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The placenta as a model for understanding the origin and evolution of vertebrate organs

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How organs originate and evolve is a question fundamental to understanding the evolution of complex multicellular life forms. Vertebrates have a relatively standard body plan with more or less the same conserved set of organs. The placenta is a comparatively more recently evolved organ, derived in many lineages independently. Using placentas as a model, we discuss the genetic basis for organ origins. We show that the evolution of placentas occurs by acquiring new functional attributes to existing tissues, changes in the patterning and development of tissues, and the evolution of novel cell types. We argue that a diversity of genomic changes facilitated these physiological transformations and that these changes are likely to have occurred during the evolution of organs more broadly. Finally, we argue that a key aspect to understanding the evolutionary origin of organs is that they are likely to result from novel interactions between distinct cell populations.

Organs are complex structures that compartmentalize biological processes, and are composed of multiple tissue and cell types. Individual organs are distinguished by their differences in form and function, so it is probably unhelpful to come up with a general definition of 'an organ'; however, understanding how complex structures evolve is a question fundamental to evolutionary biology[1]. Organ evolution is an intriguing puzzle to evolutionary biologists because it requires alterations to multiple tissues and is underpinned by many genetic changes[2,3].

Vertebrates, and in particular gnathostomes, have a highly conserved body plan that is composed of a relatively limited set of homologous organs. Given that vertebrates arose more than 500 million years ago, the organs comprising their body plan have ancient evolutionary origins. Furthermore, many organs pre-date the origin of vertebrates, including the brain, kidney and through-gut (see Fig. 1 for a summary of organ origins). Although soft tissue can fossilize, the functional characteristics and molecular underpinnings of its functions do not. Therefore, understanding how organs originate requires comparative studies of extant organisms. One way to overcome these challenges is to study the evolution of an organ that has evolved more recently, has been derived in multiple independent lineages, and exists in intermediate forms in extant taxa.

The placenta is an organ formed by the sustained apposition or fusion of fetal membranes and parental tissue for physiological exchange[4]. Placentas are an excellent model for investigating the evolution of complex organs because they have evolved independently multiple times, evolved relatively recently in some lineages, and are present in intermediate forms in extant taxa[5,6]. In amniote vertebrates, placentas form from the apposition of the Müllerian canal (female reproductive tract) and extraembryonic membranes of embryos. In amphibians and fish, however, placentas form from a broader diversity of structures including ovaries and skin appendages (Box 1).

Placentas evolve for a variety of reasons (Box 2), and have arisen in the context of the evolution of live birth (viviparity) more than 137 times in vertebrates[7,8] (Fig. 2), although the exact phylogeny of squamate viviparity is still controversial[9,10]. In some lineages placentas have evolved relatively recently[11]. Placental diversity also includes a variety of intermediate forms in extant taxa. These intermediate forms include species that rely on both placental and egg yolk for nutrient provisioning, a diversity in the kind of nutrients that can be transferred across the placenta, and species with intermediate degrees of structural complexity in the placenta. The molecular biology and evolution of vertebrate placental structures has largely been studied in three groups: mammals, squamate reptiles and teleost fish (Fig. 2 and Box 1).

Here we review the literature on the genetic processes that contributed to the evolution of placentas in vertebrates, and discuss the role of these processes in vertebrate organ evolution more broadly. There is a current expansion in interest regarding the evolution of placental structures in vertebrates. In particular, the use of new molecular tools in non-model organisms, including next-generation sequencing of RNA, has resulted in substantial new insights in this field[12]. While the field has generated new findings into the evolution of placental structures and functions, the significance of these findings for the evolution of complex organs in general has yet to be synthesized.

Understanding the evolution of a new organ

The evolution of a new organ in animals typically involves two processes: the acquiring of new functional potential in a tissue, which we refer to as a functional innovation[13,14]; and the evolution of a novel structure (akin to a new body part), which we refer to as a structural novelty[14–16]. While the evolution of new organs can proceed through subfunctionalization (that is, specialization to perform a subset of functions performed by a multifunctional ancestral structure), often novel structures also involve the acquisition of new functions. Nevertheless, the processes that lead to functional innovations and structural novelties can be fundamentally different and disassociated[15].

Functional innovations (the acquisition of new functional potential) require evolutionary changes in the activity of cells in a tissue. These are primarily achieved by changes to the physical and chemical properties of specific cell types and their physiological activities. As cellular functions are achieved by the production of proteins, functional innovations often occur after mutations that result in

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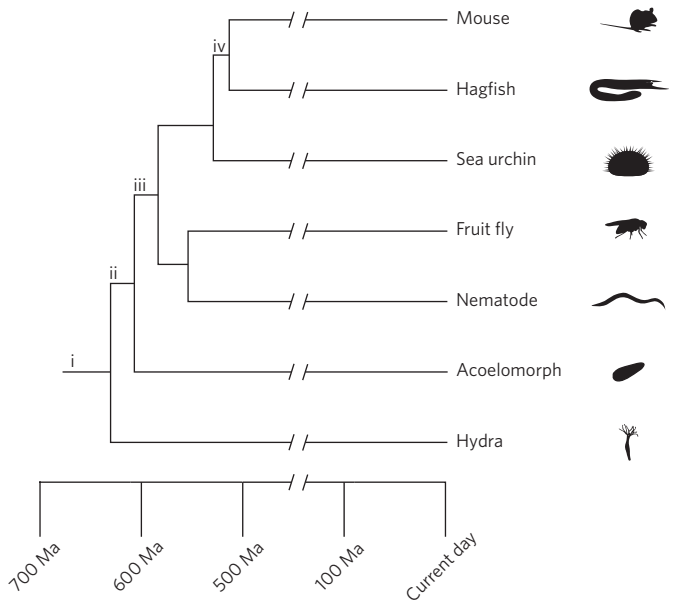

Figure 1 | Major vertebrate organs have ancient origins. The gut was one of the first organs, arising in the stem animal lineage (i). Brains and a through-gut evolved in stem bilaterians (ii)[88–90]. The excretory organs, kidneys and nephridia, are likely to have evolved from a homologous organ in the nephrozoan lineage (iii)[88,91]. Livers evolved in stem vertebrates (iv). Ma, million years ago.

changes in protein production in those cell types (for example, mutations that alter gene regulation from the transcriptional to post-translational level) and changes to the coding sequences in genes resulting in proteins with different functional attributes.

Evolutionary novelties (the acquisition of novel structures) occur by changes in the patterning of tissues through differential gene expression so that a novel developmentally individualized body part arises[17]. Tissue patterning occurs by the coordinated production of cells of specific types and their signalling. The cell types that form are controlled by a core set of transcription factors and regulatory molecules[18]. Structures that have unique core regulatory networks are able to evolve while minimizing pleiotropic effects in other tissues[19].

Together functional innovations and structural novelties are central themes to the origin of new organs. We discuss the molecular processes that facilitate the evolution of new functions and structures in the placentas of vertebrates.

How functional innovations arose during placenta evolution

In the twentieth century, biologists predicted that organismal complexity would be encoded by increased genetic material in the genome. DNA quantification and genome sequencing led researches to the surprising discovery that genome size and gene number do not correlate with organismal complexity[20]. Comparative genomics has allowed us to identify that, broadly speaking, the majority of coding gene families present in complex animals such as ourselves are shared with the genomes of sponges, and were probably present in the ancestral metazoan genome[21]. In fact, most gene families in bilaterian developmental pathways are shared with the earliest metazoan and even protozoan genomes[22]. Given the ancient origins of most vertebrate gene families, a question remains: how are these ancient genes used to facilitate the functions acquired during the evolution of a novel organ?

The functional properties of cells are largely derived from the proteins present within them, and hence the genes that encode the proteins. For a tissue to acquire a new function, cells must change the abundance, structure and/or activity of proteins produced. Therefore, the mechanisms by which placental innovation can arise include repurposing proteins already expressed in the tissue (co-option), recruiting the expression of genes normally expressed elsewhere in the organism (recruitment), and introduction of novel genes to the genome of the organism, either by gene duplication, by horizontal transfer (for example, from retroviral insertions) or by *de novo* gene evolution, as reviewed in ref. [23].

Co-option of genes. Functional innovations occur in tissues that have pre-existing functional capabilities. New functional potential can arise in organs by utilizing already expressed genes in novel ways. In vertebrates, placentas evolve from pre-existing tissues, such as the uterus and chorioallantoic membrane. Hence, to understand the evolution of placental functions, it is important to identify the genes expressed in the ancestral tissue from which the placental structure is derived. In the ancestral amniote, the uterine tissue had two prominent functions: to deposit the eggshell around the embryonic membranes and to transport the fertilized egg from the ovaries to the external environment[24,25]. Once the egg is laid, the extraembryonic membranes of this ancestor exchanged respiratory gases with the external environment, absorbed calcium from the eggshell, and played some role in hormonal signalling for embryonic development, including steroidogenesis[25–27]. These findings imply that for amniotes, the extraembryonic tissues that evolve into placental tissues were already organs of gas and nutrient exchange as well as endocrine organs. Therefore, the origin of a placenta was not a radical deviation from the ancestral functional role of these tissues, but rather a shift in the physiological context in which these capabilities are employed. Repurposing of the cellular mechanisms of ancestral functions is an important process that resulted in the evolution of new functions in placentas.

A classic example of this mechanism is the evolution of placental calcium transport in squamates[8], in which placentas evolve from the apposition of extraembryonic membranes with maternal uterine tissue. Oviparous reptiles rely on calcium from the eggshell to support embryonic growth and development. In oviparous squamates (the ancestral condition), uterine glands deposit calcium and other shell compounds to build the eggshell following ovulation and fertilization. In viviparous lizards and snakes, the eggshell is reduced or completely lost, and therefore these species require a uterine source of calcium[28]. During the evolution of viviparity, retention of uterine shell glands supports calcium transport to embryos through gestation, and so placental calcium transport has evolved by repurposing an existing process from the ancestral condition[29]. In the embryonic portion of the placenta, calcium transporters that were involved in the pathway for incorporating eggshell calcium are co-opted to support uptake of calcium from the uterine lumen[30]. Therefore, in squamates, placental calcium transport has evolved by altering the timing of expression of the already existing calcium transport machinery, with the exception that calcium is not stored in the eggshell. We predict that placental calcium transport has evolved by losing or deactivating the processes that result in the precipitation of calcium around the egg.

The repurposing of existing tissues and physiological capabilities during the evolution of new organs is fundamental to understanding the evolvability of organs. Squamates offer the ideal model for understanding this process, because placental structures always evolve from homologous tissues—the uterus and chorioallantoic and yolk sac membranes.

Recruitment of genes. Changes in the form of organisms can occur by changes in the expression of genes rather than the sequence of those genes[31,32]. In particular, tissues can have new functional potential by recruiting the expression of genes ancestrally expressed elsewhere in the organism[33]. Gene expression recruitment can occur incrementally, with each gene adding functional potential to the

Box 1 | Major lineages for which the evolution of placentas have been studied from a developmental or molecular perspective, including their advantages and limitations for inferring mechanistic processes.

Mammals. These include the most studied placental animals. There are several great mammalian model animals that allow rigorous investigation of placental function at all biological levels, in ways that are not possible in other taxa. However, placentation arose in the ancestral therian mammal approximately 130 million years ago, and research on oviparous monotremes is sparse, largely due to the difficulties in accessing them.

Squamates (lizards and snakes). These contain the largest number of independent derivations of placentation. While there has been no quantitative analysis of the instances in which the shell membrane has been completely lost allowing apposition of maternal and fetal tissues, there have been 115 independent origins of viviparity[7]. In most squamates, embryonic nutrition is provided by egg yolk and not via the placenta. Substantial placental nourishment is present in as few as six viviparous lineages. However, in all viviparous taxa that have been studied, there is measurable exchange of calcium, water and respiratory gases between the uterus and chorioallantoic membrane. There is a large range of ages of placental origins, with some lineages evolving placentas relatively recently; for example, there are four species that have both viviparous and oviparous populations. There are taxa with intermediate levels of placental complexity, and placental structures in amniotes develop from homologous structures (uterus, chorioallantois and choriovitelline membrane). Genomic resources in non-mammals are limited but expanding[27,93].

Fishes. Placentas largely form from tissues that are not homologous to placental structures in amniotes. Given the diversity of structures involved, comparative studies can separate developmental processes that support placenta evolution from the developmental histories from which placental tissues are derived. Across viviparous fishes there is a diversity of modes by which fetuses are nourished through development; these include egg yolk, placentotrophy and a range of non-placental forms of matrotrophy (such as oophagy and cannibalism of other developing embryos). However, in many species there are clearly defined placentas that form from the apposition of embryonic membranes and parental tissues. In poeciliid fishes, placentas form from the apposition of the ovarian follicle wall and yolk sac of the embryo. Placental nutrient transfer occurs in the ovaries, a tissue that ancestrally transfers nutrients to growing follicles—therefore matrotrophy utilizes the pre-existing mechanisms for egg production. Placentation has evolved rapidly in this group, with multiple degrees of matrotrophy existing in various lineages. In syngnathid fishes, placentas form from modifications to the ventral skin integument. Placentation occurs in males, which means that the hormonal regulation of pregnancy is de-coupled from normal female reproductive cycling.

Box 2 | An introduction to why placentas evolve.

Why placentas evolve is a separate question to how they evolve, but we feel that to fully understand the context of this manuscript it is important to have a brief summary of why they evolve.

Developing embryos have a requirement for respiratory gases, nutrients and waste removal. For live birth (viviparity) to evolve, offspring must be able to accommodate their needs inside a parent, and any exchange that previously occurred between the egg and the external environment must now occur between the embryo and the parental tissue. The apposition of parental and embryonic tissues forming a placenta is one way to meet these embryonic needs.

The reasons that viviparity evolves differ between organisms, but ultimately, viviparity allows for a greater ability of parental manipulation of development. In squamates, viviparity allows mothers to manipulate developmental temperature through basking, which is an important driver of offspring fitness, especially in cold climates[94]. In other taxa, viviparity offers greater protection of embryos from predation[95].

In some taxa, placentas evolve to supply substantial amounts of nutrients to embryos through pregnancy (placentotrophy). In amniotes, only a few of the viviparous lineages have evolved placentotrophy: mammals and approximately six lineages of skink lizards. Placentotrophy has evolved more frequently in fish lineages. The adaptive explanations for why organisms may shift to a placentotrophic mode of embryonic nutrition are diverse and more work is needed to separate hypotheses. However, the transition is expected to occur in environments where resources are abundant and predictable[96,97]. Furthermore, it is important to note that in viviparous organisms, placentotrophy is not the only mechanism to increase post-fertilization fetal nutrition. In fishes and amphibians, a diversity of non-placental forms of matrotrophy have evolved.

Parent–offspring conflict can arise during pregnancy as a result of differences in the desired allocation of resources to individual offspring[98]. In particular, the pregnant parent may wish to share their resources between offspring to maximize their lifetime reproductive success, while embryos may want to maximize their own resource intake[99]. Successive innovations in embryos to manipulate parental provisioning and in parents to manage resources provides an adaptive explanation for why many structures and functions in placental tissues may have arisen, and is the most strongly supported argument for the diversity of placental structures in eutherian mammals[100].

tissue, or through the recruitment of entire pre-existing regulatory landscapes[34]. Gene expression recruitment was a major mode of functional specialization of the uterus to facilitate pregnancy in therian mammals and reptiles[35–37]. Gene expression recruitment can occur by changes in *cis*-regulatory regions of the DNA proximate to the gene or changes to other genes that regulate gene expression (*trans*-regulatory elements)[38].

Comparative transcriptomic analyses of the oviduct in tetrapods shows that viviparity in mammals was correlated with the recruitment of more than a thousand genes to mammalian uterine tissue[35]. Gene ontology analysis shows that recruited genes are involved in immune modulation, metabolism and mammalian reproduction. Further, uterine defects were an over-represented phenotype in mouse knockouts of recruited genes[35]. These results suggest that gene expression recruitment probably facilitated the many functional innovations of the therian Müllerian canal, including placental nutrient transport and tissue remodelling for pregnancy[39].

***Cis*-regulatory element evolution.** For genes to evolve placenta-specific gene expression profiles, they would require the modification

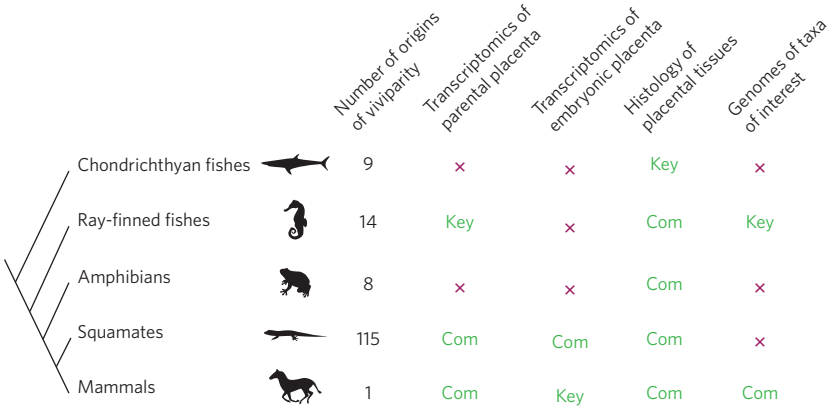

Figure 2 | Phylogenetic relationships of vertebrates in lineages in which the evolution of placental structures has been studied. Against each taxa, we have indicated the number of independent origins of viviparity[7] and the types of data that have currently been collected for these taxa. A cross indicates no currently published data of this kind for this group of organisms. 'Com' indicates that comparative data has been collected for this lineage from placental and aplacental species, while 'Key' indicates that this data has been collected for key species only, and not in both placental and aplacental species.

or *de novo* generation of *cis*-regulatory elements (for example, gene promoters or enhancers). While the mechanisms by which *cis*-regulatory elements can arise and evolve has been reviewed extensively[38], studies on the evolution of the trophoblast and uterine endometrium have provided insights on the role of transposons in transcriptome evolution.

Transposons are short sequences of DNA that when transcribed are capable of copying and inserting themselves in another region of the genome[40]. Transposons can contain their own regulatory elements, and once inserted near other genes they can induce the expression of that gene, resulting in the gene being expressed at times when it was not ancestrally expressed [41]. Retroelements can therefore introduce variation in gene expression that can be selected on, resulting in the evolution of new gene expression profiles in tissues. Retroelement insertion and subsequent modification by nucleotide substitutions resulted in expression of endometrial expression of prolactin in eutherian mammals[42]. Ultimately, transposons supported the origin of placental functions and diversification within eutherian lineages by acting as sources of regulatory variation[35,43]. This source of variation has directly resulted in the recruitment of genes to the trophoblast and endometrium in eutherian mammals[35,44].

While placental gene expression recruitment by transposons has not been documented outside of therian mammals, transposons are pervasive across the tree of life. Transposons occur in the genomes of most organisms and can be hyper-abundant in the genomes of mammals, squamates and birds[41,45,46]. The expression of transposon-related genes has been noted in the uterus and chorioallantoic membrane of squamates, as well as in the seahorse brood pouch[27,36,47]. Furthermore, lizards and snakes (unlike mammals) have accumulated transposon genes within the vicinity of important developmental genes, such as the HOX clusters, which in most vertebrates exclude transposable elements[48,49]. This raises the potential that transposon-mediated changes to gene regulation may have been more important for the evolution of viviparity in squamates, and are a worthy avenue of future research.

Protein-coding gene evolution. The evolution of new cellular processes in a tissue, that is, processes that do not occur in the ancestral organism, typically arise by the evolution of novel protein-coding genes, or the evolution of proteins by changes in gene sequence. While many placental functions appear to have arisen by modifications to pre-existing cellular functions, as described in the sections above, it is important to ask whether placental functions have been derived, in part, by the origin of new cellular processes. Innovative functions in tissues can emerge after new genes arise in the genome. These genes can arise by many processes including exon shuffling, transposable-element domestication, lateral gene transfer, frame shifts, and gene duplication (reviewed in ref. [50]). The two sources that have been frequently reported as occurring in placenta evolution are retroelement domestication and gene duplication[33,51].

Gene families are groups of paralogous genes that have arisen by gene duplications, and gene family expansion is an increase in the number of genes belonging to a particular gene family by duplication of one or more members. Gene duplications are usually the result of replication errors during cell division[52], as well as through retrotransposition, and occur on average once per gene per 100 million years[53]. When duplicated genes are retained, either the two gene copies take on a subset of the roles of the original gene (sub-functionalization) or one copy adopts a new function (neo-functionalization)[52]. Many gene families containing members with placenta-specific expression have been identified, including proteases, hormones and transcription factors[33]. Cathepsins are cysteine proteases, and are essential for mammalian placental function[54]. In rodents and primates, the number of cathepsin genes is expanded—mice have 18 cathepsin genes and humans have 11[54]. These patterns of gene family expansion are consistent at some taxonomic levels; for example, mice and rats have cathepsin genes that other mammals do not[55], but there does not appear to be gene families that are consistently expanded across all mammals[33].

How structural novelties derived during placenta evolution

The evolution of a novel organ typically involves both the functional innovations to support the organ's function and a novel structure with which this function is associated. In each lineage for which a placenta has evolved, it has evolved by modification of pre-existing structures (Fig. 3). The placenta in amniotes forms from both modified extraembryonic membranes and the uterine tissue of the mother[5] (Fig. 3a). In fish, a variety of parental structures are utilized in the formation of a placenta, including modifications to the skin in seahorses[47] or the ovaries in poecillid fishes (Fig. 3b,c)[6]. While the placenta has evolved by modification of pre-existing tissues, novelties can arise within the confines of these larger-scale identities—for example, in cattle, small patches of the endometrium, called caruncles, become specialized for nutrient transport by interacting with the allantois resulting in increased uterine folding and epithelial cell changes. Novelties of this kind can involve modifications to tissue patterning, vascular development, tissue size and surface area, and the evolution of novel cell types. By understanding how these structural changes evolved, we can understand the fundamental mechanisms that support placental evolution.

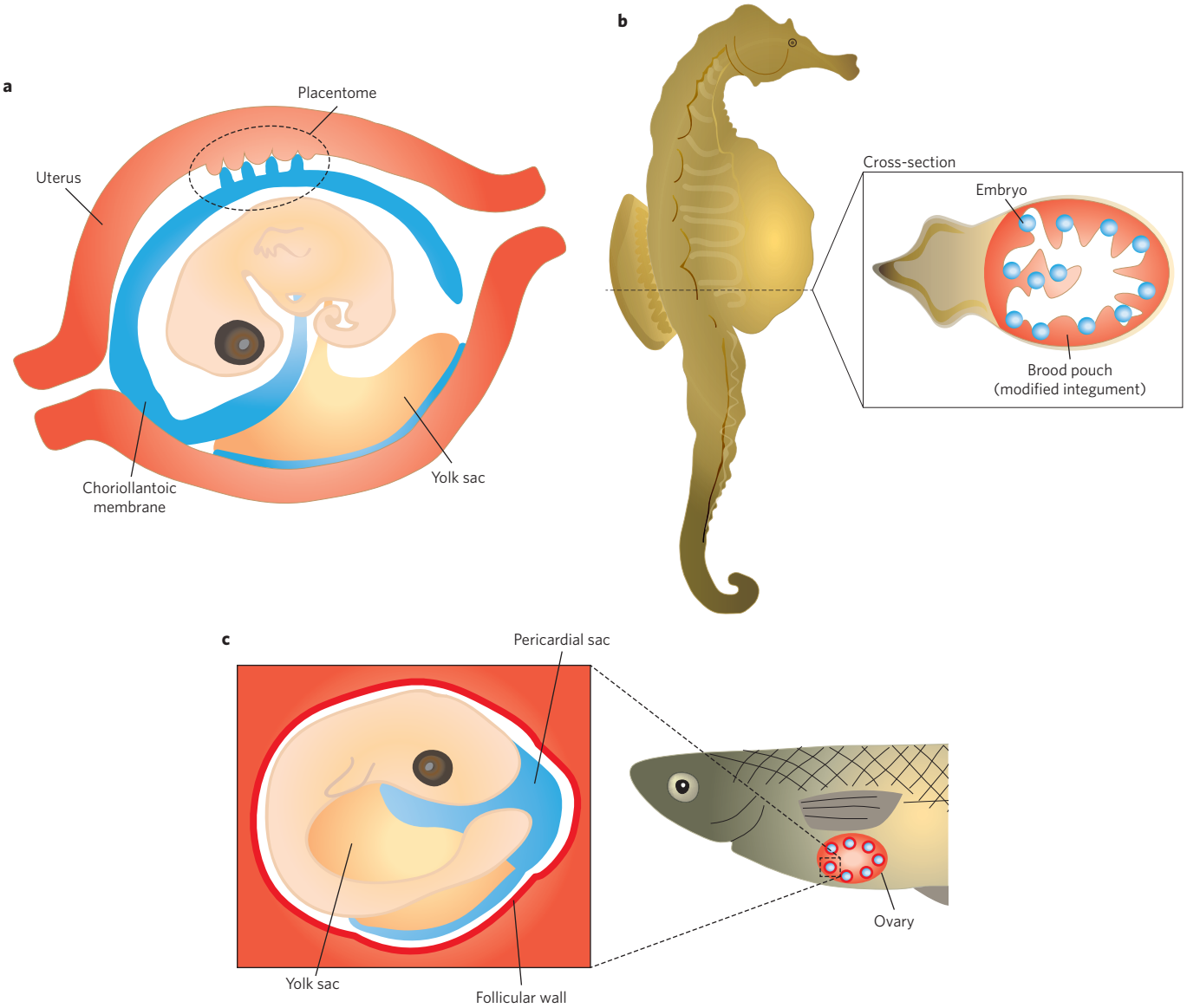

Figure 3 | A small selection of the diverse parental and embryonic tissues involved in placental development in vertebrates. a, Placentation in squamates always involves the maternal uterine tissue, and some combination of the chorioallantoic and yolk sac membranes. **b**, In male seahorses, a placenta forms from outgrowths of the integument (that then form a brood pouch) and the external tissues of the developing embryo. Adapted with permission from ref. [60], Wiley. **c**, In poeciliid fishes, a placenta forms from the follicular wall and exposed embryonic membranes including the yolk sac endoderm and pericardial sac. Adapted with permission from ref. [92], Wiley.

Tissue remodelling through rearrangement of existing cell types. The ancestral uterine tissue in amniotes, is relatively homogeneous[24]. However, in some viviparous amniotes, several specializations of the uterine tissues developed. These specializations perform different placental functions, including gas exchange and nutrient transfer[7,8,57]. In many instances, the morphological novelties identified in viviparous mammals are mirrored in independently derived viviparous lineages of reptiles[5]. Of particular interest is the placentome, which is a placental specialization consisting of a folding of the uterine lining and chorioallantoic membrane (Fig. 3a). Placentomes have been independently derived in at least four placentotrophic skink lizards and eutherian mammals[7]. Folding in the placentome results in greater surface area of the uterine epithelia, where secretory epithelial cells develop, which is expected to maximize the nutrient transfer capabilities of the placenta[5]. While the genetic basis of placentome development has not been elucidated, it represents an excellent system to study the evolution of novel organ morphologies, as it has evolved independently in multiple lineages and, in some cases, closely related species exist with and without placentomal structures, such as in the lizard genus *Chalcides*[58].

The placenta (that is, trophoblast and endometrium) of eutherians is one of the most variable structures in mammals[57]. Even between closely related species, there can be large differences in placental patterning. Placental structures vary in three important ways: the degree of intimacy between maternal and embryonic tissues, the shape of the intimate surface, and the manner in which maternal and embryonic tissues interdigitate[59]. An important structural novelty is the shape of the intimate placental surface, with placentas being either diffuse (placenta is intimate across the entire surface of the endometrium and trophoblast), cotyledonary (numerous discrete button-like placentas form across the uterine surface), zonary (an intimate placenta forms as a band around the uterus), or discoid (the placenta forms as a single regionalized disc). The discoid placenta forms at a single embryonic pole and is structurally and

physiologically similar to the placentomal structure observed in some lizards. While the diversity of placental specializations represents a good system to understand the evolution of novel structures in organisms, little work has been done to understand the genetic basis of these novelties.

In syngnathid fishes (a group of ray-finned fishes that includes seahorses and pipe fish), eggs develop following attachment to the skin integument on the underside of males. In some syngnathids, a brood pouch forms from an outgrowth on the surface of the ventral skin, and supports the attached eggs[60]. Brood pouches have evolved independently in at least two syngnathid lineages[60], providing a great system to understand the evolutionary mechanisms behind the derivation of a complex morphological structure. Across syngnathid fishes the complexity of the brood pouch varies; in some species, eggs simply attach to the epithelium of the ventral surface, in others permanent skin outgrowths form flaps that partially cover the eggs, while in seahorses, eggs are fully enclosed in a pouch structure that allows the brood pouch fluid to be regulated[61,62]. Increasing brood pouch complexity is matched by increasing epidermal complexity with the simplest brood pouches showing no sign of specialized cell types, while in complex pouches dermal tissue is interspersed with pavement cells (the highly filamentous cells typical of fish epidermis), mitochondria-rich cells, and flame cone cells[62].

How new developmental patterning is achieved in the embryonic and parental component of the placenta of non-mammals has yet to be elucidated, and is an important direction for future research. This is an especially interesting question in the seahorse, because pregnancy occurs in males, and therefore pregnancy-specific novel structures cannot rely on pre-existing hormonal cues associated with ovulation and corpus luteum maintenance, which is the case in mammals. While the G protein coupled oestrogen receptor GPER1 is upregulated in the brood pouch during pregnancy[47], and isotocyn (the fish homologue of oxytocin) induces parturition-like pouch contractions in non-pregnant male seahorses[63], little is known about hormonal cycling through reproduction in syngnathid fishes.

Novel cell types. Although new mechanisms for patterning existing tissue structures can result in changes in organ complexity, evolutionary novelties can be further developed if gene regulation of cells in the novel organ are in part de-coupled from gene regulation of other cells in the organism[19,64,65]. De-coupling of these gene regulatory processes allows gene regulation in the novel organ to evolve to some degree independently from other tissues[66]. While there are constraints that make it difficult to separate a coding gene from its regulatory elements, the introduction of new tissue-specific *cis*-regulatory elements can allow for individuation of gene expression in a given tissue. If novel mechanisms for regulating gene expression are derived in a discrete population of cells, these cells can be described as a new cell type[18]. Cell types and their behaviour are at the core of the structure and function of organs, and therefore understanding how novel cell types evolve is fundamental to understanding the evolution of organs[67]. The sister cell type model can be used to understand the origin of novel cell types, whereby cell types arise by changes in the gene regulatory network of an ancestral cell type, resulting in two daughter cell types. This model is supported by transcriptome studies that show that normal cell types have relationships consistent with a tree structure[68].

In amniotes, the endometrium is composed of multiple tissue layers: the luminal epithelium (which lines the uterine lumen), the glandular epithelium and the stroma. In most eutherian mammals, the endometrium undergoes decidualization during the reproductive cycle, where endometrial stromal fibroblasts differentiate into a new cell type, the decidual endometrial stromal cell. Decidual stromal cells facilitate uterine implantation, modulate the immune system, and are necessary for the progression of pregnancy[69]. Transcriptomic analyses can be used to infer the relationships between cell types by comparing the gene expression profiles of different cell types and comparing their transcription profile distances[68]. These analyses suggest that decidual stromal cells are sister cell types with endometrial stromal fibroblasts[70]. Marsupial endometrial stromal cells are homologous to eutherian endometrial stromal cells, but decidual stromal cells are unique to eutherian mammals[71].

The changes that supported the origin of decidual stromal cells in eutherians have yet to be elucidated, but some features are known. Importantly, several key changes to the gene regulatory network of the cell types occurred. Endometrial stromal cells in eutherians have a multi-layered gene regulatory network with progesterone receptor PGR and the transcription factor TFAP2C representing the top layer of gene regulation; in turn these genes regulate the transcription factors HOXD11, GATA2 and FOXO1[70]. In marsupials, where decidualization of endometrial cells does not occur, there is no stromal expression of FOXO1 protein[71]. In humans, FOXO1 is essential for the development of decidual stromal cells[72]. During decidual differentiation, FOXO1 interacts with a number of other proteins, most notably PGR, HOXA11 and CEBPB. Two of these transcription factors, HOXA11 and CEBPB, acquired a novel allosteric activity necessary for decidual gene expression coincidental with the origin of the decidual cell type[44,73]. Hence the evolution of a novel cell type, at least in the case of decidual cells, not only involves recruitment of genes but also the evolution of novel transcription factor complexes called core regulatory complexes (CoRCs)[18]. These CoRCs function as integrators of various signalling inputs to obtain a unitary gene regulatory output. In the case of decidual cells, they integrate progesterone and cyclic AMP–protein kinase A (cAMP–PKA) signalling to activate decidual gene expression.

Within the seahorse brood pouch, several novel cell types have evolved, including modified secretory flame cone cells, which appear to have no homologous cell types in other syngnathid fishes[62]. The gene expression changes that occur during pregnancy in seahorses and pipe fish have been characterized in several species[47,74]. In the seahorse, several regulators of transcription are differentially expressed through the reproductive cycle. Of particular interest is the transcriptional activator CALCOCO1, which is significantly upregulated at all stages of pregnancy in the seahorse brood pouch relative to non-pregnant states[47]. CALCOCO1 is a co-activator of the glucocorticoid receptor NR3C1, which is expressed in the seahorse brood pouch consistently throughout the reproductive cycle. By co-activating NR3C1, CALCOCO1 could result in divergent gene expression profiles in specific cell types through pregnancy. CALCOCO1 was not identified as a differentially expressed gene during reproduction in *Syngnathus floridae* and *Syngnathus scovelli*, which lack flame cone cells in pouch tissue[74]; however, it is not clear whether this was a limitation of this study's design. CALCOCO1 is a good candidate for a regulator of cell type identity in the seahorse brood pouch, but more work is needed to understand the evolution of novel cell types in these placental tissues. This example also illustrates the point emerging from the research on decidual cells that novel cell type identities arise, in part, through the evolution of novel transcription factor complexes[73].

While decidual stromal cells are unique to eutherians, the evolution of novel cell types is an important feature of placenta evolution in other lineages as well. Additional potential examples of novel cell types are the uterine natural killer (uNK) cells and the extravillous trophoblast cells in anthropoid apes. Research into the underlying gene regulatory networks that support the evolution of novel placental cell types in non-mammals is a poorly explored research area important for understanding the evolutionary origin of novel organs.

Tissue-to-tissue interaction and placental novelty. Placentas form as a result of apposition of two distinct tissues, one parental and one embryonic. In eutherians, maternal–embryo signalling in the placenta is vital for implantation, decidualization and placental

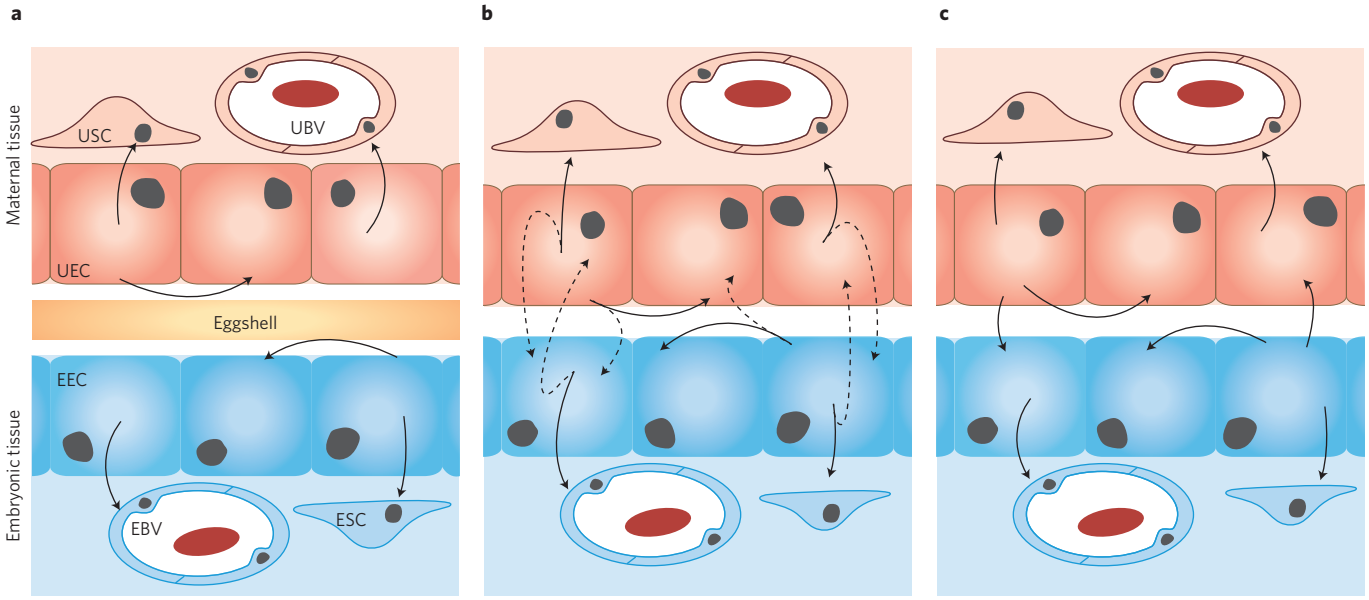

Figure 4 | How placental intimacy can result in new signalling processes supporting the evolution and origin of an organ. Here we have used amniote viviparity as an example. **a**, In the ancestral condition, fetal and maternal tissue are separated by a physical barrier in the form of an eggshell. Signalling (solid arrows) is confined to the organism in which those signals are generated. **b**, In species that have recently lost their eggshell, signals produced by the embryo to signal to other embryonic cells can spuriously diffuse into maternal tissue (dashed arrows) and impact maternal tissue development, and vice versa. These cross-organism signals are likely to impact the morphological and functional characteristics of the placenta. **c**, Through subsequent evolution, we expect a reduction in signalling processes that are detrimental to fitness. The evolution of cross-organism signalling will occur by pruning the complex signalling processes that occurred during the initial stage of placenta formation. This will result in a new set of signalling processes that includes bidirectional signalling between maternal and fetal cells. UEC, uterine epithelial cell; USC, uterine stromal cell; UBV, uterine blood vessel; EEC, embryonic epithelial cell; ESC, embryonic stromal cell; EBV, embryonic blood vessel.

differentiation[75,76]. Signalling is bidirectional: endometrial signalling contributes to implantation success, embryonic and trophoblast development, while embryonic signalling contributes to maternal receptivity to implantation, endometrial growth and rates of nutrient transfer by the mother. Crosstalk between mother and embryo is regulated by a suite of signalling molecules including hormones, cytokines, growth factors and transcription factors[77,78].

While the crosstalk between maternal and embryonic tissues in eutherian mammals is complex, involving many different gene products, these processes arose by modifications to signalling processes that were already present. Before the evolution of viviparity, the chorioallantoic membrane of amniotes was an endocrine organ, producing a diversity of hormones, growth factors and signalling molecules[27] (Fig. 4a). Once the shell membrane was sufficiently reduced, signalling molecules produced by the embryo would have the potential to impact the uterus (Fig. 4b). A likely consequence of this signalling is increased uterine angiogenesis in viviparous lizards, which have greater uterine vasculature than oviparous taxa. The chorioallantoic membrane of amniotes ancestrally produces large amounts of a potent angiogenic factor, VEGF[27]. Uterine angiogenesis in lizards can be regulated by embryonic signals during pregnancy[79], and one of these signals is likely to be embryonic production of VEGF. This is just one example of how tuning ancestral signalling processes can result in new placental developmental processes.

With the novel apposition of maternal and embryonic tissues in a placenta, both maternal and embryonic tissues are now presented with a new 'signalling environment' (Fig. 4b). The signalling molecules produced by each tissue, and their effect on the newly apposed tissue, has the potential to result in new signalling pathways that can impact placental development. This provides potential for the derivation of new cell–cell interaction networks for the evolution of novel structures (Fig. 4c).

Another interaction that occurs following the formation of a placenta is that with the immune system. The fetus is a semi-allograft, containing genetic material from two parents, which can be recognized and potentially targeted by the child-bearer's immune system. As a result of these immune consequences, various placental novelties have arisen in eutherian mammals. In particular, a pro-inflammatory reaction has been recruited to facilitate implantation, which may be a co-option of the ancestral recognition of the embryo as a foreign tissue, and an anti-inflammatory period of pregnancy has arisen and endures throughout the majority of pregnancy[80]. In reptiles, there does not appear to be a concerted immune response nor a strong silencing of the immune system[36,81]. In seahorses, there is some evidence of immune modulation during pregnancy, but it is not clear if this is a response to the fetus as an allograft, or is a mechanism to protect embryos from infection, as the brood pouch is intermittently opened to seawater throughout gestation[47]. More work is needed in non-mammals to understand the role of the immune system in the evolution of placental structure and function.

Novel tissue interactions and vertebrate organ evolution

Developmentally almost all organs form following the interaction of two distinct tissue populations[82,83]. In early vertebrate development, gastrulation establishes three distinct cell populations (ectoderm, mesoderm and endoderm), and successive organogenesis largely occurs by interactions between various epithelia and mesenchyme derived from the mesoderm. In these cases, the use of multiple tissue layers is not simply to add structural complexity to the organ as it develops—the tissues interact to establish regulatory feedback loops that are essential for establishing tissue and cell type identity. We propose that novel interactions of distinct tissues may act as a primer for the evolution of new organs, because they bring together a new combination of signalling processes leading to a perturbation

of the ancestral tissue homeostasis, which can be re-equilibrated by further evolution of the resulting structure (Fig. 4).

Most vertebrate organs develop following epithelium–mesenchyme interactions. These include both skin appendages such as hair and feathers as well as internal body organs such as the kidney and liver[84,85]. In many cases the interaction between these two tissue layers is fundamental to not only the continued development of the organ, but also its specification as it develops. For example, the development of discrete organs in the gut (such as the stomach and small intestine) is achieved by differences in epithelium and mesenchyme signalling along the gut axis[86].

The placenta both develops from and evolved as a result of the interaction of two distinct tissues. In amniotes, the placenta forms as a result of interactions between the maternal uterine mucosa and embryonic membranes; in fishes, the tissues vary but include the paternal integument and the yolk sack in syngnathids, and the ovarian follicle wall and the pericardial and yolk sac membranes in poeciliids[87]. The origin of the placenta as a new organ required the interaction of parental and fetal tissues, and we propose that tissue–tissue interactions are not just important for placenta development, but were also important for the origin of other vertebrate organs.

Take-home messages

The placenta is an organ formed by the interaction of parental and embryonic tissues. Placentas have arisen many times in diverse taxa from diverse tissues, but in each derivation key changes occur to support placental functions, such as nutrient transfer and gas exchange.

We have discussed the different animal models in which placenta evolution has been studied. While mammalian placentation is the best studied, a lack of repeated derivations of placentation in this group makes it impossible to answer some evolutionary questions. Squamates offer a system to study the repeated origin of placentation based on homologous structures, as there have been many independent derivations in this group. Fishes allow us to address different evolutionary questions, specifically how placentas evolve in non-homologous tissues, as placental structures in fishes form from a diversity of parental tissues.

We argue that in order to understand the origin and evolution of the placenta it is necessary to identify how evolutionary novelties and functional innovations are derived. We suggest that these processes could form the basis of models that attempt to more broadly explain the evolution of organs in animals.

This contribution demonstrates how, perhaps counterintuitively, even complex integrated biological structures can evolve through simple piece-wise changes.

The placenta, similar to other organs, develops from the interaction of distinct cell populations, and it is signalling between these cell populations that supports the differentiation and development of the organ. We propose that tissues interacting in new ways is a likely route to the evolution of new organs.

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Author contributions

O.W.G. wrote the manuscript. O.W.G. and G.P.W. developed ideas for, edited and approved the final version of the paper.

Additional information

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Competing interests

The authors declare no competing financial interests.