Haploid animal cells

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DEVELOPMENT AT A GLANCE

Haploid animal cells

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ABSTRACT

Haploid genetics holds great promise for understanding genome evolution and function. Much of the work on haploid genetics has previously been limited to microbes, but possibilities now extend to animal species, including mammals. Whereas haploid animals were described decades ago, only very recent advances in culture techniques have facilitated haploid embryonic stem cell derivation in mammals. This article examines the potential use of haploid cells and puts haploid animal cells into a historical and biological context. Application of haploid cells in genetic screening holds promise for advancing the genetic exploration of mammalian genomes.

KEY WORDS: Haploid cells, ES cells, Genetic screening, Transgenics, Genomic imprinting, X inactivation

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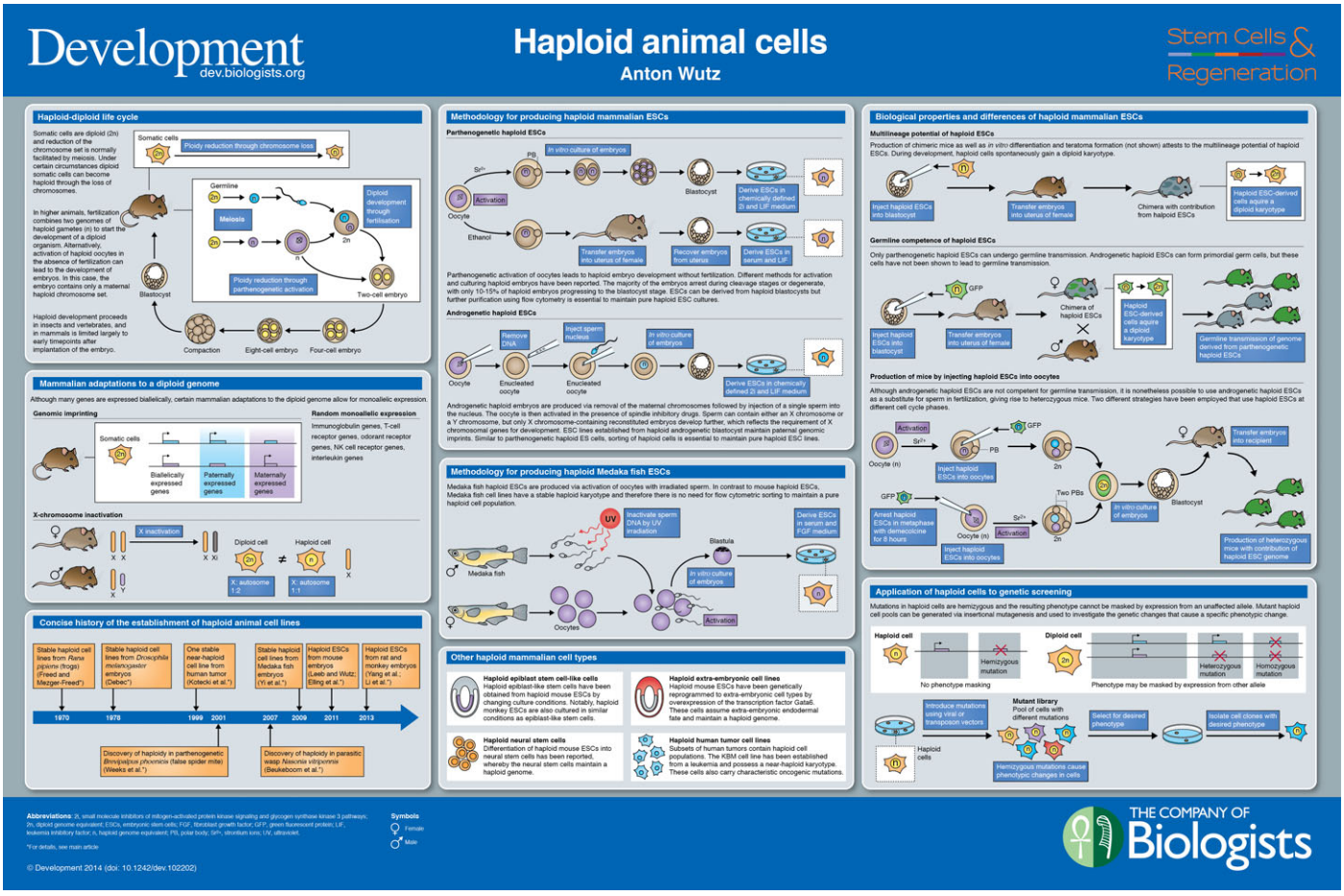
Introduction

Diploidy (from the Greek word diplous, meaning ‘double’) is a term used to denote the existence of two sets of chromosomes within a cell or an organism. Diploid genomes are typical among most living animals, and haploidy (a single set of chromosomes) is generally limited to the gametes. This is in sharp contrast to plants that possess significant haploid phases in their life cycle. One benefit of a largely diploid genome in animals may be increased fitness, which could result from the ability to mask mutations in the diploid but not haploid genome. However, this same effect also incurs long-term disadvantages that result from less efficient selection, which leads to an accumulation of mutations. It is in this light that mechanisms in animal genome evolution might have arisen to counteract long-term degeneration. Mammalian adaptions to the diploid genome include genomic imprinting, random monoallelic expression, and X chromosome inactivation. In some insect species, haploidy in males provides an effective means by which to purge a genome of mutations before transmission to the next generation (White, 1984). This likely serves to maintain or improve fitness over long evolutionary time periods.



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Only rare cases of haploid animals have been observed, and these are limited to invertebrates. Most notably, haploid mite and wasp species have both been described (Beukeboom et al., 2007; Weeks et al., 2001). In vertebrates, haploid forms do not occur naturally but have been experimentally produced, for example in fish and in mice. Haploid development can be initiated in fish species by destroying genomic DNA in either one of the gametes prior to fertilization (Brandhorst and Corley-Smith, 2004; Wiellette et al., 2004). Haploid zebrafish embryos progress through organogenesis but rarely reach mature stages (Wiellette et al., 2004). Haploid mammals can be generated in a similar manner by activation of eggs or by transplanting sperm nuclei into enucleated oocytes by micromanipulation, resulting in the production of parthenogenetic and androgenetic embryos, respectively (Graham, 1966; Modliński, 1975). Parthenogenesis in mammals refers to development from oocytes without fertilization, whereas androgenesis is development from only the paternal genome and requires destruction or elimination of the oocyte genome. In addition to these methods, bisection of mouse zygotes has been shown to lead to haploid embryo development (Tarkowski and Rossant, 1976). Haploid mouse embryos develop through the blastocyst stage with reduced efficiency; they can implant but development appears to become progressively delayed. Haploid cells have been detected up to the egg cylinder stage of embryos (Kaufman, 1978).

Haploid embryonic stem cells (ESCs) can be derived and maintained in culture from a number of different animal species. The artificial generation of haploid Medaka fish embryos enabled the derivation of the first haploid ESCs, demonstrating that haploid genomes can be maintained in proliferating cell cultures (Yi et al., 2009; Yuan et al., 2013). Recently, a number of studies have derived ESCs from mammalian parthenogenetic and androgenetic haploid embryos. Initial studies were focused on mouse (Elling et al., 2011; Leeb and Wutz, 2011; Li et al., 2012; Yang et al., 2012); however, similar techniques have also been applied to monkey (Yang et al., 2013) and rat haploid ESC derivation (Li et al., 2013). These cell lines have been shown to maintain a haploid karyotype under appropriate culture conditions. Advances in culture conditions and flow cytometric cell sorting technology are key factors to the success of haploid ESC derivation.

Haploid cells derived from mammalian embryos

Haploid ESCs maintain the typical characteristics of mouse ESCs and proliferate with similar kinetics to diploid ESCs. Gain of a diploid karyotype is associated with successive passages, which most likely occurs through suppression of cytokinesis (Leeb et al., 2012). For this reason, long-term culture of haploid ESCs requires enrichment for the haploid karyotype every 3 to 5 passages via flow cytometric cell sorting. The developmental potential of haploid mouse ESCs has been assessed using *in vitro* differentiation, teratoma formation assays and production of chimeric mice (Elling et al., 2011; Leeb and Wutz, 2011; Li et al., 2012; Yang et al., 2012). Both parthenogenetic and androgenetic haploid ESCs can give rise to differentiated cells in all of these assays, whereby differentiation is accompanied by gain of a diploid karyotype. Chimeric mice from parthenogenetic haploid ESCs show transmission through the female germline (Leeb et al., 2012), whereas formation of primordial germ cells from haploid androgenetic ESCs has been documented [although these cells did not undergo germline transmission (Li et al., 2012; Yang et al., 2012)]. This difference might be grounded in differential imprinting of androgenetic compared with pathenogenetic haploid ESCs, whereby androgenetic cells maintain paternal rather than maternal imprints. This makes them suitable for artificial fertilization experiments whereby a haploid androgenetic ESC nucleus is transplanted into an unfertilized oocyte (Li et al., 2012; Yang et al., 2012). This procedure has been shown to lead to the development of normal heterozygous mice with one copy of the genome derived from the haploid ESC. Recently, it has also been shown that haploid parthenogenetic mouse ESCs can substitute for the maternal genome in semi-cloning experiments (Wan et al., 2013). In principle, both routes can be envisioned for introducing genetic modifications into mice. In rats, the situation is different as it has been shown that androgenetic haploid rat ESCs can produce germline competent chimeric rats as well as substitute for sperm in artificial fertilization experiments (Li et al., 2013).

Attempts to obtain haploid cell types other than ESCs have met with different results. Although the derivation of haploid epiblast-like stem cells and haploid neural stem cells has been reported from androgenetic haploid ESCs (Li et al., 2012), an independent study of parthenogenetic haploid ESCs revealed strong inclination to diploidization in these same lineages (Leeb et al., 2012). By contrast, forced differentiation of haploid parthenogenetic ESCs into extra-embryonic fates by Gata6 was found to be associated with maintenance of a mainly haploid karyotype (Leeb et al., 2012). Although there is little selective pressure for a diploid genome in the pre-implantation embryo (Leeb and Wutz, 2012), the requirement for a diploid genome in somatic differentiated cells is unknown.

Haploid cells in human tumors

There are several reports of human tumors in which the chromosome number has been severely reduced (Aspberg et al., 1995; Flagiello et al., 1998; Gibbons et al., 1991; Sukov et al., 2010). These strong hypodiploid or near-haploid tumors occur with low frequency across a range of different tumor types. It has been suggested that a haploid karyotype might facilitate the loss of tumor suppressive genes in a single hit and thereby select for haploid genomes (Bovée et al., 2000; Safavi et al., 2013). However, it remains unclear by which mechanism a reduction of chromosome number is achieved. In some cases, the haploid cells can undergo a subsequent gain in ploidy to become either diploid again or polyploid, with largely homozygous markers (Bovée et al., 2000; Safavi et al., 2013). Gain in ploidy could lead to an underestimation of haploidization rates in human tumors, as these genomes are not readily identifiable using standard karyotpic analyses and instead require molecular typing (Onodera et al., 1992; Safavi et al., 2013). Notably, a near haploid tumor cell line has been established in culture from a leukemia (Kotecki et al., 1999). The resulting cells, called KBM cells, are mainly haploid and possess a (9,22) chromosomal translocation (BCR-ABL) in addition to extra copies of chromosome 8 and 15. A further derived subclone has since lost the duplication of chromosome 15 and has been maintained in culture over many passages with a mainly haploid karyotype (Kotecki et al., 1999). Subsequent experiments have resulted in an adherent cell line, HAP1, that has lost the additional copy of chromosome 8 (Carette et al., 2011). This cell line is mainly haploid but also has reduced karyotypic stability. Taken together, the observations of near-haploid cells in human tumors suggest that a haploid genome is compatible with somatic cell survival and proliferation, at least after transformation.

Applications of haploid cells in genome exploration and genetics

A primary interest in haploid mammalian cells lies in their use in forward genetics experiments. In a haploid genome, mutations are hemizygous and therefore phenotypes are immediately exposed.

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This is a distinct advantage when screening of a large pool of random mutations towards a specific phenotype. By contrast, phenotypic screening of mutations in diploid cells is difficult when recessive mutations are considered. In animals, breeding to homozygosity is required but in cells no readily available method is known. Haploid screens have been pioneered in near-haploid human tumor cells (Carette et al., 2009) and more recently also applied to haploid mouse ESCs (Elling et al., 2011; Leeb and Wutz, 2011; Li et al., 2010). Both cell systems have distinct strengths and weaknesses. For example, screening in human tumor cells can identify genes in a clinically relevant genome (Birsoy et al., 2013; Carette et al., 2009; Carette et al., 2011; Guimaraes et al., 2011; Reiling et al., 2011; Rosmarin et al., 2012), but tumor-specific genetic aberrations and oncogenic signals could interfere with the particular aim of a genetic screen. The stable haploid karyotype of human tumor cells and ease of culture are additional technical advantages over haploid mouse ESCs. Haploid mouse ESCs can provide a largely intact genome and broad differentiation potential, which could in principle allow screening in developmentally relevant pathways and in a wide range of cell types (Leeb et al., 2014). The eventual gain of a diploid karyotype during differentiation of the genetically altered haploid ESCs would then result in homozygous mutations amenable to phenotypic screening. Consideration of which haploid cell system to use will depend on the particular aim and expectations of the screen.

To date, reported haploid cell screens have applied insertional mutagenesis using viral (Carette et al., 2009; Elling et al., 2011) or transposon-derived vectors (Leeb and Wutz, 2011; Li et al., 2010). This procedure simplifies the identification of mutated genes through isolation of the genomic insertion site of the insertional mutagen. High mutagenic effect is achieved by gene-trap type insertional vectors (Friedel and Soriano, 2010). In addition to mutagenic screening strategies, reporter systems for investigating pathway activity have been employed more recently, and hold potential for investigating molecular interactions and pathways in mammalian cells (Lee et al., 2013). Haploid mouse ESCs can also be derived from genetically modified mouse strains and open up the possibility of tailor-made screening approaches for specific genetic backgrounds.

Perspectives

Haploid animal cells provide an exciting tool for studying how genomes function under different ploidy conditions, and also a powerful tool for genetic screening. It is remarkable that haploid and diploid ESCs appear to proliferate with approximate equal kinetics (Elling et al., 2011; Leeb and Wutz, 2011; Yi et al., 2009). However, an intrinsic inclination to gain a diploid genome over time in culture suggests a greater stability of the diploid karyotype. In mouse ESCs, this is surprising as dosage compensation is not initiated and genomic imprinting appears to play a minor role (Leeb and Wutz, 2012). In the short time since their discovery, haploid mammalian cells cultured *in vitro* have already contributed to gene discovery in the context of targeted forward genetics screens, establishing this system as a robust and dependable screening tool. In the future, haploid cell systems will provide an additional or alternative option to existing technology based on RNA interference or the recently introduced CRISPR-Cas9 technology (Wang et al., 2014). The use of genetically modified androgenetic haploid ESCs instead of sperm in nuclear transfer experiments holds great promise for the creation of genetically modified animals, e.g. non-human primates, to model human disease. Recent reports on the derivation of haploid ESCs from monkey (Yang et al., 2013) and rat embryos (Li et al., 2013) highlight the potential for haploid cell techniques in a range of mammalian species. It can be anticipated that haploid ESCs will be derived from different mammalian species in the future, thereby making different mammalian genomes amenable to genetic screening. Progress in establishing haploid cell cultures also makes one wonder whether derivation of human haploid ESCs could be accomplished.

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Competing interests

The author has no conflict of interest related to the content of this article but would like to make it known that a patent application has been published that covers the derivation of haploid ESCs.

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Development at a Glance

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