The *Drosophila* SNR1 (SNF5/INI1) Subunit Directs Essential Developmental Functions of the Brahma Chromatin Remodeling Complex

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The *Drosophila melanogaster* Brahma (Brm) complex, a counterpart of the *Saccharomyces cerevisiae* SWI/SNF ATP-dependent chromatin remodeling complex, is important for proper development by maintaining specific gene expression patterns. The SNR1 subunit is strongly conserved with yeast SNF5 and mammalian INI1 and is required for full activity of the Brm complex. We identified a temperature-sensitive allele of *snr1* caused by a single amino acid substitution in the conserved repeat 2 region, implicated in a variety of protein-protein interactions. Genetic analyses of *snr1^{E1}* reveal that it functions as an antimorph and that *snr1* has critical roles in tissue patterning and growth control. Temperature shifts show that *snr1* is continuously required, with essential functions in embryogenesis, pupal stages, and adults. Allele-specific genetic interactions between *snr1^{E1}* and mutations in genes encoding other members of the Brm complex suggest that *snr1^{E1}* mutant phenotypes result from reduced Brm complex function. Consistent with this view, SNR1^{E1} is stably associated with other components of the Brm complex at the restrictive temperature. SNR1 can establish direct contacts through the conserved repeat 2 region with the SET domain of the homeotic regulator Trithorax (TRX), and SNR1^{E1} is partially defective for functional TRX association. As truncating mutations of *INI1* are strongly correlated with aggressive cancers, our results support the view that SNR1, and specifically the repeat 2 region, has a critical role in mediating cell growth control functions of the metazoan SWI/SNF complexes.

The coordination between cell proliferation and terminal differentiation is of critical importance during the development of multicellular organisms. The metazoan SWI/SNF-related protein complexes function in both of these processes in a broad range of tissues by altering gene expression at specific promoters through ATP-dependent chromatin remodeling (32). The *Saccharomyces cerevisiae* SWI/SNF complex is a ~2-MDa multisubunit assembly capable of ATP-dependent chromatin remodeling both in vitro and in vivo (53). Highly related complexes have also been identified in flies (23, 48) and mammals (73). Among the SWI/SNF complex proteins, none appears to be capable of sequence-specific DNA binding; thus, the precise mechanism for targeting the SWI/SNF complexes to their respective gene targets in vivo is not well understood. The complex can interact with transcription factors to facilitate SWI/SNF function at specific promoters (53). Interactions have been observed with steroid hormone receptors; EKLF (erythroid Kruppel-like factor), which activates the β-globin locus; C/EBPβ, which activates transcription of myeloid genes (35); c-Myc (13, 65); and HRX (56), a homolog of *Drosophila melanogaster* Trithorax (TRX) that regulates expression of the homeotic genes. Depending on the chromatin context of the binding site, some transcription factors may initially bind to

their recognition site and then recruit SWI/SNF to alleviate chromatin repression at the promoter, or conversely, the factor may require SWI/SNF activity for binding. While there is presently very little known about what types of protein interactions might promote such recruitment, several complex subunits may function coordinately to mediate these associations (46). Further, mounting evidence suggests that the various SWI/SNF and related complexes might function in both gene activation and repression (22, 64).

Studies of the fly Brm and mammalian hBrm/Brg1 complexes have revealed widespread and important biological roles in development (45, 66). In addition to transcriptional activation, the complex functions in regulating cell cycle progression through interactions with the retinoblastoma (Rb) protein and/or cyclin-cdk complexes (7).

The purified Brm complex contains at least eight proteins, including BRM, MOR, SNR1, and OSA (15, 48). While *brm*, *mor*, and *osa* were identified in genetic screens as dosage-dependent activators of homeotic gene transcription, counteracting the repressive effects of the Polycomb group of genes (31), none of the other Brm complex genes was identified in any genetic screen. The *snr1* gene (*SNF5-Related-1*) was identified (23) by molecular means based on its similarity to the human *INI1/SMARCB1/hSNF5* gene, which encodes a core subunit of the hBrm and Brg1 complexes. Both *snr1* and *INI1* are strongly conserved with *SNF5*, which encodes an essential component of the yeast SWI/SNF complex, and SNR1, INI1, and SNF5 are required for full function of their respective complexes in vivo and in vitro (23, 38, 39, 55). While *brm*, *mor*, and *osa* have dosage-sensitive mutant phenotypes in combination with other gene mutations that can be examined in the

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imaginal tissues, such as leg and wing disks (8, 9, 24, 67), the existing *snr1* null mutations do not display significant dosage sensitivity in most genetic assays, apparently inconsistent with SNR1 serving as a core subunit of the Brm complex and making analysis of this essential gene very difficult. Thus, the *snr1* gene product is either in excess of that required to assemble the Brm complex, has unidentified functions independent of the complex, or has a limited role at a subset of target genes and thus is dispensable for some Brm complex functions. To help distinguish among these possibilities, we carried out a large-scale noncomplementation chemical mutagenesis screen to identify conditional mutant alleles of *snr1* (77), and we report here the molecular and genetic characterization of *snr1^{E1}*, which exhibits recessive lethality at 29°C and a variety of temperature-dependent phenotypes. Our genetic and biochemical characterizations of *snr1^{E1}* reveal that *snr1* has essential functions in the development of many tissues including the peripheral nervous system, eye, and wing veins, and surprisingly, it is also required for sustained adult viability. Further, our analyses provide strong links among *snr1* function, chromatin remodeling, and the regulation of cell proliferation, confirming its predicted role in restricting cell growth during normal development.

MATERIALS AND METHODS

Fly stocks. All stocks were maintained on a standard cornmeal medium at 18°C, except for *snr1^{E1}*/TM6B flies, which were maintained at 29°C. The *snr1^{E1}* and isogenic *red*, *e*/*red*, *e* strains were generated as described elsewhere (76). All other fly strains, markers, and special chromosomes used were as described in Flybase (http://flybase.bio.indiana.edu).

Molecular analysis of the *snr1^{E1}* mutation. Genomic DNA was isolated (23) from *snr1^{E1}*/*snr1^{E1}* adult flies at 18°C. Specific primers flanking the *snr1* open reading frame were used for PCR amplification. Products were cloned and fully sequenced on an ABI3700 sequencer (BioResource Center, Cornell University, Ithaca, N.Y.).

Viability and mutant phenotype analyses. The viability of homozygous *snr1^{E1}* flies at 18, 25, and 29°C was determined by crossing balanced heterozygotes and then determining the percentage of nonbalanced flies eclosed relative to siblings. The developmental period at which lethality first occurred was determined by crossing flies for 24 h at 18°C, turning them into fresh bottles, and collecting eggs every 24 h over 3 days. The crosses were then shifted to 29°C for 4 additional days, and eggs were similarly collected. Following hatching (~24 h after egg laying), first-instar larvae were transferred to vials and allowed to develop at either 18 or 29°C. As controls, progeny from each cross were also maintained at the original temperature. The number of individual larvae and pupae of each genotype was counted at different developmental stages. Homozygous *snr1^{E1}* white prepupae raised at 18°C were collected and shifted to 29°C for the remainder of development to determine the precise lethal phase during metamorphosis according to the staging of Bainbridge and Bownes (3). Adult longevity was measured by collecting newly emerged adult flies (<8 h posteclosion at 18°C) and placing them in vials at 29°C. The number of dead flies in each vial was scored every 24 h to determine the percent viability per genotype each day.

Wings were dissected in 100% isopropanol, placed in DPX mounting fluid (Fluka Chemika), and examined on a Zeiss Axioscope microscope at 100× magnification. Adult cuticles were examined by mounting them in Hoyer's medium. For scanning electron microscopy analysis flies were dehydrated in acetone and examined on a JEOL JSM5800LV scanning electron microscope at 10 kV.

Biomass differences in weight, size, and cell number were determined from progeny raised at 18°C from self-crosses, including (i) *snr1^{E1}*/TM3, (ii) *snr1^{E1}*/*red*, *e*, and (iii) *w^−* ; *P*[*snr1^+*]/CyO; *snr1^{E1}*/TM3. Male progeny were collected each day and aged an additional 3 days, and then pools of 10 flies (>100 flies/genotype) were weighed to determine the average weight per fly. Wing size and cell number determinations were made for five wings of each genotype. For wing area measurements, 40× digital images were outlined with Adobe Photoshop (v5.0), and the number of pixels corresponding to each wing was determined and converted into square millimeters by dividing the number of pixels in the wing by the known number of pixels in a square millimeter at the same magnification.

Digital images were generated at 200× magnification from the same five wings, and the number of hairs in a constant premeasured area of each wing was counted to determine cell number (42).

Temperature shift analysis. *snr1^{E1}*/TM6B flies were crossed at either 18 or 29°C and turned into fresh vials every 24 h. Eggs laid within each 24-h period were aged at the original incubation temperature for the appropriate length of time in 24-h increments and then shifted to the opposite temperature and allowed to develop. Alternatively, flies were raised continuously at 18°C and then shifted to 29°C for 24 h at specific intervals prior to downshifting to 18°C and allowing for the completion of development. The number of *snr1^{E1}*/*snr1^{E1}* adult flies was scored relative to balanced siblings for each 24-h interval to determine percent lethality. The number of wings showing ectopic veins in *snr1^{E1}*/+ flies was scored and compared to the total number of *snr1^{E1}*/+ wings at each time point. Developmental staging at 18°C was as follows: embryogenesis, 0 to 24 h; first larval instar, 24 to 72 h; second larval instar, 72 to 120 h; third larval instar, 120 to 216 h; pupation, 216 to 456 h; eclosion (adults), >456 h. The relative developmental staging at 29°C was 0 to 24, 24 to 48, 48 to 72, 72 to 120, 120 to 192, and >192 h. As *snr1^{E1}*/*snr1^{E1}* larvae have a 48- to 72-h developmental delay during the third-larval-instar stage, viability data from each shift during and after that stage were accordingly adjusted.

The stability and accumulation of SNR1^{E1} were measured following each shift by Western blotting with extracts prepared from pupae prior to eclosion that correlated with the shift points (18 to 29°C) or from pupae raised continuously at 18 or 29°C. Fractionated proteins were probed with affinity-purified anti-SNR1, with chemiluminescent detection with horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Labs, West Grove, Pa.).

Protein interaction studies. Immunoprecipitations were carried out with embryonic or pupal extracts (23). Following precipitation with affinity-purified rabbit anti-SNR1 antibodies, proteins were fractionated on sodium dodecyl sulfate–4 to 12% polyacrylamide gels, transferred to nitrocellulose, and probed with affinity-purified rabbit anti-BRM (77), mouse anti-OSA (15), or rat anti-SNR1 (23). Detection was performed with appropriate horseradish peroxidase-conjugated secondary antibodies with enhanced chemiluminescence (Supersignal; Pierce Chemical).

Yeast interaction trap assays were used to define the regions of SNR1 that associated with the SET domain of TRX. Full-length SNR1 (amino acids [aa] 44 to 370) and deletion derivatives were produced as fusions to the GAL4 activation domain (GAL4AD) in pACTII and verified by Western blotting with anti-GAL4 antibodies (Babco Inc.). A C-terminal portion of TRX (aa 3534 to 3759), including the entire SET domain (aa 3610 to 3759), was fused to the GAL4 DNA binding domain in pAS1-CYH (Clontech, Palo Alto, Calif.). The SNR1^{E1} (G256D) fusion protein was generated by replacing a portion of the wild-type *snr1* gene (*Bam*HI-*Sal*I) with the corresponding fragment cloned from *snr1^{E1}*. Yeast strains of opposite mating types (*Y187*, *Y190*) carrying each fusion were mated to produce diploids. Interactions were assayed by growth on His(−) medium supplemented with 25 mM 3-aminotriazole and measurement of *lacZ* activity (Bio-Rad, Hercules, Calif.). Each combination was tested a minimum of three times, and the results were averaged. Nonbiased PCR mutagenesis of full-length GALAD-SNR1 was carried out as described previously (72) followed by gap repair to identify residues important for SET domain contacts. Noninteracting GAL4AD-SNR1* fusions were identified by an inability to grow on His(−) plates after the appropriate matings. Selected isolates were assayed for *lacZ* activity, tested for production of full-length SNR1 protein by Western blotting, and then subcloned and sequenced.

RESULTS

Identification of a *snr1* conditional allele. We previously identified P-element-induced *snr1* null mutations (*snr1^{R3}*, *snr1^{SR21}*) that truncated the protein at aa 131, removing the region conserved among all SNF5 family members (23, 77). We also carried out a large-scale chemical mutagenesis noncomplementation screen and identified four new alleles of *snr1* including one temperature-sensitive (TS) mutant (*snr1^{E1}*) that displayed nearly complete recessive lethality at the 29°C restrictive growth temperature, an intermediate lethality at 25°C, and viability at 18°C (Fig. 1A) (Table 1). Homozygous *snr1^{E1}* flies exhibited a 48-h developmental delay at both the permissive and restrictive temperatures that appeared in the third-larval-instar stage and persisted throughout the remainder of
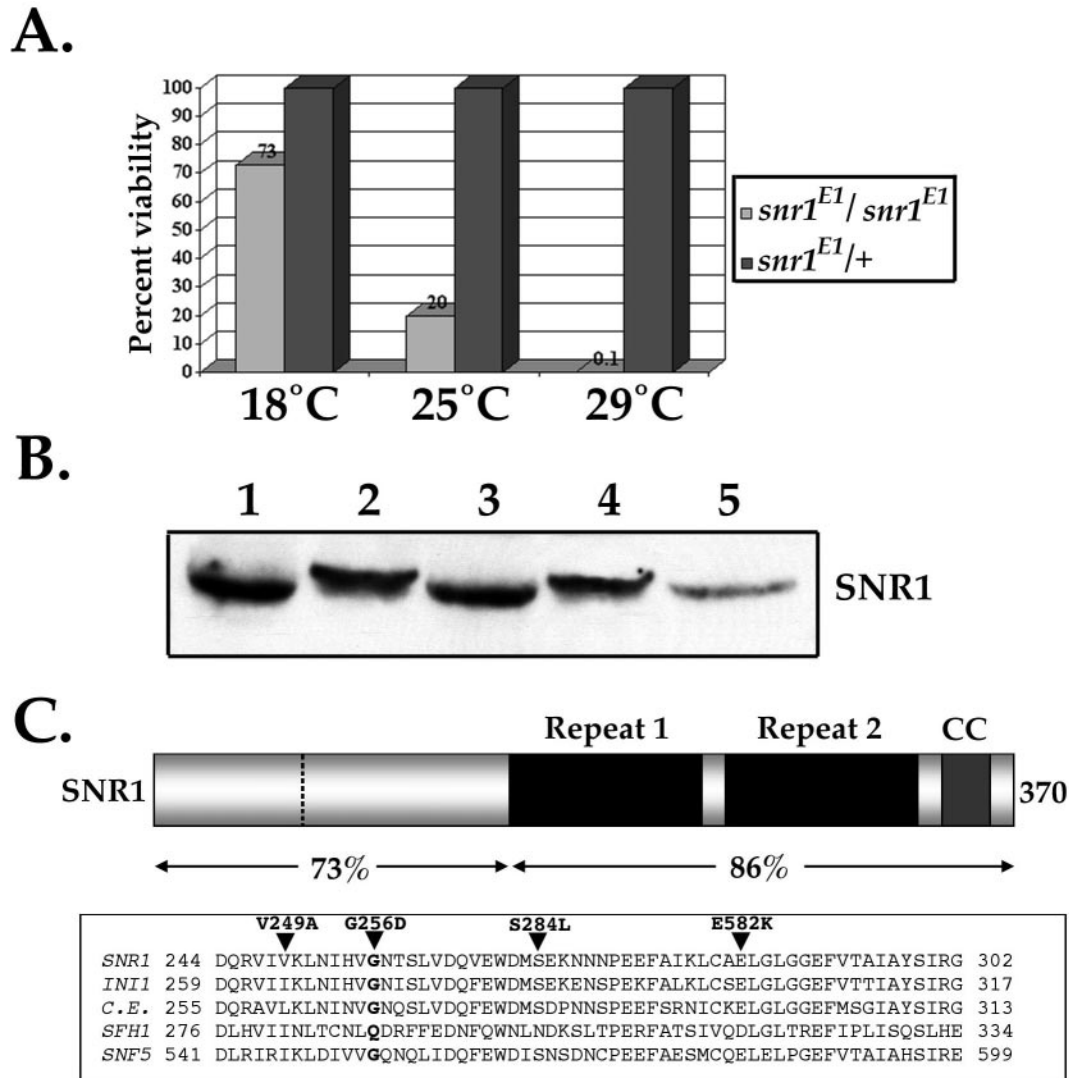
FIG. 1. Temperature sensitivity and molecular characterization of *snr1^E1*. (A) Percent viability of *snr1^E1/snr1^E1* and *snr1^E1/+* sibling flies at different incubation temperatures. (B) Western blot of pupal-stage extracts (100 μg). SNR1 (lanes 1 and 3), SNR1^E1 (lanes 2 and 4), and SNR1^R3 (lane 5) at 18°C (lanes 1 and 2) and 29°C (lanes 3 to 5) were probed with anti-SNR1 antibodies. The SNR1^E1 protein displayed a reduced mobility relative to that of the wild type. (C) SNR1 protein structure. Conserved domains include repeat 1, repeat 2, and a coiled-coil region (CC). The dashed line indicates the intron location. The amino acid similarity between SNR1 and INI1 is indicated below the diagram. Boxed is an alignment of repeat 2 from SNF5-family proteins, including SNR1 (*D. melanogaster*), INI1 (*Homo sapiens*), CeSNF5 (*Caenorhabditis elegans*), and SNF5 and SFH1 (*S. cerevisiae*). Shown are the known positions of affected repeat 2 residues in SNR1^E1 (G256D) and mutations in INI1 (S284L), SNF5 (E582K), and SFH1 (D317K).

development. A wild-type genomic transgene copy of *snr1* fully restored viability to both the *snr1* null mutations (77) and *snr1^E1* homozygotes at 29°C, demonstrating that the temperature sensitivity was due to disruption of normal *snr1* function (data not shown). However, unlike null alleles, full rescue of *snr1^E1* required two copies of the transgene. SNR1 protein levels and stability were identical in both wild-type and *snr1^E1* mutants at both the permissive and restrictive temperatures as determined by immunoblot analysis with pupal extracts (Fig. 1B). We therefore conclude that the *snr1^E1* mutation functions as an antimorph. A single guanine-to-adenine transition was the only detectable mutation associated with the *snr1^E1* allele (Fig. 1C), which resulted in a glycine-to-aspartic acid alteration (G256D) within the highly conserved (~90%)

repeat 2. A glycine at this position is found in all SNF5-related proteins except SFH1 (Fig. 1C), which is a core component of the RSC complex (11, 12).

snr1 function is required throughout development. Expression of the *snr1* gene is widespread, with ubiquitous nuclear distribution of the encoded protein in early syncytial embryos that becomes more restricted to late-dividing and differentiating tissues, including the central nervous system and brain (23). There is also a strong maternal contribution of mRNA, and zygotic expression showed modest fluctuations in accumulation throughout development. Null alleles of *snr1* exhibit early larval lethality with no obvious defects, presumably owing to high levels of *snr1* mRNA deposited into the oocyte that provide sufficient *snr1* product to complete embryogenesis.

TABLE 1. Lethal-phase analysis of $snr1^{E1}$ homozygous
and heterozygous flies

Genotype (maternal, paternal) and temp (°C)[a]	No. observed at each stage:			
	Egg	Larva	Pupa	Adult
+/+, +/+				
18	185	164	156	154
29	95	76	70	69
$snr1^{E1}/snr1^{E1}$, +/+				
18	80	61	57	57
29	124	101	87	87
+/+, $snr1^{E1}/snr1^{E1}$				
18	121	92	89	88
29	191	80	77	77
$snr1^{E1}/snr1^{E1}$, $snr1^{E1}/snr1^{E1}$				
18	213	104	73	15
29	172	17	17	0
$snr1^{E1}/+$, $snr1^{E1}/+$				
29	246	217	204	136

[a] Incubation temperature at which the progeny were raised.
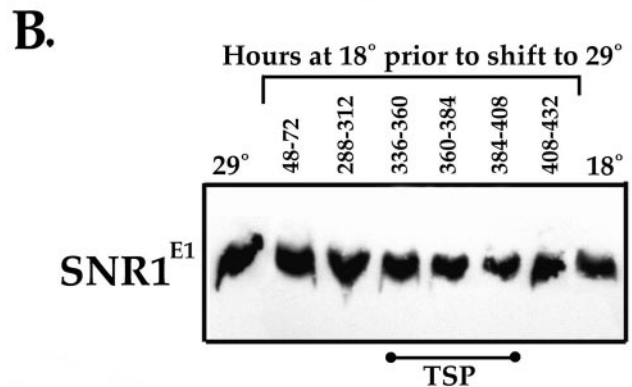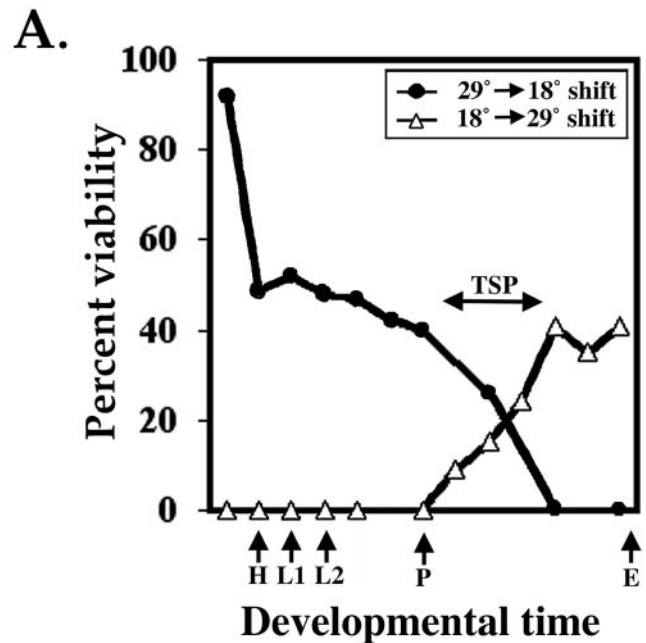

FIG. 2. TS period and lethal phase of $snr1^{E1}$. (A) TS periods (TSP) were observed during embryogenesis and pupal stages. H, hatching; L1, first larval instar; L2, second larval instar; P, pupariation; E, eclosion. (B) SNR1^{E1} synthesis and stability following temperature shift. Shown are results of Western blotting with anti-SNR1 against equivalent extract amounts from homozygous $snr1^{E1}$ pupae raised at 18 or 29°C or shifted at the times indicated. Time points corresponding to the TSP shown in panel A are indicated by the bar.

We determined the developmental stage at which the TS $snr1^{E1}$ allele caused recessive lethality. Balanced or homozygous flies were mated at 18°C for 3 days and then shifted to 29°C for an additional 4 days. Embryos were collected and allowed to develop at the temperature at which they were laid (18 or 29°C). Although homozygous $snr1^{E1}$ females were fertile, males exhibited a reduced fertility at 29°C. Progeny from homozygous parents exhibited embryonic lethality at both temperatures, though the effect was more significant at 29°C (Table 1). Mutant embryos were examined, and most had secreted a larval cuticle, indicating late embryonic lethality. Moreover, the embryonic lethality was rescued with a wild-type paternal copy of $snr1$ at both temperatures. Homozygous $snr1^{E1}$ progeny produced from heterozygous parents exhibited late-pupal-stage lethality when raised at 29°C. A significant proportion of $snr1^{E1}$ homozygous flies raised at 18°C exhibited late-pupal lethality when both parents were homozygous for the $snr1^{E1}$ allele. As the $snr1^{E1}$ mutant exhibits biphasic lethality, including critical periods during embryogenesis and pupal development, and an $snr1$-null mutant is lethal during larval growth, we can conclude that $snr1$ and Brm complex function is required throughout development.

To more precisely define the temporal requirements for $snr1$, we performed a series of temperature shift experiments. Heterozygous and homozygous $snr1^{E1}$ embryos were collected, reared at either 18 or 29°C, and later shifted to the opposite temperature at different stages for the remainder of development. Figure 2A shows the percentage of $snr1^{E1}$ homozygous adult flies that emerged following the shift at each developmental stage relative to their heterozygous siblings. The data show that there is a continuous requirement for $snr1$ function throughout development, with critical points during late embryogenesis and again during pupal growth. Shifts to 29°C at any point until midpupal stages resulted in almost complete lethality of $snr1^{E1}$ homozygotes. In contrast, $snr1^{E1}$ flies shifted from 29 to 18°C at any time prior to the midpupal stage had viability restored. We examined the accumulation and stability

of the SNR1^{E1} protein following temperature shift by Western blotting with extracts prepared from late-staged pupae (Fig. 2B). As controls, we also examined SNR1^{E1} from pupae raised continuously at 18 or 29°C and found that the SNR1^{E1} protein was stable and produced at wild-type levels following the shift at all stages examined. Thus, the SNR1^{E1} protein appears to be defective for function rather than synthesis or stability.

Homozygous $snr1^{E1}$ larvae raised continuously at 18°C were shifted to 29°C for 24-h intervals and then downshifted to 18°C to complete development to better define the critical TS period (Fig. 3A); thus, each time point represents the percentage of viable adults that emerged when shifted to the restrictive temperature for a discrete 24-h period. Shifts to 29°C prior to or immediately following pupariation ($\pm$24 h) resulted in almost complete lethality (Fig. 3A). This sensitive developmental pe-
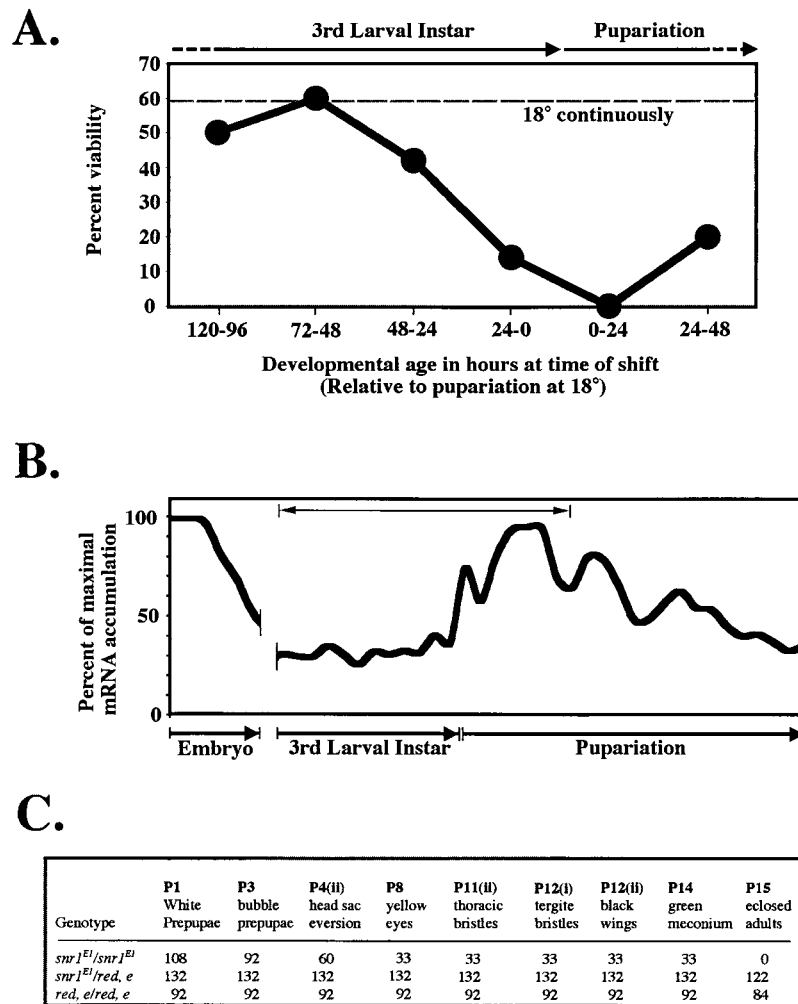
FIG. 3. Essential function of SNR1 during metamorphosis. (A) Temperature shift of $snr1^{E1}$ revealed a critically sensitive period during the transition from larval to pupal development. Flies (0- to 24-h collections) were raised at 18°C until the times indicated (expressed as hours of development relative to pupariation), shifted to 29°C for 24 h, and then shifted back to 18°C for the remainder of development. The percentage of emerged $snr1^{E1}$ homozygous adults relative to control siblings was calculated following each shift point and for flies raised continuously at 18°C (dashed line). The approximate developmental stage (at 18°C) relative to each shift point is indicated above the graph. (B) RNA accumulation of *snr1* during development. Northern blot analysis of *snr1* was performed with specific riboprobes hybridized to developmentally staged mRNA from wild-type flies raised at 25°C. Hybridization signals were quantified and plotted relative to maximal accumulation observed in 0- to 4-h embryos. The line above the graph represents the approximate developmental time period that correlates with the time points shown in panel A. (C) Homozygous $snr1^{E1}$ pupae exhibit broad lethality during metamorphosis at 29°C. Prepupae (P1) of the genotypes indicated were collected and allowed to complete development (P15). Values represent the numbers of individuals from the original cohort that displayed the morphological marker characteristic of the pupal stage indicated (3).

riod coincides with significant increases in wild-type *snr1* mRNA accumulation (Fig. 3B) and precisely corresponds with the onset of metamorphosis in flies that leads to the final elaboration of the adult structures. Consistent with these data, we observed a significant (70% of total) pupal lethality during the early stages of pupal metamorphosis subsequent to head eversion when flies were raised continuously at 29°C (Fig. 3C). Importantly, pupae that survived beyond these early stages (P4 to 8) continued to develop, although no adults emerged and dissection of the noneclosed pupae revealed only minor bristle defects (data not shown). The early pupal lethality is significantly later than that observed for strong loss-of-function mutations in other Brm complex genes, including null alleles of *snr1*, which result in death during late embryogenesis or early

larval stages and suggests a previously unknown essential requirement for *snr1* function for events that occur during metamorphosis. These pupal stages are associated with the last major cell proliferation and differentiation events that occur prior to eclosion (emergence of adults) and closely correlate with the time of new Brm complex synthesis.

Adult requirement for *snr1* function. Brm complex components, including *snr1*, are expressed in both adult males and females; in males there has been no obvious role for the complex, while in females it appears to be required for oogenesis (18, 24). Ectopic expression of *snr1* deletions suggested a broad functional role in sustaining adult viability (77). To address this question directly, newly emerged $snr1^{E1}/snr1^{E1}$ or $snr1^{E1}/snr1^{R3}$ adult flies raised at 18°C were shifted to 29°C to measure
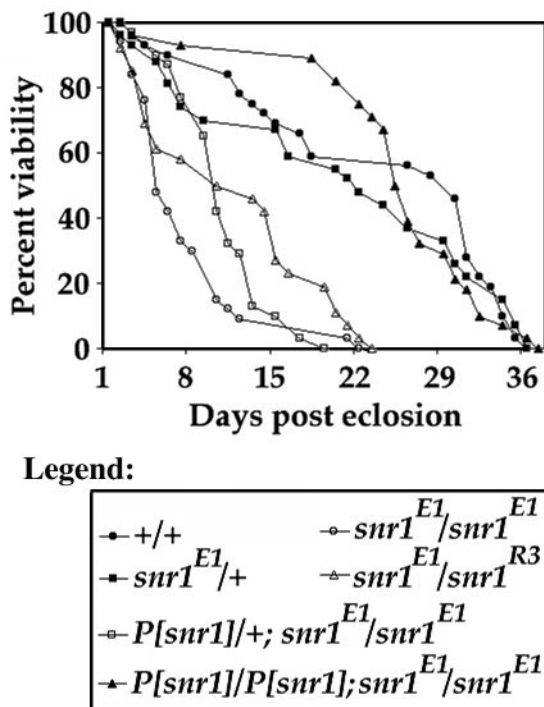
FIG. 4. *snr1* is required for sustained adult viability. Shown are the results of a comparison of longevity between wild-type and *snr1* mutant flies. Flies of each indicated genotype were incubated at 29°C, and the number of dead flies was scored every 24 h.

effects on life span (Fig. 4). Homozygous $snr1^{E1}$ adults and $snr1^{E1}/snr1^{R3}$ transheterozygotes showed dramatic reductions in longevity compared to both control and $snr1^{E1}$ heterozygous flies. The shortened life span was apparent immediately following the shift to 29°C with less than 30% viability at 7 days compared with ~90% for wild-type flies at the same temperature. The viability for $snr1^{E1}$ homozygous flies maintained at 18°C was 80% at 7 days postemergence (data not shown). Normal 29°C viability was obtained by increasing *snr1* dosage with a genomic rescue transgene, with two copies restoring full viability. These results demonstrate a previously unknown requirement for *snr1* function in sustained adult viability.

Broad requirement for *snr1* function in developing tissues. Adult $snr1^{E1}$ homozygous flies raised at 18°C exhibited a range of mutant phenotypes, including eye and wing disruptions and an increased body size. Eye morphology was frequently affected (12%), with reduced ommatidia, duplicated interommatidial bristles, and general head tissue reduction (Fig. 5B to D). The eye phenotypes displayed a broad expressivity, ranging from a weak disruption of the posterior portion of the eye and head tissues to significant reductions of eye size (Fig. 5B). The frequency and severity of these disruptions were increased

(26%) when $snr1^{E1}$ was heterozygous over a null allele ($snr1^{R3}$), and these phenotypes were completely rescued by the presence of a genomic transgene, indicating a functional requirement for *snr1* during normal proliferation and differentiation of eye tissue.

Adult $snr1^{E1}$ homozygous flies also frequently displayed duplication of dorsal thoracic macrochaetae (79%) and ectopic mechanosensory bristles along the L1 and L3 longitudinal wing veins (data not shown). These phenotypes were similar to the bristle duplications observed in the eyes and in somatic clones produced with a null allele (77) and were rescued by the presence of a single wild-type copy of *snr1*, confirming a critical role for *snr1* in peripheral nervous system development. Another frequently observed $snr1^{E1}$ phenotype was ectopic anterior A4 pigmentation in adult $snr1^{E1}/snr1^{R3}$ male flies (67%; Fig. 5F), consistent with disruptions in homeotic gene expression. This phenotype was also rescued by increasing *snr1* dosage and completely suppressed in combination with a null allele of *brm* (data not shown). In addition, $snr1^{E1}/snr1^{E1}$ and $snr1^{E1}/snr1^{R3}$ adults were flightless regardless of incubation temperature, suggesting an inability to coordinate flight muscle movement.

Heterozygous $snr1^{E1}$ adult flies showed a temperature-dependent ectopic vein phenotype (Fig. 6B). The penetrance ranged from 33% among flies reared at 18°C ($n = 482$) to 71% at 29°C ($n = 464$). The penetrance was significantly enhanced in $snr1^{E1}/snr1^{E1}$ mutant flies (85% at 18°C, $n = 159$), including incomplete longitudinal veins posterior to both the L4 and L5 veins (Fig. 6C). The highest penetrance and expressivity were observed for heterozygous $snr1^{E1}/snr1^{R3}$ flies reared at 29°C (98%, $n = 187$) (Fig. 6D). Importantly, the vein phenotypes were fully rescued by increasing the *snr1* gene dosage (Fig. 6E and F) (Table 2), indicating that the extra veins were caused by the loss of functional *snr1*.

The wing veins are formed from the imaginal disk epithelium during the late larval and early pupal stages, coincident with the TS lethal period for the $snr1^{E1}$ allele and the time of new Brm complex synthesis. As the $snr1^{E1}$ vein phenotype is temperature dependent, we carried out shift experiments to determine the precise time of *snr1* function in regulating vein development. We observed a short sensitive period from early to midpupal development (Fig. 6J), indicating that *snr1* acts on wing vein development during much of the early pupal period.

Brm complex interactions in wing patterning. Expression of dominant-negative versions of *brm*, *osa* (15, 24), and *snr1* (77) can affect wing vein patterning, and the overexpression phenotypes are sensitive to the dosage of other Brm complex genes. We therefore examined the effect of decreasing the dosage of known Brm complex genes on the $snr1^{E1}$ wing vein phenotype. Consistent with a dominant-negative phenotype, the $snr1^{E1}$ phenotype was strongly enhanced by reduction in *snr1* gene dosage with a null allele ($snr1^{R3}$), with almost every fly showing extensive ectopic veins (Table 2). Heterozygous

FIG. 5. *snr1* is required for proper eye and abdomen development. Scanning electron micrographs (150×) of adult eyes from flies raised at 18°C are shown. (A) Wild type; (B) $snr1^{E1}/snr1^{E1}$; (C) $snr1^{E1}/snr1^{R3}$. Note the reduced eye size, irregularly shaped ommatidia, and fused or missing interommatidial bristles in panels B and C. (D) Magnified (2,000×) view of an eye from an $snr1^{E1}/snr1^{E1}$ fly showing duplicated and fused interommatidial bristles. (E and F) Abdominal pigmentation in similarly aged adult males. Shown are lateral views of wild-type (E) and $snr1^{E1}/snr1^{R3}$ (F) flies raised at 25°C with segments A2 to A6 indicated. Arrows in panel F indicate ectopic pigmentation in the anterior portions of the A3 and A4 tergites.
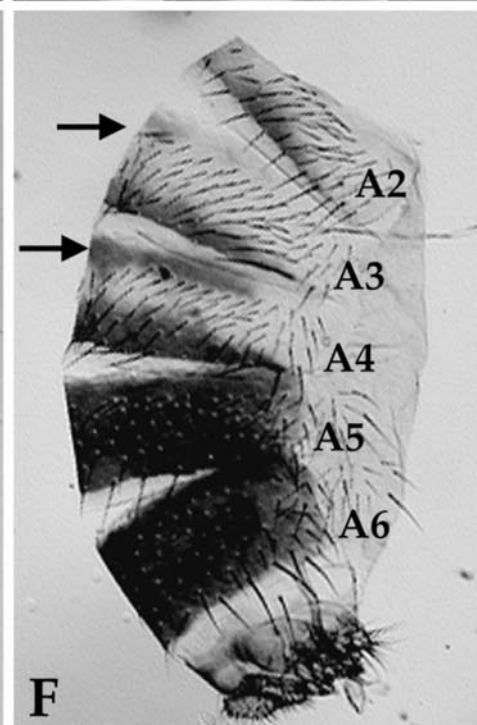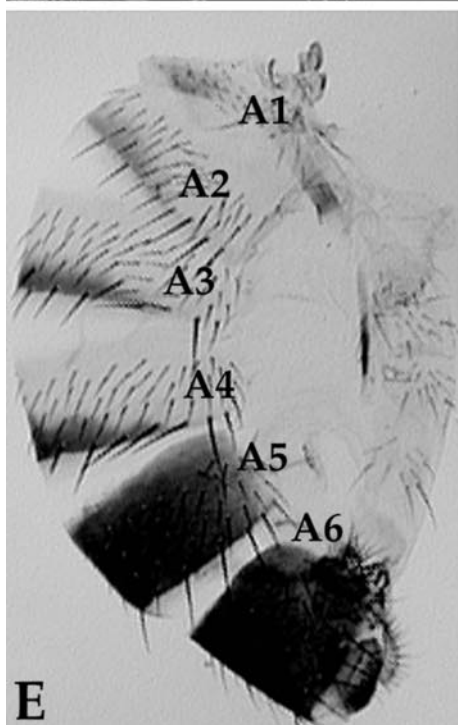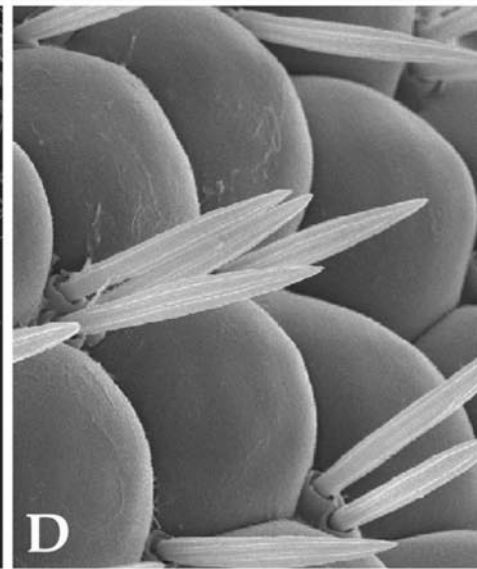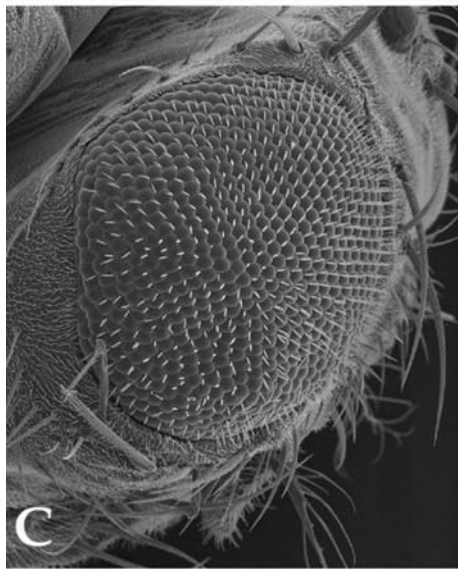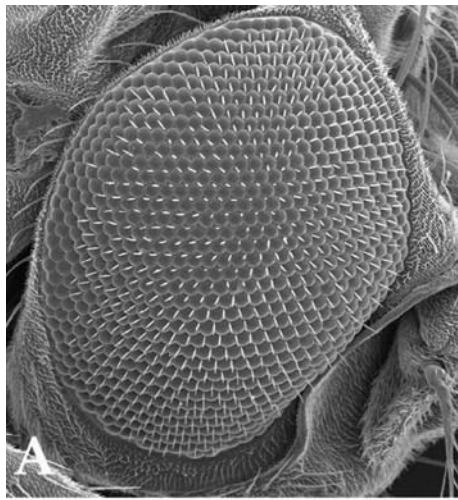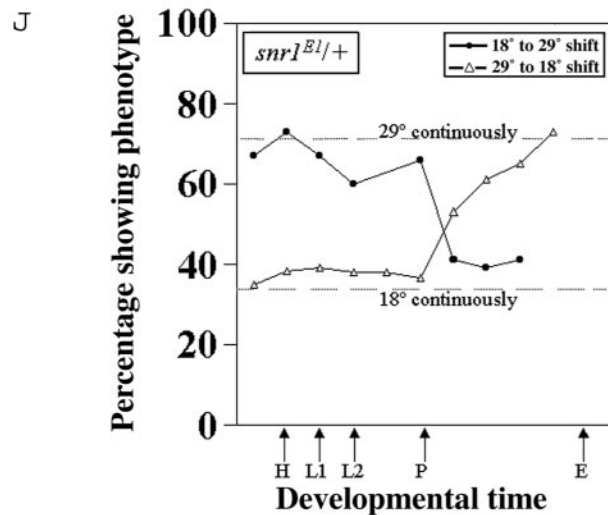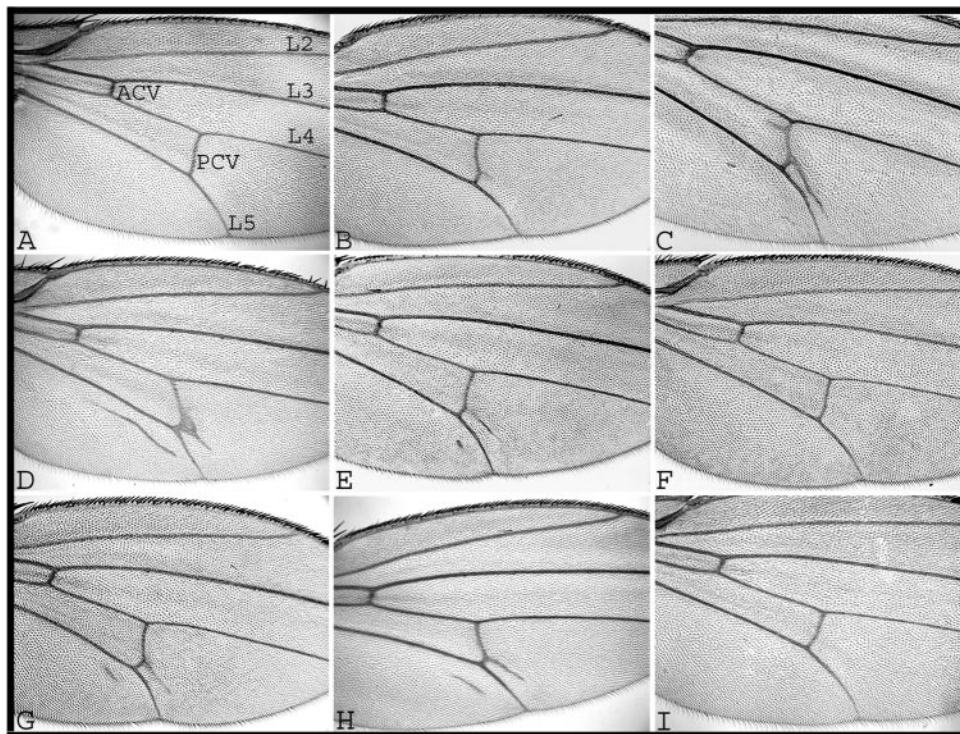
FIG. 6. *snr1* is required for proper wing vein development. (A to I) Wings (100× magnification) shown are from wild-type (A), *snr1^{E1}*/+ (B), *snr1^{E1}*/*snr1^{E1}* (C), *snr1^{E1}*/*snr1^{R3}* (D), *P*[*snr1$^+$*]/+; *snr1^{E1}*/*snr1^{R3}* (E), *P*[*snr1$^+$*]/*P*[*snr1$^+$*]; *snr1^{E1}*/*snr1^{R3}* (F), *snr1^{E1}*/*mor^1* (G), *snr1^{E1}*/*osa^2* (H), and *snr1^{E1}*/*brm^2*, *snr1^{R3}* (I) flies. Wings in panels A to C were from flies raised at 18°C, and those in panels D to I were from flies raised at 29°C. Longitudinal wing veins L2, L3, L4, and L5; anterior cross vein (ACV); and posterior cross vein (PCV) are indicated in panel A. Note the ectopic veins emanating from the posterior cross vein and posterior to L5 in panels B to E, G, and H. (J) Temperature shift showing a critical requirement for SNR1 function during early pupal development coincident with vein formation. Heterozygous *snr1^{E1}*/+ flies were shifted to 18 or 29°C at the stages indicated (Fig. 2).

hypomorphic mutations in *osa* and *mor* also enhanced the severity of the *snr1^{E1}* extra vein phenotype (Table 2) (Fig. 6G and H). While point mutations in *osa* (*osa^1* and *osa^2*) enhanced the wing phenotype, an insertion that truncates the protein (*osa^{eld}* or *osa^{13}*) (70) had no strong effect. It was previously suggested that the point mutations in *osa* allowed for defective or nonfunctional proteins to be produced that could competitively interfere with wild-type OSA in the Brm complex (70).

Similarly, allele-specific interactions were observed with heterozygous *mor^1*, but not a deficiency of *mor*, perhaps due to the removal of other genes in the deficiency that modified the phenotype. Although there are no available specific alleles of genes encoding the Brm complex subunits BAP60 and BAP111, heterozygous deficiencies that removed these genes also showed only modest effects on the *snr1^{E1}* wing phenotype (Table 2). Importantly, deficiencies previously identified

TABLE 2. Enhancement and suppression of $snr1^{E1}$ wing vein phenotypes by members of the Brm complex

Genotype	No. of wings examined	Severity of phenotype[a]		
		−	+	++
$snr1^{E1}/+$	391	33	67	0
$snr1^{E1}/snr1^{R3}$	268	1	1	98
$P[snr1^+]/+; snr1^{E1}/snr1^{R3}$	129	14	50	36
$P[snr1^+]/P[snr1^+]; snr1^{E1}/snr1^{R3}$	147	66	26	8
$snr1^{E1}/osa^1$	58	19	60	21
$snr1^{E1}/osa^2$	100	18	47	35
$snr1^{E1}/osa^{00090-eld}$	116	39	61	0
$snr1^{E1}/mor^1$	80	4	63	33
$snr1^{E1}/Df(3R)sbd^{105}, p^p, Ubx^{bx-1}, sr^1, e^s [mor]$	86	21	79	0
$snr1^{E1}/Df(2L)cl-h3 [E(brm)25D-26B]$	40	0	0	100[b]
$snr1^{E1}/Df(3L)ZN47 [E(brm)64E1-65C]$	38	42	32	26
$snr1^{E1}/brm^2$	278	91	9	0
$snr1^{E1}/brm^2, snr1^{R3}$	82	10	40	50
$snr1^{E1}, brm^2/snr1^{R3}$	72	4	31	65
$brm^{K804R}/+; snr1^{E1}/+$	88	24	76	0
$brm^{K804R}/brm^{K804R}; snr1^{E1}/+$	70	99	1	0
$snr1^{E1}/Df(3L)brm^{11}$	174	21	79	0
$Df(1)c246; snr1^{E1} [BAP60]$	64	47	53	0

[a] Phenotypes (at 29°C) were scored as follows: two or more ectopic veins, ++; one ectopic vein, +; and wild-type vein pattern, −. Numbers are expressed as percentages of the total wings examined for each genotype.

[b] $Df(2L)cl-h3/+$ heterozygotes exhibit a fully penetrant ectopic wing vein phenotype. In combination with $snr1^{E1}$, the ectopic wing vein phenotype was strongly enhanced relative to control sibling flies, with the strongest effects posterior to the L5 longitudinal vein.

as enhancers of the BRM^{K804R} dominant-negative lethality [$E(brm)25D-26B$ and $E(brm)64E1-65C$] strongly enhanced the $snr1^{E1}$ phenotype. These deficiencies are believed to include genes essential for brm function in vivo (48).

Surprisingly, both a brm^2 null allele and the brm^{K804R} dominant-negative (24) allele suppressed the vein phenotype of $snr1^{E1}$, as well as the strong ectopic veins associated with the $snr1^{E1}/snr1^{R3}$ combination at 29°C (Table 2) (Fig. 6I). The suppression was comparable when brm^2 was recombined onto either the $snr1^{E1}$ or $snr1^{R3}$ chromosome. The ability of the brm^{K804R} transgene to suppress the $snr1^{E1}$ phenotype was dependent on the dosage of the transgene (Table 2). The

BRM^{K804R} protein is efficiently incorporated into the Brm complex, disrupting the ATPase function of BRM, though not complex formation or stability, and thus functions as a dosage-sensitive dominant-negative (15, 24, 49). Our results indicate that reduced brm function in the wing could compensate for the reduced $snr1$ function, suggesting that the $snr1^{E1}$ wing phenotype was possibly due to derepression of Brm complex activity in non-vein-forming regions. These data suggest that SNR1 might serve to restrict Brm complex ATP-dependent activities in specific cells while assisting these activities in other cells.

The SNR1^{E1} protein is present in Brm complexes. As reduced brm function could suppress the mutant phenotypes of both $snr1^{E1}/+$ and $snr1^{E1}/snr1^{R3}$ flies, one likely possibility was that the SNR1^{E1} protein functioned as a dominant-negative to interfere with normal Brm complex function at specific targets. We addressed this possibility by first determining whether SNR1^{E1} was incorporated into the Brm complex. Our highly specific affinity-purified SNR1 antibodies (10, 23, 76) were capable of efficiently coprecipitating BRM and OSA from wild-type embryo extracts, confirming that the SNR1 antibodies were capable of precipitating the Brm complex (Fig. 7A). As the SNR1^{E1} protein is stable, we performed coimmunoprecipitation experiments with extracts prepared from both wild-type and $snr1^{E1}$ homozygous pupae raised at 29°C. We found that the SNR1^{E1} protein was incorporated into the Brm complex as determined by coimmunoprecipitation of both BRM and OSA (Fig. 7B). Consistent with our genetic data indicating that mutant alleles of other Brm complex genes could affect the $snr1^{E1}$ phenotypes, our coimmunoprecipitation data revealed that SNR1^{E1} functions as a weak dominant-negative, impinging on SNR1-dependent functions of the Brm complex.

Although little is known regarding the identity of bona fide cellular targets of the Brm complex, the homeotic genes are strong candidates, possibly via cooperation with TRX (8, 9, 28, 31, 67, 70, 77). We previously showed with the $snr1^{R3}$ null allele that $snr1$ interacted with trx in the abdomen (23). To determine whether the $snr1^{E1}$ phenotypes were related to disrupted trx
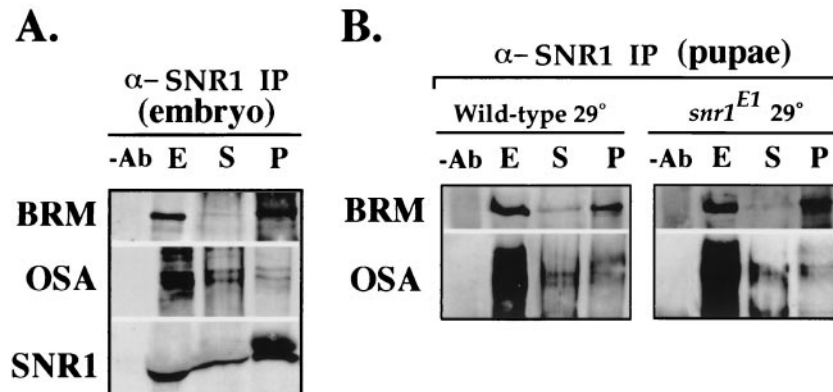

FIG. 7. SNR1^{E1} stably associates with components of the Brm complex. (A) Immunoprecipitation (IP) with affinity-purified anti-SNR1 antibodies (C. B. Zraly et al., submitted for publication) demonstrates association with BRM and OSA in wild-type embryo extracts. Lanes: −Ab, G-Sepharose control with no primary antibody (lane represents 50% of pelleted material); E, 100 μg of extract; S, supernatant fraction (lane represents 20% of input protein extract); P, pellet fraction (lane represents 50% of coimmunoprecipitated proteins). Western blots were probed with affinity-purified rabbit anti-BRM (77), mouse monoclonal anti-OSA (15), or rat anti-SNR1 (23). (B) Immunoprecipitation (IP) with anti-SNR1 from extracts prepared with wild-type or $snr1^{E1}$ homozygous pupae raised at 29°C. Lanes are as indicated for panel A.
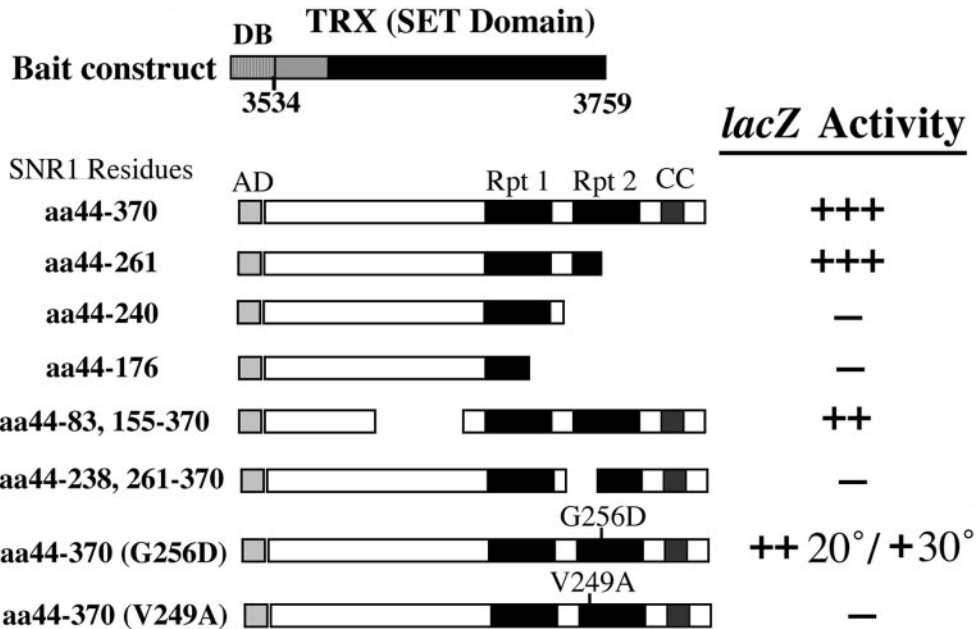
FIG. 8. SNR1[E1] is partially defective for associations with TRX. (A) Genetic interactions between *snr1*[E1] and *trx*. The *snr1*[E1] allele was maternally provided in every cross. Adult flies of each genotype were scored for viability and held-out wings at 18 or 29°C. Values are expressed as the percentages of total adults observed versus number expected for combinations involving *snr1*[E1] and *trx*[E2] and percentages of total adults observed with held-out wings. (B) Yeast two-hybrid interaction tests between SNR1 derivatives fused to the GAL4AD and the SET domain (TRX) fused to the GAL4 DNA binding domain. The amino acid regions of SNR1 and TRX included in each fusion are indicated. Interaction ability was determined by *lacZ* activity (+++, 80 to 100% of full length; ++, 50 to 80%; +, 30 to 50%; −, <30%).

function, we generated a *trx*[E2], *snr1*[R3] recombinant and examined interactions with *snr1*[E1] (Fig. 8A). *snr1*[E1]/*snr1*[R3] flies exhibited a held-out wing phenotype similar to those for *brm*/*mor* and *brm*/*osa* interactions, which resemble a loss of *Antp* function (9). A mutation in *trx* amplified the *snr1* held-out wing

phenotype, and there was a significantly enhanced lethality (17% of expected progeny); although we did not observe enhancement of a *trx* abdomen phenotype, nor did we observe any effects on sex comb number on the male prothoracic leg with *snr1*[E1], which is often used as an assay for *trx* function (31,

66). This allele-specific interaction suggested that the SNR1[E1] protein might affect localized protein interactions, as SNR1 was shown previously to be capable of forming direct contacts with the SET domain of TRX (42).

To further explore the allele-specific genetic interactions between $snr1^{E1}$ and trx, we examined the association between SNR1 and TRX in more detail by yeast interaction trap analyses (Fig. 8B). A full-length SNR1 protein (aa 44 to 370) fused to the GAL4AD was capable of strong and specific interactions with the SET domain of TRX (aa 3534 to 3759). Deletions of SNR1 revealed that the repeat 2 region was necessary for the observed association. The full-length (aa 44 to 370) SNR1[E1] protein containing the G256D change within repeat 2 was expressed in yeast fused to GALAD and verified by immunoblotting (data not shown). The SNR1[E1] protein showed temperature-dependent association with the isolated SET domain (20 > 30°C); even at the lower temperature, the interaction was modestly diminished relative to the wild-type SNR1 protein. Though interactions between SNR1 and SET are reduced by the G256D change, they are only moderately affected, suggesting that other nearby residues might provide more direct contacts or stabilize the interaction. To help identify specific SNR1 residues important for the interaction, we carried out a nonbiased PCR mutagenesis screen. As a result of several rounds of screening, we identified a single valine-to-alanine conversion at aa 249 within repeat 2 that completely abolished the two-hybrid interaction between SNR1 and the SET domain. The mutant protein was fully stable by Western blot analysis and functional for control interactions (data not shown). The critical V249 residue resides within 7 aa of the G256D in SNR1[E1], supporting our view that the repeat 2 region was necessary to mediate specific associations and that SNR1 can form direct associations with TRX.

SNR1 functions to restrict cellular proliferation. Homozygous $snr1^{E1}$ flies raised at 18°C were significantly larger than either their heterozygous siblings or control flies (Fig. 9). Male flies generated from independent crosses were examined and scored for body mass and cell number. Body mass was calculated from at least 100 similarly aged male flies of each genotype, and cell number was scored by counting cells in a defined area of the wing, as each wing cell is known to secrete only one hair during development (42). As controls, heterozygous siblings were scored from crosses that included either a balancer chromosome or the parental chromosome on which the $snr1^{E1}$ allele was generated and crosses in which either one or two copies of the $snr1$ rescue transgene were included. Both body mass and cell number were significantly ($P < 10^{-4}$) greater among the $snr1^{E1}$ flies than among controls, and this effect was ameliorated in a dosage-dependent manner by introducing wild-type copies of $snr1$. The significantly greater cell number observed in the wings of homozygous $snr1^{E1}$ flies can be extrapolated to the increased biomass associated with flies of the same genotype and suggests that the larger size of $snr1^{E1}$ mutant flies is likely due to increased cell proliferation. Hence, a normal function of $snr1$ appears to be restricting cell growth in the wing and possibly acting to repress cell proliferation in the entire organism.

DISCUSSION

We report here the characterization of the only identified conditional mutation among genes encoding the metazoan SWI/SNF chromatin remodeling complexes. A TS recessive lethal allele of the highly conserved *Drosophila snr1* gene has allowed new insights into the developmental functions of the Brm chromatin remodeling complex, including strong in vivo evidence that SNR1 has an essential role in restricting normal cell growth. The biological importance of *snr1*, and its human counterpart *INI1*, appears to be critical, as both are essential for viability and development. Our present studies have identified previously unknown functions for *snr1* during late stages of embryogenesis, for tissue patterning events including proper eye and wing vein formation and for sustained adult viability. Recently it was shown that *INI1*, but no other mammalian hBrm or Brg1 complex gene, is disrupted in nearly every confirmed case (~90%) of aggressive rhabdoid tumors that appear most often in early childhood (58, 71), and *INI1* deletions are frequently associated with chronic myeloid leukemia (29). Further, murine *INI1* is required for embryogenesis as early as the peri-implantation stage (E3.5), and embryonic stem cell-derived heterozygous adult mice are predisposed (>30%) to soft tissue and nervous system carcinomas (33). Thus, *INI1* is a tumor suppressor and both *INI1* and *snr1* have unique and possibly direct roles in restricting cell growth, either independently or as components of their respective Brm complexes.

The $snr1^{E1}$ mutation genetically functions as an antimorph. The mutant phenotypes associated with $snr1^{E1}$ are consistent with diminished *snr1* function and likely reflect altered Brm complex activities. Our conclusions are based on several lines of evidence: (i) the SNR1[E1] protein is stable and associates with other Brm complex subunits even at the restrictive temperature; (ii) $snr1^{E1}$ heterozygous phenotypes can be modified by allele-specific interactions with mutations in genes encoding other Brm complex components; (iii) the $snr1^{E1}$ phenotypes are sensitive to *snr1* dosage, being more severe in combination with a null allele and suppressed by increasing *snr1* dosage by using rescue transgenes; and (iv) both the embryonic and pupal lethal phases associated with $snr1^{E1}$ are significantly different from null mutants of *snr1*, which die during early larval development. The embryonic lethality was observed only when both parents were homozygous for $snr1^{E1}$, consistent with a disruption of Brm complex functions in late embryos (8, 9, 24, 70), whereas the late lethality of $snr1^{E1}$ homozygotes derived from heterozygous parents implicates essential Brm complex functions during metamorphosis, coinciding with the last major proliferation and differentiation events prior to the emergence of adults.

Our coimmunoprecipitation data revealed that other Brm complex subunits, BRM and OSA, were also stable in an $snr1^{E1}$ mutant and that both could be coprecipitated with SNR1 antibody at equal efficiency in either wild-type or $snr1^{E1}$ mutant backgrounds. Moreover, the $snr1^{E1}$ mutant phenotypes could be strongly suppressed by a brm^{K804R} dominant-negative transgene, whose product is stably incorporated into the Brm complex (24). While it is formally possible that $snr1^{E1}$ results in reduced complex stability, our studies are consistent with the view that $snr1^{E1}$ affects a postassembly step of Brm complex
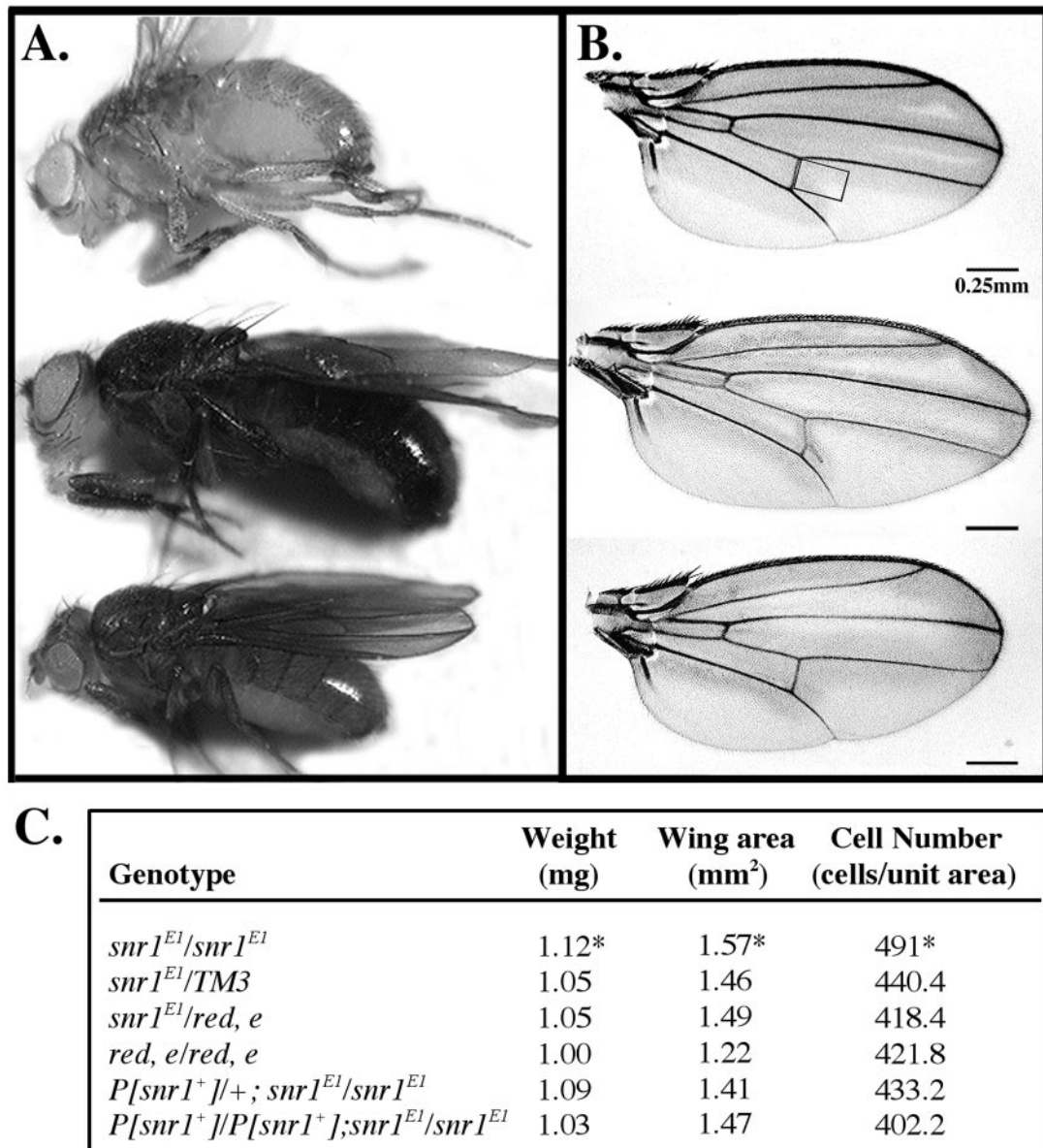
FIG. 9. *snr1* functions to restrain cell growth and proliferation. (A) Male $snr1^{E1}/snr1^{E1}$ flies (middle) are larger than either the parental *red, e* (top) or rescued $P[snr1^+];snr1^{E1}/snr1^{E1}$ (bottom) flies raised at 18°C. Similarly aged flies were photographed in the same optic field for comparison. (B) Wing size comparisons among flies of the same genotypes shown in panel A. The boxed region in the wild-type *red, e* wing (top) represents the area examined from all wings for cell number analyses. The wings shown were photographed at a 40× magnification. (C) Biomass differences among $snr1^{E1}$ flies with various gene doses. The average weight, wing area, and cell number for sibling male flies and standard deviations were determined. *, values for $snr1^{E1}/snr1^{E1}$ in all categories are significantly different from those for all other genotypes in the same category. Probability values for $snr1^{E1}/snr1^{E1}$ flies relative to parental *red, e* flies ranged from $P < 10^{-3}$ to $P < 10^{-4}$ in the same category.

functions rather than disruption or destabilization of complex assembly. This contrasts with studies of SNF5, the yeast homolog of SNR1, showing that conditional mutations affecting the repeat 2 region (*snf5-51ts*; E582K), but not the repeat 1 region (*snf5-83bts*; D475N), affected complex assembly as measured by both coprecipitation of other subunits and gel fractionation (27). In *snf5-51ts* mutant extracts, SWI/SNF complex stability was significantly affected at both the permissive and restrictive temperatures and SNF2/SWI2 was not efficiently coprecipitated with the SNF5^{E582K} protein. Further, diminished stability of yeast SWI3 (a homolog of *Drosophila* MOR)

was observed in a strain carrying disruptions of *SWI1* and *SWI2* (52). However, similar to the *brm*^{K804R} mutation, disrupting the ATPase function of the yeast SWI/SNF complex with a dominant-negative *SWI2* mutant has no significant effect on complex assembly (51).

Developmental functions of SNR1 and the Brm complex. *brm* is required for embryogenesis (8), and the expression patterns of *brm* and *snr1* are nearly identical (23, 24). *snr1*-null mutants die during larval growth, suggesting that SNR1 is not required for all Brm complex functions in embryos. However, our analyses of $snr1^{E1}$ showed that *snr1* was required during

embryogenesis, as are conserved components of the Brm complex, *brm*, *mor*, and *osa*. We conclude, therefore, that *snr1* is required for a subset of embryonic functions of the complex and that the maternal contribution of *snr1* is normally sufficient to complete these functions.

Brm complex components are widely expressed throughout development, with elevated levels observed during pupal stages. Late requirements for Brm complex functions in specific tissue patterning have been inferred from the use of overexpression of dominant-negative transgenes and mosaic clone analyses. Our present studies provide strong evidence for a sustained essential role of *snr1* in late cell growth and patterning events. Some of the late *snr1*^{E1} mutant phenotypes are consistent with loss of steroid hormone activity. Pupal case defects observed in *snr1*^{E1} homozygous pupae (our unpublished data) are similar to those observed in some ecdysone receptor mutants (4) as well as early ecdysone-regulated genes, such as *E74* (25). In addition, fluctuating RNA levels of *snr1* generally coincide with rising titers of ecdysone. In light of observed interactions between mammalian hormone receptors and the SWI/SNF complexes (47, 53), it is likely that the Brm complex assists in regulating events during *Drosophila* metamorphosis, possibly in collaboration with the ecdysone receptor. Consistent with this view, we have observed interactions between *snr1*^{E1} and some ecdysone receptor mutants suggestive of reduced receptor functions (our unpublished observations). Homozygous *snr1*^{E1} flies raised at 29°C rapidly lose viability during early metamorphosis (pupariation), and transient temperature pulse-shifts (18 to 29 to 18°C) show strong sensitivity coincident with the transition from larval to pupal development. As homozygous adults at 18°C are unable to fly and dissected pupae (29°C) show ectopic sensory bristles, it is likely that *snr1* (and presumably the Brm complex) is required for aspects of nerve or muscle cell function or development, supporting results for heterozygous *INI1* chimeric mice that revealed a strong predisposition (~30%) to develop tumors affecting the central nervous system (33).

A requirement for Brm complex activity to regulate cell growth during eye development has been shown by overexpression of OSA or dominant-negative BRM^{K804R} that resulted in severe reductions in eye size (15, 49). Further, the OSA overexpression phenotype could be enhanced by mutations in *snr1* and *mor* (15). One possibility is that the Brm complex might impact eye development by affecting cell division, as studies have linked *snr1* and the Brm complex to regulation of S phases in the eye imaginal disk through interactions with cell cycle regulators (61). It is interesting, therefore, that dominant interference of *DmCdk2* function (a cyclin-dependent kinase specific to S-phase progression) also leads to disrupted eyes and duplicated ommatidial bristle phenotypes (34) nearly identical to those seen for our *snr1*^{E1} mutants. The duplicated ommatidial bristles and ectopic mechanosensory bristles on the thorax present in *snr1*^{E1} mutants also appear similar to the phenotypes associated with somatic loss of both *snr1* (77) and *brm* activities (24), as well as alterations in *Notch* pathway genes in early pupae (30). Thus, it is possible that the Brm complex is involved in regulation of *Notch* pathway or other neurogenic genes, and the duplicated bristle phenotypes may not be a direct result of reductions in mitotic divisions but rather of impairment of neurogenic cell fates.

SNR1 can modulate Brm complex activities both positively and negatively. We and others previously showed that a null allele of *snr1* could modestly enhance a *brm* loss-of-function phenotype in the prothoracic region and that *snr1* could function synergistically with other Brm complex genes in eye development, including enhancement of an OSA overexpression phenotype (10, 15, 23). However, there is no identified function for *snr1* in leg development (77), unlike *brm*, *mor*, and *osa* (31, 67), consistent with our phenotypic analysis of *snr1*^{E1}.

The positions of the wing veins are initially determined during the late third-instar larval stage, and this positional information is subsequently refined and patterns are maintained during the late larval and early pupal periods (5, 6, 21). The BRM ATPase activity is required for the formation of the wing veins (15, 24), perhaps analogous to its function in homeotic gene regulation. For example, the Brm complex may be important for the transcriptional activation or maintenance of genes required for vein formation, such as the highly conserved *rhomboid* gene that encodes a serine protease and is expressed in all vein primordia where it functions to activate the epidermal growth factor receptor signaling pathway (reviewed in reference 41). The ectopic vein phenotype observed in *snr1* mutants extends our previous knowledge of Brm complex function in wing development by identifying a critical temporal and position-specific requirement for *snr1* function and suggesting that *snr1* might function antagonistically to *brm* in specific cells of the wing. The *snr1*^{E1} ectopic vein phenotype is both temperature and *snr1* dose dependent, and the TS period precisely corresponds to early pupal wing patterning events. The appearance of ectopic wing veins in an *snr1*^{E1} mutant is generally similar to phenotypes caused by ectopic expression of *rhomboid* (62, 63) or the transforming growth factor β homolog *dpp* (20, 21). The restricted temporal function is significant in that OSA-containing Brm complexes are required for repression of the fly transforming growth factor β homolog *decapentaplegic* (*dpp*) (16), and *dpp* function is required in early pupal wings for the proper formation of the veins. Thus, a specific functional requirement for SNR1 to regulate *dpp* expression or other vein-promoting genes in the wing may explain the presence of extra veins in *snr1*^{E1} mutants (our unpublished observations).

Reducing the dosage of functional *snr1* enhances the *snr1*^{E1} vein phenotype while increasing *snr1* gene dosage or reducing Brm complex ATP-dependent activity has the effect of suppressing the *snr1*^{E1} vein phenotype. Specifically, the ectopic wing vein phenotype associated with heterozygous *snr1*^{E1}/+ was strongly enhanced by a null allele of *snr1* and gene-specific mutations in *mor* and *osa*, as well as deficiencies defined as enhancers of a *brm* dominant-negative (48). Deficiencies or null-allele mutants that reduce the gene dosage of some Brm complex components have little detectable effect on the *snr1*^{E1} phenotype, suggesting that modest reduction in Brm complex or individual subunit abundance alone is not sufficiently limiting. There are no reported phenotypes associated with the heterozygous null mutants, although in a few specific cases null mutations in some Brm complex genes can display dosage-sensitive genetic interactions. By contrast, in *trans*-heterozygous combination with either a *brm*² null allele or the *brm*^{K804R} dominant-negative, we observed strong suppression of the vein phenotype associated with *snr1*^{E1}. As the SNR1^{E1} protein ap-

pears to be incorporated into the Brm complex, the suppression of the ectopic wing vein phenotype by reducing (though not eliminating) *brm* suggests that one in vivo function of SNR1 is to restrict or modulate Brm complex activity in specific tissues or cells.

In support of this conclusion, studies of the *Drosophila* BRM-related ISWI protein revealed that individual subunits of chromatin remodeling complexes can affect the in vitro biochemical properties of the complex distinct from the recombinant ATPase subunit alone (37). Our data are consistent with the view that SNR1 functions antagonistically to restrict Brm complex activity in non-vein-forming wing tissues during early pupal development. One possibility is that SNR1 may serve to regulate Brm complex gene targets in vivo through associations with vein-specific repressors. By analogy, the yeast SWI/SNF complex can be targeted to the histone *HTA1-HTB1* promoter in association with the HIRp repressors, possibly through interactions with SNF5 (22). Another possibility is that SNR1 (as well as MOR and OSA) could serve to regulate the gene activation functions of the Brm complex in certain cells. Consistent with this latter possibility, OSA function is normally limiting in the wing, with overexpression giving rise to morphological changes that were suppressed by decreasing BRM levels (15). As SNR1^{E1} functions as a weak dominant-negative, then decreasing wild-type *snr1* should exacerbate the *snr1^{E1}* mutant phenotype and decreasing the amount of functional Brm complex through a *brm* null mutation should suppress the phenotype, precisely as we observed. Additional genetic and biochemical tests will be required to distinguish among these and other possibilities.

While components of the Brm complex are present in male and female adult flies, the only previously known function was a requirement for proper oogenesis. It is interesting, therefore, that *snr1^{E1}* females are fertile at 29°C, implying that SNR1 has a limited role in oogenesis, perhaps as a stabilizing subunit of the Brm complex. An unexpected finding was a novel requirement for *snr1* function in adult longevity, as *snr1^{E1}* adult flies exhibited significantly shortened life spans at 29°C (<7 days). Misexpression of the trx-G group gene *ash1* also caused reduced longevity (36), and *ash1* genetically interacts with *brm* (68). ASH1 is a component of a large (~2-MDa) complex distinct from the Brm complex (48); it is not known whether SNR1 is a component of the Ash1 complex, nor have genetic interactions been identified, perhaps not surprising since null alleles of *snr1* weakly interact with *brm* mutants. As *ash1* is important for *trx* function (57, 68) and SNR1 physically and genetically interacts with TRX, it may be possible that ASH1 can also form transient complexes with SNR1 in vivo. Thus, the decreased life span observed for *snr1^{E1}* mutant flies may reflect diminished Brm complex activity or some other complex, such as Ash1, required for sustained adult gene transcription.

SNR1 functions in restricting cell growth. A primary phenotype of the *snr1^{E1}* mutants is an increased mitotic index, suggesting a direct role in controlling cell proliferation. Addition of a wild-type copy of *snr1* suppressed the growth defects, arguing against secondary recessive mutations. Further, no obvious growth defects were observed in dissected imaginal tissues, the distribution of SNR1^{E1} appeared normal, and there was no aberrant mitotic activity or chromosome morphology in larval brain tissues (our unpublished observations). In correspondence with the *snr1^{E1}* TS period, the defect in cell growth regulation occurred during pupal metamorphosis, which is characterized by rapid cell division and differentiation of larval tissues into adult structures (26). Further, disruption of *INI1*, but no other Brm complex gene, is associated with aggressive soft tissue cancers affecting renal and nervous system tissues as well as hematopoietic cell lineages (58, 59). Importantly, no recurrent lesions are found at other loci in these tumors, suggesting that loss of *INI1* is primarily responsible for tumorigenesis.

The loss of normal cell proliferation control associated with the *snr1^{E1}* mutant raises the possibility that SNR1 and the Brm complex might have a direct role in regulating aspects of the cell cycle. The mammalian Brm complexes directly interact with several transcription factors required for growth and development, as well as cell cycle regulatory proteins including CycE/CDK2, retinoblastoma (Rb), and histone deacetylases (60, 69, 75). In addition, BRG1, one of the two mammalian counterparts to BRM, is required for RAS-mediated transformation of SW13 cells (44). The *Drosophila* Brm complex can form stable associations with the CycE/CDK2 heterodimer that controls entry into S phase, and mutations in Brm complex genes can suppress the rough eye phenotype associated with a hypomorphic *cyclinE* allele (10). Thus, the Brm complex appears to function downstream of CycE/CDK2 to restrict S-phase entry. Our present studies demonstrate that SNR1 is important to mediate this aspect of Brm complex function, as the SNR1^{E1} protein remains tightly associated with the Brm complex even at the restrictive temperature and a single copy of the mutant allele is sufficient to elicit the proliferation defect. Another potential explanation for the *snr1^{E1}* growth defects is the disruption of cellular apoptosis, leading to increased cell proliferation. The mammalian GADD34 protein, implicated in DNA damage-induced apoptosis and regulation of cell growth, physically associates with INI1 (2). Although the contact residues involved are unknown and there is no obvious GADD34 counterpart in flies, it is intriguing that members of the SWI2/SNF2 family of ATPases are proteins that have roles in DNA repair and replication (50), suggesting that modification or disruption of protein-DNA interactions may be required for diverse cellular activities and that multiprotein chromatin remodeling complexes may assist these functions. Thus, while the precise role SNR1/INI1 plays in restricting cell growth is unclear, one likely possibility is that loss of SNR1/INI1 function results in the inability to recruit Brm complexes to critical in vivo gene targets.

SNR1 functions to target some Brm complex activities. The SNF5-related family of proteins share strong similarity within their C termini, with considerable homology among two imperfect repeats, termed repeat 1 and repeat 2. The repeat sequences have been shown previously to contribute to complex stability and function (1, 13, 17, 27, 40, 43, 74). The repeat regions have also been implicated in mediating contacts between the complex and cellular factors, suggesting a possible targeting role for SNF5-family proteins (13, 46, 65). Both SNR1 and INI1 can physically interact with the homologous transcription factors TRX and HRX, which regulate Hox gene expression in flies and mammals (56). Disruption of *trx* and *HRX* leads to homeotic phenotypes, and misregulation of *HRX* function has been strongly linked to the majority of infant

acute leukemias (14), possibly through involvement with *INI1*. While a null allele of *snr1* enhanced a *trx* loss-of-function phenotype in the abdomen (23), the *snr1*[E1] mutant showed only modest dosage-dependent interactions. However, the held-out wing phenotype observed in *trans*-heterozygotes is strongly reminiscent of homeotic loss-of-function phenotypes and is remarkably similar to *brm/osa* and *brm/mor* as well as *trans*-heterozygous *brm* allele interactions (8, 70).

While TRX was shown to associate with overexpressed SNR1 in vivo, SNR1 is not a stable component of the embryonic TAC1 complex (TRX acetylation complex), which contains TRX, the acetyltransferase dCBP, and the antiphosphatase protein SBF1 (54). We have shown that SNR1 and TRX can form direct contacts mediated through the TRX SET domain and the highly conserved SNR1 repeat 2 region. Although the contact between the SET domain and SNR1[E1] is moderately affected in yeast two-hybrid assays, we were unable to detect any reduction in the in vivo association measured by coprecipitation (our unpublished observations), suggesting that TRX likely interacts with other Brm complex components in vivo. The affected amino acid in SNR1[E1] resides within seven residues of a critically important valine (V249) that, when mutated to an alanine, resulted in the complete loss of SNR1 association with SET in a two-hybrid assay. Our results suggest that, at the restrictive temperature, the SNR1[E1] protein may compromise interactions with TRX through local alterations in SNR1 protein structure. However, it is not known if conformational changes in SNR1[E1] affect interactions among the other Brm complex subunits that might adversely influence the ability of TRX to properly regulate a subset of its targets. Importantly, not all targets of TRX or the Brm complex are affected by SNR1[E1], suggesting that SNR1 is essential for some, but not all, Brm complex activities. Although TRX can form some functional associations with the Brm complex in the presence of SNR1[E1], the interaction between the SNR1 repeat 2 region and the SET domain is important for normal development. As SNR1 is not a component of the TAC1 complex, this implies that the interactions must be highly regulated, perhaps transiently. In light of this possibility, it is intriguing that SBF1 also contacts TRX through the SET domain, and this association may be important for growth regulation (19). For example, forced expression of mammalian SBF1 in cultured cells resulted in competitive interference of endogenous SET domain interactions with dual-specificity phosphatases (such as myotubularin) leading to oncogenic transformation of NIH 3T3 cells and impaired differentiation of myoblasts (19). While the effect of SNR1[E1] on the formation or function of the TAC1 complex is presently unknown, one possibility is that reduced interaction between SNR1[E1] and the SET domain of TRX may lead to inappropriate TAC1 function.

Thus, it appears that SNR1/INI1 is necessary to target specific chromatin remodeling functions of the metazoan Brm complexes in vivo through interactions with cellular factors mediated through the repeat regions. Further examination of the response of cellular targets to disruptions of Brm complex activities by SNR1[E1] will be important to link chromatin remodeling and specific developmental processes.

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