it was necessary to have all stocks homozygous for the deletion allele, Adh^{fn6} (BENYAJATI *et al.* 1982) at the endogenous *Adh* locus. Toward this end, a *b Tft* $Inr\text{-}a^{hd1}$ chromosome was recombined with Adh^{fn6} *cn* by selection of w^a/Y; + $Inr\text{-}a^{hd1}/SM6a$ individuals among crosses to w^a/Y; *In(2LR)Gla, Gla/SM6a*. These were used to establish a w^a; Adh^{fn6} $Inr\text{-}a^{hd1}/SM6a$ stock. The presence of Adh^{fn6} was confirmed by backcrossing to a w^a; Adh^{fn6} *cn*; ry^{506} stock and demonstrating the lack of ADH activity compared to Canton S wild type.

For comparison of ADH activity from the construct with and without *Inr-a*, the following crosses were made. Males of a stock w^a; *T(2:3)Cy0, Tb ch*; *TM3, Ser*/+ were crossed to females of two strains: w^a; Adh^{fn6} *cn*; ry^{506} and w^a; Adh^{fn6} $Inr\text{-}a^{hd1}/SM6a$. The respective males, w^a/Y; Adh^{fn6} *cn*/*T(2:3)Cy0, Tb ch*; + and w^a/Y; Adh^{fn6} $Inr\text{-}a^{hd1}/T(2:3)Cy0$, *Tb ch*; + were crossed to the third chromosomal insert of the *w* promoter-*Adh* reporter (9g12). At the same time, the w^a; Adh^{fn6} *cn*; ry^{506} stock was grown to serve as a background control. The three crosses were grown at 25° and late pupae harvested in groups of ten and frozen at −80° until extracted for ADH determination.

RESULTS

To isolate mutations in genes altering accumulation of *white* product, the *white-apricot* (w^a) allele was used. This allele produces a leaky, hypomorphic phenotype, which allows detection of modulations of *white* expression. The molecular lesion is well defined, being a parallel insertion of the retrotransposon, *copia*, into the second intervening sequence (GEHRING and PARO 1980; BINGHAM and JUDD 1981; O'HARE *et al.* 1984).

In initial screens, described in MATERIALS AND METHODS, mutagenesis via hybrid dysgenesis yielded a mutation mapping to chromosome 2 that darkened w^a and was a recessive lethal. Despite its origin in hybrid dysgenic crosses, the mutation is stable and cannot be reverted by supplying *P* element transposase with the Δ2-3 chromsome described by ROBERTSON *et al.* (1988). The mutant gene is referred to as *Inverse regulator-a* (*Inr-a*) and the particular allele as $Inr\text{-}a^{hd1}$. We use the term inverse regulator to denote the type of dosage effect and to distinguish it from other regulatory loci.

Phenotypic and spectrophotometric quantification of *Inr-a* effects on *white*: A balanced stock was constructed that was w^a; $Inr\text{-}a^{hd1}/SM6a$. Females of this stock were mated to white-eyed, *y w*/*Y* males. This produced a progeny segregating for *Inr-a*/+ and +/+. The F_1 females have only one functional copy of w^a. The normal one-dose males exhibit dosage

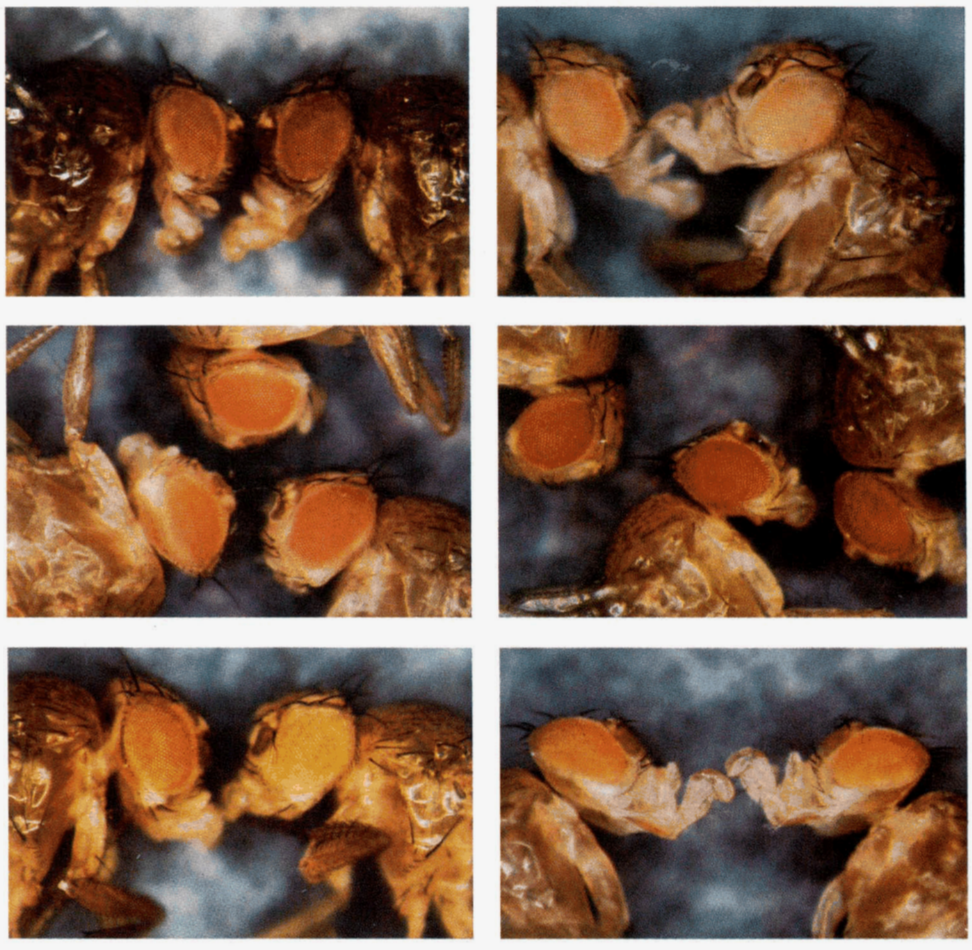

FIGURE 2.—(a) Top left, comparison of w^a with *Inr-a vs.* normal sib. Normal male on left, *Inr-a*/+ male on right. (b) Top right, comparison of w^{sp55} with *Inr-a vs.* normal sib. Normal male on left, *Inr-a*/+ on right. (c) Middle left, dosage series of *Inr-a*$^+$ in presence of w^a. Top, normal two dose; lower right, one dose; lower left, three doses. (d) Middle right, dosage series of *Inr-a* in presence of the *cis*-regulatory mutant w^{sp2}. Left to right, one, two, three doses of *Inr-a*$^+$. (e) Lower left, comparison of construct F4–1 with *Inr-a* and normal sib. Left *Inr-a*/+; right, ++. (f) Lower right, comparison of *Adh* promoter-*white* construct with *Inr-a vs.* normal sib. Left, *Inr-a*/+; right, ++.

compensation and are thus approximately twice as dark as the w^a/w females (MULLER 1932, 1950). The F_1 females of w^a/w; *Inr-a*/+ genetic constitution had an eye color similar to the w^a/Y; *SM6a*/+ males. Because there is a twofold reduction of pigment in the females due to having only a single copy of w^a, but the *Inr-a*^{hd1}/+ flies were as dark as the dosage-compensated normal w^a males, the level of elevation of expression by *Inr-a*^{hd1} is approximately twofold. Figure 2 (top left) shows the effect of *Inr-a* on the *apricot* allele of *white*.

To further quantitate the effect of *Inr-a* on w^a, w^a/Y; *Inr-a*^{hd1}/*SM6a* males were crossed to w^a diploid females, progeny sorted according to sex and *Inr-a*, and the total pigment determined spectrophotometrically. The data are shown in Table 1. The heterozygous *Inr-a*/+ genotype had approximately twice the pigment level of +/+ (*SM6a*/+) in both sexes. In contrast, a similar test on the *white-spotted* (w^{sp}) allele, a non-responsive (see below) cis regulatory mutant, showed no difference.

TABLE 1

Pigment assays of *Inr-a*

Genotype		*n*	O.D. 480	Ratio
w^a;	*Inr-a*/+ male	4	0.219 ± 0.010	1.84
w^a;	+/+ male	4	0.119 ± 0.009	
w^a;	*Inr-a*/+ female	4	0.241 ± 0.006	2.06
w^a;	+/+ female	4	0.117 ± 0.002	
w^{sp};	*Inr-a*/+ male	3	0.409 ± 0.008	1.05
w^{sp};	+/+ male	3	0.390 ± 0.014	
w^a;	*Inr-a*/+/+ 3N female	3	0.252 ± 0.004	1.46
w^a;	+/+/+ 3N female	3	0.173 ± 0.005	

Pigment assays were performed on freshly collected flies as described in MATERIALS AND METHODS. The w^a male and females are from the same cultures. The w^{sp} and triploid conparisons are from crosses to w^{sp} and triploid females, respectively. The comparisons for w^a within the respective ploidies are in segregating backgrounds, which are different for the diploid and triploid crosses and therefore should not be compared. The O.D. 480 values are means ± standard error on *n* number of assays for each genotype. The difference between the means of the w^a male, female and 3N female comparisons is significant in statistical tests at the 99% confidence level. The difference between the means of the w^{sp} comparison is not significant.

To test the effect of the *Inr-a* mutant in triploids, w^a/Y; *Inr-a/SM6a* diploid males were crossed to *C(1)RM*, y^2 *sc* w^a *ec/FM6* triploid females. Among the triploid progeny, the *Inr-a* mutant triploid females (*Inr-a*/+/+) were compared to the normal triploid females (+/+/+), both of which are homozygous for w^a. The *Inr-a*^{hd1} flies had an increase of 46% relative to their normal siblings. The presence of the *Inr-a* mutation darkens the eye color in both ploidies but to different degrees depending on the relative number of functional *Inr-a* copies. Therefore, the mutation produces a level of response equivalent to the predicted inverse effect in both diploid and triploid flies. The normal diploid and triploid w^a females have a very similar eye color when generated from the same cross in this background.

Genetic and cytological localization: For further characterization, the mutant was localized on the genetic and cytological maps. For this, *Inr-a*^{hd1} was crossed to a w^a; *al b c sp*^{2} chromosome and backcrossed to the same. This marker stock has the *apricot* monitor on the *X* chromosome and recessive mutants spanning the length of chromsome *2*. This genetic mapping placed *Inr-a*^{hd1} between *black* (*b*) and *curved* (*c*), approximately 3 cM proximal to the latter. Another recombinant chromosome *b Tft* (*Tuft*) *Inr-a*^{hd1} was produced and used to map *Inr-a* distal to two markers in the right arm of *2*: *straw* (*stw*) 4.7 cM, and *bloated* (*blo*) 3.7 cM. *Inr-a*^{hd1} was also recombined with two dominant alleles of *vestigial* (*vg*), that are associated with inversions, namely vg^U and vg^W. *Inr-a*^{hd1} shows 1.32 cM recombination with vg^U (9 recombinants/682 progeny) and 0.3 cM recombination with vg^W (1 recombinant/313 progeny). The vg^U inversion has breakpoints in 49D and 50C of the polytene chromosome map while vg^W has breakpoints in 48A and 49D (WILLIAMS *et al.* 1990). The *Inr-a*^{hd1} vg^W chromosome was used to map *Inr-a* 3.0 cM distal to *cinnabar* (*cn*).

To localize *Inr-a* on the polytene chromosome map, the mutant was tested for complementation of the recessive lethality against all available deficiencies distal to *cinnabar* (43E) and proximal to *curved* (52C4-52E3). Each of the tested chromosomes (see Table 2 and MATERIALS AND METHODS) survived as a heterozygote with *Inr-a*^{hd1} and none of them suppress the phenotype of w^a. Recombination between the *b Tft Inr-a*^{hd1} chromosome and the deficiency, *Df(2R)eve*$^{1.27}$ (46C3-4; 46C9-11) placed *Inr-a* distal to the deficiency. The summation of these data place *Inr-a* between 46D and 47D in the right arm of chromosome *2*.

Does the mutant map to a region that inversely affects *white*? An additional criterion for characterization of *Inr-a* as an inverse regulator would be that the gene lies in a position in the genome that reduces the expression of the *white* locus when made trisomic. To test this, a population of flies was generated that was *white-apricot* and segregating for individuals with the normal two doses and others with three copies of the region surrounding *Inr-a*. The translocation *T(Y;2)G44* has polytene chromosome segment from 44C to 50B instead into the *Y* chromosome and the complementary deficiency is balanced against the second chromosome balancer, *SM1*, *Cy* (LINDSLEY *et al.* 1972; L. CRAYMER, personal communication). These breakpoints surround the cytological region to which *Inr-a* has been localized. When crossed as a male to a chromosomally normal stock, the *Y* insertion and complementary deficiency form one class of gamete that is euploid and that results in viable progeny. The heterozygous deficiency alone is not viable. The *Y* insertion plus the *SM1* chromosome form the other class of gamete, which is duplicated for 44C to 50B, and which creates a segmentally trisomic zygote when joined with a normal gamete at fertilization. In most backgrounds, the trisomics survive to the adult stage at a low but sufficiently great frequency to assay phenotypically. The pigment level of the trisomic flies was reduced relative to the diploid (see Figure 2, middle left). The reduction is not as great as in heterozygous w^a/w^{1118} females, which represents a 50% reduction of *white* function. An inverse effect would reduce the pigment level to 67% of normal. Thus, the *Inr-a* mutation does in fact lie in a region of the genome that appropriately affects the expression of w^a.

To test whether the effect of the heterozygous mutation would cancel the trisomic effect, the mutation and the segmental trisomic were combined in the

TABLE 2

Deficiencies tested for complementation with *Inr-a*

Deficiency	Cytology	Source
Tp(2;3)N1-22	46B2;46D1	J. PARO
Df(2R)eve$^{1.27}$	46C3-4;46C9-11	Indiana
Df(2R)en-A	47D3;48A	Indiana
Df(2R)en-B	47E3-6;48A4-B2	Indiana
Df(2R)en-30	48A3-4;48C6-8	Indiana
Df(2R)vg^{135}	48DE;49DE	C.-T. WU
Df(2R)vg^{C}	49B2-3;49E7-F1	C.-T. WU
Df(2R)vg^{B}	49C1-2;49E2-6	C.-T. WU
Df(2R)vg^{D}	49D3-4;50A2-3	C.-T. WU
Df(2R)L^{+R48}	50F2-3;51B3	B. BAKER
Df(2R)TRIX	51A1-2;51B6	B. BAKER
Df(2R)XTE-58	51E3-4;52A13-B1	R. MACINTYRE
Df(2R)XTE-11	51E3-4;52A6-10	R. MACINTYRE
Df(2R)XTE-18	51E3-4;52C9-D1	R. MACINTYRE
Df(2R)628.12A	52C4;52E3	W. GELBART
Df(2R)WMG268-18	52A9-10;52D12-15	R. MACINTYRE

Further descriptions of the deficiencies can be found in LINDSLEY and ZIMM (1987). The genetic mapping described in the text and the exclusion from the above deficiencies places *Inr-a* between 46D and 47D.

same class of fly. This required the introduction of an independent marker on the normal chromosome 2 to facilitate classification of all progeny types, as further explained in MATERIALS AND METHODS. There are four classes of viable males produced: (1) w^a/*YG44*; *Inr-a*/*Df(2R)G44* have only one functional copy of the regulator present and were the darkest class, (2) w^a/*YG44*; *Inr-a*/+ have two functional copies of the regulator and were equivalent to the normal w^a phenotype, (3) w^a/*YG44*; *SM6a*/*Df(2R)G44* also have two functional copies and were similar to the last class in pigment intensity and (4) w^a/*YG44*; *SM6a*/+ has three copies and were the lightest phenotype present in the cross. These experiments demonstrate that the trisomic effect and the *Inr-a* mutant effect cancel. The amelioration of the mutant effect by the addition of an extra copy of the region indicates that the mutant is a loss of function allele as opposed to gain of function.

To further confirm the relationship of the *Inr-a* mutation with these trisomic effects, selected *white* alleles were tested for their response to the trisomic condition. Males of the *T(Y;2)G44* stock were crossed to females of w^a, w^{bl}, w^{a3}, w^{sp2}, w^{sp4}, w^{sp81d5} and w^e. The first three are lesions in the structural portion of the gene, which respond to the *Inr-a* mutants, and the latter four represent the two types of cis-regulatory lesions that fail to respond to *Inr-a* (see below). The segmental trisomic reduced the expression of the first three, but had no discernible effect on the *white-spotted* alleles and *white-eosin*. Therefore, the trisomic effect on *white* follows an analogous allele specificity as *Inr-a*, being effective on structural gene lesions but not on regulatory mutants. The summation of the above observations suggests that the trisomic effect and that of the *Inr-a* mutation are due to the same locus. The lack of effect of the dosage series of functional copies of *Inr-a* on the cis-regulatory mutant, w^{sp2}, is shown in Figure 2, middle right.

The segmentally trisomic region that reduces the expression of *white-apricot* was further defined by use of overlaps of translocations between a marked *Y* chromosome and sites along proximal 2R (see MATERIALS AND METHODS). This approach used selections from the translocation collection of LINDSLEY *et al.* (1972). Of the four regions tested, two trisomics reduced the expression of *apricot*. One of these regions coincides with the genetic localization of *Inr-a* described above (46A-47E) (Table 3).

Induction of additional *Inr-a* alleles: The original allele of *Inr-a* was selected by its phenotypic elevation of pigment levels of w^a. Despite extensive outcrossing to the standard w^a stock, the original mutant chromosome could not be made homozygous, suggesting that *Inr-a* might be a recessive lethal. To test this possibility, new alleles were sought.

TABLE 3

Localization of trisomic enhancement of *white-apricot*

Maternal parent	Paternal parent	Trisomic segment	*white-apricot* effect
T(Y;2)R155	T(Y:2)L23	43C-45F	None
T(Y;2)L23	T(Y;2)A24	45F-46A	None
T(Y;2)A24	T(Y;2)B107	46A-47E	Reduced pigment
T(W;2)B107	T(Y;2)L110	47E-50C	Reduced pigment

The crosses involved converting the respective translocation from the collection of LINDSLEY *et al.* (1972) to the attached *X*, *C(1)RM*, carrying the markers *yellow*^{2} (*y2*), *scute* (*sc*), *white-apricot* (w^a) and *echinus* (*ec*). These females were used as the maternal parent in crosses to more distally broken translocations to generate a progeny in which there are euploid females that have the normal chromosome 2 from the maternal parent and the paternal Y;A translocation. The segmentally trisomic sibling sisters inherit the 2^Y chromosome from the maternal parent and the Y^2 and balancer (marked by *Curly* wing mutation) chromosomes from the paternal parent. Therefore the euploid/trisomic comparison can be made from the normal winged females versus the Curly winged females. The region that overlaps the genetic localization of *Inr-a* reduces the pigment level (46A-47E).

The protocol is described in MATERIALS AND METHODS. Two new EMS-induced alleles were recovered that act as recessive lethals as heterozygotes with the original dysgenic allele. These new *Inr-a* mutant alleles also elevate the expression of w^a as a heterozygote in outcrosses to w^a, confirming that the recessive lethality and the inverse effect upon *white* are properties of the same lesions.

In a gamma irradiation mutagenesis (see MATERIALS AND METHODS), a number of autosomal mutations were selected that elevated the expression of w^a. Of those located to the second chromosome, four failed to complement the recessive lethality of *Inr-a*. In this case, new mutants were selected on the basis of elevated pigment levels of w^a, and each exhibits a recessive lethality itself, or in combination with other preexisting *Inr-a* alleles. In a gamma irradiation of w^{bl}, an additional allele was recovered. Furthermore, in an EMS mutagenesis of the *Enhancer of* w^a locus (BIRCHLER and HIEBERT 1989), three new alleles of *Inr-a* were recovered on the *SM1* balancer chromosome based on the criteria of elevation of pigment levels and failure to complement the recessive lethality of *Inr-a*^{hd1}. Thus, selection of new alleles by either elevation of w^a pigment levels or by failure to complement the recessive lethality will also select for the other characteristic. The alleles are listed in Table 4.

Lethal phase: To determine the stage at which *Inr-a* alleles are recessive lethal, two different alleles (*hd*^{1} and *γb*) were outcrossed individually to Oregon R and Canton S wild strains. The F_1 *Inr-a*/+ progeny from the outcrosses were mated together and the number of larvae hatching from this cross determined. Greater than 99% of the eggs hatched. The expectation for embryonic lethality is approximately 75% hatch rate. A determination of per cent eclosion

TABLE 4

Alleles of *Inr-a*

Designation	Source	Comments
Inr-a^{hd1}	Hybrid dysgenesis Harwich P × *w^a*	Recovered as a suppressor of *w^a*; not revertable by Δ2-3
Inr-a^{EMS1}	EMS	Generated on *al b c sp^2* as recessive lethal
Inr-a^{EMS2}	EMS	Generated on *al b c sp^2* as recessive lethal
Inr-a^{EMS3}	EMS	Generated on *SM1* as suppressor of *w^a*
Inr-a^{EMS4}	EMS	Generated on *SM1* as suppressor of *w^a*
Inr-a^{EMS5}	EMS	Generated on *SM1* as suppressor of *w^a*
Inr-a$^{\gamma b}$	Gamma irradiation	Recovered as a suppressor of *w^a*
Inr-a$^{\gamma c}$	Gamma irradiation	Recovered as a suppressor of *w^a*
Inr-a$^{\gamma d}$	Gamma irradiation	Recovered as a suppressor of *w^a*
Inr-a$^{\gamma sc}$	Gamma irradiation	Recovered as a suppressor of *w^{bl}*

of pupae gave comparable rates. Therefore, it is concluded that *Inr-a* is lethal during larval periods.

Tissue and developmental specificity: The product of the *white* locus is necessary for deposition of pigment in the eyes, ocelli, testes and malpighian tubules. The malpighian tubules and testes from adults were examined in a population bearing a wild-type allele of *white* (Canton-S), but segregating for *Inr-a^{hd1}* and *SM6a*. *Inr-a^{hd1}* males invariably had darker malpighian tubules and testes than their *SM6a* siblings after three days of aging at 25 C, indicating that *Inr-a* shows a phenotypic effect in these tissues. The ocelli are also coordinately affected with the eyes.

The effect of *Inr-a* mutations on pigment levels in larval malpighian tubules was also examined. Males heterozygous for *Inr-a^{hd1}* and *T(1;2) sc(19), y$^+$*, were constructed. The *scute(19)* translocation has a wild-type allele of the *X*-linked *y* locus translocated to the second chromosome. These males were crossed to a *y* w$^+$ strain, and malpighian tubules from larvae segregating for y$^+$ and *Inr-a* were scored. Malpighian tubules derived from *Inr-a*; *y*, larvae were darker in color than those from their siblings, indicating that *Inr-a* produces an effect during third-instar as well as in later stages.

Allele specificity of *Inr-a*: Because the *Inr-a^{hd1}* mutant was selected with *w^a*, it was possible that the suppressing effect on the *apricot* allele was by interaction with the retrotransposon, *copia* (*e.g.*, RABINOW and BIRCHLER 1989; BIRCHLER and HIEBERT 1989; BIRCHLER, HIEBERT and RABINOW 1989). To test this possibility, the *Inr-a^{hd1}* allele was examined for interaction with a series of *white* hypomorphs that include lesions in the structural portion, as well as in the regulatory region of the gene. Among these were point mutants, deletions and transposable element insertions. The physical lesion for all these alleles is known and is therefore useful in identifying the *white* locus sequences required for interaction with modifier loci. If *Inr-a^{hd1}* were involved in regulating *white*, one would expect that some *white* alleles with mutations in the 5′, *cis*-acting regulatory sequences might not respond. If, on the other hand, *Inr-a* regulated some aspect of *copia* expression, then one would expect it to interact specifically with *w^a*. If the mutant were in a gene involved in pigment production, then all alleles at *white* should be affected. To conduct this analysis, a stock was constructed that was *w^{1118}*; *Inr-a^{hd1}/Cy0*. The *white* allele, *w^{1118}*, is a deletion of sequences at the 5′ end of the gene that extend into the coding region (HAZELRIGG, LEVIS and RUBIN 1984). Males from this stock were crossed to virgin females of the 26 molecularly defined alleles of *white*. The F_1 were compared for the intensity of pigment in the *Inr-a/+ vs. Cy0/+* siblings of the same age. The results of these crosses are summarized in Table 5.

Inr-a^{hd1} elevated the expression of *w^a* and three partial revertants. It is also effective upon ten different alleles that have lesions of various types in the structural portion of *white*. In contrast, *Inr-a^{hd1}* has no effect upon a series of *white* alleles that have regulatory defects. Among these are the *white-spotted* series (*w^{sp}*, *w^{sp2}*, *w^{sp4}*, *w^{sp81d5}*), which have a reduced level of pigment that is irregular in distribution over the surface of the eye and is darker in males than in females. These mutants are insertions or deletions in the region of *white* that is implicated in serving as a transcriptional enhancer (DAVISON *et al.* 1985).

Another group tested were revertants of the original *w^1* mutation (*w^e*, *w^{apl}*, *w^h*, *w^{IR2}*). This mutation is due to an insertion of a *Doc* retroposon into the 5′ leader sequence of the *white* mRNA (DRIVER *et al.* 1989). Four characterized revertants are secondary insertions into *Doc* (O'HARE *et al.* 1991). The first two revertants, *w^e* and *w^{apl}*, do not exhibit dosage compensation between males and females. That is, most *white* alleles are similar in the amount of pigment between the two sexes, despite a difference in gene copy number. The two non-dosage compensating alleles, *w^e* and *w^{apl}*, reflect the gene copy, the males being lighter than females. These two have altered regulatory properties and are unaffected by *Inr-a*. The other two are dosage compensated and both are elevated in expression by *Inr-a^{hd1}*, although less so than other affected alleles.

Another nonresponding allele is *white-ivory*, *w^i*, which is a duplication of part of the first intron to exon two (KARESS and RUBIN 1982). It has very low levels of pigment and fails to exhibit dosage compen-

TABLE 5

Alleles of *white* tested with the *Inr-a*

Allele	Interaction	Lesion	Reference
w^a	+	*Copia* insertion in second intron	GEHRING and PARO (1980), BINGHAM and JUDD (1981), BINGHAM, LEVIS and RUBIN (1981)
w^{a59kl} (apricot revertant)	+	Solo copia LTR	CARBONARE and GEHRING (1985)
w^{aRM} (apricot revertant)	+	Transposable element insertion in *copia* 5′ LTR	MOUNT, GREEN and RUBIN (1988)
w^{aRR4h} (apricot revertant)	+	I element insertion in *copia* 3′ LTR	MOUNT, GREEN and RUBIN (1988)
w^{cf} (coffee)	+	Point	ZACHAR and BINGHAM (1982)
w^{a3} (apricot-3)	+	Point	ZACHAR and BINGHAM (1982)
w^{a2} (apricot-2)	+	Point	ZACHAR and BINGHAM (1982)
w^{sat} (satsuma)	+	Point	ZACHAR and BINGHAM (1982)
w^{col} (colored)	+	Point	ZACHAR and BINGHAM (1982)
w^{flwx} (Brownex)	+	Point	ZACHAR and BINGHAM (1982)
w^{bl} (blood)	+	Antiparallel retrotransposon insertion in intron 2	ZACHAR and BINGHAM (1982), BINGHAM and CHAPMAN (1986)
w^{bf} (buff)	+	B104 transposable element insertion in intron 4	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984), O'HARE, LEVIS and RUBIN (1983)
w^{IR1}	+	I element insertion	SANG *et al.* (1984)
w^{a4} (apricot-4)	+	BEL insertion into intron 2	ZACHAR and BINGHAM (1982), GOLDBERG *et al.* (1983)
w^{i} (ivory)	0	Duplication of intron 1, exon 2	KARESS and RUBIN (1982), COLLINS and RUBIN (1983), O'HARE *et al.* (1984)
w^{c} (crimson)	0	FB transposable element revertant of w^{i}	COLLINS and RUBIN (1982), O'HARE *et al.* (1984), LEVIS, COLLINS and RUBIN (1982)
w^{sp} (spotted)	0	B104 insertion in 5′ regulatory region	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984)
w^{sp4} (spotted-4)	0	Deficiency in 5′ *cis*-regulatory region	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984)
w^{sp2} (spotted-2)	0	Deficiency in 5′ *cis*-regulatory region	ZACHAR and BINGHAM (1982)
w^{sp81d5} (spotted-81d5)	0	Deficiency in 5′ *cis*-regulatory region	DAVISON *et al.* (1985)
w^{sp55}	−	Transposable element near 5′ structural gene	ZACHAR and BINGHAM (1982)
w^{e} (eosin)	0	Transposable element reversion of w^{1} (*Doc* element)	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984), HAZELRIGG (1987), O'HARE *et al.* (1991)
w^{h} (honey)	+/0	B104 insertion *Doc* element of w^{1}	ZACHAR and BINGHAM (1982), O'HARE *et al.* (1984, 1991)
w^{apl} (apricot-like)	0	P-M hybrid dysgenic revertant of w^{1} (*Doc* element)	C. MCELWAIN (personal communication), O'HARE *et al.* (1991)
w^{IR2}	+/0	I element revertant of w^{1} (*Doc* element)	SANG *et al.* (1984), O'HARE *et al.* (1991)
z w^{a}	+	*copia* insertion in second intron	GEHRING and PARO (1980), BINGHAM and JUDD (1981)
z $Dp(1;1)w$, w^{+61e19}	0	Duplication of *white* locus sequences with *zeste*	GREEN (1963)
Canton-S +	+ (testes)	None	

An increase in pigment is designated by +, a reduction by − and no effect by 0 in the interaction column.

sation. The basis of the regulatory phenotype is unknown.

The *white-spotted 55* allele is a transposon insertion near the 5′ end of the gene (ZACHAR and BINGHAM 1982). It has very low levels of pigment that is uniform but with the unusual regulatory phenotype of being darker in males than females. Interestingly, *Inr-a* reduces the amount of pigment in these flies (Figure 2, top right).

The essence of the allele specificity is that pigment levels in flies carrying *white* alleles with lesions in the structural gene are elevated in the presence of the $Inr\text{-}a^{hd1}$ mutation. Alleles due to lesions in the 5′ *cis*-acting regulatory region of *white*, however, are unaffected, as are alleles that fail to dosage compensate. Thus, alteration of *white* regulation blocks its interaction with the *Inr-a* mutation. The conclusion can be drawn that $Inr\text{-}a^{hd1}$ exerts its effect upon the *white* locus via the regulatory sequences.

***zeste-Inr-a* combination:** *zeste* is a gene that exerts a

regulatory effect upon the *white* locus in a synapsis dependent manner (BINGHAM and ZACHAR 1985; BICKEL and PIRROTTA 1990). Under most circumstances, *zeste* requires two adjacent copies of *white* to be effective. The sequences of *white* that are required for interaction with *z* are located in the 5′ regulatory region affected by the *spotted* alleles (HAZELRIGG, LEVIS and RUBIN 1984; DAVISON *et al.* 1985). To test the response of *white* in the presence of both *zeste* and *Inr-a*, the w^{1118}; *Inr-a/SM6a* males were crossed to females that were *z Dp(1;1)*w^{61e19}/*z Dp(1;1)* w^{61e19}. This stock has a duplication of *white* locus sequences such that males that result from this cross are *zeste* in phenotype (GREEN 1963). No difference was found between the classes of flies segregating for the *Inr-a* mutation in a *zeste* background. This indicates that when *zeste* is effective upon *white*, the action of *Inr-a* is blocked.

The w^{DZL} allele is caused by a Foldback transposable element upstream of the normal regulatory sequences of *white* (LEVIS, COLLINS and RUBIN 1982; BINGHAM and ZACHAR 1985), and produces a zeste-like phenotype in the presence of a wild type allele of *zeste* (BINGHAM 1980). This *white* allele was tested for interaction with *Inr-a* by crossing +/Y; *Inr-a*^{hd1}/*Cy0, Cy* males to females homozygous for w^{DZL}. The males are nearly wild type and cannot be scored for modulations of pigment. The females, however, have a reduction in pigment that is intermediate between the prototypical *zeste* phenotype and wild type. Among these females, those that were *Inr-a*/+ were darker than their sisters that were +/+. When the cross was repeated using females homozygous for *z* and w^{DZL}, all the progeny have a typical zeste phenotype. In this case, the *Inr-a* flies are indistinguishable from their siblings. The partial reduction in function caused by w^{DZL} does not eliminate an *Inr-a* response but the combination with *zeste* gives the same result as with a wild-type allele at *white*.

***Suppressor of white-spotted-Inr-a* combination:** A second gene implicated in regulating the *white* locus is *suppressor of white-spotted* (*su*(w^{sp})) (DAVISON *et al.* 1985). Mutants at this locus slightly suppress the phenotype of the *white-spotted* series, which are lesions altering sequences in the transcriptional enhancer. The suppressor locus and *white* are both on the *X* chromosome. A combination of a suppressed *white-spotted* (*su*(w^{sp}) w^{sp}) and *Inr-a*^{hd1} was produced to test whether the suppressed condition would allow the *Inr-a* mutation to produce an effect. No phenotypic differences between *Inr-a*^{hd1}/+ and segregating siblings, that were +/+; (*SM6a*/+), were observed. Thus the suppressor does not restore the ability to respond to *Inr-a*.

Response of truncated white constructs: The observation that regulatory mutants of *white* do not respond to *Inr-a* (or in the case of w^{sp55} do so differently) and that the *zeste* regulatory effect on *white* also eliminates any *Inr-a* response, argues that the inverse regulator works via interaction with the *white* promoter, directly or indirectly. As an additional test, *Inr-a* was combined with a promoter fusion construct that replaces the *white* promoter with that of the *alcohol dehydrogenase* gene driving *white* structural sequences and reintegrated into the Drosophila germline (kindly provided by JANICE FISCHER). The transformed construct is on the third chromosome and carried in the presence of a *white* null mutation (BIRCHLER, HIEBERT and PAIGEN 1990). The flies have a low level of pigment at eclosion that increases with age. When this stock is crossed to w^{1118}; *Inr-a*^{hd1}/*SM6a*, the segregating progeny do not differ in the intensity of pigment (Figure 2e). This experiment suggests that the 5′ *cis*-acting regulatory region of *white* is required for the interaction with *Inr-a*. Trisomics produced by the insertional translocation, G44, as described above, also have no effect upon this construct.

A series of *white* constructs truncated at different points of the promoter has been generated by LEVIS, HAZELRIGG and RUBIN (1985) and by PIRROTTA, STELLAR and BOZZETTI (1985). Most of these still produce nearly saturated levels of pigment and cannot be phenotypically tested with *Inr-a*. However, the constructs that cut at the *Sca* restriction site 360 bp 5′ to the start of transcription in the Canton S wild-type allele and some that cut at the *Bgl* site (1039 bp 5′ to the transcriptional start) are sufficiently limiting on pigment deposition for such an assay (LEVIS, HAZELRIGG and RUBIN 1985). All of the tested *Bgl* constructs were reduced in expression by the G44 trisomics and all of those not saturated at the diploid level were elevated in pigment level by *Inr-a*/+ (Table 6). Of the six different *Sca* construct insertions tested with *Inr-a*, only two are elevated in expression (Figure 2f). These same two insertions are reduced in expression by the G44 trisomics. This suggests that at least a portion of the *white* promoter that is required to respond to *Inr-a* resides between the *Sca* site and the *white* structural gene, because some inserts are still capable of responding. The position of the *Sca* construct within the genome apparently influences this ability. Also examined were two constructs with the majority of the large first intron of *white* removed, *3-76a* (BIER *et al.* 1989) and *mini-white* (PIRROTTA 1988). Both respond to *Inr-a*, indicating that the deleted sequences in the first large intron are not required for *Inr-a* interaction.

The final test of the requirement of the *white* promoter for interaction with *Inr-a* involved construction of a *white* promoter-*Adh* reporter fusion. This construct was tested for the ability of the *white* promoter to confer a response to *Inr-a* upon ADH. The details

TABLE 6

Interaction of *Inr-a* with modified *white* constructs reintroduced into the genome

Construct	Insertion site	Pigment intensity alteration		Reference
		Inr-a mutant	G44 trisomic	
F4-1	57B	Elevated	Reduced	LEVIS, HAZELRIGG and RUBIN (1985)
F3	64B	None	None	LEVIS, HAZELRIGG and RUBIN (1985)
F4-2	21D	None	None	LEVIS, HAZELRIGG and RUBIN (1985)
F4-3	97B	None	ND	LEVIS, HAZELRIGG and RUBIN (1985)
F2	86C	Elevated	Reduced	LEVIS, HAZELRIGG and RUBIN (1985)
F1	50A	None	ND	LEVIS, HAZELRIGG and RUBIN (1985)
E1	19F	ND	Reduced	LEVIS, HAZELRIGG and RUBIN (1985)
E2	12B	ND	Reduced	LEVIS, HAZELRIGG and RUBIN (1985)
E4	ND	Elevated	ND	LEVIS, HAZELRIGG and RUBIN (1985)
E5	2A	ND	Reduced	LEVIS, HAZELRIGG and RUBIN (1985)
E6	57B	Elevated	ND	LEVIS, HAZELRIGG and RUBIN (1985)
E7	94D	Elevated	Reduced	LEVIS, HAZELRIGG and RUBIN (1985)
E8	47D	ND	Reduced	LEVIS, HAZELRIGG and RUBIN (1985)
mini-white (mw^+)	X	Elevated	ND	PIRROTTA (1988)
3-76a	X	Elevated	ND	BIER *et al.* (1989)

Construct designations are from LEVIS, HAZELRIGG and RUBIN (1985), PIRROTTA (1988) and BIER *et al.* (1989). Crosses to assay the effect of *Inr-a* and trisomy for 44C-50C on the various constructs are described in MATERIALS AND METHODS. The F series has 360 bp remaining before the transcriptional start site while the E series has 1039 bp. The *mini-white* and 3-76a constructs have most of the large first intron removed. The effects are described as elevated, reduced, none, or not determined (ND).

of the fusion construction are given in MATERIALS AND METHODS. Briefly, a 2.5 kilobase *HphI* fragment carrying *white* regulatory sequences and part of the 5′ leader was fused to a restriction site within the sequences encoding the mRNA 5′ leader of *alcohol dehydrogenase*. The construct was reintroduced to the Drosophila germline of an *Adh* null strain (Adh^{fn6} *cn*; ry^{506}). The transformants exhibit ADH activity in the eyes, Malpighian tubules and testes, where *white* is normally expressed. The ADH activity per individual is highest in pupae–a developmental stage with high *white* expression (FJOSE *et al.* 1984). Further details of the expression, dosage compensation properties and transvection effects of this construct will be published elsewhere.

The *Inr-a* chromosome was recombined with Adh^{fn6} and tested for a response of the *white-Adh* fusion construct (designated 9g12) inserted into chromosome *3*. The *Adh* null chromosome and the null-*Inr-a* recombinant were crossed in parallel to a complex translocation-balancer, *T(2:3) Cy0, Tb ch*, that carries the dominant larval/pupal marker *Tubby*. F_1 heterozygous males were then crossed to a homozygous third chromosomal insert line (9g12). The non-*Tubby* pupae, which represent the *Adh* null individuals, were collected from the respective crosses to give +/+; 9g12/+ and *Inr-a*/+; 9g12/+ genotypes. Pupae were also collected from the Adh^{fn6}; ry^{506} recipient strain as a background control for nonspecific dehydrogenase activity. When alcohol dehydrogenase activity was determined from these three classes of pupae, the *Inr-a*/+ class had 263% of the +/+ strain above the background control (Table 7). These results are consistent with the inverse effect being mediated via the promoter sequences of *white* (including a short segment of the 5′ leader).

Genetic specificity: To determine what other types of genes, if any, were affected by this mutant, $Inr\text{-}a^{hd1}$ was introduced with the second chromosome balancer, *SM6a*, to stocks homozygous for a variety of hypomorphic alleles at selected loci affecting eye color, bristles and wings. Hypomorphic alleles are required in order to assay for modulation of expression. After construction of these stocks, progenies were compared that were segretating for $Inr\text{-}a^{hd1}$/+ and *SM6a*/+. None of the loci examined were affected by *Inr-a* (see Table 8).

Because *Inr-a* has a dosage effect and could potentially be implicated as involved with dosage compensation of the *white* locus and because it fails to affect alleles at *white* that do not exhibit dosage compensation, a test was performed on three *X*-encoded enzymes, as well as a third chromosomal locus, to examine whether *Inr-a* affected the *X* chromosome specifically. None of the enzymes examined were quantitatively modulated by *Inr-a* (see Table 9). This indicates that *Inr-a* does not affect *X* chromosome dosage compensation generally, but does not rule out a specific involvement with this aspect of *white* locus function.

***Inr-a* inversely affects *white* messenger RNA levels:** Because the *Inr-a* mutation had been demon-

TABLE 7

Effect of *Inr-a* on the *white* promoter-*Adh* reporter construct (9g12)

Genotype	*n*	ADH activity mean ± SE (O.D. 340)	Protein estimate mean ± SE (O.D. 595)	ADH/protein ± SE	ADH/protein − background
Adh^{fn6};ry^{506} (background)	9	0.136 ± 0.005	0.586 ± 0.013	0.233 ± 0.010	0
Adh^{fn6};ry^{506}, 9g12 +/+	9	0.169 ± 0.003	0.600 ± 0.009	0.282 ± 0.006	0.049
Adh^{fn6};ry^{506}, 9g12 *Inr-a*/+	9	0.222 ± 0.006	0.617 ± 0.017	0.362 ± 0.013	0.129

Mean values ± standard error represent the optical density readings of ADH enzyme activity and Bradford protein determination or their individual ratios on nine (n = 9) samples each consisting of ten pupae recovered from crosses described in the text. The Adh^{fn6}; ry^{506} stock serves as a background of general dehydrogenase activity in such concentrated samples. Above the background level, the *Inr-a*/+ pupae had 263% as much ADH activity as +/+ when corrected to total protein and 261% when corrected to a per individual basis. The difference between the ADH/protein means of 9g12; *Inr-a*/+ *vs.* +/+ is significant in statistical tests at the 99% confidence level.

TABLE 8

Hypomorphic and neomorphic mutations tested for interaction with *Inr-a*

Locus	Allele
glass	gl^2
pink	p^6
vermilion	v^1, v^{24}
rudimentary	r^{sp1}, r^{29}
purple	pr^1
cut	ct^1
forked	f^1
Kinked	*Ki*
Notch	*split*
Tuft	*Tft*
vestigial	vg^{Ultra}

Loci are described in LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM (1987). The effect was none in all cases.

strated to interact via the sequences within the regulatory region of *white*, the effect of its mutations on accumulation of *white* mRNA was examined. RNA derived from two different populations of adults segregating for the *Inr-a* mutation and a balancer chromosome demonstrated no discernible difference in accumulation of *white* and control RNAs (*rudimentary*, *vermilion*) on four separate Northern transfers (Figure 3). This result suggests that either that *Inr-a* does not affect total *white* RNA or that its major effect is at earlier stages of development, when the majority of pigment is deposited, in an analogous manner to *zeste*, which affects *white* RNA levels in pupae but not in adults (BINGHAM and ZACHAR 1985).

To test the response on the RNA level at earlier developmental stages, when *white* is more strongly expressed (FJOSE *et al.* 1984), an analysis of *white* mRNA was performed on a segregating population using the EMS2 induced allele of *Inr-a* in the presence of the Canton-S allele of *white*. Total RNA was prepared from larvae and pupae from two segregating populations. The first involved a comparison of *al b* $Inr\text{-}a^{EMS2}$ *c* sp^2/+ versus *T(2;3) Cy0*; *Tb ch*/+ and the second involved the chromosome on which the mutant was induced (*al b c* sp^2)/+ versus the *T(2:3) Cy0*; *Tb ch*/+ configuration as a control. Developmentally staged samples were collected from wandering third-instar larvae, 0–24-hr-old pupae, intermediate pupae and late pupae. Size fractionated and transferred RNA was probed with single stranded antisense *white* RNA on three separate blots. A similar probe for the *rp49* locus was used as a loading control. An example is shown in Figure 4. There is an elevation of *white* mRNA levels in larvae and in mid pupae in the *Inr-a*/+ individuals compared to +/+ as determined by

TABLE 9

Test of effect of *Inr-a* on *X* and autosomally encoded enzyme activities

Enzyme	Chromosome	Enzyme activity	
		$Inr\text{-}a^{hd1}$/+	*SM6a*/+
Glucose-6-phosphate dehydrogenase (G6PDH)	*X*	2.94 ± 0.03	3.29 ± 0.16
6-Phosphogluconate dehydrogenase (6PGDH)	*X*	2.28 ± 0.06	2.04 ± 0.08
β-Hydroxy acid dehydrogenase (BHAD)	*X*	3.29 ± 0.10	3.74 ± 0.22
Isocitrate dehydrogenase (IDH)	*3*	6.32 ± 0.11	5.78 ± 0.13
Protein		1.37 ± 0.02	1.25 ± 0.01
DNA		2.34 ± 0.09	2.13 ± 0.08

Values are means ± standard error of five assays per genotype. None of the paired means are significantly different in statistical tests at the 95% level of confidence. Enzyme activity is expressed as micromoles of NADH or NADPH × 10^2 produced per ml in the reactions described in MATERIALS AND METHODS. Protein is expressed as mg/ml. DNA is expressed as μg/ml. The two classes of males assayed were from a cross of an attached *X* (*C(1)DX*, *y w f*/*Y*) stock with isogenic Oregon R wild-type autosomes by w^a/*Y*; $Inr\text{-}a^{hd1}$/*SM6a* individuals. The structural gene for IDH is on the third chromosome and those for G6PDH, 6PGDH and BHAD are on the *X*.

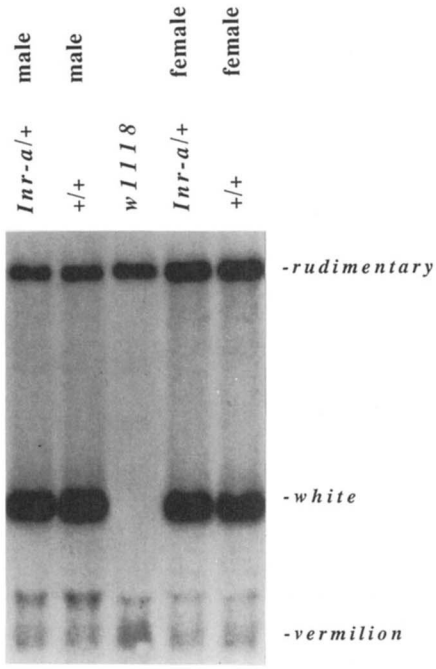

FIGURE 3.—RNA transfer analysis of an adult segregating population of +; *Inr-a*/+ versus ++ probed with *white* (*w*), *rudimentary* (*r*) and *vermilion* (*v*). Male and female preparations are shown for both genotypes. The position of the *w*, *r* and *v* RNAs is noted. The *white* deficiency strain, w^{1118}, serves as a negative control. Further details are given in the text.

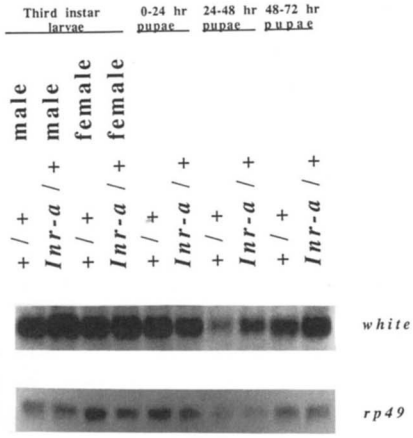

FIGURE 4.—RNA transfer analysis of larval and pupal segregating populations probed with *white* and *rp49*. Total RNA isolated from the noted genotypes and developmental stages was separated and hybridized with an antisense *w* cDNA probe followed by an *rp49* probe as a loading control. The *Inr-a*/+ class has greater levels of *w* RNA in third instar larvae but the greatest difference is in the later pupal stages. Further details of the cross are given in the text and MATERIALS AND METHODS.

densitometric scans (Table 10). One of the blots was also probed with antisense *Adh*, which showed no difference between genotypes in the stages in which *Adh* was expressed (not shown). The control blot in which the comparison was of the chromosome of origin (*al b c* sp^2)/+ *vs.* the *Tb* balancer/+ showed no discernible differences in *white* RNA levels (not shown).

***Inr-a* coordinately affects the messenger RNA levels of the *white*, *brown* and *scarlet* loci:** Two genes affecting eye color have been shown to have DNA sequences similar to *white*, these being *brown* (*bw*) on chromosome *2*, and *scarlet* (*st*) on chromosome *3* (DREESEN, JOHNSON and HENIKOFF 1988; TEARLE *et al.* 1989). The products of these three genes are thought to be involved in the transport of pigment precursors from the hemolymph into the appropriate cell types (DREESEN, JOHNSON and HENIKOFF 1988). The expression pattern of the three genes is quite similar and may be coordinately regulated. We examined the possibility that *Inr-a* serves as such a coordinate regulator of these genes by estimating *bw* and *st* mRNA quantity from the cross described above. Duplicate RNA transfers were probed with a *bw* cDNA (Figure 5) and a second set with a *st* genomic clone (Figure 6). In all cases, an *rp49* probe was used as a control for gel loading. Densitometric scans of an example of the *bw* and *st* blots indicate an increase in mRNA accumulation for these two genes in the presence of *Inr-a* mutants during similar developmental stages as those during which *white* is affected (Table 10). The strongest effects are found in larval females and mid pupae. It is interesting to note that in the larval male/female comparison of *brown*, the male levels are approximately doubled. A sexual dimorphism of autosomal genes, when present, usually is manifested as a higher male expression whereas autosomal expression is generally reduced in trisomic *X* metafemales (BIRCHLER, HIEBERT and KRIETZMAN 1989). Despite different developmental fluctuations in strength of the response, the RNA levels from all three pigment genes appear to be affected by *Inr-a*.

DISCUSSION

The results of the present experiments demonstrate that the action of the regulatory "balance" phenomena found in aneuploidy studies (BIRCHLER 1979, 1981; BIRCHLER and NEWTON 1981; BIRCHLER, HIEBERT and PAIGEN 1980; DEVLIN, HOLM and GRIGLIATTI 1982, 1988; DEVLIN, GRIGLIATTI and HOLM 1984; KLOSE and PUTZ 1983; REICHERT 1986; WHATLEY *et al.* 1984) can be reduced to single genes. This suggests that many consequences of chromosomal variation such as aneuploid syndromes, dosage compensation and the inverse effect are the result of changes in the stoichiometry of factors from the multiple regulatory networks operating in eukaryotic cells.

The inverse effect is caused by specific genes: The rationale for the present experiments was to test whether mutants could be induced that would mimic the inverse effect, which was originally defined in aneuploidy studies. It was potentially the case that the inverse effect could be brought about by an imbalance of the generalized cellular regulatory machinery or

TABLE 10

Densitometric values of RNA transfers of *Inr-a* and normal probed with *white*, *scarlet* and *brown*

Genotype and stage	*w*	*rp49*	*w*/*rp49*	*Inr-a*/+ +	*st*	*rp49*	*st*/*rp49*	*Inr-a*/+ +	*bw*	*rp49*	*bw*/*rp49*	*Inr-a*/+ +/+
Male, 3rd instar, *Inr-a*/+	2013	1725	1.17	2.21	588	464	1.27	1.08	2641	832	3.17	1.31
Male, 3rd instar, *Tb*/+	1530	2906	0.53		640	543	1.18		2457	1017	2.42	
Female, 3rd instar, *Inr-a*/+	2028	1347	1.51	1.91	633	449	1.41	1.40	1242	685	1.81	2.51
Female, 3rd instar, *Tb*/+	1760	2221	0.79		677	668	1.01		749	1052	0.72	
Early pupae, *Inr-a*/+	1205	803	1.50	1.05	1510	211	5.45	1.45	304	478	0.64	0.74
Early pupae, *Tb*/+	1664	1161	1.43		1312	324	4.05		624	724	0.86	
Mid pupae, *Inr-a*/+	542	375	1.45	1.96	367	107	3.43	2.30	1000	315	3.17	1.30
Mid pupae, *Tb*/+	239	324	0.74		146	98	1.49		560	230	2.43	
Late pupae, *Inr-a*/+	1526	1450	1.05	1.33	1490	487	3.06	1.22	2240	848	2.64	1.35
Late pupae, *Tb*/+	921	1165	0.79		1096	437	2.51		1492	739	1.95	

Numbers represent arbitrary values from densitometric scans of *w*, *rp49*; *st*, *rp49* and *bw*, *rp49* probed transfers. For the respective comparisons, a *w*/*rp49*, *st*/*rp49* and *bw*/*rp49* ratio was calculated. These values were used to compare *Inr-a*/+ *vs.* +/+ genotypes.

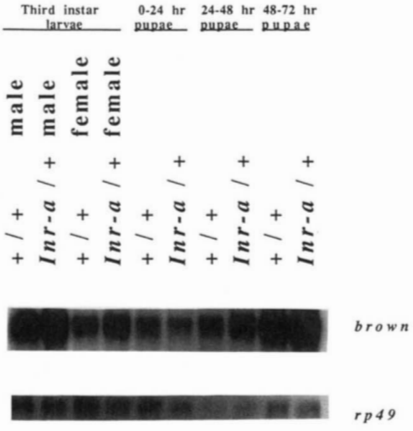

FIGURE 5.—RNA transfer analysis of larval and pupal segregating populations probed with *brown* and *rp49*. Total RNA isolated from the noted genotypes and developmental stages was separated and hybridized with antisense probes of *brown*, followed by *rp49* as a loading control. *Inr-a* elevates the levels of *brown* RNA in larvae, more so in females than in males, which overall shows greater expression. *Inr-a* also elevates *brown* RNA in the later pupal stages. Further details of the cross are given in the text and MATERIALS AND METHODS.

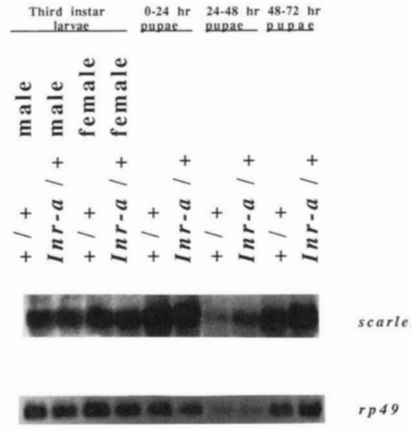

FIGURE 6.—RNA transfer analysis of larval and pupal segregating populations probed with *scarlet* and *rp49*. RNA samples used and described in Figure 4 were hybridized with antisense probe from *scarlet* and *rp49*, as a loading control. *Inr-a* shows the strongest elevation on *scarlet* RNA in the later pupal stages.

by some type of homeostatic mechanism. In this paper, the gene, *Inr-a*, is described, which exerts a coordinate inverse regulatory effect upon mRNA levels of the *white*, *brown* and *scarlet* loci. The finding of single gene mutants in inverse regulators with specificity for structurally and functionally related target genes together with the accumulated aneuploid data suggests that the balance phenomena are the cumulative effects of specific and potentially overlapping regulatory networks.

The properties of *Inr-a* are as follows. When only one functional copy of the gene is present, the expression of *white* is elevated approximately twofold. The mutation maps to a region of the genome that when present as a trisomic reduces the level of *white* expression. The introduction of a single mutant *Inr-a* copy into a triploid elevates the expression of *white* to approximately 3/2 the normal triploid level, yet the phenotypes of normal diploid and triploid females are quite similar.

The promoter sequences of *white* are required to respond to *Inr-a* mutants, as was found to be the case by extensive allele specificity analysis and by the fact that a promoter fusion construct containing the regulatory sequences of *alcohol dehydrogenase* (*Adh*) and the structural portion of *white* was not affected by *Inr-a*. The reciprocal construct of the *white* promoter and a short segment of the 5′ leader driving an *Adh* reporter produced elevated *Adh* levels when only one functional copy of *Inr-a* is present. The *Adh* promoter-*white* structural fusion demonstrates that the *white* promoter is *necessary* for interaction with *Inr-a*, while

the *white* promoter-*Adh* structural fusion construct experiment delimits the sequences that are *sufficient* for interaction. These experiments define the sequences and conditions of *white* that are required to produce this type of regulatory response but do not address whether the effect is produced by direct interaction of the product of *Inr-a* with *white* or by some indirect means.

Complexity of promoter interactions: The *white-spotted* mutations represent an interesting set of lesions at the *white* locus. Their phenotype is a mottled one that in general exhibits greater levels of pigment in males than females. They also partially complement other hypomorphic alleles, such as w^a, giving a solid dark orange phenotype (GREEN 1959).

The transduced truncated copies of *white* that are missing the *spotted* region do not have a phenotype typical of the spotted mutations (LEVIS, HAZELRIGG and RUBIN 1985). Instead, they are darker and not mottled, resembling in many respects the complementation phenotype described above. This suggests that the *spotted* mutations represent more than a loss of function but rather that the lesions, which are all deletions or insertions, have an antagonistic *cis* effect on the functioning of the promoter.

The paradox that exists for the phenotype of the *spotted* mutations and of the deletion constructs, extends to the interaction with *Inr-a*. None of the *spotted* mutants are phenotypically modulated by *Inr-a*. The cis-inactivation of the promoter in the *spotted* lesions prevents a response to *Inr-a* but the *Bgl* constructs and some of the *Sca* are affected. This suggests that the *spotted* lesions prevent the interaction of *Inr-a* (directly or indirectly) with another region of the promoter or that multiple sites in the promoter are required to mediate a response to *Inr-a*.

Coordinate regulation: The product of the *white* gene is required for the deposition of the brown pigments (ommochromes) as well as the red (pteridines) (SULLIVAN and SULLIVAN 1975). The ommochromes are present in both the primary and secondary pigment cells within each ommatidium, while the pteridines are localized in the secondary pigment cells. The sequence of *white* shows homology to membrane associated ATP binding proteins from bacteria (MOUNT 1987; DREESEN, JOHNSON and HENIKOFF 1988). Also required for deposition of the pteridines in various tissues is the product of the *brown* locus. This gene also shows DNA sequence homology to membrane associated ATP binding proteins (DREESEN, JOHNSON and HENIKOFF 1988). These findings, as well as evidence for genetic interactions between *brown* and *white*, led the latter authors to propose that the products of these two genes function as units of a multimeric permease, involved in precursor uptake for pteridine biosynthesis.

The product of the scarlet (*st*) locus is required for the deposition of ommochromes. Mutations in *scarlet* affect the same tissues as *white* in that they both are required for the uptake of brown pigment precursors in the eyes, ocelli, and Malpighian tubules. DNA sequence analysis of *scarlet*, also reveals a similarity to the *white* gene (TEARLE *et al.* 1989). It is therefore likely that *white*, *brown* and *scarlet* are functionally related as members of a gene family involved in pigment precursor uptake (DREESEN, JOHNSON and HENIKOFF 1988).

The coordinate functions and expression of *white*, *brown* and *scarlet* suggested that they are coordinately regulated. *Inr-a* was originally identified as exerting a regulatory effect on *white* by several criteria. The most striking effect on the messenger RNA level is observed in the pupal stage. Levels of *brown* and *scarlet* RNA also reveal a similar elevation at the earlier developmental periods. Whether *Inr-a* is involved in regulating these genes at all developmental stages but only exhibits a developmentally specific rate limiting effect remains to be determined. Nevertheless, it appears that *Inr-a* is a coordinate regulator of these functionally related genes.

The coordinate effect upon these three loci, but not upon other loci tested, implies that the balance phenomena in aneuploids is the cumulative effect of many regulatory genes. Dosage sensitivity of these genes is not necessarily selected for, but is merely a reflection of their mechanism of action. Autosomal dosage compensation is undoubtedly not selected to cope with aneuploidy, which is a laboratory constructed condition. If every structural gene is regulated by a set of interacting stoichiometrically sensitive factors, the balance phenomena found in aneuploids versus euploids would result.

In spite of the fact that *Inr-a* produces a negative dosage effect upon the expression of *white*, we do not view it as a repressor in the standard sense. First, the strongest effect of *Inr-a* on *white* RNA levels occurs at the developmental stage at which *white* is relatively very active (pupae). Secondly, the *white* alleles and truncated promoter constructs that fail to respond to *Inr-a* are reduced in overall expression rather than increased. These observations suggest that the mechanism of *Inr-a* action is distinct from that of a classically defined repressor.

As previously suggested, the existence of multiple dosage sensitive regulatory genes for any one structural gene predicts that the stoichiometry of components of a regulatory complex can determine the quantitative expression of structural genes (BIRCHLER and NEWTON 1981; BIRCHLER, HIEBERT and KRIETZMAN 1989; BIRCHLER, HIEBERT and PAIGEN 1990). The observation that multiple aneuploid series in a particular species can produce a direct or inverse

effect upon a single monitored structural gene is consistent with this view (BIRCHLER and NEWTON 1981; DEVLIN, HOLM and GRIGLIATTI 1988). Mutant screens as described above have proven successful in our laboratory in identifying other loci with similar properties. Further investigation will lead to elucidation of the full spectrum of regulatory genes that contribute to the balance phenomena.

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