The *Caenorhabditis elegans* vulva: A post-embryonic gene regulatory network controlling organogenesis

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The *Caenorhabditis elegans* vulva is an elegant model for dissecting a gene regulatory network (GRN) that directs postembryonic organogenesis. The mature vulva comprises seven cell types (vulA, vulB1, vulB2, vulC, vulD, vulE, and vulF), each with its own unique pattern of spatial and temporal gene expression. The mechanisms that specify these cell types in a precise spatial pattern are not well understood. Using reverse genetic screens, we identified novel components of the vulval GRN, including *nhr-113* in vulA. Several transcription factors (*lin-11*, *lin-29*, *cog-1*, *egl-38*, and *nhr-67*) interact with each other and act in concert to regulate target gene expression in the diverse vulval cell types. For example, *egl-38* (Pax2/5/8) stabilizes the vulF fate by positively regulating vulF characteristics and by inhibiting characteristics associated with the neighboring vulE cells. *nhr-67* and *egl-38* regulate *cog-1*, helping restrict its expression to vulE. Computational approaches have been successfully used to identify functional *cis*-regulatory motifs in the *zmp-1* (zinc metalloproteinase) promoter. These results provide an overview of the regulatory network architecture for each vulval cell type.

genetic regulatory networks | nematode | transcriptional regulation

Complex interactions of signaling molecules, transcription factors, and effector genes direct spatial and temporal patterning during organogenesis (1). The differentiation and morphogenesis of the *Caenorhabditis elegans* vulva is useful for studying the gene regulatory network (GRN) of larval stage organogenesis due to its invariant cell lineage, its amenability to genetic manipulation, and the availability of reporter genes with many spatial and temporal expression patterns in the seven vulval cell types (2). In ref. 3, we described a regulatory network of interactions between a set of evolutionarily conserved transcription factors and an array of genes expressed in the differentiated cells of the *C. elegans* vulva. Here, we briefly review vulval development and aspects of the provisional GRN directing its organogenesis. We then describe additional pairwise *trans*-regulatory interactions, including the results of RNAi screens and a *cis* regulatory analysis of *zmp-1* that together help refine our network model, and infer common network themes, such as boundary formation, combinatorial control, and stable feedback loops. These additional data support the hypothesis that overall network architecture is unique for each of the vulval cell types.

The life cycle of *C. elegans* consists of four larval stages (L1–4) and an adult stage, with each stage separated by a molt (2). The *C. elegans* vulva is derived postembryonically from six vulval precursor cells (VPCs) termed P3.p-P8.p. All VPCs are competent to receive an inductive signal from a specialized somatic gonadal cell, the anchor cell (AC), during the L2 stage. P6.p, which is closest to the AC, is induced to generate the 1° vulval lineage, producing the inner cells of the vulva. The P5.p and P7.p cells generate 2° vulval lineages, producing the outer cells of the vulva. P3.p, P4.p, and P8.p are uninduced and adopt the 3° fate, and fuse to the hypodermal syncytium hyp7. The L4 stage vulva comprises 22 differentiated cells that are descendents of P5.p, P6.p, and P7.p, and that are of seven different types: vulA, vulB1, vulB2, vulC, vulD, vulE, and vulF [supporting information (SI) Fig. S1]. EGF, Notch, and Wnt signaling pathways specify which VPCs generate the 1° and 2° lineages, but we are just now identifying the network of transcription factors that control cell-fate differentiation in the seven vulval cell types (2).

The *C. elegans* vulva allows passage of sperm and eggs by connecting the uterus to the outside environment (2). Each vulval cell type has specialized roles that contribute to vulval function and morphology. For example, vulF cells, the innermost vulval cells, contact the AC and the uterus; they are the target for AC invasion, thus creating the vulval-uterine connection required for egg-laying (4). Vulval muscles that regulate egg-laying connect to the vulva between vulC and vulD, and the outermost portion of the vulva comprises the vulA cells, which attach the vulva to the hypodermis (2). The unique patterns of gene expression in each of the vulval cell types are likely responsible for their individual properties. Comparison of the vulval GRN to those in other organisms is necessary for expanding our knowledge of organ development.

Results and Discussion

Functional Roles of Gene Expression During Vulval Differentiation. Much is known about the signaling network that establishes the pattern of vulval cell differentiation, but our understanding of the GRN that specifies the terminal seven vulval cell types is limited (2). Five transcription factors (*lin-11*, *lin-29*, *cog-1*, *egl-38*, and *nhr-67*) are major regulators of cell-fate determination and morphogenesis in the vulva. *lin-11* encodes a LIM homeodomain protein, consisting of a homeodomain and two specialized LIM-type zinc-fingers (5). LIM homeodomain family members play roles in differentiation and pattern formation in arthropods and vertebrates (6, 7). *lin-29*, a C2H2-type zinc finger, plays a role in many events occurring at the larva to adult transition, including terminal differentiation of the seam cells (8), morphogenesis (9), and formation of the vulval-uterine-seam cell connection (10). *cog-1* encodes a Nkx6.1/6.2 homeoprotein transcription factor (11); vertebrate Nkx6.1 proteins are involved in neuronal and pancreatic endocrine cell formation (12, 13). *egl-38* encodes a Pax2/5/8 protein, which are known to be involved in organogenesis; e.g., mouse *Pax2* mediates nephrogenesis (14, 15). *nhr-67* is an ortholog of *Drosophila melanogaster tailless* (*tll*) (16), a conserved nuclear hormone receptor necessary for *Drosophila* embryogenesis and neuronal development (17). The

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Table 1. Expression of *dhs-31*, *egl-26*, *lin-3*, *lin-11*, *pepm-1* and *unc-53* in the vulva.

Reporter	Mutation	vulA	vulB1	vulB2	vulC	vulD	vulE	vulF	*n*
dhs-31	+	0	100	100	0	100	0	0	31
dhs-31	*lin-11*(*n389*)	0	**0***	**0***	0	**0***	0	0	28
dhs-31	*lin-29*(*sy292*)	0	**0***	**0***	0	**0***	0	0	42
egl-26	+	0	61	30	0	61	30	0	37
egl-26	*lin-11*(*n389*)	0	**0***	**1***	0	**0***	**1***	0	39
lin-3	+	0	0	0	0	0	0	98	26
lin-3	*lin-11*(*n389*)	0	0	0	0	0	0	**7***	22
lin-3	*lin-29*(*n333*)	0	0	0	0	0	0	81	18
lin-11	+	41	92	90	81	100	0	0	38
lin-11	*lin-29*(*sy292*)	**5***	**43***	**59†**	**30***	**58***	0	0	52
pepm-1	+	0	0	0	91	91	95	95	22
pepm-1	*lin-11*(*n389*)	0	0	0	**0***	**0***	**0***	**0***	50
pepm-1	*lin-29*(*sy292*)	0‡	0‡	0‡	67‡	67‡	67‡	67‡	55
unc-53	+	0	0	0	100	0	0	0	Many
unc-53	*lin-29*(*sy292*)	0	0	0	80	0	0	0	20

Percentages of cells that express *dhs-31*::*YFP*, *egl-26*::*GFP*, *lin-3*::*GFP*, *lin-11*::*GFP*, *pepm-1*::*GFP* and *unc-53*::*GFP*. Boldface indicates *P* values are significantly different than wild type. *, $P = 0.000$; †, $P = 0.002$.
‡*pepm-1*::*GFP* is not detectable until the end of the fourth larval stage, at which point the vulva has already protruded in *lin-29(sy292)* mutants, making it difficult to distinguish between the vulval cell types.

pattern of vulval cell types is specified by the differential interactions of the transcription factors that operate within each cell (2).

We have identified 30 genes dynamically expressed in specific subsets of the cells of the mature *C. elegans* vulva (Table S1 has a complete list). These genes encode transcription factors, guidance cues, proteases, structural proteins, signaling molecules, and novel proteins with unknown function. The physiological relevance is known for several genes. For example, *egl-17*, which encodes a fibroblast growth factor (FGF), is necessary for migration of the sex myoblasts to the vulva (18). Induction of the uterine uv1 cells depends on the epidermal growth factor (EGF) family member *lin-3* (19). *bam-2* (neurexin-related transmembrane protein) and *syg-2* (transmembrane Ig superfamily protein) are required for vulval innervation (20, 21). *sqv-4*, which encodes an UDP-glucose dehydrogenase-related protein, is involved in the structural integrity and morphology of the vulva (22). The significance of some of the genes expressed in the vulva is not yet known, including the *Drosophila empty spiracles* (*ems*) homolog *ceh-2* (23), a cadherin-related protein encoded by *cdh-3* (24), and *zmp-1*, which encodes a MT4-MMP-related zinc metalloproteinase (23, 25). Genetic perturbations that result in altered expression patterns of these effector genes are helpful in elucidating the regulatory network.

Several additional genes with detectable expression in the mature vulva, including two putative transcription factors, have been identified since we last described the vulval GRN (3). The Pax2/5/8 gene *pax-2* is expressed exclusively in the vulD cells (16). *pax-2* is the result of a recent duplication of *egl-38* (26). *egl-38* has been identified as a regulator of cell-fate specification in the *C. elegans* vulva (3, 15, 19). *lin-39* encodes a Hox protein that is an ortholog of *Drosophila Sex combs reduced* (*Scr*) (27, 28). During the late L3 stage, *lin-39* expression increases in the vulA precursor cells; this expression persists in vulA until late L4 (29). The Patched-related protein DAF-6 is expressed in vulE and vulF (30). Hao *et al.* (31) reported the expression of seven *hedgehog*-related genes in the vulva: *grd-5* in vulB and vulD, *grd-12* in vulC, *grl-4* in vulA and vulB, *grl-10* in vulA and vulB, *grl-15* in vulB, vulC, vulD and vulE, *grl-25* in vulA, and *grl-31* in vulF. Last, *nas-37* (32), which encodes a metalloprotease, is expressed in vulB (data not shown).

Conserved Regulatory Strategies in the Vulval GRN. In ref. 3, we described how the expression patterns of a subset of reporter constructs are affected in transcription factor mutant backgrounds. We have increased the number of known interactions more than twofold, from 15 to 36, and identified many of the regulatory relationships among the transcription factors (Table 1, Fig. S2, and Table S2). This network includes strategies that are shared by other GRNs. For example, the cell-type specific expression of *cog-1* appears to be restricted to the vulC and vulD cells by a variety of mutual and autoregulatory controls (16). In a second example, *lin-11* is necessary for vulA-specific expression of *nhr-67* (16). In turn, *nhr-67* is necessary for the expression of the vulA effector gene *zmp-1*. The differentiated state of vulA may be further stabilized, because *nhr-67* is positively autoregulated in vulA. This is an instance where multiple positive inputs, including feedback loops, ensure the maintenance of a terminal cell fate.

Other network strategies that are present in the vulva include combinatorial control circuits. *egl-17* expression in vulF is perturbed by neither *cog-1* nor *egl-38* mutations (16). However, *egl-17* expression is derepressed in vulF in *cog-1; egl-38* double mutants. This redundancy could ensure proper execution of cell fate. Finally, negative autoregulation, as in *cog-1* in vulA, vulB, vulE, and vulF and *nhr-67* in vulC, vulD, vulE, and vulF, appears to be a fundamental strategy used in the vulval GRN (16). Negative autoregulation has been found to accelerate gene circuit response time and assists in making quick cell fate decisions (33).

LIN-29 and LIN-11 Interact to Determine Vulval Cell Fate. By examining the effects of specific transcription factor mutations on a wider array of genes expressed in the mature vulva, we have found increased complexity in some of their roles. We had hypothesized that *lin-29* is a temporal regulator of gene expression, because it is required for vulval gene expression at the mid to late L4 stage (*egl-17* in vulC and vulD, *ceh-2* in vulC, and *zmp-1* in vulD and vulE) (3, 23), and functions in several developmental processes at the L4 stage to adult transition (8–10, 34). However, in a *lin-29* mutant background the mid-L4 expression of *lin-3* in vulF cells is not abolished, whereas expression of *dhs-31*, which is initiated in gravid adults, is abolished (Table 1 and Fig. S2).

LIN-29 is necessary for wild-type levels of *lin-11* transgene expression (Table 1) and is thus a key regulator of *lin-11*. In addition to genes described in ref. 3, *lin-11* is required for *dhs-31*, *egl-26*, *lin-3* and *pepm-1* expression (Table 1). Thus, of the known vulval transcriptional regulators, *lin-11* exhibits the broadest effect (3). *egl-17* during L3 and *cdh-3* in vulF are the only vulva

expression patterns not abolished in a *lin-11* background. Reporter constructs are powerful tools for identifying genes' spatial and temporal expression patterns; however, reporter gene constructs might well lack relevant regulatory motifs. Consequently, we might be missing some regulatory connections.

The interplay between *lin-11*, *lin-29* and their downstream targets is complex. *lin-11*::*GFP* expression is not abolished in a *lin-29* mutant background, and several of the gene expression patterns perturbed by loss of *lin-11* are not affected in a *lin-29* deficient background (Fig. S2). LIN-29 might act in concert with another, as yet unidentified, factor to ensure the proper temporal and spatial expression of the general cell-fate regulator LIN-11. Considering LIN-29's role as a regulator of developmental timing, it could serve as a temporal input, whereas another factor serves as the spatial input.

Identification of Additional Components of the Vulval GRN. Transcription factors identified by forward genetic screens are biased by ascertainment toward those with strong, nonredundant effects, and those that affect multiple aspects of vulval development. Two categories of transcription factors are relatively more difficult to identify in genetic screens. Mutations that result in lethality or other severe defects will not be identified for roles that a partial loss of function mutation might reveal in postembryonic development. Conversely, genes with more subtle developmental phenotypes are also more likely to be overlooked. We identified genes in both these categories by RNA interference (RNAi) screens of 508 transcription factors (Table S3). One of these screens was conducted in a *ceh-2*::*YFP* background, a readout for vulB fate during the L4 stage, and identified *nhr-67* (*tailless*) (16). Because *nhr-67* deletion mutants die as young larvae, its role in vulval development was not identified. RNAi often causes a partial-loss-of-function phenotype instead of a null phenotype. It can also serve as a temporal or conditional downregulator of gene function. In the case of *nhr-67*, the larval lethality phenotype was bypassed, because RNAi was administered to L1 larvae, thus making its vulval phenotypes visible.

The second screen was conducted in a *zmp-1*::*GFP* background, focusing on perturbations of vulA expression. This screen also identified *nhr-67*, and the orphan nuclear hormone receptor *nhr-113* as positive regulators of *zmp-1* expression in vulA cells. *nhr-113* might have a narrow role in vulval organogenesis, because *nhr-113* RNAi has no effect on the regulation of several other genes: *cdh-3, ceh-2, dhs-31, lin-3*, or *pepm-1* (data not shown). These results, however, show that RNAi screens can identify new components of GRNs.

Differentiation of Discrete Fates in the 1° Vulval Lineage. The 1° lineage of the vulva generates four vulE and four vulF cells. Signals from the AC and Wnt are required for proper specification of these cell fates (25). The GRN, however, which acts downstream of these intercellular signals to guide differentiation of vulE and vulF fates, is not known. *egl-38*::*GFP* is detectable solely in the vulF cells (35); however, analysis of the mutant *egl-38* with *nhr-67* RNAi and mutant *cog-1* shows that *egl-38* functions in vulF cells and inhibits both *ceh-2* and *egl-17* expression in vulE (16) and vulF (3). Consequently, *egl-38*, *cog-1*, and *nhr-67* enforce spatial boundaries by preventing the 2° cell-fate associated genes *ceh-2* and *egl-17* from being expressed in 1° cells (Fig. S2, vulE and vulF).

It was speculated that the vulF cells, which are nearer the AC, have higher levels of *nhr-67* activity versus *cog-1*, whereas vulE cells have higher levels of *cog-1* than *nhr-67* (16). The recent availability of an *egl-38*::*GFP* reporter has allowed us to dissect further the GRN controlling the vulE versus vulF fates. *egl-38* expression in the vulF cells is positively regulated by *nhr-67* (Fig. 1). This *egl-38* expression is necessary for specification of the uv1 fate via regulation of the vulF specific gene *lin-3*, thus allowing

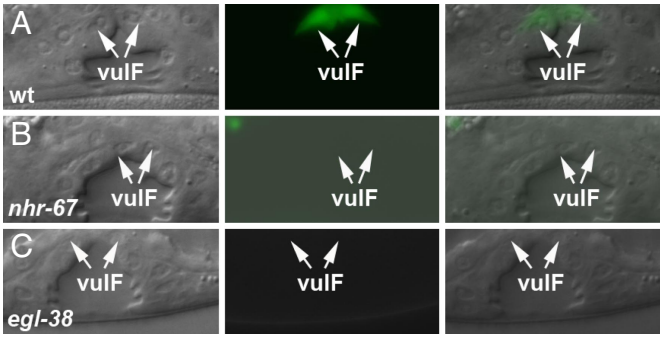

Fig. 1. *egl-38* expression in the vulF cells is dependent on several regulatory inputs. (*A–C*) Nomarski (*Left*), fluorescence (*Center*) and overlaid (*Right*). All animals displayed carry the *egl-38*::*GFP* [*guEx877*] transgene in their background. (*A*) In wild-type animals, *egl-38* is detected exclusively in the vulF cells (arrows). (*B*) *nhr-67* RNAi results in the abolition of *egl-38* expression in the vulF cells (arrows). (*C*) *egl-38(n578)* mutants lose the ability to positively autoregulate their expression levels in vulF (arrows).

for the proper development of a vulval-uterine connection (19). Conversely, *zmp-1* is expressed in vulE but not vulF, and *egl-38* is required for inhibiting *zmp-1* expression in the vulF cells (3). In vulE cells, where *egl-38* expression is absent, *lin-3* is not expressed and *cog-1* activated expression of *zmp-1* is observed (19). *egl-38* expression is unaffected in a *cog-1(sy275)* background, but in an *egl-38(n578)* background *egl-38*::*GFP* expression is decreased, suggesting that *egl-38* positively autoregulates in vulF (Fig. 1). Therefore, *egl-38* appears to stabilize vulF fate by repressing vulE characteristics and by reinforcing its own expression (Fig. 2).

In the vulE and vulF cells, *cog-1* and *nhr-67* negatively regulate both each other and themselves (16). We speculated that this

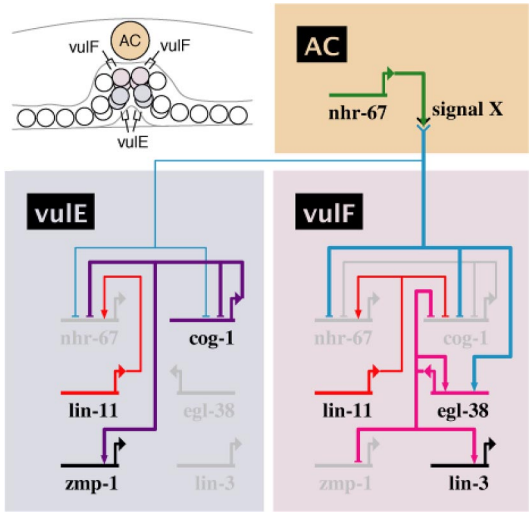

Fig. 2. Differentiation of vulE vs. vulF. The positions of the vulE and vulF cells relative to the anchor cell (AC) are shown. vulF is closer spatially than vulE to the AC. The network diagram was generated using BioTapestry Editor, Version 2.1.0 (www.biotapestry.org) (44). Linkages with arrowheads represent positive inputs and linkages with bar-heads represent repressor inputs for target gene expression. Black font indicates detectable expression levels and gray font indicates no detectable expression. This model presumes that *nhr-67* acts in the AC to differentiate between vulE and vulF cells. Signal X could be Ras, Wnt, or some other signaling pathway. The blue linkage in vulF is indicated by a thicker line than the blue linkage in vulE because it is hypothesized that vulF receives higher levels of signal X-mediated *nhr-67* signal from the AC. The thick purple and pink linkages highlight differences in the network architecture between the vulE and vulF cells, respectively.

Table 2. Expression of mutated *zmp-1* enhancers in the vulva

Construct	vulA, % (*n*)	vulC, % (*n*)	vulD, % (*n*)	vulE, % (*n*)	vulF, % (*n*)
mk50–51	86 (28)	0 (35)	0 (35)	89 (35)	0 (35)
103/4Δ	**34* (29)**	0 (41)	0 (41)	**0* (41)**	0 (41)
105/6Δ	72 (32)	4 (46)	**22† (46)**	**30* (46)**	7 (46)
107/8Δ	**15* (27)**	0 (31)	0 (31)	**6* (31)**	0 (31)

Shown are percentage of cells that express the indicated constructs. Values in bold indicate *P* values that are significantly different than wild type. *, $P = 0.000$; †, $P = 0.004$.

might allow 1° cells to rapidly switch their fates upon altered intra- and intercellular inputs. Presumptive vulE cells can induce uterine uv1 fate specification in the absence of vulF cells, thus ensuring the establishment of a proper vulval-uterine connection (19). These observations fit a model in which presumptive vulE and vulF cells are bipotential and positional cues help specify their fates. In this model, vulE, would then be biased for increased *nhr-67* activity in the absence of vulF. *egl-38* levels would thus increase, and vulF characteristics would be activated, whereas vulE characteristics would be inhibited.

Dissection of *zmp-1* Regulatory Elements. The vulA cells occupy a unique position as they form the outermost ring of cells and fuse to the surrounding hypodermal syncytium. *zmp-1* (zinc metalloproteinase) is first expressed in vulD and vulE cells beginning at the late L4 stage and in the vulA cells at the L4 to adult transition (23). vulA-specific expression of *zmp-1* is initiated in a different temporal window than its expression in vulD and vulE, which is particularly interesting because, in a *lin-29* background, vulD and vulE expression is abolished, but vulA expression is unaffected. Because *lin-29* affects early *zmp-1* expression (vulD and vulE) but not late expression (vulA), and because *lin-29* temporally regulates gene expression, it seems likely that modular *cis*-regulatory elements drive *zmp-1* expression.

A 386-bp fragment of upstream sequence (mk50–51) is sufficient to drive vulA- and vulE- but not vulD-specific expression of *zmp-1* (36). We analyzed sequences upstream of *zmp-1* and its orthologs in *C. briggsae* (CBG09053) and *C. remanei* (CRE04503), using Cistematic (37), which carries out motif-finding and phylogenetic footprinting (Table 2 and Fig. S3) and identified three motifs within the mk50–51 *zmp-1* enhancer element and a fourth motif five basepairs upstream of the 5′ end. We deleted the instances of the motifs and analyzed their effects on *zmp-1* reporter expression. Deletion of element 103/4 decreased vulA expression and abolished vulE expression. Previous *cis*-regulatory studies did not identify an element competent to drive vulA expression in the absence of vulE. Deletion of element 107/8 decreased vulA and vulE expression. Deletion of element 105/6, however, resulted in ectopic expression of mk50–51 *zmp-1*::*GFP* in vulC, vulD, and vulF. Thus, these elements likely act as positive (103/4 and 107/8) and negative (105/6) regulatory sites for controlling of *zmp-1* expression.

Conclusion

The regulatory architecture of the vulval GRN differs in all seven cell types. We postulate that this accounts for the differences in vulval cell fate, function, and morphology. Development of the *C. elegans* vulva utilizes several types of gene regulatory strategies that have been identified in other networks. For example, COG-1 participates in a network of mutual inhibitions with NHR-67 in the 1°-lineage derived vulval cells to differentiate between the vulE and vulF fates. This is reminiscent of the cross-inhibitory interaction of COG-1 and DIE-1 in the *C. elegans* ASE neurons (38). Vertebrate COG-1 homologs, the homeodomain proteins Nkx6.1 and Nkx6.2, might act in a similar manner (3). These proteins interact with the transcription factors Dbx1 and Dbx2 in a network of mutual inhibitions to specify motor neuron and interneuron fates during neural tube development (13). In another example, *lin-11* function is necessary for EGL-17 (FGF) expression in the vulva. An analogous network interaction is present during heart development in mice. Isl1, which like *lin-11* is a LIM homeodomain transcription factor, is required for the expression of FGFs (39).

We also describe new interactions within the vulva that may aid in the understanding of analogous regulatory interactions in other transcriptional networks, because the majority of the transcription factors and effectors present in this GRN have relatives in a diverse array of organisms. These interactions include positive regulation of *lin-11* by the heterochronic transcription factor LIN-29, *lin-3* (EGF) by LIN-11, and *egl-38* (Pax2/5/8) by NHR-67 (*tailless*). Our increased knowledge of the roles of transcription factors, such as *lin-11* (LIM homeodomain) and *egl-38* (Pax2/5/8) may help to further characterize other GRNs. For example, a LIM homeodomain protein (*Lim1*) and a Pax2/5/8 protein (*Pax2*) are involved in murine kidney development (40). Thus, the GRN that directs nephrogenesis in mice appears to share at least two components with the vulval network in *C. elegans*. Our understanding of the kidney morphogenesis GRN in mammals may be enhanced by investigating the mouse orthologs of other components of the vulval GRN.

New approaches are needed to elucidate all of the constituents of the complex regulatory architecture that directs organogenesis. Until now, most of the key players in vulval organogenesis have been isolated using traditional mutagenesis screens. These types of screens, however, are often limited to identifying only those factors with severe phenotypes. To account for the regulation of all genes that are expressed and function during vulva development it is apparent that other members of the vulval GRN are yet to be identified. There are transcription factors with major and minor effects. For example, LIN-11 is required in two tissues for egg-laying and regulates gene expression in multiple vulval cell types. By contrast, using an RNAi screen we were able to identify NHR-113, a factor that is possibly only required for fine tuning gene expression in the vulA cells.

Analysis of the effects of various genetic perturbations on vulval organogenesis has revealed detailed spatial and temporal distinctions in the regulation of diverse yet related cell types. Our approach provides for precise and accurate study of gene expression in an intact organism and unveils the distinct network architecture in the different cell types. Further dissection of the genomic network within the differentiated cell types would extend our knowledge of vulval organogenesis and could also provide further insights into organogenesis in other systems.

Materials and Methods

Genetics and RNAi. *C. elegans* strains were grown and constructed using standard protocols. The *nhr-67* RNAi feeding protocol was described in ref. 16.

Generation of Reporter Transgenes. *syEx1009, syEx1091* and *syEx1018* were generated by deleting one motif each, 103/4 (5′-CGAGTACGTTTACAC-3′), 105/6 (5′-GTACGTATTGCTT-3′), or 107/8 (5′-AGAAAAAGTAGAAGG-3′), respectively, from the mk50–51 (36) construct and replacing the motif with a *Sac*II restriction site. These constructs were then individually injected into the gonads of *unc-119(ed4); him-5(e1490)* animals (41), using *unc-119*(+) (42) and pBSK+ (Stratagene). *syEx756* and *syEx1012* were generated by injecting the constructs pNP10 (43) and mk50–51 (36), respectively, into a *unc-119(ed4); him-5(e1490)* background.

Microscopy. 3 mM levamisole was used to anesthetize transgenic animals for observation using Nomarski DIC optics.

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