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**The Neural Border: Induction, Specification and Maturation
of the territory that generates Neural Crest cells.**

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Key words.

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Abstract

The neural crest is induced at the edge between the neural plate and the nonneural ectoderm, in an area called the neural (plate) border, during gastrulation and neurulation. In recent years, many studies have explored how this domain is patterned, and how the neural crest is induced within this territory, that also participates to the prospective dorsal neural tube, the dorsalmost nonneural ectoderm, as well as placode derivatives in the anterior area. This review highlights the tissue interactions, the cell-cell signaling and the molecular mechanisms involved in this dynamic spatiotemporal patterning, resulting in the induction of the premigratory neural crest. Collectively, these studies allow building a complex neural border and early neural crest gene regulatory network, mostly composed by transcriptional regulations but also, more recently, including novel signaling interactions.

Introduction

During animal development, the ectoderm, i.e. the outer embryonic germ layer, progressively undergoes a refined regional patterning essential for the development of many cells and organs. In the dorsalmost aspect of the gastrula and neurula-stage vertebrate embryo, the ectoderm forms the neural plate, which generates the neural tube and ultimately the central nervous system. More laterally and ventrally, the nonneural ectoderm forms the epidermis and the associated glands. At an early stage of ectoderm patterning, the frontier between the neural plate ectoderm (NP) and the nonneural ectoderm (NNE) is not a sharp line but rather a broad area called the neural (plate) border (NB). Definitive cell fates are not yet segregated in this area and the NB domain contributes to the dorsal neural tube, the neural crest (NC), the NNE and, anteriorly, the placode progenitors. This region has been defined by lineage tracing experiments and by the expression of a combination of marker genes.

Many vertebrate-specific anatomical features derive from neural border progenitors through the development of neural crest cells and placodes. The NC gives rise to most neurons and glia of the peripheral nervous system, to a large part of the craniofacial skeleton and mesenchyme, to the melanocytes and other pigment cells, to the outflow tract of the heart, and to adrenal medulla cells. Placodes form the olfactory epithelium, the inner ear, some neurons of the cranial ganglia, and the lens of the eye. Therefore, during their early development, the NC and placode cells share the ability to migrate and generate multiple cell types including sensory neurons and glial cells (for an extensive comparison between these two populations see Baker and Bronner-Fraser, 2001; Schlosser, 2008). Understanding the multi-step Gene Regulatory Network (GRN) that governs NB and early NC development is critical for understanding the molecular mechanisms underlying human diseases, notably affecting pigmentation, craniofacial development, peripheral nervous system and heart development. This review presents the current understanding on NB development, including NB induction and specification during early development, and the induction and segregation of the NC cell fate.

Characterization of the neural-nonneural ectoderm border.

At the beginning of gastrulation, i.e. at st. 10.5 in *Xenopus laevis* (Nieuwkoop and Faber, 1994), or at st. 3-4HH in chick embryos (Hamburger and Hamilton, 1951), the NB is not outlined by visible morphological boundaries. Therefore, it has been defined by cell labeling experiments and by reference to a combination of gene expression patterns. Early cell tracing assays using lipophilic dyes (such as diI, diO) were done in gastrula-stage chick embryos at the prospective level of the otic vesicle, in order to mark small groups of neighboring cells: these experiments demonstrate that cells of the NB region contribute to epidermis, placode, neural crest and neural tube (Streit et al., 2002). Similar dye labeling was combined with time-lapse imaging in the NB area of st. 4HH chick embryos. This shows that the precursors of NC and of dorsal neural tube are intermingled early on and become segregated around the 2-somites stage (st. 7HH) in the rostral region (Ezin et al., 2009). This segregation involves extensive morphogenetic movements including convergent extension and cell intermixing (Ezin et al., 2009). NC precursors convergent extension movements are also observed in zebrafish embryos (Hochgreb-Hägele and Bronner-Fraser, 2013).

The observation that multiple cell fates are obtained by labeling small groups of early NB cells raises the question whether the NB is composed of multipotent precursors whose fate will be progressively restricted during development, or whether it is already formed early on by a salt-and-pepper array of cells specified towards distinct fates. At early gastrulation stage, the NB emerges as a separate territory that can be defined by its expression of *pax3/7* in frog, chick and lamprey (Monsoro-Burq et al., 2005; Basch et al., 2006; Nikitina et al., 2008). Several other less restricted markers such as *tfap2a*, *msx1/2*, *hes4* and *zic1* (Suzuki et al., 1997; Mizuseki et al., 1998; Tribulo et al., 2003; Monsoro-Burq et al., 2005; Hong et al., 2007; Luo et al., 2003; Nichane et al., 2008; De Crozé et al., 2011) overlap in this domain (Figure 1). Supplemental Table 1 describes the most studied NB markers, their expression patterns and their regulators, in the main vertebrate species that have been studied, namely frog, chick and fish embryos. Some NC and placode markers are indicated as well.

Recently, co-immunostaining for neural plate (Sox2), NB (Pax7), NB and NNE (Tfap2a, Msx1/2), and placode progenitors (Six1) has shown that these markers extensively overlap in individual cells at the NB of early gastrula stage chick embryos (Roellig et al., 2017). This co-expression of multiple markers is maintained later on, during neurulation, until neural tube closure. Assuming that each of these markers is a faithful predictor of the distinct cell fates, this study indicates that the NB is not divided into sharply defined fate-restricted regions prior to neural tube closure. However, there is a clear predominance of neural plate-NC progenitors (Sox2+/Pax7+/Six1-) over neural plate-PPE progenitors (Sox2+/Six1+/Pax7-), suggesting a preferential emergence of NC among neural plate progenitors. A stochastic variation in gene expressions in NB cells population before their final commitment cannot be excluded, as this was shown for erythroid progenitors (Richard et al., 2016). Overall, although the NB could be interpreted as a broad competence domain for dorsal neural tube, NNE, NC and placodes, it presents some preferential association of cell fates which are

reminiscent of the binary competence model proposed by Schlosser in which the NC and the placodes are induced separately, the NC on the neural side of the NB and placode cells on the nonneural ectodermal side of the NB (Schlosser, 2008; Pieper et al., 2012).

Altogether, these studies have helped refining the notion of neural border in the last 15 years, and paved the way for functional studies aimed at defining the roles of each candidate molecule, either secreted signals or transcription regulators, during NB development. The transcriptional factors (TFs) essential for the establishment of the NB, hence also for further for NC and placode formation, are named NB specifiers (or NB-TFs), while the ones involved in NC development, but not earlier NB formation, are named NC specifiers (Meulemans and Bronner-Fraser, 2004).

Tissues interactions involved in neural border induction.

As many other tissues in the embryo, the NB develops under the control of a combination of signaling molecules secreted from the adjacent tissues. Many studies done between 1990 and 2005 have identified signals impacting NC and placode induction. They can now be reinterpreted more precisely, according to their effect on either NB or NC induction, using the developmental stages and the markers that were analyzed. During gastrulation, the prospective NB territory has three neighboring tissues: the nonneural ectoderm, the neural plate and the underlying mesoderm. In the plane of the ectoderm, the experimental juxtaposition of NNE and NP tissues induces NC marker expression (*snail2*), followed by NC cell formation, produced mainly by NP, in axolotl, frog or chick (Moury et al., 1990; Selleck and Bronner-Fraser, 1995; Mancilla and Mayor, 1996; Schlosser, 2006, Pieper et al., 2012). Planar signals in the ectoderm are accompanied by vertical signaling from the dorsolateral marginal zone (DLMZ), i.e. the early precursor of paraxial and intermediate mesoderm. These signals induce NB and NC markers in the frog pluripotent ectodermal cells of the blastocoel roof, also named "animal caps" (Bang et al., 1999; Bonstein et al, 1998; Monsoro-Burq et al., 2003). *In vivo*, the DLMZ underlies the NB during its specification, at early gastrula stage, and is necessary for NB formation as its experimental ablation precludes the development of NB and NC (Bonstein et al., 1998; Marchant et al., 1998; Steventon et al., 2009). The secreted signals involved in those tissue interactions seem conserved between different vertebrate species since the chick paraxial mesoderm induces *msx1* and *pax3* in frog ectoderm (Bang et al., 1999).

Several signaling pathways cooperate to induce the neural border.

Many experimental studies using *in vitro* tissue recombination, *in vivo* grafting, pharmacological assays, gain and loss of function assays and genetic epistasis analysis have established that a cooperation of BMP, FGF and Wnt signals is involved in NB specification. Other signals, such as Notch signaling and Sonic Hedgehog (Shh) signaling have not yet been implicated at these early stages of ectoderm patterning.

a. The BMP signaling gradient across the ectoderm positions the neural border lateral to the neural plate.

During early gastrulation, BMP antagonists, such as chordin, noggin, cerberus and follistatin, induce the neural plate from the dorsal ectoderm (Harland, 2000; Pla and Monsoro-Burq, 2015). While BMPs are secreted by the ventral non-neural ectoderm and ventral mesoderm, BMP antagonists diffuse from the involuting dorsal mesoderm, thus generating a morphogenetic gradient of BMP activity in the ectoderm: high BMP signaling in the ventral ectoderm promotes the epidermal fate and represses the neural fate while low BMP activity dorsally allows neural specification (Smith et al., 1993; Sasai et al., 1994; Wilson and Hemmati-Brivanlou, 1995). The ectoderm region with intermediate BMP activity, assessed by moderate levels of pSmad1/5/8 or chordin immunostaining, in chick, fish or frog embryos, corresponds to the NB position (Faure et al., 2002; Wu et al., 2011; Plouhinec et al., 2014). This balanced BMP activity is essential for NB specification. *In vivo* chordin depletion abolishes NB (*zic1*) and NC (*snail2*) specifiers' upregulation (Marchal et al., 2009; Steventon et al., 2009). Conversely, *zic1*, which is expressed both in NB and NP, is upregulated by BMP inhibition in frog embryos (Mizuseki et al., 1998; Marchal et al., 2009; Hong et al., 2007), while intermediate levels of BMP signaling activate *zic3*, *pax3*, *msx1* and *hes4* in frog and zebrafish, resulting in positioning the NB along the dorsal-ventral body axis (Suzuki et al., 1997; Tribulo et al., 2003; Brugger et al., 2004; Nichane et al., 2008a; Garnett et al., 2012). Downstream of BMP signaling, *dlx3*, activated by high BMP levels, and *dlx5*, triggered by attenuated BMP levels, cooperate and further fine-tune BMP signaling (Luo et al., 2001; Woda et al., 2003). This could be mediated by a balance between Dlx and Msx opposing activities as shown in zebrafish embryos (Phillips et al., 2006). However, the experimental modulation of BMP signaling, in animal cap ectoderm explants, is not sufficient to induce NB and NC markers with the high expression levels detected *in vivo* (Saint-Jeannet et al., 1997; LaBonne and Bronner-Fraser, 1998; Villanueva et al., 2002). This observation has triggered the search for the additional signals cooperating with BMPs in whole embryos.

b. FGF and WNT signaling cooperate with intermediate BMP levels to promote NB formation.

Before gastrulation, FGF signaling is required for neural induction in frog and chick embryos as it sensitizes the NNE to respond to neural induction, using both BMP-dependent and BMP-independent mechanisms (Streit et al., 2000; Wilson et al., 2000; Delaune et al., 2005; Marchal et al., 2009). At the time of NB specification, several FGF ligands, including FGF3, FGF4, FGF8 are expressed in the DLMZ (Christen and Slack, 1997; Monsoro-Burq et al., 2003). FGF signaling is necessary for the induction of most NB specifiers *in vivo*, namely *pax3*, *hes4*, *tfap2a* and *msx1*, and activates their expression in isolated animal cap ectoderm without further neuralization by BMP antagonists or mesoderm induction (Wilson et al., 2000; Monsoro-Burq et al., 2003; Monsoro-Burq et al., 2005; Nichane et al. 2008, De Croz   et al., 2011; Garnett et al., 2012, Stuhlmiller and Garcia-Castro, 2012).

Similarly to FGFs, Wnt signaling was shown to directly neuralize the NNE by inhibiting BMP4 expression in animal caps assays (Baker et al., 1999). During anterior-posterior axis patterning, both FGFs and Wnts, especially Wnt3a, act in the neural tube and paraxial mesoderm, promoting posterior patterning and repressing anterior fates (McGrew et al., 1995; Glinka et al., 1998; Kiecker and Niehrs, 2001). Wnt3a and Wnt8 are expressed in the DLMZ and many different Wnt ligands are present in the NNE (Garriock et al., 2007). The canonical Wnt- β catenin signaling is necessary for NB and NC induction in frog, fish and chick embryos *in vivo*. This activity is independent from Wnt general posteriorizing function (Chang and Hemmati-Brivanlou 1997; Saint-Jeannet et al., 1997; LaBonne and Bronner-Fraser, 1998; Monsoro-Burq et al., 2005; Shimizu et al., 2005, Wu and Klein 2005; Hong et al., 2008; Elkouby et al., 2010; Garnett et al., 2012). In further agreement with the need for active canonical Wnt signals for NB formation, AWP1, which stabilizes β catenin, is crucial for NB and NC induction (Seo et al., 2013). Wnt signaling in the NB ectoderm activates the expression of several NB-TFs, such as *tfap2a*, *gbx2*, *pax3* and *msx1* as direct targets, as well as novel players such as apolipoprotein C-I that is also necessary for NB specification (Li et al., 2009; De Crozé et al., 2011, Yokota et al., 2017). The comparison of β catenin direct targets and NB transcriptome signature in frog embryos indicates a number of novel candidates, potentially regulated by Wnt- β catenin signaling in the NB (Kjolby et al., 2016, Plouhinec et al., 2017).

c. A complex integration of BMP, Wnt and FGF signals regulates the onset of the various neural border specifiers.

Altogether, the studies described above indicate that a combination of three main signaling pathways, BMP, Wnt and FGF, is required to induce the NB. It had long remained debated whether FGF signals were acting directly on ectoderm cells to trigger NB induction, or whether FGF signals acted indirectly, by promoting Wnt signaling from the underlying mesoderm, which would in turn activate NB/NC formation in the ectoderm. Elegant work in zebrafish embryos demonstrates that *pax3a* activation requires all three signals directly, for a response in the ectoderm (Garnett et al., 2012). In zebrafish, *zic3* plays a similar role as *zic1* in other models. In contrast to *pax3a* regulation, *zic3* activation at the NB is achieved by combining attenuation of BMP activity and Wnt signaling. In the case of *zic3*, FGF signals are redundant with Wnt activity. The action of redundant signals could contribute to provide robustness in the NB induction network (Garnett et al., 2012). In frog embryos, other NB specifiers such as *tfap2a* and *msx1* are both activated by FGF signaling and are direct targets of the Wnt- β catenin pathway (de Crozé et al., 2011). While *tfap2a* is activated by FGF in a β catenin-dependent manner, it remains unclear if both inputs are required independently for *msx1* activation (de Crozé et al., 2011).

Additionally, as shown for neural induction, it is likely that the NB cell response to the three patterning pathways involves both direct transcriptional integration on target genes regulatory

elements, and cross-modulation at the level of the intracellular transduction pathways, by posttranslational modifications. Such signaling cross-talk has been demonstrated for Wnt- β catenin and BMP signaling at the level of GSK3 and Smads; for Lef1/ β catenin and Smad4/Smad1; for BMP and FGF by the negative regulation of Smads by MAPK acting downstream of FGF receptors (Hussein et al., 2003; Fuentealba et al., 2007; Stuhlmiller and Garcia-Castro, 2012). Moreover, each pathway may further modulate the activity of another signaling pathway: for example, the Wnt/PCP pathway receptor Ror2 upregulates the expression of the BMP ligand Gdf6 which in turn is essential for NB/NC formation (Reichert et al., 2013; Schille et al., 2016a). Finally, in addition to the three most studied pathways, retinoic acid (RA) signaling is also implicated in early NC specification in frog gastrulas, likely acting as a general neural posteriorizing pathway (Villanueva et al., 2002).

Feed-forward interactions between the NB-TFs stabilize the neural border domain.

Epistasis experiments shed light on the genetic interactions triggering NB establishment, mainly focusing on events downstream of canonical Wnt signaling in frog embryos. At early gastrulation stage, the effects of Wnts are primarily mediated by Tfap2a and Gbx2. Both are directly activated by Wnt- β catenin signaling and turn on *pax3* and *msx1* expression *in vivo* and in pluripotent ectoderm neuralized by BMP antagonists (Li et al., 2009; de Croz  et al., 2011). Interestingly, Tfap2 directly activates *pax3* and is sufficient to compensate the inhibition of Wnt signaling as Tfap2 gain of function restores *pax3* expression after β catenin depletion *in vivo* (de Croz  et al., 2011). Subsequently, an active feed-forward transcriptional regulatory loop is set-up between the NB-TFs, as they cross-regulate each other's expression during neurulation (Figure 2B). *In vivo*, the depletion of either Tfap2a, Gbx2, Msx1, Pax3, Hes4, or Zic1 alters the expression of all other NB specifiers, resulting in deficient NC induction (Monsoro-Burq et al., 2005; Sato et al., 2005; Nichane et al., 2008a; Li et al., 2009; de Croz  et al., 2011). These cross-regulations can be interpreted as the establishment of a stabilization loop locking up the NB fate upstream of NC formation. Altogether, these experiments done in frog embryos allow building the first steps of the NB-GRN (Figure 2B).

Similar experiments conducted in lamprey, fish and chick early embryos demonstrate that the NB-GRN is largely conserved across vertebrate evolution, although in teleost fish embryos, because of the additional genome duplication, double loss of functions may be needed to reproduce the early phenotypes observed in frog embryos (Basch et al., 2006; Li et al., 2007; Nikitina et al., 2008). However, a few species-specific differences have been noted, involving the replacement of a given gene by a paralog gene ("paralog switch"). This is the case for *pax3* and *pax7*, two phylogenetically close paralogs of the Pax gene family (McCauley and Bronner-Fraser, 2002; Monsoro-Burq, 2015). *Pax3* is the most spatially restricted early NB marker in frog embryos, whereas *pax7* is the early player in chick gastrulas, indicating that their expression and function has been swapped in the ectoderm generating the NB during evolution, although both genes are co-expressed in the dorsal neural tube in

both species later on. Moreover, in *Xenopus laevis*, Pax7 is expressed in the paraxial mesoderm, and is involved in NB specification by the DLMZ, while Pax3 activity is required in the prospective NB ectoderm (Maczkowiak et al., 2010). In this case, the two paralog proteins are no longer functionally equivalent since Pax3 and Pax7 cannot compensate for each other's loss of function *in vivo* (Maczkowiak et al., 2010). This differs from the observations in mouse embryos where *pax3* mutants exhibit defects in cardiac and trunk NC, while cranial NC is normal, and where Pax7 can substitute for Pax3 function in trunk NC development (Relaix et al., 2004).

As a whole however, similar regulations establish the early NB-GRN during gastrulation of vertebrate embryos. In turn, the NB-TFs control the onset of neural crest induction in this territory, at the end of gastrulation and during the neural plate stage.

The neural crest is specified within the neural border during late gastrulation and neural plate stage.

The neural border cell state is transitory since the cells in this territory will be further specified into NC and placodes, as well as NNE and dorsal neural tube. In frog neural fold stage neurulas, NC and placode precursors are spatially restricted, with placodes arising anteriorly and laterally to the NC (Glavic et al., 2004). From stage 13 onwards (i.e. after the end of gastrulation), in *Xenopus laevis* embryos, the competence to become NC is restricted to the neural tissue, whereas the competence to become placodes is restricted to the NNE (Pieper et al., 2012). A similar restriction for the placode fate is observed in late zebrafish gastrulas (Kwon et al., 2010). **In chick embryos, there is a preferential association of NC markers with neural plate markers although co-expressions of combinations of neural plate, NC, placode and NNE markers is maintained in a small subset of cells late in neurulation (Roellig et al., 2017).**

NC specification occurs with activation of a new set of genes that are present neither in neural plate, nor in nonneural ectoderm, placodes and hatching gland: the NC-TFs, namely *snail2*, *foxd3* and SoxE family genes, *Sox8*, *Sox9* and *Sox10*. In frog embryos, a stereotyped sequential activation of these early NC markers is observed: *sox9* is expressed slightly earlier (from stage 11 onwards) than *snail2* and *foxd3*, which are activated earlier (from st. 12-12.5) than *Sox 10* (from st. 13/14 onwards) (Vallin et al., 2001; Spokony et al., 2002; Honore et al., 2003; Aoki et al., 2003; Figure 1B). This indicates that NC specification is a multistep process, the early induction step (induction of *sox9*, *snail2* and *foxd3*) being followed by a maturation step with the induction of late NC markers such as *sox10* and *twist1*, as well as other cellular events preparing EMT such as cadherin expression switch between epithelial cadherin (*ecadherin* or *cdh1*) and *ncadherin* or *cadherin11*. Ultimately, NC specification and maturation are followed by EMT of NC cells and migration towards various locations in the embryo, and their differentiation into multiple derivatives. Most of the aforementioned genes are conserved and needed for NC specification and development in the main vertebrate models analyzed so far (Suppl. Table 1). The ectopic expression of these genes induces ectopic NC induction

from neural progenitors in the neural tube (Dottori et al., 2001; Cheung et al., 2003; Cheung et al., 2005). However, *Snail* genes seem dispensable for NC development in mouse embryos (Murray and Gridley, 2006).

a. The NB-TFs cooperate to induce the premigratory neural crest.

In vivo, a large number of genes and secreted factors are required for the onset of the NC program, the induction phase, at the neural plate stage. In the last few years, it has become important to identify the genes that were not only essential for NC induction *in vivo*, but also that were sufficient to activate NC ectopically, outside of the neural border context. These factors would thus trigger NC development in the targeted cells, potentially overriding the original fate of the cells, independently from the cooperation with other factors present at the NB. So far, most regulators, such as the NB specifiers described above, taken individually, are important to activate the NC-GRN in the context of the neural border but fail to do so in ectopic locations *in vivo* or in isolated pluripotent ectoderm *in vitro* (e.g. in animal cap assay): for example, *Msx1* is necessary for *snail2* and *foxd3* expression *in vivo*, but *Msx* ectopic expression enlarges the NC territory without creating a novel separate NC area either in the neural plate or in the nonneural ectoderm (Tribulo et al., 2003). However, in combination with secreted neural and neural border inducers such as the BMP antagonists *noggin* and *chordin* or such as Wnts, the NB specifiers broadly activate the NC program ectopically, indicating that they cooperate with other factors also activated at the NB. To identify and understand these cooperations and their implications for the architecture of NB/NC-GRN, a core regulatory module acting as a minimal NC-inducing genetic program was searched for among NB specifiers. When compared to the activity of 10 candidate NB specifier pairs, the co-expression of the two NB-TF *Pax3* and *Zic1* was identified as a minimal and efficient NC-inducing program (Monsoro-Burq et al., 2005; Sato et al., 2005; Hong and Saint-Jeannet 2007; Milet et al., 2013).

During neurulation, *Pax3* and *Zic1* expression patterns become more restricted to the NB and the neural folds compared to their patterns during NB specification in gastrula-stage embryos (Hong and Saint-Jeannet, 2007). While both *Pax3* and *Zic1* are required for NC induction *in vivo*, an appropriately balanced relative activity of these two factors is critical to obtain the NC fate: higher *Pax3* activity orients cells towards a hatching gland fate and inhibits preplacodal ectoderm (PPE) development, whereas higher *Zic1* levels triggers a PPE destiny. *Zic1 acts in a non-cell autonomous manner, by the intermediate of retinoic acid signaling* (Hong and Saint-Jeannet, 2007; Jaurena et al., 2015). Furthermore, in ectopic induction assays, *Pax3* and *Zic1* cooperation is sufficient to fully specify NC cells from the induction step to EMT, migration and differentiation into multiple derivatives, either from nonneural ventral ectoderm or from vegetal blastomeres normally fated to form endoderm *in vivo*, or from pluripotent ectoderm *in vitro* (Milet et al., 2013). Chick extraembryonic ectoderm also initiates expression of a few NC specifiers' genes upon activation of *Pax3* and *Zic1* (Milet et al., 2013).

Because the frog pluripotent ectoderm cells rely on their yolk content for growth and proliferation, the animal cap assay is done in a growth factor-free saline medium. Since Pax3 and Zic1 trigger a complete NC developmental program in those conditions, this suggests that all the needed molecular and cellular interactions are elicited in the context of this isolated pluripotent ectoderm tissue. This includes downstream secondary signaling, such as for example Wnt signals or Tfp2a activity which are further required downstream of Pax3 and Zic1 to induce NC specifiers (Monsoro-Burq et al., 2005, Sato et al., 2005; De Crozé et al., 2011, Milet et al., 2013). Similar activities have been described in chick embryo trunk NC (Simoes-Costa et al., 2015). The activation of this novel minimal program is now largely used as a standard assay to activate the NC-GRN and further study NC development downstream of NB formation (Gutkovich et al., 2010; Buitrago-Delgado et al., 2015; Geary and LaBonne, 2018). We propose that Pax3 and Zic1 cooperation acts as a critical bottleneck within the larger and more complex current NB/NC-GRN described elsewhere (e.g. Simoes-Costa and Bronner, 2015). We thus describe the position of Pax3 and Zic1 by a novel "Hourglass model of NC induction" presented in Figure 2A. In this model, the early NB-GRN acts as an upstream feed-forward positive loop, activating a balanced expression of Pax3 and Zic1, which in turn cooperate and trigger the downstream NC-GRN. With this model, we suggest that there are critical bottlenecks within the current NB/NC-GRN and that the activation of both Pax3 and Zic1 in the NB territory is one of them. The "Hourglass model of NC induction" is based on the properties of Pax3 and Zic1, the best characterized combination of only two transcription factors sufficient to activate the downstream NC-GRN and functional NC migration and differentiation.

In this context of isolated pluripotent ectoderm induced into NC, many Pax3 and Zic1 direct targets have been characterized. This includes all the genes defining the usual premigratory NC signature such as *foxd3*, *snail2*, *sox8*, *sox9* and *twist1* (Figure 2C, Bae et al., 2014; Plouhinec et al., 2014). The sequential dynamics of activation of each gene mirrors the *in vivo* situation, indicating that the NC-GRN triggered *in vitro* is likely very close to the *in vivo* one (Milet et al., 2013). Pax3 and Zic1 activities are not redundant in the NC-GRN activation. For example, Meis3 is a homeobox transcription factor involved in NC and hindbrain primary neurons development. *Meis3* expression is activated by Pax3 and Zic1, but Meis3 does not relay Pax3 or Zic1 activity symmetrically: the loss of NC caused by Zic1 knock-down can be rescued by Meis3, but not the one caused by Pax3 knock-down, showing that this gene is the main effector among Zic1 targets but not among Pax3 targets (Gutkovich et al., 2010). Another example is the differential activation of cell-cell adhesion molecules regulating EMT: while Pax3 activates *ncadherin* expression, Zic1 is essential for transcriptional repression of *ecadherin/cdh1* (Milet et al., 2013).

Some targets of Pax3 and Zic1 are of particular interest. Znf703 (or Nlz1), a zinc finger transcription factor, recently characterized as a downstream target of Pax3 and Zic1, acts as an activator of *snai2* and *sox10* and it restricts *sox2* domain of expression (Hong et al., 2017). The tyrosine kinase receptor Pdgfra (Plouhinec et al., 2014; Bae et al., 2014) has been recently implicated

in NC migration (Bahm et al., 2017). Pax3 and Zic1 also activate *anosmin1* expression an extracellular matrix protein involved in the specification of both NC and placode development (Bae et al., 2018). In chick embryos, the Anosmin1 binds in FGF, BMP and Wnt ligands, thus potentially modulating signaling pathways critical for further NC development (Endo et al., 2012). Importantly, Pax3 and Zic1 cooperation directly activates a whole "EMT module" defined by a group of transcription factors commonly named EMT-TFs, *snai2*, *ets1*, and *twist1*. This situation contrasts with the alternative possible mechanism of a sequential and indirect activation of factors important for the EMT. Accordingly, animal cap ectoderm induced into NC readily undergoes EMT and migration *in vitro*, as well as *in vivo* albeit migration occurs with a slower velocity (Figure 2B; Milet et al., 2013; Plouhinec et al., 2014). Finally, it remains unknown if the synergistic effects of Pax3 and Zic1 on some of their targets are mediated by direct simultaneous interactions on target genes regulatory elements, such as those described during myogenesis (Himeda et al., 2013).

Pax3 and Zic1 cooperate with other factors present in the pluripotent animal cap ectoderm, such as Wnt signals and Tfap2a: Tfap2a is required downstream Pax3/Zic1 activity and directly binds *sox10* regulatory sequences in zebrafish embryos (De Crozé et al., 2011; Van Otterloo, 2012). The full complement of factors cooperating with these two "core" NC inducers remains to be defined. In chick embryos, Wnt signaling is mediated by its downstream target Axud1, which is required for *foxd3*, *sox9*, *sox10* and *ets1* upregulation. Axud1 directly interacts with Pax7 and Msx1 and controls their binding to the enhancers of NC specific genes (Simoes-Costa et al., 2015). Other NB-TFs are involved in the activation of subsets of NC specifiers, such as cMyb controlling *snail2* and *ets1* expression directly but not *sox9* and *foxd3* activation (Betancur et al., 2014). In sum, despite the impressive number of novel regulations described in the last few years, the regulatory and epistasis interactions between all these NB-TFs and NC-TFs remain largely to explore, to provide a completely validated network.

Finally, as anticipated for a complex process involving the spatial and temporal coordination of multiple gene activations and repressions, the epigenetic chromatin state is tightly regulated during NC specification. The accessibility of transcription factors to their target regulatory sequences is precisely controlled to regulate the timing of the NC genetic cascade. JMJD2A, a histone lysine demethylase, controls *sox10* de-repression by H3K9me3 demethylation (Strobl-Mazzulla et al., 2010). NSD3, a lysine methyltransferase upregulated just before chick NC specification, is required for *sox10* and *snail2* upregulation (Jacques-Fricke et al., 2014). NSD3 specifically dimethylates H3K36 in *sox10* regulatory sequences, but not in *snail2* regulatory elements. Chd7, a chromatin remodeler of the SWI-SNF superfamily, activates *snail2* and *sox9* expression in zebrafish and human NC, but Chd7 is not needed for *foxd3* expression in zebrafish embryos (Bajpai et al., 2010; Asad et al., 2016). Together, these studies indicate that the activation of each NC specifier gene requires a specific combination of epigenetic remodellers, as observed for their regulation by specific groups of NB-TFs.

b. Specification and maintenance of premigratory neural crest by secreted signals.

When NB fragments are isolated from *Xenopus laevis* neurulas shortly after the initial NC induction, and grown *in vitro*, they do not maintain the expression of the NC specifier genes: this indicates that the NC fate is not yet determined and that non-cell-autonomous maintenance mechanisms are acting during this early stage (Steventon et al., 2009). In animal cap ectoderm induced to NC by Pax3 and Zic1, cell dissociation abrogates NC induction (Monsoro-Burq et al., 2005). This indicates that non-cell-autonomous paracrine signaling is important in this minimal NC induction assay. Similarly to the regulations described during NB induction, secreted signals could originate from the paraxial mesoderm or the adjacent neural and NNE ectoderm, or could be produced by the NB itself for a paracrine mechanism. Accordingly, a continuous Wnt activity is important for NC induction and maintenance, downstream of NB specifiers in frog and chick, in particular for the direct activation of *snail2* and *tfap2a* (Vallin et al., 2001; Sasai et al., 2001; Garcia-Castro et al., 2002; ; Lewis et al., 2004; Monsoro-Burq et al., 2005; Sato et al., 2005; Carmona-Fontaine et al., 2007; de Crozé et al., 2011; Yokota et al., 2017). In the case of FGF signaling, Meis3, acting downstream of Pax3 and Zic1, activates *fgf3* and *fgf8* expression, thus further promoting NC induction (Aamar and Frank, 2004; Elkouby et al., 2010; Gutkovitch et al., 2010). Such positive feedback loops are likely to occur in multiple instances during NC formation: for example Sox9 induces *wnt3a* expression in chick embryos (Cheung et al., 2003).

The precise spatial and temporal modulation of signaling pathways' activity participates in NC maturation prior to EMT. For instance, in frog, the Wnt/PCP pathway, acting through Ror1/2 and Dishevelled/Par1 is necessary for NC specification (Ossipova and Sokol, 2011). This pathway is implicated in cell polarity and may be linked to the role of the small GTPase RhoV (Guemar et al., 2007). In chick, the RhoB GTPase is also activated in premigratory NC by LSox5 and plays a role in NC EMT although in this case, the link with Wnt signaling remains unknown (Liu and Jessell, 1998; Perez-Alcala et al., 2004). In both cases, Wnt/PCP-regulated cell polarity and cytoskeletal rearrangements may be important for the NC aggregation during their specification and EMT onset.

BMP signaling is a second major pathway used reiteratively during NC development. Phospho-Smad levels are increased at the border of the neural plate and in the neural folds, at the time of NC maturation in frog, chick and zebrafish embryos (Faure et al., 2002; Sakai et al., 2005; Wu et al., 2011; Reichert et al., 2013; Schille et al., 2016). In fish embryos, Gdf6a is an important BMP ligand, while Cvl2, an extracellular BMP-binding protein is involved in the increased BMP signaling (Reichert et al., 2013). BMP signaling upregulation is also controlled by SNW1 both in frog and zebrafish, acting upstream of the BMP receptors and controlling NC specification (Wu et al., 2011). In contrast, the downregulation of BMP signaling promotes placode specification (Ahrens and Schlosser, 2005; Litsiou et al., 2005; Kwon et al., 2010). In addition to BMP modulation, SNW1 also modulates canonical Wnt signaling levels and could therefore coordinate BMP and Wnt signaling during NC specification (Wang et al., 2010).

In addition to the better explored BMP and Wnt pathways, several studies indicate the participation of other signalisations. FGF signaling is also involved at neural plate stage, notably with FGF3 and FGF8, both upregulated by *Meis3* (Elkouby et al., 2010). Endothelin signaling is activated in response to Pax3, Msx1 and Zic1 (Bonano et al., 2008; Bae et al., 2014; Plouhinec et al., 2014); the ligand Edn1 diffuses from the underlying intermediate mesoderm and binds to Ednra. This signaling is crucial to maintain NCC identity and promote their survival. Notch signaling has a complex regulation and participates in the balance between the expansion of the NC progenitor pool and NC maturation and EMT: Hes4 stimulates proliferation and survival of immature NC progenitors, at the expense of late NC specifier induction (*sox10* delayed induction), and attenuates BMP signaling (Nagatomo and Hashimoto, 2007; Nichane et al., 2008a; Nichane et al., 2008b). In a second step, progressive NC maturation is triggered by Hes4, by the following complex negative feedback loop: (i) Hes4 activates *delta1* expression in cells adjacent to the prospective NC cell by a non-cell autonomous mechanism; (ii) upon Delta1 binding, Notch signaling is activated in the NC cell triggering *id3* induction; (iii) Id3 interacts with Hes4 by protein-protein interactions, which promote in NC maturation and *sox10* induction (Nichane et al., 2008a; Nichane et al., 2008b). In zebrafish embryos, Notch/Delta signaling also promotes NC specification at the expense of neural specification by inhibiting Neurogenin1 (Cornell and Eisen, 2002).

Altogether, multiple signaling pathways interact during NC induction and maintenance. As previously stressed out during NB specification, it is likely that these multiple regulations are integrated at the level of the regulatory elements of the NC specifiers genes: for example, *snail2* is a direct target of Pax3 and its regulatory regions contains both TCF/LEF and Smad1 binding sites, suggesting its potential simultaneous regulation by both Wnt and BMP signaling (Sakai et al., 2005; Plouhinec et al., 2014).

Furthermore, post-transcriptional regulations, although still poorly understood, could be also involved in NC specification. Ribosome modifications by the CUL3 ubiquitin ligase associated with the adaptor KBTBD8 contribute to *snail2* and *sox10* activation and neural plate genes inhibition (*sox2*) in frog embryos and during human ES cells differentiation into NC (Werner et al., 2015). Small RNAs are increasingly implicated in post-transcriptional regulations: in NB/NC development, several microRNAs have been involved but the study of their precise roles is only at the beginning (Ward et al., 2018).

Finally, negative regulations either limit NC to the NB, or promote neural and placode fates outside the NB. Hence, in contrast to the pathways described above, Shh signals produced by the notochord inhibit NC specification, thus limiting NC expansion ventrally in the neural tube (Selleck et al., 1998). Conversely, DNMT3a represses the neural fate in the dorsal neural folds: DNMT3a is a DNA methyltransferase, repressing the neural genes *sox2* and *sox3* (Hu et al., 2012). In zebrafish, PRDM1a is expressed in the NB and inhibits *olig4* that controls interneurons development, thus PRDM1a safeguards the prospective NC domain (Hernandez-Lagunas et al., 2011). Another series of

negative molecular cross-talk separate NC from PPE. While Gbx2 promotes NC formation, it inhibits the expression of the placodal promoting-gene *six1* (Li et al., 2009). In contrast, PRDM12 trimethylates the histone residue H3K9 on regulatory sequences of *foxd3* in the PPE domain, repressing its expression (Matsukawa et al., 2015). To promote the placode fate, BMP and Wnt signaling need to be downregulated, while active FGF activity is required (Litsiou et al., 2005; Kwon et al., 2010). In zebrafish, Dlx3b activates *bambi-b*, which repress BMP signaling (Reichert et al., 2013). On the other hand, Dlx3 inhibits Nkx6.3, a NC inducing factor, while Nkx6.3 inhibits Dlx3 (Woda et al., 2003; Zhang et al., 2014). This mutual antagonism between two genes demonstrates how NC and placode GRNs markedly diverge at this stage.

Conclusions and Perspectives

In the past 15 years, many molecular events controlling the formation of the neural border have been interconnected, resulting in building a NB-GRN that has greatly advanced the initial hypothetical scaffold proposed in 2004 by Meulemans and Bronner-Fraser. From this proposal, many transcriptional regulations have been validated *in vivo* and a host of novel factors have been added, both for NB and for NC, resulting in one of the most advanced vertebrate GRN described. Despite these efforts, many genetic interactions remain untested. Transcriptomics and epigenomic analyses, applied to the neural crest, either as a small cell population, or in isolated single cell sequencing will be immensely valuable to organize this network on the large scale. Such analyses will be applied to NB or NC selected *in vivo* (Plouhinec et al., 2017) or induced in a variety of assays (Bae et al., 2014, Plouhinec et al., 2014; Radaban et al. 2013), raising the question of potential differences between these assays and the *in vivo* situation. Comparing the different conditions, related to the cellular phenotypes will be critical to better understand the complexity of the parameters controlling NB and NC development.

So far, most regulators of the NB/NC-GRN are secreted signals and transcriptional regulators, either TFs or epigenetic modifiers. Several of them, however, also control other cellular parameters, directly or indirectly, such as differential cell adhesion, cell cycle regulation, cell competence to signaling, among others. A recent study has evidenced how the NB/NC GRN controls AKT signaling by upregulating an AKT non-conventional regulator, PFKFB4 (Figuereido et al., 2017). This regulation is essential for the maturation of NC after its induction, and timely activation of *twist1* and *ncadherin*, required for EMT and migration in the NC cells. Finally, how NB and NC specification is synchronized with the morphogenetic movements of neurulation remains a mystery. It should be pointed out that some proteins that are implicated in NB specification, such as Pax3, Grhl3 or Zic genes, are also necessary for neural tube closure in mouse (Kimura-Yoshida et al., 2015; Nikolopoulou et al., 2017). It is likely that they interact within a largely different GRN in this other context.

The NB territory is present in vertebrates but also in chordates: in the tunicate *Ciona intestinalis* the NB gives rise to pigmented cells and migratory neuronal progenitors generating bipolar tail neurons (Stolfi et al., 2015). More generally, a juxtaposition of peripheral nervous system progenitors with a central nervous system neuroderm is common among animals, including Protostomians. Many orthologous genes, such as *msx*, *pax*, *zic* and *znf703*, are involved in the development both of the lateral neuroblasts of the nematode *Caenorhabditis elegans* and of the vertebrate NB territory (Li et al., 2017). The co-expression of these genes, however, is not sufficient to obtain NC induction in those animals, indicating that evolutionary modifications have occurred in vertebrates. Among the genes involved in the early NB/NC-GRN in vertebrates, *tfap2* is of particular interest as it is involved upstream of NB induction and plays reiterative subsequent roles throughout NC development, in NC induction and NC migration (De Crozé et al., 2011). *Tfap2* also acts upstream of NB and NC specifying genes in lamprey, a basal vertebrate (Nikitina et al., 2008), indicating its ancestral function in vertebrates. In protostomians (*Drosophila*) and chordates (*Amphioxus*), which do not form NC, *Tfap2* is involved in ectodermal patterning, a function also conserved in vertebrate ectoderm (Kerber et al., 2001; Luo et al., 2002; Meulemans and Bronner-Fraser, 2002). The *tfap2* gene has been duplicated several times during evolution from chordates to vertebrates, giving rise to five different paralogous genes in the vertebrate genome. Orthologous gene complementation experiments show that, despite the lack of NC in this animal, *Amphioxus tfap2* can promote NC development in fish embryos. This suggests that the major parameter explaining *tfap2* contribution to the emergence of NC in vertebrates is likely the evolution of the gene regulatory sequences rather than coding sequence modifications (Van Otterloo et al., 2012). Downstream of *Tfap2*, the control of *sox10* expression has probably evolved before the split between urochordates and vertebrates, but cooperation with other *Tfap2* targets is necessary to trigger complete NC cells formation. As for *sox10* itself, it is believed to have evolved by neo-functionalization (Van Otterloo et al., 2012). When the other end of the vertebrate evolutionary tree is considered, NB and NC formation are not well understood in mammals. Many of the genes implicated in these processes in fish, frog or chick are dispensable in the mouse (Barriga et al., 2015). Several hypotheses could account for these discrepancies, such as a highly divergent GRN in mammals, or gene redundancy mechanisms involving different paralogous genes. Moreover, as revealed by the studies in fish, frog and chick embryos, the NB/NC-GRN cascade starts at early gastrulation, a developmental stage challenging to study in mammals.

Due to obvious ethical reasons and rarity, studies on human embryos are very limited. Expression patterns confirm that some major players of the NB/NC-GRN defined in other vertebrates, namely *tfap2a*, *pax3*, *sox9*, and *sox10*, are also expressed in humans (Better et al., 2010). Many teams have developed various strategies to derive NC from human stem cells, either embryonic (ESC) or induced (iPSC) grown *in vitro*. The knowledge on molecular and signaling interactions, accumulated by the studies on other vertebrate embryos *in vivo*, is used for these *in vitro* protocols (Chambers et al.,

2009; Bajpai et al., 2010; Menendez et al., 2011; Rada-Iglesias et al., 2012; Chambers et al., 2012; Mica et al., 2013; Leung et al., 2016). When NC induction is obtained by activating the canonical Wnt pathway, NB markers expression precedes the onset of NC markers (Leung et al., 2016), suggesting that cellular models recapitulate the main steps of the NB/NC-GRN. *The *in vitro* production of human NC-like cells highly resembling the human NC generated *in vivo*, provides a unique tool to explore the mechanisms of human neurocristopathies and to test possible therapies.*

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REFERENCES

- Aamar, E., Frank, D., 2004. *Xenopus* Meis3 protein forms a hindbrain-inducing center by activating FGF/MAP kinase and PCP pathways. *Development* 131, 153–163.
<https://doi.org/10.1242/dev.00905>
- Ahrens, K., and Schlosser, G., 2005. Tissues and signals involved in the induction of placodal Six1 expression in *Xenopus laevis*. *Dev. Biol.* 288, 40–59
- Aoki, Y., Saint-Germain, N., Gyda, M., Magner-Fink, E., Lee, Y.H., Credidio, C., Saint- Jeannet, J.P., 2003. Sox10 regulates the development of neural crest-derived melanocytes in *Xenopus*. *Dev. Biol.* 259, 19-33
- Asad, Z., Pandey, A., Babu, A., Sun, Y., Shevade, K., Kapoor, S., Ullah, I., Rajan, S., Scaria, V., Bajpai, R., Sachidanandan C., 2016. Rescue of neural crest-derived phenotypes in a zebrafish CHARGE model by Sox10 downregulation. *Hum. Mol. Genet.* 25, 3539-54
- Aybar, M.J., Nieto, A., Mayor, R., 2003. Snail precedes Slug in the genetic cascade required for the specification and migration of the *Xenopus* neural crest. *Development* 130, 483–494.
<https://doi.org/10.1242/dev.00238>
- Bae, C.-J., Hong, C.-S., Saint-Jeannet, J.-P., 2018. Anosmin-1 is essential for neural crest and cranial placodes formation in *Xenopus*. *Biochem. Biophys. Res. Commun.* 495, 2257–2263.
<https://doi.org/10.1016/j.bbrc.2017.12.127>
- Bae, C.-J., Park, B.-Y., Lee, Y.-H., Tobias, J.W., Hong, C.-S., Saint-Jeannet, J.-P., 2014. Identification of Pax3 and Zic1 targets in the developing neural crest. *Dev. Biol.* 386, 473–83.
<https://doi.org/10.1016/j.ydbio.2013.12.011>

- Bahm, I., Barriga, E. H., Frolov, A., Theveneau, E., Frankel, P., Mayor, R., 2017. PDGF controls contact inhibition of locomotion by regulating N-cadherin during neural crest migration. *Development*, 144, 2456–2468. <https://doi.org/10.1242/dev.147926>
- Bajpai, R., Chen, D. a., Rada-Iglesias, A., Zhang, J., Xiong, Y., Helms, J., Chang, C.P., Zhao, Y., Swigut, T., Wysocka, J., 2010. CHD7 cooperates with PBAF to control multipotent neural crest formation. *Nature* 463, 958–962. <https://doi.org/10.1038/nature08733>
- Baker, C.V.H., Bronner-Fraser, M., 2001. Vertebrate cranial placodes. I. Embryonic induction. *Dev. Biol.* 232, 1–61. <https://doi.org/10.1006/dbio.2001.0156>
- Baker, J.C., Beddington, R.S.P., Harland, R.M., 1999. Wnt signaling in *Xenopus* embryos inhibits Bmp4 expression and activates neural development. *Genes Dev.* 13, 3149–3159.
- Bang, A.G., Papalopulu, N., Goulding, M.D., Kintner, C., 1999. Expression of Pax-3 in the lateral neural plate is dependent on a Wnt-mediated signal from posterior nonaxial mesoderm. *Dev. Biol.* 212, 366–80. <https://doi.org/10.1006/dbio.1999.9319>
- Barenbaum, M., Bronner, M.E., 2013. Identification and dissection of a key enhancer mediating cranial neural crest specific expression of transcription factor, Ets-1. *Dev. Biol.* 382, 567–575. <https://doi.org/10.1016/j.ydbio.2013.08.009>
- Barriga, E. H., Trainor, P. A., Bronner, M., Mayor, R., 2015. Animal models for studying neural crest development: is the mouse different ? *Development*, 142, 1555–1560. <https://doi.org/10.1242/dev.121590>
- Basch, M.L., Bronner-Fraser, M., García-Castro, M.I., 2006. Specification of the neural crest occurs during gastrulation and requires Pax7. *Nature* 441, 218–22. <https://doi.org/10.1038/nature04684>
- Bellmeyer, A., Krase, J., Lindgren, J., LaBonne, C., 2003. The protooncogene c-myc is an essential regulator of neural crest formation in *xenopus*. *Dev. Cell* 4, 827–39.
- Betters, E., Liu, Y., Kjaeldgaard, A., Sundström, E., García-Castro, M.I., 2010. Analysis of early human neural crest development. *Dev. Biol.* 344, 578–92. <https://doi.org/10.1016/j.ydbio.2010.05.012>
- Bonano, M., Tríbullo, C., De Calisto, J., Marchant, L., Sánchez, S.S., Mayor, R., Aybar, M.J., 2008. A new role for the Endothelin-1/Endothelin-A receptor signaling during early neural crest specification. *Dev. Biol.* 323, 114–129. <https://doi.org/10.1016/j.ydbio.2008.08.007>
- Bonstein, L., Elias, S., Frank, D., 1998. Paraxial-fated mesoderm is required for neural crest induction in *Xenopus* embryos. *Dev. Biol.* 193, 156–168. <https://doi.org/10.1006/dbio.1997.8795>
- Borchin, B.E. and T. Barberi, Chapter Fifteen - The Use of Human Pluripotent Stem Cells for the In Vitro Derivation of Cranial Placodes and Neural Crest Cells, in *Current Topics in Developmental Biology*, A.T. Paul, Editor. 2015, Academic Press. p. 497-514.
- Brugger, S.M., Merrill, A.E., Torres-Peraza, J.F., Wu, N., Ting, M., Maxson, R.E., 2004. A phylogenetically conserved cis-regulatory module in the *Msx2* promoter is sufficient for BMP-dependent transcription in murine and *Drosophila* embryos. *Development* 131, 5153–5165. <https://doi.org/10.1242/dev.01390>

- Brugmann, S.A., Pandur, P.D., Kenyon, K.L., Pignoni, F., Moody, S.A., 2004. Six1 promotes a placodal fate within the lateral neurogenic ectoderm by functioning as both a transcriptional activator and repressor. *Development* 131, 5871–5881. <https://doi.org/10.1242/dev.01516>
- Buckingham, M., & Rigby, P. W. J., 2014. Gene Regulatory Networks and Transcriptional Mechanisms that Control Myogenesis. *Dev. Cell.* 25, 225–38 <https://doi.org/10.1016/j.devcel.2013.12.020>
- Buitrago-Delgado, E., Nordin, K., Rao, A., Geary, L., LaBonne, C., 2015. Shared regulatory programs suggest retention of blastula-stage potential in neural crest cells. *Science* 348, 1332–1335. <https://doi.org/10.1126/science.aaa3655>
- Carmona-Fontaine, C., Acuna, G., Ellwanger, K., Niehrs, C. and Mayor, R., 2007. Neural crests are actively precluded from the anterior neural fold by a novel inhibitory mechanism dependent on Dickkopf1 secreted by the prechordal mesoderm. *Dev. Biol.* 309, 208–221.
- Chambers, S.M., Fasano, C.A., Papapetrou, E.P., Tomishima, M., Sadelain, M., Studer, L., 2009. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat. Biotechnol.* 27, 275–280.
- Chambers, S.M., Yuchen, Q., Mica, Y., Lee, G., Zhang, X.J., Studer, L., 2012. Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. *Nat. Biotechnol.* 30, 715–720. <https://doi.org/10.1038/nbt.2249>
- Chang, C., Hemmati-Brivanlou, A., 1998. Neural Crest Induction by Xwnt7B in *Xenopus*. *Dev. Biol.* 194, 129–134. <https://doi.org/10.1006/dbio.1997.8820>
- Cheung, M., Briscoe, J., 2003. Neural crest development is regulated by the transcription factor Sox9. *Development* 130, 5681–5693. <https://doi.org/10.1242/dev.00808>
- Christen, B., Slack, J., 1997. FGF-8 is associated with anteroposterior patterning and limb regeneration in *Xenopus*. *Dev. Biol.* 192, 455–466.
- Cornell, R.A., Eisen, J.S., 2002. Delta/Notch signaling promotes formation of zebrafish neural crest by repressing Neurogenin 1 function. *Development* 129, 2639–48. <https://doi.org/10.1126/science.282.5394.1711>
- Dady, A., Duband, J., 2017. Cadherin interplay during neural crest segregation from the non-neural ectoderm and neural tube in the early chick embryo. *Dev. Dyn.* 1–47. <https://doi.org/10.1002/dvdy>
- De Crozé, N., Maczkowiak, F., Monsoro-Burq, A.H., 2011. Reiterative AP2a activity controls sequential steps in the neural crest gene regulatory network. *Proc. Natl. Acad. Sci. U. S. A.* 108, 155–60. <https://doi.org/10.1073/pnas.1010740107>
- Degenhardt, K.R., Milewski, R.C., Padmanabhan, A., Miller, M., Singh, M.K., Lang, D., Engleka, K.A., Wu, M., Li, J., Zhou, D., Antonucci, N., Li, L., Epstein, J.A., 2010. Distinct enhancers at the Pax3 locus can function redundantly to regulate neural tube and neural crest expressions. *Dev. Biol.* 339, 519–527. <https://doi.org/10.1016/j.ydbio.2009.12.030>
- Delaune, E., Lemaire, P., Kodjabachian, L., 2005. Neural induction in *Xenopus* requires early FGF signalling in addition to BMP inhibition. *Development* 132, 299–310. <https://doi.org/10.1242/dev.01582>

- Dottori, M., Gross, M.K., Labosky, P., Goulding, M., 2001. The winged-helix transcription factor Foxd3 suppresses interneuron differentiation and promotes neural crest cell fate. *Development* 128, 4127–4138. <https://doi.org/10.1016/j.devcel.2012.07.006>
- Endo, Y., Ishiwata-Endo, H., Yamada, K.M., 2013. Extracellular Matrix Protein Anosmin Promotes Neural Crest Formation and Regulates FGF, BMP and WNT activities. *Dev. Cell.* 23, 305–316. <https://doi.org/10.1016/j.devcel.2012.07.006>
- Endo, Y., Osumi, N., Wakamatsu, Y., 2002. Bimodal functions of Notch-mediated signaling are involved in neural crest formation during avian ectoderm development. *Development* 129, 863–873.
- Ezin, A.M., Fraser, S.E., Bronner-Fraser, M., 2009. Fate map and morphogenesis of presumptive neural crest and dorsal neural tube. *Dev. Biol.* 330, 221–236. <https://doi.org/10.1016/j.ydbio.2009.03.018>
- Faure, S., de Santa Barbara, P., Roberts, D.J., Whitman, M., 2002. Endogenous Patterns of BMP Signaling during Early Chick Development. *Dev. Biol.* 244, 44–65. <https://doi.org/10.1006/dbio.2002.0579>
- Figueiredo, A., Maczkowiak F., Borday, C., Pla, P., Sittewelle, M., Pegoraro, C., Monsoro-Burq, A.H., 2007. PFKFB4 control of AKT signaling is essential for premigratory and migratory neural crest formation. *Development* 134, 4183–94. <https://doi.org/10.1242/dev.157644>
- Fishwick, K. J., Kim, E., Bronner, M. E., 2012. ILF-3 is a regulator of the neural plate border marker Zic1 in chick embryos. *Dev. Dyn.*, 241, 1325–1332. <https://doi.org/10.1002/dvdy.23809>
- Fuentealba, L.C., Eivers, E., Ikeda, A., Hurtado, C., Kuroda, H., Pera, E.M., De Robertis, E.M., 2007. Integrating patterning signals: Wnt/GSK3 regulates the duration of the BMP/Smad1 signal. *Cell* 131, 980–93. <https://doi.org/10.1016/j.cell.2007.09.027>
- Garcia-Castro, M. I., Marcelle, C. and Bronner-Fraser, M., 2002. Ectodermal Wnt function as a neural crest inducer. *Science* 297, 848–851.
- Garnett, A.T., Square, T.A., Medeiros, D.M., 2012. BMP , Wnt and FGF signals are integrated through evolutionarily conserved enhancers to achieve robust expression of Pax3 and Zic genes at the zebrafish neural plate border. *Development* 139, 4220–4231. <https://doi.org/10.1242/dev.081497>
- Garriock, R.J., Warkman, A.S., Meadows, S.M, D'Agostino, S., Krieg P.A., 2007. Census of vertebrate Wnt genes : isolation and developmental expression of Xenopus Wnt2, Wnt3, Wnt9a, Wnt9b, Wnt10a, and Wnt16. *Dev. Dyn.* 236, 1249–58. <https://doi.org/10.1002/dvdy.21156>
- Geary, L., LaBonne, C. 2018. FGF mediated MAPK and PI3K/Akt Signals make distinct contributions to pluripotency and the establishment of Neural Crest. *eLife*, 7, e33845. <https://doi.org/10.7554/eLife.33845>
- Glavic, A., Silva, F., Aybar, M.J., Bastidas, F., Mayor, R., 2003. Interplay between Notch signaling and the homeoprotein Xiro1 is required for neural crest induction in Xenopus embryos. *Development* 131, 347–359. <https://doi.org/10.1242/dev.00945>

- Glavic, A., Honoré, S.M., Feijóo, C.G., Bastidas, F., Allende, M.L., Mayor, R., 2004. Role of BMP signaling and the homeoprotein *iroquois* in the specification of the cranial placodal field. *Dev. Biol.* 272, 89–103. <https://doi.org/10.1016/j.ydbio.2004.04.020>
- Glinka, A., Wu, W., Delius, H., Monaghan, A. P., Blumenstock, C., & Niehrs, C., 1998. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature*, 391, 357–362. <https://doi.org/10.1038/34848>
- Gouti, M., Briscoe, J., Gavalas, A., 2011. Anterior hox genes interact with components of the neural crest specification network to induce neural crest fates. *Stem Cells*, 29, 858–870. <https://doi.org/10.1002/stem.630>
- Guémar, L., de Santa Barbara, P., Vignal, E., Maurel, B., Fort, P., Faure, S., 2007. The small GTPase RhoV is an essential regulator of neural crest induction in *Xenopus*. *Dev. Biol.* 310, 113–128. <https://doi.org/10.1016/j.ydbio.2007.07.031>
- Gutkovich, Y.E., Ofir, R., Elkouby, Y.M., Dibner, C., Gefen, A., Elias, S., Frank, D., 2010. *Xenopus* Meis3 protein lies at a nexus downstream to Zic1 and Pax3 proteins, regulating multiple cell-fates during early nervous system development. *Dev. Biol.* 338, 50–62. <https://doi.org/10.1016/j.ydbio.2009.11.024>
- Hamburger, V., and Hamilton, H. L., 1951. A series of normal stages in the development of the chick embryo. *J. Morphol.* 88, 49–92.
- Harland, R., 2000. Neural Induction. *Curr. Opin. Genet. Dev.* 10, 357–362. [https://doi.org/10.1016/S0959-437X\(00\)00096-4](https://doi.org/10.1016/S0959-437X(00)00096-4)
- Hassler, C., Cruciat, C.-M., Huang, Y.-L., Kuriyama, S., Mayor, R., Niehrs, C., 2007. Kremen is required for neural crest induction in *Xenopus* and promotes LRP6-mediated Wnt signaling. *Development* 134, 4255–4263. <https://doi.org/10.1242/dev.005942>
- Heeg-Truesdell, E., LaBonne, C., 2006. Neural induction in *Xenopus* requires inhibition of Wnt-beta-catenin signaling. *Dev. Biol.* 298, 71–86. <https://doi.org/10.1016/j.ydbio.2006.06.015>
- Hernandez-Lagunas, L., Powell, D.R., Law, J., Grant, K. a., Artinger, K.B., 2011. Prdm1a and olig4 act downstream of Notch signaling to regulate cell fate at the neural plate border. *Dev. Biol.* 356, 496–505. <https://doi.org/10.1016/j.ydbio.2011.06.005>
- Himeda, C.L., Barro, M. V, Emerson, C.P., 2013. Pax3 synergizes with Gli2 and Zic1 in transactivating the Myf5 epaxial somite enhancer. *Dev. Biol.* 1–8. <https://doi.org/10.1016/j.ydbio.2013.09.006>
- Hochgreb-Hägele, T., Bronner, M.E., 2013. A novel FoxD3 gene trap line reveals neural crest precursor movement and a role for FoxD3 in their specification. *Dev. Biol.* 374, 1–11. <https://doi.org/10.1016/j.ydbio.2012.11.035>
- Hoffman, T.L., Javier, A.L., Campeau, S.A., Knight, R.D., Thomas F Schilling, 2007. Tfap2 Transcription Factors in Zebrafish Neural Crest Development and Ectodermal Evolution. *J. Exp. Zool. B. Mol. Dev. Evol.* 308B, 679–691. <https://doi.org/10.1002/jez.b>
- Hong, C., Saint-Jeannet, J.-P., 2007. The Activity of Pax3 and Zic1 Regulates Three Distinct Cell Fates at the Neural Plate Border. *Mol. Biol. Cell* 18, 2192–2202. <https://doi.org/10.1091/mbc.E06>

- Hong, C.-S., Devotta, A., Lee, Y.-H., Park, B.-Y., Saint-Jeannet, J.-P., 2014. Transcription factor AP2 epsilon (Tfap2e) regulates neural crest specification in *Xenopus*. *Dev. Neurobiol.* <https://doi.org/10.1002/dneu.22173>
- Hong, C.-S., Park, B.-Y., Saint-Jeannet, J.-P., 2008. Fgf8a induces neural crest indirectly through the activation of Wnt8 in the paraxial mesoderm. *Development* 135, 3903–3910. <https://doi.org/10.1242/dev.026229>
- Hong, C.-S., Saint-Jeannet, J.-P., 2017. Znf703, a novel target of Pax3 and Zic1, regulates hindbrain and neural crest development in *Xenopus*. *Genesis* e23082. <https://doi.org/10.1002/dvg.23082>
- Hu, N., Strobl-Mazzulla, P., Sauka-Spengler, T., Bronner, M.E., 2012. DNA methyltransferase3A as a molecular switch mediating the neural tube-to-neural crest fate transition. *Genes Dev.* 26, 2380–2385. <https://doi.org/10.1101/gad.198747.112>
- Huang, H.Y., Dai, E.S., Liu, J.T., Tu, C.T., Yang, T.C., et al. 2009. The embryonic expression patterns and the knockdown phenotypes of zebrafish ADP- ribosylation- like factor-6 interacting protein gene. *Dev. Dyn.* 238, 232–240
- Hussein, S.M., Duff, E.K., Sirard, C., 2003. Smad4 and β -catenin co-activators functionally interact with lymphoid-enhancing factor to regulate graded expression of Msx2. *J. Biol. Chem.* 278, 48805–48814. <https://doi.org/10.1074/jbc.M305472200>
- Jacques-Fricke, B.T., Gammill, L.S., 2014. Neural crest specification and migration independently require NSD3-related lysine methyltransferase activity. *Mol. Biol. Cell.* <https://doi.org/10.1091/mbc.E13-12-0744>
- Janssens, S., van den Broek, O., Davenport, I.R., Akkers, R.C., Liu, F., Veenstra, G.J.C., Hoppler, S., Vleminckx, K., Destrée, O., 2013. The Wnt signaling mediator tcf1 is required for expression of foxd3 during *Xenopus* gastrulation. *Int. J. Dev. Biol.* 57, 49–54. <https://doi.org/10.1387/ijdb.120191kv>
- Jaurena, M. B., Juraver-Geslin, H., Devotta, A., Saint-Jeannet, J. P., 2015. Zic1 controls placode progenitor formation non-cell autonomously by regulating retinoic acid production and transport. *Nature Communications*, 6. <https://doi.org/10.1038/ncomms8476>
- Ka man Kam, M., Cheung, M., Jiang, J., Wai, W., Cheng, C., Wai, E., Sat, Y., Kwong, P., Tam, H., Chi, V., Lui, H., 2014. Homeobox b5 (Hoxb5) regulates the expression of Forkhead box D3 gene (Foxd3) in neural crest. *Int. J. Biochem. Cell Biol.* 55, 144–152. <https://doi.org/10.1016/j.biocel.2014.09.002>
- Kee, Y., Bronner-Fraser, M., 2005. To proliferate or to die: Role of Id3 in cell cycle progression and survival of neural crest progenitors. *Genes Dev.* 19, 744–755. <https://doi.org/10.1101/gad.1257405>
- Kerber B, Monge I, Mueller M, Mitchell PJ, Cohen SM., 2001. The AP-2 transcription factor is required for joint formation and cell survival in *Drosophila* leg development. *Development* 128, 1231–1238
- Kerosuo, L., Bronner, M.E., 2016. cMyc Regulates the Size of the Premigratory Neural Crest Stem Cell Pool. *Cell Reports* 17, 2648–2659. <https://doi.org/10.1016/j.celrep.2016.11.025>

- Khadka, D., Luo, T., & Sargent, T. D., 2006. Msx1 and Msx2 have shared essential functions in neural crest but may be dispensable in epidermis and axis formation in *Xenopus*. *Intl. J. Dev. Biol.*, 50, 499–503. <https://doi.org/10.1387/ijdb.052115dk>
- Khudyakov, J., Bronner-Fraser, M., 2009. Comprehensive spatiotemporal analysis of early chick neural crest network genes. *Dev. Dyn.* 238, 716–723. <https://doi.org/10.1002/dvdy.21881>
- Kiecker, C., Niehrs, C., 2001. A morphogen gradient of Wnt/ β -catenin signaling regulates anteroposterior neural patterning in *Xenopus*. *Development* 128, 4189–4201
- Kimura-Yoshida, C., Mochida, K., Ellwanger, K., Niehrs, C., Matsuo, I., 2015. Fate Specification of Neural Plate Border by Canonical Wnt Signaling and Grhl3 is Crucial for Neural Tube Closure. *EBioMedicine* 2, 513–527. <https://doi.org/10.1016/j.ebiom.2015.04.012>
- Kjolby, R. A. S., Harland, R. M. 2017. Genome-wide identification of Wnt/ β -catenin transcriptional targets during *Xenopus* gastrulation. *Dev. Biol.*, 426, 165–175. <https://doi.org/10.1016/j.ydbio.2016.03.021>
- Kos, R., Reedy, M. V, Johnson, R.L., Erickson, C.A., 2001. The winged-helix transcription factor FoxD3 is important for establishing the neural crest lineage and repressing melanogenesis in avian embryos. *Development* 128, 1467–1479.
- Krispin, S., Nitzan, E., Kassem, Y., Kalcheim, C., 2010. Evidence for a dynamic spatiotemporal fate map and early fate restrictions of premigratory avian neural crest. *Development* 137, 585–95. <https://doi.org/10.1242/dev.041509>
- Kuriyama, S., Lupo, G., Ohta, K., Ohnuma, S.-I., Harris, W. a, Tanaka, H., 2006. Tsukushi controls ectodermal patterning and neural crest specification in *Xenopus* by direct regulation of BMP4 and X-delta-1 activity. *Development* 133, 75–88. <https://doi.org/10.1242/dev.02178>
- Kwon, H.J., Bhat, N., Sweet, E.M., Cornell, R.A., Riley, B.B., 2010. Identification of early requirements for preplacodal ectoderm and sensory organ development. *PLoS Genet.* 6. <https://doi.org/10.1371/journal.pgen.1001133>
- LaBonne, C., Bronner-Fraser, M., 1998. Neural crest induction in *Xenopus*: evidence for a two-signal model. *Development* 125, 2403–2414.
- Leung, A.W., Murdoch, B., Salem, A.F., Prasad, M.S., Gomez, G.A., Garcia-Castro, M.I., 2016. WNT/ β -catenin signaling mediates human neural crest induction via a pre-neural border intermediate. *Development* 143, 398–410.
- Lewis, J.L., Bonner, J., Modrell, M., Ragland, J.W., Moon, R.T., Dorsky, R.I., Raible, D.W., 2004. Reiterated Wnt signaling during zebrafish neural crest development. *Development* 131, 1299–1308.
- Li, B., Kuriyama, S., Moreno, M., Mayor, R., 2009. The posteriorizing gene Gbx2 is a direct target of Wnt signalling and the earliest factor in neural crest induction. *Development* 136, 3267–3278. <https://doi.org/10.1242/dev.036954>
- Li, W., Cornell, R.A., 2007. Redundant activities of Tfap2a and Tfap2c are required for neural crest induction and development of other non-neural ectoderm derivatives in zebrafish embryos. *Dev. Biol.* 304, 338–354. <https://doi.org/10.1016/j.ydbio.2006.12.042>

- Li, Y., Zhao, D., Horie, T., Chen, G., Bao, H., Chen, S., Liu, W., Horie, R., Liang, T., Dong, B., Feng, Q., Tao, Q., Liu, X., 2017. Conserved gene regulatory module specifies lateral neural borders across bilaterians. *Proc. Natl. Acad. Sci.* 114, E6352–E6360.
<https://doi.org/10.1073/pnas.1704194114>
- Light, W., Vernon, A.E., Lasorella, A., Iavarone, A., LaBonne, C., 2005. *Xenopus* Id3 is required downstream of Myc for the formation of multipotent neural crest progenitor cells. *Development* 132, 1831–41. <https://doi.org/10.1242/dev.01734>
- Litsiou, A., Hanson, S., Streit, A., 2005. A balance of FGF, BMP and WNT signalling positions the future placode territory in the head. *Development* 132, 4051–4062.
<https://doi.org/10.1242/dev.01964>
- Locascio, A., Manzanares, M., Blanco, M.J., Nieto, M.A., 2002. Modularity and reshuffling of Snail and Slug expression during vertebrate evolution. *Proc. Natl. Acad. Sci.* 99, 16841–16846.
<https://doi.org/10.1073/pnas.262525399>
- Luo, T., Lee, Y.-H., Saint-Jeannet, J.-P., Sargent, T.D., 2003. Induction of neural crest in *Xenopus* by transcription factor AP2alpha. *Proc. Natl. Acad. Sci. U. S. A.* 100, 532–537.
<https://doi.org/10.1073/pnas.0237226100>
- Luo, T., Matsuo-Takasaki, M., Lim, J.H., Sargent, T.D., 2001. Differential regulation of *Dlx* gene expression by a BMP morphogenetic gradient. *Int. J. Dev. Biol.* 45, 681–684.
- Luo, T., Matsuo-Takasaki, M., Sargent, T.D., 2001. Distinct roles for distal-less genes *dlx3* and *dlx5* in regulating ectodermal development in *xenopus*. *Mol. Reprod. Dev.* 60, 331–337.
<https://doi.org/10.1002/mrd.1095>
- Luo T, Matsuo-Takasaki M, Thomas ML, Weeks DL, Sargent TD., 2002. Transcription factor AP-2 is an essential and direct regulator of epidermal development in *Xenopus*. *Dev. Biol.* 245, 136–144
- McCauley, D.W., Bronner-Fraser, M., 2002. Conservation of Pax gene expression in ectodermal placodes of the lamprey. *Gene* 287, 129–139
- Maczkowiak, F., Matéos, S., Wang, E., Roche, D., Harland, R., Monsoro-Burq, A.H., 2010. The Pax3 and Pax7 paralogs cooperate in neural and neural crest patterning using distinct molecular mechanisms, in *Xenopus laevis* embryos. *Dev. Biol.* 340, 381–96.
<https://doi.org/10.1016/j.ydbio.2010.01.022>
- Mancilla, A., and Mayor, R., 1996. Neural crest formation in *Xenopus laevis*: mechanism of Xslug induction. *Dev. Biol.* 177, 580–589.
- Marchal, L., Luxardi, G., Thomé, V., Kodjabachian, L., 2009. BMP inhibition initiates neural induction via FGF signaling and *Zic* genes. *Proc. Natl. Acad. Sci. U. S. A.* 106, 17437–17442.
- Marchant, L., Linker, C., Ruiz, P., Guerrero, N., Mayor, R., 1998. The inductive properties of mesoderm suggest that the neural crest cells are specified by a BMP gradient. *Dev. Biol.* 198, 319–329
- Mašek, J., Machoň, O., Kořínek, V., Taketo, M.M., Kozmik, Z., 2016. Tcf7l1 protects the anterior neural fold from adopting the neural crest fate. *Development* 143, 2206–2216.
<https://doi.org/10.1242/dev.132357>

- Matsukawa, S., Miwata, K., Asashima, M., Michiue, T., 2015. The requirement of histone modification by PRDM12 and Kdm4a for the development of pre-placodal ectoderm and neural crest in *Xenopus*. *Dev. Biol.* 399, 164–176. <https://doi.org/10.1016/j.ydbio.2014.12.028>
- McGrew, L. L., Lai, C. J., Moon, R. T., 1995. Specification of the Anteroposterior Neural Axis through Synergistic Interaction of the Wnt Signaling Cascade with noggin and follistatin. *Dev. Biol.*, 172, 337–342. <https://doi.org/10.1006/dbio.1995.0027>
- McLarren, K.W., Litsiou, A., Streit, A., 2003. DLX5 positions the neural crest and preplacode region at the border of the neural plate. *Dev. Biol.* 259, 34–47. [https://doi.org/10.1016/S0012-1606\(03\)00177-5](https://doi.org/10.1016/S0012-1606(03)00177-5)
- Menendez, L., Yatskievych, T.A., Antin, P.B., Dalton, S., 2011. Wnt signaling and a Smad pathway blockade direct the differentiation of human pluripotent stem cells to multipotent neural crest cells. *Proc. Natl. Acad. Sci. U.S.A.* 108, 19240–19245.
- Meulemans, D., Bronner-Fraser, M., 2002. Amphioxus and lamprey AP-2 genes: implications for neural crest evolution and migration patterns. *Development* 129, 4953–4962.
- Meulemans, D., Bronner-fraser, M., 2004. Gene-Regulatory Interactions Review in Neural Crest Evolution and Development. *Dev. Cell* 7, 291–299.
- Mica, Y., Lee, G., Chambers, S.M., Tomishima, M.J., Studer, L., 2013. Modeling neural crest induction, melanocyte specification, and disease-related pigmentation defects in hESCs and patient-specific iPSCs. *Cell Rep.* 3, 1140–1152.
- Milet, C., Maczkowiak, F., Roche, D.D., Monsoro-Burq, A.H., 2013. Pax3 and Zic1 drive induction and differentiation of multipotent, migratory, and functional neural crest in *Xenopus* embryos. *Proc. Natl. Acad. Sci. U. S. A.* 110, 5528–33. <https://doi.org/10.1073/pnas.1219124110>
- Mizuseki, K., Kishi, M., Matsui, M., Nakanishi, S., Sasai, Y., 1998. *Xenopus* Zic-related-1 and Sox-2, two factors induced by chordin, have distinct activities in the initiation of neural induction. *Development* 125, 579–587.
- Monsoro-Burq, A.-H., Fletcher, R.B., Harland, R.M., 2003. Neural crest induction by paraxial mesoderm in *Xenopus* embryos requires FGF signals. *Development* 130, 3111–24. <https://doi.org/10.1242/dev.00531>
- Monsoro-Burq, A.-H., Wang, E., Harland, R., 2005. Msx1 and Pax3 cooperate to mediate FGF8 and WNT signals during *Xenopus* neural crest induction. *Dev. Cell* 8, 167–78. <https://doi.org/10.1016/j.devcel.2004.12.017>
- Monsoro-Burq, A-H., 2015. PAX transcription factors in neural crest development. *Semin. Cell Dev. Biol.* 44, 87-96. <https://doi.org/10.1016/j.semcdb.2015.09.015>
- Moury, J.D., Jacobson, A.G., 1990. The origins of neural crest in the axolotl. *Dev. Biol.* 141, 243–253.
- Murko, C., Bronner, M.E., 2017. Tissue specific regulation of the chick Sox10E1 enhancer by different Sox family members. *Dev. Biol.* 422, 47–57. <https://doi.org/10.1016/j.ydbio.2016.12.004>

- Murray, S. A. and Gridley, T., 2006. Snail family genes are required for left-right asymmetry determination, but not neural crest formation, in mice. *Proc. Natl. Acad. Sci. USA* 103, 10300-10304
- Nagatomo, K.-I., Hashimoto, C., 2007. *Xenopus* hairy2 functions in neural crest formation by maintaining cells in a mitotic and undifferentiated state. *Dev. Dyn.* 236, 1475–83. <https://doi.org/10.1002/dvdy.21152>
- Nakata, K., Koyabu, Y., Aruga, J., Mikoshiba, K., 2000. A novel member of the *Xenopus* Zic family, Zic5, mediates neural crest development. *Mech. Dev.* 99, 83–91. [https://doi.org/10.1016/S0925-4773\(00\)00480-9](https://doi.org/10.1016/S0925-4773(00)00480-9)
- Nichane, M., de Crozé, N., Ren, X., Souopgui, J., Monsoro-Burq, A.H., Bellefroid, E.J., 2008a. Hairy2-Id3 interactions play an essential role in *Xenopus* neural crest progenitor specification. *Dev. Biol.* 322, 355–367. <https://doi.org/10.1016/j.ydbio.2008.08.003>
- Nichane, M., Ren, X., Souopgui, J., Bellefroid, E.J., 2008b. Hairy2 functions through both DNA-binding and non DNA-binding mechanisms at the neural plate border in *Xenopus*. *Dev. Biol.* 322, 368–380. <https://doi.org/10.1016/j.ydbio.2008.07.026>
- Nieuwkoop, P.D., Faber, J., 1994 in *Normal Table of Xenopus laevis* (Daudin). Garland (New York), 3rd edition.
- Nikitina, N., Sauka-Spengler, T., Bronner-Fraser, M., 2008. Dissecting early regulatory relationships in the lamprey neural crest gene network. *Proc. Natl. Acad. Sci. U. S. A.* 105, 20083–20088. <https://doi.org/10.1073/pnas.0806009105>
- Nikolopoulou, E., Galea, G. L., Rolo, A., Greene, N. D. E., Copp, A. J., 2017. Neural tube closure: cellular, molecular and biomechanical mechanisms. *Development*, 144, 552–566. <https://doi.org/10.1242/dev.145904>
- Nitzan, E., Krispin, S., Pfaltzgraff, E.R., Klar, A., Labosky, P. a, Kalcheim, C., 2013. A dynamic code of dorsal neural tube genes regulates the segregation between neurogenic and melanogenic neural crest cells. *Development* 140, 2269–79. <https://doi.org/10.1242/dev.093294>
- Nordin, K., LaBonne, C., 2014. Sox5 Is a DNA-binding cofactor for BMP R-Smads that directs target specificity during patterning of the early ectoderm. *Dev. Cell* 31, 374–382. <https://doi.org/10.1016/j.devcel.2014.10.003>
- Ossipova, O., Sokol, S.Y., 2011. Neural crest specification by noncanonical Wnt signaling and PAR-1. *Development* 138, 5441–50. <https://doi.org/10.1242/dev.067280>
- Patthey, C., Edlund, T., Gunhaga, L., 2009. Wnt-regulated temporal control of BMP exposure directs the choice between neural plate border and epidermal fate. *Development* 136, 73–83. <https://doi.org/10.1242/dev.025890>
- Patthey, C., Gunhaga, L., Edlund, T., 2008. Early development of the central and peripheral nervous systems is coordinated by Wnt and BMP signals. *PLoS One* 3, 2–11. <https://doi.org/10.1371/journal.pone.0001625>
- Perez-Alcala, S., Nieto, M.A., Barbas, J.A., 2004. LSox5 regulates RhoB expression in the neural tube and promotes generation of the neural crest. *Development* 131, 4455–4465. <https://doi.org/10.1242/dev.01329>

- Phillips, B.T., Kwon, H.J., Melton, C., Houghtaling, P., Fritz, A., Riley, B.B., 2006. Zebrafish *msxB*, *msxC* and *msxE* function together to refine the neural-nonneural border and regulate cranial placodes and neural crest development. *Dev. Biol.* 294, 376–390.
<https://doi.org/10.1016/j.ydbio.2006.03.001>
- Pieper, M., Ahrens, K., Rink, E., Peter, a., Schlosser, G., 2012. Differential distribution of competence for panplacodal and neural crest induction to non-neural and neural ectoderm. *Development* 139, 1175–1187. <https://doi.org/10.1242/dev.074468>
- Pla, P., Monsoro-Burq, A.-H.. *Xenopus Embryo: Neural Induction*. In: Wiley John & Ltd Sons, editor. eLS. Chichester, UK: John Wiley & Sons, Ltd; 2015. pp. 1–10.
<https://doi.wiley.com/10.1002/9780470015902.a0000731.pub2>
- Plouhinec, J.-L., Roche, D.D., Pegoraro, C., Figueiredo, A.L., Maczkowiak, F., Brunet, L.J., Milet, C., Vert, J.-P., Pollet, N., Harland, R.M., Monsoro-Burq, A.H., 2014. Pax3 and Zic1 trigger the early neural crest gene regulatory network by the direct activation of multiple key neural crest specifiers. *Dev. Biol.* 386, 461–72. <https://doi.org/10.1016/j.ydbio.2013.12.010>
- Plouhinec, J.-L., Zakin, L., Moriyama, Y., De Robertis, E.M., 2013. Chordin forms a self-organizing morphogen gradient in the extracellular space between ectoderm and mesoderm in the *Xenopus* embryo. *Proc. Natl. Acad. Sci. U. S. A.* 110, 20372–9. <https://doi.org/10.1073/pnas.1319745110>
- Plouhinec, J.L., Medina-Ruiz, S., Borday, C., Bernard, E., Vert, J.P., Eisen, M.B., Harland, R.M., Monsoro-Burq, A.H., 2017. A molecular atlas of the developing ectoderm defines neural, neural crest, placode, and nonneural progenitor identity in vertebrates. *PLoS Biol.* 15, 1–44.
<https://doi.org/10.1371/journal.pbio.2004045>
- Prasad, M.S., Sauka-Spengler, T., LaBonne, C., 2012. Induction of the neural crest state: Control of stem cell attributes by gene regulatory, post-transcriptional and epigenetic interactions. *Dev. Biol.* 366, 10–21. <https://doi.org/10.1016/j.ydbio.2012.03.014>
- Rada-Iglesias, A., Bajpai, R., Prescott, S., Brugmann, S.A., Swigut, T., Wysocka, J., 2012. Epigenomic annotation of enhancers predicts transcriptional regulators of human neural crest. *Cell Stem Cell* 11, 633–48. <https://doi.org/10.1016/j.stem.2012.07.006>
- Ragland, J.W., Raible, D.W., 2004. Signals derived from the underlying mesoderm are dispensable for zebrafish neural crest induction. *Dev. Biol.* 276, 16–30.
<https://doi.org/10.1016/j.ydbio.2004.08.017>
- Reichert, S., Randall, R.A., Hill, C.S., 2013. A BMP regulatory network controls ectodermal cell fate decisions at the neural plate border. *Development* 140, 4435–4444.
<https://doi.org/10.1242/dev.098707>
- Relaix, F., Rocancourt, D., Mansouri, A., Buckingham, M., 2004. Divergent functions of murine Pax3 and Pax7 in limb muscle development. *Genes Dev.* 18, 1088–1105
- Richard, A., Boullu, L., Herbach, U., Bonnafoux, A., Morin, V., Vallin, E., Guillemin, A., Papili Gao, N., Gunawan, R., Cosette, J., Arnaud, O., Kupiec, J.J., Espinasse, T., Gonin-Giraud, S., Gandrillon, O., 2016. Single-Cell-Based Analysis Highlights a Surge in Cell-to-Cell Molecular Variability Preceding Irreversible Commitment in a Differentiation Process. *PLoS Biol.* 14, 1–35. <https://doi.org/10.1371/journal.pbio.1002585>

- Roellig, D., Tan-Cabugao, J., Esaian, S., Bronner, M.E., 2017. Dynamic transcriptional signature and cell fate analysis reveals plasticity of individual neural plate border cells. *Elife* 6, 1–24. <https://doi.org/10.7554/eLife.21620>
- Rossi, C.C., Kaji, T., Brük Artinger, K., 2009. Transcriptional control of Rohon-Beard sensory neuron development at the neural plate border. *Dev. Dyn.* 238, 931–43. <https://doi.org/10.1002/dvdy.21915>.Transcriptional
- Saint-Jeannet, J.P., He, X., Varmus, H.E., Dawid, I.B., 1997. Regulation of dorsal fate in the neuraxis by Wnt-1 and Wnt-3a. *Proc. Natl. Acad. Sci. U. S. A.* 94, 13713–13718. <https://doi.org/10.1073/pnas.94.25.13713>
- Sakai, D., Tanaka, Y., Endo, Y., Osumi, N., Okamoto, H., Wakamatsu, Y., 2005. Regulation of *Slug* transcription in embryonic ectoderm by b-catenin-Lef/Tcf and BMP-Smad signaling. *Dev. Growth Differ.* 47, 471–482. <https://doi.org/doi:10.1111/j.1440-169x.2005.00821.x>
- Sakai, D., Suzuki, T., Osumi, N., Wakamatsu, Y., 2006. Cooperative action of Sox9, Snail2 and PKA signaling in early neural crest development. *Development* 133, 1323–1333. <https://doi.org/10.1242/dev.02297>
- Sasai, Y., Lu, B., Steinbeisser, H., Geissert, D., Gont, L. K., and De Robertis, E. M., 1994. *Xenopus* chordin: a novel dorsalizing factor activated by organizer-specific homeobox genes. *Cell* 79, 779–790.
- Sasai, N., Mizuseki, K., Sasai, Y., 2001. Requirement of FoxD3-class signaling for neural crest determination in *Xenopus*. *Development* 128, 2525–2536.
- Sato, T., Sasai, N., Sasai, Y., 2005. Neural crest determination by co-activation of Pax3 and Zic1 genes in *Xenopus* ectoderm. *Development* 132, 2355–63. <https://doi.org/10.1242/dev.01823>
- Sela-Donenfeld, D., Kalcheim, C., 1999. Regulation of the onset of neural crest migration by coordinated activity of BMP4 and Noggin in the dorsal neural tube. *Development* 126, 4749–4762
- Schille, C., Bayerlová, M., Bleckmann, A., Schambony, A., 2016a. Ror2 signaling is required for local upregulation of GDF6 and activation of BMP signaling at the neural plate border. *Development*. <https://doi.org/10.1242/dev.135426>
- Schille, C., Heller, J., Schambony, A., 2016b. Differential requirement of bone morphogenetic protein receptors Ia (ALK3) and Ib (ALK6) in early embryonic patterning and neural crest development. *BMC Dev. Biol.* 16, 1. <https://doi.org/10.1186/s12861-016-0101-5>
- Schlosser, G., 2008. Do vertebrate neural crest and cranial placodes have a common evolutionary origin? *BioEssays* 30, 659–672. <https://doi.org/10.1002/bies.20775>
- Selleck, M.A., García-Castro, M.I., Artinger, K.B., Bronner-Fraser, M., 1998. Effects of Shh and Noggin on neural crest formation demonstrate that BMP is required in the neural tube but not ectoderm. *Development* 125, 4919–4930.
- Seo, J.-H., Park, D.-S., Hong, M., Chang, E.-J., Choi, S.-C., 2013. Essential role of AWP1 in neural crest specification in *Xenopus*. *Int. J. Dev. Biol.* 57, 829–36. <https://doi.org/10.1387/ijdb.130109sc>

- Shi, J., Severson, C., Yang, J., Wedlich, D., Klymkowsky, M.W., 2011. Snail2 controls mesodermal BMP/Wnt induction of neural crest. *Development* 138, 3135–3145.
<https://doi.org/10.1242/dev.064394>
- Shimizu, T., Bae, Y.K., Muraoka, O., and Hibi, M., 2005. Interaction of Wnt and caudal-related genes in zebrafish posterior body formation. *Dev. Biol.* 279, 125–141.
- Simões-Costa, M., Stone, M., Bronner, M.E., 2015. Axud1 Integrates Wnt Signaling and Transcriptional Inputs to Drive Neural Crest Formation. *Dev. Cell* 34, 544–554.
<https://doi.org/10.1016/j.devcel.2015.06.024>
- Smith, W.C., A.K. Knecht, M. Wu, and R.M. Harland. 1993. Secreted noggin protein mimics the Spemann organizer in dorsalizing *Xenopus* mesoderm. *Nature* 361, 547–549.
- Spokony, R.F., Aoki, Y., Saint-Germain, N., Magner-Fink, E., Saint-Jeannet, J.-P., 2002. The transcription factor Sox9 is required for cranial neural crest development in *Xenopus*. *Development* 129, 421–432.
- Sridharan, J., Harembaki, T., Weinstein, D.C., 2018. Cloning and spatiotemporal expression of *Xenopus laevis* Apolipoprotein CI. *PLoS One* 13, e0191470.
<https://doi.org/https://doi.org/10.1371/journal.pone.0191470>
- Steventon, B., Araya, C., Linker, C., Kuriyama, S., Mayor, R., 2009. Differential requirements of BMP and Wnt signalling during gastrulation and neurulation define two steps in neural crest induction. *Development* 136, 771–779. <https://doi.org/10.1242/dev.029017>
- Steventon, B., Mayor, R., 2012. Early neural crest induction requires an initial inhibition of Wnt signals. *Dev. Biol.* 365, 196–207. <https://doi.org/10.1016/j.ydbio.2012.02.029>
- Stolfi, A., Ryan, K., Meinertzhagen, I. a., Christiaen, L., 2015. Migratory neuronal progenitors arise from the neural plate borders in tunicates. *Nature* 527, 371–374.
<https://doi.org/10.1038/nature15758>
- Streit, A., 2002. Extensive Cell Movements Accompany Formation of the Otic Placode. *Dev. Biol.* 249, 237–254. <https://doi.org/10.1006/dbio.2002.0739>
- Stuhlmiller, T.J., Garcia-Castro, M.I., 2012. FGF/MAPK signaling is required in the gastrula epiblast for avian neural crest induction. *Development* 139, 289–300. <https://doi.org/10.1242/dev.070276>
- Suzuki, A., Ueno, N., Hemmati-Brivanlou, A., 1997. *Xenopus* msx1 mediates epidermal induction and neural inhibition by BMP4. *Development* 124, 3037–3044.
- Teng, L., Mundell, N. a., Frist, a. Y., Wang, Q., Labosky, P. A., 2008. Requirement for Foxd3 in the maintenance of neural crest progenitors. *Development* 135, 1615–1624.
<https://doi.org/10.1242/dev.012179>
- Tribulo, C., Aybar, M.J., Nguyen, V.H., Mullins, M.C., Mayor, R., 2003. Regulation of Msx genes by a Bmp gradient is essential for neural crest specification. *Development* 130, 6441–6452.
<https://doi.org/10.1242/dev.00878>
- Tsuji, S., Cho, K.W.Y., Hashimoto, C., 2003. Expression pattern of a basic helix-loop-helix transcription factor Xhairy2b during *Xenopus laevis* development. *Dev. Genes Evol.* 213, 407–411. <https://doi.org/10.1007/s00427-003-0338-4>

- Tu, C.T., Yang, T.C., Huang, H.Y., Tsai, H.J., 2012. Zebrafish *Arl6ip1* is required for neural crest development during embryogenesis. *PLoS One* 7. <https://doi.org/10.1371/journal.pone.0032899>
- Uy, B.R., Simoes-Costa, M., Koo, D.E.S., Sauka-Spengler, T., Bronner, M.E., 2015. Evolutionarily conserved role for *SoxC* genes in neural crest specification and neuronal differentiation. *Dev. Biol.* 397, 282–292. <https://doi.org/10.1016/j.ydbio.2014.09.022>
- Vallin, J., Thuret, R., Giacomello, E., Faraldo, M.M., Thiery, J.P., Broders, F., 2001. Cloning and Characterization of Three *Xenopus* Slug Promoters Reveal Direct Regulation by *Lef/β-Catenin* Signaling. *J. Biol. Chem.* 276, 30350–30358. <https://doi.org/10.1074/jbc.M103167200>
- Van Otterloo, E., Li, W., Garnett, a., Cattell, M., Medeiros, D.M., Cornell, R. a., 2012. Novel *Tfap2*-mediated control of *soxE* expression facilitated the evolutionary emergence of the neural crest. *Development* 139, 720–730. <https://doi.org/10.1242/dev.071308>
- Villanueva, S., Glavic, A., Ruiz, P., Mayor, R., 2002. Posteriorization by FGF, Wnt, and Retinoic Acid Is Required for Neural Crest Induction. *Dev. Biol.* 241, 289–301. <https://doi.org/10.1006/dbio.2001.0485>
- Wahlbuhl, M., Reiprich, S., Vogl, M.R., Bösl, M.R., Wegner, M., 2012. Transcription factor *Sox10* orchestrates activity of a neural crest-specific enhancer in the vicinity of its gene. *Nucleic Acids Res.* 40, 88–101. <https://doi.org/10.1093/nar/gkr734>
- Wakamatsu, Y., Endo, Y., Osumi, N., & Weston, J. A., 2004. Multiple roles of *Sox2*, an HMG-box transcription factor in avian neural crest development. *Dev. Dyn.*, 229, 74–86
- Wang, W. Der, Melville, D.B., Montero-Balaguer, M., Hatzopoulos, A.K., Knapik, E.W., 2011. *Tfap2a* and *Foxd3* regulate early steps in the development of the neural crest progenitor population. *Dev. Biol.* 360, 173–185. <https://doi.org/10.1016/j.ydbio.2011.09.019>
- Wang, Y., Fu, Y., Gao, L., Zhu, G., Liang, J., Gao, C., Huang, B., Fenger, U., Niehrs, C., Chen, Y.G., Wu, W., 2010. *Xenopus* *skip* modulates Wnt/β-catenin signaling and functions in neural crest induction. *J. Biol. Chem.* 285, 10890–10901. <https://doi.org/10.1074/jbc.M109.058347>
- Werner, A., Iwasaki, S., McGourty, C. A., Medina-Ruiz, S., Teerikorpi, N., Fedrigo, I., Rape, M., 2015. Cell-fate determination by ubiquitin-dependent regulation of translation. *Nature*, 525, 523–527. <https://doi.org/10.1038/nature14978>
- Wilson, S.I., Graziano, E., Harland, R., Jessell, T.M., Edlund, T., 2000. An early requirement for FGF signalling in the acquisition of neural cell fate in the chick embryo. *Curr. Biol.* 10, 421–9.
- Woda, J.M., Pastagia, J., Mercola, M., Artinger, K., 2003. *Dlx* proteins position the neural plate border and determine adjacent cell fates. *Development* 130, 331–342. <https://doi.org/10.1242/dev.00212>
- Wu, M.Y., Ramel, M.C., Howell, M., Hill, C.S., 2011. *SNW1* is a critical regulator of spatial BMP activity, neural plate border formation, and neural crest specification in vertebrate embryos. *PLoS Biol.* 9. <https://doi.org/10.1371/journal.pbio.1000593>
- Yokota, C., Åstrand, C., Takahashi, S., Hagey, D.W., Stenman, J.M., 2017. Apolipoprotein C-I mediates Wnt/*Ctnnb1* signaling during neural border formation and is required for neural crest development. *Int. J. Dev. Biol.* 61, 415–425. <https://doi.org/10.1387/ijdb.160399cy>

Zhang, Z., Shi, Y., Zhao, S., Li, J., Li, C., Mao, B., 2014. *Xenopus* Nkx6.3 Is a neural plate border specifier required for neural crest development. PLoS One 9. <https://doi.org/10.1371/journal.pone.0115165>

Zhao, T., Gan, Q., Stokes, A., Lassiter, R.N.T., Wang, Y., Chan, J., Han, J.X., Pleasure, D.E., Epstein, J.A., Zhou, C.J., 2014. β -catenin regulates Pax3 and Cdx2 for caudal neural tube closure and elongation. Development 141, 148–57. <https://doi.org/10.1242/dev.101550>

Figure legends.

Figure 1: Expression dynamics of selected genes during neural border and neural crest early development.

A) Expression of the some of the most commonly used markers for NB, NNE, NP and placodes.

Upper row: Dorsal views of frog embryos at neural plate stage (stage 14, Nieuwkoop and Faber, 1994), stained for a selection of genes by whole-mount *in situ* hybridization: *pax3*, *zic1* and *msx1* are expressed at the NB, *Six1* main expression delineates the preplacodal ectoderm (PPE) anteriorly, *sox2* is expressed in the neural plate and *epk* marks the non neural ectoderm. Note the partial overlap between *pax3* and *sox2* in the lateral neural plate corresponding to the future dorsal part of the neural tube; and of *Zic1* and *Six1* in the PPE. Scale bar: 500 μ m. **Lower row:** Cross-sections of neural plate stage 14 frog embryos stained by whole-mount *in situ* hybridization for the indicated genes. *Pax3*, *zic1* and *hes4* are expressed in the NB, *sox2* is expressed in the neural plate with a partial overlap with *pax3* in the lateral neural plate and *epk* is expressed in the non-neural ectoderm. Scale bar: 100 μ m.

B) The comparison of the sequential temporal activation of selected genes in the NB/NC territory outlines the progressive specification of NB and NC in frog and chick embryos during gastrulation and neurulation. References for frog: *AP2a* (de Croz   et al., 2011), *Id3* (Light et al., 2005; Kee and Bronner-Fraser, 2005), *Pax3* (Bang et al., 1999 ; Monsoro-Burq et al., 2005), *Gbx2* (Li et al., 2009), *Dlx5* (Luo et al., 2001; Steventon et al., 2012), *Hes4* (Tsuji et al., 2003; Glavic et al., 2003), *Zic1* (Mizuseki et al., 1998; Nakata et al., 1998), *Snail1* (Aybar et al., 2003), *c-myc* (Bellmeyer et al., 2003), *Zic5* (Nakata et al., 2000), *Sox9* (Spokony et al., 2002), *Ednra* (Bonano et al., 2008), *Snail2* (Mayor et al., 1995; Aybar et al., 2003), *Foxd3* (Sasai et al., 2001), *Sox10* (Aoki et al. 2003; Honore et al., 2003), *Anosmin-1* (Bae et al., 2018), *Tfap2e* (Hong et al., 2014). References for chick: *Pax7* (Basch et al., 2006); *c-myc* (Khudyakov et al., 2009; Keruoso et al., 2016) ; *c-myb* (Betancur et al., 2014) ; *DNMT3a* (Hu et al., 2012) ; *Snail2* (Nieto et al., 1994; Endo et al., 2002) ; *NSD3* (Jacques-Fricke et al., 2014) ; *Foxd3* (Kos et al., 2001) ; *Msx1* (Khudyakov et al., 2009) ; *LSox5* (Perez-Alcala et al., 2004) ; *Ets1* (Barenbaum and Bronner, 2013) ; *Sox10* (Cheng et al., 2000)

Figure 2: The Hourglass model of NC induction: upstream and downstream of Pax3/Zic1. (A)

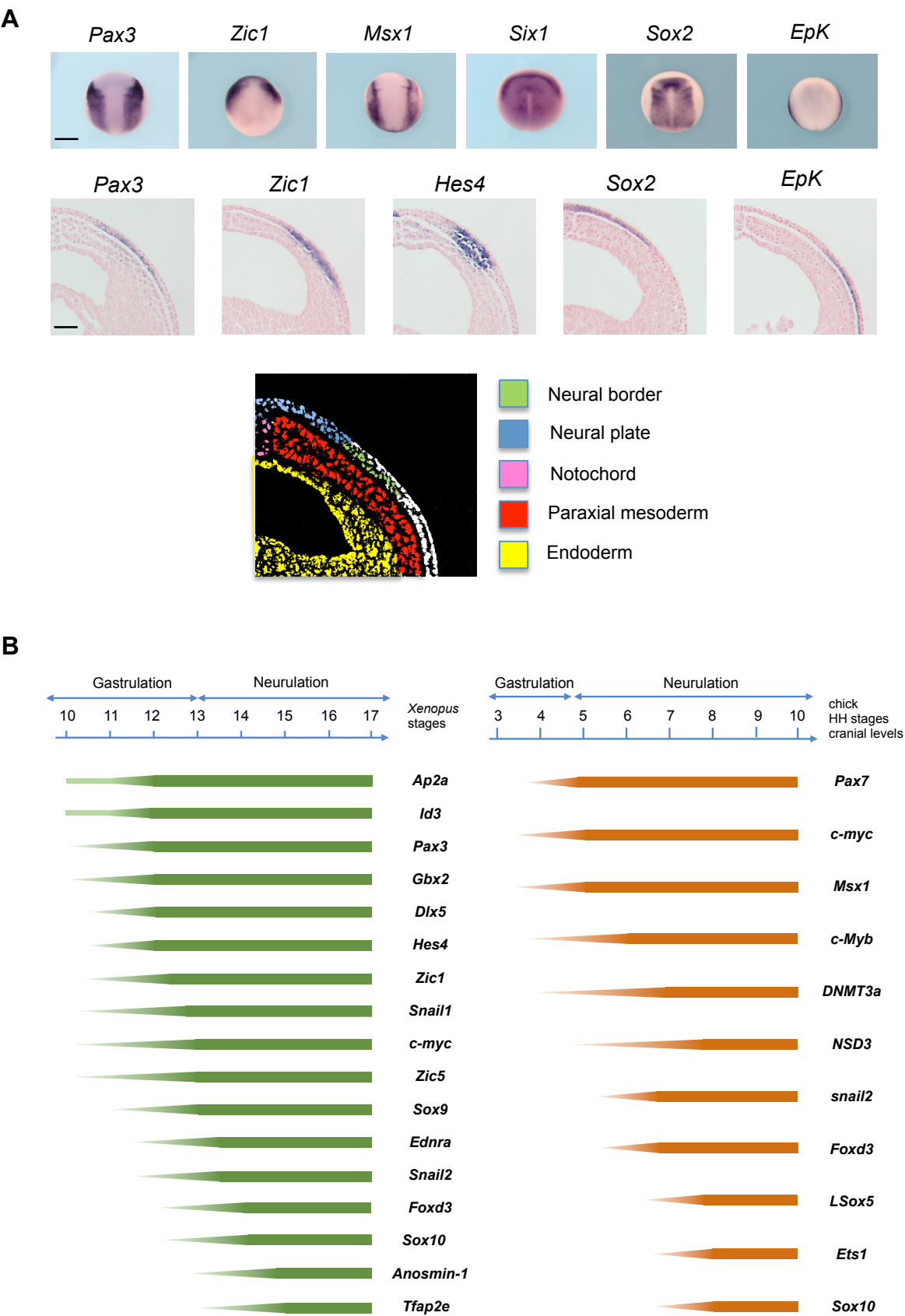
General presentation of the "Hourglass model" for NB/NC-GRN, divided into a neural border module upstream of the activation of the two essential TFs Pax3 and Zic1, and a downstream module, describing Pax3 and Zic1 targets in subsequent NC induction. **(B)** Through a combination of Wnt, FGF and modulated BMP signals, NB-TFs are activated and mutually enhance each other's expression, creating a feed-forward NB stabilization loop, culminating with the robust and balanced activation of Pax3 and Zic1. **(C)** In turn, the balanced expression of Pax3 and Zic1 is necessary and sufficient to trigger all downstream aspects of NC development. The Pax3/Zic1-dependent GRN presented here has been validated in animal caps assays *in vitro* and frog whole embryos *in vivo*. The main regulations are shown, including the direct activation of the EMT-TFs *snail2* and *twist1* (*ets1*,

also direct target EMT-TF is not shown). Direct regulations are shown with solid lines, other regulatory connections with dashed lines. References: Bonano et al., 2008; Marchal et al., 2009; Li et al., 2009 ; Gutkovitch et al., 2010 ; Bae et al., 2014; Plouhinec et al., 2014.

Supplementary materials

Supplemental Table 1 : Expression, upstream regulation and main functions of genes of interest for NB development. Informations demonstrated in frog, chick and zebrafish are shown in green, red and blue, respectively. Informations demonstrated in multiple models are shown in black.

Figure 1
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Gene frog chick zebrafish	Expression pattern	Upstream	Downstream	References
<i>anosmin-1</i>	In NB during NC specification	Activated by Pax3 and Zic1	Necessary for normal NC and sensory placode development Enhances FGF signaling, inhibits BMP and Wnt signaling	Endo et al., 2013 ; Bae et al., 2018
<i>apoc1</i>	In NB and NC	Activated by canonical Wnt signaling	Required for NB and NC specification	Yokota et al., 2017
<i>arl6ip1</i>	Expressed ubiquitously		Necessary for complete NC specification	Huang et al., 2009; Tu et al., 2012
<i>bambi-b</i>	In PPE	Activated by Dlx3b	Inhibits NC specification and promotes PPE specification by inhibiting BMP signaling	Reichert et al., 2013
<i>cadherin 6b</i>	In NB, after Pax7 and Msx1 but before NCC specifiers such as Snail2	Activated by BMP signaling		Sela-Donnenfeld and Kalcheim, 1999; Dady and Duband, 2017
<i>chd7</i>	Expressed in neural plate, NC and PPE in neurula		Interacts with PBAF complex. Binds regulatory sequences of Sox9 and Twist and control their expressions. Controls Snail2 and Sox9 expression	Bajpai et al., 2010; Asad et al., 2016
<i>c-myb</i>	In NB then in NC		Activates Gbx2, Six1, Twist, Pax7, Snail2 and Ets1 expression but not Sox9 and Foxd3. Inhibits Zic1	Betancur et al., 2014
<i>c-myc</i>	In NB and NC	Activated by Apolipoprotein C-I	Activates Id3. Interacts with Miz1. Regulates the size of neural crest stem pool	Bellmeyer et al., 2003; Keruoso and Bronner, 2016; Yokota et al., 2017
<i>cvl2</i>	Ventro-dorsal gradient during gastrulation then in NC territory		Binds BMP and enhance BMP signaling which is necessary for NC induction	Reichert et al., 2013
<i>delta1</i>	Around NC territory	Activated by Xiro1, Hes4 and Stat3 Repressed by Snail1	Through Notch, represses neurogenin-1 expression Through Notch, Stimulates NC proliferation and differentiation Activates Id3	Cornell and Eisen, 2002; Glavic et al., 2003; Nichane et al., 2008b
<i>dlx3</i>	Before gastrulation, expressed in the entire ectoderm. Then during gastrulation, expression is progressively restricted to the NNE and its expression is lower in NB and absent in the neural plate	Depends on high BMP levels. Inhibited by Nkx6.3	Represses neural fates and promotes non-neural competence. Activates bambi which downregulates BMP signaling (necessary for PPE induction)	Luo et al., 2001; Woda et al., 2003 ; Rossi et al., 2009 ; Pieper et al., 2012; Reichert et al., 2013 ; Zhang et al., 2014
<i>dlx5</i>	Expressed in the xenopus NB whereas in chick, it is expressed in NNE and more faintly in NB. Expressed in NC	Depends on attenuated BMP levels Induced by BMPs	Represses neural markers and non-cell autonomously activate NB markers. Can activate PPE marker Six4.	Luo et al., 2001; Steventon et al. 2012, Pera et al., 1999; Streit, 2002; McLarren et al., 2003

<i>dnmt3a</i>	In NB		Represses Sox2 and Sox3 by methylating CpG islands	Hu et al., 2012
<i>ednra</i>	In late NB and NC	Depends on Msx1 expression in vivo Activated by Pax3 and Zic1	Required for NC specification. Inhibits apoptosis in NC.	Bonano et al. 2008; Bae et al., 2014
<i>ets1</i>	In cranial and vagal NC	Activated by Pax7 and Msx1. Depends on cMyb activity	Activates directly Sox10 expression	Betancur et al., 2010, Barembaum and Bronner, 2013 ; Betancur et al., 2014
<i>foxd3</i>	In NC (and already in NB in zebrafish)	Wnt signals are necessary via Axud1. Activated by Pax3/Zic1, by Msx1, Hoxb5, Snail2 and Sox9. Downregulated by PRDM12	Maintains multipotency of NC	Sasai et al., 2001; Tribulo et al., 2003 ; Sato et al., 2005 ; Wang et al., 2011 ; Nitzan et al., 2013 ; Bae et al., 2014 ; Ka Man Kam et al., 2014; Simoes-Costa et al., 2015; Matsukawa et al., 2015
<i>gata2</i>	In non-neural ectoderm	Activated directly by BMP signaling	Required for placodal and epidermal fate	Luo et al., 2001 ; Pieper et al., 2012
<i>gbx2</i>	In a broad region including NB at st12. At st. 16, Gbx2 is expressed in NC domain, and is excluded from PPE.	Activated directly by Wnt signaling Depends on cMyb activity	Upregulates Pax3 and Msx1	Li et al., 2009 Betancur et al., 2014
<i>hes4</i>	In NB and NC	Activated by intermediate BMP levels, FGF and Wnt signaling Downstream effector of Delta/Notch interaction	Maintains NB cells in a proliferative and non-differentiated state by repressing p27 ^{xic1} expression. Required for NC induction downstream of BMP and FGF signaling	Tsuji et al., 2003; Glavic et al., 2003; Nagatomo and Hashimoto, 2007; Nichane et al., 2008a
<i>id3</i>	In the non neural ectoderm then restricted to the NB	Direct target of c-myc	Inhibits cell cycle inhibitor p27 ^{xic1} Represses Sox10	Kee and Bronner-Fraser, 2005; Light et al., 2005
<i>krm2</i>	In NC	Activated by canonical Wnt signaling	Enhances Wnt signaling by binding to LPR6 and enhancing its cell surface levels	Hassler et al., 2007
<i>nkx6.3</i>	In the non neural ectoderm and NB	Inhibited by Dlx3	Inhibits Dlx3. Necessary for Pax3, Zic1 and Msx1 normal expression.	Zhang et al., 2014
<i>nsd3</i>	Expressed in neural plate and more strongly in NB/NC		Dimethylates H3K26. Required for Sox10 and Snail2 activation	Jacques-Fricke et al., 2014
<i>notch</i>	In neural plate and NB		With Delta, represses neurogenin-1 expression With Delta, Stimulates NC proliferation and differentiation Activates Id3	Cornell and Eisen, 2002; Glavic et al., 2003; Nichane et al., 2008b
<i>meis3</i>	In neural plate	Activated by Pax3/Zic1 Activated in the ectoderm by mesodermal Wnt3a	Activates FGF expression. Required for hindbrain neural and NC development	Aamar and Frank, 2004; Elkouby et al., 2010; Gutkovitch et al., 2010
<i>msx1</i>	In non neural ectoderm and NB then restricted to neural border at neurula stages	Activated by intermediate levels of BMP. Direct target of BMP signaling. Activated by Wnt and	Necessary for Snail2 expression. Upregulates Ednra. Interacts directly with Axud to bind	Suzuki et al., 1997; Tribulo et al., 2003; Brugger et al., 2004 ; Monsoro-Burq et al., 2005 ; Phillips et al., 2006 ; Bonano et al.,

		FGF signaling Activated by Gbx2, TFAP2a, Nkx6.3	enhancers of NC specific genes	2008 ; Li et al., 2009 ; DeCrozé et al., 2011 Simoes-Costa et al., 2015 ; Zhang et al., 2016
<i>msx2</i>	In NB	Immediate downstream target of BMP signaling. BMPR1a/ALK3 and BMPR1b/ALK6 are both involved	Necessary for expression of Snail2	Suzuki et al., 1997; Tribulo et al., 2003; Brugger et al., 2004 ; Phillips et al., 2006 ; Khadka et al., 2006 ; Schille et al., 2016
<i>pax3</i>	In NB and NC	Integration of BMP, Wnt and FGF signals. Activated by Gbx2 and directly by TFAP2a	At high doses activates Xhe (hatching gland marker). With Zic1 activates NC specification genes. Directly activates Snail2	Bang et al., 1999 ; Monsoro-Burq et al., 2005 ; Hong et al., 2007 ; Garnett et al., 2012 ; Bae et al., 2014 ; Plouhinec et al., 2014
<i>pax7</i>	In NB Faintly expressed in neural anterior plate then reinforced in brain and truncal paraxial mesoderm	Depends on cMyb activity	Necessary for Snail2, Sox9 and Sox10 expression	Basch et al., 2006 ; Maczkowiak et al., 2010 ; Betancur et al., 2014
<i>pfkfb4</i>	In neural plate then in NB and NC		Necessary for ectodermal patterning, NC specification through Akt signaling	Pegoraro et al., 2013; Pegoraro et al., 2015; Figueiredo et al., 2017
<i>prdm1a</i>	In NB (from 70% epiboly stage)	Repressed by Notch	Directly activates Sox10, Foxd3 and TFAP2a, inhibits olig4 who promotes neural fate	Rossi et al., 2009 ; Hernandez-Lagunas et al., 2011 ; Powell et al., 2013
<i>prdm12</i>	In preplacodal ectoderm	Activated by BMP and repressed by Wnt signaling	Repress expression of Foxd3, Snail2 and Sox8 by promoting histone H3K9 trimethylation	Matsukawa et al., 2015
<i>rhov</i>	In late NB and early anterior NC domain	Activated by Wnt canonical pathway	Necessary for Sox9, Sox10, Snail2 and Twist expression	Guemar et al., 2007
<i>six1</i>	Expressed in PPE	Depends on cMyb activity. Downregulated by BMP and Wnt signaling and activated by FGF signaling at the time of PPE specification	Necessary for placode development	Brugmann et al., 2004; Litsiou et al., 2005 ; Betancur et al., 2014
<i>snail1</i>	In NB and NC		Necessary for NC induction	Aybar et al., 2003
<i>snail2</i>	In NC	Controlled directly by Wnt and BMP signaling Depends on cMyb activity. Activated by Sox9 Directly activated by Pax3 Expression repressed by PRDM12	Activates Twist Controls EMT	Vallin et al., 2001; Sakai et al., 2005; Sakai et al., 2006; Betancur et al., 2014 ; Plouhinec et al., 2014 ; Matsukawa et al., 2015
<i>snw1</i>	Neural plate (except medial region), NB and NC		Enhances BMP signalisation (acts upstream BMP receptors). Modulates Wnt signaling during NC specification	Wang et al., 2010; Wu et al., 2011
<i>sox2</i>	Neural plate	Inhibited by BMP signaling	Inhibits NC development	Wakamatsu et al., 2004
<i>sox5</i>	Expressed in ectoderm then restricted to NB and NC		Enhances NC production Activates RhoB and Sox10	Perez-Alcala et al., 2004 Nordin and LaBonne, 2014

	In NC (Sox5L)		expression. Interacts with Sox5 Associates with Smad1 and regulates BMP targets specificity	Murko et al., 2017
sox8	In NC	Expression, activated by Pax3/Zic1, repressed by PRDM12	Interacts with Sox5 and activates Sox10	Hong and Saint-Jeannet, 2005; Bae et al., 2014 ; Kastuyama et al., 2015; Murko et al., 2017
sox9	In NC	Repressed by Id3	Activates Snail2, Cad6B, Cad7, and directly Sox10. Required for cranial neural crest development. Required for trunk NC survival. Represses neural progenitor markers	Spokony et al., 2002 ; Saint-Germain et al., 2004 ; Cheung and Briscoe, 2003; Light et al., 2005 ; Cheung et al., 2005 ; Betancur et al., 2010 ;
sox10	In NC	Activated by BMP signaling, Wnt signaling (mediated by Axud), TFAP2e, and directly by Sox5, Sox8, Sox9, Ets1, c-Myb. Repressed by Id3	Necessary for NC maintenance, survival and proliferation	Cheng et al., 2000 ; Honore et al., 2003 ; Perez-Alcala et al., 2004 Light et al., 2005 ; Betancur et al., 2010, Aoki et al., 2013, Reichert et al., 2013, Betancur et al., 2014, Simoes-Costa et al., 2015; Murko et al., 2017
sox11	Expressed early in the NB		Essential for NC development in zebrafish and Xenopus	Uy et al., 2015
tfap2a	In ectoderm before gastrulation then upregulated in NB during early gastrulation. Not expressed in NB in zebrafish	A direct target of FGF and Wnt signaling	Induces NC when misexpressed in neural plate. Activates Pax3, Hes4 and Msx1 expressions. Binds directly to Pax3 promoter.	Shen et al., 1997; Hoffmann et al., 2007; Li and Cornell, 2007; deCrozé et al., 2011; Wang et al., 2011
tfap2e	In NB and NC. Not in NNE contrary to TFAP2a	Activated by Pax3 and Zic1	Necessary for Sox10 and Snail2 expression	Bae et al., 2014 ; Hong et al., 2014
twist	In NC	Activated by Snail2, FoxD3, Chd7, RhoV, cMyb Directly activated by Pax3	Controls EMT	Aoki et al., 2003; Aybar et al., 2003 ; Sasai et al., 2001 ; Meulemans and Bronner-Fraser, 2004 ; Guemar et al., 2007 ; Bajpai et al., 2010 ; Betancur et al., 2014 ; Plouhinec et al., 2014 ; Bae et al., 2014
xiro1	In late gastrula, in neural plate (except the midline) and less strongly in NB		Represses Bmp4 expression and activates Hes4, Msx1 and Delta1 expressions	Glavic et al., 2003
zic1	Neural territory then restricted to the NB	Depends on BMP attenuation Activated by ILF-3	Activates RALDH2 (retinoic acid signaling). Activates Six1 non-cell autonomously via retinoic acid signaling. With Pax3 activates NC specification genes	Mizuseki et al.,1998; Hong et al., 2007 ; Marchal et al., 2009 ; Hong et al., 2001; Fishwick et al., 2012 ; Bae et al., 2014 ; Plouhinec et al., 2014; Jaurena et al., 2015
zic5	In NB. Stronger anteriorly. Then in NC	Activated by Pax3 and Zic1. Expression promoted by FGF	Required for NC development	Nakata et al., 2000; Monsoro-Burq et al., 2003 ; Bae et al., 2014
znf703	Neural plate (except midline), NB, NNE posteriorly to hindbrain. Decreases in the NNE during NC specification	Activated by Pax3 and Zic1	Necessary for Sox 10 and Snail2 expression but not of FoxD3	Hong and Saint-Jeannet, 2017