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Inducing your neighbors to become like you: cell recruitment in developmental patterning and growth

LUIS M. MUÑOZ-NAVA#, MARYCRUZ FLORES-FLORES# and MARCOS NAHMAD*

Department of Physiology, Biophysics, and Neurosciences, Center for Research and Advanced Studies of the National Polytechnical Institute (Cinvestav-IPN), Mexico

ABSTRACT Cell differentiation, proliferation, and morphogenesis are generally driven by instructive signals that are sent and interpreted by adjacent tissues, a process known as induction. Cell recruitment is a particular case of induction in which differentiated cells produce a signal that drives adjacent cells to differentiate into the same type as the inducers. Once recruited, these new cells may become inducers to continue the recruitment process, closing a feed-forward loop that propagates the growth of a specific cell-type population. So far, little attention has been given to cell recruitment as a developmental mechanism. Here, we review the components of cell recruitment and discuss its contribution to development in three different examples: the *Drosophila* wing, the vertebrate inner ear, and the mammalian thyroid gland. Finally, we posit some open questions about the role of cell recruitment in organ patterning and growth.

KEY WORDS: *cell recruitment, patterning, growth, Drosophila wing, inner ear, thyroid*

Introduction

In developmental biology, induction is defined as a two-component process that includes an *inducer* cell or tissue that produces a non-cell autonomous signal (*induction signal*); and a *responder* cell or tissue that is able to receive and respond to the induction signal (an ability referred as *competence*; Waddington, 1940; Gilbert and Barresi, 2016). Historically, the pre-molecular concept of induction attracted attention after the studies of Hans Spemann and Hilde Mangold in the 1920s (Spemann and Mangold, 1924; see Gilbert, 1996 for a historical review). Nowadays, induction has been described in a plethora of developing systems: from vulval cell determination in *C. elegans* (Schindler and Sherwood, 2013) to organizer centers that specify whole developmental programs in amphibian embryos (De Robertis, 2006; Martínez-Arias and Steventon, 2018) to vascular smooth-muscle cell differentiation in mice (Manderfield *et al*., 2012).

Induction interactions can be further classified depending on the nature of the induction signal and the type of response that it drives. For example, the induction signal may be *paracrine* and activate different cell types in a concentration-dependent manner, as in morphogen signaling gradients (Rogers and Schier, 2011; Sagner and Briscoe, 2017); or it can be a *juxtacrine* interaction that requires cell-to-cell contacts, as in Notch-dependent lateral induction (Sjöqvist and Andersson, 2019). Sometimes, the inducer can drive the responder to become a new inducer, thereby propagating the induction cascade either to other cells (*sequential induction*), or to the original inducer (*reciprocal induction*). Generally, the induction response contributes to the establishment of a particular cell fate (Perrimon *et al*., 2012), but it could also drive different cellular behaviors such as proliferation (Oesterle *et al*., 1997; Paterno and Gillespie, 1989; Fresno Vara *et al*., 2001; Cheesman *et al*., 2011), migration (Bauer *et al*., 1994; Arnold *et al.,* 2008; Cerrizuela *et al*., 2018), or polarization (Wallingford *et al*., 2000; Yang and Mlodzik, 2015; see Basson, 2012 for a review).

Inductive assimilation or cell recruitment is a particular case of induction in which the responder cell differentiates into the same fate of the inducer (Baena-López and García-Bellido, 2003; Zecca and Struhl, 2007a). In a cell recruitment process, we will refer to the inducer cell as *recruiter* and the responder cell as *recruitable*, whereas the induction signal will be referred as *recruitment signal* (Fig. 1). In recruitable cells, a general aspect of the response to the recruitment signal is the activation of the same transcription factor

Abbreviations used in this paper: D/V, dorsal/ventral; Ds, dachsous; *fj*, *four-jointed*; Ft, fat; Jag1, jagged1; NICD, notch intracellular domain; Nkx2.1, NK2 homeobox gene 1; Sd, scalloped; *vg*, *vestigial*; Wg, wingless; Wts, warts; Yki, yorkie.

***Address correspondence to:** Marcos Nahmad. Avenida Instituto Politécnico Nacional 2508, Colonia San Pedro Zacatenco, Delegación Gustavo A. Madero, Mexico City, 07360; Mexico. Tel: +52-55-5747-3800 x 3362. E-mail: mnahmad@fisio.cinvestav.mx - web: www.nahmadlab.com - https://orcid.org/0000-0001-6300-5608

#Note: These authors contributed equally to this work.

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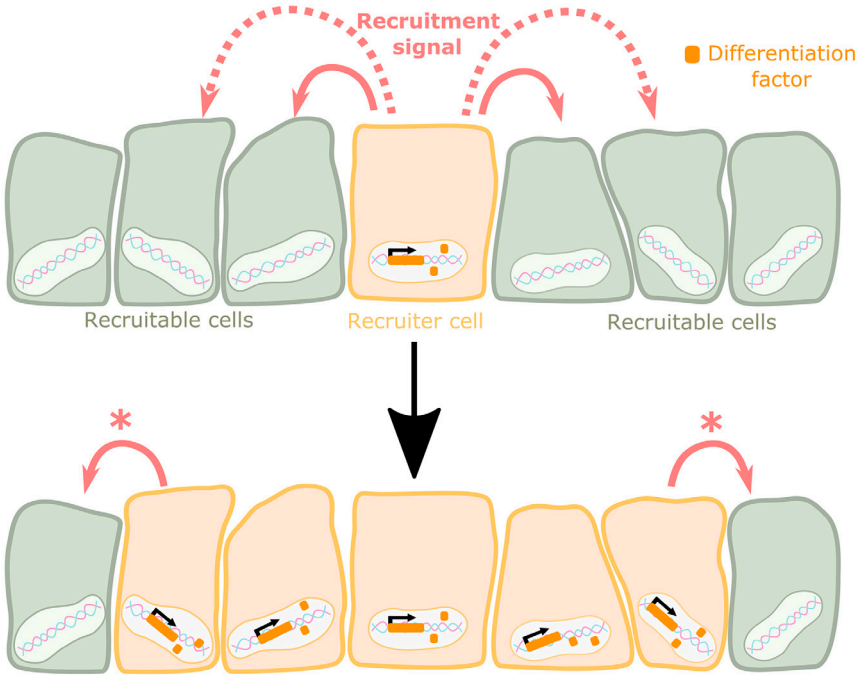

Fig. 1. Components of cell recruitment. *A recruiter cell (orange) that expresses a differentiation factor (orange squares), produces and sends a recruitment signal (pink arrows) to neighboring cells (green) that are competent to respond to the signal (recruitable cells). The recruitment signal may be juxtacrine (solid arrows) or paracrine (dotted arrows). Upon reception of the recruitment signal, recruitable cells activate the same differentiation factor as the recruiter cell. These newly-recruited cells may become recruiters themselves, propagating the recruitment signal in a sequential process (asterisks indicate that this is an optional component).*

that defines the recruiter's cell fate (*differentiation factor*; Fig. 1, orange squares). But other properties of cell recruitment vary from case to case. For example, the recruitment process could be local or act at a distance depending on whether the recruitment signal is juxtacrine or paracrine (Fig. 1, solid *vs.* dotted arrows). In some cases, the differentiation factor itself activates the recruitment signal, turning recruitable cells into recruiters and the process becomes sequential (Fig. 1, asterisks). Examples of cell recruitment have been reported in *Drosophila* wing development (Baéna-López and García-Bellido, 2003; Zecca and Struhl, 2007a; Zecca and Struhl, 2010), as well as in the developing vertebrate inner ear (Morrison *et al.*, 1999; Kiernan *et al.*, 2005; Kiernan *et al.*, 2006), thyroid gland (Fagman *et al.,* 2006; Lania *et al.*, 2009), kidney (Lindström *et al.,* 2018), and heart (Alfano *et al.*, 2019). However, several questions about the molecular and mechanistic aspects of cell recruitment remain open in each of these systems. Here, we review and compare some examples of cell recruitment under the concepts defined above and discuss how they contribute to developmental patterning and growth.

Cell recruitment drives patterning and growth in the *Drosophila* wing disc

The best-studied example of cell recruitment is the establishment of wing fate in the fruit fly, *Drosophila melanogaster* (Baéna-López and García-Bellido, 2003; Zecca and Struhl, 2007a; Zecca and Struhl, 2010; Muñoz-Nava *et al.*, 2020; Fig. 2). In *Drosophila*, appendages such as wings, legs, eyes, and antennae develop from larval precursor tissues known as imaginal discs. In the wing imaginal disc, not all the cells are committed to the wing fate itself. For example, cells from the notum are destined to the adult thorax, whereas other cells determine the hinge of the adult wing (Fig. 2A). Wing fate in *Drosophila* is determined by the selector gene, *vestigial* (*vg*), which is expressed in a particular area of the disc known as the wing pouch (Williams *et al.,* 1991; Williams *et al.,* 1993; Fig. 2A). *vg* expression originates in a narrow stripe of cells abutting the Dorsal/Ventral (D/V) boundary in response to Notch signaling (Irvine and Vogt, 1997; Klein and Martínez-Arias, 1999; Fig. 2A). The Vg pattern then expands in response to the Wingless (Wg) and Decapentaplegic (Dpp) signaling gradients (Kim *et al.,* 1996; Klein and Martínez-Arias, 1999), and cell proliferation (Pérez *et al.,* 2011). Although Wg and Dpp act as morphogens, neither of these signaling gradients appear to reach the edge of the wing pouch (Restrepo *et al.*, 2014; Chaudhary *et al.,* 2019). Therefore, it was unclear how the Vg pattern covers the whole wing pouch by the end of the third larval instar (Fig. 2A). Using genetic mosaics, previous studies showed that Vg expressing cells can propagate the activation of *vg* in a non-cell autonomous manner and proposed that a cell recruitment mechanism is taking place (Baena-López and García-Bellido, 2003; Zecca and Struhl, 2007a). We recently expanded these findings by using rapid fluorescent-reporter tools to directly visualize newly-recruited cells and showed that cell recruitment does take place in normal wing development (Muñoz-Nava *et al.*, 2020).

In this example of recruitment, *vg* is the differentiation factor that defines the recruitment process: recruiters are Vg-expressing cells located at the edges of the Vg pattern (Fig. 2B, orange cell), whereas recruitable cells are those that do not express Vg but are located within the rest of the wing pouch (Fig. 2B, green cells). The recruitment signal is driven by the polarization of the protocadherins Fat (Ft) and Dachsous (Ds) that form heterotypical bonds across the membranes of adjacent cells (Zecca and Struhl, 2010; Fig. 2B, inset i). The polarization signal is created by an asymmetric distribution of Ft-Ds bonds across their plasma membrane (Brittle *et al.*, 2012). In the wing pouch, Ft is uniformly expressed, but Ds is expressed in a gradient with low levels in Vg-expressing cells and high levels in non-Vg cells; this is because Vg (together with the TEAD transcription factor, Scalloped [Sd]) transcriptionally represses *ds* (Zecca and Struhl, 2010; Fig. 2B, inset ii). In addition, the Vg-Sd complex transcriptionally activates *four-jointed* (*fj*; Fig.

2B, inset ii), a gene encoding for a Golgi kinase that phosphorylates Ft and Ds (Fig. 2C, inset 1). Ft-Ds complexes are stable at the membrane when Ft is phosphorylated, and unstable when Ds is phosphorylated (Moscona and Monroy, 2017; Fig. 2C, inset 2). Before the recruitment signal, Ft and Ds are similarly expressed and no polarization occurs since Ft-Ds bonds are distributed uniformly throughout the cell membrane (Fig. 2B, inset i). Upon activation of the recruitment signal at the edges of the Vg domain (Fig. 2C), Vg-expressing cells produce high levels of Fj and low levels of Ds, biasing the formation and stabilization of Ft-Ds bonds at the recruitment front (Fig. 2C, inset 2). In recruitable cells, the polarization of Ft-Ds bonds results in the polarization of the atypical myosin Dachs (D); D then sequesters the kinase Warts (Wts) to the membrane, where it cannot phosphorylate Yorkie (Yki), the transcriptional factor downstream of the Wts-Hippo pathway (Misra and Irvine, 2016; Fig. 2D, inset 3; compare to Fig. 2B, inset iii). Unphosphorylated Yki binds Sd and the Yki-Sd complex enters the nucleus (Goulev *et al.*, 2008), where it promotes *vg* expression transcriptionally (Zecca and Struhl, 2010; Fig. 2D, inset 3) resulting in the recruitment of a new cell into the Vg domain (Fig. 2D). The cell recruitment process drives the continuous expansion of the Vg pattern and contributes to about 20% of total wing

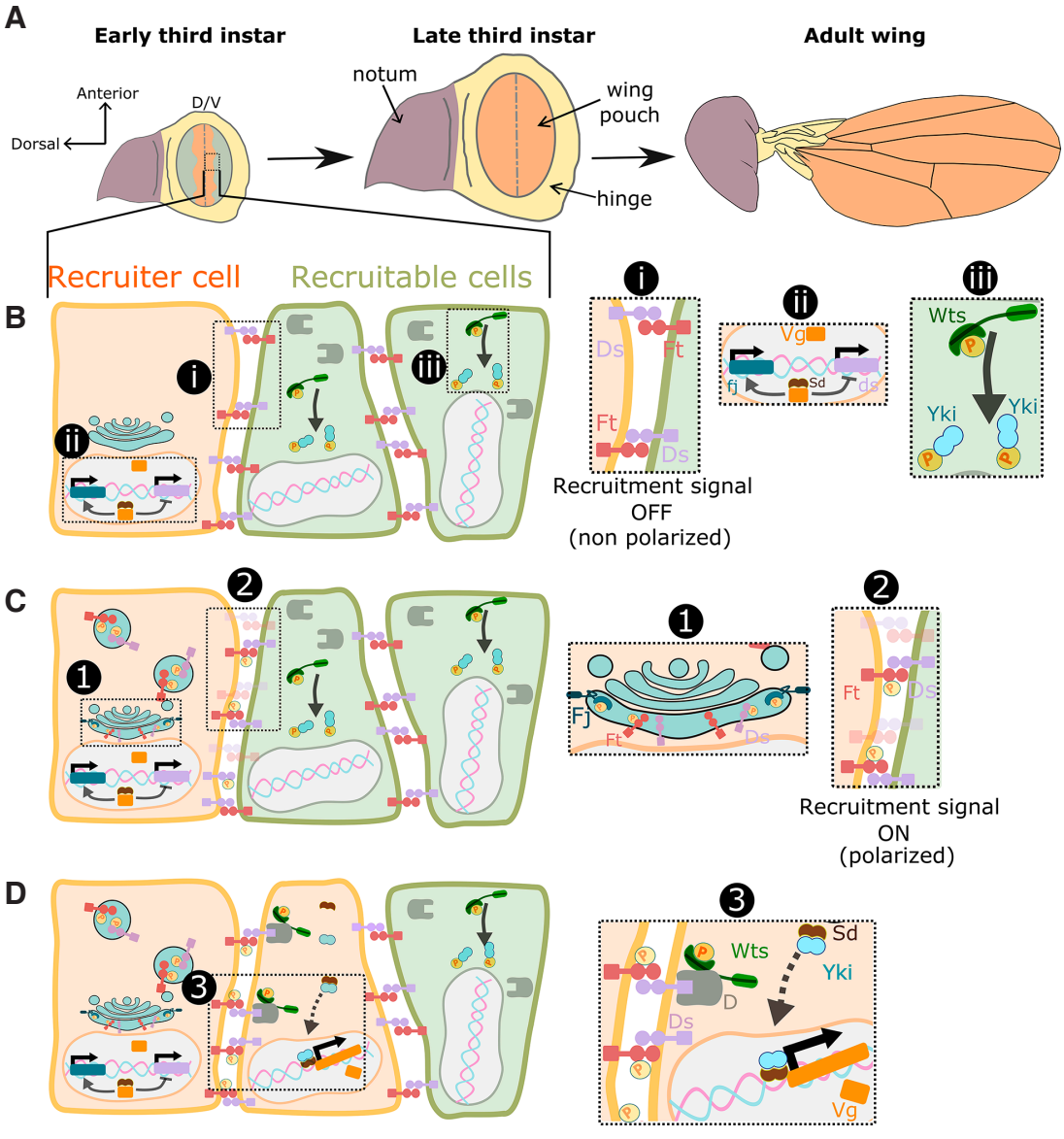

Fig. 2. Cell recruitment in the *Drosophila* wing disc. (A) *Cartoon of the* Drosophila *wing imaginal disc during early and late third larval instar, and the adult wing. Colored regions depict domains that correspond to specific structures in the adult wing. The Vg pattern (orange) is limited to a narrow stripe of cells abutting the D/V border in the early third instar and covers the whole wing pouch by the end of the third instar. Note that the Vg pattern determines the wing blade in the adult.* **(B)** *Amplification of three cells at the edge of the Vg pattern* prior to the start of the recruitment process. *Pre-recruitment conditions are as follow: Ft-Ds (light purple and red symbols) bonds in neighboring cells are distributed in a non-polarized manner across the plasma membrane of neighboring cells (inset i); in the recruiter cell (orange), Vg and Sd are just turned on, they enter the nucleus as a complex (orange and brown symbols) and begin to transcriptionally activate the* fj *gene (dark blue rectangle) and repress the* ds *gene (light purple rectangle; inset ii). All cells ubiquitously express Ft. In recruitable cells (green), the Wts-Hippo pathway is on and this results in phosphorylated Wts (green symbol; a P associated with a symbol represents it is phosphorylated), which then phosphorylates Yki (light blue symbol) and retains it at the cytoplasm (inset iii).* **(C)** *Amplification of three cells at the edge of the Vg pattern* at the time when recruiter cells are sending the recruitment signal. *Recruitment signal is activated by the phosphorylation of Ft and Ds at the Golgi by the Fj kinase (dark blue symbol; inset 1). Phosphorylated Ft – Unphosphorylated Ds bonds are stable at the membrane, while other Ft-Ds bonds are unstable (shaded; inset 2). This results in the polarization of Ft-Ds bonds at the boundary shared by the recruiter and the recruitable cells (recruitment front).* **(D)** *Amplification of three cells at the edge of the Vg pattern at the time when recruitable cells are interpreting the recruitment signal. Polarization of Ft-Ds results in the translocation of the D myosin (gray symbol) to the recruitment front (inset 3). D then sequesters Wts to the plasma membrane where it cannot longer phosphorylates Yki (inset 3). Unphosphorylated Yki binds Sd and enters the nucleus where they activate* vg *transcriptionally, completing the recruitment process (green cell turned into an orange cell).*

size (Muñoz-Nava *et al.*, 2020). Therefore, cell recruitment is a patterning-driven mechanism of growth in this system.

Despite this molecular and mechanistic understanding of cell recruitment in the *Drosophila* wing, several questions remain to be investigated. First, what determines the rate of cell recruitment? Since Vg controls the recruitment signal by transcriptionally repressing *ds* and activating *fj* (Fig. 2B, inset ii), it is possible that the strength of Ft-Ds polarization depends on Vg levels in the recruiter cell. Testing this hypothesis requires genetic manipulation of Vg levels, coupled with a time-lapse analysis of the recruitment process. Second, it is unclear what is the relationship between cell proliferation and cell recruitment. We showed that one mechanism couldn't rescue the other, suggesting that these mechanisms additively contribute to growth in this system (Muñoz-Nava *et al.*, 2020), but whether cell proliferation and cell recruitment rates are coupled in some way under normal circumstances remain to be investigated.

Finally, if a feed-forward signal is in place ensuring self-propagation of the Vg pattern, what is limiting cell recruitment in this system? Cells require basal levels of Vg in order to be competent for recruitment (Zecca and Struhl, 2007b), but how these levels are confined to the cells in the pouch is unclear. On the other hand, the *Drosophila* Tbx6 subfamily of genes *dorsocross* (*doc*)

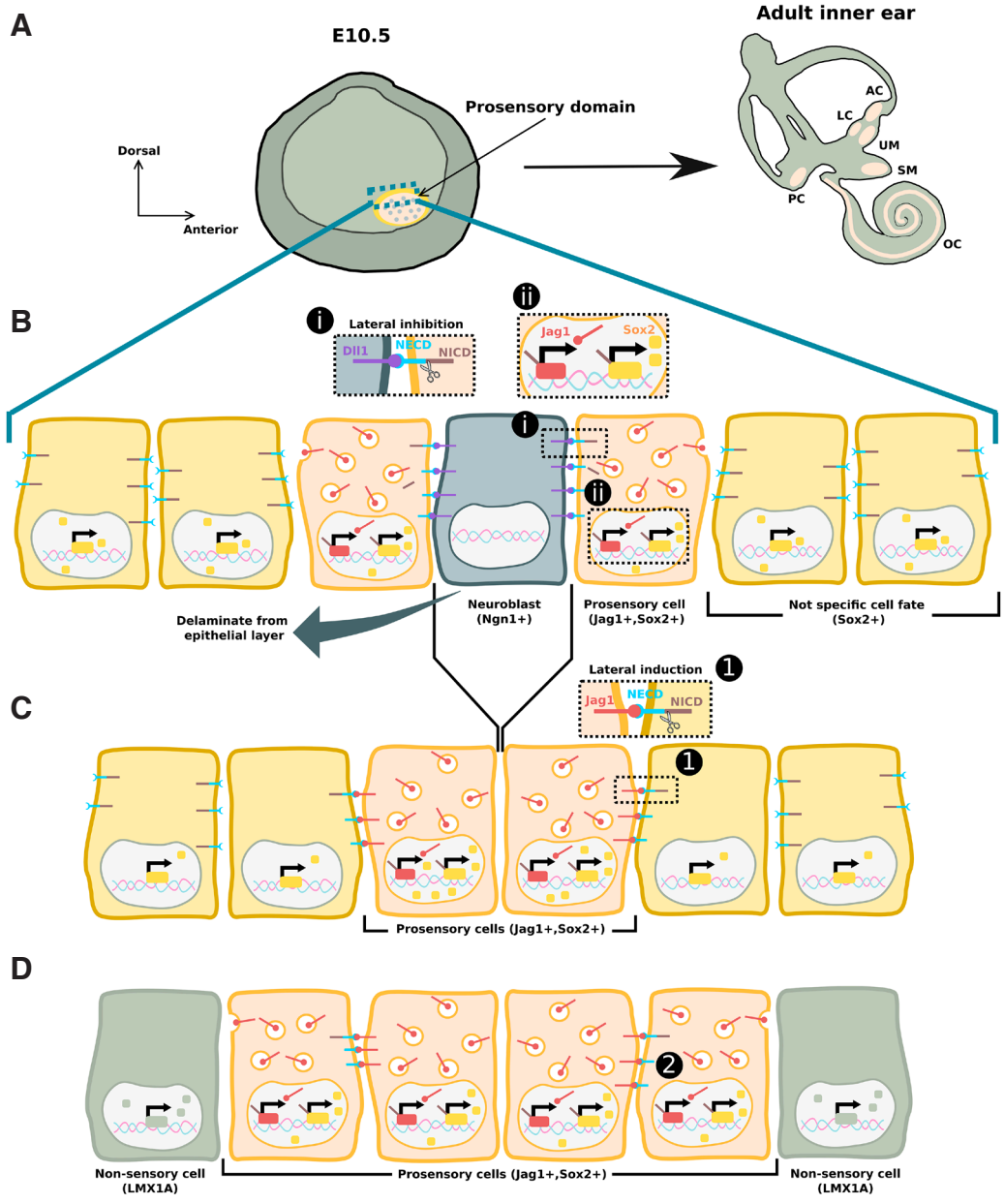

Fig. 3. Cell recruitment in the mouse inner ear. (A) *At E10.5, patches of cells in the otocyst transiently express Sox2 (yellow), but only a subset of these cells (referred as prosensory domains; marked in orange) will become sensory organs. These patches also contain neuroblasts (grey dots). In the mouse there are six of these prosensory domains that give rise to the following sensory organs in the adult inner ear: Anterior Crista (AC), Posterior Crista (PC), Lateral Crista (LC), Utricular Macula (UM), Saccular Macula (SM), and Organ of Corti (OC).* **(B)** *Amplification of cells at the edge of the prosensory domain prior to the start of the recruitment process. Ngn1-expressing neuroblasts express Dlt1 (purple symbols) that binds the NECD (light blue symbols) from neighboring cells, activating the cleavage of the NICD (brown symbols; inset i). This process activates Notch-dependent lateral inhibition in neighboring cells and results in the transcriptional activation of Jag1 (red rectangle) and Sox2 (yellow rectangle; inset ii). These cells (orange) become the first recruiters. Neuroblasts are then delaminated from the epithelial layer.* **(C)** *Amplification of cells at the edge of the prosensory domain at the time when recruiter cells are sending the recruitment signal. Prosensory cells (orange) that express high levels of Jag1 and Sox2 signal to neighboring cells through Notch signaling (and possibly through another Sox2-dependent mechanism as well; not shown). Particularly, Jag1 (red symbols) bind the NECD in neighboring (recruitable) cells (yellow), activating the cleavage of the NICD (inset 1).* **(D)** *Amplification of cells at the edge of the prosensory domain at the time when recruitable cells are interpreting the recruitment signal. Cleavage of the NICD in recruitable cells result in the feed-forward expression of Jag1 and Sox2 [marked with a number 2; same as inset ii, in (B)] completing the recruitment process (yellow cell turned into an orange cell). This process propagates for as long as transient Sox2 expression lasts. Once Sox2 levels drop, cells activate the expression of Lmx1a and differentiate into non-sensory cells (green).*

are expressed at the pouch-hinge boundary and Doc has been shown to transcriptionally repress *vg* (Sui *et al.*, 2012). Therefore, Doc might act as a break to limit the propagation of cell recruitment into the hinge.

Cell recruitment of prosensory cells in the development of the vertebrate inner ear

The inner ear of vertebrates has evolved to sense sound, linear/angular acceleration, and in some cases, magnetic fields (Biesel *et al.*, 2005; Wu and Dickman, 2011; Duncan and Fritzsch, 2012). A common feature in all these sensory functions is the specification of patches of prosensory cells during the development of the inner ear, a process known as prosensory specification (Hartman *et al.*, 2010). The molecular mechanism underlying prosensory specification has been studied in chick, fish, and mouse embryos, and in all cases they appear to rely on Notch signaling (Adam *et al.*, 1998; Haddon *et al.*, 1998; Lewis *et al.*, 1998). In mouse embryos, the inner ear develops from complex morphogenetic events in which the otic placode invaginates into a vesicle known as the otocyst (Wu and Kelley, 2012). At E10.5, prosensory patches are specified in the otocyst giving rise to six sensory organs (Fig. 3A). First, prosensory precursor cells are prevented from becoming Neurogenin1 (Ngn1)-expressing neuroblasts by an evolutionary-conserved mechanism known as lateral inhibition (Adam *et al.*, 1998; Haddon *et al.*, 1998; Eddison *et al.*, 2000): neuroblasts produce Delta-1, which binds to the Notch Extracellular Domain (NECD) in neighboring cells and induces the cleavage of its intracellular domain (Notch Intracellular Domain [NICD]; Fig. 3B, inset i), repressing the neuroblast fate. Neuroblasts are then delaminated from the epithelial layer (Fig. 3B) and two populations are derived from the remaining cells: prosensory cells that require the continuous expression of the Notch ligand, Jagged1 (Jag1; Morrison *et al.*, 1999; Kiernan *et al.*, 2006), as well as the HMG-box

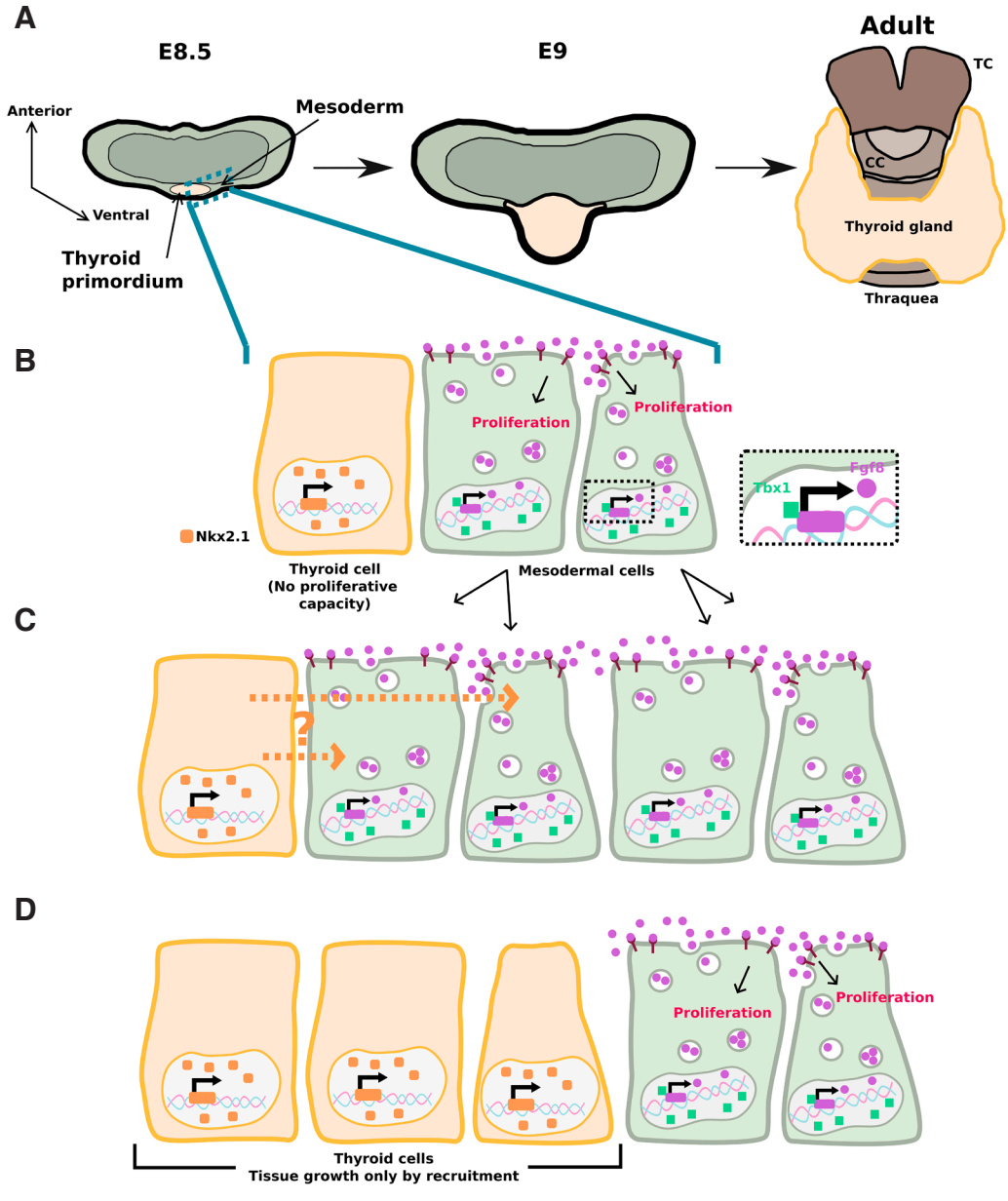

Fig. 4. Cell recruitment in the mouse thyroid gland. (A) *A small set of cells in the thyroid primordia (orange) grow dramatically between E8.5 and E9 without cell proliferation at the expense of mesoderm cells (light green). These cells become the thyroid gland, which surrounds the trachea (brown) in the adult. (TC, Thyroid Cartilage; CC, Cricoid Cartilage).* **(B)** *Amplification of cells at the edge of the thyroid primordia prior to the start of the recruitment process. Cells in the thyroid primordia (recruiter cells, orange) are defined by the expression of Nkx2.1 (orange squares), whereas mesodermal cells (recruitable cells, light green), marked by the expression of Tbx1 (green squares). Tbx1-expressing cells promote their proliferation in a non-cell autonomous manner by transcriptionally activating the gene (purple square) of Fgf8 (purple dots; inset).* **(C)** *Amplification of cells at the edge of the thyroid primordia at the time when recruiter cells are sending the recruitment signal. An unknown recruitment signal (represented by orange arrows) is sent from thyroid from mesoderm cells. It is unclear if this is a juxtacrine or a paracrine signal.* **(D)** *Amplification of cells at the edge of the thyroid primordia at the time when recruitable cells are interpreting the recruitment signal. Upon reception of the recruitment signal, cells become thyroid cells by activating Nkx2.1 (light green cells turned into orange cells). Since thyroid cells have no proliferative capacity, growth of this organ depends on the proliferation of mesoderm cells that are competent for cell recruitment.*

TABLE 1

COMPARISON OF RECRUITMENT PROCESSES IN DIFFERENT SYSTEMS

	Drosophila wing	Vertebrate inner ear	Mammalian thyroid
Differentiation factor(s) (recruiter cell type)	Vg (wing)	Jag1 and Sox2 (sensory organs)	Nkx2.1 (thyroid gland)
Recruitment signal (molecular players)	Ft-Ds polarization, coupled to inhibition of Wts-Hippo pathway	Jag/Notch lateral induction (Other: via Sox2?)	?
Recruitment signal range	Cell-to-cell	Cell-to-cell	?
Sequential recruitment	Yes	Yes	?
Competence factors (recruitable cells)	Low Vg	Transient Sox2	?
Contribution to organ growth	20 %	?	100% (relaying on cell proliferation of recruitable cells)
Inhibitors of recruitment	Doc	Lmx1a (cLmx1b)	Tbx1?

transcription factor, Sox2 (Kiernan *et al.*, 2005); and non-sensory cells that express the Notch signaling antagonist, LIM homeobox transcription factor 1-alpha (Lmx1a, Koo *et al.*, 2009). At first, Sox2 is expressed in all sensory and non-sensory precursors (Gu *et al.*, 2016; Steevens *et al.*, 2019; Fig. 3B), perhaps through Wnt signaling activity (Jayasena *et al.*, 2008). All these Sox2-expressing cells are competent to the prosensory fate (Hartman *et al.*, 2010; Pan *et al.*, 2010), but only those that maintain Notch signaling activity are specified into sensory cells (Daudet *et al.*, 2007; Mann *et al.*, 2017; Brown *et al.*, 2020). Therefore, the default state of these cells is the non-sensory fate and they require Notch-dependent lateral induction in order to become prosensory cells.

We argue that the lateral induction process involved in prosensory specification in the developing inner ear is indeed a cell recruitment mechanism. The first recruiter cells are defined by the lateral inhibition signal sent by neuroblasts that result in NICD-dependent transcriptional activation of Jag1 and Sox2 (Fig. 3B, inset ii). Upon delamination of the neuroblasts from the epithelium, Jag1 in recruiter cells binds and activates Notch in neighboring cells, propagating the recruitment signal laterally into recruitable cells (Fig. 3C, inset 1). Upon reception of the signal, NICD activates Jag1 and Sox2, and a new prosensory cell is recruited (Fig. 3D). These new prosensory cells then become recruiters and acquire the ability to propagate the feed-forward expression of Jag1 and Sox2 to their new neighbors. Thus, cell recruitment in this system is a cell-to-cell, sequential process.

Several questions remain open about this recruitment mechanism that deserve attention in future studies. Cell recruitment of prosensory cells via Notch activity prevents the expression of cLmx1b (the homolog of Lmx1a) in chicken embryos, suggesting that in addition to inducing the prosensory fate, cell recruitment also prevents cells to become non-sensory cells (Mann *et al.*, 2017). Moreover, Lmx1a overexpression experiments suggest that cells already expressing high levels of Lmx1a antagonize Notch signaling and are not able to be recruited (Mann *et al.*, 2017). Therefore, the extent of the recruitment process depends on counteracting Notch signaling and Lmx1a expression. An interesting hypothesis is that the early, but transient expression of Jag1 and Sox2 prevents early expression of Lmx1a and facilitates the initiation of lateral induction; however, as the transient expression of Jag1 and Sox2 drops, cells that have not been already recruited acquire Lmx1a expression and become non-competent for cell recruitment. These dynamics could limit the range of the recruitment process and define the final size of the sensory organs. In addition, the molecular mechanism by which Lmx1a and Notch signaling antagonize each other remains unclear.

Another interesting observation in this system is that ectopic Sox2 can induce itself non-autonomously, in a Jag1-independent manner (Pan *et al.*, 2013). The identity of the feed-forward recruitment signal initiated by Sox2 remains unknown, but suggests that Jag1-Notch signaling and Sox2 may drive, in parallel, the propagation of prosensory cells in the vertebrate inner ear. Perhaps, these apparently redundant mechanisms of recruitment provide some sort of robustness to the growth of the prosensory domain.

Cell recruitment contributes to growth of the developing mammalian thyroid gland

The thyroid gland develops from the pharyngeal endoderm and then undergoes growth and several morphogenetic events (Nilsson and Fagman, 2017; Fig. 4A). In the mouse, the early thyroid primordium, defined by NK2 homeobox 1 (Nkx2.1) expression, appears at embryonic day 8.5 (E8.5) and grows without cell proliferation (Fig. 4B, orange cell), suggesting that the formation of the thyroid bud occurs by recruitment of cells from outside the thyroidal placode (Fagman *et al.*, 2006). Tbx1, a member of the family of T-box transcription factors (Baldini *et al.*, 2017), is expressed in subpharyngeal mesoderm and promotes the proliferation of recruitable cells in an Fgf8-dependent manner (Lania *et al.*, 2009; Fig. 4B, inset). Lack of mesoderm-expressed Tbx1 reduces cell proliferation of potential thyroid precursors, but Fgf8 expression (using an *Fgf8* knock-in into the *Tbx1* locus) rescues the size of the Nkx2.1^{+} population at E10.5 (Lania *et al.*, 2009). Together, these results suggest that in this system, thyroid precursors, marked by the expression of the differentiation factor Nkx2.1, induce the expansion of the thyroid in a proliferation-independent manner, whereas Tbx1 expands the population of recruitable cells through Fgf8 signaling (Fig. 4C-D). However, the identity and nature (cell-to-cell or action at a distance) of the recruitment signal (Fig. 4C, arrows), as well as how this recruitment signal results in the expression of Nkx2.1 (Fig. 4D) have not been elucidated.

From the examples presented here, this system is the one in which the molecular mechanisms of cell recruitment are less understood. However, since recruiter cells in the thyroid primordium do not proliferate, cell recruitment is the leading mechanism of growth in this organ, relaying on the proliferation of recruitable cells. An interesting aspect of this system is that the balance of recruitment rate *vs.* proliferation rate of the recruitable population should determine the size of the thyroid gland. In particular, when the recruitment process extinguishes the population of recruitable cells, the thyroid primordium arrests its growth. This poses thyroid development as a very attractive model system to investigate organ growth control.

Outlook

Cell recruitment is a widespread mechanism during development, but it has received little attention in developmental biology. In this article, we provided a clear definition of the components involved in a cell recruitment process (Fig. 1) and discuss three examples in which cell recruitment participates as an induction process (Figs. 2-4). A comparison of the recruitment components

of these examples is presented in Table 1.

Of the examples presented here, the *Drosophila* wing is the only system in which the term 'cell recruitment' has been used and recognized in the primary literature. We hope that this article provides the conceptual framework to identify cell recruitment processes in other systems.

Future research on these and other models will address important aspects about how cell recruitment contributes to developmental patterning and growth, and how cell recruitment interacts with other developmental processes such as morphogenesis. One important question is to quantitatively determine what is the relative contribution of cell recruitment to organ growth. For example, cell recruitment has a modest contribution to growth in the *Drosophila* wing (20% of the adult size; Muñoz-Nava *et al*., 2020), but in the developing mammalian thyroid it appears to be the main contributor of growth (Fagman *et al*., 2006; Lania *et al*., 2009). The integration of cell recruitment with other developmental mechanisms, such as cell proliferation, cell growth, and apoptosis will provide a better understanding of how organs develop and attain a robust size and shape.

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