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Phylogenetic evidence for the modular evolution of metazoan signalling pathways

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Communication among cells was paramount to the evolutionary increase in cell type diversity and, ultimately, the origin of large body size. Across the diversity of Metazoa, there are only few conserved cell signalling pathways known to orchestrate the complex cell and tissue interactions regulating development; thus, modification to these few pathways has been responsible for generating diversity during the evolution of animals. Here, we summarize evidence for the origin and putative function of the intracellular, membrane-bound and secreted components of seven metazoan cell signalling pathways with a special focus on early branching metazoans (ctenophores, poriferans, placozoans and cnidarians) and basal unikonts (amoebozoans, fungi, filastereans and choanoflagellates). We highlight the modular incorporation of intra- and extracellular components in each signalling pathway and suggest that increases in the complexity of the extracellular matrix may have further promoted the modulation of cell signalling during metazoan evolution. Most importantly, this updated view of metazoan signalling pathways highlights the need for explicit study of canonical signalling pathway components in taxa that do not operate a complete signalling pathway. Studies like these are critical for developing a deeper understanding of the evolution of cell signalling.

This article is part of the themed issue 'Evo-devo in the genomics era, and the origins of morphological diversity'.

1. Introduction

Multicellularity has evolved numerous times [1], but has only resulted in the expansive diversification of complex organisms in six lineages: basidiomycete and ascomycete fungi, red algae, brown algae, plants and animals. Many factors influenced the transition from unicellular to multicellular life; important among them were the evolution of adhesion that permitted daughter cells to remain in physical contact following replication [2], and the division of labour that enabled the spatial segregation of incompatible/diverse cell functions and enabled the evolution of specialized somatic cell types [3]. Adherent colonies of cells have arisen numerous times and, indeed, classical cell–cell adhesion proteins evolved long before the origin of Metazoa [4], implying that adhesion alone is insufficient to drive the evolution of diversity in metazoans. The origin of cell–cell communication through intercellular signalling pathways enabled cells to communicate their identity to their neighbours, and was essential for stimulating the rise of multicellularity. Without the ability to communicate information among individual cells, a colonial organism can only be a cluster of autonomous units; thus, the evolution of the basement membrane played a critical role in facilitating communication among cells as it provided a scaffold for the retention and transmission of extracellular ligands and inhibitors. The combined evolution of intercellular communication and an extracellular means to transmit signals permitted the evolution of long-range policing of cell identity, promoting the evolution of truly multicellular life where only aggregates had succeeded before. Here, we review recent advancements in our understanding of the evolution of major cell signalling pathways in non-bilaterian unikonts (see glossary)

and the origin of the basement membrane as a means to facilitate long-range signal propagation. This review highlights the modular evolution of intracellular, membrane-bound and secreted components of these conserved pathways and points to the evolution of secreted inhibitory molecules as key regulators of pathway diversity.

2. Cell–cell communication and the evolution of signal transduction pathways

Across Metazoa, seven signalling pathways appear to play similar and essential roles in regulating animal development: the transforming growth factor β (TGF-β) pathway, canonical WNT (cWNT) signalling, nuclear receptors (NRs), receptor tyrosine kinase (RTK) signalling, the Notch/Delta pathway, Hedgehog signalling and the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway. These seven highly conserved signalling pathways transduce extracellular signalling into transcriptional regulation of target genes and are responsible for facilitating the specification of cells differentiating from a proliferative progenitor during embryogenesis. Thus, these signalling pathways may be representative of the ancestral mechanisms permitting the division of labour that resulted in the origin of novel cell types and the evolution of complex multicellular life. However, understanding how the origin of these seven pathways may have influenced the diversification of Metazoa requires an understanding of the functions of these pathways in diverse metazoan lineages. In particular, understanding the developmental context in which they acted to influence the cell biology of ancestral taxa is critical for reconstructing the events that led to the rise of the canonical/functional pathways and the origin of complex multicellular life.

Recent efforts to combine molecular (e.g. transcriptomics) and developmental techniques [5] have provided an opportunity to use expression data as a first attempt to imply function rather than relying on only presence/absence data from genomic studies. Despite having only limited data from several critical taxa, we take a first step towards integrating genomic and developmental studies from diverse non-bilaterian metazoans to attempt to reconstruct ancestral functions of several key signal transduction cascades. First, we summarize the presence/absence of pathway components in cnidarians, placozoans, sponges and ctenophores as well as in the unicellular lineages that preceded the origin of metazoans (choanoflagellates, filastereans, fungi and amoebozoans, where possible). We then summarize available data regarding the developmental role of these genes to explore their ancestral functions. There have been several previous reviews of cell signalling in non-bilaterians [6–9], and we refer the reader to these and additional resources throughout. With this review, we aim to comprehensively integrate genomic and developmental data from ctenophores and other emerging non-bilaterian models with previous observations of signal pathway evolution to build a richer model of metazoan evolution.

3. Using ctenophores to reconstruct the cell biology of the ancestral metazoan

Inferences about the factors that promoted the evolutionary diversification of metazoans are anchored in the phylogeny used to describe the relationships among metazoans. The sequencing of the sponge genome [10] enabled new predictions about the nature of cell biology in what was considered at the time to be the earliest diverging metazoan. Several studies now support ctenophores as sister to the rest of Metazoa [11–13], necessitating a re-review of the evolution of cell signalling pathways in non-bilaterians. Although much progress has been made in understanding the developmental biology of ctenophores in the past decade, there is still much to be learned from increased sampling of this lineage, and we hope this review stimulates renewed interest in characterizing the developmental biology of ctenophores. Notably absent from our developmental surveys below are data from placozoans. As there has been little advancement in efforts to spawn and successfully rear placozoan embryos through development [14], little is known about the spatial distribution of gene expression in these animals, and there is little opportunity to test hypotheses raised by the recent sequencing of the genome and proteome of *Trichoplax adhaerens* [15,16]. Conversely, there have been numerous recent advancements in our understanding of the evolution and development of diverse sponge taxa [17–19]. Considering sponges to be sister to the rest of Bilateria, Muller [20] suggested that the ancestral metazoan likely had cell adhesion molecules with intracellular signalling capabilities, gradient-forming growth factors, and immune and nervous systems. Given that ctenophores are now largely accepted to be the earliest diverging lineage of metazoans (figure 1), we ask: how does this list of putative ancestral metazoan characteristics change if the ancestral metazoan is reconstructed under the new phylogenetic framework?

4. Pathways that unite metazoans

(a) Transforming growth factor β signalling

The mechanism underlying TGF-β signalling has been reviewed in detail [21,22] as has the bone morphogenetic protein (BMP) pathway explicitly [23]. Briefly, this pathway becomes activated when a ligand (TGF-β type or BMP type) binds to a receptor complex (heterodimer of type I and type II receptors) to translocate a SMAD-based intracellular cascade into the nucleus activating or repressing transcription (figure 1*a*). Now known to be unique to Metazoa [24–26], a full complement of TGF-β signalling components (TGF-β and BMP ligands, their receptors, and numerous SMAD cofactors) has been found in cnidarians [27–29], placozoans [15], sponges [30] and, most recently, the ctenophores *Mnemiopsis leidyi* [31] and *Pleurobrachia bachei* (table 1). Non-metazoan unikonts lack not only the ligands, receptors and SMAD cofactors [17,24–26], but also most of the inhibitors. This pathway is a clear example of a metazoan innovation [25], and the presence of all elements together in ctenophores suggests that this system was actively transducing extracellular signals into transcriptional responses in the ancestral metazoan. Notably, both ctenophores and sponges appear to lack most of the secreted antagonists to this pathway, including chordin (CHRD), follistatin (FST), noggin (NOG; but see [17]), and members of the CAN family (cerberus, DAN, gremlin), as well as the intracellular cofactors that inactivate SMAD signalling downstream of receptor activation (SARA and SKI/SNO). This suggests that modulation of TGF-β signalling through

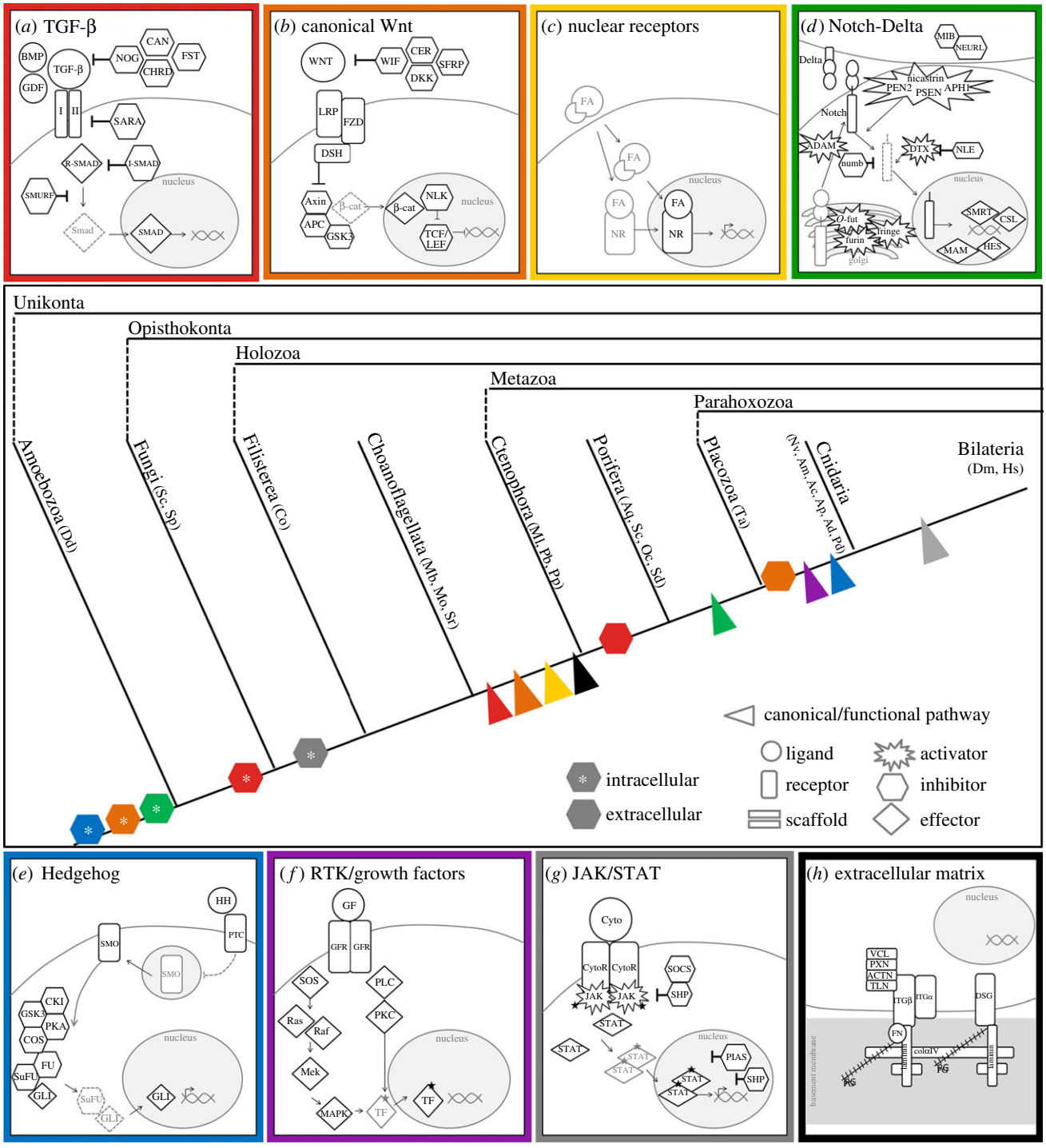

Figure 1. Schematic of the major components of the seven conserved metazoan signalling pathways: TGF-β (red), canonical Wnt (orange), nuclear receptor (yellow), Notch/Delta (green), Hedgehog (blue), RTK/growth factor (violet) and JAK/STAT (grey), and the components that comprise the basement membrane and adhesome complex (black). The origin of the canonical/functional pathway and the origin of secreted/extracellular and intracellular inhibitors are indicated on the cladogram. Abbreviations for taxa represented by each lineage are indicated in the glossary. The evolutionary origin of the canonical/functional version of each pathway is indicated at the node where conserved function has been identified experimentally.

selective inhibition in specific cells or at specific stages of development may have evolved as a means to diversify the output of this signalling pathway after its origin.

In bilaterians, TGF-β signalling plays important roles in the promotion of cell and tissue fate, immunity and, embryonically, this pathway is known to be an essential regulator or dorsal/ventral axis specification [25]. The BMP arm of the TGF-β signalling is asymmetrically expressed along the directive axis (orthogonal to the anterior/posterior axis) of the sea anemone *Nematostella vectensis* [38,53,54] and has been implicated in defining the bilateral axis of symmetry in cnidarians [39,43]. Furthermore, ectopic expression of NvSMAD (a SMAD homologue isolated from *Nematostella vectensis*) in *Xenopus* during development has demonstrated that the Nv homologue of this gene is sufficient to recapitulate the ventralized phenotype generated by vertebrate homologues in similar studies [40]. Similarly, using *in situ* hybridization, Adamska *et al*. [30] identified asymmetrical expression of TGF-β along the larval swimming axis in sponge embryos and Pang *et al*. [31] revealed that TGF-β

Table 1. TGF-β signalling pathway components across unikonts. EC, extracellular; IC, intracellular; MB, membrane bound.

									Metazoa									
			Amoebozoa	Fungi	Fungi	Filasterea	Choanoflagellata	Choanoflagellata	Ctenophora	Ctenophora	Porifera	Porifera	Placozoa	Cnidaria	Cnidaria	Cnidaria	Bilateria	Bilateria
Component	type	location	Dd[a]	Sc	Sp	Co	Mb, Mo	Sr	Ml	Pb, Pp,	Aq, Sd	Oc	Ta	Nv	Ad, Ac, Am, Ap, Pd	Hm, Hv, Pc	Dm	Hs
TGFβ/GDF[a]	ligand	EC																
BMP5-8	ligand	EC																
TGFR	receptor	MB																
BMP1/TLD	activator	EC							*	*	*	*		*		*		
R-SMAD/CoSMAD (SMAD 1/5, 2/3 , 4)	activator	IC																
I-SMAD (SMAD 6/7)	Inhibitor	IC							*	*		*	*	*	*	*		
NOG	inhibitor	EC																
CHRD	inhibitor	EC										*						
FST	inhibitor	EC											*					
CAN – CER	inhibitor	EC																
CAN – DAN/NBL1	inhibitor	EC								*			*		*	*		
CAN – GREM	inhibitor	EC												*	*	*		
SMURF	inhibitor	IC			*	*	*	*		*	*	*	*	*	*	*		
SKI/SNO	inhibitor	IC												*				
SARA	scaffold	IC												*	*			
References			[32,33]	[32,33]	[32,33]	[17,26,34]	[17,24,25,35]	[17,35]	[11,31]	[13]	[10,17,30,33,36,37]	[17,32,33]	[15,25]	[38–42]	[28,43–46]	[43,47–52]	[21,22]	[21,22]

[a]See glossary for gene and species abbreviations.

*Homologues identified by reciprocal BLAST against the human NR database; accession numbers available in electronic supplementary material. White boxes—no homologue reported in the literature or detected by BLAST. Grey boxes—putative/degenerate homologue. Black boxes—homologue present. The origin of the canonical/functional pathway is indicated by the bold line.

pathway genes are expressed asymmetrically along all three axes in *M. leidyi*. The non-overlapping expression patterns of TGF-β ligands in early ctenophores suggest these genes may be under the control of maternally deposited RNAs/proteins. Together, these developmental/functional data provide strong evidence for a conserved role for TGF-β signalling in axis specification throughout Metazoa.

In addition to this role in axis specification, Matus *et al*. [41] propose that the co-expression of transcripts from multiple TGF-β signalling components in the early endoderm of *N. vectensis* suggests an important role for this pathway in patterning germ layer identity. Indeed, studies of spatial expression of TGF-β components across basal metazoans have uncovered multiple roles for TGF-β signalling in the development and maintenance of differentiated cell types, including: regenerating head cells, tentacles, the oral nerve ring and adult endo/gastrodermal cells in cnidarians [47,48,55,56] and the embryonic pigment ring in sponges [30]. Finally, pharmacological studies in the facultatively symbiotic sea anemone *Aiptasia pallida* suggest a potential role for TGF-β in facilitating the interaction between the cnidarian host and algal symbiont [57]. Together, these patterns suggest that this pathway may have had an original function in transducing maternally derived molecules into developmental signals regulating axis specification, but clearly this pathway was been co-opted, spatially and temporally, to regulate several aspects of larval and adult tissue identity.

(b) Canonical WNT signalling

The WNT signalling pathways are known to play diverse roles in the cell/tissue biology of metazoans (reviewed by [58]). cWNT signalling is initiated by binding of the extracellular WNT ligand to its receptor complex composed of frizzled (FZD) and lipoprotein receptor-related protein 5/6 (LRP5/6) (figure 1*b*). This activates an intracellular signal transduction cascade through which dishevelled (DSH) inhibits glycogen synthase kinase 3 (GSK-3) leading to the translocation of β-catenin (β-cat) to the nucleus where it complexes with the transcription factors T-cell-specific transcription factor/lymphoid enhancer binding factor (TCF/LEF) to regulate gene expression. Like TGF-β signalling, cWNT signalling appears to be a metazoan innovation as the ligands, receptors and intracellular transducers are largely excluded from pre-metazoan taxa (table 2). While the genome of the amoebozoan *Dictyostelium discoideum* encodes a putative orthologue of β-cat [59], several FZD-like receptors [60] and an orthologue of GSK-3 [71], with the exception of GSK-3, no cWNT pathway genes are encoded in the genomes of other unikonts. This suggests either the convergent evolution of FZD-like proteins in amoebozoans and metazoans or that these components were lost after the divergence of amoebozoans and re-evolved at the base of Metazoa. Cnidarians have not only a complete cWNT pathway, but also have a surprising diversity of WNT ligands [65,72]; by contrast, sponges and placozoans have orthologues of FZD, LRP5/6, GSK-3 and β-cat but

Table 2. Canonical WNT signalling pathway components across unikonts. EC, extracellular; IC, intracellular; MB, membrane bound.

									Metazoa									
			Amoebozoa	Fungi	Fungi	Filasterea	Choanoflagellata	Choanoflagellata	Ctenophora	Ctenophora	Porifera	Porifera	Placozoa	Cnidaria	Cnidaria	Cnidaria	Bilateria	Bilateria
Component	type	location	Dd[a]	Sc	Sp	Co	Mb, Mo	Sr	Ml	Pb, Pp	Aq, Sd	Oc	Ta	Nv	Ac, Ad, Am, Ap, Pd	Hm, Hv, Pc	Dm	Hs
WNT[a]	ligand	EC																
FZD	receptor	MB																
LRP	receptor	MB								*			*	*				
DIXDC	activator	IC								*			*	*	*	*		
DSH	effector	MB																
β-cat	effector	IC																
TCF/LEF1	effector	IC																
SFRP	inhibitor	EC	*							*								
GSK-3	inhibitor	IC		*	*													
APC	inhibitor	IC							*			*	*		*			
Axin	inhibitor	IC										*						
DKK-1	inhibitor	EC																
WIF-1	inhibitor	EC														*		
NLK	inhibitor	IC									*					*		
References			[26,32,52,59,60,61]	[32]	[32]	[17,26]	[17,24,35]	[17,35]	[62]	[13,63]	[17,36,64]	[10,17,32]	[15,16]	[27,65–67]	[46]	[29,49,52,55,65,68,69]	[67,70]	[70]

[a]See glossary for gene and species abbreviations.

*Homologues identified by reciprocal BLAST against the human NR database; accession numbers available in electronic supplementary material. White boxes—no homologue reported in the literature or detected by BLAST. Grey boxes—putative/degenerate homologue. Black boxes—homologue present. The origin of the canonical/functional pathway is indicated by the bold line.

only few WNT ligands (4 and 3, respectively) [10,15,30]. Pang *et al*. [73] found a nearly complete set of cWNT pathway genes present in the ctenophore *M. leidyi* but, like sponges and placozoans, ctenophores have only few (4) WNT ligands [62,73]. As was seen for the TGF-β pathway, cWNT signalling in ctenophores, sponges and placozoans appears to lack inhibitory control—conspicuously absent from ctenophore, sponge and placozoan genomes are WNT inhibitory factor (WIF), dickkopft (DKK) genes and cerberus (CER), which are important inhibitors of cWNT signalling in cnidarians and bilaterians, and axin, which is part of the β-cat degradation complex, is missing from both published ctenophore genomes. Finally, both sponges and ctenophores have fewer ligands and receptors for the cWNT pathway than do cnidarians and bilaterians and the sponge paralogues appear to be the result of independent duplication of an ancestral gene in the sponge lineage [10]. Thus, sponges appear to have undergone their own WNT ligand expansion (albeit, a more limited one) sometime after they diverged from the rest of Metazoa.

cWNT signalling plays diverse roles in cell/tissue development and homeostasis. Although this pathway has been implicated in patterning of the primary axis in some bilaterians [63], the earliest developmental role of cWNT appears to be defining the site of gastrulation (the blastopore) and establishing this tissue as an embryonic organizing centre [74–76]. Among cnidarians, cWNT signalling has been shown to facilitate the establishment of embryonic [65,77,78] and adult [29,72] organizing centres in both anthozoan and hydrozoan model systems. Although functional studies in sponges are lacking, studies of WNT gene expression *in situ* suggest that this pathway is also asymmetrically expressed along the larval swimming axis and during development of the adult axis (defined by the aquiferous system) [36,79,80]. Treatment with pharmacological activators of cWNT signalling (e.g. LiCl) in sponges also point to a possible role for this pathway in specifying the epithelia in adult sponges [63], though clearly functional studies of cWNT signalling in sponges are much needed. Together, these data suggest that cWNT-regulated specification of the blastopore and the subsequent defining of the anterior/posterior axis were early features of metazoan development but that this pathway may have been co-opted to play a role in specification of larval and adult tissues in some lineages. Although gastrulation has not been observed in placozoans [14] and the only identifiable axis in these animals appears to be a dorsal/ventral axis, the genome of *T. adhaerens* encodes a full complement of WNT/β-cat signalling components [15]. It is intriguing to consider what organizing centre or potential axis may be revealed if analyses of spatial gene expression are one day performed in these animals.

Using whole mount *in situ* hybridization, Pang *et al*. [31] described the expression of cWNT pathway genes in *M. leidyi*. Surprisingly, WNT genes were detected only late in development, after the onset of gastrulation. With the use of RNAseq, WNT transcripts were detected much earlier in the development of *P. bachei* [13]. No functional studies of cWNT signalling have been performed in ctenophores, so it is unclear if these early expressed WNTs play a homologous role in the specification of the ctenophore blastopore. Interestingly, pharmacological treatment with cWNT activators (e.g.

LiCl and alsterpaullone) has little effect on early development in *M. leidyi* (K Pang and MQ Martindale, personal observations). Alternatively, the asymmetrical division of maternally loaded proteins and/or the ratio of nuclear material to cytoplasm have been suggested to be important drivers of early embryonic cell fate in ctenophores [81]. How the WNT/β-cat pathway interacts with these cytological determinants (e.g. nuclear : cytoplasm ratio) is not known but studies of this type could reveal an ancestral mechanism for the activation of the cWNT pathway in ctenophores. It has been suggested that the overlapping expression domains of three different WNT pathways—cWNT, WNT/planar cell polarity (PCP) and WNT/Ca^{2+}—in cnidarians suggests these pathways work synergistically to pattern the primary axis in this lineage. Curiously, ctenophores lack the essential components of the PCP pathway [11,82], suggesting this synergistic effect of multiple WNT signalling pathways on axis specification must have arisen after ctenophores split from the lineage giving rise to sponges and parahoxozoans.

(c) Nuclear receptor signalling

NRs are transcription factors that translate ligand binding directly into activation or repression of transcription. NRs can be activated by binding a hydrophobic ligand (e.g. fatty acid and steroid), complexing with other proteins, or undergoing post-translational modification, after which they bind DNA directly to regulate (activate or repress) the expression of target genes (figure 1*c*). There are two conserved functional domains diagnostic of NRs: the DNA binding domain responsible for transcriptional regulation and the ligand binding domain to which hydrophobic ligands bind. NRs are thought to have evolved from ancestral molecules that did not require activation by a ligand [83,84]; thus, the expansive diversity in this group of proteins may have evolved with the independent origins of numerous diverse ligand binding capabilities [85]. Unlike the other cell signalling mechanisms discussed here, NRs act as both the receptor and the effector molecule, providing the most direct link between the extracellular signal and the transcriptional response. Pathways of this type may be the easiest to evolve because they rely on only few signalling components; the massive expansion of the NR gene family in nematodes provides support for this hypothesis [86]. Conversely, the small number of nodes that comprise this pathway means there are fewer opportunities for co-option/modification of this pathway once it evolves.

Like the TGF-β and cWNT pathways, NRs evolved in the stem metazoan. These receptors have been described from cnidarians [87], placozoans [15,88], sponges [89,90] and ctenophores [91], but are missing from choanoflagellates [24], filastereans [26,35], fungi [92] and amoebozoans (table 3). Over 17 NRs have been identified among cnidarians, many of which lack clear orthology with bilaterian NRs but have clear orthologues in other cnidarians, suggesting that this gene family expanded through several rounds of cnidarian-specific duplication events [87,91]. Srivastava *et al.* [15] identified four NRs from placozoans, orthologues of: hepatocyte nuclear factor 4 (HNF4), retinoid X receptor (RXR), an NR2 family member, and one that appears to lack orthology with other available sequences. Baker [88] also described an oestrogen-related receptor (family NR3) from *T. adhaerens* but indicated that it contained a hormone binding pocket that was too small to accommodate a hormone, suggesting it may not function the way NR3 family receptors do in bilaterians. Among sponges, two NRs have been identified from *Amphimedon queenslandica*, *Suberites domuncula* and *Oscarella carmela*—one, NR2, appears to be an orthologue of HNF4 and is found only in *A. queenslandica* and *O. carmela*; the second, apparently sponge-specific, is found in *A. queenslandica*, *S. domuncula* and *O. carmela* [85,91]. Three NR orthologues have been identified from ctenophores (two from *M. leidyi* and one from *P. bachei*), all of which appear to be orthologues of the NR2 family but none of which has a canonical DNA binding domain. This suggests that the DNA binding domain (and, likely, the ability to regulate transcription) may have arisen as a feature of NRs only after ctenophores diverged from the rest of Metazoa [91].

Three classes of NRs have been identified, based on their mode of activation; one group becomes active (and activates transcription) upon binding a hydrophobic ligand, another requires no ligand and is constitutively active, and a third exclusively acts to repress the function of other NRs [85]. All three classes of NRs are represented among bilaterians, and the functional output of these signalling molecules is accordingly diverse. Perhaps most famously, this group of signalling molecules includes the retinoic acid receptors, which are known to play a critical role in establishing morphogen gradients and regulating cell identity during development of bilaterians [93]. Only few studies of NR function have been performed in non-bilaterian animals. Notably, signalling through the RXR receptor has been examined in several species of cnidarian; exogenous application of retinoic acid has been shown to play a role in promoting metamorphosis in the jellyfish *Aurelia aurita* [94] and regulating the development of the eyes in the box jelly *Tripedalia cystophora* [95]. Further, the COUP-TF transcription factor (NR2 family) is an important regulator of neurogenesis in hydrozoans [96,97]. While Reitzel & Tarrant [87] demonstrated differential expression of NRs through developmental time in *N. vectensis*, without spatial analysis of gene expression it is difficult to hypothesize what role these diverse receptors may play in embryogenesis of the sea anemone. The two NRs identified from the demosponge *A. queenslandica* also appear to be functional during development. The HNF4 homologue is expressed in the ciliated cells of the ectoderm before they are specified to become choanocytes [90] and functional studies suggest that both sponge NRs bind fatty acids but the outcome of this interaction differs. While NR1 activates transcription in response to ligand binding, NR2 appears to repress transcription [85]. Thus, at least two different mechanisms of NR-mediated cell signalling appear to have been present before the divergence of sponges. Although NR signalling has not been characterized in the ctenophores, whole mount *in situ* hybridization in *M. leidyi* suggests that transcripts for these receptors are expressed only after the onset of gastrulation [91]. Thus, it seems NRs may not play a prominent role in the earliest specification of cell identity in ctenophores.

5. Parahoxozoan pathways

(a) Notch/Delta signalling

The Notch/Delta signalling cascade is unlike other signalling mechanisms described herein inasmuch as both Notch and its

Table 3. Nuclear receptor signalling pathway components across unikonts. IC, intracellular.

									Metazoa									
			Amoebozoa	Fungi	Fungi	Filasterea	Choanoflagellata	Choanoflagellata	Ctenophora	Ctenophora	Porifera	Porifera	Placozoa	Cnidaria	Cnidaria	Cnidaria	Bilateria	Bilateria
Component	type	location	Dd[a]	Sc	Sp	Co	Mb, Mo	Sr	Ml	Pb, Pp	Aq, Sd	Oc	Ta	Nv	Ac, Ad, Am, Ap, Pd	Hm, Hv, Pc	Dm	Hs
NR1[a]	receptor/effector	IC																
NR2	receptor/effector	IC									*	*						
NR3	receptor/effector	IC																
NR4	receptor/effector	IC																
NR5	receptor/effector	IC																
NR6	receptor/effector	IC																
lineage specific	receptor/effector	IC									*	*						
References				[92]	[92]	[26,34]	[24,26,35]	[35]	[91]	[13,91]	[10,85,89–91]		[15,85,88,91]	[85,87,91]	[83,85,91]	[91]	[85]	[85]

[a]See glossary for gene and species abbreviations.

*Homologues identified by reciprocal BLAST against the *Amphimedon queenslandica* NR database; accession numbers available in electronic supplementary material. White boxes—no homologue reported in the literature or detected by BLAST. Grey boxes—putative/degenerate homologue. Black boxes—homologue present. The origin of the canonical/functional NR pathway is considered to be coincident with the origin of metazoan NR homologs and is indicated by the bold line.

canonical ligand (Delta) are membrane-bound proteins; thus, this system behaves as a juxtacrine signalling system, transducing signals between adjacent cells (see [98] for a thorough review of Notch/Delta signalling in bilaterians). Binding of the ligand (Delta) catalyses cleavage of the Notch intracellular domain that translocates to the nucleus to effect transcriptional change (figure 1*d*). Beyond the ligand (Delta) and receptor (Notch), the proteases that process the Notch preprotein (furin, O-fut and fringe) and liberate the intracellular domain from the extracellular domain (ADAM-type metalloproteases and the γ-secretase complex) are critically important for effective signalling. Outside of Bilateria, Notch homologues have been found in cnidarians [27,99], placozoans [11,15], sponges [17] and ctenophores [11,13]. Notch-like proteins have also been described from the choanoflagellate *Monosiga brevicollis* and the filasterean *Capsaspora owczarzaki*, though these proteins lack key functional domains including the Delta interaction domain (DSL) in the former and the epidermal growth factor (EGF) domains in both taxa [26]. While Delta has been described from each of these taxa as well, analysis of the Delta-like molecule found in *M. brevicollis* and *C. owczarzaki* suggests this protein may interact with other EGF domain-containing proteins but likely does not interact with the Notch-like proteins in these taxa [26]. Similarly, the Delta protein described in ctenophores may not function equivalently to the canonical Delta ligand from other taxa because it is missing key diagnostic domains [11]. Only few of the numerous molecules involved in Notch/Delta signalling are metazoan-specific (including both Notch and Delta; table 4). Specifically, many of the enzymes associated with this pathway (e.g. ubiquitin ligases and ADAM-type proteases) were present before the origin of Metazoa, suggesting Notch/Delta signalling may have evolved by co-option of an existing intracellular cascade [100].

Notch/Delta signalling functions primarily in promoting differential cell identity in subpopulations of adjacent cells. Two main developmental roles have been described for this pathway: lateral inhibition promoting one of two possible fates and boundary mechanisms that induce a third cell fate at the interface between two other cell fates [100]. The mechanism of Notch/Delta signalling appears to depend on stochastic variation in the abundance of both the ligand and receptor [103]. Notch/Delta signalling pathways are known to be functional in cnidarians and bilaterians but the roles of these pathways may be quite different in these two lineages. Pharmacological studies using the γ-secretase inhibitor DAPT have identified a role for Notch/Delta signalling in neural specification across cnidarians [99,104,105], but knockdown experiments in the sea anemone *N. vectensis* suggest that cnidarians use the non-canonical Notch pathway, independent of the hairy/enhancer of split (HES) transcription factors, in this process [106]. Curiously, cnidarians have orthologues of HES genes (indeed, HES genes are found throughout Metazoa), suggesting that the canonical Notch/HES signalling pathway may be used in a context other than neural specification in non-bilaterian metazoans. In sponges, Notch and Delta are expressed in adjacent tissues during embryonic development and the downstream transcription factors have been shown to have neurogenic effects when expressed during development in *Xenopus* and *Drosophila* [107]. *In situ*, Notch/Delta signalling seems to regulate cell identity in globular cells of *A. queenslandica* [107]. Thus, in both cnidarians and sponges, Notch/Delta signalling promotes the differentiation of specialized cell fate during early embryogenesis. The putative function of this pathway has not been examined extensively in ctenophores but preliminary pharmacological assays using the γ-secretase inhibitor DAPT resulted in no detectible effects on development in *M. leidyi* (K Pang and MQ Martindale, personal

Table 4. Notch/Delta signalling pathway components across unikonts. AC, adjacent cell; IC, intracellular; MB, membrane bound.

													Parahoxozoa					
			Amoebozoa	Fungi	Fungi	Filasterea	Choanoflagellata	Choanoflagellata	Ctenophora	Ctenophora	Porifera	Porifera	Placozoa	Cnidaria	Cnidaria	Cnidaria	Bilateria	Bilateria
Component	type	location	Dd[a]	Sc	Sp	Co	Mb, Mo	Sr	Ml	Pb, Pp	Aq, Sd	Oc	Ta	Nv	Ac, Ad, Am, Ap, Pd	Hm, Hv, Pc	Dm	Hs
Notch[a]	receptor	MB																
Delta	ligand	MB														*		
O-fut	activator	IC					*	*										
Fringe	activator	IC										*						
Furin	activator	IC																
ADAM17	activator	IC					*	*				*				*		
ADAM10	activator	IC						*				*			*			
DTX	activator	IC						*				*	*	*				
PSEN	activator	MB						*				*						
Nicastrin	activator	MB						*				*				*		
APH1	activator	MB						*	*	*		*						
PEN2	activator	MB						*		*								
CSL	effector	IC						*				*				*		
MAM	effector	IC												*	*			
SMRT/NCOR2	effector	IC										*			*			
HES	effector	IC										*						
Numb	inhibitor	IC							*	*		*	*	*		*		
NEURL	inhibitor	AC												*				
MIB	inhibitor	AC							*	*		*		*		*		
NLE	inhibitor	IC	*	*	*			*				*						
References			[32,100]	[32,100]	[32,100,101]	[17,26]	[11,17]	[17]	[11]	[13,100]	[11,17,100,102]	[17,32,100]	[11,15,100]	[11,27,100]	[27,46]	[11,99]	[98]	[11,100]

[a]See glossary for gene and species abbreviations.

*Homologues identified by reciprocal BLAST against the human NR database; accession numbers available in electronic supplementary material. White boxes—no homologue reported in the literature or detected by BLAST. Grey boxes—putative/degenerate homologue. Black boxes—homologue present. The origin of the canonical/functional pathway is indicated by the bold line.

observations). Although ctenophores lack a complete Delta ligand [11], abundant research from bilaterians supports a functional role for Delta-like ligands in tissue specification during development. What this pathway may regulate in ctenophores is unknown, but the fact that ctenophores seem to be insensitive to γ-secretase is intriguing and leads us to conclude that the canonical/functional Notch/Delta pathway evolved after ctenophores diverged from other metazoans.

6. Pathways restricted to Cnidaria + Bilateria

(a) Hedgehog signalling

The hedgehog (HH) pathway regulates many aspects of cell and tissue identity during bilaterian development (see [108] for review). Upon binding of the HH ligand to the patched (PTC) receptor, constitutive inhibition of the smoothened (SMO) receptor is relaxed, leading to the translocation of SMO to the cell membrane (figure 1*e*). Insertion of SMO into the plasma membrane enhances the interaction between SMO and the intracellular HH signalling components and results in the release of the transcription factor GLI from the signalling complex and leads to its translocation into the nucleus to modulate transcription. HH signalling is well known to operate through the establishment of a ligand concentration gradient; thus, cells responding to HH far from the source of the ligand respond differently from those near the source. The HH pathway is an interesting model for the modular evolution of cell signalling pathways; while a complete set of HH signalling components (ligand, dispatched transmembrane transporter, both PTC and SMO receptors, and the GLI transcription factor) is restricted to the group containing cnidarians and bilaterians (table 5), there is clear evidence for the origin of individual pathway components well before this. Both the 'Hedge' and 'Hog' domains that define the ligand arose before the origin of the complete *HH* gene [111]. Indeed, choanoflagellates, sponges and cnidarians have proteins with Hedge domains that lack the associated Hog domain characteristic of the true HH ligand [11]. Even without a Hog domain, these so-called *Hedgling* genes contain many functional domains (e.g. von willebrand factor, cadherin and EGF domains) suggesting they are capable of interacting with many other signalling components [30].

Ctenophores lack the orthologues of several HH pathway components. Orthologues of both *HH* and *Hedgling* ligands are missing from the genomes of *M. leidyi* and *P. bachei*, as are the PTC and SMO receptors [11]. The phylogenetic patterns represented in table 5 suggest that the lack of PTC and *Hedgling* orthologues may represent secondary loss in ctenophores, indicating that these molecules may have

Table 5. Hedgehog signalling pathway components across unikonts. EC, extracellular; IC, intracellular; MB, membrane bound.

														Cnidaria + Bilateria				
			Amoebozoa	Fungi	Fungi	Filasterea	Choanoflagellata	Choanoflagellata	Ctenophora	Ctenophora	Porifera	Porifera	Placozoa	Cnidaria	Cnidaria	Cnidaria	Bilateria	Bilateria
Component	type	location	Dd[a]	Sc	Sp	Co	Mb, Mo	Sr	Ml	Pb, Pp	Aq, Sd	Oc	Ta	Nv	Ac, Ad, Am, Ap, Pd	Hm, Hv, Pc	Dm	Hs
HH[a]	ligand	EC													*			
Hedge only	domain	EC												*	*			
Hint/Hog only	domain	EC							*					*	*			
PTC	receptor	MB				*	*	*				*			*	*		
DISP	activator	MB							*	*		*			*	*		
FU/STK36	activator	IC	*						*	*			*		*	*		
SMO	effector	MB	*									*		*	*			
GLI	effector	IC							*	*					*			
KIF7/COS	inhibitor	IC							*	*	*		*	*	*	*		
SuFU	inhibitor	IC										*			*	*		
PKA	inhibitor	IC	*	*	*				*	*			*		*	*		
HHIP	inhibitor	IC				*						*			*	*		
CKI	inhibitor	IC	*	*	*				*	*					*	*		
References			[32]	[32]	[32]	[11,17,26]	[11,91,109]	[11,35]	[11,15,110]	[13]	[11,17,64]	[11,17,26]	[17,32]	[11,15,16]	[11,66]	[11]	[11,108]	[11]

[a]See glossary for gene and species abbreviations.

*Homologues identified by reciprocal BLAST against the human NR database; accession numbers available in electronic supplementary material. White boxes—no homologue reported in the literature or detected by BLAST. Grey boxes—putative/degenerate homologue. Black boxes—homologue present. The origin of the canonical/functional pathway is indicated by the bold line.

experienced relaxed selection associated with loss of a specific pathway or function during the divergence of ctenophores from rest of Metazoa. Understanding how PTC and *Hedgling* orthologues function in filastereans and choanoflagellates is critical to understanding the evolution of the canonical pathway from these early precursors. Furthermore, while *M. leidyi* does have an orthologue of the GLI transcription factor, this gene encodes only four of the five diagnostic zinc finger domains [110], thus it is unknown whether this transcription factor is functional. Interestingly, placozoans do not have orthologues of any of the essential components of HH signalling [11,15] but given their position between sponges and cnidarians, this likely represents a lineage-specific loss of the pathway.

The role of *HH* genes in bilaterian development has been well characterized as they are known to play a crucial role in establishing tissue-specific organizers (e.g. the limb ZPA and neural floor plate) during embryogenesis [112]. Outside of Bilateria, cnidarians are the only metazoans to have true *HH* orthologues and their role in development has been studied only by whole mount *in situ* hybridization in the sea anemone *N. vectensis* [66]. The overlapping expression of the PTC receptor and GLI transcription factor in the endodermal mesenteries and their proximity to the expression of both *HH* genes suggests this pathway may be regulating the development of endodermal cell identity in cnidarians. Although they are not true *HH* orthologues, *Hedgling* genes are also expressed during development in cnidarians and sponges [30]. Indeed, the overlapping/abutting expression of TGF-β, WNT and *Hedgling* revealed by *in situ* hybridization during formation of the pigment ring in *A. queenslandica* and the endodermal mesenteries in *N. vectensis* [19,30] is reminiscent of the overlapping expression of TGF-β/WNT/*HH* expression during bilaterian organ specification and suggests that *Hedgling* and *HH* may play similar regulatory roles in non-bilaterians. Understanding the putative differences between the roles of *HH* and *Hedgling* in cnidarians and the function of *Hedglings* in sponges and choanoflagellates would shed much-needed light on the origin/evolution of this protein family and functional studies would be particularly useful in this context. Ctenophores encode a Hog domain, but there is no evidence of Hedge domains in this lineage [11]. Neither the spatial/temporal expression of this Hog-domain containing transcript nor the putative function of the protein it encodes has been studied in ctenophores; thus, the putative role that HH pathway genes play in the development of ctenophores remains elusive.

(b) Receptor tyrosine kinase/growth factor signalling

Protein tyrosine kinases (PTKs) function to phosphorylate other proteins, enabling them to manipulate (activate/inhibit) the function of other proteins. RTKs are one large class of PTKs that have evolved a transmembrane domain and transduce the binding of extracellular signals (e.g. growth factors, cytokines and amino acids) into altered phosphorylation states of their targets. Binding of the ligand induces dimerization and stabilization of the receptor in the cell membrane. This stabilization of the RTK activates an intracellular signalling pathway, typically the Ras/Raf/MAPK protein kinase pathway, resulting in phosphorylation of a target transcription factor that can then move into the nucleus to promote or repress transcription (figure 1*f*).

Table 6. RTK signalling pathway components across unikonts. IC, intracellular; MB, membrane bound.

														Cnidaria + Bilateria				
			Amoebozoa	Fungi	Fungi	Filasterea	Choanoflagellata	Choanoflagellata	Ctenophora	Ctenophora	Porifera	Porifera	Placozoa	Cnidaria	Cnidaria	Cnidaria	Bilateria	Bilateria
Component	type	location	Dd[a]	Sc	Sp	Co	Mb, Mo	Sr	Ml	Pb, Pp	Aq, Sd	Oc	Ta	Nv	Ac, Ad, Am, Ap, Pd	Hm, Hv, Pc	Dm	Hs
EGFR/ERBB (Class I)[a]	receptor	MB										*						
INSR (Class II)	receptor	MB						*				*			*	*		
PDGFR (Class III)	receptor	MB									*				*			
FGFR (Class IV)	receptor	MB											*		*			
VEGFR (Class V)	receptor	MB																
MET/HGFR (Class VI)	receptor	MB									*	*						
TRK (Class VII)	receptor	MB												*				
EPHR (Class VIII)	receptor	MB										*			*			
AXL/UFO (Class IX)	receptor	MB									*	*						
ALK/LTK (Class X)	receptor	MB																
TIE (Class XI)	receptor	MB																
ROR (Class XII)	receptor	MB									*				*			
DDR (Class XIII)	receptor	MB										*			*			
RET (Class XIV)	receptor	MB										*	*					
PTK7/CCK4 (Class XV)	receptor	MB										*			*			
RYK (Class XVI)	receptor	MB										*	*					
MUSK (Class XVII)	receptor	MB							*	*	*			*	*	*		
lineage specific	receptor	MB																
Ras/Raf	effector	IC																
MAPK	effector	IC																
References			[116–119]	[116,120,121]	[121]	[26,116]	[35,115,116,122]	[35]	[11]	[13]	[116]	[123]	[17,32]	[124]		[125–127]	[116,124]	[116,124]

[a]See glossary for gene and species abbreviations.

*Homologues identified by reciprocal BLAST against the human NR database; accession numbers available in the electronic supplementary material. White boxes—no homologue reported in the literature or detected by BLAST. Grey boxes—putative/degenerate homologue. Black boxes—homologue present. The origin of the canonical/functional pathway is indicated by the bold line.

Certain members of the RTK family signal through alternative means, relying instead on the activation of phospholipase C (PLC) and phosphorylation of target genes by protein kinase C (PKC) or even through the JAK/STAT pathway (described below). The diversity of RTKs is matched by the equally diverse mechanisms by which they transduce signals and the numerous roles they play in cell biology [113]. Similar to the expansive repertoire of NR paralogues described above, this may suggest the evolutionary expansion of RTKs resulted from duplication followed by modifications to the ligand binding domain that allowed them to respond to diverse extracellular cues. Accordingly, the intracellular kinase domain of RTKs exhibits greater conservation than the extracellular ligand-binding domains.

Bilaterians have dozens of family members, but the origin of RTKs predates the dawn of Metazoa as numerous RTKs have been described from choanoflagellates [114,115] and filastereans [116]. The genomes of amoebozoans and fungi encode PTKs but not RTKs (table 6) [117]. A comparison of the protein domains in RTKs from filastereans, choanoflagellates and metazoans suggests that the pre-metazoan RTKs lack homologues in other lineages, suggesting RTKs underwent expansive diversification independently in each lineage before the origin of Metazoa [116]. Clear homologues of bilaterian RTKs first appear in the Ctenophora with the emergence of an EPHR in *M. leidyi* [11] and an EGFR/ERBB receptor in *P. bachei* [13] and expanded considerably in sponges and cnidarians. Indeed, analysis of the *A. queenslandica* genome identified approximately 150 RTKs from sponges [10], only some of which appear to have ligands that are orthologous to other metazoan RTK ligands (e.g. EGFR, EPHR and INSR). Thus, the origin of the bilaterian family of RTKs did not preclude further evolution of lineage-specific RTKs [123,125] and it will be interesting to learn whether ctenophores have their own lineage-specific RTKs. A novel RTK family—venus kinase receptor (VKR)—was recently identified in *N. vectensis* as well as several bilaterian lineages but is absent from sponges and *Hydra*. A search of the *M. leidyi* genome for the diagnostic VFT domain of VKR-related proteins failed to find any significant matches in this ctenophore, suggesting VKRs also evolved after ctenophores and sponges diverged from the cnidarian/bilaterian ancestor [126]. Also absent from ctenophores and sponges is the FGFR family of RTKs [128], which is known to have many important roles governing the development of cell and tissue identity in Cnidarians and Bilaterians. Finally, despite the lack of conserved RTK orthologues among basal unikonts, the intracellular effectors of RTK signalling (i.e. Ras/Raf/MAPK) were present in amoebozoans. Again, this signalling pathway seems to have evolved by co-option of an existing intracellular signalling mechanism after the origin of the ligand/receptor.

While the bilaterian RTK family is expansive [124] and well known for promoting cell differentiation during tissue patterning, the role of RTKs in directing the development of non-bilaterians is poorly understood. Fibroblast growth factor (FGF) orthologues first emerged before cnidarians diverged from bilaterians. In *N. vectensis*, FGF signalling was assayed using a combination of descriptive and functional techniques and was shown to regulate the development of the aboral sensory organ (apical tuft) in planula larvae [129–131]. Recent studies of *Hydra* have identified a putative role for FGF in maintenance of the interstitial stem cell lineage and for defining the termini of the animal, whereas another growth factor (vascular endothelial growth factor, VEGF) was shown to be involved in specification of neurons [132,133]. Lemon, an orthologue of protein tyrosine kinase-like 7 (PTK7/CCK4) family RTKs from *Hydra vulgaris*, is expressed in interstitial stem cells and upregulated during times of gametogenesis [127]. Combined, these data suggest that RTKs play numerous diverse roles in regulating cell specification and tissue morphogenesis in cnidarians, supporting the origin of functional RTK signalling before the cnidarian/bilaterian split. In addition to *bona fide* FGFs, FGF-like proteins have been identified from choanoflagellates, ctenophores, sponges, placozoans and cnidarians [128]. These proteins are characterized by a β-trefoil fold and group more closely with *bona fide* FGFs than with any other protein family, but the potential role of these proteins in the developmental biology of these taxa is unknown. FGFR-like proteins were recently characterized in cnidarians and chordates and appear to be expressed in early development [134], suggesting this putative FGF-like pathway may have a role in directing cell/tissue specification or aspects of morphogenesis. The presence of putative RTK homologues in ctenophores, sponges and placozoans suggests that these pathways may operate (canonically or not) in these taxa as well, but this remains to be empirically tested.

7. Bilaterian-specific signalling pathways

(a) Janus kinase/signal transducer and activator of transcription signalling

The JAK/STAT signalling cascade plays an essential role in cell signalling in bilaterians by transducing secreted peptide signals (typically cytokines) into transcriptional activation responses (see [135,136] for recent reviews). Intracellular JAK proteins become activated by complexing with transmembrane receptors in the cytokine receptor class I/II families. This interaction leads to the phosphorylation and dimerization of STAT transcription factors in the cytoplasm that then undergo translocation to the nucleus, where they activate transcription of target genes (figure 1*g*). Inactivation of the pathway is accomplished through dephosphorylation by SH2-domain containing protein tyrosine phosphatases (SHPs) or through negative regulation by molecules including suppressor of cytokine signalling (SOCS) and protein inhibitor of activated STAT (PIAS). Studies in *Drosophila* revealed a non-canonical JAK signalling pathway responsible for modification of heterochromatin architecture, revealing the potential for this pathway to affect many genes beyond the specific targets of STAT activation [135].

The complete repertoire of proteins comprising the canonical signalling pathway (cytokines, cytokine receptors, JAK, STAT, SHP and SOCS) is restricted to the Bilateria as there are no clear orthologues of JAK in other Unikonts. As was the case with hedgehog signalling, however, many of the intracellular components of JAK/STAT signalling were present before the evolution of the canonical/functional pathway (table 7). A proto-JAK first appeared before the origin of Porifera, but JAK-like proteins in sponges and cnidarians both lack a pseudo PTK domain [137]. The evolutionary history of STAT proteins may be slightly older than that of JAK. STAT-like transcription factors have been reported from all major lineages of unikonts; however, most of these molecules lack the C-terminal transactivation (TAD) domain. Despite this, the cnidarian STAT-like protein appears to complex with nuclear factor kappa-B (NFκB) to serve as part of the rudimentary immune system [140]. This suggests that STAT-like proteins may have engaged in transcriptional regulation before becoming associated with the JAK signalling cascade. Clearly, the involvement of ancestral STAT-like proteins and their partners (including proto-JAKs) in cell–cell signalling has not been sufficiently studied in any of the non-bilaterians. This pathway, however, appears to be one of the few/unusual examples whereby the negative regulator evolved before the full pathway as SOCS, PIAS and SHPs all evolved long before the origin of canonical JAKs and STATs. Understanding what other functions these inhibitory molecules play in organisms that lack the full JAK/STAT pathway may indeed reveal novel protein–protein interactions and point to previously uncharacterized signalling pathways.

8. Tying the room together: the role of the extracellular matrix in cell–cell communication

The evolution of the extracellular matrix (ECM) was pivotal in permitting the evolution of large complex organisms as it allowed the directed transmission of extracellular signals among cells. The ECM is now known to play a critical role in cell–cell signalling [141], and much progress has been made in characterizing the evolutionary history of the ECM and animal epithelia [142–144]. Epithelia are not unique to metazoans; indeed, the fruiting body of the amoebozoan *D. discoideum* forms a polarized epithelium in its multicellular stage, using a catenin-based system of cell–cell adhesion [145,146]. Although cell–cell adhesion evolved before the origin of Holozoa, the rise of multicellularity did not occur until much later. The origin of the ECM and its ability to facilitate the development of diffusion gradients likely provided a significant selection advantage at the origin of complex life. Here, we focus specifically on two aspects of ECM: the evolution of the basement membrane, which provided the infrastructure for the development of signal gradients, and the evolution of the adhesome complex (integrin and its associated molecules), which provided a means to keep cells attached to the ECM.

Essential components of the basement membrane include type IV collagen (ColαIV), the multiple subunits of laminin (LAMA, LAMB1 and LAMC), and both membrane-bound and free/secreted heparan sulfate proteoglycans (HSPGs; e.g. glypican and perlecan; figure 1*h*). Several components of the

Table 7. JAK/STAT signalling pathway components across unikonts. EC, extracellular; IC, intracellular; MB, membrane bound.

																Bilateria	
			Amoebozoa	Fungi	Fungi	Filasterea	Choanoflagellata	Choanoflagellata	Ctenophora	Ctenophora	Porifera	Porifera	Placozoa	Cnidaria	Cnidaria	Cnidaria	Bilateria	Bilateria
Component	type	location	Dd[a]	Sc	Sp	Co	Mb, Mo	Sr	Ml	Pb, Pp	Aq, Sd	Oc	Ta	Nv	Ac, Ad, Am, Ap, Pd	Hm, Hv, Pc	Dm	Hs
Cyto[a]	ligand	EC																
CytoR	receptor	MB																
JAK[b]	activator	IC										*						
STAT[c]	effector	IC				*		*		*	*	*		*	*			
PIAS	inhibitor	IC						*	*	*	*	*	*	*	*	*		
SOCS	inhibitor	IC										*			*			
SHP	inhibitor	IC				*		*	*	*	*	*			*	*		
References			[26,32,137,138]	[32,138]	[32,138]	[26,34,116,138]	[26,34,115,137,138]	[138]	[138]	[13,138]	[13,138]	[32,138]	[15,16,138]	[13,137,138]	[138]	[138,139]	[135–137]	[135–137]

[a]See glossary for gene and species abbreviations.

[b]JAK homologues were identified by the presence of the following domains: FERM, SH2 and at least one PTK.

[c]Non-bilaterian STAT homologues were identified by the presence of the following domains: N-terminal, alpha coil, DNA binding, and SH2; the TAD domain is clearly a bilaterian novelty [137].

*Homologues identified by reciprocal BLAST against the human NR database; accession numbers available in the electronic supplementary material. White boxes—no homologue reported in the literature or detected by BLAST. Grey boxes—putative/degenerate homologue. Black boxes—homologue present.

ECM are known to facilitate growth factor gradient formation. In particular, fibronectin (FN) facilitates contact between the ECM and cell surface molecules (including cytokine receptors, HSPGs, integrins and dystroglycans), providing a functional link among these proteins and making it an essential component of extracellular signal transduction in bilaterians. Furthermore, perlecan is known to stabilize secreted peptide ligands (cytokines, FGFs and VEGFs), increasing the efficacy of FGF [141] and hedgehog signalling [112] in bilaterians. The canonical/complete basement membrane is a metazoan-specific novelty, as clear ColαIV and laminin orthologues first appear in the Ctenophora but the adhesome complex (integrin and its intracellular partners vinculin, α-actinin, talin and paxilin) dates back to the origin of Holozoa as both α and β integrins have been identified from filastereans (table 8). This suggests that integrins may have played a role in intracellular signalling long before they became associated with the ECM and, further, that the ECM may have evolved scaffolding properties first (through the integration of structural proteins such as laminin and collagen) and developed the ability to act as a signalling centre only after the origin of proteoglycans and linker proteins such as fibronectin.

Combinations of *in vivo* and *in vitro* assays have revealed the numerous important roles cnidarian basement membrane plays in directing cell differentiation, cell migration and tissue regeneration (see [155] for a review of these processes in *Hydra*). However, because the focus of these studies has largely been on hydrozoans, little is known about the development of the basement membrane in other cnidarians. Likewise, studies of the spatial expression and/or function of basement membrane transcripts in sponges are lacking. The situation with sponges is a particularly unusual one as homoscleromorphs appear to be the only sponges to secrete a basement membrane [156]. Considering that basement membrane-specific HSPGs also arose before the divergence of sponges and parahoxozoans, future studies aimed specifically at assessing the signalling capacity of the basement membrane in homoscleromorph sponges would be extremely informative. Basement membrane is known to underlie both epithelia in ctenophores [157] but again, the development of this structure has not been studied. Assaying stage-specific transcriptomes from *M. leidyi* throughout early development suggests that the expression of both ColαIV and laminins peaks just after gastrulation, coincident with the development of the mesoglea (LS Babonis and MQ Martindale, personal observations). Neither FN nor any of the basement membrane-specific HSPGs are encoded in the genome of *M. leidyi*, suggesting that the basement membrane of ctenophores may play only a structural role, rather than a role in cell signalling. In *Drosophila melanogaster*, DPP (the homologue of BMP 2/4) is known to bind directly to ColαIV in the basement membrane without the aid of HSPGs [158]. Both BMPs and ColαIV are present in ctenophores just after gastrulation. It would be interesting to learn if this direct relationship between ColαIV and secreted ligands for the WNT/TGF-β pathways also functions in ctenophores, suggesting it was present in the stem metazoan.

Using *in situ* hybridization and quantitative PCR, integrins have been shown to be expressed throughout all life stages in the coral *Acropora millepora* and the hydrozoan *Podocoryne carnea* and specifically upregulated in the endoderm during the invagination movements of gastrulation in the former [154,159]. Furthermore, integrin expression was maintained throughout the dynamic morphogenic processes

Table 8. ECM components across unikonts. EC, extracellular; IC, intracellular; MB, membrane bound.

										Metazoa									
			Amoebozoa	Fungi	Fungi	Filasterea	Choanoflagellata	Choanoflagellata	Ctenophora	Ctenophora	Porifera	Porifera	Placozoa	Cnidaria	Cnidaria	Cnidaria	Bilateria	Bilateria
Component	type	location	Dd[a]	Sc	Sp	Co	Mb, Mo	Sr	Ml	Pb, Pp	Aq, Sd	Oc	Ta	Nv	Ac, Ad, Am, Ap, Pd	Hm, Hv, Pc	Dm	Hs
HSPG2[a]	scaffold	EC													*			
AGRN	scaffold	EC																
NID	scaffold	EC													*			
ColαIV	scaffold	EC													*			
DSG	scaffold	MB																
FN	effector	EC																
LAMA	scaffold	EC								*								
LAMB1	scaffold	EC								*								
LAMC	scaffold	EC							*	*								
ITGα	receptor	MB							*	*		*			*			
ITGβ	receptor	MB							*	*		*						
PXN	effector	IC			*		*		*	*		*			*	*		
ACTN1	effector	IC						*	*	*	*	*	*	*	*	*		
TLN	effector	IC						*	*	*		*			*			
VCL	effector	IC							*	*		*			*	*		
References			[24,32,34,61,143]	[32]	[32]	[17,26,143,147,148]	[17,35,143,147–151]	[17,35,143,147–149,151]	[147,152]	[152]	[10,147–149]	[143,147,153]	[15,143,148,149]	[143,147–149]	[147]	[143,147,149,154]	[142,143,147,148]	[142,143,147,148]

[a]See glossary for gene and species abbreviations.

*Homologues identified by reciprocal BLAST against the human NR database; accession numbers available in the electronic supplementary material. White boxes—no homologue reported in the literature or detected by BLAST. Grey boxes—putative/degenerate homologue. Black boxes—homologue present. The origin of the canonical/complete basement membrane is indicated by the bold line.

associated with transdifferentiation and cell migration in *P. carnea* [154] but in *N. vectensis*, integrins seem to be specifically upregulated during regeneration [160]. Thus, studies of quantitative and spatial expression suggest that integrins play many diverse roles in the cell and tissue biology of cnidarians. Transduction of integrin-mediated signals into upregulated DNA synthesis was also demonstrated in the sponge *S. domuncula* [161], confirming that the inward transmission of signals from ECM to nucleus was functional before the origin of Porifera. Integrin signalling during development of ctenophores has not been studied but it is reasonable to hypothesize that intracellular signalling through integrins is likely operational in these animals as they possess the complete set of intracellular binding partners. Whether ctenophore integrins also play a role in facilitating embryonic morphogenesis or regeneration is not known. Studies explicitly examining the effect of perturbed integrin expression during ctenophore development are necessary to evaluate these ideas.

9. Commonalities in the evolution of novel proteins and novel pathways

Although changes in the spatial and temporal expression of existing genes are sufficient to induce novel expression patterns and signal new outcomes, the de novo evolution of new/novel molecules is also a critical driver of novel cell functions. Duplication and divergence may be the primary source of novel genes [162], but domain/exon shuffling is a well-established means for linking historically disparate functional domains into a single molecule [163]. Exon shuffling and gene fusion are two prominent ways in which the pathways described here have evolved. In the case of each pathway, functional domains necessary for protein–protein interactions arose long before the origin of the molecule itself. Indeed, the hedgehog pathway is an excellent example of this—hedge and hog domains are present in the lineages leading up to the Metazoa but the complete *hedgehog* gene did not arise until the stem lineage giving rise to Cnidaria + Bilateria [109]. Likewise, there is very good evidence for the piecewise assembly of genes in the JAK/STAT pathway [137], Notch/Delta pathway [100] and the evolution of the ECM [142]. Another striking pattern to be drawn from this summary is the timing of evolution of the components of each pathway. Figure 1 shows clearly that intracellular pathway components tend to arise before the membrane-bound and secreted components. Furthermore, it seems that the evolution of a 'canonical pathway' is largely coincident with the origin of the ligand, although evidence of individual pathway components before the evolution of a canonical pathway underscores the need for additional functional studies in diverse unikont systems. Together, these data support previous assertions that cell–cell communication evolved by co-option of existing intracellular pathways by novel extracellular and membrane-bound modulators [10,116].

10. Summary/outlook

To conclude, we return to Müller's [20] reconstruction of the ancestral metazoan but consider the new data gathered from ctenophores. Like Müller, we think it is reasonable to assume the ancestral metazoan had cell adhesion molecules, intracellular and intercellular signalling capabilities, and immune and nervous systems. In contrast, we suggest that the ability to form growth factor gradients evolved after ctenophores split from the rest of Metazoa. Perhaps more importantly, this review highlights the need for more explicit study of the ancestral components of canonical cell signalling pathways in ctenophores and other non-bilaterian taxa. Studies aimed at understanding complexity often start with a hypothesis derived from observations of bilaterians; thus, these studies focus on understanding when the parts of the bilaterian cell signalling systems first arose and when they were assembled into the canonical pathways represented by modern bilaterians. If, however, we are to understand how evolution produced these signalling pathways, we need explicit studies of the functions of pathway components in basal taxa before they acquired the role they play in bilaterians. Although challenging, these studies have the potential to reveal novel biochemical features of pathway components and the mechanisms by which gene functional domains evolve. These studies are essential for understanding the evolution of complex multi-component signalling pathways and the evolution of novel cell communication systems in general. Studying these interactions may well reveal biochemical properties of proteins that have gone overlooked as a result of our focus on bilaterian biology.

11. Methods

In cases where pathway components were not available in the literature, we used BLAST to search for homologues. Query sequences were collected from the human NR protein database (NCBI accession numbers provided in electronic supplementary material, file S1). To identify putative homologues in other taxa, we performed reciprocal BLAST searches against the NR protein databases (NCBI) for most of the taxa listed in tables 1–8. To identify homologues from *M. leidyi*, we queried human sequences against the Protein Models 2.2 database at NHGRI (https://research.nhgri.nih.gov/mnemiopsis/blast/), for *P. bachei*, we queried against the Unfiltered_Gene_Models database at NeuroBase (http://neurobase.rc.ufl.edu/pleurobrachia/blast?view=blast), and for *O. carmela*, we queried the OCAR_T-PEP_130911 database at Compagen (http://www.compagen.org/blast.html). Alignments were inspected by eye and putative homologues (with low support) are indicated separately from clear homologues in tables 1–8. In cases where our BLAST results conflicted with previous observations, the results of our BLAST searches are provided.

Authors' contributions. L.S.B. wrote the manuscript and performed BLAST searches. M.Q.M. evaluated and edited the manuscript for content. Both authors contributed to conceptualization of the study and gave final approval for publication.

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Glossary

Unikonta	the monophyletic group composed of amoebozoans, fungi, filastereans, choanoflagellates and metazoans.
Opisthokonta	the monophyletic group composed of fungi, filastereans, choanoflagellates and metazoans.
Holozoa	the monophyletic group composed of filastereans, choanoflagellates and metazoans.
Metazoa	the monophyletic group composed of ctenophores, sponges, placozoans, cnidarians and bilaterians.
Parahoxozoa	the monophyletic group composed of placozoans, cnidarians and bilaterians.

Gene abbreviations

ADAM	a disintegrin and metalloproteinase domain
ACTN1	α-actinin
AGRN	agrin
ALK/LTK	anaplastic lymphoma tyrosine kinase, leukocyte tyrosine kinase
APC	adenomatous polyposis coli
APH-1	anterior pharynx defective homolog 1, γ-secretase subunit
AXL/UFO	AXL/unidentified function
β-cat	β-catenin
BMP	bone morphogenic protein
CAN	cerberus/DAN/gremlin family
CER	cerberus
CHRD	chordin
CKI	casein kinase 1
ColαIV	type IV α-collagen
COS	costal
CSL	CBF1, suppressor of hairless, LAG-1
Cyto	cytokine
CytoR	cytokine receptor
DAN/NBL1	neuroblastoma suppressor of tumorigenicity 1 isoform 1
DDR	Discoidin domain receptor
DAPT	N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester; γ-secretase inhibitor
DISP	dispatched receptor
DIXDC	dixin domain containing
DKK	dickkopft
DPP	decapentaplegic, *Drosophila* homologue of BMP
DSG	dystroglycan
DSL	delta interaction domain
DSH	disheveled
DTX	deltex
ECM	extracellular matrix
EGF	epidermal growth factor
EGFR/ERBB	EGF receptor, erythroblastic leukemia viral oncogene
EPHR	ephrin receptor
FA	fatty acid
FGF	fibroblast growth factor
FGFR	FGF receptor
FN	fibronectin
FST	follistatin
FU	fused
FZD	frizzled

GDF	growth/development factor
GF	growth factor
GFR	growth factor receptor
GLI	glioma-associated oncogene
GREM	gremlin
GSK-3	glycogen synthase kinase 3
HES	hairy and enhancer of split
HH	hedgehog
HHIP	hedgehog interacting protein
HNF4	hepatocyte nuclear factor 4
HSPG2	heparin sulfate proteoglycan 2/perlecan
INSR	insulin receptor
ITG	integrin α/β
JAK	janus kinase
LAMA	laminin subunit α
LAMB1	laminin subunit β
LAMC	laminin subunit γ
LRP	low density lipoprotein receptor-related protein
MAM	mastermind
MAPK	mitogen activated protein kinase
MEK	MAPK/ERK kinase
MET/HGFR	met proto-oncogene, hepatocyte growth factor receptor
MIB	mindbomb
MUSK	muscle-specific kinase
NEURL	neuralized
NFκβ	nuclear factor κ-β
NLE	Notchless
NLK	nemo-like kinase
NOG	noggin
NR	nuclear receptor
NvSMAD	a SMAD homologue isolated from *Nematostella vectensis*
O-fut	o-fucosyltransferase
PCP	planar cell polarity
PDGFR	platelet-derived growth factor receptor
PEN2	presenilin enchancer 2, γ-secretase subunit
PG	proteoglycan
PIAS	protein inhibitor of activated STAT
PKC	protein kinase C
PKA	phosphokinase A
PLC	phospholipase C
PSEN	presenilin-1, γ-secretase subunit
PTC	patched receptor
PTKs	protein tyrosine kinases
PTK7/CCK4	protein tyrosine kinase 7, colon carcinoma kinase 4
PXN	paxillin
Ras/Raf	proto-oncogene serine/threonine kinase
RET	rearranged during transfection proto-oncogene
ROR	receptor tyrosine kinase-like orphan receptor
RTK	receptor tyrosine kinase
RXR	retinoid X receptor
RYK	related to receptor tyrosine kinase
SARA/ ZFYVE9	SMAD anchor for receptor activation
SFRP	secreted frizzled receptor protein
SH2	SRC homology 2 domain
SHP	SH2 domain protein tyrosine phosphatase
SKI/SNO	SKI proto-oncogene/SKI-related gene
SMAD	mothers against decapentaplegic homologue
SMO	smoothened receptor
SMRT/ NCOR2	nuclear receptor co-repressor
SMURF	SMAD specific E3 ubiquitin protein ligase
SOCS	suppressor of cytokine signalling
SOS	SOS Ras/Rac guanine nucleotide exchange factor 1
STAT	signal transducer and activator of transcription
STK36	serine/threonine kinase 36 (homologue of fused)
SuFU	suppressor of Fused
TAD	C-terminal transactivation domain
TCF/LEF	transcription factor/lymphoid enhancer binding factor
TF	transcription factor
TGF-β	transforming growth factor β
TGFR	TGF-β receptor
TIE	tyrosine kinase with immunoglobulin-like and EGF-like domains
TLD	tolloid/BMP-1
TLN	talin
TRK	tropomyosin receptor kinase
VCL	vinculin
VEGF	vascular endothelial growth factor
VEGFR	VEGF receptor
VFT	venus fly trap domain
VKR	venus kinase receptor
WIF	WNT inhibitory factor
WNT	wingless-type MMTV integration site
ZPA	zone of polarizing activity

Species abbreviations

Dd	*Dictyostelium discoideum*
Sc	*Saccharomyces cerevisiae*
Sp	*Schizosaccharomyces pombe*
Co	*Capsaspora owczarzaki*
Mb	*Monosiga brevicollis*
Mo	*Monosiga ovata*
Sr	*Salpingoeca rosetta*
Ml	*Mnemiopsis leidyi*
Pb	*Pleurobrachia bachei*
Pp	*Pleurobrachia pileus*
Aq	*Amphimedon queenslandica*
Oc	*Oscarella carmela*
Sd	*Suberites domuncula*
Ta	*Trichoplax adhaerens*
Nv	*Nematostella vectensis*
Ac	*Acropora cervicornis*
Ap	*Acropora palmata*
Ad	*Acropora digitifera*
Am	*Acropora millepora*
Pd	*Pocillopora damicornis*
Hm	*Hydra magnipapillata*
Hv	*Hydra vulgaris*
Pc	*Podocoryne carnea*
Dm	*Drosophila melanogaster*
Hs	*Homo sapiens*