

# THE GENE NETWORK DETERMINING DEVELOPMENT OF *DROSOPHILA MELANOGASTER* MECHANORECEPTORS

**Bukharina T.A.<sup>\*1</sup>, Katokhin A.V.<sup>1,2</sup>, Furman D.P.<sup>1,2</sup>**

<sup>1</sup>Institute of Cytology and Genetics, SB RAS, Novosibirsk, 630090, Russia; <sup>2</sup>Novosibirsk State University, Novosibirsk, 630090, Russia

\* Corresponding author: e-mail: bukharina@bionet.nsc.ru

**Key words:** gene network, proneural cluster, *achaete-scute complex (AS-C)*, signaling pathways

## SUMMARY\*

*Motivation:* The integration and comprehension of the experimental data on the genetic control of the development of *Drosophila melanogaster* mechanoreceptors accumulated so far require a formalized representation of the data in question in the context of a gene network for their further analysis and modeling of morphogenesis molecular mechanisms.

*Results:* This work reports reconstruction of the gene network Neurogenesis: Determination, describing determination of the precursor cells for drosophila mechanoreceptors and analysis of the information contained herein. The main functional elements with this gene network are detected, and the general principles underlying its function are considered.

*Availability:* <http://www.mgs.bionet.nsc.ru/mgs/gnw/genenet/viewer/>

## INTRODUCTION

Mechanoreceptors (external sensory organs) are elements of the *Drosophila* peripheral nervous system. By now, a considerable volume of the information related to the regulation of mechanoreceptor development has been accumulated (see, for example, reviews by Ghysen, Thomas, 2003; Lai, Orgogozo, 2004; and references therein). Certain key moments of this process are known, and individual stages of the mechanoreceptor development are modeled (Marnellos, Mjolsness, 1998; Meir *et al.*, 2002); however, understanding of the integral pattern of their development requires systematization of the available data and their representation in a unified formalized form. The GeneNet technology allows this demand to be met via a reconstruction of the gene network of mechanoreceptor morphogenesis. This forms the background for a future construction of the model reproducing adequately not only the development of various sensory organs, but also the overall peripheral nervous system. This work reports the first stage in the development of mechanoreceptors—determination of the cells of proneural clusters.

## METHODS AND ALGORITHMS

The gene network was reconstructed using the GeneNet technology, developed at the Laboratory of Theoretical Genetics with the Institute of Cytology and Genetics SB RAS (Ananko *et al.*, 2005). The gene network Neurogenesis: Determination was constructed based on annotation of 132 scientific publications and at the moment comprises the

information about 187 objects, including 31 genes, 54 proteins and protein complexes, 85 interrelations between the network components and 3 processes.

## RESULTS

Each mechanoreceptor consists of four cells, which originate from one precursor cell (SOP, Sensory Organ Precursor cell)<sup>11</sup> via two sequential divisions. Individualization of these SOP from the cells of imaginal discs is the determinative moment in the morphogenesis of mechanoreceptors; this proceeds in two stages, each having its individual genetic support.

**The first stage** involves the separation of proneural cluster—a group of cells, each displaying a potential of developing into SOP. The leading part here is assigned to the so-called proneural genes, constituents of the *achaete-scute* (*AS-C*) complex. The topology of proneural clusters is determined by interaction between the regulatory elements located within the complex with transcription factors, the proteins of the EGFR (Epidermal Growth Factor Receptor) signaling pathway, and the products of *u-shaped*, *iroquois*, and *pannier* genes (Culi *et al.*, 2001).

The binding of EGFR to the ligand SPITZ initiates a cascade of events leading to activation of *pointed* gene transcription. In turn, two POINTED isoforms further play the role of transcription activators for the proneural genes (Fig. 1a).

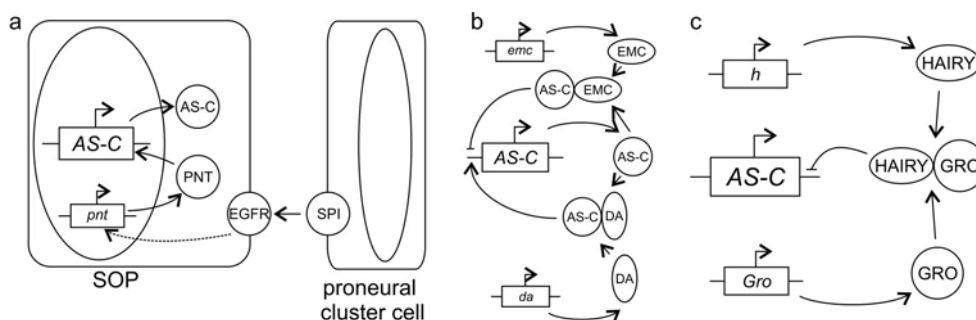


Figure 1. A scheme of the processes involved in regulation of the first stage in mechanoreceptor development: *a* – EGFR signal pathway; *b*, *c* – expression regulation of proneural genes. (*b*) The heterodimers of AS-C and DA proteins enhance the expression of proneural genes, while the heterodimers of AS-C and EMC are involved in a negative expression regulation of proneural genes. (*c*) The negative regulation of AS-C gene expressions by the repressor complex HAIRY/GRO.

Another positive regulator of *AS-C* gene expressions is the product of gene *daughterless* (*da*), a protein of bHLH type. It forms heterodimeric complexes with AS-C proteins; these heterodimers are capable of binding to E boxes (CANNTG) in the regulatory regions of the corresponding genes, thereby activating their expression (Fig. 1b). On the other hand, the heterodimers of AS-C proteins and the product of *extramacrochaete* (EMC) gene are involved in a negative regulation of *AS-C* gene expression, since EMC, an HLH-type protein, lacks the DNA-binding basic domain. Competing with DA for binding AS-C proteins, EMC decreases the transcription level of the genes of this complex. The protein product of *hairy* gene, HAIRY, is the repressor for

<sup>11</sup> **The following abbreviations are used:** *AS-C* – *achaete-scute* complex; *SOP* – *Sensory Organ Precursor cell*; *EGFR* – *Epidermal Growth Factor Receptor*; *PNT* – *pointed*; *DA* – *daughterless*; *EMC* – *extramacrochaete*; *GRO* – *groucho*; *h* – *hairy*; *E(spl)-C* – *Enhancer of split complex*; *D* – *Delta*; *N* – *Notch*; *N<sub>id</sub>* – *NOTCH intracellular domain*.

the transcription activity of *AS-C* genes. It needs cofactors, in particular, GRO, the product of gene *groucho*, to fulfill its regulatory activity (Fig. 1c).

**The second stage** is connected with a more precise SOP location within the proneural cluster, which occurs during lateral inhibition, controlled by the products of genes from Notch signaling pathway (Ghysen, Thomas, 2003). The rest cells of proneural cluster develop into epidermal cells. The mandatory condition for SOP separation within the cluster is the threshold level of AS-C proteins in this cell.

Insignificant distinctions in the expression levels of *AS-C* genes result in the different concentrations of NOTCH and DELTA proteins, thereby triggering the molecular mechanism of intercellular interactions—the lateral inhibition. Consequently, the necessary level of AS-C proteins is reached in only one cell of the proneural cluster; this is the particular cell that subsequently becomes the SOP of mechanoreceptor. In the neighbor cells, genes *AS-C* are repressed, and these cells develop into ectodermal cells.

The central part of the Notch signaling pathway is represented by NOTCH receptor, its ligand DELTA, and transcription factor SUPPRESSOR OF HAIRLESS, whose target is the genes of *Enhancer of split (E(spl)-C)* complex. The Notch signaling pathway functions in the following manner. The ligand DELTA, localized to the SOP surface, binds to the NOTCH receptor, localized to the membrane of a cell adjacent to SOP. The action of a number of proteins causes the cleavage of NOTCH intracellular domain ( $N_{id}$ ), which, once it enters the cell, forms the complex with SUPPRESSOR OF HAIRLESS. The resulting complex activates the transcription of *E(spl)-C* genes, whose products repress the transcription of their own targets, *AS-C* genes (Fig. 2).

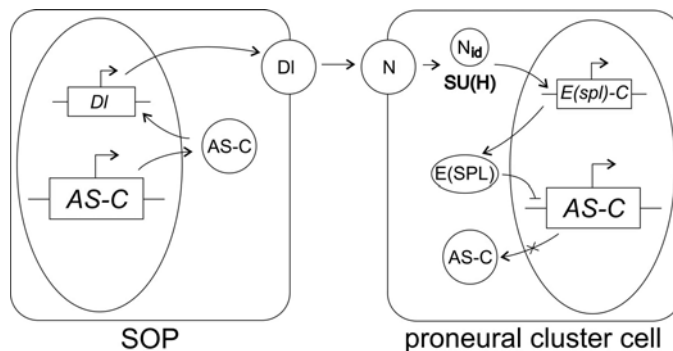


Figure 2. A scheme of expression regulation of the AS-C complex genes via the NOTCH signaling pathway.

Concurrently, the Notch signaling pathway ceases stimulating the proneural determination of cells of the cluster via the EGFR signaling pathway.

Then each SOP divides twice, giving rise to socket, shaft, and sheath cells and to bipolar neuron. Differentiation of these cells is controlled by the Notch signaling pathway and an ensemble of genes, where *numb* and *Suppressor of Deltex* are the main components (Rhyu *et al.*, 1994).

## CONCLUSIONS

Analysis of the reconstructed gene network Neurogenesis: Determination allowed the authors to detect the key components and molecular genetic processes: 1) genes *AS-C*, whose expression is necessary for determining the position of mechanoreceptor on drosophila body; 2) the mechanism enhancing the transcription of *AS-C* genes, which allows the proteins AS-C to reach the threshold concentrations via a self-regulation by DA/AS-C heterodimers; 3) the mechanisms repressing the transcription of *AS-C* genes with

involvement EMC/AS-C heterodimers and transcription factor HAIRY; and 4) the signaling pathways EGFR and Notch, providing transducing of signaling information between the cells of proneural cluster. The direction of proneural cluster cell development depends on the interactions of all described molecular mechanisms of regulation of key component – AS-C genes –transcription both inside cell and between the cells of proneural cluster.

## ACKNOWLEDGEMENTS

The work was supported by the Program “The Origin and Evolution of the Biosphere” (state contract No. 10104-34/P-18/155-270/1105-06-001/28/2006-1), Program of the Basic Research of the RAS Presidium “Molecular and Cellular Biology” (10.4), the interdisciplinary integration project on the basic research of the SB RAS no. 119, and the Program of the Federal Agency for Science and Innovations (state contract No. 02.467.11.1005, project no. IT/KP No. 5/001). The authors are grateful to G.B. Chirikova for translating this paper into English.

## REFERENCES

- Ananko E.A., Podkolodny N.L. *et al.* (2005) GeneNet in 2005. *Nucl. Acids Res.*, **33**, D425–D427.
- Culi J., Martin-Blanco E., Modolell J. (2001) The EGF receptor and N signaling pathways act antagonistically in *Drosophila* mesothorax bristle patterning. *Development*, **128**, 299–308.
- Ghysen A., Thomas R. (2003) The formation of sense organs in *Drosophila*: a logical approach. *Bioassays*, **25**, 802–807.
- Lai E.C., Orgogozo V. (2004) A hidden program in *Drosophila* peripheral neurogenesis revealed: fundamental principles underlying sensory organ diversity. *Dev. Biol.*, **269**, 1–17.
- Marnellos G., Mjolsness E. (1998) A gene network approach to modeling early neurogenesis in *Drosophila* Pac. *Symp. Biocomput.*, 30–41.
- Meir E., von Dassow G. *et al.* (2002) Robustness, flexibility, and the role of lateral inhibition in the neurogenic network. *Curr. Biol.*, **12**, 247–349.
- Rhyu M.S., Jan L.Y., Jan Y.N. (1994) Asymmetric distribution of numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. *Cell*, **76**, 477–491.