

# UC Davis

## UC Davis Previously Published Works

### Title

DPPA2, DPPA4, and other DPPA factor epigenomic functions in cell fate and cancer

### Permalink

<https://escholarship.org/uc/item/2h62p7p9>

### Journal

Stem Cell Reports, 16(12)

### ISSN

2213-6711

### Authors

Klein, Rachel Herndon  
Knoepfler, Paul S

### Publication Date

2021-12-01

### DOI

10.1016/j.stemcr.2021.10.008

Peer reviewed

# DPPA2, DPPA4, and other DPPA factor epigenomic functions in cell fate and cancer

Rachel Herndon Klein<sup>1,2,3</sup> and Paul S. Knoepfler<sup>1,2,3,\*</sup>

<sup>1</sup>Department of Cell Biology and Human Anatomy, University of California, Davis, CA 95616, USA

<sup>2</sup>Institute of Pediatric Regenerative Medicine, Shriners Hospital for Children Northern California, Sacramento, CA 95817, USA

<sup>3</sup>Genome Center, University of California, Davis, CA 95616, USA

\*Correspondence: [knoepfler@ucdavis.edu](mailto:knoepfler@ucdavis.edu)

<https://doi.org/10.1016/j.stemcr.2021.10.008>

## SUMMARY

Many gene networks are shared between pluripotent stem cells and cancer; a concept exemplified by several DPPA factors such as DPPA2 and DPPA4, which are highly and selectively expressed in stem cells but also found to be reactivated in cancer. Despite their striking expression pattern, for many years the function of DPPA2 and DPPA4 remained a mystery; knockout of *Dppa2* and *Dppa4* did not affect pluripotency, but caused lung and skeletal defects late in development, long after *Dppa2* and *Dppa4* expression had been turned off. A number of recent papers have further clarified and defined the roles of these important factors, identifying roles in priming the chromatin and maintaining developmental competency through regulating both H3K4me3 and H3K27me3 at bivalent chromatin domains, and acting to remodel chromatin and facilitate reprogramming of somatic cells to induced pluripotency. These findings highlight an important regulatory role for DPPA2 and DPPA4 at the transitional boundary between pluripotency and differentiation and may have relevance to the functions of DPPA2 and 4 in the context of cancer cells as well.

## INTRODUCTION

There are striking similarities between the gene networks involved in regulating pluripotency and those aberrantly activated in cancer, a finding that will have implications for many stem cell-related regenerative medicine treatments as well as for cancer therapies that must effectively target the generally slow-cycling cancer stem cells. Developmental pluripotency factors 2 and 4 (DPPA2 and DPPA4) are key examples of transcriptional regulators active in both pluripotent and cancer cells. They underlie the connections between these two physiological cell states. Indeed, DPPA2 was originally named embryo/cancer sequence (ESCA) based on the identification of high levels of expression in embryonic stem cells (ESCs) and its reactivation in certain types of cancer cells (John et al., 2008).

DPPA2 and DPPA4 were tested for their ability to reprogram somatic cells to induced pluripotent stem cells (iPSCs) in the Yamanaka laboratory's screens due to their high and selective expression in ESCs (Takahashi and Yamanaka, 2006). Neither DPPA2 nor DPPA4 made the final list of factors minimally required for reprogramming of somatic cells to iPSC; however, many studies continued to use them as

markers of both pluripotency in general and successful reprogramming due to their strikingly selective expression pattern in pluripotent stem cells (Hu et al., 2010; Zhu et al., 2017).

Despite their early identification as strong markers of pluripotency, the exact functions and molecular mechanisms of action of DPPA4 and DPPA2 have remained unclear for many years. The protein structures and domains provided little insight (Maldonado-Saldivia et al., 2007), and standard approaches, including gene knockout, produced more questions than they answered (Madan et al., 2009; Nakamura et al., 2011). In recent years, however, several key studies have outlined and characterized an important role for DPPA2 and DPPA4 in modulating the transition between pluripotency and differentiation through interactions with chromatin-modifying complexes and have identified overlapping regulatory functions for these factors in both stem and cancer cells. Additionally, the number of papers reporting the activation of DPPA2 and DPPA4 in different kinds of cancer continues to increase.

## HOW ARE DPPA2/4 REGULATED?

*Dppa2* and *Dppa4* are located in tandem on chromosome 3 in humans and on chromosome 16 in mice, suggesting they are coordinately expressed and regulated (Madan et al., 2009). Studies have revealed a combination of chromatin modifications and transcription factors that regulate the *Dppa2* and *Dppa4* locus. Indeed, ChIP-seq data on chromatin modifications identifies a strong enhancer region located equidistant between the two genes that is bound by OCT4, SOX2, and NANOG (Madan et al., 2009) in stem cells. DNA methylation has also been shown to be important to regulation of the region: like a number of other pluripotency regulators, the *Dppa4/Dppa2* region remains highly methylated during reprogramming until the cells reach pluripotency, at which point methylation is removed and *Dppa2/4* are expressed (Lee et al., 2014).

Additional studies indicate that both the *Dppa4* and the *Dppa2* promoters are demethylated in the germline and remain demethylated in the developing embryo until day 7.5, when their expression drops off as their promoters



are remethylated (Eckersley-Maslin et al., 2019). Further evidence of regulation through chromatin modification comes from a study showing that Setdb1, a histone H3K9 methyltransferase, binds to *Dppa2* and downregulates expression during primordial germ cell differentiation (Mochizuki et al., 2018). This downregulation is necessary for proper differentiation and gamete formation (Mochizuki et al., 2018), and suggests that H3K9me3 is an important mechanism for downregulating *Dppa2*, and possibly *Dppa4* as well, during differentiation.

The nuclear receptor NR5A1 binds near the transcription termination site of the *Dppa4* gene and within the *Dppa2* gene (Yamauchi et al., 2020). NR5A1 plays a role in inducing a naive chromatin state in human pluripotent stem cells and functional studies have revealed that DPPA2 and DPPA4 are downstream mediators of NR5A1 activity in stem cells (Yamauchi et al., 2020). Intriguingly, NR5A1 is also a key regulator of the development of the gonads and adrenal glands (Anamthathmakula et al., 2019). In the developing embryo, *Dppa2/4* expression persists the longest in the gonadal tissue (Maldonado-Saldivia et al., 2007), suggesting *Dppa2* and *Dppa4* may also be regulated by NR5A1 in the developing gonads and possibly in germ cells.

DPPA2 and DPPA4 function is additionally regulated at the protein level through a number of mechanisms. Several lines of evidence, including ChIP-seq and protein interaction studies, indicate that DPPA2 and DPPA4, which share 32% homology at the amino acid level (Madan et al., 2009), heterodimerize. Notably, knockdown of *Dppa2* affects DPPA4 protein but not mRNA levels, and knockdown of *Dppa4* reduces DPPA2 protein levels (Eckersley-Maslin et al., 2019; Tung et al., 2013), indicating protein stabilization potentially occurs through heterodimerization of DPPA2 and DPPA4.

Another interesting study identified two isoforms of DPPA4; one isoform lacks the SAP domain, which is thought to be important for DNA binding and nuclear localization (De Iaco et al., 2019). The truncated isoform is most highly expressed during zygotic genome activation (ZGA) relative to the full-length version, which is thought to be of maternal origin (De Iaco et al., 2019). In mouse embryonic stem cells (mESCs) only the full-length version is detected (De Iaco et al., 2019), suggesting the isoform lacking the SAP domain has a particular function only during ZGA.

While post-translational modification of DPPA2 and DPPA4 is not fully understood, a recent study showed that DPPA2 is sumoylated by PIAS4 (Yan et al., 2019). Sumoylation of a protein can change the binding surfaces and potential interacting partners. It has been described as the "glue" that helps transcription factors and epigenetic complexes locate and bind to one another to regulate gene

expression (Theurillat et al., 2020). Sumoylation of DPPA2 appears to inhibit its role in activating ZGA (Theurillat et al., 2020; Yan et al., 2019), potentially through disruption of multiprotein complexes, and points to protein modification as an additional level of DPPA2/4 regulation.

## OTHER DPPA FACTORS ALSO HAVE ROLES IN PLURIPOTENCY AND CANCER

There are several other DPPA factors that have also been shown to have related roles in pluripotent cells, early development, and cancer. DPPA3 is a key factor in oocyte development, where it helps maintain the hypomethylation of the oocyte genome that is subsequently required for ZGA after fertilization (Li et al., 2018). In stem cells, DPPA3 has also been shown to regulate the transition between naive and primed ESC states, acting downstream of LIN28 (Sang et al., 2018). Similar to *DPPA2* and *DPPA4*, *DPPA3* is also overexpressed in certain cancers. In hepatocellular carcinoma (HCC), *DPPA3* expression promotes demethylation of both *MYCN* and *GLI1*, two factors that shift HCC toward a progenitor identity (Yan et al., 2021).

Like other DPPA factors, DPPA5 also appears to act at cellular transitions: overexpression of *DPPA5* enhances reprogramming efficiency, in part through regulation of NANOG stability (Qian et al., 2016). *DPPA5* also regulates reconstitution ability in hematopoietic stem cells after a bone marrow transplant (Miharada et al., 2014). Intriguingly, *DPPA5* exerts this function through suppression of endoplasmic reticulum (ER) stress, a role that has not, as of yet, been described for other DPPA factors. Further research into the functions of DPPA factors will likely shed light upon the complex roles and relationships between these key developmental genes.

## DPPA2/4 PLAY CRUCIAL ROLES IN MODULATING THE TRANSITIONS TO AND FROM PLURIPOTENCY

Despite the high expression level of *Dppa2* and *Dppa4* in stem cells, they are not essential for maintenance of pluripotency. *Dppa2* knockout ESCs do not have any discernible defects in pluripotency and maintain normal pluripotency-associated gene expression but have decreased proliferation. In addition, several other genes are downregulated in the knockouts compared with wild-type (WT) ESCs, including numerous genes involved in gametogenesis like *Ddx4*, *Mael*, and *Syce1* (Nakamura et al., 2011). Surprisingly, neither *Dppa4* knockout ESCs nor *Dppa2/Dppa4* double-knockout ESCs show any obvious defects in pluripotency or proliferation (Madan et al., 2009; Nakamura et al., 2011).

What is perhaps the most intriguing finding regarding *Dppa2* and *Dppa4* is that despite the knockout of one or



both factors having minimal effect in stem cells, striking defects are observed *in vivo* in mice at very late stages of development, and many knockout pups die soon after birth due to respiratory failure (Madan et al., 2009; Nakamura et al., 2011). The main phenotypic effects of *Dppa2* or *Dppa4* knockout were observed in the lungs and skeletal system. The lungs of *Dppa2* and *Dppa4* knockouts had a thickening of the mesenchyme that appeared only after E16.5 and failed to inflate after birth (Madan et al., 2009; Nakamura et al., 2011). *Dppa2* knockout mice also had decreased alveolar spaces (Madan et al., 2009). Skeletal defects in *Dppa4*<sup>-/-</sup> pups included decreased ossification and abnormal fusing of the thoracic ribs (Madan et al., 2009).

*Dppa2* and *Dppa4* knockouts also somehow later caused expression changes in a modest number of genes in the lung tissue, despite neither *Dppa2* nor *Dppa4* being expressed at this stage. Developmental transcription factors *Gata4*, *Nkx2-5*, *Pitx1*, and *Pitx2* were affected in *Dppa2* knockout lungs (Nakamura et al., 2011). Oddly, the *Dppa2* and *Dppa4* double knockout appears to be less lethal in mice than single knockout of *Dppa2* (Nakamura et al., 2011). As *Dppa2* and *Dppa4* expression persists the longest in gonadal tissues during development, defects in germ cell production could be caused by knockout; however, functional germ cells can be produced in both the *Dppa2* and the *Dppa4* single and double knockout mice (Madan et al., 2009).

The valuable information gained from these early studies seems to point to a role for DPPA2 and DPPA4 at the transition from pluripotency to differentiation, rather than directly regulating aspects of pluripotency itself. In this context, high expression of *Dppa2* and *Dppa4* may be a mechanism of preparing, or "poising," stem cells for future developmental stages.

One recent line of research on DPPA2 and DPPA4 function has focused on these factors' roles in the regulation of ZGA, the process by which gene expression is activated in the very early embryo. This process is believed to occur during the transition between the one- and the two-cell stages in mice, and between the four- and the eight-cell stages in human embryos, although some embryonic genes are activated earlier (De Iaco et al., 2019). In mESC culture, a subset of cells have properties of a two-cell stage embryo (2C cells), including activation of ZGA regulators, and have been used to characterize the role of transcriptional regulators, including DPPA2/4, in ZGA (De Iaco et al., 2019).

*Dppa2* and *Dppa4* were identified in a screen for factors that regulate ZGA in somatic cell nuclear transfer (SCNT), along with the transcription factor *Dux*. The researchers found, however, that transient overexpression of *Dppa2* or *Dppa4* was not able to improve SCNT efficiency, sug-

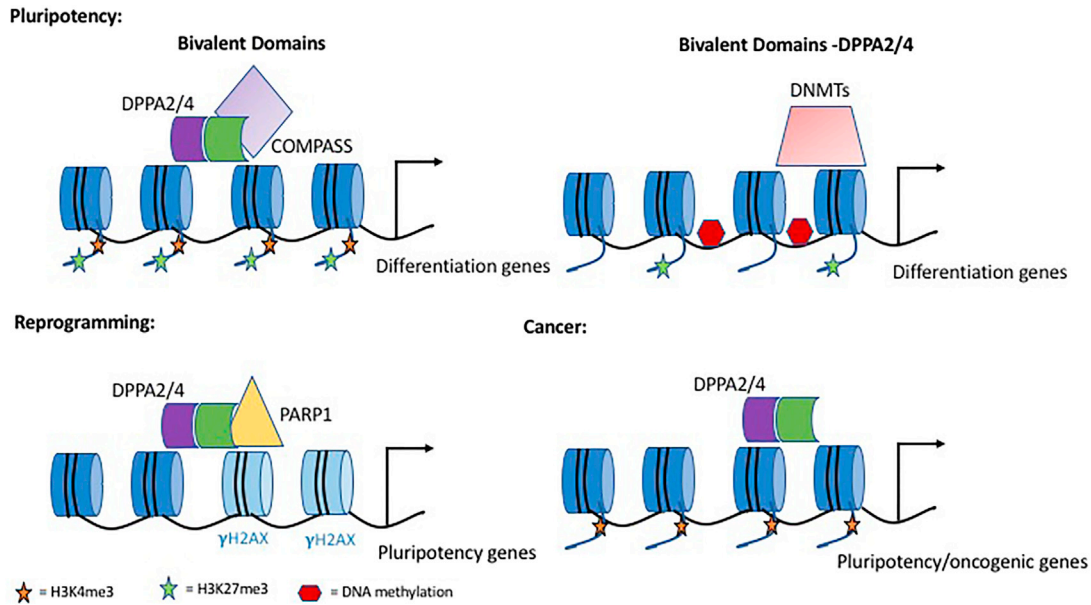
gesting that the effects of *Dppa2* and *Dppa4* on ZGA may be time dependent, or may rely on other interacting factors (Yang et al., 2020).

Another recent paper found that knockout of *Dppa2* or *Dppa4* in mESCs completely eliminates the 2C cells from the population (De Iaco et al., 2019), a finding that is intriguing given that *Dppa2* and *Dppa4* knockout have very little effect on bulk populations of mESCs. The study further found that the transcription factor *Dux*, which is expressed in a wave during ZGA and has been shown to activate many of the genes involved in the process, is a direct target of DPPA2 and DPPA4 (De Iaco et al., 2019; Eckersley-Maslin et al., 2019). In addition to their activation of *Dux*, DPPA2 and DPPA4 also activate the LINE-1 retrotransposons that are turned on during ZGA and negatively regulate *Dux* levels, helping to achieve the brief pulse of *Dux* expression that is necessary for ZGA (De Iaco et al., 2019).

These findings would be consistent with a model that suggests most *Dppa2/4* knockout mice are able to survive the initial stages of embryogenesis because maternally deposited (WT) *Dppa2/4* transcripts are present and able to successfully activate the zygotic genome; however, a recent and yet to be published study found that maternally deposited *Dppa2* and *Dppa4* are not required for *Dux* activation during ZGA (Chen et al., 2021). A second recent preprint also found that *Dppa2* and *Dppa4* are not required for ZGA to occur, however the offspring of *Dppa4* knockout females had a lower survival rate than the offspring of *Dppa4* knockout males (Kubinyecz et al., 2021). These results suggest that maternally deposited *Dppa4* is not essential for ZGA but is still important for offspring survival. Further studies will be needed to fully resolve and define the roles of *Dppa2* and *Dppa4* in the early embryo.

Building on the idea that other factors interact with DPPA2/4 to regulate ZGA, a link between DPPA2 and SMARCA5, the ATPase subunit in the ISWI chromatin remodeling complex, was identified and characterized in a recent study that used a CRISPR-based screening approach to search for novel regulators of ZGA (Alda-Catalinas et al., 2020). The study's findings suggest a model whereby DPPA2 acts, at least in part, downstream of SMARCA5 to regulate gene activation during ZGA (Alda-Catalinas et al., 2020).

Further contributing to our knowledge of the role of DPPA2/4 in ZGA, ChIP-seq and RNA-seq studies demonstrate that DPPA2 and DPPA4 bind to and regulate a gene called alkaline phosphatase placental-like 2 (*Alpl2*), which is upregulated during ZGA and has been shown to have roles in folate metabolism, a pathway that is crucial for ensuring proper DNA methylation among many other cellular functions (Li et al., 2020).



**Figure 1. DPPA2 and DPPA4 interact with chromatin factors to regulate the transitions between different cell states.**

In pluripotent stem cells, DPPA2 and DPPA4 interact with the COMPASS complex to maintain H3K4me3 levels in bivalent domains at a key subset of differentiation-related gene targets. Bivalency prevents DNA methylation from occurring at these genes prior to differentiation and lineage commitment. Loss of DPPA2 or DPPA4 can interfere with this priming of the chromatin, resulting in premature DNA methylation and improper silencing of key developmental genes, leading to defects at later developmental time points. DPPA2 and DPPA4 expression significantly increases the efficiency of reprogramming somatic cells to iPSCs. These two factors heterodimerize and also interact with PARP1 to promote opening of the chromatin during reprogramming through a unique trajectory involving the DNA damage response. DPPA2 and DPPA4 are overexpressed in a number of cancer types. Their expression is associated with the reactivation of a subset of shared pluripotency and oncogenic genes.

Additional evidence for DPPA2 and DPPA4 functioning at transition stages comes from the study by Hernandez et al., where overexpression of both *Dppa2* and *Dppa4* had a strong effect on increasing reprogramming efficiency (Hernandez et al., 2018). They found that this effect is likely to be mediated through the DPPA2/4 regulation of  $\gamma$ H2AX. *Dppa2/4* overexpression causes  $\gamma$ H2AX levels to increase during reprogramming, and knockdown of  $\gamma$ H2AX reduces the reprogramming efficiency of *Dppa2/4*-overexpressing cells (Hernandez et al., 2018). The authors further showed that  $\gamma$ H2AX is deposited through the DNA damage response (DDR) pathway in *Dppa2/4*-overexpressing cells through the activity of PARP1 (Figure 1) (Hernandez et al., 2018). These findings, combined with transcriptome analysis of reprogramming of cells overexpressing *Dppa2/4*, revealed the surprising finding that reprogramming in *Dppa4/2*-overexpressing cells occurs through an alternative trajectory, including the upregulation of the DDR pathway. *Dppa2/4* overexpression-mediated reprogramming was also characterized by faster downregulation of somatic genes that are direct DPPA2/4 targets and quicker activation of mitochondrial genes involved in upregulating glycolysis and downregu-

lating oxidative phosphorylation (Hernandez et al., 2018). A link between DPPA4 and metabolic pathways has also been documented in cancer (Li et al., 2019), suggesting a functional connection between the metabolic states in stem and cancer cells that is regulated by the same factor, DPPA4.

## DPPA2 AND DPPA4 REGULATE EPIGENETIC MARKS DURING EARLY DIFFERENTIATION

Two recent studies published together have added substantially to our understanding of the function of DPPA2 and DPPA4 in the transition between pluripotency and differentiation through characterization of the epigenetic state of *Dppa2/4*-deficient cells (Eckersley-Maslin et al., 2020; Gretarsson and Hackett, 2020). In the first of these studies, DPPA2 and DPPA4 were identified in a screen as having a strong effect on focal sites of DNA methylation, but knockout of either did not have a substantial effect on global methylation levels (Gretarsson and Hackett, 2020). *Dppa2* or *Dppa4* knockdown led to hypermethylation at an identified set of genes (Gretarsson and Hackett, 2020).





These sites of focal hypermethylation are located at CpG islands that normally remain unmethylated throughout development (Gretarsson and Hackett, 2020). In *Dppa2* or *Dppa4* knockout mESCs, these aberrantly methylated sites are maintained through the early stages of differentiation, when a significant number of promoters and LINES also become hypermethylated in the knockout cells (Gretarsson and Hackett, 2020). Regions that are hypermethylated in *Dppa2/4* knockout are often bound by DPPA2 and have high levels of H3K4me<sub>3</sub>, which are lost upon *Dppa2* knockout (Figure 1) (Gretarsson and Hackett, 2020).

As demonstrated in previous studies, *Dppa2* and *Dppa4* knockout had only a modest effect on gene expression in mESCs and did not affect pluripotency or the ability to begin differentiation, however as differentiation proceeds, more differences in gene expression in *Dppa2*- and *Dppa4*-deficient cells are evident, including in particular an inability to activate genes of the mesodermal lineage (Gretarsson and Hackett, 2020). Many of these genes had differential methylation in their promoters in *Dppa2/4* knockout in earlier stages (Gretarsson and Hackett, 2020). Further differentiation along the endodermal lineage reveals the failure of key genes to activate, even after developmental points where *Dppa2* is no longer expressed (Gretarsson and Hackett, 2020).

A second study found that *Dppa2/4* double-knockout ESCs show delays in forming embryoid bodies (EBs) and were slow both to turn off pluripotency genes and to turn on differentiation genes (Eckersley-Maslin et al., 2020). Building from previous work showing an enrichment of DPPA2/4 at bivalent domains in ESCs, this paper identified a reduction in H3K4me<sub>3</sub> and H3K27me<sub>3</sub> signals at a subset of these bivalent domains in the double-knockout ESCs and revealed that DPPA2 and DPPA4 interact with members of the COMPASS and Polycomb complexes, which catalyze H3K4me<sub>3</sub> and H3K27me<sub>3</sub>, respectively (Eckersley-Maslin et al., 2020). They found that DPPA2/4-dependent bivalent promoters failed to recruit COMPASS or Polycomb complex members and had reduced chromatin accessibility as well as increased DNA methylation levels (Figure 1) (Eckersley-Maslin et al., 2020). *Dppa2/4* knockdown also reduced H3K4me<sub>3</sub> levels in DNMT family triple-knockout ESCs, indicating that loss of bivalency precedes DNA methylation changes in *Dppa2/4* depleted cells (Eckersley-Maslin et al., 2020). Altogether these findings point to a role for DPPA2 and DPPA4 in maintaining H3K4me<sub>3</sub> levels and, to a lesser extent, H3K27me<sub>3</sub> levels through interactions with members of the COMPASS and Polycomb complexes at a subset of bivalent domains in ESCs, thereby inhibiting DNA methylation of the regions, which allows for these genes to become active with proper timing during differentiation.

## DPPA2/4 PROTEIN-PROTEIN INTERACTIONS

Protein-protein interaction studies have identified specific DPPA2- and DPPA4-interacting partners and provided insights into DPPA2 and DPPA4 functions in various contexts. A recent proteomics study found that *Dppa4* binds to ERBB3-binding protein 1 (EBP1) in pluripotent cells, an interaction that appears to depend on the SAP domain of DPPA4 (Somanath et al., 2018). DPPA2 also bound EBP1. In this study, DPPA4 in addition bound DNMT1, LIN28a, and OCT4 (Somanath et al., 2018). The work was followed up in an additional paper that demonstrated that DPPA4 and OCT4 directly interact with each other in a pluripotent context and bind to a shared set of sites not bound by SOX2 or NANOG (Klein et al., 2018). The genes cobound by OCT4 and DPPA4 are highly enriched in functional categories relating to cancer and cell signaling, particularly Wnt and MAPK signaling (Klein et al., 2018).

Interestingly, several studies have suggested an interaction between DPPA2 or DPPA4 and members of the Polycomb complex (Oliviero et al., 2015), however many of these interactions were not able to be validated in coimmunoprecipitation experiments, suggesting the interactions may be chromatin dependent. In other cases the interactions did not appear to involve the canonical Polycomb complex (Eckersley-Maslin et al., 2020; Oliviero et al., 2015).

Fitting with the role of DPPA2 and DPPA4 in reprogramming of somatic cells to hiPSCs, an interesting interaction has been observed between HP1 $\gamma$  and DPPA4 in the context of murine iPSC generation. Depletion of HP1 $\gamma$  was shown to have different effects on iPSC reprogramming depending on the timing of knockdown: early depletion of HP1 $\gamma$  resulted in a decrease in the number of successful iPSCs, whereas late depletion of HP1 $\gamma$  increased the efficiency of reprogramming (Zaidan et al., 2018). Pull-down assays identified pluripotent specific interactions between HP1 $\gamma$  and DPPA4, DPPA2, and also OCT4 (Zaidan et al., 2018), further providing evidence of DPPA4-OCT4 interactions outside of the canonical pluripotency complex. This also suggests that interactions between these factors are important in regulating the acquisition of pluripotency.

## DPPA2/4 IN CANCER

Despite the restriction of *DPPA2/4* expression to pluripotent cells under normal conditions, *DPPA2/4* are reactivated in several cancer types, a specific connection between pluripotency and cancer that was poorly characterized until recently. *DPPA4* is reactivated in bladder, colon, and prostate cancers, as well as in some



non-small cell lung cancers (NSCLCs) (Li et al., 2019; Zhang et al., 2015). In colorectal cancer, *DPPA2* expression strongly correlated with tumor invasion and with more advanced stages of cancer progression (Ghodsi et al., 2015). In NSCLC, the level of *DPPA4* was also strongly associated with disease progression. High levels of *DPPA4* promoted alterations in glycolysis, including increased glucose utilization, lactate production, and LDH activity, that enhanced proliferation in NSCLC (Li et al., 2019). Links between *DPPA4* and alterations in metabolism have also been described in the context of reprogramming somatic cells to pluripotency (Hernandez et al., 2018), suggesting that *DPPA4* mediation of metabolic pathways is a function shared between different cellular states.

While some studies had documented *DPPA2/4* reactivation in various cancer types, the role of *DPPA4* in tumorigenesis was first established by Tung et al. (Tung et al., 2013). By screening a human ES cell cDNA library, they identified *DPPA4* as having oncogenic potential and showed that *DPPA4*-transduced cells have tumor properties, including increased proliferation and anchorage-independent growth *in vitro* and tumor-forming ability *in vivo* (Tung et al., 2013). Gene expression analysis pointed to a potential link between *DPPA4* transformation and the p53 pathway (Tung et al., 2013). Interestingly, they found that *DPPA2* had relatively lower oncogenic activity compared with *DPPA4*, and no synergy in transformation was observed between *DPPA2* and *DPPA4* overexpression (Tung et al., 2013).

A more recent study profiled *DPPA4* binding by ChIP-seq in pluripotent and oncogenic contexts (Klein et al., 2018). In an oncogenic context, *DPPA4* binds to and represses the cell-cycle inhibitor gene *Cdkn2c*, and binds and activates the transcription-factor-coding gene *Etv4*, which promotes proliferation and invasiveness of cancer cells (Klein et al., 2018). Surprisingly, *DPPA4* appears to regulate several genes, including *Etv4*, in opposite directions in an oncogenic compared with a pluripotent context (Klein et al., 2018), suggesting other chromatin factors may influence the mechanism of action for *DPPA4* and determine whether it exerts activating or repressing functions.

Further support for potential differences in the roles of *DPPA4* in a pluripotent and oncogenic context come from the findings that while bivalent domains are most prevalent in pluripotent stem cells, they occur infrequently in cancer. In addition, regions that were bivalent in stem cells are often associated with hypermethylation of associated gene promoters in cancer. Given this, *DPPA2/4* overexpression in cancer does not seem to fit with the roles established for *DPPA4* in stem cells, where it promotes bivalency and inhibits DNA methylation. It may be that the physiological context and available cofactors both affect the functions of *DPPA4* and its family members.

For instance, in cancer *DPPA2/3/4* may still have a role in mediating DNA methylation, but in this context this activity is targeted to tumor suppressor genes.

Another possibility is that *DPPA4* functions in oncogenesis in a manner similar to its role in reprogramming, where it acts to move cells toward a more pluripotent state. Like in reprogramming, *DPPA4* could interact with DDR pathway members to help alter the chromatin at the overlapping sets of genes that can serve pluripotent and oncogenic functions, depending on the context in which they are reactivated. Future studies looking for activation of DDR pathway members associated with *DPPA4* expression in cancer could help shed light on this possibility.

## CONCLUSIONS

Since their identification, attempts at comprehensive characterization of *DPPA2* and *DPPA4* have been thwarted by the many unexpected findings and apparent contradictions about these factors. For example, they are highly expressed in ESCs but not required for pluripotency (Madan et al., 2009; Nakamura et al., 2011), they are not expressed during lung or skeletal development but are required for these processes to occur normally (Madan et al., 2009; Nakamura et al., 2011), and they associate with chromatin but do not behave as classical transcription factors (Masaki et al., 2010). Recently, many high-quality studies have come together to characterize a role for *DPPA2* and *DPPA4* at the transitions between pluripotency and differentiation, where they maintain the chromatin in a state that permits proper differentiation (Eckersley-Maslin et al., 2020; Gretarsson and Hackett, 2020; Hernandez et al., 2018). Together, they also facilitate the reverse process of reprogramming somatic cells to pluripotency (Hernandez et al., 2018). Both in progenitor cells and in cancer they regulate proliferation and metabolic pathways (Hernandez et al., 2018; Klein et al., 2018; Li et al., 2019; Tung et al., 2013), findings that can potentially provide insights into the shared physiology of stem and cancer cells.

Despite the significant advances in recent years, many questions about *DPPA2* and *DPPA4* function remain. A number of studies have documented heterodimerization between *DPPA2* and *DPPA4* (Eckersley-Maslin et al., 2020; Hernandez et al., 2018), but it is not clear whether the majority of *DPPA2* and *DPPA4* in the cell exists in heterodimers, or whether *DPPA2* and *DPPA4* have separate and unique roles at certain gene targets. Recent studies that show a role for *DPPA2* and *DPPA4* in maintaining H3K4me3 at a subset of targets in stem cells (Eckersley-Maslin et al., 2020; Gretarsson and Hackett, 2020), raise intriguing questions about whether *DPPA2* and *DPPA4* continue this function during oncogenesis.



Future studies will likely address such questions and further define the functions of DPPA2 and DPPA4.

## AUTHOR CONTRIBUTIONS

P.K. and R.H.K. wrote and edited the manuscript.

## CONFLICTS OF INTEREST

The authors declare no competing interests.

## ACKNOWLEDGMENTS

This work was supported by NIH grant 1R01NS106878-01A1 to P.K.

## REFERENCES

Alda-Catalinas, C., Bredikhin, D., Hernando-Herraez, I., Santos, F., Kubinyecz, O., Eckersley-Maslin, M.A., Stegle, O., and Reik, W. (2020). A single-cell transcriptomics CRISPR-activation screen identifies epigenetic regulators of the zygotic genome activation program. *Cell Syst.* *11*, 25–41.e9.

Anamthathmakula, P., Miryala, C.S.J., Moreci, R.S., Kyathanahalli, C., Hassan, S.S., Condon, J.C., and Jeyasuria, P. (2019). Steroidogenic factor 1 (Nr5a1) is required for sertoli cell survival post sex determination. *Sci. Rep.* *9*, 4452.

Chen, Z., Xie, Z., and Zhang, Y. (2021). DPPA2 and DPPA4 are dispensable for mouse zygotic genome activation and preimplantation development. *bioRxiv* <https://doi.org/10.1101/2021.08.19.457017>.

De Iaco, A., Coudray, A., Duc, J., and Trono, D. (2019). DPPA2 and DPPA4 are necessary to establish a 2C-like state in mouse embryonic stem cells. *EMBO Rep.* *20*, e47382.

Eckersley-Maslin, M., Alda-Catalinas, C., Blotenburg, M., Kreibich, E., Krueger, C., and Reik, W. (2019). Dppa2 and Dppa4 directly regulate the Dux-driven zygotic transcriptional program. *Genes Dev.* *33*, 194–208.

Eckersley-Maslin, M.A., Parry, A., Blotenburg, M., Krueger, C., Ito, Y., Franklin, V.N.R., Narita, M., D'Santos, C.S., and Reik, W. (2020). Epigenetic priming by Dppa2 and 4 in pluripotency facilitates multi-lineage commitment. *Nat. Struct. Mol. Biol.* *27*, 696–705.

Ghodsi, M., Jafarian, A.H., Montazer, M., Sadeghian, M.H., and Forghanifard, M.M. (2015). Diagnostic clinical relevance of developmental pluripotency-associated 2 (DPPA2) in colorectal cancer. *Int. J. Surg.* *13*, 193–197.

Gretarsson, K.H., and Hackett, J.A. (2020). Dppa2 and Dppa4 counteract de novo methylation to establish a permissive epigenome for development. *Nat. Struct. Mol. Biol.* *27*, 706–716.

Hernandez, C., Wang, Z., Ramazanov, B., Tang, Y., Mehta, S., Dambrot, C., Lee, Y.W., Tessema, K., Kumar, I., Astudillo, M., et al. (2018). Dppa2/4 facilitate epigenetic remodeling during reprogramming to pluripotency. *Cell Stem Cell* *23*, 396–411.e8.

Hu, J., Wang, F., Yuan, Y., Zhu, X., Wang, Y., Zhang, Y., Kou, Z., Wang, S., and Gao, S. (2010). Novel importin-alpha family member

Kpna7 is required for normal fertility and fecundity in the mouse. *J. Biol. Chem.* *285*, 33113–33122.

John, T., Caballero, O.L., Svobodová, S.J., Kong, A., Chua, R., Browning, J., Fortunato, S., Deb, S., Hsu, M., Gedye, C.A., et al. (2008). ECSA/DPPA2 is an embryo-cancer antigen that is coexpressed with cancer-testis antigens in non-small cell lung cancer. *Clin. Cancer Res.* *14*, 3291–3298.

Klein, R.H., Tung, P.Y., Somanath, P., Fehling, H.J., and Knoepfler, P.S. (2018). Genomic functions of developmental pluripotency associated factor 4 (Dppa4) in pluripotent stem cells and cancer. *Stem Cell Res* *31*, 83–94.

Kubinyecz, O., Santos, F., Drage, D., Reik, W., and Eckersley-Maslin, M.A. (2021). Maternal Dppa2 and Dppa4 are dispensable for zygotic genome activation but important for offspring survival. *bioRxiv* <https://doi.org/10.1101/2021.09.13.460183>.

Lee, D.S., Shin, J.Y., Tonge, P.D., Puri, M.C., Lee, S., Park, H., Lee, W.C., Hussein, S.M., Bleazard, T., Yun, J.Y., et al. (2014). An epigenomic roadmap to induced pluripotency reveals DNA methylation as a reprogramming modulator. *Nat. Commun.* *5*, 5619.

Li, H., Long, C., Xiang, J., Liang, P., Li, X., and Zuo, Y. (2020). Dppa2/4 as a trigger of signaling pathways to promote zygote genome activation by binding to CG-rich region. *Brief. Bioinform.* *22*, bbaa342.

Li, L., Wang, Y., Wang, Q., Qu, J., Wei, X., Xu, J., Suo, F., and Zhang, Y. (2019). High developmental pluripotency-associated 4 expression promotes cell proliferation and glycolysis, and predicts poor prognosis in non-small-cell lung cancer. *Mol. Med. Rep.* *20*, 445–454.

Li, Y., Zhang, Z., Chen, J., Liu, W., Lai, W., Liu, B., Li, X., Liu, L., Xu, S., Dong, Q., et al. (2018). Stella safeguards the oocyte methylome by preventing de novo methylation mediated by DNMT1. *Nature* *564*, 136–140.

Madan, B., Madan, V., Weber, O., Tropel, P., Blum, C., Kieffer, E., Viville, S., and Fehling, H.J. (2009). The pluripotency-associated gene Dppa4 is dispensable for embryonic stem cell identity and germ cell development but essential for embryogenesis. *Mol. Cell Biol.* *29*, 3186–3203.

Maldonado-Saldivia, J., van den Bergen, J., Krouskos, M., Gilchrist, M., Lee, C., Li, R., Sinclair, A.H., Surani, M.A., and Western, P.S. (2007). Dppa2 and Dppa4 are closely linked SAP motif genes restricted to pluripotent cells and the germ line. *Stem Cells* *25*, 19–28.

Masaki, H., Nishida, T., Sakasai, R., and Teraoka, H. (2010). DPPA4 modulates chromatin structure via association with DNA and core histone H3 in mouse embryonic stem cells. *Genes Cells* *15*, 327–337.

Miharada, K., Sigurdsson, V., and Karlsson, S. (2014). Dppa5 improves hematopoietic stem cell activity by reducing endoplasmic reticulum stress. *Cell Rep.* *7*, 1381–1392.

Mochizuki, K., Tando, Y., Sekinaka, T., Otsuka, K., Hayashi, Y., Kobayashi, H., Kamio, A., Ito-Matsuoka, Y., Takehara, A., Kono, T., et al. (2018). SETDB1 is essential for mouse primordial germ cell fate determination by ensuring BMP signaling. *Development* *145*, dev164160.





- Nakamura, T., Nakagawa, M., Ichisaka, T., Shiota, A., and Yamanaka, S. (2011). Essential roles of ECAT15-2/Dppa2 in functional lung development. *Mol. Cell Biol.* *31*, 4366–4378.
- Oliviero, G., Munawar, N., Watson, A., Streubel, G., Manning, G., Bardwell, V., Bracken, A.P., and Cagney, G. (2015). The variant Polycomb Repressor Complex 1 component PCGF1 interacts with a pluripotency sub-network that includes DPPA4, a regulator of embryogenesis. *Sci. Rep.* *5*, 18388.
- Qian, X., Kim, J.K., Tong, W., Villa-Diaz, L.G., and Krebsbach, P.H. (2016). DPPA5 supports pluripotency and reprogramming by regulating NANOG turnover. *Stem Cells* *34*, 588–600.
- Sang, H., Wang, D., Zhao, S., Zhang, J., Zhang, Y., Xu, J., Chen, X., Nie, Y., Zhang, K., Zhang, S., et al. (2018). Dppa3 is critical for Lin28a-regulated ES cells naïve–primed state conversion. *J. Mol. Cell. Biol.* *11*, 474–488.
- Somanath, P., Bush, K.M., and Knoepfler, P.S. (2018). ERBB3-Binding protein 1 (EBP1) is a novel developmental pluripotency-associated-4 (DPPA4) cofactor in human pluripotent cells. *Stem Cells* *36*, 671–682.
- Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* *126*, 663–676.
- Theurillat, I., Hendriks, I.A., Cossec, J.C., Andrieux, A., Nielsen, M.L., and Dejean, A. (2020). Extensive SUMO modification of repressive chromatin factors distinguishes pluripotent from somatic cells. *Cell Rep.* *32*, 108146.
- Tung, P.Y., Varlakhanova, N.V., and Knoepfler, P.S. (2013). Identification of DPPA4 and DPPA2 as a novel family of pluripotency-related oncogenes. *Stem Cells* *31*, 2330–2342.
- Yamauchi, K., Ikeda, T., Hosokawa, M., Nakatsuji, N., Kawase, E., Chuma, S., Hasegawa, K., and Suemori, H. (2020). Overexpression of nuclear receptor 5A1 induces and maintains an intermediate state of conversion between primed and naive pluripotency. *Stem Cell Rep.* *14*, 506–519.
- Yan, Q., Zhang, Y., Fang, X., Liu, B., Wong, T.L., Gong, L., Liu, S., Yu, D., Liu, M., Jiang, L., et al. (2021). PGC7 promotes tumor oncogenic dedifferentiation through remodeling DNA methylation pattern for key developmental transcription factors. *Cell Death Differ.* *28*, 1955–1970.
- Yan, Y.L., Zhang, C., Hao, J., Wang, X.L., Ming, J., Mi, L., Na, J., Hu, X., and Wang, Y. (2019). DPPA2/4 and SUMO E3 ligase PIAS4 oppositely regulate zygotic transcriptional program. *PLoS Biol.* *17*, e3000324.
- Yang, L., Liu, X., Song, L., Di, A., Su, G., Bai, C., Wei, Z., and Li, G. (2020). Transient Dux expression facilitates nuclear transfer and induced pluripotent stem cell reprogramming. *EMBO Rep.* *21*, e50054.
- Zaidan, N.Z., Walker, K.J., Brown, J.E., Schaffer, L.V., Scalf, M., Shortreed, M.R., Iyer, G., Smith, L.M., and Sridharan, R. (2018). Compartmentalization of HP1 proteins in pluripotency acquisition and maintenance. *Stem Cell Rep.* *10*, 627–641.
- Zhang, M., Cui, F., Lu, S., Lu, H., Xue, Y., Wang, J., Chen, J., Zhao, S., Ma, S., Zhang, Y., et al. (2015). Developmental pluripotency-associated 4: a novel predictor for prognosis and a potential therapeutic target for colon cancer. *J. Exp. Clin. Cancer Res.* *34*, 60.
- Zhu, L.F., Chen, Q.R., Chen, S.Z., Wang, L.Y., Luo, X.F., Ren, J.H., Yuan, X.H., Wu, X.Q., Zeng, Y.L., Xiao, M., et al. (2017). The construction and identification of induced pluripotent stem cells derived from acute myelogenous leukemia cells. *Cell. Physiol. Biochem.* *41*, 1661–1674.