A systems biology approach highlights the role of GSK3-beta in the regulation of PDX1 by IL1-beta in pancreatic beta cell.

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ABSTRACT: A network model describing the response of proliferation (PDX1) to inflammation (IL1-Beta) in beta cell is described. The model is automatically extracted using an automated path-tracing algorithm from a large beta cell network based on integration of extensive literature. A mathematical model based on mass action kinetics is formulated for this network model. A steady-state simulation of the mathematical model shows that PDX1 decreases as IL1-beta is increased, saturating at high IL1-beta levels. Measurements on mouse pancreatic beta cell line (NIT-1) confirmed this behavior. Further simulations showed that under conditions of GSK3-beta inhibition, the response of PDX1 to IL1-beta reverses to increasing behavior, again saturating at high IL1-beta levels; further, the PDX1 levels were lower in this case. The saturation levels in both of these cases are comparable. Again this behavior was confirmed by measurements. This study highlights the role of GSK3-beta in the switching of PDX1 response to IL1-beta.

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Materials and Methods

Cell culture and treatment

NIT1 cells (ATCC) were cultured in Ham's F12 media (Sigma-Aldrich) supplemented with 15% FBS, 2 mM Glutamine, 100 U penicillin and 100 µg/ml of streptomycin (All reagents from GIBCO, USA). For experiments, NIT-1 cells were seeded at a density of 100,000 cells/well in 24-well plates and incubated for 72 h.

RNA isolation, reverse transcription and quantitative real time polymerase chain reaction (qPCR)

NIT1 cell line was cultured under either control or different concentrations of IL1b for 72h in presence of GSK3b inhibitor (LiCl, 10mM) or control (NaCl, 10mM). After incubation, total RNA was extracted from the cells using Trizol reagent (Sigma, St. Louis, MO, USA), and was used as a template for cDNA synthesis with reverse transcriptase and random hexamer primers (ABI, CA, USA). Quantification of gene expression was done using SYBR Green PCR Master Mix (SsoFast™ EvaGreen, Bio-Rad) using the Bio-Rad CFX96 thermal cycler. Gene analyzed in this study was PDX1. 18S RNA was used as a housekeeping gene.

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Materials and Methods

We took a pancreatic β cell network that was built in-house from literature curated evidence and selected 2 entities namely IL-1β and PDX-1. Using a breadth first search (BFS) algorithm we obtained a directed acyclic graph (DAG) between IL-1β and PDX-1. We further simplified this DAG eliminating trivial links to get a simplified DAG shown in panel 4 (called D[IL1B,PDX1]).

Using mass action kinetics we modeled the dynamics of D[IL1B,PDX1] using single body production and decay terms (namely σ and d terms) generating ODE's. We obtained the steady state expression of PDX-1 (x_2) as a function of IL-1β (x_1) and other parameters like rate constants and production and decay terms. The ODE system and steady state expression for PDX-1 are shown in panel 4.

Simulation of this system was performed by perturbing IL-1β and measuring the steady state level of PDX-1. This was performed under both normal GSK3β levels and inhibited GSK3β levels. Since exact values of the parameters are unknown we used placeholder values for low, normal and high conditions.

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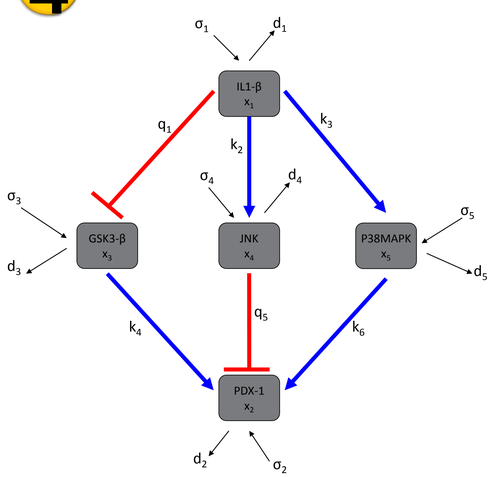
DAG between IL-1β and PDX-1



ODE system representing the network

$$\frac{dx_1}{dt} = \sigma_1 - d_1 x_1,$$
$$\frac{dx_2}{dt} = \sigma_2 - d_2 x_2 + k_4 x_3 + k_6 x_5 - q_5 x_4,$$
$$\frac{dx_3}{dt} = \sigma_3 - d_3 x_3 - q_1 x_1,$$
$$\frac{dx_4}{dt} = \sigma_4 - d_4 x_4 + k_2 x_1,$$
$$\frac{dx_5}{dt} = \sigma_5 - d_5 x_5 + k_3 x_1.$$

$$x_2 = \frac{d_4(k_6 q_1 x_1(\sigma_5 + k_3 x_1) + d_5(k_4\sigma_3 + q_1\sigma_2 x_1) + d_3(d_5\sigma_2 + k_6(\sigma_5 + k_3 x_1)))}{d_5(d_3 + q_1 x_1)(d_2 d_4 + q_5(\sigma_4 + k_2 x_1))}$$

Steady State Representation of PDX-1 in terms of IL-1 β

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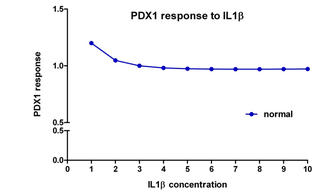
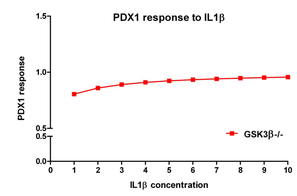
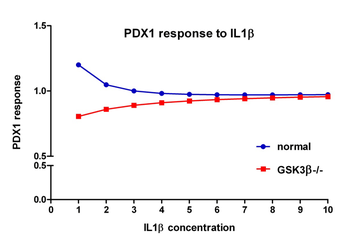
Results

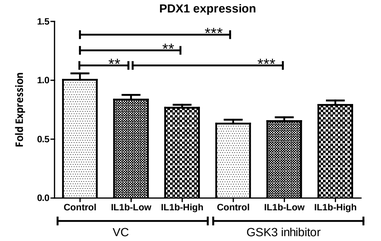


Simulation results of PDX-1 response to increasing IL-1β under normal GSK3β levels



Simulation results of PDX-1 response to increasing IL-1β under inhibited GSK3β levels





Comparison of Simulation results (left) and experimental results (right) shows that under normal GSK3β levels increasing IL-1β decreases PDX-1 levels while under inhibited GSK3β condition the behavior is reversed

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Conclusions

- With GSK3β inhibited PDX-1 levels decrease
- Simulation results predict that under normal GSK3β conditions increased IL-1β reduces PDX-1 levels
- Simulation also shows that under inhibited GSK3β levels increased IL-1β switches behavior and leads to an increase in PDX-1 levels
- At very high IL-1β levels PDX-1 levels are independent of GSK3β levels.
- Experimental results corroborate these findings.

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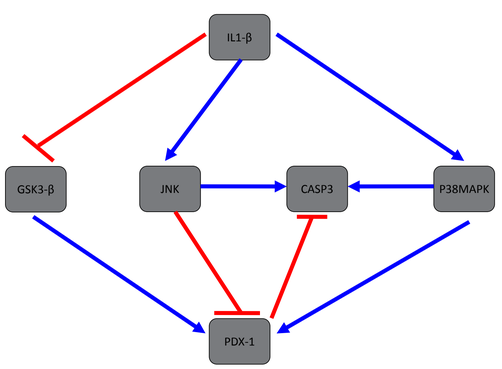
Future Work



We also plan to extend the network to include CASP3 and thus model the effect of increasing inflammation (IL1β) on apoptosis in pancreatic β cells, an important physiological outcome in T2DM