Developmental Cell
Perspective

Haploidy in Humans: An Evolutionary and Developmental Perspective

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Although haploidy has not been observed in vertebrates, its natural occurrence in various eukaryotic species that had diverged from diploid ancestors suggests that there is an innate capacity for an organism to regain haploidy and that haploidy may confer evolutionary benefits. Haploid embryonic stem cells have been experimentally generated from mouse, rat, monkey, and humans. Haploidy results in major differences in cell size and gene expression levels while also affecting parental imprinting, X chromosome inactivation, and mitochondrial metabolism genes. We discuss here haploidy in evolution and the barriers to haploidy, in particular in the human context.

Introduction

The genetic material of all eukaryotes is organized in the form of distinct chromosomes that reside within the cell nucleus. Diploidy, defined as having two homologous sets of chromosomes per nucleus, is the most common mode of ploidy in multicellular organisms and all mammals (Perrot et al., 1991). Diploid genomes are at the basis of sexual reproduction (Goodenough and Heitman, 2014), as they are the direct outcome of combining two complete haploid sets of chromosomes at mating. From an evolutionary point of view, diploidy provides several key advantages, including the potential to increase genetic variation through the random segregation and recombination of chromosomes during meiosis (Roze, 2009). Moreover, carrying two copies of each allele imperatively affords protection against deleterious phenotypes, as in cases where a mutation occurs in a single allele of an essential gene, while the second allele remains intact and can serve as backup (Perrot et al., 1991). Furthermore, de novo mutations can be corrected based on the sequence of the normal allele by endogenous DNA repair mechanisms (Kondrashov and Crow, 1991).

Nevertheless, haploidy is prevalent in various eukaryotic species that had diverged from diploid ancestors (Otto and Jarne, 2001), demonstrating the capacity to regain haploidy (meaning that diploidy is not irreversible), as well as suggesting that haploidy may bear potential evolutionary benefits by its own nature. Notably, natural haploidy has not been documented in vertebrates, including mammals. The strict absence of haploid genomes in mammals raises a fundamental question: does a haploid mammalian genome in itself pose a sufficient developmental barrier simply because lacking half of the genetic material does not allow proper cellular and developmental functions, or is mammalian haploidy obscured due to constraints that rely on a diploid life cycle? The discovery of haploid embryonic stem cells (ESCs) from several mammalian species (Elling et al., 2011; Leeb and Wutz, 2011; Li et al., 2014; Yang et al., 2013), including the recent derivation of haploid human ESCs (Sagi et al., 2016a), provides new opportunities for addressing basic questions on ploidy requirements in mammalian development and evolution, and particularly in humans.

Haploidy in Evolution

Different eukaryotes have strikingly diverse numbers of chromosomal copies, or ploidies (n). Aside from diploidy (2n), polyploidy (having more than two chromosomal sets per nucleus) is a feature of various species (Comai, 2005), including the allotetraploid frog *Xenopus laevis* (Schmid et al., 2015). Polyploidy is highly frequent and variable in plants (Adams and Wendel, 2005; Soltis et al., 2015), as exemplified by the triploid (3n) banana (genus *Musa*) (Heslop-Harrison and Schwarzacher, 2007), tetraploid (4n) cotton (genus *Gossypium*) (Wendel and Richard, 2003), hexaploid (6n) wheat (genus *Triticum*) (Shewry, 2009), and octoploid (8n) and decaploid (10n) strawberry species (genus *Fragaria*) (Liston et al., 2014).

Polyploidy arises from an increase in the number of complete sets of chromosomes. Conversely, the reverse transition, i.e., from diploidy to haploidy (1n), requires that the number of chromosomal copies is reduced by half, usually through meiosis. Remarkably, numerous organisms across eukaryotic phylogeny are viable as haploids, carrying only one set of chromosomes in at least some part of their life cycle and/or one of the sexes.

Since diploidy is the direct consequence of sexual reproduction, haploidy is the only possible chromosomal complement in unicellular eukaryotes that are exclusively asexual, such as certain protists and fungi (Figure 1). However, many unicellular eukaryotes are also capable of sexual reproduction, whereby haploid individuals are produced by meiosis and diploidy is re-established through mating (Goodenough and Heitman, 2014). Taken together, haploidy is rather prevalent in unicellular eukaryotes such as yeast (Lee et al., 2010), yet much less common in multicellular organisms.

Even so, natural haploidy does occur across different clades of invertebrates (Figure 1). Haplodiploidy, a reproductive strategy in which males are haploid and females are diploid, is common in invertebrates. For example, in nematodes of the order *Oxyurida*, males are haploids as they develop from unfertilized eggs (Adamson, 1990), but the best examples of haploid multicellular organisms are probably demonstrated in insects (Normark, 2003). In species of the order *Hymenoptera* (which

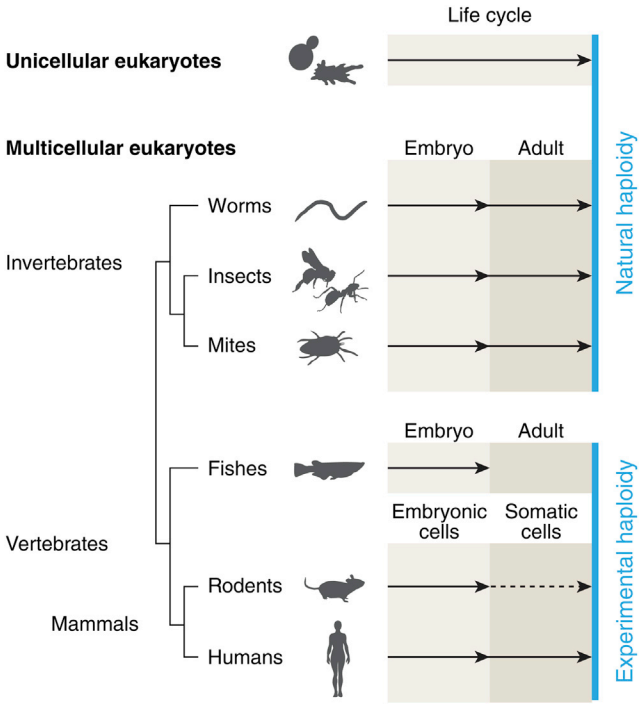


Figure 1. Haploidy in Eukaryote Evolution
Haploidy is common in unicellular eukaryotes but rare in multicellular organisms. Natural haploidy occurs in invertebrates including worms, insects (such as ants and bees), and mites. In vertebrates, haploidy can be achieved experimentally in fish embryos. In mammals, haploid ESCs have been derived from a few species including mouse and rat, and more recently also human. Haploid ESCs can differentiate into haploid somatic cells, as shown mainly in human. Phylogenetic relation is indicated (not to scale).

includes bees, ants and wasps), females are diploid by virtue of sexual reproduction, whereas males are mostly parthenogenetic (i.e., maternally descendant) haploids that develop either from unfertilized eggs or following elimination of the paternal genome after fertilization (Normark, 2003). In these organisms, male haploidy may have been favored due to several evolutionary advantages. First, the generation of males does not require other males; a single queen can produce both male and female offspring by different reproductive modes. In addition, deleterious recessive mutations are unmasked in males, resulting in lethality that enables removal of mutations in essential genes from the gene pool. Interestingly, as observed in several ant species, haploid males can also arise through androgenesis (carrying only a paternal genome) by elimination of the maternal genome following fertilization (Fournier et al., 2005; Ohkawara et al., 2006; Pearcy et al., 2011). This example stresses the significance of haploid genomes in male ants, whether they are achieved through parthenogenesis or androgenesis. Outstandingly, in mites of the species *Brevipalpus phoenicis*, most females are haploid and parthenogenetic (Weeks et al., 2001).

In vertebrates, natural haploidy has not been documented. However, haploid embryogenesis can be induced experimentally, as was demonstrated in fish by the generation of haploid zebrafish (*Danio rerio*) embryos, capable of survival for several days (Walker, 1999), as well as the in vitro derivation of pluripotent ESCs from early medaka fish embryos (*Oryzias latipes*) (Yi et al., 2009) (Figure 1). Importantly, haploid adult fish have not been reported.

Haploidy in Mammals

Deviation from diploidy is highly frequent in tumorigenesis as genetic instability and chromosomal aberrations are important hallmarks of cancer (Davoli and de Lange, 2011; Gordon et al., 2012). In most cases, a tumor cell will become aneuploid (having an irregular number of chromosomes) due to gain or loss of chromosomes, but whole-genome duplications resulting in polyploidy may also occur. Notably, in rare cases, chromosome loss can be extremely extensive, ending up in near-haploidy. Such a case allowed the isolation of the near-haploid leukemic human cell line KBM7 (Carette et al., 2009; Kotecki et al., 1999). Although these cells are transformed and harbor additional genomic alterations (including the *BCR-ABL1* translocation), they were the first human cells with a haploid-like genome to be propagated in culture.

The only haploid mammalian cells that occur normally are mature germ cells, namely the oocyte and sperm, which become haploid as a consequence of reductive nuclear division in meiosis (Clift and Schuh, 2013). As opposed to meiotically derived haploid cells of unicellular organisms, haploid germ cells are terminally differentiated cells incapable of mitotic self-renewal, and whose genomes fuse during fertilization to generate a new diploid organism. Nevertheless, haploid oocytes are able to commence cleavage and early embryonic development without fertilization, generating parthenogenetic embryos that carry an exclusively maternal genome.

Parthenogenesis may occur spontaneously in women, but the resulting embryos cannot survive to term due to the absence of a paternal genome (which is discussed later in more detail). Instead, human parthenogenetic embryonic cells continue to divide and generate benign ovarian tumors called teratomas (Linder et al., 1975; Surti et al., 1990). Importantly, diploid ovarian teratomas can be completely homozygous (Stelzer et al., 2011), suggesting development from a haploid oocyte that had later undergone endoreduplication.

Parthenogenesis can also be induced in culture by exposing an oocyte to chemically or electrically mediated activation that mimics fertilization. Notably, parthenogenetic mouse blastocysts formed by artificial activation of haploid oocytes often contain a mixture of haploid and diploid cells (Tarkowski et al., 1970), indicating that activated mammalian oocytes start dividing and differentiating as haploid cells, but these cells may become diploid in the process. The notion that haploidy may persist after parthenogenesis has recently led to the isolation of haploid mammalian ESCs from parthenogenetic blastocysts, first from mouse (*Mus musculus*) (Elling et al., 2011; Leeb and Wutz, 2011), and later also from rat (*Rattus norvegicus*) (Li et al., 2016) and monkey (*Macaca fascicularis*) (Yang et al., 2013) (Figure 1). Similarly, haploid androgenetic ESCs have also been derived by replacing the maternal genome of the oocyte with the paternal genome of the sperm (Li et al., 2012, 2014; Yang et al., 2012). Haploid ESCs readily proliferate as undifferentiated cells in culture, yet it was proposed that mouse and monkey haploid ESCs cannot be differentiated into mature somatic haploid cells either in vitro or in vivo (Elling et al., 2011; Leeb and Wutz, 2011; Leeb et al., 2012; Yang et al., 2013), suggesting that haploidy poses a barrier for generating somatic cells

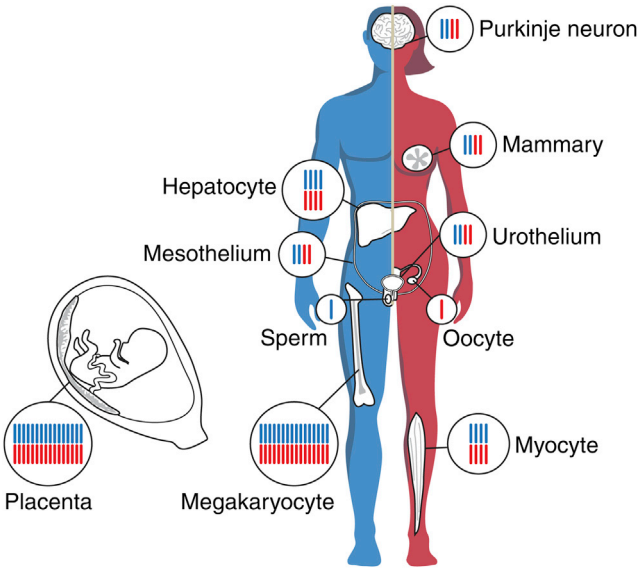


Figure 2. Variation of Ploidy in the Human Body
Most human cell types are diploid, but deviation from diploidy occurs throughout the body. Polyploid cells arise in the extraembryonic placenta, as well as in somatic tissues such as the brain, bone marrow, muscle, liver, mammary glands, and other epithelial tissues. In contrast, the only haploid cells are the egg and sperm.

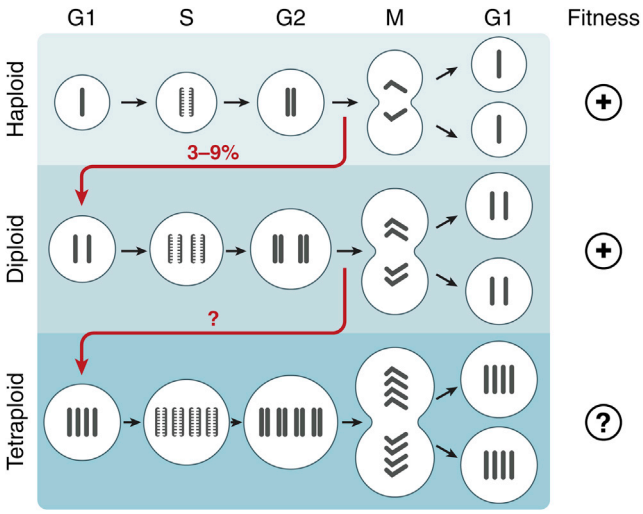


Figure 3. Diploidization and Polyploidization of Human ESCs
Haploid human ESCs can either self-renew, producing two haploid daughter cells, or undergo diploidization by endoreduplication at a rate of about 3%–9% cells per cell cycle. The frequency at which diploid cells convert into tetraploid cells is unknown. Based on their similar proliferation rates, haploid and diploid ESCs likely feature comparable fitness, but the relative fitness of tetraploid cells remains to be determined.

in mammals. However, it has been recently reported that haploid mouse ESCs may retain haploidy after differentiation into neurons (Xu et al., 2017) (Figure 1). Importantly, the difficulty of haploid ESCs to differentiate is not due to their uniparental nature, as upon diploidization these cells can contribute to the development of chimeric animals, as well as colonizing the germline (Leeb et al., 2012).

Haploid Human ESCs

Recently, we have isolated haploid human ESC lines of parthenogenetic origin (Sagi et al., 2016a, 2016b) (Figure 1). In our study, mature human oocytes were activated and subsequent extrusion of the second polar body resulted in a haploid egg with a single maternal pronucleus (Sagi et al., 2016a). These eggs can efficiently develop to the blastocyst stage, allowing derivation of human parthenogenetic ESC lines (Kim et al., 2007; Paull et al., 2013; Revazova et al., 2007). Although previous characterization of such cell lines suggested that they were completely diploid (Paull et al., 2013), we speculated that they may have originated from haploid cells that became diploid in culture, and that rare haploid cells might persist among a majority of diploid cells. We therefore searched for a minority of haploid cells within the diploid populations of multiple human parthenogenetic ESC lines, and indeed identified cell lines in which a small fraction of haploids was preserved (Sagi et al., 2016a). Subsequently, these haploid ESCs were isolated by chemical modification with a viable DNA stain followed by fluorescence-activated cell sorting on the basis of a haploid DNA content (Sagi et al., 2016a, 2016b).

Most intriguingly, haploid human ESCs are not restricted to the undifferentiated state, but can also differentiate into a variety of mature somatic cell type lineages while retaining their haploid genome (Figure 1). In vitro, the haploid human cells differentiated into embryoid bodies with various differentiated haploid cell derivatives expressing specific marker genes of ectodermal (such as brain and skin), mesodermal (such as muscle and kidney), and endodermal tissues (such as liver, pancreas, lung, and intestine), and could also be directed to differentiate into haploid human neurons, cardiomyocytes, and pancreatic cells (Sagi et al., 2016a). Moreover, these cells also formed teratomas in vivo upon injection into immunodeficient mice, with haploid somatic cells organized in defined tissue structures such as the gut epithelium (Sagi et al., 2016a). Most recently, additional haploid human parthenogenetic ESC lines have been derived and shown to differentiate into somatic haploid cells (Zhong et al., 2016).

Diploidization and Polyploidization of Human Cells

Humans are fundamentally diploid, yet variation in ploidy can be observed in different body cells. Unlike duplication of single chromosomes, which causes aneuploidy, polyploidy involves duplication or fusion of entire genomes. Polyploidization is not uncommon in normal human somatic cells (Davoli and de Lange, 2011) (Figure 2). In fetal development, the extraembryonic placenta contains trophoblast giant cells that can reach up to 64n, and multiple cell types in the adult body are also polyploid, including hepatocytes (liver cells), myocytes (muscle cells), Purkinje cells (in the brain), and lactating mammary cells (in the mammary gland) (Davoli and de Lange, 2011). In addition, both the mesothelium and urothelium (membrane linings of thoracic and abdominal cavities and of the urinary tract, respectively) can also be polyploid (Biesterfeld et al., 1994). Perhaps the most impressive example of a polyploid somatic cell is the megakaryocyte, named after its "large nucleus" which can reach 128n before giving rise to multiple platelets (Machlus and Italiano, 2013). The mechanisms underlying polyploidy vary across cell

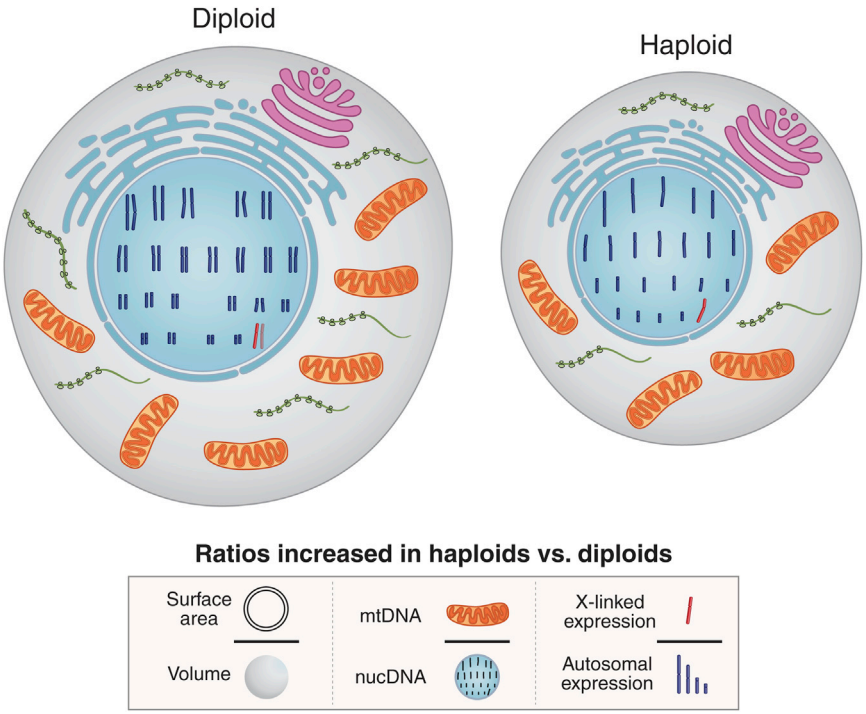


Figure 4. Comparison between Haploid and Diploid Human ESCs
Schematic representation of haploid and diploid human ESCs. The volume of haploid ESCs is smaller than that of diploid ESCs, but the surface area to volume ratio is higher in haploids. Haploid cells also have a higher ratio of mtDNA to nuclear DNA (nucDNA), suggesting a relative increase in mitochondrial abundance. In addition, haploid cells have one X chromosome that remains active, whereas diploid cells often inactivate one of their two X chromosomes. Thus, the gene expression ratio between the X chromosome and autosomes is also higher in haploid cells compared with diploid cells.

types; e.g., polyploidization in muscle cells occurs through cell fusion, but in megakaryocytes it is driven by repeated endoreduplication (Davoli and de Lange, 2011).

Whereas polyploidization affects diploid genomes, diploidization is the parallel process affecting haploid genomes. Since mature germ cells are the only haploid cells that occur normally in humans, physiological diploidization is observed upon the fusion of egg and sperm at fertilization. As described above, both natural and artificial parthenogenesis can also initiate from a haploid human oocyte, generating haploid embryonic cells that gradually become diploid over the course of cell divisions. Diploidization also occurs in haploid human ESCs in culture in a fairly rapid rate (Figure 3). We estimated that 3%–9% of human haploid ESCs become diploid over one cell cycle (Sagi et al., 2016a), and therefore their maintenance demands occasional haploid cell enrichment by cell sorting (Sagi et al., 2016b). Haploid and diploid mouse ESCs have similar growth rates (Elling et al., 2011), suggesting that rather than a growth advantage of diploids over haploids, diploidization may be the main cause for the dilution of haploid cells in the population. Mechanistically, diploidization of haploid mouse ESC results from endoreduplication as a consequence of an aberrant cell cycle, and apparently not from cell fusion (Leeb et al., 2012). Accordingly, accelerating the transition to the G2/M phase by a small molecule can decrease the rate of diploidization in haploid mouse ESCs (Takahashi et al., 2014). It is still unknown which genes and drugs can influence the dynamics of diploidization of haploid human ESCs.

It is entirely unclear why haploid cells are diluted by diploid cells, while diploid cells are not diluted by tetraploid cells by a similar process (Figure 3). It may be that even if diploid cells become tetraploid in culture, in analogy to the diploidization of haploid cells, the rate of polyploidization is very low. Importantly, viable tetraploid mouse and human pluripotent cells have been generated by cell fusion of ESCs and somatic cells, and although these cells are capable of self-renewal and differentiation (Cowan et al., 2005; Tada et al., 2001), diploid cells might still have a significant growth advantage over tetraploid cells in culture. Otherwise, if the rate of tetraploidization were substantial and tetraploids did not have a selective disadvantage compared with diploids, the entire culture would likely become polyploid over time.

The Barriers for Being a "Haploid Human"

The surprising ability of haploid human ESCs to differentiate into mature somatic cells raises fundamental questions about the barriers that haploidy (but not diploidy) poses for human development. As discussed above, previous studies concluded that undifferentiated haploid mouse ESCs have a difficulty to differentiate into somatic haploid cells either in vitro or in vivo (Elling et al., 2011; Leeb and Wutz, 2011; Leeb et al., 2012; Xu et al., 2017). However, these cells sustain the developmental potential of pluripotent cells, as once they diploidize or are used as substitutes for germ cells at fertilization they can support embryogenesis and germline transmission (Leeb et al., 2012; Li et al., 2012; Yang et al., 2012).

In contrast, haploid human ESCs can readily adopt somatic cell fates of all three embryonic germ layers in vitro and in vivo while remaining haploid (Sagi et al., 2016a). They can undergo either spontaneous or directed differentiation using the same protocols designed for the differentiation of diploid cells (Sagi et al., 2016a), demonstrating that haploid and diploid cells have similar responses to given differentiation cues within comparable time frames. Differentiated haploid cells also exhibit the morphological, molecular, and functional changes observed in their diploid counterparts, emphasizing that haploid and diploid somatic cells are highly similar. Furthermore, the differentiation and tissue organization of haploid human cells in vivo resembles those observed with diploid cells (Sagi et al., 2016a).

The differentiation capacity of haploid human ESCs brings up issues regarding the barriers of haploidy in humans. Although haploid and diploid human ESCs are comparable in terms of

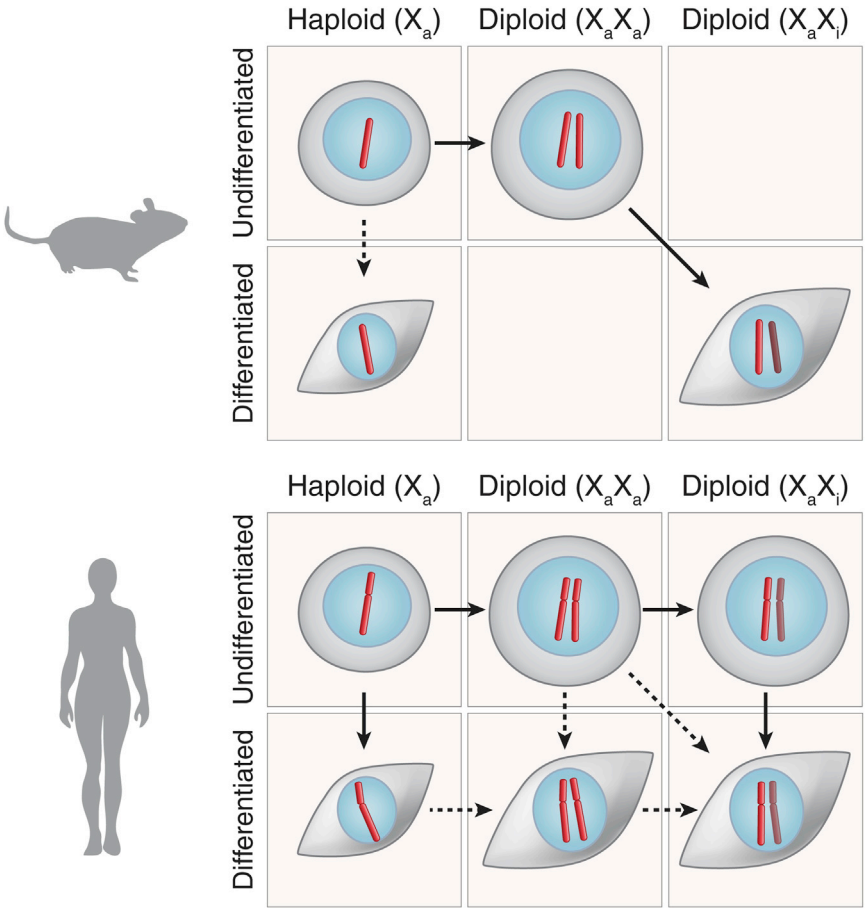


Figure 5. X Chromosome Inactivation Status and Differentiation Propensity of Haploid and Diploid ESCs in Mouse and Human

In mouse, haploid ESCs have one X chromosome, which is transcriptionally active, and they may differentiate into haploid somatic cells. These haploid ESCs can diploidize into cells carrying two active X chromosomes, and are able to differentiate concomitantly with X chromosome inactivation (XCI). In human ESCs, the status of XCI is more variable than in mouse. Haploid cells, carrying a single active X chromosome, can exist in both the undifferentiated and differentiated states, suggesting that dosage compensation is not required for differentiation. Diploid cells may retain two active X chromosomes or display XCI either as undifferentiated or differentiated cells. Solid and dash lines represent demonstrated and presumed conversions, respectively.

proliferation and differentiation, they also display several inherent differences that might help explain these barriers (Figure 4).

DNA and RNA Levels and Cell Size

By definition, haploid human ESCs have half the amount of nuclear DNA (nucDNA) of diploid cells, comprising a karyotype of 23 chromosomes rather than 46. Notably, this reduction in DNA content also correlates with a marked decrease in RNA expression levels (Sagi et al., 2016a) (Figure 4). The volume of haploid cells corresponds to about 60% of the volume of their diploid counterparts, whereas their cell surface area is about 70% of that of diploid cells. This results in a higher surface area to volume ratio in haploids compared with diploids (Figure 4), suggesting that regulatory mechanisms may act in haploid cells to compensate for this difference. For example, maintaining a certain density of membrane proteins in haploids and diploids (a function of surface area) would likely be achieved differently than maintaining a comparable concentration of cytoplasmic proteins (a function of volume).

Mitochondrial Abundance

Despite having half the nucDNA of diploid cells, haploid ESCs seem to have about two-thirds of their mtDNA (Sagi et al., 2016a), meaning that the ratio of mtDNA to nucDNA is higher in haploids (Figure 4). An elevated mtDNA content may correspond to an increased abundance of mitochondria in the cell, which would potentially affect mitochondrial metabolism. Oxidative phosphorylation is one of the major metabolic pathways occurring in mitochondria, and the proteins comprising the different subunits of the oxidative phosphorylation complexes are encoded in both mtDNA and nucDNA (Quirós et al., 2016). The relative increase in mtDNA probably underlies the upregulation of all mitochondrially encoded oxidative phosphorylation genes observed in haploid cells (Sagi et al., 2016a). Many oxidative phosphorylation genes encoded in the nucleus are also expressed at higher levels in haploid cells (Sagi et al., 2016a), likely reflecting a compensatory response to the discrepancy in the mtDNA to nucDNA ratio. It is plausible that the relatively higher expression of mitochondrial genes in haploid cells serves to achieve higher levels of oxidative phosphorylation, suggesting that the haploid state might be associated with increased energy demands.

X Chromosome Inactivation

Another difference between haploid and diploid ESCs is related to X chromosome inactivation (XCI), a developmental process which allows dosage compensation of X-linked genes between males and females, despite having different numbers of X chromosomes (one and two, respectively) (Deng et al., 2014; Schulz and Heard, 2013). As a result, one of the X chromosomes in female somatic cells is transcriptionally active (X_a), whereas the second X chromosome undergoes epigenetic silencing that renders it inactive (X_i). XCI also results in a fixed ratio of gene expression between the X chromosomes and autosomes regardless of sex (X:A = 1:2). Diploid female human ESCs can display variable XCI states, being X_aX_a or, as observed more often, X_aX_i (Bruck and Benvenisty, 2011; Silva et al., 2008). Haploid cells have only one X chromosome (similar to male diploid cells), which must remain active due to the essentiality of X-linked genes (Sagi et al., 2016a). Thus, the X:A expression ratio is higher in X_a haploid ESCs than in X_aX_i diploid ESCs with XCI (1:1 compared with 1:2) (Figure 4). Importantly, this dosage imbalance between the autosomes and X chromosome in undifferentiated haploid human cells also persists in haploid human somatic cells (Sagi et al., 2016a).

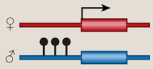
Haploidy-related feature	Affected feature	Type of effect	
		Qualitative	Quantitative
Uniparental origin 	Imprinted genes	+++	+++
	↓		
	Target genes	–	+++
X-chromosome regulation 	X:A expression ratio	–	++
	↓		
	Autosomal target genes	–	++
Mitochondrial abundance 	Oxidative phosphorylation	–	+

Figure 6. Barriers for Haploidy in Human Development

Uniparental origin, X chromosome regulation, and mitochondrial abundance are haploidy-related features that confer gene expression differences between haploid and normal diploid cells. Whereas the absence of a complete set of imprinted genes due to uniparental origin is a qualitative difference (a gene is either expressed or not expressed), the other differences are quantitative, representing relative changes in the expression levels of specific genes. "All-or-none" qualitative differences are predicted as more developmentally restrictive.

Mouse and human haploid cells are markedly different with regard to their XCI status before and after differentiation (Figure 5). Haploid human ESCs differentiate into haploid somatic cells more readily than their mouse counterparts (Elling et al., 2011; Leeb and Wutz, 2011; Leeb et al., 2012; Sagi et al., 2016a; Xu et al., 2017). Moreover, diploid female mouse ESCs have two active X chromosomes, and they undergo XCI only upon differentiation (the X_aX_a state and differentiation are mutually exclusive). In humans, however, undifferentiated diploid female ESCs can exist in both the X_aX_i and X_aX_a states (Bruck and Benvenisty, 2011; Silva et al., 2008). Diploid somatic human cells are normally X_aX_i, but the X_aX_a state might also persist in differentiation (Figure 5). Taken together, these observations illustrate that, compared with mouse cells, human cells are much less constrained with respect to the possible co-existence of different states of ploidy, XCI, and differentiation. In agreement with this notion, X chromosome regulation is highly divergent during early development in mouse and human (Okamoto et al., 2011; Petropoulos et al., 2016). In particular, the long non-coding RNA *XIST*, which initiates inactivation of one of the two X chromosomes on differentiation, is not expressed in the inner cell mass (ICM) of female mouse blastocysts, but is expressed from both X chromosomes in the female human ICM. In human ICM cells, both X chromosomes remain active despite *XIST* expression, but X-linked gene levels are nonetheless compensated to conform with those in males by an unknown mechanism (Petropoulos et al., 2016). Interestingly, the in vivo X_aX_a *XIST*-expressing state is not recapitulated in conventional human ESCs in vitro, providing additional evidence for the relative flexibility of XCI in human cells.

The basis for the observed disparity in differentiation capacity between mouse and human haploid ESCs has yet to be resolved. The regulation of X chromosome gene expression has been proposed as the underlying cause for the challenge to differentiate mouse haploid cells (Leeb and Wutz, 2013). The tight coupling between differentiation and XCI in female mouse ES (Figure 5) leads to X chromosome dosage compensation in differentiated cells. This in vitro process closely resembles XCI during early mouse development, which is considered essential for proper embryogenesis (Schulz and Heard, 2013). Haploid mouse cells, whose single-copy X chromosome remains active, would not be able to bring about dosage compensation. Because the regulation of XCI in female human ESCs is not as strict as in the mouse (Figure 5), it is possible that distinct states of dosage compensation are tolerated in undifferentiated human cells. Moreover, this dosage imbalance does not seem to act as a barrier for the differentiation and survival of haploid human somatic cells.

Parental Imprinting

The genomes of normal diploid human cells consist of 23 maternal chromosomes and 23 paternal chromosomes. By definition, haploid human cells harbor only one of these parental sets, and are thus considered uniparental. In mammals, parental genomes are functionally non-equivalent; the contributions of both the maternal and paternal genomes are essential for proper embryogenesis (McGrath and Solter, 1984; Surani et al., 1984). This co-dependence is conferred by parental imprinting, a process by which parent-specific epigenetic patterns are differentially established in oocytes and sperm and direct the allele-specific expression of imprinted genes in offspring according to parental origin (Barlow and Bartolomei, 2014; Ferguson-Smith, 2011). Uniparental development and asexual reproduction are therefore restricted by imprinting dysregulation, as imprinted genes normally expressed from a given parental allele would not be expressed in uniparental cells. Notably, uniparental disomy or the absence of specific chromosome regions encoding imprinted genes can manifest as developmental imprinting disorders such as Prader-Willi syndrome, Angelman syndrome, and Russell-Silver syndrome (Peters, 2014).

Imprinting is one of the best examples for a diploidy-dependent evolutionary constraint, as its emergence was not only contingent on diploidy but it also reinforced diploidy (and sexual reproduction) in mammals. Consequently, imprinting prevents not only uniparental development but also the development of a haploid organism. Indeed, human parthenogenesis does not allow proper embryogenesis, yet it is compatible with pluripotency both in vivo and in vitro, as demonstrated by the generation of ovarian teratomas (Linder et al., 1975; Surti et al., 1990) and the differentiation potential of parthenogenetic pluripotent stem cells (Mai et al., 2007; Revazova et al., 2007; Stelzer et al., 2011), including haploid parthenogenetic ESCs (Sagi et al., 2016a).

Haploidy-Related Differences in Development

It is clear that haploid human ESCs can differentiate into haploid somatic cells despite their uniparental origin and their inability to

balance the dosage of X-linked genes. This notion highlights an important distinction between the differentiation potential of pluripotent cells in development and in culture: whereas proper imprinting and XCI are both essential during development, their dysregulation is insufficient to impede ESC differentiation. Nonetheless, outlining the specific extents to which these phenomena affect the biology of haploid cells enables speculation on their relative contributions to the developmental barriers associated with haploidy (Figure 6).

In haploid cells, the absence of an entire chromosome set from one of the parents results in major differences in the transcriptome of haploids versus normal diploids cells: a complete subset of parent-specific imprinted alleles is absent, leading to an "all-or-none" qualitative difference in gene expression and, in turn, further results in quantitative alteration in the genome-wide expression levels of additional target genes, which are normally regulated by the missing imprinted genes (Figure 6).

The inherent inability to undergo XCI and achieve dosage compensation in haploid cells also has a quantitative influence on their transcriptome, but not in an "all-or-none" manner (Figure 6). First, the expression ratio between X-linked genes and autosomal genes is higher in haploid cells compared with normal diploid cells, leading to a wide-ranging imbalance in gene dosage. Second, many autosomal genes are regulated directly or indirectly by genes encoded on the X chromosome, rendering the transcriptome of cells with only one active X chromosome significantly different from that of cells with two active X chromosomes (Bruck et al., 2013).

The differential regulation of oxidative phosphorylation genes and the different mtDNA content add further quantitative differences between haploids and diploids (Figure 6). However, since the regulation of mitochondrial metabolism can differ across cell types and under different conditions in normal development, it is less likely to act as a major haploidy-related barrier.

The accumulation of qualitative and quantitative differences in the molecular signature of haploid cells makes them considerably distinct from normal diploid cells. We propose that although haploid cells retain the ability to differentiate, their unique gene expression profile, and mainly the lack of expression of an entire set of parentally imprinted genes, prevents them from contributing to a haploid human.

Conclusions and Outlook

The recent derivation of haploid human ESCs and their potential to differentiate into haploid somatic cells pave the way for new avenues in evolutionary-developmental research. From an evolutionary point of view, haploid human ESCs place humans among the already-existing repertoire of haploid cells and organisms. The viability of haploid undifferentiated and differentiated human cells suggests that haploidy per se is not a major barrier for asexual reproduction in humans, emphasizing the critical developmental role of diploidy-dependent evolutionary constraints. We speculate that parental imprinting in particular poses one of the most significant developmental blocks in that regard.

It is still unknown how haploid human ESCs can readily differentiate into haploid somatic cells, whereas their rodent counterparts have a difficulty to do so. In that regard, haploid cells and their diploid derivatives may serve as valuable tools for studying X chromosome regulation in humans compared with mice. In addition, the rather rapid diploidization rate observed in haploid mammalian ESCs raises intriguing questions about the potential of diploids to further convert into polyploids, and about the genes and cellular pathways involved in endoreduplication in development and disease. Aside from the value of haploid human ESCs in understanding human development, they may also play a role in future procedures of reproductive medicine, providing a putative source for haploid gametes. Yet, the most obvious usefulness of haploid human ESCs is in the context of haploid genetics, enabling simplified loss-of-function screening on a human genetic background (for a broader view on the biomedical applications of haploid human ESCs, see Yilmaz et al., 2016). We anticipate that haploid human ESCs will serve as useful tools for studying human genetics, development, and pathology in a wide range of research fields.

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