Regulation of cell differentiation by Eph receptor and ephrin signaling

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Abbreviations: Eph receptor, Erythropoietin-producing hepatocellular carcinoma cell receptor; ephrin, Eph receptor interacting protein; FGF, Fibroblast growth factor; IGF-1, Insulin-like growth factor-1; JNK, c-Jun N-terminal kinase; MAPK, Mitogen activated protein kinase; NFAT, Nuclear factor of activated T-cells; p120GAP, GTPase activating protein; RGS3, Regulator of G-protein signaling 3; STAT3, Signal transducer and activator of transcription 3; TAZ, Tafazzin; TCR, T cell receptor; TEC, Thymic epithelial cell; TGF, Transforming growth factor; ZHX2, Zinc fingers and homeboxes 2

There is increasing evidence that in addition to having major roles in morphogenesis, in some tissues Eph receptor and ephrin signaling regulates the differentiation of cells. In one mode of deployment, cell contact dependent Eph-ephrin activation induces a distinct fate of cells at the interface of their expression domains, for example in early ascidian embryos and in the vertebrate hindbrain. In another mode, overlapping Eph receptor and ephrin expression underlies activation within a cell population, which promotes or inhibits cell differentiation in bone remodelling, neural progenitors and keratinocytes. Eph-ephrin activation also contributes to formation of the appropriate number of progenitor cells by increasing or decreasing cell proliferation. These multiple roles of Eph receptor and ephrin signaling may enable a coupling between morphogenesis and the differentiation and proliferation of cells.

Introduction

The generation of organized tissues during development requires coordinated mechanisms which regulate the differentiation of cells at the appropriate time and place. A central role is played by local cell-cell signaling which upregulates specific sets of genes that underlie the differentiated state. The generation of a pattern of distinct regions or cell types within the tissue can involve diffusible signals which induce different fates in a concentration-dependent manner, and cell contact dependent signals which influence the differentiation of their immediate neighbors. A key question is how the spatial organization of cell types that arises from these interactions is maintained during tissue growth and morphogenesis, in which cell division and intercalation can potentially scramble the pattern. A number of mechanisms involving the control of cell adhesion, cortical tension or cell repulsion have been uncovered which stabilize tissue organization by driving segregation and restricting intermingling between distinct cell populations.1-3

Eph receptors and ephrins are major players in morphogenesis, in which they act to establish and maintain the organization of cell types or of regional domains within tissues.4-7 With some exceptions, Eph receptors and ephrins fall into 2 binding specificity classes in which EphA receptors bind to GPI-anchored ephrinAs, and EphB receptors to transmembrane ephrinBs.5,7 The ability of Eph receptor and ephrin proteins to each transduce signals enables bi-directional signaling at the interface of complementary Eph-ephrin expression, or co-activation within cells expressing both Eph receptor and ephrin. The control of cell migration by Eph-ephrin interactions involves a complex network of protein phosphorylation cascades, as well as other intracellular pathways, which regulate assembly of the actin cytoskeleton and/or the strength of cell adhesion.5,8,9 Eph receptor and ephrin signaling can have contrary effects, such as promoting or restricting cell migration, which can differ for EphA-ephrinA compared to EphB-ephrinB activation.8,10

In many contexts in which Eph receptor and ephrin signaling has been studied, it is reasonable to assume that they are effectors of morphogenesis expressed downstream of transcription factors that regulate cell identity. Direct evidence has been obtained, for example, in studies of the regulation of Eph receptor expression in hindbrain segmentation,11-15 in which Eph-ephrin interactions restrict the intermingling of cells between segments. Another well-studied example is regulation of the graded expression of Eph receptors and ephrins in the retina,16-21 which underlies topographic mapping of axons to the appropriate location in the brain. This hierarchical relationship ensures that cells with a specific identity are maintained at the correct location, and that a neuron at a specific position projects its axon to the appropriate target. However, in some tissues Eph-ephrin activation can itself regulate cell differentiation through signal transduction pathways which lead to changes in gene expression. As well as providing a cell contact dependent signaling mechanism for the control of cell differentiation, this may enable a direct coupling between cell type specification and the maintenance of cell positioning.

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This review will focus on roles which have been uncovered for the control of cell differentiation by Eph-ephrin signaling, and the implications of these findings for the coordination of developmental mechanisms.

Primary Versus Secondary Effects on Cell Differentiation

Before discussing roles of Eph-ephrin signaling in the regulation of cell differentiation, it is important to point out a potential difficulty that affects some approaches. In experiments in which the disruption of Eph or ephrin function leads to an altered location of cell or tissue types, this could expose cells to a new environment in which other signals affect their differentiation. This is a significant problem in the nervous system, where radial and sometimes tangential migration of cells from the neural epithelium is an integral aspect of neuronal differentiation. It has therefore been recognized in a number of studies that it is difficult to distinguish primary effects of Eph-ephrin signaling from those which may be secondary to changes in cell migration or tissue organization, for example following gene knockout or transient blocking of function. Similar considerations apply to roles of Eph-ephrin signaling in thymocyte development, in which the progression of differentiation is linked to migration through thymic stroma where they interact with thymic epithelial cells. Evidence that Eph-ephrin signaling directly regulates cell differentiation has been obtained by several strategies, including in vivo studies in which cell positioning is not altered, or by identifying the intracellular pathway(s) that links Eph-ephrin activation to the regulation of gene expression. Another valuable approach comes from using unclustered or clustered Eph-Fc/ephrin-Fc fusion proteins as blocking or activating reagents, respectively, in in vitro cell culture; a caveat to some studies is that dimeric Fc fusions can induce weak activation rather than block and that clustered Fc proteins can activate a different signaling response from membrane-bound ligand. A related difficulty concerns the effects of altered Eph-ephrin signaling on cell proliferation. While this can be direct, for example through regulation of the MAPK pathway, an increase in cell proliferation could in some cases be due to a compensatory mechanism secondary to apoptotic loss of cells.

Regulation of Cell Fate in Ascidian Embryogenesis

Clear evidence for roles of Eph-ephrin signaling in the control of cell differentiation has come from studies in a primitive chordate, the ascidian Ciona intestinalis. At early stages of Ciona development, some cell divisions generate daughter cells which have a distinct fate. An example is the generation of a pair of sibling cells, one of which becomes a notochord cell and the other a neural cell. Previous work had shown that activation of the MAPK pathway by FGF signaling has a key role in regulating this cell fate decision: high MAPK activation specifies a notochord fate, whereas low MAPK activation specifies a neural fate. However, the FGF ligand is expressed widely, including in the mother cell of the notochord and neural cells, raising the question of how the difference in fate is established. Elegant studies revealed that the key factor is ephrinA, which is expressed in adjacent ectoderm precursors that interact with one but not the other sibling. The sibling cell which is not adjacent to an ephrinA-expressing cell maintains high MAPK activity and thus acquires a notochord fate (Fig. 1A). In contrast, in the sibling adjacent to an ephrinA-expressing cell, Eph receptor activation inhibits the MAPK pathway through p120GAP, creating low MAPK activity and thus a neural fate (Fig. 1A). MAPK inhibition by Eph activation also contributes to specification of the adjacent epidermal cells, in cooperation with Admp and Gdf signals that repress neural genes which might otherwise be induced by residual MAPK activity. Studies of endomesoderm differentiation revealed that ephrinA-mediated inhibition of FGF-activated MAPK also generates the asymmetric fate of mesoderm (low MAPK) and endoderm (high MAPK) cells. Likewise, the inhibition of MAPK activity by ephrinA activation of Eph receptor contributes to the choice of neuronal subtype in the motor ganglion.

These studies have shown that in Ciona an ephrin underlies cell fate choice in multiple lineages by acting as a cell contact dependent signal which inhibits the signaling pathway of the fusible Fgf ligand. Since inhibition of the MAPK pathway is a common effect of Eph receptor activation, these findings raise the prospect that analogous mechanisms occur more widely where the level of MAPK activity is involved in cell fate regulation. Indeed, as will be discussed below, Eph receptor mediated inhibition of the MAPK pathway plays such a role in neural progenitors, keratinocytes and thymocytes. Likewise, studies in cell culture suggest that suppression of the MAPK pathway by EphA receptor activation enables IGF-1 signaling to induce myogenic differentiation. Since in other contexts Eph receptors instead activate the MAPK pathway, there could also be a distinct relationship in which Eph receptors synergise or have overlapping functions with Fgf signaling.

Borders and Boundary Cells in the Vertebrate Hindbrain

Another example of roles of Eph-ephrin signaling in cell fate choice comes from studies of the vertebrate hindbrain. The hindbrain is subdivided into a series of segments, each of which has a distinct anteroposterior identity and forms a sharp compartment border with its neighbors. At the interface of the segments, specialized boundary cells form which have distinct properties from non-boundary regions of the hindbrain. Boundary cell formation is marked by expression of a number of genes, including the Notch modulator Rfng, which is required to maintain boundary cells as progenitors by inhibiting their differentiation to neurons. Cell transplantation experiments revealed that interactions between odd and even numbered segments underlie both the restriction of cell intermingling and the formation of boundary cells at the interface of segments. Since suppression
of boundary cell formation does not affect border sharpness, it seems that boundary cells are not required for the restriction of cell intermingling between segments. Recent studies suggest that in zebrafish, hindbrain boundary cells act as a source of semaphorin chemorepellants, which by positioning fgf20-expressing neurons help to establish the stereotyped organization of neurogenesis within segments. These findings raise the question of the mechanism that underlies boundary cell formation and whether this is coordinated with border sharpening.

Loss and gain of function experiments have shown that Eph-ephrin signaling is able to drive cell segregation and is required to transform the initial fuzzy interface of hindbrain segments to a sharp border. Cell segregation involves both Eph-ephrin interactions at the segment border and mechanisms acting within the segments. The mechanism(s) by which Eph-ephrin signaling sharpens the hindbrain borders is not known; a recent study has implicated Eph-ephrin mediated regulation of actomyosin cables, but as these form several hours after Eph-

Figure 1. Examples of the regulation of cell differentiation by Eph receptor and ephrin signaling. (A) Control of notochord vs. neural differentiation in Ciona. High level MAPK activation induces a notochord fate, whereas low level activation induces a neural fate. The generation of different fates is established by widespread FGF signaling which activates MAPK, and localized Eph receptor activation by ephrinAd which inhibits MAPK. (B) Borders and boundary cells in the vertebrate hindbrain. Complementary expression of Eph receptors and ephrins underlies both the segregation of cells which sharpens segment borders and the generation of specialized boundary cells. This dual role ensures a precise localization of boundary cells which are involved in patterning of hindbrain segments. (C) Regulation of the balance of cell types in bone remodelling. Eph receptor and ephrin signaling within the osteoclast and osteoblast lineages inhibits or promotes cell differentiation. The same signals, such as ephrinB1 and ephrinB2, can have opposite effects on osteoclast versus osteoblast differentiation. Eph-ephrin signaling may also occur between the 2 cell lineages and help to couple their differentiation. (D) Regulation of the proliferation and differentiation of neural progenitors. The generation of the correct number of neuronal cell types requires control of the proliferation of neural progenitor cells and the onset of their differentiation. Eph receptor and ephrin signaling has diverse and context-dependent roles in which it promotes or inhibits the proliferation or differentiation of progenitors.
both the sharpening of the border and the induction of Wingless border, this is coordinated by Notch activation which regulates Eph-ephrin induced boundary cell induction in patterning. Such knockdowns lead to a reorganisation of neurons and neurogenesis in the hindbrain, highlighting the importance of Eph-ephrin induced boundary cell induction in patterning.

These findings reveal an Eph-ephrin mediated coupling between the sharpening of hindbrain borders and the induction of boundary cells, and consequently a precise localization of the signaling source (Fig. 1B). This situation is analogous to the wing imaginal disc of the fruit fly, in which the sharpening of compartment borders is important for localization of boundary signaling centers. In the case of the dorsoventral compartment border, this is coordinated by Notch activation which regulates both the sharpening of the border and the induction of Wingless signal.

Balancing Cell Types in Bone Remodelling

The homeostatic process of bone renewal, termed remodelling, requires the bone matrix which is locally resorbed by osteoclasts to be replaced by the same amount of new bone generated by osteoblasts. This balancing act involves a coupling between the generation of osteoclasts and osteoblasts. Numerous factors have been implicated in such coupling, including TGF-β and IGF-1 growth factors which are embedded in bone matrix, as well as secreted intercellular signals such as interleukins and semaphorins. Eph receptors and ephrins have emerged as further regulators of the differentiation of osteoclasts and osteoblasts.

Initial evidence came from several gene knockout studies which found that EphB and ephrinB1 signaling is required for correct skeletal development. Further insights were obtained in detailed studies showing that EphB4 is expressed by osteoclasts, whereas ephrinB2 is expressed by osteoclasts downstream of the transcription factor NFAT which is required for osteoclast differentiation. By use of cell culture assays, it was found that reverse signaling through ephrinB2 inhibits NFAT expression and osteoclast differentiation, whereas EphB4 forward signaling promotes osteoblast differentiation. These findings suggest a model in which ephrinB2-EphB4 interactions between the 2 cell lineages contribute to the shift from bone removal to bone production during remodelling. Although ephrinB2 knockout mice have no overt defect in bone remodelling, this is due to functional compensation by ephrinB1 expressed by osteoclasts, which can bind to EphB2 expressed by osteoblasts.

It is currently unresolved whether Eph-ephrin signaling directly contributes to coupling during remodelling, since it is unclear to what extent there is contact between cells of the osteoclast and osteoblast lineages at the relevant time and place. Other studies have shown that ephrinB2 is expressed not only by osteoclasts but also by osteoblasts, and thus interactions within the osteoblast lineage can activate EphB4. The results of blocking experiments using soluble EphB4 suggest that EphB4-ephrinB2 signaling is required for late steps of osteoblast differentiation, which may then indirectly affect osteoclast differentiation. Likewise, it has been shown that reverse signaling due to EphB2-ephrinB1 interactions within the osteoblast lineage promote osteoblast differentiation, in part through nuclear translocation of the transcriptional co-activator TAZ. A similar situation may exist within the osteoclast lineage in which co-expression of ephrinA2 and EphA2 has been implicated in promoting osteoclast differentiation.

Intriguingly, A-class versus B-class Eph-ephrin signaling has opposite roles in the control of osteoclast differentiation, suggesting that there is a switch from the promotion of differentiation by ephrinA2-EphA2 to inhibition by ephrinB1 and ephrinB2. This dynamic regulation also involves other signals acting in parallel, such as semaphorin family members which promote or inhibit the differentiation of osteoclasts or osteoblasts. Taken together, these findings have revealed that Eph-ephrin signaling contributes to the regulation of osteoclast and osteoblast cell differentiation (Fig. 1C), although it is currently unclear to what extent this involves interactions between as well as within the 2 cell lineages.

Proliferation and Differentiation of Neural Progenitors

Eph receptors and ephrins have emerged as one of the signaling systems which contributes to another balancing act: the generation of neurons during vertebrate development, and in adult stem cell niches, while maintaining a sufficient number of progenitor cells throughout the period that neurogenesis is occurring. This is achieved by control of the rate of proliferation of the progenitor cell pool, as well as of the transition to neuronal differentiation.

A number of studies have uncovered roles of Eph-ephrin signaling in promoting the proliferation of neural progenitor cells, including EphB-ephrinB in the lateral ventricles; EphA-ephrinA5 in the hippocampus; and activation of EphA4 by ephrinB1 in the cerebral cortex. Consistent with this, EphA4 knockout leads to decreased proliferation of progenitor cells in adult neurogenic niches, albeit this may be secondary to premature neuronal differentiation. In contrast, in other contexts Eph-ephrin signaling inhibits neural progenitor proliferation, mediated for example by ephrinA2 reverse signaling in the adult lateral ventricle; ephrinA2/ephrinA3 activation of EphA7 forward signaling in non-neurogenic regions of the brain; ephrinB3 and EphB3 in the subventricular zone; and ephrinB3-EphB1 in the hippocampus. Progenitor cell number can also be limited by apoptosis, for example promoted by ephrinA5-EphA7 interactions in the cerebral cortex. Furthermore, ephrinA2/ephrinA3, EphA4, and reverse signaling through ephrinB1 inhibit neuronal differentiation and thus help maintain the progenitor state. These latter studies have implicated a number of different
pathways downstream of ephrinB1 in progenitor maintenance, including antagonism of G protein signaling by RGS5,84,85 binding of ephrinB1 cytoplasmic fragment to the ZHX2 transcriptional repressor,87 and downregulation of the microRNA mir-124 which otherwise would promote neuronal differentiation.86 Taken together, these studies suggest that Eph-ephrin signaling contributes both to the maintenance of neural progenitors and to promotion or inhibition of their proliferation.

A related picture has emerged in an adult neural stem cell niche, in which the neuronal progenitors are astrocytes, and require interactions with ependymal cells to be maintained.88 An important feature of the niche is plasticity in cell type, in which following a lesion astrocytes can become ependymal cells, which in turn can generate niche astrocytes. EphB2 is expressed in ependymal cells downstream of Notch activation and is required to maintain ependymal cell identity. Since ependymal cells express ephrinB ligands, this implicates Eph-ephrin interactions within this cell lineage in the maintenance of cell identity. EphB2-ephrinB interactions thus modulate the plasticity of cell fate within the stem cell niche by restraining the transdifferentiation that can occur between ependymal cells and astrocytes.88

Finally, other studies have found roles of Eph-ephrin signaling in promoting neuronal differentiation, for example EphA signaling in the telencephalon,89 and activation of EphB4 in hippocampal progenitors by ephrinB2 expressed in astrocytes.90 Consistent with a neurogenic role, ephrinB1 promotes the in vitro differentiation of human embryonic stem cells to form dopaminergic neurons.91 Thus, as found for their functions in axon guidance,8,10 the roles of Eph-ephrin signaling in the control of neural progenitor cell proliferation and differentiation are context dependent and contrary (Fig. 1D). The differences in cell response are presumably due to different regulation of downstream effectors, such as activation or inhibition of the MAPK pathway.80,89

**Proliferation and Differentiation of Keratinocytes**

The skin is another tissue in which Eph-ephrin signaling has been implicated in the control of cell proliferation and differentiation. Proliferating keratinocytes located in basal layers give rise to post-mitotic differentiating cells, which progressively move to more superficial layers, eventually undergoing specialized cell death to form a cornified outer layer. An important component of this differentiation process is the modification of intercellular junctions to form an impermeable stratified epithelium. The results of blocking experiments suggest that both A and B class Eph-ephrin signaling has a role in decreasing the proliferation of keratinocytes.92 Furthermore, studies in cell culture suggest that activation of EphA2 by ephrinA1 promotes the differentiation of keratinocytes, in part by upregulating the expression of a desmosomal cadherin, desmoglein1.93 In addition to contributing to early steps of epidermal morphogenesis, desmoglein1 promotes keratinocyte differentiation by suppressing the MAPK pathway downstream of EGF receptor signaling.94 Since EphA2 activation itself transiently inhibits MAPK,93 this suggests a model in which EphA2 signaling initiates differentiation, which is then sustained by upregulation of desmoglein1. Since EphA2 and other EphA receptors are expressed throughout the forming epithelium, whereas ephrinA1 is present in basal layers,95 it seems likely that EphA-ephrinA activation occurs through homotypic interactions between basal keratinocytes.

Although defects in epidermal development have not been found in EphA and ephrinA gene knockouts, this may be due to the overlaps in the expression and function of multiple family members.95 However, transcriptome profiling studies suggest that different ephrinA ligands can induce different sets of genes during keratinocyte differentiation in cell culture: ephrinA3, ephrinA4 and ephrinA5 induce a very similar set of genes, whereas ephrinA1 and ephrinA2 each induce a related but distinct set of genes.96 The differences presumably reflect preferential binding of the ephrinAs for EphA receptors that have distinct downstream signaling. Intriguingly, reverse signaling through ephrinBs also promotes epidermal gene expression,97 but unlike EphA activation this occurs after a 24 h delay, suggesting that it acts through intermediary factors. It will be important to uncover whether EphB forward signaling, or co-activation of Eph receptors and ephrins, has similar or distinct effects on keratinocyte differentiation.

**Differentiation and Survival of Thymocytes**

Regulation of the differentiation and survival of thymocytes to form mature T cells is central to the development and function of the immune system. Key regulatory steps occur in the signaling interactions of thymocytes with the 3-dimensional network of thymic epithelial cells (TECs) as they migrate through the stroma of the thymus. There is increasing evidence that Eph-ephrin interactions within the thymus are required for multiple aspects of thymocyte development.26 One of these roles is in establishing the correct architecture of the thymic epithelial network, which is disrupted in EphA4 mutants,98 EphB2 and EphB3 mutants,27 and following treatment of organ cultures with ephrinB1-Fc.24 This morphogenetic function of Eph-ephrin interactions is likely to compromise the interactions between thymocytes and TECs and thus have secondary effects on thymocyte development.26 Importantly, it was found that knockouts of EphA4 and of EphB receptors which disrupt the thymic epithelium lead to a block of distinct steps of thymocyte development.98,99 Furthermore, there is good evidence that Eph-ephrin signaling between TECs and thymocytes has more direct effects, in which it regulates both the differentiation and survival of thymocytes.25,26,100 For example, the effect of conditional inactivation of ephrinB1 and/or ephrinB2 in TECs or in thymocytes reveals roles in TEC maturation as well as autonomously in thymocytes,101 although another study found no effect on thymocyte development following inactivation of ephrinB2 in TECs.102 Experiments in which forward and reverse signaling are manipulated suggest that the balance of EphB and ephrinB signaling is important for T cell differentiation.25

At least in part, Eph-ephrin signaling acts by modulating T Cell Receptor (TCR) signaling which regulates critical steps of thymocyte development; for example, EphB1 and EphB6
associate with TCR and increase activation of some downstream signaling pathways, whereas activation of the JNK or MAPK pathways downstream of TCR is inhibited by EphB6 and EphA receptors, respectively. In another mechanism, reverse signaling through ephrinB1 and ephrinB2 synergizes with interleukin-6 in the activation of STAT3, which is required to inhibit apoptosis and enable maturation of thymocytes. Since EphB2 promotes the adhesive interaction of thymocytes with TECs, Eph-ephrin interactions may also contribute to thymocyte development by enabling other signals to act.

Transcriptional and Post-Translational Regulation of Cell Responses

Many studies have elucidated mechanisms by which Eph receptor and ephrin signaling contribute to morphogenesis, in which the cellular responses to activation can be rapid and are triggered by signal transduction pathways which lead, for example, to phosphorylation of cytoskeletal and adhesion proteins. Likewise, cell proliferation responses to Eph-ephrin signaling involves post-translational regulation by intracellular signaling cascades. Thus the simplest hypothesis is that Eph-ephrin signaling regulates cell positioning and proliferation through pathways that act post-translationally, whereas distinct pathways which regulate gene expression control the differentiated state of cells. This has been addressed in several studies which have carried out genome-wide analysis of changes in gene expression following Eph-ephrin activation.

Activation of EphB2/EphB3 by ephrinB1 has a crucial role in the fusion of palatal shelves by stimulating cell proliferation which is required for shelf outgrowth. A proteomic analysis of palatal cells in which EphB receptors are activated with clustered ephrinB1-Fc found changes in the phosphorylation of many proteins, including components involved in cell adhesion, migration and proliferation. Importantly, these include members of the MAPK pathway, and this pathway is essential for the cell proliferation response to ephrinB1. Analysis of the transcriptome one hour after activation with ephrinB1 revealed that only few genes are upregulated, but prominent among these are known targets of the MAPK pathway which act as feedback regulators. This study therefore suggests that in the palatal shelves, post-translational regulation by the MAPK pathway has a key role in the control of cell proliferation by EphB receptor activation, with transcriptional changes serving to modulate the level of MAPK activation.

A different situation has been revealed for the roles of Eph-ephrin signaling in intestinal crypts. Wnt signaling occurs at high levels in the base of the crypt, where it drives cell proliferation and expression of EphB2 and EphB3, whereas ephrinB1 and ephrinB2 expression is upregulated in differentiating cells as Wnt activity declines toward the apex of the crypt. Analysis of EphB2/EphB3 double gene knockouts showed that the complementary expression of EphB receptors and ephrinBs maintains the organization of proliferating progenitor cells in the base and differentiated cells in the apex. Intriguingly, inactivation of EphB2 plus EphB3 also leads to decreased proliferation of progenitor cells in the crypt. Since the change in proliferation could be secondary to the altered location of progenitor cells, blocking or activation of EphB receptors was carried out for a short period such that tissue organization is not affected. These manipulations too were found to alter cell proliferation, with EphB activation contributing around 50% of the proliferative activity. Since decreasing EphB expression enables the movement of differentiating cells toward the ephrinB-expressing apex, EphB activation thus couples cell proliferation and migration. Transcriptomic analysis following acute blocking of EphB receptors revealed decreased expression of many genes associated with cell proliferation, but as this occurred only at late stages (12 h) it is likely to be secondary to the decreased number of cycling cells. In contrast, there was altered expression of genes associated with cytoskeletal regulation at early (3 h) as well as later stages following blocking EphB activation. Furthermore, different downstream pathways were found to be required for the cell proliferation and migration responses: EphB2 kinase activity is required to promote cell proliferation and acts via Abl to regulate cyclinD1 levels, whereas EphB2 kinase-independent signaling mediated by phosphoinositide 3-kinase is required for cell positioning.

These findings suggest that Eph-ephrin signaling regulates cell proliferation in the palate and intestinal crypts through protein phosphorylation cascades which act post-translationally rather than through the regulation of gene expression. It is intriguing that in the crypts Eph-ephrin signaling changes the expression of genes encoding cytoskeletal regulators as there is no evidence for roles in regulating cell differentiation in this tissue. The regulation of gene expression is thus presumably a component of the cell response that underlies cell migration rather than due to an altered differentiated state. In principle, such changes in gene expression could mediate feedback that alters the migration response of cells to Eph-ephrin signaling. Similarly it has been found that EphB activation segregates Schwann cells from fibroblasts by increasing the level of Sox2 transcription factor, which in turn affects the subcellular localization of N-Cadherin. Likewise, Eph-ephrin signaling may modulate synaptic plasticity through cross-talk that increases NMDA receptor dependent gene expression, as well as through numerous post-translational pathways. This suggests a model in which Eph-ephrin signaling acts not only through post-translational mechanisms in morphogenesis, but also by regulating the expression level of genes that control cell adhesion and the cytoskeleton.

Common Themes and Future Directions

Studies of the roles of Eph-ephrin signaling in the spatial and temporal control of cell differentiation have revealed 2 general scenarios. In multiple cell lineages in ascidian embryogenesis, and in the hindbrain, Eph-ephrin interactions induce a distinct fate at the interface of 2 cell populations. This is seen strikingly in ascidian development, where different cell fates arise from the combination of widespread FGFR activation by diffusible FGF...
ligand and cell contact dependent Eph receptor activation by an ephrin. In a related situation, Eph-ephrin interactions between distinct cell types can regulate the progression of cell differentiation, for example interactions of thymocytes with TECs and of neural progenitors with astrocytes. On the other hand, Eph-ephrin interactions can act within cell lineages, for example in the control of cell differentiation in bone remodelling, neurogenesis and keratinocyte differentiation. It will be interesting to know whether, as found for axon guidance,\textsuperscript{116-119} co-activation or cis-inhibition of signaling plays a role in these responses to overlapping Eph receptor and ephrin expression. In some tissues Eph-ephrin signaling mediates regulatory interactions both between and within specific cell types, for example in the nervous system and perhaps in bone remodelling.

Several different hierarchical relationships between cell identity and Eph-ephrin expression and function have been uncovered. Studies of the intestinal crypt give insights into the interplay that can occur when Eph-ephrin expression is regulated as a component of cell identity. In this tissue, the amount of Wnt signaling regulates cell differentiation and proliferation, as well as the expression level of EphB and ephrinB proteins. EphB signaling in turn controls the positioning of cells along the crypt as they differentiate, and also regulates cell proliferation in parallel with Wnt signaling. A different situation occurs in the hindbrain, in which Eph-ephrin signaling acts downstream and upstream of different aspects of cell identity. Transcription factors that specify segment identity regulate the complementary expression of Eph receptors and ephrins, which in turn mediate boundary cell specification at the interface of Eph and ephrin expression. Since Eph-ephrin signaling also sharpens the segment borders, this ensures a precise localization of boundary cells. It will be important to elucidate whether such direct coupling between inducing a cell type and maintaining its location occurs more widely where Eph-ephrin signaling regulates cell differentiation. Another type of relationship occurs in osteoclast precursors, where a key transcription factor for differentiation, NFAT, upregulates ephrinB2 expression, and reverse signaling through ephrinB2 decreases NFAT expression. This enables a feedback loop in which ephrinB2 activation limits osteoclast cell differentiation.

Further work is required to disentangle the potential dual role of Eph-ephrin signaling in regulating cell differentiation and maintenance of cell location. A key question is the identity of the pathways by which Eph-ephrin signaling regulates gene expression to control cell differentiation, and whether these are distinct or overlap with those that control cell migration. Several transcription factors and pathways have been found which link Eph receptor and ephrin signaling to the control of cell differentiation, and further links may come from studies of other targets of Eph-ephrin activation which are known regulators of gene expression.\textsuperscript{4,120,121} A related question is how Eph-ephrin signaling leads to diverse migration, proliferation and/or differentiation responses, perhaps through expression of different mediators or targets of signal transduction in different cell types. Important insights into the mechanisms of Eph receptor and ephrin signaling in cell differentiation and other cell responses are likely to emerge from further proteome and transcriptome-wide studies of the post-translational modifications and genes regulated by Eph-ephrin activation in different tissues.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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