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Topic (Subject): Gene regulation

Title: TRANSCRIPTIONAL REGULATION OF GLUCOSE DEPENDENT INSULINOTROPIC PEPTIDE

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Abstract:

Glucose dependent Insulinotropic Peptide (GIP), an enteric hormone produced by intestinal K cells in response to glucose preserves glucose homeostasis acting at multiple levels and has high potential as therapeutic target for diabetes/obesity. Transcriptional control of GIP synthesis remains obscure. OBJECTIVE To understand the control of GIP transcription in enteroendocrine (EE) cells via Wnt (through TCF/LEF--catenin) and Protein Kinase A (PKA). Experimental model: STC-1 cell line (from a mouse EE tumor) METHODS Arrays and qRT/PCR for presence/abundance of transcription factors, co-transfections and Luciferase (LUC) assays to evaluate GIP promoter activity. Gel retardation (EMSA) to measure STC-1 extracts binding affinity. RESULTS 1. We cloned 1921 bp from the 5´flanking region of the mouse GIP gene including 946 bp of the promoter. 2.In silico analysis reveals similarities (well conserved TCF/LEF binding sites) and differences (lacks strong cAMP responsive elements (CRE) targeted by PKA) with the closely related promoter of proglucagon (gcg). 3. Inhibition of Wnt effector GSK3 with LiCl strongly increased GIP promoter activity (LUC assays) and endogenous GIP transcripts (qRT/PCR). 4. Promoter deletions co-transfected with an expression vector for -catenin reveal three TCF/LEF sites that mediate a 16 fold transcriptional activation: T1 and T2 sites at the 5´untranslated region; T5 in the proximal promoter and strikingly conserved in the gcg promoter. 5. EMSA experiments confirm TCF/LEF proteins from EE cells binding and preference for the T5 site followed by T1 and T2. 6. Directed mutagenesis confirmed the importance of these sites for GIP transcriptional activation. 7. As expected since there is no CRE in the GIP promoter, PKA activation does not affect GIP transcriptional activity but increased 18 fold the expression of gcg control promoter. 8. Co-transfection of PKAc with -catenin multiply by 5 the  catenin induction revealing an unexpected synergism. Since there is no CRE in the GIP promoter this synergism might derive from Wnt-PKA crosstalk at another level and we are currently working to define it. CONCLUSSION We define Wnt-dependent GIP expression in EE cells and report a Wnt/PKA synergism independent of CRE sites.

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