Lateral inhibition in the *C. elegans* vulva – a dialog

Scientist 1 (curious developmental biologist). Why does the lin-12 mutant make all secondary cells in the pnp equivalence group?

Scientist 2 (worm expert). In the *Caenorhabditis elegans* vulval precursor cell (Pn.p) equivalence group, the *lin-12* gene plays a crucial role in cell fate determination. The *lin-12* gene encodes a Notch receptor that mediates lateral inhibition, a mechanism through which cells within an equivalence group communicate to diversify their fates. In this context, *lin-12* helps specify which cells will adopt primary (1°) and secondary (2°) fates within the Pn.p cells.

In wild-type *C. elegans*, one Pn.p cell (typically P6.p) adopts the 1° fate and is flanked by cells adopting the 2° fate, while others remain in a tertiary (3°) or non-vulval state. The decision between 1° and 2° fates is governed by interactions involving both EGF (Epidermal Growth Factor) signaling, which promotes the 1° fate, and *lin-12*-mediated Notch signaling, which promotes the 2° fate in neighboring cells. Essentially, the cell receiving the highest EGF signal adopts the 1° fate, while those receiving Notch signaling from the 1° cell adopt the 2° fate.

In a *lin-12* mutant, this Notch signaling is disrupted, meaning cells no longer receive the lateral inhibition cues necessary to block the 1° fate in neighbors. Without functional *lin-12*, the system loses the lateral inhibition mechanism, so the other Pn.p cells do not receive signals to reinforce a 2° fate in response to a neighboring 1° cell. Consequently, they all adopt the default 2° fate in response to the broader environmental context rather than differentiating into primary and tertiary fates. This mutant phenotype highlights the importance of *lin-12* signaling in regulating distinct cell fates within the Pn.p equivalence group and ensuring the proper patterning of the vulval cells.

Scientist 1 (curious developmental biologist). What does "the broader environmental context" mean? in a lin-12 mutant, all the cells take secondary fates, even if the anchor cell is ablated, so this suggests that lin-3 from the anchor cell is not required to enable lin-12 activity.

Scientist 2 (worm expert). you're correct, and this observation points to an important aspect of *lin-12*'s function and its independence from *lin-3* EGF signaling in certain contexts. The term "broader environmental context" can indeed be misleading here if it implies the need for *lin-3* signaling. In reality, the *lin-12* pathway can function autonomously within the Pn.p equivalence group to mediate cell-cell interactions that establish secondary fates, even without external cues from the anchor cell.

In a *lin-12* mutant, all cells in the equivalence group adopt the secondary (2°) fate, even in the absence of the anchor cell, which produces *lin-3* EGF. This finding suggests that *lin-12* signaling is self-sustained within the Pn.p group and doesn't strictly require *lin-3*

input for enforcing the secondary fate. Instead, *lin-12* activity is regulated by lateral inhibition within the equivalence group, meaning that cells in close proximity signal to each other to coordinate their fates. In a wild-type context, one cell receiving high *lin-3* from the anchor cell adopts the primary fate, while lateral inhibition through *lin-12* prevents neighboring cells from also adopting this primary fate, resulting in a coordinated mix of primary and secondary fates.

In the absence of functional *lin-12*, all cells default to the secondary fate because *lin-12*-mediated lateral inhibition, which would normally limit the secondary fate to specific cells, is absent. Therefore, the Pn.p cells lack the necessary signals to diversify into both primary and tertiary fates and instead adopt the 2° fate as a default in response to cell-intrinsic and local cues, regardless of anchor cell presence. This underscores that *lin-12* signaling acts independently of *lin-3* in enforcing the lateral inhibition necessary to establish fate diversity within the Pn.p equivalence group.

Scientist 1 (curious developmental biologist). But this explanation doesn't address the tertiary fate: if lin-12 is already active in all the pnps, and doesn't require the lin-3 signal, how is the teritary fate maintained in the wildtype.

Scientist 2 (worm expert). You're right to highlight the tertiary (3°) fate, which introduces an additional layer of complexity in the *C. elegans* vulval equivalence group. In the wild-type, the tertiary fate arises when a Pn.p cell does not receive sufficient *lin-3* EGF signal from the anchor cell and is also not receiving *lin-12* activation strong enough to push it into a 2° fate. Thus, the 3° fate represents a "default" state in the absence of strong *lin-3* and *lin-12* signals, rather than an actively enforced state.

Here's a breakdown of how the fates are maintained in wild-type conditions:

1. **Primary (1°) fate**: The P6.p cell, closest to the anchor cell, receives the highest concentration of *lin-3* EGF signal. This signal promotes the primary (1°) fate in P6.p, which overrides *lin-12* activity, suppressing the secondary fate.
2. **Secondary (2°) fate**: Cells adjacent to the 1° cell (typically P5.p and P7.p) are indirectly exposed to *lin-3* but receive lateral inhibition signals from P6.p through *lin-12* signaling. This lateral inhibition from the 1° cell via *lin-12* directs them to adopt the secondary (2°) fate.
3. **Tertiary (3°) fate**: In the more distal Pn.p cells (e.g., P3.p, P4.p, and P8.p), *lin-3* signal from the anchor cell is weak or absent, and lateral inhibition through *lin-12* is minimal. Without sufficient *lin-3* activation to trigger the 1° fate or *lin-12* signaling to push the 2° fate, these cells default to the tertiary (3°) fate, which is essentially a non-vulval fate.

In a *lin-12* mutant, however, *lin-12*-mediated lateral inhibition is disrupted. Without this inhibitory signal, all cells default to a secondary (2°) fate, as there is no mechanism to

distinguish or maintain the 3° state. Therefore, in the wild type, the tertiary fate is indirectly maintained by the absence of *lin-3* and *lin-12* signals, which allows cells to stay in a quiescent, non-vulval state. This intricate interplay ensures that, in wild-type conditions, only specific cells adopt the 1° and 2° fates, while others remain in the 3° fate as a result of insufficient signaling.

Scientist 1 (curious developmental biologist). does this mean in that the secondary cells inhibit their neighbors from secondary fate in the wildtype, so either primary or tertiary are read out, depending on whether lin-3 is there?

Scientist 2 (worm expert). Yes, that's exactly right! In the wild-type, *lin-12* signaling from cells adopting the secondary (2°) fate does indeed inhibit neighboring cells from also adopting the secondary fate. This lateral inhibition mechanism helps ensure that the neighboring cells either adopt the primary (1°) or tertiary (3°) fate, depending on the presence and strength of the *lin-3* EGF signal from the anchor cell.

Here's how this works:

1. **Primary (1°) fate**: The cell closest to the anchor cell (typically P6.p) receives the strongest *lin-3* signal, which activates pathways promoting the 1° fate. This 1° cell then sends *lin-12*-mediated lateral inhibition signals to its immediate neighbors, which pushes those cells toward the secondary (2°) fate rather than the primary fate.
2. **Secondary (2°) fate**: Cells that are adjacent to the 1° cell (usually P5.p and P7.p) are indirectly influenced by *lin-3* but receive stronger lateral inhibition signals through *lin-12*. This lateral inhibition from the 1° cell's *lin-12* signaling suppresses the potential for a primary fate in these neighboring cells, thereby solidifying the secondary fate in them. These 2° cells, in turn, exert a weaker *lin-12* inhibitory influence on their own neighbors.
3. **Tertiary (3°) fate**: For cells further from the anchor cell, both the *lin-3* and *lin-12* signals are weak or absent. These distal cells (e.g., P3.p, P4.p, and P8.p) don't receive enough *lin-3* to push toward a primary fate and aren't under significant lateral inhibition from neighboring cells adopting the 2° fate. Therefore, they default to the tertiary (3°) fate, remaining in a non-vulval state.

So in summary, the 2° cells indeed inhibit their neighbors from adopting the secondary fate. This inhibition sets up a pattern in which cells close to a strong *lin-3* source (from the anchor cell) adopt primary or secondary fates, while those further away adopt tertiary fates due to the absence of both strong *lin-3* and *lin-12* signals. This tightly coordinated interplay between *lin-3* signaling and *lin-12* lateral inhibition maintains the precise pattern of 1°, 2°, and 3° fates in the Pn.p equivalence group.