Neural crest mechanosensors: seeing old proteins in a new light

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Abstract

Mechanical forces exerted on neural crest cells control their collective migration and differentiation. This Perspective discusses our current understanding of neural crest mechanotransduction during cell migration and differentiation. Additionally, we describe proteins that have mechanosensitive functions in other systems, such as mechanosensitive G protein-coupled receptors, mechanosensitive ion channels, cell-cell adhesion and cell-matrix interacting proteins; and highlight that these same proteins have in the past been studied in neural crest development from a purely signalling point of view. We propose that future studies elucidate the mechanosensitive functions these receptors may play in neural crest development and integrate this with their known molecular role.

Introduction:

The mechanics of embryonic development

Embryonic development is accompanied by growth, movement, and tissue re-arrangement, generating mechanical forces that the cells can sense. Supracellular forces in vertebrate development emerge from very early stages. For example, tissue compaction in mouse embryos initiates at the 8-cell stage (Ziomek and Johnson, 1980). Tissue compaction is driven by cell-cell contact expansion (Li *et al.*, 2009; Turlier and Maître, 2015) and pulsed actomyosin contractility (Maître *et al.*, 2015), which altogether impact the adhesion and surface tension at a cell and tissue level (Turlier and Maître, 2015; Özgüç *et al.*, 2022). The forces generated during tissue compaction in mouse embryos are required for lineage segregation and blastocyst formation (Stephenson, Yamanaka and Rossant, 2010; Chan *et al.*, 2019). In zebrafish embryos, patterns of compaction-extension are distributed along the dorsal and ventral hemispheres (Bhattacharya *et al.*, 2021) and drive the dynamic re-distribution of cells during convergent extension and epiboly (Yin *et al.*, 2008; Bhattacharya *et al.*, 2021; Thomson, Muresan and Steventon, 2021).

Tissue mobilisation and re-arrangement during vertebrate body axis elongation is another example in which there are drastic changes to tissue-scale mechanical properties. In zebrafish, a fluid-like behaviour is detected in the anterior mesodermal progenitor zone during body axis elongation (Mongera *et al.*, 2018; Banavar *et al.*, 2021). Tissue fluidity is accompanied by persistent supracellular stresses, guiding tissue movement (Mongera *et al.*, 2018). In contrast, the posterior presomitic mesoderm undergoes a jamming transition (from a fluid to a solid behaviour), which is required to maintain an organised tissue architecture and support the coordinated movement of the migrating mesodermal progenitor zone (Mongera *et al.*, 2018). Jamming during body axis elongation is regulated by the assembly/disassembly of N-cadherin adhesion proteins (Mongera *et al.*, 2018). However, in airway epithelial cells, during branching morphogenesis, the mechanism of unjamming transition relies on increased cell propulsion forces, independent of cell-cell adhesions (Mitchel *et al.*, 2020). Further research is required to understand the mechanisms driving changes in tissue topology during development, including jamming-unjamming transitions.

In recent years, mechanical forces have been identified as a trigger and guidance cue for the collective migration and differentiation of neural crest (NC) cells (Barriga *et al.*,

2018; Zhu *et al.*, 2019; Shellard and Mayor, 2021), thereby positioning the NC as a great model for biomechanical studies. Specifically, higher cell density of the head mesoderm in *Xenopus* embryos leads to tissue stiffening, which can be detected by NC cells, triggering the initiation of collective NC migration (Barriga *et al.*, 2018). Furthermore, *Xenopus* NC cells establish their migratory path by following a self-generated stiffness gradient across the adjacent placodal tissue (Shellard and Mayor, 2021). In addition to cell migration, NC differentiation in mice is affected by the stiffness of the substrate, with stiff substrates favouring smooth muscle differentiation as opposed to glial differentiation in soft matrices (Li *et al.*, 2011; Zhu *et al.*, 2019). In summary, mechanical stimuli drive collective migration and differentiation of NC cells. However, the mechanism by which the NC senses the mechanical forces is not fully understood. This Perspective explores the different families of plasma membrane mechanosensors and discusses their potential role in NC mechanotransduction.

Plasma membrane mechanosensors are transmembrane proteins that can be activated or modified by physical forces (Le Roux *et al.*, 2019) and transform a mechanical force into a biochemical response through mechanotransduction (Tschumperlin, 2011). Forces applied to cell-cell and cell-matrix proteins, such as cadherins and integrins, can be transduced to the cytoskeleton via catenin and focal adhesion proteins, respectively (Leckband and de Rooij, 2014; Sun, Guo and Fässler, 2016). Transmembrane receptors, such as mechanosensitive G-protein coupled receptors (GPCR) and mechanosensitive (MS) ion channels, control the traffic of a myriad of molecules to the cell, including ions (Coste *et al.*, 2010), hormones (Wang *et al.*, 2015; Xu *et al.*, 2019), metabolites (Tan *et al.*, 2017), and neurotransmitters (Betke, Wells and Hamm, 2012).

The mechanism by which mechanosensitive GPCRs and most MS ion channels detect and transduce forces is poorly understood. However, research in this field is rapidly growing. In recent years, several GPCRs and ion channels have been identified as mechanosensitive (O'Neil and Heller, 2005; Coste *et al.*, 2010; Storch, Mederos y Schnitzler and Gudermann, 2012). Interestingly, several mechanosensitive GPCRs and ion channels have been previously studied in the NC, and are known to be essential for NC formation, migration, or differentiation (Clouthier *et al.*, 1998; Ruest and Clouthier, 2009; Schuurs-Hoeijmakers *et al.*, 2012; Hutson *et al.*, 2017; Camargo-

Sosa *et al.*, 2019; Canales Coutiño and Mayor, 2021b). Nonetheless, at the time these GPCRs and ion channels were studied in the NC, there was no evidence of their role as mechanosensors.

This Perspective discusses the four families of plasma membrane mechanosensors: GPCRs, MS ion channels, cell-cell adhesion, and cell-matrix interacting proteins. We highlight mechanosensitive GPCRs and ion channels required for NC development. We discuss how their recently discovered role as mechanosensors might affect what we know about the activation of these receptors in the NC. Additionally, we discuss how the expression of several mechanosensitive GPCRs and ion channels in the NC might expand our knowledge of NC mechanosensitivity and its response to mechanical forces during embryonic development.

The mechanics of the neural crest

The neural crest (NC) is a multipotent embryonic cell population specified at the border of the neural plate in vertebrates (Mayor and Theveneau, 2013). At the onset of cell migration, the NC undergoes an epithelial to mesenchymal transition (EMT), which initiates their collective migration in a directional path (Shellard and Mayor, 2019). Once they reach their target tissue, NC cells differentiate into a subset of specialised cells, including the connective tissue that forms the jaw and other facial structures, heart outflow tract, dorsal root ganglia, and melanocytes, among others (Szabó and Mayor, 2018). Defects in NC formation, migration, or differentiation lead to craniofacial defects and several other developmental diseases collectively termed neurocristopathies (Bolande, 1974).

In recent years, the effect of mechanical forces on NC development has begun to be unravelled. An *in vivo* study on *Xenopus* embryos described the impact of head mesoderm cell density and stiffening on cranial NC migration (Barriga *et al.*, 2018). Barriga *et al.* measured the apparent elasticity of the head mesoderm, the tissue adjacent to the NC, by *in vivo* atomic force microscopy (iAFM) at different developmental stages. In non-migratory NC, the mesoderm has an apparent soft stiffness (50 Pa) (Figure 1A). At later stages, the elasticity of the mesoderm increases (150 Pa) due to a rise in cell density. NC cells adjacent to the mesoderm can sense and respond to the mechanical force generated by mesoderm stiffening via focal

adhesion proteins, triggering epithelial to mesenchymal transition (EMT) and NC collective migration (Figure 1A'). Failure of mesoderm stiffening completely abrogates the initiation of NC migration (Barriga *et al.*, 2018). A role for substrate stiffness has also been shown for the migration on enteric NC in chicken and mouse embryos (Chevalier *et al.*, 2016), which indicates that enteric NC is also mechanosensitive; however, their mechanism of action might defer from cranial NC.

Although the initiation of NC migration is triggered by an increase in tissue stiffening, its direction of migration in *Xenopus* embryos is partly established by attractive signals from placode cells, the tissue ventrally adjacent to the cephalic NC (Figure 1A-A'). Placode cells release chemoattractants that NC follow as they migrate (Theveneau *et al.*, 2013). However, a recent study has shown that a mechanical cue from the placodes also contributes to NC directional migration (Shellard and Mayor, 2021). NC cells dynamically soften the adjacent placode cells during migration via N-cadherin interaction; thereby, NC cells self-generate a stiffness gradient that they follow during embryonic development (Shellard and Mayor, 2021). Generation of ectopic stiffness gradients or the absence of a stiffness gradient (e.g., constant stiffness level) results in erratic NC migration *in vivo* (Shellard and Mayor, 2021).

NC directional migration is also established by negative signals from the lateral tissues, which release several Rac1 inhibitor proteins, e.g., semaphorins, ephrins, and Slit/Robo (Theveneau and Mayor, 2012). Rac1 inhibition leads to a collapse in actin-based protrusions, blocking the invasion of NC cells into nearby lateral tissues (Bajanca *et al.*, 2019). A recent study shows that in addition to extracellular repellents, NC cells regulate Rac1 activity via the mechanosensitive ion channel Piezo1 (Canales Coutiño and Mayor, 2021b). Piezo1 knockdown in migratory NC cells abrogates inhibitory semaphorin 3A and 3F signals, leading to uncontrolled NC cell invasion *in vitro* and *in vivo* (Canales Coutiño and Mayor, 2021b), indicating that a precise level of Piezo1 activity is required for normal NC migration. Therefore, coordination between biochemical signals (released from adjacent tissues) and extracellular forces (sensed by mechanosensitive receptors in the NC) control organised collective behaviour during NC migration.

Additionally, studies on human multipotent neural crest stem cells (NCSCs), derived from induced pluripotent stem cells (iPSC), indicate that mechanical forces can also regulate NC cell differentiation. iPSC-NCSCs cultured on stiff hydrogels (1 GPa) differentiate into smooth muscle cells after three days, whereas NCSCs cultured on soft hydrogels (15 kPa) differentiated into Schwann cells (Figure 1B) (Li *et al.*, 2019). Moreover, mechanical strain can suppress the differentiation of NCSCs into Schwann glial cells (Li *et al.*, 2011, 2012). The same differentiation pattern was observed when polymer-embedded NCSC grafts were implanted into rat carotid arteries. After three months of implantation, NCSCs differentiated into smooth muscle cells near the stiffer-outer surface of the polymer grafts; in contrast, NCSCs differentiated into glial cells in the softer hydrogel (Li *et al.*, 2019). Overall, these studies consistently show a preference for smooth muscle over glial cell differentiation when NCSCs are cultured on stiff substrates.

Cell types derived from NC stem cells are also responsive to substrate stiffness, which affects their differentiation and gene expression patterns. Boundary cap neural crest stem cells preferentially differentiate into astroglia when grown in 1 kPa gels (Han, Baltriukienė and Kozlova, 2020). However, cell differentiation is inhibited in both softer (0.5 kPa) and stiffer (7 kPa) gels (Han, Baltriukienė and Kozlova, 2020), suggesting a specific window of substrate stiffness is required for the differentiation of boundary cap neural crest stem cells. Epidermal NC cells show differential gene expression when cultured on hydrogels of different stiffness; NC cells grown on 1 kPa gels preferentially express the NC marker SOX-10, while softer gels enhance the expression of glial cell line-derived neurotrophic factor, neurotrophin-3, and vascular endothelial growth factor (Pandamooz *et al.*, 2020).

In summary, the NC is a mechanosensitive tissue, and mechanical signals coordinate NC migration and differentiation during embryonic development. However, little is known about how NC cells detect these forces and transform them into biochemical signals through mechanotransduction. With the field of mechanobiology rapidly growing, several transmembrane receptors that were previously thought to be exclusively activated by chemical signals are now known to be mechanosensitive. Several of these cell membrane mechanoreceptors are expressed in NC cells and have been studied in the context of NC migration and differentiation. Loss of some of

these receptors in the NC leads to craniofacial defects, indicating that their activity is required for normal embryonic development. In the following sections, we describe these mechanosensors, emphasising their mechanical activation, which has not been explored in the neural crest to date.

Cell membrane mechanosensors

The cell plasma membrane constitutes the limit between the cell contents and the extracellular space. Cell integrity, trafficking, and communication with neighbours are regulated through the plasma membrane (Gonzalez Jr. and Scheller, 1999; Grecco, Schmick and Bastiaens, 2011). Plasma membrane receptors sense changes in the extracellular composition. Transmembrane proteins that are sensitive to mechanical forces are essential for mechanotransduction, and these include members of the GPCRs, MS ion channels, cell-cell and cell-matrix adhesion proteins (Figure 2A).

Several GPCRs and MS ion channels that are now identified as mechanosensitive can be simultaneously activated by ligands, temperature, or many different stimuli (Storch, Mederos y Schnitzler and Gudermann, 2012; Hutson *et al.*, 2017). As the number and diversity of mechanosensitive proteins increase, the definition of a mechanosensor can become blurred. Here, a mechanosensitive receptor or channel is considered if: *i*) there is evidence of conformational changes upon cell membrane deformations in the presence of a mechanical force, *ii*) a detectable biochemical response is generated upon mechanical force in the absence of a ligand or non-mechanical stimuli, *iii*) expression of the mechanosensor confers mechanosensitivity (points *i* and *ii*) to non-responsive cells. However, several MS ion channels are referred to as indirectly mechanosensitive. These channels are not activated when a force is applied to them; instead, they are activated downstream of another mechanosensitive receptor; we will discuss them in section 2.2.

G-protein coupled receptors

G-protein coupled receptors (GPCRs) form the most diverse family of transmembrane channels and are the most common target for drug treatments (Rosenbaum, Rasmussen and Kobilka, 2009; Sriram and Insel, 2018). GPCRs mediate most cellular responses to hormones (Wang *et al.*, 2015; Xu *et al.*, 2019), metabolites (Tan *et al.*, 2017), neurotransmitters (Betke, Wells and Hamm, 2012), and other signals.

Additionally, GPCRs have been identified as sensitive to voltage (Ben-Chaim *et al.*, 2006) and mechanical forces (Storch, Mederos y Schnitzler and Gudermann, 2012). GPCRs propagate extracellular signals into cells by coupling with heterotrimeric guanine-nucleotide-binding regulatory proteins (G proteins) (Milligan and Kostenis, 2006), which are formed by α, β and γ subunits (Kim *et al.*, 2020). G proteins of α subunits are the most widely studied and are divided into four groups according to their structural and functional similarities: Gαs, Gαi/o, Gαq/11, and Gα12/13 (Mizuno and Itoh, 2009; Syrovatkina *et al.*, 2016).

Receptors coupled to the Gαq/11 (G(q/11)) subunit have been described as mechanosensitive and are activated by membrane stretch in a ligand-independent manner (Mederos y Schnitzler *et al.*, 2008). The mechanism of mechanical activation has been attributed to the cytoplasmic C-terminal helix 8 (H8) of G(q/11) receptors, which undergo specific patterns of conformational change upon mechanical stimuli (Erdogmus *et al.*, 2019). Transfer of H8 to non-responsive GPCRs confers mechanosensitivity, while removing H8 precludes mechanosensitivity (Erdogmus *et al.*, 2019). However, it is unclear whether this is the only mechanism by which GPCRs respond to mechanical stimuli.

Mechanical activation of GPCRs leads to a canonical G(q/11) response involving phospholipase C (PLC) activity (Drissi *et al.*, 1998). PLC catalyses the conversion of phosphatidylinositol 4,5-bisphosphate (IP2) into the Ca^{2+}-mobilizing second messenger inositol 1,4,5-trisphosphate (IP3), and the protein kinase-activating second messenger diacylglycerol (DAG) (Harden *et al.*, 2011) (Figure 2B). Therefore, the activation of mechanosensitive GPCRs indirectly leads to the regulation of Ca^{2+} levels via PLC-mediated MS ion channel activation (Figure 2B). Additionally, DAG signalling activates protein kinase C (PKC) (Figure 2B), which triggers several signalling cascades by phosphorylating serine and threonine residues in many target proteins (reviewed in Newton, 2018)

Examples of mechanosensitive G(q/11) GPCRs include endothelin (ETA) receptors (Mederos y Schnitzler *et al.*, 2008), sphingosine 1-phosphate receptors (S1PR) (Jung *et al.*, 2012), and parathyroid hormone 1 receptors (PTH1R) (Zhang, Frangos and Chachisvilis, 2009). The expression and function of these receptors have been

described for NC cells, and NC-derived cells. Loss of PTH1R impairs differentiation into osteogenic cells (Liu *et al.*, 2020). Aberrant PTH1R expression is associated with several jaw malformations and diseases (Houpis *et al.*, 2010; Richman, 2019). Mice deficient in ETA receptors show severe craniofacial deformities and defects in the cardiovascular outflow tract (Clouthier *et al.*, 1998). Similarly, several studies found that knockdown of ETA, specifically within NC tissue in zebrafish, leads to fusions between upper and lower jaw cartilages (Ruest and Clouthier, 2009; Clouthier, Garcia and Schilling, 2010; Camargo-Sosa *et al.*, 2019). Furthermore, ETA inhibition in Xenopus embryos interferes with early NC formation (Bonano *et al.*, 2008). S1PR receptors are found expressed in mouse migratory neural crest cells (Meng and Lee, 2009). They are also detected in neural crest derivatives such as enteric neurons and branchial arches (Meng and Lee, 2009). Craniofacial defects are observed in zebrafish harbouring mutations in S1PR (Balczerski *et al.*, 2012).

In conclusion, although many mechanosensitive GPCRs have been demonstrated to play important roles in neural crest development, whether they are activated by mechanics in the neural crest remains to be investigated.

Mechanosensitive Ion channels

Mechanosensitive (MS) ion channels include a wide variety of ion-permeable transmembrane channels that can be activated by mechanical forces (Canales Coutiño and Mayor, 2021a). Members of the TRP superfamily and Piezo1 channels are the most characterised MS ion channels in eukaryotic cells. They allow the entry of divalent ions, such as Ca^{2+} and Mg^{2+}, into the cell from the extracellular space in response to membrane deformations (Coste *et al.*, 2010). TRP channels can be activated by different stimuli such as temperature (Feng, 2014), chemicals (Putney Jr, 1999), pH changes (Holzer, 2009), low cation levels (store-operated) (Ong, de Souza and Ambudkar, 2016) and mechanical stress (Liu and Montell, 2015). In contrast, Piezo1 channels are the only mechanosensitive ion channels known to be activated exclusively by mechanical forces (Coste *et al.*, 2010).

Studies of Piezo1 channels have linked the effect of mechanical forces on several biological processes, including cell proliferation (Gudipaty *et al.*, 2017), cell extrusion (Eisenhoffer *et al.*, 2012), cell differentiation (Pathak *et al.*, 2014), axon guidance

(Koser *et al.*, 2016), and cell migration (McHugh *et al.*, 2012), among others. Migratory NC cells in *Xenopus* embryos express Piezo1 and require precise control of Piezo1 activity for normal NC migration (Canales Coutiño and Mayor, 2021b). *In vitro*, activation of Piezo1 leads to inhibition of NC migration, while inhibition of Piezo1 increases the speed of cell migration via Rac1 activation (Canales Coutiño and Mayor, 2021b). Accordingly, inhibition of Piezo1 in *Xenopus* migratory NC cells *in vivo* leads to invasion of the NC cells into the adjacent tissue (Canales Coutiño and Mayor, 2021b); this suggests that a precise level of Piezo1 activation is required at the border of the NC tissue to prevent invasion. As Piezo1 is exclusively activated by mechanical forces (Saotome *et al.*, 2018; Lin *et al.*, 2019), stresses generated between the NC and adjacent tissues could be the mechanical force required for Piezo1 activation in the NC migratory cells, since increased tension and stresses are detected at tissue boundaries across different models and species (reviewed in Heer and Martin, 2017).

Mechanosensitive TRP channels have two main mechanisms of mechanical activation. i) Mechanosensitive TRP channels, such as TRPC6, TRPV2 and TRPV4, can be activated directly by stretch and tension applied to the lipid bilayer (Muraki *et al.*, 2003; Dyachenko *et al.*, 2009; Mamenko *et al.*, 2015; Lee *et al.*, 2017) (Figure 2C). ii) TRP channels can be activated downstream from mechanosensitive G-protein coupled receptors that signal through phospholipase C (PLC) (Drissi *et al.*, 1998) (Figure 2B). TRPV4 and TRPM7 are two examples of mechanosensitive TRP channels that have been previously studied in NC and embryonic development. Interestingly, members of the TRP family of MS ion channels contain IP3 binding domains that lead to channel activation (Putney Jr, 1999).

TRPV4 is the most characterised TRP member, and it can be activated by osmotic pressure and shear stress, leading to an influx of Ca^{2+} ions into the cell (Liedtke *et al.*, 2000; Gao *et al.*, 2011; Mamenko *et al.*, 2015). Additionally, ankyrin repeats located in the N-terminus of TRPV4 directly interact with the actin filaments (F-actin) of the cytoskeleton (Liedtke *et al.*, 2000). Therefore, TRPV4-mediated mechanotransduction depends on Ca^{2+} signalling and binding to the actin cytoskeleton. In zebrafish embryos, cranial NC migration is regulated by TRPV4, in cooperation with PACS, a membrane traffic regulator (Youker *et al.*, 2009; Schuurs-Hoeijmakers *et al.*, 2012). Knockdown of TRPV4 and PACS in zebrafish embryos leads to NC migration defects

and craniofacial deformations (Schuurs-Hoeijmakers *et al.*, 2012). Additionally, specific overactivation of TRPV4 in cranial and cardiovascular NC in chick and zebrafish embryos leads to severe developmental defects (Hutson *et al.*, 2017). It indicates that a threshold of TRPV4 activity is required for normal NC development and that TRPV4 activity is needed for both cranial and cardiac NC tissue.

TRPM7 was identified as mechanosensitive in mesenchymal stromal cells (Liu *et al.*, 2015), HeLa cells (Numata, Shimizu and Okada, 2007) and rat odontoblasts (Won *et al.*, 2018). TRPM7 is part of a subfamily of ion channels that acts as a bifunctional channel. In addition to their permeability to divalent ions, TRPM7 channels contain kinase domains in their C-terminal segment and act as enzymatic regulators (Runnels, Yue and Clapham, 2001). The kinase domain of TRPM7 induces phosphorylation of many downstream targets, including annexin A1 (Dorovkov, Kostyukova and Ryazanov, 2011), calpain II (Su *et al.*, 2010), myosin II (Clark *et al.*, 2008), and SMAD2 (Zhong *et al.*, 2018). Moreover, unlike other families of MS ion channels, which prefer Ca^{2+} regulation, TRPM7 has a higher sensitivity to Mg^{2+} (Zou *et al.*, 2019). Thereby, TRPM7 increase the diversity of biological responses of MS ion channels. TRPM7 expression is required for embryonic development (Jin *et al.*, 2008, 2012). Knockdown of TRPM7 specifically within trunk NC in mice results in blockage of melanocyte and dorsal root ganglion neuron differentiation (Jin *et al.*, 2012), indicating a role for TRPM7 in trunk NC differentiation.

In summary, the role of several mechanosensitive ion channels on different aspects of NC development has been unequivocally established. Furthermore, it has been demonstrated that Piezo1, which is specifically activated by mechanics, is required for NC migration. It is expected that future studies will demonstrate the mechanical role of other mechanosensitive channels on NC development.

Cell adhesion proteins

Cell adhesion proteins allow cell anchorage to neighbour cells and the extracellular matrix (ECM). Changes in tissue tension, cell contractility, ECM stiffness and composition can be sensed and transmitted through adhesion proteins. Mechanotransduction via adhesion proteins leads to cytoskeletal re-arrangement via

physical interactions formed with the actin cytoskeleton, the recruitment of actin-binding proteins, such as vinculin, or the activation of the Rho family of small GTPases mediated by guanosine exchange factors (GEFs).

Cell-cell adhesion

Cell-cell adhesions allow for tissue integrity and communication. NC cells establish multiple cell-cell adhesions, including cadherin based and connexin junctions (Monier-Gavelle and Duband, 1995; Xu *et al.*, 2001; Kuriyama *et al.*, 2014; Scarpa *et al.*, 2015). The cadherin-catenin proteins are the most characterised cell-cell adhesion mechanosensors. Cadherins are transmembrane proteins that form homotypic interactions necessary for cell-cell adhesion. Intracellularly, cadherin binds to p120 catenin, β-catenin, and other proteins to form the adherens junction (AJ) complex (Figure 2A, D). AJs formed with E-cadherin, N-cadherin, or VE-cadherin have been established as mechanosensors in different experimental models (Coon *et al.*, 2015; Bays *et al.*, 2017; Labernadie *et al.*, 2017).

The AJ forms a link to the cortical actin filaments (F-actin) through α-catenin (Drees *et al.*, 2005), which ultimately connects the cytoskeleton of two neighbouring cells (Figure 2D). Vinculin recruitment by β and α-catenins stabilise AJ binding to F-actin and can further potentiate cadherin-mediated mechanotransduction (Peng *et al.*, 2010; Yao *et al.*, 2014) (Figure 2D). Additionally, AJs can sense intrinsic forces generated by actomyosin contractility and transfer them to neighbouring cells (Rauzi *et al.*, 2008; Martin *et al.*, 2010). Ultimately, AJs can further propagate cell-generated forces over long distances within a tissue (Barry, Wang and Leckband, 2015). Moreover, AJ strength and rigidity can modify the mechanical properties of a tissue by altering its stiffness, which neighbouring tissues can sense via cell-matrix adhesions (Ganz *et al.*, 2006; Tsai and Kam, 2009).

At the onset of NC cell migration, a cadherin switch from E-cadherin to N-cadherin is required to initiate epithelial to mesenchymal transition (EMT), which triggers NC migration (Duband *et al.*, 1995; Scarpa *et al.*, 2015). The loss of E-cadherin also leads to re-distribution of forces, repolarisation of actin protrusions, and contact inhibition of locomotion (Scarpa *et al.*, 2015), which contributes to NC directional collective behaviour. Additionally, cell-cell adhesion remodelling of the placode cells by NC cells

is required for modifying the mechanical properties of the placode tissue, allowing for a stiffness gradient of the placodes and collective durotaxis of NC cells (Shellard and Mayor, 2021). Changes in the mechanical properties of tissues adjacent to the NC are detected by cell-matrix interactions.

Cell-matrix adhesion

Cells bind to the extracellular matrix through integrin proteins. Intracellularly, integrins connect to the actin cytoskeleton by focal adhesion (FA) proteins, such as talin, paxillin, and vinculin. The integrin/FA complex provides an important channel for cell communication with its microenvironment. FAs are one the most widely studied cellular mechanosensors, able to detect mechanical stimuli from the ECM and transform them to downstream biochemical signalling. There are three main mechanisms triggered by FA-mediated mechanotransduction. *i*) signalling proteins, such as kinases and phosphatases, are recruited to the FA complex; some examples include Src, focal adhesion kinase (FAK), integrin-linked kinase (ILK), and receptor-like tyrosine phosphatase α (RPTP-α) (Figure 2E) (Cary and Guan, 1999; Mitra and Schlaepfer, 2006). *ii*) structural FA proteins, such as talin, paxillin, vinculin and zyxin, directly link integrin proteins to the actin cytoskeleton in a similar fashion to cell-cell adhesion proteins (Figure 2E) (Thompson *et al.*, 2014; Legerstee *et al.*, 2019). iii) the small Rho GTPases: RhoA, Rac and CDC42 can be activated downstream of FAs through GEFs (Scales and Parsons, 2011) (Figure 2E).

Communication of the ECM to the cytoskeleton of the cell through the integrin-FA complex is essential for NC attachment to the ECM, establishment of contact inhibition of locomotion and for collective migration *in vivo* (Desban and Duband, 1997; Testaz, Delannet and Duband, 1999; Roycroft *et al.*, 2018). Furthermore, as mentioned in section 2, mechanotransduction by FA proteins is required to trigger EMT and initiate NC migration *in vivo* (Barriga *et al.*, 2018). Additionally, FA proteins are necessary for NC cells to probe the stiffening of the placode cells during migration, thereby migrating following a durotaxis gradient *in vivo* (Shellard and Mayor, 2021).

FA proteins have been shown to co-localise with Piezo1 in HEK293 (Jetta *et al.*, 2021) and *Drosophila* gliomas (Chen *et al.*, 2018), suggesting a potential interaction between the two mechanosensors. It remains to be explored whether FA proteins

interact with Piezo1 in NC cells. It is also unknown how the different simultaneous mechanical forces are detected and discerned throughout NC development and whether the other mechanosensitive proteins expressed in NC cells have an active role in detecting forces and mechanotransduction of NC cells during embryonic development.

Concluding remarks

The expression and functionality of several cell membrane mechanosensors are required in NC cells during development, and their loss leads to craniofacial malformations and other developmental defects (Clouthier *et al.*, 1998; Ruest and Clouthier, 2009; Balczerski *et al.*, 2012). A functional role of the mechanosensitive ion channel Piezo1 in NC migration *in vivo* (Canales Coutiño and Mayor, 2021b) indicates that NC cells can detect mechanical forces via receptors other than cadherins and integrins. Specifically, in the case of GPRCs and MS ion channels, their activation and function during NC development have only been studied from a biochemical point of view. However, it is now known that receptors such as ETA, S1PR, PTH1R, and channels TRPV4 and TRPM7 can be activated by mechanical forces in the absence of ligands or other stimuli (Liedtke *et al.*, 2000; Mederos y Schnitzler *et al.*, 2008; Jung *et al.*, 2012). It remains to be investigated whether these proteins have a functional mechanosensory role in the NC. Given that processes such as NC differentiation and migration *in vivo* are regulated by mechanical stimuli (Barriga *et al.*, 2018; Li *et al.*, 2019; Shellard and Mayor, 2021), we hypothesize that mechanical forces could activate these transmembrane proteins during NC development. It is likely that many developmental processes, including neural crest formation, migration and differentiation, are initiated and regulated by cell membrane mechanosensors. Future research should explore how specific mechanical signals are detected and transduced by the NC at different stages during development. These processes are likely to be conserved in other developmental contexts.

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Declaration of Interests

The authors declare no competing interests

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Figure legends

Figure 1. Effect of mechanical forces on neural crest cells. (A-A') Illustration of sagittal sections of *Xenopus laevis* embryos at a non-migratory NC stage (stage 13) (A) and at the onset of NC cell migration (stage 20) (A'). As the mesoderm proliferates during embryonic development, the apparent elasticity of the tissue increases. Non-migratory NC cells adjacent to the mesoderm sense the increase in mesoderm stiffness through focal adhesion (FA) proteins, which triggers epithelial to mesenchymal transition (EMT) and NC collective migration. Additionally, migratory neural crest cells sense a stiffness gradient across the placode tissue and direct their migration via durotaxis **(B)** Neural crest stem cells (NCSC) derived from induced pluripotent stem cells (iPSC) cultured on gels of different stiffness undergo specific cell differentiation patterns. NCSC cultured on stiff gels (1 GPa) differentiate into smooth muscle cells after 3 days. NCSC cultured on soft gels (15 kPa) differentiate into Schwann glial cells after 3 days.

Figure 2. Cell membrane mechanosensors expressed by neural crest cells. (A) Illustration of two migratory neural crest (NC) cells which are forming cell-cell and cell-matrix contacts. Each mechanosensor is specified by a red star and a number, which are described in more detail in (B-E). **(B)** Mechanosensitive G-protein coupled receptors (GPCR). Upon mechanical activation, GPCRs activate phospholipase C (PLC) signalling. PLC mediates i) the conversion of IP2 to IP3, which can activate mechanosensitive ion channels and lead to increased Ca^{2+} levels; ii) the conversion of IP2 into membrane bound diacyl glycerol (DAG), which activates proteinase kinase C (PKC) and signalling downstream of this enzyme. **(C)** Mechanosensitive (MS) ion channels. Mechanical activation of TRP and Piezo1 channels allows entry of Ca^{2+} and Mg^{2+} ions and to the activation of signalling $Ca^{2+/}Mg^{2+}$-dependant signalling pathways. **(D)** Cadherin based cell-cell adhesions. Cell membrane tension by intrinsic actomyosin contractility or extracellular forces can be detected by cadherin-based junctions, which leads to actin remodelling via α-catenin unfolding and/or vinculin recruitment. **(E)** Integrin-focal adhesion complex. Forces exerted by the extracellular matrix are sensed by integrins proteins and propagated by focal adhesions (FA). Mechanotransduction via FA proteins leads to: i) the recruitment of signalling kinases (Src and FAK), ii) modification of stress fibres via vinculin binding, iii) small GTPase activation through guanosine exchange factors (GEFs).

Figure 1

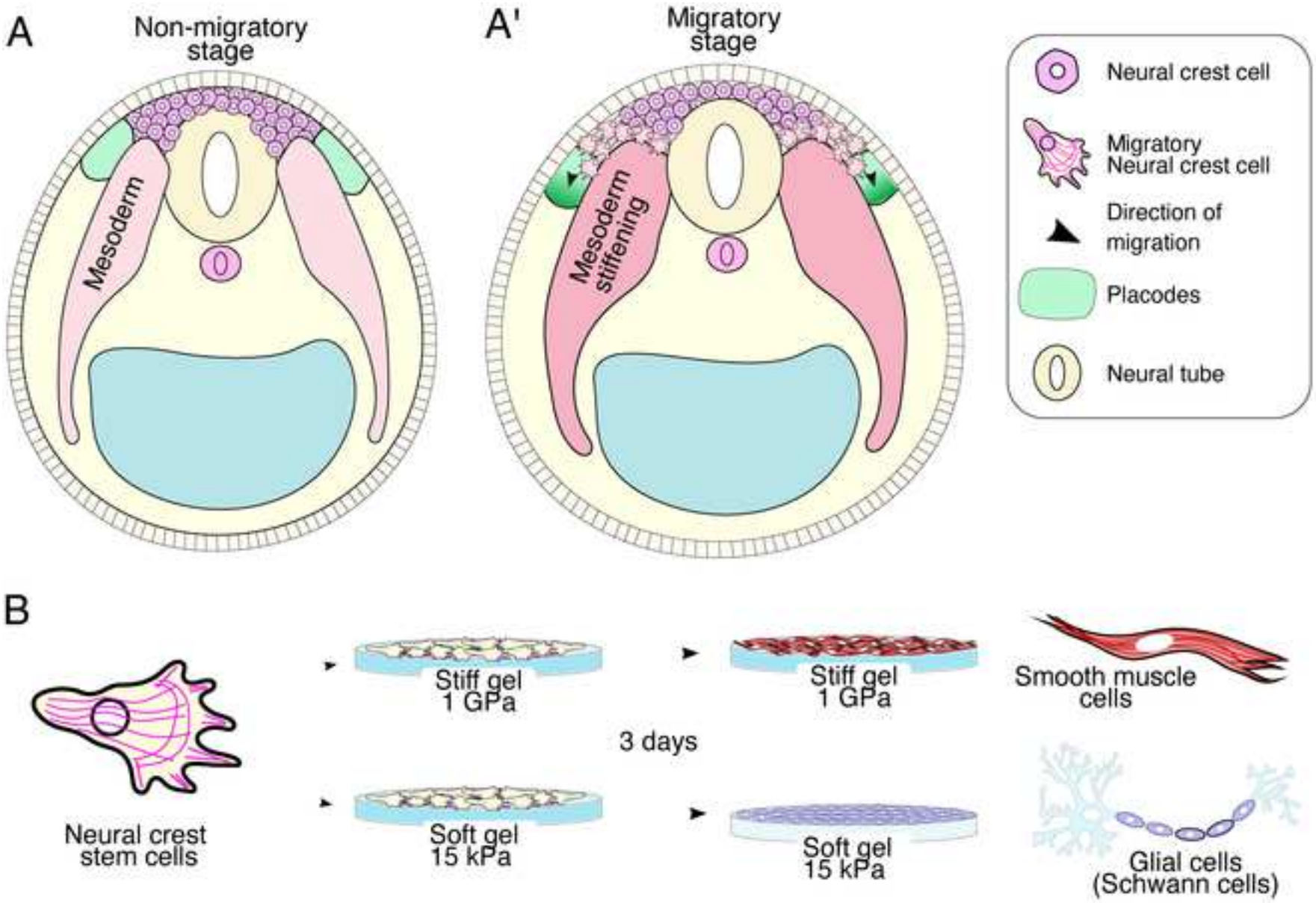

Figure 2