Causes and consequences of chromosome segregation error in preimplantation embryos

Cayetana Vázquez-Diez and Greg FitzHarris

1Centre de Recherche du Centre Hospitalier de l’Université de Montréal, Montréal, Québec, Canada and
2Département d’Obstétrique-Gynécologie, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada

Correspondence should be addressed to G FitzHarris; Email: greg.fitzharris@umontreal.ca

Abstract

Errors in chromosome segregation are common during the mitotic divisions of preimplantation development in mammalian embryos, giving rise to so-called ‘mosaic’ embryos possessing a mixture of euploid and aneuploid cells. Mosaicism is widely considered to be detrimental to embryo quality and is frequently used as criteria to select embryos for transfer in human fertility clinics. However, despite the clear clinical importance, the underlying defects in cell division that result in mosaic aneuploidy remain elusive. In this review, we summarise recent findings from clinical and animal model studies that provide new insights into the fundamental mechanisms of chromosome segregation in the highly unusual cellular environment of early preimplantation development and consider recent clues as to why errors should commonly occur in this setting. We furthermore discuss recent evidence suggesting that mosaicism is not an irrevocable barrier to a healthy pregnancy. Understanding the causes and biological impacts of mosaic aneuploidy will be pivotal in the development and fine-tuning of clinical embryo selection methods.

Introduction: the advent of the mosaic embryo

Preimplantation development is initiated by the fusion of highly specialised gametes, the sperm and the oocyte, resulting in the formation of a totipotent zygote. Faithful execution of the first several cell divisions after fertilisation is fundamental to the establishment of a healthy pregnancy. Following fertilisation, the male and female genomes form pronuclei in the zygote, whose subsequent breakdown marks the onset of the first mitotic division. Preimplantation mitotic cell divisions are ‘reductive’, meaning unaccompanied by cellular growth, thereby producing progressively smaller cells (Fleming & Johnson 1988, Tsichlaki & FitzHarris 2016). Simultaneously, numerous developmentally important events take place. For example, zygotic genome activation at the 4- to 8-cell stage in humans and 2- to 4-cell stage in mouse means that the embryo no longer relies exclusively on maternally stockpiled mRNAs and proteins and can synthesise these factors from the embryonic genome (Niakan et al. 2012, Lee et al. 2014). Compaction at the 8-cell stage brings the dividing cells into close adherence with each other, and cavitation at the 16- to 32-cell stage creates a fluid-filled cavity marking arrival at the blastocyst stage of development (Fleming & Johnson 1988). Blastocyst formation is also accompanied by the clear delineation of the first two cell fate lineages in the embryo; the trophoderm which will give rise to extraembryonic structures, and the inner cell mass which will constitute all embryonic tissues (Morris & Zernicka-Goetz 2012, Niakan et al. 2012, Rossant 2016) (Fig. 1). This complex and coordinated series of events all take place as the embryo travels down the fallopian tubes from the ovary en route to the implantation site, the endometrium.

Thus, preimplantation development presents a highly unusual cellular context in which to execute such a critical succession of cell divisions.

Given that the early mitoses form the small number of cells from which the entire organism develops, one might imagine that these mitoses should be heavily safeguarded to ensure genetic fidelity is maintained. Rather, early mammalian development is synonymous with cell division errors. It has long been recognised that aneuploidy, when cells have an abnormal number of chromosomes, may be linked to reduced fertility since genetic aberrations are common in tissues from spontaneous miscarriages and most unisomy and trisomy aneuploid karyotypes are non-viable (Hassold & Hunt 2001, Jones & Lane 2013, Webster & Schuh 2017). Whole-embryo single chromosome copy number aberrations are predominantly due to chromosome segregation defects in oocyte meiosis, which markedly increase with advanced maternal age (Hassold & Hunt 2001). However, the introduction and development of improved culture methods and assisted reproduction...
technologies such as in vitro fertilisation (IVF) in the last four decades provided an opportunity to study the chromosomal status of cells during early human development, and led to the realisation that whilst meiotically derived whole-embryo aneuploidies occur in some embryos, ‘mosaic’ aneuploidy, where only a subset of blastomeres within an embryo are aneuploid, is more prevalent (Taylor et al. 2014). This phenomenon was first reported in 1993 using simple Fluorescent in situ hybridisation (FISH) to label and count the copies of a limited number of chromosomes, and subsequent studies using whole-genome hybridisation and modern sequencing approaches have revealed single chromosome gains or losses as the predominant genetic anomaly in mosaic embryos, occurring in up to 90% of embryos, depending upon the study (Delhanty et al. 1993, Munné et al. 1993, 2017, Echten-Arends et al. 2011, Vera-Rodriguez & Rubio 2017). Additionally, polyploid and segmental mosaic aneuploidies are observed, albeit at much lower incidences (Echten-Arends et al. 2011) (Fig. 2). Chromosomal mosaicism has also been reported in non-human primate, porcine, bovine and murine embryos, suggesting it may be a wide-reaching phenomenon (Dupont et al. 2010, Elaimi et al. 2012, Hornak et al. 2012, 2016, Bolton et al. 2016). Chromosomal mosaicism must originate from mitotic errors during preimplantation development (Mantikou et al. 2012, Taylor et al. 2014), but why the early mammalian embryo is inherently susceptible to mitotic errors, and precisely how these errors come about, is very poorly understood.

Many excellent studies have used observed chromosome complements in spare embryos from the clinical setting to attempt to extrapolate the series of events that lead to embryo mosaicism (Coonen et al. 2004, Mantikou et al. 2012, Fragouli et al. 2013, Taylor et al. 2014). However, understanding the aetiology of mitotic errors requires visualisation of the events in real time, and interventional experiments to probe the role of molecular players – experiments that are hard to tackle in the clinical setting. Therefore, the underlying cellular mechanisms through which chromosome segregation errors arise in early human development remain mostly elusive. Since many reviews of clinical literature cover the incidence and characteristics of preimplantation mosaicism (Echten-Arends et al. 2011, Taylor et al. 2014,
Munné et al. 2017), we here discuss what is known about the mechanisms of cell division in mammalian embryos, highlighting recent key advances that point towards perspectives on the aetiology and impact of mitotic chromosome segregation errors in early embryos, and then go on to consider their consequences. For reviews of other types of genomic errors occurring in embryos such as segmental aneuploidies, chromosomal rearrangements, microdeletions and duplications, see (Capalbo et al. 2017, Morin et al. 2017, Treff & Franasiak 2017).

The mechanics of chromosome segregation in mammalian embryos

Form and function of the mammalian spindle

Accurate chromosome segregation at the time of cell division is essential to preserve genetic integrity and is achieved through a highly coordinated series of events. As cells enter mitosis, the nuclear envelope breaks down and chromatin becomes further condensed into mitotic chromosomes. Simultaneously, microtubule-organising centres (MTOCs) form, usually around two separate pairs of centrioles that nucleate and promote the polymerisation of spindle microtubules. Kinetochores, complex multi-protein structures, become fully assembled at centromeric regions of chromosomes and act as a binding platform for spindle microtubules. Thus, by the stochastic and directed attachment of microtubules emanating from two MTOCs results in the formation of a fusiform spindle, along whose equator chromosomes become progressively attached and aligned (Compton 2000, Heald & Khodjakov 2015, Petry 2016).

Following the correct alignment and attachment of chromosomes, cohesins complexes affixing sister chromatids are cleaved, allowing replicated sister chromatids to be separated and pulled towards apart in a process termed anaphase. In most cells, anaphase comprises two components that co-ordinately separate the chromosomes, termed anaphase A and anaphase B. Anaphase A consists of a shortening of kinetochore-bound microtubules, thereby lessening the distance from the chromosome to the pole. Anaphase B describes a spindle elongation that separates the spindle poles and thus further separates the chromosomes (Maiato...
& Lince-Faria 2010, Asbury 2017). Key to accurate segregation of chromosomes is that they should be correctly attached at the bipolar spindle, with sister kinetochores-binding microtubules emanating from opposite spindle poles. Errors in attachment in somatic cells lead to segregation error and aneuploidy (Cimini et al. 2001, Thompson & Compton 2011). It is thus perhaps unsurprising that most cells possess mechanisms for ensuring correct kinetochore-microtubule attachment. Extensive work in somatic cells has elucidated two major and interconnected mechanisms that operate to prevent chromosome segregation errors during mitosis; kinetochore-microtubule error correction (‘error correction’), and the spindle assembly checkpoint (SAC), both of which are outlined below. Anaphase is followed by the partitioning of the cytoplasm (a process termed cytokinesis) and decondensation of mitotic chromosomes and formation of daughter nuclei (termed telophase) giving rise to two daughter cells with newly formed interphase nuclei (Fig. 3).

**Spindle idiosyncrasies in the early mammalian embryo**

The mechanisms of mitosis have been intensely studied in a variety of cellular contexts, demonstrating that even slight variations in spindle dynamics may result in dramatic chromosome segregation defects (Thompson et al. 2010). The reductive divisions of early development are highly idiosyncratic, and understanding spindle behaviour in this cellular system cannot rely upon extrapolation from somatic cells. Importantly, a small number of recent studies employing live imaging approaches to understand how spindles are assembled in embryos, largely utilising mouse as a model for mammalian embryogenesis, have begun to reveal some of the challenges faced by embryo cell divisions.

In the context of dramatically changing cell size, with each division approximately halving cell size, how spindle structure is regulated during embryo mitosis presents an interesting cell biological conundrum. In general, spindle length is considered to be controlled by two classes of forces; the dynamics of the microtubules and associated motor proteins as well as the physical properties of chromosomes are termed ‘intrinsic’ influences, whereas ‘extrinsic’ influences describe external influences on the spindle, such as whether the cell size limits the length of the spindle (Dumont & Mitchison 2009, Goshima & Scholey 2010, Levy & Heald 2012). The spindle in the zygote is substantially shorter than the diameter of the cell, suggesting length regulation is entirely intrinsic. But from the second mitosis onwards, as cell size decreases, spindle size approaches the diameter of the cell, indicating extrinsic regulation (Courtois et al. 2012, Yamagata & FitzHarris 2013), as has been seen

![Figure 3](image-url) Chromosome segregation during mitosis and mechanisms preventing errors. Top: Schematic of the major physical events during mitosis. Condensation of the interphase nucleus into mitotic chromosomes (blue) as the nuclear envelope breaks down and microtubule-organising centers (MTOCs) emerge (red). This is followed by MTOC clustering, bipolar spindle assembly and chromosome attachment to spindle microtubules (red). This is followed by chromosome congression and alignment at the metaphase plate as correct kinetochore-microtubule attachments are establish. Sister chromatids are pulled apart during anaphase and separation of the cytoplasm during cytokinesis ensues the formation of daughter cells. Bottom left: The error correction mechanism relies on the enrichment of Chromosomal Passenger Complex (CPC), the activity of Aurora kinases B and C and downstream effectors to destabilise incorrect kinetochore (yellow)-microtubule attachments, such as merotelic attachments. Bottom right: Spindle Assembly Checkpoint (SAC) proteins are recruited to unattached kinetochores, catalysing the formation of the mitotic checkpoint complex (MCC) which acts to prevent the metaphase-to-anaphase transition.
in lower vertebrate embryos (Wühr et al. 2008, Good et al. 2013). Cytoplasmic removal experiments in mouse illustrated this point elegantly; moderate reductions in cell size during the first few cell divisions, when the spindle is far shorter than the cell, has little effect upon spindle length. Contrastingly, cytoplasmic removal in later preimplantation divisions (4–8 cell and onwards), when spindle length is similar to cell length, shortens the spindle (Courtois et al. 2012). Similarly, whereas 1- and 2-cell embryos exhibit a pronounced anaphase B spindle elongation, the extent and speed of spindle elongation during anaphase is decreased thereafter as the size of the cell becomes a limiting factor to spindle elongation (Yamagata & FitzHarris 2013). Hence, reductive divisions impose ever-changing limits on spindle structure and dynamics during preimplantation development. Curiously, the spindle in the first mitotic division is proportionally smaller than in the second division, despite a far greater cell size (Courtois et al. 2012, Yamagata & FitzHarris 2013), suggesting spindle length during the first mitosis is subject to different, perhaps more meiotic-like, regulatory mechanisms, than later divisions. Whether this is mediated by remnants of cytosstatic factor components that may persist in the first mitosis, remains to be seen (Kubiak & Ciemerych 2001, Maller et al. 2002). The shift in the mode of spindle length regulation in metaphase and anaphase described above exemplifies an emerging theme of cell division during early development; that progression from zygote to blastocysts is accompanied by gradual developmental shifts, rather than an abrupt switch in the way in which blastomeres approach a canonical mode of mitosis. Another such example is that maintenance of spindle bipolarity in metaphase is critically dependent on the mitotic motor kinesin-5 during the first three mitosis but not later divisions, reflecting a shift in the role of the motor from its oocyte role, where it is essential in metaphase, to its somatic cell role, where it is not (FitzHarris 2009). How changing cell size may impact the occurrence of segregation errors is further discussed in the context of error avoidance pathways below.

**Unusual centriole behaviour in early development**

Centrioles form the focal point of the spindle-organising centrosome in most mammalian cells. Since one centriole pair is inherited by each daughter cell, centrioles must replicate each S-phase to provide two centriole pairs in the subsequent mitosis (Loncarek & Khodjakov 2009, Nigg & Raff 2009). Making sure that centriole replication happens once-and-only-once is important, since over-replication causes multipolar spindles, which cause chromosome instability as seen in cancer cells (Kwon et al. 2008, Ganem et al. 2009, Gönczy 2015). To avoid too many centrioles after fertilisation, oocytes of most species degrade their centrioles. In many species, including humans, a single centriole and centrosome is thus inherited from the sperm at fertilisation. Pronuclear removal experiments in tripolar human zygotes and somatic cell nuclear transfer in mouse zygotes demonstrated that the sperm or donor centrosome is functional and directs spindle assembly in the first mitotic divisions (Palermo et al. 1994, Van Thuan et al. 2006, Kalatova et al. 2015). However, little is known about how these function during the remainder of early human preimplantation development, and some intriguing observations allude that centriole dysregulation may occur in embryos. Firstly, studies of fresh and vitrified human embryos have reported multipolar spindles and tri-directional anaphases (Chatzimeletiou et al. 2012, Vera-Rodriguez et al. 2015), which in somatic cells is symptomatic of too many centrosomes (Ganem et al. 2009). Secondly, a recent genome-wide association study identified single-nucleotide polymorphisms in the sequence of PLK4, a key regulator of centriole duplication, to be associated with embryonic mitotic errors (McCoy et al. 2015). Thus, the idea that centrosome/centriole dysregulation contributes to human embryo mosaicism requires further attention.

In the mouse embryo, the role and regulation of centrioles is even more intriguing. In addition to the oocyte lacking centrioles, the mouse sperm also destroys its centrioles during the elongating spermatid phase (Sathanathan 1997), such that most of mouse preimplantation development then occurs in the absence of classical centrosomes until the ~64-cell stage, when centrioles mysteriously emerge (Gueth-Hallonet et al. 1993, Courtois et al. 2012, Howe & FitzHarris 2013). This unexplained series of events uncovers another gradual shift in spindle microtubule behaviour in embryos. Spindle assembly in the first few mitoses was seen to rely on recruiting several cytoplasmic MTOCs, which form a multipolar spindle, that is later focused and achieves bipolarity, reminiscent of meiotic spindles (Schatten et al. 1986, Gueth-Hallonet et al. 1993, Schuh & Ellenberg 2007), but from the eight-cell stage, whilst centrioles and canonical centrosomes are still absent, spindle assembly is mediated by MTOCs that arise exclusively at the nuclear periphery and along the spindle in multipolar intermediates (Courtois et al. 2012). This MTOC clustering within the spindle during mid-preimplantation development was shown to be dependent on the Augmin complex, distinct from its role in other systems (Watanabe et al. 2016). Additionally, PLK4 has been shown to be essential for bipolar spindle assembly in mouse preimplantation embryos, despite the fact that centrioles are not present, further indicating non-canonical roles of well-characterised spindle proteins in early development (Coelho et al. 2013, Arquint & Nigg 2016). Although centrioles later emerge at blastocyst stage, it remains unclear whether they are fully functional, since they appear then to lack...
canonical microtubule-organising ability in interphase (Howe & FitzHarris 2013). To summarise, multiple lines of evidence both from mouse and human embryos highlight the importance of understanding mechanisms of centriole and centrosome function in preimplantation development, and multiple clues indicate that the way in which they function is likely distinct from better-studied somatic cells. Further work is needed to elucidate how centrosome function is regulated during development.

**How it all goes wrong: the dynamics of errors in embryos**

**Micronuclei as a possible catalyst for mosaicism**

Manifold studies of the in vitro cultured human embryos have attempted to use the observed chromosome complements of blastomeres at various developmental stages to extrapolate how chromosome segregation errors originated in those embryos. These analyses resulted in various potential explanations as to how mosaicism might arise including non-disjunction of sister chromatids at anaphase, disappearance of a sister chromatid (often termed ‘anaphase lag’ in the human embryo literature) and inappropriate repeated occurrence of a single chromatid (sometimes referred to as endoreplication). Whilst these studies highlight important principles of how errors could arise, unravelling the true nature of missegregation will require coupling current and emerging genetic screening techniques, such as next-generation genome sequencing, with direct observation of errors to determine the temporal and genetic dynamics of mosaic aneuploidy in the early embryo. Perhaps unsurprisingly, live imaging experiments of chromosome segregation errors are yet to be presented in human embryos. However, recent live imaging of chromosome segregation errors in mouse embryos suggest that micronuclei, small additional nuclei containing one or few chromosomes separate from the main nucleus which, importantly, are well known to be prevalent in human embryos, may play a critical and previously unrecognised role in the generation of mosaicism (Fragouli et al. 2013, Vázquez-Diez et al. 2016).

By extensive imaging of embryonic mitoses in mouse it was shown that micronuclei arise because of single sister chromatids that remain separate from the main group in anaphase (termed ‘lagging chromosomes’), which then form their own nuclear membrane in the resulting daughter cells (Fig. 4 and Video 1.). Following encapsulation into micronuclei, the chromosomes become extensively damaged and lose centromeric identity indicated by the centromere marker CENP-A, and therefore, fail to assemble a kinetochore and be segregated by the spindle during subsequent cell divisions (Vázquez-Diez et al. 2016). A similar series of events has been observed in somatic cells (Guerrero et al. 2010, Crasta et al. 2012, Huang et al. 2012, Zhang et al. 2015a), and it is thought that the defective nuclear

![Figure 4](https://www.reproduction-online.org)
envelope on the micronucleus, leading to failed nuclear compartmentalisation, underpins the DNA damage to the chromosome enclosed (Hatch et al. 2013). Strikingly, in embryos, the same heavily damaged chromosome re-appears as a micronucleus following each subsequent cell division (Vázquez-Diez et al. 2016). Although live imaging of this type in human embryos is yet to be reported, human embryo immunofluorescence data show the occurrence of single anaphase lagging chromosomes, micronuclei bearing high levels of DNA damage and lacking centromeric identity, and micronucleus-like chromosomes in mitosis devoid of kinetochore staining (Kort et al. 2016). These findings strongly allude that the same sequence of events likely occurs in human embryos. Importantly, as elaborated in Box 1, this series of events provides a mechanism for the high occurrence of single chromosome copy number variation in mosaic embryos, and, provide for the first time a coherent explanation as to why single chromosome losses outnumber gains in early embryos (Box 1, Fig. 5). This mechanism may be specific to early embryos, as micronuclei are typically re-incorporated back into the main nucleus in other cell types (Crasta et al. 2012, Hatch et al. 2013, Leibowitz et al. 2015, Zhang et al. 2015, Ly et al. 2017).

Video 1
H2BRFP MajSatTALEmClover-expressing embryo, showing lagging anaphase chromosomes result in micronuclei. View video from the online version of the article available at https://doi.org/10.1530/REP-17-0569.

Chromosomal abnormalities unexplained by micronucleus formation

Other chromosomal composition abnormalities have been described in preimplantation embryos including ploidy defects and complex or ‘chaotic’ abnormalities, albeit at lower frequencies (Echten-Arends et al. 2011). Furthermore, nucleation status abnormalities such as binucleation and multinucleation are also common in preimplantation embryos (Roen et al. 2003). Also commonly observed in human preimplantation embryos, is tetraploidy, when a cell has double the normal number of chromosomes. How tetraploidy emerges is not clear but could conceivably arise from cell fusion, although cytokinesis failure and mitotic slippage – where the cells exit mitosis in the absence of sister chromatid separation – are more plausible explanations. In the human embryo, these tetraploid conditions are likely accompanied by extra centrosome copies, which could lead to multipolar spindle formation and multidirectional anaphases, both scenarios resulting in aneuploidy and multinucleation. Whether binucleated and tetraploid embryos exhibit multipolar metaphases and tri-directional cell divisions

BOX 1. Micronucleus formation and inheritance can explain the most prevalent forms of mosaic aneuploidy in embryos

Live imaging in mouse embryos revealed that after the formation of micronuclei by a lagging chromosome (Fig. 4), the micronucleus is unilaterally inherited at each subsequent cell division (Vázquez-Diez et al. 2016). This can explain two of the most commonly observed features of mosaicism in the clinic:

Firstly, these events provide a coherent explanation for single chromosome losses, which are by far the most common genetic abnormality present in embryos – mosaic unisomies being up to seven times more frequent than trisomies (Coonen et al. 2004, Taylor et al. 2014) (Fig. 5). During cell division, a lagging chromosome causes a single chromated to be incorporated into a micronucleus in only one of the two daughter cells. In a balanced scenario, the lagging chromosome initially forms a micronucleus in the correct daughter cell, such that the complete chromosome complement is present taking account both the main nucleus and micronucleus. However, although both sister blastomeres are initially formally euploid, the main nucleus of the micronucleus-containing cell harbours a single chromosome copy loss. In the subsequent division of that cell, the daughter cell that does not receive the MN will necessarily be hypoploid (Fig. 5, left panel). Alternatively, if the lagging chromosome generates a micronucleus in the daughter cell whose main nucleus already contains a euploid chromosome content (i.e. the initial segregation error is unbalanced), then the micronucleus-free sister blastomere will lack a single chromosome. Subsequently, all of the progeny of this cell will display single chromosome loss (Fig. 5, right panel). Thus, regardless of the initial ‘direction’ of lagging chromosomes, micronucleus inheritance provides a potential explanation for a high incidence of single chromosome losses.

Secondly, micronucleus inheritance can explain single chromosome gains, the second most common genetic abnormality in mosaic embryos (Echten-Arends et al. 2011, Taylor et al. 2014). Specifically, if the lagging chromosome forms a micronucleus in the daughter cell whose main nucleus already harbours a complete chromosome complement, this results in a single (micronucleus-enclosed) chromosome gain (Fig. 5, right panel). Whilst such single chromosome gains are usually attributed to a classical non-disjunction event, wherein an extra sister chromatid arises within a single newly forming nucleus (Echten-Arends et al. 2011, Mantikou et al. 2012, Taylor et al. 2014), lagging chromosomes leading to the formation of micronuclei in non-complementary cells should have a similar impact.

Importantly, note that in the literature, single chromosome losses have often been attributed to ‘anaphase lag’, without the mechanism for this being clear. Within our model, the lagging anaphase chromosome acts as a trigger point by generating a micronucleus, but it is the clonal propagation of the blastomeres following a micronucleus formation event (rather than the lagging chromosome per se) that accounts for the high incidence of cells exhibiting a chromosome loss.

Finally, note that this model unexpectedly provides a possible mechanism for aneuploid cells to ‘self-correct’. During cell division of a trisomic cell, if the additional chromated is enclosed in a micronucleus, the micronucleus-free daughter of that (formally aneuploid) cell will be euploid and should therefore have the potential to generate a clone of euploid blastomeres (Fig. 5, right panel, arrows). Whilst some preimplantation genetic screening approaches can detect DNA within micronuclei on at least some occasions, how reliable micronucleus detection is by such methods after several cell divisions is unclear and will be important to clarify.
has not been confirmed. However, in human somatic cells, the SAC delays mitosis in the presence of multipolar spindles (Kwon et al. 2008), and aneuploid human embryos show delayed first mitotic divisions, together alluding that super-numerary centrioles may exist (Vera-Rodriguez et al. 2015).

**Why it all goes wrong: the embryo as a permissive environment for errors?**

Given the importance of maintaining genetic fidelity, it is perhaps unsurprising that most mammalian cells possess multiple mechanisms for averting errors. Indeed, in somatic tissues the rate of aneuploidy is thought to be only 2% (Knouse et al. 2014). Given the high incidence of mosaic aneuploidy and prevalence of mitotic errors in mammalian preimplantation development, it has long been suggested such mechanisms may be compromised or absent during mitotic cleavage divisions (Echten-Arends et al. 2011, Mantikou et al. 2012, Taylor et al. 2014, Albertini 2016). Here, we discuss the limited data on whether and how these pathways function in early embryos.

**Kinetochore-microtubule attachment error correction**

The error correction mechanism acts locally on mis-attached chromosomes and involves the targeted recruitment of factors that will destabilise improper kinetochore-microtubule attachments. This mechanism is mediated by the chromosomal passenger complex (CPC), a multi-protein assembly typically composed of Aurora B, INCENP, Survivin and Borealin, which localises to centromeres during prometaphase (Musacchio & Salmon 2007, Krenn & Musacchio 2015). At mis-attached chromosomes, Aurora B kinase phosphorylates several kinetochore components (Ndc80, Hec1, Knl1 and Dsn1) to reduce their microtubule-binding affinity. Furthermore, Aurora B also recruits kinesin-13 family members KIF2B and MCAK/KIF2C, which have microtubule depolymerising activities (Krenn & Musacchio 2015).

The error correction mechanism disrupts improper kinetochore-microtubule attachments, promoting the formation of new, correct, end-on attachments. Error correction is particularly important for the detection and correction of syntelic (where both sister kinetochores are bound to microtubules emanating from the same spindle pole) and merotelic attachments (in which a single kinetochore is bound to microtubules originating from both spindle poles) (Fig. 3).

Several recent lines of evidence reveal embryo-specific particularities of the error correction pathway that could conceivably contribute to the increased rates of missegregation in early embryos. Whilst in somatic cells CPC error correction function is mediated by Aurora B kinase (Krenn & Musacchio 2015), recent studies suggest a major role for a less-well-characterised Aurora kinase C. Human and murine preimplantation embryos exhibit higher relative abundance of Aurora C in early development, with Aurora B abundance increasing by blastocyst stage, both in mouse and human embryos (Avo Santos et al. 2011, Schindler et al. 2012, Li et al. 2017). Moreover, knockout of Aurora B kinase in mouse embryos results in
normal developmental rates but fail to develop beyond blastocyst stage (Fernández-Miranda et al. 2011). On the other hand, Aurora C knockout significantly impairs development, reducing blastocyst formation rates, and knockdown of both Aurora B and C together results in increased rates of mitotic arrest and chromosome segregation defects (Fernández-Miranda et al. 2011), perhaps suggesting a more significant role of Aurora C kinase in error correction in embryonic mitoses (Fernández-Miranda et al. 2011, Schindler et al. 2012). Additional evidence implicating Aurora kinases in regulation of embryonic mitosis comes from the identification of two Aurora B and C single-nucleotide variants in humans, one of which is associated with reduced rates of aneuploidy in women advanced maternal age and which promotes correct chromosome alignment when expressed in mouse oocytes (Nguyen et al. 2017). Further studies using immunofluorescence and chemical inhibition approaches in human trionuclear and diploid embryos have revealed an unusual CPC component localisation in zygotes due to increased activity of Aurora-activator Haspin kinase (van de Werken et al. 2015). Determining how this unexpected expression pattern of Aurora kinases regulates downstream CPC effectors such as KIF2B and MCAK to modulate chromosome segregation dynamics and microtubule interactions at the kinetochore, will be important to establish the role of these kinesins in the early embryo.

The spindle assembly checkpoint (SAC)

The SAC is a near-ubiquitous signalling pathway that operates to arrest cells in metaphase of mitosis until all chromosomes have been successfully attached. SAC signalling is initiated at unattached kinetochores through the recruitment proteins such as MPM1, MAD1, MAD2, BUB1, BUB3 and BUBR1. These in turn catalyse the production of the mitotic checkpoint complex (MCC), composed of MAD2/BUBR1/BUB3/CDC20 (Musacchio & Salmon 2007, Lara-Gonzalez et al. 2012). Kinetochore-produced MCC acts as a diffusible signal in the cytoplasm that targets the APC/C to inhibit its ubiquitin ligase activity and prevent the degradation of securin and cyclin B, hence preventing cohesin cleavage and delaying anaphase onset (Musacchio & Salmon 2007, Lara-Gonzalez et al. 2012, Collin et al. 2013). The SAC coordinates achievement of correct kinetochore-microtubule attachment of all chromosomes with sister chromatid separation and mitotic exit (Fig. 3). It is perhaps unsurprising therefore that absent SAC activity has recurrently been forwarded as a candidate explanation for the high incidence of mitotic errors in embryos (Echten-Arends et al. 2011, Mantikou et al. 2012, Fragouli et al. 2013, Taylor et al. 2014). In support of such an idea, SAC component transcript levels are low during human cleavage stages (Wells et al. 2005).

A recent study in human embryos revealed that the spindle poison nocodazole can arrest cell divisions in mitosis, which is a first line of evidence that the SAC might in fact operate (Jacobs et al. 2017). Whether these effects are a bona fide indicator of SAC function remains to be determined and will require direct investigation of SAC components. In mouse, deletion of SAC components Mad2 or BubR1 subtly affects preimplantation development, but direct information of SAC activity is currently lacking (Dobles et al. 2000, Wang et al. 2004). In the meantime, there are strong clues that, analogous to error correction, the function of the SAC may be non-canonical. Significantly, landmark recent studies revealed that in the mouse oocyte, the SAC not only surveys attachment of kinetochores to spindle microtubules, but unexpectedly also specifically arrests meiosis-I in response to DNA damage (Collins et al. 2015, Marangos et al. 2015, Lane et al. 2017). Interestingly, DNA damage during mitosis has been shown to directly alter microtubule dynamics in somatic cells (Bakhoun et al. 2014). Whether DNA damage may influence spindle dynamics and SAC function in mammalian embryos remains elusive. Classic studies of SAC function in other vertebrate embryos, including Xenopus and zebrafish, demonstrated that early cleavage divisions occur in the absence of a functional SAC, which appears later at the mid-blastula transition (Hara et al. 1980, Zhang et al. 2015b). Indeed, a role for changing cell size in SAC function has been underlined by elegant recent studies. In C. elegans embryos, it was shown that early embryos possess a weak checkpoint, and the SAC becomes increasingly robust as development progresses and cells become smaller (Galli & Morgan 2016). Analogously, in mouse oocytes, experimental manipulation of oocyte size found that larger cells had less robust SAC function (Kyogoku & Kitajima 2017, Lane & Jones 2017). Whether a similar cell size-dependent shift in SAC function operates in the early mammalian embryo and might contribute to the error-prone nature of the mammalian embryo has not yet been tested.

Consequences of errors: is mosaicism such a bad thing?

The impact of mosaicism on embryo quality

In the light of the prevalence of mosaic aneuploidy in mammalian preimplantation development, it is essential to understand its impact on developmental potential and embryo viability, particularly in the clinical setting. Whilst early studies of early embryo mosaicism relied on FISH to analyse the presence or absence of a restricted number of chromosomes, the advent of advanced methods such as comparative genomic hybridisation and so-called next-generation sequencing to assess all chromosomes has improved
our understanding of the impact of mosaicism (Munné et al. 2017). Blastocysts classified mosaic after biopsy and analysing a moderate number of cells (often ~5 or so) are less likely to implant and more likely to miscarry than ‘euploid’, controls. Importantly, however, the same study also provides strong evidence indicating mosaic embryos can be viable (Munné et al. 2017). Specifically, diploid–aneuploid mosaic embryos had implantation rates comparable to euploid embryos, and blastocyst with up to 60% aneuploidy in biopsied cells resulted in successful pregnancy outcomes (Fragouli et al. 2017, Munné et al. 2017). These findings suggest that mosaic aneuploidy does not necessarily need development potential. One caveat of this conclusion is that biopsy studies are naturally hampered by random selection of cells from embryos, of which a large proportion likely comprise at least some aneuploid cells (Vanneste et al. 2009). Importantly, it is becoming increasingly clear that preimplantation genetic screening results of a few cells is not necessarily a reliable judge of the extent of mosaicism within an embryo (Gleicher et al. 2016, Albertini 2016). Nonetheless, the emerging view is that, clinically, embryos judged to be likely mosaic are embryos of intermediate favourability for patient transfer – less favourable than euploid, more than uniformly aneuploid embryos – but consistency of practice between clinics remains lacking (Munné et al. 2017).

Detailed and elegant experiments in mouse corroborate the notion that mosaic embryos are not necessarily doomed, and provide the beginnings of an explanation. Embryo transfer experiments using two different mouse models of mosaicism demonstrated that degree of mosaicism does not affect implantation rates (Lightfoot et al. 2006, Bolton et al. 2016). Furthermore, embryos with moderate but not extensive levels of mosaicism can fully develop to term (Bolton et al. 2016), suggesting a ‘threshold’ degree of mosaicism that could be tolerated provided that a critical number of healthy inner cell mass cells are preserved. Indeed, it has been proposed that the mammalian foetus is derived from as little as three inner cell mass cells (Markert & Petters 1978), leading to the hypothesis that only a small portion of euploid cells are necessary to sustain human foetal development – a notion that is supported by the observation that frozen-thawed human embryos that have lost almost half of blastomeres during the cryopreservation procedure are still viable and result in live births (Zheng et al. 2008). This may be explained by two major non–mutually exclusive mechanisms – the preferential proliferation of euploid cells and the negative selection of aneuploid cells in the inner cell mass (Echten-Arends et al. 2011, Fragouli et al. 2013). Live imaging experiments in mouse embryos revealed that some aneuploid cells within the inner cell mass undergo apoptosis, presumably to increase the proportion of euploid cells, though no evidence was seen of aneuploid cells being ‘directed’ to the trophectoderm as previously posited (Bolton et al. 2016). Increased rates of apoptosis were also seen in mosaic embryos, such that the embryo cylinder from embryo chimaeras is largely composed of normal cells by embryonic day 7.5 (Lightfoot et al. 2006, Bolton et al. 2016). Coherently, in human embryos, rates of mosaicism are lower in blastocyst than morula stages and are further reduced post-implantation (Echten-Arends et al. 2011, Mantikou et al. 2012, Taylor et al. 2014). Thus, whilst much is left to be learned about the consequences and fate of aneuploid cells in the mosaic embryo, evidence to date suggests that apoptotic pathways and other mechanisms may act in a targeted cell-type-specific manner before and after implantation to remove abnormal cells from mosaic embryos (Albertini 2016) and that provided sufficient euploid cells are present in the inner cell mass to form the embryo proper, mosaic aneuploidy can be compatible with healthy, full-term development, and live birth.

An unexpected advantage: mosaicism as an evolutionary benefit?

As described earlier, it appears increasingly plausible that mosaic aneuploidy is, perhaps counter-intuitively, a normal feature of preimplantation development across multiple mammalian species (Dupont et al. 2010, Hornak et al. 2012, 2016, Mizutani et al. 2012, Bolton et al. 2016, Vázquez-Diez et al. 2016). In which case, it becomes reasonable to ask; could chromosome missegregation in the early embryo present some positive biological benefit?

Unlike other animals which produce tens to hundreds of embryos ex vivo, mammals produce fewer offspring. Due to the physical and time investment in gestation, lactation and nursing required by their offspring, mammalian reproduction should be selective of the parental genomes, environment and maternal health status. One possible way to achieve such selection could be to exploit the highly atypical environment of preimplantation embryo development that likely poses strains on cellular resources of embryo during development. Having mitotic divisions that are inherently error-prone and sensitive to environmental perturbations may be physiologically advantageous since upon undesired situations such as poor maternal health, low gamete quality or deleterious environment (poor nutrition for example), a ‘mosaicism threshold’ can be surpassed and chances of full-term development in an unfavourable biological context are reduced. In other words, the mosaicism threshold could serve as a sensor for whether an ongoing pregnancy is desirable. Alternatively, it was recently suggested that increasing oocyte aneuploidy with maternal age may serve a positive benefit in humans by causing an extended period of subfertility in the 40s, prior to the complete cessation of ovulatory cycles (menopause) which occurs.
at ~50. Since the loss of all oocytes is associated with a decline in oestrogen that precipitates osteoporosis and other changes detrimental to general health, age-related oocyte aneuploidy potentially provides a mechanism to allow older females to maintain a baseline level of oestrogen that beneficial to health, whilst averting pregnancy (Sirard 2011). This notion is consistent with the well-known grandmother hypothesis, wherein older females are considered to increase their chances of passing genes to future generations by helping raise their children’s offspring, rather than undergoing further potentially hazardous pregnancies (Hawkes et al. 1998, Alvarez 2000, Hawkes & Coxworth 2013). Within such a model one might imagine that an inherently fragile ooplasm susceptible to increased missegregation in the face of age-related ovarian changes may be crucial, and that segregation errors in the embryo shortly after fertilisation are simply a manifestation of this fragility. Whilst much of the above is speculative, we consider it at least conceivable that mosaicism of the preimplantation embryo may not be purely a pathology, but may play a positive role that is yet to be fully realised.

Concluding remarks

Mosaic aneuploidy is a common phenomenon in mammalian preimplantation embryos that arises due to errors in mitosis during embryonic divisions. The underlying mechanisms responsible for chromosome segregation errors are yet to be fully elucidated but the emerging theme is that the unique cellular environment and idiosyncrasies of cell division in the early embryo are at the heart of the story. For this reason, unravelling the aetiology of these errors cannot rely on extrapolation from somatic cells and other traditional systems, but will require direct examination in mammalian embryos, and the advent of advanced imaging systems to observe these events will no doubt advance our understanding. Furthermore, it becomes increasingly clear that whilst a high level of mosaic aneuploidy is detrimental to embryo viability, some levels can be compatible with successful pregnancy and full-term development, but the biological reasons for this remain far from clear. We envisage a continuing shift in how mosaicism is viewed and interpreted in the clinical context in the coming years.

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/REP-17-0569.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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