The functional diversity of the POUV-class proteins across vertebrates

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POUV is a relatively newly emerged class of POU transcription factors present in jawed vertebrates (Gnathostomata). The function of POUV-class proteins is inextricably linked to zygotic genome activation (ZGA). A large body of evidence now extends the role of these proteins to subsequent developmental stages. While some functions resemble those of other POU-class proteins and are related to neuroectoderm development, others have emerged de novo. The most notable of the latter functions is pluripotency control by Oct4 in mammals. In this review, we focus on these de novo functions in the best-studied species harbouring POUV proteins—zebrafish, Xenopus (anamniotes) and mammals (amniotes). Despite the broad diversity of their biological functions in vertebrates, POUV proteins exert a common feature related to their role in safeguarding the undifferentiated state of cells. Here we summarize numerous pieces of evidence for these specific functions of the POUV-class proteins and recap available loss-of-function data.

1. Introduction

The POUV class consists of a group of proteins that harbour the POU domain and are expressed mainly during early embryogenesis [1,2]. The most famous member of this class is Oct4, a key factor for induction and maintenance of pluripotency [3–5]. Considering the impact of this protein on these processes, it can be assumed that Oct4 orthologues exhibit strong conservation among multicellular organisms. Surprisingly, though, members of the POUV class were found only in vertebrates and, remarkably, only in jawed vertebrates (Gnathostomata), from cartilaginous fishes to human, and not in lampreys, for example [6,7]. In our previous review, we focused on the structural features of Oct4 and the POUV class and the role of these proteins in pluripotency induction and zygotic genome activation (ZGA) [8]. We concluded that although POUV-class proteins make a major contribution to ZGA in anamniotes (zebrafish, Xenopus), they are overtaken by proteins such as Nfya and Dux in Placentalia development. Nevertheless, POUV proteins from all studied vertebrates are characterized by numerous functions in embryogenesis following ZGA. Some of these functions, which are performed by Pou5f3 in zebrafish and Xenopus, are indicative of the origin of POUV from the POUIII class and are related to neuroectoderm development—midbrain–hindbrain boundary establishment, embryo integrity and neuro-progenitors maintenance [9–14]. Mammals possess Pou5f1 (Oct4), a protein known primarily as a key regulator of pluripotency—the undifferentiated state that endows cells the ability to become endo-, ecto- and mesoderm [3,15–17]. Interestingly, both Pou5f3 and Pou5f1 have a common function in posterior (trunk and tail) body extension [9,11,18,19], a feature that may be indicative of the role of POUV in maintenance of the undifferentiated state. This state is needed for regulative development of vertebrates and for prevention of premature differentiation to one or another trajectory. However, in some cases, Oct4 at least does not
2. POUV-class origin

The POU domain consists of the combination of a POU-specific subdomain (POUs), a linker and a homeodomain (POUh) [1]. POUs emerged after the divergence of choano- flagellate and animals, but before the divergence of sponges and eumetazoans. Thus, POUs are not present in plants and fungi, and like other metazoan-specific proteins such as Six and Pax, they appear to have contributed to animal multicellularity [20,21]. The POUV-class proteins appeared approximately 450 million years ago in some jawed ancestor (Gnathostomata) and across different taxa, represented by two orthologues—Pou5f1 (Oct4) and Pou5f3 (previously known as pou2) (figure 1) [6,22,23]. The evolution of the POUV class was reviewed in detail elsewhere [7,24,25]. All studied jawed vertebrates bear at least one of these orthologues and demonstrate an early lethal phenotype upon Pou5f1 or Pou5f3 factor knockout [17,26,27]. Considering that POUV-class members are involved in important processes during early embryonic development, it is surprising that they appeared during evolution only relatively recently [28].

The appearance of POUV in vertebrates could be related to global rearrangements in the genome, resulting in the emergence of principally new organisms. Whole-genome duplications (WGDs), which occurred before the origin of vertebrates, is an example of such a rearrangement [29–32]. Two subsequent WGDs took place between tunicates and lampreys [33,34] and perhaps account for the switching from a ‘mosaic’ type of development to a ‘regulative’ development. The former type of development is typical for most invertebrates while the latter applies to all vertebrates. However, the absence of any POUVs in the lamprey’s genome [35] does not support the role of WGDs in the origin of the POUV class. Thus, unless lampreys had POUV genes and then lost them, this class likely appeared by simple duplication of some POUIII gene (figure 1) [36]. This is confusing in light of the relatively similar early development of lampreys and zebrafish [37,38] as well as the indispensability of POUV-class proteins for embryogenesis of all vertebrates except lampreys (discussed below). Nevertheless, other POU-domain classes are widely distributed across multicellular organisms (classes I, III, IV and VI) probably first appearing at the dawn of Metazoa development and, now present in species ranging from sponges to human [20,21,36]. It is unlikely that these proteins perform POUV functions because they are not related to early embryogenesis [39]. Members of the POUIII class, which is probably the ancestor of POUV, usually serve as regulators of neuroectoderm development [40–45]. Interestingly, the emergence of Nanog is also associated with jawed vertebrates, as Nanog has been found as early as in Osteichthyes [46]. Of note, the SoxB class, which in cooperation with POUV proteins regulates early development and maintenance of pluripotency, has existed much longer than the POUV class and was already present in sponges [28,47,48]. Also, the partnership between Sox and POUV proteins could have existed longer than the Sox-POUV partnership, even as early as in invertebrates. For example, it was proposed that the cooperation between the HMG-containing Dichaete and the POU protein vrl occurs during Drosophila neurogenesis [49–51]. On the other hand, in vivo data (ChIP-seq) show that DNA-dependent formation of the Sox–Oct dimer is more typical for POUV-class proteins, while other POU factors prefer binding to DNA as homodimers [52–55].

Although it is generally accepted that participation of POU-domain proteins in pluripotency is a privilege of Gnathostomata, several reports have attempted to address roles of these proteins in stem cell function in invertebrates [56–59]. It was shown that the cnidarian POU-containing protein Pln, which is likely to be a POUV-class member, is expressed in the embryo and adult stem cells (i-cells) [59]. Interestingly, these cells could be positively stained with anti-human Oct4 antibodies. However, considering that Pln is not even an orthologue of Oct4 but rather a paralogue from another POU class, this observation casts some doubts. Oocytes, embryos, primordial germ cells and some branchial sac cells in the tunicate Botryllus schlosseri are stained positively by
anti-Oct4 antibodies [56], which is also inconsistent with the absence of Oct4 orthologues in tunicates. The study of planarian stem cells has revealed evolutionary conservation of a gene network governing pluripotency between these organisms and mammals, including genes affecting both Oct4 and Oct4 target expression [58]. Considering the ambiguity of this data, additional research on the potential stem cell function of POU-domain proteins is needed. To date, involvement of other proteins such as Piwi and Vasa in invertebrate stem cell functions represents a more likely scenario [60].

3. Biological functions of POUV proteins in anamniotes

3.1. Zebrafish

These animals are the most studied early Gnathostomata across vertebrates. Their POUV member Pou5f3 is known to be a regulator of neuroectoderm and endoderm development, as well as an organizer of dorsoventral patterning and gastrulation, acting via regulation of cell motility. Maternally expressed Pou5f3 is present in the zygote, whereas embryonic Pou5f3 begins to be expressed in the blastoderm and becomes restricted to the epiblast. Subsequently, Pou5f3 is expressed in the midbrain and hindbrain at late gastrulation and early somitogenesis (figure 2) [9,61]. While there is no evidence of Pou5f3 expression in zebrafish primordial germ cells (PGCs) [62], medaka fish PGCs were shown to harbour expression of this protein [63,64].

Early Pou5f3 research in zebrafish showed that this protein is necessary for establishment and maintenance of the midbrain–hindbrain boundary (MHB) organizer [9,10,18]. MHB is responsible for proper neuroectoderm patterning and differentiation, and it is dependent on Fgf8, Wnt1 and Pax2.1 to exert its function. These markers demonstrated strong decline in expression upon Pou5f3 knockdown and consequently, several morphological defects in neurogenesis were observed—no MHb, a smaller midbrain, and fewer neurons in the spinal cord. Defects in trunk and tail development during Pou5f3 knockdown were also noted—abnormal somite morphology and variable tail length [9,18]. Furthermore, using embryos with maternally and zygotically knocked-out Pou5f3 (MZspg), MHb defects became more pronounced; however, an earlier phenotype characterized by gastrulation delay and endoderm loss was discovered [26,65]. It was shown that Pou5f3 maintains Nodal-dependent Sox32 (cas) expression; together, Pou5f3 and Sox32 activate Sox17 transcription, a requirement for endoderm development. Pou5f3 and Sox32 bind cis-regulatory modules B and C of Sox17 gene, respectively, and act synergistically [66]. Accordingly, MZspg mutants had reduced Sox32 (cas) and Sox17 levels and failed to develop endoderm tissue. Gastrulation delay is also a distinctive feature of MZspg. By the time wild-type embryos reach 30% epiboly, the mutants reach only the dome stage [26]. This abnormality is related to defects in cytoskeleton, cell adhesion, and cell behaviour in MZspg [67]. It was shown that both upward and downward intercalations during gastrulation are significantly affected in Pou5f3-null embryos [68]. These abnormalities in cell motility are related to disturbances in E-cadherin endosomal trafficking; while this molecule is used during epiboly in wild-type embryos, in MZspg it accumulates on the plasma membrane and interferes with cell motility. E-cadherin endocytosis is controlled by Pou5f3-dependent EGF expression and thus, ectopic EGF mRNA could rescue E-cadherin distribution in MZspg [69]. Pou5f3 deficiency has also shown an influence on cell viability during gastrulation and is related to mych activity [70]. Using ChIP-seq analysis, Kotkamp et al. showed that transcription of both mych and myclb is directly regulated by Pou5f3. MZspg mutants showed increased apoptosis during gastrulation, and this phenotype was partially rescued by ectopic...
mych expression, whereas combined mych and p53 overexpression completely attenuated apoptosis in MZspg [70]. The authors also showed a specific role for Pou5f3 in activating Klf2a, Klf2b and Klf17 during the establishment of the extraembryonic envelope layer (EVL) and ectoderm. In the case of the ventral ectodermal domain, Pou5f3 acts together with BMP to mediate Klf2a and Klf2b expression [71]. Finally, there is the well-known function of Pou5f3 in the dorsoventral patterning of the zebrafish embryo, again achieved through collaboration with BMP signalling [62,72,73]. MZspg mutants are characterized by dorsalization and ventral expression of BMP antagonists Gsc, Chd and Nog1. Pou5f3 promotes ventralization through activation of Bmp2b, Bmp4, Bmp7, Vox, Vent and other factors. Ventralization is at least partly achieved via the Alk8-TGFbeta receptor, as receptor overexpression was shown to rescue Bmp2b and Bmp4 activation. It was also demonstrated that Pou5f3 directly regulates Vox transcription through a specific regulatory element [73].

Considering that lampreys resemble zebrafish in early development but have no POUV analogues, it is surprising that this new class of proteins in zebrafish has acquired so many functions. It seems that this may be due, at least partially, to the functionality newly emerged Pou5f3 class in zygotic genome activation [52,74,75], and some of the discussed defects in MZspg mutants could be related to failure to initiate the expression of one or several key developmental regulators. Therefore, the major mutant phenotype is characterized by gastrulation delay and consequent absence of endoderm—and may be caused by genome activity shutdown. At the same time, the listed defects in neurogenesis may indicate the Pou5f3 origin—the neuroectoderm regulators of the POUV class.

3.2. Xenopus

Ambthixs, like fish, are amniotes and have similar early development. However, the function of POUV proteins in these animals is different from and sometimes even opposite to that in zebrafish. Of note, Xenopus, unlike fish and mammals, does not express Nanog, and Ventx is likely to serve the role of Nanog in this species [46]. The POUV class in Xenopus is represented by three Pou5f3 homologues—Pou5f3.1 (Oct91/Xlpou91), Pou5f3.2 (Oct25/Xlpou25) and Pou5f3.3 (Oct60/Xlpou60). In addition to their role in genome activation [76], these Pou5f3 proteins perform several functions in neurogenesis and cell integrity. Their expression pattern is different throughout early embryogenesis. Pou5f3.3 is maternally expressed and is downregulated in blastula and undetected early during gastrulation [11]. Pou5f3.1 and Pou5f3.2 are expressed after ZGA in animal and marginal blastula zones and are then expressed during gastrulation (not in involuting cells) and in the developing neural tissue (figure 2) [11]. Pou5f3.1 (Oct91, Xlpou91), which completely rescues Oct4-null mouse embryonic stem cells (ESCs) [11], is not maternally expressed and functions after genome activation. However, like mouse Oct4, this protein is found in Xenopus PGCs [77].

Like zebrafish, in Xenopus, POUV proteins play a notable role in neurogenesis. Pou5f3.1 and Pou5f3.2 downregulation leads to a decline of neural markers Fgf8, En2 and Krox20, as well as upregulation of organizer (Cer, Gsc, Chordin) and endoderm (Sox17, Mixer, Endodermin) markers [11]. This observation is unexpected due to the crucial role of POUV in endoderm formation of the evolutionarily older zebrafish and in primitive endoderm formation of the evolutionarily more recent mammals (discussed below). On the other hand, the authors speculate that their results may indicate a conserved role of POUV-class proteins in prevention of premature commitment, which is supported by the ability of these proteins to rescue the self-renewal of mouse ESCs [11]. The participation of Pou5f3.1 and Pou5f3.2 in neurogenesis occurs in part through activation of Cich and Sipl1, as overexpression of these two proteins rescues Pou5f3.1-knockdown embryos [12]. Interestingly, Pou5f3.1 and Pou5f3.2 promote maintenance of the neuro-progenitor state rather than neuro-differentiation. Cooperativity with SoxB1-class proteins leads to inhibition of epidermis formation but expanded neural tube formation [14,78]. Co-injection of Pou5f3.1 and SoxB1 into blastomeres leads to the appearance of neuron-filled protrusions at the tailbud stage [14]. Interactions of POUV and SoxB1 in the maintenance of neural progenitors is thus reminiscent of interactions between Oct4 and Sox2 in mammals in pluripotency control. Inhibition of ectodermal formation is thought to occur through BMP suppression by Pou5f3.2 [79]. Xenopus Pou5f3 proteins have been found to inhibit posterior neural fate. FGF-induced Sal14 suppresses their activity and thus promotes spinal cord formation, as Sal14 knockdown leads to Pou5f3 upregulation and loss of spinal cord tissue [80]. Of note, this is not true for overall posterior extension, as other studies indicate that Pou5f3 depletion also leads to posterior body truncation [11,76]. Moreover, this phenotype is characteristic of both zebrafish and mammals (discussed below).

There is also a documented function of Pou5f3 proteins in inhibition of mesendoderm formation [27,78]. These proteins act in opposition to both activin/nodal and FGF signalling through inhibition of VegT/beta-catenin, Gsc and Mix2 [12,81,82]. Of note, Xbra, a marker of mesendoderm progenitors, is differentially dependent on Pou5f3 knockdown: it is downregulated upon knockdown of all Pou5f3 proteins (PVD2 in the article) [13] while upregulated during gastrulation upon Pou5f3.1 knockdown [12,13]. It was also shown that Pou5f3s could act as a repressor of Nodal/TGF-beta signalling by direct DNA binding of Foxh1 targets. Pou5f3 binding motifs were found in Foxh1 ChIP-seq data, and Pou5f3 knockdown led to upregulation of Gsc and Nodal2, both controlled by Foxh1 [83]. Interestingly, mouse Oct4 behaves like its Xenopus orthologues, as its overexpression also leads to inhibition of mesendoderm differentiation in Xenopus [78]. Also, Xenopus Pou5f3.1 can rescue Oct4-deficient mouse ESC self-renewal [11] and mouse Oct4 can substitute for Pou5f3 in zebrafish development [84]. Therefore, one could conclude that POUV proteins have not undergone any principal structural changes throughout vertebrate evolution but acquired their functions according to the species-specific developmental context. Due to the differential regulatory environment, POUV protein functions may have different effects. For example, in zebrafish, POUV induces endoderm formation and in mammals, it induces primitive endoderm formation, whereas in Xenopus, POUV suppresses mesendoderm formation (figure 2). At the same time, the abovementioned functions in inhibition of mesendoderm formation and posterior neural tissue formation confirm a role for POUV in prevention of premature differentiation.

In addition to the described functions of Pou5f3 proteins during neuroectoderm and mesendoderm specification, these
proteins play an important function in embryo integrity [13]. Livigni et al. compared conserved POUV targets in Xenopus, mouse and human, and revealed that evolutionarily conserved genomic targets are related to cell adhesion. Improved knockdown of all Pou5f3 mRNAs in this work led to complete embryo disaggregation at the neurula stage, while injection of Pou5f3.1, Pou5f3.2, or mouse Oct4 mRNA rescued this phenotype. Those authors considered the following: (1) conserved POUV genome targets are associated with cell adhesion; (2) E-cadherin overexpression partially rescues Pou5f3 (PVD2) knockdown in Xenopus and blocks differentiation of mouse ESCs in the absence of the Oct4; and (3) Pou5f3.1 and Pou5f3.2 could rescue the self-renewal of mESCs at least partially by maintaining E-cadherin expression. The authors thus speculated that an ancient role of POUV-class proteins is to block delamination itself and to support an undifferentiated state via ‘uncommitted ectodermal epithelium’ [13].

4. Biological functions of the POUV proteins in amniotes

Amniotes are characterized by the presence of amnion during embryogenesis. Amnion is a liquid-filled structure that allows the embryo to develop in an out-of-water environment. These taxa include reptiles, birds and mammals. Unfortunately, there are just a few pieces of information about POUV proteins in non-mammalian amniotes [85]. It is known that (1) both Pou5f1 and Pou5f3 are present in turtles, (2) only Pou5f1 is present in snakes and lizards, and (3) only Pou5f3 is present in crocodiles and birds (figure 1). Pou5f1 expression was observed in the posterior segment of the snake embryo, suggesting that Pou5f1 participates in trunk elongation during snake development [86]. Early mammals such as monotremes and marsupials have both Pou5f1 and Pou5f3. Other mammals harbour Pou5f1 (Oct4) while some (rodents and primates) also have the relatively newly emerged Pou5f2, which is expressed in male germ cells [7,22,87]. Chicken Pou5f3, like Nanog, is expressed at a high level in chicken ESCs (cESCs), and, like mouse Oct4, its expression is downregulated upon retinoic acid treatment [88]. During early chicken development, Pou5f3 was found first in the epiblast and, to a limited extent, in hypoblast in the pre-streak embryo stage; then in the primitive streak, ectoderm, and mesoderm during gastrulation; and finally, in the neural tube, underlying the mesoderm, and in PGCs, but not in endoderm [89]. This expression pattern of Pou5f3 is more reminiscent of its orthologues in zebrafish and Xenopus rather than Pou5f1 expression in mammals, as one would expect.

Unlike the case for zebrafish and Xenopus, POUV research in mammals is performed by using not only an animal genetics approach but also cultured pluripotent stem cells or cellular reprogramming into a pluripotent state. Despite the longstanding comprehensive research on these proteins in mouse and human, new data continues to emerge and change our view of POUV functions in these species. While most studies underlie the function of Oct4 in mammals as a gatekeeper of the undifferentiated pluripotent state, data on Oct4 function in early differentiation begins to accumulate.

In mammalian ontogenesis, Oct4 is first detected in oocytes, and after fertilization, its transcription begins before the 8-blastomere stage; after trophoderm segregation, Oct4 is detected in the inner cell mass (ICM) at embryonic day 3.5 (E3.5). Oct4 becomes transiently upregulated in the primitive endoderm (PrE) and is expressed in the pluripotent epiblast before (E4.5) and after implantation (E5.5–E8.0). During gastrulation and with the onset of somitogenesis, Oct4 expression is downregulated and subsequently becomes restricted to PGCs (figure 2) [2,90–92]. Oct4 is downregulated during spermatogenesis, but spermatogonial stem cells remain positive for Oct4. Oct4 is not detected at the early stages of oogenesis but is re-expressed during the growth phase of primary oocytes and is present until fertilization [93–95]. Oct4 is a key marker of cultured pluripotent stem cells, with two major stem cell types identified: the classic so-called ‘naive’ cells, which are ESCs that correspond to and could be obtained from the epiblast before implantation [15,16,96,97]; and the ‘primed’ epiblast stem cells (EpiSCs), which correspond to the epiblast after implantation [98,99]. In the past few years, an intermediate ‘formative’ pluripotent stem cell type, which corresponds to E5.5 epiblast and to cultured epiblast-like stem cells (EpiLCs), was identified and shown to have an ability to differentiate into germ cells [100–103]. Loss of pluripotency correlates with Oct4 downregulation in epiblast and thus, EpiSCs could be obtained up to E8.0 [92]. EpiSCs and EpiLCs/EpiSCs differ from each other by the presence of specific markers, signalling and culture conditions [102,104]. While ESCs are mainly dependent on leukemia inhibitory factor (LIF), EpiLCs/EpiSCs require bFGF/Activin for self-renewal [98,99,105]. The Pou5f1 gene is subject to complex transcriptional regulation: it has three key regulatory elements—the distal enhancer (approx. 2 kb 5' from TSS), the proximal enhancer (approx. 1 kb 5' from TSS), and the proximal promoter—targets of a variety of transcriptional regulators [2,90,106–108]. The distal enhancer is active in the pluripotent epiblast of pre-implantation embryos and, as expected, in its cultured counterparts, ESCs, while the proximal enhancer is active in the epiblast of post-implantation embryos and the cells derived thereof, EpiSCs [109]. An additional enhancer element, which is located within the first intron of Pou5f1 gene, was found to be active in naive human ESCs. This enhancer is conserved across placental animals but it is not active in mouse ESCs [110].

4.1 Oct4 functions in mammals before implantation

Early functional studies pointed to an Oct4 role as an antagonist to Cd2x during morula separation into ICM (Oct4+) and trophoderm (TE, Cd2x2+) [4,17,111]. Nichols et al. showed that while Oct4-null blastocysts were initially established, they gave rise to only trophoblast giant cells in outgrowth experiments and no implanted mutant embryos were found at E5.5 [17]. A further study by Niwa et al. with a regulatable Oct4 transgene system showed that less than two-fold down-regulation of Oct4 in ESCs led to ESC differentiation into trophoblast, while comparable Oct4 upregulation drove ESC differentiation into primitive endoderm (PrE) and mesoderm [4]. This study pointed to Oct4 roles in differentiation to both extraembryonic cell types—TE and PrE—via unknown mechanisms. Another work with Oct4 knockdown in ESCs showed upregulation of Cd2x, Hand1, Eomes and Mash2 mRNAs [112]. Further research revealed an important role for Cd2x in trophoblast stem cell specification and maintenance, while Oct4 downregulation induced trophoblast differentiation normally in Cd2x-null cells [111,113]. However,
more recent in vitro and in vivo studies brought to light an alternative view of the role of Oct4 levels in lineage choice. It was clearly shown that in ESCs, Oct4 level could be reduced two-fold [114], and even seven-fold, resulting in the emergence of a robust naive pluripotent state [5]. Those cells with constitutively low Oct4 level could be maintained in both defined N2B27 and serum-containing media without LIF and additional inhibitors. The authors also showed that Oct4 overexpression resulted in differentiation of ESCs into all three embryonic lineages [5]. Nonetheless, elimination of Oct4 in ESCs led to loss of pluripotency—first, ‘naive’ markers were downregulated, then, trophodermal genes were upregulated [115]. Interestingly, at early timepoints after rapid Oct4 depletion via an auxin-inducible degron approach, Nanog binding to its genomic targets was enhanced, within both Oct4-occupied and Oct4-free regions, ruling out the possibility of physical competition between Oct4 and Nanog [115]. The derivation of maternal and zygotic Oct4-knockout mouse embryos surprisingly revealed that TE-ICM segregation and epiblast specification proceeded normally without Oct4; however, PrE formation was abolished [116–118]. These studies showed several interesting facts. First, the formation of Nanog-positive pluripotent epiblast is not affected, and at E3.0-E4.0 stage, the average number of outside and inside cells is similar between wild-type and Oct4-null embryos. Second, Cdx2 mRNA level is elevated as early as E4.5 in Oct4-knockout embryos, pointing to the notion that reciprocal interaction between Oct4 and Cdx2 is needed for the maintenance rather than the establishment of the ICM and TE. Third, initial PrE marker Gata6 expression is not affected; however, further Sox17 and Gata4 activation with subsequent PrE maturation is not observed in Oct4 mutants. Finally, a recent study by Stirparo et al. showed that in Oct4-deficient ICM of an early blastocyst (E3.5), the TE markers Gata2, Gata3, Eomes, but not Cdx2, are upregulated [119]. Moreover, the authors pointed to failure of activation of both epiblast- and PrE-specific genes in late blastocyst (E4.5), decline in P-STAT3 level and glycolytic gene activity, and upregulation of genes associated with autophagy and lysosomes, which is most likely a response of energy-insufficient metabolism [119]. Of note, in line with the results of Livigni et al. on the conserved role of POUV proteins in cell adhesion [13], an enrichment in modulated genes associated with cell adhesion and tight junction formation was observed in mutant E4.5 blastocysts [119]. Considering that Pou5f3 is important for zebrafish endoderm formation was observed in mutant E4.5 blastocysts [119]. These studies showed several interesting facts. First, the formation of Nanog-positive pluripotent epiblast is not affected, and at E3.0-E4.0 stage, the average number of outside and inside cells is similar between wild-type and Oct4-null embryos. Second, Cdx2 mRNA level is elevated as early as E4.5 in Oct4-knockout embryos, pointing to the notion that reciprocal interaction between Oct4 and Cdx2 is needed for the maintenance rather than the establishment of the ICM and TE. Third, initial PrE marker Gata6 expression is not affected; however, further Sox17 and Gata4 activation with subsequent PrE maturation is not observed in Oct4 mutants. Finally, a recent study by Stirparo et al. showed that in Oct4-deficient ICM of an early blastocyst (E3.5), the TE markers Gata2, Gata3, Eomes, but not Cdx2, are upregulated [119]. Moreover, the authors pointed to failure of activation of both epiblast- and PrE-specific genes in late blastocyst (E4.5), decline in P-STAT3 level and glycolytic gene activity, and upregulation of genes associated with autophagy and lysosomes, which is most likely a response of energy-insufficient metabolism [119]. Of note, in line with the results of Livigni et al. on the conserved role of POUV proteins in cell adhesion [13], an enrichment in modulated genes associated with cell adhesion and tight junction formation was observed in mutant E4.5 blastocysts [119]. Considering that Pou5f3 is important for zebrafish endoderm development but is dispensable for Oct4-deficient mESC maintenance, it would be interesting to investigate whether zebrafish Pou5f3 could rescue PrE maturation in Oct4-deficient mouse embryos.

Initial work by Niwa et al. [4] showed another interesting feature: artificial expression of Oct4 to wild-type level in ESCs, together with LIF withdrawal, led to differentiation of ESCs into PrE, as did Oct4 overexpression in the presence of LIF [4]. LIF is a member of the IL-6 family of cytokines. Its binding to its target receptor promotes STAT3 phosphorylation, which in turn regulates Oct4 expression through the occupation of the Pou5f1 distal enhancer [105,120]. The data by Niwa et al. suggest that LIF/p-STAT3 are needed to not only activate Oct4 transcription but also provide a regulatory context supportive of Oct4’s role as a pluripotency gatekeeper rather than a lineage specifier. Oct4 dimerizes with Sox2, but Oct4 can also dimerize with Sox17 on the so-called compressed motifs near PrE genes [121,122]. Oct4 dephosphorylation at T343 amino acid leads to a shift in Oct4 dimerization preference, from Oct4-Sox2 dimers to Oct4-Sox17 dimers [123]. Thus, one can hypothesize that, directly or indirectly, LIF/p-STAT3 signalling modulates Oct4 activity, for example, by phosphorylation to favour Oct4 interaction with Sox2 instead of Sox17. The model can explain, for example, the robust pluripotent state of ESCs with low Oct4 level [5,114]—the decrease of the total amount of Oct4 protein may shift the equilibrium toward the phosphorylated form. However, this idea is not supported by the observation that ESCs can be maintained with seven-fold downregulated Oct4 level, even without LIF [5]. On the other hand, the ‘LIF/p-STAT3 context’ hypothesis agrees with another recent study from the same research group [124]. Following the generation of mouse chimeras by introducing ESCs with constitutive Oct4 expression, the authors could successfully obtain Oct4-expressing MEFs. These cells could be reprogrammed into iPSCs simply by addition of LIF and simultaneous transfection with IL6 and IL6 receptor-encoding constructs—a/i.e. by LIF/p-STAT3 pathway activation [124]. Interestingly, while STAT3-knockout embryos at E3.5 demonstrate normal morphology, as well as normal Cdx2, Oct4, and Nanog expression, those at E4.5 consist almost entirely of Cdx2-positive (but Oct4- and Nanog-negative) cells and very few Gat6-positive cells [120]. Of note, it appears that p-STAT3 and Oct4 regulation is consistent with the previously discussed work showing that p-STAT3 level declines in response to Oct4 knockdown in the mouse blastocyst [119]. The role of Oct4 in PrE development is also related to Fgf4 activation. Oct4 plus Sox2 together occupy the Fgfl enhancer and drive Fgf4 transcription [125]. Secreted by pluripotent epiblast, Fgf4 binds to the Fgf receptors Fgrf1 and Fgrf2, thereby activating the MAPK/ERK-signalling pathway and driving the PrE differentiation programme [126,127]. Inhibition of the FGF pathway leads to the conversion of all ICM cells into Nanog-positive epiblast cells at E4.5 [128], while addition of exogenous Fgf4 induces the conversion of ICM cells into Gat6-positive PrE cells [129].

Though the role of the Oct4 in pre-implantation development is well studied in mouse, some differences in Oct4 function are seen across other placental animals. During bovine and human Oct4-null blastocyst development, ICM–TE segregation was found to occur as in mouse but without Nanog activation [130]. It was also shown that Oct4 knockout leads to some difficulties in expansion of human blastocyst: 47% (8 of 17) of control Cas9-injected embryos developed to the blastocyst stage, whereas 19% (7 of 37) of Oct4-targeted-Cas9 embryos matured to this stage [131]. There are also significant differences in the role of FGF during epiblast–PrE segregation. While activation or inhibition of the FGF pathway in mouse is critical for PrE or epiblast establishment, respectively, it has little role in bovine and no role in human pre-implantation development [132,133].

4.2. Oct4 functions in mammals after implantation

There is also substantial evidence about the importance of Oct4 in cell differentiation within the embryo proper. Based on zebrafish and Xenopus Pou5f3 studies and a one report showing that under serum-free conditions, Oct4 upregulation leads to ESC differentiation into neuroectoderm, it is possible that Oct4 plays a similar role in the neuroectoderm
development of mammals [134]. However, most studies point to a key role of Oct4 in mesendoderm specification. Upregulation of Oct4 via transgenesis or by TGF-beta induction triggered differentiation toward the cardiac lineage [135]. While Sox2 and Oct4 cooperate to maintain the pluripotent state in ESCs, they act oppositely during ESC differentiation into neuroectoderm (NE) and mesendoderm (ME), respectively. At initial steps of specification of both mouse and human ESCs, Sox2 and Oct4 levels are strongly predictive of subsequent ME and NE fate—i.e. before the expression of corresponding markers [136,137]. During differentiation of mouse ESCs, Oct4 suppresses the NE state and does not colocalize with Sox1, while Sox2 antagonizes ME (Brachyury+) specification. It was also shown that ME specification is driven by Nac1 in cooperation with Oct4 and that NE specification is driven by Tcf3 and Sox2, as Nac1 and Tcf3 downregulation compromised the corresponding differentiation [138]. In human ESCs, robust Oct4 expression, in cooperation with BMP signalling, led to ME specification, while Oct4 knockdown, along with BMP repression, promoted the NE state [139]. Using mouse primed Episcs, Yu et al. demonstrated that inhibition of FGF signalling leads to Oct4 downregulation, abrogation of ME markers, and subsequent NE differentiation by a default mechanism that does not require additional factors in the media [140].

These results, along with the observation that the ME factor Gata3 can substitute for Oct4 in Yamanaka’s cocktail for fibroblast reprogramming toward iPSCs, suggest that pluripotency may be maintained by antagonism of NE and ME specifiers [141,142]. Shu et al. proposed a ‘seesaw’ model that postulates that a delicate balance between Oct4 and Sox2 levels prevents differentiation into NE or ME [141]. However, this hypothesis was refuted by several facts, as discussed in a recent work by Velychko et al. [143]. First, forced expression of Oct4 could rescue the pluripotent state in Sox2-knockout ESCs [144]. Second, Velychko and colleagues showed that only KSM—that is, the reprogramming cocktail without Oct4—could reprogram fibroblasts into iPSCs, and that Oct4 and Gata3 work primarily to enhance proliferation during reprogramming [143]. Finally, to the best of our knowledge, there were no successful attempts to obtain stable ESC lines with substitution of Oct4 and Sox2 by ME and NE factors, for example, by Brachury and Sox1, respectively.

An interesting difference is observed in Oct4 partner choice during PGC maturation in mouse and human. Sox2 is expressed throughout the establishment of murine PGCs [100], whereas Sox17 substitute for Sox2 in human PGCs [145]. As it was pointed above, the Sox17-Oct4 complex occupies compressed motifs, which are 1 bp shorter that the canonical Sox2-Oct4 motif [121]. Analyses of open chromatin during hPGC maturation have revealed the compressed Sox-Oct motif occurs across active DNA elements [146,147]. As Sox17 is associated with endoderm specification, the rationale for Oct4 partner switching from Sox2 to Sox17 in human PGC development remains unclear. This may have to do with differences in the mechanism of germ cell differentiation between mouse and human. While mouse germ cells are induced from the murine primed EpiLCs, human ESCs are supposed to go through the so-called pre-mesendoderm condition [148].

Although in vitro works usually point to rather definite roles for Oct4 in the specification of one or another cell type of the embryo proper, in vivo data are more comprehensive. Depletion of Oct4 in PGCs by Cre/loxP gene targeting led to apoptosis of these cells between E9.5 and E10.5, pointing to a specific Oct4 role in PGC viability and maturation [94]. Further development of germ cells differentially requires Oct4 expression. Spermatogonial stem cells depend on Oct4 for their self-renewal, and Oct4 knockdown leads to a reduced ability to colonize the seminiferous tubules [149]. ZP3-dependent Oct4 depletion in growing oocytes do not affect oocyte maturation and embryogenesis immediately after fertilization (discussed above) [116]. In other works, DeVeale et al. and Mulas et al. used conditional systems that allowed them to knock out Oct4 after embryo implantation and thus, to dissect Oct4 function in the early gastrulating embryo [19,150]. Oct4 depletion from approximately E7.0 onward led to multiple defects—craniorachischisis, posterior truncation, random heart tube orientation, defective somitogenesis and failed anterior–posterior orientation. Mulas et al. demonstrated that in Oct4-null epiblasts, Nanog expression was elevated and endoderm was expanded at the expense of mesoderm [150]. An interesting observation of this work is that beating structures were successfully obtained from Oct4-depleted embryos, pointing to a successfully launched mesendoderm programme and, again, contradicting the view that Oct4 is a factor indispensable for differentiation into ME. However, the epithelial-to-mesenchymal transition was impaired due to E-cadherin upregulation and overall, the anterior–posterior axis was abnormal. DeVeale et al. also pointed to an Oct4 role in primitive streak (mesendoderm) proliferation and suggested a conserved Oct4 function in posterior extension, consistent with the observed posterior truncations in both zebrafish and Xenopus POUV mutants [11,18,65]. The data is also in accordance with the demonstrated Oct4 role in regulation of the trunk length in snakes and mice. While prolonged Oct4 expression in mice (via Cdx2 enhancer-driven posterior expression up to E12.5) led to an abnormal increase in number of ribs, exceptionally long snake trunks might be a result of heterochronic changes in Oct4 activity during body axis extension [86].

Finally, a very recent study revealed that Oct4 is reactivated in premigratory cranial neural crest cells (CNCCs) at early somitogenesis (E8.0), endowing CNCCs with the pluripotent state [151]. CNCCs present a specific population of cells with ectodermal origin and are responsible for craniofacial skeleton development—not only neurons and glia but also bone, cartilage, and muscles. In their study, Zalc et al. demonstrated that Oct4 ablation at E7.5 leads to complete absence of the front nasal mass. The pluripotent state was confirmed by the observation that in the absence of Oct4, neural derivatives of CNCCs developed normally, while ectomesenchyme maturation was affected. Furthermore, ATAC-seq of Oct4-positive CNCCs revealed that these cells clustered with Episcs, while the Oct4-positive trunk cells, which were also present during early somitogenesis, did not [151]. Notably, artificially maintained constitutive Oct4 levels do not prevent any type of differentiation during chimera formation up to E12.5 of mouse development [5,124]. Overall, as we discussed earlier with an example of LIF/p-STAT3 signalling, it appears that Oct4 is a context-dependent transcription factor. In some cases, it safeguards pluripotency, while in other cases, it maintains proliferation or at least does not prevent differentiation.
There were numerous reports about Oct4 expression in different somatic cells of the adult organism, prompting Lengner et al. [152], using a Cre/lox-based genetic approach, to examine the role of Oct4. The authors found that Oct4 does not play a role in the compartments of several somatic tissues, such as intestinal epithelium, bone marrow (haematopoietic and mesenchymal lineages), hair follicle, brain and liver [152]. The investigation appeared to settle the debate about whether Oct4 functions outside of the mammalian germline (epiblast and PGCs); however, a recent study again sparked the debate. In that study, investigators relied on a similar Cre/lox-based genetic approach and the same Oct4<sup>lox</sup> mouse line and demonstrated that Oct4 is induced in mouse atherosclerotic lesions. Oct4 expression was observed in smooth muscle cells (SMCs) and thought to promote a specific atheroprotective SMC phenotype switch involved in the formation of a protective fibrous cap. SMC-specific depletion of Oct4 led to an increase in the size of atherosclerotic lesions and, consequently, in reduced lumen size, increased necrotic core area, and increased intraplaque haemorrhage [153]. It might be that under specific pathological conditions, adult somatic cells can engage Oct4 function, and the reported case with atherosclerotic SMCs might not be unique in that respect.

5. Conclusion

Despite the huge amount of data about POUV-class proteins, there are still a lot of questions about their origin and mechanisms of their action. Absence of any POUV member in lampreys complicates our understanding about the emergence of this class. Considering the similar early development of lamprey and zebrafish, and the early lethal phenotype of zebrafish with Pou5f3 knockout, it is unclear how lampreys develop without POUV. Future works should look to uncover whether there are some alternative regulatory mechanisms that enable normal development in the absence of POUV or that other proteins present in these organisms control the same processes.

It appears that the main function of the POUV-class proteins is maintenance of the undifferentiated state through activation of their genome targets. Though there was some evidence about their role in transcriptional repression, it was also shown that specific protein fusion making only the active form of Xenopus Pou5f3 or mouse Oct4 is sufficient for performing the whole range of functions [154]. The involvement of POUV proteins in different processes enables maintenance of the undifferentiated state. During Xenopus neuroectoderm development, Pou5f3 function is related to the maintenance of neural progenitors [14] and prevention of ectodermal differentiation [79]. The conservative role of POUV in posterior extension [11,18,19] also indicates that proteins of this class prevent immature differentiation, delaying the onset of further body patterning, for example, by prevention of Hox genes activation [86]. Finally, as the most notable member of the POUV class, Oct4 functions in maintenance and induction of the undifferentiated state, thus being a key determinant of mammalian pluripotent stem cells [3,17,155,156].

The facts indicate that the mechanisms of POUV-class protein functioning are species and stage dependent. As we have previously discussed [8], these proteins are inextricably linked to zygotic genome activation in zebrafish and Xenopus, and, to a limited extent, in mammals. The ability of murine Oct4 to rescue Pou5f3-deficient zebrafish in early development [84] or Xenopus Pou5f3 to substitute for Oct4 in mouse ESCs [11] indicates that POUV class homologues did not undergo significant structural changes. At the same time, Pou5f3 from zebrafish could not rescue the pluripotent properties of murine ESCs [11]. Moreover, while Pou5f3 is needed for endoderm establishment in zebrafish [26,65], Pou5f3 orthologues suppress mesendoderm maturation in Xenopus [11,27]. Additionally, POUV could exert a different function in the same species depending on the developmental stage. A striking example of context-dependent activity is the behaviour of murine Oct4 during embryogenesis. While murine Oct4 maintains the pluripotency of the epiblast [17,119] and induces this cell state in CNCCs [151], when introduced artificially, it does not prevent any type of differentiation at subsequent development up to E12.5 [124]. Moreover, when Oct4-expressing MEFs were isolated at this stage, they were successfully reprogrammed into iPSCs only via LIF/p-STAT3 pathway activation. It appears that to arrive at a better understanding of the POUV-class protein function, one should take into consideration their functional amino acids and the regulatory environment engaged depending on the developmental stage.

We have also previously discussed that the functional novelty of the POUV class is the ability to dimerize in vivo with proteins of the Sox8 class. This property was immediately linked to participation of POUV members in ZGA (figure 3) [75,76]. It is most likely that POUV-class proteins have emerged from some of the POUIII-class proteins, which are known to be regulators of neuroectodermal development. Taking this into account, it appears that the function of zebrafish and Xenopus Pou5f3 in neurogenesis is an evolutionary inherited feature. The Pou5f1 orthologue (Oct4), which is present in mammals, has probably lost this feature, as it acts primarily in pluripotency control [5] and mesendoderm development [137,150]. Nevertheless, murine ESCs, which harbour only this Pou5f1 orthologue, are characterized by epithelial morphology, E-cadherin expression, and the default capacity for neuroectodermal differentiation [157]. These features, along with the ancient role of POUV-class proteins in preventing differentiation by securing unrestricted ectodermal epithelium [13], point to a close link between ectoderm and cells expressing POUV, enhancing our understanding of the nature of cellular pluripotency.

Figure 3. Functional diversity between POUIII-class ancestor and POUV-class orthologues—Pou5f1 and Pou5f3.

Data accessibility. This article has no additional data.

Authors’ contributions. E.I.B.: conceptualization, funding acquisition, writing—original draft; A.N.T.: conceptualization, project administration, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.
References

1. Herr W et al. 1988 The POU domain: a large conserved region in the mammalian pit-1, oct-1, oct-2, and Caenorhabditis elegans unc-86 gene products. Genes Dev. 2, 1513-6

2. Wu G, Scholer HR. 2014 Role of Oct4 in the early embryo development. Cell Regen. 3, 10

3. Takahashi K, Yamanaka S. 2006 Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663–676. (doi:10.1016/j.cell.2006.07.024)

4. Niwa H, Miyazaki J-i, Smith AG. 2000 Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat. Genet. 24, 372–376. (doi:10.1038/74199)

5. Radzisheuskaya A, Le Bin Chia G, Dos Santos RL, Shimeld SM, Degnan BM. 2008 A defined Oct4 level governs cell state transitions of pluripotency entry and differentiation into all embryonic lineages. Nat. Cell Biol. 10, 579–590. (doi:10.1038/ncb2742)

6. Frankenberg S, Pask A, Renfree MB. 2010 The evolution of class V POU domain transcription factors in vertebrates and their characterisation in a marsupial. Dev. Biol. 337, 162–170. (doi:10.1016/j.ydbio.2009.10.017)

7. Onichtchouk D. 2016 Evolution and functions of Oct4 homologs in non-mammalian vertebrates. Biochim. Biophys. Acta 1859, 770–779. (doi:10.1016/j.bbamem.2016.03.013)

8. Bakhmet EI, Tomilin AN. 2021 Key features of the POU transcription factor Oct4 from an evolutionary perspective. Cell. Mol. Life Sci. 78, 7339–7353. (doi:10.1007/s00018-021-03975-8)

9. Belting H-G et al. 2001 Spiel Ohne grenzen/pou2 is required for establishment of the zebrafish midbrain-hindbrain boundary organizer. Development 128, 4165–4176. (doi:10.1242/dev.128.21.4165)

10. Reim G, Brand M. 2002 Spiel-ohne-grenzen/pou2 is required during establishment of the zebrafish early neural development. Development 129, 917–933. (doi:10.1242/dev.129.917)

11. Morrison GM, Brickman JM. 2006 Conserved roles for Oct4 homologues in maintaining multipotency during early vertebrate development. Development 133, 2011–2022. (doi:10.1242/dev.02362)

12. Snir M, Ofrir R, Elias S, Frank D. 2006 Xenopus laevis POU91 protein, an Oct3/4 homologue, regulates competence transitions from mesoderm to neural cell fates. EMBO J. 25, 3664–3674. (doi:10.1038/sj.emboj.7601238)

13. Livigni A et al. 2013 A conserved Oct4/POU-V-dependent network links adhesion and migration to progenitor maintenance. Curr. Biol. 23, 2233–2244. (doi:10.1016/j.cub.2013.09.048)

14. Archer TC, Jin J, Casey ES. 2011 Interaction of Sox1, Sox2, Sox3 and Oct4 during primary neurogenesis. Dev. Biol. 350, 429–440. (doi:10.1016/j.ydbio.2010.12.013)

15. Evans MJ, Kaufman MH. 1981 Establishment in culture of pluripotential cells from mouse embryos. Nature 292, 154–156. (doi:10.1038/292154a0)

16. Martin GR. 1981 Isolation of a pluripotent cell line derived from mouse embryos grown in culture. Nature 290, 136–138. (doi:10.1038/290136a0)

17. Nichols J, Zevnik B, Anastassiads K, Niwa H, Klemm-Nebenius D, Chambers I, Scholer H, Smith A. 1998 Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell 95, 379–391. (doi:10.1016/S0092-8674(00)01769-9)

18. Burgess S, Reim G, Chen W, Hopkins N, Brand M. 2002 The zebrafish Spiel-Ohne-Grenzen (Spg) gene encodes the POU domain protein Pou2 related to mammalian Oct4 and is essential for formation of the midbrain and hindbrain, and for pre-gastrula morphogenesis. Development 129, 905–916. (doi:10.1242/dev.129.4.905)

19. Deveale B et al. 2013 Oct4 is required ~E7.5 for proliferation in the primitive streak. PLoS Genet. 9, e1003957. (doi:10.1371/journal.pgen.1003957)

20. Larroux C, Luke GN, Koopman P, Rokhsar DS. 2001 Early evolution of metazoan transcription factors in vertebrates and their characterisation in a marsupial. Dev. Biol. 234, 223–237. (doi:10.1016/S0012-1606(01)00478-3)

21. Degnan BM, Vervoort M, Larroux C, Richards GS. 2008 Oct4 is required and functions in the endoderm specification cascade. Curr. Biol. 18, 48–55. (doi:10.1016/j.cub.2003.11.022)

22. Cao Y, Knöchel S, Donov C, Mieethe J, Kaufmann E, Knöchel W. 2004 The Pou factor Oct-25 regulates the Xvent-2B gene and counteracts terminal differentiation in Xenopus embryos. J. Biol. Chem. 279, 43–473. (doi:10.1074/jbc.M407544200)

23. Hemmrich G, Bosch TC. 2008 Compagen, a comparative genomics platform for early branching metazoan animals, reveals early origins of genes regulating stem-cell differentiation. Bioessays 30, 1010–1018. (doi:10.1002/bies.200813)

24. Ohno S. 1970 Evolution by gene duplication. New York, NY: Springer.

25. Holland PW, Garcia-Fernandez J, Williams NA, Sidow A. 1994 Gene duplications and the origins of vertebrate development. Dev. Suppl. 125–133.

26. Hokamp K, McVeyacht A, Wolfe H. 2003 The 2R Hypothesis and the Human Genome Sequence. J. Struct. Funct. Genom. 3, 95–110. (doi:10.1023/A:1022661917301)

27._leave_11400357

28. 2012 Pou5f1/oct4 in pluripotency and progenitor maintenance. Cell Regen. 11-56. (doi:10.1038/292154a0)

29. Bayramov A V, Ermakova GV, Kucheryavyy AV, Zoraya AG. 2019 Lampreys, “Living Fossils,” in Research on Early Development and Regeneration in Vertebrates. Russ. J. Dev. Biol. 49, 327–338. (doi:10.1134/s1018-0159-0159-9)

30. Holland LZ, Ocampo Daza D. 2018 A new look at an old question: when did the second whole genome duplication occur in vertebrate evolution? Genome Biol. 19, 209. (doi:10.1186/s13059-018-1559-0)

31. Smith JJ et al. 2018 The sea lamprey germine genome provides insights into programmed genome rearrangement and vertebrate evolution. Nat. Genet. 50, 270–277. (doi:10.1038/s41588-017-0036-1)

32. Gold DA, Gates RD, Jacobs DK. 2014 The early expansion and evolutionary dynamics of POU class genes. Mol. Biol. Evol. 31, 3136–3147. (doi:10.1093/molbev/msu245)

33. Bayramov AV, Ermoakova GV, Kucheryavyy AV, Zoraya AG. 2019 Lampreys, “Living Fossils,” in Research on Early Development and Regeneration in Vertebrates. Russ. J. Dev. Biol. 49, 327–338. (doi:10.1134/s1018-0159-0159-9)

34. Miyashita T, Green SA, Bronner ME. 2018 Comparative development of cyclostomes. In Evolution and development of fishes (eds Z Johanson, C Underwood, M Richter), pp. 30–58. Cambridge, UK: Cambridge University Press.

35. Zhao F-Q. 2013 Octamer-binding transcription factors: genomics and functions. Front. Biosci. (Landmark Ed) 18, 1051–1071. (doi:10.2741/4162)
controls zygotic gene activation in vertebrates. Science 341, 1005–1009. (doi:10.1126/science.1242527)

53. Jerabek S et al. 2016 Changing POU dimerization preferences converts Oct6 into a pluripotency inducer. *EMBO Rep.* 18, 319–333. (doi:10.1525/embrep.201642958)

54. Malik V et al. 2019 Pluripotency reprogramming by competent and incompetent POU factors uncovers temporal dependency for Oct4 and Sox2. Nat. Commun. 10, 3477. (doi:10.1038/s41467-019-11054-7)

55. Tan DS et al. 2021 Directed evolution of an enhanced POU reprogramming factor for cell fate engineering. *Mol. Biol. Evol.* 38, 2854–2868. (doi:10.1093/molbev/msab075)

56. Rosner A, Mozeeva E, Rinkevich Y, Lapidot Z, Rinkevich B. 2009 Vasa and the germ line lineage in a colonial urchinate. *Dev. Biol.* 331, 113–128. (doi:10.1016/j.ydbio.2009.04.025)

57. Mashanov VS, Zueva OR, Garcia-Arraras JE. 2015 Expression of pluripotency factors in echinoderm embryos. *Cell Tissue Res.* 359, 521–536. (doi:10.1007/s00441-014-2040-4)

58. Önal P et al. 2012 Gene expression of pluripotency determinants is conserved between mammalian and planarian stem cells. *EMBO J.* 31, 2755–2769. (doi:10.1038/emboj.2012.110)

59. Millane RC, Kanska J, Duffy DJ, Seoighe C, Onichtchouk D, Driever W. 2014 Pou5f1/Oct4 transcription factor promotes cell survival via direct activation of mych expression during zebrafish gastrulation. *Plas ONE* 9, e92356. (doi:10.1371/journal.pone.0092356)

60. Kotkan K, Kur E, Wendik B, Polok B, Ben-Dor S, Onichtchouk D, Driever W. 2014 Pou5f1/Oct4 promotes cell survival via direct activation of mych expression during zebrafish gastrulation. *Plas ONE* 9, e92356. (doi:10.1371/journal.pone.0092356)

61. Lai AG, Abouaker AA. 2018 EvolveRegen in animals: time to uncover deep conservation or convergence of adult stem cell development and regenerative processes. *Dev. Biol.* 433, 118–131. (doi:10.1016/j.ydbio.2017.10.010)

62. Takeda H, Matsuoka T, Oki T, Miyagawa T, Amanuma H. 1994 A novel POU domain gene, zebrasf-pou2: expression and roles of two alternatively spliced twin products in early development. *Genes Dev.* 8, 45–59. (doi:10.1101/gad.8.1.45)

63. Reim G, Brand M. 2006 Maternal control of vertebrate dorsoventral axis formation and embryo by the POU domain protein Spg/Pou2/Oct4. *Development* 133, 2575–2770. (doi:10.1242/dev.02391)

64. Sánchez-Sánchez R, Camp E, García-España A, Leal-Tassias A, Muller JL. 2010 Medaka Oct4 is expressed during early embryo development, and in primordial germ cells and adult gonads. *Dev. Dyn.* 239, 672–679. (doi:10.1002/dvdy.22298)

65. Liu R, Li M, Li Z, Hong N, Xu H, Hong Y. 2015 Medaka Oct4 is essential for pluripotency in blastula formation and ES cell differentiation. *Stem Cell Rev. Rep.* 11, 11–23. (doi:10.1007/s12105-014-9523-2)

66. Reim G, Mizoguchi T, Stainier DY, Kikuchi Y, Brand M. 2004 The POU Domain Protein Spg (pou2/Oct4) is essential for endoderm formation in cooperation with the HMG domain protein casanova. *Cell Dev.* 6, 91–101. (doi:10.1016/S1534-5807(03)00396-4)

67. Chan T-M, Chao C-H, Wang H-D, Yu Y-J, Yuh C-H. 2009 Functional analysis of the evolutionarily conserved cis-regulatory elements on the sox17 gene in zebrasf. *Dev. Biol.* 326, 456–470. (doi:10.1016/j.ydbio.2008.11.010)

68. Lachnit M, Kur E, Driever W. 2008 Alterations of the cytoskeleton in all three embryonic lineages contribute to the epiboly defect of Pou5f1/Oct4 deficient MZspg zebrasf embryos. *Dev. Biol.* 315, 1–17. (doi:10.1016/j.ydbio.2007.10.008)

69. Song S, Eckerle S, Onichtchouk D, Mans J, Nitschke R, Driever W. 2013 Pou5f1/Oct4-dependent EGF expression controls c-adherin endocytosis, cell adhesion, and zebrasf embryo movements. *Dev. Cell* 24, 486–501. (doi:10.1016/j.devcel.2013.01.016)

70. Kotkan K, Mössner R, Allen A, Onichtchouk D, Driever W. 2014 Pou5f1/Oct4 dependent Klf2a, Klf2b, and Klf17 regulatory sub-network contributes to EVL and ectoderm development during zebrasf embryogenesis. *Dev. Biol.* 385, 433–447. (doi:10.1016/j.ydbio.2013.10.025)

71. Khan A, Nakamoto A, Okamoto S, Tai M, Nakayama Y, Kobayashi K, Kawamura A, Takeda H, Yamau k K. 2012 Pou2, a class V POU-type transcription factor in zebrasf, regulates dorsalventral patterning and convergent extension movement at different blastula stages. *Mech. Dev.* 129, 219–235. (doi:10.1016/j.mod.2012.07.007)

72. Belting HG, Wendik B, Lunde K, Leischnering M, Mössner R, Driever W, Onichtchouk D. 2011 Pou5f1 contributes to dorsalventral patterning by positive regulation of vola and modulation of fghta expression. *Dev. Biol.* 356, 323–336. (doi:10.1016/j.ydbio.2011.05.060)

73. Lee MT, Bonneau AR, Takacs CM, Bazini AA, Divito KR, Fleming ES, Giraldez AJ. 2013 Nanog, Pou5f1 and Sox81 activate zygotic gene expression during the maternal-zygotic transition. *Nature* 503, 360–364. (doi:10.1038/nature12632)

74. Veil M, Yampolsky LY, Grning B, Onichtchouk D. 2019 Pou5f1, Sox81, and Nanog remodel chromatin on high nucleosome affinity regions at zygotic genome activation. *Genome Res.* 29, 383–395. (doi:10.1101/gr.240572.118)

75. Gentsch GE, Spruce T, Owens ND, Smith JC. 2019 Maternal pluripotency factors initiate extensive chromatin remodelling to predefine first response to inductive signals. *Nat. Commun.* 10, 4269. (doi:10.1038/s41467-019-12263-w)
87. Andersen B, Pearse RV, Schlegel PN, Cichon Z, Aires R, Jurberg AD, Leal F, Nóvoa A, Cohn MJ, Nakanoh S, Agata K. 2019 Evolutionary view of TGFbeta/Foxh1 regulation of the early trunk length diversity. Proc. Natl. Acad. Sci. USA 116, 259–267. (doi:10.1073/pnas.1901375116)

88. Young JJ, Kjolby RAS, Kong NR, Monica SD, Harland SM, Schonemann MD, Bardin CW, Rosenfeld MG. 1993 Germ line cell survival. Nature 360, 481–484. (doi:10.1038/360481a0)

89. Plusa B, Hadjantonakis AK. 2014 Embryonic stem cells with features of formative pluripotency. Cell 159, 649–661. (doi:10.1016/j.cell.2014.08.040)

90. Brow A, Niwa H, Yoshikawa H, Tomishige K, Muroya J, Stahl M, Rogers D. 1988 Inhibition of pluripotency gene expression in mouse embryos. Nature 336, 688–690. (doi:10.1038/336688a0)

91. Takebayashi-Suzuki K, Arita N, Murasaki E, Suzuki A. 2016 Oct4 is a key regulator of vertebrate germ cell survival. Dev. Biol. 413, 294–295. (doi:10.1016/j.ydbio.2016.03.028)

92. Osorno R et al. 2012 The developmental dismantling of pluripotency is reversed by ectopic Oct4 expression. Development 139, 2288–2298. (doi:10.1242/dev.078071)

93. Pesce M, Wang X, Wolgemuth DJ, Scholer HR. 1998 Differential expression of the Oct-4 transcription factor during mouse germ cell differentiation. Mech. Dev. 71, 89–98. (doi:10.1016/S0925-4773(98)00002-1)

94. Kehler J et al. 2004 Oct4 is required for primordial germ cell survival. EMBO Rep. 5, 1078–1083. (doi:10.1038/sj.embor.7400279)

95. Hong TK, Song JH, Lee SB, Do JT. 2021 Germ cell derivation from pluripotent stem cells for understanding in vitro gametogenesis. Cells 10, 1889. (doi:10.3390/cells10081889)

96. Boroviak T, Loos R, Bertone P, Smith A, Nichols J. 2014 The ability of inner-cell-mass cells to self-renew as embryonic stem cells is acquired following epiblast specification. Nat. Cell Biol. 16, 516–528. (doi:10.1038/nclerb2965)

97. Pluta B, Hadjantonakis AK. 2014 Embryonic stem cell identity grounded in the embryo. Nat. Cell Biol. 16, 502–504. (doi:10.1038/nclerb2984)

98. Brons IGM et al. 2007 Derivation of pluripotent epiblast stem cells from mammalian embryos. Nature 448, 191–195. (doi:10.1038/nature05950)

99. Tesar PJ, Chenoweth JG, Brook FA, Davies TJ, Evans EP, Mack DL, Gardner RL, Mckay RDG. 2007 New cell lines from mouse epiblast share defining features with human embryonic stem cells. Nature 448, 196–199. (doi:10.1038/nature05972)

100. Hayashi K, Ohta H, Kurimoto K, Aramaki S, Saitou M. 2011 Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. Cell 146, 519–532. (doi:10.1016/j.cell.2011.06.052)

101. Kinoshita M, Barber M, Mansfield C, Cui Y, Spindlove L, Stirparo GG, Dietmann S, Nichols J, Smith A. 2020 Capture of mouse and human stem cells with features of formative pluripotency. Cell Stem Cell. 28, 453–471. (doi:10.1016/j.stem.2020.11.005)

102. Kinoshita M, Smith A. 2018 Pluripotency Deconstructed. Dev. Growth Different. 60, 44–52. (doi:10.1111/dgd.12419)

103. Yu L et al. 2020 Derivation of Intermediate Pluripotent Stem Cells Amenable to Primordial Germ Cell Specification. Cell Stem Cell. 28, 550–567. (doi:10.1016/j.stem.2020.11.003)

104. Morgani S, Nichols J, Hadjantonakis AK. 2017 The many faces of Pluripotency: in vitro adaptations of a continuum of in vivo states. BMC Dev. Biol. 17, 7. (doi:10.1186/s12861-017-0150-4)

105. Smith AG, Heath JK, Donaldson DD, Wong GG, Moreau J, Stahl M, Rogers D. 1988 Inhibition of pluripotency gene expression in mouse embryos by purified polypeptides. Nature 336, 688–690. (doi:10.1038/336688a0)

106. Bakhmet E et al. 2019 HnRNP-K targets open chromatin in mouse embryonic stem cells in concert with multiple regulators. Stem Cells, 1018–1029. (doi:10.1002/stem.3025)

107. Minucci S, Botquin V, Yeom YI, Dey A, Sylvester I, Zand DJ, Ohbo K, Ozato K, Scholer HR. 1996 Retinoic acid-mediated down-regulation of Oct4/3 coincides with the loss of promoter occupancy in vivo. EMBO J. 15, 888–899. (doi:10.1002/97804700146071.004232.4)

108. Young RA. 2011 Control of the embryonic stem cell state. Cell 144, 940–954. (doi:10.1016/j.cell.2011.01.032)

109. Choi HW, Joo JH, Hong YJ, Kim JS, Song H, Lee JW, Wu G, Scholer HR, Do JT. 2016 Distinct enhancer activity of Oct4 in naïve and primed mouse pluripotency. Stem Cell Rep. 7, 911–926. (doi:10.1016/j.stemcr.2016.09.012)

110. Pastor WA et al. 2018 TASFC2 regulates transcription in human naive pluripotency by opening enhancers. Nat. Cell Biol. 20, 553–564. (doi:10.1038/s41556-018-0089-0)

111. Niwa H, Toyooka Y, Shimozato D, Strumpf D, Takahashi K, Yagi R, Rossant J. 2005 Interaction between Oct3/4 and Cdx2 determines trophoderm differentiation. Cell 123, 917–929. (doi:10.1016/j.cell.2005.08.040)

112. Ivanova N, Dubrin R, Lu R, Kotenko L, Levarse J, Decoste C, Schafer X, Lun Y, Lemsiccha IR. 2006 Dissecting self-renewal in stem cells with RNA interference. Nature 442, 533–538. (doi:10.1038/nature04915)

113. Tolkunova E, Cavalieri F, Eckardt S, Reinbold R, Christenson LK, Scholer HR, Tomilin A. 2006 The caulidal-related protein Cdx2 promotes trophoblast differentiation of mouse embryonic stem cells. Stem Cells 24, 139–144. (doi:10.1634/stemcells.2005-0240)

114. Karwacki-Neisius V et al. 2013 Reduced Oct4 expression directs a robust pluripotent state with distinct signaling activity and increased enhancer occupancy by Oct4 and Nanog. Cell Stem Cell 12, 531–545. (doi:10.1016/j.stem.2013.04.023)

115. Bates LE, Alves MRP, Silva JCR. 2021 Auxin-degron system identifies immediate mechanisms of OCT4 and NAN. Stem Cell Rep. 16, 1818–1831.

116. Wu G et al. 2013 Establishment of totipotency does not depend on Oct4A, Nat. Cell Biol. 15, 1089–1097. (doi:10.1038/ncb2816)

117. Frum T, Halboizen M, Wang C, Amiri H, Robson P, Ralston A. 2013 Oct4 cell-autonomously promotes primitive endoderm development in the mouse blastocyst. Dev. Cell 25, 610–622. (doi:10.1016/j.devcel.2013.05.004)

118. Le Bin GC et al. 2014 Oct4 is required for lineage priming in the developing inner cell mass of the...
Suppression of Erk signalling promotes ground state pluripotency in the mouse embryo. Development 136, 3215–3222. (doi:10.1242/dev.038893)

129. Yamanaka Y, Lanner F, Rossant J. 2010 FGF signalling during mouse blastocyst formation is not dependent on Fgf signalling. Dev. Biol. 361, 358–363. (doi:10.1016/j.ydbio.2011.10.030)

130. Aksoy I et al. 2006 Oct-3/4 dose dependently regulates specification of embryonic stem cells toward a cardiac lineage and early heart development. Dev. Cell 11, 535–546. (doi:10.1016/j.devcel.2006.07.013)

131. Shu J et al. 2018 ERK inhibition promotes neuroectodermal precursor commitment by blocking self-renewal and primitive streak formation of the embryo. Stem Cell Res. Ther. 9, 2. (doi:10.1186/s13287-017-0750-8)

132. Nichols J, Silva J, Roode M, Smith A. 2009 Stimulation of Erk signalling promotes ground state pluripotency via STAT3 signaling and metabolic mechanisms. Proc. Natl Acad. Sci. USA 108, e2008890118. (doi:10.1073/pnas.2008890118)

133. Kuijk EW, Van Tol LTA, Van De Velde H, Wubbolts R, Welling M, Geijten B, Roelen BAJ. 2012 The roles of FGFR and MAP kinase signalling in the segregation of the epiblast and hypoblast cell lineages in bovine and human embryos. Development 139, 871–882. (doi:10.1242/dev.076688)

134. Shimosaki K, Nakashima K, Niwa H, Taga T. 2003 Involvement of Oct3/4 in the enhancement of neuronal differentiation of ES cells in neurogenesis-inducing cultures. Development 130, 2505–2512. (doi:10.1242/dev.00476)

135. Ziemedine D et al. 2006 Oct-3/4 dose dependently regulates specification of embryonic stem cells toward a cardiac lineage and early heart development. Dev. Cell 11, 535–546. (doi:10.1016/j.devcel.2006.07.013)

136. Thomson M, Liu SJ, Zou L-N, Smith Z, Meissner A, Ramanathan S. 2011 Pluripotency factors in embryonic stem cells regulate differentiation into germ layers. Cell 145, 875–889. (doi:10.1016/j.cell.2011.05.017)

137. Valcourt JR, Huang RE, Kundu S, Venkatasubramanian D, Kingston RE, Ramanathan S. 2021 Modulating mesendoderm competence during human germ layer differentiation. Cell Rep. 37, 109990. (doi:10.1016/j.celrep.2021.109990)

138. Malleshaiah M, Padi M, Ruel P, Quackenbush J, Martinez-Arias A, Gunawardena J. 2016 Nac1 coordinates a sub-network of pluripotency factors to regulate embryonic stem cell differentiation. Cell Rep. 14, 1181–1194. (doi:10.1016/j.celrep.2015.12.101)

139. Wang Z, Oron E, Nelson B, Razis S, Ivanova N. 2021 Transcriptional activation by TFAP2C-Regulated OCT4 in mouse somatic cells with lineage specifiers. Cell Stem Cell 25, 403–415. (doi:10.1016/j.stem.2020.07.020)

140. Cherepanova OA et al. 2016 Activation of the pluripotency factor OCT4 in smooth muscle cells is atheroprotective. Nature Med. 22, 657–665. (doi:10.1038/nm.4109)

141. Rosner MH, Vigano MA, Ozato K, Timmons PM, Poire F, Rigby PKJ, Staudt LM. 1990 A POU domain transcription factor in early stem cells and germ cells of the mammalian embryo. Nature 343, 686–692. (doi:10.1038/344454a0)

142. Montserrat N et al. 2017 Principles of early human development and germ cell program from conserved model systems. Nature 546, 416–420. (doi:10.1038/nature22812)

143. Dann CT, Alvareado AL, Molyneux LA, Denard BS, Garbers DL, Porteus MH. 2008 Spermatogonial stem cell self-renewal requires OCT4, a factor downregulated during retinoic acid-induced differentiation. Stem Cells 26, 2928–2937. (doi:10.1634/stemcells.2008-0134)

144. Mulas C, Chia G, Jones KA, Hodgson AC, Stripo GG, Nichols J. 2018 Oct4 regulates the embryonic axis and coordinates exit from pluripotency and germ layer specification in the mouse embryo. Development 145, dev159103. (doi:10.1242/dev.159103)

145. Zalc A, Sinha R, Gutali GS, Wesche DJ, Dasszuk P, Swigut T, Weissman IL, Wysoka J. 2021 Reactivation of the pluripotency program precedes formation of the cranial neural crest. Science 371, eabb4776. (doi:10.1126/science.aabb4776)

146. Lengner CJ, Camargo FD, Hochadelinger K, Welstead GG, Zaidi S, Gokhale S, Scholer HR, Tomilin A, Jaenisch R. 2007 Oct4 expression is not required for mouse somatic stem cell self-renewal. Cell Stem Cell 1, 403–415. (doi:10.1016/j.stem.2007.07.020)

147. Cherepanova OA et al. 2016 Activation of the pluripotency factor OCT4 in smooth muscle cells is atheroprotective. Nature Med. 22, 657–665. (doi:10.1038/nm.4109)

148. Rose M, Viganò MA, Oztarik B, Timmons PM, Poire F, Rigby PKJ, Staudt LM. 1990 A POU domain transcription factor in early stem cells and germ cells of the mammalian embryo. Nature 343, 686–692. (doi:10.1038/344454a0)
**Abbreviations**

POU  name of transcription factor class, which name is originated by first letters of the names of the founding members of the family—mammalian Pit1, Oct1, Oct2, and C. elegans Unc86

ZGA   zygotic genome activation

POUs  POU-specific subdomain

POUh  POU-homeodomain

WGD   whole-genome duplication

PGCs  primordial germ cells

MHB   midbrain hindbrain boundary

MZspg maternally and zygotically knocked-out Pou5f3 (abbreviation for studies with zebrafish)

PVD2  ‘POUV-depleted 2’, designation of the morpholino antisense oligos combination for POUV-proteins knockdown in *Xenopus* [13]

ICM   inner cell mass

PrE   primitive endoderm

TE    trophoderm

ESCs  embryonic stem cells

EpiSCs epiblast stem cells

EpiLCs epiblast-like stem cells

LIF   leukaemia inhibitory factor

NE    neuroectoderm

ME    mesendoderm

CNCCs cranial neural crest cells