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Review

The SynMuv genes of *Caenorhabditis elegans* in vulval development and beyond

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Abstract

For a nonessential diminutive organ comprised of only 22 nuclei, the *Caenorhabditis elegans* vulva has done very well for itself. The status of the vulva as an overachiever is in part due to its inherent structural simplicity as well as to the intricate regulation of its induction and development. Studies over the past twenty years have shown the vulva to be a microcosm for organogenesis and a model for the integration of complex signaling pathways. Furthermore, many of these signaling molecules are themselves associated with cancer in mammals. This review focuses on what is perhaps the most intriguing and complex story to emerge from these studies thus far, the role of the Synthetic Multivulval (SynMuv) genes in controlling vulval cell-fate adoption. Recent advances have led to a greater mechanistic understanding of how these genes function during vulval development and have also identified roles for these genes in diverse developmental processes.

Keywords: Development; Vulva; Review; *lin-35*/Rb; Genetic redundancy; Transcriptional repression; SynMuv

An overview

Hermaphrodites of *Caenorhabditis elegans* are self-fertilizing, and their embryos begin development *in utero*. The vulva is therefore not required for fertility, nor is it required for viability. These are among several features that have made studies of the vulva particularly fruitful, providing insight into cell–cell interactions and morphogenesis and the genes involved in these processes. In many cases, these genes have been found to encode signaling molecules required elsewhere in the worm, sometimes for vital processes. The ability to isolate non-null alleles of vital genes that have specific effects on vulval development exemplifies the facility of genetic analysis in this organism. Despite the wealth of information generated by these studies, an unanswered question has been the role of the Synthetic Multivulval (SynMuv) genes in controlling vulval cell fates. New studies have shed light on this phenotype and have provided additional information on the diverse functions carried out by these genes.

The art and science of laying an egg

The process by which a hermaphrodite worm produces its egg-laying and mating organ can be divided into four basic stages, some of which overlap temporally (for in-depth reviews on vulval development, see Felix, 2005; Sommer, 2001; Sternberg, 2005; Sundaram, 2005). In the first stage, six vulval precursor cells (VPCs) are generated from progenitor cells in the L1 larva following a series of coordinated cell migrations and divisions. The newly produced VPCs are located along the ventral mid-line of the animal and are designated (from anterior to posterior), P3.p, P4.p, P5.p, P6.p, P7.p, and P8.p (Fig. 1). In order to maintain VPC status, these cells must retain their independence by not fusing with hyp7, a large syncytium that forms much of the hypodermis or skin of the worm. This is accomplished in part through a Hox gene, *lin-39*, that inhibits expression of *eff-1*, a gene that promotes cell fusion; in *lin-39* loss-of-function (LOF) mutants, VPCs aberrantly express *eff-1* and prematurely fuse with the hypodermis, rendering them

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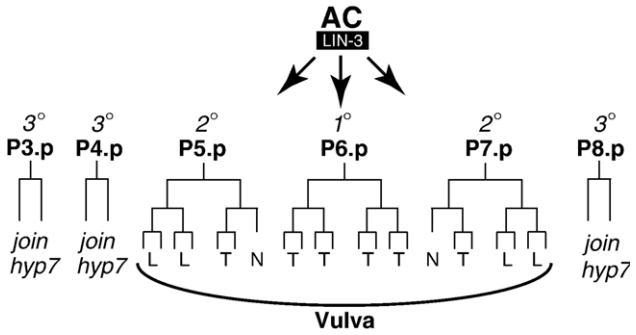

Fig. 1. The six VPCs and their normal fates represented as cell divisions. The vulva is composed of the eight descendants of P6.p, which has executed the 1° fate, the seven descendants of P5.p, which has executed the 2° fate, and the seven descendants of P7.p, which has also executed the 2° fate, but in a manner that is mirror symmetric to P5.p. Fusing with hyp7, the descendants of P3.p, P4.p, and P8.p do not contribute to the vulva. The cell divisions occur in the third larval stage following induction by LIN-3 (arrows), which is expressed by the anchor cell (AC) of the somatic gonad. L (longitudinal) cell divisions indicate an orientation plane that is parallel to the anterior–posterior axis of the animal, whereas T (transverse) divisions are orthogonal to this axis; N indicates non-dividing cells of the secondary lineage.

incapable of responding to vulval induction signals later in development (Clandinin et al., 1997; Maloof and Kenyon, 1998; Shemer and Podbilewicz, 2002).

In the second stage, three of the six VPCs, P5.p, P6.p, and P7.p (P5–7.p), become formally committed to the vulval cell fate. Acquisition of the vulval fate follows exposure of P5–7.p to an EGF-like ligand, LIN-3, during the L3 stage. The source of the inducing signal, the somatic gonad anchor cell, is located just dorsal to P6.p (Fig. 1). A number of lines of evidence indicate that LIN-3 forms a graded signal with P6.p experiencing the highest concentration of LIN-3, followed by the adjacent anterior and posterior cells, P5.p and P7.p, which receive lower doses (Sternberg, 2005). The more distal VPCs (P3.p, P4.p, and P8.p) presumably see even lower levels of LIN-3 that are not sufficient (under normal circumstances) to support the induction of vulval cell fates, resulting in these cells fusing with the hyp7 syncytium. In P5–7.p, binding of LET-23, a receptor tyrosine kinase, by LIN-3 results in the activation of a canonical Ras/MAPK intracellular signaling pathway that ultimately leads to the expression of genes that are required for vulval cell differentiation.

The third stage of vulval development is characterized by P5–7.p initiating a set of invariant cell divisions, leading to the generation of 22 nuclei arranged in a mirror symmetric pattern along the anterior/posterior and left/right axes (Fig. 1). Specifically, P6.p executes the so-called 1° fate, producing eight cells, whereas P5.p and P7.p execute 2° fates and produce seven cells each. The correct implementation of 1° versus 2° fates is accomplished through a complex network of interactions and involves at least three major signaling pathways, including Ras-MAPK, Notch, and Wnt (Felix, 2005; Sternberg, 2005; Sundaram, 2005). A number of other factors, including putative transcriptional regulators, have also been shown to play roles during this stage and are thought to act downstream of the signaling pathways.

The final, most complex, and currently least understood step of vulval development involves the many morphogenetic events that coincide with and follow the execution of the vulval cell divisions, leading to a fully functional organ comprised of seven ring-shaped, multinucleate cells. This step also encompasses events that are required for correct association of the vulval cells with certain adjacent muscles, neurons, and cells of the somatic gonad. Recently, it has been shown that connection of the vulva to the uterus requires the functions of a *C. elegans* Fos homolog (Sherwood et al., 2005), suggesting that studies addressing the mechanisms of vulval morphogenesis, much like those focused on vulval induction, will reveal many interesting molecules that are relevant to human biology and disease.

The ABCs of SynMuv

For the *C. elegans* field, the 1980s and 1990s saw vulval development obtain growth industry status as many labs, led by the groundbreaking work of Bob Horvitz and colleagues (Ferguson and Horvitz, 1985; Ferguson et al., 1987; Sternberg and Horvitz, 1986; Trent et al., 1983), conducted in-depth searches for mutations affecting vulval cell induction. These searches quickly led to an appreciation for two opposing phenotypes: some mutations consistently caused too many of the VPCs to be induced – the Multivulval (Muv) phenotype – and some mutations caused too few of the VPCs to be induced – the Vulvaless (Vul) phenotype. Among the initial mutations isolated was a gain-of-function (GOF) mutation in *let-60*/Ras that led to animals containing ectopic vulvae, the result of abnormal induction events occurring in P3.p, P4.p, and P8.p (in addition to P5–7.p, where LET-60 normally acts) (Beitel et al., 1990; Han and Sternberg, 1990). In contrast, loss-of-function (LOF) mutations in *lin-3* or in *let-23*, the gene encoding its receptor, were found to produce animals where the VPCs were grossly under-induced, often leading to the complete absence of vulval tissue in adults (Aroian et al., 1990; Hill and Sternberg, 1992).

In one curious case, it was noted that the observed hyper-induction of VPCs in a particular strain (originally isolated by Sydney Brenner) (Davison et al., 2005) was the result of two unlinked recessive mutations; neither mutation on its own was capable of causing the Muv phenotype (Ferguson and Horvitz, 1985; Horvitz and Sulston, 1980; Sulston and Horvitz, 1981). This is the genetic equivalent of lightening striking twice, albeit in this case at two discrete locations in the genome. The requirement that two genes be mutant in order to observe Muv animals (Fig. 2) meant that the phenotype in this strain was "synthetic," a product of genetic synergy. The responsible genes, *lin-8* and *lin-9*, were to serve as the paradigm for the many synthetic multivulval (SynMuv) mutants that would follow, and their apparent genetic redundancy, which is a straightforward interpretation of a synthetic phenotype, was inferred to result from a functional overlap of the two gene products. Another Muv mutant isolated from the early screens, *lin-15*, was later shown to carry a lesion that led to the inactivation of two adjacent genes, *lin-15a* and *lin-15b*, neither of which was capable of producing a Muv phenotype when inactivated singly (Clark et al., 1994; Huang et al., 1994).

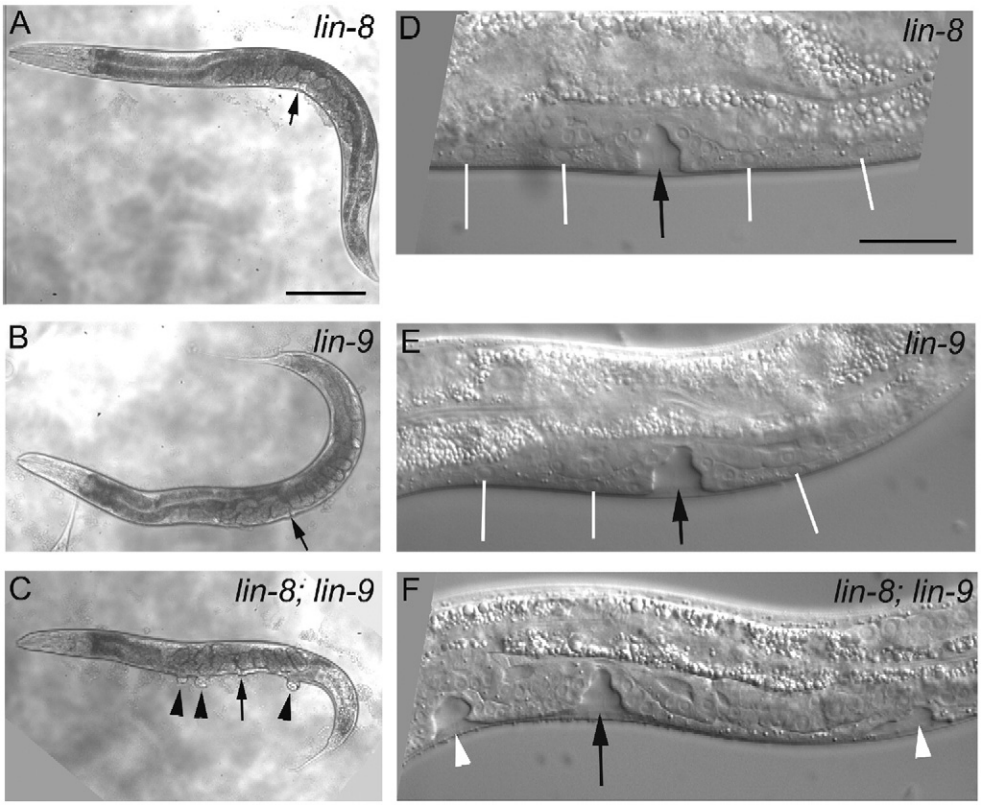

Fig. 2. The SynMuv phenomenon. (A–C) Adult hermaphrodites. Phase optics. Scale bar, 100 μm. The normal vulva of a *lin-8* mutant (A), a *lin-9* mutant (B), and a *lin-8; lin-9* double mutant (C) is indicated with an arrow. Ectopic pseudovulvae, the hallmark of the SynMuv phenotype, protrude from the double mutant (arrowheads). (D–F) Hermaphrodites in the fourth larval stage. Nomarski optics. Scale bar, 20 μm. Normal vulvae derived from P5-P7.p are indicated with an arrow. In the *lin-8* mutant (D) and the *lin-9* mutant (E), daughter nuclei (white lines) of P4.p and P8.p have not contributed to the vulva. Improper induction of P4.p and P8.p in the double mutant (F) results in the development of extra vulval tissue (white arrowheads). The descendants of P3.p are not shown in panels D to F.

In order to find additional SynMuv genes, the Horvitz lab carried out screens in the *lin-8* and *lin-9* single mutant backgrounds for extragenic second-site mutations that led to the appearance of the Muv phenotype (Ferguson and Horvitz, 1989). Several genes, in addition to *lin-8*, were found to be synthetically Muv with *lin-9*. These have been assigned as class A genes (Table 1). Likewise, several genes were designated as class B, because, like *lin-9*, they were synthetically Muv with *lin-8*. At least 24 genes are now known to belong to this class (Table 1). Although this classification scheme has caveats (see below), in general the Muv phenotype results when any class A gene mutation is combined with any class B gene mutation. In contrast, being doubly mutant for just class A or just class B genes usually does not confer Muv. The mechanistic implication of these results was that members within each class were somehow functioning together, either in a pathway or molecular complex, whereas the two different SynMuv classes, being functionally redundant, were most likely acting in parallel.

More recently, a class C SynMuv status has been proposed for four genes that are likely to participate in a conserved transcriptional regulatory complex (Table 1 and also see below), one of which, *trr-1*, was initially identified through its interactions with the class A gene, *lin-15a* (Ceol and Horvitz, 2004). In the case of *trr-1*, strong interactions with class A genes and moderate interactions with the class B group led to the notion of a third separate class whose functions are redundant with both the A and B classes. For the other proposed class C genes, however, the genetic interactions observed with either the A or B classes were considerably weaker. For example, an *ssl-1* mutation failed to show interactions with *lin-15a*, and *epc-1(RNAi)* showed only very weak interactions with *lin-15b*, perhaps raising some question as to the precise functional classification of these genes (Ceol and Horvitz, 2004).

It is also important to stress that not all of the reported SynMuv genes have been confirmed by multiple groups or are necessarily even accurately described. In some cases, the evidence for SynMuv status is relatively weak and may reflect a peripheral (or redundant) role for these genes in one of the SynMuv pathways or possibly in some other process connected to vulval development. For example, although *lin-40* was initially suggested to act as a Class A gene, further studies have not supported this finding but have indicated a more general role in vulval patterning and morphogenesis (Chen and Han, 2001; Solari and Ahringer, 2000). Furthermore, certain SynMuv genes may defy clear-cut categorization. One example is *hpl-2*, which shows broad interactions with the class A genes but also interacts with a subset of the class B group at elevated temperatures (Coustham et al., 2006; Couteau et al., 2002). Another example is *lin-13*, which shows properties of a class B mutant at 15 °C but is Muv as a single mutant at 25 °C (Ferguson and Horvitz,

Table 1
The SynMuv genes of *C. elegans*, arranged by class

Gene	Description	Complex [a]	References
Class A			
lin-8	Novel, acidic, nuclear		1, 2
lin-15a	Novel, hydrophilic		1, 3, 4
lin-38	Unknown		1
lin-56	Unknown		5
smo-1 [b]	SUMO, ubiquitin-related peptide		6
uba-2 [b]	E1B, SUMO activating enzyme		6
Class B			
dpl-1	DP family, forms complex with E2Fs	E D M	7
efl-1	E2F family of transcriptional regulators	E D M	7
gei-4	Coiled-coil and Q/N-rich domains		6
hda-1	Histone deacetylase	N M	8
hpl-2 [c]	Heterochromatin protein 1 (HP1), CHROMO domain	E	9, 10
let-418	Mi-2/CHD3, DNA helicase	N	11, 12
lin-9	Novel, conserved, similar to *Drosophila* Mip130	D M	1, 13
lin-13	C2H2 zinc finger, LXCXE motif		1, 14
lin15b	Novel		1, 3, 4
lin-35	Similar to human pocket proteins pRb, p107, p130	E D M	8
lin-36	Novel, hydrophilic, nuclear		1, 15
lin-37	Novel, hydrophilic, similar to *Drosophila* Mip40	D M	1, 16
lin-52	Novel, conserved in *Drosophila* and vertebrates	D M	5, 17
lin-53	Similar to human Rb associated protein 48, RbAP48	E N D M	8
lin-54	Cysteine-rich, similar to *Drosophila* Mip120	D M	5, 6, 16
lin-61	MBT repeats, similar to human L(3)MBT	M	6
lin-65	Novel, acidic		6, 17
mep-1	C2H2 zinc finger and Q/N-rich domains		6, 17
met-2	Predicted histone H3 lysine-9 methyltransferase	E	6
tam-1	RING finger and B-box domains		18
tra-4	C2H2 zinc finger domains, similar to human PLZF		19
ubc-9 [c]	E2, SUMO conjugating enzyme		6
E01A2.4	Similar to human NFκB activating protein		6
W01G7.3	Similar to human RNA polymerase II subunit J		6
Class C			
epc-1	Similar to enhancer of Polycomb-like, HAT-associated	T	20
mys-1	MYST histone acetyltransferase, CHROMO domain	T	17, 20
ssl-1	SWI/SNF ATPase homolog	T	20
trr-1	Similar to mammalian TRRAP, HAT-associated	T	17, 20
RTK-Ras			
ark-1 [d]	Ack family of nonreceptor tyrosine kinases		17
gap-1 [d]	GTPase activating protein		17
sli-1 [d]	E3, RING finger ubiquitin ligase, similar to hCBL		17

1989; Melendez and Greenwald, 2000), an attribute also observed for certain alleles of *hpl-2* (Couteau et al., 2002). These findings suggest that *lin-13* and *hpl-2* may possess properties of more than one SynMuv class, or perhaps that they act through an additional mechanism that is independent of the SynMuvs to control VPC induction. In addition, because the temperature-sensitive Muv phenotype is observed for null alleles of both *hpl-2* and *lin-13*, it would follow that the underlying process regulated by these genes is itself temperature sensitive. This latter finding is also consistent with the long-standing observation that many combinations of SynMuv genes exhibit a markedly higher penetrance of the Muv phenotype at higher temperatures, the specific meaning of which remains unclear (Ceol and Horvitz, 2004; Ferguson and Horvitz, 1989).

Molecular identities: the connection to transcription and chromatin regulation

To date, the molecular identities of at least 34 SynMuv genes have been reported (Table 1). The clear overriding theme to emerge from the molecular characterization of members from all three SynMuv classes is one of *transcriptional regulation*. This is exemplified by the large class B group, which includes a *C. elegans* E2F ortholog, *efl-1*, and its conserved binding partner, *dpl-1*/Dp (Ceol and Horvitz, 2001). Another class B gene, *lin-35*, encodes the *C. elegans* Retinoblastoma (pRb)/pocket protein ortholog (Lu and Horvitz, 1998), which has been shown to form complexes with E2Fs in other systems to inhibit E2F-target gene transcription (Frolov and Dyson, 2004; Harbour and Dean, 2000; Korenjak et al., 2004; Macaluso et al., 2006). Furthermore, *C. elegans* homologs of a number of transcriptional repressors that are recruited to E2F sites by pRb have also been shown to act as class B genes (Table 1). These include the nucleosome remodeling and histone deacetylase (NuRD) complex components *hda-1*, *lin-53*/RbAp48 (Lu and Horvitz, 1998), and *let-418*/Mi-2 (Von Zelewsky et al., 2000), as well as the putative H3K9 methyltransferase, *met-2* (Poulin et al., 2005), and the HP1 homolog, *hpl-2* (Coustham et al., 2006; Couteau et al., 2002). In addition, a number of class B genes have been shown to be components of the *C. elegans* DRM (for

Notes to Table 1:
References: 1, Ferguson and Horvitz (1989); 2, Davison et al. (2005); 3, Clark et al. (1994); 4, Huang et al. (1994); 5, Thomas et al. (2003); 6, Poulin et al. (2005); 7, Ceol and Horvitz (2001); 8, Lu and Horvitz (1998); 9, Couteau et al. (2002); 10, Coustham et al. (2006); 11, Solari and Ahringer (2000); 12, Von Zelewsky et al. (2000); 13, Beitel et al. (2000); 14, Melendez and Greenwald (2000); 15, Thomas and Horvitz (1999); 16, Harrison et al. (2006); 17, Ceol et al. (2006); 18, Hsieh et al. (1999); 19, Grote and Conradt (2006); 20, Ceol and Horvitz (2004); 21, Korenjak et al. (2004); 22, Lewis et al. (2004); 23, Lipsick (2004); 24, Frolov and Dyson (2004); 25, Macaluso et al. (2006).

[a] Putative assigned protein complexes based on biochemical studies in *C. elegans* and other systems. Abbreviations are: D, dREAM/DRM; E, E2F-Rb; M, MybMuvB; N, NuRD; T, Tip60. For specific references regarding these complexes, see 16, 20–25.

[b] Also shows a SynMuv phenotype with other class A genes.

[c] Also shows a SynMuv phenotype with other class B genes.

[d] Shows principal or exclusive SynMuv interactions with members of the class A group.

DP, Rb, MuvB) complex, which is orthologous to the dREAM (for the *Drosophila* RBF, E2F, and Myb interacting protein) transcriptional regulatory complex of *D. melanogaster* (Table 1; Harrison et al., 2006; Korenjak et al., 2004; Lewis et al., 2004; Lipsick, 2004). Despite the considerable overlap in the subunit compositions of the DRM and NuRD complexes, subtle differences in the phenotypes of strains containing mutations in some of the non-overlapping components suggest that they possess distinct functions (Harrison et al., 2006). Lastly, a number of class B genes not currently associated with either the DRM or NuRD complexes contain motifs associated with transcriptional regulatory functions (Table 1).

The identities of the class C genes also indicate an involvement in chromatin-level transcriptional regulation, as these include putative components of the mammalian Tip60 chromatin remodeling complex (Ceol and Horvitz, 2004). Curiously, in contrast to the class B genes, which are primarily thought to function as transcriptional repressors, the established enzymatic activity of the Tip60 complex is H4 histone acetylation, which is associated with transcriptional activation (Sapountzi et al., 2006). One possible interpretation of this finding is that the class B and C SynMuvs may directly target distinct sets of genes (also see below).

The molecular functions of the class A genes remain the most mysterious. Sequence data have not provided much insight, and two of the genes have yet to be cloned (Table 1). At least one of the class A proteins, LIN-8, is present in nuclei and is predicted to be charged, suggesting a role in transcriptional regulation. RNAi feeding screens have also identified several genes that show interactions with the class B SynMuvs; RNAi of *smo-1* and *uba-2* led to a SynMuv phenotype in a *lin-15b* mutant background, although somewhat weaker interactions with a class A gene, *lin-15a*, were also observed (Table 1; interactions with class C genes were not reported; Poulin et al., 2005). *smo-1* encodes SUMO, and *uba-2* is a presumed SUMO activating enzyme. This same study also found that *ubc-9*, a SUMO conjugating enzyme, displayed interactions with both the class A and B groups. Although protein modification by SUMO has been shown to regulate a diverse set of cellular targets, many of the known substrates include transcription factors, histones, and histone modifying enzymes (reviewed by Gill, 2004, 2005). These findings further support the general conclusion that many or most SynMuv genes likely function at the level of transcriptional regulation.

Also consistent with the role of SynMuv genes in chromatin-level regulation are recent demonstrations that LOF of numerous genes with likely roles in chromatin remodeling can efficiently suppress the Muv phenotypes of multiple class A, B, and C combinations (Andersen et al., 2006; Cui et al., 2004, 2006b; Wang et al., 2005). These include the nucleosome remodeling complex (NURF) factors *isw-1* and *nurf-1*, as well as putative components of *C. elegans* NuA4, SWT1, and COMPASS complexes. Furthermore, many of these suppressors were also able to ameliorate several additional phenotypes associated with class B SynMuv genes (also see below), indicating that they are acting globally to antagonize class B SynMuv activities.

An additional category to emerge from the genetic screens are LOF mutations in *ark-1*, *gap-1*, and *sli-1* (Ceol et al., 2006), all of which are known to act as negative regulators of the vulval-inducing RTK-Ras-MAPK signaling pathway (Table 1; Ceol et al., 2006; Sternberg, 2005). Furthermore, it was previously shown that when coupled to an amber suppressor (*sup-7*), a GOF mutation in *let-60*/Ras also behaved as a class B SynMuv gene (Ferguson and Horvitz, 1989). That such mutations might predispose animals to a Muv phenotype is not unexpected. What is curious is that these mutations specifically show genetic interactions with the class A and C SynMuv genes. Although the meaning of this is currently unclear, it suggests that there are significant functional differences between the SynMuv groups and that the expression of the SynMuv phenotype could potentially arise through more than a single underlying mechanism.

It is worth noting that although a few of the identified SynMuv genes appear to be nematode specific, many appear to have orthologs in diverse phyla. A good example comes from studies on the *Drosophila* Myb-MuvB transcriptional regulatory complex, which was found to contain the fly orthologs of EFL-1, DPL-1, LIN-35, LIN-53, LIN-9, LIN-54, LIN-37, LIN-52, HDA-1, and LIN-61 (Table 1; reviewed by Lipsick, 2004). In addition, studies in mammalian cells have also identified the human LIN-9 ortholog, hLin-9, as a nuclear chromatin-associated protein (Gagrica et al., 2004). These studies also went on to show that hLin-9 physically interacts with human pRb, is required for pRb to induce a senescence-like state in Saos-2 cells, and can inhibit oncogenic transformation of NIH3T3 cells via a mechanism that is dependent on the presence of wild-type pRb. It will be interesting to see the extent to which sequence-related SynMuv genes in various species have conserved molecular and cellular functions.

Towards solving the mechanism

Although it took the field about 17 years to arrive at a convincing mechanistic understanding of the SynMuv phenotype, strong hints about how the SynMuvs were working came from studies conducted more than 16 years ago. In 1990, Robert Herman and Edward Hedgecock demonstrated by mosaic analysis that the repressive effect of the two *lin-15* genes on vulval development occurred by a nonautonomous mechanism, as the presence or absence of *lin-15* functions in the VPCs themselves did not correlate with the acquisition of excess vulval cell fates (Herman and Hedgecock, 1990). Also, although short of definitive, this work strongly indicated that the focus for *lin-15* function might be the hyp7 syncytium, a tissue that lies adjacent to the VPCs. Mosaic analysis also provided strong evidence that *lin-37*, a class B gene, was also functioning non-autonomously via the hypodermis (Hedgecock and Herman, 1995). Together with other results, these findings led to the model that the hypodermis was emitting a signal that somehow led to the inhibition of vulval fates in the VPCs. In wild-type hermaphrodites, the presence of sufficient LIN-3 signal from the anchor cell would override the repression by hyp7 and lead to the induction of P5–7.p but not of P3.p, P4.p,

and P8.p. In the SynMuv double mutants, repression by hyp7 was attenuated or abolished such that all six VPCs could take on vulval fates, even in the absence of the anchor cell signal.

There were, however, conflicting observations. Autonomous function for a number of SynMuv genes was inferred when these genes were found to be expressed in the VPCs, although they were found to be expressed in other tissues as well. Also, mosaic analysis of *lin-36* indicated that this class B gene acts within the VPCs (Thomas and Horvitz, 1999). In addition, genetic epistasis analysis of SynMuv mutants with LOF mutations in the RTK-Ras-MAPK pathway (which cause Vul phenotypes as single mutants) strongly indicated that RTK-Ras-MAPK signaling was required for the expression of the SynMuv phenotype, as triple compound mutant animals were typically missing vulval tissue. A somewhat surprising exception to this was the upstream ligand in the pathway, LIN-3, where it was found that a *lin-3* allele (*n378*) did not suppress the SynMuv phenotype of several tested class A and B SynMuv combinations (Ceol and Horvitz, 2001; Ferguson et al., 1987; Lu and Horvitz, 1998), although this allele did weakly suppress the Muv phenotype of *trr-1; lin-15b* mutants (Ceol and Horvitz, 2004). The failure to see strong suppression with *lin-3* was not, however, completely unexpected, because the SynMuv phenotype is not dependent on the presence of the anchor cell (Ferguson et al., 1987). On the basis of these findings, it was suggested that the SynMuv genes function autonomously within the VPCs to antagonize the downstream effects of RTK-Ras-MAPK signaling at the level of transcription. In this model, the absence of SynMuv gene activity would remove an inhibitory input that normally represses the transcriptional targets of RTK-Ras-MAPK signaling in the VPCs. Furthermore, in this scenario, efficient VPC induction could be accomplished by basal (non-ligand-induced) levels of RTK-Ras-MAPK signaling, provided that the repressive SynMuv input was removed.

Key work reported in two recent papers has largely resolved these issues and has led to a convincing and simple explanation of the SynMuv phenotype. In a careful analysis, Toshia Myers and Iva Greenwald demonstrated that *lin-35* functions within the hypodermis to inhibit vulval cell induction (Myers and Greenwald, 2005). Because these studies used tissue-specific promoters to drive *lin-35* expression in addition to mosaic analysis, several possible caveats associated with the mosaic technique were avoided. The strong conclusion from this work is that *lin-35* functions nonautonomously in hyp7 to control vulval fates and not in the VPCs themselves. Moreover, their observations were completely consistent with the pioneering mosaic analysis by Herman and Hedgecock described above.

In a series of elegant experiments, Mingxue Cui and colleagues have now shown that many Class A and B SynMuv genes are likely to function within the hyp7 syncytium to repress ectopic expression of the VPC inducer, LIN-3 (Cui et al., 2006a). In this model, the absence of SynMuv function leads to the improper secretion of LIN-3 from the hypodermis, leading to the induction of P3.p, P4.p, and P.8p, the VPCs that are normally not induced by LIN-3 from the anchor cell (Fig. 3).

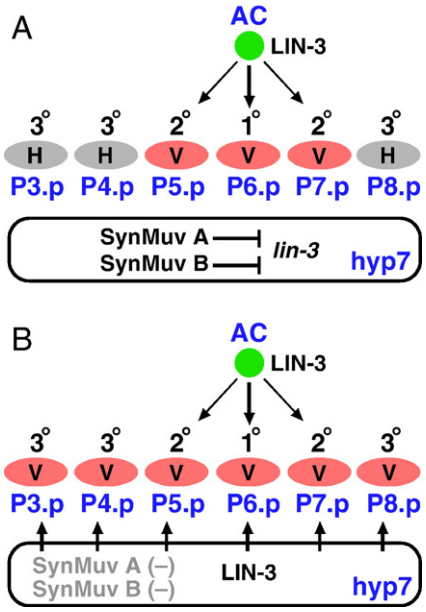

Fig. 3. A model of the SynMuv mutant phenotype (Cui et al., 2006a). Vulval induction in wild-type (A) and SynMuv-mutant (B) animals. In wild type, the anchor cell is the only source of the LIN-3 inducing signal. This leads to P5.p, P6.p, and P7.p acquiring the vulval cell fate (V), whereas P3.p, P4.p, and P8.p adopt a default hypodermal fate (H). In SynMuv mutants, the hypodermal hyp7 syncytium secretes ectopic LIN-3 leading to additional VPCs acquiring the vulval cell fate.

Evidence in support of this mechanism includes the finding that inhibiting *lin-3* expression specifically in the hypodermis (using RNAi-based methods) is sufficient to suppress the SynMuv phenotype. In addition, *lin-3* mRNA abundance was found to be increased in class A and B double mutant animals but not in single mutants, demonstrating that this regulation occurs at the level of transcription and that two classes are functionally redundant for this activity. Given these results, a question arises as to why the *lin-3(n387)* allele was unable to suppress efficiently the SynMuv phenotype in the previous reports. The answer appears to be that this particular allele of *lin-3*, although it strongly impairs induction of the VPCs by the anchor cell, may have significant residual activity in cells other than the anchor cell. This interpretation is consistent with the fact that *n387* cannot be a null allele, because null alleles of *lin-3* are lethal (Ferguson and Horvitz, 1985; Hill and Sternberg, 1992).

What still remain to be determined are how the various SynMuv genes specifically act to repress the expression of *lin-3* transcription and the manner by which class A and class B pathways or complexes are functionally redundant with each other. One straightforward possibility is that the A and B classes act directly on the *lin-3* locus to mediate transcriptional repression. This, however, is unlikely to be the case for the class C genes, given their presumed roles in transcriptional activation. Alternatively, the SynMuv genes, including in some cases genes from within the same class, may act on a number of distinct targets whose functions are ultimately integrated to control *lin-3* levels. If the latter is the case, it could prove quite difficult to

trace the individual contributions of each SynMuv gene to *lin-3*, since these effects could in some cases be indirect. It may also be the case that some SynMuv genes act through an alternate (non-LIN-3-associated) mechanism, although direct evidence for this is currently lacking. Finally, it is possible that some of the recent functions ascribed to the class B SynMuv genes, such as mediation of the RNAi response (see below), may also turn out to be relevant to the SynMuv phenotype. However, the observation that not all class B SynMuv genes function in these processes makes this possibility less likely.

Multiple functions for SynMuv genes

Given the observation that many SynMuv genes are broadly expressed throughout development and that the VPCs comprise less than one percent of the total somatic cell number in a *C. elegans* adult, an obvious question to follow in the wake of the vulval studies has been, what are these genes doing in other tissues? In several cases, additional functions for SynMuv genes were already evident based on the phenotypes of strong LOF mutations or RNAi studies. For example, mutations affecting *dpl-1* and *efl-1* were identified in screens for maternal-effect lethal mutations. Their inactivation leads to the mislocalization of a zinc finger protein, MEX-5, resulting in abnormal patterns of differentiation in the early embryo (Page et al., 2001). Another example is *lin-9*, where null mutants show highly penetrant defects in the development of hermaphrodite and male reproductive systems (Beitel et al., 2000). In many other cases, however, lack of an overt phenotype, even in presumed null mutants, has failed to shed light on additional roles the SynMuv genes may play during development.

Of particular interest to many labs has been the class B gene, *lin-35*/Rb, the human ortholog of which is known to be functionally inactivated in the majority of human cancers (Sherr and McCormick, 2002). In the case of *lin-35*, lack of an obvious phenotype in the null mutant motivated screens for new mutations displaying *lin-35*-synthetic phenotypes that were distinct from those affecting vulval induction (Fay et al., 2002). This approach, along with work from van den Heuvel and colleagues, revealed a function for *lin-35* in the control of cell proliferation during larval development (Boxem and van den Heuvel, 2001), a role that is known to be well conserved in other systems (Giacinti and Giordano, 2006). For example, loss of *lin-35* can substantially suppress the growth defects of cyclin D (*cyd-1*) and *cdk-4* mutants, and mutations in *lin-35* show strong synthetic genetic interactions with *cki-1* and *fzr-1*, which are known to function as negative regulators of the cell cycle in other systems (Boxem and van den Heuvel, 2001; Fay et al., 2002). Furthermore, cell cycle regulation appears to be the shared function of a number of the class B SynMuv genes, including several phylogenetically well-conserved genes such as *lin-36* and *lin-9* that had not been previously implicated in this process (Boxem and van den Heuvel, 2002; Fay et al., 2002). As noted above, hLin-9 associates with both chromatin and pRb in humans and may function in tumor suppression (Gagrica et al., 2004). Additionally, similar to *C. elegans*, a homozygous LOF mutation in hLin-9 can partially suppress the phenotype of homozygous *cdk4* mutant mice and also leads to the upregulation of E2F target genes in *cdk4* mutant cells in culture (Sandoval et al., 2006; White-Cooper et al., 2000). Furthermore, *Drosophila aly/lin-9* has been shown to regulate meiotic cell cycle progression and the terminal differentiation of male gametes (White-Cooper et al., 2000). Finally, human LIN-9 was recently shown to directly promote expression of ~12 genes important for the G2/M cell cycle transition, a function that it carries out in association with B-MYB (Osterloh et al., 2007).

The synthetic screen approach, as well as the work of other laboratories, has also led to the identification of roles for *lin-35* (and other class B genes) in somatic gonad development (Bender et al., 2004, 2007; Voutev et al., 2006), organogenesis of the pharynx (Fay et al., 2003, 2004), and larval growth, viability, and nutritional uptake (Cardoso et al., 2005; Chesney et al., 2006; Cui et al., 2004) (Fay lab, unpublished results). Recent work has also revealed non-redundant functions for several class B genes, including *lin-35*, in repressing the expression of germline-specific genes in the soma and damping down the RNAi response in both somatic and germline tissues (Lehner et al., 2006; Unhavaithaya et al., 2002; Wang et al., 2005). In addition, several class B genes have been shown to promote the somatic expression of repetitive transgenic arrays (Hsieh et al., 1999), possibly by repressing the expression of germline-specific silencing factors in the soma.

In most cases, the basis for these non-vulval phenotypes is unknown, although the majority of these defects appear not to be connected to cell cycle control. It is also unclear if the functions identified for the class B SynMuv genes in *C. elegans* are conserved in other species and phyla. Nevertheless, the finding that SynMuv genes act to repress ectopic growth factor expression in *C. elegans* has led to the suggestion that this may constitute part of the tumor suppressing activity of pRb (and potentially other class B gene orthologs) in mammals. Similar arguments have also been made regarding the repression of germline-specific genes in the soma and for the observed roles for class B genes in tissue organization and morphogenesis. Furthermore, the observation that hLin-9 can inhibit oncogenic transformation in tissue culture cells and that it may carry out this activity with pRb in a manner that is independent of cell cycle regulation lends support to these ideas (Gagrica et al., 2004). It is undoubtedly the case that further studies in *C. elegans* and other systems will reveal many additional redundant and non-redundant functions for the SynMuv genes, a number of which may have important implications for human biology and health.

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