Summary

Embryonic stem (ES) cells are pluripotent and have the capacity to differentiate into every cell type of the adult body. Therefore, they have great potential in regenerative medicine, but also they provide an excellent tool to study embryonic development.

One of the challenges in developmental biology is to understand how neural induction occurs and how the neural tissue is patterned along the antero-posterior (A/P) body axis. Though much of our present understanding of these processes comes from experiments in amphibia, more and more evidence suggests that two steps are important. First, neural induction occurs by default in the primitive ectoderm and the newly formed neural tissue is anterior in character. Hereafter, a series of caudalizing factors can posteriorize this anterior neurectoderm to obtain all regional neural cell types of the A/P axis.

In this thesis mouse ES cells were used to study the processes of neural induction and neural patterning. Neural induction was shown to occur spontaneously in serum free media, without the addition of inductive factors, suggesting that neural induction occurs by default in the mouse as well. One differentiation system using mono-adherent cultures in the specified serum free medium N2B27 was characterized further to analyze the different steps of neural differentiation.

To study the earliest phases of neural patterning, a gain-of-function/loss-of-function approach using mouse ES cells and a combination of reverse transcription and Real-Time PCR was designed. In this system, it was shown that Fgf2 has the strongest caudalizing potential of all Fgfs tested, reducing the expression level of forebrain markers such as *Foxg1* (also known as *Bf1*), while elevating the expression of posterior markers such as *Hoxc9*. Furthermore, also Bmp4 and Wnt3a, but not Wnt1, were shown to caudalize the neurectodermal cells. The effect of the antagonists of these factors was also examined and though Dkk1 and Noggin clearly have an effect that opposes that of Wnt3a and Bmp4 respectively on most of the caudal markers analyzed, no clear upregulation of the anterior markers could be observed. The patterning effect of SU5402, an Fgf receptor inhibitor, was rather limited and only a small upregulation of the anterior markers was

detectable. Furthermore, it was shown that Activin A and Nodal do not have a caudalizing effect on neural differentiated ES cells, while the inhibition of the Activin type I receptors, clearly induced anteriorization of the cells. Based on these findings, a novel factor with a strong encephalic regionalization potential was identified: Gdf11. This is the first time that this factor is shown to be implicated in the early regionalization of the brain.

All together, our data confirms that also in the mouse, two steps are involved in neural patterning: induction of anterior neural tissue, which occurs by default, and posteriorization to obtain all regional cell fates along the antero-posterior axis. We showed that while Fgf4, Fgf8 and Wnt1 have no strong patterning effect, Fgf2, Wnt3a and Bmp4 are strong posteriorizing factors. Furthermore Gdf11 was identified to have strong patterning effect, causing a regionalization from the level of the forebrain to the level of the hindbrain.