Regulation of Oct4 in stem cells and neural crest cells

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Abstract
During embryonic development, cells gradually restrict their developmental potential as they exit pluripotency and differentiate into various cell types. The POU transcription factor Oct4 (encoded by Pou5f1) lies at the center of the pluripotency machinery that regulates stemness and differentiation in stem cells, and is required for reprogramming of somatic cells into induced pluripotent stem cells (iPSCs). Several studies have revealed that Oct4 and other stemness genes are also expressed in multipotent cell populations such as neural crest cells (NCCs), and are required to expand the NCC developmental potential. Transcriptional regulation of Oct4 has been studied extensively in stem cells during early embryonic development and reprogramming, but not in NCCs. Here, we review how Oct4 is regulated in pluripotent stem cells, and address some of the gaps in knowledge about regulation of the pluripotency network in NCCs.

KEYWORDS
craniofacial defects, neural crest cells, Oct4, pluripotency, reprogramming, stem cells

1 | INTRODUCTION

Neural crest cells (NCCs) are a unique, ectoderm-derived, multipotent cell population that give rise to both ectodermal derivatives such as neurons and glia and ectomesenchymal derivatives such as chondrocytes and osteocytes (reviewed in Le Douarin, Creuzet, Couly, & Dupin 2004; Cebra-Thomas et al., 2013). Understanding the molecular mechanisms that govern NCC multipotency may unlock the etiologies of many developmental anomalies that arise during neural crest development, including craniofacial defects, cardiac defects, and pediatric cancers, such as neuroblastoma (T. S. Sato et al., 2019). We are just beginning to understand some of the key molecular factors that regulate mammalian NCC development. Several studies have suggested that NCCs express the pluripotency machinery to expand their developmental potential and give rise to ectomesenchymal derivatives such as chondrocytes and osteocytes (Scherbo & Monsoro-Burq, 2020; Zalc et al., 2021). Thus, the gene regulatory networks of pluripotent embryonic stem cells (ESCs) likely have essential roles in NCCs.

Pluripotent cells of the mammalian epiblast express the core transcription factors, Oct4, Sox2, and Nanog,
which define and maintain the pluripotent state (Osorno & Chambers, 2011). During differentiation, expression of Oct4 and other pluripotency genes is lost as lineage-specific gene programs are activated. Exit from pluripotency and lineage segregation is carefully coordinated by several signaling cues and transcriptional factors. Differentiation into different lineages was once considered a unidirectional process; pluripotent stem cells at the peak of Waddington’s landscape traveled downhill toward their ultimate cell fate, progressively restricting their developmental potential along the way (Waddington, 1957). This paradigm was challenged in 1962 when Sir John Gurdon generated a fully functional tadpole by transplanting the nucleus of a differentiated intestinal epithelium cell from an adult frog into an enucleated unfertilized egg (Gurdon, 1962). This landmark result suggested that a fully differentiated cell retains the genetic memory and potential to give a complete organism when placed in an appropriate environment. Thirty years later, somatic cell nuclear transfer technology (SCNT) was applied to mammals to clone Dolly the sheep (Wilmut, Schnieke, McWhir, Kind, & Campbell, 2007).

This work laid the groundwork for Yamanaka and Takahashi, who discovered that the introduction of four transcription factors, Oct4, Sox2, Klf-4, and c-Myc (OSKM), could convert a somatic cell into an induced pluripotent stem cell (iPSC; Takahashi & Yamanaka, 2006). Several groups have since identified different combinations of pluripotency factors sufficient to reprogram somatic cells (reviewed in Takahashi & Yamanaka, 2016). These studies fundamentally changed our concept of cellular plasticity by showing that differentiated cells could dedifferentiate in the presence of critical components of the pluripotency program. Later work showed that differentiated cells can also transdifferentiate—transform directly into another differentiated cell type without first passing through a pluripotent state (reviewed in Grath & Dai, 2019). Collectively, these groundbreaking findings created new avenues in regenerative biology and revealed insights into mechanisms of dedifferentiation that can occur in normal development and disease. Here, we review different mechanisms and factors that regulate the pluripotency network in stem cells and development, with a focus on Oct4.

2 | MAIN

2.1 | Regulation of the Oct4 pluripotency network in ESCs

Transcription factors regulate cell fate decisions by acting as molecular switches to activate or silence gene expression programs during development. A study in mouse embryonic stem cells (mESCs) showed that a two-fold increase in Oct4 expression pushed mESCs to differentiate into primitive endoderm and mesoderm, whereas loss of Oct4 expression caused differentiation into trophectoderm (Niwa, Miyazaki, & Smith, 2000), suggesting that the levels of pluripotency transcription factors must be tightly maintained for stem cell self-renewal. Reprogramming of somatic cells into iPSCs requires ectopic expression of pluripotent factors both in vivo and in vitro. Important to clinical translation, the delivery of pluripotency factors for in vivo reprogramming needs to be tightly regulated as it can lead to tumor formation (Simpson, Olova, & Chandra, 2021). Thus, it is not surprising that Oct4 levels are regulated by several mechanisms to maintain pluripotency in ESCs (Table 1).

2.1.1 | The DNA methylation status of Oct4 regulatory elements

Direct reprogramming to a pluripotent state requires genome-wide changes in chromatin composition, such as global DNA demethylation (Maherali et al., 2007; Mikkelsen et al., 2008). Integrative genomic analysis during reprogramming revealed that DNA hypermethylation of pluripotency-related genes resulted in inefficient reprogramming in partially reprogrammed cell lines (Mikkelsen et al., 2008). Indeed, inhibition of DNA methylation using 5-aza-cytidine in the partially reprogrammed cell lines resulted in a rapid and stable transition to a complete pluripotent state (Mikkelsen et al., 2008). Oct4 transcription is regulated by at least three well-studied sites; the proximal promoter, the proximal enhancer, and the distal enhancer (Niwa, 2007; Yeom et al., 1996). Consistent with an important role in maintaining pluripotency, the DNA of these regulatory elements was found unmethylated in ESCs and methylated in somatic cells (Hattori et al., 2004). During somatic cell reprogramming, the methylation status of the Oct4 locus directly correlated with reprogramming efficiency and selection of iPSCs (Mikkelsen et al., 2008).

DNA methyltransferases (Dnmt) are a family of enzymes that catalyze the transfer of methyl groups to DNA. Dnmt3a and Dnmt3b were sufficient and required to methylate the proximal enhancer and promoter elements of Oct4 (Figure 1) and Nanog during the differentiation of mESCs (J. Y. Li et al., 2007). Moreover, complete loss of Dnmt3a and Dnmt3b in mouse embryos resulted in abnormal expression of Oct4 and Nanog at E9.5 (both genes are silenced by E9.5 in wild-type embryos; J. Y. Li et al., 2007). In contrast to Dnmt enzymes, the ten-eleven translocation (Tet) proteins, TET1 and TET2, played an
| Stem genes | Regulators | Class | Mechanism | Model | Reference |
|------------|------------|-------|-----------|-------|-----------|
| Oct4 and Nanog | Dnmt3a and Dnmt3b | DNA methyltransferases | DNA methylation of proximal enhancer and promoter | mESCs/mouse embryos | J. Y. Li et al. (2007) |
| Oct4, Nanog | Tet1 | DNA demethylases | Facilitates DNA demethylation of Oct4 and prevents hypermethylation of Nanog promoter | mESCs | Gao et al. (2013); Ito et al. (2010) |
| Oct4 | G9a | Histone methyltransferase | Promotes methylation of H3K9 residues at the Oct4 promoter and recruits Dnmt3a for further DNA methylation | Mouse embryos/mouse neural stem cells | Feldman et al. (2006); Ma et al. (2008) |
| Oct4 | Jhdm2a | Histone demethylase | Global DNA demethylation resulted in Oct4 reactivation | Mouse embryos/mouse neural stem cells | Ma et al. (2008) |
| Nanog, Oct4, Tbx3, Esrrb, Bmp4, Tcl1, Klf4, and Klf5 | Paf1c-Set1 complex | Histone methyltransferase | Maintains active H3K4me3 at the promoters which results in further recruitment of histone acetyltransferases for active H3K27ac | mESCs | Ding et al. (2009) |
| Oct4 | SF-1/NR5A1 and LRH-1/NR5A2 | Orphan nuclear receptors | Maintains Oct4 expression possibly by recruiting activating chromatin remodelers and transcription factors | Human embryonal carcinoma NCCIT cells, embryonic carcinoma P19 cells, and mESCs | Gu, Goodwin, et al. (2005); Yang et al. (2007) |
| Oct4, Nanog and Sox2 | GCNF/NR6A1 | Orphan nuclear receptors | Negatively regulates Oct4 expression by recruiting Dnmt enzymes and promoting DNA methylation of proximal enhancer and promoter | mESCs and mouse embryo | Fuhrmann et al. (2001); Gu et al. (2006); Gu, Goodwin, et al. (2005); Gu, LeMenuet, et al. (2005); N. Sato et al. (2006); Yang et al. (2007) |
| Oct4 | COUP-TFII/ NR2F2 | Orphan nuclear receptors | Negatively regulates Oct4 expression by binding to the proximal promoter | mESCs and mouse embryo | Rosa and Brivanlou (2011) |
| Oct4, Sox2, and Nanog | Sox2-Oct4 complex | Pluripotency factors | Binds to cis-regulatory enhancer elements to activate transcription | mESCs, hESCs, mouse embryos | Chew et al. (2005); Kuroda et al. (2005); Okumura-Nakanishi et al. (2005); Tomioka et al. (2002) |
essential role in initiating DNA demethylation in mESCs (Gao et al., 2013; Ito et al., 2010; Koh et al., 2011; Tahiliani et al., 2009). Tet1 and Tet2 were highly expressed in mESCs consistent with a globally demethylated state in naïve pluripotency (Ito et al., 2010; Koh et al., 2011). Loss of Tet1 disrupted ES cell self-renewal and differentiation into different lineages consistent with the role of Oct4 in maintaining pluripotency and lineage specification (Ito et al., 2010; Koh et al., 2011; Takahashi & Yamanaka, 2006). Loss of Tet1 also resulted in decreased RNA and protein levels of Nanog, Oct4, and Sox2 in ESCs (Ito et al., 2010). Tet1 replaced Oct4 in OSKM-mediated reprogramming of mouse embryonic fibroblasts (MEFs), while MEFs treated only with the three SKM factors failed to reprogram (Gao et al., 2013). Indeed, the addition of Tet1 to OSKM-mediated reprogramming significantly increased Oct4 reactivation by enhancing demethylation of the enhancer and promoter regions (Gao et al., 2013). Together, these results suggest that the DNA methylation status of the Oct4 promoter must be carefully regulated by the opposing roles of Dnmt and Tet enzymes in ESCs and during reprogramming.

2.1.2 | Dynamic changes in histone methylation of the Oct4 regulatory elements

A comprehensive analysis of transcriptional and epigenetic changes during reprogramming revealed that DNA
methylation changes at promoters of genes occurred late during reprogramming (Polo et al., 2012). Pluripotency genes such as Oct4 and Nanog became demethylated at later stages as well (Polo et al., 2012). Chromatin analysis of active H3K4me3 (trimethylation of the 4th lysine residue of histone H3 protein) and repressive H3K27me3 (trimethylation of the 27th lysine residue of histone H3 protein) histone methylation marks revealed dynamic changes in histone methylation patterns at the promoters of genes consistent with changes in the transcriptome during early stages of reprogramming (Polo et al., 2012). Oct4 and Nanog, which were bivalently (H3K4me3 and H3K27me3) marked in MEFs, acquired H3K4me3 marks accompanied by a loss of H3K27me3 marks before DNA demethylation occurred at the promoter (Polo et al., 2012). These results suggest that changes in histone methylation precede DNA demethylation and are crucial in determining the transcriptional activation status of the Oct4 locus during reprogramming.

Upon exit from pluripotency, active H3K4me3 histone modifications were removed from the Oct4 promoter (Topalovic, Schwirtlich, Stevanovic, & Mojsin, 2017), followed by the addition of repressive histone marks, such as H3K9me3 (trimethylation of the 9th lysine residue of histone H3 protein) and H3K27me3 (Feldman et al., 2006; K. Wang, Chen, Chang, Knott, & Cibelli, 2009), and further stabilized by DNA methylation to repress Oct4 transcription (Mikkelsen et al., 2008). H3K9me3 remains a major barrier to cellular reprogramming. Chromatin marked by H3K9me3 remains inaccessible to most regulatory factors and transcriptional machinery, in contrast to other heterochromatin markers, such as H3K27me3, where DNA remains accessible to general transcription factors and a paused RNA polymerase (reviewed in Becker, Nicetto, & Zaret, 2016). Suppression of the CAF-1 complex (which maintains H3K9me3) in MEFs resulted in the activation of the endogenous Oct4 promoter independent of OSKM-induced reprogramming (Cheloufi et al., 2015). These results suggest that removal of H3K9me3 marks from the Oct4 promoter may be the first critical epigenetic change required during reprogramming, followed by removal of H3K27me3 and DNA demethylation.

Histone methyltransferase and demethylase enzymes are responsible for maintaining the histone methylation and demethylation status, respectively. The histone methyltransferase G9a induces methylation of H3K9 residues, resulting in heterochromatin formation and transcriptional repression (Tachibana, Sugimoto, Fukushima, & Shinkai, 2001; Tachibana et al., 2002). Knocking down G9a enhanced reprogramming of neural cells by speeding up Oct4 reactivation (Ma, Chiang, Ponnusamy, Ming, & Song, 2008). In contrast, the overexpression of histone demethylase Jhdm2a, which promotes transcriptional activation by catalyzing demethylation of H3K9 residues (Yamane et al., 2006), increased Oct4 reactivation during the reprogramming of neural cells (Ma et al., 2008). These results suggest that the coordinated activity of opposing histone-modifying enzymes regulates the reactivation of Oct4 expression during reprogramming (Figure 1). Furthermore, G9a was required for H3K9 methylation followed by DNA methylation of the Oct4 promoter (Feldman et al., 2006). In the absence of G9a, DNMT3A was not recruited to the Oct4 locus (Feldman et al., 2006). These results further support the idea that histone methylation changes precede DNA methylation of the Oct4 locus. It appears that the down-regulation of G9a during the early stages of reprogramming remains the key to Oct4 reactivation and efficient reprogramming (Feldman et al., 2006). While extensive studies have been done to determine how G9a regulates different physiological processes and its downstream targets, little is known about the factors that regulate G9a expression.

After removal of repressive histone and DNA methylation marks during reprogramming, the Oct4 proximal promoter must be modified with active histone marks to maintain Oct4 expression in iPSCs (Polo et al., 2012). A genome-wide screen led to the identification of factors within the Paf1 complex that had the strongest effect on Oct4 expression and played an important role in maintaining ESC identity (Ding et al., 2009). Paf1C was bound to promoters of many pluripotency genes, including Oct4, and served as a platform to attract other histone modifiers (Ding et al., 2009). Knocking down Paf1C decreased levels of H3K4me3 at pluripotency genes such as Oct4 with no changes at lineage-specific genes (Ding et al., 2009). Simultaneous knockdown of Paf1C with Set1 complex (maintains H3K4me3) resulted in the loss of Oct4 expression (Ding et al., 2009). These results suggest that Paf1C controls pluripotency by directly maintaining H3K4me3 levels at promoter regions of pluripotency genes, including Oct4, by synergistically working with the Set1 complex (Figure 1). H3K4me3 can also recruit downstream effectors, including chromatin remodelers which can lead to further gene activation and repression (reviewed in Berger, 2007). For instance, H3K4me3 recruits the histone acetyltransferase protein NuA3 (acetylates histone H3 protein) to increase chromatin accessibility (Martin, Grimes, Baetz, & Howe, 2006; Figure 1). Indeed, histone acetylation is generally associated with transcriptionally active genes (reviewed in Kuo & Allis, 1998). Genome-wide analysis of histone acetylation and methylation modifications revealed that the pluripotency factor genes such as POU5F1 (OCT4), SOX2, and NANOG showed hyperacetylation of H3K27
Regulation of Oct4 is a sequential process governed by several genetic, epigenetic, and noncoding factors. Oct4 locus was silenced in the somatic state by DNA and histone methylation (H3K9me3 and H3K27me3) marks maintained by epigenetic modifiers such as Dnmt3a (J. Y. Li et al., 2007), G9a (Feldman et al., 2006; Ma et al., 2008), and Ezh2 (P. G. Hawkins & Morris, 2010). These methylases were recruited to the Oct4 locus by orphan NRs such as GCNF (Fuhrmann et al., 2001; Gu et al., 2006; Gu, Goodwin, et al., 2005; Gu, LeMenuet, et al., 2005; N. Sato et al., 2006; Yang et al., 2007) and COUP-TFII (Rosa & Brivanlou, 2011) and lncRNAs (P. G. Hawkins & Morris, 2010). Activation of the Oct4 locus was regulated by demethylating enzymes such as Jhdm2a (Ma et al., 2008) and Tet proteins (Gao et al., 2013; Ito et al., 2010). Removal of inactive methylation marks increased accessibility of the Oct4 locus for other regulatory factors such as Paf1C, Set1 complex, and NuA3 which increased active methylation marks (H3K4me3 and H3K27ac) at the Oct4 regulatory elements (Ding et al., 2009). Addition of active methylation groups and recruitment of other transcriptional regulators such as Sall4 (Zhang et al., 2006), LRH-1, and SF-1 (Gu, Goodwin, et al., 2005; Yang et al., 2007) promoted active transcription of the Oct4 locus. Oct4 was posttranscriptionally regulated by miRNAs such as miR-145, resulting in the downregulation of Oct4 levels during differentiation (Xu et al., 2009).
residues (acetylation of the 27th lysine residue of histone H3 protein or H3K27ac) in hESCs which was lost upon differentiation and replaced by H3K27me3 (R. D. Hawkins et al., 2011). Collectively, these studies suggest that Paf1C and H3K4me3 can play a crucial role in determining the status of Oct4 transcription and maintaining the balance between stemness and differentiation in ESCs. Future studies investigating mechanisms regulating epigenetic modifiers in the pluripotent state may clarify the crosstalk controlling the dynamic chromatin accessibility at the Oct4 locus.

2.1.3 Coregulation of the Oct4 locus by nuclear receptors

The cis-regulatory elements near Oct4 contain binding sites for several orphan nuclear receptors (NRs), which maintain Oct4 expression in ESCs or inhibit expression during differentiation and embryogenesis (Fuhrmann et al., 2001; Gu, Goodwin, et al., 2005; Yang et al., 2007). For example, the orphan NR steroidogenic Factor 1 (SF-1/NR5A1), activated Oct4 transcription in pluripotent teratoma cells and was essential during late organogenesis (Yang et al., 2007). However, during early embryogenesis, another orphan NR, liver receptor homolog-1 (LRH-1/NR5A2), directly regulated Oct4 expression (Gu, Goodwin, et al., 2005). LRH-1 and Oct4 were co-expressed in the epiblast and ESCs (Gu, Goodwin, et al., 2005). Loss of LRH-1 resulted in the loss of Oct4 expression and early embryonic death (Gu, Goodwin, et al., 2005; Figure 1). Further investigation is required to determine the precise mechanisms by which SF-1 and LRH-1 positively regulate Oct4 expression in ESCs. In contrast, orphan NRs such as germ cell nuclear factor (GCNF/NR6A1), negatively regulated Oct4 during differentiation (Fuhrmann et al., 2001; Gu, LeMenuet, et al., 2005). Retinoic acid-induced differentiation of GCNF knockout ESCs resulted in the loss of repression of Oct4, Nanog, and Sox2 (Gu, LeMenuet, et al., 2005). GCNF binding silenced Oct4 expression by recruiting DNA methylating enzymes such as DNMT3A/B to the proximal enhancer and promoter during differentiation (Gu, Le Menuet, Chung, & Cooney, 2006; N. Sato, Kondo, & Arai, 2006; Figure 1). In ESCs, OCT4 repressed expression of the orphan NR, Coup-TFII/NR2F2, which repressed Oct4 transcription by binding to the proximal promoter during differentiation, forming a feedback loop (Mullen, Gu, & Cooney, 2007; Rosa & Brivanlou, 2011). Together, these studies highlight the critical role of orphan NRs in maintaining the pluripotent and differentiated states of ESCs by regulating Oct4 transcription.

2.1.4 Feedback regulation between signaling pathways and core pluripotency network

The transcription factors that comprise the core pluripotency network often form complexes with each other to coregulate transcription of their target genes as well as to autoregulate themselves and each other by feedback regulation. How these factors synergize to maintain the pluripotent state in ESCs remains understudied. Functional studies in mESCs have revealed that the SOX2-OCT4 complex occupies and coregulates Oct4, Sox2, and Nanog by forming an autoregulatory circuitry (Chew et al., 2005; Kuroda et al., 2005; Okumura-Nakanishi, Saito, Niwa, & Ishikawa, 2005; Tomioka et al., 2002). Overexpression of Nanog along with the histone demethylase Jhmd2a significantly improved endogenous Oct4 reactivation and the reprogramming efficiency of NSCs (Ma et al., 2008). Indeed, Oct4, Sox2, and Nanog comprised the group of pluripotency genes that were expressed later during the second wave of transcriptional changes and conferred a stable pluripotency state during reprogramming (Polo et al., 2012). This category of genes included several other DNA-binding factors, such as Sall4 (Polo et al., 2012). In an effort to identify a combination of transcription factors for somatic cell reprogramming, devoid of OSKM, a study identified a seven-factor (7F) system that could reprogram somatic cells into iPSCs with similar high quality and efficiency (B. Wang et al., 2019). This 7F system included the pluripotency gene Sall4, which, remarkably, was required for both opening and closing of chromatin during reprogramming (B. Wang et al., 2019). Functional assays indicated that SALL4 activated Oct4 expression by binding to its distal enhancer element in ES cells (Zhang et al., 2006; Figure 1). These results suggest that the interconnected autoregulatory loop formed by pluripotency factors plays an important role in the maintenance of pluripotency and efficient somatic cell reprogramming.

The core pluripotency network is tightly regulated by extrinsic signaling pathways governing different pluripotency states. The pluripotent state of naïve mESCs is regulated by LIF, BMP, Wnt, and FGF signaling pathways, while that of primed EpiSCs is governed by TGFβ and FGF signaling pathways (reviewed in Mossahebi-Mohammadi, Quan, Zhang, & Li, 2020). These signaling pathways bring developmental cues directly to the core pluripotency circuitry to maintain the balance between stemness and differentiation in ESCs and during early mouse development (reviewed in Mossahebi-Mohammadi et al., 2020). For instance, T-cell factor-3 (Tcf3), a terminal component of the Wnt signaling pathway, maintained the balance between stemness and
differentiation in mESCs (Cole, Johnstone, Newman, Kagey, & Young, 2008). Tcf3 knockout mESCs showed an increase in the expression of the core pluripotency genes—Oct4, Nanog, and Sox2 (Cole et al., 2008), suggesting an important role of Tcf3 in coordinating exit from pluripotency. TCF3 was shown to occupy its promoter and that of the core pluripotency genes to form an autoregulatory feedback network (Cole et al., 2008). These studies highlight the fact that the pluripotency network is tightly regulated by a complex network involving multiple different signaling cues and transcription factors.

2.1.5 | Noncoding RNAs in Oct4 regulation

A significant portion of transcripts are not translated into proteins and are referred to as noncoding RNAs. Advances in RNA-sequencing techniques and functional assays have expanded our knowledge about different classes of noncoding RNAs and their critical function in development and disease. However, relatively little is known about noncoding RNAs that can activate Oct4 transcription. MicroRNAs (miRNAs) post-translationally silence gene expression by targeting mRNA transcripts, and they can also activate transcription by binding to enhancers and promoters (reviewed in Catalanotto, Cogoni, & Zardo, 2016). Screening for double-stranded RNAs (dsRNAs) that can bind to the Oct4 promoter region led to the identification of a synthetic small activating RNA (saRNA), dsOCT4-622, that activated and increased Oct4 transcription in human adipose-derived stem cells (J. Wang et al., 2015). While this study holds promise in developing small RNA-mediated reprogramming of somatic cells, it remains unknown if there are any endogenous small RNAs such as miRNAs that can activate Oct4 transcription during development and reprogramming. miRNAs can positively regulate Oct4 expression indirectly by targeting other genes as well. For instance, miR-302 promoted the self-renewal of human embryonic stem cells (hESCs) by targeting cell-cycle regulator AKT1 and maintaining OCT4 expression (H. L. Li et al., 2016). Pluripotency genes are also targets of miRNA-mediated silencing during differentiation. Luciferase reporter assays showed that OCT4, SOX2, and KLF4 were directly targeted by miRNA mir-145 in hESCs (Xu, Papagiannakopoulos, Pan, Thomson, & Kosik, 2009; Figure 1). In contrast, a long intergenic non-protein coding RNA (lincRNA), linc-ROR, and Oct4-pseudogene 5 acted as miRNA sponges and prevented core pluripotency transcription factors such as OCT4, NANOG, and SOX2 from mir-145-mediated suppression in hESCs (Bai et al., 2015; Y. Wang et al., 2013). Altogether, these results suggest a broader role for noncoding RNAs in regulating the expression of core pluripotency network genes.

Long noncoding RNAs (lncRNAs) are another class of noncoding RNAs that can modulate transcription programs by recruiting epigenetic modifiers at gene loci, serving as scaffolds for protein complex assembly and function, regulating miRNA activity, and affecting transcript stability and maturation (reviewed in Statello, Guo, Chen, & Huarte, 2021). Genome-wide transcriptome analysis in combination with chromatin occupancy analysis of pluripotency transcription factors in mESCs identified lncRNAs that were directly targeted by Oct4 and Nanog (Sheik Mohamed, Gaughwin, Lim, Robson, & Lipovich, 2010). In turn, these IncRNAs modulated the expression of Oct4 and Nanog in a regulatory feedback loop (Sheik Mohamed et al., 2010). How these IncRNAs regulate pluripotency circuitry remains elusive. A lncRNA antisense to Oct4-pseudogene 5, as-Oct4-pg5, suppressed transcription of Oct4 in MCF-7 breast cancer cell line (P. G. Hawkins & Morris, 2010). The lncRNA as-Oct4-pg5 directed histone methyltransferase Ezh2 to the Oct4 promoter resulting in increased H3K27me3 levels and heterochromatin formation at the Oct4 locus (P. G. Hawkins & Morris, 2010). Further investigation is required to determine how as-Oct4-pg5 recruits Ezh2 to the Oct4 promoter, perhaps by RNA–protein interactions. In general, the role of IncRNAs in the context of reprogramming and Oct4 regulation requires further investigation.

2.2 | Neural crest cells challenge the accepted paradigm of cellular potential during development

Following specification at the neural plate border (NPB), NCCs undergo epithelial-to-mesenchymal transition (EMT), delaminate, and migrate throughout the embryo, giving rise to cells that contribute to several tissues in the body, such as the craniofacial skeleton, heart, enteric nervous system, melanocytes, and smooth muscle (Couly, Grapin-Botton, Colley, Ruhn, & Le Douarin, 1998; Crea-izzo, Godt, Leatherbury, Conway, & Kirby, 1998; Le Douarin, 1982; Le Douarin & Smith, 1988; Le Douarin & Teillet, 1973; Le Lièvre & Le Douarin, 1975; Le Lièvre, Schweizer, Ziller, & Le Douarin, 1980). Based on the origin along the anterior/posterior body axis, NCCs are divided into four subpopulations: cranial, vagal, trunk, and sacral NCCs. Cranial and trunk NCCs are the only subpopulations of the neural crest known so far that can give rise to ectomesenchymal cells, such as chondrocytes
and osteocytes (reviewed in Cebra-Thomas et al., 2013; Le Douarin et al., 2004).

NCCs are unique to vertebrates; however, the transcriptional circuitry that regulates NCC development is not constrained to vertebrates and is found in invertebrates as well. So, how did vertebrates evolve to expand the developmental potential of NCCs in order to form a highly advanced “head,” endowing them with a greater advantage in the ecosystem compared to invertebrates? A recent study applied phylogenetic analysis and functional studies to reveal that evolution of the VENTX/NANOG gene family endowed NCCs with multipotency in vertebrates (Scerbo & Monsoro-Burq, 2020).

Prior studies had shown that pluripotency factors such as pouV (Oct4), ventx (Nanog), and sox2 were expressed in the neural plate border region during development in Xenopus (frog) embryos (Morrison & Brickman, 2006; Rogers, Archer, Cunningham, Grammer, & Casey, 2008; Scerbo et al., 2012). Knocking down these pluripotency factors resulted in anterior neural defects and posterior truncations consistent with the contribution of NCCs to these tissues (Morrison & Brickman, 2006; Rogers et al., 2008; Scerbo et al., 2012). Further studies showed that pluripotency factors such as Oct4, Sox2, and Nanog were expressed in mouse NCCs and alluded to the role of pluripotency genes in the development and differentiation of NCCs (Hagiwara et al., 2014; Kikuchi et al., 2011). These studies used P0-Cre (Schwann cell-specific protein)/Floxed-EGFP mice to isolate EGFP+ cells from the iris stromal region (Kikuchi et al., 2011) or the craniofacial, and trunk region (Hagiwara et al., 2014) to perform sphere formation assay in order to assess their stem cell-like properties. Immunofluorescence staining (detects and visualizes protein expression) revealed expression of the pluripotency factors such as OCT4, NANOG, SOX2, and NESTIN in the spheres (Hagiwara et al., 2014; Kikuchi et al., 2011). Later, a study in mouse trunk NCCs showed that OCT4, NANOG, and SOX2 co-occupied regulatory elements of genes involved in the formation of trunk NCCs such as Foxd3, Sox9, and Sox10 in a Wnt signaling-dependent manner (Fujita, Ogawa, & Ito, 2016). Knockdown of these pluripotency factors decreased expression levels of Foxd3 and Sox10 in trunk NCCs, suggesting a role for the pluripotency network in regulating NCC development (Fujita et al., 2016). Collectively, these studies highlighted the role of pluripotency factors in regulating NCC genes and maintenance of NCCs. However, it was still unclear whether the pluripotency factors were expressed in NCCs in vivo and during what stage of embryonic development.

The NCC gene regulatory network comprises several transcription factors, and it is well accepted that NCCs represent a highly heterogeneous cell population that expresses a combination of different transcription factors (Simões-Costa & Bronner, 2015). Techniques such as in situ hybridization and immunolabeling have allowed investigators to look at transcript and protein expression of pluripotency and NCC genes in intact embryonic tissue, but with relatively limited quantitative resolution. To visualize the transcriptional heterogeneity in NCCs, a recent study coupled multiplex single-molecule fluorescence in situ hybridization with machine learning to examine the expression of NCC and pluripotency genes at a single-cell resolution in vivo in chicken embryos (Lignell, Kerosuo, Streichan, Cai, & Bronner, 2017). The study revealed that not all cells with a neural crest gene signature expressed pluripotency genes (Lignell et al., 2017). Only one of the cell clusters in the dorsal neural tube with a premigratory NCC gene signature expressed pluripotency genes (Lignell et al., 2017). Interestingly, lateral to these cells there was another population of cells that expressed pluripotency genes but had a neural gene signature (Lignell et al., 2017). These results revealed that not all the cells in the dorsal neural folds that give rise to NCCs may express the pluripotency genes. The question remained whether the expression of pluripotency factors was required for the formation of both ectodermal and ectomesenchymal NCC derivatives?

The role of pluripotency factors in regulating NCC proliferation was further strengthened by a study in mouse cranial NCCs in association with folate metabolism (Mohanty et al., 2016). Folate deficiency results in neural tube defects (NTDs), such as spina bifida and anencephaly; hence, folate supplementation is highly recommended to women of reproductive age (Centers for Disease Control and Prevention, n.d.). One of the key questions in the field is to understand how folate metabolism regulates the development of neural tube and prevents NTDs. Functional studies showed that supplementation of folate rescued the proliferation and differentiation defect of NCCs in a mouse model of embryonic NTDs (Ichinose et al., 2010, 2012; Nakazaki et al., 2008). FRα is a receptor for folic acid which upon binding of folic acid, translocates to the nucleus and acts as a transcription factor to regulate downstream genes (Mohanty et al., 2016). Treatment of a cranial NCC (cNCC) line, O9-1, with folic acid, increased expression of pluripotency factors, including Oct4, Sox2, Klf4, and Trim71 (Mohanty et al., 2016). Chromatin-binding assays revealed that the FRα bound to regulatory elements of these pluripotency genes following FA treatment and promoted active transcription. This was further confirmed by enrichment of active H3K27ac modifications and p300 occupancy at enhancer elements of these pluripotency genes (Mohanty et al., 2016). FRα also downregulated miRNAs such as miR-138 and let-7 that targeted Oct4 and Trim71, respectively (Mohanty et al., 2016).
et al., 2016). In turn, knocking down Oct4 and Trim71 prevented folate mediated rescue of NCC proliferation defect in the NTD mouse model (Mohanty et al., 2016), which suggested that expression of pluripotency factors is required to maintain proliferation of NCCs during development.

Recent studies in Xenopus and mouse provided definitive evidence supporting the role of the pluripotency factors Oct4 and Nanog in NCC specification andectomesenchyme development. Knocking down ventx2 (Nanog) in Xenopus embryos disrupted early NCC specification and differentiation into ectomesenchyme without affecting NCC migration or differentiation into melanocyte and sensory lineages (Scerbo & Monsoro-Burq, 2020). The ventx2 mutants displayed reduced craniofacial skeleton (Scerbo & Monsoro-Burq, 2020). The study further showed that ventx2 could promote the expression of other pluripotency genes in early NCCs in cooperation with NCC/NB-specific transcription factors (Scerbo & Monsoro-Burq, 2020). Corroborating these results, ablation of Oct4 function in cNCCs in mouse embryos caused craniofacial defects such as loss of frontonasal mass and absence of nasal processes (Zalc et al., 2021). Similar to the study in Xenopus (Scerbo & Monsoro-Burq, 2020), the ectodermal derivatives of cNCCs, such as neurons and glia, remained unaffected by loss of Oct4 expression (Zalc et al., 2021). Together, these results demonstrated the role of the pluripotency network in endowing NCCs with ectomesenchymal potential across species.

Traditional views of embryonic development follow a unidirectional trajectory down the Waddington landscape. Cellular potential becomes restricted as a totipotent zygote develops into a pluripotent mouse epiblast (or blastula in Xenopus), and finally into the lineage-specified (ectoderm, mesoderm, and endoderm) gastrula, defined by gradual loss of expression of the pluripotency factors. However, NCCs (arising from NPB in the ectoderm) seem to contradict this notion as they express pluripotency factors that endow them with broader developmental potential beyond their origin to give rise to the ectomesenchyme. A recent model suggests that the precursors of NCCs in the ectoderm reactivate the pluripotency network to reprogram and roll back up the Waddington landscape to give rise to ectomesenchyme. The reactivation model is an alternative, yet complementary update of an earlier model which suggested retention of pluripotency features in NCCs from the earlier embryonic stages that would promote multipotency.

The retention model is supported by studies that identified several NCC regulatory factors that played an important role in maintaining pluripotency in ESCs and NCC development, such as Myc, Id3, Sox5, Tf-AP2, Ets1, FoxD3, and Snail1 in Xenopus (Bellmeyer, Krase, Lindgren, & LaBonne, 2003; Buitrago-Delgado, Nordin, Rao, Geary, & LaBonne, 2015; Cartwright et al., 2005; Light, Vernon, Lasorella, Iavarone, & LaBonne, 2005; Nordin & LaBonne, 2014; Ying, Nichols, Chambers, & Smith, 2003). In situ hybridization revealed that these genes were broadly expressed in the pluripotent blastula stage and were gradually restricted to the NPB (Bellmeyer et al., 2003; Buitrago-Delgado et al., 2015; Cartwright et al., 2005; Light et al., 2005; Nordin & LaBonne, 2014; Ying et al., 2003). Functional studies revealed that these factors maintained expression of the core pluripotency factors from the blastula (Buitrago-Delgado et al., 2015). When NCC transcription factors were over-expressed in early blastula explants, pluripotency was retained, as revealed by the ability of the cells to form mesoderm and interestingly, to endoderm as well. In contrast, overexpression in late explants had limited developmental potential (Buitrago-Delgado et al., 2015). Collectively, these results showed that the NCC lineage is specified before gastrulation and has greater plasticity beyond the ectoderm and the ectomesenchyme than previously appreciated.

Further supporting evidence for the retention model came from studies in various other model systems such as avian (chick; Basch, Bronner-Fraser, & Garcia-Castro, 2006; Patthey, Edlund, & Gunhaga, 2009; Patthey, Gunhaga, & Edlund, 2008; Prasad, Uribe-Querol et al., 2020), rabbit (Betters, Charney, & Garcia-Castro, 2018), and a human model based on differentiation of hESCs into NCCs (Gomez et al., 2019; Leung et al., 2016; Prasad, Charney, Patel, & Garcia-Castro, 2020). Similar studies using explants showed that an intermediate region in the chick epiblast when dissected out and grown in neutral conditions displayed NCC fate specification, assessed by expression of NCC regulatory markers and a migratory phenotype (Basch et al., 2006; Prasad, Uribe-Querol et al., 2020). These explants did not express neuroectodermal or mesodermal markers suggesting a direct origin from the pluripotent state (Prasad, Uribe-Querol et al., 2020). A similar finding was observed in an in vitro neural crest differentiation model using hESCs (Gomez et al., 2019; Leung et al., 2016; Prasad, Charney, et al., 2020). Transcriptome analysis during differentiation revealed that within 6 hours of Wnt activation, there was a significant upregulation of NCC genes such as PAX3, PAX7, MYB, ZIC3, and GBX2 (Gomez et al., 2019; Leung et al., 2016; Prasad, Charney, et al., 2020). Expression of many pluripotent genes was seen until 24 hr with very low expression of ectodermal genes, suggesting direct specification of NCC lineage from a pluripotent state (Gomez et al., 2019; Leung et al., 2016; Prasad, Charney, et al., 2020). While these studies provide evidence for NCC specification
before gastrulation, whether cells expressing NCC markers in the epiblast (or blastula) can give rise to terminally differentiated ectomesenchyme derivatives such as chondrocytes and osteocytes remains unclear.

The development of single-cell transcriptomics has allowed us to follow differentiation across various cell lineages of the embryo over time which has helped us better understand cellular heterogeneity in a more unbiased manner. Using single cell gene expression analysis, researchers profiled whole *Xenopus* embryos from the zygote stage to the tailbud stage and revealed that an intermediate stage of *Xenopus* development (between the blastula and the neural crest) had a neuroectoderm gene signature rather than a pluripotent-NCC signature (Briggs et al., 2018). These results suggested limited retention of the pluripotency factors in cNCCs from the blastula stage (Briggs et al., 2018). Moreover, the low expression level of pluripotency genes postgastrulation was not limited to the ectoderm but was also present in mesoderm and endoderm, which would have been difficult to detect from in situ hybridization (Briggs et al., 2018). The low levels of transcripts detected for the pluripotency genes could be transcripts from earlier stages that may not have undergone degradation yet.

To address these contradictory findings, a recent study investigated whether the pluripotency program is reactivated or maintained from the pluripotent epiblast stage in cNCC precursors using a transgenic fluorescent reporter mouse and single-cell RNA-sequencing (Zalc et al., 2021). The authors found that precursors of cNCCs reactivated canonical pluripotency factors such as *Oct4*, *Nanog*, *Sox2*, and *Klf4*, with *Oct4* being one of the most enriched factors based upon transcriptomic data (Zalc et al., 2021). Using an *Oct4-GFP* reporter mouse, the authors observed that *Oct4* was highly expressed in the pluripotent epiblast stage of development (embryonic day E7.5) before downregulation, as the embryo became specified into different germ layers during gastrulation (E7.5–E7.75; Zalc et al., 2021). *Oct4* was then reactivated in the cNCC precursor cells (late E7.75, when the first two somites are formed), which gave rise to cNCCs that generated ectomesenchymal derivatives (Zalc et al., 2021). This finding was consistent with a study that examined the expression of various *Oct4* homologs in *Xenopus* embryos during development (Morrison & Brickman, 2006). That study showed by in situ hybridization that *Xipou19* and *Xipou25* (*Oct4*) were de novo activated in the developing anterior neural tissue and posterior neural tube, as no transcripts were detected at intermediate stages (Morrison & Brickman, 2006). Loss of *Oct4* function in cNCCs caused craniofacial defects due to a decrease in proliferation and an increase in apoptosis of migratory cNCCs (Zalc et al., 2021). In contrast, cNCC-derived neurons and glia remained unaffected in *Oct4* mutants (Zalc et al., 2021).

Developmental cell fate decisions are accompanied by dynamic changes in chromatin. Pluripotency is associated with an open chromatin structure, which gradually acquires repressive histone and DNA marks, resulting in compacted chromatin and gene silencing in differentiated cells. During reprogramming, the unwinding of tight chromatin by exogenous factors promotes an epigenetic state that supports activation of the endogenous pluripotency program (reviewed in Apostolou & Hochedlinger, 2013). Comparison of accessible chromatin regions between *Oct4*+ cNCC precursors, mESCs, epiblast-like cells (EpiLcs), and epiblast stem cells (EpiSCs) revealed that the *Oct4*+ cNCC precursors resembled EpiSCs (Zalc et al., 2021). Open chromatin regions specific to cNCC precursors were associated with genes involved in NCC development and differentiation, glial cell differentiation, and cranial skeletal system development (Zalc et al., 2021). Interestingly, these genes were expressed at a lower level in *Oct4*+ cNCC precursors but increased later in delaminating and migratory NCCs (Zalc et al., 2021). Collectively, these observations suggest that an *Oct4*-centered pluripotency program is reactivated in mouse cNCC precursors to achieve two goals: (a) to open the locked chromatin state and expand the differentiation potential of cNCCs beyond the ectoderm; (b) to prime the chromatin landscape of cNCCs for future activation of migratory and differentiation programs (Zalc et al., 2021). However, one caveat of this study is the use of an *Oct4-GFP* reporter mouse which is an indirect measurement of *Oct4* protein expression. Thus, the level of *Oct4* protein present after *Oct4* transcriptional reactivation remains unknown. This is important because pluripotency factors associate as a complex to regulate the expression of their downstream target genes (Chew et al., 2005; Kuroda et al., 2005; Okumura-Nakanishi et al., 2005; Tomioka et al., 2002). Since the *Oct4-GFP* reporter mouse follows *Oct4* expression indirectly through the expression of GFP, it remains unclear whether *Oct4* is associated with other pluripotency cofactors to regulate transcription in cNCCs.

Another recent study in *Xenopus* revealed that *ventx2* (Nanog) expression in NCCs is essential for the formation of ectomesenchyme (Scherbo & Monsoro-Burq, 2020). Consistent with the study in mice (Zalc et al., 2021), functional studies revealed that the gain of *ventx2* activity in late but not early gastrula stage embryos promoted NCC identity by enhancing expression of the NCC regulatory and pluripotency network genes in the NPB region (Scherbo & Monsoro-Burq, 2020). Together, these studies support the idea that NCCs have the potential to reprogram into a pluripotent state during development, and...
furthermore, reactivation of the pluripotency circuit is required to form the ectomesenchyme. It is worth noting that collecting early embryos with accurate staging (E7.5 vs. E7.75 vs. E8.0 in mouse/early vs. late blastula in Xenopus) can be challenging, thus, contributing to variability in some of the results presented in these studies. Whether the pluripotency network is retained from an earlier stage or reactivated in NCCs remains controversial due to some of the technical challenges and species-specific differences (Table 2), which warrants further investigation (Figure 2). Nevertheless, it is now well established, across various species and using different investigative tools, that expression of the pluripotency factors in NCCs is essential for the formation of ectomesenchyme in vertebrates (Table 2). Oct4 has been shown to have a regulatory role in not just maintaining the pluripotent state but in orchestrating fate choice and tissue organization during development as well. A study showed that conditional deletion of Oct4 in the epiblast cells resulted in the disruption of embryonic axis patterning and failure to differentiate into the germ layers (Mulas et al., 2018). Further investigation is required to determine the mechanisms that regulate the Oct4 pluripotency program in NCCs. Understanding how the pluripotency network regulates the expansion of differentiation potential of NCCs is likely to inform our understanding of common craniofacial birth defects. Finally, in the following section, we highlight research that would

| Pluripotency genes | Model | Stage of development when expression of pluripotency genes was reported | Reference |
|--------------------|-------|------------------------------------------------------------------------|-----------|
| Oct4, Nanog, and Klf4 | Chick | HH9 stage (seven somites), cranial dorsal neural tube region | Lignell et al. (2017) |
| Oct4, Nanog, and Sox2 | Human ESC differentiated into NCCs | First 24 hr of differentiation | Prasad, Charney, et al. (2020) |
| Oct4, Nanog, and Sox2 | Mouse NCCs derived using Schwann cells- specific Cre (iris stromal region, craniofacial, and trunk region) | >E9.5 (in vitro sphere formation assay) | Hagiwara et al. (2014); Kikuchi et al. (2011) |
| Oct4, Nanog, and Sox2 | Mouse trunk NCCs | E9.5 (NCCs migrated out from neural tube explants) | Fujita et al. (2016) |
| Oct4, Sox2, Klf4, and Trim71 | Mouse cranial NCCs (neural tube explants and O9-1 cell line) | NCCs migrated from E10.5 neural tube explants and O9-1 cells cNCC line | Mohanty et al. (2016) |
| Oct4, Sox2, Nanog, Klf4 | Mouse Epiblast, NPB | | Zalc et al. (2021) |
| PouV (Oct4), Ventx (Nanog), and Sox2 | Xenopus NPB (postgastrulation) | | Morrison and Brickman (2006); Rogers et al. (2008); Scerbo et al. (2012) |
| PouV (Oct4), Ventx (Nanog), Sox2, Myc, Id3, TAP2, Ets1, FoxD3, and Snail1 | Xenopus Blastula stage and NPB (postgastrulation) | | Bellmeyer et al. (2003); Buitrago-Delgado et al. (2015); Cartwright et al. (2005); Light et al. (2005); Nordin and LaBonne (2014); Ying et al. (2003) |
| PouV (Oct4), Ventx (Nanog), Myc, Id3, TAP2, Ets1, FoxD3, and Snail1 | Xenopus Blastula and NPB | | Briggs et al. (2018) |
| Oct4 | Xenopus Anterior neural tissue and posterior neural tube | | Morrison and Brickman (2006) |
| Ventx2 (Nanog) | Xenopus Late gastrula stage | | Scerbo and Monsoro-Burq (2020) |
NCCs can follow alternate paths to express the Oct4-centered pluripotency program and expand their developmental potential (Adapted from Zalc et al., 2021). Waddington landscape depicting alternate paths that ectoderm cells may use to expand their developmental potential. Heterogeneity in Oct4 expression may lead to a fate bias of NCCs into ectodermal or ectomesenchymal lineages.

clarify the extent to which pluripotency regulatory mechanisms in stem cells can apply to NCC development.

### 2.3 Common mechanisms regulate the pluripotency network in NCCs and ESCs

NCCs are specified in the ectoderm through concerted activities of different signaling pathways such as Wnt, BMP, Notch, and FGF signaling (reviewed in Simões-Costa & Bronner, 2015). These pathways also play an important role in governing naïve ESC versus primed EpiSC pluripotency states during early embryonic development as mentioned above (reviewed in Mossahebi-Mohammadi et al., 2020). However, the role of extrinsic signaling pathways in activating the NCC specification program and in regulating the pluripotency network in NCC precursors is incompletely understood. Assessment of chromatin accessibility in mouse embryos revealed that the chromatin landscape of Oct4+ cNCC precursors resembled that of EpiSCs (Zalc et al., 2021). The pluripotent state of mouse EpiSCs was shown to be governed by TGFβ and FGF signaling pathways (reviewed in Mossahebi-Mohammadi et al., 2020); whether these signaling pathways regulate Oct4 transcription in NCC precursors needs further investigation. These pathways may bring signaling cues to the pluripotency network in NCCs to activate downstream NCC specification genes. Oct4 expression is regulated by two enhancers: a distal enhancer and a proximal enhancer, which were differentially active in the inner cell mass and epiblast stages, respectively (Yeom et al., 1996). The question that then naturally arises is if the proximal enhancer has a dominant role in regulating Oct4 expression in NCC precursors similar to EpiSCs.

Orphan NRs which bind to gene regulatory elements and recruit other factors to regulate gene transcription is relatively less well understood in NCCs. As mentioned previously, GCNF suppressed Oct4 expression in pluripotent stem cells (Fuhrmann et al., 2001). Consistent with the gradual shutdown of pluripotency and onset of differentiation, GCNF was found to be expressed in all three germ layers of the late mouse epiblast stage (E7.5–E7.75; Chung et al., 2001; Dennis, 2008). Strong GCNF expression was observed in the dorsal neural folds, throughout the anterior neuroepithelium, at E8.5 (Chung et al., 2001; Dennis, 2008), consistent with dynamic changes in Oct4 expression (Zalc et al., 2021). Several studies have analyzed GCNF expression during early mouse embryonic development (E7.5–E10.5); however, to date, no study has carefully looked at its expression in the anterior neural folds between the E7.75 and E8.0 stage, when the first two somites are formed. It is this late gastrula stage when Oct4 and ventx2 (Nanog) were shown to be reactivated in the NPB region in mouse and Xenopus embryos, respectively (Scherbo & Monsoro-Burq, 2020; Zalc et al., 2021). Additional studies are required to determine if GCNF levels are downregulated in the anterior neuroepithelium at the late gastrula stage to allow reactivation of the pluripotency network in NCC precursors.

Some groups have reported a positive role of GCNF in regulating differentiation of NCC progenitors (Dennis, 2008), which can be correlated with upregulation of GCNF expression in the anterior neural folds at E8.5 (Chung et al., 2001; Dennis, 2008). Loss of GCNF expression resulted in NCC-related defects (Dennis, 2008). GCNF mutants exhibited an expanded neural plate due to an increase in proliferation of neural progenitors and failure of NCCs to migrate from the neuroepithelium, suggesting that GCNF is required for the transition from precursors to migratory cNCCs (Dennis, 2008). These results are consistent with the gain of ventx2 activity in late gastrula stage Xenopus embryos, which resulted in a lateral expansion of the NCC domain (Scherbo & Monsoro-Burq, 2020). Putative binding sites for GCNF were identified in the promoters of genes responsible for EMT in NCCs (Dennis, 2008). Altogether, these studies suggest that GCNF might have a role in coordinating exit from pluripotency and activation of pluripotent-NCC specification programs in NCC precursors. In contrast, LRH-1 positively regulated Oct4 expression and was highly expressed in the pluripotent epiblast (Gu, Goodwin, et al., 2005); however, its expression pattern...
during early somitogenesis in the anterior neural folds remains unknown. Further investigation is required to determine how orphan NRs such as GCNF and LRH-1 regulate the pluripotency network to initiate NCC specification programs.

The distal Oct4 enhancer which is active in the naïve pluripotent state may also have a role in Oct4 regulation in NCC precursors. Sall4, a pluripotency transcription factor, is highly expressed in ES cells and activates Oct4 by binding to its distal enhancer element (Zhang et al., 2006). Neuromesodermal progenitors (NMPs) give rise to neural and paraxial mesodermal progenitors in the trunk and tail during embryonic development (Tahara et al., 2019). Loss of Sall4 in NMPs led to accelerated differentiation of NMPs toward the neuronal lineage at the expense of presomitic mesoderm (Y. Wang et al., 2013). Sall4 mutants displayed developmental defects such as a truncated tail and disorganized vertebrae (Tahara et al., 2019). Some of the trunk NCCs which are biased toward neuronal fate were shown to arise in zebrafish from posterior NMP-neural cells in the tailbud (Lukoseviciute, Mayes, & Sauka-Spengler, 2021). It will be interesting to see if NMPs that give rise to trunk NCCs express different levels of Sall4 compared to the rest of the NMP population. Strong Sall4 expression was seen in craniofacial structures such as the frontal nasal structure, lower jaw, and first branchial arch between E8.5 and E10.5 (Kohlhase et al., 2002; Tahara, Kawakami, Zhang, Zarkower, & Kawakami, 2018). However, the role of Sall4 in the development of these structures remains poorly understood. Thus, Sall4 is a candidate factor to regulate Oct4 in NCCs and merits further investigation. However, it is to be noted that Sall4 expression in NMPs, and craniofacial structures may just be a progenitor marker and have nothing to do with the regulation of the pluripotency network.

Along with changes in gene expression and epigenetic profile, expression levels of miRNAs also change during reprogramming (Polo et al., 2012). OSK-induced reprogramming of MEFs resulted in stochastic activation of two pluripotent miRNA clusters, miR-302 and miR-290 (Parchem et al., 2014). In contrast, OSK + Sall4-mediated reprogramming induced a uniform sequential activation of miR-302 followed by miR-290 during late stages of somatic cell reprogramming and a 10-fold increase in reprogramming efficiency (Parchem et al., 2014). These results suggest that somatic cells can follow alternative paths during reprogramming, governed by the expression of transcription factors and miRNAs. This paradigm may also extend to the regulation of the pluripotency program in NCCs. The miR-302/367 cluster of miRNAs can reprogram somatic cells into iPSCs without any additional expression of transcription factors (Anokye-Danso et al., 2011). Oct4 and miR-302 are co-expressed in the epiblast during early embryonic development and in ESCs (Card et al., 2008). MiR-302 was broadly expressed in the embryo at the epiblast stage until E9.5 (Parchem et al., 2015, 2014) and remained expressed in cNCCs during specification, delamination, and migration (Parchem et al., 2015). Since miR-302 positively regulated Oct4 expression in hESCs (H. L. Li et al., 2016), miR-302 may have a role in Oct4 regulation in cNCC precursors as well. Moreover, OCT4, SOX2, and NANOG were shown to transcriptionally regulate miR-302 expression by binding to the promoter region of the miR-302 cluster in hESCs (Card et al., 2008).

Lin28a is another pluripotency factor that was strongly expressed in the cranial neural folds, premigratory and early migratory NCCs in the chick embryo (Simoes-Costa & Bronner, 2016; Simões-Costa, Tan-Cabugao, Antoshechkin, Sauka-Spengler, & Bronner, 2014). Lin28a was known to be part of the pluripotency network that maintains stemness and reprograms somatic cells into iPSCs (Yu et al., 2007; Zhang et al., 2016). Lin28a was shown to be involved in maintaining NC stem cell identity and promoting NCC multipotency in chick embryos (Bhattacharya, Rothstein, Azambuja, & Simoes-Costa, 2018). Expression of Lin28a promoted differentiation of NCCs into multiples cell types, such as neurons, glia, melanocytes, chondroblasts, and smooth muscle cells (Bhattacharya et al., 2018), suggesting a role for Lin28a in maintaining stem cell properties of NCCs. Lin28a has been shown to regulate genes posttranscriptionally by inhibiting the let-7 family of miRNAs (Newman, Thomson, & Hammond, 2008). Functional studies showed that lin28a regulated NCC development in a let-7 dependent manner (Tahara et al., 2019). Disruption of the lin28a-let-7 axis by loss of Lin28a and gain of let-7 resulted in downregulation of NCC and stem cell genes that contained let-7 target sites (Bhattacharya et al., 2018). Let-7 was also shown to form a feedback regulatory loop with Lin28a (Bhattacharya et al., 2018). Furthermore, the enhancer that drove the expression of Lin28a in neural crest cells contained four TCF/LEF (downstream factors of Wnt signaling pathway) binding sites (Bhattacharya et al., 2018). Disruption of the binding sites resulted in complete loss of enhancer activity and knocking down Wnt1 and Wnt4 resulted in decreased Lin28a expression and increased levels of mature let-7 (Bhattacharya et al., 2018). Together, these results suggest that the pluripotency factor Lin28a maintains multipotency in NCCs by preventing let-7 mediated repression of the pluripotency network regulated by Wnt signaling. These studies suggest that NCCs may co-opt autoregulatory feedback circuitry of pluripotent transcription factors, miRNAs, and signaling pathways to
regulate the pluripotency network and expand their developmental potential (Table 1).

As development progresses and NCCs migrate and differentiate into different cell types, the pluripotency program must be shut off, as it can lead to aberrant tumor formation. Interestingly, overexpression of miR-145, which directly targeted OCT4, SOX2, and KLF4 in hESCs, resulted in differentiation of hESCs into ectoderm and mesoderm lineages and not endoderm or tumor formation. Hence, we speculate that miR-145 might be involved in downregulating Oct4 transcript levels in NCCs as they delaminate and migrate to give rise to ectodermal and ectomesenchymal derivatives. Further investigation is needed to determine the miRNA profile of NCCs at a single-cell resolution, and how the pluripotency network can be regulated by different miRNAs during NCC development.

3 | CONCLUSION

This review highlights some of the mechanistic programs that may be co-opted by NCCs during development, to regulate the pluripotent state and expand their developmental potential. Several factors may synergistically or antagonistically regulate Oct4 expression in NCCs by feedback regulation, such as epigenetic modifiers, non-coding RNAs, signaling factors, and transcription factors. Transcriptional heterogeneity of NCCs is believed to be important for expanded developmental potential and later diversification and differentiation into various cell types. Thus, it will be important to investigate the interplay between pluripotency network genes and regulatory factors mentioned in this review in individual cells. One key question is whether different NCCs express different levels of the core pluripotency factors and if these factors coregulate the same or different sets of target genes to control heterogeneity. Pluripotency transcription factors and miRNAs expressed in NCCs may also form a feedback regulatory loop and co-regulate downstream lineage-specific genes. Further investigation is required to determine the crosstalk between the pluripotency state and fate specification in NCCs at single-cell resolution. Lastly, chromatin accessibility remains a major barrier for reactivation of the pluripotency program and efficient somatic cell reprogramming. Future studies are required to examine the heterogeneity of expression of epigenetic machinery in NCCs and how these modifiers differentially regulate histone modifications at NCC lineage specification genes. These studies can determine whether the ectodermal and ectomesenchymal lineage genes are differentially regulated in Oct4+ NCCs by chromatin accessibility. Indeed, differences in histone modifications can reflect the timing of expression of lineage-specific genes resulting in cell fate bias toward ectodermal or ectomesenchymal lineages in NCCs. Further investigation into these mechanisms will provide exciting new insights into reprogramming and NCC development. These studies will further our understanding of how reprogramming of cNCCs can be utilized as a therapeutic tool for treating and preventing patients with craniofacial defects.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

Anokye-Danso, F., Trivedi, C. M., Juhr, D., Gupta, M., Cui, Z., Tian, Y., ... Morrisey, E. E. (2011). Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell, 8(4), 376–388. https://doi.org/10.1016/j.stem.2011.03.001

Apostolou, E., & Hochedlinger, K. (2013). Chromatin dynamics during cellular reprogramming. Nature, 502(7472), 462–471. https://doi.org/10.1038/nature12749

Bai, M., Yuan, M., Liao, H., Chen, J., Xie, B., Yan, D., ... Feng, Y. (2015). OCT4 pseudogene 5 upregulates OCT4 expression to promote proliferation by competing with miR-145 in endometrial carcinoma. Oncology Reports, 33(4), 1745–1752. https://doi.org/10.3892/or.2015.3763

Basch, M. L., Bronner-Fraser, M., & García-Castro, M. I. (2006). Specification of the neural crest occurs during gastrulation and requires Pax7. Nature, 441(7090), 218–222. https://doi.org/10.1038/nature04684

Becker, J. S., Nicetto, D., & Zaret, K. S. (2016). H3K9me3-dependent heterochromatin: Barrier to cell fate changes. Trends in Genetics, 32(1), 29–41. https://doi.org/10.1016/j.tig.2015.11.001

Bellmeyer, A., Krase, J., Lindgren, J., & LaBonne, C. (2003). The protooncogene c-myc is an essential regulator of neural crest formation in Xenopus. Developmental Cell, 4(6), 827–839. https://doi.org/10.1016/s1534-5807(03)00160-6
Berger, S. L. (2007). The complex language of chromatin regulation during transcription. *Nature*, 447(7143), 407–412. https://doi.org/10.1038/nature05915

Betters, E., Charney, R. M., & Garcia-Castro, M. I. (2018). Early specification and development of rabbit neural crest cells. *Developmental Biology*, 444, S181–S192. https://doi.org/10.1016/j.ydbio.2018.06.012

Bhattacharya, D., Rothstein, M., Azambuja, A. P., & Simoes-Costa, M. (2018). Control of neural crest multipotency by Wnt signaling and the Lin28/PI3K-PI3K axis. *eLife*, 7, e40556. https://doi.org/10.7554/eLife.40556

Briggs, J. A., Weinreb, C., Wagner, D. E., Megason, S., Peshkin, L., Kirschner, M. W., & Klein, A. M. (2018). The dynamics of gene expression in vertebrate embryogenesis at single-cell resolution. *Science*, 360(6392), eaar5780. https://doi.org/10.1126/science.aar5780

Buitrago-Delgado, E., Nordin, K., Rao, A., Geary, L., & LaBonne, C. (2015). NEURODEVELOPMENT. Shared regulatory programs suggest retention of blastula-stage potential in neural crest cells. *Science*, 348(6241), 1332–1335. https://doi.org/10.1126/science.aaa3655

Card, D. A., Hebbar, P. B., Li, L., Trotter, K. W., Komatsu, Y., Mishina, Y., & Archer, T. K. (2008). Oct4/Sox2-regulated miR-302 targets cyclin D1 in human embryonic stem cells. *Molecular and Cellular Biology*, 28(20), 6426–6438. https://doi.org/10.1128/MCB.00359-08

Cartwright, P., McLean, C., Sheppard, A., Rivett, D., Jones, K., & Dalton, S. (2005). LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism. *Development*, 132(5), 885–896. https://doi.org/10.1242/dev.01670

Catalano, C., Cogoni, C., & Zardo, G. (2016). MicroRNA in control of gene expression: An overview of nuclear functions. *International Journal of Molecular Sciences*, 17(10), 1712. https://doi.org/10.3390/ijms17101712

Cebra-Thomas, J. A., Terrell, A., Branyan, K., Shah, S., Rice, R., Gyi, L., ... Gilbert, S. F. (2013). Late-emigrating trunk neural crest cells in turtle embryos generate an osteogenic ectomesenchyme in the plastron. *Developmental Dynamics*, 242(11), 1223–1235. https://doi.org/10.1002/dvdy.24018

Centers for Disease Control and Prevention. Folic acid helps prevent serious birth defects of the brain and spine. Retrieved from https://www.cdc.gov/nbcdd/d/folicacid/features/folic-acid-helps-prevent-some-birth-defects.html

Cheloufi, S., Elling, U., Hopfgartner, B., Jung, Y. L., Murn, J., Ninova, M., ... Hochdedinger, K. (2015). The histone chaperone CAP-1 safeguards somatic cell identity. *Nature*, 528(7581), 218–224. https://doi.org/10.1038/nature15749

Chew, J. L., Loh, Y. H., Zhang, W., Chen, X., Tam, W. L., Yeap, L. S., ... Ng, H. H. (2005). Reciprocal transcriptional regulation of Pou5f1 and Sox2 via the Oct4/Sox2 complex in embryonic stem cells. *Molecular and Cellular Biology*, 25(14), 6031–6046. https://doi.org/10.1128/MCB.25.14.6031-6046.2005

Chung, A. C., Katz, D., Pereira, F. A., Jackson, K. J., DeMayo, F. J., Cooney, A. J., & O’Malley, B. W. (2001). Loss of orphan receptor germ cell nuclear factor function results in ectopic development of the tail bud and a novel posterior truncation. *Molecular and Cellular Biology*, 21(2), 663–677. https://doi.org/10.1128/MCB.21.2.663-677.2001

Cole, M. F., Johnstone, S. E., Newman, J. J., Kagey, M. H., & Young, R. A. (2008). Tcf3 is an integral component of the core regulatory circuitry of embryonic stem cells. *Genes & Development*, 22(6), 746–755. https://doi.org/10.1101/gad.1642408

Couly, G., Grapin-Botton, A., Coltey, P., Ruhin, B., & Le Douarin, N. M. (1998). Determination of the identity of the derivatives of the cephalic neural crest: Incompatibility between Hox gene expression and lower jaw development. *Development*, 125(17), 3445–3459.

Creazzo, T. L., Godt, R. E., Leatherbury, L., Conway, S. J., & Kirby, M. L. (1998). Role of cardiac neural crest cells in cardiovascular development. *Annual Review of Physiology*, 60, 267–286. https://doi.org/10.1146/annurev.physiol.60.1.267

Dennis, J. F. (2008). Induction and patterning of neural crest cells in the developing mouse embryo: Roles for Genf and Hhat (Dissertation). University of Kansas, Lawrence, KS. Retrieved from https://kuscholarworks.ku.edu/handle/1808/5366

Ding, L., Paszkowski-Rogacz, M., Nitzsche, A., Slabicki, M. M., Heninger, A. K., de Vries, I., ... Buchholz, F. (2009). A genome-scale RNAi screen for Oct4 modulators defines a role of the Pafl complex for embryonic stem cell identity. *Cell Stem Cell*, 4(5), 403–415. https://doi.org/10.1016/j.stem.2009.03.009

Feldman, N., Gerson, A., Fang, J., Li, E., Zhang, Y., Shinkai, Y., ... Bergman, Y. (2006). G9a-mediated irreversible epigenetic inactivation of Oct-3/4 during early embryogenesis. *Nature Cell Biology*, 8(2), 188–194. https://doi.org/10.1038/ncb1353

Fuhrmann, G., Chung, A. C., Jackson, K. J., Hummelke, G., Baniahmad, A., Sutter, J., ... Cooney, A. J. (2001). Mouse germ line restriction of Oct4 expression by germ cell nuclear factor. *Developmental Cell*, 1(3), 377–387. https://doi.org/10.1016/s1534-5807(01)00038-7

Fujita, K., Ogawa, R., & Ito, K. (2016). CHD7, Oct3/4, Sox2, and Nanog control FoxD3 expression during mouse neural crest-derived stem cell formation. *The FEBS Journal*, 283(20), 3791–3806. https://doi.org/10.1111/febs.13843

Gao, Y., Chen, J., Li, K., Wu, T., Huang, B., Liu, W., ... Gao, S. (2013). Replacement of Oct4 by Tet1 during iPSC induction reveals an important role of DNA methylation and hydroxymethylation in reprogramming. *Cell Stem Cell*, 12(4), 453–469. https://doi.org/10.1016/j.stem.2013.02.005

Gomez, G. A., Prasad, M. S., Sandhu, N., Shelar, P. B., Leung, A. W., & Garcia-Castro, M. I. (2019). Human neural crest induction by temporal modulation of WNT activation. *Developmental Biology*, 449(2), 99–106. https://doi.org/10.1016/j.ydbio.2019.02.015

Grath, A., & Dai, G. (2019). Direct cell reprogramming for tissue engineering and regenerative medicine. *Journal of Biological Engineering*, 13, 14. https://doi.org/10.1186/s13036-019-0144-9

Gu, P., Goodwin, B., Chung, A. C., Xu, X., Wheeler, D. A., Price, R. R., ... Cooney, A. J. (2005). Orphan nuclear receptor LRH-1 is required to maintain Oct4 expression at the epiblast stage of embryonic development. *Molecular and Cellular Biology*, 25(9), 3492–3505. https://doi.org/10.1128/MCB.25.9.3492-3505.2005

Gu, P., Le Menuet, D., Chung, A. C., & Cooney, A. J. (2006). Differential recruitment of methylated CpG binding domains by the orphan receptor GCNF initiates the repression and silencing of Oct4 expression. *Molecular and Cellular Biology*, 26(24), 9471–9483. https://doi.org/10.1128/MCB.00898-06
Hagiwara, K., Obayashi, T., Sakayori, N., Yamanishi, E., Kohlhase, J., Heinrich, M., Liebers, M., Fröhlich Archangelo, L., Hawkins, P. G., & Morris, K. V. (2010). Transcriptional regulation...
Ma, D. K., Chiang, C. H., Ponnusamy, K., Ming, G. L., & Song, H. (2008). G9a and Jhdm2a regulate embryonic stem cell fusion-induced reprogramming of adult neural stem cells. Stem Cells, 26(8), 2131–2141. https://doi.org/10.1634/stemcells.2008-0388

Maherali, N., Sridharan, R., Xie, W., Utitak, J., Eminli, S., Arnold, K., ... Hochedlinger, K. (2007). Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell, 1(1), 55–70. https://doi.org/10.1016/j.stem.2007.05.014

Martin, D. G., Grimes, D. E., Baetz, K., & Howe, L. (2006). Methylation of histone H3 mediates the association of the NuA3 histone acetyltransferase with chromatin. Molecular and Cellular Biology, 26(8), 3018–3028. https://doi.org/10.1128/MCB.26.8.3018-3028.2006

Mikkelsen, T. S., Hanna, J., Zhang, X., Ku, M., Wernig, M., Schorderet, P., ... Meissner, A. (2008). Dissecting direct reprogramming through integrative genomic analysis. Nature, 454(7200), 49–55. https://doi.org/10.1038 nature07056

Mohanty, V., Shah, A., Allender, E., Siddiqui, M. R., Monick, S., ... Wang, X. (2018). miR-302 regulates the embryonic axis and coordinates exit from pluripotency and germ layer specification in the mouse embryo. Development, 145(12), dev159103. https://doi.org/10.1242/dev.159103

Mullen, E. M., Gu, P., & Cooney, A. J. (2007). Nuclear receptors in regulation of mouse ES cell pluripotency and differentiation. PPAR Research, 10, 61563. https://doi.org/10.1155/2007/61563

Nakazaki, H., Reddy, A. C., Mania-Farnell, B. L., Shen, Y. W., Ichii, S., McCabe, C., ... Mayanil, C. S. (2008). Key basic helix-loop-helix transcription factor genes Hes1 and Ngn2 are regulated by Pax3 during mouse embryonic development. Developmental Biology, 316(2), 510–523. https://doi.org/10.1016/j.ydbio.2008.01.008

Newman, M. A., Thomson, J. M., & Hammond, S. M. (2008). Lin-28 interaction with the Let-7 precursor loop mediates regulated microRNA processing. RNA, 14(8), 1539–1549. https://doi.org/10.1261/rna.1155108

Niwa, H. (2007). How is pluripotency determined and maintained? Development, 134(4), 635–646. https://doi.org/10.1242/dev.02787

Niwa, H., Miyazaki, J., & Smith, A. G. (2000). Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nature Genetics, 24(4), 372–376. https://doi.org/10.1038/74199

Nordin, K., & LaBon, E. (2014). Sox5 is a DNA-binding cofactor for BMP R-Smads that directs target specificity during patterning of the early ectoderm. Developmental Cell, 31(3), 374–382. https://doi.org/10.1016/j.devcel.2014.10.003

Okumura-Nakanishi, S., Saito, M., Niwa, H., & Ishikawa, F. (2005). Oct-3/4 and Sox2 regulate Oct-3/4 gene in embryonic stem cells. The Journal of Biological Chemistry, 280(7), 5307–5317. https://doi.org/10.1074/jbc.M410015200

Osorno, R., & Chambers, I. (2011). Transcription factor heterogeneity and epiblast pluripotency. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 366(1575), 2230–2237. https://doi.org/10.1098/rstb.2011.0043

Parchem, R. J., Moore, N., Fish, J. L., Parchem, J. G., Braga, T. T., Shenoy, A., ... Blelloch, R. (2015). miR-302 is required for timing of neural differentiation, neural tube closure, and embryonic viability. Cell Reports, 12(5), 760–773. https://doi.org/10.1016/j.celrep.2015.06.074

Parchem, R. J., Ye, J., Judson, R. L., LaRussa, M. F., Krishnakumar, R., Blelloch, A., ... Blelloch, R. (2014). Two miRNA clusters reveal alternative pathways in late-stage reprogramming. Cell Stem Cell, 14(5), 617–631. https://doi.org/10.1016/j.stem.2014.01.021

Pattay, C., Edlund, T., & Gunhaga, L. (2009). Wnt-regulated temporal control of BMP exposure directs the choice between neural plate border and epidermal fate. Development, 136(1), 73–83. https://doi.org/10.1242/dev.025890

Pattay, C., Gunhaga, L., & Edlund, T. (2008). Early development of the central and peripheral nervous systems is coordinated by Wnt and BMP signals. PLoS One, 3(2), e1625. https://doi.org/10.1371/journal.pone.0001625

Polo, J. M., Anderssen, E., Walsh, R. M., Schwarz, B. A., Neffzer, C. M., Lim, S. M., ... Hochedlinger, K. (2012). A molecular roadmap of reprogramming somatic cells into iPSCs. Cell, 151(7), 1617–1632. https://doi.org/10.1016/j.cell.2012.11.039

Prasad, M. S., Charney, R. M., Patel, L. J., & García-Castro, M. I. (2020). Distinct molecular profile and restricted stem cell potential defines the prospective human cranial neural crest from embryonic stem cell state. Stem Cell Research, 49, 102086. https://doi.org/10.1016/j.scr.2020.102086

Prasad, M. S., Uribe-Querol, E., Marquez, J., Vadasz, S., Yardley, N., Sherar, P. B., ... García-Castro, M. I. (2020). Blas tula stage specification of avian neural crest. Developmental Biology, 458(1), 64–74. https://doi.org/10.1016/j.ydbio.2019.10.007

Rogers, C. D., Archer, T. C., Cunningham, D. D., Grammer, T. C., & Casey, E. M. (2008). Sox3 expression is maintained by FGF signaling and restricted to the neural plate by vent proteins in the Xenopus embryo. Developmental Biology, 313(1), 307–319. https://doi.org/10.1016/j.ydbio.2007.10.023

Rosa, A., & Brivanlou, A. H. (2011). A regulatory circuitry comprised of miR-302 and the transcription factors OCT4 and NR2F2 regulates human embryonic stem cell differentiation. The EMBO Journal, 30(2), 237–248. https://doi.org/10.1038/emboj.2010.319

Sato, T. S., Hanada, A., Priya, S., Watal, P., Becker, R. M., & Sato, Y. (2019). Neurocristopathies: Enigmatic appearances of neural crest cell-derived abnormalities. Radiographics, 39(7), 2085–2102. https://doi.org/10.1148/rg.2019190086

Sato, N., Kondo, M., & Araki, K. (2006). The orphan nuclear receptor GCNF recruits DNA methyltransferase for Oct-3/4 silencing.
Tahiliani, M., Koh, K. P., Shen, Y., Pastor, W. A., Bandukwala, H., Brudno, Y., ... Rao, A. (2009). Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science, 324(5929), 930–935. https://doi.org/10.1126/science.1170116

Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 126(4), 663–676. https://doi.org/10.1016/j.cell.2006.07.024

Takahashi, K., & Yamanaka, S. (2016). A decade of transcription factor-mediated reprogramming to pluripotency. Nature Reviews. Molecular Cell Biology, 17(3), 183–193. https://doi.org/10.1038/nrm.2016.8

Tomioka, M., Nishimoto, M., Miyagi, S., Katayanagi, T., Fukui, N., Niwa, H., ... Okuda, A. (2002). Identification of Sox-2 regulatory region which is under the control of Oct-3/4-Sox-2 complex. Nucleic Acids Research, 30(14), 3202–3213. https://doi.org/10.1093/nar/gkf435

Topalovic, V., Schwirtlich, M., Stevanovic, M., & Mojsin, M. (2017). Histone modifications on the promoters of human OCT4 and NANOG genes at the onset of neural differentiation of NT2/D1 cells. Biochemistry. Biokhimiya, 82(6), 715–722. https://doi.org/10.1134/S000629710600086

Waddington, C. H. (1957). The strategy of the genes. Crows Nest, NSW, Australia: George Allen & Unwin, Ltd.

Wang, K., Chen, Y., Chang, E. A., Knott, J. G., & Cibelli, J. B. (2009). Dynamic epigenetic regulation of the Oct4 and Nanog regulatory regions during neural differentiation in rhesus nuclear transfer embryonic stem cells. Cloning and Stem Cells, 11(4), 483–496. https://doi.org/10.1089/csc.2009.0019

Wang, J., Huang, V., Ye, L., Bárcena, A., Lin, G., Lue, T. F., & Li, L. (2015). Identification of small activating RNAs that enhance endogenous OCT4 expression in human mesenchymal stem cells. Stem Cells and Development, 24(3), 345–353. https://doi.org/10.1089/scd.2014.0290

Wang, B., Wu, L., Li, D., Liu, Y., Guo, J., Li, C., ... Pei, D. (2019). Induction of pluripotent stem cells from mouse embryonic fibroblasts by Jdp2-Jhdm1b-Mkk6-Glis1-Nanog-Essrb-Sall4. Cell Reports, 27(12), 3473–3485. https://doi.org/10.1016/j.celrep.2019.05.068

Wang, Y., Xu, Z., Jiang, J., Xu, C., Kang, J., Xiao, L., ... Liu, H. (2013). Endogenous miRNA sponge lincRNA-RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal. Developmental Cell, 25(1), 69–80. https://doi.org/10.1016/j.devcel.2013.03.002

Wilmut, I., Schnieke, A. E., McWhir, J., Kind, A. J., & Campbell, K. H. (2007). Viable offspring derived from fetal and adult mammalian cells. Cloning and Stem Cells, 9(1), 3–7. https://doi.org/10.1089/clo.2006.0002

Xu, N., Papagiannakopoulos, T., Pan, G., Thomson, J. A., & Kosik, K. S. (2009). MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. Cell, 137(4), 647–658. https://doi.org/10.1016/j.cell.2009.02.038

Yamane, K., Toumazou, C., Tsukada, Y., Erdjument-Bromage, H., Tempst, P., Wong, J., & Zhang, Y. (2006). JHDM2A, a JmJC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor. Cell, 125(3), 483–495. https://doi.org/10.1016/j.cell.2006.03.027

Yang, H. M., Do, H. J., Kim, D. K., Park, J. K., Chang, W. K., Chung, H. M., ... Kim, J. H. (2007). Transcriptional regulation of human Oct4 by steroidogenic factor-1. Journal of Cellular
Yeom, Y. I., Fuhrmann, G., Ovitt, C. E., Brehm, A., Ohbo, K., Gross, M., ... Schöler, H. R. (1996). Germline regulatory element of Oct-4 specific for the totipotent cycle of embryonal cells. *Development (Cambridge, England)*, 122(3), 881–894.

Ying, Q. L., Nichols, J., Chambers, I., & Smith, A. (2003). BMP induction of id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell*, 115(3), 281–292. https://doi.org/10.1016/s0092-8674(03)00847-x

Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., ... Thomson, J. A. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science*, 318(5858), 1917–1920. https://doi.org/10.1126/science.1151526

Zalc, A., Sinha, R., Gulati, G. S., Wesche, D. J., Daszczuk, P., Swigut, T., ... Wysocka, J. (2021). Reactivation of the pluripotency program precedes formation of the cranial neural crest. *Science*, 371(6529), eabb4776. https://doi.org/10.1126/science.abb4776

Zhang, J., Ratarasirinrawoot, S., Chandrasekaran, S., Wu, Z., Ficarro, S. B., Yu, C., ... Daley, G. Q. (2016). LIN28 regulates stem cell metabolism and conversion to primed pluripotency. *Cell Stem Cell*, 19(1), 66–80. https://doi.org/10.1016/j.stem.2016.05.009

Zhang, J., Tam, W. L., Tong, G. Q., Wu, Q., Chan, H. Y., Soh, B. S., ... Lim, B. (2006). Sall4 modulates embryonic stem cell pluripotency and early embryonic development by the transcriptional regulation of Pou5f1. *Nature Cell Biology*, 8(10), 1114–1123. https://doi.org/10.1038/ncb1481

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