Available online at www.sciencedirect.com
ScienceDirect
Mitochondrion 7 (2007) 289–296
Mitochondrion
www.elsevier.com/locate/mito

Review

Mitochondria in stem cells

Thomas Lonergan [a,*], Barry Bavister [a], Carol Brenner [b]

[a] Department of Biological Sciences, University of New Orleans, 2000 Lakeshore Drive, New Orleans, LA 70148, USA
[b] Departments of Physiology and Obstetrics and Gynecology, Wayne State University School of Medicine, 540 E. Canfield, Detroit, MI 48201, USA

Received 29 December 2006; received in revised form 6 April 2007; accepted 11 May 2007
Available online 23 May 2007

Abstract

The current status of knowledge about mitochondrial properties in mouse, monkey and human embryonic, adult and precursor stem cells is discussed. Topics include mitochondrial localization patterns, oxygen consumption and ATP content in cells as they relate to the maintenance of stem cell properties and subsequent differentiation of stem cells into specific cell types. The significance of the perinuclear arrangement of mitochondria, which may be a characteristic feature of stem cells, as well as the expression of mitochondrial DNA regulatory proteins and mutations in the mitochondrial stem cell genome is also discussed.

Keywords: Mitochondria; Stem cells; Biochemical applications

1. Introduction

Stem cells have potential for numerous biomedical applications, including therapeutic cell replacement to repair damaged body organs, as tools for studying genetic defects and testing drugs, and as models for studying cell differentiation and early development. Scientists will need to have a thorough understanding of basic stem cell properties before such high stakes goals can be achieved.

There are three types of stem cells: the pluripotent embryonic stem (ES) cells that have the potential to differentiate into any type of body cell, the multipotent cells derived from adult tissue including umbilical cord blood and amniotic fluid which can differentiate into a limited number of cell types of their own lineage, e.g., mesoderm only, and the precursor cells which are adult stem cells committed to differentiation. For a review of the somewhat complicated classification scheme for adult stem cell and precursor cells, see Raff (2003). Investigators need to carefully catalog and compare the properties of these three types of cells. While it is possible that they share common properties, it is also likely that significant differences will be found.

While stem cell research has progressed rapidly since the initial report of human ES cell isolation (Thomson et al., 1998), the majority of published articles have focused primarily on three general areas. Many reports have examined the expression of various genes that are believed to indicate the pluripotency status of stem cells (so-called "stemness"), such as alkaline phosphatase, Oct 4 or SSEA 4. Researchers must verify that the expression of such markers is stable for each cell line during prolonged periods of cell culture in order to verify that the pluripotent condition has not been lost. Another major focus involves physiological cell culture and microenvironment conditions that lead to uniform directed differentiation of stem cells into specific cell types. The third research thrust focuses on reducing possible xenogenic complications arising from cell culture techniques. Human ES cells are typically grown on a layer of mitotically-inactive fibroblasts (commonly mouse fetal fibroblasts), called a "feeder layer", in the presence of fetal calf serum supplements. Although this approach has been moderately successful in supporting proliferation of ES cells, it is fraught with problems. For example, it has been demonstrated that mouse cell surface antigens have translocated into the plasma membrane of some ES cell lines

* Corresponding author.
E-mail address: tlonera@uno.edu (T. Lonergan).

1567-7249/$ - see front matter
doi:10.1016/j.mito.2007.05.002

(Martin et al., 2005), and the potential for transfer of murine or bovine viruses into human ES cells also exists (Tailor et al., 1999). These xenogenic concerns will preclude use of many human ES cell lines for therapeutic purposes. To avoid these complications, several laboratories have reported some success in culturing ES cells either on human fibroblasts, without using any feeder layer, and/or without fetal serum (Richards et al., 2002; Hovatta et al., 2003; Amit et al., 2004; Heins et al., 2004; Rosler et al., 2004; Valbuena et al., 2006), but these culture conditions are technically challenging and not yet widely used. There is the possibility that growing ES cells without feeder layers will not constitute superior growth conditions (Richards et al., 2004), and there is the possibility that cells acquire different biological properties under such culture conditions.

The fundamental importance of stem cell stability in culture conditions, and the consequent maintenance of pluripotency or multipotency, has led to searches for indicators of what is commonly referred to as "stemness". Expression of such markers might indicate not only if the stem cells are normal, but also whether they are fully capable of differentiation. There is a serious lack of research on functional cellular markers of stem cells, such as ultrastructural morphology, metabolic profiles or cell signaling pathways. General features of stem cell function need to be identified so that deviations from the normal pattern could then be used to eliminate defective cell lines from further use. Such properties will also be important in comparing cell lines among different laboratories. The phenotypic stability of ES lines cultured for prolonged periods of time must also be addressed because they may not be chromosomally stable after prolonged cell culture passage in the absence of feeder cells (Ludwig et al., 2006).

While most stem cell studies have focused on the activity of the nuclear genome, characteristics of the mitochondrial genome have been largely ignored. Cells from embryos created by *in vitro* fertilization (IVF) procedures, the source of ES cells, have been reported to exhibit various forms of mitochondrial DNA (mtDNA) mutations, and it is not known if metabolic functions of stem cells are affected by high copy number of mtDNA point mutations or mitochondrial deletions (Gibson et al., 2006). There is a distinct possibility that many of the ES cell lines currently available for study do not represent high quality stem cells. Considering that mutations in mitochondrial DNA have been linked to a wide range of disorders, including diabetes, cardiovascular disease and cancer (Chinney et al., 2002; Liu et al., 2002; Maitra et al., 2005; Birch-Machin, 2006), it is surprising that the mitochondrial properties of stem cells have been largely overlooked. These concerns raise the possibility that the use of aberrant stem cell lines, whether from embryonic or adult sources, for therapeutic cell replacement could lead to the development of cancer. ES cells and cancer cells share several traits, including unlimited self renewal capabilities and the ability to generate a diverse range of other cell types. The possible presence of stem cell populations in tumors (Huntley and Gilliland, 2005; Clarke and Fuller, 2006) has many implications for the diagnosis and treatment of cancers, since the most effective way to eliminate the disease would be to target cancer stem cells for destruction. Thus, mtDNA anomalies could have widespread implications for the biomedical applications of stem cells as well as for studies on their behavior *in vitro*.

In this review, we examine mitochondrial properties in early-stage embryos, the source of ES cells, as well as in adult stem cells and differentiating precursor cells.

2. Mitochondrial localization in early-stage embryos and stem cells

ES cells are derived from the inner cell mass (ICM) of the pre-implantation blastocyst stage (Fig. 1). Descriptions of mitochondria in a number of human and mouse ES cell lines using electron microscopy report that these cells have few mitochondria with poorly developed cristae (Sathananthan et al., 2001; Baharvand and Matthaei, 2003; Oh et al., 2005; Cho et al., 2006). The human ES cell line HSF6 was reported as having few mitochondria that tend to localize in small perinuclear groups (see below) (St. John et al., 2005). When HSF6 cells were removed from feeder layers and allowed to differentiate for one week, the resulting cells showed numerous larger mitochondria with distinct cristae. The observations of ES cells having few mitochondria are consistent with reports of lower mitochondrial mass and

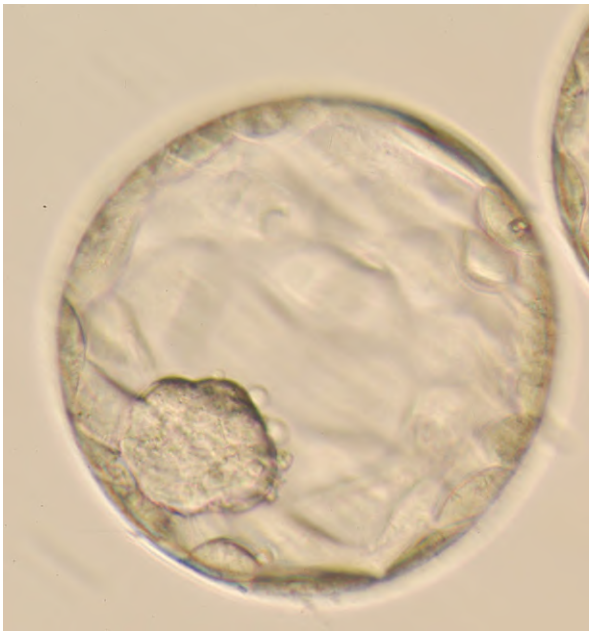

Fig. 1. Human IVF blastocyst at approximately the 100 cell stage. The outer layer of cells is the trophectoderm layer that contributes to the placenta. The ball of cells at the lower left of the image is the inner cell mass (ICM) from which the fetus develops or from which the embryonic stem cells are derived upon ICM removal followed by culturing *in vitro*. Courtesy of Dr. Richard Scott, Reproductive Medicine Associates of New Jersey, Morristown, NJ, USA.

mtDNA content in both the human HSF6 cell line (St. John et al., 2005) and several newly created human cell lines (Cho et al., 2006), in which mtDNA content was quantified using real-time PCR. In both reports, the numbers of mtDNA copies increased as cells were allowed to differentiate, and in the HSF6 cell line, differentiation resulted in cells with numerous larger mitochondria with distinct cristae.

A pattern for the cytoplasmic location of mitochondria, as viewed using fluorescent probes, is beginning to emerge. Because of the continuum of development from fertilization through ES cells, it has been suggested that the mitochondrial characteristics of the pre-implantation embryo might have much in common with those of ES cells (Bavister, 2006). In several species, including hamster (Barnnett et al., 1996), mouse (Batten et al., 1987), monkey (Squirrell et al., 2003) and human (Wilding et al., 2001), the clustering of mitochondria around the pronuclei of fertilized oocytes and around the nuclei of cleaveage stage embryos (Fig. 2) has led to the idea that a perinuclear arrangement of mitochondria might be a cellular marker for "stemness" (Lonergan et al., 2006). Such an arrangement was reported for the human HSF6 cell line (St. John et al., 2005), cells of the human SNU line series (Cho et al., 2006), an adult human hematopoietic cell line (Piccoli et al., 2005), and for a rhesus monkey adult mesenchymal cell line (ATSC line) (Lonergan et al., 2006). Patterns of mitochondrial localization can be quantified by measuring the fluorescence intensity of labeled mitochondria from the edge of the nucleus to the edge of the plasma membrane (Fig. 3b and c). Using this approach, the proportions of the population exhibiting three distinct patterns – perinuclear, homogeneous/random or aggregated/fused (Fig. 3d–f) – were measured in the ATSC line (Lonergan, 2006). This cell line is known to spontaneously differentiate into adipose-like cells over several passages (Fig. 3a), and changes in mitochondrial localization can be monitored during the process of differentiation. Early passage stem cells (P11) showed 85% perinuclear mitochondrial localization, but as cultures progressed to higher passage numbers (P17) over a six month period, this frequency decreased to 18% as cells began to differentiate into adipocyte-like cells that predominantly exhibited an aggregated or fused mitochondrial pattern. The mitochondria in the early and mid passage numbers were described as threadlike. That the aggregated/fused mitochondrial morphology truly represents mitochondrial localization was verified by labeling with an antibody against oxidative phosphorylation (OXPHOS) complex I. The percentage of cells with the aggregated pattern correlated to the percentage of cells identified as presumptive adipocytes. The report by Lonergan et al. (2006) suggests that periodically measuring the percentage of cells with a perinuclear mitochondrial arrangement might serve as method to monitor the maintenance of "stemness" in stem cell populations, at least in adult stem cell lines. Additional cell lines need to be examined using this approach to determine the validity of this predictive marker.

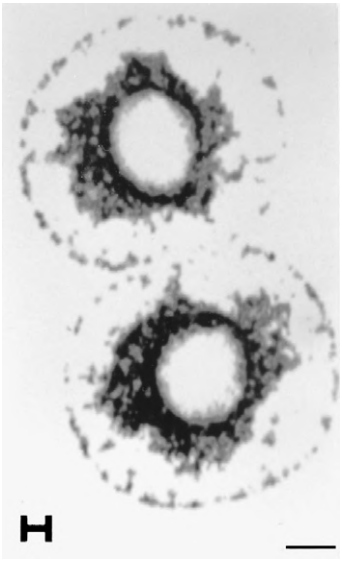

Fig. 2. Hamster two-cell stage embryo stained with Rhodamine 123. Mitochondria are clustered around each nucleus. Bar equals 10 μm. Reproduced with permission from Barnett et al. (1996).

3. Metabolic characterizations of stem cells

Prior to embryo implantation and subsequent *in vivo* vascularization, embryonic cells are contained in a hypoxic environment within the uterine lumen (Fisher and Bavister, 1993). Because this environment is not conducive to ATP synthesis through OXPHOS, the embryonic cells rely on anaerobic metabolism to meet their energy demands (Brown, 1992). It is therefore not surprising that ES cells have few mitochondria that lack cristae development. It has been shown that the ATP content of blastomeres is correlated to their mitochondrial content (Van Blerkom et al., 2000). This finding is consistent with data showing that there is limited mtDNA replication during mammalian pre-implantation development (Spikings et al., 2007), hence there is a progressive decrease in mtDNA content in the blastomeres during cleavage stages. However, the mitochondrial genome undergoes significant replication during implantation of the blasotocyst (Thundathil et al., 2005), and once gastrulation occurs cells replicate their mtDNA to match the OXPHOS demands of differentiating cells (St. John et al., 2005).

There are only two reports of ATP content in stem cells, one for a human ES line (Cho et al., 2006), and the other for the monkey adult ATSC cell line (Lonergan et al., 2006). The ATP content in both cell lines was lower when cells were characterized as stem-like, but upon differentiation the ATP increased fourfold in the human cell line and fivefold in the monkey cell line. An elevation in ATP content per cell may therefore signal a loss of stemness and the subsequent onset of differentiation.

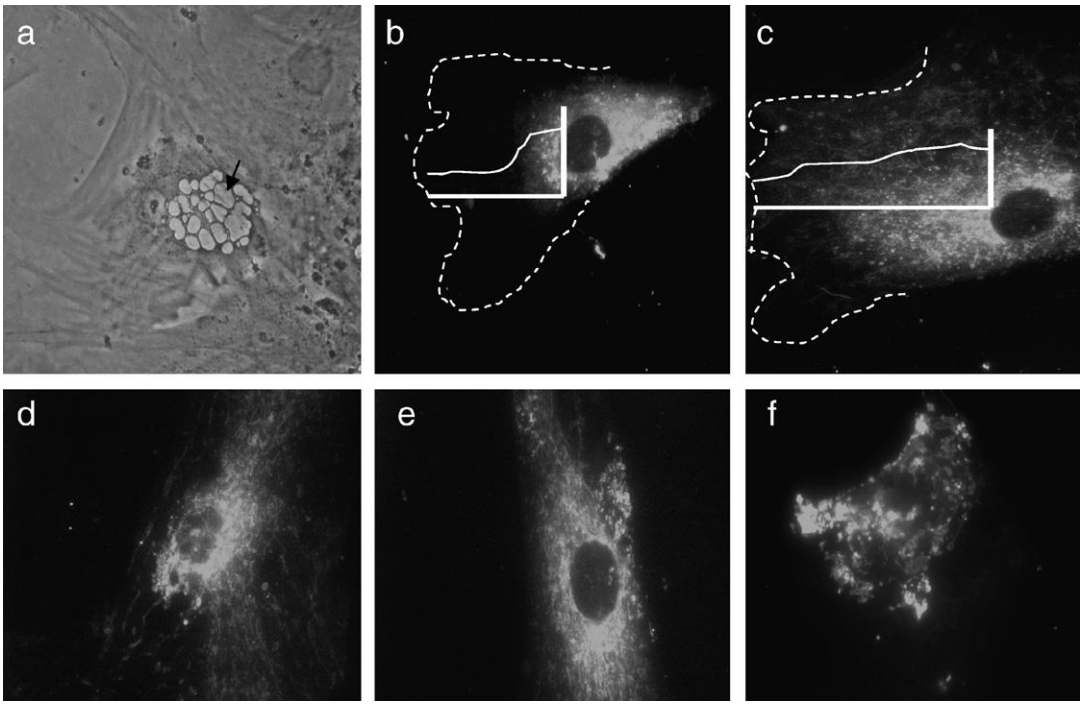

Fig. 3. Images of adult rhesus stromal stem cells. (a) Phase contrast image (400×) of a passage 14 stem cell that has differentiated or deteriorated into a cell containing numerous lipid droplets (arrow). (b) Measurement of Mitotracker fluorescence intensity in a passage 11 cell with perinuclear mitochondrial arrangement (600×). The cell periphery is outlined (dotted line). Cells with a predominantly perinuclear arrangement of mitochondria have a very high fluorescence intensity near the nucleus which drops precipitously toward the cell periphery (graph inset). Because there is such a large variation in intensity at the two ends of the transect line, the standard deviation is numerically high, resulting in a low fluorescence intensity/standard deviation ratio. (c) In a cell with a homogeneous distribution of mitochondria (passage 14, 600×), the fluorescence intensity is relatively constant along the transect line (graph inset) and the standard deviation is numerically low, resulting in a higher ratio. (d) Representative image of a passage 11 perinuclear mitochondrial arrangement (600×), (e) image of a passage 14 homogeneous mitochondrial arrangement (600×), and (f) image of passage 17 an aggregated mitochondrial arrangement (600×). Reproduced with permission from Lonergan et al. (2006).

There are three reports of oxygen consumption rates for stem cells, and a fourth report using JCP-1 to identify the presence of high potential mitochondria, which is an indirect indicator of oxygen consumption rates. All of these studies used either adult cell lines or precursor cells, but a clear pattern has yet to emerge. Human pre-adipocytes were reported to have a lower rate of oxygen consumption compared to mature adipocytes (von Heimburg et al., 2005), a pattern also reported for human hematopoietic cells (Piccoli et al., 2005). Two other reports suggest that the rate of oxidative phosphorylation is higher in undifferentiated adult stem cells compared to their differentiated counterparts. Neuronal stem cells isolated from 9- to 10-week-old human fetuses form neurospheres in culture (Plotnikov et al., 2006). Stem cells in younger, smaller neurospheres had more high-potential mitochondria, as judged by JC-1 staining, compared to older, larger neurospheres (Plotnikov et al., 2006). The reduction in JC-1 staining in the mature cells might be the result of lower oxygen levels in the center of the neurosphere. Similar results were reported for the rates of oxygen consumption, measured with a Clark-type electrode, using the ATSC rhesus monkey adult cell line cultured in 5% CO_2 in air. The cultures with a higher percentage of stem cells, as judged by a predominantly perinuclear arrangement of mitochondria, had a tenfold higher rate of oxygen consumption compared to the more differentiated cells.

Oxygen consumption rates for ES cells have not been reported. As previously stated in this review, the early embryo from which ES cells are derived, exists in a hypoxic environment. The cell culture conditions typically reported for ES cells consist of 5% CO_2 in air (~20% O_2). Reports that oxygen supply can alter oxygen-dependent gene expression in cells, including differentiating embryonic mouse and human cells (Gassman et al., 1996; Wartenberg et al., 2001; Harvey et al., 2004), suggest that future studies using ES cells should be conducted at lower oxygen tensions (e.g., 5% CO_2 in 1–5% O_2), and compared to results in the traditional culture condition of 5% CO_2 in air (Harvey, 2007).

4. The significance of perinuclear arrangement of mitochondria

The possible functional significance of the perinuclear arrangement of mitochondria in stem cells deserves further examination. Numerous types of differentiated cells have also been reported to exhibit perinuclear arrangements of mitochondria, including fibroblasts (Yaffe, 1999), pancreatic ascinar cells (Johnson et al., 2003; Bruce et al., 2004),

and astrocytes and neurons (Collins et al., 2002). The addition of ES and adult stem cells to this list reopens the question "Is there a benefit for some cells having a perinuclear arrangement of mitochondria in comparison to cell types that do not?" The mitochondrion and nucleus interact for at least three possible reasons. First, the DNA coding for most of the approximately 1500 mitochondrial-related genes resides in the nuclear genome. The mitochondrial genome codes for thirteen polypeptides that are subunits of the mitochondrial OXPHOS complexes, along with twenty-two tRNAs used in mitochondrial proteins synthesis, and the 12s and 16s mitochondrial rRNAs. Transcripts for the vast majority of the mitochondrial polypeptides are translated in the cytoplasm and imported into the mitochondria. For example, mtDNA replication requires an RNA primer generated by mitochondrial transcription factor A (TFAM) (Larsson et al., 1998) and is coordinated by catalytic DNA polymerase gamma (POLG) and accessory POLYG2 subunits (Gray and Wong, 1992). The transport of polypeptides like POLG into the mitochondria might be more efficient if the mitochondria are clustered around the nucleus. A second reason for perinuclear clustering of mitochondria might be for energy transfer. The import and export of macromolecules across the nuclear pores is thought to involve the energy-dependent Ran monomeric G protein transport system. Positioning the mitochondria near the nucleus might provide the energy for this process in a more efficient manner. Thirdly, it is speculated that a perinuclear arrangement of mitochondria might buffer the nucleus from fluctuations in Ca^{2+} levels occurring in the cytoplasm (Park et al., 2001; Bruce et al., 2004; Giannucci et al., 2003). However, all of these functional explanations for mitochondrial clustering are purely speculative. Studies are needed to ascertain how and why this arrangement might benefit cells in general, and stem cells in particular. Furthermore, it has always been assumed that the cellular positioning of mitochondria is coordinated by their association with microtubules. With the exception of fibroblasts (Yaffe, 1999), it is not known whether this is a general feature of cells. This issue should also be examined in the several cell types in which a perinuclear arrangement has been documented.

5. Expression of mtDNA regulatory proteins in early stage embryos and stem cells

For most species, it is not known at what stage of early embryo development mtDNA replication begins. In mice, reports suggest that no mtDNA replication occurs in the early cleavage stages (Ebert et al., 1988; Piko and Taylor, 1987) although one report suggests a short burst of mtDNA replication in the mouse two-cell stage with no corresponding increase in mtDNA copy number (McConnell and Petrie, 2004). mtDNA replication has been reported in the blastocyst stage in both the mouse (Thundathil et al., 2005) and cattle (McConnell and Petrie, 2004). Several protein factors encoded by the nuclear genome regulate or are involved in mtDNA replication. These factors include polymerase gamma (PolG), mitochondrial DNA helicase (twinkle), mitochondrial single-stranded binding protein, mitochondrial transcription factors, such as TFAM, and other nuclear factors that indirectly regulate TFAM expression, such as PGC1-α, and NRF-1. The expression of TFAM and POLG in competent and incompetent porcine embryos, determined by brilliant cresyl blue staining, was assessed from the two cell stage to the expanded blastocyst stage using both real-time PCR and immunohistochemistry, concomitantly measuring mtDNA copy number using real-time PCR (Spikings et al., 2007). Increased POLG expression was not detected in the ICM until the expanded blastocyst stage, however a small but consistent increase in TFAM gene transcription was observed from the morula to the expanded blastocyst stage. TFAM was present at very low levels throughout the cytoplasm at all stages suggesting that only low levels of TFAM are needed for mtDNA replication.

There are two studies in human ES lines reporting that the expression of mitochondrial transcription factors increases only slightly during differentiation, even though both mitochondrial mass and the number of mtDNA copies increased (St. John et al., 2005; Cho et al., 2006). This observation appears to be inconsistent with the observed accumulation of mitochondrial mass as ES cells differentiate, and this discrepancy has yet to be explained.

6. Mitochondrial mutations in oocytes, embryos and stem cells

A growing concern in the area of Assisted Reproductive Technology is that mitochondrial mutations and deletions have been found at high frequencies in oocytes and embryos, and these may be passed on to the derived ES cell lines and subsequent differentiated tissues (Harvey et al., 2007). The extent to which these mitochondrial mutations perturb mitochondrial functions is unclear, but it has been suggested that mitochondrial instability and lack of repair mechanisms may be associated with poor ooctye and embryo quality in older women whose oocytes are more likely to have significant chromosomal abnormalities (Munne et al., 1995; Harvey et al., 2007).

The mtDNA deletion ΔmtDNA4977, also called the "common deletion", was found to be present in rhesus monkey oocytes and embryos. Germinal vesicle oocytes obtained from necropsied rhesus ovaries which were never stimulated by exogenous follicle stimulating hormone (FSH) or human chorionic gonadotropin (hCG) were compared to oocytes obtained from stimulated follicles and with IVF embryos derived from these oocytes. Twenty-one percent of the germinal vesicle-stage oocytes exhibited the common deletion compared to 71% of the oocytes from gonadotrophin-stimulated ovaries and embryos derived by IVF (Gibson et al., 2006). It was concluded that stimulation with FSH and hCG causes mtDNA replication errors resulting in higher frequencies of the common deletion.

This conclusion implies that mitochondrial replication must be occurring during oocyte maturation which is contrary to current beliefs. These results also have profound implications for primate ES cell lines that are typically derived from *in vitro*-produced embryos using oocytes from gonadotropin-stimulated ovaries.

The presence of the common deletion was also examined in three rhesus monkey ES cell lines derived from *in vitro*-produced embryos, and compared to an ES cell line from an *in vivo*-generated rhesus (Gibson et al., 2006). The results were compared to a rhesus adult bone marrow stromal cell line (BMSC) and the rhesus ATSC stromal adult stem line, as well as an immortalized version of the ATSC line created by transfection with a retroviral vector resulting in the expression of telomerase activity (ATSC-TERT) (Kang et al., 2004). The common deletion was detected, but not quantified, in the *in vivo*-derived R4 cell line and the three ORMES cell lines derived from IVF-derived embryos. The ATSC cell line expressed the common deletion both at earlier passages, in which cells were thought to be undifferentiated as judged by a low incidence of triglyceride accumulation, as well as at higher passages in which the majority of cells had visible triglyceride droplet accumulation. The BMSC adult stromal cell line showed high levels of the common deletion in passages 3, 10 and 30. It is not known whether the *in vivo*-derived R4 ES cell line exhibited the common deletion prior to the establishment of the cell line. While these studies are preliminary, an enormous advantage of studying ES cell lines in the rhesus monkey is that ES lines prepared from *in vitro*- vs. *in vivo*-created embryos can be compared (Pau and Wolf, 2004; Bavister et al., 2005). No such comparison can be done with human ES cell lines because of the unavailability of *in vivo*-derived embryos. An accumulation of mtDNA mutations, resulting in detectable cytochrome c oxidase deficiency, has also been reported for adult human colonic stem cells (Greaves et al., 2006). One colonic crypt stem cell with a mtDNA mutation has the potential to expand and replace the entire colonic crypt stem cell population. In view of these observations, mtDNA mutations and deletions should be routinely evaluated for all stem cell lines as part of their normal characterization during prolonged periods of cell culture. Mutations in the mitochondrial genome, could have numerous consequences, including disruption of ATP production, altered cell division rates, premature differentiation or failure to differentiate with subsequent apoptosis (van Blerkom et al., 1995, 2000; Chinnery et al., 2002).

7. Future directions

Little is known about the mechanisms regulating mtDNA replication, repair mechanisms or transcription during the differentiation of ES cells. Numerous questions need to be addressed in order to develop a full understanding of the role of mitochondrial activity in stem cells. Important questions include: (1) When does mtDNA replication occur during the differentiation process, and does this correlate with an increase in mtDNA copy number? (2) Does the timing of mtDNA replication correlate with the attainment of functional metabolic activity, and do differentiated cells, such as heart or nerve cells, intended for clinical therapy and maintained in cell culture for prolonged periods of time, retain their full metabolic capabilities or do such cells suffer from any metabolic impairment? (3) During the differentiation process, when is POLG expression upregulated? (4) Are patterns of expression for POLG and TFAM consistent across a variety of ES and adult stem cell lines or are they specific for pluripotent cells or for particular cell lineages? (5) What is the impact of mtDNA mutation frequency on ES cell colony growth rate, differentiation capacity and cancer potential? (6) If mitochondrial-based metabolism is altered in any way, what are the consequences for stem cell growth rates and their ability to differentiate? Answers to these and similar fundamental questions are needed before stem cells can be considered as agents for alleviating human disease.

References

Amit, M., Shariki, C., Margulets, V., Itskovitz-Eldor, J., 2004. Feeder layer and serum free culture of human embryonic stem cells. Biol. Reprod. 70, 837–845.

Baharvand, H., Matthaei, K.I., 2003. The ultrastructure of mouse embryonic stem cells. Reprod. Biomed. Online 7, 330–335.

Barnett, D.K., Kimura, J., Bavister, B.D., 1996. Translocation of active mitochondria during hamster preimplantation embryo development studies by confocal laser scanning microscopy. Dev. Dyn. 205, 64–72.

Batten, B.E., Albertini, D.F., Ducibella, T., 1987. Patterns of organelle distribution in mouse embryos during preimplantation development. Am. J. Anat. 178, 204–213.

Bavister, B.D., Wolf, D.P., Brenner, C.A., 2005. Challenges of primate embryonic stem cell research. Cloning Stem Cells 7, 82–94.

Bavister, B.D., 2006. The mitochondrial contribution to stem cell biology. Reprod. Fertil. Dev. 18, 829–838.

Birch-Machin, M.A., 2006. The role of mitochondria in ageing and carcinogenesis. Clin. Exp. Dermatol. 31, 548–552.

Brown, G.C., 1992. Control of respiration and ATP synthesis in mammalian mitochondria and cells. Biochem. J. 284, 1–13.

Bruce, J.I.E., Giovannucci, D.R., Blinder, G., Shuttleworth, T.J., Yule, D.I., 2004. Modulation of $[Ca^{2+}]_i$ signaling dynamics and metabolism by perinuclear mitochondria in mouse parotid acinar cells. J. Biol. Chem. 279, 12909–12917.

Chinnery, P.F., Samuels, D.C., Elson, J., Turnbull, D.M., 2002. Accumulation of mitochondrial DNA mutations in ageing, cancer and mitochondrial disease: is there a common mechanism? Lancet 360, 1323–1325.

Cho, Y.M., Kwon, S., Pak, Y.K., Seol, H.W., Choi, Y.M., Park, D.J., Park, K.S., Lee, H.K., 2006. Dynamic changes in mitochondrial biogenesis and antioxidant enzymes during the spontaneous differentiation of human embryonic stem cells. Biochem. Biophys. Res. Commun. 348, 1472–1478.

Clarke, M.F., Fuller, M., 2006. Stem cells and cancer: two faces of eve. Cell 124, 1111–1115.

Collins, T.J., Berridge, M.J., Lipp, P., Bootman, M.D., 2002. Mitochondria are morphologically and functionally heterogeneous within cells. EMBO J. 21, 1616–1627.

Ebert, K.M., Liem, H., Hecht, N.B., 1988. Mitochondrial DNA in the mouse preimplantation embryo. J. Reprod. Fertil. 82, 145–149.

Fisher, B., Bavister, B.D., 1993. Oxygen tension in the oviduct and uterus of rhesus monkeys, hamsters and rabbits. J. Reprod. Fertil. 99, 673–679.

Gassman, M., Fandrey, J., Bichet, S., Wartenberg, M., Marti, H.H., Bauer, C., Wenger, R.H., Acker, H., 1996. Oxygen supply and oxygen-dependent gene expression in differentiating embryonic stem cells. Proc. Natl. Acad. Sci. USA 93, 2867–2872.

Giannucci, B.J., Blinder, D.R., Shuttleworth, T.J., Yule, D.I., 2003. Modulations of $[Ca2+]_i$ signaling dynamics and metabolism by perinuclear mitochondria in mouse parotid acinar cells. J. Biol. Chem. 279, 12909–12917.

Gibson, T.C., Pei, Y., Quebedeaux, T., Brenner, C.A., 2006. Mitochondrial DNA deletions in primate embryonic and adult stem cells. Reprod. Biomed. Online 12, 101–106.

Gray, H., Wong, T.W., 1992. Purification and identification of subunit structure of the human mitochondrial polymerase. J. Biol. Chem. 267, 5835–5841.

Greaves, L.C., Preston, S.L., Tadrous, P.J., Taylor, R.W., Barron, M.J., Oukrif, D., Leedham, S.J., Deheragoda, M., Sasieni, P., Novelli, M.R., Jankowski, J.A., Turbull, D.M., Wright, N.A., McDonald, S.A., 2006. Mitochondrial DNA mutations are established in human colonic stem cells, and mutated clones expanded by crypt fission. Proc. Natl. Acad. Sci. USA 103, 714–719.

Harvey, A.J., Kind, K.L., Pantaleon, M., Armstrong, D.T., Thompson, J.G., 2004. Oxygen-regulated gene expression in bovine blastocysts. Biol. Reprod. 7, 1108–1119.

Harvey, A.J., Gibson, T.C., Quebedeaux, T.M., Brenner, C.A, 2007. Impact of assisted reproductive techniques: a mitochondrial perspective from the cytoplasmic transplantation. Curr. Top. Dev. Biol. 77, 230–251.

Harvey, A.J., 2007. The role of oxygen in ruminant preimplantqtion embryo development and metabolism. Anim. Reprod. Sci. 98, 113–128.

Heins, N., Englund, M.C., Sjoblom, C., Dahl, U., Tonning, A., Bergh, C., Lindahl, A., Hanson, C., Semb, H., 2004. Derivation, characterization, and differentiation of human embryonic stem cells. Stem Cells 22, 367–376.

Hovatta, O., Mikkola, M., Gertow, K., Stromberg, A.M., Inzunza, J., Hreinsson, J., Rozell, B., Blennow, E., Andang, M., Ahrlund-Richter, L., 2003. A culture system using human foreskin fibroblasts as feeder cells allows production of human embryonic stem cells. Hum. Reprod. 18, 1404–1409.

Huntley, B.J.P., Gilliland, D.G., 2005. Leukemia stem cells and the evolution of cancer stem-cell research. Nat. Rev. Cancer 5, 311–321.

Johnson, P.R., Dolman, N.J., Vaillant, C., Peterson, O.H., Tepkin, A.V., Erdemli, G., 2003. Non-uniform distribution of mitochondria in pancreatic ascinar cells. Cell Tissue Res. 313, 37–45.

Kang, S.K., Putnam, L., Dufour, J., Ylostalo, J., Jung, J.S., Bunnell, B.A., 2004. Expression of telomerase extends the lifespan and enhances osteogenic differentiation of adipose tissue-derived stromal cells. Stem Cells 22, 1356–1374.

Larsson, N.G., Wang, J., Wilhelmsson, H., Oldfors, A., Rustin, P., Lewandoski, M., Barsh, G.S., Clayton, D.A., 1998. Mitochondrial transcription factor factor A is necessary for mtDNA maintenance and embryogenesis in mice. Nat. Genet. 18, 231–236.

Liu, L., Trimarchi, J.R., Smith, P.J., Keefe, D.L., 2002. Mitochondrial dysfunction leads to telomere attrition and genomic instability. Aging Cell 1, 40–46.

Lonergan, T., Brenner, C., Bavister, B., 2006. Differentiation-related changes in mitochondrial properties as indicators of stem cell competence. J. Cell Physiol. 208, 149–153.

Ludwig, T.E., Levenstein, M.E., Jones, J.M., Berggren, W.T., Mitchen, E.R., Frane, J.L., Crandall, L.J., Daigh, C.A., Conrad, K.R., Piekarczyk, M.S., Llanas, R.A., Thomson, J.A., 2006. Derivation of human embryonic stem cells in defined conditions. Nat. Biotechnol. 24, 185–187.

Maitra, A., Arking, D.E., Shivapurkar, M., Ikeda, M., Stastny, V., Kassauei, K., Sui, G., Cutler, D.J., Liu, Y., Brimble, S.N., Noaksson, K., Hyliner, J., Schulz, T.C., Zeng, X., Freed, W.J., Crook, J., Abraham, S., Colman, A., Sartipy, P., Matsui, S., Carpenter, M., Gazdar, A.F., Rao, M., Chakravarti, A., 2005. Genomic alterations in cultured human embryonic stem cells. Nat. Genet. 37, 1099–1103.

Martin, M.J., Muotri, A., Gage, F., Varki, A., 2005. Human embryonic stem cells express an immunogenic nonhuman sialic acid. Nat. Med. 11, 228–232.

McConnell, J., Petrie, L., 2004. Mitochondrial DNA turnover occurs during preimplantation development and can be modulated by environmental factors. Reprod. Biomed. Online 9, 418–424.

Munne, S., Alikani, M., Tomkin, G., Grifo, J., Cohen, J., 1995. Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. Fertil. Steril. 64, 382–391.

Oh, S.K., Kim, H.S., Ahn, H.J., Soel, H.W., Kim, Y.Y., Park, Y.B., Yoon, C.J., Kim, D.-W., Kim, S.H., Moon, S.Y., 2005. Derivation and characterization of new human embryonic stem cell lines: SNUhES1, SNUhES2, and SNUhES3. Stem Cells 23, 211–219.

Park, M.K., Ashby, M.C., Erdemil, G., Peterson, O.H., Tepikin, A.V., 2001. Perinuclear, perigranular and sub-plasmalemmal mitochondria have distinct functions in the regulation of cellular calcium transport. EMBO J. 20, 1863–1874.

Pau, K.Y., Wolf, D.P., 2004. Derivation and characterization of monkey embryonic stem cells. Reprod. Biol. Endocrinol. 2, 41–52.

Piccoli, C., Ria, R., Scrima, R., Cela, O., D'Aprile, A., Boffoli, D., Falzetti, F., Tabilio, A., Capitanio, N., 2005. Characterization of mitochondrial and extra-mitochondrial oxygen consumpting reactions in human hematopoietic stem cells. J. Biol. Chem. 280, 26467–26476.

Piko, L., Taylor, K.D., 1987. Amounts of mitochondrial DNA and abundance of some mitochondrial gene transcripts in early mouse embryos. Dev. Biol. 123, 364–374.

Plotnikov, E. Yu., Marei, M.V., Podgornyi, O.V., Aleksandrova, M.A., Zorov, D.B., Sukhikh, G.T., 2006. Functional activity of mitochondria in cultured neural precursor cells. Bull. Exp. Biol. Med. 141, 142–146.

Raff, M. 2003. Adult Stem Cell Plasticity: Fact or Artifact? In: Schekman, R., Goldstein, L. Rossant, J. (Eds.), Ann. Rev. Cell Dev. Biol., Annual Reviews, Palo Alto, CA, USA, pp. 1–22.

Richards, M., Fong, C.Y., Chan, W.K., Bongso, A., 2002. Human feeders support prolonged undifferentiated growth of human inner cell masses and embryonic cells. Nat. Biotechnol. 20, 933–936.

Rosler, E.S., Fisk, G.J., Ares, X., Irving, J., Miura, T., Rao, M.S., Carpenter, M.K., 2004. Long-term culture of human embryonic stem cells in feeder-free conditions. Dev. Dyn. 229, 259–274.

Sathananthan, H., Pera, M., Trounson, A., 2001. The fine structure of human embryonic stem cells. Reprod. Biomed. Online 4, 56–61.

Spikings, E.C., Alderson, J., St.John, J.C., 2007. Regulated mitochondrial DNA replication during oocyte maturation is essential for successful porcine embryonic development. Biol. Reprod. 76, 327–335.

Squirrell, J.M., Schramm, R.D., Paprocki, A.M., Wokosin, D.L., Bavister, B.D., 2003. Imaging mitochondrial organization in living primate oocytes and embryos using multiphoton microscopy. Microsc. Microanal. 9, 190–201.

St. John, J., Ramalho-Santos, J., Gray, H., Petrosko, P., Rawe, V.Y., Navara, C.S., Simerly, C.R., Schatten, G., 2005. The expression of mitochondrial DNA transcription factors during early cardiomyocyte in vitro differentiation from human embryonic stem cells. Cloning Stem Cells 7, 141–153.

Tailor, C.S., Nouri, A., Lee, C.G., Koazk, C., Kabat, D., 1999. Cloning and characterization of a cell surface receptor for xenotropic and polytropic murine leukemia viruses. Proc. Natl. Acad. Sci. USA 96, 927–932.

Thomson, J.A., Itskovitz-Elder, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., Jones, J.M., 1998. Embryonic stem cell lines derived from human blastocysts. Science 282, 1145–1147.

Thundathil, J., Filion, F., Smith, J.C., 2005. Molecular control of mitochondrial function in preimplantation mouse embryos. Mol. Reprod. Dev. 71, 405–413.

Valbuena, D., Galan, A., Sanchez, E., Poo, M.E., Gomez, E., Sanchez-Luengo, S., Melguizo, D., Garcia, A., Ruiz, V., Moreno,

R., Pellicer, A., Simon, C., . Derivation and characterization of three new Spanish human embryonic stem cell lines (VAL-3, -4, -5) on human feeder and in serum-free conditions. Reprod. Biomed. Online 13, 875–886.

Van Blerkom, J., Davis, P.W., Lee, J., 1995. ATP content of human oocytes and developmental potential and outcome after in-vitro fertilization and embryo transfer. Hum. Reprod. 10, 415–424.

Van Blerkom, J., Davis, P., Alexander, S., 2000. Differential mitochondrial distribution in human pronuclear embryos leads to disproportionate inheritance between blastomeres: relationship to microtubular organization, ATP content and competence. Hum. Reprod. 15, 2621–2633.

Von Heimburg, D., Hemmrich, K., Zachariah, S., Staiger, H., Pallua, N., 2005. Oxygen consumption in undifferentiated versus differentiated adipogenic mesehcnymal precursor cells. Respir. Physiol. Neurobiol. 146, 107–116.

Wartenberg, M., Frederike, F.D., Ling, F.C., Acker, H., Hescheler, J., Sauer, H., 2001. Tumor-induced angiogenesis studied in confrontation cultures of multicellular tumor spheroids and embryoid bodies grown from pluripotent embryonic stem cells. FASEB J. 15, 995–1005.

Wilding, M., Dale, B., Marino, M., di Matteo, L., Alviggi, C., Pisaturo, M.L., Lombardi, L., DePlacido, G., 2001. Mitochondrial aggregation patterns and activity in human oocytes and preimplantation embryos. Hum. Reprod. 16, 909–917.

Yaffe, M., 1999. The machinery of mitochondrial inheritance and behavior. Science 283, 1493–1497.