Transcription factor interactions and target gene specificity in Wnt signaling

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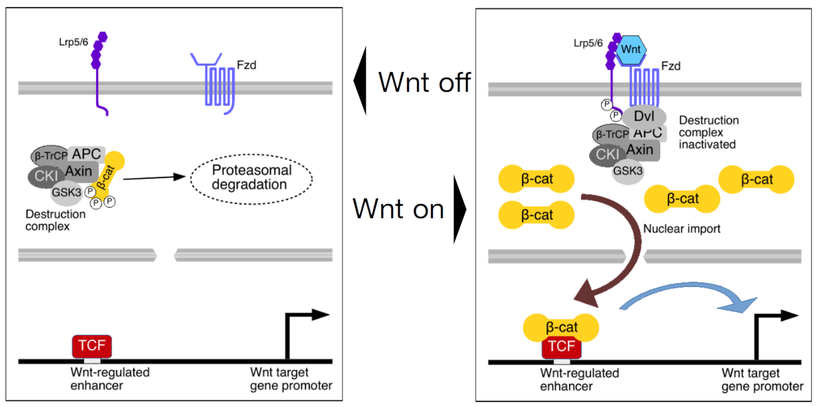
β-catenin stabilisation activates Wnt target genes



TCFs are considered the primary mediators of Wnt transcriptional responses

Wnt target genes are tissue and cell type-specific

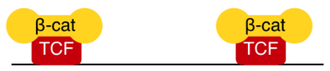
Tissue	Tissue-specific targets	Broadly expressed targets
Small intestine	Human defensins 5/6	Axin2, Lgr5, c-Myc
Liver	Glycogen synthase	Axin2, Lgr5
Nail bed	Krt14	Axin2, Lgr6

TCF/β-catenin-mediated activation drives the transcription of diverse genes with great spatiotemporal specificity

Even within the same tissue, different cell types can express different Wnt target genes

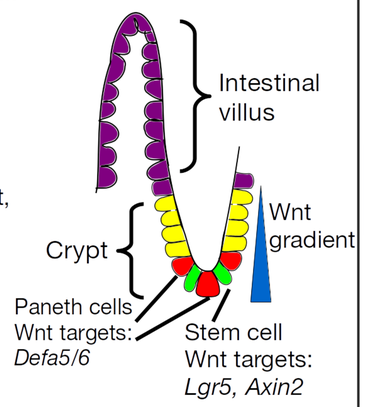
In the intestinal epithelium, both stem and Paneth cells are in a high Wnt environment, but show different Wnt-responsive gene expression programmes

Conventional TCF-only model of a Wnt-regulated enhancer:



Model suggested by target gene diversity:

Wnt target gene diversity in the small intestine

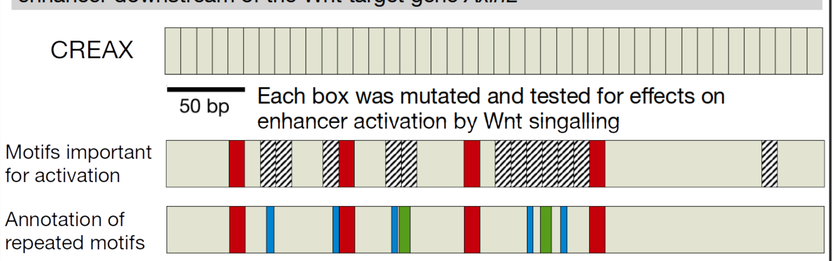


Additional transcription factors (TFs) may regulate Wnt responses by:
- Affecting TCF recruitment
- Modulating other cooperative signalling inputs
- Co-activating specific sets of enhancers based on their expression status

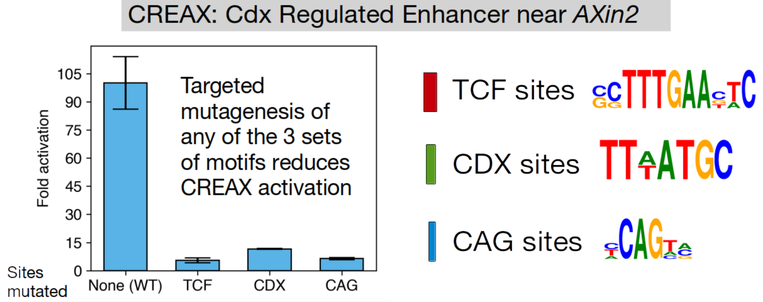
How do we identify and characterise additional TFs co-regulating Wnt target enhancers?

Using cis-regulatory information to characterise additional regulators of Wnt target genes

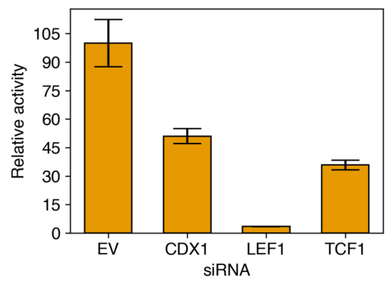
A systematic mutagenesis screen with a luciferase reporter of CREAX, an enhancer downstream of the Wnt target gene *Axin2*



CREAX: Cdx Regulated Enhancer near *AXin2*



TCF and CDX TFs directly bind to and regulate CREAX activity

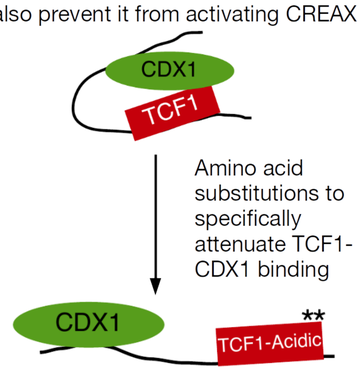


Depletion of multiple TCF family members or CDX1 with RNAi reduces CREAX reporter activity

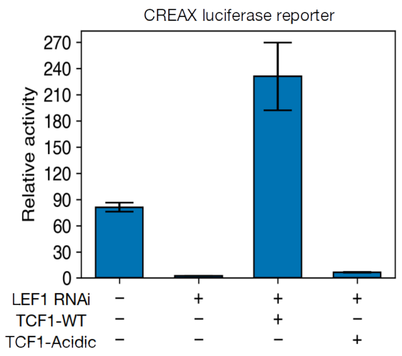
Additionally, with ChIP, an enrichment of TCF and CDX protein binding can be seen at the CREAX locus compared to neighbouring regions

A TCF/CDX interaction is essential for activating CREAX

Mutations in TCF1 which attenuate CDX1 binding also prevent it from activating CREAX



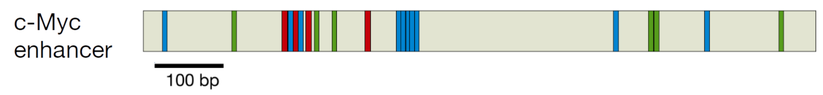
No binding → No transcription

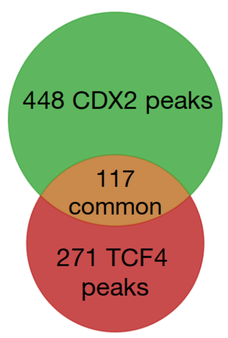


TCF/CDX/CAG binding site grammar is conserved in other enhancers

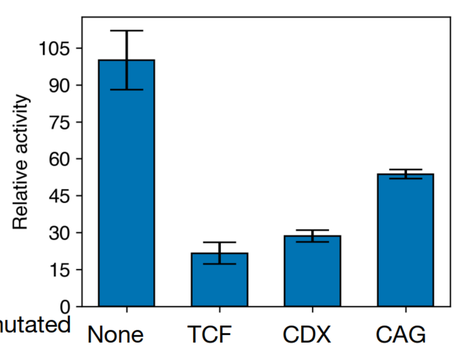
Previous studies in colorectal cancer cell lines have identified significant overlaps in genomic loci bound by TCF and CDX proteins

A Wnt-responsive enhancer of the human c-Myc gene was predicted to have TCF, CDX, and CAG sites





LS174T cell ChIP-chip dataset from Verzi et al , 2010



Enhancer activity is reduced by mutating TCF, CDX, or CAG sites

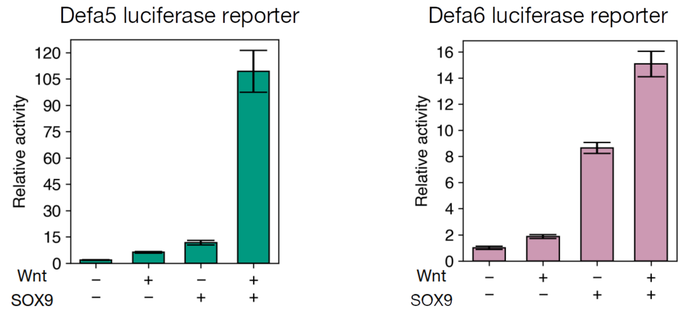
Model: Regulation of broadly-expressed Wnt target genes

- Even broadly-expressed Wnt target genes require the action of multiple different transcription factors to respond to Wnt signalling
- The formation of TCF-CDX complexes is an important step in activating a subset of Wnt target genes
- TCFs do not work in isolation

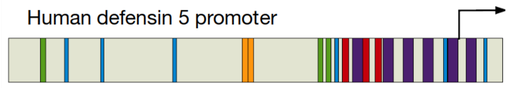
Wnt and SOX9 synergistically activate some cell type-specific Wnt target genes

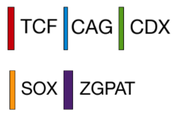
The transcription factor SOX9 is essential for Paneth cell differentiation

SOX9 and Wnt signalling synergistically work together to activate Paneth cell Wnt target genes Defa5/6

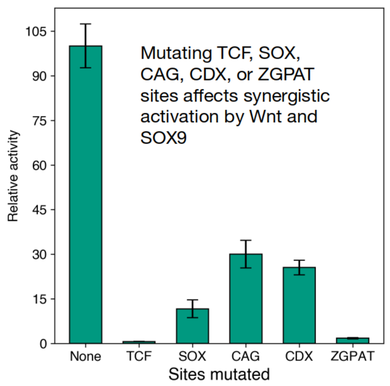


SOX binding sites are required for synergistic activation in addition to TCF/CDX/CAG sites

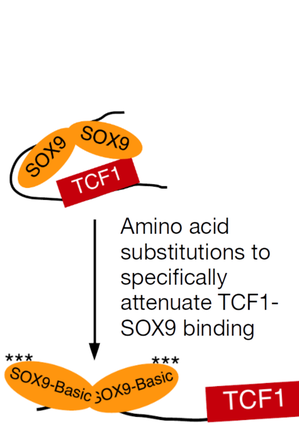


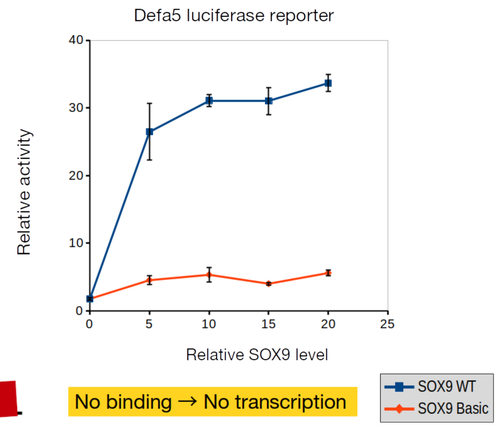


Functionally important CAG sites and binding sites for TCF, SOX, CDX, and the zinc finger transcription factor ZGPAT can be found in these promoters



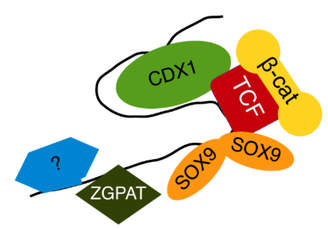
Protein-protein interactions between TCFs and SOX9 are essential for activation





No binding → No transcription

Model: Regulation of cell type-specific Wnt target genes



- Involve more regulators for tissue specificity
- Multiple different transcription factor complexes at work in the cell
- Complex formation with TCFs is a general feature of co-factors

Acknowledgements

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