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lin-35/Rb Cooperates With the SWI/SNF Complex to Control *Caenorhabditis elegans* Larval Development

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ABSTRACT

Null mutations in *lin-35*, the *Caenorhabditis elegans* ortholog of the mammalian Rb protein, cause no obvious morphological defects. Using a genetic approach to identify genes that may function redundantly with *lin-35*, we have isolated a mutation in the *C. elegans psa-1* gene. *lin-35; psa-1* double mutants display severe developmental defects leading to early larval arrest and adult sterility. The *psa-1* gene has previously been shown to encode a *C. elegans* homolog of yeast SWI3, a critical component of the SWI/SNF complex, and has been shown to regulate asymmetric cell divisions during *C. elegans* development. We observed strong genetic interactions between *psa-1* and *lin-35* as well as a subset of the class B synMuv genes that include *lin-37* and *lin-9*. Loss-of-function mutations in *lin-35*, *lin-37*, and *lin-9* strongly enhanced the defects of asymmetric T cell division associated with a *psa-1* mutation. Our results suggest that LIN-35/Rb and a certain class B synMuv proteins collaborate with the SWI/SNF protein complex to regulate the T cell division as well as other events essential for larval growth.

MEMBERS of the retinoblastoma tumor suppressor family of proteins (pRb, p107, and p130) play a fundamental role in cell cycle control, apoptosis, and development (reviewed by Morris and Dyson 2001; Classon and Harlow 2002). A major cellular target of pRb is the E2F family of transcription factors that regulate the expression of genes required for G_1 to S phase transition as well as many genes unconnected to cell cycle control (Dyson 1998; Stevaux and Dyson 2002). The formation of Rb-E2F complexes at the promoters of cell cycle genes blocks transcriptional activation by E2F's. In addition, Rb-E2F complexes can actively repress transcription through the recruitment of histone deacetylase (HDAC; Brehm *et al.* 1998; Luo *et al.* 1998; Magnaghi-Jaulin *et al.* 1998), histone methylase (Robertson *et al.* 2000; Nielsen *et al.* 2001; Pradhan and Kim 2002), and members of the nucleosome remodeling complex (SWI/SNF; Dunaief *et al.* 1994; Strober *et al.* 1996; Zhang *et al.* 2000). Transfection studies indicate that class I HDACs and SWI/SNF members are recruited by pRb and may coordinate the sequential expression of E2F target genes to regulate the progression through G_1 and S phases (Zhang *et al.* 2000). A genetic screen for modifiers of an E2F overexpression phenotype in the Drosophila eye identified enhancer mutations in *Osa*, *Brahma*, and *Moira* genes, which encode homologs of SWI1, SWI2, and SWI3, respectively, suggesting that the SWI/SNF complex downregulates E2F activity (Staehling-Hampton *et al.* 1999). First identified in the yeast *Saccharomyces cerevisiae*, SWI/SNF is a 2-MD multisubunit assembly that is highly conserved in eukaryotes. The SWI/SNF complex contributes to the regulation of gene expression by locally altering the structure of chromatin. Depending on the context, it can be involved in either transcriptional activation or repression (reviewed by Martens and Winston 2003). Growing genetic and molecular evidence indicate that subunits of the SWI/SNF complex act as tumor suppressors in humans and mice (reviewed by Klochendler-Yeivin *et al.* 2002). In *Caenorhabditis elegans*, PSA-1/SWI3 and PSA-4/SWI2 have been shown to play a role in the asymmetric division of the hypodermal T cell during larval development (Sawa *et al.* 2000).

In contrast to Rb knockout mutations in flies and mice (for a review of mammalian studies see Lipinski and Jacks 1999), strong loss-of-function (lf) mutations in *lin-35/Rb* cause relatively subtle effects on viability and development in *C. elegans* (Lu and Horvitz 1998; Boxem and van den Heuvel 2002; Fay *et al.* 2002). To uncover the functions of *lin-35* during *C. elegans* development, we have carried out a genetic screen to isolate mutations that are synthetically lethal with *lin-35(n745)* (Fay *et al.* 2002, 2003). Briefly, the strain used for the screen was homozygous for a strong loss-of-function mutation in *lin-35(n745)*, but carried an extrachromosomal array (*kuEx119*) containing copies of the wild-type *lin-35* gene and a ubiquitously expressed green fluorescent protein (GFP) reporter. Typically, extra-

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chromosomal arrays fail to be transmitted to a certain percentage of progeny from parents that carry the array (*kuEx119* is transmitted to ~70% of self-progeny). After mutagenesis, mutant animals that are synthetically lethal with *lin-35* were identified by their complete dependence on *kuEx119* for viability. Using this strategy, our laboratories have previously reported the identification of mutations in *fzr-1*, a regulatory subunit of the APC proteasome (Fay *et al.* 2002), and mutations in *ubc-18*, the *C. elegans* homolog of human UBCH7 (Fay *et al.* 2003). *lin-35*; *fzr-1* double mutants display a hyperproliferation phenotype, while *lin-35*; *ubc-18* double mutants are defective at an early stage of pharyngeal morphogenesis. Here we describe our analysis of another mutation (*ku355*) isolated by this screen that shows defects that differ from that of *fzr-1* and *ubc-18*.

MATERIALS AND METHODS

Strains: Nematodes were cultured using standard techniques (Sulston and White 1980). Mutations used were as follows: LGI, *lin-35(n745)*; LGII, *lin-8(n111)*; LGIII, *lin-36(n766)* and *lin-37(n758)*; LGV, *fog-2(q71)*, *psa-1(ku355)*, *psa-1(os22)*, *rol-9 (sc148)*, and *unc-51(e369)*; LGX, *lin-15(n765ts)*, *lin-15b(n744)*, and *lin-15a(n767)*. The Hawaiian strain CB4856 was used in the single-nucleotide polymorphism (SNP) mapping. In addition, we used a strain containing an extrachromosomal array, *kuEx119* [*lin-35(+)*, *sur-5::GFP*; Fay *et al.* 2002]. GFP marker strains used were JK2868 [*qIs56* (IV or V; *lag-2::GFP*; Blelloch *et al.* 1999)], JR767 [*SCM::GFP* (Terns *et al.* 1997)], and JM63 [*elt-2::GFP* (Fukushige *et al.* 1998)].

Isolation and mapping of *psa-1(ku355)*: *psa-1(ku355)* was isolated in a genetic screen for identifying mutations that cause synthetic phenotypes with *lin-35(n745)* in *C. elegans* (Fay *et al.* 2002). *psa-1(ku355)* was then mapped to *LGV*. Three-factor crosses using marker mutations in *fog-2* and *rol-9* further defined the map position of *psa-1(ku355)* to the region between *fog-2* and *rol-9*. Among the progeny of hermaphrodites of genotype *fog-2(q71) rol-9(sc148)/psa-1(ku355)*, 4 of 12 Rol non-Fog recombinants segregated *psa-1(ku355)*. The strain *rol-9(sc148) psa-1(ku355)* and the Hawaiian strain CB4856 were used in a standard SNP mapping procedure to place *psa-1(ku355)* in a 96-kb region between *fog-2* and the polymorphisms [vl30h01.s1@572,t,38] on the cosmid ZC15.

RNAi analysis: RNAi was performed essentially as described (Kamath *et al.* 2003). Feeding vectors containing *lin-35*, *lin-36*, *lin-53*, *hda-1*, and *lin-15A* were previously described (Fay *et al.* 2002). Feeding vectors containing *lin-37*, *lin-9*, *let-418*, C01G8.9, F01G4.1/*psa-4*, ZK1128.5, R07E5.3, Y71H2AM.17, F26D10.3, *act-1*, *act-2*, *act-3*, *act-4*, and *act-5* were obtained from an RNAi library from the United Kingdom Human Genome Mapping Project Resource Centre (HGMP-RC). For *psa-1*, a 1055-bp *Sal*I-*Sac*I fragment from the *psa-1* cDNA clone yk257g1 was subcloned into vector pPD129.36. For C18E3.2, a 1-kb PCR fragment of genomic DNA corresponding to nucleotides 26,000–27,000 of C18E3 (part of C18E3.2 coding region) was subcloned into vector pPD129.36. For ZK616.4, a 1369-bp PCR fragment of genomic DNA corresponding to nucleotides 2903–4272 of ZK616.4 (part of ZK616.4 coding region) was subcloned into vector pPD129.36. In most experiments, we placed synchronized L1 stage worms (parents or P0) onto a plate seeded with a bacterial strain carrying specific RNAi plasmids. We started scoring the phenotypes in the P0 or the F_1 generation 3 days later.

Embryonic lethal and larval arrest phenotypes: For embryonic lethality, individual L4 larvae were transferred to fresh plates every day for 3 consecutive days, and the eggs laid on the plates were scored for hatching. For the larval arrest phenotype, the percentage of arrest animals was determined 60 hr after hatching. The larval stage of the arrest was evaluated mainly by the size and shape of the gonad.

Cell lineage analysis, microscopy, and dye-filling assays of the phasmids: Cell morphology and lineage in living animals were observed using Nomarski optics (Herman and Horvitz 1994). Phasmid dye filling as an indicator for the normal T cell polarity was scored as previously described (Herman and Horvitz 1994). Briefly, adult animals were soaked in a cold solution of M9 salts containing 0.4 mg/ml of fluorescein isothiocyanate (FITC) for 4 hr, washed three times with cold M9 salts, and then transferred to a seeded agar plate at room temperature. For quantification and microphotography, dye filling was evaluated using a Zeiss Standard microscope equipped with epifluorescence. Generally, the amphids and phasmids of N2 animals were filled with the dye. In contrast, in *psa-1(ku355)* animals, the amphids were filled with the dye, but the phasmids often were not. Occasionally, neither the amphids nor the phasmids in N2 animals were filled with the dye. For this reason, we counted as phasmid defective only those individuals that showed amphid dye filling but no phasmid filling.

RESULTS

***psa-1(ku355)* and *lin-35(n745)* cause a synthetic larval arrest phenotype :** We initiated our study of the *psa-1* gene by isolating the *psa-1(ku355)* allele in a genetic screen in *C. elegans* for mutations that cause synthetic mutant phenotypes with *lin-35* (Fay *et al.* 2002, 2003). At 20°, *lin-35(n745); psa-1(ku355)* animals that carried an extrachromosomal array containing the *lin-35(+)* gene were viable. In contrast, 98% of the *lin-35; psa-1* animals that failed to inherit the array arrested at the first larval stage (L1; Table 1), and those *lin-35; psa-1* animals that reached adulthood were small and sterile ($n = 50$; Figure 1; Table 1). In addition, 16% of *lin-35; psa-1* double mutants failed to progress through embryogenesis ($n = 840$). The arrested L1 animals tended to be curled and inactive, although no obvious pharyngeal morphological defects were observed in these animals. It was previously reported that *lin-35(n745)* single mutants are viable but show a substantial reduction in brood sizes when compared with wild type (Lu and Horvitz 1998; Table 1). At 20°, *psa-1(ku355)* single mutants were also viable but grew slowly and had reduced brood sizes (Table 1). In addition to larval arrest and embryonic lethality, *psa-1(ku355)* animals also displayed other pleiotropic phenotypes including extra vulval cell inductions (Muv, 1%, $n = 309$; Figure 2), egg-laying defectiveness (Egl, <1%, $n = 400$), and protruding vulvae (Pvl, 100%, $n > 300$). The Egl and Pvl phenotypes, but not the Muv phenotype, were mentioned previously to be associated with another *psa-1* allele (Sawa *et al.* 2000). Furthermore, closer examination using a *lag-2::GFP* marker revealed that one arm of the gonad failed to elongate properly in 37% of *psa-1(ku355)* animals ($n = 200$) and

TABLE 1

Viability of *lin-35* and *psa-1* mutants

	Temperature (°)	% embryonic lethality (*n*)	% larval arrest[a] (*n*)	% sterile (*n*)	Average brood size (*n*)
N2 (wild type)	20	0 (500)	0 (500)	0 (50)	270 ± 20 (30)
lin-35(n745)	20	0 (830)	1 (830)	3 (100)	104 ± 45 (10)
lin-35(n745)	15	0 (838)	1 (838)	6 (50)	90 ± 40 (10)
psa-1(ku355)	20	15 (866)	17 (736)	0 (50)	28 ± 20 (30)
psa-1(ku355)	15	9 (824)	3 (748)	0 (50)	141 ± 40 (30)
lin-35; psa-1	20	16 (840)	98 (700)	100 (50)	ND
lin-35; psa-1	15	10 (929)	10 (731)	6 (50)	53 ± 15 (10)
N2 RNAi control[b]	20	0 (200)	0 (200)	0 (200)	ND
N2; *lin-35 (RNAi)*	20	0 (300)	0 (280)	1 (50)	ND
psa-1 RNAi control[b]	20	14 (260)	13 (240)	0 (50)	ND
psa-1; lin-35 (RNAi)	20	15 (281)	95 (239)	6 (50)	ND

ND, not done; *n*, number of animals checked.
[a] Percentage of hatched animals arrested at larval stages.
[b] Bacteria HT115 with pPD129.36 was used as RNAi control.

that gonad arms folded in abnormal forms in 52% of the animals ($n = 200$; Figure 2). All these phenotypes associated with *psa-1(ku355)* were temperature sensitive (Table 1 and data not shown). The larval arrest and sterile phenotypes associated with *lin-35(n745); psa-1(ku355)* double mutants were also temperature sensitive (Table 1).

To further investigate the cause of the synthetic larval arrest of *lin-35; psa-1* double mutants, we assayed *psa-1* and *lin-35; psa-1* mutants for proliferation defects. We failed to detect any hyperproliferation defects in both the single and the double mutants. We also failed to observe any proliferation defects in seam and intestinal cell lineages as well as gonadal distal-tip cells (DTCs) in both the single mutants and the double mutants, using a seam-cell-specific GFP marker (*SCM::GFP*; gift of J. Rothman, UCSB), a gut-cell-specific marker (*elt-2::GFP*; Fukushige *et al.* 1998), and a DTC-expressing *lag-2::GFP* marker (Blelloch *et al.* 1999). Therefore, there is no evidence that the synthetic larval arrest in double mutants is caused by proliferation defects.

***ku355* is an allele of *psa-1* encoding a homolog of the yeast SWI3:** We mapped *psa-1(ku355)* to a 96-kb region between *fog-2* and the cosmid ZC15 using the genetic markers and the SNPs (see materials and methods). This region contains 16 predicted genes including *psa-1* (Y113G7B.23). Complementation tests indicated that *psa-1(ku355)* failed to complement *psa-1(os22)*. Sequencing of the *psa-1* coding region of the genomic DNA from the *psa-1(ku355)* identified a missense mutation that leads to the replacement of leucine with proline at the amino acid position 86. This proline residue is completely conserved among homologs from different organisms.

The predicted PSA-1 protein is similar to the yeast SWI3 and its homologs in other organisms (Sawa *et al.* 2000). In yeast, Drosophila, and humans, the SWI3 family of proteins is known to be a component of the SWI/SNF complexes that are involved in remodeling chromatin structure by destabilizing the histone-DNA interaction (Kingston and Narlikar 1999). Rb interacts with certain SWI/SNF complexes to coordinate the sequential expression of E2F target genes to regulate progression through G_1 and S phases (Zhang *et al.* 2000). Previously, it has been shown that *psa-1* and other components of the SWI/SNF complex are required for asymmetric T cell divisions in *C. elegans* (Sawa *et al.* 2000).

To further investigate the synthetic phenotype of complete loss of both *lin-35* and *psa-1* genes, we inacti-

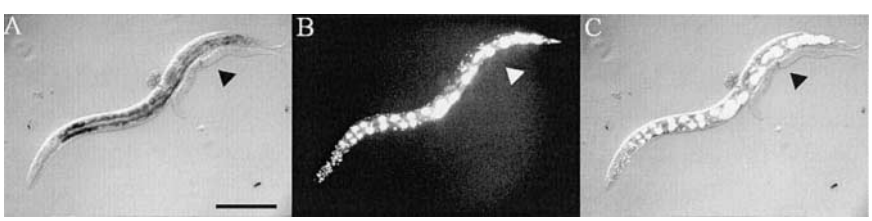

Figure 1.—Larval arrest caused by a synergistic effect of mutations in both *lin-35* and *psa-1*. Differential interference contrast (A), corresponding GFP fluorescence (B), and merged (C) images of *lin-35(n745); psa-1 (ku355)* animals with and without an extrachromosomal array containing wild-type copies of *lin-35*. The large adult with GFP fluorescence contains the array *kuEx119*. Arrowhead indicates the position of an arrested *lin-35*; *psa-1* larva that lost the extrachromosomal array. Bar, 100 μm.

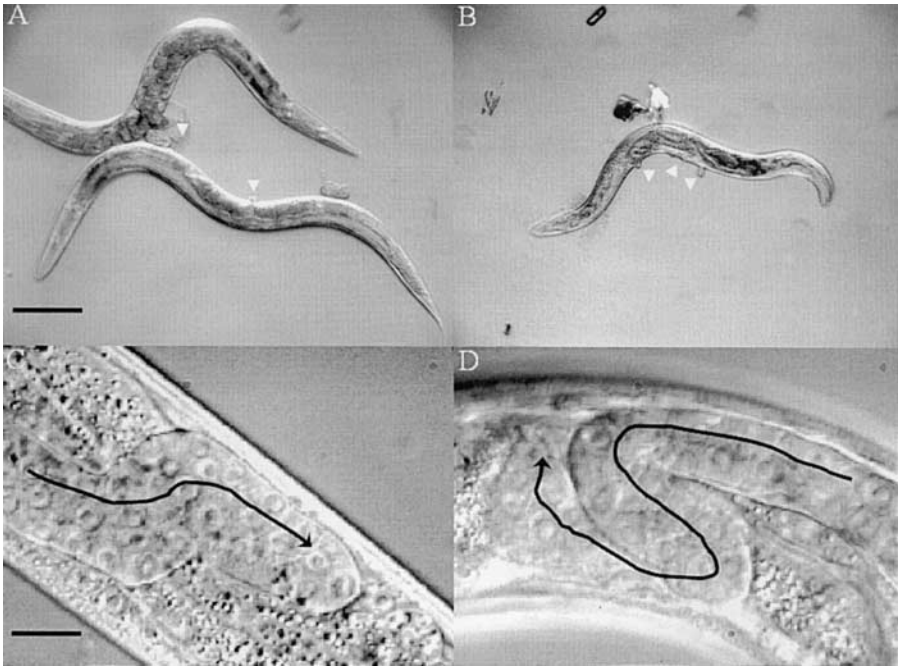

FIGURE 2.—Phenotypes of *psa-1(ku355)* at 20°. (A) Adult animal with a distinct protruding vulva (Pvl). (B) Adult animal with a Multivulva phenotype (Muv). Arrowheads indicate vulval tissue. (C and D) Animals with an abnormal U-turn of the gonad. Anterior is to the left. Bars, 100 μm in A (for A and B) and 10 μm in C (for C and D).

vated the *psa-1* gene in both wild-type and *lin-35(n745)* animals using the RNAi feeding method (see MATERIALS AND METHODS). When synchronized L1 wild-type animals were fed with *psa-1* dsRNA containing bacteria, a sterile phenotype associated with these P0 animals was observed (Table 2). However, when *lin-35(n745)* animals were treated the same way, a larval arrest phenotype was observed (Table 2). These animals arrested at the third (L3) or the fourth (L4) larval stage as determined by the size and shape of the gonad ($n = 80$).

TABLE 2

Identification of *C. elegans* homologs of subunits of the SWI/SNF complex and their RNAi phenotype in N2 and *lin-35(n745)*

S. cerevisiae[a]	*Drosophila*	*C. elegans*	S/I%[b]	wt N2[c] (*n*)	*lin-35(n745)*[c] (*n*)
Swi1/Adr6	ELD/OSA	C01G8.9	31/10	P0: Ste Pvl (9)	P0: Ste Pvl (70)
Swi2/Snf2	BRM	PSA-4	58/43	F_1: WT (>300)	F_1: WT (>300)
Swi3	MOIRA	PSA-1	37/30	P0: Ste Pvl (9)	P0: Lva (80)
Snf5	SNR1	R07E5.3	66/50	P0: Ste Pvl (9)	P0: Ste Pvl (74)
Swp73	BAP60	C18E3.2	61/45	F_1: Stp Pvl (241)	F_1: Lva (92)
Swp73	BAP60	ZK1128.5	64/49	F_1: Stp Pvl (245)	F_1: Lva (98)
Arp9	BAP55	ZK616.4	69/44	F_1: Stp Pvl (260)	F_1: Lva (115)
	BAP111	Y71H2AM.17	25/16	F_1: Stp Pvl (210)	F_1: Lva (120)
	BAP74	F26D10.3	89/80	P0: Lva (9)	P0: Lva (80)
Arp7	ACT1/2	ACT-1	99/97	P0: Ste Pvl (9)	P0: Ste Pvl (76)
	ACT1/2	ACT-2	99/97	P0: Ste Pvl (9)	P0: Ste Pvl (78)
	ACT1/2	ACT-3	99/97	P0: Ste Pvl (9)	P0: Ste Pvl (60)
	ACT1/2	ACT-4	99/97	P0: Ste Pvl (9)	P0: Ste Pvl (66)
	ACT1/2	ACT-5	97/93	P0: Ste Pvl (9)	P0: Ste Pvl (70)

[a] In *Saccharomyces cerevisiae*, there are three more subunits, Snf6, Snf1, Swp29, which are not present in Drosophila and *C. elegans*.

[b] S, similarity; I, identity. Whole protein sequences of the *C. elegans* homologs were compared to the counterparts of Drosophila using the MacVector program.

[c] Synchronized L1 worms (P0) were placed on RNAi plates and their phenotypes were scored. If those P0's were not sterile, their progeny's (F_1) phenotypes were scored. For wild type, three P0's were placed on each plate. For the *lin-35* mutant, ~24 worms were placed on each plate. Experiments for all samples were done in triplets. All phenotypes listed are 100% penetrant. For RNAi in the *lin-35* background, partial Ste/Pvl phenotypes were observed in P0's for C18E3.2, ZK1128.5, Zk616.4, and Y71H2AM.17. Ste, sterile; Stp, sterile progeny; Pvl, protruding vulvae; Lva, larval arrest; *n*, number of animals examined.

***lin-35* interacts with other components of the SWI/SNF complex:** To further confirm that the SWI/SNF complex and *lin-35/Rb* collaboratively regulate larval development in *C. elegans*, we examined the possibility that loss of function of other components of the SWI/SNF complex may lead to synthetic phenotypes with *lin-35(n745)*. A BLAST search of the *C. elegans* genome identified the homologs for a number of subunits of the SWI/SNF complex in Drosophila and yeast (Table 2). In the case of Swp73/BAF60, two *C. elegans* proteins, C18E3.2 and ZK1128.5, which are 45 and 49% identical to the Drosophila homologs, were identified. There are also five actin proteins in *C. elegans* that display high homology to Drosophila ACT1/2. In addition, the predicted nematode protein C01G8.9 displays weak homology to the Drosophila ELD/OSA protein.

We performed RNAi analysis with the genes coding for potential SWI/SNF subunits in both wild-type and *lin-35 (n745)* animals to test for synthetic mutational effects. *psa-1(RNAi)* in wild-type animals led to a sterile (Ste) and Pvl phenotype in the P0 worms. RNAi of four other genes, C18E3.2, ZK1128.5, ZK616.4, and Y71H2AM.17, led to Pvl and sterile phenotypes in the F_1 generation in the wild-type background. However, no larval arrest phenotype was observed when RNAi was applied to wild-type worms for any of these genes. In contrast, RNAi of all five of these genes produced the synthetic larval arrest phenotype in the *lin-35(n745)* background (Table 2). When RNAi was applied to *lin-35(n745)* animals (Table 2), 100% of larval arrest of P0 worms (for *psa-1*) or of the F_1 generation (for the other four genes) was observed. These results indicate that *lin-35/Rb* cooperates with the SWI/SNF complex to control *C. elegans* larval development.

The results are more complicated for several other genes, the five actin genes, C01G8.9, R07E5.3, and F26D10.3. RNAi of these genes produced a complete sterile phenotype or complete larval arrest in the P0 animals in both the wild-type and the *lin-35(n745)* background (Table 2). One exception was that RNAi of *psa-4*, encoding a homolog of yeast Swi2/Snf2 and Drosophila *brm*, failed to produce any obvious phenotype either in the wild-type or in the *lin-35* mutant background. In the *C. elegans* genome, there are >20 proteins with the Snf2 motif. It is possible that some of these proteins have redundant functions with PSA-4.

***psa-1* interacts with a subset of synMuv B genes:** *lin-35* is a member of a large synthetic Multivulva (synMuv) gene family. synMuv genes are commonly assigned to one of two classes, A or B. In general, animals carrying mutations in genes from either class undergo normal vulval development, as do most double mutants containing two mutations in the same class. However, animals containing mutations in both classes (A and B) display a Muv phenotype, the result of extra vulval cell inductions (Ferguson and Horvitz 1989). To determine the spectrum of *psa-1* genetic interactions, we inactivated synMuv genes in both wild-type and *psa-1* mutants by the RNAi feeding method and assayed for the larval arrest phenotype (Table 3). RNAi of the two class B genes, *lin-9* and *lin-37* (Beitel *et al.* 2000; Boxem and van den Heuvel 2002), in *psa-1(ku355)* animals produced a highly penetrant larval arrest phenotype similar to that observed in *lin-35*; *psa-1* double mutant. In the control experiment, no larval arrest was observed in the wild-type background. RNAi of the RbAp46/48 homolog, *lin-53* (Lu and Horvitz 1998), histone deactylase, *hda-1* (Lu and Horvitz 1998; Solari and Ahringer 2000; Dufourcq *et al.* 2002), and *let-418* (von Zelewsky *et al.* 2000; Solari and Ahringer 2000) rendered all P0's of wild-type animals sterile (for *lin-53* and *hda-1*) or complete larval arrest in the F_1 generation (for *let-418*), thus preventing the test of the synthetic interaction between these three genes and *psa-1*. RNAi treatment of another class B gene, *lin-36* (Thomas and Horvitz 1999), and a class A gene, *lin-15a*, failed to produce the larval arrest phenotype in either the *psa-1* mutant or wild-type animals (Table 3). To further verify the RNAi results, we also constructed double mutants containing *psa-1(ku355)* and a mutation in *lin-37*, *lin-36*, or *lin-15a*. At 20°, *psa-1; lin-37* double-mutant animals displayed 100% larval arrest, while double-mutant animals of *psa-1* with *lin-36* or *lin-15a* were indistinguish-

TABLE 3

Genetic interactions between *psa-1* and synMuv genes

	n	% larval arrest[a]
N2 (wild type)	500	0
psa-1(ku355)	736	17
lin-35(n745); psa-1(ku355)[b]	800	98
N2; *lin-35(RNAi)*	280	0
psa-1(ku355); lin-35(RNAi)	239	95
N2; *lin-37(RNAi)*	270	0
psa-1(ku355); lin-37(RNAi)	254	84
lin-37(n758)	805	0
psa-1(ku355); lin-37(n758)[c]	740	100
N2; *lin-9(RNAi)*	280	0
psa-1(ku355); lin-9(RNAi)	271	93
N2; *lin-36(RNAi)*	250	0
psa-1(ku355); lin-36(RNAi)	260	15
lin-36(n766)	946	0
psa-1(ku355); lin-36(n766)	250	15
N2; *lin-15a(RNAi)*	280	0
psa-1(ku355); lin-15a(RNAi)	220	17
lin-15a(n767)	691	0
psa-1(ku355); lin-15a(n767)	240	15
lin-8(n111)	640	0
psa-1(ku355); lin-8(n111)	300	17

[a] Percentage of hatched animals arrested at larval stages. The experiments were performed at 20°.

[b] *lin-35*; *psa-1* animals were the progeny of *lin-35*; *psa-1*; *kuEx119*.

[c] *lin-37*; *psa-1* embryos were grown at 15° for 6 hr and then shifted to 20°.

able from *psa-1* single mutants with respect to larval development (Table 3). Concurrently, we also constructed a double mutant between *psa-1(ku355)* and *lin-8(n111)* and found no synthetic larval arrest phenotype associated with it (Table 3).

Several synMuv genes, including *lin-35*, *lin-36*, and *lin-9*, are involved in the repression of cell proliferation and regulation of the G_1 to S phase transition (Boxem and van den Heuvel 2002). In contrast, *lin-37*, *let-418*, *lin-53*, *hda-1*, and synMuv A genes (including *lin-15a*, *lin-8*, and *lin-38*) do not appear to function in cell cycle control (Boxem and van den Heuvel 2002). We have shown here that *lin-36, lin-8*, and *lin-15a* mutations do not cause the synthetic larval arrest phenotype with the *psa-1* mutation, as do *lin-35*, *lin-37*, and *lin-9*. Taken together, these results are consistent with an idea that the synthetic larval arrest phenotype may not result from perturbation in cell cycle control *per se*, although these results firmly rule out this possibility.

Mutations in a subset of synMuvB genes enhanced the T cell lineage defects of *psa-1(ku355)*: In wild-type males and hermaphrodites, the bilaterally symmetric T cells divide during the early L1 stage to produce a hypodermal cell (T.aa) that joins the large hyp7 syncytium, a hypodermal blast cell that divides later in a sex-specific manner (T.ap), and a group of neural cells (derived from T.p). Cells T.paa and T.pap are the phasmid socket cells PHso1 and PHso2, which function to provide channels for the phasmid neurons PHA and PHB to communicate with the environment (Sulston and White 1980). When animals are soaked in the fluorescent dyes FITC or DiO, the two neurons of each of the two phasmids, as well as six of the eight amphidial neurons in the head, take up these dyes, an effect that can be visualized in living animals (Herman and Horvitz 1994). Abnormalities in the T cell lineage will cause defects in phasmids and there will often be no dye filling in the phasmids position. Therefore dye-filling assays of phasmids are well-established methods used to detect abnormalities in the T cell lineage (Herman and Horvitz 1994; Zhao *et al.* 2002).

Previously *psa-1* was shown to be required for asymmetric T cell division in *C. elegans*; at 18°, 16% of *psa-1 (os22)* animals had T cell lineage defects (Sawa *et al.* 2000). Since inactivation of *lin-35*, *lin-37*, and *lin-9* in the *psa-1(ku355)* background produced a dramatic larval arrest phenotype, we investigated the possibility of these genes interacting with *psa-1* in regulation of asymmetric T cell division. As RNAi of these synMuv genes in the *psa-1(ku355)* animals produced nearly 100% larval arrest at 20°, we performed the dye-filling assay on the animals beyond the fourth larval stages at 18°. A total of 50% of the phasmids in *psa-1(ku355)* animals were found to be filled with FITC (Table 4). In contrast, only 11% of the phasmids in the *lin-35(n745); psa-1(ku355)* animals at the same stage were filled with FITC (Table 4). After inactivating *lin-35*, *lin-37*, and *lin-9* in *psa-1(ku355)* animals by RNAi, a very low percentage of the phasmids were found to be filled with FITC (Table 4). We also determined the T cell lineage in the *psa-1(ku355)* single and *lin-35; psa-1* double mutants at 18°. Two of the seven *psa-1* mutant animals examined displayed abnormal T cell lineage, while four of the six *lin-35; psa-1* mutant animals displayed abnormal T cell lineage (Figure 3). These results are consistent with the idea that these class B synMuv genes play a role in the regulation of T cell divisions.

TABLE 4

Genetic interactions between *psa-1* and synMuvB genes in regulating T cell division

	n	% phasmids filling with FITC[a]
N2 (wild type)	200	99
psa-1(ku355)	120	50[b]
lin-35(n745); psa-1(ku355)	100	11[c]
N2; *lin-35(RNAi)*	150	99
psa-1(ku355); *lin-35(RNAi)*	150	24[d]
N2; *lin-37(RNAi)*	120	98
psa-1(ku355); *lin-37(RNAi)*	100	29[d]
N2; *lin-9(RNAi)*	125	99
psa-1(ku355); *lin-9(RNAi)*	150	25[d]

[a] Animals were grown at 18°.
[b] $P < 0.001$. *P*-value was derived from comparing data from *psa-1* mutants with those from N2.
[c] $P < 0.001$. *P*-value was derived from comparing data from *psa-1(ku355); lin-35(n745)* with those from *psa-1(ku355)* alone.
[d] $P < 0.01$. *P*-values were derived from comparing data from *psa-1(ku355)* following RNAi of three different synMuv genes with those from *psa-1(ku355)* alone.

***psa-1* does not function as a class A or class B synMuv gene, but its mutation enhances the Muv phenotype of *lin-15(n765)*:** Only 1% of the *psa-1(ku355)* single mutants displayed a Muv phenotype ($n = 309$). To determine if *psa-1* acts as a class A or class B synMuv gene, we constructed double mutants containing *psa-1(ku355)* and either a class A or a class B synMuv mutation. For double mutants with class A mutations, *lin-15a(n767)* and *lin-8(n111)* that display no Muv phenotype on their own were used. Double mutants containing either of these class A synMuv mutations exhibited no synthetic Muv phenotype (data not shown), indicating that *psa-1* is not a class B synMuv gene. For double mutants with class B mutations, *lin-35(n745)*, *lin-37(n758)*, *lin-36 (n766)*, and *lin-15b(n744)* were used. No synthetic Muv phenotype was observed in any of these double-mutant strains, further indicating that *psa-1* is not a class A synMuv gene. However, *psa-1(ku355)* displayed a significant interaction with a hypomorphic mutation in the *lin-15(n765)* (Ferguson and Horvitz 1989). The *lin-15* locus is an operon containing a class A synMuv gene, *lin-15a*, and a class B synMuv gene, *lin-15b* (Clark *et al.* 1994; Huang *et al.* 1994). *lin-15(n765)* is a temperature-sensitive allele

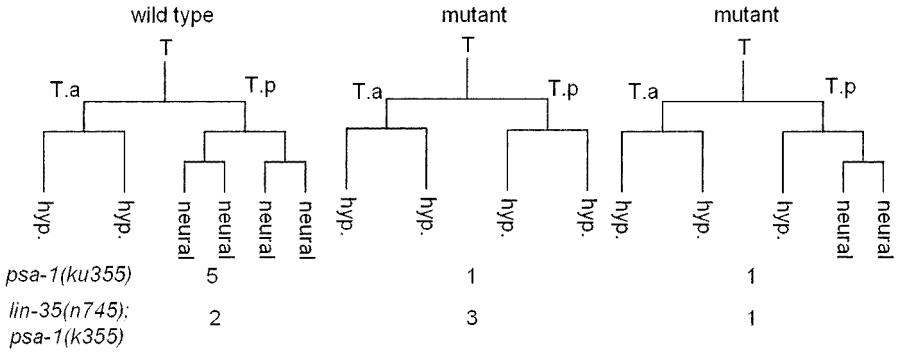

FIGURE 3.—Abnormal T cell lineages in *psa-1(ku355)* and *lin-35(n745); psa-1(ku355)* mutants during the L1 stage. All animals were grown at 18°. The numbers of animals that showed the corresponding lineages are indicated below the diagrams. The fate of cells was determined by nuclear morphology (HERMAN and HORVITZ 1994). hyp, hypodermal.

that causes a strong Muv phenotype (100%, $n = 300$) at 20°, but displays only 27% of Muv phenotype ($n = 324$) at 15°. The severity of the Muv phenotype of this allele at different temperatures reflects the relative level of *lin-15* gene activity (HSU and MEYER 1994). At 15°, 78% of *psa-1(ku355); lin-15(n765)* double mutants were Muv ($n = 400$), suggesting that *psa-1* may play an important role in *lin-15* gene expression or the *lin-15*-mediated repression of vulval differentiation.

DISCUSSION

Using a genetic screen to identify mutations that are synthetically lethal with *lin-35/Rb*, we have isolated a mutation in *psa-1*, a gene encoding a central component of the SWI/SNF complex. Strong loss-of-function mutations in *lin-35* cause only subtle effects on viability and larval development (LU and HORVITZ 1998; FAY *et al.* 2002). A reduction-of-function mutation in *psa-1* causes the low-penetrance larval arrest. However, *lin-35*; *psa-1* double-mutant animals are almost completely arrested in the L1 larval stage. Inactivation of several other components of the SWI/SNF complex in the *lin-35(n745)* background by RNAi also causes a synthetic larval arrest phenotype. These results revealed a previously unknown function of *lin-35/Rb* in larval development and indicated that *lin-35/Rb* functions concertedly with the SWI/SNF complex. The expression patterns of both *lin-35* and *psa-1* genes are also consistent with their roles in larval development. LIN-35 has been determined to be expressed throughout development in most or all cell types by antibody staining (LU and HORVITZ 1998). Through the use of a functional *psa-1::GFP* construct, *psa-1* has been shown to be expressed ubiquitously in the nuclei of all somatic cells (SAWA *et al.* 2000).

Genetic studies in Drosophila showed that the SWI/SNF complex downregulates E2F activity (STAEHLING-HAMPTON *et al.* 1999). These studies imply that, like Rb family members, SWI/SNF proteins function in the regulation of E2F activity. Transfection studies indicate that class I HDACs and SWI/SNF are recruited by Rb and may coordinate the sequential expression of E2F target genes to regulate progression through G_1 and S phases (ZHANG *et al.* 2000). Our study presented here shows for the first time that Rb functions in a redundant manner with the SWI/SNF complex to regulate animal development *in vivo*. As we have obtained no evidence for cellular hyperproliferation in *lin-35; psa-1* double mutants, the synthetic larval arrest does not appear to be caused by overexpression of E2F cell cycle targets. Interestingly, however, E2F's have been reported to regulate a number of non-cell cycle genes (ISHIDA *et al.* 2001; MULLER *et al.* 2001) and has been shown in *C. elegans* to play a role in the establishment of early asymmetries in the embryo (PAGE *et al.* 2001).

SAWA *et al.* (2000) have already reported that the SWI/SNF complex is involved in regulating T cell division. *egl-27*, which encodes a protein homologous to MTA1 (a component of NURD complex), has also been found to be required for T cell division (HERMAN *et al.* 1999). The observation that the loss-of-function mutation in *lin-35/Rb* strongly enhanced the T cell division defects in a *psa-1* mutant allele suggests that *lin-35/Rb* may play a role in T cell division. Since *lin-35(lf)* alone has no defects in T cell division, we propose that *lin-35* and the SWI/SNF complex may function redundantly to affect the expression of a common set of targets. It has been shown that T cell division is regulated by Wnt signaling (HERMAN *et al.* 1995; SAWA *et al.* 1996; ROCHELEAU *et al.* 1999; ZHAO *et al.* 2002). It is also known that Wnt signaling and synMuv pathways are both involved in vulval induction in *C. elegans* (GLEASON *et al.* 2002). The elucidation of the interaction between *lin-35* and Wnt signaling in T cell division could be crucial in understanding how *lin-35* acts in asymmetric cell divisions.

The exact cause of the synthetic larval arrest of *lin-35; psa-1* double mutant is presently unknown. While a *lin-35* mutation can enhance the defects in the T cell asymmetric division of a *psa-1* mutant, these defects alone cannot be responsible for the larval arrest. On the basis of the pleiotropic phenotypes of these mutant animals, it is hard to assign the cause of the synthetic larval arrest to one specific developmental event. The discovery of common targets of *lin-35/Rb* and the SWI/SNF complex will be a key step toward understanding the mechanism of the synthetic larval arrest.

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