Identification of Chromosome Inheritance Modifiers in *Drosophila melanogaster*

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ABSTRACT

Faithful chromosome inheritance is a fundamental biological activity and errors contribute to birth defects and cancer progression. We have performed a *P*-element screen in *Drosophila melanogaster* with the aim of identifying novel candidate genes involved in inheritance. We used a "sensitized" minichromosome substrate (*J21A*) to screen ~3,000 new *P*-element lines for dominant effects on chromosome inheritance and recovered 78 *S*ensitized *c*hromosome *i*nheritance *m*odifiers (*Scim*). Of these, 69 decreased minichromosome inheritance while 9 increased minichromosome inheritance. Fourteen mutations are lethal or semilethal when homozygous and all exhibit dramatic mitotic defects. Inverse PCR combined with genomic analyses identified *P* insertions within or close to genes with previously described inheritance functions, including *wings apart-like* (*wapl*), *centrosomin* (*cnn*), and *pavarotti* (*pav*). Further, lethal insertions in *replication factor complex 4* (*rfc4*) and *GTPase-activating protein 1* (*Gap1*) exhibit specific mitotic chromosome defects, discovering previously unknown roles for these proteins in chromosome inheritance. The majority of the lines represent mutations in previously uncharacterized loci, many of which have human homologs, and we anticipate that this collection will provide a rich source of mutations in new genes required for chromosome inheritance in metazoans.

ACCURATE chromosome inheritance is a dynamic and multifactorial process (Rieder and Salmon 1998). In mitotic prophase chromosomes become condensed and sister chromatids are held together at centric heterochromatin and along the chromosome arms. As mitosis progresses the chromosome arms and centromeres associate with microtubules radiating from centrosomes and chromosomes congress to the metaphase plate due to the action of motor proteins that result in balanced poleward and antipoleward forces. The spindle assembly checkpoint apparatus monitors this process and sister chromatids segregate to opposite poles only after all the chromosomes have aligned at the plate and attached to the spindle. The kinetochore, a specialized proteinaceous structure, contains checkpoint proteins as well as proteins required for spindle attachment, chromosome congression, and segregation (Dobie *et al.* 1999). Following cytokinesis, chromosomes must undergo decondensation and DNA replication before chromosome division can be repeated. Mitotic missegregation is associated with tumor progression and cancer (Mitelman 1994). A further level of complexity is added in germ cells where homologous chromosomes pair and segregate in meiosis I and sister chromatids remain associated until meiosis II. Errors in meiotic chromosome segregation can result in aneuploid zygotes, which are associated with birth defects such as Down syndrome (Hook 1985).

Studies performed in diverse organisms have been crucial in the identification of genes involved in chromosome segregation (Pluta *et al.* 1995). However, a more complete understanding will require the identification and characterization of components that govern chromosomal processes during the cell cycle. The fruit fly *Drosophila melanogaster* is an excellent model system for higher eukaryotic chromosome inheritance. The identification and analysis of proteins involved in chromosome inheritance are facilitated by the availability of robust molecular-genetic tools and mutation screening approaches. As is true for humans and higher eukaryotes in general, Drosophila displays diverse types of chromosome cycles and cell divisions during development. Furthermore, Drosophila centromeres share many structural and functional similarities (*e.g.*, large amount of DNA, kinetochore structure, heterochromatic location and attachment to several microtubules) with those of mammalian cells. Finally, genome sequence analyses revealed that two-thirds of positionally cloned human disease genes have significant homologs in Drosophila (Adams *et al.* 2000). Therefore, information derived from studies on chromosome inheritance in Drosophila is likely to be relevant to human chromosome inheritance and elucidation of the causes of aneuploidy.

The Drosophila minichromosome *Dp(1;f)1187* (*Dp 1187*) is a unique tool for studying chromosome inheritance. *Dp1187* is derived from the *X* chromosome and is

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not required for viability (Murphy and Karpen 1995b; Williams *et al.* 1998). It is only 1.3 Mb in size, is transmitted normally through mitosis and meiosis, and binds known kinetochore proteins, demonstrating that it contains a fully functional centromere as well as all other inheritance components. The small size of the minichromosome has allowed detailed restriction mapping of the entire minichromosome using pulsed-field gel electrophoresis and Southern analysis (Le *et al.* 1995; Sun *et al.* 1997). Transmission studies of X-ray-induced deletion derivatives of *Dp1187* identified a 420-kb region essential for chromosome transmission (Murphy and Karpen 1995b; Sun *et al.* 1997). One deletion derivative from this study, *J21A*, contains only 290 kb of centric heterochromatin, corresponding to two-thirds of the *cis*-acting DNA sequences required for normal centromere function, and is inherited only half as well as larger derivatives. Previous studies demonstrated that *J21A* transmission is dominantly affected by mutations in genes required for inheritance, whereas the inheritance of normal chromosomes is unaffected (Murphy and Karpen 1995a; Cook *et al.* 1997). *J21A* inheritance is sensitized to the reduced dosage of genes involved in different aspects of inheritance, including spindle components (Cook *et al.* 1997), antipoleward forces (Murphy and Karpen 1995a), sister chromatid cohesion (Lopez *et al.* 2000), and overall chromosome architecture (see discussion).

Here we describe the results of a screen designed to search for new genes involved in chromosome inheritance by identifying mutations that dominantly affect *J21A* inheritance. A screen using inheritance of *J21A* as a dosage-sensitive substrate allowed the recovery of mutations that would otherwise be undetectable as heterozygotes and/or lethal as homozygotes (Figure 1). *P*-element mutagenesis was chosen for this screen due to the ease with which a "transposon-tagged" gene can be identified using inverse PCR amplification of the flanking DNA (Gloor *et al.* 1993; Spradling *et al.* 1999), which greatly facilitates subsequent molecular-genetic analysis. We have isolated 78 *S*ensitized *c*hromosome *i*nheritance *m*odifier (*Scim*) lines that exhibit significantly altered levels of *J21A* inheritance. Comparison of the DNA sequences flanking the *P* elements to the complete euchromatic sequence of Drosophila (Adams *et al.* 2000) allowed us to identify several known genes, many of which have chromosome inheritance-related functions. The majority of lines represent mutations in previously uncharacterized loci, many of which have human homologs. We show that this collection identified novel genes involved in various aspects of inheritance, such as centromere structure and function, chromosome movement (motor proteins), chromosome architecture (sister chromatid cohesion, condensation, and replication) and cell-cycle regulation (checkpoint proteins or the anaphase promoting complex).

MATERIALS AND METHODS

Drosophila stocks and culture: The *SM1* and *TM3* balancer chromosome and *y;ry* stocks are described by Cook *et al.* (1997). The strain containing the *SUPor-P* (*sup*pressor-*P*) element on the *CyO* balancer chromosome is described by Roseman *et al.* (1995). The *P* element was mobilized using *P[ry*$^{+}\Delta$*2-3]*(99B) transposase on the *TMS* balancer chromosome (Robertson *et al.* 1988; Figure 2A). The genotypes of the GFP balancer chromosome lines are *w*; *In(2LR)noc*^{4L}*sco*^{rv9R}, b^{1}/*CyO*, *P*{w^{+mC} = *ActGFP*}*JMR1* for chromosome *2*, *w*; Sb^{1}/*TM3*, *P*{w^{+mC} = *ActGFP*}*JMR2*, Ser^{1} for chromosome *3*, and *FM7i*, *P*{w^{+mC} = *ActGFP*}*JMR3*/*C(1)DX*, f^{1} for the *X* chromosome (see http://flybase.bio.indiana.edu/). Flies were grown on standard corn meal/agar media at 25°. The *Scim* loci were numbered and ordered in Table 3 relative to the position of the *P* insertions on polytene chromosomes. In a minority of lines the polytene locations are slightly out of order, in particular those on the *X* chromosome, due to more precise information provided by the genome sequencing project.

Recovery of insertions on the *X* chromosome: The mobilization-generating crosses were performed in vials as a precaution against recovering multiple lines from the same insertion event. This involved setting up >10,000 vials, which made the collection of virgin females containing new mobilization events impractical. Eleven individual loci on the *X* chromosome (Tables 2 and 3) were recovered by collecting y^{+};*ry* nonvirgin females and crossing in *J21A* (Figure 2B). Males carrying the *P* element and *J21A* (y^{+};ry^{+}) were selected and outcrossed to *y;ry* virgin females. Incorporating this extra generation allowed selection of y^{+};ry^{+} virgin females in the next generation that carried the new *P* insertion and *J21A*, which could be transmission tested in the normal fashion. Insertions in the *Y* chromosome were not tested for transmission defects because the transmission tests were performed in females (Figure 2B). However, we established ~170 lines that exhibit variegated expression of the *yellow* (y^{+}) marker on the *P* element. These insertions represent a collection of insertions within heterochromatin, some of which are on the *Y* chromosome (K. W. Dobie, C. M. Yan and G. H. Karpen, unpublished data).

Monosome transmission assay: The monosome transmission assay has been described in Cook *et al.* (1997). A one-tailed Student's *t*-test demonstrated that lines exhibiting an average of <22% or >37% transmission are significantly different ($P < 0.05$) from the normal 27% transmission for *J21A* (data not shown). If a line met the above transmission criteria using up to three vials per line, the transmission test was repeated with 10–15 vials to verify the results (Figure 2B). A stock was made if a line still exhibited <22% or >37% transmission; 78 lines met this criteria.

Inverse PCR: Genomic DNA preparation, digests, and ligations were performed using standard methods (Gloor *et al.* 1993; Spradling *et al.* 1999). DNA from each line was digested separately using three restriction enzymes (*Hpa*II or *Hha*I or *Hae*III) to maximize the generation of 5′ and 3′ flanking DNA. Primers tgaaccactcggaaccatttgagcga (KWD2) and cgatcgggac caccttatgttatttcatcat (GK36) were used to amplify from the 5′ end of *SUPorP* while primers ccagattggcgggcattcacataagt (KWD4) and GK36 were used to amplify from the 3′ end. Amplified DNA bands were cut from agarose gels and reamplified before sequencing using ABI377 automated sequencers (Perkin-Elmer, Norwalk, CT).

Blast search strategy: Sequence data was analyzed using the Berkeley *Drosophila* Genome Project (BDGP) WU-BLAST 2.0 and National Center for Biotechnology Information (NCBI) Advanced BLAST servers. Initial searches were performed using a BLASTN search of the BDGP nonredundant (nr) DNA database. This provided a rich source of sequence matches

with large genomic clones (20–350 kb), known Drosophila genes, expressed sequence tags (ESTs), and *P* insertions from other screens [Enhancer-Promoter (EP; Rørth 1996) or lethal *P* lines (Spradling *et al.* 1999)]. At least one large clone was obtained for every line that produced inverse PCR sequence data. This facilitated searches in BDGP using 5 kb of sequence surrounding the insertion site (2.5 kb either side) to identify neighboring genes, ESTs, and other *P* elements. These 5-kb blocks and ESTs were used to search for homologs in other species by performing a BLASTX search of the NCBI nr database. Sequence matches with Drosophila ESTs indicate that the *P* insertion is close to or within an expressed sequence and homology with DNA flanking other lethal *P* insertions suggests that our insertion is close to or within a gene that is essential for viability. Protein accession numbers for similar human genes for Drosophila *wapl*, *grp*, *Gli*, *cnn*, *pav*, *eIF-4E*, *Gap1*, and *JIL-1* were directly available from FlyBase reports (http://flybase.bio.indiana.edu/) while the Online Mendelian Inheritance in Man (OMIM) database (within the FlyBase reports) was used for *Fim*, *Rab5*, *Hr39*, *His4*, *Sca*, *LanA*. Similar human sequences for the novel loci were determined using the Genome Annotation Database of Drosophila (GadFly: http://flybase.bio.indiana.edu/) and LocusLink (http://www.ncbi.nlm.nih.gov/LocusLink/).

Stage of lethality and cytological analysis of mitotic defects: Embryo collections were performed on apple juice plates supplemented with yeast paste to encourage egg laying. The stage of lethality was determined using standard procedures and by normalizing to *inter se* crosses using control nonlethal +/P, +/*SM1*, and +/*TM3* lines. A line was classified as lethal if it exhibited <5% of the expected number of P/P (nonbalancer) flies and semilethal if it exhibited between 5% and 50% of the expected number of P/P flies (Ashburner 1989). Homozygous lethal and semilethal lines were crossed with green fluorescent protein (GFP) balancer chromosome lines, which enabled discrimination of P/GFP and P/P larvae using a Zeiss Axiophot fluorescence microscope equipped with a fluorescein filter. Larval neuroblast squashes were prepared using a standard method (Gatti *et al.* 1994) with some modifications. Neuroblasts were fixed in 45% acetic acid followed by 60% acetic acid for 45 sec each. Squashes were performed in 60% acetic acid and chromosomes were stained in 1 μg/ml 4′,6-diamidino-2-phenylindole diluted in Vectashield (Vector Laboratories, Burlingame, CA). Chromosome defects were examined using a ×63 lens and a ×1.25 optivar on a Zeiss Axiophot fluorescence microscope and were examined independently by two investigators.

RESULTS

A sensitized *P*-element screen to identify dominant mutations that affect chromosome inheritance: The *J21A* minichromosome is transmitted to only 27% of the progeny in a monosome transmission assay, corresponding to half the frequency observed for a normal monosome. Previous studies demonstrated that *J21A* transmission is more sensitive than the sex chromosomes or autosomes to heterozygous mutations in genes known to be important for mitosis and meiosis (Murphy and Karpen 1995b; Cook *et al.* 1997), and we used this strategy (Figure 1) to identify new mutations that dominantly affect *J21A* transmission.

The *SUPor-P* element was used to generate the mutations since the presence of two Suppressor of Hairy Wing [Su(Hw)] binding sites enhances its mutagenic properties (Roseman *et al.* 1995). *SUPor-P* was mobilized off the *CyO 2* chromosome and ~3500 mobilizations were recovered with the *P* element inserted in a different chromosome. This strategy enabled us to target the entire Drosophila genome (*X*, *Y*, second, third, and fourth chromosomes) with *P*-element insertions (Figure 2A; materials and methods). We were unable to test ~500 lines due to insertions in the *Y* chromosome (transmission tests were performed in females) or culture failure. Each of the 3000 remaining lines was tested for dominant effects (increases or decreases) on *J21A* transmission (Figures 1 and 2B). Statistical analyses indicated that lines exhibiting *J21A* transmission to <22% or >37% of progeny differ significantly from normal and warranted further analyses (see materials and methods). Seventy-eight lines were recovered with altered *J21A* transmission and were named *Scim*, for *S*ensitized *c*hromosome *i*nheritance *m*odifiers (Table 1). Sixty-nine lines exhibited significantly reduced transmission of *J21A*, ranging from 9 to 21%. In addition, 9 lines were recovered that significantly increased *J21A* transmission, ranging from 38% to as high as 51% (completely stable) transmission. The lines that exhibited increased transmission could represent an interesting class of mutations in cell-cycle regulatory genes or genes that repress proteins involved in inheritance (see discussion). Fourteen lines were lethal or semilethal when homozygous for the *P* element. Thus, at least 18% (14 out of 78) of the mutations affect genes that are important for viability and also strongly influence minichromosome inheritance.

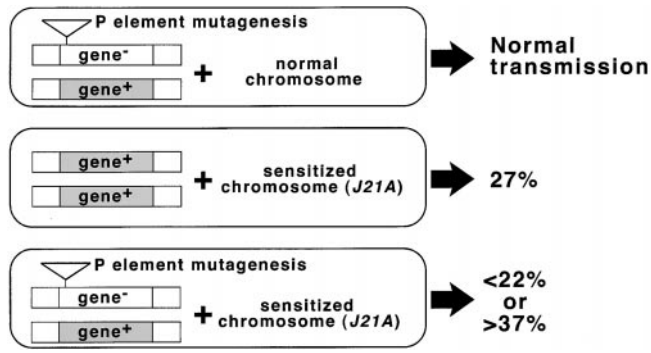

Figure 1.—Dominant interaction between a *P*-element-induced mutation and a sensitized minichromosome. Inheritance of *J21A* was used as a sensitized assay to detect dominant mutations that affect chromosome inheritance. *J21A* is only 580 kb and exhibits moderate instability in a monosome transmission assay; it is transmitted to only 27% of the progeny, half of the normal 50% transmission exhibited by larger, monosomic minichromosomes and the 100% transmission displayed by the disomic autosomes and sex chromosomes.

Most *P* insertions are in previously characterized genes with demonstrated roles in chromosome inheritance: Insertion sites are transposon tagged after *P*-element mutagenesis, which facilitated molecular analysis of the

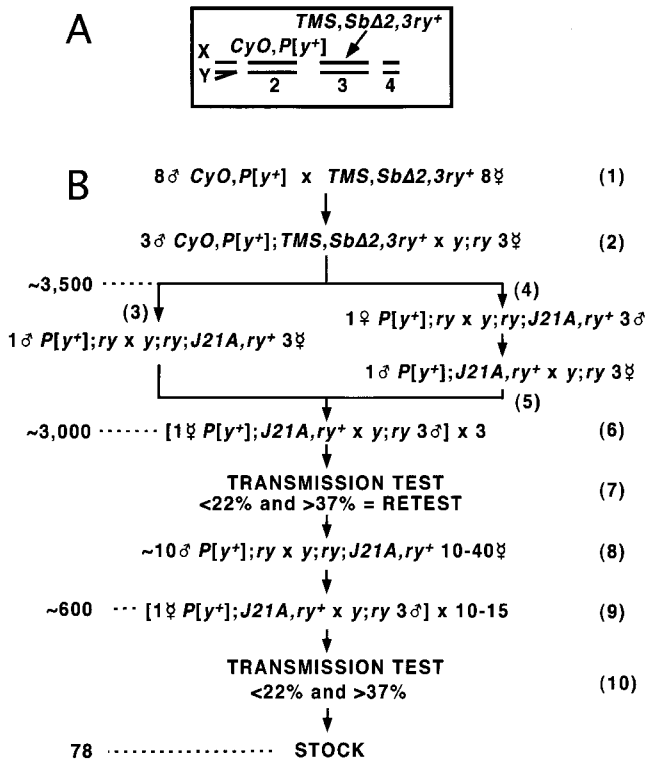

FIGURE 2.—A screen for sensitized chromosome inheritance mutations using *P*-element mutagenesis. (A) A schematic of the Drosophila genome. *SUPor-P* (ROSEMAN *et al.* 1995) was mobilized from the *CyO* chromosome using *TMS,SbΔ2,3ry*$^+$. (B) An outline of the multiple generations in the screen. (1) *CyOP[y*$^+$*]* males containing SUPor-P were crossed with *TMS,SbΔ2,3ry*$^+$ virgin females containing the transposase activity. (2) A pilot study demonstrated no difference in *SUPor-P* mobilization frequency between males or females. Therefore we mobilized the *SUPor-P* from males because *CyOP[y*$^+$*]; TMS,SbΔ2,3ry*$^+$ males were more convenient to collect than *CyOP[y*$^+$*]; TMS,SbΔ2,3ry*$^+$ virgin females and *y;ry* virgin females were relatively plentiful. (3 and 4) New *SUPor-P* insertions were collected by selecting for *P[y*$^+$*]* and against the *CyO* and *TMS* chromosomes. (4) *X* chromosome insertions were recovered by collecting nonvirgin females (see MATERIALS AND METHODS). This was possible because the nonvirgin females remated with *y;ry; J21A,ry*$^+$ males and produced offspring with the appropriate phenotype (5). (3 and 4) *J21A* was crossed into the *SUPor-P*-induced mutant background. (6) Three virgin *y*$^+$*;ry*$^+$ (and therefore containing *P[y*$^+$*]* and *J21A*) females were collected for each *SUPor-P* line and three individual transmission tests were performed by outcrossing each female to *y;ry* males in individual vials. (7) The average transmission rate was calculated from the three vials. If a line exhibited <22% or >37% *ry*$^+$ transmission then it was retained and retested. (8 and 9) The retests were essentially a repeat of steps 3 and 6, only with 10–15 vials per line instead of only 3. (10) Seventy-eight lines retested with significantly interesting transmission rates. These were established as balanced stocks and subjected to further genetic and molecular analyses.

mutated loci. Inverse PCR was used to generate *P*-element flanking DNA sequence and we capitalized on the recent maturation of Drosophila genome sequencing projects (ADAMS *et al.* 2000) to position 90% (70 out of 78) of the insertions in the genome. This approach enabled us to divide the collection into *P* insertions associated with previously characterized (Table 2) or novel (Table 3) loci.

We recovered 22 *P* insertions within or close to the open reading frame (ORF) of 18 known Drosophila genes (Table 2; Figure 3A). We positioned the *P* insertion relative to the ORF for all the known loci and demonstrated that the majority of the *P* insertions have inserted within or close to the 5′ end of the gene, especially the 5′ untranslated region (UTR; Figure 3A). The preference for *P* elements to insert close to the start of transcription of genes has been documented previously (SPRADLING *et al.* 1995; LIAO *et al.* 2000) and is confirmed by this study. In some cases the *P* element could have hopped in and out of a different locus that is responsible for the effect on *J21A* transmission; however, previous analyses demonstrate that the deviant *J21A* transmission phenotype is most likely associated with the *P* element in most or all of these loci (SPRADLING *et al.* 1995).

The recovery of a dominant mutation in the screen suggests that its normal product is dose limiting and important for chromosome inheritance. We identified *P* insertions associated with 11 genes that have previously documented or direct roles in chromosome inheritance or cell-cycle regulation, or encode proteins that can logically be connected to these processes: *wings-apart like* (*wapl*, VERNI *et al.* 2000), *centrosomin* (*cnn*), *pavarotti* (*pav*, LOGARINHO and SUNKEL 1998), *grapes*/CHK1 (*grp*), *histone H4* (*His4*, KEDES 1979), *JIL-1* kinase (JIN *et al.* 1999), *Domina* (*Dom*, STRÖDICKE *et al.* 2000), *replication factor complex-4* (*rfc4*, HARRISON *et al.* 1995), *GTPase-activating protein* (*Gap1*, SEVERIN *et al.* 1997), *Rab-protein 5* (*Rab5*, NIELSEN *et al.* 1999), and *nanos* (*nos*, DESHPANDE *et al.* 1999; Table 2; Figure 3A). The recovery of genes involved in chromosome architecture, sister chromatid cohesion, replication, spindle dynamics/organization, and cell-cycle regulation is significant because it demonstrates an enrichment for loci with direct roles in cell division and chromosome inheritance. Most of these loci have been previously mutated to homozygous lethality, yet many of our *P* insertions are homozygous viable (Table 2). Thus, many of our mutations are likely to be hypomorphic, consistent with the general tendency of *P* insertions to partially inhibit gene function (SPRADLING *et al.* 1999), and the percentage of homozygous lethal mutations (18%) is most likely an underestimate of the percentage of loci required for viability.

We also recovered mutations in five genes that are likely to play indirect roles in inheritance including *Eukaryotic initiation factor-4E* (*EIF-4E*, HERNANDEZ *et al.* 1997), *Fimbrin* (*Fim*, ADAMS *et al.* 1991), *bifocal* (*bif*, BAHRI *et al.* 1997), *out at first* (*oaf*, BERGSTROM *et al.* 1995), and *scabrous* (*sca*, LEE *et al.* 1998; Table 2; Figure 3A). The functions of these loci and how they might impact mini-

TABLE 1

Dominant modifiers of *J21A* inheritance

	No. of lines	Transmission (% of progeny)	No. homozygous lethal[a] (%)
Decreased transmission	69	9–21	11 (16)
Increased transmission	9	38–51	3 (33)
Total	78		14 (18)

[a] The homozygous lethals include semilethal lines (see MATERIALS AND METHODS).

chromosome inheritance will be examined in detail in the DISCUSSION.

A small subset of insertions were recovered in genes with no obvious role in inheritance including *Gliotactin* (*Gli*), *Hormone receptor-like in 39* (*Hr39*), and *laminin A* (*LanA*; Table 2; Figure 3A). Isolation in the sensitized minichromosome screen could uncover previously unknown functions for these proteins, or the recovery of these insertions could represent background "noise" associated with all genetic screens. Finally, three *P* insertions (*Scim1*, *Scim2*, and *Scim3*) are associated with mobile genetic elements (*mdg3*, *gypsy*, *YOYO*) and therefore have not been positioned precisely within the genome (Table 2). For example, it has been estimated that the *mdg3* element is present at 15–17 sites on different chromosomes (ILYIN *et al.* 1980). The transmission defects in *Scim1*, *Scim2*, and *Scim3* and the lethal phenotype in *Scim1* are likely due to disruptions in as yet unidentified neighboring loci.

Some of the mutations display a complex insertion pattern. First, one line contained two *P* insertions, one within the first intron of *grp* (Table 2; Figure 3A) and the other within a novel multiple insertion locus at 23A7-B1 (*Scim12*[4], Table 3; Figure 3B and see below). It will

TABLE 2

Dominant modifiers of *J21A* inheritance in known loci

Line	T.T. (%)	Location	Insertion site	Stage of lethality	Human GenBank accession no.
			Insertions in known loci		
wapl^{Scim}	19	2D6	Intron 2	—	D87450
bif^{Scim}	16	10D1-2		—	—
Fim^{Scim}	19	16A1-2		—	L05491, M22299, M34427
Rab5^{Scim}	21	22E1-2	5′ UTR	First instar	M28215
oaf$^{Scim\text{-}a}$	20	22F3		—	—
oaf$^{Scim\text{-}b}$	20	22F3		—	—
Gli^{Scim}	19	35D4		—	AH003534, M16541
grp$^{Scim[a]}$	17	36A6-7	Intron 1	—	AF016582
Hr39^{Scim}	18	39C1-3		—	SEG_D84206S
His4^{Scim}	20	39D	50 bp 5′ of start	—	HSHU4
Sca$^{Scim\text{-}a}$	17	49D1-3		—	D49353, D63160
Sca$^{Scim\text{-}b}$	20	49D1-3		—	D49353, D63160
cnn^{Scim}	17	50A3-6	Intron 1	—	AB020673, X69292
rfc4^{Scim}	21	64A10	Exon 1	Second instar/pupal	—
pav^{Scim}	18	64B2-7	120 bp 5′ of start	Embryonic	X67155
LanA^{Scim}	14	65A10-11		—	X58531
eIF-4E$^{Scim\text{-}a}$	13	67B1-2	Intron 1	Third instar	M15353
eIF-4E$^{Scim\text{-}b}$	17	67B1-2	Intron 1	Third instar	M15353
Gap1$^{Scim\text{-}a}$	18	67D2-3	480 bp 5′ of start	—	X89399
Gap1$^{Scim\text{-}b}$	10	67D2-3	Intron 1	Embryonic/first instar[b]	X89399
JIL-1^{Scim}	19	68A4-5	5′ UTR	—	AF074393, AF090421
nos^{Scim}	17	91F4-5	260 bp 5′ of start + 3′	Embryonic[b]	—
			Insertions in mobile elements		
Scim1 (mdg3)	10	2		Third instar[b]	
Scim2 (gypsy)	20	2		—	
Scim3 (YOYC)	20	2		—	

T.T., transmission test data.

[a] There are two *P* insertions in this line; one within *grp* (Figure 3A) and one within a novel locus at 23A7-B1 (*Scim12*[4], Table 3; Figure 3B).

[b] Semilethal. See MATERIALS AND METHODS for derivation of human accession no.

TABLE 3

Dominant modifiers of *J21A* inheritance in novel loci

Line	T.T. (%)	Location	Stage of lethality	Clone accession no.	Human GenBank accession no.
Scim4	20	3A4	—	AE003424	[a]
Scim5	21	16C7-10	—	AE003506	[a]
Scim6	20	8C11-13	—	AE003446	[b]
Scim7	18	20A1	—	AE003574	[a]
Scim8^{1}	19	11B17-18	—	AE003490	[b]
Scim8^{2}	20	11B17-18	—	AE003490	[b]
Scim9	17	2B17-18	Third instar[c]	AE003422	[a]
Scim10	21	6C11-13	—	AE003438	AB018303, AF221712
Scim11	25	19F1-2	—	AE003568	[a]
Scim12^{1}	*51*	23A7-B1	—	AE003582	[b]
Scim12^{2}	21	23A7-B1	—	AE003582	[b]
Scim12^{3}	18	23A7-B1	—	AE003582	[b]
Scim12^{4}	17	23A7-B1	—	AE003582	[b]
Scim12^{5}	*40*	23A7-B1	Embryonic/larval	AE003582	[b]
Scim12^{6}	*40*	23A7-B1	Embryonic/larval	AE003582	[b]
Scim12^{7}	*39*	23A7-B1	—	AE003582	[b]
Scim12^{8}	19	23A7-B1	—	AE003582	[b]
Scim13^{1}	19	26A6-B1	—	AE003582	[b]
Scim13^{2}	*39*	26A6-B1	—	AE003582	[b]
Scim14^{1}	19	28A5-6	—	AE003618	[a]
Scim14^{2}	21	28A5-6	—	AE003618	[a]
Scim15^{1}	9	30D1	—	AE003626	X54938
Scim15^{2}	14	30D1	—	AE003626	X54938
Scim16	21	31E2-3	—	AE003628	[b]
Scim17	16	33B7-8	—	AE003634	[b]
Scim18	18	38C8-9	—	AE003666	[b]
Scim19	17	39B3-4	—	AE003669	[b]
Scim20	21	42A13-14	—	AE003784	[b]
Scim21	20	42A13-16	—	AE003789	[b]
Scim22	*38*	42C3-4	—	AE003789	AL050389
Scim23	19	44A7-B1	—	AE003838	D87436
Scim24	19	47C5-6	Third instar	AE003828	U75309
Scim25	15	50F6	Second instar	AE003815	[b]
Scim26	14	50F4	—	AE003815	[b]
Scim27	21	54B17-C1	—	AE003803	Z50150
Scim28	*43*	57A10-B1	—	AE003791	[a]
Scim29	14	58F2-3	—	AE003458	[b]
Scim30	19	84D14-E1	—	AE003676	[b]
Scim31	*45*	86A2-4	Third instar	AE003686/*Dom*	Y11739
Scim32^{1}	18	87C9-D1	—	AE003697	X77244
Scim32^{2}	14	87C9-D1	—	AE003697	X77244
Scim33	18	91A2-3	—	AE003722	[b]
Scim34	10	91F4-7	—	AE003725	L75847
Scim35	22	92F5-6	—	AE003732	[b]
Scim36	14	97D14-15	—	AE003758	M93285
Scim37	18	98B7-8	—	AE003764	[a]

T.T., transmission test data; high transmitters are in italics. We have been unable to localize one of the high transmitters and it is not represented in the table. *Scim12*^{5} and *Scim12*^{6} are also lethal over the *SM1*, *SM5*, and *CyO* balancer chromosomes. *Scim11* and *Scim35* exhibited a bimodal distribution of *J21A* inheritance and were retained as stocks even though they were outside of the standard <22% or >37% cutoff. See MATERIALS AND METHODS for derivation of human accession no.

[a] No Drosophila ESTs.

[b] There are Drosophila ESTs but no significant homology was found.

[c] Semilethal.

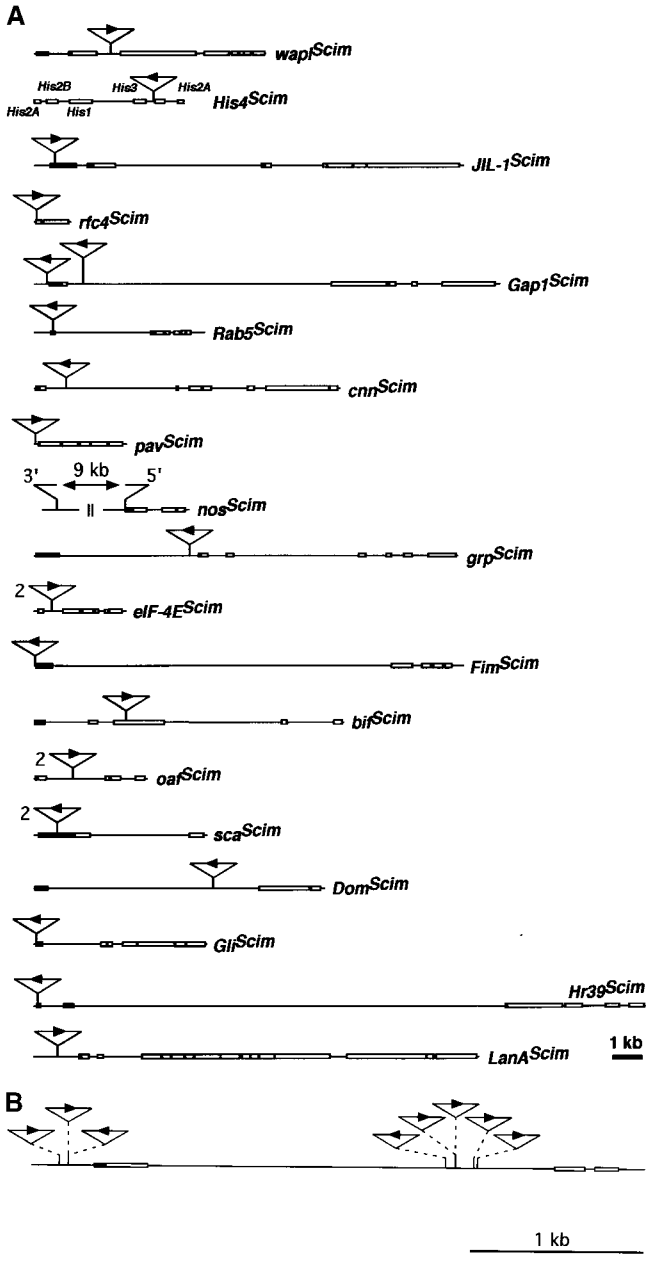

FIGURE 3.—(A) The ORFs of 19 Drosophila loci are presented. Exons are depicted as boxes; the 5′ UTRs are solid boxes. *P* elements are represented by triangles and the orientation is indicated by an arrow (5′ to 3′). Loci with two *P* insertions at an identical position (*oaf*, *sca*, and *eIF-4E*) are indicated by a 2 next to the *P*-insertion site. The ORFs are to scale. (B) A map of eight *P* insertions within a novel 3-kb locus. The *P*-insertion sites and predicted ORF were established by aligning two ESTs and the *P*-insertion flanking sequences with the genomic clone AE003582 (Table 3). The lines are (left to right) $Scim12^{1}$ (51%), $Scim12^{2}$ (21%), $Scim12^{3}$ (18%), $Scim12^{4}$ (17%), $Scim12^{5}$ (40%), $Scim12^{6}$ (40%), $Scim12^{7}$ (39%), and $Scim12^{8}$ (19%).

be necessary to separate the two insertions by recombination to determine whether one or both of these loci are responsible for the transmission defect. Second, *Scim31* is a *P* insertion within the first intron of *Dom*, which likely plays a role in chromatin structure (STRÖDICKE *et al.* 2000). The *P* insertion is relatively far from the start of transcription for *Dom* (~6 kb 3′, Figure 3A) when compared with the other insertions and ORFs described here, and sequence analysis has identified novel ESTs that span the insertion site. The inheritance defect may be due to a disruption in *Dom* and/or a putative novel locus represented by the ESTs. Third, analysis of genomic sequence flanking nos^{Scim} demonstrated that the 5′ and 3′ parts of the *P* element appear separated by 9 kb of genomic DNA and that the 5′ region of the *P* element is ~260 bp 5′ of the start of transcription for *nos* (Table 2; Figure 3A). There was no evidence for an ORF around the 3′ region of the *P* element. One explanation for this unusual arrangement is that the *P* element underwent an imprecise excision that separated the 5′ and 3′ ends.

In sum, of the 18 previously characterized genes isolated as dominant modifiers of *J21A* transmission, 11 genes have direct roles in chromosome architecture or inheritance (56%) and 5 other genes may have an indirect role (28%). Fourteen (78%) of the known Drosophila loci have recognizable homologs in humans (Table 2), suggesting that these genes may affect human chromosome inheritance.

The majority of the collection comprises *P* insertions in novel loci: The insertion sites for 46 other lines representing 34 independent loci have also been identified (Table 3). Eighty percent (37 out of 46) of these lines are associated with ESTs, and 32% (12 out of 37) of these ESTs have similar sequences in humans (Table 3). However, we have not identified any known Drosophila genes associated with these lines after extensive analysis of the *P*-insertion sites. Based on the precedent set by the insertions in known loci, a large fraction (>50%) of these novel genes should play roles in chromosome inheritance and cell-cycle regulation.

Single independent alleles were recovered for 28 of these novel genes (Table 3). Of particular interest are *Scim34* (10% *J21A* transmission), *Scim26*, *Scim29*, and *Scim36* (14% transmission), and *Scim28* (43% transmission). In addition, four novel loci were recovered with two independently isolated *P* insertions (Table 3). $Scim15^{1}$ and $Scim15^{2}$ are intriguing because they exhibit the lowest (9%) and third lowest (14%) *J21A* transmission rates (Table 3). The *P* insertions are in the same orientation at the same site in 30D1. $Scim8^{1}$ and $Scim8^{2}$ have inserted in the same orientation on the *X* chromosome and a novel EST is associated with the insertion site. $Scim13^{1}$ and $Scim13^{2}$ have inserted in opposite orientations at the same site and are associated with novel ESTs. Surprisingly, they exhibit very different primary *J21A* transmission rates (19 *vs.* 39%, respectively; Table 3), which may be due to the inverted orientation of the insertions. $Scim14^{1}$ and $Scim14^{2}$ have insertions in opposite orientations at the same site; this site is rich with *P* insertions isolated in other screens, including a

lethal *P*-element line. Given that hypomorphic insertions were recovered in known loci, the homozygous viable *P* insertions in *Scim14*^{1} and *Scim14*^{2} may be associated with a locus important for both chromosome inheritance and organismal viability.

We recovered eight independent insertions at 23A7-B1, which surprisingly includes four low and four high transmitting lines (*Scim12*^{1}–*Scim12*^{8}; Table 3). Analysis of inverse PCR sequence identified a large genomic clone (AE003582) and two ESTs that positioned the *P* insertions relative to a putative ORF (Figure 3B). The eight insertions are grouped as two clusters separated by ~2.5 kb; three insertions are ~100 bp 5′ of the CAAT and TATA boxes while five are located between the predicted first and second exons. Conceptual translation of the locus does not contain any signature motifs and database searches suggest that the locus is novel. An epitope-tagged cDNA expressed in S2 embryonic tissue culture cells localizes to the nucleus but is not found on metaphase chromosomes (K. W. Dobie, C. D. Kennedy and G. H. Karpen, unpublished data).

Eight additional lines remain unlocalized to a specific region of the genome because obtaining sequence data from the flanking regions was unsuccessful, potentially due to deletions or rearrangements in the *P*-element sequence or the absence of relevant restriction sites in the flanking DNA. Determination of the genes associated with these insertions requires more intensive cloning approaches.

Homozygous lethal *P* insertions in known loci exhibit mitotic chromosome defects: We recovered insertions in four genes with previously documented abnormal mitotic phenotypes associated with null mutations: *wapl* (Verni *et al.* 2000), *cnn* (Megraw *et al.* 1999), *pav* (Adams *et al.* 1998), and *grp* (Fogarty *et al.* 1997; Sibon *et al.* 2000). Our insertions associated with *cnn*, *wapl*, and *grp* are not lethal when homozygous for the *P* insertion and likely represent hypomorphic alleles (Table 2). We extended the analysis of mitotic phenotypes to the lethal insertions in known loci whose effects on mitotic chromosome behavior have not previously been reported (*rfc4*, *Gap1*, *eIF-4E*, and *Rab5*). Mitotic chromosomes prepared from all four homozygous lethal lines exhibited a range of dramatic defects (Figure 4). *rfc4*^{Scim} neuroblasts demonstrated fragmented metaphase and anaphase figures (Figure 4, D and E). While individual chromosomes were easily identified in control metaphase figures (Figure 4A), the individual chromosomes in *rfc4*^{Scim} homozygotes were difficult to identify and some regions of the chromosome arms exhibited aberrant condensation (Figure 4, D and E). The lethal insertion in *Gap1*$^{Scim\text{-}b}$ resulted in precocious sister chromatid separation and aberrant anaphase figures (Figure 4, F and G). Given that *Gap1* is likely involved in spindle formation (see discussion), we suspect that precocious sister chromatid separation in homozygous mutants may be due to an inability of the chromosomes to segregate

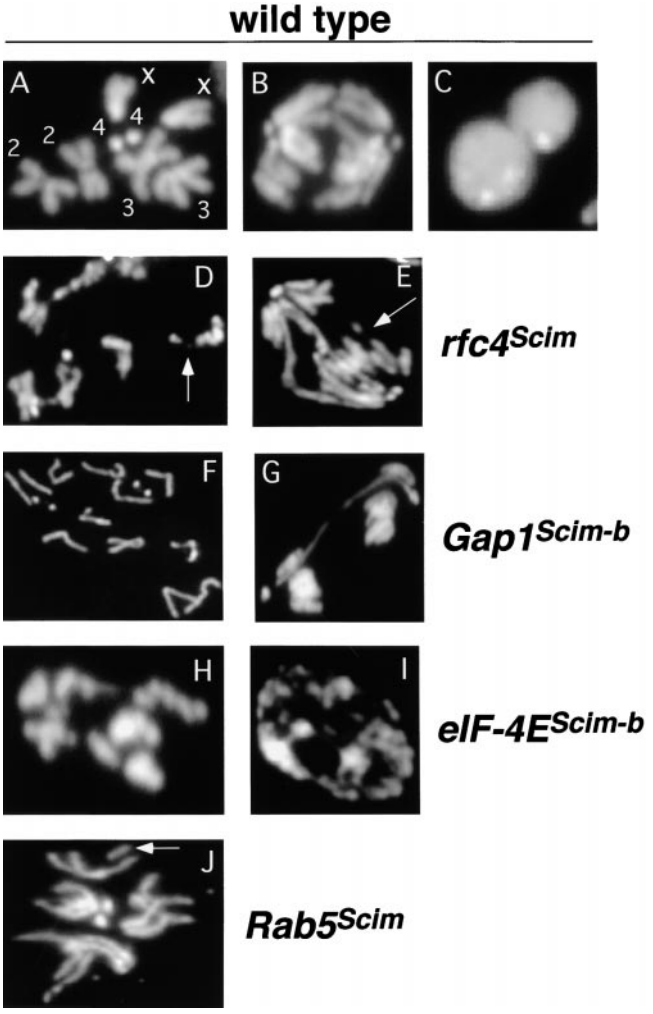

Figure 4.—Mitotic chromosome defects in known loci. Wild-type metaphase (A), anaphase (B), and interphase (C) figures are presented. The metaphase *X*, second, and third chromosomes are indicated in A and the two small dots in the center are the fourth chromosomes. Figures depicting the predominant defects in the mutant lines are presented: *rfc4*^{Scim} metaphase (D) and anaphase (E), *Gap1*$^{Scim\text{-}b}$ metaphase (F) and anaphase (G), *eIF-4E*$^{scim\text{-}b}$ metaphase (H) and interphase (I), and *Rab5*^{Scim} metaphase (colcemid treated; J). Fragmented chromosomes are indicated by the arrows in D, E, and J. See text for additional details and interpretations.

to the poles correctly at anaphase. These phenotypes are satisfying because they represent what one might predict from mutations associated with *rfc4*^{Scim} and *Gap1*$^{Scim\text{-}b}$ (see discussion). Thus, it is possible to make predictions about gene function from the chromosome phenotypes associated with some of the novel loci (see below).

Few mitotic figures were present in neuroblast squashes prepared from the *eIF-4E* and *Rab5* lines, indicating that the mitotic index is extremely low. The most obvious phenotype associated with the insertions in *eIF-4E* was fragmented interphase nuclei that were two to four

times the diameter of wild-type nuclei (Figure 4I). Again, in the rare mitotic figures, the individual chromosome morphology was disrupted and the chromosomes appeared hypocondensed (Figure 4H). We were unable to find any mitotic figures in six slides prepared from the insertion in *Rab5*. Colcemid treatment allowed the identification of a few mitotic figures, all of which were grossly disrupted, exhibiting chromosome fragmentation (Figure 4J). The extreme phenotypes associated with *eIF-4E* and *Rab5* may reflect the general functions of these loci, and the effects on chromosome architecture may indicate an indirect role in chromosome inheritance or a general effect on cellular health.

In summary, we have characterized previously unknown mitotic defects associated with homozygous lethal *P*-induced mutations in four known loci. We conclude that homozygous *P*-induced mutations in the collection result in characteristic defects in endogenous chromosome inheritance and that the effects of the mutations are not limited to the minichromosome.

Homozygous lethal *P* insertions in novel loci exhibit mitotic chromosome defects: We extended our analysis of mitotic chromosomes in larval neuroblasts to mutations in six novel loci that were homozygous lethal and observed characteristic mitotic defects associated with all of these mutations. Two novel loci exhibited similar but distinctive patterns of precocious sister chromatid separation. *Scim25* has a *P* insertion associated with a novel locus at 50F6 (Table 3). This line exhibits a very low mitotic index and partial loss of sister chromatid cohesion in some mitotic figures (Figure 5A). The chromosomes lacked cohesion at heterochromatic regions, but the sister chromatids do not separate completely; instead they remain attached by strands of chromatin (Figure 5A). The fourth chromosomes appeared as "dumbbells" due to the partial loss of cohesion and the sister chromatids of the *Y* chromosome were partially separated (Figure 5A). This phenotype is very similar to that described for *wapl* (Verni *et al.* 2000) and suggests that the different locus disrupted in *Scim25* may have a function in maintaining heterochromatin architecture and sister chromatid cohesion/separation. Further, interphase nuclei appeared disintegrated and some mitotic figures were clumped together (Figure 5B). These defects may represent downstream phenotypes that are induced by precocious loss of cohesion in previous divisions. The *P* insertion in *Scim9* is associated with a novel locus at 2B17-18 (Table 3). Although the mitotic index appears normal, some metaphase figures exhibit partial or complete sister chromatid separation (Figure 5, C and D). This phenotype is similar to that observed for $Gap1^{Scim\text{-}b}$ (see above), suggesting a role for *Scim9* in microtubule dynamics or initiation/maintenance of sister chromatid cohesion.

Scim31 has a homozygous lethal *P* insertion within the first intron of *Dom* (Table 3; Figure 3). The insertion within this locus results in a unique phenotype; although

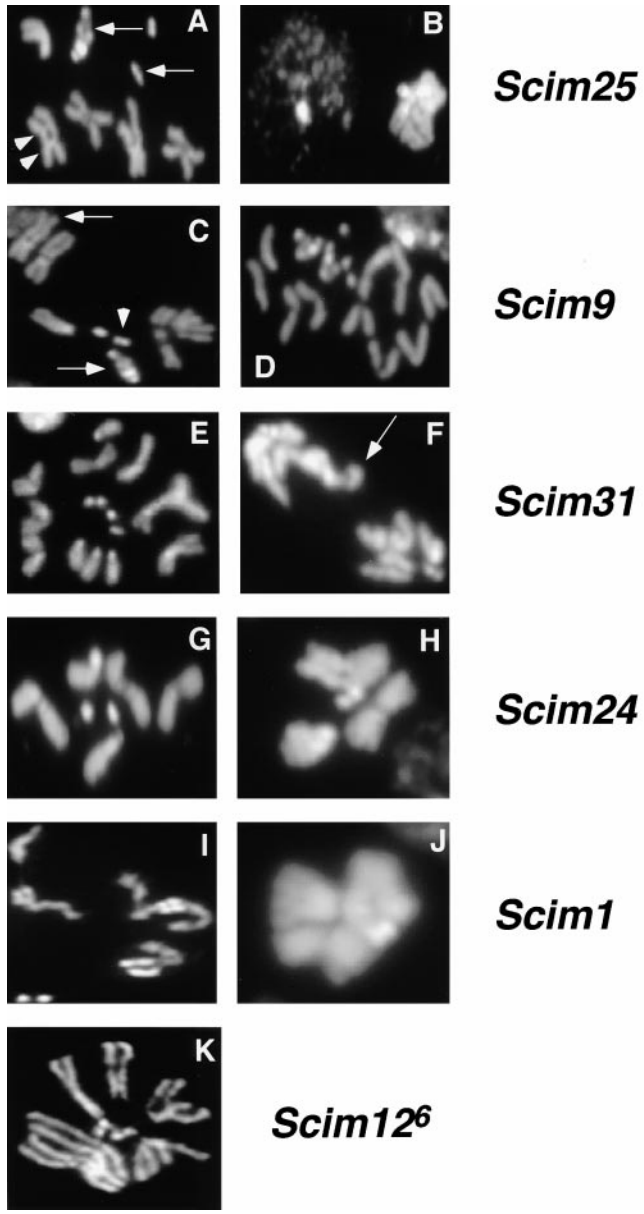

Figure 5.—Mitotic chromosome defects in novel loci. Representative figures depicting the predominant defects are presented for mutant lines: *Scim25* metaphases (A and B) and interphase nucleus (B), *Scim9* metaphases (C and D), *Scim31* metaphase (E) and anaphase (F), *Scim24* metaphases (G and H), *Scim1* metaphases (I and J), and $Scim12^{6}$ metaphase (colcemid treated; K). The arrowheads in A indicate strands of chromatin that link the sister chromatids, and the arrows indicate a *Y* chromosome with partial separation of sisters. In C the sister chromatids in one of the second chromosomes and the *Y* chromosome are partially separated (arrows) and one of the fourth chromosomes appears larger than the other, as though the sister chromatids are starting to separate (arrowhead). In F, a lagging chromosome is evident (arrow) and only seven sister chromatids, rather than the expected eight, are present at the lower right pole. See text for additional details and interpretations.

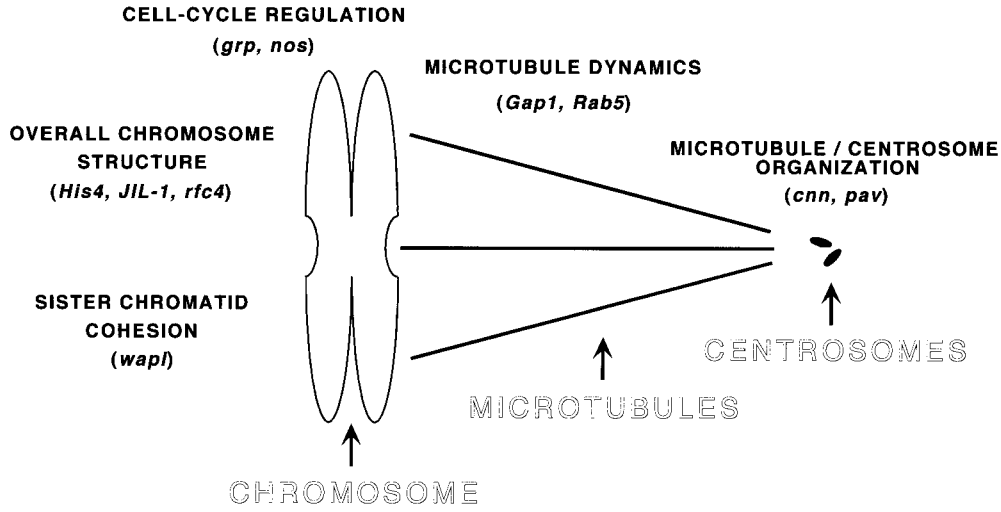

FIGURE 6.—Model representing processes involved in chromosome inheritance and associated genes recovered in the screen.

the mitotic index appears normal, a large number of the mitotic figures are polyploid (Figure 5E). Some anaphase figures exhibited missegregation of chromatids, likely representing early stages in the progression to polyploidy (Figure 5F). Significant aneuploidy was also observed (data not shown), which would be expected to accompany this type of segregation defect. Interestingly, *Scim31* is one of the high transmitting lines and, as mentioned earlier, we have identified novel ESTs associated with the *P* insertion within the *Dom* ORF. The relationship between the homozygous lethal phenotype and the increase in minichromosome inheritance is under further investigation.

Three more novel loci exhibited mitotic defects in homozygotes. The homozygous lethal *P* insertion in *Scim24* resulted in a lower than normal mitotic index and some mitotic figures exhibited aneuploidy and/or decondensed chromosomes (Figure 5, G and H). Further, many of the nuclei appeared disintegrated, similar to the *Scim25* phenotype. The *P* insertion in *Scim1* is associated with a *mdg3* retrotransposon and the insertion is homozygous lethal (Table 2). Mitotic chromosomes exhibited several defects including disintegrated chromosome arms, hypocondensed centric heterochromatin, and sister chromatid separation (Figure 5I). A high proportion of mitotic figures were so hypocondensed that it was difficult to distinguish individual chromosomes (Figure 5J). Finally, *Scim12*^{5} and *Scim12*^{6} are lethal insertions within the multiple insertion locus at 23A7-B1 (Table 3). Again, colcemid treatment was required to find any mitotic figures, all of which exhibited aberrant metaphases and sister chromatid separation (Figure 5K).

In summary, homozygous mutant animals from all six novel loci exhibited different mitotic defects, demonstrating that the novel loci also play important roles in the inheritance of endogenous chromosomes.

DISCUSSION

Here we describe the results of a sensitized *P*-element screen for genes that have dominant effects on sensitized minichromosome inheritance in Drosophila. Previous analyses demonstrated that inheritance of the *J21A* minichromosome derivative is sensitive to mutations in genes known to be important for inheritance. We recovered 78 lines that exhibit altered *J21A* inheritance; 69 lines display significantly decreased transmission and 9 lines exhibit significantly increased transmission. The use of *P* elements as the mutagenic agent combined with inverse PCR enabled us to generate and isolate genomic DNA flanking 90% of the *P*-element insertion sites. The completion of the euchromatic Drosophila genome sequence (ADAMS *et al.* 2000) and analysis of the flanking sequences enabled us to divide the collection into two groups. We identified *P* insertions within or close to 18 known Drosophila genes. We have mutagenized genes involved in overall chromosome architecture/organization (*His4* and *JIL-1*), DNA replication (*rfc4*), sister chromatid cohesion (*wapl*), microtubule dynamics (*Gap1* and *Rab5*), spindle organization (*cnn* and *pav*), and cell-cycle regulation (*nos* and *grp*; Figure 6). Null mutations in 4 of these genes (*cnn*, *pav*, *wapl*, and *grp*) have previously been shown to result in mitotic abnormalities. It is unlikely that recovery of so many loci with chromosome-related functions is due to chance, demonstrating that the collection is enriched for genes that promote inheritance. In addition to these 18 previously characterized genes, we identified 46 lines representing 34 individual loci at known locations in the genome representing mutations in novel loci. On the basis of the analysis of the previously characterized loci, we predict that >50% of the insertions in novel loci (>17 genes) will also have direct roles in different chromosome inheritance processes. Eighteen percent of the lines are lethal or semilethal when homozygous for the

P element and exhibit dramatic and distinctive mitotic chromosome defects, demonstrating that these loci play vital and different roles in inheritance (Figure 6).

Why is J21A inheritance compromised in diverse heterozygous mutant backgrounds? The frequency of chromosome transmission to progeny represents the cumulative transmission efficiency throughout development, including at least 17 mitotic plus 2 meiotic divisions (Ashburner 1989). As is true for other metazoans, Drosophila displays diverse types of chromosome cycles and cell divisions, including multiple rapid divisions without cellularization during early embryonic development, somatic and germline mitoses, and sex-specific patterns of meiosis I and II. Chromosomes must segregate in all these divisions and processes in order to be inherited properly. *J21A* is transmitted to 40 or 27% of the progeny when inherited from males or females, respectively (Murphy and Karpen 1995b). Brooding experiments in males demonstrated that *J21A* is stably transmitted in male germline stem cell mitosis and thus loss must be restricted to preblastoderm mitosis and/or meiosis (Murphy and Karpen 1995b; Williams *et al.* 1998). The simplest explanation is that the greater female instability observed for *J21A* is due to loss in meiosis (Murphy 1998).

Cytological studies demonstrate that *J21A* binds the outer kinetochore protein ZW10 (Williams *et al.* 1998), the centric cohesion protein MEI-S332 (Lopez *et al.* 2000), and CID, the functional ortholog of CENP-A, a centromere-specific histone H3-like protein (M. Blower and G. H. Karpen, unpublished results). If *J21A* contains at least a partially functional kinetochore, why then is *J21A* inheritance sensitized? Within these divisions, chromosomes have to accomplish replication, cohesion, condensation, division (congression and separation), and decondensation. The small size of *J21A per se* likely predisposes sensitivity to a heterozygous mutant background for several reasons. First, *J21A* inheritance is particularly sensitive to reduced levels of kinesin-like proteins (KLPs) that function in spindle organization and cytokinesis. The Drosophila KLP family includes *no distributive disjunction* (*nod*), *non-claret disjunction* (*ncd*), and *kinesin-like protein 3A* (*klp3A*), and all three genes have very dramatic dominant effects on *J21A* inheritance (Murphy and Karpen 1995a; Cook *et al.* 1997). The small size of *J21A* and/or a limited amount of centric heterochromatin likely renders it more susceptible to falling off a compromised spindle. Furthermore, centrosomes are not present in female meiosis I, and such anastral spindle formation appears to initiate from the chromosomes rather than the poles (Hawley and Theurkauf 1993; Karpen and Endow 1998). We screened for effects on the sensitized minichromosome in females, and the small size of *J21A* may make it defective in spindle initiation in response to heterozygosity for mutations in spindle components. Second, the lack of substantial amounts of centric heterochromatin compromises heterochromatin-specific functions such as cohesion (Lopez *et al.* 2000) and pairing (Dernburg *et al.* 1996; Karpen *et al.* 1996). Centric cohesion is specially regulated in meiosis I (Moore and Orr-Weaver 1998), and *J21A* may be particularly sensitive to partial loss of centric cohesion proteins in this division. Third, *J21A* may be sensitive to the dosage of proteins involved in overall chromosome structure and DNA replication because its small size renders it susceptible to factors that influence chromosome architecture and processing, such as limited origins of replication. The diversity of inheritance functions encoded by the loci isolated in this screen (Figure 6) demonstrates the utility of the *J21A* sensitized minichromosome assay in identifying genes involved in many different inheritance processes.

The sensitized screen identified genes known to be involved in chromosome architecture and inheritance: Mutations in *wapl* result in an increase in *X* chromosome nondisjunction during female meiosis and partial separation of all sister chromatids at heterochromatic regions in mitotic chromosomes (Verni *et al.* 2000). In addition, *wapl* is a dominant suppressor of position-effect variegation (PEV), the heterochromatin-induced gene silencing of normally euchromatic genes (Wakimoto 1998). These phenotypes imply a role for WAPL in achiasmate chromosome segregation during meiosis, which is heterochromatin dependent (Dernburg *et al.* 1996; Karpen *et al.* 1996), and pairing between the heterochromatic portions of all the sister chromatids during mitosis. It is likely that inheritance of *J21A* is more sensitive to a mutation in *wapl* than the *X*, second, and third chromosomes, because they have intact centromeres and large amounts of heterochromatin. We suspect that our collection of mutations will likely contain other genes with roles in heterochromatin biology. Thus, a useful secondary screen is to test if our *P* insertions enhance or suppress heterochromatin-induced PEV. In addition, it will be useful to determine the cytological basis for *J21A* loss in *wapl* mutants, which may allow us to dissect which heterochromatic functions are related to inheritance.

We recovered a *P* insertion associated with one of the *histone H4* (*His4*) genes. There are five classes of major histone genes that are grouped as a unit (*His2A*, *His2B*, *His1*, *His3*, and *His4*) and, in Drosophila, the histone unit is repeated ~100-fold to achieve sufficient expression for the enormous task of packaging the genome (Kedes 1979). The *P* insertion in $His4^{Scim}$ appears to be close to a copy of *His4* at the edge of the histone cluster (data not shown; BDGP), which may represent a differentially expressed or alternative form of H4. In budding yeast, genetic (Smith *et al.* 1996) and molecular (Meluh *et al.* 1998) analyses have demonstrated that histone H4 interacts with Cse4p, the centromere-specific histone H3-like protein, and that this interaction is required for the formation of centromeric chromatin and faithful chromosome inheritance. Inheritance of *J21A* might be

particularly sensitive to mutations in genes required for centromere formation because it lacks one-third of the functional centromere. Further analysis will utilize a minichromosome deletion series to determine whether this mutation interacts genetically with the centromere (Murphy and Karpen 1995a; Cook *et al.* 1997; Williams *et al.* 1998).

JIL-1 is localized on chromosomes throughout the cell cycle in Drosophila, on the gene-rich interband regions of larval polytene chromosomes, and is enriched twofold on the hypertranscribed male *X* chromosome compared to autosomes (Jin *et al.* 1999). Its phosphorylation properties and cytological localization suggest that JIL-1 is a chromosomal kinase involved in regulating the chromatin structure of regions of the genome that are actively transcribed. We speculate that a mutation in *JIL-1* could affect *J21A* inheritance either through the regulation of a gene or genes required for inheritance or by directly altering overall chromatin structure. *J21A* inheritance may be particularly sensitive to effects on chromatin structure because it has a greatly reduced amount of heterochromatin.

Harrison *et al.* (1995) have described the cloning of *rfc4* (*rfc40*) in Drosophila and demonstrate that the gene encodes a 40-kD protein, suggesting that *rfc4* is the gene for one of the small subunits of the Drosophila replication factor C (RFC) complex. The RFC complex is required for loading proliferating cell nuclear antigen onto DNA, which in turn tethers the polymerase to the DNA template during synthesis (Mossi *et al.* 1997). The mutation in $rfc4^{Scim}$ may compromise the assembly of the RFC complex and result in a block at S phase. In heterozygotes, *J21A* maintenance may be more sensitive to the dose of replication factors because it is much smaller and contains a higher than usual proportion of heterochromatin, which replicates late in S phase. Incomplete or delayed replication of *J21A* would reduce its ability to be transmitted intact during mitosis. Analysis of chromosome morphology in homozygous larvae from $rfc4^{Scim}$ demonstrated dramatic and characteristic chromosome defects associated with this line that are consistent with aberrant replication. The recovery of *rfc4* demonstrates the benefit of a sensitized screen to uncover essential loci that have little or no effect on endogenous chromosomes as heterozygous mutations, and this mutation will be an important tool in future analyses of replication in Drosophila.

The sensitized screen identified genes known to be involved in spindle organization/function: Mutations that perturb spindle organization and/or cytokinesis have a dramatic impact on *J21A* and endogenous chromosomes inheritance. *Centrosomin* (CNN) is required for localization of the other centrosomal proteins γ-tubulin, CP60, and CP190 and for the assembly of functional centrosomes. The cnn^{Scim} *P* insertion may reduce the levels of CNN to a phenocritical level, such that mitotic spindles are sufficient to segregate full-sized chromosomes but are compromised to a degree that results in loss of *J21A*. Megraw *et al.* (1999) demonstrated that mitotic spindle defects in *cnn* mutants occur in a cumulative fashion and that some mitotic spindles look completely normal; furthermore, CP190 and γ-tubulin are present at low levels at these centrosomes. This implies that functional centrosomes can still form even in a *cnn* mutant background. Ultimately the embryos die at around cycle 12 before cellularization can occur. cnn^{Scim} is not lethal when homozygous, implying that it is a hypomorphic mutation; the cumulative effects of a *cnn* mutation combined with hypomorphy of the *P* insertion may explain why *J21A* is lost in our *P*-insertion background while the other chromosomes are not.

Pavarotti (PAV) is a member of the KLP superfamily of microtubule motor proteins that are required for centrosome organization, spindle assembly, and chromosome movement (Moore and Endow 1996). Inheritance of *J21A* appears to be particularly sensitive to reduced levels of the KLPs *nod*, *ncd*, and *klp3A* (Murphy and Karpen 1995a; Cook *et al.* 1997). *J21A* inheritance may be compromised in these mutant backgrounds because *J21A* does not contain all the *cis*-acting sequences required for normal inheritance. For example, a partially defective spindle may enhance loss of a partially defective centromere because it binds fewer microtubules, in comparison to a normal centromere. Another possibility is that *J21A* inheritance may be particularly compromised due to the greatly reduced size and a decreased capacity to bind chromokinesins that interact all along chromosome arms and are thought to mediate antipoleward forces (Afshar *et al.* 1995; Murphy and Karpen 1995a).

The sensitized screen identified known genes involved in neural development or with actin-related functions: We recovered at least four *P* insertions (two in *oaf* and two in *sca*) in genes with potential roles in neural development in Drosophila (Bergstrom *et al.* 1995; Lee *et al.* 1998). Why were two genes with potential roles in neural development recovered in a screen for genes involved in chromosome inheritance? There is a strong precedent for defects in early chromosome inheritance causing abnormal neural development. Several mutations have been described in Drosophila with peripheral nervous system (PNS) development defects (Kania *et al.* 1995; Salzberg *et al.* 1997) that result from abnormal chromatid decatenation (*barr*, Bhat *et al.* 1996), spindle formation (*pav*, Adams *et al.* 1998), and cytokinesis (*pav*, Adams *et al.* 1998; *pbl*, Prokopenko *et al.* 1999). Thus, while some of our insertions are in genes with documented roles in PNS development, they may have primary roles in inheritance. Analysis of mitotic chromosomes from lines with null mutations (imprecise excisions) is necessary to test this hypothesis.

We also recovered mutations in two genes (*bif* and *fim*) that function in the organization of the actin cytoskeleton. BIF co-localizes with actin as early as cycle 10 in preblastoderm embryos in defined cytoplasmic domains (Bahri *et al.* 1997). The co-localization of BIF

with actin at early stages of embryogenesis may be significant for chromosome inheritance (see below). Yeast FIMBRIN (*SAC6*) is lethal when overexpressed and cells exhibit an abnormal distribution of actin with defects in cytoskeletal organization (Adams *et al.* 1991). Why were genes encoding proteins associated with actin recovered in our screen for inheritance mutations? Drosophila embryos undergo 13 rapid cell divisions (syncytial divisions) without cellularization. The organization of the actin cytoskeleton is essential for correct distribution of syncytial nuclei during this period (Foe *et al.* 1993). Mutations in proteins that interact with actin may affect the architecture of the actin cytoskeleton during early embryogenesis and have an indirect impact on chromosome inheritance.

The sensitized screen recovered mutations in genes with diverse biological roles: It is not immediately evident why mutations in *gli*, *Hr39*, and *lamA* were recovered in a screen for chromosome inheritance mutations. Briefly, gliotactin is a transmembrane protein involved in the establishment of the blood/nerve barrier (Auld *et al.* 1995); *Hr39* (also known as DHR39 or FTZ-F1beta) is a member of the Drosophila nuclear hormone receptor family (Horner *et al.* 1995); Laminin A is localized to the basement membrane and has been shown to be involved in growth cone guidance of axons (Garcia-Alonso *et al.* 1996). It is possible that some mutations reflect the random noise that accompanies most screens; for example these insertions may have resulted from "hit-and-run" events, which result in mutations at loci unlinked to the final resting site of the *P* element. Alternatively, these loci may have as yet undescribed functions in inheritance.

How could mutations dominantly increase *J21A* transmission? Most of our lines (88%) exhibit significantly reduced levels of transmission. This would be expected because *P*-element mutagenesis should result in reduced levels of gene expression. In most cases perturbation of a particular aspect of inheritance would result in reduced *J21A* transmission. However, mutations in genes that encode repressor functions may result in, for example, misexpression of a protein required for proper spindle attachment to the kinetochore. Mutations in such a repressor gene may rescue *J21A* transmission by allowing more spindle microtubules to attach to the compromised centromere. Mutations in genes that result in a metaphase delay may also result in high transmission. For example, mutations in a regulator of the metaphase to anaphase checkpoint might result in a delay of the cell cycle and allow time for more faithful inheritance of *J21A*. Therefore, this small subset of the collection (six individual loci) represents a very interesting class of genes that warrant further analysis.

The majority of the collection represents *P* insertions in novel loci: The identification of *P* insertions in previously characterized genes predicts the cellular functions (Figure 6) that are likely to be encoded by the novel genes isolated in this screen. We estimate that >50% of the 34 novel loci will have roles in these functions, as well as other essential inheritance functions such as kinetochore structure, microtubule capture, and chromosome congression. Indeed, we have demonstrated that all of the six independent homozygous lethal or semilethal mutations in novel loci exhibit dramatic mitotic chromosome defects. The identification of genomic clones, ESTs, and other *P* insertions for many of these loci will greatly facilitate further analyses.

Broad genetic screens performed in Drosophila have had an enormous impact on the field that they were designed to investigate and also on other fields and in other organisms (Sandler *et al.* 1968; Baker and Carpenter 1972; Nüsslein-Volhard *et al.* 1984; Kania *et al.* 1995; Salzberg *et al.* 1997; Sekelsky *et al.* 1999). We anticipate that the results of this screen will allow the analyses of novel gene products that are required in multicellular eukaryotes for spindle formation, cell-cycle regulation, chromosome structure, and centromere structure and function. The fact that most of the genes identified in this screen have significant human homologs suggests that further analyses will elucidate the roles these proteins play in human chromosome inheritance. At least two of the genes identified in the screen ($wapl^{Scim}$ and *Scim25*) may have relevance to a human genetic disorder. Patients with Roberts syndrome exhibit growth retardation, craniofacial malformations, and tetraphocomelia (Van Den Berg and Francke 1993). Affected individuals exhibit chromosomes with a "railroad-track appearance" that look very similar to the *wapl* (Verni *et al.* 2000) and *Scim25* mutant phenotypes. In sum, discoveries from this screen will surely broaden our understanding of how chromosomes and the cellular machinery are orchestrated to promote chromosome inheritance in multicellular eukaryotes and should ultimately inform us of the causes and consequences of human disorders associated with aneuploidy, such as birth defects and cancer.

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