Homeoprotein transduction in neurodevelopment and physiopathology

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Homeoproteins were originally identified for embryonic cell–autonomous transcription activity, but they also have non–cell-autonomous activity owing to transfer between cells. This Review discusses transfer mechanisms and focuses on some established functions, such as neurodevelopmental regulation of axon guidance, and postnatal critical periods of brain plasticity that affect sensory processing and cognition. Homeoproteins are present across all eukaryotes, and intercellular transfer occurs in plants and animals. Proposed functions have evolutionary relevance, such as morphogenetic activity and sexual exchange during the mating of unicellular eukaryotes, while others have physiopathological relevance, such as regulation of mood and cognition by influencing brain compartmentalization, connectivity, and plasticity. There are more than 250 known homeoproteins with conserved transfer domains, suggesting that this is a common mode of signal transduction but with many undiscovered functions.

Cell-cell recognition is the basis for the initiation and evolution of multicellularity. From this point of view, such recognition must convey positional information for controlling cell differentiation and organ formation according to position. Homeobox genes, which were first discovered through their role in position-dependent morphogenesis, are at the forefront in this process (1). Besides multicellularity, another important recognition process is mating type, and in some unicellular eukaryotes, such as yeast or unicellular green algae, mating type is encoded by homeobox genes (2–4). However, in either case, most homeoproteins encoded by the homeobox genes have been regarded as purely cell-autonomous transcription factors that regulate the expression of secreted morphogenetic signals acting through classical transduction mechanisms.

Non–cell-autonomous activity of homeoproteins was serendipitously discovered during the course of experiments in which the 60–amino acid–long DNA binding domain (the homeodomain) of Antennapedia was injected into cells. The homeodomain added to culture medium in control experiments was spontaneously internalized by live cells, with direct addressing to cytoplasm and nucleus (5). Building on this first observation, it was demonstrated soon after that several full-length homeoproteins can be transferred between cells [reviewed in (6)]. While we will describe below current knowledge of the internalization and secretion mechanisms, it must be emphasized that the homeodomain, which is a very highly conserved structure (1, 4, 7–9), is necessary and sufficient for transfer both in plants and in animals (10–12). This is probably why almost all homeoproteins tested so far (~150) have demonstrated intercellular passage, both in vitro and in vivo (13).

Homeoprotein transfer would have remained an eccentric observation had efforts not been made to identify important physiological functions of this unexpected signaling mechanism. Complicating this goal is that the transfer motifs are within the homeodomain sequence, making it impossible to genetically disrupt transfer without modifying DNA binding and, thus, cell-autonomous activities (12). A strategy to circumvent this difficulty involves minigenes encoding secreted single-chain antibodies (scFvs) (14). The antibodies expressed and secreted in vivo block homeoproteins only in the extracellular space, thus leaving intact their cell-autonomous functions. This strategy was very useful in elucidating the direct non–cell-autonomous functions of a limited number of homeoproteins across five animal species (summarized in Table 1).

ESTABLISHED FUNCTIONS IN BRAIN DEVELOPMENT

Guidance and migration, the retinotectal example

Migration and guidance are of special interest in the context of positional information. Any migrating entity, say a cell or a growth cone (here considered as a neuron leading edge), needs to know which direction to take, a choice dictated, in part, by the original position and the lineage of the cell body. Several models explaining directed migration have been proposed, primarily implicating the presence of attractive and repulsive cues either attached to the substratum or diffusing into the environment (15). In this context, only those few morphogens bound to carriers following secretion have distant targets; otherwise, they remain trapped within the extracellular matrix, particularly through specific binding to proteoglycans, and do not diffuse freely (16–18).

One of the most popular examples of guidance is that of retinal axons onto the superior colliculus (SC; mammal) or optic tectum (lower vertebrates). The system is more complex than summarized below, with several Ephrin ligand and Eph receptor subtypes and redundancy, but the principle holds that the nasotemporal Eph receptor gradient at the surface of the growth cones and the anteroposterior gradient of EphrinA at the surface of the tectum/SC provides a way to limit the advance of the temporal axons onto the posterior territory (19–21).

Graded expression of Engrailed homeoprotein in the tectum/SC is established by the expression of several factors, including WNT1, FGF8, and PAX family members, at the hindbrain/midbrain boundary and is required for proper positioning of nasal and temporal retinal axons along the anteroposterior axis of the tectum/SC (22). The regulation of positioning was first attributed to the Ephrin/Eph interactions according to the graded expression of EphrinA5, which

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is under Engrailed cell-autonomous transcriptional control (23–26). However, neutralizing Engrailed in the extracellular space leads temporal axons to invade the posterior tectum, in frog and chick (Fig. 1) (27). The proposed mechanism is that Engrailed once internalized by the growth cones regulates the translation of mitochondrial mRNAs leading to ATP synthesis, secretion, and degradation into adenosine. Adenosine and Ephrin signaling pathways interact, inducing grow cone collapse when a combined concentration threshold of extracellular EN1/2 and EphrinA5 has been reached (27–29). (C) EphrinA5 signaling is enhanced by EN1/2 presence. This very simple scheme illustrates how EN1/2 addition increases the sensitivity of the response to low EphrinA5 concentration. In the presence of EN1/2, the growth cone response takes place at much lower EphrinA5 concentrations, allowing for growth cones to orient themselves along a shallow morphogen gradient.

Figure 1 illustrates some key messages to retain from these experiments. First, extracellular graded Engrailed distribution reflects the intracellular distribution, which means that the protein does not diffuse after its secretion (27). This local retention is probably, in part, electrostatic because of the negatively charged environment of the cell surface but may also involve specific interactions with glycosaminoglycans (GAGs), as shown for VAX1 and OTX2 (31–33). A second point is the importance of cosignaling between adenosine, EphrinA5, and Engrailed (29). Such cosignaling was also found between Engrailed and decapentaplegic (DPP) in the fly wing disk (34) and between PAX6 and netrin in the neural tube (35). A final point of interest is the importance of local translation. In the in vitro turning assay with frog retinal axons, EN2 binds the translation initiation factor eIF4E once internalized and regulates the initiation of translation (28).
Cerebral cortex plasticity

Critical periods of plasticity are windows of time in postnatal development during which a particular neural circuit can be shaped by a specific sensory input (experience) and after which the changes are normally very limited. Ocular dominance is a well-studied critical period that shapes primary visual cortex (V1) output according to the input levels from each eye (40). If the inputs are imbalanced, perhaps due to eye misalignment (strabismus) or congenital cataract, then amblyopia will ensue with impaired visual acuity that cannot be cured during adulthood (41). This disorder affects 2 to 5% of the human population but can be reversed in children if treated before heightened plasticity closes (by ~7 years of age). Critical periods exist for diverse processes of increasing complexity, including, for example, filial imprinting, sensory processing, motor skills, language, and emotional control, and they typically occur in cascade across different neural circuits (42–44). The mechanisms involved seem to be repeated across these diverse brain regions, be they for the triggers of critical period onset, the manifestation of plasticity, or the closure and consolidation of circuitry.

Critical period onset is driven by the maturation of fast-spiking GABAergic (γ-aminobutyric acid–releasing) inhibitory neurons that express parvalbumin (PV), leading to a shift in excitatory/inhibitory circuit balance and to various molecular changes in the extracellular matrix permissive to plasticity (42). PV cell maturation requires appropriate stimulus coupled with specific molecular signals, including OTX2. This homeoprotein exhibits non–cell-autonomous activity in V1, as its locus is completely silent throughout the postnatal cortex (45). It is expressed along the visual pathway, from the retina (in retinal pigmented epithelium, photoreceptor cells, and bipolar cells) to the dorsal lateral geniculate nucleus (in GABAergic interneurons) to the SC (in principal cells). It is also highly expressed in the choroid plexus, an epithelium within brain ventricles that produces cerebrospinal fluid. When OTX2 levels are reduced in either the retina or choroid plexus, OTX2 protein levels are concomitantly reduced in V1 PV cells, PV expression is negatively affected, and V1 plasticity can be altered (45, 46). However, given that such manipulations can induce confounding cell-autonomous effects in either the retina or choroid plexus, methods involving extracellular blocking peptides or antibodies were developed to selectively target non–cell-autonomous functions (14, 31, 45). OTX2 transfer into V1 not only triggers ocular dominance critical period onset but also is implicated in closure and consolidation, with constant OTX2 transfer into PV cells maintaining a nonplastic state in the adult (14, 31, 46). This high sensitivity of PV cell maturation state to OTX2 levels evokes a two-threshold model of OTX2 accumulation in PV cells, whereby a first OTX2 concentration threshold initiates opening and a second one initiates closure (Fig. 2A) (6). For example, OTX2 protein infusion in V1 accelerates both critical period onset and closure (45), while a 20% reduction in OTX2 accumulation
is sufficient to reopen ocular dominance plasticity in the adult mouse and cure experimental amblyopia (31, 46). This two-threshold hypothesis is supported by genetic mouse models that knock down Otx2 expression at the source or that sequester extracellular OTX2 protein in cerebrospinal fluid or around PV cells through conditional expression of a secreted single-chain OTX2 antibody (14).

While the mechanism of transfer from the choroid plexus to cortex has yet to be revealed, likely involving dedicated carriers, it is clear that the specificity of OTX2 for PV cells is dependent on its interaction with GAGs. The GAG-binding motif in OTX2 favors binding to disulfated GAGs present in perineuronal nets (PNNs) that surround PV cells (31). PNNs are dense extracellular matrices composed of hyaluronic acid, link proteins, and chondroitin sulfate proteoglycans (CSPGs) that accumulate and condense around select neurons (47, 48). Along with myelination, PNNs are an important feature of critical period closure that limit PV cell synaptic physiology, buffer ions and reactive species, and interact with signaling molecules. A genetic point mutation in the GAG recognition motif of OTX2 that decreases affinity for disulfated CSPGs results in reduced transfer specificity into PV cells and results in mice with delayed critical period timing (49). This mouse model supports the hypothesis that critical period mechanisms are recapitulated across brain regions; not only is V1 plasticity timing affected but also primary auditory cortex (A1) and medial prefrontal cortex (mPFC) plasticity timing (Fig. 2B). Thus, OTX2 is continually secreted into cerebrospinal fluid and accumulates specifically in PV cells throughout the cortex when retained by their PNNs.

OTX2 transfer into PV cells initiates a feedback loop, as it activates the expression of the PNNs that promote its accumulation (50). Various PNN molecules, including CSPGs and matrix-modifying proteases, are activated by OTX2, possibly by direct regulation of translation or by downstream changes in transcription (49, 51). This tight coupling between OTX2 levels, PV cell maturation, and PNN expression suggests that OTX2 can regulate bistable PV cell states that oscillate between permissive and consolidated. OTX2 also targets epigenetic programs (Fig. 2C). At a concentration level between the two thresholds that is compatible with plasticity, OTX2 activates the expression of GADD45 family proteins (52), which are implicated in regulating DNA methylation. Both OTX2-dependent juvenile critical period onset and induced adult plasticity result in the up-regulation of Gadd45 and in changes of gross indicators of chromatin organization (Fig. 2D). Epigenetic programming affects critical period timing, and its deregulation is implicated in autism spectrum disorders (53).

**TRANSFER MECHANISMS**

**Crossing membranes**

Homeoproteins are not unique in their ability to exit or enter cells, as unconventional protein secretion pathways have been described for several fully mature cytosolic or nuclear proteins that lack canonical secretion signals (54). EN2 secretion shares steps with type I unconventional protein secretion, originally described for FGF2 (55) and HIV Tat proteins (56). This secretion occurs by direct translocation through the plasma membrane and requires interaction with phosphatidylinositol diphosphate (PIP2) at the inner membrane leaflet (Fig. 3). However, while FGF2 (57) and Tat (58) oligomerize to create pores for their secretion, EN2 does not oligomerize, suggesting that it uses a different translocation mechanism following PIP2 binding. Nuclear magnetic resonance studies reveal that negatively charged membrane mimetics induce conformational modifications of the homeodomain that promote its insertion directly into the membrane hydrophobic core (59). EN2 internalization also requires binding to PIP2 (60), suggesting that exit and entry use very similar membrane translocation mechanisms (Fig. 3). Given the high conservation of the homeodomain sequence, EN2 transfer mechanism, studied in cell culture assays, is hypothesized to be similar for most homeoproteins that traffic.

**Homeoprotein intracellular journey**

Homeoproteins do not have classic secretion signals and traffic through the nucleus before secretion. For example, secretion is prevented if EN2 is retained in the nucleus by deleting its nuclear export sequence (61, 62). Plant KN1 homeoprotein is competent for transfer between plant cells in vivo through plasmodesmata (10, 63) and shows a similar requirement for nuclear import (10). KN1
homeodomain also transfers between mammalian cells, and deleting the nuclear localization sequence abolishes secretion (11). From an evolutionary perspective, the animal/plant comparisons suggest that the passage of KN1 through plasmodesmata involves similar mechanisms operating in animals. However, the nuclear factors and cytosolic transport mechanism for homeoprotein secretion remain unknown. One hypothesis is that they use intracellular vesicular flow to reach the plasma membrane, given that EN2 strongly interacts with cholesterol and negatively charged phospholipids and associates with caveolae-like vesicles (64).

**Homeoprotein extracellular journey**

Extracellular homeoproteins bind the plasma membrane before entering cells. Similar to all DNA-binding proteins, homeoproteins are prone to electrostatic interactions with nucleic acids and also interact with negatively charged carbohydrates at the cell surface, particularly GAGs that may play the same role as PIP2 plays for secretion (31). Interaction with GAGs would help concentrate homeoproteins at the cell surface and trigger internalization, as first suggested by early in vitro experiments demonstrating that homeodomain internalization is higher in neurons than in fibroblasts (5). Similar to most morphogens, homeoproteins do not travel far in vivo and signal to neighboring cells (34), although a counterexample is provided by the brain-wide distribution of OTX2 after secretion from the choroid plexus into the cerebrospinal fluid, where OTX2 is likely bound to a carrier (46, 50). Cortical distribution of OTX2 also highlights another role of homeoprotein-GAG interaction, as OTX2 specifically accumulates in cortical PV cells thanks to high-affinity binding to GAGs within their PNNs (31). The GAG-binding motif in OTX2 overlaps with the first helix of the homeodomain, and similar sequences are present in several homeoproteins (6), suggesting the existence of a “sugar code” for homeoprotein-cell recognition that guides transfer specificity and non–cell-autonomous activity.

**Generalization of homeoprotein intercellular transfer**

The capacity of a large number of homeoproteins to transfer is less of an open question despite the initial focus on only few homeoproteins. Non–cell-autonomous in vivo activity has been reported for EN1/2, PAX6, OTX2, KN1, several HOXs, and VAX1 (10, 27, 34, 35, 45, 65–70), while all in vitro studies addressing the cellular mechanisms of transfer were done mainly with EN2. The hypothesis that most homeoproteins transfer in vivo is now supported by systematic assays involving 160 diverse homeoproteins in which intracellular transfer was confirmed for more than 150 homeoproteins both in HeLa cells and in mouse embryonic brain (13). However, an important issue that remains less well understood is the regulation of secretion and internalization, which is likely distinct for each homeoprotein. As discussed above, regulation may be through nuclear import/export, phosphorylation, vesicular transport, binding to PIP2, and recognition by complex sugars. For internalization, specific target cell recognition is necessary when the protein is in the extracellular milieu, as in the case of OTX2. However, if homeoproteins are secreted in the vicinity of the target cells, then their very limited diffusion may ensure specificity, as seen in the optic tectum or wing epidermis. Last, as discussed above, homeoproteins may co signal with other classical pathways, as shown for EN1/2 (27, 29, 34) and PAX6 (35). If so, the regulation of homeoprotein signal transduction must include that of the cosignaling classical pathways.

Last, an unexpected consequence of homeoprotein internalization is the possibility to use them as therapeutic proteins, as shown for OTX2 in a mouse model of glaucoma (71) and for EN1/2 in rodent and nonhuman primate models of Parkinson’s disease (72, 73). A single injection of homeoprotein has long-lasting protective effects best explained, at least in the case of EN1/2, by an ability to restore heterochromatin marks and to fight genomic instability (74, 75).

**TWO HYPOTHETICAL FUNCTIONS OF EVOLUTIONARY RELEVANCE**

Among the hypothetical functions of homeoprotein signaling, two are of interest in the context of evolution. How can homeoproteins be considered true morphogens under the terms proposed by Turing (76), and can homeoprotein signaling be considered the first sexual exchange during the mating of unicellular eukaryotes (77)?

**Turing morphogens**

In The Chemical Basis of Morphogenesis (76), Turing proposed that two morphogens in the presence of a catalyst allow for the creation
of heterogeneities within an originally unified field. For this to happen, the two morphogens must have different diffusion constants and autoactivating properties and must be reciprocal inhibitors. During compartmentalization, two homeoproteins expressed on either side of a future boundary have the two latter properties while the first one (different diffusion constants) is not necessary as they are not uniformly distributed. Their initial graded distributions are under the control of classical morphogens, fitting different models derived from the “French Flag” one proposed by L. Wolpert (78). In the example shown in Fig. 4, two opposing gradients of EMX2 and PAX6 define the positioning of the visual and sensory areas in the developing mouse cortex at embryonic day 11 (79). Modifying the dosage equilibrium between the Emx2 and Pax6 pushes the boundary backward or forward (79, 80). This is also the case for several boundaries, such as the midbrain/hindbrain boundary defined by the OTX and GBX2 (81, 82), the pallium/subpallium boundary defined by GSH2 and PAX6 (83, 84), or the Zona Limitans Intra-thalamica defined by OTX1/2 and IRX1 (85). In the latter reports, boundary positioning by “opposing” homeoproteins is entirely explained by their cell-autonomous activities. However, based on the demonstration of homeoprotein non–cell-autonomous activity, including the role of extracellular PAX6 in the regulation of Cajal-Retzius cell migration in the developing mouse cortex (70), we speculate that homeoprotein diffusion may also participate in forming boundaries. Accordingly, a mathematical model shows that adding short-distance homeoprotein diffusion to the classical French Flag model allows for the formation of straight and stable boundaries (86). This displacement of boundaries by changes in the expression of abutting homeoproteins, be it cell autonomous or not, is of evolutionary importance, as it allows for changes affecting the volume of cortical and subcortical regions, not necessarily implying a modification of the global size of the brain, sometimes with behavioral consequences, as shown by the increased locomotor activity of mice with a shifted midbrain/hindbrain boundary (87).

**Mating types in unicellular eukaryotes**

“Sex” recognition in unicellular eukaryotes has been widely studied in *Saccharomyces cerevisiae* and in green algae *Chlamydomonas reinhardtii*, where mating types are defined by specific homeoproteins, such as Mat-alpha1 and Mat-alpha2 in *Saccharomyces* or GSM1 and GSP1 in *Chlamydomonas* (3, 88). Differences exist between the two species, but mating strategies have many points in common. Using *C. reinhardtii* as an example, this green algae proliferates as haploid mt+ (expressing GSP1) and mt− (expressing GSM1) cells until food deprivation induces loss of cell wall followed by conjugation (89). Conjugation or haplotype fusion, triggered by adhesion molecules at the surface of flagella, allows for the formation of GSM1:GSP1 dimers that activate zygotic gene expression. The zygote remains dormant until food supply increases and meiotic divisions take place, yielding a new generation of dividing haploid cells. GSM1 and GSP1 belong to the Knotted and Bell family of homeoproteins, respectively, raising the possibility that they can transfer between gametes before fusion (63). Accordingly, de novo protein synthesis, which is required to maintain flagellar adhesion during gamete activation (90), would depend on GSM1 and/or GSP1 transfer before cell fusion (91, 92).

**PATHOPHYSIOLOGICAL AND EVOLUTIONARY PERSPECTIVES**

There is not a physiology for health and another for illness; most pathological states stem from derailed physiological processes. Thus, the discovery of novel physiological pathways can increase our understanding of some pathologies and lead to original therapeutic approaches. An example is the observed regulation of cerebral cortex plasticity through non–cell-autonomous OTX2 activity.

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**Fig. 5. Cooperative OTX2 signaling to mature cortical PV cells.** For V1 ocular dominance critical period onset, visual activity stimulated by light (1; green box) coupled with OTX2 passage (2) is required to activate PV cell maturation (3), which initiates PNN assembly (green circle arrows) (45). OTX2 from cerebrospinal fluid (blue box) is captured by PNNs (4) and accelerates PV cell maturation and PNN assembly (14, 46). Dark rearing (1; red box) reduces V1 input and delays PV cell maturation and PNN assembly (red circle arrows). In behavioral paradigms, physiological signals (1; green box) or, respectively, stress (1; red box) increases (or, respectively, reduces) expression of *Otx2* and its targets in the VTA (2) and choroid plexus (96, 97). It is hypothesized that this activation (or reduction) affects the activation of PV cell maturation (3 and 4; pink) in various limbic cortical structures. Green and red arrows show up- and down-regulation, respectively, of either neuronal activity, protein transduction, or gene expression.
Reducing OTX2 transfer to PV cells reopens plasticity in adult mice and can cure them from experimental amblyopia (31, 46). Whether these findings represent a possible advance in our understanding of the etiology of psychiatric diseases is worth considering. As discussed above, cerebral cortex and subcortical nuclei evolution is a question not only of global size but also of changes in connectivity (93) and in the positioning of boundaries, with the relative increase of the anterior parts of the brain in relation to the posterior ones, particularly within the primate clade (94, 95). As such, one can anticipate commonalities in regulatory mechanisms among all cortical functions, including mood and cognition.

Commonalities suggest that critical period regulation in V1 is recapitulated in higher-order cortices. This hypothesis has received recent support from studies showing that maternal separation in mice between a 10-day critical period, from postnatal day 10 to postnatal day 20, can induce permanent anxiety and depressive phenotypes (96). In the non-resilient offspring, Ottx2 expression is down-regulated in the ventral tegmental area (VTA) during maternal separation while anxiety can be reversed in the adult by viral Ottx2 expression in the VTA, which demonstrates an important cell-autonomous role for OTX2 within the VTA. In a similar maternal separation paradigm, anxiety was accompanied by increased choroid plexus OTX2 expression and altered transfer in ventral hippocampus PV cells, which suggests a parallel non–cell-autonomous role for OTX2 (97). In ocular dominance plasticity, antagonizing OTX2 synthesis in the retina during the critical period delays PV cell maturation in V1 (45). By drawing a parallel between the systems, we hypothesize that the VTA plays a similar role in activating cortical and subcortical regions involved in mood regulation, as the retina plays for activating V1 maturation (Fig. 5). In both cases, OTX2 regulates the maturation of the “periphery” (VTA and retina) through cell-autonomous activity and regulates the maturation of cortical PV cells non–cell-autonomously to ensure the proper shift in excitatory/inhibitory balance in the corresponding cortex. Many psychiatric diseases may be routed in biological and/or environmental adversity experienced during critical periods. Schizophrenic patients show defective PNN maturation in the dorsolateral prefrontal cortex (98) and disrupted cortical inhibition, leading to reduced high-power oscillations (99). DNA samples from maltreated children reveal a correlation between depression and the methylation status of OTX2 and its downstream targets (100), strengthening the hypothesis that OTX2 is an important modulator of mental health.

Because homeoproteins are present in all eukaryotes, including unicellular eukaryotes (7), their signaling properties must be very ancient. Pursuing the idea that homeoproteins signal during unicellular eukaryote mating, one can propose that they are at the origin of organ diversification in the first multicellular organisms. In this context, if we adopt the idea that multicellular organisms do not derive from cleavages taking place in multinucleated cells but from the aggregation of individual cells followed by compartment specialization (101), then slime molds provide an interesting model. Dictyostelium aggregation is promoted upon starvation by cAMP (cyclic adenosine monophosphate) signaling, and the aggregates produce a slug with three main anteroposterior domains: prestalk A (pstA) cells, prestalk O (pstO) cells, and anterior-like cells (ALCs), which represent 10, 10, and 80% of the body, respectively (102). pstO and ALC cells are in equilibrium and form a boundary that can be shifted to the advantage of the pstO compartment by knocking out DdHbx-2, a homeobox gene expressed only in the ALC and pstA compartments, very similarly (Fig. 4) to what was proposed for boundary positioning in the developing brain with homeoproteins behaving like Turing morphogens (76).

CONCLUSIONS

Homeobox genes are expressed by all eukaryotic cells, which makes it likely that they were also expressed earlier in the progeny of the Last Universal Common Ancestor. This means that homeoprotein signaling may have preceded all classical types of signaling, which is consistent with secretion and internalization, requiring no specific apparatus or receptors. Hence, homeoproteins may have been very primitive signaling entities, getting across the plasma membranes to non–cell-autonomously regulate translation, transcription, or epigenetic marks. If so, other signaling mechanisms based on agonist-receptor interactions would have been later selected for their ability to add robustness to homeoprotein signaling. Homeoproteins can signal with classical signaling molecules, as shown for EN1/2 and Ephrin in axon guidance (27), PAX6 and Netrin for oligodendrocyte precursor migration (35), and Engrailed and DPP in the fly anterior cross vein formation (34).

While there are clearly established functions associated with direct non–cell-autonomous homeoprotein activity (Table 1), we have described only a few of them and, in a more speculative mood, proposed others that remain hypothetical. Considering that there are more than 250 homeoproteins, as compared to the 23 members of the FGF family (103), for example, our present knowledge might be the tip of the iceberg. If all homeoproteins that can transfer actually do so with physiological relevance, then homeoprotein transfer might represent, at least quantitatively, the most common mode of signal transduction.

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