Aim 1: Engrailed, a major actor in neurodevelopment.
(*Project leader: F. Maschat*)

The knowledge of the regulatory networks involved in neuronal differentiation will help our understanding of molecular mechanisms involved in the construction of a nervous system. The identification of determining factors in neuronal differentiation using Drosophila model, might be particularly informative since mammalian cortex neurogenesis was found to be very similar to the Drosophila central nervous system, and might imply the same genes. Our study is linked to this topic, since it concerns genes that have been clearly involved in nervous system diseases in humans. Indeed, *engrailed* (*en*) gene is upstream several developmental processes and is implicated in neurodegenerative diseases, such as Parkinson or autism.

- We analysed the molecular mechanisms required by this homeoprotein to construct a mature embryonic nerve cord, in Drosophila. One of the most fascinating aspects of the nervous system development is the establishment of stereotypic neuronal networks. Our previous studies identified that the Engrailed (EN) transcription factor is playing a central role in the formation of the posterior commissures (PC), and in particular in the correct growth and navigation of the axons. We identified that the transcription factor Gooseberry-Neuro (GsbN) (ortholog of *Pax* genes) is a cofactor of EN (Colomb *et al.,* 2008), and together they orchestrate the formation of the PC (Figure 1). By their action in neural stem cells, they ensure the future growth of the axons, but also their pathfinding, though different molecular mechanisms that we attempted to dissect.

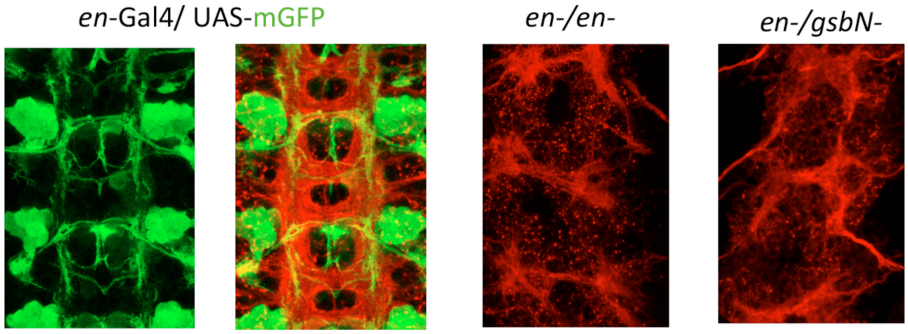

Figure 1: EN positive neurons send their axons preferentially through PC. PC are missing in *en* mutants or *en/gsbN* transheteroygotes.

- In order to better understand EN-GsbN action, and the mechanisms involved in commissure formation, we identified putative targets of EN-GsbN regulation by combining two methods: i) by transcriptional profiling of *en-/gsbn-* transheterozygous with missing PC (Figure 1), compared to wild-type; and ii) by "ChIP on chip" experiments to identify putative direct targets of EN and of GsbN. This allowed the identification of 175 putative target genes. Among 23 of these targets, 13 show a clear neural phenotype in interaction with *en* and/or *gsbn* mutations. This allowed puzzling out how, EN and GsbN are orchestrating axonal growth and guidance to form PC, by acting at 3 levels: i) on sequential control of attractive and then repulsive signaling cues to ensure the contralateral projection of the commissural axons, (ii) on translation of some mRNAs to the relevant time, (iii) on glial cells behavior that will act as guidepost cells for developing axons (*Article submitted*).

- We also identified a non-autonomous action of EN from the glia. Indeed, apart its function as a transcription factor, EN homeoprotein also presents a role as a diffusible signal, a function identified in vertebrates that we also found in Drosophila (Layalle et al., 2011).

- Therefore our working model (Figure 2) is that a secretion of EN from the glia might occur. Once in the growth cones, EN signal might activate local translation of latent mRNAs that have been activated by EN-GsbN in NBs and that are involved in axon guidance. As described in vertebrates, this might involve an interaction with EIF4E (which we verified genetically). This action of EN from the glia might explain a process whereby axonal growth and pathfinding are connected and whereby growth cones can receive information from the glia when they cross their paths.

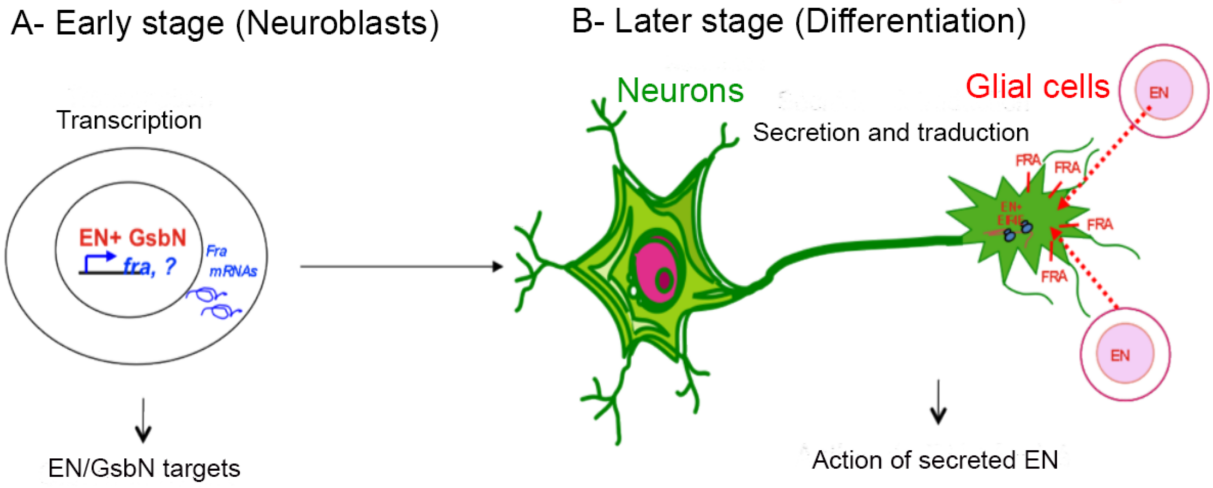

Figure 2: Model of action of EN: EN could be secreted from the glia and internalized in the growth cones, where it will interact with EIF4E to activate translation of latent mRNAs that are accumulated in the growth cones, such as *frazzled*. This mechanism could explain how axon growth and guidance are connected.

Related publications *:*

- Bonneaud, N.*, Layalle S.*, (*co-authors), Colomb, S., Jourdan, C., Severac, D., Dantec. C., Nègre, N. and Maschat, F. Control of nerve cord formation by Engrailed and Gooseberry-Neuro: a multi-step, coordinated process". *Submitted.*
- Layalle, S., Volovitch, M. Mugat, B. Bonneaud, N. Parmentier, ML., Prochiantz A., Joliot, A., Maschat, F. (2011) Engrailed homeoprotein acts as a signaling molecule in the developing fly. *Development.* 138:2315-2323. Evaluation in top 2% (Faculty of 1000: 2011. F1000.com/10832956)
- Colomb S, Joly W, Bonneaud N, Maschat F (2008) A Concerted Action of Engrailed and Gooseberry-Neuro in Neuroblast 6-4 Is Triggering the Formation of Embryonic Posterior Commissure Bundles. PLoS ONE 3(5): e2197. doi:10.1371/journal.pone.0002197
- Joly, W., Mugat, B., Maschat, F. (2007) Engrailed controls the organisation of the ventral nerve cord through frazzled regulation. *Developmental Biology.* 301, 542-554.