Permanent embryo arrest: molecular and cellular concepts

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Developmental arrest is one of the mechanisms responsible for the elevated levels of embryo demise during the first week of in vitro development. Approximately 10–15% of IVF embryos permanently arrest in mitosis at the 2- to 4-cell cleavage stage showing no indication of apoptosis. Reactive oxygen species (ROS) are implicated in this process and must be controlled in order to optimize embryo production. A stress sensor that can provide a key understanding of permanent cell cycle arrest and link ROS with cellular signaling pathway(s) is p66Shc, an adaptor protein for apoptotic response to oxidative stress. Deletion of the p66Shc gene in mice results in extended lifespan, which is linked to their enhanced resistance to oxidative stress and reduced levels of apoptosis. p66Shc has been shown to generate mitochondrial H₂O₂ to trigger apoptosis, but may also serve as an integration point for many signaling pathways that affect mitochondrial function. We have detected elevated levels of p66Shc and ROS within arrested embryos and believe that p66Shc plays a central role in regulating permanent embryo arrest. In this paper, we review the cellular and molecular aspects of permanent embryo arrest and speculate on the mechanism(s) and etiology of this method of embryo demise.

Keywords: anti-apoptosis; p66Shc; ROS; telomere; mitochondria

Introduction

One of the features of in vitro-produced (IVP) mammalian embryos is the high frequency of early developmental failure thought to be brought on by sub-optimal culture environments (Johnson and Nasr-ESfahani, 1994; Betts and King, 2001; Favetta et al., 2004a,b). Fewer than 50% of all in vitro fertilized (IVF) embryos reach the blastocyst stage of development (Xu et al., 1992) with many of these unable to sustain development following embryo transfer (Farin et al., 2001). Data generated from the European registers by European Society of Human Reproduction and Embryology (ESHRE) indicate that the clinical pregnancy rates per aspiration and per embryo transfer were 26.6 and 30.1%, respectively, for all human IVF cycles in 2004 (Andersen et al., 2008). The reasons for this high rate of embryo demise remains unclear, but it has been proposed as a protective mechanism for preventing further development of abnormal, poor-quality embryos. Almost half of all arrested human embryos display chromosomal abnormalities (Almeida and Bolton, 1998), and significantly more chromosomal aberrations are observed, alongside delayed development, for IVP bovine embryos compared to their in vivo-derived counterparts (Kawarsky et al., 1996; Viuff et al., 1999). Blastomeres with characteristic features of apoptosis, including nuclear and cytoplasmic fragmentation, have been detected in both in vitro- and in vivo-derived embryos, indicating that high levels of apoptosis might play a role in early embryo death (Hardy, 1997; Kamjoo et al., 2002). The in situ terminal uridine deoxyxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, which detects fragmented DNA, has identified a greater incidence of apoptotic nuclei in cultured bovine blastocysts compared with those derived in vivo (Gjorret et al., 2003). Interestingly, no morphological or biochemical signs of apoptosis has been observed during the early cleavage stages of embryogenesis (Byrne et al., 1999; Matwee et al., 2000, 2001; Betts and King, 2001; Hardy et al., 2001; Gjorret et al., 2003). It is at this early developmental stage that ~15% of all IVF bovine embryos are permanently arrested in a senescence-like state (Fig. 1). Our preliminary results have revealed that roughly 10% of all human embryos produced by IVF or intracytoplasmic sperm injection permanently arrest at the early cleavage stages in culture and that 40% of patients exhibit at least one arrested embryo per treatment cycle indicating that this is a common phenomenon in humans as well (D. H. Betts, unpublished results). In this review we discuss what permanent early embryo arrest is, the role of reactive oxygen species (ROS) and the possible molecular mechanism(s) involved in mediating this event, and speculate on the causative role of telomere integrity in this context.

Permanent embryo arrest is a non-apoptotic event

Programmed cell death, or apoptosis, is a feature of both IVP and in vivo-derived preimplantation/preattachment stage embryos (Hardy, 1997; Matwee et al., 2000; Kamjoo et al., 2002). Apoptosis, a mechanism to rid the early embryo of unwanted or damaged cells, if extensive, could also lead to developmental failure. In spite of this effective program, the ability of an early embryo to undergo apoptosis appears to be developmentally regulated (Betts and King, 2001;
Hayflick who proposed that most proliferating somatic cell types permanently stop dividing after a limited number of population doublings (Hayflick and Moorhead, 1961). Senescent cells display distinct morphological and gene expression profiles of ‘aged’ cells that accumulate in vivo in tissues with increasing age (Herbig et al., 2006). In vivo senescence may contribute to organismal aging as a consequence of its proposed role as a potent tumor suppressor mechanism (Di Micco et al., 2007). Cellular senescence is thought to be a DNA damage-response to oncogene-induced DNA replication stress and/or to telomere disruption, both converging on the p53 DNA damage signaling pathway (Bartek et al., 2007). We have previously shown that permanent replication arrest of cultured bovine somatic cells is associated with telomere shortening (Betts et al., 2008), increased levels of serine 20-p53 phosphorylation, and elevated levels of oxidative damage (Favetta et al., 2004a). Since cellular senescence can occur prematurely under conditions of elevated oxidative stress (Toussaint et al., 2000; von Zglinicki, 2000) and can be activated by disruption of the telomere structure itself, even at maximally long telomere lengths (Li et al., 2003; Stewart et al., 2003), we hypothesize that arrested mammalian embryos permanently stop dividing at the 2- to 4-cell stage of early development by a similar stress signaling pathway.

Permanent embryo arrest is dependent on the time of first cleavage, with ~15% of embryos arrested at the 2- to 4-cell stage if their first cleavage event was at 32 hpi, while only ~1% of embryos were arrested among those cleaved at 26–28 hpi (Favetta et al., 2004b). Embryos cultured in 20% oxygen conditions display significantly elevated levels of intracellular ROS and higher frequencies of permanent embryo arrest compared with the embryos produced under 5% oxygen atmospheres (Favetta et al., 2007b). Unlike senescent fibroblasts (Favetta et al., 2004a), no significant differences in mRNA and protein levels of the tumor suppressor p53 are observed in both arrested and late cleaving embryos compared with their early cleaving counterparts (Favetta et al., 2004b, 2007a,b). However, like senescent fibroblasts (Favetta et al., 2004b, 2007a,b). However, like senescent fibroblasts (Favetta et al., 2004b, 2007a,b).
et al., 2004a), significantly higher levels of the oxidative stress adapter protein p66Shc have been detected in arrested embryos (Favetta et al., 2004b, 2007a,b). These results further substantiate the view that permanent embryo arrest may be mediated by the production of intracellular ROS.

The involvement of oxygen tension and ROS in permanent embryo arrest

ROS are implicated in the induction of apoptosis (Stone and Yang, 2006) and cellular senescence (Passos et al., 2007). Specificity could be determined by the intensity of the pro-oxidant challenge since the treatment of different primary cells with increasing doses of exogenous hydrogen peroxide (H$_2$O$_2$) induces proliferation, senescence or apoptosis, respectively (Chen et al., 2000). Somatic cell proliferation is inhibited by high-oxygen culture environments (Balin et al., 2002) as well as by treatment with H$_2$O$_2$ (Chen and Ames, 1994). Conversely, low-oxygen conditions and antioxidant treatments prolong the proliferative lifespan of cell cultures (Packer and Fuehr, 1977; Poot, 1991).

Among the various culture conditions and exogenous factors that lead to elevated production of ROS in embryos, including the traces of metallic cations, visible light and amino oxidases, the in vitro oxygen tension is the most studied and the easiest to control (Guerin et al., 2001). Earlier studies have demonstrated the detrimental effects of culturing embryos of various species under atmospheric (~20%) oxygen concentrations (Pabon et al., 1989; Nagao et al., 1994; Dumoulin et al., 1999) and the beneficial effects of reducing the oxygen tension (Dumoulin et al., 1999; Hashimoto et al., 2000; Orsi and Leese, 2001) and co-culturing with somatic cells to provide an oxidant ‘buffering’ system (Xu et al., 1992; Nagao et al., 1994). Reducing the oxygen concentration of embryo culture from 20 to 5% enhances embryo development to the blastocyst stage and increases their total cell counts (Batt et al., 1991; Nagao et al., 1994; Gardner and Lane, 1996), probably by reducing the proportion of cells undergoing apoptosis (Van Soom et al., 2002). Enhanced embryo development under lower oxygen conditions is thought to be attributable to improved embryo metabolism (Khurana and Wales, 1989; Du and Wales, 1993) and decreased ROS production leading to reduced oxidative stress (Guerin et al., 2001).

A direct relationship between increased H$_2$O$_2$ production and elevated embryo fragmentation has also been documented, suggesting that ROS may induce apoptosis in embryos (Yang et al., 1998). In addition, it has been noted that embryos cultured in 20% oxygen conditions sustain a 10-fold increase in intracellular H$_2$O$_2$ levels and a 2-fold increase in the frequency of permanent embryo arrest at the 2- to 4-cell stage, compared with embryos cultured in 5% oxygen tensions (Favetta et al., 2007b).

More recently, we have observed a dose-dependent increase in permanent embryo arrest after exposure of 2- to 4-cell embryos to H$_2$O$_2$ and that this oxidant-induced embryo arrest can be abrogated by exposure to an antioxidant, PEG-Catalase (D. H. Betts, unpublished results). Although oxidative stress appears to signal a permanent senescence-like state in 2- to 4-cell bovine embryos, exposure of oocytes and late-cleavage stage embryos (8- to 16-cells, morulae and blastocysts) to exogenous H$_2$O$_2$ imposes a dose-dependent increase in apoptosis (D. H. Betts, unpublished results), suggesting that ROS-mediated events are developmentally regulated. Intracellular ROS levels are significantly more abundant in 2- to 4-cell arrested embryos (D. H. Betts, unpublished results) and in an embryo population that exhibit higher frequencies of permanent embryo arrest (Favetta et al., 2007b). Concentration of non-toxic ROS, via alterations in the reduction-oxidation (redox) state, is considered to be an important mechanism that regulates cellular functions including energy production and proliferation in early embryos by way of redox-sensitive transcription factors (Harvey et al., 2002). Interestingly, oxygen tension has recently been shown to alter gene expression in blastocysts (Harvey et al., 2004, 2007) possibly through epigenetic mechanisms (Islam and Mendelson, 2006). It is intriguing to speculate that permanent embryo arrest may be activated directly by ROS/oxygen-mediated gene expression. It is well established that the elevated ROS levels generate damage to cells/embryos through increased lipid peroxidation (Nasr-Esfahani et al., 1990; Nasr-Esfahani and Johnson, 1992), and protein oxidation, and induce DNA strand breaks (Guerin et al., 2001; Orsi and Leese, 2001), including telomeric DNA (Petersen et al., 1998). All of these are also observed features of senescent somatic cells.

Does telomere dysfunction play a role in embryo arrest?

The signaling of permanent cell cycle arrest has long been attributed to the critical shortening of telomeres(s), the repetitive DNA sequences (TTAGGG)$^n$ and associated proteins located at the ends of mammalian chromosomes (Harley et al., 1990, 1992; Allsopp et al., 1992, 1995; Vaziri, 1997; Vaziri and Benchimol, 1998). Telomere shortening can be overcome by the de novo synthesis of telomeric DNA by telomerase (Greider and Blackburn, 1985), a multi-subunit reverse transcriptase that uses its RNA component (TERC) to align itself to the chromosomal ends and as a template for the synthesis of telomeric sequences (Collins and Greider, 1995; Collins et al., 1995). High levels of telomerase activity have been detected in germline tissues, cells of renewal tissues, cancer cells and immortalized cell lines but not in most somatic tissues (Kim et al., 1994; Harle-Bachor and Boukamp, 1996; Wright et al., 1996; Betts and King, 1999). Ectopic expression of the telomerase catalytic subunit (TERT) extends replicative lifespan while preserving long telomere lengths and normal karyotypes in diploid somatic cells (Bodnar et al., 1998; Thomas et al., 2000). Conversely, late generation mice lacking the telomerase RNA (nTERC--;--) component display shortened telomeres, chromosome abnormalities and exhibit infertility, increased apoptosis and a decreased cell proliferation in the testis, bone marrow and spleen (Blasco et al., 1997; Lee et al., 1998). Together, these results demonstrate that telomers provide chromosomal stability and regulate the proliferative capacity of cells in vivo and in vitro.

We have previously shown that the permanent cell growth arrest of cultured bovine somatic cells is associated with telomere shortening (Betts et al., 2008), increased levels of serine 20-p53 phosphorylation, and elevated levels of oxidative damage (Favetta et al., 2004a). Celluar senescence occurs prematurely under the conditions of elevated oxidative stress (von Zglinicki, 2000, 2002) and can be activated by disruption of the telomere structure itself, even at maximally long telomere lengths (Li et al., 2003; Stewart et al., 2003). Mild hyperoxia and even normoxic (20% oxygen) culture environments inhibit the proliferation of human fibroblasts and increase telomere shortening/damage by causing single-stranded breaks specifically within telomeric DNA (von Zglinicki et al., 1995; Petersen et al., 1998; Sitte et al., 1998). ROS damage to the telomeres accumulates due to cellular deficiencies in the repair of such damage (Petersen et al., 1998). Single-stranded telomeric DNA contributes significantly to telomere shortening (Makarov et al., 1997; Petersen et al., 1998), but also acts as a trigger of p53-dependent cell cycle arrest and cell death (von Zglinicki, 1998; Saretzki et al., 1999). Telomeres therefore act as sentinels that signal cell cycle arrests by the accumulation of single-stranded telomeric DNA above a certain threshold in length and/or amount that abrogates a functional telomere structure. Indeed,
experimental disruption of the telomerase catalytic subunit (hTERT) in proliferating normal diploid somatic cells disrupts telomere structure (telomere uncapping) and subsequently activates an early senescence phenotype (Masutomi et al., 2003). ROS-mediated disruption of the telomere structure may explain the high rates of developmental arrest of IVF embryos and the stochastic variation in division capacity within somatic cells cultures (Sozou and Kirkwood, 2001). Conversely, under high-oxidative stress conditions, TERT is reversibly excluded from the nucleus, where it co-localizes with mitochondria, possibly providing protection through improved mitochondrial metabolism and reduced ROS-generation (Ahmed et al., 2008). Interestingly, we have localized TERT in proliferating 2-cell bovine embryos as large punctate foci reminiscent of the mitochondria staining patterns observed in early cleavage-stage embryos (D. H. Betts, unpublished results). Also, our preliminary results from pharmacologically treating bovine embryos with telomerase inhibitors have shown an increased incidence of permanent embryo arrest (D. H. Betts, unpublished results). There is now supporting evidence that telomere-dysfunction induced senescence/apoptosis is triggered by the production of mitochondrial ROS (Liu et al., 2002a,b,c; Liu et al., 2003; Passos et al., 2007). Co-localization of phosphorylated histone γ-H2A.X fluorescence, a marker of DNA damage, to the telomeres indicates telomere dysfunction-induced foci (Herbig et al., 2004, 2006). We have observed γ-H2A.X foci in the arrested 2-cell embryos but no staining in their proliferating counterparts (D. H. Betts, unpublished results; Fig. 1E and F). Telomere and/or mitochondrial dysfunction could explain the ‘pre-mature’ arrest states that arise in vivo or in vitro after exposure to oxidative stresses for cells and embryos that possess relatively long telomere lengths (Betts and King, 2001; Favetta et al., 2004a,b; Kurz et al., 2004).

The role of the stress adaptor protein p66Shc in embryo arrest

At the molecular level, the response of somatic cells to oxidative stress appears to involve the tumor suppressor protein p53, which recognizes free radical-induced DNA damage (Migliaccio et al., 1999; Sharpless and DePinho, 2002). p53 leads cells to either die through apoptosis, or to arrest in response to a variety of cellular stresses, such as DNA damage, hypoxia, oxidative stress, excessive mitogenic stimuli or denuded telomeres (Donehower, 2002; Sharpless and DePinho, 2002). At low concentrations, ROS can modulate p53, but are also suggested to be downstream mediators of p53 in p53-dependent apoptosis (Johnson et al., 1996). Although we have detected significantly elevated levels of p53 in senescent bovine fibroblasts (Favetta et al., 2004a), our studies have also shown that p53 might not play a significant role during early embryo development (Matwee et al., 2000; Favetta et al., 2004b). This was further confirmed by more recent studies (Velez-Pardo et al., 2007). It would be interesting to examine the isoforms of p53 (p63 and p73) as possible inducers of early embryo arrest.

A stress sensor that can link intracellular ROS levels with permanent arrest of replication in cultured cells and embryos is p66Shc, a newly identified protein belonging to the Shc family of adaptors for signal transduction in mitogenic and apoptotic-responses (Pinton and Rizzuto, 2008). p66Shc is a splice variant of p52Shc/p46Shc, a cytoplasmic signal transducer Shc family involved in mitigating proliferation signals from activated receptors to Ras (Pellicci et al., 1992). Deletion of p66Shc in mice results in approximately a 30% increase in lifespan because of a greater resistance to oxidative stress and reduction in p53-mediated apoptosis (Migliaccio et al., 1999). The observations that p66Shc is required for early mitochondrial responses to oxidative challenge including mitochondrial fragmentation and suppression of Ca$^{2+}$ signal propagation (Pinton et al., 2007) and that forhead/FOXO activity is regulated by intracellular ROS in a p66Shc-dependent, mitochondrial and extra-mitochondrial manner (Fig. 2) suggest that intracellular H$_2$O$_2$/ROS might also provide specific signaling functions in cellular senescence (Nemoto and Finkel, 2002). Upon serine-36 phosphorylation, p66Shc is translocated into the mitochondrial intermembrane space where it interacts with reduced cytochrome c to produce H$_2$O$_2$ and to open the permeability transition pores which allow the generation and release of ROS into the cytosol (Orsini et al., 2004; Giorgio et al., 2005; Pinton et al., 2007). This p66Shc-mediated intracellular ROS production may facilitate permanent replication arrest in cells/embryos at modest ROS levels and induce apoptosis at high ROS doses (Fig. 2). The activation of permeability pores in a subpopulation of mitochondria may provide the means for the embryo to regulate mitochondrial metabolism and/or remove impaired mitochondria by triggering their autophagic degradation (Elmore et al., 2001; Hajneczky and Hoek, 2007). Morphologically good-quality embryos have mitochondria localized at the nuclear periphery; however, they are also distributed at the outside edges of cytoplasm in granular and clumped aggregates (Neganova et al., 2000; Wilding et al., 2001). In contrast, in slow developing or blocked embryos, the distribution pattern is more uniform with dense mitochondria accumulated around the nuclei while the marginalized cytoplasmic clusters of mitochondria and mitochondrial activity remain strikingly reduced (Neganova et al., 2000; Wilding et al., 2001). These observations point to a possible connection between cytoskeletal organization and the activity of motor proteins involved in mitochondrial transport (Neganova et al., 2000). The mitochondrial aggregation patterns closely parallel the p66Shc distribution pattern observed in proliferating cells and arrested 2- to 4-cell embryos (Favetta et al., 2004b, 2007a,b). It would be interesting to perform co-localization studies of activated p66Shc with MitoTracker$^\text{TM}$ staining of mitochondria in embryos to correlate p66Shc staining intensities with trans-membrane potentials. It would be equally interesting to test whether or not the serine-threonine kinase mTOR is present in arrested embryos since it inhibits autophagy (Meijer and Codogno, 2006) and augments mitochondrial metabolism and ROS generation (Nemoto et al., 2006). This mTOR-regulation of mitochondrial autophagy/metabolism may also be under the control of the extra-mitochondrial p66Shc pathway (Fig. 2). This line of events has been linked to the activation of Akt, which leads to the phosphorylation of Forkhead (FOXO) transcription factors (Nemoto and Finkel, 2002). Subsequent reduction in forkhead-dependent transcriptional activity (Kops et al., 1999; Takaishi et al., 1999; Nemoto and Finkel, 2002) provides for cell survival and resistance to apoptosis (Kops et al., 2002; Nemoto and Finkel, 2002), which are requirements for permanent cell cycle arrest.

Although low levels of p66Shc are detected in post-mitotic cells (Conti et al., 1997, 2001), we have demonstrated significantly elevated levels of p66Shc mRNA and protein in senescent bovine fibroblasts and permanently arrested embryos in the absence of apoptosis (Favetta et al., 2004a,b). These elevated levels of p66Shc are associated with more extensive oxidative damage and the production of intracellular ROS (Favetta et al., 2004a,b, 2007a,b). It is interesting that early cleavage-stage embryos, which are more or less transcriptionally quiescent, have rising p66Shc mRNA levels as the duration of arrest increases (Favetta et al., 2004b). The high levels of p66Shc in arrested embryos may be a result of faulty degradation of maternal mRNAs. A recent report from Richard Schultz’s group has revealed through global gene expression profiling that many maternal transcripts are not properly degraded during maturation of aged (low quality) oocytes (Pan et al., 2008). Gene products such as p66Shc may be present at sufficient quantities in low-quality cleavage-stage...
embryos prior to embryonic genome activation to elicit various cellular processes including permanent cell cycle arrest. The detection of increased levels of the cyclin-dependent kinase inhibitor p27 in arrested cleavage-stage human embryos (Civico et al., 2002) further supports this assumption.

Likewise, increasing levels of p66Shc have been detected in aging human diploid fibroblasts and exposure to oxidative stress has been shown to induce greater levels of p66Shc in cells from aged individuals relative to their younger counterparts (Pandolfi et al., 2005). Elevated quantities of the cell cycle regulator p21waf1/cip1 have been detected upon p66Shc activation in epidermal growth factor-stimulated A431 cells (Sato et al., 2002). Oxidant or antioxidant treatment of embryos at different embryonic stages modulates the occurrence of permanent embryo arrest or apoptosis depending on the stage of development at which the embryos were treated (D. H. Betts, unpublished data). These observations suggest that ROS-induced embryo arrest is developmentally regulated. Furthermore, our recent observation that RNA interference knockdown of p66Shc in bovine embryos significantly diminishes the occurrence of permanent embryo arrest (Favetta et al., 2007a) supports the hypothesis that p66Shc regulates the senescence-signaling pathway in cells and embryos. Moderate production of ROS/H2O2 (oxidative stress) by mitochondrial p66Shc may mediate global changes in gene expression/activation and the induction of oxidative telomeric damage resulting in permanent arrest of growth in cells and blastomeres (Fig. 2).

Figure 2: p66Shc is proposed to regulate a ROS-mediated, telomere dysfunction pathway that signals permanent embryo arrest. Extracellular stressors such as H2O2 or intracellular mitochondrial ROS production can activate various kinases that subsequently activate p66Shc (serine-36 phosphorylation) leading to its mitochondrial translocation and p66Shc-mediated ROS production and release from the mitochondria that can be partially detoxified by antioxidants. Oxidative stress can also activate the p66Shc-Akt-FOXO pathway, which leads to the activation/inactivation of the forkhead family (FOXO) of transcription factors by post-translational modifications. The effects of acetylation and deacetylation of FOXO appear to be promoter specific, altering (up- or down-regulation) the expression of various genes that will promote permanent cell cycle arrest. Although high levels of intracellular ROS can lead to necrosis or apoptosis, moderate levels of ROS can accelerate telomere shortening and/or cause telomere-uncapping leading to a DNA damage response that activates permanent cell cycle arrest. This cyclic pattern of ROS-mediated activation of p66Shc leads towards continual intracellular ROS production and mitochondrial dysfunction, allowing for a cellular environment favoring mitochondria autophagy or senescence-activation (anti-apoptosis) via a retrograde response and/or other Ca2+ -dependent signaling pathways (not shown).

**Why embryo arrest?**

The question that still comes up is: why do early embryos, equipped with such a potent apoptotic mechanism to rid itself of damaged or unwanted cells, have this alternative permanent-arrest state? The early cleavage divisions are under maternal control (Braude et al., 1988), using transcripts accumulated during oogenesis. Depending on the level of specific maternal stores and the typically low gene expression profiles of most genes, early cleavage embryos, which are sensitive to their micro-environment, including suboptimal culture conditions, may signal permanent embryo arrest at this stage of development as a means to prevent further development of low-quality (abnormal/damaged) embryos (Betts and King, 2001). Embryo arrest may be another mechanism to prevent further development of certain chromosomally abnormal embryos (Almeida and Bolton, 1998), and/or embryos that fail to activate embryonic genomes (Artley et al., 1992). This is especially relevant since early cleavage embryos, which display a high proportion of undifferentiated mitochondria (Sathananthan and Trounson, 2000), are not subject to the scrutiny of cell cycle checkpoints (Hartwell and Weinert, 1989). Alternatively, p66Shc-mediated embryo arrest may represent a failed attempt to regulate mitochondrial trans-membrane potential to compensate for metabolic over-reactivity resulting in the overproduction of ROS.

Germ cells show high levels of telomerase (Betts and King, 1999) and it has long been held that the telomere length is reset in gametes...
(Kozik et al., 1998; Baird et al., 2006) to ensure that each generation begins life with its complement of telomeres intact. Surprisingly, recent studies have shown that there is considerable telomere lengthening in early post-fertilization stages of embryo development (Schaeftlein et al., 2004; Liu et al., 2007). Telomeres of mouse oocytes are shorter compared with their somatic cells counterparts; but, they are predominantly elongated in the early cleavage stages presumably by telomere sister-chromatid exchanges (Liu et al., 2007). Permanent embryo arrest could be the result of a checkpoint mechanism that evaluates the ability of an embryo to establish the correct telomere length and structure (telomere integrity) at the outset of development since proper telomere length and structure have long-lasting implications for health and reproduction (Liu et al., 2002a,b;c; Epel et al., 2004, 2006; Aydos et al., 2005; Keefe et al., 2005, 2006).

**Future perspectives**

Entry into a permanently arrested state is likely determined by an embryos’ ability to protect and lengthen its telomeres (TERT and other telomere-associated proteins), its potential to combat ROS (anti-oxidants) and its capacity to regulate the p66Shc pathway. Oxidative stress activation of p66Shc, which induces H2O2/ROS generation and mitochondrial dysfunction, may be a key player in the positive feedback signaling pathway required for permanent cell cycle arrest. Other investigators have suggested that p66Shc polymorphism may be linked to longevity (Ventura et al., 2002; Mooijaart et al., 2004) and may even be associated with fertility in humans. P66Shc appears to regulate mitochondrial metabolism in a way that modulates the quantity of ROS released into the cytosol. Depending on when p66Shc is activated during preimplantation development and on the amount of ROS generated, the oxidative stress-induced telomere uncapping may lead to permanent embryo arrest or apoptosis. Telomere length has been recently used to predict developmental competence of human embryos (Keefe et al., 2005). Therefore, genetic or pharmacological modification of the p66Shc pathway by promoting stress resistance could have direct implications for various age-related diseases and infertility in humans (Napoli et al., 2003; Francia et al., 2004; Graiani et al., 2005; Berry et al., 2006; Tootsava et al., 2007). The observation that ~50% of human embryos arrested at the 2- to 4-cell stage display a normal karyotype (Almeida and Bolton, 1998) indicates that technical means to combat permanent cell cycle arrest could alleviate some cases of age-related infertility and that these embryos, originally deemed to be non-viable biological waste, could serve as a less ‘controversial’ source for embryonic stem cells for use in regenerative medicine or in the very least additional means to study human embryonic stem cell biology.

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