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Inducing your neighbors to become like you: Cell recruitment and its contribution to developmental patterning and growth

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Abbreviations: vestigial (vg); Dorsal/Ventral (D/V); Wingless (Wg); Decapentaplegic (Dpp); Fat (Ft); Dachsous (Ds); Scalloped (Sd); four-jointed (fj); Dachs (D); Warts (Wts); Yorkie (Yki); dorsocross (doc); Notch Extracellular Domain (NECD); Notch Intracellular Domain (NICD); Jagged1 (Jag1); LIM homeobox transcription factor 1-alpha (Lmx1a); NK2 homeobox gene 1 (Nkx2.1).

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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.
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; see 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; Spemann, 1938; 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 inducer itself (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; Cerriuella 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 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 recruitment process becomes sequential (Fig. 1, asterisks). Examples of cell
recruitment have been reported in both vertebrate and invertebrate development such as in the Drosophila wing (Baéna-López and García-Bellido, 2003; Zecca and Struhl, 2007a; Zecca and Struhl, 2010), as well as in the vertebrate inner ear (Morrison et al., 1999; Kiernan et al., 2005; Kiernan et al., 2006), thyroid (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

Perhaps 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). 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, Baena-López and García-Bellido, and then Zecca and Struhl, 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 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 (ff; 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 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 ff (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)* 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 acceleration, 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 a set of complex morphogenetic events in which the otic placode invaginates into a vesicle known as the otocyst (Wu and Kelley, 2012). Six sensory organs are derived from prosensory patches that originate in the otocyst at E10.5 (Fig. 3A). First, prosensory precursor cells are prevented to becoming Neurogenin1 (Ngn1)-expressing neuroblasts by an evolutionarily-conserved mechanism known as lateral inhibition (Adam et al., 1998; Haddon et al., 1998; Eddison et al., 2000) in which Delta-1 produced in neuroblasts 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 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., 2013), 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 in a recruitable cell, 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 the thyroid precursors, but Fgf8 expression (using an Fgf8 knock-in into the Tbx1 locus) rescues the size of the Nkx2.1+ population of cells 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 the 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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References

ADAM, J., MYAT, A., LE ROUX, I., EDDISON, M., HENRIQUE, D., ISH-HOROWICZ, D., LEWIS, J. (1998). Cell fate choices and the expression of Notch, Delta and Serrate homologues in the chick inner ear: Parallels with Drosophila sense-organ development. Development 125, 4645–4654.
ALFANO, D., ALTOMONTE, A., CORTES, C., BILIO, M., KELLY, R.G., BALDINI, A. (2019). Tbx1 regulates extracellular matrix-cell interactions in the second heart field. Hum. Mol. Genet. 28, 2295–2308.

ARNOLD, M., HIRSCHFELD-WARNEKEN, V.C., LOHMÜLLER, T., HEIL, P., BLÜMMEL, J., CAVALCANTI-ADAM, E.A., LÓPEZ-GARCÍA, M., WALTHER, P., KESSLER, H., GEIGER, B., SPATZ, J.P. (2008). Induction of cell polarization and migration by a gradient of nanoscale variations in adhesive ligand spacing. Nano Lett. 8, 2063–2069.

BAENA-LOPEZ, L.A. and GARCÍA-BELLIDO, A. (2003). Genetic requirements of vestigial in the regulation of Drosophila wing development. Development 130, 197–208.

BALDINI, A., FULCOLI, F.G., and ILLINGWORTH, E. (2017). Tbx1: Transcriptional and Developmental Functions. Current Topics in Developmental Biology (Elsevier Inc.). 122:223-243

BASSON, M.A. (2012). Signaling in cell differentiation and morphogenesis. Cold Spring Harb. Perspect. Biol. 4, 1–21.

BAUER, D. V., HUANG, S., MOODY, S.A. (1994). The cleavage stage origin of Spemann’s Organizer: Analysis of the movements of blastomere clones before and during gastrulation in Xenopus. Development 120, 1179–1189.

BEISEL, K.W., ROCHA-SANCHEZ, S.M., MORRIS, K.A., NIE, L., FENG, F., KACHAR, B., YAMOAH, E.N., FRITZSCH, B. (2005). Differential expression of KCNQ4 in inner hair cells and sensory neurons is the basis of progressive high-frequency hearing loss. J. Neurosci. 25, 9285–9293.

BRITTLE, A., THOMAS, C., and STRUTT, D. (2012). Planar polarity specification through asymmetric subcellular localization of fat and dachsous. Curr. Biol. 22, 907–914.

BROWN, R.M., NELSON, J.C., ZHANG, H., KIERNAN, A.E., GROVES, A.K. (2020). Notch-mediated lateral induction is necessary to maintain vestibular prosensory identity during inner ear development. Dev. Biol. 462, 78-84.

CERRIZUELA, S., VEGA-LÓPEZ, G.A., PALACIO, M.B., TRÍBULO, C., AYBAR, M.J. (2018). Gli2 is required for the induction and migration of Xenopus laevis neural crest. Mech. Dev. 154, 219–239.
CHAUDHARY, V., HINGOLE, S., FREI, J., PORT, F., STRUTT, D., BOUTROS, M. (2019). Robust Wnt signaling is maintained by a Wg protein gradient and Fz2 receptor activity in the developing Drosophila wing. Development 146, dev174789.

CHEESMAN, S.E., NEAL, J.T., MITTGE, E., SEREDICK, B.M., GUILLEMIN, K. (2011). Epithelial cell proliferation in the developing zebrafish intestine is regulated by the Wnt pathway and microbial signaling via Myd88. Proc. Natl. Acad. Sci. U. S. A. 108, 4570–4577.

DAUDET, N., ARIZA-MCNAUGHTON, L., LEWIS, J. (2007). Notch signalling is needed to maintain, but not to initiate, the formation of prosensory patches in the chick inner ear. Development 134, 2369–2378.

DUNCAN, J.S., FRITZSCH, B. (2012). Evolution of sound and balance perception: Innovations that aggregate single hair cells into the ear and transform a gravistatic sensor into the organ of corti. Anat. Rec. 295, 1760–1774.

EDDISON, M., LE ROUX, I., LEWIS, J. (2000). Notch signaling in the development of the inner ear: Lessons from Drosophila. Proc. Natl. Acad. Sci. U. S. A. 97, 11692–11699.

FAGMAN, H., ANDERSSON, L., and NILSSON, M. (2006). The developing mouse thyroid: Embryonic vessel contacts and parenchymal growth pattern during specification, budding, migration, and lobulation. Dev. Dyn. 235, 444–455.

FRESNO VARA, J.A., DOMÍNGUEZ CÁCERES, M.A., SILVA, A., MARTÍN-PÉREZ, J. (2001). Src family kinases are required for prolactin induction of cell proliferation. Mol. Biol. Cell 12, 2171–2183.

GILBERT, S.F. and BARRESI, M.J.F. (11th edition) (2016). Developmental Biology. Sinauer Associates, Inc.

GILBERT, S.F. (1996). A brief history of premolecular induction studies. Semin. Cell Dev. Biol. 7, 67–76.

GOULEV, Y., FAUNY, J.D., GONZALEZ-MARTI, B., FLAGIELLO, D., SILBER, J., ZIDER, A. (2008). SCALLOPED Interacts with YORKIE, the Nuclear Effector of the Hippo Tumor-Suppressor Pathway in Drosophila. Curr. Biol. 18, 435–441.
GU, R., BROWN, R.M., HSU, C.W., CAI, T., CROWDER, A.L., PIAZZA, V.G., VADAKKAN, T.J., DICKINSON, M.E., GROVES, A.K. (2016). Lineage tracing of Sox2-expressing progenitor cells in the mouse inner ear reveals a broad contribution to non-sensory tissues and insights into the origin of the organ of Corti. Dev. Biol. 414, 72–84.

HADDON, C., JIANG, Y.J., SMITHERS, L., LEWIS, J. (1998). Delta-Notch signalling and the patterning of sensory cell differentiation in the zebrafish ear: Evidence from the mind bomb mutant. Development 125, 4637–4644.

HARTMAN, B.H., REH, T.A., and BERMINGHAM-MCDONOGH, O. (2010). Notch signaling specifies prosensory domains via lateral induction in the developing mammalian inner ear. Proc. Natl. Acad. Sci. 107, 15792–15797.

HEBERLEIN, U., SINGH, C.M., LUK, A.Y., DONOHOE, T.J. (1995). Growth and differentiation in the Drosophila eye coordinated by hedgehog. Nature. 373, 709-711.

IRVINE, K.D., and VOGLT, T.F. (1997). Dorsal-ventral signaling in limb development. Curr. Opin. Cell Biol. 9, 867–876.

JAYASENA, C.S., OHYAMA, T., SEGIL, N., GROVES, A.K. (2008). Notch signaling augments the canonical Wnt pathway to specify the size of the otic placode. Development 135, 2251–2261.

JEON, S.J., FUJIOKA, M., KIM, S.C., and EDGE, A.S.B. (2011). Notch signaling alters sensory or neuronal cell fate specification of inner ear stem cells. J. Neurosci. 31, 8351–8358.

KIERNAN, A.E., PELLING, A.L., LEUNG, K.K.H., TANG, A.S.P., BELL, D.M., TEASE, C., LOVELL-BADGE, R., STEEL, K.P., CHEAH, K.S.E. (2005). Sox2 is required for sensory organ development in the mammalian inner ear. Nature 434, 1031–1035.

KIERNAN, A.E., XU, J., and GRIDLEY, T. (2006). The notch ligand JAG1 is required for sensory progenitor development in the mammalian inner ear. PLoS Genet. 2, 27–38.

KIM, J., SEBRING, A., ESCH, J.J., KRAUS, M.E., VORWERK, K., MAGEE, J., and CARROLL, S.B. (1996). Integration of positional signals and regulation of wing formation and identity by Drosophila vestigial gene. Nature 382, 133–138.
KLEIN, T., and MARTÍNEZ-ARIAS, A.M. (1998). Different spatial and temporal interactions between Notch, wingless, and vestigial specify proximal and distal pattern elements of the wing in Drosophila. Dev. Biol. 194, 196–212.

KLEIN, T., MARTÍNEZ-ARIAS, A. (1999). The Vestigial gene product provides a molecular context for the interpretation of signals during the development of the wing in Drosophila. Development 126, 913–925.

KOO, S.K., HILL, J.K., HWANG, C.H., LIN, Z.S., MILLEN, K.J., WU, D.K. (2009). Lmx1a maintains proper neurogenic, sensory, and non-sensory domains in the mammalian inner ear. Dev. Biol. 333, 14–25.

LANIA, G., ZHANG, Z., HUYNH, T., CAPRIO, C., MOON, A.M., VITELLI, F., and BALDINI, A. (2009). Early thyroid development requires a Tbx1-Fgf8 pathway. Dev. Biol. 328, 109–117.

LEWIS, A.K., FRANTZ, G.D., CARPENTER, D.A., DE SAUVAGE, F.J., GAO, W.Q. (1998). Distinct expression patterns of notch family receptors and ligands during development of the mammalian inner ear. Mech. Dev. 78, 159–163.

LINDSTRÖM, N.O., DE SENA BRANDINE, G., TRAN, T., RANSICK, A., SUH, G., GUO, J., KIM, A.D., PARVEZ, R.K., RUFFINS, S.W., RUTLEDGE, E.A., ET AL. (2018). Progressive Recruitment of Mesenchymal Progenitors Reveals a Time-Dependent Process of Cell Fate Acquisition in Mouse and Human Nephrogenesis. Dev. Cell 45, 651-660.e4.

LIU, Z., WALTERS, B.J., OWEN, T., BRIMBLE, M.A., STEIGELMAN, K.A., ZHANG, L.L., LAGARDE, M.M.M., VALENTINE, M.B., YU, Y., COX, B.C., ET AL. (2012). Regulation of p27 kip1 by Sox2 maintains quiescence of inner pillar cells in the murine auditory sensory epithelium. J. Neurosci. 32, 10530–10540.

MANDERFIELD, L.J., HIGH, F.A., ENGLEKA, K.A., LIU, F., LI, L., RENTSCHLER, S., and EPSTEIN, J.A. (2012). Notch activation of Jagged1 contributes to the assembly of the arterial wall. Circulation 125, 314–323.

MANN, Z.F., GÁLVEZ, H., PEDRENO, D., CHEN, Z., CHRYSOSTOMOU, E., ŽAK, M., KANG, M., CANDEN, E., DAUDET, N. (2017). Shaping of inner ear sensory organs through antagonistic interactions between Notch signalling and Lmx1a. Elife 6, 1–29.
MARTÍNEZ-ARIAS, A., and STEVENTON, B. (2018). On the nature and function of organizers. Development 145, dev159525.

MISRA, J.R., and IRVINE, K.D. (2016). Yamana Couples Fat Signaling to the Hippo Pathway. Dev. Cell 39, 254–266.

MORRISON, A., HODGETTS, C., GOSSLER, A., HRABÉ DE ANGELIS, M., LEWIS, J. (1999). Expression of Delta1 and Serrate1 (Jagged 1) in the mouse inner ear. Mech. Dev. 84, 169–172.

MOSCONA A. A. and MONROY A. (1ST edition) (2017). Current Topics in Developmental Biology. Elsevier Inc.

MUÑOZ-NAVA, L.M., ALVAREZ, H.A., FLORES-FLORES, M., CHARA, O., and NAHMAD, M. (2020). Cell Recruitment Drives Growth of the Drosophila Wing by Overscaling the Vestigial Expression Pattern. Dev. Biol. 462, 141–151.

NEVES, J., PARADA, C., CHAMIZO, M., and GIRÁLDEZ, F. (2011). Jagged 1 regulates the restriction of Sox2 expression in the developing chicken inner ear: A mechanism for sensory organ specification. Development 138, 735–744.

NILSSON, M., and FAGMAN, H. (2017). Development of the thyroid gland. Dev. 144, 2123–2140.

OESTERLE, E.C., TSUE, T.T., RUBEL, E.W. (1997). Induction of cell proliferation in avian inner ear sensory epithelia by insulin-like growth factor-I and insulin. J. Comp. Neurol. 380, 262–274.

PAN, W., JIN, Y., STANGER, B., and KIERNAN, A.E. (2010). Notch signaling is required for the generation of hair cells and supporting cells in the mammalian inner ear. Proc. Natl. Acad. Sci. U. S. A. 107, 15798–15803.

PAN, W., JIN, Y., CHEN, J., ROTTIER, R.J., STEEL, K.P., and KIERNAN, A.E. (2013). Ectopic expression of activated notch or SOX2 reveals similar and unique roles in the development of the sensory cell progenitors in the mammalian inner ear. J. Neurosci. 33, 16146–16157.

PATERNO, G.D., GILLESPIE, L.L. (1989). Fibroblast growth factor and transforming growth factor beta in early embryonic development. Prog. Growth Factor Res. 1, 79–88.
PÉREZ, L., BARRIO, L., CANO, D., FIUZA, U.-M., MUZZOPAPPA, M., MILÁN, M. (2011). Enhancer-PRE communication contributes to the expansion of gene expression domains in proliferating primordia. Development 138, 3125–34.

PERRUIMON, N., PITSOULI, C., SHILO, B.Z. (2012). Signaling mechanisms controlling cell fate and embryonic patterning. Cold Spring Harb. Perspect. Biol. 4, 1–18.

RESTREPO, S., ZARTMAN, J.J., BASLER, K. (2014). Coordination of patterning and growth by the morphogen DPP. Curr. Biol. 24, R245–R255.

DE ROBERTIS, E.M. (2006). Spemann’s organizer and self-regulation in amphibian embryos. Nat. Rev. Mol. Cell Biol. 7, 296–302.

ROGERS, K.W., and SCHIER, A.F. (2011). Morphogen Gradients: From Generation to Interpretation. Annu. Rev. Cell Dev. Biol. 27, 377–407.

SAGNER, A., and BRISCOE, J. (2017). Morphogen interpretation: concentration, time, competence, and signaling dynamics. Wiley Interdiscip. Rev. Dev. Biol. 6, 1–19.

SCHINDLER, A.J., and SHERWOOD, D.R. (2013). Morphogenesis of the Caenorhabditis elegans vulva. Wiley Interdiscip. Rev. Dev. Biol. 2, 75–95.

SJÖQVIST, M., and ANDERSSON, E.R. (2019). Do as I say, Not(ch) as I do: Lateral control of cell fate. Dev. Biol. 447, 58–70.

STEEVENS, A.R., GLATZER, J.C., KELLOGG, C.C., LOW, W.C., SANTI, P.A., KIERNAN, A.E. (2019). SOX2 is required for inner ear growth and cochlear nonsensory formation before sensory development. Dev. 146.

STRUTT, D.I., WIERSDORFF, V., MLODZIK, M. (1995). Regulation of furrow progression in the Drosophila eye by cAMP-dependent protein kinase A. Nature 373, 705–709.

SUI, L., PFLUGFELDER, G.O., SHEN, J. (2012). The Dorsocross T-box transcription factors promote tissue morphogenesis in the Drosophila wing imaginal disc. Development 139, 2773–2782.

WALLINGFORD, J.B., ROWNING, B.A., VOGELL, K.M., ROTHBÄCHER, U., FRASER, S.E., HARLAND, R.M. (2000). Dishevelled controls cell polarity during Xenopus gastrulation. Nature 405, 81–85.
WILLIAMS, J. A, BELL, J.B., and CARROLL, S.B. (1991). Control of Drosophila wing and haltere development by the nuclear vestigial gene product. Genes Dev. 5, 2481–2495.

WILLIAMS, J. A, Paddock, S.W., and CARROLL, S.B. (1993). Pattern formation in a secondary field: a hierarchy of regulatory genes subdivides the developing Drosophila wing disc into discrete subregions. Development 117, 571–584.

WU, D.K., and KELLEY, M.W. (2012). Molecular mechanisms of inner ear development. Cold Spring Harb. Perspect. Biol. 4.

WU, L.Q., DICKMAN, J.D. (2011). Magnetoreception in an avian brain in part mediated by inner ear lagena. Curr. Biol. 21, 418–423.

YANG, Y., MLODZIK, M. (2015). Wnt-Frizzled/Planar Cell Polarity Signaling: Cellular Orientation by Facing the Wind (Wnt). Annu. Rev. Cell Dev. Biol. 31, 623–646.

ZECCA, M., and STRUHL, G. (2007a). Recruitment of cells into the Drosophila wing primordium by a feed-forward circuit of vestigial autoregulation. Development 134, 3001–3010.

ZECCA, M., and STRUHL, G. (2007b). Control of Drosophila wing growth by the vestigial quadrant enhancer. Development 134, 3011–3020.

ZECCA, M., and STRUHL, G. (2010). A Feed-Forward Circuit Linking Wingless, Fat-Dachsous Signaling, and the Warts-Hippo Pathway to Drosophila Wing Growth. PLoS Biol. 8, e1000386.

Tables and Figure Legends

**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).
**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).

**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).

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

| Differentiation factor(s) (recruiter cell type) | Drosophila wing | Vertebrate inner ear | Mammalian thyroid |
|------------------------------------------------|-----------------|----------------------|------------------|
|                                                 | 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 expression | Transient Sox2      | ? |
| Contribution to organ growth                    | 20 %            | ?                    | 100% (relaying on cell proliferation of recruitable cells) |
| Inhibitors of recruitment                       | Doc             | Lmx1a (cLmx1b)       | Tbx1?            |
Figure 4

A

E8.5

Anterior

Ventral

Thyroid primordium

Mesoderm

E9

Adult

Thyroid gland

Thraquea

B

Thyroid cell
(No proliferative capacity)

Mesodermal cells

Nkx2.1

C

D

Thyroid cells

Tissue growth only by recruitment

Proliferation