The role of the *Caenorhabditis elegans* anterior *Hox* gene *ceh-13* in controlling cell migration and fusion

Ph.D. Theses

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Budapest
2010
Introduction and aims

Hox genes encode evolutionary conserved transcriptional factors which control cell fates along the anteroposterior axis during animal development. They are characterized by clustering at a single genomic site and colinearity between their domains of function and genomic map position along the body axis of the embryo. The homologous clusters of Hox genes can be found in all bilaterians and they play similar roles in controlling animal patterning. In humans, the malfunction of Hox genes can result in serious morphological abnormalities and early lethality. I have studied the role of the anterior and middle paralog Hox genes in cell migration and cell fusion as well as their interactions with each other in controlling these developmental processes using the soil nematode Caenorhabditis elegans as a genetic model organism.

The C. elegans Hox cluster consists of 6 genes which represent 4 canonical Hox homologous groups: the anterior homolog, ceh-13, the central homolog lin-39 and mab-5, and the posterior homolog genes egl-5, nob-1 and php-3. The C. elegans Hox cluster differs in some characteristics from its counterparts in other organisms in that the anterior ortholog, ceh-13 is positioned downstream of the medial-group gene lin-39 on the chromosome due to an inversion. The functional consequence of this unusual genomic organisation remains unknown. Essential embryogenesis and viability requires only the anterior paralog ceh-13 and the posterior paralog nob-1. The other members of the nematode Hox cluster function during postembryonic development, only.

The developmental function of the anterior ortholog ceh-13 is the less known among the C. elegans Hox genes. The absence of ceh-13 activity results in embryonic lethality (mutants exhibit serious morphological abnormalities), yet a small percent of the ceh-13(-) mutants are able to develop into fertile adults. Furthermore, the embryonic expression of ceh-13 is not restricted to the anterior body region but overlaps with the expression domains of other Hox paralogs, especially with that of the central homolog lin-39 and mab-5. These data raise the possibility that there might be a genetic interaction (potential functional redundancy) between ceh-13 and the middle-group Hox paralogs. However, the interaction of ceh-13 with other members of the C. elegans Hox cluster is not known. There is no information about the role of ceh-13 during postembryonic development, either.

During my PhD work I have studied the genetic interactions of ceh-13 with other
members of the *C. elegans* Hox cluster, especially with the medial homolog genes *lin-39* and *mab-5*. Furthermore, I have investigated the role of *ceh-13* in postembryonic developmental processes which are known to be controlled by *lin-39* and *mab-5*. I have also examined the sequential similarity and phylogenetic relation of the *C. elegans* Hox paralogs using bioinformatic tools.

I was also involved in a project which focused on studying the transcriptional regulation of the medial homolog Hox gene *lin-39* during hermaphrodite vulva development. Within this project, I have investigated the genetic interactions of *tra-1* (the terminal regulator of the nematode sex determination gene cascade) and the *synMuv* (*synthetic Multivulva*) genes. These genes are known to control the expression of *lin-39* which acts as the central regulator of vulva development.

**Methods**

**Expression analysis**

I used translational fusional HOX::GFP reporter constructs to study and compare the expression patterns of the *C. elegans* Hox paralogs. Transgenic strains carrying *ceh-13::gfp*, *LIN-39::GFP*, *MAB-5::GFP* or *EGL-5::GFP* constructs were kindly provided by Dr Vellai Tibor, Prof. Alex Hajnal, Prof. Cynthia Kenyon and Henrique B. Ferreira. I have constructed translational PHP-3::GFP and NOB-1::GFP reporter constructs, which were transformed by microinjection and integrated by UV irradiation on order to create stable transgenic worms. Transgenic strains were back-crossed at least eight times.

I have studied the expression of Hox genes in *comma stage* and *two fold stage* embryos and in L1-L2 stage larvae.

**Mutant analysis and rescue experiments**

For mutant analyses, mutant strains were obtained from the CGC (*Caenorhabditis elegans*
Genetics Center). To study genetic interactions between *ceh-13*, *lin-39* and *mab-5* and between *tra-1* and the *synMuv* genes, I created double and triple mutant strains and examined the mutant phenotypes exhibited by these strains. For the *Hox* rescue experiments, I introduced a translational LIN-39::GFP reporter construct (the extra copy of *lin-39*) as well as the *mab-5(e1751gf)* mutation into *ceh-13(sw1)* null mutant genetic background to study their effect on the survival and developmental abnormalities of *ceh-13(-)* mutant animals.

**RNA interference**

In some experiments, the mutant phenotypes caused by the inactivation of certain *Hox* genes made it impossible to use genetic mutations. In such cases, I used RNAi (feeding method) to reduce gene function. dsRNA specific to the mRNA of the gene of interest was produced by a bacterial strain (HT115) previously transformed by the recombinant vector pPD129.36 carrying the sequence of the gene to be inactivated. Inducible RNAi agar plates were seeded with the specific HT115 bacterial strain described above, then 3-5 L3 stage larvae were transferred onto the plates. The phenotype of F1 progeny was observed. RNAi experiments were carried out at 25 ºC.

**Cell migration and cell fusion**

To study the role of the anterior and middle paralog *Hox* genes in cell migration I used MEC-7::GFP and TAX-4::GFP reporter constructs which are expressed in different neuronal cell lineages. By crossing, I created transgenic strains carrying MEC-7::GFP or TAX-4::GFP constructs in single and double *Hox(-)* mutant genetic backgrounds. In these strains, I scored the number, position and the axongrowth of different neurons, as compared to wild type.

To examine the effect of *Hox* genes on the fusion of epidermal cell, I used a AJM-1::GFP reporter to visualize cell boundaries. I examined the number and the fusion pattern of P ectodermal blast cells, Pn.p daughters and their descendant that make up the hermafrodite vulva tissue, and also that of the V and seam cells in single and double *Hox(-)* mutant strains versus to wild-type background.
Phylogenetic analysis

For clustering the *C. elegans* Hox genes we applied the Bayesian phylogenetic method and used the 177 nucleotide long homedomain part of sequences. To calculate the tree, we used MrBayes v3.1.2 software. Aminoacid sequences of the *C. elegans* HOX proteins were also aligned by using the ClustalW software (available at the European Bioinformatics Institute's website: [http://www.ebi.ac.uk/Tools/clustalw2/index.html](http://www.ebi.ac.uk/Tools/clustalw2/index.html)).

Results

Characterization of the pleiotropic Ceh-13(-) mutant phenotype and ceh-13 expression pattern:

- *ceh-13* affects the morphogenesis of the anterior, middle and posterior body regions of the animal
- several aspects of the pleiotropic Ceh-13(-) mutant phenotype resemble to the phenotypic defects caused by the inactivation of other Hox paralogs
- in embryos and early (L1-L2) stage larvae *ceh-13* is expressed all along the anteroposterior body axis; in certain cell types ceh-13 expression persists during adulthood as well
- the embryonic expression of *ceh-13* overlaps with the expressional domain of all other members of the *C. elegans* Hox cluster; in later developmental stages there is an overlap between the expressional domains of *ceh-13*, *lin-39* and *mab-5*

Genetic interaction of *ceh-13* with *lin-39* and *mab-5*:

- simultaneous inactivation of *ceh-13* and *lin-39* or *ceh-13* and *mab-5* results in a synthetic phenotype which differs from the phenotype of the corresponding single mutants
• ceh-13(sw1)lin-39(RNSi) and lin-39(n1760)ceh-13(RNSi) animals exhibit a fully penetrant (100%) embryonic lethality, while mab-5(e1239)ceh-13(RNSi) animals a 94% penetrant embryonic lethal phenotype – these mutant phenotypes are more severe and occur with a much higher penetrance than in either of the single mutants or RNSi worms

• in ceh-13(sw1)mab-5(e1751gf) double mutant and ceh-13(sw1)LIN-39::GFP worms embryonic or early larval lethality is less penetrant and the percent of escapars which are able to develop to adulthood is much higher, as compared to ceh-13(sw1) single mutants

• extra copies of lin-39 or a gain-of-function mutation of mab-5 is able to suppress the morphological abnormalities, small body size, slow growth and movement defects caused by ceh-13 deficiency [in ceh-13(sw1)mab-5(e1751gf) double mutant and ceh-13(sw1)LIN-39::GFP animals]

The role of ceh-13 in postembryonic developmental processes controlled by lin-39 and mab-5:

• in ceh-13(-) mutants the position of the Q descendant neurons is altered both at the anterior and posterior body parts

• inactivation of ceh-13 results in the mispositioning and axonal outgrowth defects of the ALM and PLM neurons, and also of cells marked by a TAX-4::GFP neuronal fate marker

• ceh-13(sw1) mutant hermafrodites exhibit an abnormal fusion pattern of the Pn.p epidermal cells at both anterior and posterior body regions

• ceh-13(sw1) mutation causes defects in the fusion pattern and adhesion of the P ectodermal precursor cell, V and seam-cells

• in ceh-13(sw1)mab-5(e1751gf) double mutants, a gain-of-function mutation of mab-5 is able to suppress the cell positioning and cell fusion defects caused by ceh-13 deficiency

• ceh-13(sw1) mutant hermafrodites show various vulva mutant phenotypes (including Vulvaless, Protruded vulval morphology, Multivulva); an abnormal (asymmetric) development of the vulval structure can also be observed using an AJM-1::GFP
riporter

- *ceh-13(sw1)* mutation reduces the average number of induced vulval cells in *synMuv AB* double mutant genetic backgrounds, in which vulval induction is overactivated.

**Sequencial similarity and phylogeny of the *C. elegans* Hox genes:**

- multiple/pair-wise sequence alignment reveals higher similarity among the genes *ceh-13, lin-39* and *mab-5*, as well as among *egl-5, nob-1* and *php-3* than between the two groups.
- phylograms generated by clustering the *C. elegans* Hox genes show that the middle *Hox* paralogs *lin-39* and *mab-5* are more closely related to the anterior gene *ceh-13* than to the posterior paralogs *egl-5, nob-1* and *php-3*.

**Genetic interaction of *tra-1* and the *synMuv* genes in regulating vulva development:**

*tra-1* encodes the terminal transcription factor of the nematode sex-determination pathway. Vulva is a hermaphrodite-specific tissue, the development of which is negatively regulated by the (redundant) *synMuv* pathways.

- *tra-1(e1099)* mutation or *tra-1* RNSi treatment increases the average vulva number in *synMuv AB* double mutant genetic backgrounds.
- *fem-3* RNSi (the inactivation of *fem-3* causes the hyperactivation of *tra-1*) results in the reduction of the average vulva number in *synMuv A(-) B(-)* double mutants.
- inactivation of *tra-1* results in a Muv phenotype in *synMuv A(-)* mutant genetic background.

**Conclusions**

I have studied the role of the *C. elegans* anterior *Hox* gene *ceh-13* in postembryonic developmental processes as well as its genetic interaction with other members of the *C.*
elegans Hox cluster, especially with the middle Hox paralogs lin-39 and mab-5.

By phenotypic analysis, I showed that the inactivation of the anterior ortholog, ceh-13 causes morphological abnormalities not only at the anterior but also at the middle and posterior body parts of the mutant animals. These defects resemble to those observed in other Hox(-) mutants. I constructed specific translational fusion HOX::GFP reporter constructs to compare the expression patterns of C. elegans Hox paralogs. This analysis revealed that the embryonic expression of ceh-13 overlaps with the expressional domains of all the other members of the C. elegans Hox cluster. In larval stages the expression domain of ceh-13 overlaps with that of lin-39 and mab-5. These data together with the results of the Hox phenotype analysis suggest that ceh-13 affects cell fates not only at the anterior but also at the middle and posterior body parts. Thus, ceh-13 represents an exception from the rule of colinearity.

I showed that ceh-13 genetically interacts with lin-39 and mab-5 in controlling embryonic development. Furthermore, extra copies of lin-39 and a gain-of-function mutation of mab-5 are able to suppress the developmental defects caused by ceh-13 deficiency. These results suggest a partial functional redundancy between these Hox genes. LIN-39 and MAB-5 proteins are able to substitute CEH-13 function in controlling morphogenesis and viability. This may explain the presence of ceh-13(-) null mutant escapers in the population.

I also studied the role of ceh-13 in postembryonic developmental processes which are known to be controlled by lin-39 and mab-5. I found that ceh-13 influences the migration of Q neuronal descendants and the fusion of Pn.p epidermal cells with the hypodermis. ceh-13 also affects the hermaphrodite vulva development. The effect of ceh-13 on cell fate specification is obvious in both anterior and mid-posterior body regions where its functional domain overlaps with that of lin-39 and mab-5.

Data from multiple sequence alignment (using bioinformatic tools) revealed that the closest paralogs of ceh-13 within the C. elegans Hox cluster are lin-39 and mab-5.

The Hox clusters of bilaterians arose by the sequential tandem duplications of an ancient “ProtoHox” ancestor. An early gene duplication event of this „ProtoHox” gene might have given rise to the ancestors of the anterior and posterior paralogous groups. The sequence similarity and partial functional redundancy among ceh-13, lin-39 and mab-5 support our hypothesis that the middle Hox paralogs evolved from an anterior ancestor during the evolution of the Hox clusters. Together, my findings may help to understand better how C. elegans Hox genes function during development and how they may have emerged during evolution.
Publications
