Méghane Sittewelle, Anne H Monsoro-Burq (2018 Jun 4)

**AKT signaling displays multifaceted functions in neural crest development.**

*Developmental biology*: S144-S155 : [DOI : S0012-1606(17)30660-7](https://doi.org/S0012-1606(17)30660-7)

**Summary**

AKT signaling is an essential intracellular pathway controlling cell homeostasis, cell proliferation and survival, as well as cell migration and differentiation in adults. Alterations impacting the AKT pathway are involved in many pathological conditions in human disease. Similarly, during development, multiple transmembrane molecules, such as FGF receptors, PDGF receptors or integrins, activate AKT to control embryonic cell proliferation, migration, differentiation, and also cell fate decisions. While many studies in mouse embryos have clearly implicated AKT signaling in the differentiation of several neural crest derivatives, information on AKT functions during the earliest steps of neural crest development had remained relatively scarce until recently. However, recent studies on known and novel regulators of AKT signaling demonstrate that this pathway plays critical roles throughout the development of neural crest progenitors. Non-mammalian models such as fish and frog embryos have been instrumental to our understanding of AKT functions in neural crest development, both in neural crest progenitors and in the neighboring tissues. This review combines current knowledge acquired from all these different vertebrate animal models to describe the various roles of AKT signaling related to neural crest development in vivo. We first describe the importance of AKT signaling in patterning the tissues involved in neural crest induction, namely the dorsal mesoderm and the ectoderm. We then focus on AKT signaling functions in neural crest migration and differentiation.

Patrick Pla, Anne H Monsoro-Burq (2018 Jun 1)

**The neural border: Induction, specification and maturation of the territory that generates neural crest cells.**

*Developmental biology*: S36-S46 : [DOI : S0012-1606(18)30136-2](https://doi.org/S0012-1606(18)30136-2)

**Summary**

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.
Summary

Wnt proteins form a family of highly conserved secreted molecules that are critical mediators of cell-cell signaling during embryogenesis. Partial data on Wnt activity in different tissues and at different stages have been reported in frog embryos. Our objective here is to provide a coherent and detailed description of Wnt activity throughout embryo development. Using a transgenic Xenopus tropicalis line carrying a Wnt-responsive reporter sequence, we depict the spatial and temporal dynamics of canonical Wnt activity during embryogenesis. We provide a comprehensive series of in situ hybridization in whole-mount embryos and in cross-sections, from gastrula to tadpole stages, with special focus on neural tube, retina and neural crest cell development. This collection of patterns will thus constitute a valuable resource for developmental biologists to picture the dynamics of Wnt activity during development.

Summary

While the external vertebrate body plan appears bilaterally symmetrical with respect to anterior-posterior and dorsal-ventral axes, the internal organs are arranged with a striking and invariant left-right asymmetry. This laterality is important for normal body function, as alterations manifest as numerous human birth defect syndromes. The left-right axis is set up very early during embryogenesis by an initial and still poorly understood break in bilateral symmetry, followed by a cascade of molecular events that was discovered 20 years ago in the chick embryo model. This gene regulatory network leads to activation of the pitx2 gene on the left side of the embryo which ultimately establishes asymmetric organogenesis of the heart, gut, brain, and other organs. In this review, we highlight the crucial contributions of the avian model to the discovery of the differential transcriptional cascades operating on the Left and Right sides, as well as to the physiological events operating upstream of asymmetric gene expression. The chick was not only instrumental in the discovery of mechanisms behind left-right patterning, but stands poised to facilitate inroads into the most fundamental aspects that link asymmetry to the rest of evolutionary developmental biology.
Characterization of Pax3 and Sox10 transgenic Xenopus laevis embryos as tools to study neural crest development.

*Developmental biology* : S202-S208 : [DOI : 10.1222-1606(17)30693-0](https://doi.org/10.1222-1606(17)30693-0)

**Summary**

The neural crest is a multipotent population of cells that originates a variety of cell types. Many animal models are used to study neural crest induction, migration and differentiation, with amphibians and birds being the most widely used systems. A major technological advance to study neural crest development in mouse, chick and zebrafish has been the generation of transgenic animals in which neural crest specific enhancers/promoters drive the expression of either fluorescent proteins for use as lineage tracers, or modified genes for use in functional studies. Unfortunately, no such transgenic animals currently exist for the amphibians Xenopus laevis and tropicalis, key model systems for studying neural crest development. Here we describe the generation and characterization of two transgenic Xenopus laevis lines, Pax3-GFP and Sox10-GFP, in which GFP is expressed in the pre-migratory and migratory neural crest, respectively. We show that Pax3-GFP could be a powerful tool to study neural crest induction, whereas Sox10-GFP could be used in the study of neural crest migration in living embryos.

Year of publication 2017


*A molecular atlas of the developing ectoderm defines neural, neural crest, placode, and nonneural progenitor identity in vertebrates.*

*PLoS biology* : e2004045 : [DOI : 10.1371/journal.pbio.2004045](https://doi.org/10.1371/journal.pbio.2004045)

**Summary**

During vertebrate neurulation, the embryonic ectoderm is patterned into lineage progenitors for neural plate, neural crest, placodes and epidermis. Here, we use Xenopus laevis embryos to analyze the spatial and temporal transcriptome of distinct ectodermal domains in the course of neurulation, during the establishment of cell lineages. In order to define the transcriptome of small groups of cells from a single germ layer and to retain spatial information, dorsal and ventral ectoderm was subdivided along the anterior-posterior and medial-lateral axes by microdissections. Principal component analysis on the transcriptomes of these ectoderm fragments primarily identifies embryonic axes and temporal dynamics. This provides a genetic code to define positional information of any ectoderm sample along the anterior-posterior and dorsal-ventral axes directly from its transcriptome. In parallel, we use nonnegative matrix factorization to predict enhanced gene expression maps onto early and mid-neurula embryos, and specific signatures for each ectoderm area. The clustering of spatial and temporal datasets allowed detection of multiple biologically relevant groups (e.g., Wnt signaling, neural crest development, sensory placode specification, ciliogenesis, germ layer specification). We provide an interactive network interface, EctoMap, for exploring synexpression relationships among genes expressed in the neurula, and suggest several
strategies to use this comprehensive dataset to address questions in developmental biology as well as stem cell or cancer research.

Ana Leonor Figueiredo, Frédérique Maczkowiak, Caroline Borday, Patrick Pla, Meghane Sittewelle, Caterina Pegoraro, Anne H Monsoro-Burq (2017 Oct 18)

**PFKFB4 control of AKT signaling is essential for premigratory and migratory neural crest formation.**


**Summary**

Neural crest (NC) specification comprises an early phase, initiating immature NC progenitors formation at neural plate stage, and a later phase at neural fold stage, resulting in a functional premigratory NC that is able to delaminate and migrate. We found that the NC gene regulatory network triggers upregulation of (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4) during this late specification phase. As shown in previous studies, PFKFB4 controls AKT signaling in gastrulas and glycolysis rate in adult cells. Here, we focus on PFKFB4 function in NC during and after neurulation, using time-controlled or hypomorph depletions. We find that PFKFB4 is essential both for specification of functional premigratory NC and for its migration. PFKFB4-depleted embryos fail to activate and late NC specifiers, and exhibit severe migration defects resulting in craniofacial defects. AKT signaling mediates PFKFB4 function in NC late specification, whereas both AKT signaling and glycolysis regulate migration. These findings highlight novel and essential roles of PFKFB4 activity in later stages of NC development that are wired into the NC gene regulatory network.


**Implication of thyroid hormone signaling in neural crest cells migration: Evidence from thyroid hormone receptor beta knockdown and NH3 antagonist studies.**

*Molecular and cellular endocrinology* : [DOI : S0303-7207(16)30372-0](https://doi.org/S0303-7207(16)30372-0)

**Summary**

Thyroid hormones (TH) have been mainly associated with post-embryonic development and adult homeostasis but few studies report direct experimental evidence for TH function at very early phases of embryogenesis. We assessed the outcome of altered TH signaling on early embryogenesis using the amphibian Xenopus as a model system. Precocious exposure to the TH antagonist NH-3 or impaired thyroid receptor beta function led to severe malformations related to neurocristopathies. These include pathologies with a broad spectrum of organ dysplasias arising from defects in embryonic neural crest cell (NCC) development. We identified a specific temporal window of sensitivity that encompasses the...
emergence of NCCs. Although the initial steps in NCC ontogenesis appeared unaffected, their migration properties were severely compromised both in vivo and in vitro. Our data describe a role for TH signaling in NCCs migration ability and suggest severe consequences of altered TH signaling during early phases of embryonic development.

Year of publication 2015

Anne H Monsoro-Burq (2015 Jun 3)
PAX transcription factors in neural crest development.

Summary

The nine vertebrate PAX transcription factors (PAX1-PAX9) play essential roles during early development and organogenesis. Pax genes were identified in vertebrates using their homology with the Drosophila melanogaster paired gene DNA-binding domain. PAX1-9 functions are largely conserved throughout vertebrate evolution, in particular during central nervous system and neural crest development. The neural crest is a vertebrate invention, which gives rise to numerous derivatives during organogenesis, including neurons and glia of the peripheral nervous system, craniofacial skeleton and mesenchyme, the heart outflow tract, endocrine and pigment cells. Human and mouse spontaneous mutations as well as experimental analyses have evidenced the critical and diverse functions of PAX factors during neural crest development. Recent studies have highlighted the role of PAX3 and PAX7 in neural crest induction. Additionally, several PAX proteins – PAX1, 3, 7, 9 – regulate cell proliferation, migration and determination in multiple neural crest-derived lineages, such as cardiac, sensory, and enteric neural crest, pigment cells, glia, craniofacial skeleton and teeth, or in organs developing in close relationship with the neural crest such as the thymus and parathyroids. The diverse PAX molecular functions during neural crest formation rely on fine-tuned modulations of their transcriptional transactivation properties. These modulations are generated by multiple means, such as different roles for the various isoforms (formed by alternative splicing), or posttranslational modifications which alter protein-DNA binding, or carefully orchestrated protein-protein interactions with various co-factors which control PAX proteins activity. Understanding these regulations is the key to decipher the versatile roles of PAX transcription factors in neural crest development, differentiation and disease.

Caterina Pegoraro, Ana Leonor Figueiredo, Frédérique Maczkowiak, Celio Pouponnot, Alain Eychène, Anne H Monsoro-Burq (2015 Jan 20)
PKF4 controls embryonic patterning via Akt signalling independently of glycolysis.
Nature communications : 5953 : DOI : 10.1038/ncomms6953

Summary

How metabolism regulators play roles during early development remains elusive. Here we
show that PFKFB4 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4), a glycolysis regulator, is critical for controlling dorsal ectoderm global patterning in gastrulating frog embryos via a non-glycolytic function. PFKFB4 is required for dorsal ectoderm progenitors to proceed towards more specified fates including neural and non-neural ectoderm, neural crest or placodes. This function is mediated by Akt signalling, a major pathway that integrates cell homeostasis and survival parameters. Restoring Akt signalling rescues the loss of PFKFB4 in vivo. In contrast, glycolysis is not essential for frog development at this stage. Our study reveals the existence of a PFKFB4-Akt checkpoint that links cell homeostasis to the ability of progenitor cells to undergo differentiation, and uncovers glycolysis-independent functions of PFKFB4.

**Year of publication 2014**


**The Tumor-Suppressor WWOX and HDAC3 Inhibit the Transcriptional Activity of the β-Catenin Coactivator BCL9-2 in Breast Cancer Cells.**

*Molecular cancer research : MCR : 902-12 : DOI : 10.1158/1541-7786.MCR-14-0180*

**Summary**

The WW domain containing oxidoreductase (WWOX) has recently been shown to inhibit of the Wnt/β-catenin pathway by preventing the nuclear import of disheveled 2 (DVL2) in human breast cancer cells. Here, it is revealed that WWOX also interacts with the BCL9-2, a cofactor of the Wnt/β-catenin pathway, to enhance the activity of the β-catenin-TCF/LEF (T-cell factor/lymphoid enhancer factors family) transcription factor complexes. By using both a luciferase assay in MCF-7 cells and a Xenopus secondary axis induction assay, it was demonstrated that WWOX inhibits the BCL9-2 function in Wnt/β-catenin signaling. WWOX does not affect the BCL9-2-β-catenin association and colocalizes with BCL9-2 and β-catenin in the nucleus of the MCF-7 cells. Moreover, WWOX inhibits the β-catenin-TCF1 interaction. Further examination found that HDAC3 associates with BCL9-2, enhances the inhibitory effect of WWOX on BCL9-2 transcriptional activity, and promotes the WWOX-BCL9-2 interaction, independent of its deacetylase activity. However, WWOX does not influence the HDAC3-BCL9-2 interaction. Altogether, these results strongly indicate that nuclear WWOX interacts with BCL9-2 associated with β-catenin only when BCL9-2 is in complex with HDAC3 and inhibits its transcriptional activity, in part, by inhibiting the β-catenin-TCF1 interaction. The promotion of the WWOX-BCL9-2 interaction by HDAC3, independent of its deacetylase activity, represents a new mechanism by which this HDAC inhibits transcription.

Cecile Milet, Anne Helene Monsoro-Burq (2014 Mar 19)

**Dissection of Xenopus laevis neural crest for in vitro explant culture or in vivo transplantation.**

*Journal of visualized experiments : JoVE : DOI : 10.3791/51118*
Summary

The neural crest (NC) is a transient dorsal neural tube cell population that undergoes an epithelium-to-mesenchyme transition (EMT) at the end of neurulation, migrates extensively towards various organs, and differentiates into many types of derivatives (neurons, glia, cartilage and bone, pigmented and endocrine cells). In this protocol, we describe how to dissect the premigratory cranial NC from Xenopus laevis embryos, in order to study NC development in vivo and in vitro. The frog model offers many advantages to study early development; abundant batches are available, embryos develop rapidly, in vivo gain and loss of function strategies allow manipulation of gene expression prior to NC dissection in donor and/or host embryos. The NC explants can be plated on fibronectin and used for in vitro studies. They can be cultured for several days in a serum-free defined medium. We also describe how to graft NC explants back into host embryos for studying NC migration and differentiation in vivo.

Year of publication 2013

Caterina Pegoraro, Anne H Monsoro-Burq (2013 Sep 7)
Signaling and transcriptional regulation in neural crest specification and migration: lessons from xenopus embryos.

Summary

The neural crest is a population of highly migratory and multipotent cells, which arises from the border of the neural plate in vertebrate embryos. In the last few years, the molecular actors of neural crest early development have been intensively studied, notably by using the frog embryo, as a prime model for the analysis of the earliest embryonic inductions. In addition, tremendous progress has been made in understanding the molecular and cellular basis of Xenopus cranial neural crest migration, by combining in vitro and in vivo analysis. In this review, we examine how the action of previously known neural crest-inducing signals [bone morphogenetic protein (BMP), wingless-int (Wnt), fibroblast growth factor (FGF)] is controlled by newly discovered modulators during early neural plate border patterning and neural crest specification. This regulation controls the induction of key transcription factors that cooperate to pattern the premigratory neural crest progenitors. These data are discussed in the perspective of the gene regulatory network that controls neural and neural crest patterning. We then address recent findings on noncanonical Wnt signaling regulation, cell polarization, and collective cell migration which highlight how cranial neural crest cells populate their target tissue, the branchial arches, in vivo. More than ever, the neural crest stands as a powerful and attractive model to decipher complex vertebrate regulatory circuits in vivo.

Jean-Louis Plouhinec, Daniel D Roche, Caterina Pegoraro, Ana Leonor Figueiredo, Frédérique Maczkowiak, Lisa J Brunet, Cécile Milet, Jean-Philippe Vert, Nicolas Pollet, Richard M Harland,
Anne H Monsoro-Burq (2013 May 30)

**Pax3 and Zic1 trigger the early neural crest gene regulatory network by the direct activation of multiple key neural crest specifiers.**


**Summary**

Neural crest development is orchestrated by a complex and still poorly understood gene regulatory network. Premigratory neural crest is induced at the lateral border of the neural plate by the combined action of signaling molecules and transcription factors such as AP2, Gbx2, Pax3 and Zic1. Among them, Pax3 and Zic1 are both necessary and sufficient to trigger a complete neural crest developmental program. However, their gene targets in the neural crest regulatory network remain unknown. Here, through a transcriptome analysis of frog microdissected neural border, we identified an extended gene signature for the premigratory neural crest, and we defined novel potential members of the regulatory network. This signature includes 34 novel genes, as well as 44 known genes expressed at the neural border. Using another microarray analysis which combined Pax3 and Zic1 gain-of-function and protein translation blockade, we uncovered 25 Pax3 and Zic1 direct targets within this signature. We demonstrated that the neural border specifiers Pax3 and Zic1 are direct upstream regulators of neural crest specifiers Snail1/2, Foxd3, Twist1, and Tfap2b. In addition, they may modulate the transcriptional output of multiple signaling pathways involved in neural crest development (Wnt, Retinoic Acid) through the induction of key pathway regulators (Axin2 and Cyp26c1). We also found that Pax3 could maintain its own expression through a positive autoregulatory feedback loop. These hierarchical inductions, feedback loops, and pathway modulations provide novel tools to understand the neural crest induction network.

Caterina Pegoraro, Frederique Maczkowiak, Anne H Monsoro-Burq (2013 Apr 30)

**Pfkfb (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase) isoforms display a tissue-specific and dynamic expression during Xenopus laevis development.**


**Summary**

Pfkfb (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase) enzymes are bi-functional enzymes encoded by four different genes (pfkfb1, pfkfb2, pfkfb3, pfkfb4) in vertebrates. They are involved in the regulation of glycolysis: they catalyze the synthesis and the degradation of F-2,6-BP (fructose-2,6-bisphosphate), the most potent allosteric activator of phosphofructokinase 1 (Pfk1), a key glycolytic enzyme. By producing F-2,6-BP, Pfkfb enzymes allow glycolysis to proceed, while by degrading F-2,6-BP they block glycolysis. As major regulators of glycolysis, Pfkfb enzymes are involved in cancer: tumor cells have a higher glycolytic rate compared to normal cells, even in the presence of adequate oxygen levels (Warburg effect) and several cancer cell lines express elevated levels of Pfkfb enzymes. Glycolysis is also important for energy and metabolite production in proliferating cells. In embryos, however, the role of glycolysis and the expression of glycolysis regulators remain to be explored. Here, we provide a phylogenetic analysis of Pfkfb enzymes in...
vertebrates, and we detail the expression pattern of pfk1, pfkfb1, pfkfb2, pfkfb3, and pfkfb4 genes in Xenopus laevis embryos. We show that pfkfb transcripts expression is overlapping at blastula and gastrula stages and that from neurulation to tadpole stages, they display tissue-specific, complementary and dynamic expression patterns.