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A Deficiency Screen for Dominant Suppressors of Telomeric Silencing in Drosophila

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ABSTRACT

Heterochromatin is a specialized chromatin structure in chromosomal regions associated with repeated DNA sequences and low concentrations of genes. Formation of heterochromatin is determined in large part by enzymes that modify histones and structural proteins that bind to these modified histones in a cooperative fashion. In Drosophila, mutations in genes that encode heterochromatic proteins are often dominant and increase expression of genes placed into heterochromatic positions. To find components of telomeric heterochromatin in Drosophila, we screened a collection of autosomal deficiencies for dominant suppressors of silencing of a transgene at the telomere of chromosome *2*L. While many deficiency chromosomes are associated with dominant suppressors, in the cases tested on chromosome *2* the suppressor mapped to the *2*L telomere, rather than the deficiency. We infer that background effects may hamper the search for genes that play a role in telomeric heterochromatin formation and that either very few genes participate in this pathway or mutations in these genes are not dominant suppressors of telomeric position effect. The data also suggest that the *2*L telomere region plays a major role in telomeric silencing.

TELOMERES are structures at the ends of linear chromosomes that are required for chromosome stability. They allow the linear DNA molecules to complete the replication of chromosome ends. Telomeres also cap chromosome ends, which would otherwise resemble DNA double-strand breaks. In addition, telomeres form a domain of transcriptionally repressed chromatin.

A prominent characteristic of telomeres is heterochromatin-like organization of surrounding chromatin. Silencing [termed *t*elomeric *p*osition *e*ffect (TPE)] is observed when genes are placed near telomeres in *Trypanosoma brucei* (Horn and Cross 1995; Rudenko *et al.* 1995), *Saccharomyces cerevisiae* (Gottschling *et al.* 1990; Palladino and Gasser 1994), *Schizosaccharomyces pombe* (Nimmo *et al.* 1994), *Drosophila melanogaster* (Gehring *et al.* 1984; Hazelrigg *et al.* 1984; Karpen and Spradling 1992; Levis *et al.* 1993; Wallrath and Elgin 1995), and humans (Baur *et al.* 2001). Such a widespread conservation of telomeric silencing among eukaryotes suggests that it is fundamental to telomere function. Indeed, telomere length maintenance and TPE in yeast appear to be tightly connected (Kyrion *et al.* 1993; Park *et al.* 2002).

Telomeres typically consist of a tandem array of GC-rich telomeric DNA repeats specified by copying of the template sequence within the telomerase RNA. These DNA repeats bind a set of sequence-specific DNA-binding proteins that, through separate domains, bind additional proteins to assemble an inferred higher-order complex nucleated on the telomeric DNA repeats (Blackburn 2001). In *S. cerevisiae*, where TPE is most extensively studied (Dubrana *et al.* 2001; Shore 2001), Rap1p binds to multiple sites within the telomeric repeats and, together with chromosome end-binding proteins yKu70p and yKu80p, recruits the silent information regulation silencing complex (Kyrion *et al.* 1993; Moretti *et al.* 1994; Boulton and Jackson 1998; Laroche *et al.* 1998).

Chromosome ends in *D. melanogaster* do not terminate in an array of simple repeats that is synthesized by telomerase, as in other species. Instead, Drosophila uses two families of non-long terminal repeat retrotransposons, *HeT-A* and *TART*, to elongate its chromosome ends (Mason and Biessmann 1995). Proximal to the terminal retrotransposon array Drosophila telomeres carry several kilobases of complex satellites, termed *t*elomere-*a*ssociated *s*equences (TAS), which exhibit sequence similarities among themselves (Karpen and Spradling 1992; Walter *et al.* 1995) and structural similarities to TAS in other eukaryotes (Pryde *et al.* 1997). Despite the fact that Drosophila does not possess arrays of simple repeats, such as those that bind Rap1p, Drosophila shares the property of telomeric silencing with other organisms. Reporter genes exhibit repressed and variegated expression when inserted into Drosophila telo-

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meric regions (Gehring *et al.* 1984; Hazelrigg *et al.* 1984; Levis *et al.* 1985; Karpen and Spradling 1992; Tower *et al.* 1993; Roseman *et al.* 1995; Wallrath and Elgin 1995; Mason *et al.* 2000, 2003a). As all variegating telomeric transgenes analyzed to date are embedded in or lie adjacent to TAS (Karpen and Spradling 1992; Levis *et al.* 1993; Wallrath and Elgin 1995; Cryderman *et al.* 1999; Marin *et al.* 2000; Golubovsky *et al.* 2001), TAS appears to play a role in telomeric silencing. This was demonstrated directly using a transgenic approach (Kurenova *et al.* 1998). In Drosophila, variegated repression of telomeric transgenes resembles *p*osition-*e*ffect *v*ariegation (PEV), the clonal inactivation of a euchromatic gene that has been positioned close to or within centric heterochromatin (Weiler and Wakimoto 1995). TPE, however, appears to be qualitatively different from PEV, because genetic modifiers of PEV, including the presence of an extra *Y* chromosome, have no effect on repression of transgenes inserted into TAS sequences (Talbert *et al.* 1994; Wallrath and Elgin 1995; Cryderman *et al.* 1999). Many suppressors of PEV in Drosophila are found to encode components of the repressive multimeric protein complex bound to centric heterochromatin or enzymes involved in their modification (Weiler and Wakimoto 1995).

Another well-known example of genetic silencing in Drosophila, developmentally regulated gene silencing, is mediated by proteins encoded by the Polycomb group (PcG) of genes (Pirrotta 1995). Many mutations in PcG genes, however, do not affect TPE. Exceptions are the weak suppression by certain alleles of *Psc*, *Su(z)2*, and possibly a few other loci, and the stronger suppression by the small deficiency, $Su(z)2^5$, which deletes *Su(z)2*, and *Psc* (Cryderman *et al.* 1999; Boivin *et al.* 2003). Thus, while a repressive chromatin complex is likely formed at Drosophila telomeres, components of this complex remain unknown.

We recently characterized the molecular structure of $P\{w^{var}\}$, a variegating insertion of a genomic *white* gene in the *2*L telomere (Gehring *et al.* 1984; Golubovsky *et al.* 2001). Unlike other repressed telomeric reporter genes, the transgene in $P\{w^{var}\}$ is located precisely between the terminal retrotransposon array and TAS (Golubovsky *et al.* 2001). $P\{w^{var}\}$ is very sensitive to its context; changes in the structure of the telomere region, such as *HeT-A* additions to the chromosome terminus, terminal deficiencies, gradual loss from the chromosome end due to incomplete replication, and loss of the *2*L TAS region on the homolog, can be identified easily by changes in eye color. Considering the sensitivity of $P\{w^{var}\}$ to changes at the *2*L telomere *in cis* as well as *in trans*, we reasoned that this insertion and its derivatives might be a sensitive model for selection of *trans*-modifiers of TPE.

Here we report the results of a screen for dominant *trans*-acting TPE modifiers on autosomal deficiency chromosomes maintained at the Bloomington Drosophila stock center (http://flystocks.bio.indiana.edu/). While many of the second chromosomes tested carried suppressors of TPE, in every case examined in detail the suppressor mapped to the *2*L tip, rather than to the site of the deficiency. In addition, several of these chromosomes fail to hybridize a *2*L TAS probe *in situ*, and some fail to complement lethal mutations at *l(2)gl*, a gene very close to the *2*L telomere. While the third chromosome deficiencies were not characterized in detail, these results indicate that genetic background effects may be a serious complication when analyzing the ability of extant mutants to suppress TPE. They also confirm reports (Golubovsky *et al.* 2001) that deficiencies of the *2*L telomere strongly suppress silencing of a reporter gene in the homologous tips.

MATERIALS AND METHODS

Drosophila crosses: Drosophila stocks were maintained and crosses were performed at 25° on cornmeal, molasses medium with dry yeast added to the surface. The $y^1\ w^{67c23};\ P\{w^{var}\}$ stock has been described recently (Golubovsky *et al.* 2001), and $P\{w^{var}\}KR3\text{-}2$ is a stable "brown-red" variant of $P\{w^{var}\}$. Other genetic markers and special chromosomes are described by Lindsley and Zimm (1992) and/or FlyBase (FlyBase Consortium 2003). $Su(z)2^5$ was kindly supplied by L. L. Wallrath, and Psc^1 was a generous gift of S. Ronsseray.

Suppression of telomeric silencing: Deficiency (Df) chromosomes obtained from the Bloomington stock center were tested for suppression of TPE by crossing $y\ w^{67c23};\ P\{w^{var}\}KR3\text{-}2$ females to Df/Balancer males and scoring the eye color of $y\ w^{67c23};\ P\{w^{var}\}KR3\text{-}2$ sons with and without the Balancer. Males with a light orange eye color were designated nonsuppressor. Males with darker eye color were considered to carry a suppressor. At least five males of each genotype were examined before a determination was made. If the eye color of Df males overlapped the color of Balancer males, a more careful comparison was made. At least six Df and at least six Balancer males, 8–48 hr old, were arranged in order according to eye color, and the Mann-Whitney rank order test was used to identify suppressors using the tables in Mendenhall (1971).

If Balancer males had dark eyes, Df/Balancer males were crossed with $y\ w^{67c23}$; Balancer females (*Sco/SM1* for chromosome *2* deficiencies; *Sb/TM6* for chromosome *3* deficiencies), and the deficiency chromosome was retested for a suppressor phenotype. In some cases repeated backcrosses to $y\ w^{67c23}$; Balancer females were required to get a consistent result. To identify a suppressor on the Df chromosome the resulting Balancer males were required to have orange eyes, while Df males had dark eyes. That is, the suppressor must segregate with the Deficiency chromosome to be considered. In the first test for suppression of TPE, a parallel cross of $y\ w^{67c23}$ females to Df/Balancer males was made to control for the presence of cryptic *white* insertions on the deficiency chromosomes.

Ambiguities in the reported deficiency breakpoints made the identification of putative sites of dominant suppressors problematic. For purposes of constructing a map, we made the assumption that the deficiencies on a chromosome with a suppressor phenotype include the region of ambiguity, but the nonsuppressor deficiencies do not. This overestimates the number and extent of putative suppressor sites. Breakpoints reported by FlyBase (FlyBase Consortium 2003) are used here, as these positions are determined by genetic as well as

cytological data. The proportion of the genome uncovered by the deficiencies tested was estimated by counting euchromatic bands; *i.e.*, regions 40, 41, 80, and 81 were not counted.

Lethal complementation tests: Complementation tests were made by crossing *l(2)gl/SM1* females to Df/*Cy* balancer males in small mass matings. After 4 days parents were transferred to a second vial. F_1 progeny were counted through day 17 or until at least two straight-winged flies were recovered. Thus, viability is defined operationally as two adult test flies. Crosses lacking straight-winged progeny were repeated until there were at least 60 Cy progeny or, if the two Cy classes could be distinguished, until at least 20 of each class emerged. Independent tests were made with two alleles of *l(2)gl* in different genetic backgrounds, $l(2)gl^{26}$ and $l(2)gl^{M1}$. The latter is a terminal deficiency for *2*L TAS that acquired a lethal allele of *l(2)gl* by terminal erosion (A. Y. Konev and J. M. Mason, unpublished results). A deficiency chromosome must fail to complement both alleles to be considered to carry a mutation for *l(2)gl*.

Recombination mapping: Second chromosome deficiencies were combined with $y\ w^{67c23}$ by crossing deficiency males to $y\ w^{67c23}$; *Sco/SM1* females and then mating Sco^+ Cy F1 brothers and sisters and selecting for y and w progeny in the F_2.

A stock of *al S* $wg^{Sp\text{-}1}$ *Tft* nw^B Pin^{Yt}/*CyO* was obtained from the Bloomington stock center and males from this stock were crossed to $y\ w^{67c23}$ females to replace the *X* chromosome. As the multiply marked chromosome had poor viability in the presence of $y\ w^{67c23}$ and *Cy*, the line was maintained by backcrossing $y\ w^{67c23}$; + females to $y\ w^{67c23}$; *al S* $wg^{Sp\text{-}1}$ *Tft* nw^B Pin^{Yt}/+ males each generation. Only second chromosome modifiers were mapped by recombination, because multiply marked third chromosomes from the stock center carried dominant suppressors of telomeric silencing.

For mapping studies, $y\ w^{67c23}$; Df/*SM1* females were crossed to $y\ w^{67c23}$; *al S* $wg^{Sp\text{-}1}$ *Tft* nw^B Pin^{Yt}/+ males. To map lethal mutations on the deficiency chromosome, the F_1 Cy^+ multiply marked females were backcrossed to $y\ w^{67c23}$; Df/*SM1* males, and F_2 Cy^+ progeny were scored for the dominant visible markers. To map the suppressors on the deficiency chromosome, the F_1 Cy^+ multiply marked females were crossed to $y\ w^{67c23}$; $P\{w^{var}\}KR3\text{-}2$ *al* males, and progeny were scored for eye color as well as the other visible chromosome *2* markers. Test crosses consisted of small mass matings. As the $P\{w^{var}\}KR3\text{-}2$ chromosome carries *al*, but the deficiency chromosomes do not, *al* could be used as a marker for mapping the suppressors, but not the lethals. At least 100 chromosomes were counted to map the suppressors, and 100 Cy^+ chromosomes were counted to map the lethals.

***In situ* hybridization:** Salivary chromosome squashes of larvae from deficiency stocks were prepared according to Kurenova *et al.* (1998). The balancer breakpoints were used as cytological markers to identify the *2*L telomere region. A 6-kb fragment of the *2*L TAS array (Kurenova *et al.* 1998) was used as probe, and the *2*L TAS array on the balancer acted as a hybridization control. To confirm that hybridization occurred with the balancer rather than with the deficiency chromosome, several deficiencies were retested from a $y\ w^{67c23}$; Df/*SM1* stock, where the *SM1* balancer chromosome is known to hybridize strongly to the *2*L TAS probe.

RESULTS

A screen for suppression of telomeric silencing: To inquire into the existence of suppressors of TPE in Drosophila and simultaneously map their positions, we screened the autosomal deficiency kits from the Bloomington stock center for a suppressor phenotype. $y\ w^{67c23}$; $P\{w^{var}\}KR3\text{-}2$ females were mated to Df/Balancer males and the eye color of $y\ w^{67c23}$ males with and without the Balancer was determined. Males with a light orange eye color were considered to lack a suppressor of TPE; males with darker eye color were considered to carry a suppressor (Figure 1; Table 1). As the $P\{w^{var}\}KR3\text{-}2$ insert carries a w^+ telomeric transgene, thus necessitating a null *white* allele on the *X* chromosome, it was not possible to test most *X* chromosome deficiencies in this assay. We, therefore, tested only autosomal deficiencies. In the discussion below, deficiencies are referred to by their sequence numbers in Table 1.

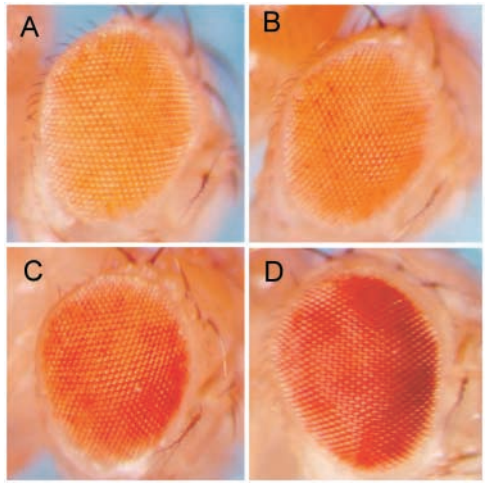

Figure 1.—Eye color phenotypes of suppressors of telomeric silencing. All photographs show hemizygous $P\{w^{var}\}KR3\text{-}2$. Equivocal suppressors, labeled "+" in Table 1, have a phenotype that overlaps the nonsuppressed phenotype and are not shown here. (A) The absence of a suppressor of TPE, labeled "−" in Table 1. (B) A weak suppressor of TPE, labeled ++ in Table 1. (C) A moderate suppressor of TPE, labeled +++. (D) A strong suppressor of TPE, labeled ++++.

To start, the standard deficiency kits were used to screen the maximum fraction of the genome with the minimum number of deficiencies. We assumed that all null alleles would have the same phenotype, and thus deficiencies on chromosomes that do not have a suppressor phenotype identify regions devoid of dosage-sensitive suppressor genes. As regions of potential interest were identified, additional deficiencies were obtained to verify and refine the position of a potential suppressor. Within the limits of the stock center collection, we tested deficiencies for any given locus until we found a chromosome that did not have a suppressor phenotype. This led to unequal coverage of the genome, with some regions tested several times.

Some of the deficiencies could not be tested. Thirty deficiency chromosomes carried cryptic *white* genes that became obvious only in a control cross to $y\ w^{67c23}$; + females that was run in parallel with the test cross.

TABLE 1

Deficiency chromosomes tested for suppression of telomeric silencing

Df no.	Breakpoints[a]	Name	Suppression[b]	Call[c]
1	21A1; 21B6–7	*Df(2L)net-PM47C*	++	FP4
2	21A1; 21B7–8	*Df(2L)net-PMF*	+	FP4, -5
3	21A1–4; 21B3	*Df(2L)net18*	++	FP4, -5
4	21A1–4; 21B4	*Df(2L)net62*	++	FP4, -5
5	21C1; 21C7	*Df(2L)al*	++	Region 1
6	21C3–4; 21C6–8	*Df(2L)BSC16*	−	
7	21C8–D1; 22A8–B1	*Df(2L)S2*	+++	FP4
8	21D1–2; 21E1–2	*Df(2L)ast4*	+++	FP4, -5
9	21D1–2; 22B2–3	*Df(2L)ast2*	+	FP6
10	21D2; 21F3–22A1	*Df(2L)S3*	+	FP6
11	21E3–4; 22B5–7	*Df(2L)frtz17*	−	
12	22A1; 22B6–9	*D(2L)frtz11*	++	FP1
13	22A2–3; 22B7	*D(2L)frtz19*	++	FP1
14	22A2–3; 22D5–E1	*Df(2L)dp-79b*	−	
15	22A3; 22B3	*Df(2L)frtz14*	+	FP1, -6
16	22A3–4; 22C1–2	*Df(2L)frtz25*	+++	FP1
17	22A6; 22B9	$Df(2L)J69^{L}H56^{R}$	++	FP1
18	22F1–2; 23A2	*Df(2L)dpp-d14*	−	
19	22F3–4; 23C3–5	*Df(2L)C144*	−	
20	22F4; 23A1	*Df(2L)D20*	++	FP1, -4
21	23A3–4; 23D4–6	*Df(2L)JS13*	++	Region 2
22	23C1–2; 23E1–2	*Df(2L)JS17*	++	Region 2
23	23C3–5; 23D1–2	*Df(2L)JS32*	−	
24	23D2; 23E3	*Df(2L)S2590*	++	FP2, -4
25	23F2–3; 23F6–24A1	*Df(2L)tim-02*	−	
26	24A2; 24D4	*Df(2L)ed1*	−	
27	24C3; 25A2	*Df(2L)ed-dp*	+	FP6
28	24E1; 25A2	*Df(2L)M24F-B*	−	
29	24E3; 25A7	*Df(2L)sc19-3*	−	
30	24E4; 25B2	*Df(2L)dp-h25*	−	
31	24F1–2; 25C5	*Df(2L)sc19-6*	−	
32	25A5; 25D6	*Df(2L)sc19-5*	++	Region 3
33	25D2–3; 26B2–5	*Df(2L)cl-h3*	−	
34	25F3–26A1; 26D3–11	*Df(2L)E110*	−	
35	26D3–E1; 26F4–7	*Df(2L)BSC6*	−	
36	26D10–E1; 27C1	*Df(2L)BSC7*	++	Region 4
37	27B2; 27F1–2	*Df(2L)spd-j2*	−	
38	27C5–9; 28B3–4	*Df(2L)J-H*	−	
39	27E; 28C1–4	*Df(2L)spd*	+	FP6
40	27E2; 28D1	*Df(2L)XE-3801*	+++	FP4
41	28B2; 28D1	*Df(2L)XE-2750*	+	FP6
42	28DE (within)	*Df(2L)Trf-C6R31*	++	FP4
43	28E4–7; 29B2–C1	*Df(2L)TE29Aa-11*	−	
44	29C1–2; 30C8–9	*Df(2L)N22-14*	++	FP4
45	29C3–5; 30C8–9	*Df(2L)N22-5*	++	Regions 5, 6
46	29E2–F1; 30C2–4	*Df(2L)TE30Cb-1*	−	
47	30A1–2; 30D1–2	*Df(2L)N22-3*	++	Region 6
48	30A3–5; 30C5	*Df(2L)30A-C*	++	Region 6
49	30A9–B1; 30D2–F4	*Df(2L)gamma7*	+++	FP4
50	30D1–F6; 31F1–5	*Df(2L)Mdh*	−	
51	31B1; 32A1–2	*Df(2L)J2*	−	
52	31D1–11; 31E7	*Df(2L)J27*	−	
53	32D1; 32F1–3	*Df(2L)FCK-20*	−	
54	32F1–3; 33F1–2	*Df(2L)Prl*	+	FP5, -6
55	33A1; 33B1–2	*Df(2L)esc-P2-0*	+	FP6
56	33A1; 33B2	*Df(2L)esc10*	−	
57	33A1; 33E	*Df(2L)esc-P3-0*	−	
58	33B3; 34A1–2	*Df(2L)prd1.7*	−	

(*continued*)

TABLE 1

(Continued)

Df no.	Breakpoints[a]	Name	Suppression[b]	Call[c]
59	34B7–12; 34E3	*In(2L)b82a1*	++	Region 7
60	34C1; 35C1	*Df(2L)b87e25*	++	FP2, -5
61	34C4; 35A4	*Df(2L)b80e3*	−	
62	34D1–2; 35C1	*Df(2L)64j*	+++	FP1
63	34D3–4; 35C1	*Df(2L)fn30*	++++	FP1
64	34D4; 34E3	*Df(2L)b88f32*	++	FP1
65	34E2; 35B3–4	*Df(2L)fn7*	+++	FP1
66	34E3; 35D2–5	*Df(2L)el80f1*	+	FP1, -6
67	34E4–34F1; 35C3	*Df(2L)noc11*	−	
68	34E5–F1; 35C3	*Df(2L)A263*	−	
69	34F2–5; 35C4	*Df(2L)osp38*	++	FP1
70	34F4; 35C3	*Df(2L)fn5*	−	
71	34F4–5; 35D4–5	*Df(2L)fn1*	++++	FP1
72	34F5; 35B2	*Df(2L)el81i1*	−	
73	34F5; 35B10	*Df(2L)TE35BC-31*	−	
74	34F5–35A4; 35D2	*Df(2L)do1*	−	
75	35A1–4; 35C1–3	*Df(2L)A400*	−	
76	35A4–B1; 35B2	*Df(2L)fn2*	+	FP1
77	35B1; 35F1	*Df(2L)A446*	−	
78	35B3; 35E6	*Df(2L)osp29*	−	
79	35B4–6; 35E1–2	*Df(2L)TE35BC-24*	−	
80	35D1; 36A6–7	*Df(2L)r10*	++	Region 8
81	35F6–12; 36D	*Df(2L)cact-255rv64*	+++	FP5
82	36A8–9; 36F1	*Df(2L)H20*	−	
83	36C2–4; 37B9–10	*Df(2L)TW137*	−	
84	36E4–F1; 38A6–7	*Df(2L)TW50*	+++	FP1
85	36F7–9; 37B2–7	*Df(2L)TW3*	−	
86	36F7–9; 37D1–2	*Df(2L)VA16*	++	FP1
87	37B2–8; 37C5	*Df(2L)hk-UC2*	−	
88	37B2–10; 38D2–5	*Df(2L)pr-A16*	−	
89	37B2–8; 37E2	*Df(2L)TW158*	++	FP1
90	37B9–10; 37D1–2	*Df(2L)TW130*	++	FP1
91	37B9–10; 37D5	*Df(2L)VA23*	++	FP1
92	37C1; 37F5	*Df(2L)VA17*	++	FP1
93	37C2–5; 38B2–C1	*Df(2L)VA12*	+++	FP1
94	37C2–7; 38C1–2	*Df(2L)Sd77*	−	
95	37D2; 38A1	*Df(2L)E55*	+++	FP1
96	37D2–5; 38A6–B2	*Df(2L)Sd37*	−	
97	37D2–5; 39A4–7	*Df(2L)pr-A14*	++	FP1
98	37D6–E1; 38E6–9	*Df(2L)TW2*	++	FP1
99	37E2–4; 39D1	*Df(2L)TW12*	−	
100	37E2–F1; 38B5–C1	*Df(2L)TW9*	+++	FP1
101	38A1; 39D3–E1	*Df(2L)TW84*	+	FP1, -6
102	38A1; 39F1	*Df(2L)TW65*	++	Region 9
103	38A3–4; 38B6–C1	*Df(2L)pr-A20*	++	FP1
104	38A7–B1; 39C2–3	*Df(2L)TW1*	++	FP1
105	38B3–6; 40A3	*Df(2L)pr-M1*	+	FP6
106	38E2; 39E7	*Df(2L)DS6*	−	
107	40h35; 40h38L	*Df(2L)C'*	−	
108	h38R; h46	*Df(2R)M41A10*	+	FP6
109	h42–h43; 42A2–3	*In(2R)bw^{VDe2L}Cy^{R}*	+	FP6
110	h44–h46; 41B1–41F11	*Df(2R)M41A8*	+++	FP4, -5
111	h44–h46; 42A1–2	*Df(2R)M41A4*	++	FP4
112	41BC; 42A16–B1	*Df(2R)nap14*	+	FP6
113	41D2–E1; 42B1–3	*Df(2R)nap1*	−	
114	41F3–4; 42A3–9	*Df(2R)17I*	++	FP1
115	42A1–2; 42E6–F1	*Df(2R)nap9*	−	
116	42A1–19; 42E2–7	*Df(2R)cn88b*	−	

(*continued*)

TABLE 1

(Continued)

Df no.	Breakpoints[a]	Name	Suppression[b]	Call[c]
117	42B3–4; 43E18	*Df(2R)ST1*	++	FP1
118	42B4–C1; 43F–44A1	*Df(2R)cn87e*	−	
119	42C1–7; 43F5–8	*Df(2R)pk78s*	−	
120	42C2; 42D2–3	*Df(2R)42*	−	
121	42C2–7; 43D1–7	*Df(2R)Drl-rv17*	+	FP1, -6
122	42E; 44C1	*Df(2R)cn9*	++	FP1
123	42E1–4; 43C3	*Df(2R)Drl-rv3*	++	FP1
124	43A3; 43F6	*Df(2R)P32a*	+	FP1, -6
125	43C5; 44B6–C1	*Df(2R)cn83c*	+	FP1, -6
126	43C7; 43F2–8	*Df(2R)cn-S6*	−	
127	43E7–18; 44B4–5	*Df(2R)CA53*	−	
128	43F; 44D3–8	*Df(2R)H3C1*	−	
129	44C1–2; 44E1–4	*Df(2R)44CE*	−	
130	44D1–4; 44F12	*Df(2R)H3E1*	−	
131	44D2–E1; 45B8–C1	*Df(2R)Np3*	−	
132	44F11; 45C1	*Df(2R)Np4*	++	FP1
133	44F11; 45D9–E1	*Df(2R)Np5*	+	FP1, -6
134	44F2–3; 45C6	*Df(2R)Np1*	−	
135	45A6–7; 45E2–3	*Df(2R)w45-30n*	+	FP4
136	45A9–10; 45D5–8	*Df(2R)w73-1*	−	
137	45C8; 45D8	*Df(2R)wun-GL*	++	FP1
138	45C8–D10; 45D9–E1	*Df(2R)w45-19g*	++	FP1
139	45D3–4; 45F2–6	*Df(2R)BSC29*	−	
140	46A1–4; 46C3–12	*Df(2R)B5*	+	FP6
141	46C1–2; 46E1–2	*Df(2R)X3*	−	
142	46C2; 47A1	*Df(2R)X1*	−	
143	46C3–4; 46C9–11	*Df(2R)eve*	+++	FP1, -2
144	46F1; 47A10	*Df(2R)12*	++	FP2
145	46F1; 47B9	*Df(2R)stan2*	++	FP3
146	47A3; 47E	*Df(2R)E3363*	−	
147	47D3; 48A5	*Df(2R)en-A*	+	FP6
148	47E3; 48A5–B2	*Df(2R)en-B*	++	FP2, -4
149	48A1; 48B5	*Df(2R)en-SFX31*	+++	FP3
150	48A1–2; 48B–C1	*Df(2R)en28*	+	FP3, -6
151	48A3; 48C6–8	*Df(2R)en30*	−	
152	48C5–D1; 48D5–E11	*Df(2R)BSC39*	−	
153	48E; 49A	*Df(2R)CB21*	+++	FP2, -5
154	49A; 49E1–2	*Df(2R)vg135*	+	FP5, -6
155	49B2–3; 49E2	*Df(2R)vg-C*	−	
156	49C1–2; 49E6	*Df(2R)vg-D*	++	Region 10
157	49C1–4; 50C23–D1	*Df(2R)CX1*	+++	FP4
158	49D3–4; 50A2	*Df(2R)vg-B*	+	FP6
159	50C21–23; 50D1–5	*Df(2R)50C-101*	−	
160	50E6–F1; 51E2–4	*Df(2R)BSC11*	+++	FP4
161	50F6–9; 51B3	*Df(2R)L48*	−	
162	51A2; 51B6	*Df(2R)trix*	++	FP1
163	51A5; 51C1	*Df(2R)03072*	−	
164	51C3–7; 51E7–11	*Df(2R)14*	−	
165	51D1–2; 51E5	*Df(2R)XTE-58*	−	
166	51D3–E1; 52D1	*Df(2R)XTE-18*	−	
167	51F13; 52F8–9	*Df(2R)Jp4*	+++	Region 11
168	52A13–14; 52F10–11	*Df(2R)Jp5*	+++	Region 11
169	52A9–10; 52D9–15	*Df(2R)WMG*	−	
170	52D3; 53A1	*Df(2R)Jp6*	++	Region 11
171	54E8–F1; 55B9–C1	*Df(2R)Pcl7B*	−	
172	54F2; 56A1	*Df(2R)RM2-1*	+++	FP3
173	55A1; 55C1–3	*Df(2R)Pcl11B*	−	
174	55A1; 55F1–2	*Df(2R)PC4*	−	

(*continued*)

TABLE 1

(Continued)

Df no.	Breakpoints[a]	Name	Suppression[b]	Call[c]
175	55C1–2; 56B1–2	*Df(2R)C29*	++	FP3
176	55D2–E1; 56B2	*Df(2R)PC66*	++	FP3
177	55E6–F3; 56C1	*Df(2R)P34*	++	FP2
178	56D7–E3; 56F9–12	*Df(2R)BSC22*	+	FP6
179	56F 5; 56F15	*Df(2R)173*	−	
180	56F 5; 56F15	*Df(2R)017*	−	
181	56F 9–11; 57D12	*Df(2R)AA21*	+	FP3, -6
182	56F12–14; 57A4	*Df(2R)BSC19*	−	
183	57A3; 57B1	*Df(2R)exu2*	+++	FP2
184	57A6; 57B6	*Df(2R)D4*	−	
185	57B1; 57B13–14	*Df(2R)E2*	−	
186	57B4; 58B1–2	*Df(2R)Pu-D17*	−	
187	57D2–8; 58D1	*Df(2R)Egfr5*	−	
188	58B1–2; 58E4	*Df(2R)X58-7*	−	
189	58B3; 59A1	*Df(2R)X58-8*	−	
190	58C3–7; 58D6–8	*Df(2R)X58-3*	−	
191	58D1–2; 59A1	*Df(2R)X58-12*	+++	Region 12
192	59A1–3; 59B1–2	*Df(2R)59AB*	++	Region 12
193	59A1–3; 59D1–4	*Df(2R)59AD*	−	
194	59D 4–8; 59D9–E1	*Df(2R)vir-12*	−	
195	59D 8; 60A7	*Df(2R)bw-S46*	+	FP6
196	59D11; 59F6–8	*Df(2R)bw-HB132*	+	FP4
197	59E; 60A1	*Df(2R)egl2*	++	FP1
198	59E1; 59F6	*Df(2R)bw5*	+	FP6
199	59E1; 60C7–D1	$Df(2R)bw^{VDe2L}Px^{KR}$	−	
200	59F1; 59F5	*Df(2R)egl3*	++	FP1
201	59F3; 60A8–16	*Df(2R)G10-7-5*	−	
202	59F6; 60A12–16	*Df(2R)or-BR11*	−	
203	60B8–10; 60D1	*Df(2R)Px1*	−	
204	60C6; 60D9–10	*Df(2R)Px2*	−	
205	60E6; 60F1–2	*Df(2R)ES1*	++	FP2, -4
206	60E1–2; 60E6	*Df(2R)Dll-MP*	++	FP4
207	60E10; 60F5	*Df(2R)Kr10*	++	FP4
208	60E6–9; 60E11	*Df(2R)M60E*	−	
209	60E9; 60F1	*Df(2R)gsb*	+	FP3, -6
210	60F2; 60F5	*Df(2R)Kr14*	−	
211	61A; 61D3	*Df(3L)emc-E12*	−	
212	61A1; 61B	*Df(3L)B71*	−	
213	61C1–4; 61F3	*Df(3L)Ar12-1*	+	FP6
214	61C3–4; 61E	*Df(3L)Ar11*	+	FP6
215	61C4; 62A8	*Df(3L)Ar14-8*	+	FP6
216	61D3–E1; 61F5–8	*Df(3L)bab-PG*	−	
217	61F8; 62A3–5	*Df(3L)ru-22*	+	FP6
218	62A10–B1; 62C4–D1	*Df(3L)R-G5*	−	
219	62A10–B1; 62D2	*Df(3L)Aprt-1*	−	
220	62B9; 62E7	*Df(3L)R-G7*	−	
221	63C1; 63D3	*Df(3L)HR232*	−	
222	63C1–2; 63F1–2	*Df(3L)1227*	++	FP1
223	63C6; 63F7	*Df(3L)HR119*	−	
224	63E2; 64B17	*Df(3L)GN50*	−	
225	63F6–7; 64C13–15	*Df(3L)GN24*	−	
226	64; 65B5–C1	*Dr(3L)CH39*	−	
227	64B–C; 65B5–C1	*Df(3L)CH18*	−	
228	64C; 65C	*Df(3L)ZN47*	−	
229	64E1–13; 65C1–D6	*Df(3L)v65c*	++	FP1
230	65A; 65E1	*Df(3L)W5.4*	−	
231	65A2; 65E1	*Df(3L)XDl98*	−	
232	65D4–5; 65E4–6	*Df(3L)BSC27*	−	

(*continued*)

TABLE 1

(Continued)

Df no.	Breakpoints[a]	Name	Suppression[b]	Call[c]
233	65E1–12; 66A17	*Df(3L)RM5-2*	−	
234	65F3; 66B10	*Df(3L)pbl-X1*	−	
235	66A17–20; 66C1–5	*Df(3L)ZP1*	−	
236	66B12–C1; 66D2–4	*Df(3L)BSC13*	++	Region 13
237	66B8–9; 66C9–10	*Df(3L)66C-G28*	+++	Region 13
238	66C7–10; 66C7–10	*Df(3L)66C-I65*	−	
239	66E1–6; 66F1–6	*Df(3L)Scf-R6*	−	
240	66E3–4; 66F1–2	*Df(3L)Scf-R11*	−	
241	66F5; 66F5	*Df(3L)Rdl-2*	−	
242	66F5; 67B1	*Df(3L)29A6*	++	Region 14
243	67A2; 67D13	*Df(3L)AC1*	+	FP6
244	67E1–2; 68C1–2	*Df(3L)lxd6*	−	
245	68A2; 69A1	*Df(3L)vin5*	−	
246	68C8; 69B4–5	*Df(3L)vin7*	−	
247	69A4–5; 69D4–6	*Df(3L)eyg-C1*	−	
248	69B1–5; 69D1–6	*Df(3L)iro-2*	−	
249	69D; 69D	*Df(3L)8ex25*	+	FP1, -6
250	69D2; 69E3–5	*Df(3L)E44*	−	
251	69D4–5; 69F5–7	*Df(3L)BSC10*	−	
252	69F3–4; 70C3–4	*Df(3L)C190^{l}-Ubx^{42TR}*	−	
253	70C2; 72A1	*Df(3L)D-5rv12*	−	
254	70C2–6; 70E1	*Df(3L)fz-CAL*	−	
255	70D2; 70E8	*Df(3L)fz-D21*	−	
256	70D2; 71E4–5	*Df(3L)fz-M21*	−	
257	70E; 71F	*Df(3L)Brd6*	++	FP1
258	71A1–2; 71C1–2	*Df(3L)Brd15*	−	
259	71C2–3; 72B1–C1	*Df(3L)XG-5*	−	
260	71C3; 71E5	*Df(3L)BK10*	−	
261	72A2; 72D10	*Df(3L)th102*	−	
262	72A3–4; 72D1–5	*Df(3L)brm11*	−	
263	72C1; 73A4	*Df(3L)st-f13*	−	
264	72D10–11; 73D1–2	*Df(3L)st-b11*	−	
265	73A3; 74F1–4	*Df(3L)81k19*	−	
266	74D3–75A1; 75B2–5	*Df(3L)BSC8*	−	
267	75B10; 75C5	*Df(3L)W4*	−	
268	75C1–2; 75F1	*Df(3L)Cat*	−	
269	75F10–11; 76A1–5	*Df(3L)fz2*	−	
270	76A7–B1; 76B4–5	*Df(3L)BSC20*	−	
271	76B; 76F	*Df(3L)XS2182*	−	
272	76B; 77A	*Df(3L)XS543*	−	
273	76B1–2; 76D5	*Df(3L)kto2*	++	FP1
274	76B4; 76D3	*Df(3L)XS705*	−	
275	76B4; 77B	*Df(3L)XS-533*	−	
276	76B6; 77C1	*Df(3L)XS572*	−	
277	77A1; 77D1	*Df(3L)rdgC-co2*	−	
278	77B7–9; 77F1–5	*Df(3L)ri-79c*	++	Region 15
279	77E2; 78A4	*Df(3L)ri-XT1*	−	
280	77F3; 78C8–9	*Df(3L)ME107*	−	
281	78C5–6; 78E3–79A1	*Df(3L)Pc-2q*	−	
282	79C; 79E1–8	*Df(3L)Ten-m-AL29*	−	
283	79D3–E1; 79F3–6	*Df(3L)HD1*	−	
284	79E1–2; 79E1–8	*Df(3L)Ten-m-AL1*	−	
285	79E5–F1; 80A2–3	*Df(3L)BSC21*	−	
286	79F; 80A	*Df(3L)Delta1AK*	−	
287	80Fb–g	*Df(3L)3-52*	+	FP6
288	80Fd–e	*Df(3R)6-61*	+	FP6
289	80Ff–g	*Df(3L)8A-80*	−	
290	80Fg–j	*Df(3L)1-166*	−	
291	80Fh–j	*Df(3L)2-66*	+	FP6
292	80Fj	*Df(3L)2-30*	+	FP6

(*continued*)

TABLE 1

(Continued)

Df no.	Breakpoints[a]	Name	Suppression[b]	Call[c]
293	81 Fab	*Df(3R)4-75*	−	
294	81F; 82F10–11	*Df(3R)2-2*	−	
295	81F3–6; 82F5–7	*Df(3R)ME15*	+	FP6
296	82A5–6; 82E4	*Df(3R)Z1*	−	
297	82C4; 82F3	*Df(3R)110*	−	
298	82D5; 82F3–6	*Df(3R)6-7*	+	FP1, -6
299	82F3–4; 82F10–11	*Df(3R)3-4*	−	
300	82F8–10; 83A1–3	*Df(3R)e1025-14*	−	
301	83B7–C1; 83C6–D1	*Df(3R)BSC47*	−	
302	83E1–2; 84B1	*Df(3R)WIN11*	++	Region 16
303	83E3; 84B1	*Df(3R)Dfd13*	+++	Region 16
304	84A1; 84B1	*Df(3R)9A99*	−	
305	84A1–2; 84B1–2	*Df(3R)Scr*	−	
306	84A6–B1; 84D4–D9	*Df(3R)roe*	−	
307	84C1–2; 84E1	*Df(3R)dsx2M*	−	
308	84C8; 84F6	*Df(3R)dsx29*	−	
309	84D 4–6; 85B6	*Df(3R)p712*	++	FP1
310	84D 8; 85B3–5	*Df(3R)dsx37*	−	
311	84D 8–9; 85A1–2	*Df(3R)dsx11*	−	
312	84D11; 84E8	*Df(3R)dsx15*	−	
313	84E8–9; 85B6	*Df(3R)p40*	++	FP1
314	84F1; 85A6–B9	*Df(3R)p13*	−	
315	84F2; 85A5–7	*Df(3R)CA3*	++	FP1
316	85A2; 85C1–2	*Df(3R)p-XT103*	−	
317	85D8; 85E10–13	*Df(3R)by10*	−	
318	85D11–13; 85F6	*Df(3R)by62*	+++	Region 17
319	85D12; 85E10	*Df(3R)GB104*	−	
320	86C1; 87B5	*Df(3R)M-Kx1*	−	
321	86E2–3; 87C6–7	*Df(3R)T-32*	−	
322	86F1–2; 87C6–7	*Df(3R)T-10*	++	FP1
323	87B12; 87E8	*Df(3R)ry615*	−	
324	87D2; 87F2	*Df(3R)ry27*	−	
325	87E–F; 88B	*Df(3R)Cbx^{TwtL}-Ubx^{KM5R}*	+	FP1, -6
326	87E1; 87F11	*Df(3R)I26c*	−	
327	87F1; 87F15	*Df(3R)urd*	−	
328	87F12–14; 88C2	*Df(3R)red31*	−	
329	88A2; 88C1–D1	*Df(3R)red1*	−	
330	88B1; 88C2	*Df(3R)red-P93*	−	
331	88E7–13; 89A1	*Df(3R)ea*	+	FP6
332	88F7; 89A11–13	*Df(3R)Po4*	−	
333	89A1–2; 89A11–13	*Df(3R)Po2*	++	FP1
334	89A1–2; 89A11–13	*Df(3R)Po3*	−	
335	89A8; 89B3	*Df(3R)Exel7327*	−	
336	89B4; 89B10	*Df(3R)sbd45*	−	
337	89B5; 89C	*Dr(3R)sd104*	−	
338	89B5–6; 89E2–3	*Df(3R)bxd100*	+	FP1, -6
339	89B7–8; 89E7–8	*Df(3R)P115*	−	
340	89E1–89F4; 91B1–B2	*Df(3R)DG2*	−	
341	89E2–3; 90A	*Df(3R)C4*	++	FP1
342	89E2–3; 90D	*Df(3R)RD31*	−	
343	90F1–2; 91F5	*Df(3R)Cha7*	−	
344	91A2–B3; 91F13–92A1	*Df(3R)Cha1a*	−	
345	91F1–2; 92D3–6	*Df(3R)D1-BX12*	−	
346	92B3; 92F13	*Df(3R)H-B79*	−	
347	93B2–13; 94A3–8	*Df(3R)e-N19*	−	
348	93B6; 93D3–4	*Df(3R)e-R1*	−	
349	93C6; 94A1–4	*Df(3R)e-GC3*	+	FP1, -6
350	93E–F; 94C–D	*Df(3R)5C1*	+	FP1, -6

(*continued*)

TABLE 1

(Continued)

Df no.	Breakpoints[a]	Name	Suppression[b]	Call[c]
351	93F11–14; 94D10–13	*Df(3R)hh*	−	
352	95A5–7; 95C10–11	*Df(3R)mbc-30*	++	Region 18
353	95A5–7; 95D6–11	*Df(3R)mbc-R1*	−	
354	95D7–11; 95F15	*Df(3R)crb-F89-4*	−	
355	95D11–E2; 96A2	*Df(3R)crb87-4*	−	
356	96A1; 96A20–25	*Df(3R)Ubx^{7LL}ats^{R}*	−	
357	96A2–7; 96D2–4	*Df(3R)slo8*	−	
358	96F1; 97B1	*Df(3R)Espl3*	−	
359	96F10–11; 96F11	*Df(3R)Espl22*	−	
360	96F12–14; 97C4–5	*Df(3R)ME61*	−	
361	97A; 98A1–2	*Df(3R)T1-P*	−	
362	97E3; 98A5	*Df(3R)D605*	−	
363	98D3–7; 98D3–7	*Df(3R)M15*	−	
364	98E3; 99A6	*Df(3R)3450*	−	
365	98F14; 99E2–3	*Df(3R)R133*	−	
366	99A6; 99C1	*Df(3R)01215*	−	
367	99D1–2; 99E1	*Df(3R)X3F*	−	
368	99F1–2; 100B5	*Df(3R)tll-g*	−	
369	100A2; 100C2–3	*Df(3R)tll-e*	+	FP6
370	100D1; 100D3–4	*Df(3R)awd-KRB*	−	
371	100D1–2; 100E–F	*Df(3R)faf-BP*	−	
372	100D2; 100F5	*Df(3R)04661*	−	

[a] Breakpoints are as determined by FlyBase Consortium (2003); otherwise breakpoints were supplied by the Bloomington stock center.

[b] −, nonsuppressor; +, equivocal suppressor with a phenotype that overlaps the nonsuppressed phenotype; ++, weak suppressor; +++, moderate suppressor; ++++, strong suppressor (see Figure 1).

[c] Determination of suppressor on the deficiency chromosome. FP1, false positive because the region of the deficiency is covered by one or more nonsuppressing deficiencies; FP2, false positive because the suppressor maps to the tip of *2*L rather than to the site of the deficiency; FP3, false positive because the region of the deficiency is covered by a combination of nonsuppressing deficiencies and deficiencies with suppressors that map to the *2*L tip; FP4, assumed false positive because the *2*L TAS is missing by *in situ* hybridization; FP5, assumed false positive because the deficiency chromosome fails to complement lethal mutations of *l(2)gl*; FP6, the suppressor cannot be adequately tested because the phenotype overlaps wild type. Regions refer to map positions as shown in Figures 3 and 4.

Twenty-two others required a duplication for viability. Six stocks were insufficiently marked to allow us to easily distinguish the deficiency from the balancer chromosome. In total, we tested 372 deficiency chromosomes for suppression of TPE, 210 for chromosome *2*, and 162 for chromosome *3*.

As chromosomes were tested, the deficiencies were aligned on a cytogenetic map to identify sites of potential suppressor genes. With the assumption that deficiencies with a nonsuppressor phenotype identified regions devoid of suppressor genes, it quickly became obvious that many of the tested chromosomes carried suppressors that are not within the bounds of the deficiencies being tested. These were deemed to be false positive results (Table 1; FP1). With a high frequency of false positives (58/149 = 0.39), it became necessary to verify the suppressors on as many of the deficiency chromosomes as possible. Therefore, all second chromosome deficiencies with suppressor phenotypes were chosen for further analysis, but we could obtain data on only 40 (Table 2). The primary difficulty in obtaining useful data was due to the health of the deficiency flies; many stocks had such poor viability on outcrossing that they could not be tested further.

Meiotic recombination mapping of TPE suppressors: The most informative test, and the most demanding in terms of the health of deficiency-bearing individuals, was genetic mapping of the suppressor using a second chromosome marked with the recessive mutation *al* and the dominant mutations *S wg$^{Sp\text{-}1}$ Tft nw^{B} Pin^{Yt}*. This is one of the few multiply marked chromosomes at the Bloomington stock center useful for recombination studies that did not carry a suppressor of TPE. No such third chromosome could be found; thus the third chromosome suppressors were not mapped genetically. In this assay, *y w^{67c23}*; Df/*SM1* females were crossed to *y w^{67c23}*; *al S wg$^{Sp\text{-}1}$ Tft nw^{B} Pin^{Yt}*/+ males, and Cy^{+} F_1 multiply marked females were mated with *y w^{67c23}*; *P{w^{var}}KR3-2 al* males. F_2 progeny were scored for the visible markers as well as eye color.

TABLE 2

Tests to distinguish whether a suppressor is at the *2*L telomere or the named deficiency

Df no.[a]	Genetic Map Position[b]	*2*L TAS	*l(2)gl* lethal complementation	Call[c]
7	—	Weak	Viable	FP4
8	—	No	Lethal	FP4, -5
20	—	No	Viable	FP1, -4
21	—	Yes	Viable	Region 2
24	0.4 (256)	No	Viable	FP2, -4
36	—	Yes	Viable	Region 4
40	—	Weak	Viable	FP4
42	—	Weak	Viable	FP4
44	—	Weak	Viable	FP4
48	—	Yes	Viable	Region 6
49	—	No	Viable	FP4
59	—	Yes	Viable	Region 7
60	0.2 (442)	Yes	Lethal	FP2, -5
80	—	Yes	Viable	Region 8
81	—	—	Lethal	FP5
102	—	Yes	Viable	Region 9
110	—	No	Lethal	FP4, -5
111	—	Weak	Viable	FP4
135	—	Weak	—	FP4
137	—	Yes	Viable	FP1
138	—	Yes	Viable	FP1
143	0.4 (382)	—	Viable	FP1, -2
144	0.4 (145)	Yes	Viable	FP2
145	—	Yes	Viable	FP3
148	0.4 (210)	Weak	Viable	FP2, -4
149	—	Yes	—	FP3
153	0.4 (674)	—	Lethal	FP2, -5
157	—	Weak	Viable	FP4
160	—	No	Viable	FP4
167	—	Yes	Viable	Region 11
172	—	Yes	Viable	FP3
176	—	Yes	Viable	FP3
177	−0.1 (350)	Yes	Viable	FP2
183	0.1 (336)	—	Viable	FP2
191	—	Yes	Viable	Region 12
192	—	Yes	Viable	Region 12
196	—	Weak	—	FP4
205	0.4 (332)	No	Viable	FP2, -4
206	—	No	Viable	FP4
207	—	Weak	Viable	FP4

[a] This number corresponds to the Df number in column 1 of Table 1.
[b] Number in parentheses is the number of chromosomes tested. —, not tested.
[c] Abbreviations are as defined in Table 1.

The suppressors on nine deficiency chromosomes were mapped by meiotic recombination (Table 2). In each case the suppressor was inseparable from or to the left of *al*, the left-most marker, which is located at 0.4 on the genetic map. In no case was there any evidence for a suppressor at the site of the deficiency. There may be some question about the separation of the suppressor on the *Df(2L)S2590* (Df 24 in Table 1) chromosome from the deficiency itself, as this deficiency removes a region in 23DE, close to the *2*L tip. The suppressor, however, clearly maps to the left of *S* (three crossovers/256 chromosomes), while the deficiency clearly is to the right of *S*, which is at 21EF. Thus, all nine cases constitute false positives (Table 1, FP2). As these tests clearly separated the suppressors from the deficiencies, further regions devoid of suppressors were identified, and more deficiencies could be eliminated as causing a suppressor phenotype (Table 1, FP3).

The suppressors on most deficiency chromosomes could not be mapped genetically. Among the deficiency chromosomes with a suppressor phenotype, 44 were deemed to be neither FP1 nor FP3. Of these, 5 deficien-

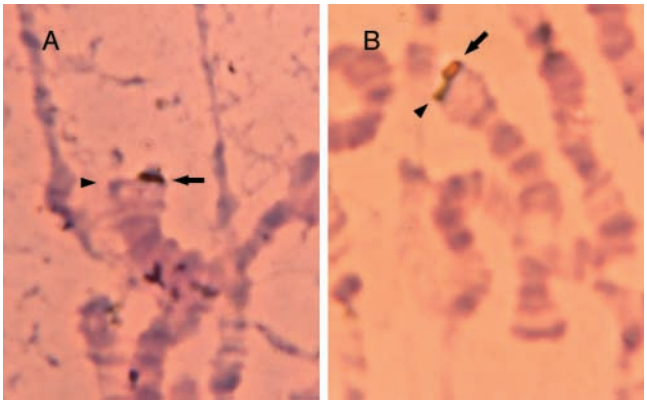

FIGURE 2.—Identification of *2*L TAS on deficiency chromosomes. Arrows point to TAS on the balancer chromosomes. Arrowheads point to the site where TAS should be on the deficiency chromosomes. (A) Df 20/*Gla*. (B) Df 157/*SM1*. Deficiency designation is as in Table 1.

cies are to the left of *al* and could not be separated from the *2*L tip, 4 are too sick to attempt genetic mapping, 1 died in our lab and at the Blooming stock center and could not be tested, 16 are inviable or sterile in combination with *y* w^{67c23}; *SM1*, 4 are inviable or sterile in combination with *y* w^{67c23}; *al S* $wg^{Sp\text{-}1}$ *Tft* nw^{B} Pin^{Y}, 4 have suppressor phenotypes too weak to map, and 8 were mapped.

2L TAS on deficiency chromosomes: We have shown previously (GOLUBOVSKY *et al.* 2001; MASON *et al.* 2003b) that silencing of brown-red variants of *P{*w^{var}*}* is suppressed by a complete, or even a partial, deficiency of the *2*L TAS array on the homolog. A recent search for radiation-induced suppressors of telomeric silencing on chromosome *2* produced almost exclusively deficiencies of *2*L TAS (A. Y. KONEV and J. M. MASON, unpublished results). We, therefore, tested 36 deficiency chromosomes for the presence of *2*L TAS by *in situ* hybridization (Table 2). Eight of these showed no evidence for the presence of *2*L TAS, and 10 others showed only weak hybridization to the *2*L TAS probe (Figure 2). Thus, a substantial proportion of the suppressor chromosomes lack much or all of the *2*L TAS array and are considered to be false positives (Table 1, FP4). This proportion could be even higher than these data indicate, because a partial deficiency for the TAS repeat sufficient to cause suppression of TPE may not be obvious from *in situ* hybridization. Consistent with this idea, three chromosomes, Df 60, 144, and 177, with suppressors that map to the tip of *2*L, suggesting a disruption of *2*L TAS (GOLUBOVSKY *et al.* 2001; MASON *et al.* 2003b), show relatively strong hybridization to TAS. Some of the deficiencies in Table 2 could not be tested for *2*L TAS, because they did not produce satisfactory salivary chromosome spreads.

Lethal complementation: Many of the identified deficiencies for *2*L TAS are also mutant for the adjacent gene, *l(2)gl* (GOLUBOVSKY *et al.* 2001). We, therefore, crossed the chosen deficiencies to *l(2)gl* mutants to inquire into the ability of the deficiency chromosomes to complement the lethality associated with this locus. Two alleles of *l(2)gl* in different genetic backgrounds were chosen to obviate genetic background effects. The deficiency chromosome must be lethal in combination with both alleles to show a failure to complement. Most of the deficiencies tested complemented both alleles of *l(2)gl*. Five deficiencies did not complement either allele. Two deficiencies (Df 8 and Df 110) failed to complement the *l(2)gl* mutations and also failed to hybridize the *2*L TAS probe. Df 153 failed to complement the *l(2)gl* mutations, carries a suppressor that mapped to the *2*L tip, but was not tested for the presence of *2*L TAS. Interestingly, Df 60 fails to complement the *l(2)gl* mutations and carries a suppressor that mapped to the tip of *2*L, but it also hybridizes to *2*L TAS. Given the evidence that a partial or complete deletion of *2*L TAS acts as a suppressor of TPE silencing, these latter results suggest that the deletion need not show a discernible decrease in *2*L TAS hybridization to exhibit a suppressor phenotype. Finally, Df 81 fails to complement the *l(2)gl* mutations, but could not be tested in the other assays. Given the results on the other deficiencies, we believe that this gives a strong reason to doubt that the suppressor on this chromosome is a result of the deficiency itself, and this chromosome should be considered a false positive (FP5) until demonstrated otherwise. Failure to complement *l(2)gl* mutations and to hybridize strongly to the *2*L TAS probe *in situ* suggests a suppressor at the tip of *2*L, but does not unequivocally demonstrate that a deficiency does not uncover a suppressor. Thus, these deficiencies (FP4 and FP5) were dropped from further consideration, rather than used to identify regions devoid of suppressors.

Given the high frequency of false positive results, we feel uncomfortable assigning sites of potential TPE suppressors on the basis of equivocal results. We, therefore, chose to ignore deficiencies associated with a suppressor phenotype that overlaps wild type (Table 1, FP6).

A map of potential suppressors of telomeric silencing: In several regions with potential suppressor deficiencies the ambiguity surrounding the deficiency breakpoints of nonsuppressing deficiencies raised the possibility that the latter deficiencies might overlap. Overlaps would eliminate the ambiguous regions as potential sites of TPE suppressors. We, therefore, looked for genetic evidence for overlaps. The FLYBASE CONSORTIUM (2003) reports that complementation tests between deficiencies and gene mutations indicate that Df 174 and 177 in cytological region 55 overlap, and Df 300 and 301 in region 84 also overlap, thus eliminating two potential sites of a suppressor of TPE. We conducted lethal complementation tests between Df 189 and 193 in region 59 and found that they complement, indicating that these two deficiencies do not overlap. Complementation tests indicate that Df 269 and 270 overlap, eliminating a potential suppressor in region 76.

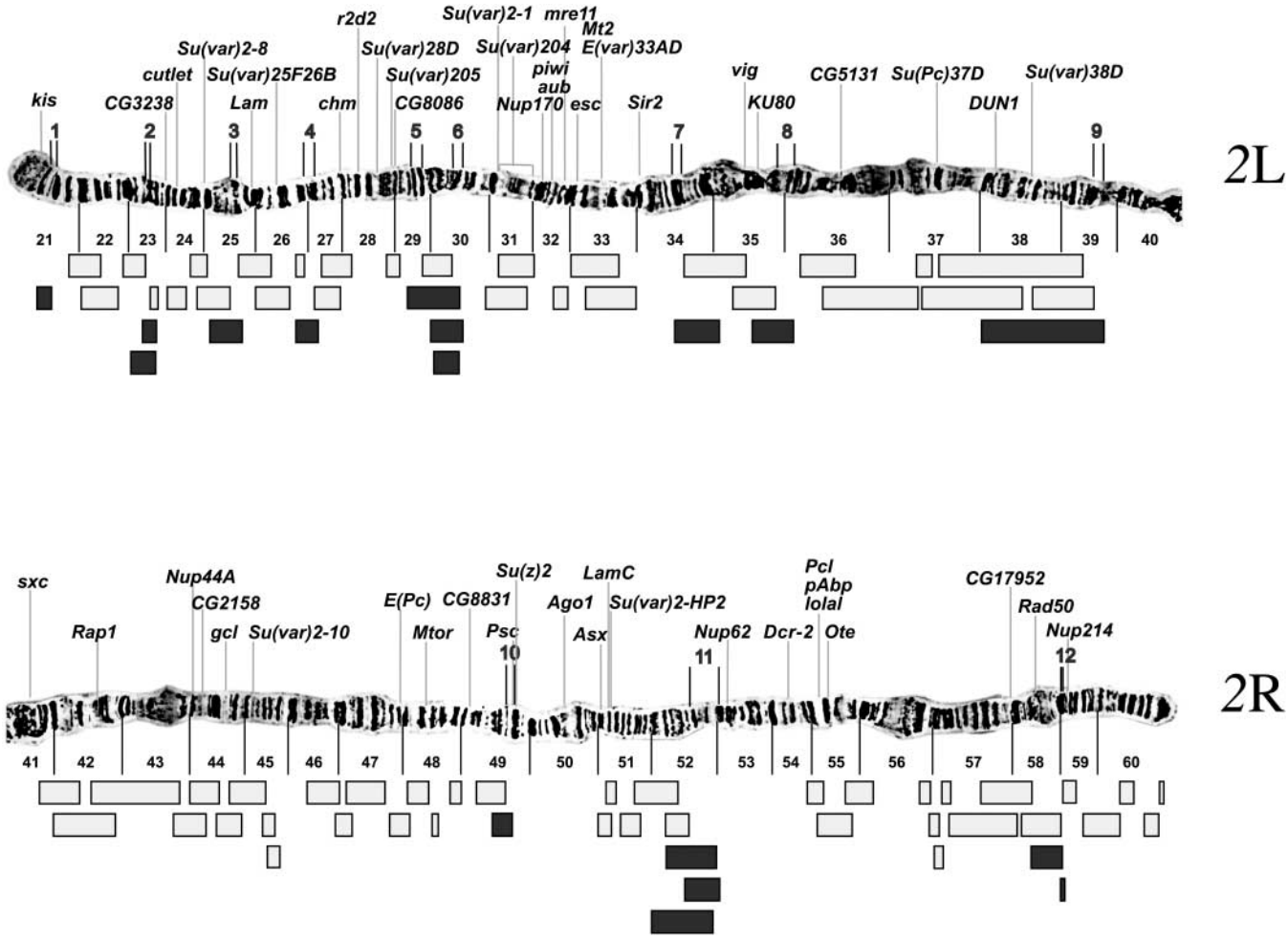

FIGURE 3.—Deficiencies for chromosome *2* and their ability to suppress telomeric silencing. Open rectangles below the polytene chromosome represent deficiencies without the ability to suppress TPE. Solid rectangles show deficiencies on chromosomes that suppress TPE. The minimum number of nonsuppressing deficiencies covering the full genetic distance is shown; *i.e.*, redundant deficiencies are not shown. False positive results are not shown. Symbols shown above the chromosome are genes whose mutations exhibit effects on gene expression or genes that are homologous of yeast genes that encode telomeric proteins. Numbers above the chromosome indicate sites identified by the deficiencies as potential sites of suppressors of TPE.

After eliminating false positive and potential false positive results, a map was constructed to identify chromosomal regions that may contain suppressors of TPE (Figures 3 and 4). Twelve sites of potential TPE suppressors were identified on chromosome *2*, and 6 on chromosome *3*. Given the high frequency of false positive results and the inability to test all of the deficiency chromosomes adequately, these are probably high estimates for the actual number of suppressors on these chromosomes. As deficiencies for *2*L TAS are strong TPE suppressors (GOLUBOVSKY *et al.* 2001; A. Y. KONEV and J. M. MASON, unpublished results), and deficiencies for other autosomal telomeres may not exhibit the same phenotype (M. D. GOLUBOVSKY, S. PRASAD and J. M. MASON, unpublished results), the estimate for the number of suppressor sites on chromosome *2* may be especially high.

To ask whether potentially interesting genes might lie in the regions identified, we placed selected categories of genes on the same map. These include suppressors and enhancers of PEV, PcG, and trithorax group (trxG) genes; genes necessary for RNAi; homologs of genes that encode yeast telomeric proteins; genes that encode components of the nuclear lamin and nuclear pores; and genes that encode post-translational histone modifiers. Of 108 autosomal genes examined, 2 fell into potentially interesting sites identified by the deficiencies. These are *Psc* on chromosome *2*, and *gpp*, the homolog of yeast *DOT1*, on chromosome *3*. The sites of potential TPE suppressors, as defined here by the deficiencies, encompass ~5% of the autosomal genome. On the basis of random sampling of the tested loci, we would have expected 6 genes to be in potentially interesting sites. We found 2, suggesting that these "hits" may be due to chance.

Several reports (CRYDERMAN *et al.* 1999; KURENOVA

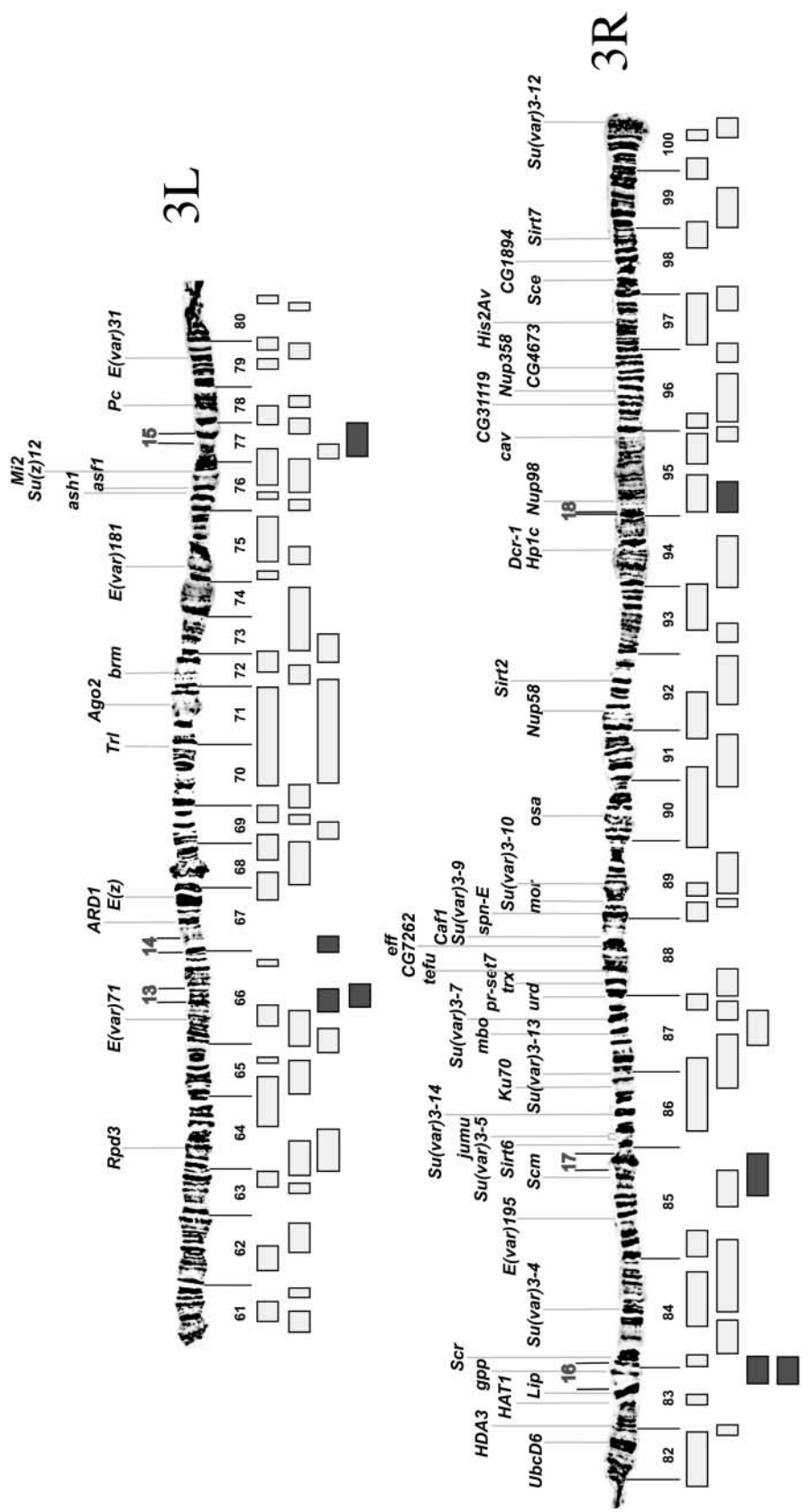

FIGURE 4.—Deficiencies for chromosome *3* and their ability to suppress telomeric silencing. Symbols are the same as for Figure 3.

et al. 1998; Boivin *et al.* 2003) have implicated the deficiency $Su(z)2^5$ as a suppressor of TPE. We, therefore, tested it for suppression and mapped the suppressors and lethals on this chromosome. A strong suppressor was found to be inseparable from *al*. A second suppressor in the stock could not be mapped easily in the presence of the strong suppressor. This second suppressor segregated independently of *al* and did not segregate with either the *X* chromosome or chromosome *3*. Thus, it may be on *2*R [near *Su(z)2*] or chromosome *4*. There were also multiple lethals on the $Su(z)2^5$ chromosome. One mapped to the tip of *2*L and failed to complement mutations for *l(2)gl*. Another lethal appeared to map to the *Su(z)2* locus. Most of the tested chromosomes were noncrossover, however, and detailed mapping was not pursued. The $Su(z)2^5$ chromosome also failed to hybridize to the *2*L TAS. By these assays, it appears that one suppressor is associated with the *2*L telomere and not related to the $Su(z)2^5$ deficiency, although we cannot exclude the possibility that the second suppressor is a result of the deficiency of the *Su(z)2* locus.

A recent report (Boivin *et al.* 2003) also implicates Psc^1, a mutation in another locus uncovered by the $Su(z)2^5$ deficiency, as a suppressor of TPE. We found that the suppressor on this chromosome maps to the tip of *2*L, not to the *Psc* locus. This chromosome, however, complements the lethality of *l(2)gl* mutations.

DISCUSSION

As part of a systematic search for genes in Drosophila that play a role in TPE, we screened the Bloomington autosomal deficiency kits for dominant suppressors of telomeric silencing. Of 372 deficiencies tested, 149 chromosomes gave a positive response. The suppressors on 124 (83%) of the latter are not associated directly with the deficiency itself, but appear to be due to a second mutation on the deficiency chromosome. Ignoring deficiencies on chromosomes with a suppressor phenotype that overlaps wild type, we are left with 25 deficiencies that identify 18 potential sites of TPE suppressors. On chromosome *2*, where there were more deficiency chromosomes with a suppressor phenotype and more tools to characterize them, 80% of the suppressors (67/84) were determined to be false positives, while on chromosome *3* more than half (10/18) were false positives. Given the high frequency of false positive results and the inability to adequately test all of the deficiency chromosomes, we may have overestimated the number of suppressor genes.

2L TAS plays a role in TPE: All nine of the suppressors mapped by meiotic recombination are at or near the *2*L telomere. *In situ* hybridization studies indicated that 18 of 36 deficiency chromosomes tested lacked all or most of the *2*L TAS array, independent of the position of the deficiency. Given published observations that even partial deficiencies for *2*L TAS may have strong suppressing effects on TPE (Golubovsky *et al.* 2001), and the present data showing that for half (3/6) of the chromosomes tested in both assays the suppressor that mapped to the *2*L telomere region did not show a discernible decrease in TAS hybridization, this proportion (18/36) is likely an underestimate of the number of suppressor chromosomes that lack at least part of the *2*L TAS. Taken together, these data reinforce and extend previous suggestions that *2*L TAS plays a major role in TPE (Golubovsky *et al.* 2001; Mason *et al.* 2003b). It is possible that the suppressive effect of *2*L TAS deficiencies on silencing of $P\{w^{var}\}KR3\text{-}2$ is the result of homologous interactions. These deficiencies, however, also suppress TPE at nonhomologous telomeres, while the converse is not true; Df of *3*R TAS do not suppress TPE at *2*L (M. D. Golubovsky, S. Prasad and J. M. Mason, unpublished results). Thus, we believe that deficiencies for *2*L TAS have a global impact on telomeric silencing.

*2*L TAS hybridizes *in situ* with both the *2*L and *3*L chromosome tips, but not with the tips of *X*L, *2*R, or *3*R (Mechler *et al.* 1985; Walter *et al.* 1995), suggesting a similar sequence for the former two TAS arrays. Sequencing of BACs derived from the Drosophila Genome Project also indicates strong similarities between *2*L and *3*L TAS arrays (A. Villasante, personal communication). We speculate that deficiencies for *3*L TAS may have a suppressor phenotype similar to that seen with deficiencies for *2*L TAS, and that deficiencies of *3*L TAS may be responsible for the high frequency of false positives we find on chromosome *3*. The difference in frequency between false positives on chromosomes *2* and *3*, that is, presumptive *2*L and *3*L TAS deficiencies, is consistent with the observation that *l(2)gl* mutants exist at a high frequency in natural populations (Golubovsky 1978) and that these mutations are primarily terminal (*i.e.*, TAS) deficiencies (Mechler *et al.* 1985; Walter *et al.* 1995). It is possible that the terminal *2*L region is more susceptible to loss, or that heterozygous deficiencies for the *2*L tip region have a selective advantage (Golubovsky 1978).

The role of known genes on TPE: In an effort to ask whether other suppressors of genetic silencing may act on telomeres, we compared the map positions of Su(var) and PcG genes, as well as their opposites, E(var) and trxG genes, with the loci identified by the deficiencies. RNAi and histone modification may play a role in heterochromatin formation; we therefore considered genes that control these two processes. The position of telomeres in the nucleus, and especially proximity to nuclear pores, is important for telomeric silencing in yeast (Gotta *et al.* 1996; Galy *et al.* 2000). We, therefore, examined genes that encode the structural components of the nucleus, including nuclear pores, and lamin, as well as homologs of yeast genes that encode telomere-specific proteins. On the basis of the size of

the sites of potential suppressors of TPE and the number of genes considered, and assuming random positions for these genes, we would have expected approximately six genes to lie in these sites; only two (*Psc* and *gpp*) fell within these sites. Therefore, the positions of these genes within sites of potential suppressors is not a strong indicator that the genes thus identified are important for telomeric silencing. Indeed, Cryderman *et al.* (1999) showed that several mutant alleles of *Psc* do not have suppressor phenotypes. Thus, this gene is probably not involved in telomeric silencing.

Boivin *et al.* (2003) reported that several PcG and trxG mutations have an effect on telomeric silencing. This observation seems at odds with the present report. How do we interpret the apparent discrepancy? Boivin *et al.* (2003) tested several mutant alleles for genes examined. In several cases, one or more mutations exhibited a suppressor or enhancer phenotype and other alleles for the gene did not. Standard genetic practice is to assume that phenotypic differences between alleles in different genetic backgrounds are due to background effects until proven otherwise. These authors did the opposite. Their approach seems destined to maximize the number of false positive results. We reexamined two of the three second chromosome mutations they claim suppress telomeric silencing, $Su(z)2^5$ and Psc^1, and showed that both of these mutant chromosomes have a suppressor at the tip of *2*L, even though the mutation being tested was on *2*R. Thus, the results of Boivin *et al.* (2003) require verification. Interestingly, previous results (Cryderman *et al.* 1999) showed that a Psc^1 chromosome did not have a suppressor phenotype. It should be stressed that genetic background effects can be a serious problem when dealing with mutations from different sources.

Numerous genes act to remodel and repress chromatin in heterochromatin and around euchromatic genes during development. Many of these have been identified by dominant mutations that suppress this repression. We have found relatively few potential sites for genes that have a similar effect at telomeres. There are several possible reasons for the difference. First, the 18 sites we have mapped may all identify suppressor genes. This is unlikely, because for nine of nine second chromosomes on which we mapped the suppressor by meiotic recombination, the suppressor was located at the tip of *2*L, rather than at the site of the deficiency on that chromosome. Thus, we believe that the remaining deficiencies overestimate the number of suppressor loci. Some of the remaining sites may still contain suppressor genes. The Drosophila homolog of the yeast *DOT1* gene is a candidate identified by the deficiencies. Several newly induced mutant alleles for this gene, renamed *grappa* (*gpp*), also have a suppressor phenotype (G. Shanower and P. Schedl, personal communication).

Second, we looked at the major autosomes, but the suppressors of telomeric silencing may be on the *X* or fourth chromosomes. R. Levis (personal communication), for example, has found that several mutant alleles of the *X*-linked gene *ph* exhibit a suppressor phenotype and that this phenotype is rescued by a duplication for the region. Although there may be some suppressors on the *X*, the major autosomes make up 80% of the genome and would be expected to carry a majority of suppressor genes, if they are randomly distributed. Third, autosomal suppressor genes may exist in regions that have not been uncovered by the deficiencies. Although this is possible, the deficiencies we examined span 77% of the two major autosomes. Thus, we should have found a majority of suppressor loci, if they are distributed randomly throughout the genome.

Fourth, partial elimination of the relevant proteins may not suppress TPE. It is possible that suppressors of TPE are recessive, even though many Su(var) and PcG mutations are dominant (Reuter and Wolff 1981; Sinclair *et al.* 1989; Reuter and Spierer 1992; Pirrotta 1995), and *gpp* mutations are dominant suppressors of TPE (G. Shanower and P. Schedl, personal communication).

Fifth, we may have chosen the wrong phenotype. As we do not know the mechanism of silencing, it is possible that many mutations in the process decrease, rather than increase, gene expression in telomeric regions. While we did not score for enhancers specifically, they would have been visible in our screen. Other, more subtle phenotypes are also possible.

Finally, chromatin structure in telomeric regions of Drosophila, at least in and around the TAS array, may be simple, with relatively few components. Further searches for disruption of telomeric silencing may reveal that there are, in fact, few genes that play a role in TPE.

TPE is independent of the chromosome capping complex: A few components of the telomere capping complex have recently been identified. Heterochromatin protein 1 (HP1) binds to chromosome ends in Drosophila independently of the presence of the terminal transposon array or the TAS repeats (Fanti *et al.* 1998; Siriaco *et al.* 2002). Null mutations in *Su(var)205*, the gene that encodes HP1, cause an increase in the length of the terminal *HeT-A/TART* array when heterozygous (Savitsky *et al.* 2002) and telomere fusions when homozygous (Fanti *et al.* 1998). HP1 associates with *H*P1 *o*rigin recognition complex-*a*ssociated *p*rotein (HOAP; Shareef *et al.* 2001) at chromosome ends (Badugu *et al.* 2003; Cenci *et al.* 2003). Disruption of *caravaggio* (*cav*), the gene that encodes HOAP, has an effect at telomeres similar to *Su(var)205* mutations (Cenci *et al.* 2003; P. Georgiev, personal communication). Mutations for *tefu*, the Drosophila ATM homolog, interfere with the binding of HP1 and HOAP to chromosome ends, and mutants for this gene have a phenotype similar to *Su(var)205* and *cav* mutants. It has been proposed that these genes encode components of the telomere capping complex. Mutation or deletion of none of these

genes, however, affects TPE dominantly (Cryderman *et al.* 1999 and this report). Thus, the terminal capping protein complex may act independently of any chromatin complex that plays a role in TPE.

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Note added in proof: Oikemus *et al.* (S. R. Oikemus, N. McGinnis, J. Queiroz-Machado, H. Tukachinsky, S. Takada *et al.*, 2004, Drosophila atm/telomere fusion is required for telomeric localization of HP1 and telomere position effect. Genes Dev. **18:** 1850–1861) report that mutations in *tefu*, the Drosophila ATM homologue, reduce the amount of HP1 at telomeres and cause a recessive suppression of telomeric silencing. This suggests an interaction between the telomere capping complex and telomeric silencing.

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