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Control of Vulval Cell Division Number in the Nematode *Oscheius/Dolichorhabditis* sp. CEW1

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ABSTRACT

Spatial patterning of vulval precursor cell fates is achieved through a different two-stage induction mechanism in the nematode *Oscheius/Dolichorhabditis* sp. CEW1 compared with *Caenorhabditis elegans*. We therefore performed a genetic screen for vulva mutants in *Oscheius* sp. CEW1. Most mutants display phenotypes unknown in *C. elegans*. Here we present the largest mutant category, which affects division number of the vulva precursors P(4–8).p without changing their fate. Among these mutations, some reduce the number of divisions of P4.p and P8.p specifically. Two mutants omit the second cell cycle of all vulval lineages. A large subset of mutants undergo additional rounds of vulval divisions. We also found precocious and retarded heterochronic mutants. Whereas the *C. elegans* vulval lineage mutants can be interpreted as overall (homeotic) changes in precursor cell fates with concomitant cell cycle changes, the mutants described in *Oscheius* sp. CEW1 do not affect overall precursor fate and thereby dissociate the genetic mechanisms controlling vulval cell cycle and fate. Laser ablation experiments in these mutants reveal that the two first vulval divisions in *Oscheius* sp. CEW1 appear to be redundantly controlled by a gonad-independent mechanism and by a gonadal signal that operates partially independently of vulval fate induction.

GENETIC analysis of developmental processes in animals is mostly limited to a few distant "model" species belonging to different phyla. Genetic analysis of species closely related to a model species has several goals: understanding the developmental mechanisms through phenotypic analysis (Brakefield *et al.* 1996; Sommer 1996; Sulston and Anderson 1996; Maderspacher *et al.* 1998; Sommer *et al.* 1998; Pultz *et al.* 1999), determining the genes involved at the molecular level (Beeman *et al.* 1993; Eizinger *et al.* 1999), and comparing the phenotypic spectrum that can be obtained by one-gene alterations in different species.

The nematode *Caenorhabditis elegans* is one of the genetic model systems used for developmental studies; one well-studied developmental process is vulva formation. Many evolutionary variations have been found in other nematodes, in vulval cell lineages and in the cellular interactions specifying vulva precursor cell fates (Sommer 1996; Félix 1999).

The nematode vulva is formed from precursor cells in the ventral epidermis called Pn.p cells (Figure 1). In *C. elegans*, three Pn.p precursors divide and form the vulva: P6.p adopts a specific central fate (called 1°) and P5.p and P7.p adopt lateral fates (2°). P3.p, P4.p, and P8.p are competent to adopt a vulval fate in the absence of P(5–7).p, but normally adopt a nonvulval fate (3°), dividing once and fusing to the epidermal syncytium. This centered pattern of fates is induced by a signal from the anchor cell (a gonadal cell located close to P6.p). The anchor cell signal may act in a graded fashion to induce the 1° fate at high level and the 2° fate at lower levels and is reinforced by lateral signaling between P6.p and its neighbors (Horvitz and Sternberg 1991; Katz *et al.* 1995; Simske and Kim 1995; Figure 1).

C. elegans vulval development has been studied using genetic screens for egg-laying-defective mutants. In this self-fertilizing hermaphrodite, absence of a vulva does not hinder internal fertilization and embryo development. The vulva mutations found in *C. elegans* affect distinct steps in the formation and function of the vulva (Horvitz and Sulston 1980; Sulston and Horvitz 1981; Trent *et al.* 1983; Ferguson and Horvitz 1985; Ferguson *et al.* 1987). Most of the mutations that affect vulval lineages can be interpreted as homeotic changes of vulva precursor cell fate, for example from 3° to 2° fate. We use the word homeotic with its original meaning of modular change of one part of the animal into another—here one precursor cell fate to another (Bateson 1894; Greenwald *et al.* 1983)—and not as a molecular reference to the HOM-C gene cluster. A few *C. elegans* vulva mutants affect 2° lineage polarity. Thus, *C. elegans*

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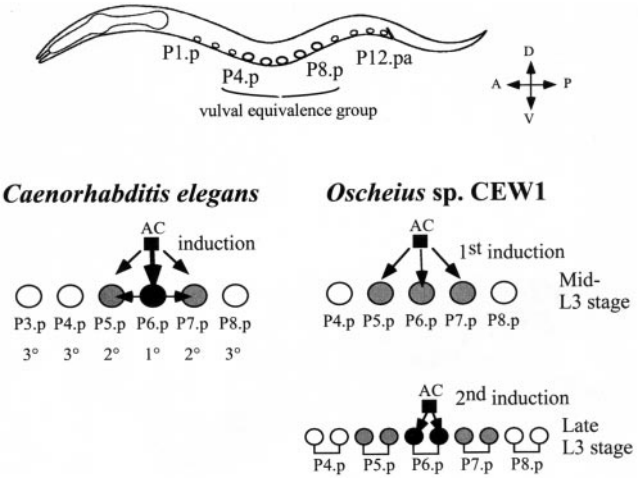

FIGURE 1.—Vulval development in *C. elegans* and *Oscheius/Dolichorhabditis* sp. CEW1. The vulva develops from ventral epidermal Pn.p cells. The vulval equivalence group is formed by P(3–8).p in *C. elegans* and P(4–8).p in *Oscheius* sp. CEW1. The final cell fate pattern is identical in both species: P6.p adopts a central vulval fate (1°, black), P5.p and P7.p a lateral vulval fate (2°, gray), and the others a nonvulval fate (3°, white). In *C. elegans*, this pattern is the result of a graded induction from the anchor cell and lateral signaling from P6.p to its neighbors. In *Oscheius* sp. CEW1, anchor cell signaling is required twice, first to induce vulval *vs.* nonvulval fates in P(5–7).p and then to induce the central fate in P6.p daughters.

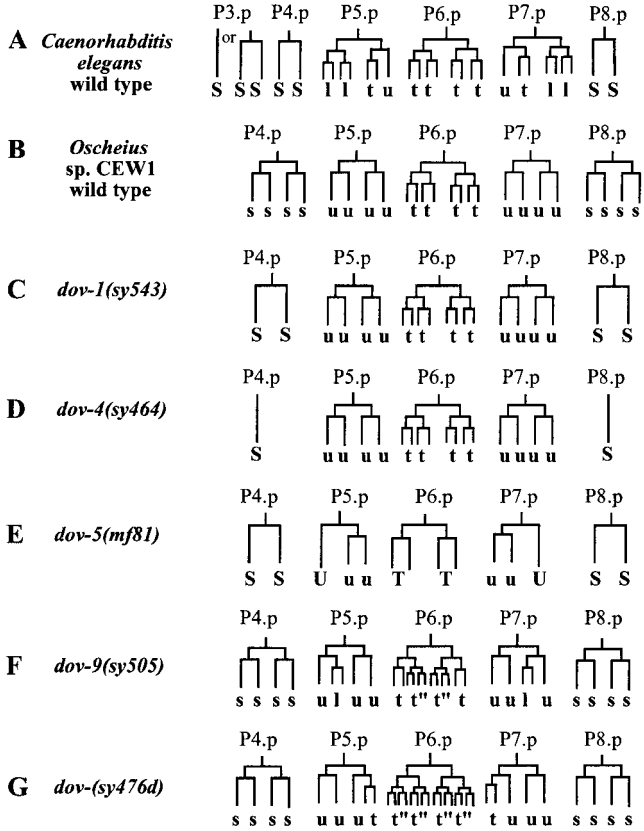

FIGURE 2.—Vulval cell lineages in wild-type and mutant *Oscheius* sp. CEW1. The wild-type *C. elegans* vulval lineage is shown for comparison. The lineage of only one typical individual is shown for each mutant (see tables for more examples). Unless otherwise indicated, the divisions are longitudinal (antero-posterior) and the anterior daughter is drawn on the left. s/S, nonvulval syncytial fate; u/U, undivided, vulval fate; t/T, transverse (left-right) division; l, longitudinal (antero-posterior) division. Capital letters indicate the absence of one division round. Primes (t″) indicate the presence of an additional round of division.

vulva mutants correspond to vulval cell lineage defects that can be correlated with Pn.p fate or polarity changes.

We screened for vulva mutants in another nematode species, *Oscheius/Dolichorhabditis* sp. CEW1. In this species, although the final vulval cell fate pattern is similar to that found in *C. elegans*, the cell interactions that build this pattern differ (FÉLIX and STERNBERG 1997). In *Oscheius* sp. CEW1, the anchor cell is required twice in two successive cell cycles: it first induces vulval *vs.* nonvulval fates in P(5–7).p and then induces the two daughters of P6.p to adopt a specific central fate (1°; Figure 1). *Oscheius* sp. CEW1 belongs to the same family as *C. elegans* (family Rhabditidae; ANDRÁSSY 1984; BLAXTER *et al.* 1998; FÉLIX *et al.* 2000). It also shows favorable features for vulva genetics: self-fertilizing hermaphrodites with facultative males and a fast life cycle. Its vulval cell lineage is simpler than that of *C. elegans* (SOMMER and STERNBERG 1995; Figure 2), which facilitates cell lineage determination.

Here we describe results from genetic screens performed in *Oscheius* sp. CEW1. We report on the largest category of vulva mutations isolated, which show defects in the number of vulval divisions but not in the overall competence and induced fates of the precursor cells (we will describe such mutations elsewhere). Our results demonstrate a difference with *C. elegans* and *Pristionchus pacificus* in the spectrum of vulva mutations recovered after mutagenesis; moreover, they show that vulval cell cycle regulation can be uncoupled from fate specification in *Oscheius* sp. and suggest the existence of an anchor cell signal inducing vulval cell divisions independently of vulval fates.

MATERIALS AND METHODS

Strain and cultures: The CEW1 strain was isolated by C. E. Winter in Sao Paulo, Brazil. It is the same biological species as the PS1131 strain used in SOMMER and STERNBERG (1995) and FÉLIX and STERNBERG (1997) and the SB128 strain described as *Rhabditis (Oscheius) tipulae* (LAM and WEBSTER 1971; SUDHAUS 1993); it will be (re)described by L. CARTA (personal communication). The (sub)genus Oscheius may need to be split in two genera (corresponding to the two Oscheius clades in SUDHAUS and FITCH 2001) and the Dolichorhabditis genus name may need to be resurrected (L. CARTA, personal communication). Because the Dolichorhabditis name has been used to describe the same strain (EVANS *et al.* 1997; BLAXTER *et al.* 1998), we sometimes use the combined name Oscheius/Dolichorhabditis. As long as the species name and the genus may be revised, we use one strain of this species as a reference, namely CEW1 (see FÉLIX *et al.* 2000).

The nematodes were cultured on *Escherichia coli* OP50 as described for *C. elegans* (Wood 1988).

Microscopy and laser ablations: The worms were mounted on a thin agar pad between a slide and a coverslip (Wood 1988) and observed by Nomarski optics with a ×100 objective mounted on a Zeiss Axioskop. Cells were ablated using the Micropoint Ablation laser system (Photonic Instruments; Epstein and Shakes 1995).

***Oscheius* sp. CEW1 genetics:** *Oscheius* sp. CEW1 reproduces through self-fertilizing hermaphrodites and facultative males like *C. elegans* (Félix *et al.* 2000). Its life cycle lasts about 5 days at 20°, 3.5 days at 23°, and 2.5–3 days at 25°. Spontaneous males are rare; some were isolated by starving plates and replating them on new food. A male-containing culture was then maintained by mating about five males with five hermaphrodites (Brenner 1974). Matings were usually performed at 23°; males produced at 25° were not as proficient in mating.

Mutagenesis: The worms were mutagenized by incubation for 4 hr in 4 ml of diluted ethyl methanesulfonate (EMS) as described in Brenner (1974) and Epstein and Shakes (1995), followed by extensive washing in M9. *Oscheius* sp. CEW1 appears less sensitive to EMS than *C. elegans*; concentrations of 100–150 mm EMS were used instead of 50 mm.

Some mutageneses were performed using trimethylpsoralen-ultraviolet irradiation (TMP-UV) as a mutagen, which preferentially creates small deletions (Yandell *et al.* 1994; Epstein and Shakes 1995). The worms were incubated for 15 min in 66 μg/ml TMP, washed two times in M9 in the dark, allowed to recover for 10 min on a bacteria-free plate and exposed for 40–60 sec to a hand-held Mineralight UVSL-25 lamp set on long wave and held at 2.5 cm from the uncovered plate. Approximately 40,000 gametes were screened after EMS mutagenesis and 10,000 after TMP-UV mutagenesis. The TMP-UV mutageneses yielded the *mf81*, *mf82*, and *mf83* alleles.

At the molt to adulthood in *Oscheius* sp., the germ line has not divided as much as in *C. elegans*. To diminish the number of gamete mitotic divisions occurring after mutagenesis, young adults were used instead of L4 larvae. Each mutagenized animal was transferred to a 90-mm plate seeded with OP50, allowed to lay about 30 F_1 eggs overnight, and removed from the plate (so that the plate would not be overcrowded at the time of screening). After 7–9 days at 25°, the F_2 generation was screened for genetic markers (Félix *et al.* 2000), egg-laying-defective phenotypes (seen as accumulation of many embryos in the uterus, instead of only 1 to 2 in wild type, or as bags of larvae) and abnormal vulval phenotypes (protruding/everted vulva, multivulva, etc.). Candidate vulva mutants were isolated. If the F_3 generation showed a partially or fully penetrant vulva phenotype, it was screened for a cellular phenotype by Nomarski optics.

Each mutation was backcrossed several times (usually five) to wild type and checked for recessivity and single-locus segregation. Genetic nomenclature is as in *C. elegans* (Horvitz *et al.* 1979) and *P. pacificus* (Sommer *et al.* 1996).

As a reference, we can estimate the coverage of the screen by the number of Uncoordinated Twitcher alleles. In *C. elegans*, the only gene to give a Twitcher phenotype is *unc-22* and its transcription unit covers over 38 kilobases (Benian *et al.* 1993), which constitutes a large target for mutagenesis (about 10-fold an average-sized gene). During our screen, we saw over 10 Twitcher mutations. We tested 4 of them and found them to be allelic, as in *C. elegans* and *P. pacificus* (Sommer *et al.* 1996). Thus, we believe that a gene with a clearly visible and nonlethal mutant phenotype could have been recovered with a high probability in our screen.

Complementation tests: Genetic complementation tests between two mutations, *m1* and *m2*, were performed by crossing heterozygous *m1*/+ males (obtained by crossing *m1*/*m1* hermaphrodites to CEW1 males) to *m2*/*m2* hermaphrodites (Brenner 1974). These hermaphrodites carried a recessive marker (Dumpy or Unc) to distinguish self- from cross-progeny. The hermaphrodite cross-progeny were scored by Nomar-

TABLE 1

Mutants with defects in P4.p and P8.p divisions

		S	SS	S ss	ssss	% standard lineage
Wild-type CEW1	P4.p	4	1	—	95	
n = 100	P8.p	—	—	—	100	95
dov-1(sy543)	P4.p	22	**76**	2	—	
n = 41	P8.p	37	**59**	2	2	0
dov-2(mf65)	P4.p	**33**	18	9	40	
n = 57	P8.p	9	5	4	82	33
dov-3(sy449)	P4.p	**29**	5	1	65	
n = 76	P8.p	**29**	16	9	46	30
dov-4(sy451)	P4.p	**22**[a]	—	—	78	
n = 39	P8.p	**32**	6	6	56	36
dov-4(sy464)	P4.p	**42**[a]	—	—	58	
n = 47	P8.p	**28**	—	—	72	49

In these mutants, P4.p and P8.p adopt a nonvulval syncytial fate, but divide abnormally. P(5–7).p fates and divisions are normal. The animals were grown at 23°. Phenotypes were scored in the L4 stage and are expressed in percentages. ssss, two divisions (wild type); SS, one division; S, no division; S ss, one division followed by a division of one of the daughters. The right column indicates the percentage of animals with the standard vulva lineage shown in Figure 2B. The major categories of defects are indicated in boldface type.

[a] In the *dov-4* mutants, the 15% (*sy451*) and 4% (*sy464*) of animals with the vulva centered on P5.p were not considered here.

TABLE 2

Ablation experiments in wild-type CEW1 and mutants with defects in P4.p and P8.p divisions

Cell ablations (time of ablation)	P4.p	P5.p	P6.p	P7.p	P8.p	No.
			Wild-type CEW1			
Intact	ssss	uuuu	tttt	uuuu	ssss	
Gonad (early L1)	ssss	ssss	ssss	ssss	ssss	5/5
AC (mid-L3)	ssss	uuuu	uuuu	uuuu	ssss	8/8[a]
P(5–7) (L1)	uuuu	—	—	—	tttt	5/6
	tttt	—	—	—	uuuu	1/6
P(5–7).p (early L2)	uuuu	—	—	—	tttt	10/19
	tttt	—	—	—	uuuu	7/19
	tttt	—	—	—	tuuu	1/19
	S	—	—	—	tttt	1/19
			dov-1(sy543)			
Intact	S/SS	uuuu	tttt	uuuu	S/SS	
	22/78%				37/61%	$n = 41$
Gonad (early L1)			Total 7–11 S			8/8
AC (mid-L3)	S/SS		Total 8–11 U		S/SS	9/9
P(5–7).p (L1)	UU	—	—	—	UU	2/7
	uuuu	—	—	—	tttu	1/7
	TT	—	—	—	TU	1/7
	uutt	—	—	—	tuuu	1/7
	uuut	—	—	—	UU	1/7
	UU	—	—	—	tuuu	1/7
P(5–7).p (L2)	UU	—	—	—	TTT	1/6
	TT	—	—	—	UU	1/6
	UT	—	—	—	TU	1/6
	UU	—	—	—	UU	1/6
	UU	—	—	—	U	1/6
	UUU	—	—	—	U	1/6
			dov-2(mf65)			
Intact	S/SS	uuuu	tttt	uuuu	S/SS	
	33/27%				9/9%	$n = 57$
P(5–7).p (early L2)	uuuu	—	—	—	tttt	8/17
	ssuu	—	—	—	tttt	2/17
	uuuu	—	—	—	tttt	2/17
	uutt	—	—	—	ttuu	1/17
	tttt	—	—	—	uuuu	1/17
	uuuu	—	—	—	tttl	1/17
	S	—	—	—	tttt	1/17
	tttt	—	—	—	S	1/17
			dov-3(sy449)			
Intact	S/SS	uuuu	tttt	uuuu	S/SS	
	29/5%				29/16%	$n = 41$
P(5–7).p (early L2)	tttt	—	—	—	uuss	6/14
	tttt	—	—	—	uuuu	4/14
	tttt	—	—	—	uu S	1/14
	tttt	—	—	—	usss	1/14
	tttt	—	—	—	ssss	1/14
	tttt	—	—	—	S	1/14
			dov-4(sy451)			
Intact	S	uuuu	tttt	uuuu	S/SS	
	22%				32/6%	$n = 39$
Gonad (early L1)	ssss		Total 12 S		ssss	3/8
	S		Total 9 S		ssss	2/8
	ssss		Total 12 S		S	1/8
	S		Total 12 S		ssss	1/8
	S		Total 11 S		ssss	1/8

(*continued*)

TABLE 2

(Continued)

Cell ablations (time of ablation)	P4.p	P5.p	P6.p	P7.p	P8.p	No.
			dov-4(sy464)			
Intact	S	uuuu	tttt	uuuu	S	
	42%				28%	$n = 47$
P(5–7).p (early L2)	tttt	—	—	—	ssss	6/17
	tttt	—	—	—	uuuu	9/17
	tttt	—	—	—	S	1/17
	S	—	—	—	tttt	1/17

The fates of the Pn.p cells, their daughters, or granddaughters are summarized as in Figure 2. t, transverse division (left-right); l, longitudinal division (antero-posterior); u, undivided; s, syncytial. A capital letter indicates the absence of one or two rounds of divisions. TT indicates that the second division is transverse (and not longitudinal, as in uuuu). Phenotypes were usually scored in the L4 stage after the completion of divisions. In some instances, the number of progeny for several Pn.p cells is indicated because their precise lineal origin could not be determined. For example, total 7–11 S indicates that 7 to 11 progeny were present and fused to the epidermal syncytium. The percentage of defects in the different mutants are summarized from Table 1 (SS and S ss categories are pooled for simplicity). AC, anchor cell. Early L1 is before Pn migration. Early L2 is during or right after V seam cell divisions (when daughter cells are still aligned along the antero-posterior axis). Mid-L3 is defined as during or after the division of the ventral uterine precursors but before that of the Pn.p cells.

[a] Data from Félix and Sternberg (1997).

ski optics at the L4 stage for the presence of mutant phenotypes. As only half of these animals received the *m1* allele, complementation was assessed by the absence of mutant phenotypes in at least 20 animals (more for partially penetrant mutations).

Complementation tests were performed between all mutations with similar phenotypes, namely on one hand all mutations with a defect of divisions (Tables 1 and 3) and on the other hand those with an excess of divisions (Table 4). Complementation tests could not be performed with *dov-(sy476)* because of its dominance and with *dov-(mf69)* because its vulva defect is so strong that it could not be mated into; we therefore did not assign them a gene number. All other mutations are recessive.

RESULTS

Determination of fates of the vulval precursor cells requires two steps of induction by the anchor cell in *Oscheius* sp. CEW1 compared to a single one in *C. elegans* (Félix and Sternberg 1997). To better understand the differences in vulval development between the two species, we performed a genetic screen for vulva mutants in *Oscheius* sp. CEW1.

In wild-type *Oscheius* sp. CEW1, P4.p and P8.p divide twice and their granddaughters adopt a nonvulval epidermal syncytial fate (noted "ssss" for *s*yncytial). P5.p and P7.p divide twice and their progeny adopt a vulval fate (noted "uuuu" for *u*ndivided): their granddaughters do not fuse to the syncytium, do not adhere to the cuticle, and form vulval tissue. P6.p granddaughters divide a third time transversally (left-right) and contact the anchor cell (noted "tttt" for *t*ransverse division; Figure 2B; Sommer and Sternberg 1995). In the wild strain CEW1, we observed some variations in this pattern: P4.p does not divide or divides only once in about 5% in the animals (Table 1).

We mutagenized the CEW1 strain and screened for vulva mutants. The mutations were backcrossed and checked for allelism by genetic complementation tests (see materials and methods). We call them *dov-* for *d*evelopment *o*f the *v*ulva.

We analyze here 20 mutations, defining 16 complementation groups, that affect cell divisions but not overall fates of the vulva precursors. These mutants can be separated into three broad phenotypic categories: (i) a specific defect in P4.p and P8.p divisions (3° lineage), (ii) a division defect of all vulval precursor cells, and (iii) an excess of divisions in the vulva lineages (Figure 2).

Mutants with a defect in P4.p and P8.p cell divisions: We found five mutants (defining four loci) that affect the division of P4.p and P8.p specifically (Table 1). In these mutants, P(5–7).p divide normally and their fates are not affected, whereas P4.p and P8.p adopt their normal nonvulval fate but with an altered division pattern. In *dov-1(sy543)* mutants, P4.p and P8.p usually divide once instead of twice, or sometimes not at all (Table 1; Figures 2C and 3B). In *dov-2(mf65)* and *dov-3(sy449)* mutants, they either do not divide at all, or sometimes divide only once. In the *dov-2* mutant, the defect is far more penetrant for P4.p than for P8.p. Finally, in *dov-4(sy451)* and *dov-4(sy464)*, P4.p and P8.p do not divide at all (with partial penetrance; Table 1; Figure 2D).

Cell ablation experiments in mutants with a defect in P4.p and P8.p cell divisions: The absence of P4.p and P8.p divisions in these mutants mimics the fate of more anterior and posterior Pn.ps that are not competent for vulval induction (like P3.p or P9.p). However, unlike

TABLE 3

Mutants with defects in P(4–8).p divisions

	P4.p	P5.p	P6.p	P7.p	P8.p	No.
Wild-type CEW1	ssss	uuuu	tttt	uuuu	ssss	
A. *dov-5(mf81)*	SS	U uu	TT	uu U	SS	1/7
A1. Intact	SS	uuuu	TT	uu U	SS	1/7
(no ablation)	S	UU	TT	uu U	SS	1/7
	S	U uu	tttt	uu U	SS	1/7
	SS	U uu	tttt	uu U	SS	1/7
	SS	UU	TT	uu U	SS	1/7
	SS	U uu	tttt	uuuu	SS	1/7
A2. Gonad ablation			Total 7 S			2/4
in L1	SS	SS	SS	SS	SS	1/4
	SS	SS	SS	SS	S	1/4
A3. AC ablation in	SS	U uu	uuuu	uu U	SS	3/8
mid-L3	S	U uu	uuuu	uu U	SS	1/8
	SS	U uu	UU	UU	SS	1/8
	SS	UU	uuuu	uuuu	SS	1/8
	S	UU	uuuu	UU	S	1/8
	SS	UU	UU	UU	S	1/8
B. *dov-6(sy482)*	SS	U uu	TT	uu U	SS	3/6
	SS	UU	tttt	uu U	SS	1/6
	SS	uu U	tttt	UU	SS	1/6
	S	U	uuuu	UU	SS	1/6
C. *dov-7(mf59)*	S	uuuu[a]	tttt	UU	ssss	1/7
	SS	uuuu	tttt	uuuu	sss	1/7
	S	uuuu	tttt	uuuu	SS	1/7
	SS[a]	uuuu[a]	tttt	uuuu[a]	ssss	1/7
	ssss	uuuu[a]	tttt	uuuu	ssss	1/7
	ssss	uuuu	TT[a]	uuuu	ssss	1/7
	SS[a]	uuuu[a]	tttt[a]	uuuu[a]	SS[a]	1/7

The animals were observed continuously to determine the cell lineage. Abbreviations are as in Table 2.
[a] The corresponding divisions occurred one larval stage later than normal (heterochronic mutant).

mutants that remove the whole competence group (S. Louvet-Vallée and M.-A. Félix, unpublished results), these mutants all form a normal vulva from P(5–7).p. We wondered whether the P4.p and P8.p division defect reflected a change of their competence or of the nonvulval 3° lineage. Vulval competence is defined as the ability to regulate and adopt a vulval fate, for example, upon P(5–7).p ablation. In these mutants as in wild type, P4.p and P8.p are competent to replace P(5–7).p. For example, P4.p does not divide in 40% of intact *dov-4(sy464)* animals (Table 1), but adopts a vulval fate in 16/17 animals after P(5–7).p ablation (18/19 in wild type; Table 2). P8.p also usually regulates, although not always in *dov-3* and *dov-4* mutants. Therefore, in these mutants, P4.p and P8.p do not undergo the two rounds of divisions characteristic of the 3° nonvulval fate but are still part of the vulval equivalence group. Interestingly, whereas after P(5–7).p ablation, P4.p and P8.p progeny are induced to central vulval fates with similar probability in the wild type, P4.p is more often central (1°) in *dov-3* and *dov-4* mutants and P8.p in the *dov-2* mutant (Table 2). This asymmetry between P4.p and P8.p may correlate with the lower penetrance of the P8.p division defect in intact *dov-2* animals and with a vulva centering defect on P5.p in *dov-4* mutants (Table 1 legend).

In the *dov-2*, *dov-3*, and *dov-4* mutants, P4.p and P8.p usually divide normally when adopting a vulval fate after P(5–7).p ablation. After gonad ablation in *dov-4* mutants, all cells of the vulval equivalence group adopt a nonvulval fate and show some division defects, with variable penetrance (Table 2). We therefore conclude that the division defect in these mutants specifically affects the nonvulval 3° fate.

In *dov-1(sy543)* mutants, however, when P4.p and P8.p adopt a vulval fate after P(5–7).p ablation, they divide either only once, or twice with a second transverse division and adhesion to the anchor cell, as if adopting the 1° fate but omitting its second cell cycle. P(5–7).p divide normally in intact animals, but show division defects when adopting a nonvulval fate after gonad ablation. Moreover, if the anchor cell is ablated in the mid-L3 stage, *i.e.*, after the first fate induction but before the second (Figure 1), they adopt a 2° vulval fate but divide abnormally (Table 2). Thus, fate specification and num-

ber of divisions of vulval precursor cells can be uncoupled and some gonadal/anchor cell signal influences the number of divisions independently of fate induction.

Mutants with a defect in P(4–8).p cell divisions: We found five mutants with a division defect that affects all vulva precursor cells (Table 3). In *dov-5(mf81)* and *dov-6(sy482)* animals, P4.p and P8.p divide once instead of twice, and sometimes not at all (Table 3, A1 and B; Figure 3C). P5.p and P7.p also fail to undergo their second division. Finally, P6.p often divides only twice; its second division is transverse (instead of longitudinal) and its progeny invaginate and connect to the uterus, suggesting that they adopt a central fate. A vulva forms, albeit with an altered morphology that results in a protruding vulva and egg-laying defects. Thus, like ablated *dov-1(sy543)* mutants (Table 2), intact *dov-5* and *dov-6* animals appear to omit the second division of all Pn.p cells without altering their overall fates.

Cell ablation experiments in mutants with a defect in P(4–8).p divisions: After gonad ablation in *dov-5* mutants, all P(4–8).p divide only once, whereas in intact animals the division defect appears more severe for P4.p and P8.p than for P(5–7).p. These experiments suggest again that a gonadal signal induces a second division in P(5–7).p (Table 3A2). We cannot distinguish here between induction of division and of vulval fate.

The inner daughters of P5.p and P7.p divide more often than their sisters in *dov-5* and *dov-6* mutants. We wondered whether they could be induced to divide by the anchor cell. After anchor cell ablation in the mid-L3 stage, P5.p and P7.p show the same asymmetric division pattern (Table 3A3). It is possible that induction of this asymmetric pattern cannot be temporally separated by ablation from fate induction of P5.p and P7.p.

Retarded heterochronic mutants: The *dov-7(mf59)*, *dov-8(mf66)*, and *dov-(mf69)* mutants display similar division defects to *dov-5* and *dov-6* (Table 3C; data not shown), as well as large defects in vulval division timing. In wild-type animals, the Pn.p cells divide in the second half of the L3 stage. In these mutants, Pn.p cell division is sometimes delayed until the L4 stage (Table 3C). The lack of divisions is probably a consequence of the delay. We wondered if this delay affected other developmental events. In the wild type, the V lateral epidermal seam cells divide shortly after each molt, except after the adult molt when they synthesize cuticular alae. In *dov-7(mf59)*, *dov-8(mf66)*, and *dov-(mf69)* mutants, we observed V seam cell divisions at the "adult" (fifth) stage and penetrant alae defects (Figure 4). The *dov-(mf69)* mutant displays stronger phenotypes; the sex myoblast precursor divisions are also delayed and additional molts could be observed. Altogether, several developmental processes occur one larval stage later in these mutants than in wild type. We conclude that they are retarded "heterochronic" mutations that affect the temporal identity of larval stages (Ambros and Horvitz 1984) and result in a delay and a defect in vulval lineages.

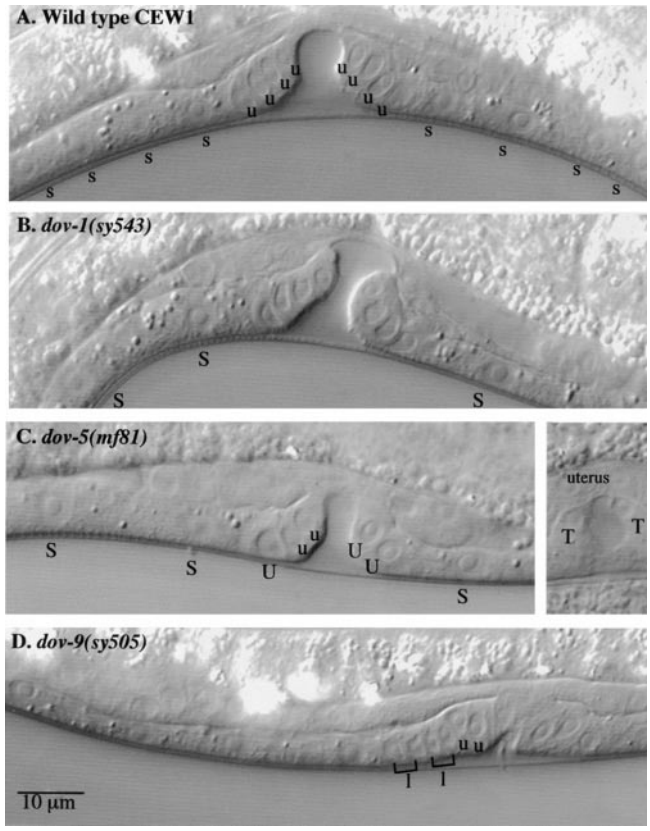

Figure 3.—Nomarski photomicrographs of the vulva in the wild-type *Oscheius* sp. CEW1 (A) and Pn.p division mutants (B–D). L4 stage animals. (A) Wild-type CEW1. The four granddaughters of P4.p, P5.p, P7.p, and P8.p are visible. The progeny of P5.p and P7.p participate in the vulval invagination (uuuu lineage), whereas that of P4.p and P8.p fuse to the epidermal syncytium (ssss). P6.p progeny is not in focus. (B) *dov-1(sy543)* mutant. P(5–7).p fates and lineages are normal; P4.p has divided once (SS); P8.p has not divided (S). (C) *dov-5(mf81)* mutant. Only the posterior daughter of P5.p has divided (U uu lineage). P7.p daughters have not divided (UU lineage). The vulval fates of P5.p and P7.p appear otherwise normal. P4.p and P8.p also show division defects. Right: P6.p underwent only two divisions and the second was transverse (TT lineage). P6.p progeny apparently adopted a central fate and connected to the uterus. Two left granddaughters of P6.p are visible in a sublateral focal plane (instead of four great-granddaughters in wild type). (D) *dov-9(sy505)* mutant. The anterior P5.p granddaughters have divided once more longitudinally (lluu lineage). Anterior is on the left; ventral is at the bottom.

In *dov-5* and *dov-6* mutants, the Pn.p divisions are not delayed and the alae are not affected. Thus, their vulval division defects do not correspond to a larval-stage heterochronic phenotype.

Mutants with an excess of vulval cell divisions: The largest category of mutants found in the screen displays an excess of cell divisions of P(5–7).p. An additional division in the P4.p and P8.p lineages was observed twice among all these mutants (noted **s′**; Table 4). These

TABLE 4

Mutants with an excess of P(5–7).p divisions

	P4.p	P5.p	P6.p	P7.p	P8.p	No.
Wild type CEW1	ssss	uuuu	tttt	uuuu	ssss	
dov-9(sy505)		**ll**uu	tt**t**″t	uu**ll**		1/11
		uu**l**u	t**t**″tt	uu**l**u		1/11
		u**l**uu	t**l**″**l**″t	uuuu		1/11
		u**l**uu	t**t**′tt	uu**l**u		1/11
		u**l**uu	t**t**″**t**″t	uuuu		1/11
		u**l**uu	tttt	uu**l**u		1/11
		uuuu	t**t**″tt	uu**l**u		1/11
		uuuu	t**o**″tt	uu**ll**		1/11
		uuuu	tttt	uu**l**u		1/11
		u**l**uu	t**t**′**t**″t	uuuu		1/11
		uuuu	tttt	uu**l**u		1/11
dov-10(mf15)		u**l**uu	t**t**″**t**″t	uuu**l**	ssss	1/13
		llll	tt**t**′t	uu**ll**	ssss	1/13
		uuuu	tt**t**′t	uuuu	ssss	1/13
		uuu**l**	t**t**″tt	uuuu	ssss	1/13
		uuuu	tttt	uu**l**u	uuuu	1/13
		uuuu	tttt	uuuu	ssss	2/13
		ssss	uuuu	t**t**″**t**″t	uuuu	1/13
		ssss	u**l**uu	o**d**″**o**″l	uuuu	1/13
		ssss	u**l**uu	t**t**″tt	uuuu	1/13
		ssss	uuuu	t**t**″tt	uuuu	1/13
		ssss	uuuu	tott	uu**l**u	1/13
		ssss	uuuu	tttt	uuuu	1/13
dov-11(mf58)		**ll**uu	t**t**″**t**′t	uu**ll**		1/8
		u**l**u**l**	t**t**″**t**″t	uuu**l**		1/8
		uuuu	t**t**″**t**″t	uuuu		1/8
		u**lll**	t**t**″**t**″t	uuuu		1/8
		llu**d**	t**t**″tt	uuuu		1/8
	s**s**′ss	uuuu	tt**t**″t	**o**uuu		1/8
	uuu**o**	t**t**′**t**″t	**l**u**ll**	ssss		1/8
		uuuu	tttt	uuuu		1/8
dov-12(mf68)		**ll**uu	tttt	uuuu		1/8
		uuu**o**	t**t**″tt	uuuu		1/8
		uuuu	tttt	uu**ll**		1/8
		u**l**uu	tttt	uuuu		1/8
	S	uuuu	tttt	uuuu		1/8
		uuuu	tttt	uuuu		3/8
dov-13(sy448)		ssuu	t**t**″**t**″t	uuss		1/10
		sssu	t**t**″**t**″t	uusu		1/10
		uuuu	t**t**″**t**″t	uuus		1/10
		llsu	t**o**″**o**″t	uuss		1/10
		sssu	t**t**″**t**″t	uuss		1/10
		sssu	t**t**″**t**″t	ssss		1/10
		uusu	t**t**″**t**″t	uuuu		1/10
		suuu	t**t**″**t**″t	uuus		1/10
		susu	t**t**″**t**″t	uuuu		1/10
		suuu	t**t**″**t**″t	uuss		1/10
dov-14(mf82)		uuuu	t**t**″**t**″t	**o**uuu		1/17
		uuuu	t**t**″**t**″t	uuuu		1/17
		uuuu	tt**t**″t	uuuu		1/17
		uu**o**u	tt**t**″t	uuuu		1/17
		uuuu	tttt	u**o**uu		1/17
		uuu**t**	t**t**″tt	uu**o**u		1/17
		uu**dd**	t**t**″tt	**tt**uu		1/17

(*continued*)

TABLE 4

(Continued)

	P4.p	P5.p	P6.p	P7.p	P8.p	No.
		uuuu	ttt″t	uuu**d**		2/17
		uuu**o**	tttt	uuuu		1/17
		uuuu	ttt′t	uuuu		2/17
		uuuu	tttt	uuuu		5/17
dov-15(mf83)		uuu**t**	t**l′o′**t	uuuu		1/7
		uuuu	o**l″**lt	**d**uuu		1/7
		uuu**o**	tott	uuuu		1/7
		uuuu	tt**″t′**t	uuuu		1/7
		uuu**d**	tt**′t′**t	uuuu		1/7
		uuuu	t**l′l**t	uu**t**u		1/7
		uuuu	tt**″o″**t	uuuu		1/7
dov-(sy476d)		uuu**t**	**t″t″t″t″**	**t**uuu		1/9
		uuuu	**t″t″t″t″**	uuuu		1/9
		uuuu	tttt	uuuu		1/9
		uuu**d**	tt**t″t″**	uuuu		1/9
		uuu**t**	**t″t″t″t″**	uuuu		1/9
	ssss′	uuuu	**t″t″t″t″**	uuuu		1/9
		uuuu	**t″t″t″t″**	uuuu		1/9
		uu**o**u	**t″t″t″t″**	**o**uuu		1/9
		uuu**t**	**t″t″**tt	**t**uuu		1/9

The letters indicate the fate and division pattern of each Pn.p granddaughter. Unless otherwise indicated, P4.p and P8.p have a wild-type phenotype. Abnormal divisions are indicated in boldface. The animals were observed continuously to determine the cell lineage. s, nonvulval syncytial fate; u, undivided, vulval fate; t, transverse (left-right) division; l, longitudinal (antero-posterior) division; o, oblique division (between longitudinal and transverse); d, division (orientation could not be determined). Primes indicate the presence of an additional round of division in the t and s lineages. t′ indicates that one of the daughters divided again, t″ that both divided again. Of *dov-10(mf15)* 36% have a vulva centered on P7.p ($n = 66$). *dov-12(mf68)* is the least penetrant mutation with 70% of vulval lineage defect ($n = 38$); it displays maternal rescue of this defect. Additional Pn.p are present in *sy476d* (probably because of a division in the L1 or L2 stage) and our assignment of Pn.p number is therefore arbitrary; only three cells were induced and the fates of P4.p and P8.p were often duplicated.

mutations also do not appear to affect the specification of vulval fates *per se*.

dov-9(sy505), *dov-10(mf15)*, *dov-11(mf58)*, and *dov-12(mf68)* mutants display similar phenotypes: some P(5,7).p granddaughters (mostly the outer ones) divide along the antero-posterior axis shortly before the third molt (noted **l** for longitudinally) and the P6.p inner great-granddaughters divide about 2 hr after their birth, in any orientation (noted **t″**; Figures 2F and 3D; Table 4). The adult vulva often protrudes slightly. Some animals form bags of larvae.

In *dov-13(sy448)* mutants, this P6.p lineage defect is fully penetrant and some granddaughters of P5.p and P7.p adopt a nonvulval fate (Table 4). The protruding vulva phenotype is highly penetrant.

In *dov-14(mf82)* and *dov-15(mf83)*, the inner P6.p granddaughter defect is associated with a low penetrance of an additional oblique or transverse division of the inner P5.p and P7.p granddaughters (Table 4). *dov-15(mf83)* presents additional uterine and vulval morphogenetic defects that result in egg-laying defects.

Finally, in the dominant *dov-(sy476d)* mutant, all P6.p great-granddaughters divide a fourth time and the inner P5.p and P7.p granddaughters sometimes divide (Figure 2G; Table 4). The protruding vulva and egg-laying phenotypes are penetrant.

Cell ablation experiments in mutants with an excess of vulval cell divisions: Induction of the central 1° fate in P6.p daughters results in a third round of division (Félix and Sternberg 1997). Therefore, we tested whether late signaling from the anchor cell was required for the additional divisions in these mutants. Anchor cell ablation in the mid-L3 stage in *dov-9(sy505)* animals prevents induction of the third P6.p division, but not of the aberrant division in P(5,7).p lineages (Table 5A). In *dov-15(mf83)*, division of the inner P(5,7).p granddaughters appears to depend on the presence of the anchor cell (Table 5B). Finally, the fourth division in the P6.p lineage of *dov-13(sy448)* mutants depends on anchor cell signaling after induction of the third division (Table 5C).

Precocious heterochronic mutants: We found two other mutations with an excess of vulval divisions, namely *dov-16(sy478)* and *dov-16(sy508)*. These muta-

TABLE 5

Ablations in mutants with an excess of P(5–7).p divisions

	P5.p	P6.p	P7.p	No.
A. *dov-9(sy505)*	**u**l**u**u	t**t′**tt	u**u**l**u**	Var.
AC⁻ in mid-L3	**u**l**u**u	**u**u**u**u	u**u**l**u**	3/8
	ul**u**u	**u**u**u**u	**u**u**u**u	1/8
	ul**u**u	**u**u**u**u	u**ull**	1/8
	lllu	**u**u**u**u	**u**u**u**u	1/8
	uu**u**u	u**d**u**u**	u**ull**	1/8
	uu**u**u	**u**u**u**u	**u**u**u**u	1/8
B. *dov-15(mf83)*	**u**u**u**t	t**t′t′**t	**u**u**u**u	Var.
Gonad⁻ in early L1	ssss	ssss	ssss	5/6
	SS	ssss	ssss	1/6
AC⁻ in mid-L3	**u**u**u**u	**u**u**u**u	**u**u**u**u	20/23
	uu**u**u	**u**u**u**u	**u**u**u**s	1/23
	uu**u**t	**u**u**u**u	**u**u**u**u	1/23
	uu**u**u	**u**u**t**u	**u**u**u**u	1/23
C. *dov-13(sy448)*	ssuu	t**t″t″**t	uuus	Var.
AC⁻ in mid-L3	uuuu[a]	uuuu	uuuu[a]	6/8
	uuuu	uuuu	uuus	1/8
	uuuu	uuuu	ssss	1/8
AC⁻	uusu	tttt	ssss	1/5
P6.p 4-cell stage	u**l**su	tttt	ssss	1/5
	sssu	tttt	usss	1/5
	ssuu	tttt	uuus	1/5
	uuuu	tddt	uuss	1/5

Abbreviations are as in Table 4. Var., variable penetrance (see Table 4); the lineage of only one typical intact animal is shown.

[a] It was difficult to score the s *vs.* u fates in the P5.p and P7.p granddaughters of these animals.

tions also affect division timing in a reverse manner compared with *dov-7(mf59)*, *dov-8(mf66)*, and *dov-(mf69)*. In *dov-16* animals, Pn.p cells often already divide at the L2 stage and reiterate some divisions one stage later, resulting in an excess of vulval divisions in P(5–7).p lineages. Fewer divisions are also seen in the 3° lineage. The morphogenetic events in the vulva occur earlier and ventral protrusions already form in the L4 stage, as well as occasional aberrant alae, suggesting that some adult features are already present (Figure 4). These *dov-16* alleles thus appear to define precocious heterochronic mutants. Although gonad development is apparently not affected, the vulval fate pattern already forms in the L2 stage. Therefore, the anchor cell is most likely already active in the L2 stage for both vulval fate inductions.

Other cell divisions in the animal: We attempted to determine whether the mutations that affect Pn.p divisions also affected divisions elsewhere in the animal. These mutations are neither lethal nor sterile or, if so, only weakly; therefore they do not affect all embryonic or gonadal divisions. Moreover, they do not affect all Pn.p divisions equally.

In some mutants with a lack of Pn.p divisions, intestinal nuclei are abnormally large. We counted these nuclei in wild-type and mutant strains. In *Oscheius* sp. CEW1 as in *C. elegans*, there are on either side of the intestine 10 cells at the L1 stage and 17 cells at the L2 stage (n = 8). In the mutants, results are more variable and are expressed as averages (with range and number of animals observed). In *dov-5(mf81)* mutants, we found 16 nuclei (15–17; n = 9) at the L2 stage; in *dov-1(sy543)* mutants, 13 nuclei (12–14; n = 14); in *dov-6(sy482)* mutants, 11 nuclei (10–12; n = 12). The number of nuclei at the L1 stage was 10 in all cases. Thus, *dov-1*, *dov-5*, and *dov-6* mutations affect intestinal divisions. By contrast, we found 17 intestinal cells in *dov-2(mf65)* and *dov-15(mf83)* mutants at the L2 stage (n = 10 for both).

We also estimated lateral epidermal cell number at the end of the L4 stage by counting nuclei in a window of a given size (data not shown). Lateral epidermal cell number is clearly affected in the *dov-6* mutant, but probably not in the *dov-2* and *dov-15* mutants.

We conclude that *dov-6(sy482)* shows division defects in different larval tissues, although it appears to specifically affect the second round of Pn.p divisions. By contrast, *dov-2(mf65)* does not affect all postembryonic lineages and appears to be relatively specific to the 3° nonvulval Pn.p lineage.

DISCUSSION

In this article, we describe the isolation and characterization of 20 mutants of the nematode *Oscheius* sp. CEW1 that affect the timing and number of Pn.p divisions without overall change in their fate. Surprisingly, most phenotypes described do not resemble any of the previously characterized vulva mutations in the model system *C. elegans*. We discuss the implications of these results concerning the control of vulval lineages in *Oscheius* sp. CEW1 compared with *C. elegans* and propose that in *Oscheius* sp., the anchor cell induces vulval divisions independently of inductions of fates *per se*.

Comparative genetics of the vulva between *C. elegans* and *Oscheius* sp. CEW1: Similar screens for egg-laying-defective mutants have been performed in two other nematodes, *C. elegans* and *P. pacificus*. In *C. elegans*, most vulval cell lineage defects show overall defects in Pn.p competence and induction (homeotic changes in Pn.p fates), or affect 2° lineage polarity, or are larval-stage heterochronic mutants (Ferguson *et al.* 1987). These screens appeared saturated for reduction-of-function mutations (Ferguson and Horvitz 1985). However, some new categories of vulva mutants were later found, for example *lin-39* (Clark *et al.* 1993) or *bar-1* in a screen for protruding vulva (PVul) phenotypes (Eisenmann *et al.* 1998). In the more distantly related nematode *P. pacificus* (family Diplogastridae), a similar screen yielded mutants affecting Pn.p competence and induction, although with a different phenotypic range compared

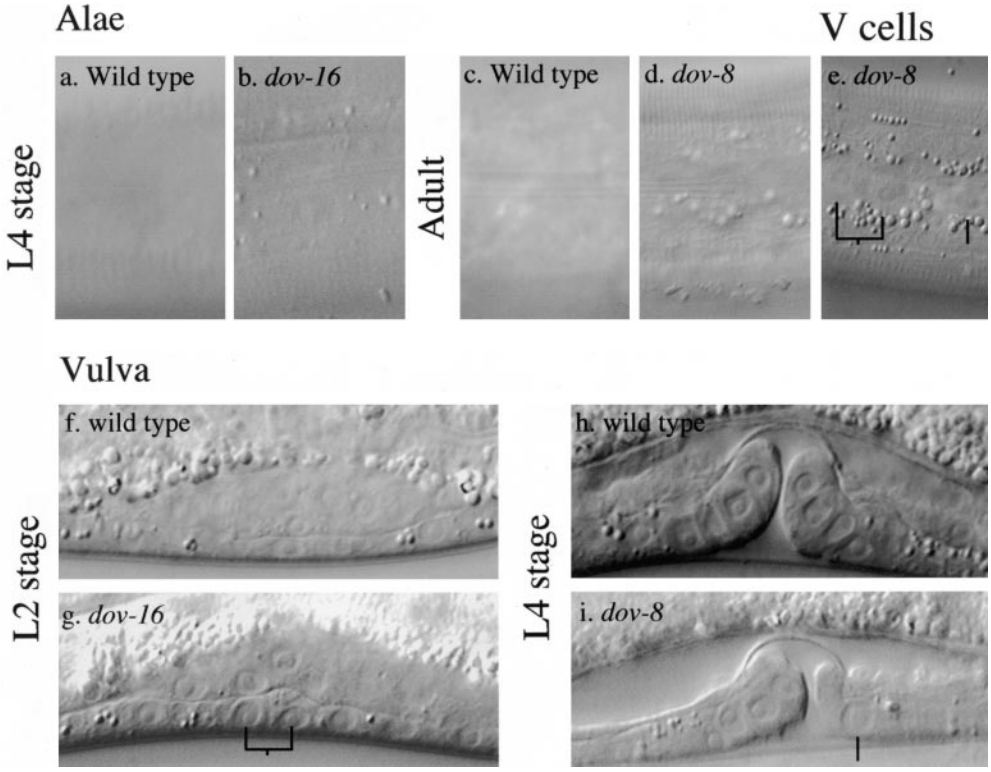

FIGURE 4.—Alae and vulva formation in wild type and in the heterochronic *dov-8* and *dov-16* mutants. The alae are cuticular components that lie on the lateral sides of the adult. (a) No alae can be seen at the L4 stage in wild type. (b) Alae are already present at the L4 stage in the precocious *dov-16* mutant. (c) Adult alae in wild type. In *Oscheius* sp., as in *C. elegans*, the V cells divide after each molt except the last one, during which they synthesize the adult alae. (d and e) The alae are interrupted in the retarded *dov-8* mutant and the holes in the adult alae correlate with ectopic divisions of the V seam cells, *i.e.*, reiteration of their larval lineage. (f and g) The Pn.p cells do not divide until the L3 stage in wild type, but already do in *dov-16* mutants (P6.p daughters are marked). (h and i) Some Pn.p divisions conversely occur in the L4 stage in the *dov-8* mutant and some Pn.p do not divide at all (P7.p in the picture).

with *C. elegans* (SOMMER *et al.* 1996; SIGRIST and SOMMER 1999).

The *Oscheius* sp. vulval screen is clearly not saturated for vulval division mutants, as most were found as single alleles per locus. However, the range of phenotypes and their relative frequency clearly differ from those found in *C. elegans*.

This difference in vulva mutant phenotypes between the two species may be partially explained if pleiotropic effects causing sterility or lethality hindered their isolation in the screens performed in *C. elegans*. A recent clonal screen in *C. elegans* actually yielded a sterile mutant with a lineage defect similar to that of *dov-5* and *dov-6* (D. FAY and M. HAN, personal communication).

Some differences in the range of vulva phenotypes produced by mutagenesis, however, result from different developmental mechanisms at play in the two species or from their level of redundancy. One major outcome of our screen is the dissociation of the mechanisms determining vulval fates and vulval division patterns in *Oscheius* sp. CEW1. It is not clear whether this dissociation is linked to the difference in vulval patterning mechanisms. It is, however, remarkable that in *Oscheius* sp. CEW1, unlike in *C. elegans*, both nonvulval (3°) and vulval Pn.p fates correspond to an identical number of cell cycles, whereas the second wave of induction on P6.p daughters (1° fate) correlates with an additional cell cycle (FÉLIX and STERNBERG 1997). Therefore, the link between vulval cell cycle and cell fate could have been altered during evolution: the second cell cycle is specified downstream of the program of Pn.p competence in *Oscheius* sp. CEW1 and downstream of anchor cell induction in *C. elegans*.

Thus, the mutability of Pn.p division patterns appears high in *Oscheius* sp. and low in *C. elegans*. Such a difference between species in the range of vulva mutant phenotypes that can be reached by a one-gene mutation is likely to have evolutionary consequences. Interestingly, the Pn.p division phenotypes are found at low penetrance in natural populations of *Oscheius* sp. but not of *C. elegans* (M. DELATTRE and M.-A. FÉLIX, unpublished results).

Heterochronic mutants in *Oscheius* sp. CEW1: temporal control of the onset of vulval divisions: *C. elegans* and *Oscheius* sp. CEW1 develop through four larval stages separated by molts. In *C. elegans*, heterochronic mutations modify larval stage identity (SLACK and RUVKUN 1997). Among those affecting early larval stages (before Pn.p divisions), *lin-4(lf)* and *lin-14(gf)* retarded mutants repeat many characteristic events of the L1 stage; Pn.p divisions are correspondingly delayed or absent (or sometimes reiterated) and vulval fates are not correctly specified (CHALFIE *et al.* 1981; AMBROS and HORVITZ 1984; EULING and AMBROS 1996a). Conversely, in *lin-*

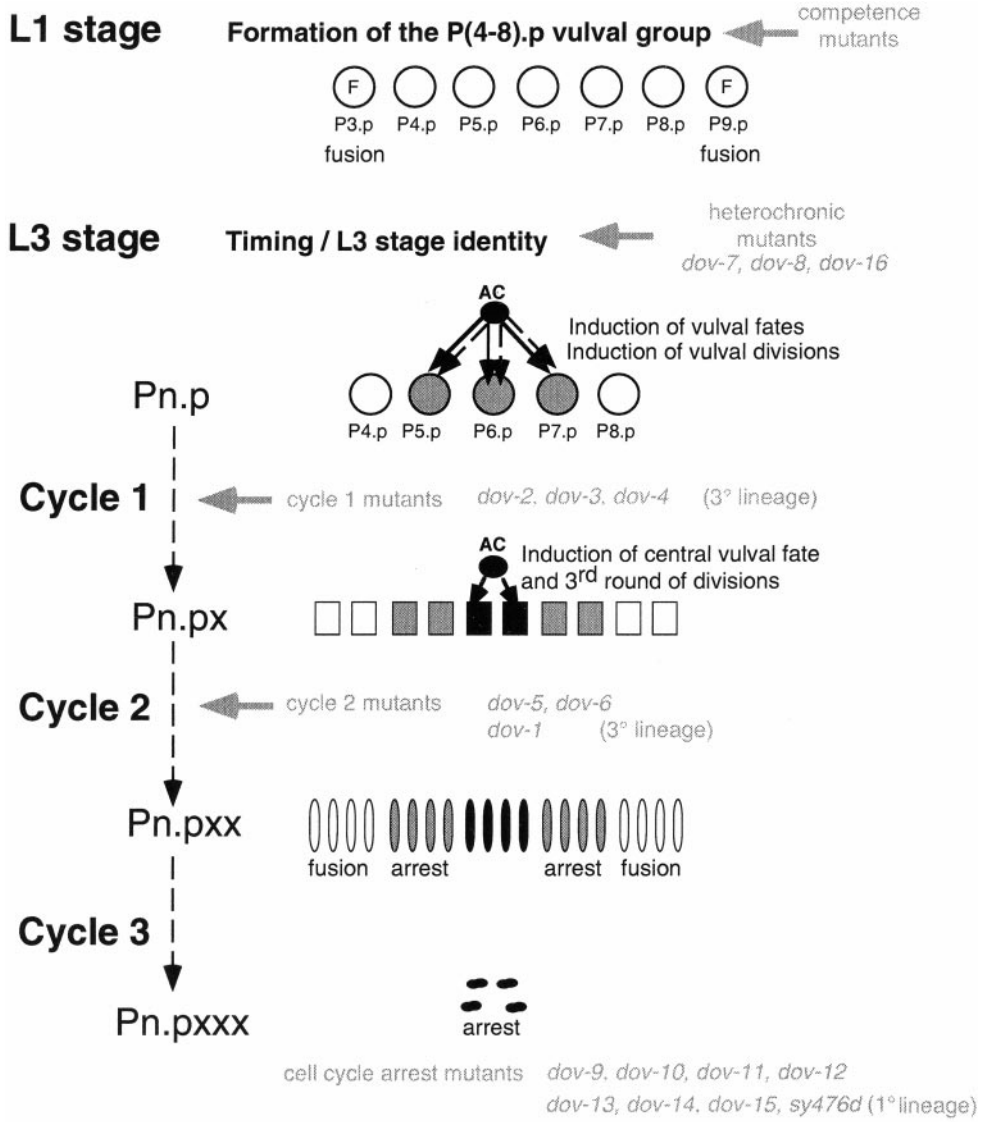

FIGURE 5.—Control of Pn.p cell divisions in *Oscheius* sp. CEW1. Fates of Pn.p cells and their Pn.px daughters are depicted with the same colors as in Figure 1. F, noncompetent cell that fuses to the syncytium; white, nonvulval fate (3°, P4.p and P8.p); gray, outer vulval fate (2°, P5.p and P7.p); black, central vulval fate (1°, P6.p). Cell identity at each cell cycle is depicted with different shapes: Pn.p, circles; Pn.px, squares; Pn.pxx, ovals; Pn.pxxx, dots. The steps at which the different mutants are defective are indicated in gray. Competence mutants suppress all Pn.p cell divisions and also affect competence (S. LOUVET-VALLÉE, unpublished data). Heterochronic mutants are defective in the timing of divisions. Cycle 1 mutants suppress the first division; those isolated affect the 3° lineage specifically. Cycle 2 mutants appear to suppress the second division of all Pn.p cells; some of them are specific for the 3° lineage. Cell cycle arrest mutants show additional divisions in specific lineages. The two latter categories can be interpreted as changes in the identity of the Pn.px cells to that of their daughters (square to oval), or conversely.

14(lf) and *lin-28(lf)* precocious mutants, Pn.p divisions occur one larval stage earlier as a result of a shortened G_1 phase and vulval fates appear to be correctly specified (AMBROS and HORVITZ 1984; EULING and AMBROS 1996b).

The heterochronic mutants of *Oscheius* sp. CEW1 resemble those of *C. elegans*. They concern nongonadal postembryonic development (vulva and sex myoblast precursors, seam cells). However, Pn.p cells are competent to be induced to vulval fates in both precocious or retarded *Oscheius* sp. heterochronic mutants, but not in retarded *C. elegans* mutants. Also, unlike in *C. elegans*, divisions are reiterated one larval stage later in the *Oscheius* sp. precocious mutants. These differences in the phenotypes of the heterochronic mutants between these two species may reflect the fact that the mutations alter different parts of the temporal control of larval stage identity (we did not analyze whether the L1 developmental events were already affected in *Oscheius* sp. heterochronic mutants). Alternatively, a similar heterochronic pathway may differently influence Pn.p competence and division in these two species.

Altogether, these heterochronic mutants suggest that in *Oscheius* sp., as in *C. elegans*, the onset of Pn.p divisions is regulated by a temporal mechanism that jointly controls several developmental events in the worm.

Dissociation of vulval cell fate and number of divisions: In *C. elegans*, vulval lineage control is dissociated from Pn.p fate specification neither in mutants nor by ablation experiments. For example, in mutants with a lowered activity of the epidermal growth factor-Ras-mitogen-actived protein kinase pathway, or after anchor cell ablation, P(3–8).p all adopt a 3° fate and this results in a lowered number of divisions compared to 2° or 1° fates (Figure 2). P(3–8).p divisions are suppressed in mutations also affecting Pn.p competence, as in the HOM-C gene *lin-39* (CLARK *et al.* 1993; WANG *et al.* 1993) and in Armadillo/*bar-1* (EISENMANN *et al.* 1998).

In *Oscheius* sp. CEW1, noncompetent Pn.p cells do not divide and P(4–8).p all divide at least twice, irrespective of their vulval fate and independently of the gonad (Table 2). P6.p progeny divide a third time upon induction of the central vulval fate (Figure 2). Therefore, a change in division number may correspond to a change in cell competence or to central vulval fate specification. We did isolate mutations that affect divisions by obviously altering competence or fate, for example, mutations that abolish the competence group (S. Louvet-Vallée and M.-A. Félix, unpublished results; Figure 5). However, the mutants described here specifically affect cell cycles and do not correspond to homeotic changes in vulval fate and competence. They define genes controlling vulval cell lineages *per se*. For example, *dov-2*, *dov-3*, and *dov-4* mutations specifically affect both divisions of P4.p and P8.p, but not their competence. Therefore, they act on cell cycle regulation downstream of the program specifying vulval competence (Figure 5).

The second Pn.p division can be genetically distinguished from the others as it is specifically affected in *dov-1(sy543)*, *dov-5(mf81)*, and *dov-6(sy482)* mutants. These mutations may thus be interpreted as changes in identity of the Pn.p daughter (Pn.px) to that of its own daughter (Pn.pxx; square to oval; Figure 5).

Furthermore, these mutants uncover a role for the anchor cell in inducing the second division. Only P4.p and P8.p divide abnormally in *dov-1* mutants. However, when the anchor cell is ablated or when Pn.p are distant from it, Pn.p cells adopting vulval fates also fail to undergo the second division in *dov-1* mutants (Table 2). This suggests that the anchor cell can induce the second division. In wild-type animals, a similar signal may exist but cannot be revealed by ablations, because it acts redundantly with the autonomous specification of P(4–8).p divisions. The *dov-1* mutation may affect this autonomous program of divisions, with its effect on P(5–7).p being rescued by the anchor cell division signal. This hypothesis of an anchor cell division signal is reinforced by the observation that in *dov-5* and *dov-6* mutants, the division defect is less penetrant for those Pn.p daughters closest to the anchor cell (Table 3). In summary, *dov-1* can be fully rescued by anchor cell signaling on P(5–7).p whereas *dov-5* and *dov-6* can be only partially rescued. The difference may be quantitative; alternatively, *dov-1* may affect only the autonomous division program, whereas *dov-5* and *dov-6* affect the second division *per se* (Figure 5). Importantly, induction of Pn.p division by the anchor cell can be uncoupled from vulval fate induction. It may involve the same or distinct molecular pathways.

Conversely, mutations with an excess of Pn.p divisions may reflect an identity change of the Pn.pxx cell to a Pn.px cell (oval to square; Figure 5), or of Pn.pxxx to Pn.pxx. P4.p and P8.p progeny are not much affected, perhaps as a result of their rapid fusion to the syncytium, which overrides cell cycle defects. The fourth division in the P6.p lineage of *dov-13(sy448)* mutants requires anchor cell signaling after the induction of the third round, which may reflect an extended competence to respond to anchor cell signaling. The additional transverse division in some P(5,7).p granddaughters in *dov-15(mf83)* apparently depends on late induction (Table 5B) and could correspond to an excess of signaling.

Alternatively, mutants with an excess of divisions may be interpreted as defective in cell cycle arrest, for example, in repression of cell cycle genes (Figure 5). Interestingly, the additional longitudinal and transverse divisions of specific P(5,7).p granddaughters resemble the wild-type lineage of other species of this family, for example, that of *C. elegans* (Sulston and Horvitz 1977; Sommer and Sternberg 1995; Figure 2). Which vulval cell lineage is ancestral is unclear, but these mutants may uncover some default program for a third division round of P5.p and P7.p in *Oscheius* sp. CEW1. By contrast, a fourth division in the P6.p lineage has never been observed, either in different species or in *C. elegans* vulva mutants.

Regulation of cell division during development: The mutations described above may act on overall cell cycle regulation or only downstream of a specific fate specification program. Some of the *Oscheius* sp. mutations affect other postembryonic cell cycles, but they do not affect all divisions. Moreover, they appear specific for some of the divisions in the Pn.p lineages.

In *C. elegans*, some mutations that affect all postembryonic cell cycles have been described. A relatively frequent class results in a sterile PVul phenotype (also seen in our screens, but not kept) or larval lethality. For example, *lin-5* is required for mitotic divisions (Albertson *et al.* 1978; Lorson *et al.* 2000). New components of postembryonic cell cycle regulation are now found by RNA interference experiments, for example, cyclin D/*cyd-1* and its associated *cdk-4* kinase (Park and Krause 1999). These mutants and knockouts cause almost complete arrest of divisions of Pn cells (the mothers of the Pn.p cells that divide in the L1 stage). Conversely, some sterile mutations like *lin-19/cul-1* (Kipreos *et al.* 1996) and RNA interference of the cyclin-dependent kinase inhibitor *cki-1* (Hong *et al.* 1998) cause hyperproliferation of all postembryonic lineages.

The *Oscheius* sp. mutants that selectively affect some postembryonic divisions may correspond to partial reduction-of-function mutations in genes controlling the basic cell cycle machinery, or to partially redundant functions, particularly in gene families such as cyclins and cdk kinases. However, the specificity of the affected cell cycle within the Pn.p lineage (for example the second cell cycle for *dov-5* and *dov-6* mutants) suggests that these genes normally play a role in coupling cell identity with cell cycle control in several places in the animal.

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