Role of SMN1 and SMNDC1 in phase separation required for the high expression of insulin and glucagon in β- and α-cells

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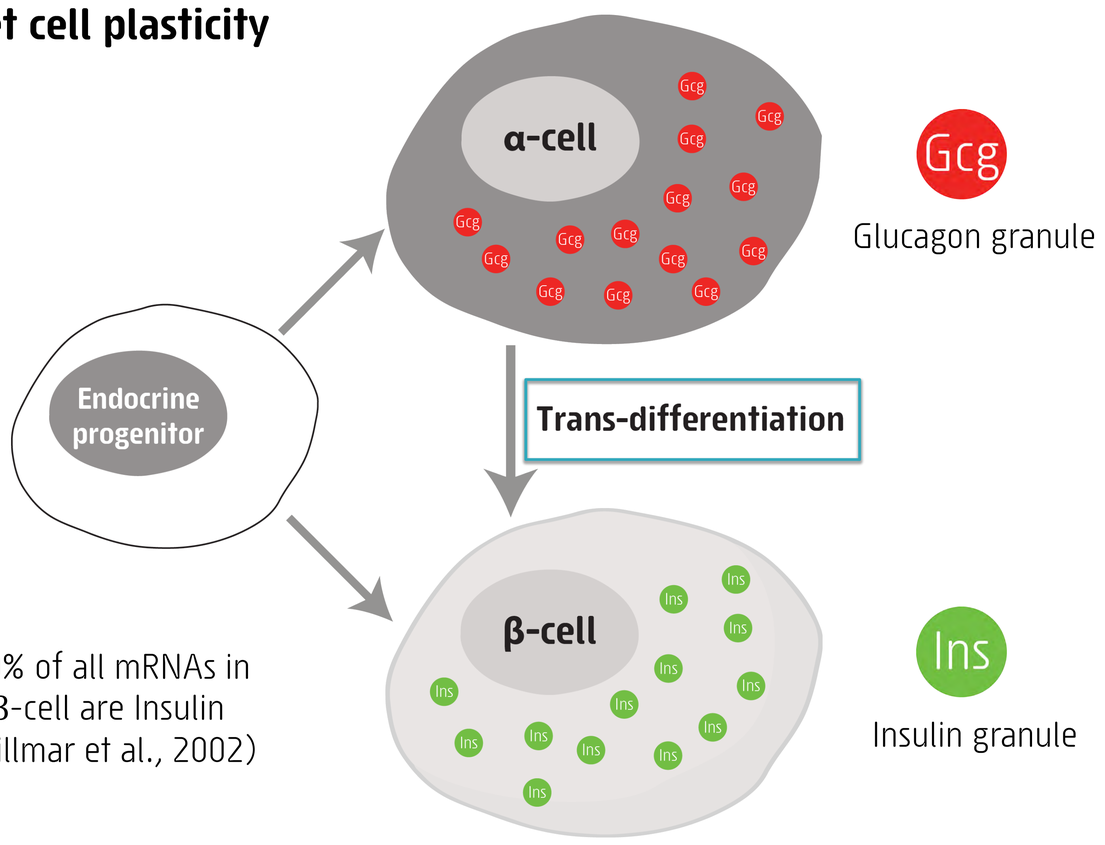
Specific Aims of my DOC project

1. Generate chemical and genetic tools for the perturbation of SMN1 and SMNDC1, doseable and with fast kinetics.
2. Establish assays to analyze subcellular phase distribution in α- and β-cells particularly at glucagon and insulin loci.
3. Investigate the role of SMNDC1 and SMN1 in the establishment and maintenance of these phases.

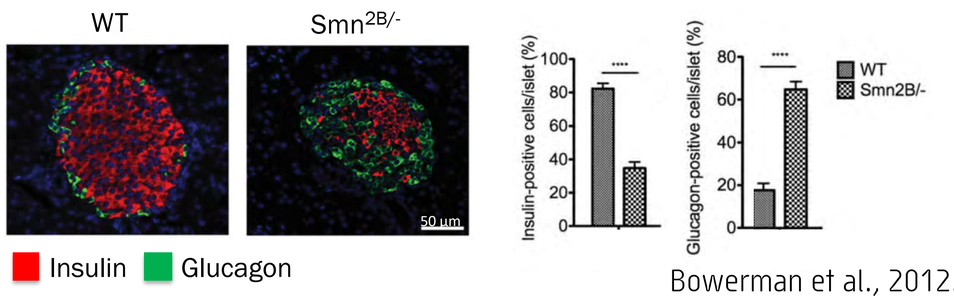
Islet cell plasticity



30% of all mRNAs in a β-cell are Insulin (Tillmar et al., 2002)

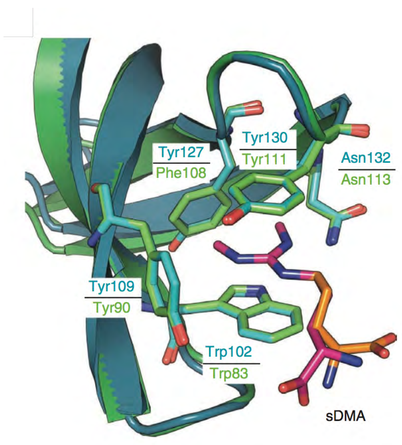
SMN1 and SMNDC1 – candidates for differentiation modulators

- Mutations in SMN1 cause changes in the endocrine cell type composition of islets



Bowerman et al., 2012.

- SMNDC1 is its paralog, both share a a very conserved Tudor domain



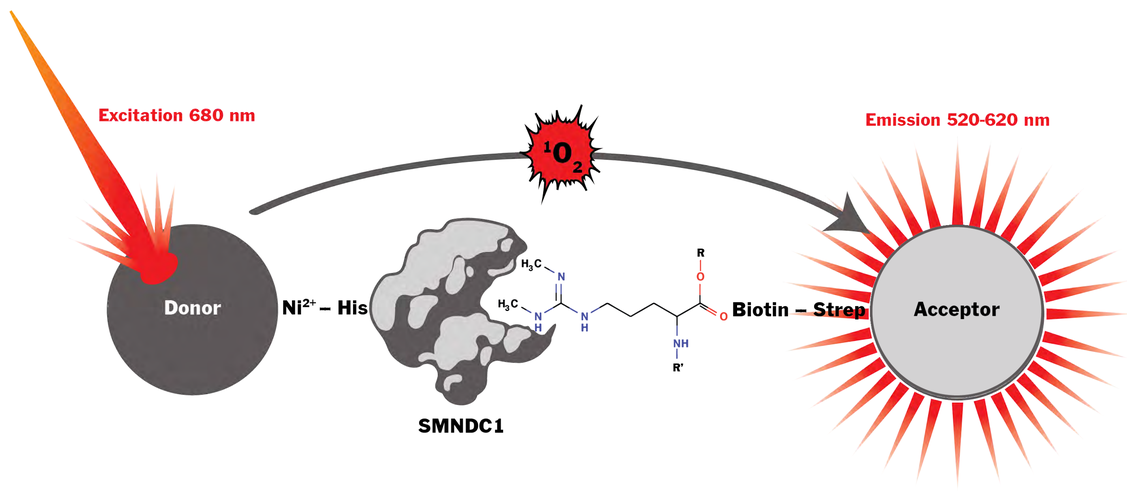
Crystal structure of the Tudor domain, taken from Tripsianes et al., 2011

SMN1 and SMNDC1 – transcriptional control via phase separation?

- Arginine methylation can affect phase separation (Qamar et al., 2018)
- SMN1's Tudor domain was shown to be required for the regulation of stress granules (Chitiprolu et al., 2018)
- Multiple layers of transcriptional regulation are controlled and affected by phase separation (Strom et al., 2017; Lu et al., 2018; Gueroussov et al., 2017; Sabari et al., 2018)

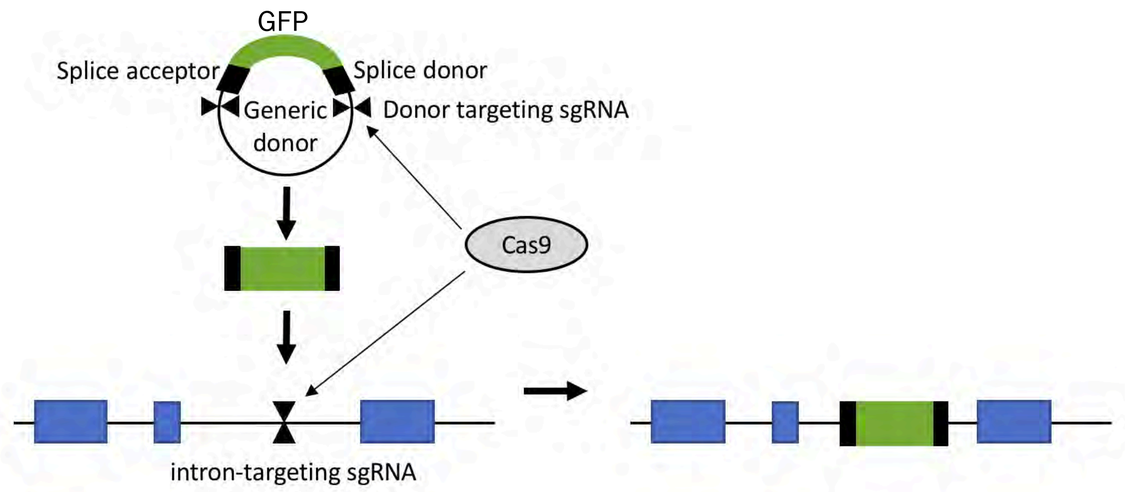
Inhibitor for SMN1's and SMNDC1's tudor domain

- An inhibitor could be used to induce trans-differentiation of alpha to insulin-producing (beta-) cells, change composition of pancreatic islets
- Employing AlphaScreen™ technology

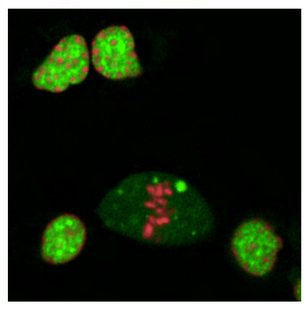


- 90,000 compounds tested
- Currently follow-up of 40 most promising hits

Intron-tag SMNDC1



- Sort for GFP-positives
- Live-cell tracking, nuclear localization of SMNDC1
- Can be used to test AlphaScreen Hits
- Co-localization assays



Nuclear co-staining with DRAQ5

- Treatment with 1,6-hexanediol destroys SMNDC1 separated phase