C. elegans ISWI and NURF301 antagonize an Rb-like pathway in the determination of multiple cell fates

Erik C. Andersen, Xiaowei Lu* and H. Robert Horvitz†

The class A, B and C synthetic multivulva (synMuv) genes act redundantly to negatively regulate the expression of vulval cell fates in Caenorhabditis elegans. The class B and C synMuv proteins include homologs of proteins that modulate chromatin and influence transcription in other organisms similar to members of the Myb-MuvB/dREAM, NuRD and Tip60/NuA4 complexes. To determine how these chromatin-remodeling activities negatively regulate the vulval cell-fate decision, we isolated a suppressor of the synMuv phenotype and found that the suppressor gene encodes the C. elegans homolog of Drosophila melanogaster ISWI. The C. elegans ISW-1 protein likely acts as part of a Nucleosome Remodeling Factor (NURF) complex with NURF-1, a nematode ortholog of NURF301, to promote the synMuv phenotype. isw-1 and nurf-1 mutations suppress both the synMuv phenotype and the multivulva phenotype caused by overactivation of the Ras pathway. Our data suggest that a NURF-like complex promotes the expression of vulval cell fates by antagonizing the transcriptional and chromatin-remodeling activities of complexes similar to Myb-MuvB/dREAM, NuRD and Tip60/NuA4. Because the phenotypes caused by a null mutation in the tumor-suppressor and class B synMuv gene lin-35 Rb and a gain-of-function mutation in let-60 Ras are suppressed by reduction of isw-1 function, NURF complex proteins might be effective targets for cancer therapy.

KEY WORDS: ISWI, NURF, Rb, Ras, C. elegans

INTRODUCTION

The ordered recruitment of factors required for proper gene expression is crucial for animal development. For example, sequence-specific transcription factors recruit a variety of protein complexes that remodel chromatin to regulate the transcription of target genes either by using the energy of ATP hydrolysis to move nucleosomes or by chemically modifying histones (Narlikar et al., 2002). These two mechanisms for chromatin remodeling have been characterized extensively in vitro (Roth et al., 2001; Smith and Peterson, 2005), but efforts to understand how each functions in the development of metazoa have just begun.

Studies of vulval development in the nematode Caenorhabditis elegans could help establish the roles of chromatin remodeling during development. The vulva of the C. elegans hermaphrodite is formed by the 22 descendants of three ectodermal blast cells (P5.p, P6.p and P7.p) located along the ventral surface of the animal (Sulston and Horvitz, 1977). P(5-7).p, three of a set of six equipotent cells called the vulval equivalence group, are induced to divide once and fuse with the nearby hypodermal syncytium (hyp7), P(3-8).p, during development. The vulva of the C. elegans animal in which no cells of the vulval equivalence group express vulval fates; by contrast, mutations that increase the function of this pathway can cause the ectopic adoption of vulval cell fates by P3.p, P4.p and P8.p, and result in a multivulva (Muv) animal (Beitel et al., 1990; Han et al., 1990; Katz et al., 1996). Loss-of-function mutations in the synthetic multivulva (synMuv) genes also can cause a Muv phenotype (Horvitz and Sulston, 1980; Ferguson and Horvitz, 1989). These genes have been grouped into three classes: A, B and C (Ferguson and Horvitz, 1989; Ceol and Horvitz, 2004). Loss-of-function mutations within any one class do not cause a Muv phenotype, whereas mutations in any two genes within two different classes cause a Muv phenotype (Ferguson and Horvitz, 1989; Ceol and Horvitz, 2004). The class A synMuv genes encode novel, nuclear components (Clark et al., 1994; Huang et al., 1994; Davison et al., 2005). Many class B synMuv genes encode homologs of transcriptional repressors and factors that remodel chromatin, including LIN-35 Rb (Lu and Horvitz, 1998), the EFL-1/DPL-1 E2F heterodimeric transcription factor (Ceol and Horvitz, 2001), the HDA-1 HDAC1, LET-418 Mi2, LIN-53 RbAp48 NuRD complex (Lu and Horvitz, 1998; Tong et al., 1998; Xue et al., 1998; von Zelewsky et al., 2000; Unhavaithaya et al., 2002) and HPL-2 Heterochromatin Protein 1 (HP1) (Couteau et al., 2002). The Drosophila melanogaster homologs of some class B synMuv proteins form a complex, identified by two different groups and called Myb-MuvB or dREAM, that is likely to repress the transcription of genes through chromatin remodeling (Korenjak et al., 2004; Lewis et al., 2004). Class C synMuv genes encode homologs of a putative Tip60/NuA4 histone acetyltransferase complex (Ceol and Horvitz, 2004). Because of these homologies, these synMuv genes negatively regulate the vulval cell fate, probably by repressing the transcription of genes that promote the expression of vulval cell fates.

In this study, we describe the identification of a C. elegans ortholog of Drosophila ISWI, called isw-1, as a suppressor of the synMuv phenotype. ISWI is an ATP-dependent chromatin-remodeling enzyme identified by homology to S. cerevisiae
Snf2/Swi2 (Elfring et al., 1994). We show that ISW-1 probably acts as a component of a Nucleosome Remodeling Factor (NURF)-like complex with the Drosophila NURF301 ortholog NURF-1 to antagonize the synMuv genes. Our observations reveal the antagonistic functions of a NURF-like chromatin remodeling complex and complexes similar to Myb-MuvB/dREAM, NuRD and TIP60/NuA4 in the determination of multiple cell fates, including the antagonistic regulation of at least one putative target of synMuv transcriptional repression.

MATERIALS AND METHODS

Strains and genetics

C. elegans strains were cultured as described previously and maintained at 20°C unless otherwise noted (Brenner, 1974). N2 (Bristol) was the wild-type strain. The following mutations and integrants were used:

- L1: dpy-5(e61), lin-35(n745), lin-33(n833, n3368), ced-4251 (Hsieh et al., 1999).
- LGI: unc-4(e120), isw-1(n4293, n4295) (this study), dpl-1(n3316) (Ceol and Horvitz, 2001), lin-8(n11, n2731) (Thomas et al., 2003), lin-56(n2728) (Thomas et al., 2003), lin-38(n751), trr-1(n3712) (Ceol and Horvitz, 2004), lin-31(n301), let-23(n622) (Katz et al., 1996), rrf-3(pk1426) (Simmer et al., 2002).
- LGII: dpy-17(e164), isw-1(n3294, n3297, n4066) (this study), lin-9(n12, n942) (Beitel et al., 2000), lin-36(n766) (Thomas and Horvitz, 1999), lin-37(n758, n2234) (Ferguson and Horvitz, 1989; Thomas et al., 2003), lin-27(n3718) (Thomas et al., 2003), lin-13(n387) (Melendez and Greenwald, 2000), hpl-2(tm1489), flr-1(tm235).
- LGIV: unc-30(e191), pyp-1(n4599) (this study), let-60(n1046) (Beitel et al., 1999), lin-1304, el275).
- LGV: unc-46(e1777), let-418(n5356, n3719), mep-1(q6600), lin-54(n3423), hda-1(e1795) (Dufourcq et al., 2002), tram-1(csc57) (Hsieh et al., 1999), lin-45(ku12) (Sundaram and Han, 1995), him-5(e490).

The following reciprocal transfections containing GFP-expressing transgenes integrated at or near the translocation breakpoints were used: hT2 [q(s48)] LGI; LGIII and nII [q(s51)] LGIV; LGV. LGV. mln1 [mls14 dpy-10(e128)] is a balancer chromosome that expresses GFP. The following mutations were provided by C. Ceol, F. Steigmeier and M. Harrison: let-418(n5356, n3719), lin-54(n3423), mep-1(n3703), flr-1(tm235) and hpl-2(tm1499) were provided by S. Miti. Those mutant alleles for which no citation is given are described by Riddle (Riddle, 1997).

RNAi analyses

RNAi by injection was performed as described by Ceol and Horvitz (Ceol and Horvitz, 2004), except single-stranded RNA molecules were annealed by incubation at 85°C for 15 minutes, then at 37°C for 30 minutes and slowly cooled to room temperature for 1 hour. RNAi of F37A4.6, the gene predicted to be within an intron of isw-1, did not suppress the synMuv phenotype of lin-53(n833); lin-15A(n767) mutants (data not shown). The following constructs were used to make dsRNA: for isw-1, yk593a10 and ykh17c10; for nurf-1, yk273g2, yk1151c7, pEA30, yk899g1, yk172b9, yk374b9, yk381c2, yk480b9, yk565d9, yk752a6, yk765d8, yk759b11, yk1030g7, yk1151c6, yk1288b1, yk1456g11, yk1691d5. Gene-specific primers were used to amplify reverse-transcribed products of PCR to confirm several nurf-1 transcripts, and a Stratagene C. elegans cDNA library also was used for PCR analyses of nurf-1 gene structures. The PCR ends of nurf-1b and nurf-1c were identified by SL1 RT-PCR, and the 5’ ends of nurf-1a, d and e are from GeneFinder (Liang et al., 2001) predictions.

Isolation of deletion alleles

Genomic DNA pools from EMS-mutagenized animals were screened for deletions using PCR as described by Ceol and Horvitz (Ceol and Horvitz, 2001). Deletion mutant animals were isolated from frozen stocks and backcrossed to the wild type at least twice. isw-1(n4066) removes nucleotides 20629 to 21932 of cosmid F37A4. nurf-1(n4293) removes 3058 to 3782, and nurf-1(n4295) removes 18656 to 19733 of cosmid F26H11. pyp-1(n4599) removes nucleotides 26777 to 28936 of cosmid C47E12. Lin-53(n3368) removes nucleotides 38104 to 38857 of cosmid K07A1.

Scoring of vulval cell fates

For trr-1(n3712) and trr-1(n3712); lin-15B(n744), vulval induction was scored during the L4 larval stage using Nomarski optics. If more than three out of the six Pn.p cells were induced, the animals were counted as Muv.

Antibody staining

We cloned a full-length isw-1 cDNA into a vector containing the coding sequence for the maltose-binding protein (MBP). Antisera recognizing ISW-1 were generated by injecting MBP:ISW-1 into two rabbits (Covance). Anti-ISW-1 antibodies were affinity purified using GST (glutathione S-transferase)-tagged ISW-1 as described by Koelle and Horvitz (Koelle and Horvitz, 1996). Embryos, larvae and adults were fixed as described by Finney and Ruvkun (Finney and Ruvkun, 1990). Affinity-purified antibodies were used at a 1:10 dilution for whole-mount staining and at 1:1000 for western blots. Horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch) were used at 1:3000 for western blots, and Alexafluor 488 (Invitrogen) was used at a 1:200 dilution for detection by whole-mount immunocytochemistry.

Determination of mutant sequences

We used PCR-amplified regions of genomic DNA to determine mutant sequences. For both isw-1 alleles, all exons and splice junction sequences were determined. All mutations were confirmed using independently derived PCR products. Sequences were determined using an ABI Prism 3100 Genetic Analyzer.

Germline transformation experiments

Germline transformation experiments were performed as described by Mello et al. (Mello et al., 1991). For rescue of the lin-53(n833); isw-1(n3294); lin-15A(n767) synMuv suppression phenotype, we injected cosmid C28G2 (30 ng/μl). 100 μg/μl of 1 kb DNA ladder (Invitrogen) was used to increase the complexity of the extrachromosomal arrays, and pTG96 (sur-5::gfp) (Yochem et al., 1998) was used as the co-injection marker at 20 ng/μl.

Suppression of non-vulval defects caused by class B synMuv mutations

Using the same exposure time, GFP expression (Tam phenotype) was quantified for each micrograph within the linear range for signal detection using the Profiler function of the OpenLab software package (Improvision). PGL-1 staining and RNAi sensitivity were scored as described by Wang et al. (Wang et al., 2005). The L1 larval arrest phenotypes of let-418(n5356) and mep-1(n3703) were scored at 25°C.

RESULTS

Loss of function of the C. elegans homolog of Drosophila ISWI causes suppression of the lin-53; lin-15A synMuv phenotype

We screened for suppressors of the lin-53(n833); lin-15A(n767) synMuv phenotype and identified two mutations, n3294 and n3297, that failed to complement for suppression of the synMuv phenotype (Fig. 1A; Table 1). We mapped the synMuv suppressor to the centromeric LGI and obtained transformation rescue of the synMuv suppression phenotype with cosmid C28G2, which contains 16 predicted genes. RNA interference (RNAi) of the
gene F37A4.8 (using either of two cDNA clones; see Materials and methods) caused suppression of the lin-53; lin-15A synMuv phenotype. Additionally, expression of an F37A4.8 cDNA driven by a dpy-7 promoter (Gilleard et al., 1997) rescued the synMuv suppression phenotype of n3294 animals (Table 1). We determined that n3294 and n3297 harbor two distinct missense mutations in F37A4.8 (Fig. 1B; see Fig. S1 in the supplementary material).

Using two independent cDNA clones and one 5′ RACE product (see Materials and methods), we determined the sequence of the full-length F37A4.8 transcript, which included the SL1 trans-splice leader sequence found in many C. elegans transcripts (Blumenthal, 1995) (Fig. 1B). F37A4.8 encodes the C. elegans homolog of Drosophila ISWI, a regulator of transcription and chromatin (Elfring et al., 1994; Deuring et al., 2000). We named F37A4.8 isw-1 (isw, ISWI-like).

ISW-1 is 60% identical to Drosophila ISWI (Elfring et al., 1994) and 69% identical to human SNF2H (Okabe et al., 1992). ISW-1 contains an AT-hook (Reeves and Nissen, 1990) and two SANT (Aasland et al., 1996) domains; each of these domains can bind DNA. Additionally, ISW-1 contains a domain similar to many helicases (the DEXD/H box) and an ATPase domain. Each are required for chromatin-remodeling activity (Corona et al., 1999). isw-1(n3294) is predicted to cause a proline-to-leucine substitution within the DEXD/H domain, implicating this domain in isw-1 function. isw-1(n3297) is predicted to cause a leucine-to-phenylalanine substitution within a non-conserved region (Fig. 1C; see Fig. S1 in the supplementary material).

isw-1(n3294) and isw-1(n3297) each conferred a fully penetrant recessive synMuv suppression and incompletely penetrant sterile phenotype (Table 1; see Table S1 in the supplementary material). By contrast, RNAi of isw-1 caused a fully penetrant synMuv suppression and sterile phenotype (Table 1; see Table S1 in the supplementary material). We isolated a deletion allele of isw-1, n4066, which removes part of the ATPase domain and most of the DEXD/H domain, causing a presumptive null phenotype. The isw-1(n4066) deletion allele caused a fully penetrant recessive sterile phenotype (see Table S1 in the supplementary material) but failed to cause strong suppression of the synMuv phenotype (Table 1). Because isw-1(n4066) homozygotes are sterile, the animals we studied were descended from isw-1(n4066) heterozygotes. Homozygous missense mutants descended from heterozygous mothers also did not have a strong synMuv suppression phenotype (Table 1), so the lack of synMuv suppression observed in isw-1 homozygotes derived from heterozygous mothers was probably caused by maternally inherited wild-type isw-1 gene product. isw-1(n4066) did perturb the synMuv suppressor function of isw-1, because this mutation failed to complement the synMuv suppression phenotype caused by each of the missense alleles (Table 1). isw-1(n3294) and isw-1(n3297) cause a decrease of isw-1 function, because each resulted in recessive suppression of the synMuv phenotype and failed to complement the phenotype conferred by a deletion of isw-1. Additionally, RNAi of isw-1 caused a synMuv suppression and sterile phenotype, so the two isw-1 missense alleles probably cause a partial loss of isw-1 gene function.
For the dominance, complementation and maternal effect tests with the missense lin-53(n833); him-5(e1490); lin-15A(n767) hermaphrodites and heterozygous hermaphrodites were scored.

‡M+ denotes that the maternally provided product might be present in these homozygous offspring.

†These animals were the non-Dpy non-GFP-positive offspring of

¶ M+ 95 (212)

n4066

‡These animals were the non-GFP-positive offspring of

†These animals were the non-GFP-positive offspring of

*M+ or M− denotes the presence (+) or absence (−) of maternal isw-1 gene product. M− animals were descended from isw-1 heterozygous mutant hermaphrodites. M+ animals were descended from isw-1 homozygous mutant hermaphrodites.

These animals were the non-Dpy non-GFP-positive offspring of dpy-5(e61) lin-15A(n767) hermaphrodites and lin-53(n833)/ hT2 [qIs48]; isw-1/hT2 [qIs48]; isw-1(n3294)−marked with dpy-5(e61) cis alleles, a

dpy-5(e61) lin-53(n833); isw-1; lin-15A(n767) males.

These animals were the non-Dpy offspring of dpy-5(e61) lin-53(n833); isw-1; lin-15A(n767) hermaphrodites and lin-53(n833)/ hT2 [qIs48]; isw-1/hT2 [qIs48]; isw-1(n3294)−marked with dpy-5(e61) cis-marked strain was used.

NURF55 orthologs, caused embryonic lethality (data not shown), and thus precluded the scoring of the synMuv suppression phenotype. The other NURF55 ortholog is a class B synMuv gene, lin-53 (Lu and Horvitz, 1998), and was not tested for synMuv suppression for this reason. Additionally, a deletion allele of pyp-1 caused larval lethality before the third larval stage (data not shown), which precluded scoring of the synMuv suppression phenotype.

Using the sequences determined from 15 independent cDNA clones (see Materials and methods), RT-PCR products and 5′ RACE products, we identified five distinct transcripts generated from the nurf-1 locus (Fig. 2A). Each transcript is predicted to encode a protein with domains similar to some of the domains of Drosophila NURF301 ortholog, pyp-1, or of one of the two NURF55 orthologs, rba-1, caused embryonic lethality (data not shown), which precluded scoring of the synMuv suppression phenotype.

Using BLAST (Altschul et al., 1990) and SMART (Sonnhammer et al., 1997) searches, we identified C. elegans orthologs of the ACF, CHRAC and NURF complex members. The ACF and CHRAC complexes share one component: ACF1. Deletion of one of the two genes encoding a C. elegans ACF1 ortholog (flt-1), RNAi of the other ortholog (H20J04.2), or both deletion and RNAi together failed to suppress the synMuv phenotype. Furthermore, RNAi of each of the remaining genes encoding CHRAC complex orthologs failed to suppress the synMuv phenotype (Table 2). In these RNAi experiments in which a failure to suppress the synMuv phenotype was observed, it remains possible that the gene plays a role in the antagonism of the synMuv genes, but this role was not seen because the gene was not inactivated sufficiently.

Only RNAi of a Drosophila NURF301 ortholog, which we named nurf-1 (NURF301-like), suppressed the synMuv phenotype (Table 2). The Drosophila NURF complex consists of ISWI, NURF301, NURF38 and NURF55 (Tsukiyama and Wu, 1995). RNAi of the sole C. elegans NURF301 ortholog, pyp-1, or of one of the two NURF55 orthologs, rba-1, caused embryonic lethality (data not shown), and thus precluded the scoring of the synMuv suppression phenotype. The other NURF55 ortholog is a class B synMuv gene, lin-53 (Lu and Horvitz, 1998), and was not tested for synMuv suppression for this reason. Additionally, a deletion allele of pyp-1 caused larval lethality before the third larval stage (data not shown), which precluded scoring of the synMuv suppression phenotype.

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Using the sequences determined from 15 independent cDNA clones (see Materials and methods), RT-PCR products and 5′ RACE products, we identified five distinct transcripts generated from the nurf-1 locus (Fig. 2A). Each transcript is predicted to encode a protein with domains similar to some of the domains of Drosophila NURF301. However, none of the identified transcripts is predicted to encode a protein with all of the domains contained in full-length NURF301 (Fig. 2B).

<table>
<thead>
<tr>
<th>Genotype in addition to lin-15A(n765)</th>
<th>Drosophila homolog(s)</th>
<th>Drosophila complex(es) in which the homolog is found</th>
<th>% Muv (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>ISWI</td>
<td>ACN, CHRAC, NURF</td>
<td>100 (546)</td>
</tr>
<tr>
<td>isw-1(n294)</td>
<td>NURF301</td>
<td>NURF</td>
<td>1 (204)</td>
</tr>
<tr>
<td>nurf-1(RNAi)</td>
<td>NURF301</td>
<td>NURF</td>
<td>1 (204)</td>
</tr>
<tr>
<td>pyp-1(RNAi)</td>
<td>NURF38</td>
<td>NURF</td>
<td>1 (204)</td>
</tr>
<tr>
<td>pyp-1(n4599) M*‡</td>
<td>NURF38</td>
<td>NURF</td>
<td>1 (204)</td>
</tr>
<tr>
<td>rba-1(RNAi)</td>
<td>NURF55</td>
<td>NURF</td>
<td>1 (204)</td>
</tr>
<tr>
<td>H20J04.2(RNAi)</td>
<td>ACF1</td>
<td>ACF, CHRAC</td>
<td>100 (158)</td>
</tr>
<tr>
<td>flt-1(tm235)</td>
<td>ACF1</td>
<td>ACF, CHRAC</td>
<td>100 (158)</td>
</tr>
<tr>
<td>fit-1(tm235), H20J04.2(RNAi)</td>
<td>ACF1</td>
<td>ACF, CHRAC</td>
<td>100 (158)</td>
</tr>
<tr>
<td>T26A5.8(RNAi)</td>
<td>CHRAC-16</td>
<td>CHRAC</td>
<td>100 (122)</td>
</tr>
<tr>
<td>Y53F4B.3(RNAi)</td>
<td>CHRAC-16</td>
<td>CHRAC</td>
<td>100 (122)</td>
</tr>
<tr>
<td>Y53F4B.3(RNAi), T26A5.8(RNAi)</td>
<td>CHRAC-14 and CHRAC-16</td>
<td>CHRAC</td>
<td>100 (122)</td>
</tr>
</tbody>
</table>

*NA, not applicable because RNAi caused lethality prior to vulval development.

‡M+ denotes that the maternally provided product might be present in these homozygous offspring.
nurf-1a encodes a protein most similar to the N terminus of NURF301 and contains domains implicated in binding DNA, including an HMGY/I domain (Reeves and Beckerbauer, 2001), a DDT domain (Doerks et al., 1993; Aasland et al., 1995), nurf-1b and nurf-1c share two exons with nurf-1a and encode proteins with similarity to the C-terminus of NURF301. Unlike nurf-1b, which encodes a protein with only a Q-rich domain, nurf-1c encodes a protein with two PHD fingers and a bromodomain. The nurf-1d and nurf-1e transcripts are initiated at different sites but encode the same predicted protein, which shares a C terminus with NURF-1C. RNAi of nurf-1a, but not of the other nurf-1 transcripts, suppressed the synMuv phenotype of lin-15AB(n765) mutants and caused sterility (Fig. 2C and data not shown).

To confirm that reduction of the nurf-1a transcript was responsible for the suppression of the synMuv phenotype, we isolated two deletion alleles of the nurf-1 locus, n4293 and n4295. nurf-1(n4293) is deleted for part of nurf-1a but not for the other nurf-1 variants. nurf-1(n4295) removes part of every nurf-1 variant except nurf-1a. nurf-1(n4293) but not nurf-1(n4295) suppressed the synMuv phenotype of lin-15AB(n765) mutants and caused sterility, both features of the isw-1 mutant phenotype (Fig. 2C and data not shown). Therefore, nurf-1a probably acts with isw-1 to promote the synMuv phenotype.

The C. elegans NURF-like genes isw-1 and nurf-1 promote the synMuv phenotypes of all synMuv mutant combinations

The C. elegans NURF-like genes might promote the ectopic vulval fates of only specific synMuv mutant combinations, e.g. the lin-53; lin-15A double mutant. To address this issue, we used RNAi to reduce the function of isw-1 or nurf-1 in a variety of synMuv double mutants. Inactivation of isw-1 or nurf-1 suppressed not only the synMuv phenotype of lin-53(n833); lin-15A(n767) animals but also the synMuv phenotype of the null double mutant combination lin-53(n368); lin-15A(n767) (Table 3). Additionally, RNAi of isw-1 or nurf-1 suppressed other class AB, BC and AC synMuv double mutant combinations (Table 3). Reduction of isw-1 function suppressed the synMuv phenotype of lin-53(n833) in combination with putative null mutations in each of the class A synMuv genes. Additionally, reduction of isw-1 function suppressed the synMuv phenotype of lin-15A(n767) in combination with putative null alleles of all identified class B synMuv genes (Table 3; see Table S2 in the supplementary material).

isw-1 and nurf-1 promote the multivulva phenotype caused by activation of the Ras pathway

A functional Ras pathway is required for expression of the synMuv phenotype (Ferguson et al., 1987; Beitel et al., 1990; Han and Sternberg, 1990). Therefore, reduction of isw-1 function might suppress the synMuv phenotype by reducing the activity of the Ras pathway. If so, isw-1 might act by promoting the activity of the Ras pathway. However unlike many mutants defective in the Ras pathway, isw-1 mutants did not have abnormalities in the expression of vulval cells.

We tested for a subtle role of isw-1 in the specification of vulval cells by asking if a weak Vul phenotype conferred by a decrease in Ras pathway activity could be enhanced by an isw-1 mutation. A lin-2 partial loss-of-function mutation, e1453, causes an incompletely penetrant Vul phenotype (Ferguson and Horvitz, 1985) and a weak
Table 3. Reduction of the C. elegans NURF-like genes complex suppresses the synMuv phenotype of multiple synMuv mutant combinations

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% Muv after control RNAi (n)</th>
<th>% Muv after isw-1 RNAi (n)</th>
<th>% Muv after nurf-1a RNAi (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lin-53(n833); lin-15A(n767)*</td>
<td>100 (319)</td>
<td>0 (353)</td>
<td>0 (128)</td>
</tr>
<tr>
<td>lin-53(n3368)<em>; lin-15A(n767)</em></td>
<td>100 (95)</td>
<td>2 (155)</td>
<td>0 (86)</td>
</tr>
<tr>
<td>lin-15A(b1763)*</td>
<td>100 (300)</td>
<td>55 (347)</td>
<td>40 (72)</td>
</tr>
<tr>
<td>lin-35(n745)<em>; lin-15A(n767)</em></td>
<td>100 (143)</td>
<td>14 (70)</td>
<td>8 (62)</td>
</tr>
<tr>
<td>lin-37(n758)<em>; lin-15A(n434)</em></td>
<td>100 (625)</td>
<td>52 (431)</td>
<td>0 (91)</td>
</tr>
<tr>
<td>lin-37(n758)<em>; lin-15A(n767)</em></td>
<td>100 (207)</td>
<td>5 (99)</td>
<td>21 (39)</td>
</tr>
<tr>
<td>lin-33(n833)<em>; lin-8(n2731)</em></td>
<td>56 (117)</td>
<td>0 (77)</td>
<td>7 (28)</td>
</tr>
<tr>
<td>lin-53(n833)<em>; lin-56(n2728)</em></td>
<td>100 (234)</td>
<td>4 (118)</td>
<td>0 (66)</td>
</tr>
<tr>
<td>trr-1(n3712)<em>; lin-15A(n767)</em></td>
<td>49 (59)</td>
<td>3 (75)</td>
<td>23 (22)</td>
</tr>
<tr>
<td>trr-1(n3712)<em>; lin-15B(n444)</em></td>
<td>100 (243)</td>
<td>1 (181)</td>
<td>6 (51)</td>
</tr>
</tbody>
</table>

The Muv phenotype caused by an increase in Ras pathway activity suggests either that a greater inhibition of the functions of isw-1 and nurf-1 is required to observe complete effects or that other factors act redundantly with the NURF-like genes to promote Ras pathway activity.

Table 4. Reduction of isw-1 and nurf-1 function suppresses non-vulval abnormalities of class B synMuv mutants

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% Muv after control RNAi (n)</th>
<th>% Muv after isw-1 RNAi (n)</th>
<th>% Muv after nurf-1a RNAi (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>let-23(n2a27g)</td>
<td>91 (57)</td>
<td>44 (88)</td>
<td>66 (27)</td>
</tr>
<tr>
<td>let-60(n1046g)</td>
<td>96 (228)</td>
<td>19 (148)</td>
<td>9 (159)</td>
</tr>
<tr>
<td>lin-1(e1275)*</td>
<td>87 (100)</td>
<td>22 (107)</td>
<td>2 (103)</td>
</tr>
<tr>
<td>lin-1(n304)*</td>
<td>100 (134)</td>
<td>100 (53)</td>
<td>100 (34)</td>
</tr>
<tr>
<td>lin-31(n301)*</td>
<td>60 (312)</td>
<td>9 (96)</td>
<td>18 (246)</td>
</tr>
</tbody>
</table>

The Muv phenotype caused by an increase in Ras pathway activity suggests either that a greater inhibition of the functions of isw-1 and nurf-1 is required to observe complete effects or that other factors act redundantly with the NURF-like genes to promote Ras pathway activity.

Several class B synMuv genes control aspects of a germline-versus-soma cell-fate decision. Specifically, the somatic cells of some class B synMuv mutants adopt a more germline-like fate: by the somatic expression of germline genes, such as PGL-1 (Kawasaki et al., 1998); by a germline-like appearance of somatic cells in mep-1 and let-418 arrested larvae (Unhavaithaya et al., 2002); by the tandem-array-modifier (Tam) phenotype of increased somatic silencing of expression from repetitive transgenes by a process similar to that which occurs in the germline (Kelly et al., 1997; Kelly and Fire, 1998; Hsieh et al., 1999); and by enhanced RNAi sensitivity, perhaps caused by ectopic expression of a germline RNA polymerase EGO-1 in the soma producing an increased RNAi effect (Wang et al., 2005).

Reduction of isw-1 and nurf-1 function suppressed abnormalities associated with defects in the germline-versus-soma cell-fate decision caused by lin-15B(n744) and lin-35(n745), including the ectopic somatic expression of the germline-expressed protein PGL-1, the Tam phenotype (see Fig. S4 in the supplementary material) and the RNAi hypersensitivity phenotype (Fig. 3A-C; data not shown). However, the reduction of isw-1 or nurf-1 function did not cause a germline desilencing of expression from repetitive transgenes (data not shown), so both genes might not be required for mechanisms of transcriptional repression in the germline. Additionally, reduction of isw-1 and nurf-1 function suppressed the mep-1 and let-418 larval-arrest phenotypes (Fig. 3D), and somatic cells did not have a germline-like appearance in isw-1; mep-1 or in nurf-1; mep-1 double mutants (data not shown). These data indicate that not only are isw-1 and nurf-1 required for the synMuv vulval phenotype but also for the ectopic adoption of germline fates in the soma caused by loss of class B synMuv gene function. Therefore, the putative synMuv complexes and the NURF-like complex might antagonize the transcription of similar sets of target genes.
DISCUSSION

*isw-1* and *nurf-1* antagonize the activities of at least the class B and C synMuv genes in the determination of *C. elegans* cell fates

The synMuv phenotype is caused by mutations in two genes in two different classes, and synMuv single mutants have a wild-type vulval phenotype (Horvitz and Sulston, 1980; Ferguson and Horvitz, 1989). The loss of *isw-1* or *nurf-1* function must antagonize a deficit in one or both of the synMuv genes in each suppressed strain to counteract the synMuv phenotype. Because the synMuv phenotypes of class AB, BC and AC double mutants were each suppressed by loss of *isw-1* or *nurf-1* function, the *C. elegans* NURF-like genes must antagonize the functions of at least two classes of synMuv genes. Defects caused by single class B mutants were suppressed by reduction of *isw-1* or *nurf-1* function (Fig. 3), and the vulval phenotype of the class C synMuv mutant *trr-1(n3712)* was suppressed (see Table S2 in the supplementary material). Thus, *isw-1* and *nurf-1* likely antagonize the activities of at least the class B and C synMuv genes. Because a functional Ras pathway is required for the synMuv phenotype, it is possible that the antagonism of the class B and C gene functions caused by reduction of *isw-1* or *nurf-1* function involves a downregulation of the Ras pathway.

*isw-1* and *nurf-1* probably are not targets of synMuv-mediated transcriptional repression, as mRNA levels of each were not increased using semi-quantitative RT-PCR analysis of *lin-53; lin-15A* and *lin-35; lin-15A* mutants compared with the wild type (data not shown). Additionally, ISW-1 levels were not increased noticeably in *lin-53; lin-15A* and *lin-15AB* mutants, based on whole-mount immunofluorescence analysis (data not shown). Therefore, the synMuv genes probably do not act through the NURF-like genes to negatively regulate the vulval cell-fate decision.

**ISW-1 and NURF-1 might be components of a NURF-like chromatin-remodeling complex involved in the *C. elegans* vulval cell-fate decision**

*C. elegans* ISW-1 probably acts as part of a NURF-like complex and not as part of a CHRAC-like or ACF-like complex to antagonize the actions of the synMuv proteins, because inhibition...
of isw-1 or nurf-1 but not inhibition of ACF or CHRAC ortholog gene functions suppressed the synMuv phenotype (Table 2). The *Drosophila* NURF complex is composed of four subunits: ISWI, NURF53, NURF55 and NURF301 (Tsukiyama and Wu, 1995). Because loss of the *C. elegans* homolog of NURF38, PYP-1, caused embryonic lethality (data not shown), we have not determined if it functions as a NURF-like complex component to antagonize the actions of the synMuv proteins. *Drosophila* NURF55 is similar to two proteins in *C. elegans*, LIN-53 and RBA-1 (72% and 53% identical, respectively). LIN-53 is a class B synMuv protein and 54% identical to its neighbor RBA-1. Strong reduction-of-function mutations in *lin-53* and *isw-1* cause opposite mutant phenotypes. Therefore, it is unlikely that LIN-53 and ISW-1 always act in the same complex. The LIN-53 homolog NURF55/RbAp48/CAF-1 is present in many chromatin-regulatory complexes, and it is possible that LIN-53 similarly acts in a number of *C. elegans* complexes, possibly both preventing and promoting the synMuv phenotype. If so, the role of LIN-53 in preventing the synMuv phenotype must be predominant, because loss-of-function mutations in *lin-53* cause a synMuv phenotype in combination with mutations in class A genes (Lu and Horvitz, 1998). Alternatively, RBA-1 might act with ISW-1 as part of a NURF-like complex. Because *rba-1* (RNAi) caused embryonic lethality (data not shown), we have not tested this possibility.

*nurf-1*, the *C. elegans* homolog of *Drosophila nurf301*, is predicted to encode at least five different proteins, each of which has some similarity to NURF301. Using deletion alleles and RNAi, we found that *nurf-1a* but not *nurf-1b*, *nurf-1c*, *nurf-1d* or *nurf-1e* was required to promote the synMuv phenotype. The region of NURF301 between the DDT domain (Doerks et al., 2001) and the C-terminal PHD fingers (Aasland et al., 1995) interacts with transcription factors required for recruitment of the NURF complex to target gene promoters (Xiao et al., 2001). The corresponding regions of NURF-1A, NURF-1B and NURF-1C differ in length and could mediate interactions with distinct sets of transcription factors to direct recruitment of the complex to different promoters. The NURF-1A region that presumably interacts with transcription factors might be responsible for recruitment of the NURF-like complex to promoters of genes required for the vulval cell-fate decision.

The functions of ISW-1 and NURF-1 might be involved in the generation of normal vulval cell fates

It is possible that the function of the putative *C. elegans* NURF-like complex is required only for the generation of ectopic vulval cell fates, e.g. in synMuv mutants, but is not involved in normal vulval development. For example, when the inhibitory actions of the synMuv proteins are absent or when the activity of the Ras pathway is increased, the NURF-like complex might promote the specification of ectopic vulval cell fates. However, we observed that *isw-1* is required not only for the Tam phenotype of class B synMuv mutants (Fig. 3B) but also for a basal level of repression of expression from the *ccIs4251* GFP reporter (see Fig. S4 in the supplementary material). This observation suggests that *isw-1* is required not only in the absence of synMuv activity but also in a wild-type synMuv background to promote expression of genes repressed by the synMuv proteins. By analogy, we propose that the putative NURF-like complex helps promote the normal generation of vulval cell fates in a wild-type synMuv background.

The *C. elegans* NURF-like complex acts antagonistically to complexes similar to Myb-MuvB/dREAM, NuRD and Tip60/NuA4 to control transcription

The *Drosophila* NURF complex slides nucleosomes along the DNA to allow access for transcription factors to bind target sequences in vitro (Hamiche et al., 1999). Both ISWI and NURF301 are required for the transcription of Hox and heat-shock genes in vivo (Deuring et al., 2000; Badenhorst et al., 2002). Therefore, the NURF complex has been hypothesized to be involved in transcriptional activation. The homologs of many class B synMuv proteins are components of at least two complexes, Myb-MuvB/dREAM and NuRD, involved in transcriptional repression (Tong et al., 1998; Xue et al., 1998; Korenjak et al., 2004; Lewis et al., 2004). Studies of *Drosophila* and mammalian cells argue that the NURF complex and the Myb-MuvB/dREAM and NuRD complexes have opposite effects on transcription.

The vulval cell-fate decision in *C. elegans* demonstrates the biological consequences of the opposing effects of the Myb-MuvB/dREAM and NuRD and the NURF chromatin-remodeling activities. We propose that a complex containing both ISW-1 and NURF-1 antagonizes one or more synMuv protein complexes in the transcriptional control of the vulval cell-fate decision. One hypothesis is that loss of transcriptional repression by the synMuv proteins causes a Muv phenotype, as a result of the increased transcription of genes that promote the vulval cell-fate decision. The NURF-like complex might be required for this ectopic expression of synMuv target genes. Alternatively, the NURF-like complex might act at targets distinct from those that are misregulated in synMuv mutants, and transcription of these genes would antagonize the activities of the synMuv proteins. The identification of the transcriptional targets of the synMuv proteins and of the NURF-like complex should help differentiate between these two hypotheses. The *Drosophila* Myb-MuvB complex copurified with sub-stoichiometric amounts of NURF complex members. The actions of and requirements for the NURF complex components for Myb-MuvB function were not investigated (Lewis et al., 2004). Perhaps NURF-like complexes bind Myb-MuvB-like complexes to directly inhibit activities of these complexes.

The antagonism of the *lin-35 Rb* and *let-60 Ras* mutant phenotypes by partial loss of *isw-1* ISWI function suggests a possible approach to cancer therapy

The functional antagonism between a NURF-like complex and synMuv repressive complexes and/or activation of the Ras pathway could be conserved in humans and be important for human cancer. The loss of Rb function is associated with the majority of human carcinomas (Adams and Kaelin, 1998), and methods to inhibit the defects of Rb-deficient cells should be beneficial as cancer treatment strategies. Additionally oncogenic forms of human Ras are involved in many cancers, especially cancers of the lung (Minamoto et al., 2000). Because a reduction of *isw-1* ISWI function can suppress defects associated with a complete loss of *lin-35 Rb* or activation of *let-60 Ras* in *C. elegans*, inhibition of the human ISW-1 homolog (SNF2H; SMARCA5 – Human Gene Nomenclature Database) might suppress the effects of Rb loss or of oncogenic Ras in human cells and hence reduce or eliminate the consequences of oncogenic mutations. SNF2H is a chromatin-remodeling enzyme (Okabe et al., 1992; Aihara et al., 1998) and might be a reasonable target for therapeutic intervention.
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Supplementary material
Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/133/14/2695/DC1

References


