

Genetic Analysis of a *Drosophila* Microtubule-associated Protein

Andrea Pereira, Jerry Doshen, Elly Tanaka, and Lawrence S. B. Goldstein

Department of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts 02138

Abstract. The 205-kD microtubule-associated protein (205K MAP) is one of the principal MAPs in *Drosophila*. 205K MAP is similar to the HeLa 210K/MAP4 family of MAPs since it shares the following biochemical properties: it is present in several isoforms, has a molecular mass of ~200 kD, and is thermostable. Furthermore, immuno-crossreactivity has been observed between mouse MAP4, HeLa 210K, and *Drosophila* 205K MAP. Currently, there is little information concerning the biological function of this group of nonmotor MAPs. We have used a classical genetic approach to try to identify the role of the 205K MAP in *Drosophila* by isolating mutations in the 205K MAP gene. An F2 lethal screen was used to acquire deficiencies of 100EF, the chromosomal loca-

tion of the 205K MAP gene. *Drosophila* bearing a homozygous deficiency for the 205K MAP region are fully viable and show no obvious phenotype. A recently developed polymerase chain reaction screen was also used to recover five *P*-element insertions upstream from the 205K MAP gene. Western blot analysis has shown that these insertions result in hypomorphic mutations of the 205K MAP gene. As was seen with animals that have no 205K MAP, these mutations appear to have no phenotype. These data unambiguously demonstrate that the 205K MAP gene is inessential for development. These results also suggest that there may exist protein(s) with redundant function that can substitute for 205K MAP.

MICROTUBULES form a large variety of functionally and structurally diverse organelles such as the spindle apparatus, the sperm axoneme, and the fibrous cytoplasmic network of the interphase cell. Understanding how these structures form and function demands an identification of all of the components involved. The extensive biochemical characterization of the basic microtubule component tubulin has revealed much information concerning the assembly of simple microtubule protofilaments. How the same basic protofilament can participate in diverse structures remains unknown. The discovery of multiple tubulin isotypes in most eukaryotic organisms (see discussion in Raff et al., 1987), suggested that different tubulins might have specific cytoskeletal functions; however, most tubulin isotypes appear capable of functioning in multiple cytoskeletal structures (Lewis et al., 1987; Lopata and Cleveland, 1987). Therefore, increasing attention has centered on a diverse class of proteins that copolymerize with tubulin. These proteins are collectively referred to as microtubule-associated proteins (MAPs).¹

MAPs have been isolated by virtue of their copurification with microtubules and have been classified according to molecular weight and in some cases antigenic cross-reactivity

(for review see Olmsted, 1986). The first MAPs to be identified were isolated from mammalian brain and include the high molecular mass MAPs of greater than 300 kD (Murphy and Borisy, 1975) and the tau proteins of 55–66 kD (Weingarten et al., 1975). Only one of the brain MAPs, MAP1c, has been functionally identified; MAP1c is a cytoplasmic dynein (Paschal and Vallee, 1987). HeLa cells have also been used as a source of MAPs since they might contain a greater amount of those MAPs that possibly function during mitosis. The principle HeLa MAPs consist of a group of three species of 200–220 kD, collectively called the HeLa 210K MAPs, and the 125K MAP (Bulinski and Borisy, 1979). Antibody localization studies of these proteins demonstrated that they are located in the spindle apparatus and/or cytoplasmic microtubules.

Although rapid advances have been made in understanding microtubule-based motors (for review see Vallee and Shpetner, 1990), the specific function of nonmotor MAPs are unknown at present. Nonetheless, several hypotheses of MAP function have been proposed based on their biochemical properties and intracellular location. For example, MAPs have been suggested to play roles in the assembly or stabilization of the spindle, spindle bridging and organization, linkage of the spindle to other filamentous systems, and microtubule bundling. Consistent with these hypotheses, expression of brain MAP2 in HeLa cells has been shown to cause microtubule bundling, although it is uncertain whether bundling is an intrinsic activity of MAP2 or results from microtubule stabilization (Lewis et al., 1989; Chapin et al.,

E. Tanaka's present address is Department of Biochemistry, University of California School of Medicine, San Francisco, CA 94143-0448.

1. Abbreviations used in this paper: A4-4, *P*[(*w*⁺,*ry*⁺)A]4-4; MAP, microtubule-associated protein; PCR, polymerase chain reaction.

1991). In addition, MAP2 and tau appear to be required for microtubule assembly or function during neurite outgrowth since reduced expression of either protein in cultured cells perturbs neurite outgrowth (Caceras and Kosik, 1990; Dinsmore and Solomon, 1991). A human MAP4 species, HeLa 210K MAP, is the only nonmotor MAP that has been implicated in mitosis (Izant et al., 1983).

In this study, we report experiments on the *in vivo* function of the *Drosophila* 205-kD MAP, designated 205K MAP. This particular MAP was initially isolated from Schneider line 2 cells of *Drosophila* and shown to be a part of both the spindle apparatus and cytoplasmic microtubule network. Isolation of the DNA sequence encoding the 205K MAP led to the identification of the chromosomal location of the corresponding structural gene by *in situ* hybridization to polytene chromosomes (Goldstein et al., 1986). By taking advantage of *Drosophila* classical genetics we have now isolated mutations in the 205K MAP gene. Surprisingly, homozygous deficiencies for this gene are viable and have no obvious phenotype. These observations suggest that there may exist gene products with overlapping function that can substitute for the 205K MAP.

Materials and Methods

Drosophila Culture

All flies were raised on standard cornmeal-molasses-agar-yeast medium supplemented with fresh yeast. Culture temperatures were 25°C or as stated.

Markers and Chromosomes

A listing of chromosome aberrations is provided below (for a more complete description see Lindsley and Grell (1968): (a) w^{1118} ; $P[w^+, ry^+]A4-4$ (abbreviated A4-4): a strain carrying a *P*-element transposon marked with w^+ and ry^+ at the telomeric end of 3R (Levis, 1989) (supplied by R. Levis). (b) $w;TM3/TM6B$: a third chromosome balancer stock in which both chromosomes carry many mutations. We principally followed *ebony* and *Tubby* on *TM6B* and *ebony* and *Stubble* on *TM3*. (c) *Birm-2; ry⁵⁰⁶*: an isogenic second and third chromosome strain containing 17 defective *P*-elements on the second chromosome (supplied by W. Engels). (d) *Sb P[ry⁺ delta2-3]-99B*: A source of *P*-element transposase activity due to a nonmobilizable *P*-element insertion located at 99B on the third chromosome (supplied by W. Engels).

PCR Screen

To screen for *P*-element insertions in the 205K MAP gene a modification of a newly described transposon mutagenesis technique was used (Ballinger and Benzer, 1989; Kaiser and Goodwin, 1990). This screen was the concerted effort of five groups sharing DNA from separate pools of mutagenized animals. The crosses were as follows. Each group mated ~1,000 *Cy/Sp; ry⁵⁰⁶ Sb P[ry⁺ delta2-3]/TM6* males to 2,000 *Birm-2/Birm-2; ry⁵⁰⁶/ry⁵⁰⁶* females. Resulting males of the genotype *Cy* or *Sp/Birm-2; ry⁵⁰⁶ Sb P[ry delta2-3]* were mated to *w/w; TM6B/TM3* females. This cross was set up using 3 males and 20 females per vial. From the next generation 10 *Cy* or *Sp/+; TM6B/ry⁵⁰⁶* males were mated to 20 *w/w; TM6B/TM3* females in individual vials. Each group set up an average of 250 vials. Parents were transferred on days 3, 6, and 9. On day 6 DNA was prepared from 50 pools of male parents; each pool contained the parents from five vials. The polymerase chain reaction (PCR) was then used to detect *P*-element insertions in the gene of interest using gene-specific primers as described below. If a PCR product was detected in any one of these pools, DNA was then prepared from half of the female progeny from each of the five separate vials. Once an individual vial was identified as having animals bearing the specific insertion, further rounds of PCR screening were conducted on smaller pools, and finally on the progeny of males mated singly to *w/w; TM6B/TM3* females.

To screen for *P*-element insertions in the 205K MAP gene, two sets of

three gene-specific primers (each set designed to serve as primers for polymerization in the opposite direction) were used together with a *P*-specific primer corresponding to the 31-bp inverted repeat sequence of the *P*-element (Ballinger and Benzer, 1989). The three gene primers correspond to nucleotide residues 295–324, 1908–1927, and 2646–2675, pointing in the 5' direction and residues 466–495, 2069–2098, and 2996–3025 pointing in the 3' direction. These number designations are relative to the beginning of the DNA sequence reported in Irminger-Finger et al. (1990). PCR reactions were conducted essentially as described in Ballinger and Benzer (1989).

Western Analysis

Whole adult flies, heads, or embryos were homogenized in boiling 1.2× Laemmli sample buffer using a ground glass homogenizer. Insoluble material was pelleted in a microfuge and the supernatant was then loaded onto a 7.5% SDS acrylamide gel (Laemmli, 1970). Proteins were transferred to nitrocellulose and analyzed as described (Yang et al., 1988). For specific detection of 205K MAP, an affinity-purified rabbit antiserum was used. The antiserum used was raised against gel-isolated *Drosophila* 205K MAP (Goldstein et al., 1986). A preparation of β -gal-205K MAP fusion protein was used to affinity purify the anti-205K MAP antisera as described (Goldstein et al., 1986). The fusion protein was isolated from *Escherichia coli* transformed with a bluescript vector containing a 205K MAP cDNA insert provided by Dr. I. Irminger-Finger (Université de Genève).

Southern Blot Analysis

Genomic DNA was prepared as described (Ballinger and Benzer, 1989) with the addition of two extractions of phenol/chloroform (1:1, vol/vol) at the end of the procedure. EcoRI-digested genomic DNA was separated on a 0.7% agarose gel and transferred to Zeta-probe membrane (Bio-Rad Laboratories, Cambridge, MA) using the alkali blotting method (Reed and Mann, 1985). After baking the blot for 2 h at 80°C, filters were hybridized by the method of Church and Gilbert (1984). The random priming method was used to label probe DNA with P^{32} (Feinberg and Vogelstein, 1984).

P-element Transformation

$P[w^+ \text{Sph}]$ was made by inserting the 12-kb SphI genomic DNA fragment shown in Fig. 1 into the SphI site of the pW8 polylinker (Klemenz et al., 1987). Several independent lines of *white⁺* transformants were recovered using the host strain *yw*.

$P[w^+ \text{MAP}]$ is a *P*-element-bearing cosmid isolated from a *D. melanogaster* cosmid library (kindly supplied by John Tamkun, University of California, Santa Cruz). This library was made using the vector pCosper which allows one to transform animals immediately upon isolation of the clone. Two independent cosmid transformants were recovered using the host strain *w;delta2-3*. A restriction map of the genomic DNA contained within pCosper is shown in Fig. 1 (labeled *genomic DNA*). The lengths of the terminal EcoRI fragments are estimated.

Results

At the outset of this project 205K MAP was believed to be required for viability for two reasons. First, 205K MAP was previously found to be associated with the spindle and interphase microtubule populations (Goldstein et al., 1986), and the structural components of such organelles would be assumed to be essential. Second, at the time this study began, 205K MAP was thought to be the *Drosophila* counterpart to vertebrate MAP4. Vertebrate MAP4 and *Drosophila* 205K MAP share biochemical properties such as size, thermostability, and ability to bind microtubules. Furthermore they share common antigenic determinants since antibodies to 205K MAP cross-react with HeLa 210K MAP and mouse MAP4 (West, R. R., K. M. Tenbarge, M. Gorman, L. S. B. Goldstein and J. B. Olmsted, 1988. *J. Cell Biol.* 107:460a.), and their intracellular distributions are also similar, both being found on spindle and cytoplasmic microtubules. Surpris-

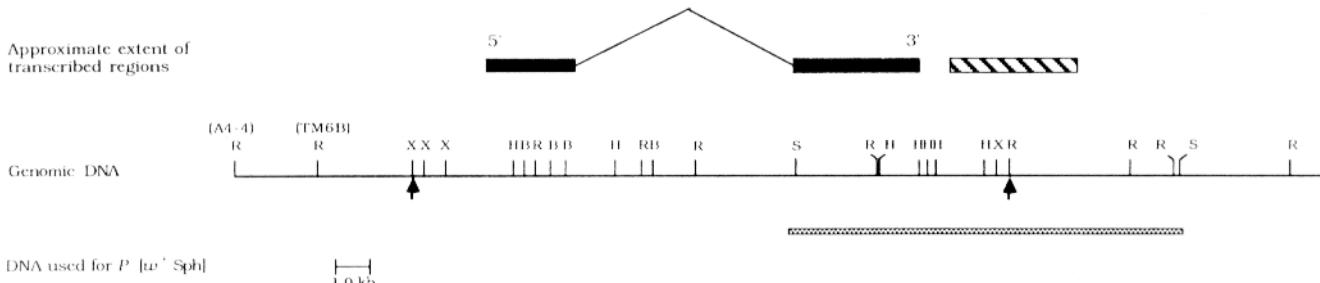


Figure 1. Organization of the 205K MAP gene (includes data from Fig. 1 of Irminger-Finger et al., 1990) and surrounding DNA. R, EcoRI; X, XbaI; H, HindIII; B, BamHI. In the area outside of the vertical arrows, only the EcoRI sites have been mapped. Solid bar indicates 205K MAP-coding regions, crosshatched bar indicates the neighboring gene, *modulo*. A4-4 and TM6B indicate the locations of polymorphic EcoRI sites in the upstream region of 205K MAP as described in the text.

ingly, recent DNA sequence analysis of the bovine, human, and mouse MAP4 genes has revealed no significant sequence identity with *Drosophila* 205K MAP (Aizawa et al., 1990; West et al., 1991). However, an essential role for MAP4 and by inference 205K MAP was suggested by an experiment with HeLa Ptk₁ cells in which microinjected anti-HeLa 210K MAP resulted in mitotic arrest (Izant et al., 1983). Based upon these data, it was predicted that 205K MAP, like HeLa 210K MAP had an essential function during mitosis and that mutations could be recovered in this gene by screening for lethality.

Mutagenesis of the 205K MAP Gene

As a first approach toward isolating mutations in the 205K MAP locus, we wished to use an F2 lethal screen to obtain deletion or rearrangement mutations. An essential prerequisite for an F2 lethal screen is a "tester" deficiency-bearing chromosome for assaying the mutagenized chromosomes. Since 100EF (the location of the 205K MAP gene) was previously uncharacterized genetically, there were no existing deficiencies of the region that could be used. To acquire the first deficiency of 100EF, the chromosome A4-4 was mutagenized. This chromosome contains a *P*-element marked with the wild-type allele of the *white* gene 7 kb upstream of the 205K MAP gene (Levis, 1989). Males of the genotype *w*;A4-4 (orange eyes) were gamma irradiated with 4,000 rads and revertants of the *white* gene on A4-4 (white eyes) were selected from the next generation. One of these revertants, *Df(3R)wrev2*, was missing the 205K MAP gene by the criteria of *in situ* hybridization and Southern blot analysis.

There are three lines of evidence that *Df(3R)wrev2* is a deletion that lacks at least 40 kb of the region including the 205K MAP gene. First, *Df(3R)wrev2* lacks an EcoRI fragment (9 kb) that is polymorphic in size among *Drosophila* stocks and hence can be followed in crosses (see Fig. 1 for location). Specifically, the 9-kb EcoRI fragment from the A4-4 parent chromosome is absent in *Df(3R)wrev2/TM6B* animals. Second, *in situ* hybridization of lambda clone 5C (which contains all but 2 kb of DNA at the 3' end of the 205K MAP gene; see Irminger-Finger et al., 1990 for description of the isolation and characterization of this clone) to chromosomes from *Df(3R)wrev2/TM6B* revealed no hybridization to the tip of chromosome 3R. Because *Df(3R)wrev2* is not cytologically visible it is probably missing <100 kb of DNA (Pereira, A., and E. Tanaka, unpublished data). Third, Southern blot analysis of *Df(3R)wrev2/TM6B* animals re-

veals no alteration in restriction fragment size using several different enzymes. This result indicates that there are no breakpoints within the 40 kb region for which probes are available (Fig. 1).

A standard F2 lethal screen was next used to obtain physical breakpoints within or near the 205K MAP gene. Initially, *Df(3R)wrev2* was used as a tester chromosome until it was discovered that this chromosome contained a recessive lethal mutation located outside of 100EF. The tester chromosome was then changed to *Df(3R)AP4*, which was isolated in this initial F2 lethal screen. Southern blot analysis of *Df(3R)AP4*, similar to that which was done for *Df(3R)wrev2* (see above), showed that like *Df(3R)wrev2*, it is a large cytologically invisible deficiency removing the region covered by the genomic walk shown in Fig. 1. Most important, this deficiency removes no vital loci other than those in the region of interest. The evidence for this latter conclusion comes from a *P*-element rescue experiment. A *P*-element cosmid construct, *P[w^r MAP]*, containing 33 kb of genomic DNA including the 205K MAP gene (see Materials and Methods), allows *Df(3R)AP4* to survive as a homozygote (Pereira, A., unpublished data). Therefore all of the recessive lethal mutations acquired using *Df(3R)AP4* must be in genes located within the 33-kb region surrounding and including the 205K MAP gene. Out of a total of ~30,000 gamma-irradiated chromosomes screened using *Df(3R)AP4*, 25 lethal mutations were recovered. Only one of these, *I(3)API* has a breakpoint in the 40-kb region for which DNA probes are available. This mutant allele appears to be an inversion with one breakpoint within the HindIII fragment located proximal to the neighboring gene *modulo* (Krejci et al., 1984) and the second breakpoint outside the area shown in Fig. 1. The other 24 deficiencies are estimated to be between 40 and 100 kb by the criteria described above.

Since this large-scale gamma-ray mutagenesis did not result in a physical breakpoint in the 205K MAP gene, a second approach was taken to mutagenize the A4-4 chromosome. The rationale behind this screen was that it might be possible to recover lethal *P*-element excision-induced rearrangements in 205K MAP. To screen for *P*-element-induced rearrangements, mosaic males of the genotype *w/Y;A4-4/ry^{s6} Sb P[ry^r delta2-3]* were individually mated to *w;TM6B/TM3* females. From each group of A4-4/TM6B males with the same eye color, one male was chosen to avoid recovering large "clusters" of an identical mutation. Single-pair matings were then set up using one *w;A4-4/TM6B* male and three *red e Df(3R)AP4/TM6B* females. The absence of

Tb⁺ progeny from this cross signaled a lethal mutation on the A4-4 chromosome in the 205K MAP region. A stock was then established from the non-*ebony* flies. Eight lethals were recovered from 1,000 chromosomes tested in this screen. These are designated *I(3)A4-4L1* through *I(3)A4-4L7*, and *Df(3R)A4-4L8*. Southern blot analysis of these eight strains revealed that several have breakpoints in or near *modulo* but none are associated with rearrangements in the 205K MAP gene.

Animals That Are Homozygous Deficient for the 205K MAP Gene Are Viable

As a result of the various mutagenesis experiments described above, two key pieces of information emerged that eventually lead to the hypothesis that the 205K MAP locus may not be vital. The first was the molecular nature of the 25 lethal mutations recovered from the ~30,000 gamma ray mutagenized chromosomes. These mutations removed the 205K MAP gene and at least 40 kb of surrounding DNA but were not associated with physical breakpoints within the 205K MAP gene. Hence, we realized that it might not be possible to isolate physical breakpoints in the 205K MAP gene with an F2 lethal screen. The second indication that the 205K MAP gene may not mutate to cause lethality was that members of the one lethal complementation group that were recovered in the region (as defined by a set of three noncomplementing EMS-induced alleles) were not rescued by a *P*-element containing the 205K MAP gene (Pereira, A., unpublished data).

To test the hypothesis that the 205K MAP gene is not required for *Drosophila* development, a *P*-element rescue experiment was conducted. This experiment was specifically designed to determine if the lethality resulting from a deficiency in the 100EF region, which lacks the 205K MAP gene and surrounding DNA, could be solely due to a requirement for the neighboring gene *modulo* during development. Therefore, a 12-kb *SphI* genomic fragment containing *modulo* (Fig. 1) was cloned into the *P*-element vector pW8 (designated *P[w⁺Sph]*) and injected into *y w* embryos. This 12-kb *SphI* fragment contains ~3.5 kb from the 3' half of the 205K MAP gene and is therefore likely to be incapable of supplying 205K MAP function. Phenotypically *white*⁺ germline transformants were recovered and tested for their ability to rescue animals that are homozygous for various lethal mutations. It was found that *P[w⁺Sph]* was able to rescue animals that were homozygous for a deficiency, *Df(3R)A4-4L8* (see above), which removed both *modulo* and the 205K MAP gene. Confirmation of this result is shown in Fig. 2 in which a Southern blot of EcoRI-digested genomic DNA from adult animals of various genotypes has been probed with the 205K MAP cDNA clone B3 (Irminger-Finger et al., 1990). Previous Southern blot analysis of different strains probed with a subclone of genomic DNA from the 5' flanking region of 205K MAP demonstrated the existence of a stably inherited upstream polymorphic EcoRI fragment (Fig. 1). This polymorphism is also detected with the cDNA probe used in Fig. 2 since a 6-kb EcoRI fragment is detected in the *TM6B* strain and a corresponding 9 kb fragment is detected in the *A4-4* strain. DNA from adults of the genotype *P[w⁺Sph];Df(3R)A4-4L8/Df(3R)A4-4L8* can be seen to be deficient for the parental *A4-4* 9-kb EcoRI fragment. The internal 5.0- and 3.0 kb EcoRI fragments are also absent from the rescued animals. The 1.7-kb EcoRI fragment shown in

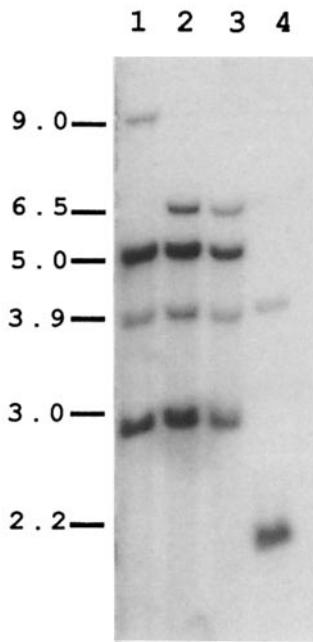


Figure 2. Southern blot of DNA from animals deficient for the 205K MAP gene. EcoRI-digested genomic DNA from adult animals was probed with the 205K MAP embryonic cDNA B3 as described in Materials and Methods (Irminger-Finger et al., 1990 for the origin and description of B3). (Lane 1) *A4-4/A4-4*; (lane 2) *Df(3R)A4-4L8/TM6B*; (lane 3) *TM6B/TM3, Sb*; (lane 4) *P[w⁺Sph]; Df(3R)A4-4L8/Df(3R)A4-4L8*.

Fig. 1 is in an intron and is therefore not detected with the cDNA probe. The 2.2- and 3.9-kb EcoRI fragments detected in the rescued strain are not from the endogenous 205K MAP gene. These two fragments are contained within the *P*-element construct. The 2.2-kb EcoRI fragment corresponds to the 2.2-kb *SphI*-EcoRI fragment at the 3' end of the 205K MAP gene in Fig. 1 (the *SphI* site in this fragment is adjacent to the EcoRI site in the *pw8* polylinker) and the 3.9-kb EcoRI fragment corresponds to the 3'-most EcoRI fragment of the 205K MAP gene (Fig. 1). We also confirmed this result by demonstrating the absence of 205K MAP immunoreactivity in the rescued animals (Fig. 4, lane 1; discussed below). Hence, the lethality caused by the *Df(3R)A4-4L8* mutation is a consequence of the absence of the *modulo* gene and not to the absence of the 205K MAP gene. This experiment therefore conclusively proves that the 205K MAP is not required for *Drosophila* development.

Isolation of *P*-element Insertions at the 205K MAP Locus

Although the 205K MAP-deficient animals rescued by *P[w⁺Sph]* could be used for a mutant phenotypic analysis, it would be difficult to prove that any abnormalities were due to the lack of 205K MAP function and not to the incomplete rescue of *modulo*. To circumvent this problem, mutations were generated that affect 205K MAP only. A recently developed procedure (Ballinger and Benzer, 1989; Kaiser and Goodwin, 1990), which does not require screening for a mutant phenotype, was used to generate mutations in the 205K MAP gene (see Materials and Methods). A hybrid dysgenesis cross was carried out as described in Robertson et al. (1988). *P*-element insertions in 205K MAP were detected by PCR amplification of genomic DNA from the mutagenized animals. A set of oligonucleotide primers that included a primer corresponding to the end of the *P*-element and six oligonucleotide primers homologous to three different regions of the 205K MAP gene were used. The gene-specific primers were designed such that a *P*-element insertion anywhere in



Figure 3. Insertion sites of *P*-elements upstream of the 205K MAP gene. The 31-terminal bp repeat element of the *P*-element is underlined and the beginning of a 205K MAP embryonic transcript is shown by the arrow. Arrows indicate the points of insertion and insertion numbers of the *P*-elements.

or near the 205K MAP gene could be detected. In the initial screen of genomic DNA from 60 pools of 250 animals each, a large number of PCR products were visible with ethidium bromide. Southern blot hybridization using a 32 P-labeled 205K MAP cDNA probe showed that the majority of these products were not amplified from the 205K MAP gene. Similar spurious amplification products were reported in the original description of this technique (Ballinger and Benzer, 1989; Kaiser and Goodwin, 1990). In our particular case, two factors were found to contribute to this problem. One was the use of three gene-specific primers together in each reaction. Once the primer that detected the 205K MAP-specific product was identified, much of the background was reduced when the other primers were removed from the reaction. The second factor was the amount of heterogeneity in the sample. As the pools became smaller the amount of background also decreased.

A screen of 15,000 mutagenized chromosomes resulted in the identification of five *P*-element insertions 5' to the 205K MAP gene. Using the same gene-specific primer, it was observed that four of these five insertions resulted in PCR products that appeared to be the same size as determined by migration in agarose gels. To determine the exact location of these four insertions, the PCR products were subcloned and their DNA sequence was determined. As can be seen in Fig. 3, all four insertions were within the same 5-bp region. The insertion closest to the gene is located 9 bp 5' to the transcription start, which was deduced from the sequence of the characterized embryonic cDNA clone. It is unknown whether this insertion site is sometimes contained in other transcripts because only one embryonic cDNA that extends this far was characterized (Irminger-Finger et al., 1990). The fifth insertion site is \sim 200 bp further 5' of this site.

Reduced Level of 205K MAP in 205K MAP Deletions and *P*-element Insertions

Since the *P*-element insertions were several hundred base pairs upstream from the 205K MAP protein-coding sequence their effect on the level of the 205K MAP could not be predicted. Western blot analysis of one of these *P*-element insertions demonstrates that these are indeed 205K MAP mutants because the level of 205K MAP protein is significantly reduced in these animals (Fig. 4). As can be seen, the amount of 205K MAP in a strain homozygous for insertion 15.6 (Fig. 4, lane 3) is greatly reduced compared to wild type (Fig. 4, lane 2). We reprobed these blots using tubulin antibodies (data not shown) and conducted densitometric analysis using tubulin as a loading control (data not shown). The results demonstrated that the amount of 205K MAP in strain 15.6 is \sim 25% of the wild-type level. As expected all of these

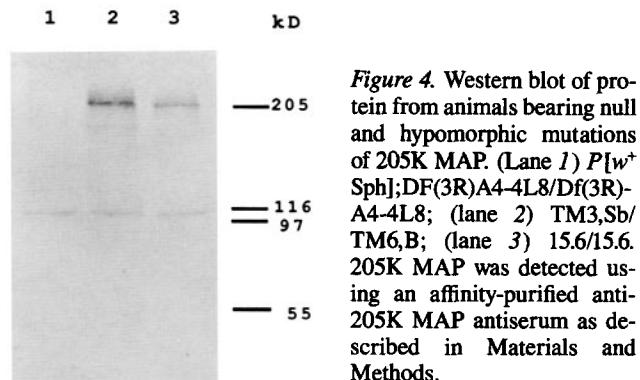


Figure 4. Western blot of protein from animals bearing null and hypomorphic mutations of 205K MAP. (Lane 1) $P[w^+ Sph];DF(3R)A4-4L8/DF(3R)-A4-4L8$; (lane 2) TM3,Sb/TM6,B; (lane 3) 15.6/15.6. 205K MAP was detected using an affinity-purified anti-205K MAP antiserum as described in Materials and Methods.

P-element insertions are homozygous viable since as described above, animals that are homozygous deficient for the 205K MAP gene are viable. Although these insertions appear to be hypomorphs, they can ultimately be used to generate simple null alleles by inducing *P*-element excision.

We also wished to verify that animals completely deficient for the 205K MAP gene ($P[w^+ Sph]; Df(3R)A4-4L8/Df(3R)-4L8$) also lack 205K MAP. By the criterion of Western blot analysis, there is no 205K MAP in these animals (Fig. 4, lane 1).

A preliminary phenotypic analysis was conducted using animals heterozygous for one *P*-element insertion, 15.6, and the 205K MAP deletion, $Df(3R)A4-4L8$. These heterozygous animals were used for these experiments for two reasons. First, the *P*-element insertion is a hypomorphic allele since it results in a reduced amount of 205K MAP; $Df(3R)A4-4L8$ should further lower the amount of 205K MAP as compared with animals with two doses of 15.6. Second, any phenotype observed using animals homozygous for 15.6 could be the result of other *P*-element insertions located elsewhere on the chromosome. Hence, we did not want to make this chromosome homozygous. A preliminary set of observations have been made on progeny from mothers of the genotype, $Df(3R)AP4/15.6$ crossed to $Df(3R)AP4/TM6B$. No effect was seen on: (a) hatching time as compared with eggs from wild-type mothers, (b) rate of development of progeny which are $Df(3R)AP4/15.6$ (phenotypically *Tubby*⁺) compared with sibling 15.6 or $Df(3R)AP4/TM6B$ progeny (*Tubby*; these animals have one dose of the 205K MAP gene), and (c) the fertility of $Df(3R)AP4/15.6$ males compared with sibling males that carry *TM6B*.

Discussion

In this study we report the isolation of mutations in the *Drosophila* 205K MAP gene. The initial mutagenesis strategy was based on the assumption that these mutations would result in lethality. After an exhaustive screen for physical breakpoints in the 205K MAP gene proved unsuccessful, we entertained the possibility that breakpoints in the 205K MAP gene could not be recovered in a lethal screen because the 205K MAP gene may not be vital. To examine this hypothesis, a *P*-element construct containing the neighboring gene, *modulo* was used to rescue the lethality caused by a larger deficiency that removed both *modulo* and the 205K MAP gene. To our surprise, animals that completely lack 205K MAP are viable.

The 205K MAP-deficient animals do not appear to have an obvious phenotype. These animals are healthy, fertile, and appear wild type in appearance and behavior. Furthermore, the homozygous null stock ($P[w^+SpH]; Df(3R)A4-4L8/Df(3R)A4-4L8$) has been now propagated for several months and exhibits no growth disadvantage. It is possible, however, that closer examination will reveal a phenotype. Alternatively, a defect may become more apparent in a different genetic background. For example, the growth rate of 205K MAP-deficient animals may be normal, but in combination with a tubulin gene deficiency might be slowed. Another possibility is that the rate of chromosome loss may be wild type in 205K MAP-deficient animals but increased when a mutation such as *mitotic loss* (Gelbart, 1974) is also present.

While this work was in progress, a second group also discovered that animals bearing homozygous deficiencies of the 205K MAP gene are viable. In an attempt to isolate mutations in the neighboring gene, *modulo*, DNA breakpoints were recovered in the 205K MAP gene (Pradel, J., personal communication). In this particular screen, animals bearing a third chromosome that contained both the A4-4 transposon as well as the mutation *mu2* were gamma irradiated (*mu2* is a mutation that causes a high frequency of terminal deletions in the progeny of irradiated animals [Mason et al., 1984]). The DNA of several resulting deletions was analyzed and two were found to have endpoints in the center of the 205K MAP gene. As we have reported for $P[w^+SpH]; Df(3R)A4-4L8/Df(3R)A4-4L8$, these animals are fully fertile and viable.

At this point it is difficult to conclude why 205K MAP is inessential for development. One possibility is that other gene products are able to substitute. However, if other species with redundant function exist, these genes are not detectable under high stringency conditions at the DNA level. At the protein level, a second species of ~100 kd, is detected with affinity-purified antiserum (Fig. 4). It is currently unknown whether this species is also a MAP. If 205K MAP mutants survive owing to redundant function, it may be possible to identify such products in a genetic screen for lethal mutations in a 205K MAP-deficient background.

An alternative explanation for the finding that 205K MAP is inessential for development is that this protein may have a subtle influence on microtubule function, but its absence does not affect animals grown under laboratory conditions, which are relatively optimal. This latter possibility is somewhat analogous to observations made on three of the chromosome instability genes in *Saccharomyces cerevisiae*. These three genes were identified independently in two separate screens; one screen was for mutations that resulted in an increased rate of chromosome loss (Hoyt et al., 1990), and the second was for mutations that displayed a supersensitivity to the antimicrotubule drug benomyl (Stearns et al., 1990). These phenotypes indicate that the chromosome instability gene products affect microtubule function, yet these genes are inessential for viability. With these results in mind, we are carrying out similar types of analyses with 205K MAP-deficient animals. Such experiments may indicate that 205K MAP does indeed have a microtubule-related function even though animals can develop without it.

Another consequence of our work is that we have additional information on a recently developed technique to generate mutations without having to assume phenotype

(Ballinger and Benzer, 1989; Kaiser and Goodwin, 1990). Hybrid dysgenesis was used to generate *P*-element insertions upstream of the 205K MAP gene, which were detected by PCR amplification of genomic DNA. The studies reported here suggest that the *P*-element mutagenesis approach may be successful for only a limited set of genes and that *P*-element insertion sites within a given region are not randomly distributed. Although screening of 15,000 chromosomes resulted in the identification of 5 *P*-element insertions in the vicinity of the 205K MAP gene, no insertions were identified in the three other genes examined (MyoD, α -spectrin, and a β -spectrin isoform). This highly nonrandom distribution is consistent with evidence from hybrid dysgenesis screens for mutant phenotypes (Kidwell, 1987). DNA sequence analysis of the five *P*-element insertions recovered suggests that insertion "hot spots" may be extremely small in molecular terms spanning ~200 bp in the case of the 205K MAP gene. Intriguingly, all of the insertions occurred within the putative promoter region of the gene.

To conclude, we have demonstrated that the 205K MAP gene is inessential for *Drosophila* development. Experiments in progress should determine whether animals deficient in 205K MAP have subtle microtubule-related defects such as an increased rate of chromosome loss. If no such phenotype is observed, a reasonable explanation is that MAPs with overlapping function are able to substitute for 205K MAP. Mutations of 205K MAP will then be invaluable in identifying these other proteins in subsequent genetic screens.

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