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Genetics

Use of second-site suppressor mutations in *Drosophila* to identify components of the transcriptional machinery

(protein interactions/conditional lethal mutants/compensatory mutants/RNA polymerase II)

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ABSTRACT **Isolation of second-site suppressor mutations provides a powerful method for identifying (*i*) genes that encode proteins that interact and (*ii*) domains within the interacting proteins that contact each other. Flies conditionally lethal because they carry mutations in the largest subunit of RNA polymerase II were mutagenized; ten million progeny were then screened for compensatory mutations. Eight intragenic and 10 extragenic suppressor mutations were recovered. Both the conditional lethality and premature termination of transcription caused by one mutation in the largest subunit of RNA polymerase II are compensated by an allele-specific suppressor mutation in the second-largest subunit of the enzyme.**

Biochemical studies have identified many transcription factors by their ability to bind RNA polymerase II (Pol-II) (1–4) and/or by their requirement in *in vitro* transcription assays (5–8). Nevertheless, the mechanism by which Pol-II recognizes and transcribes a gene is only partially understood (9, 10). These mechanisms could be further elucidated by identifying and characterizing mutations that disrupt different components in this process.

Mutations in the largest subunit of Pol-II were isolated by their ability to confer resistance to the toxin α-amanitin (11–14). It was also possible to directly induce mutations in cloned subunits of yeast Pol-II (15, 16). Once identified genetically, a gene can be further mutated by standard techniques (17–19). The mutationally altered enzyme can then be analyzed either biochemically or in intact cells or whole organisms to determine its effects on cellular processes, such as growth and development. These studies provide insights into the functioning of individual components of the transcriptional machinery.

Estimates of the number of subunits of RNA Pol-II range from 9 to 14 (15, 20). Unfortunately, it is difficult to design screens to identify mutations in subunits or accessory factors that are not cloned, as the mutant phenotypes predicted for these mutations are indistinguishable from other essential genes. To circumvent this problem, I chose to screen for second-site suppressor mutations that ameliorate the mutant phenotype of existing mutations in the largest subunit of *Drosophila* RNA Pol-II. These suppressor mutations have the implied property of identifying genes that encode proteins also used for transcription.

The largest subunit of *Drosophila* Pol-II was initially identified by the induction of a mutation that conferred resistance to α-amanitin (12, 21). Conditional lethal mutations were induced in this subunit (17, 22–24). I have used three of these heat-sensitive lethal alleles to recover 18 second-site suppressor mutations. Ten are extragenic suppressors identifying at least three genes likely to encode proteins required in the transcriptional process. These proteins might include different subunits of Pol-II and transcription factors. At least two extragenic suppressors result from mutations in the second-largest subunit of Pol-II. Their allele-specific interactions with a single conditional lethal allele of the largest subunit suggest that they identify contact points between domains of the two largest subunits of Pol-II.

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MATERIALS AND METHODS

Screen for Second-Site Suppressor Mutations. Stocks of three mutations in the largest subunit of Pol-II (*RpII215*), *v RpII215*^{E28} (17), *y RpII215*^{ts} *f* (22), and *ras v RpII215*^{WJK1} (24) (*RpII215* mutations will be referred to by their allelic designation, e.g., *E28*), were marked as indicated with different combinations of recessive-visible mutations (described in ref. 25). Multiple lines of each stock were maintained at permissive temperature in half-pint bottles containing standard cornmeal/molasses/agar yeast medium. Each bottle was treated as a separate line and tested periodically for lethality at restrictive conditions.

Flies were treated overnight with ethyl methanesulfonate (10 μl/ml) in half-pint bottles (100–500 flies per bottle). Flies were then transferred to fresh bottles and allowed to lay eggs at permissive temperature. The eggs used in F_1 screens were shifted to restrictive temperature 24–48 hr after egg laying, whereas the eggs used in F_2 screens were allowed to develop to the adult stage at permissive temperature. The resulting progeny's eggs were shifted to restrictive temperature 48 hr after egg laying.

Mapping of Second-Site Suppressors. The suppressor mutations were initially maintained by continual selection at restriction conditions. The X, second, and third chromosomes of the suppressor lines were individually replaced with balancer chromosomes for the respective chromosomes, *M-6*, *CyO*, and *TM6B* (18, 25). Chromosomal regions were further subdivided by using the following mutations for the X chromosome: *y* [0.0 centiMorgan (cM)], *w* (1.5 cM), *ec* (5.5 cM), *cv* (13.7 cM), *ct* (20.0 cM), *ras* (32.8 cM), *v* (33.0 cM), *m* (36.1 cM), and *f* (56.7 cM); and third chromosome: *ru* (0.0 cM), *h* (26.5 cM), *th* (43.2 cM), *st* (44.0 cM), *cu* (50.0 cM), *sr* (62.0 cM), *e* (70.0 cM), and *ca* (100.7 cM) (25). A given region defined by adjacent markers was tested both for the loss of suppression when replaced by the equivalent region from the marker chromosome and for compensation of the conditional lethality by this same region when separated from the rest of the initial suppressor background.

RNA Analysis. The P57 line of a modified *Hsp82* gene (*modHsp82*) (Fig. 1*A*; ref. 26) was crossed into flies mutant for different polymerase alleles. Progeny of the appropriate genotypes were raised at 19°C until the crawling third-larval instar stage and then shifted to 29°C for 48 hr. Half the resulting pupae of each genotype were heat shocked in a water bath at 36.5°C for 20 min. RNA was isolated in

Abbreviations: cM, centiMorgan; Pol-II, RNA polymerase II.

Holmes–Bonner solution (27) and analyzed by Northern (RNA) blots or by RNA probe protection, as described elsewhere (26).

RESULTS

Recovery of Second-Site Suppressors. Recovery of second-site suppressor mutations requires the screening of large numbers of mutagenized flies for rare mutations. Screening is facilitated by using conditionally lethal flies that carry a temperature-sensitive mutation in the largest subunit of RNA Pol-II. Flies mutant for one of three different conditional lethal *RpII215* alleles—*E28*, *ts*, and *WJK1*—are raised and mutagenized at permissive temperature. Their eggs (F_1 screen) or the eggs of their progeny (F_2 screen) are allowed to develop at restrictive temperature. All flies die at restrictive temperature before reaching the adult stage, except those with newly induced compensatory mutations. From 115 such screens ≈10 million flies were tested, and 18 suppressor mutations were recovered (Table 1). Ten of the 18 mutations are extragenic.

Ten suppressors map on the X chromosome, and eight map on the third chromosome. All eight third chromosome suppressors of the *RpII215* allele, *WJK1*, map to a 12-cM region between *cu* and *sr*. Seven of the eight third chromosome suppressors are homozygous viable and phenotypically wild type when separated from *WJK1*. The eighth, *S8*, is recessive lethal. Two observations suggest that both the compensatory and recessive-lethal phenotypes of *S8* result from a single lesion. (*i*) Both phenotypes map between *cu* and *sr* and appear to be inseparable by recombination. (*ii*) *WJK1* suppresses the lethality of the *S8* chromosome, as *WJK1*/+;*S8* flies are viable.

The second-largest subunit of Pol-II, encoded by the *RpII140* locus, also maps to the *cu–sr* region of the third chromosome (28). Mutations have been induced in *RpII140* by saturation mutagenesis of a deficiency of the locus (M.A.M., S. Berger, R. Zuerner, B. Hamilton, and A. Greenleaf, unpublished results). The lethality of *S8* complements all *RpII140* alleles tested, suggesting that it defines a previously unidentified gene.

Because seven of the eight third chromosome extragenic suppressors fail to elicit mutant phenotypes when homozygous, further analysis of them is difficult. Two lines of evidence suggest that the third chromosome suppressors are missense mutations that result in structurally altered proteins. (*i*) They were induced by ethyl methanesulfonate, a chemical that often produces missense mutations (29). (*ii*) Deficiencies including the loci fail to suppress *WJK1*. It should, therefore, be possible to further mutate these suppressor alleles, reverting their suppressor phenotype to non-suppression. The resulting mutations are likely to be null alleles and to cause a recessive-lethal phenotype.

Table 1. Screen for suppressor mutations

	RpII215 mutations, no. flies		
	E28	*ts*	*WJK1*
F_1 screen			
Tested	3×10^6	4×10^6	2×10^6
Suppressors	0	3	0
Extragenic	0	0	0
F_2 screen			
Tested	0.1×10^6	0.1×10^6	1×10^6
Suppressors	0	2	13
Extragenic	0	0	10

Flies from three different stocks of *RpII215* alleles, *v* $RpII215^{E28}$, *y* $RpII215^{ts}$ *f*, and *ras v* $RpII215^{WJK1}$ were mutagenized; eggs were collected at permissive temperature and either shifted to restrictive temperature (F_1) or allowed to develop to the adult stage at permissive temperature. The latter adults were transferred to new bottles; eggs were collected and shifted to restrictive temperature (F_2).

Flies carrying one of three different extragenic suppressors, *S1*, *S2*, or *S8*, were mutagenized and examined for the loss of their ability to suppress *WJK1* lethality at restrictive temperature. Six mutations were induced in *S1* (*S1R1–S1R6*; these will be collectively referred to as *S1R*), and one each was induced in *S2* (*S2R*) and *S8* (*S8R*). Although these new mutations were selected only because they fail to suppress the conditional lethality of *WJK1*, all eight also cause a recessive-lethal phenotype. The recessive lethality was inseparable by recombination from the initial suppressor phenotypes in all cases tested; therefore, the lethal phenotypes probably result from second mutational events in the same genes that cause the initial suppression phenotype. Furthermore, the initial suppressor mutations most likely encode structurally altered proteins, as is postulated above.

S1 and *S2* are two independently derived alleles of the *RpII140* locus, as all seven putative null alleles, *S1R* and *S2R*, fail to complement each other and all *RpII140* alleles tested. Both *S8* and *S8R* complement the lethality of all *RpII140* alleles tested, including *S1R* and *S2R*. *S1* and *S2* are probably alleles of the second-largest subunit of RNA Pol-II, and *S8* most likely identifies another locus that encodes a protein required for transcription. Whether the other five third chromosome suppressors are alleles of *RpII140*, *S8*, or identify additional loci remains to be determined.

Two X chromosome-linked suppressor mutations of *WJK1* map near the tip of the chromosome, a region not known to possess a subunit of RNA Pol-II. When separated from the conditional allele they compensate, neither *S3* nor *S13* display mutant phenotypes. Further genetic tests (see below) suggest that these two suppressors are independently derived mutations in the same gene.

All five of the suppressor mutations of the *RpII215* allele, *ts*, but only three of 13 *WJK1* suppressors were inseparable from the *RpII215* locus (Table 1). Chromosomes carrying these suppressor mutations are cytologically normal and do not alter recombination around the *RpII215* region. These eight suppressors are, therefore, most probably intragenic mutational events. However, none of them is a revertant of the initially altered amino acid, as all eight intragenic suppressors retain some properties of the original conditional mutations, including reduced viability.

Altered Activity of WJK1 Polymerase. To study effects of suppressor mutations on activity of conditional lethal mutant polymerase, it was first necessary to characterize the activity of polymerase in flies mutant for either of the two suppressible *RpII215* alleles, *ts* and *WJK1*. This objective was accomplished by examining their ability to accumulate Hsp82 mRNA in response to heat shock. Transcription from the *Hsp82* promoter is constitutively low but greatly stimulated by heat shock (30). Development of both *ts* and *WJK1* flies is arrested when they are reared at the restrictive temperature of 29°C (22, 24, 31); therefore, a heat pulse of 36.5°C tests the ability of their polymerase to function at restrictive temperature.

Transcription from the *Hsp82* promoter was examined in flies containing both wild-type *Hsp82* gene and modified *Hsp82* (*modHsp82*) gene introduced by transformation. The latter, *modHsp82*, (Fig. 1*A*; ref. 26) has been increased in size by insertion of ≈7.5 kilobases (kb) of DNA into its intron. In addition to a spliced 1.8-kb transcript, *modHsp82* produces a 1.4-kb truncated transcript, owing to a polyadenylylation signal in the insert (26). With various DNA probes, the relative abundance of three different transcripts from *Hsp82* promoter, truncated (1.4-kb) and full-length spliced *modHsp82* (1.8-kb) transcripts as well as endogenous *Hsp82* full-length spliced transcript (2.9 kb), can be compared in different mutant fly strains.

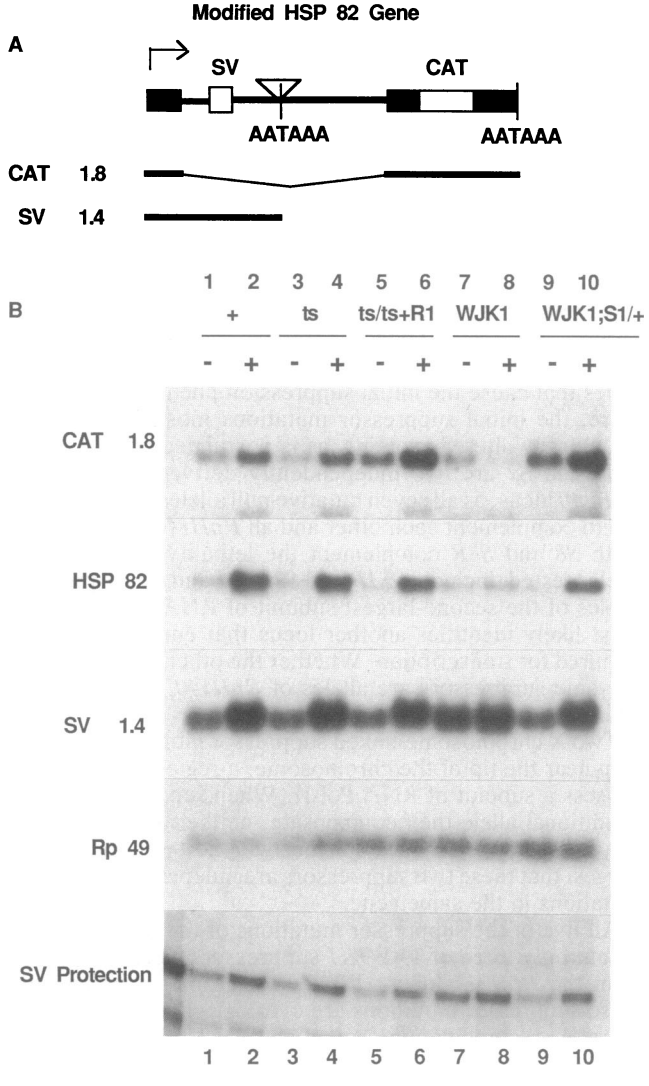

FIG. 1. WJK1 polymerase has altered ability to synthesize mRNA. (*A*) Diagram of the *modHsp82* gene described in detail elsewhere (26). Two exogenous DNA inserts were used to detect either the full-length spliced 1.8-kb RNA [chloramphenicol acetyltransferase (CAT) probe] or a 1.4-kb RNA [simian virus (SV) probe], the latter truncated near the polyadenylylation site inserted into the intron (thin line) of *Hsp82*. (*B*) One-half the pupae of each indicated genotype was heat shocked before RNA extraction (lanes 2, 4, 6, 8, and 10). *ts+R1* (lanes 5 and 6) is a suppressor mutation. Total cellular RNA was size-separated, transferred to nylon filters, and sequentially probed with CAT, SV, the endogenous *Hsp82* gene, and a clone of the ribosomal protein Rp49 (32). In addition, RNA was hybridized to a continuously labeled single-strand antisense SV probe, digested with RNase, and size-separated on 6% sequencing gel (SV protection).

Wild-type flies accumulate readily detectable amounts of all three transcripts during 20-min heat pulse at 36.5°C (Fig. 1*B*, lanes 1 and 2). In contrast, *WJK1* flies cannot accumulate either of the full-length spliced *Hsp82* mRNAs, as determined by Northern (RNA) blot analysis [Fig. 1*B*, lanes 7 and 8, chloramphenicol acetyltransferase (CAT) and *Hsp82* probes]. WJK1 polymerase appears to retain some activity, even at restrictive temperature, as the level of the 1.4-kb truncated RNA is somewhat elevated during the 20-min heat shock [Fig. 1*B*, compare lanes 7 and 8, simian virus (SV) probe]. Unexpectedly, *WJK1* flies accumulate more truncated 1.4-kb RNA before the heat pulse than do wild-type flies (Fig. 1*B*, compare lanes 1 and 7, SV probe). These two observations were confirmed by RNase protection analysis (Fig. 1*B*, SV protection).

Table 2. Allele-specific suppression

Suppressor genotype	*RpII215* mutation			
	E28	*ts*	*WJK1*	*K26*
+/+*	0.68†	0.93	0.89	0.93†
+/+	0.00†	0.00	0.00	0.00†
S1/+	0.00	0.00	0.94	0.00
S2/+	0.00	0.00	0.66	0.00
S3/+‡	0.00	0.00	0.00	0.00
S5/+	0.00	0.00	0.80	0.00
S6/+	0.00	0.00	1.22	0.00
S7/+	0.06	0.00	0.16	0.00
S8/+	0.00	0.00	0.87	0.00
S11/+	0.08	0.00	0.14	0.00
S12/+	0.10	0.00	0.27	0.00
S13/+‡	0.00	0.00	0.00	0.00
S3/Y¶	0.00	0.00	0.68	0.00
S13/Y¶	0.00	0.00	0.31	0.00

RpII215^x/FM7 females were mated to *WJK1;Sn* males at restrictive temperature (x, *RpII215* allele; *Sn*, suppressor allele). 1.00, suppression; 0.00, no suppression. Data are the ratio of *RpII215^x;Sn/+* to *WJK1/FM7;Sn/+* flies surviving to the adult stage with the following exceptions: *, Crosses at permissive temperature. †, Data from ref. 23. ‡, *RpII215^x/FM7* females were mated to *RpII215^x Sn* males with data representing the ratio of *RpII215^x/RpII215^x Sn* females to *RpII215^x/FM7* females. ¶, *RpII215^x Sn/FM7* females were mated to *RpII215^x Sn*/Y males with data representing the ratio of *RpII215^x Sn/FM7* females to *RpII215^x Sn/FM7* females.

Activity of WJK1 polymerase is restored to wild-type levels by the presence of a single copy of suppressor mutation *S1*. During 20-min heat pulse, *WJK1;S1/+* flies accumulate *modHsp82* and *Hsp82* spliced full-length RNAs and the 1.4-kb truncated RNA to levels similar to that of wild-type flies (Fig. 1*B*). Furthermore, the elevated level of the 1.4-kb truncated transcript seen in nonheat-pulsed *WJK1* flies is not present in *WJK1;S1/+* flies.

The activity of polymerase encoded by the *RpII215* allele *ts* is normal and unaffected by a single copy of the suppressor mutation *ts+R1*. Both *ts* and *ts/ts+R1* flies accumulate *Hsp82* RNAs to levels similar to those seen in wild-type flies.

All Extragenic Suppressors Are Allele Specific. The efficiency of each of the ten extragenic suppressors in rescuing flies mutant for *WJK1* and three additional conditional lethal *RpII215* alleles was measured. Results show that seven suppressor mutations only compensate the *WJK1* allele against which they were initially selected (Table 2). In contrast, the three weakest *WJK1* suppressors, *S7*, *S11*, and *S12*, also weakly suppress *E28*, although the resulting progeny are sterile. For some suppressor mutations, a single paternally introduced copy is enough to restore viability of *WJK1* flies to wild type. Note that one copy of suppressor mutation *S1* restores both activity of WJK1 polymerase and viability of *WJK1* flies to approximately wild-type levels.

A surprising result from the initial crosses used to measure suppressor efficiency (Table 2) is that neither of the X chromosome-linked suppressors *S3* and *S13* suppress *WJK1* as heterozygotes. Further crosses show that flies hemizygous (as in the last two crosses in Table 2) or homozygous for *S3 WJK1* or *S13 WJK1* are viable. Therefore, *S3* and *S13* are recessive suppressors, unlike the eight third-chromosome suppressors, which are dominant. Furthermore, lethality of *WJK1* is suppressed in *S3/S13* trans-heterozygotes, suggesting that they are alleles of the same locus.

DISCUSSION

The suppressor screen described above is modeled after the genetic dissection of bacteriophage assembly (33). This screen has been used successfully in yeast to identify genes

that interact with actin subunits (34, 35) and that regulate the cell cycle (36, 37), but until now it has not been extensively used in *Drosophila*.

I have screened for second-site suppressor mutations of three different conditionally lethal alleles of the largest subunit of RNA Pol-II. Although no suppressors of the *RpII215* allele *E28* were recovered, both *ts* and *WJK1* are readily suppressible. All 5 second-site suppressors of *ts* are intragenic; however, 10 of 13 *WJK1* suppressors are extragenic (Table 1). The implications of this difference are discussed below. All 10 extragenic suppressor mutations are allele specific, suggesting that they are functionally related to the largest subunit of Pol-II. Therefore, these mutations most likely identify components of the transcriptional machinery.

Five of the 10 *WJK1* extragenic suppressor mutations identify three different genes. *S1* and *S2* are probably alleles of the second-largest subunit of RNA Pol-II, as all seven of their revertants cause a recessive-lethal phenotype and fail to complement *RpII140* alleles. The extragenic suppressor *S8* identifies a second locus, which maps within the same 12-cM region as the second-largest subunit. *S8* and the revertant *S8R* cause recessive lethality; yet, they complement all recessive-lethal *RpII140* alleles tested. The *S8* locus is likely to identify another component of the transcriptional machinery, as it dominantly suppresses only one *RpII215* allele, *WJK1*. Furthermore, suppression is reciprocal, as *WJK1* also dominantly suppresses the recessive lethality caused by *S8*.

Two *WJK1* extragenic suppressor mutations *S3* and *S13* identify a third locus. They map to the tip of the X chromosome, a region not known to encode a subunit of RNA Pol-II. *S3* and *S13* are unusual in that both are recessive suppressor mutations, suggesting that they may be reduced or loss-of-function mutations. Although the reduced activity of a general protease might increase stability of WJK1 Pol-II, leading to a compensatory phenotype, the allele specificity of *S3* and *S13* suggests that they actually result from specific interactions between proteins used in transcription.

Shifting *WJK1* flies to restrictive temperature does not inactivate their RNA Pol-II, as is the case for a conditional allele of the largest subunit of yeast polymerase (38). The accumulation of three distinct transcripts, all using the *Hsp82* promoter, is differentially affected in *WJK1* flies at restrictive temperature (Fig. 1*B*). Therefore, altered promotion efficiency by WJK1 polymerase probably cannot account for its abnormal activity. The better explanation is that WJK1 polymerase has an increased likelihood of premature termination at restrictive temperature because the shortest (the truncated 1.4-kb RNA), but not the longer, transcripts accumulate at restrictive temperature. Several transcriptional defects could account for this termination, including a slower elongation rate, more efficient truncation, or less efficient splicing. The premature termination of transcription caused by WJK1 polymerase at restrictive conditions is ameliorated by a single copy of the suppressor mutation *S1* (Fig. 1*B*). This allele-specific compensation of the altered activity caused by WJK1 polymerase probably accounts for the efficient suppression of lethality seen in *WJK1;S1/+* flies reared at restrictive temperature (Table 2).

Hartman and Roth (39) provide a comprehensive review of mechanisms for the many kinds of suppressor–mutation interactions possible. The interactions expected between conditional alleles and allele-specific suppressor mutations may occur by a specific subset of these mechanisms. Two extreme cases of the structural disruption of a multimeric enzyme by a conditional lethal mutation are considered. (*i*) A subunit may be unable to fold correctly under restrictive conditions, preventing all contact with other subunits (i.e., the disruption is internal). (*ii*) The subunit may fold normally but be mutated so as to prevent essential interactions with other subunits, substrates, and/or associated factors under restrictive conditions (i.e., the disruption is external). These possibilities, diagrammed in Fig. 2 *B* and *C*, represent the extremes of what may be a continuum.

Second-site suppressors of conditional lethal alleles can be classified by the location of the suppressor mutation. One such class consists of intragenic suppressor mutations that restore correct intramolecular residue–residue contacts (Fig. 2*D*). The other class of mutations, extragenic suppressors, will lie in other subunits, substrates, and/or associated factors that contact the conditionally mutant subunit.

The types of suppressors recovered for a given mutation probably indicate the position within the conditionally lethal protein of the mutational event responsible for its mutant phenotype. Suppressor mutations of folding-defective conditional lethal mutations will tend to be internal (i.e., intragenic), as diagrammed in Fig. 2*D*; for some proteins, such as the tailspike of bacteriophage P22, this is the predominant conditional lethal class (40). Polypeptide-binding proteins or "molecular chaperones" (41, 42) might occur to suppress the folding-defective mutations. The *ts* allele of *RpII215* is compensated only by intragenic suppressors, suggesting a defect in folding (Fig. 2*B*). Once a folding-defective subunit is assembled at permissive conditions, it may be refractory to restrictive conditions. This situation appears so for three conditional lethal mutations in the large subunit of yeast Pol-II (43). My inability to detect alterations in the activity of polymerase encoded by the *RpII215* allele *ts* at restrictive temperature suggests that it behaves similarly (Fig. 1*B*); however, small differences in activity might not be detected by this assay.

Suppressor mutations of conditional alleles that disrupt external contacts (Fig. 2*C*) may be either intragenic or

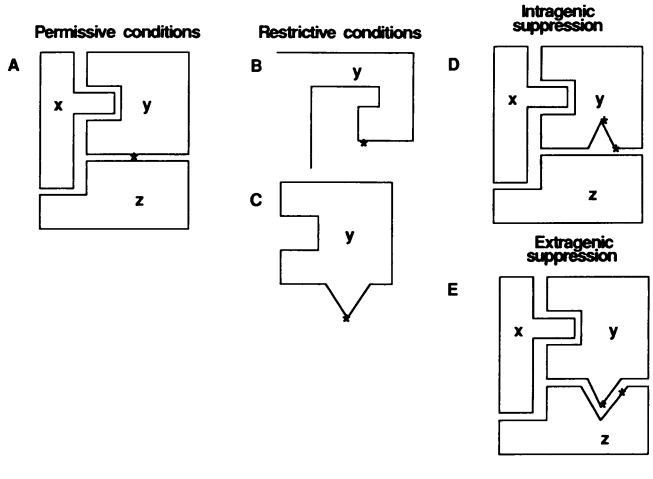

FIG. 2. Second-site suppression of an hypothetical multimeric enzyme mutated to cause conditional lethality. (*A*) At permissive conditions this heterotrimeric enzyme, possessing a conditional lethal mutation in subunit y, is functionally normal. At restrictive conditions the y subunit might cause two types of dysfunction. The first disrupts internal contacts such that the subunit fails to fold properly; therefore, assembly of the enzyme is blocked (*B*). The second alters external contacts, such that conformation of the y subunit allows normal contact with x subunit but disrupts contacts with subunit z (*C*). Second-site suppressors can also be placed into two groups. The first group consists of intragenic suppressor mutations that alter a second amino acid within y subunit, allowing normal or near-normal function of the enzyme at what was restrictive temperature (*D*). The second group consists of extragenic suppressors that compensate the defect in the original mutant subunit, permitting contact between the mutated regions of subunits y and z (*E*). Note that conditional mutations of the *B* class will primarily be compensated by suppressors of the *D* class, whereas those of the *C* class can be suppressed as diagrammed in *D* or *E*. Extragenic suppressors will likely identify genes that encode proteins contacting the original mutant subunit.

extragenic (Fig. 2 *D* and *E*, respectively). The *RpII215* allele, *WJK1*, appears of this class, as 10 of 13 suppressor mutations map outside the gene. The mode of extragenic suppressor action may result from compensatory structural changes in contact points between two or more proteins that normally contact each other during transcription (Fig. 2*E*). Support for such a model comes from the observation that a single copy of the *RpII140* allele *S1* restores both activity of WJK1 polymerase and viability of *WJK1* flies to near wild-type levels (Fig. 1*B* and Table 2).

Isolation of second-site suppressor mutations will prove valuable in identifying the network of proteins used in the transcriptional machinery of *Drosophila*. In addition, because this technique identifies interacting proteins, it could be applied to a wide range of developmental and enzymatic processes of *Drosophila* and other organisms.

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