

# Isolation and Characterization of a Putative Intestinal Stem Cell Fraction From Mouse Jejunum

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Although there have been many recent advances regarding the biology of intestinal stem cells, the field has been significantly hampered by the lack of a method to isolate these cells. Therefore, the aim of this work was to explore the hypothesis that viable intestinal stem cells can be isolated as a side population (SP) by FACS following staining with the DNA-binding dye Hoechst 33342.

Preparations of individual cells from either whole mucosa or epithelium of mouse jejunum were stained with Hoechst 33342 and propidium iodide, then sorted using FACS. Cells were characterized using fluorochrome-labeled antibodies to surface and intracellular markers and using annexin V to detect early apoptosis. Total RNA was isolated from sorted fractions and used for quantitative real-time RT-PCR to evaluate expression of cell lineage markers and the intestinal stem cell marker, Musashi-1.

Adult and neonatal jejunum contain a viable population of cells that displays the SP phenotype and is sensitive to verapamil. This population (from both mucosal and epithelial preparations) includes a CD45-negative fraction corresponding to nonhematopoietic cells and shows minimal expression of surface markers typically found on stem cells from other tissues and of intracellular markers found in mesenchymal cells. Additionally, these cells were enriched for Musashi-1, were cytokeratin positive, and survived in culture for up to 14 days.

The CD45-negative SP fraction, although not pure, represents the first successful isolation of a viable population significantly enriched in small intestinal epithelial stem cells.