Embryonic stem (ES) cells are pluripotent and therefore have the capacity to form any tissue type in the body. They are isolated and grown from blastocyte stage embryos on a feeder layer of cells and are maintained through the use of appropriate growth media. Selective culture conditions may be applied in order to direct the growth of particular types of cells. Pluripotent cells may also be derived from adult cells by using retroviral vectors to introduce appropriate pluripotency genes. The resulting cells are termed induced pluripotent stem (iPS) cells, and they are thought to be equivalent to their ES cell counterparts. We are studying the structure of pluripotent stem cells through the use of the Kock 3D Fusion Microscope (10PM) and live cell imaging techniques, and we are analyzing population characteristics by Flow Cytometry. We have been labeling pluripotent cells with organelle-specific fluorescent dyes, markers of apoptosis, and differentiation markers in order to visualize the subcellular localization of specific organelles and measure relative levels of marker expression and activity. A mitochondrial-GFP ES cell line has been developed in our lab as an imaging tool. We are focusing on correlating mitochondrial distribution and activity with ES cell apoptosis, growth, and differentiation.

**Mitochondrial Imaging**

- **Mitochondrial activity and localization:** JC-1, TMRE, MitoTracker Green
- **Apoptosis:** JC-1, TMRE, Annexin V/PI, Caspase 3, TUNEL
- **Pluripotency:** AP, Oct-4, SSEA-1
- **Differentiation:** Nestin (ectoderm), Brachyury (Mesoderm), α-fetoprotein (endoderm)

**High pluripotency, low differentiation, high mitochondrial activity, low apoptosis**

**Technology Transfer**

* Mitochondrial imaging was performed
* Mitochondrial staining of ES cells suggests that active mitochondria reside in the periphery of the colony
* Mitochondrial staining also suggests that mitochondria may exist in both singular and tubular forms
* Endogenous eGFP is far brighter, more penetrating, and more stable than exogenous mitochondrial dyes

**Conclusions/Future Plans**

* We will use the power of our mtGFPtg ES and iPS cell lines to analyze changes in mitochondrial distribution and activity in differentiating and aging pluripotent stem cells

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