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High sensitivity of *C. elegans* vulval precursor cells to the dose of posterior Wnts

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ABSTRACT

Cell competence is a key developmental property. The *Caenorhabditis elegans* vulval competence group consists of P(3–8).p, six cells aligned along the antero-posterior axis in a wide central body region. The six cells are not equal in their competence: 1) P3.p quits the competence group in half of the individuals; 2) the posterior cells P7.p and P8.p are less competent than central vulval precursor cells. Competence to adopt a vulval fate is controlled by expression of the HOM-C gene *lin-39*, and maintained through Wnt signals that are secreted from the tail in a long-range gradient. Here we quantify the LIN-39 protein profile in vulval precursor cells of early L2 stage larvae, prior to P3.p fusion and inductive signaling. We show that LIN-39 levels are very low in P3.p and P4.p, peak in P5.p and progressively decrease until P8.p. This unexpectedly centered profile arises independently from the gonad. Posterior Wnt signaling reduces LIN-39 level in the posterior cells by activating the next-posterior HOM-C gene, *mab-5*. On the anterior side, P3.p and P4.p competence and division are sensitive to the already low LIN-39 and Wnt doses; most dramatically, each of the *cwn-1/Wnt* and *egl-20/Wnt* genes show haplo-insufficience for P3.p fate. In contrast to previous results, we find that these Wnts maintain P3.p and P4.p competence without affecting their LIN-39 level. The centered vulval competence profile is thus under the control of the posterior Wnts and of cross-regulation of three HOM-C genes and prepatterns the later induction of vulval fates.

Introduction

Competence is a property of cells during development that renders them able to respond to a specific intercellular signaling event. When more than one cell is competent for a given signaling event, the group of competent cells is classically called "equivalence group". Examples of such equivalence groups are found during development of the nematode *Caenorhabditis elegans* (Kimble, 1981; Sulston and White, 1980), insect neurogenesis (Doe and Goodman, 1985; Stollewerk and Simpson, 2005), ectoderm development in the leech (Kuo and Shankland, 2004; Quigley et al., 2010) or ocellus/otolith development in the ascidian embryo (Nishida and Satoh, 1989). Traditionally in developmental biology, cell competence has been viewed as an all-or-none feature, and cells of an equivalence group are therefore considered …equivalent. We now know in several systems molecular and cellular mechanisms that specify cell competence acquisition and maintenance. With the accumulation of molecular and quantitative knowledge, it is now clear that all cells of an "equivalence group" are not necessarily equally competent (Clandinin et al., 1997; Cubas and Modolell, 1992; Katz et al., 1995; Sommer and Sternberg, 1994). We therefore favor the term "competence group" and will use it thereafter. An illustration of the inhomogeneity of competence in a field of competent cells is provided by the neurogenic precursor cells in *Drosophila melanogaster* wing imaginal disks. A bias is found in the choice of cells that adopt a sensory mother cell fate (Cubas et al., 1991), due to the expression domains of upstream regulators of pro-neural genes, such as the negative regulator *extramacrochaetae* (Cubas and Modolell, 1992; Cubas et al., 1991; Huang et al., 1995). Unequal competence can be viewed as a prepatterning event that biases the outcome of the signaling event.

A particularly favorable system to study the mechanisms and significance of unequal developmental cell competence is provided by vulval development in the nematode *C. elegans*. In this system, six cells, called P3.p to P8.p from anterior to posterior, are part of the competence group, (Fig. 1A). Each of them can adopt a vulval fate if the other cells are killed with a laser beam — an empirical definition of the competence group (Sternberg and Horvitz, 1986; Sulston and White, 1980). A gonadal cell external to the competence group, the anchor cell, sends a LIN-3/EGF signal in the late L2 to L3 larval stages, which activates a Ras/MAP kinase signaling cascade in receiving Pn.p cells. The Ras pathway in turn activates lateral signaling between the Pn.p cells through a LIN-12/Notch pathway. The anchor cell is located closest to P6.p, which adopts the 1° fate at high levels of Ras signaling. The adjacent cells P5.p and P7.p adopt a 2° fate as a result of lower EGF signal levels and lateral Notch signaling from P6.p. The remaining cells, P3.p, P4.p and P8.p, adopt a non-vulval 3° fate.

Cells of the competence group exhibit different levels of competence to respond to anchor cell induction, as assessed by a variety of

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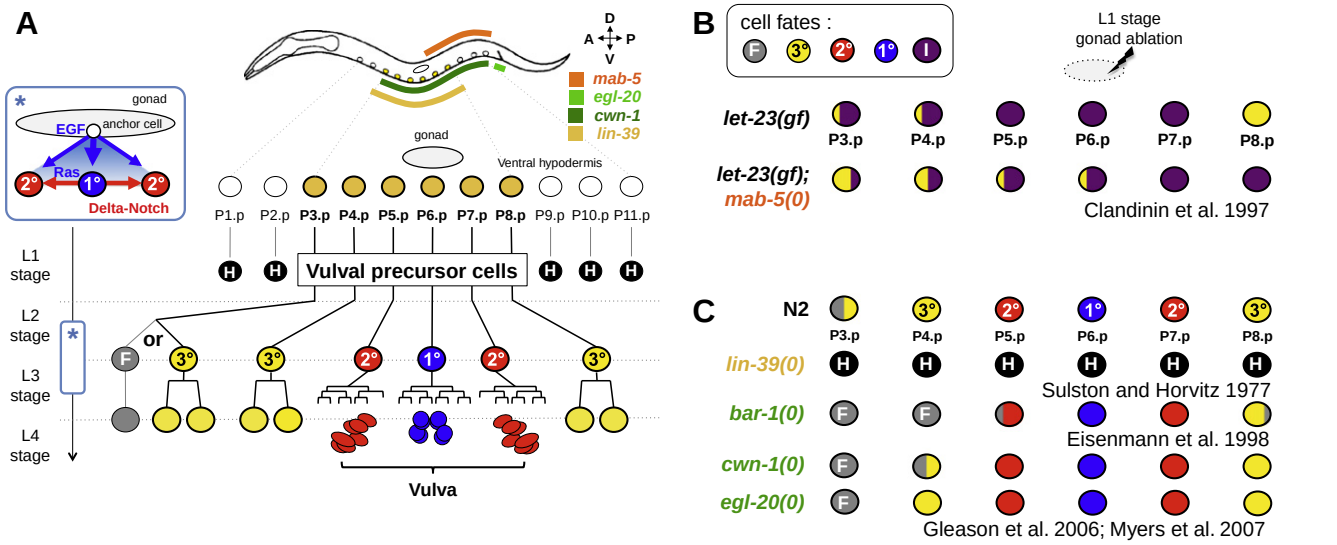

Fig. 1. Vulva development in *C. elegans*. (A) At the early L1 stage, P(3–8).p express *hox5/lin-39*, which prevents them from fusing to the hypodermal syncytium hyp7. In the L2 stage, P(3–8).p are maintained unfused by the activity of EGL-20 and CWN-1. MAB-5 activity negatively modulates vulval competence in P7.p and P8.p. The transcriptional expression domains of *lin-39/hox5*, *mab-5/hox6-8*, *egl-20/Wnt* and *cwn-1/Wnt* are indicated. Vulval fate specification among the vulval precursor cells then occurs through the combination of EGF-Ras signaling coming from the anchor cell and Delta–Notch lateral signaling between the precursor cells. In normal conditions, P(5–7).p adopt a 2°1°2° vulval cell fate pattern. The other vulval precursor cells, P(3,4,8).p, adopt a by-default 3° fate consisting in one division round and daughter cell fusion to hyp7. P3.p may either fuse to hyp7 at the late L2 stage (F fate) or adopt a 3° fate. H: Hypodermal cell, i.e. fusion to hyp7 during the L1 stage; F: Fused fate, i.e. fusion to hyp7 during the L2 stage. 3°, 2° and 1° fates are symbolized by a color code: yellow, red and blue, respectively. (B) Posterior precursor cell competence is down-regulated by *hox7/mab-5*. The relative level of competence to EGF signaling from the gonad can be assayed by laser ablation of the gonad in an EGF-receptor/*let-23* gain-of-function context. Purple: induced cell (1° or 2°). Yellow: uninduced vulval precursor cell (3°). (C) Anterior vulval precursor cell competence is maintained by the activity of the canonical Wnt pathway. The *cwn-1; egl-20* double mutant displays a phenotype similar to that of *β-catenin/bar-1* mutants. In (B, C), the colored areas in a given cell schematize the frequencies of adoption of each cell fate.

methods. First, in about half of the individuals of the *C. elegans* reference strain N2 in standard conditions, P3.p fuses to the epidermal syncytium hyp7 in the late L2 stage, which removes it from the competence group (Eisenmann et al., 1998; Sulston and Horvitz, 1977). Second, relative cell competence can be tested by removing the endogenous, spatially localized, source of LIN-3 and providing the cells with a LIN-3 dose from an ubiquitous heat-shock construct or using a gain-of-function in its receptor (Fig. 1B). Under this experimental paradigm, at intermediate EGF concentrations, P3.p and P8.p are not always induced; if EGF concentration is increased, P8.p is always induced, but not P3.p (Table 2 in Katz et al., 1995). A variation of this experimental paradigm consists in isolating one Pn.p cell (by ablating the others) and submitting it to a LIN-3 dose as above. With this protocol, P8.p is less responsive than P6.p, and P7.p is intermediate (Clandinin et al., 1997). Thus, the *C. elegans* vulva competence group is formed by P(3–8).p, with on the anterior side P3.p being less competent due to its variable L2 stage fusion to hyp7, and on the posterior side P8.p – and to a lesser extent P7.p – displaying lower competence.

The Pn.p cells are the posterior daughters of Pn cells. At hatching, the Pn cells are localized in six left–right pairs, which correspond to left–right homologs in the embryonic lineage (Sulston et al., 1983). In the L1 stage, each pair rotates and the twelve cells align along the antero-posterior axis, at which point they can be numbered from P1 to P12 from head to tail. For the central pairs (including P3/P4, P5/6), the left and right cells rotate in each direction with equal probability (Eisenmann et al., 1998; Sulston and Horvitz, 1977). Over a population of individuals, the lineage origin of P3 is thus equivalent to that of P4, the lineage origin of P5 is equivalent to that of P6, etc. Since P3.p and P4.p arise from equivalent lineages, their differential competence must depend on instructive spatial information from extracellular signals.

At the molecular level, P(3–8).p competence requires expression of the *Hox5/SexCombReduced/lin-39* gene (Wang et al., 1993): in homozygous *lin-39(null)* mutants, vulval precursor cells express *eff-1* (coding for a fusogen protein) and fuse to the epidermal syncytium hyp7, shortly after their birth in the L1 stage (Shemer and Podbilewicz, 2002). The next posterior HOM-C gene, *mab-5*, quantitatively inhibits the competence of P8.p and P7.p through an unknown mechanism (Clandinin et al., 1997; Fig. 1B).

Several factors are likely to regulate competence through the control of *lin-39* expression or activity of its protein product. They will be reviewed successively in the three next paragraphs.

Wnt signaling prevents vulval precursor cells from fusing to hyp7 in the L2 stage and thus maintains their competence until the L3 stage (Eisenmann et al., 1998; Shemer and Podbilewicz, 2002; Fig. 1C). Among the five Wnts in the *C. elegans* genome, loss-of-function mutations in two of them, *egl-20* and *cwn-1*, considerably increase P3.p and P4.p L2 fusion frequency and correspondingly reduce their division frequency (Gleason et al., 2006; Myers and Greenwald, 2007). *egl-20* is expressed in the tail and rectal region and the EGL-20 protein forms a long-range gradient from the posterior to the mid-body in L1 larvae (Coudreuse et al., 2006; Whangbo and Kenyon, 1999). *cwn-1* is expressed in the ventral nerve cord throughout larval development (Gleason et al., 2006), with a gradient of expression by the ventral neurons at early larval stages, from strong posterior levels to faint mid-body expression (Hayashi et al., 2009). As revealed by genetic mosaics using a *bar-1/β-catenin* mutation, the Wnt signal transduction pathway acts cell-autonomously in the Pn.p cells for the choice between L2 fusion versus 3° fate (Eisenmann et al., 1998; Shemer and Podbilewicz, 2002). Multiple studies have suggested that these Wnts affect vulval precursor cell competence by activating *lin-39* expression (Eisenmann et al., 1998; Gleason et al., 2002; Wagmaister et al., 2006a).

The Ras pathway also maintains competence of vulval precursor cells in the L2 stage (Eisenmann et al., 1998), before its main role in vulval fate induction in the L2 and L3 stages. Mutations reducing the activity of the Ras pathway show little effect on anterior vulval precursor cell competence and division, whereas increased Ras activity prevents P3.p fusion and increases its division frequency