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Clonal lineage analysis of the endoderm

The pancreas is significant for medical research as a deletion of its ß-cells by autoimmune attack results in type I diabetes mellitus, and aberrant ß-cell function is also a component of type II diabetes. There is therefore interest in developing ways of generating ß-cells in vitro for transplantation. Because of this, it is important to understand how the organ as a whole and this cell-type in particular develop from the endoderm. For example, tracing the lineage of mature ß-cells from progenitor cells or even pancreatic stem cells might identify a therapeutically relevant source of transplantable in vitro derived ß-cells.

I have used the laacZ-system, a powerful tool for retrospective clonal analysis, which provides many advantages compared to methods of prospective analysis, to examine cell lineage in the developing pancreas. Transgenic mice inherit a modified lacZ gene under the control of the ubiquitously expressed ROSA26 promoter. The gene contains duplication within its coding sequence resulting in a frameshift and the expression of a truncated, non-functional ß-galactosidase. Spontaneous homologous recombination events excise the duplication in this sequence and reestablish the correct reading frame. The expression of a functional ß-galactosidase in this cell and its progenitors can be visualised by means of immunohistochemistry. Since the recombination frequency is very low, this system can be used for retrospective clonal analysis: Analysis of the spatial arrangement and cell type identity of labeled cells in a library of different clones provides information about the mode of growth of the organ in general and fate choices for the different cell types (e.g. duct cells, acinari cells, islet cells in the pancreas) via antibody staining.

In the pancreas, an important issue is to find out when pancreatic progenitor cells generate the diverged exocrine and endocrine lineages. In a pilot study, some young embryos (E8.5, E10.5, E12.5) were therefore examined for endoderm-derived clones; additionally, a small pancreas clone library (a collection of dissected pancreata containing clones) of E14.5 & E15.5 embryos was generated and analyzed.

Preliminary results indicate that cells disperse isotropically at the onset of pancreatic organogenesis. A subsequent stage of semi-regionalised growth may precede almost completely coherent growth during pancreatic bud development.

In order to generate a lineage model that is representative of all events, a saturated clone library must be generated, that contains more than one clone of each possible labeling pattern at each stage. Therefore, in the future the library size must be expanded.