Note

HPL-2/HP1 Prevents Inappropriate Vulval Induction in Caenorhabditis elegans by Acting in Both HYP7 and Vulval Precursor Cells

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ABSTRACT

A current model for Caenorhabditis elegans vulval cell fate specification is that SynMuv genes act redundantly in the hyp7 hypodermal syncytium to repress the LIN-3/EGF inducer and prevent ectopic vulval induction of vulva precursor cells (VPCs). Here we show that the SynMuv gene hpl-2/HP1 has an additional function in VPCs, where it may act through target genes including LIN-39/Hox.

The Caenorhabditis elegans vulva represents a simple system in which to study how chromatin factors influence developmental processes dependent on signaling pathways. The vulva is derived from three of six equivalent vulva precursor cells (VPCs) located on the ventral side of the animal. An epidermal growth factor (EGF)-like signal from the neighboring anchor cell is transduced by a conserved RTK/Ras pathway and induces these three cells to divide and generate the vulva (Kornfeld 1997). The three other VPCs normally give rise to nonvulval cells due to the activity of three functionally redundant sets of genes, known as class A, B, and C synthetic multivulva (SynMuv) genes (Ferguson and Horvitz 1989; Ceol and Horvitz 2004). When any two redundant pathways are inactivated, these cells adopt vulval fates and produce a Muv phenotype. Most SynMuvB genes encode chromatin-associated factors, including homologs of an Rb-related complex (Lü and Horvitz 1998; Ceol and Horvitz 2004). When any two redundant pathways are inactivated, these cells adopt vulval fates and produce a Muv phenotype. Most SynMuvB genes encode chromatin-associated factors, including homologs of an Rb-related complex (Lü and Horvitz 1998; Ceol and Horvitz 2004), the Mi-2/NuRD complex (Solari and Ahringer 2000; Von Zelewsky et al. 2000), and the heterochromatin-associated protein HP1 (Couteau et al. 2002).

In embryonic extracts two distinct SynMuvB complexes have been identified: one containing LIN-35Rb and related to complexes isolated from Drosophila, and one similar to mammalian NuRD (Korenjak et al. 2004; Lewin et al. 2004). Although HPL-2/HP1 was not detected in either one of these complexes, we have shown that HPL-2 physically interacts in vivo with the SynMuvB gene LIN-13 (Couteau et al. 2006). Furthermore, in mammalian cells the malignant-brain-tumor protein L3MBTL1, a homolog of the SynMuvB gene LIN-61, was found in association with core histones, HP1, and pRB (Trojer et al. 2007).

Mosaic analysis of lin-15AB and lin-37B suggested that these SynMuv genes may act in the hyp7 syncytium surrounding the VPCs (Herman and Hedgecock 1990; Hedgecock and Herman 1995), while for lin-36, another SynMuvB gene, a site of action in the VPCs was suggested (Thomas and Horvitz 1999). More recently, tissue-specific rescue experiments were used to confirm a hyp7 cellular focus for lin-35Rb (Myers and Greenwald 2005). Within hyp7, lin-35 Rb was shown to repress ectopic expression of the VPC inducer LIN-3 (Cui et al. 2006), leading to a model in which the absence of SynMuv function causes the improper secretion of LIN-3 from the hypodermis and the ectopic induction of VPCs. Nonetheless, different SynMuv genes may have different cellular foci, consistent with their action in distinct complexes, and establishing the cellular focus of each gene remains an important issue.

Genetic data show that while HPL-2 acts as a SynMuvB gene at 20°, at higher temperatures it may act in both SynMuvA and -B pathways (Couteau et al. 2002), suggesting that it might function more broadly in the transcriptional repression of genes necessary for proper vulval development. To gain insight into how hpl-2 may interact with other SynMuv genes, we investigated its cellular focus.

HPL-2 and HPL-1 act in both HYP7 and VPCs for vulval cell fate determination: Combining a mutation in hpl-2 with mutations in SynMuvA genes results in a highly

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penetrant SynMuv phenotype at 20\degree. Because HPL-2 is ubiquitously expressed in most cell types, including VPCs and HYP7 at all stages of development (Schott et al. 2006; Figure 1), we carried out tissue-specific rescuing experiments by expressing hpl-2::cDNA under the control of the dpy-7 and lin-31 promoters, expressed in HYP7 and VPCs, respectively (Myers and Greenwald 2005). As shown in Figure 2A, using three independent lines we found that the percentage of Muv animals observed in hpl-2;lin-15A animals passed from 100% in the control strain to 5–12% when hpl-2 was expressed in HYP7. Similar results were obtained in rescuing experiments with hpl-2;lin-38A animals. Therefore, as with the Syn-MuvB genes lin-35, lin-37, and lin-15B (Herman and Hedgecock 1990; Hedgecock and Herman 1995; Myers and Greenwald 2005), hpl-2 acts in hyp7. Surprisingly, however, we found that expression of hpl-2 in VPCs using the lin-31 promoter also rescued the Muv phenotype (5–20% Muv; Figure 2). This rescue is unlikely to be due to ectopic expression of the lin-31p::hpl-2 array in other tissues, as low-copy arrays were also able to partially rescue (supplemental Figure 1). Furthermore, expression of lin-31::CFP and dpy-7::YFP constructs in...
hpl-2 animals was no different from that of wild type (supplemental Figure 2 and data not shown). Finally, loss of function of SynMuvB genes, including lin-35 and hpl-2, commonly increases silencing (the Tam phenotype; HSIEH et al. 1999; SIMONEK et al. 2007) rather than expression of transgenes in somatic cells. Altogether, our data are consistent with the possibility that HPL-2 might act in both hyp7 and the VPCs to affect vulval cell fate specification. At elevated temperatures, a second HP1 homolog, HPL-1, acts redundantly with HPL-2, but not with other SynMuv genes in vulval development (SCHOTT et al. 2006). Tissue-specific rescuing experiments showed that hpl-1 expression from either hyp7 or VPCs only partially restored normal vulval induction in hpl-1;hpl-2 mutants at 24°C (Figure 2B). These results suggest that hpl-1 is required in both hyp-7 and VPCs for full rescue or has additional functions outside of these tissues.

**HPL-2 regulates LIN-3 expression in the absence of other SynMuv mutations:** Expression of lin-3 is increased in SynMuvA;SynMuvB double mutants with respect to the single mutants or wild type (CU1 et al. 2006). To assess how lin-3 expression levels correlate with the highly penetrant Muv phenotype of hpl-2;SynMuvA mutants at 20°C and hpl-1;hpl-2 double mutants at 24°C (90–100%, Figure 2), and with the low-penetrance Muv phenotype of hpl-2 single mutants at 25°C (COUTSTHAM et al. 2006), we measured lin-3 mRNA levels in each of these contexts. hpl-2;lin-15A mutants showed a significant increase in lin-3 mRNA levels, consistent with what has been previously been observed for this and other SynMuvA;SynMuvB double mutant combinations (Figure 3; CU1 et al. 2006; ANDERSEN and HORTT 2007). Although hpl-1;hpl-2 double mutants at 24°C exhibit a more penetrant phenotype than hpl-2 single mutants at 25°C, lin-3 mRNA expression levels were increased two-to threefold in both backgrounds. Therefore, lin-3 overexpression levels alone are not predictive of the penetrance of ectopic vulval induction. This is further supported by the fact that lin-36 animals, which are non-Muv at 25°C, also showed a significant increase in lin-3 levels. lin-3 expression was reduced in lin-53 single mutants (Figure 3), consistent with the positive role previously observed with other SynMuvB genes (CU1 et al. 2006). It is, however, worth noting that in these experiments we are measuring lin-3 RNA levels from bulk RNA preparations, raising the possibility that these measurements do not accurately reflect LIN-3 protein levels in VPCs.

**TABLE 1**

| Genotype                     | % Muv (20°C) | n  |
|------------------------------|--------------|----|
| lin-15AB (empty vector)      | 100          | 113|
| lin-15AB; lin-3(RNAi)         | 7            | 109|
| hpl-2; lin-15A (empty vector)| 100          | 48 |
| hpl-2; lin-15A; lin-3(RNAi)   | 3            | 85 |
| hpl-2; lin-15A; Ex[dpy-7p::lin-3hp] | 0  | 298|
| hpl-2; lin-15A; sid-1(qt2)    | 100          | 102|
| hpl-2; lin-15A; sid-1(qt2); Ex[dpy-7p::lin-3hp] | 0  | 100|
| hpl-2; lin-15A; Ex[lin-31p::lin-3hp] | 100  | 126|

**TABLE 1**

| Genotype                     | % Muv (24°C) | n  |
|------------------------------|--------------|----|
| hpl-2; hpl-1 (empty vector)  | 99           | 115|
| hpl-2; hpl-1; lin-3(RNAi)p     | 4           | 114|
| hpl-2; hpl-1; Ex[dpy-7p::lin-3hp] | <1 | 124|
| hpl-2; hpl-1; Ex[lin-31p::lin-3hp] | 99  | 223|

Strains were grown on HT115 bacteria either carrying the empty vector or expressing lin-3 double-strand RNA. Vulval induction was scored as described in Figure 2. Ex[dpy-7p::lin-3hp] and Ex[lin-31p::lin-3hp] are extrachromosomal arrays expressing a lin-3 hairpin-forming structure in hyp7 (CU1 et al. 2006) and VPCs (DUJT et al. 2004), respectively. Data are the average of two independent lines. Experiments were performed at 20°C.

*p These animals were vulvaless due to reduction of the inducive signal from the anchor cell (AC).

† These animals are also vulvaless due to spreading of the RNAi effect from hyp7 to the AC, as previously described (CU1 et al. 2006).
LIN-3 is not likely to be a relevant target for HPL-2 in VPCs: Expression of a hairpin-forming lin-3 sequence in hyp7 was able to suppress the SynMuv phenotype observed for hyp7;lin-15A mutants at 20°C (Table 1). Suppression was also observed in a sid-1 mutant (Winston et al. 2002), which prevents the spreading of the RNA interference (RNAi) effect between cells. Therefore, as previously shown for other class A and B SynMuv genes, hpl-2 functions within hyp7 to repress lin-3 (Cut et al. 2006). Similar results were observed for hpl-1;hpl-2 mutants. An additional role for LIN-3 in VPCs has previously been proposed (Dutt et al. 2004). However, we found that lin-3 RNAi in VPCs failed to suppress the Muv phenotype of either hpl-2;lin-15A or hpl-2;hpl-1 mutants to any extent, suggesting that lin-3 is not the relevant target in VPCs. Although ectopic expression of lin-3 from hyp7 in these contexts could potentially mask any contribution from lin-3 overexpression in VPCs, the lack of even a partial rescue from lin-3 RNAi in VPCs suggests that, in VPCs, HPL-2 may act through an alternative, LIN-3-independent mechanism.

Previous studies have suggested an additional role for some SynMuv genes in repressing vulval cell fate in VPCs through repression of the lin-39 Hox gene, thereby promoting fusion with the surrounding hyp7 through the activity of the EFF-1 fusogen (Chen and Han 2001a, Shemer and Podbilewicz 2002). Interestingly, while components of the NuRD complex were found to inhibit lin-39 expression, Rb/E2F complex components were unexpectedly found to be required for normal lin-39 expression, suggesting independent functions (Chen and Han 2001a, b). More recently, the SynMuvB gene LET-418/Mi-2 was shown to directly regulate lin-39 transcription during vulval induction (Guerry et al. 2007).

We have previously shown that lin-39 expression increases in hpl-2 mutants at the time of vulval induction (Coustham et al. 2006), suggesting that lin-39 could be a relevant target of HPL-2 in VPCs. To further investigate this, we made use of functional translational lin-39::GFP fusions that include the entire lin-39 genomic sequence and recapitulate endogenous LIN-39 expression (Wagemaister et al. 2006). In wild-type animals, this construct was expressed in P5.p–P8.p and more weakly in P3.p and P4.p at the time of vulval induction. By contrast, in hpl-2 mutants we found that lin-39::GFP expression significantly increased in P4.p, P5.p, P7.p, and P8.5 (Figure 4). As previously shown for let-418/Mi-2, lin-39 RNAi experiments showed that this increase is independent of lin-3/EFG (data not shown; Guerry et al. 2007).

Altogether, our results suggest that in hyp7, hpl-2 may act with lin-35Rb and other SynMuvB genes to repress lin-39 expression, whereas in VPCs it may antagonize LIN-3/EFG signaling at the level of the transcription of additional target genes, including lin-39, independently of lin-35Rb and in conjunction with lin-36B and other components of the NuRD complex. Cellular focus studies with additional SynMuv genes will contribute to a better understanding of these mechanisms. More generally, these results show how chromatin regulatory factors may antagonize signaling pathways both cell autonomously at the level of downstream transcriptional output and cell nonautonomously to prevent inappropriate signaling.

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