Transcriptional network underlying *Caenorhabditis elegans* vulval development

Takao Inoue, Minqin Wang, Ted O. Ririe, Jolene S. Fernandes, and Paul W. Sternberg*

Division of Biology and Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA 91125

Edited by Eric H. Davidson, California Institute of Technology, Pasadena, CA, and approved February 1, 2005 (received for review November 11, 2004)

The vulval development of *Caenorhabditis elegans* provides an opportunity to investigate genetic networks that control gene expression during organogenesis. During the fourth larval stage (L4), seven vulval cell types are produced, each of which executes a distinct gene expression program. We analyze how the expression of cell-type-specific genes is regulated. Ras and Wnt signaling pathways play major roles in generating the spatial pattern of cell types and regulate gene expression through a network of transcription factors. One transcription factor (*lin-29*) primarily controls the temporal expression pattern. Other transcription factors (*lin-11*, *cog-1*, and *egl-38*) act in combination to control cell-type-specific gene expression. The complexity of the network arises in part because of the dynamic nature of gene expression, in part because of the presence of seven cell types, and also because there are multiple regulatory paths for gene expression within each cell type.

organogenesis | signaling pathways | transcription

Developmental events are driven by spatially and temporally regulated gene expression. Understanding how complex patterns of expression are produced is therefore a critical part of deciphering mechanisms of development. In general, intercellular signaling mechanisms interact with a network of transcription factors to generate cell-type-specific patterns of gene expression. The late stage of *Caenorhabditis elegans* vulval development offers a useful model in which to study this process. During this period of vulval development, seven distinct cell types are produced that express unique combinations of genes. Over the last several years, a number of genes were discovered that are expressed in cell-type and stage-specific patterns in the vulva, and several transcription factors were found to regulate these genes. In this paper, we synthesize and extend our current knowledge of this genetic network.

The *C. elegans* vulva connects the uterine lumen to the outside, allowing for passage of sperm and fertilized eggs (1). Vulval cells are generated postembryonically from precursor cells P3.p P4.p, P5.p, P6.p, P7.p, and P8.p [also called vulval precursor cells (VPC)]. During the mid-third larval (L3) stage, EGF and Notch signaling induces the middle three VPCs (P5.p, P6.p, and P7.p) to adopt vulval fates, whereas P3.p, P4.p, and P8.p fuse with the hypodermal syncytium, hyp7 (2–6).

During the late-L3 to the early-L4 stage, P5.p, P6.p, and P7.p undergo two or three rounds of cell division to produce 22 nuclei (7) (Fig. 1*A*). These nuclei are in cells of seven types (vulA, vulB1, vulB2, vulC, vulD, vulE, and vulF), as evidenced by subsequent morphogenetic movements and by the pattern of gene expression (8, 9) (Fig. 1*B*). The seven cell types that are present in the adult vulva represent specializations within the general epithelial cell class. These cells exhibit cell-type general features; for example, each expresses *ajm-1*, a component of the apical junction that connects neighboring cells in epithelial tissues (8). However, in addition, each cell type exhibits functional specializations: vulF cells, which form the innermost section of the vulva, connect directly with cells of the uterus. vulE cells form structural attachments to lateral hypodermal (seam) cells. vulC and vulD cells attach to vulval muscles that open the vulva for the passage of eggs. vulA cells form attachment to the hyp7 syncytium. It is expected that gene expression differences underlie these specializations.

Here, we are concerned with the execution of cell-type-specific gene expression programs during the late L3 and L4 stages, mostly after the terminal division of vulval cells. During this period, each cell type exhibits a cell-type-specific pattern of gene expression, and several transcription factors are known that regulate the expression of these cell-type-specific genes. We bring together our current knowledge of this system to produce the framework in which to investigate the gene regulatory network controlling vulval organogenesis.

Materials and Methods

Determination of Gene Expression Patterns. Essentially all gene expression analyses described in this paper (including data from other papers) were carried out by using *gfp* reporter transgenes. For all results, it is possible that reporter expression does not accurately reflect the expression pattern of the endogenous gene. For simplicity, we refer to the reporter by the corresponding gene name.

The expression pattern of C55C3.5 was determined by using *gfp* reporter clone pUL#G221N (I. Hope, personal communication). This plasmid was injected into *unc-119*(*ed4*) animals by using the plasmid pDP#MM016B [*unc-119*(+)] as a coinjection marker (10). Of genes listed in Fig. 1*B* and in the main text, we have not examined the expression pattern of *syg-2*, *bam-2*, and *sqv-4*. Because GFP is likely to be stable for many hours, the time at which expression is turned off is not reliably indicated by decreased GFP expression. For most genes we analyzed, GFP fluorescence persists into the adult stage.

Genotypes. For Tables 1–3, *gfp* reporter transgenes used were *ayIs4[egl-17::gfp]*, *syIs50[cdh-3::gfp]*, *syIs49[zmp-1::gfp]*, and *syIs54[ceh-2::gfp]* (9). The *egl-26::gfp* transgenic line analyzed was *kuIs36* (11). Mutations used are; *cog-1*(*sy275*), *cog-1*(*sy607*), *lin-29*(*sy292*), *lin-11*(*n389*), and *egl-38*(*n578*). Of two *cog-1* transcripts, the longer *cog-1A* transcript contains a corepressor-binding domain, whereas the shorter *cog-1B* transcript does not (12). *sy275* is a missense mutation predicted to affect both transcripts. *sy607* is a deletion that eliminates the *cog-1A* transcript. The two alleles exhibit complementary defects in vulval development (13). Although both alleles are recessive, it is not known whether the loss of *cog-1* function causes observed phenotypes. *lin-29*(*sy292*) and *lin-11*(*n389*) are strong loss-of-function alleles, and *egl-38*(*n578*) is a reduction-of-function allele. Strains were constructed by using standard methods.

Results and Discussion

Vulval Cell-Type-Specific Gene Expression. A number of genes are expressed in specific subsets of vulval cells (Fig. 1*B*). Previously

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: VPC, vulval precursor cell; L*n* stage, larval *n* stage.

*To whom correspondence should be addressed. E-mail: pws@caltech.edu.

www.pnas.org/cgi/doi/10.1073/pnas.0408122102

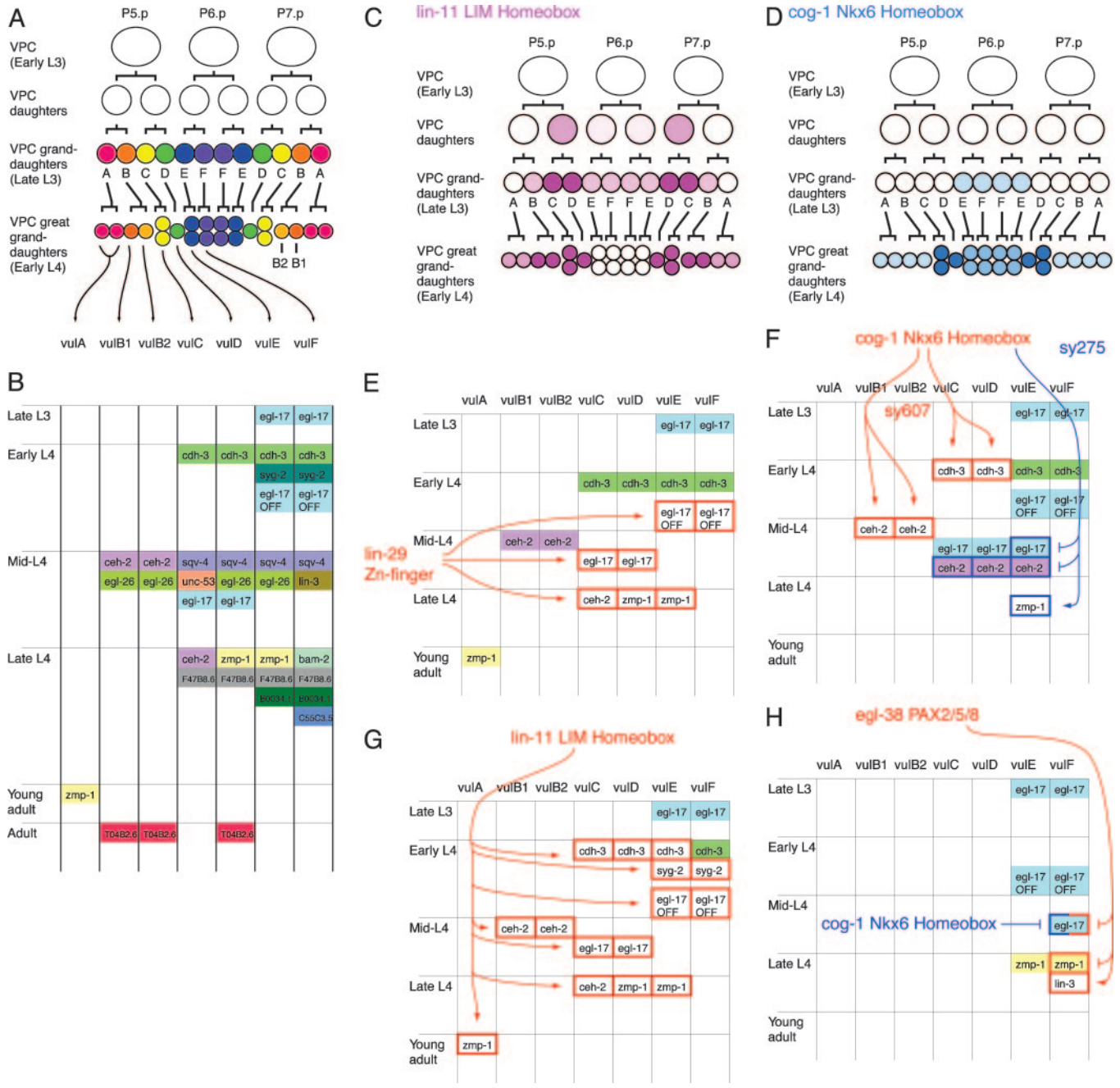

Fig. 1. The pattern of gene expression during late stages of vulval development. (*A*) An overview of vulval development. Lineal origins of 22 vulval nuclei are indicated. ''ABCDEFFEDCBA'' refer to vulval cell types vulA, vulB1, vulB2, vulC, vulD, vulE, and vulF. vulB is the only case in which a single VPC granddaughter gives rise to two cell types (8). Vulval cell nuclei at each stage are positioned as indicated (left, anterior; right, posterior). (*B*) A summary of cell-type specificity and timing of expression in the wild type (*Materials and Methods*) (9, 11, 13–21, 24–26). Boxes indicate stages at which gene expression is activated. The vertical order of events within each time block is arbitrary. For *egl-17*, vulE/vulF expression begins in P6.p (early L3) and persists in their descendants (vulE and vulF) until turned off in the early L4 stage. This inactivation, which is regulated by *lin-29* and *lin-11*, is indicated by the box marked ''*egl-17* OFF.'' *ceh-2* is expressed at a higher level in vulB1 compared with vulB2. (*C*) Expression pattern of *lin-11*. The diagrammed pattern is based on the *lin-11*::*gfp* transgene *syIs80* (26). (*D*) Expression pattern of *cog-1*. The pattern is based on the *cog-1*::*gfp* transgene *syIs63* (13). (*E*) The altered pattern of gene expression in *lin-29* mutants (9, 23) (Tables 1 and 2). White boxes with the red outlines indicate loss of expression and loss of *egl-17* down-regulation in the *lin-29* mutant. *lin-29* appears to regulate events that occur during the mid-L4 to the late L4 stage. (*F*) The altered pattern of gene expression in *cog-1* mutants (Table 3 and Fig. 2). Arrows are drawn with the assumption that both *sy607* and *sy275* phenotypes are caused by different reduction of function of the *cog-1* gene. Filled boxes with red or blue outline indicate ectopic expression. (*G*) Altered pattern of gene expression in *lin-11* mutants (26). (*H*) Altered pattern of gene expression in the *egl-38* mutant (16) (Tables 1 and 2). *egl-17* expression in vulF is observed in the *cog-1*(*sy275*); *egl-38* double mutant, suggesting a redundant repression mechanism.

described genes of this type include *cdh-3* (14), *egl-17* (15), *lin-3* (16), *zmp-1* (9, 17), *ceh-2* (9), T04B2.6 (9), F47B8.6 (9), B0034.1 (9), *unc-53* (18), *egl-26* (11), *sqv-4* (19), *bam-2* (20), and *syg-2* (21). *egl-26* was previously reported to express in vulE and vulB2 cells (11). We found that a nuclear-localized *egl-26*::*gfp* transcriptional fusion expressed in vulB1, vulB2, vulD, and vulE cells (*Materials and Methods*). The expression was somewhat variable and was observed starting from the mid-L4 stage and continuing

Table 1. Expression of *zmp-1* in vulE and vulF cells

Genotype	vulE	vulF	No. of animals
Wild type	+	−	80
lin-11	−	−	55*
lin-29	−	−	50†
cog-1 (sy275)	−	−	52
egl-38	+	+	48
lin-11; egl-38	−	−	52
lin-29; egl-38	−	−	56
cog-1; egl-38	−	−	56

*Ref. 26.
†Ref. 9.

into the adult stage. The C55C3.5 gene encoding a novel protein was previously found to express in vulval cells (I. Hope, personal communication). We found that *C55C3.5*::*gfp* was expressed in vulF cells, starting from the late-L4 and continuing into the adult stage.

Several conclusions can be drawn from Fig. 1*B*. First, all seven cell types exhibit distinct programs of gene expression, despite the fact that these cells are related by cell lineage and function. [vulB1 and vulB2 differ in the level of *ceh-2* expression but otherwise have similar expression profiles (9)]. Distinct expression profiles likely underlie distinct functions of vulval cell types. For example, *lin-3*, which encodes an EGF-related signaling protein, is expressed in vulF cells in the mid-L4 stage (16). This signal is required for a vulva-to-uterus signaling that induces a specific fate, uv1, in uterine cells adjacent to vulF.

The pattern of marker expression also reveals a strict temporal regulation of gene expression (Fig. 1*B*). For example, *cdh-3* is expressed in early L4, F47B8.6 is expressed in late L4, and T04B2.6 is expressed ≈1 day after the L4-to-adult molt (9). For *egl-17*, *ceh-2*, *zmp-1*, and *sqv-4*, the timing of gene expression is different for different vulval cells (9, 15, 19). For example, *egl-17* is expressed in vulE and vulF cells in the L3 stage and in vulC and vulD in the L4 stage.

Trans-Regulation of Vulva Gene Expression. The analysis of the regulatory network controlling the pattern of gene expression in the vulva has focused primarily on the effect of transcription factor mutations on gene expression reporter transgenes. In most cases, a direct transcriptional regulation of the target has not been demonstrated. Key results are summarized in Fig. 1 *E–H*. So far, important regulators are *lin-29* (encoding Zn-finger transcription factor; Fig. 1*E*) (9, 22, 23), *cog-1* (Nkx6 homeodomain; Fig. 1 *D* and *F*) (13), *lin-11* (LIM homeodomain; Fig. 1 *C* and *G*) (24–26), and *egl-38* (PAX 2/5/8; Fig. 1*H*) (16, 27).

Table 2. Expression of *egl-17* in vulE and vulF cells (L4)

Genotype	vulE	vulF	No. of animals
Wild type	−	−	59
cog-1 (sy275)	+++	−	46
egl-38	−	−	38
cog-1; egl-38	+++	+++	37
lin-11	—*	—*	45†
lin-29	—*	—*	43‡
lin-11; lin-29	—*	—*	38
lin-11; cog-1	—*	—*	43
lin-29; cog-1	—*	—*	40
lin-11; cog-1; egl-38	—*	—*	35
lin-29; cog-1; egl-38	—*	—*	36

*These cells express *egl-17*::*gfp* at a low level. We interpret these as the persistence of L3 expression.
†Ref. 26.
‡Ref. 23.

Table 3. Expression of *egl-17, ceh-2,* and *cdh-3* in *cog-1* mutants

Reporter	Mutations	vulA	vulB1 and vulB2	vulC	vulD	vulE	vulF
egl-17	+	0	0	100	100	0	0
egl-17	*cog-1 (sy275)*	0	0	100	92	92	0
egl-17	*cog-1 (sy607)*	0	0	93	100	0	0
ceh-2	+	0	100	0	0	0	0
ceh-2	*cog-1 (sy275)*	20	90	80	80	88	0
ceh-2	*cog-1 (sy607)*	0	0	0	0	0	0
cdh-3	+	0	0	100	100	100	100
cdh-3	*cog-1 (sy275)*	0	0	100	100	100	100
cdh-3	*cog-1 (sy607)*	0	0	14	14	71	94

Percentages of cells in mid-L4 animals that expressed *egl-17*::*gfp*, *ceh-2*::*gfp* and *cdh-3*::*gfp*. See Table 4, which is published as supporting information on the PNAS web site, for number of cells scored.

A Temporal Regulator of Gene Expression. *lin-29* is required for the expression of *egl-17* in vulC and vulD (23), *ceh-2* in vulC (9), and *zmp-1* in vulD and vulE (Fig. 1*E*, Tables 1 and 2, and Fig. 5, which is published as supporting information on the PNAS web site) (9). By contrast, *lin-29* in not required for the expression of *cdh-3* in vulC, vulD, vulE, vulF (9), *ceh-2* in vulB (9), *egl-17* in vulE and vulF (23), and *zmp-1* in vulA (9). Moreover, the expression of *egl-17* in vulE and vulF is observed during the L4 stage (23), suggesting that the mechanism that turns off *egl-17* expression in these cells is compromised (Fig. 5). These *lin-29* phenotypes are not easily explained by cell fate changes between vulval cell types but suggest a temporal regulatory defect: *lin-29* mutations cause loss of events associated with the mid-to-late L4 time points. This interpretation of these data is particularly attractive, because *lin-29* mutations are known to cause heterochronic defects in other tissues, specifically in the L4-to-adult transition in the lateral hypodermis (22, 28, 29). *lin-29* is expressed in all vulval cells, starting in the mid-L3 stage and continuing through the L4 stage (30).

Cell-Type-Specific Regulators of Gene Expression. We analyzed the effect of two *cog-1* (Nkx6.1/6.2 homeodomain) mutations on the expression of vulval-cell-specific gene expression reporters (Fig. 1*F*, Table 3, and *Materials and Methods*). *cog-1*(*sy275*) is a missense mutation in the homeodomain, and *cog-1*(*sy607*) is a small deletion that eliminates one of two *cog-1* transcripts (13). We found that in the mid-L4 stage, *cog-1*(*sy275*) caused ectopic expression of *egl-17* in vulE cells (Fig. 2) and ectopic expression of *ceh-2* in vulC, vulD, and vulE cells and loss of *zmp-1* expression in vulE cells. In contrast, *cog-1*(*sy607*) caused loss of *cdh-3* expression in vulC, vulD, and vulE cells and loss of *ceh-2* expression in vulB. These results indicate that *egl-17*, *cdh-3*, *ceh-2*, and *zmp-1* are regulated by the *cog-1* gene. Although some *cog-1* expression is observed in all vulval cells, *gfp* reporters suggest that *cog-1* is most strongly expressed in vulC and vulD and weakly in vulE and vulF, implying a cell-type-specific function (13) (Fig. 1*D*).

A somewhat similar situation is presented with *lin-11* (LIM-homeodomain) (Fig. 1 *C* and *G*). During the L4 stage, *lin-11* is expressed strongly in vulB, vulC, and vulD and weakly in other vulval cells, suggesting that *lin-11* is involved in the specification of these cell types (24, 26). However, unexpectedly, *lin-11* is cell-autonomously required for expression of most vulval genes tested, including in cells where the *lin-11* level is low (26).

egl-38 is a PAX2/5/8 transcription factor required for expres-

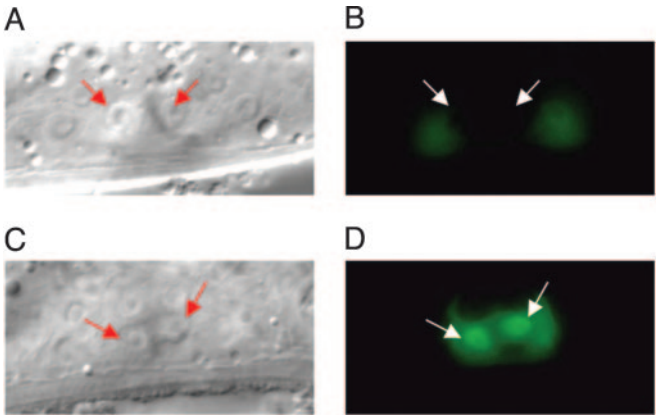

Fig. 2. Regulation of *egl-17* by *cog-1*. (*A* and *B*) Nomarski and epifluorescence images of wild-type mid-L4 animal carrying the *egl-17*::*gfp* transgene. Arrows point to vulE nuclei. vulE cells are not fluorescent. (*C* and *D*) *cog-1*(*sy275*) animals at the same stage carrying the *egl-17*::*gfp* transgene. vulE cells are fluorescent.

sion of the *lin-3* gene in vulF cells (16, 27). We found that *egl-38* represses expression of *zmp-1* in vulF cells, indicated by ectopic *zmp-1* expression in *egl-38* mutants (Fig. 1*G* and Table 2). In addition, in an *egl-38*; *cog-1* double mutant, *egl-17* is expressed in both vulE and vulF cells. Thus, *egl-38* is also capable of repressing *egl-17* expression in vulF cells, although in the wild type, this function is redundant with the *cog-1*-dependent mechanism that restricts *egl-17* expression to vulC and vulD. *egl-38* is currently the best example of cell-type-specific factors, promoting expression of some genes (*lin-3*) and repressing expression of others (*zmp-1*, *egl-17*) in a single cell type, vulF.

Regulators of the Transcription Factor Network. The transcription factor network that regulates gene expression in individual cell types must be regulated by the cell-fate-patterning mechanism that specifies each cell to a specific fate and does so in a spatially precise pattern. In the vulva, the cell types occur in a specific ABCD-EFFE-DCBA pattern (Fig. 1*A*). Although the full mechanism that establishes this pattern is not known, Wnt signals, mediated by *lin-17* (Frizzled-type Wnt receptor) and *lin-18* (Ryk-type Wnt receptor), control the anterior/posterior order of cell types among P7.p descendants (31, 32) (Fig. 3). Analysis of *cog-1* (31) and *lin-11* (25) expression in *lin-17* and *lin-18* mutants indicates that Wnt signaling establishes the correct spatial pattern of transcription factor expression. As described above (Tables 1 and 3 and Fig. 1 *F* and *G*) (26), *cog-1* and *lin-11*, in turn, control the expression pattern of *egl-17* and *cdh-3*. Patterns of *egl-17* and *cdh-3* expression observed in *lin-17* and *lin-18* mutants are consistent with high levels of *cog-1* and *lin-11* turning on the expression of these genes (31, 32). Another set of cell-fate-patterning mechanisms controlling gene expression was revealed by the analysis of vulE vs. vulF fate specification using the *zmp-1* reporter. A dominant-negative Ras or the ablation of the anchor cell disrupts the pattern of *zmp-1* expression in presumptive vulE and vulF cells, indicating that a Ras-mediated signal, probably from the anchor cell, establishes the spatial pattern of cell fates (17).

These results confirm that cell–cell communication is important in patterning cell fates, and that signaling pathways operate through the transcription factor network to control the pattern of gene expression. Expression patterns of various genes (Fig. 1 *B–D*) suggest that transcription factors are expressed in all vulval cells at different levels, whereas genes regulated by them have relatively simple on/off patterns of expression. This difference suggests that the spatial pattern becomes progressively more refined as the information is passed through the regulatory network. This progressive refinement of pattern is likely a consequence of integration of information from multiple regulatory mechanisms, such as intercellular communication and feedback regulation. Many of these disparate data inputs are likely processed at the level of cis-regulatory modules. Thus, the

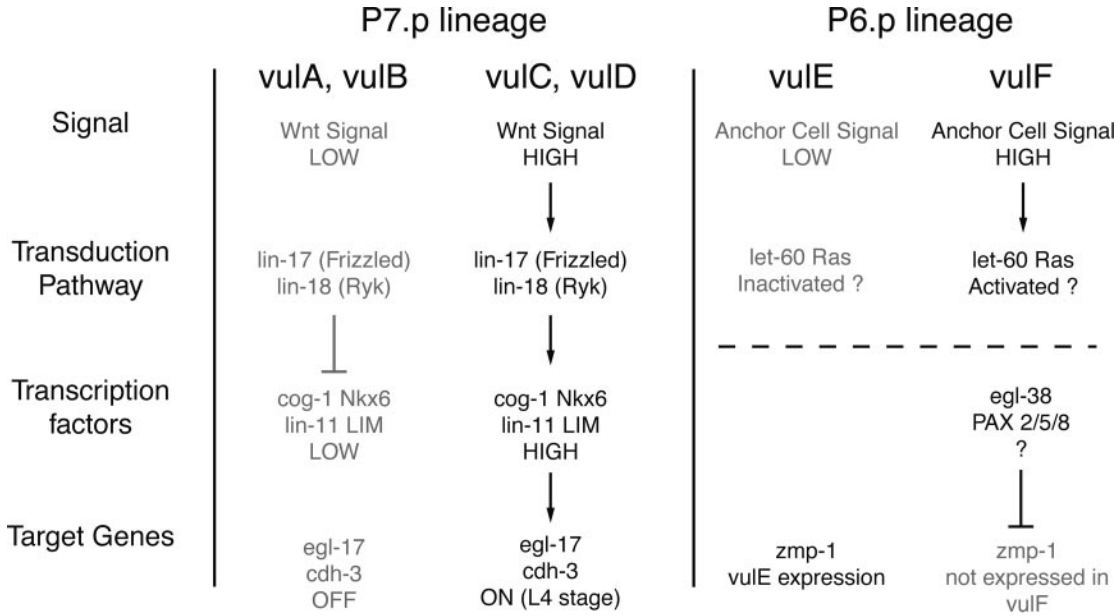

Fig. 3. Link between cell fate patterning mechanisms and gene expression. In general, inductive signals regulate transcription factor networks to regulate gene expression. In the P7.p (but not P5.p) lineage, Wnt signals transduced by *lin-17* and *lin-18* control the pattern of *cog-1* and *lin-11* expression (25, 31). *cog-1* and *lin-11* in turn regulate *egl-17* and *cdh-3* expression (Table 3) (26). It has not been determined whether *cog-1* and *lin-11* regulate each other. In the P6.p lineage, an anchor cell signal and a *let-60* Ras signal transduction pathway are required to establish the correct pattern of *zmp-1* expression pattern (17). *zmp-1* expression is also repressed in vulF by *egl-38* PAX2/5/8 (Table 1). It is not known whether the patterning mechanism acts through *egl-38*. The expression pattern of *egl-38* is also not known.

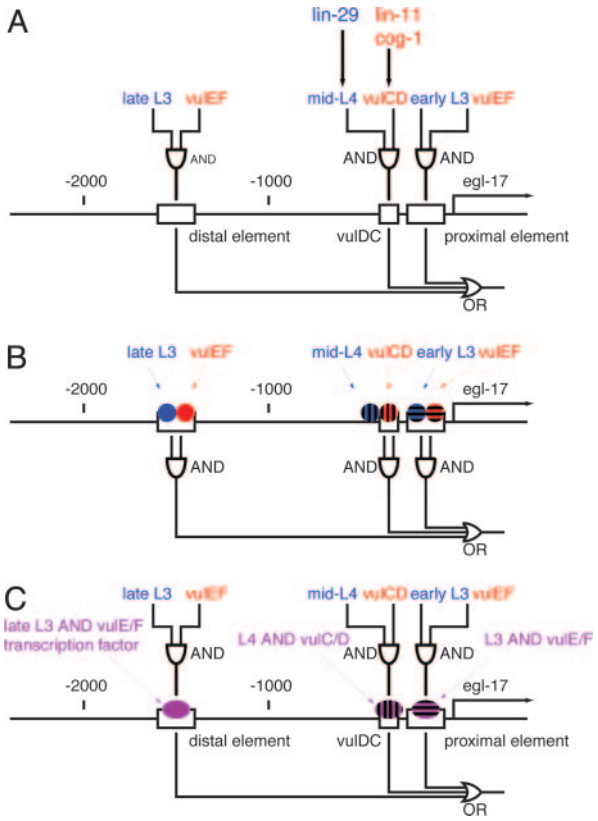

Fig. 4. cis-regulatory elements of *egl-17*. (*A*) A map of the *egl-17* 5′ regulatory region. Boxes indicate enhancer elements defined by Cui and Han (34) and Kirouac and Sternberg (33). ''AND'' and ''OR'' logic gate symbols indicate sites and logic of information integration. Temporal (blue) and spatial (red) information is integrated as indicated by the logic circuit diagram to produce the complete *egl-17* expression pattern. In one model (*B*), spatially and temporally regulated transcription factors each bind directly to the *egl-17* cis-regulatory region. The integration of information takes place on enhancer elements. In the alternative model (*C*), spatial and temporal cues are integrated at the transcription factor level. These transcription factors (purple) with both spatially and temporally restricted activity regulate each enhancer element.

spatial pattern of transcription factor effects becomes more restricted than the spatial pattern of transcription factor expression. This hypothesis is consistent with the observation that cells affected by *lin-11* and *cog-1* mutations do not correspond directly to cells that express high levels of *lin-11* and *cog-1*.

cis-Regulation of Vulva Gene Expression. cis-regulatory elements (e.g., enhancers) have been analyzed in detail for several genes expressed in the vulva, most notably *egl-17*, *cdh-3*, and *zmp-1*, using transgenic assays (33, 34). A comparative genomics analysis of the regulatory region of orthologs from *C. elegans* and *Caenorhabditis briggsae* has also proved useful.

Here, we focus on the analysis of the *egl-17* gene. As shown in Fig. 1*B*, this gene is expressed in vulE and vulF cells during the L3 stage and in vulC and vulD cells during the L4 stage. Dissection of the 5′ regulatory region revealed that there are three separable enhancer elements, two driving expression in vulE and vulF and one driving expression in vulC and vulD (33, 34) (Fig. 4*A*). Notably, each of these elements drives expression at different times. The distal vulE/vulF element drives expression in the mid-L3 to early-L4 and the proximal vulE/vulF element drives expression in the early to mid-L3 stage (34). The vulDC element drives expression in the mid-L4 stage. Thus, the expression of *egl-17* is produced by the composite activity of three discrete enhancers, each of which drives both spatially and temporally restricted pattern of expression. We propose two models for how the information that operates on these enhancers is integrated. In one model (Fig. 4*B*), temporal (blue) and spatial (red) regulators both bind directly to the *egl-17* promoter, and information integration is achieved directly on the cis-regulatory element. Alternatively, transcription factors that bind to each of these promoters may already combine temporal and spatial information (Fig. 4*C*). Our results indicate that the vulDC element regulating mid-L4 expression is likely regulated by *lin-29*, *lin-11*, *cog-1*, and *egl-38*. Additional experiments are necessary to determine the molecular mechanism of information integration.

Conclusion

The late vulval development of *C. elegans* offers an excellent system in which to investigate cell fate determination and regulation of cell-type-specific gene expression. In particular, this system combines single-cell resolution with a high degree of temporal resolution in an easily manipulated model organism. In many respects, vulval development is reminiscent of other systems in that transcription factors are expressed in overlapping domains, and the identity of each domain is established combinatorially by the presence or absence of specific subsets of these transcription factors. One interesting example with possible parallels to the vulva is the fate-specification mechanism in the vertebrate ventral neural tube (35). In this system, Nkx6.1 and Nkx6.2 homeodomain proteins (homologs of *cog-1*) interact with transcription factors Dbx1 and Dbx2 in a mutually repressive network, and different activities of repressor proteins help establish the spatial pattern of cell fates (36, 37). It is possible that *C. elegans cog-1* functions in a similar manner in the vulva.

Analysis of vulval development also highlights several features that are not necessarily evident in other systems. First, analysis of vulval development has revealed a highly complex pattern of temporal regulation, which is undoubtedly a feature of most organogenetic processes (for example, see refs. 38 and 39). The involvement of *lin-29*, a known regulator of stage-specific development in *C. elegans*, suggests that the global mechanism of temporal regulation feeds into the development of this particular organ. Additional mechanisms probably exist that control expression at other time points. Whether these other time points are regulated by a global mechanism or in an organ-autonomous manner is not yet clear.

One concept that has been invoked in analyses of cell or organ fate specification is that of ground state and selector genes. For example, in *Drosophila* appendage development, it has been proposed that a default "ground state" exists and is modified by "selector" genes to produce an antenna or a leg (40). The concept can be applied to the level of individual cell types as well (for example, ref. 41). From this point of view, the cell-type-specific transcription factors *cog-1*, *lin-11*, and *egl-38* can be thought of as selector genes for subsets of vulval cell types. What is the ground state of vulval cells in the absence of selector genes? A cell in such a state presumably will not express the cell-type-specific genes described in Fig. 1 but will retain the epithelial identity common to all vulval cells. It is unclear whether such a state has been observed in any of the mutants. Vulval cells in *lin-11* mutants lack most cell-type-specific expression but retain the ability to undergo some morphogenetic movements characteristic of vulval cells and thus may most closely resemble the ground state.

In other systems, analyses of coregulated genes have successfully identified "gene batteries" (42), sets of genes with common cis-regulatory elements that are coexpressed (for example, ref. 43). However, our understanding of vulval development is still limited, relative to the number of cell types

and the number of distinct stages that require different gene expression patterns. Consequently, within the relatively small number of functionally unrelated genes analyzed so far, genes are more likely to be regulated by distinct mechanisms. Thus, although gene batteries with multiple genes probably exist in this system, their analysis requires knowledge of more genes and a detailed understanding of which transcription factors regulate their expression.

We thank A. Fire (Stanford University, Stanford, CA) for providing GFP vectors, and I. Hope (University of Leeds, Leeds, U.K.) for providing the *C55C3.5*::*gfp* construct. We thank Ryan Baugh, Jennifer Sanders, Mihoko Kato, Steven Kuntz, Alok Saldanha, and reviewers for comments on the manuscript. We thank the *Caenorhabditis* Genetics Center (University of Minnesota, Minneapolis) for *C. elegans* strains. T.I. was supported by fellowship DRG-1646 from the Damon Runyon Cancer Research Foundation. Research was supported by the Howard Hughes Medical Institute, with which P.W.S. is an investigator.

1. Wood, W. B. (1988) *The Nematode Caenorhabditis elegans* (Cold Spring Harbor Lab. Press, Plainview, NY).
2. Kimble, J. (1981) *Dev. Biol.* **87,** 286–300.
3. Sternberg, P. W. & Horvitz, H. R. (1986) *Cell* **44,** 761–772.
4. Sternberg, P. W. (1988) *Nature* **335,** 551–554.
5. Yochem, J., Weston, K. & Greenwald, I. (1988) *Nature* **335,** 547–550.
6. Hill, R. & Sternberg, P. (1992) *Nature* **358,** 470–476.
7. Sulston, J. & Horvitz, H. R. (1977) *Dev. Biol.* **56,** 110–156.
8. Sharma-Kishore, R., White, J., Southgate, E. & Podbilewicz, B. (1999) *Development (Cambridge, U.K.)* **126,** 691–699.
9. Inoue, T., Sherwood, D., Aspöck, G., Butler, J., Gupta, B. P., Kirouac, M., Wang, M., Lee, P.-Y., Kramer, J. M., Bürglin, T., *et al*. (2002) *Mech. Dev.* **119S,** S203–S209.
10. Maduro, M. & Pilgrim, D. (1995) *Genetics* **141,** 977–988.
11. Hanna-Rose, W. & Han, M. (2002) *Dev. Biol.* **241,** 247–258.
12. Chang, S., Johnston, R. J., Jr. & Hobert, O. (2003) *Genes Dev.* **17,** 2123–2137.
13. Palmer, R. E., Inoue, T., Sherwood, D. R., Jiang, L. I. & Sternberg, P. W. (2002) *Dev. Biol.* **252,** 202–213.
14. Pettitt, J., Wood, W. & Plasterk, R. (1996) *Development (Cambridge, U.K.)* **122,** 4149–4157.
15. Burdine, R. D., Branda, C. S. & Stern, M. J. (1998) *Development (Cambridge, U.K.)* **125,** 1083–1093.
16. Chang, C., Newman, A. P. & Sternberg, P. W. (1999) *Curr. Biol.* **9,** 237–246.
17. Wang, M. & Sternberg, P. W. (2000) *Development (Cambridge, U.K.)* **127,** 5047–5058.
18. Stringham, E., Pujol, N., Vandekerckhove, J. & Bogaert, T. (2002) *Development (Cambridge, U.K.)* **129,** 3367.
19. Hwang, H. Y. & Horvitz, H. R. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 14224–14229.
20. Colavita, A. & Tessier-Lavigne, M. (2003) *Science* **302,** 293–296.
21. Shen, K., Fetter, R. D. & Bargmann, C. I. (2004) *Cell* **116,** 869–881.
22. Rougvie, A. E. & Ambros, V. (1995) *Development (Cambridge, U.K.)* **121,** 2491–2500.
23. Newman, A. P., Inoue, T., Wang, M. & Sternberg, P. W. (2000) *Curr. Biol.* **10,** 1479–1488.
24. Freyd, G., Kim, S. & Horvitz, H. R. (1990) *Nature* **344,** 876–879.
25. Gupta, B. P. & Sternberg, P. W. (2002) *Dev. Biol.* **247,** 102–115.
26. Gupta, B. P., Wang, M. & Sternberg, P. W. (2003) *Development (Cambridge, U.K.)* **130,** 2589–2601.
27. Chamberlin, H. M., Palmer, R. E., Newman, A. P., Sternberg, P. W., Baillie, D. L. & Thomas, J. H. (1997) *Development (Cambridge, U.K.)* **124,** 3919–3928.
28. Ambros, V. & Horvitz, H. R. (1984) *Science* **226,** 409–416.
29. Ambros, V. (1989) *Cell* **57,** 49–57.
30. Bettinger, J. C., Euling, S. & Rougvie, A. E. (1997) *Development (Cambridge, U.K.)* **124,** 4333–4342.
31. Inoue, T., Oz, H. S., Wiland, D., Gharib, S., Deshpande, R., Hill, R. J., Katz, W. S. & Sternberg, P. W. (2004) *Cell* **118,** 795–806.
32. Deshpande, R., Inoue, T., Priess, J. R. & Hill, R. J. (2005) *Dev. Biol.* **278,** 118–129.
33. Kirouac, M. & Sternberg, P. W. (2003) *Dev. Biol.* **257,** 85–103.
34. Cui, M. & Han, M. (2003) *Dev. Biol.* **257,** 104–116.
35. Briscoe, J., Pierani, A., Jessell, T. M. & Ericson, J. (2000) *Cell* **101,** 435–445.
36. Muhr, J., Andersson, E., Persson, M., Jessell, T. M. & Ericson, J. (2001) *Cell* **104,** 861–873.
37. Vallstedt, A., Muhr, J., Pattyn, A., Pierani, A., Mendelsohn, M., Sander, M., Jessell, T. M. & Ericson, J. (2001) *Neuron* **31,** 743–755.
38. Aurelio, O., Boulin, T. & Hobert, O. (2003) *Development (Cambridge, U.K.)* **130,** 599–610.
39. Bateman, J. M. & McNeill, H. (2004) *Cell* **119,** 87–96.
40. Casares, F. & Mann, R. S. (2001) *Science* **293,** 1477–1480.
41. Lints, R., Jia, L., Kim, K., Li, C. & Emmons, S. W. (2004) *Dev. Biol.* **269,** 137–151.
42. Davidson, E. H. (2001) *Genomic Regulatory Systems: Development and Evolution* (Academic, San Diego).
43. Wenick, A. S. & Hobert, O. (2004) *Dev. Cell* **6,** 757–770.