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" A Systems Biology Approach to Type 1 Diabetes "

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&

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Our “systems biology” vision & approach

“Combine the use of experimental models with different profiling technologies and *in silico* approaches to be followed by functional assays in order to identify novel pathways in β-cell destruction associated with T1D”

Different levels

1. Definition of clinical question to be addressed
2. Selection of experimental model and methods of choice
3. Functional analysis of identified mechanisms
4. Diabetes and global relevance?

A systems biology approach to T1D

Level 1. The Aim

T1D is characterised by an immune-mediated destruction of the β-cells of the pancreas, leading to absolute insulin deficiency and a corresponding need for exogenous insulin to preserve life.

1. Clear definition of the pathogenesis behind T1D
2. Identify predictive markers
3. Identify targets for intervention

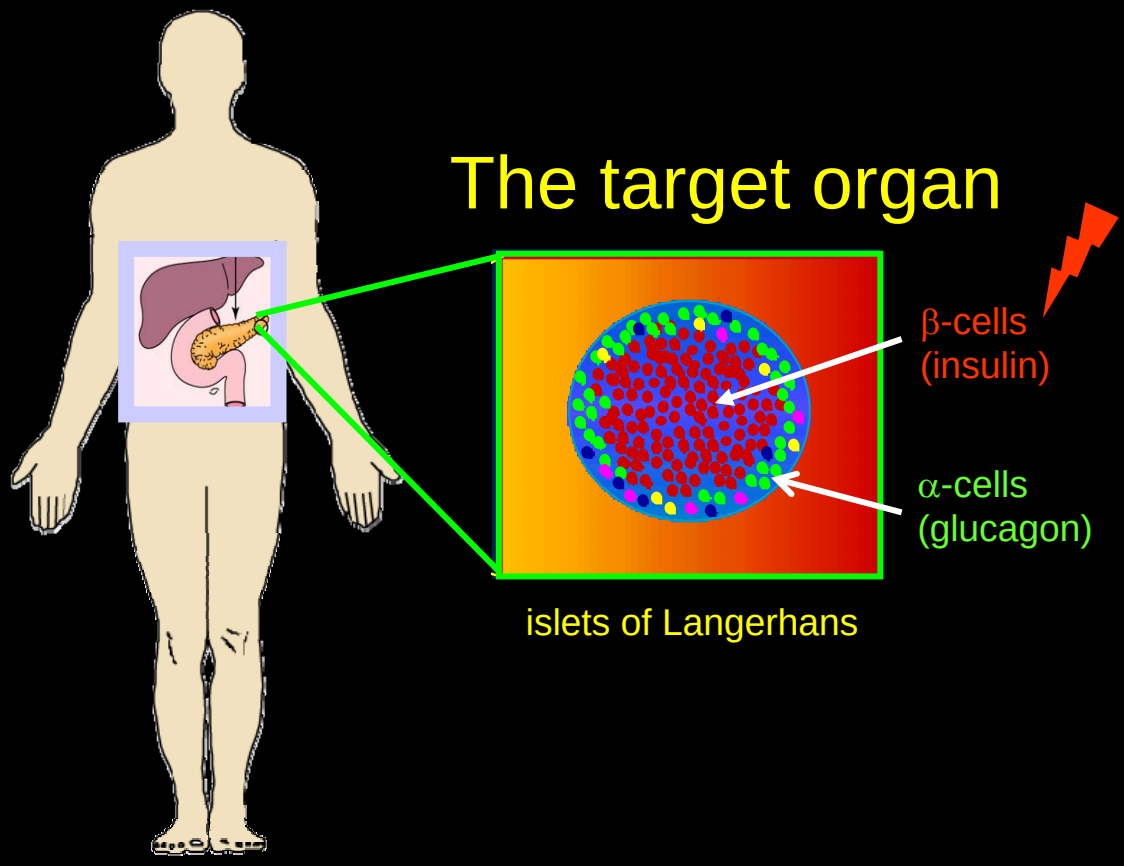
Important players

Genetics

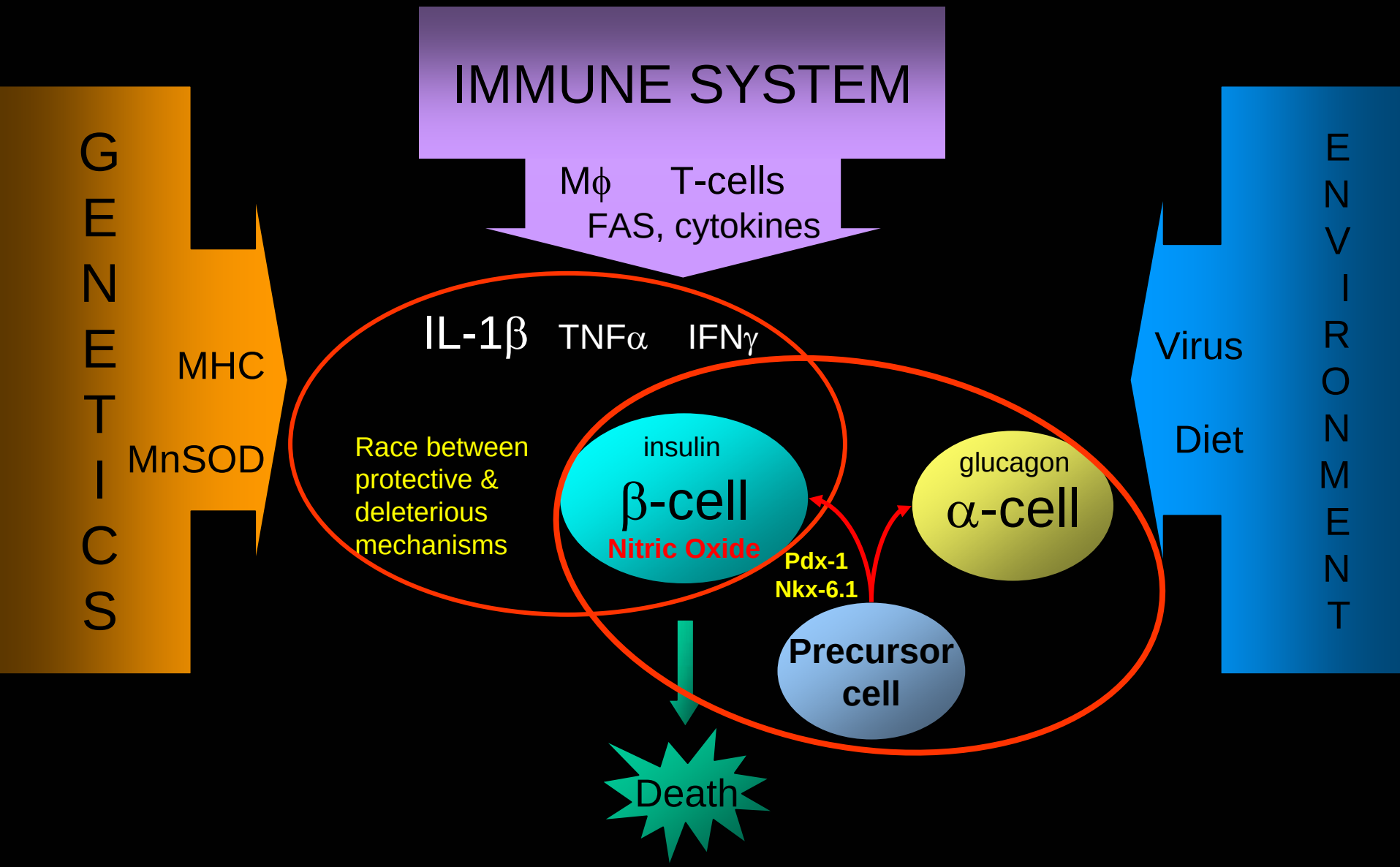
- Multifactorial disease with polygenic predisposition

Immune system

Environment

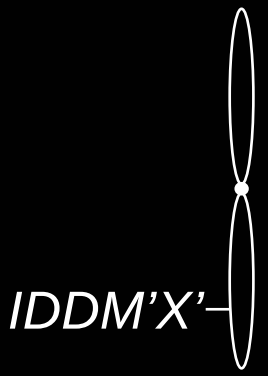


Outline of todays presentation

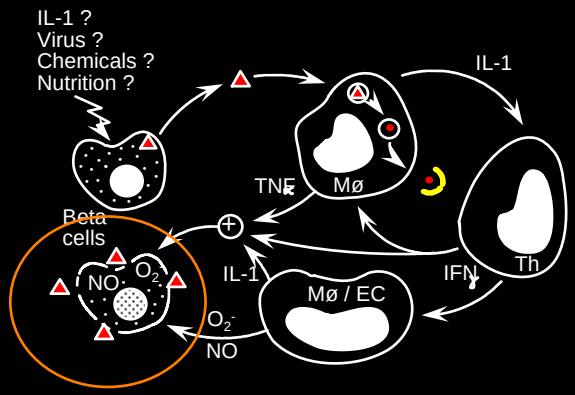


Level 2: Select the model and methods of choice - laying the puzzle by profiling

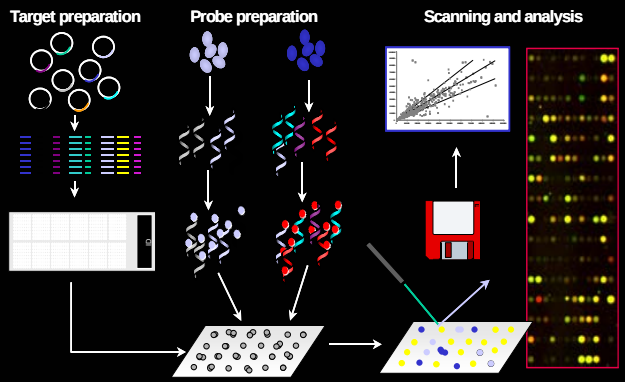
Genome approach



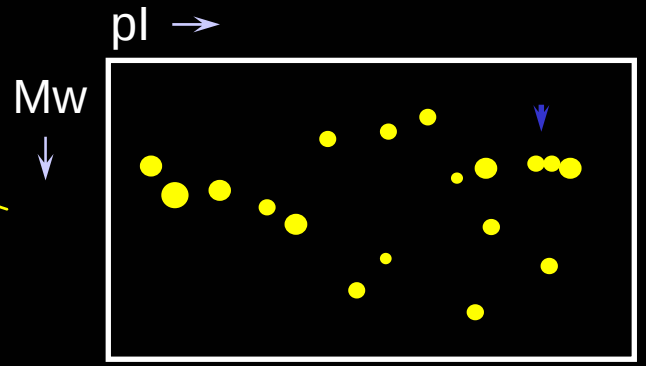
Candidate approach



Transcriptome approach



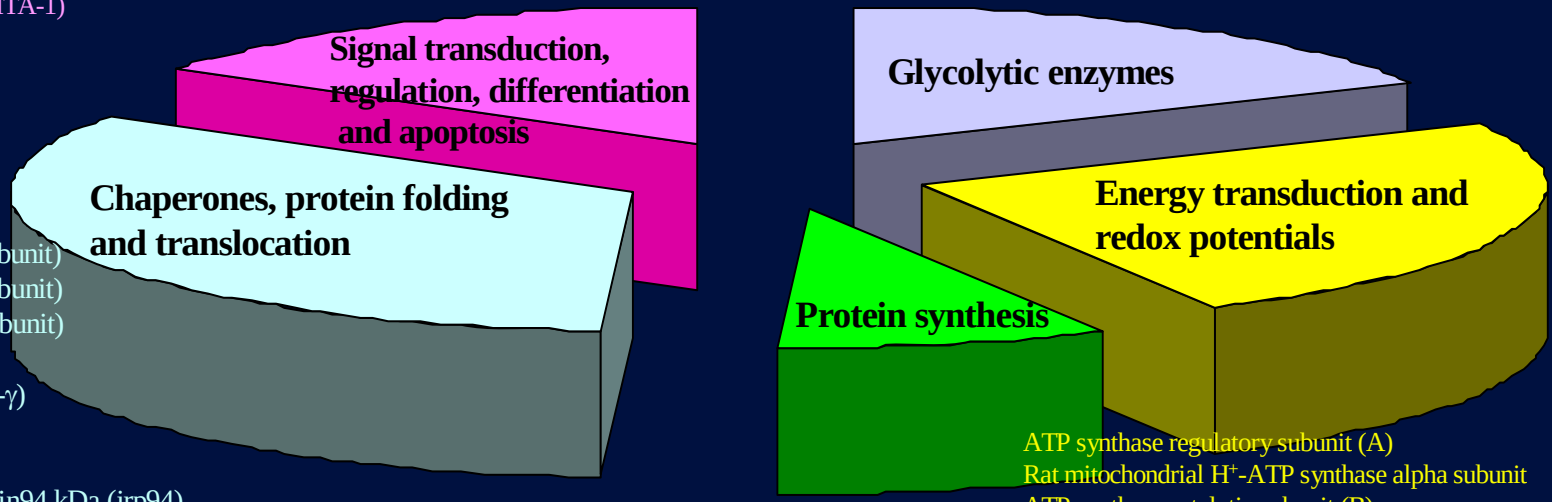
Proteome approach



Bioinformatics:

Proteome analysis of rat islets exposed to IL-1β

Phosphatidylethanolamine-binding protein (P23K)
Lamin A (split product)
Lamin B1
TGF-β receptor interacting prot. 1 (TGFrip)
14-3-3 protein-ε-isoform
Turned on after division (TOAD-64)
Neuroendocrine convertase 2 precurser (NEC2)
Metastasis assoc. protein (MTA-1)
Galectin-3, MAC-2 antigen



T-complex protein 1 (γ-subunit)
T-complex protein 1 (ε-subunit)
T-complex protein 1 (ζ-subunit)
Caldesmon, human
Tropomyosin NM4 (TMP-γ)
Annexin II
Calnexin
Ischemia-responsive protein94 kDa (irp94)
HSP90-β (HSP84)
HSP71c (Heat shock cognate 71 kD protein)
HSP70KD protein AGP-2
Mitochondrial matrix protein p1 (HSP60)
Glucose regulated protein precursor=HSPA5 (GRP78)
Mortalin (GRP75)
Protein disulfide isomerase (PDI) ER60 (GRP58)
Probable protein disulfide isomerase P5
ER protein (ERP31 precursor to ERP29)
Coatomer (δ-subunit)
Ubiquitin carboxyl-terminal hydrolase T

Elongation factor 2 (EF2, polypeptidyl – tRNA translocase)
Heterogeneous nuclear ribonucleoprotein K/ ROK
Polypyrimidine tract-binding protein (PTB)
Major vault protein (RNP protein for nucleocytoplasmic transport)
Glycyl – tRNA synthetase

6-phospho fructo-2-kinase
Fructose 1,6 - biphosphate Aldolase A
Triose phosphate isomerase (TPI1)
Glyceraldehyde-3-p-dehydrogenase (GAPDH)
Phospho-glycerate kinase
Phospho-glycerate mutase (PGAM), brain form
Enolase α/1
Pyruvate kinase M (processed psedugene)
Puruvate kinase M2, early foetal tissue
Pyruvate carboxylase

ATP synthase regulatory subunit (A)
Rat mitochondrial H^+-ATP synthase alpha subunit
ATP synthase catalytic subunit (B)
Adenylate kinase isoenzyme 2, (mitochondrial)
Transitional endoplasmic reticulum ATPase
Vacuolar ATP synthase subunit B, brain isoform
5-aminoimidazole-4-carboxamid ribonucleotide formyl transferase IMP cyclohydrolase
Acetyl-CoA acetyl transferase (ACAT2)
L-3-hydroxyacyl-CoA dehydrogenase
NADH-cytochrome B5 reductase
NADPH-cytochrome P450 reductase
Creatin kinase (ubiquitous mitochondrial form)
Glutamate dehydrogenase (GDH)
Methylmalonate-semialdehyde dehydrogenase

HU Andersen et al, Diabetes 44:1995
HU Andersen et al, Electrophoresis 18:1997
NE John et al, Diabetes 49, 2000
PM Larsen at al, Diabetes, 50, 2001
T Sparre et al, Diabetologia 45, 2002

Lots of data – what now?

Level 1 and 2 (aim, biological system and profiling methods):

- Systems biology provides a detailed and complex picture of proteins and pathways activated in β-cells or islets in response to cytokines.
  Hypothesis generating!

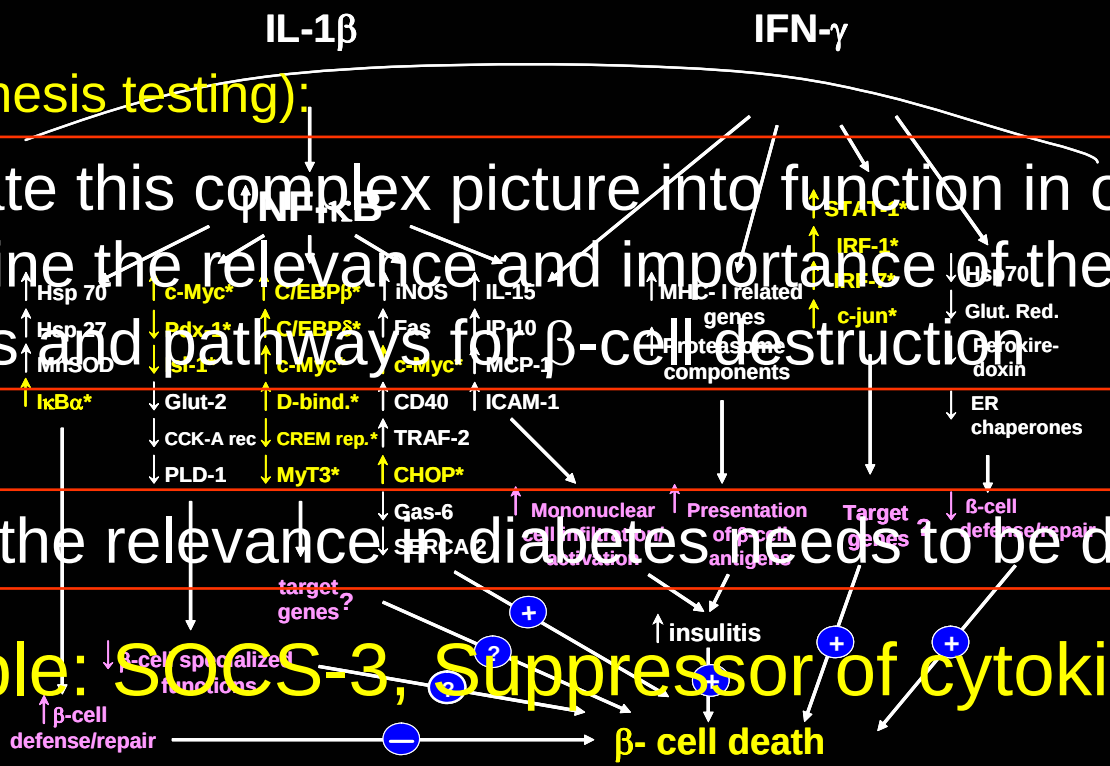
Level 3 (hypothesis testing):

- Translate this complex picture into function in order to determine the relevance and importance of the identified proteins and pathways for β-cell destruction

Level 4:

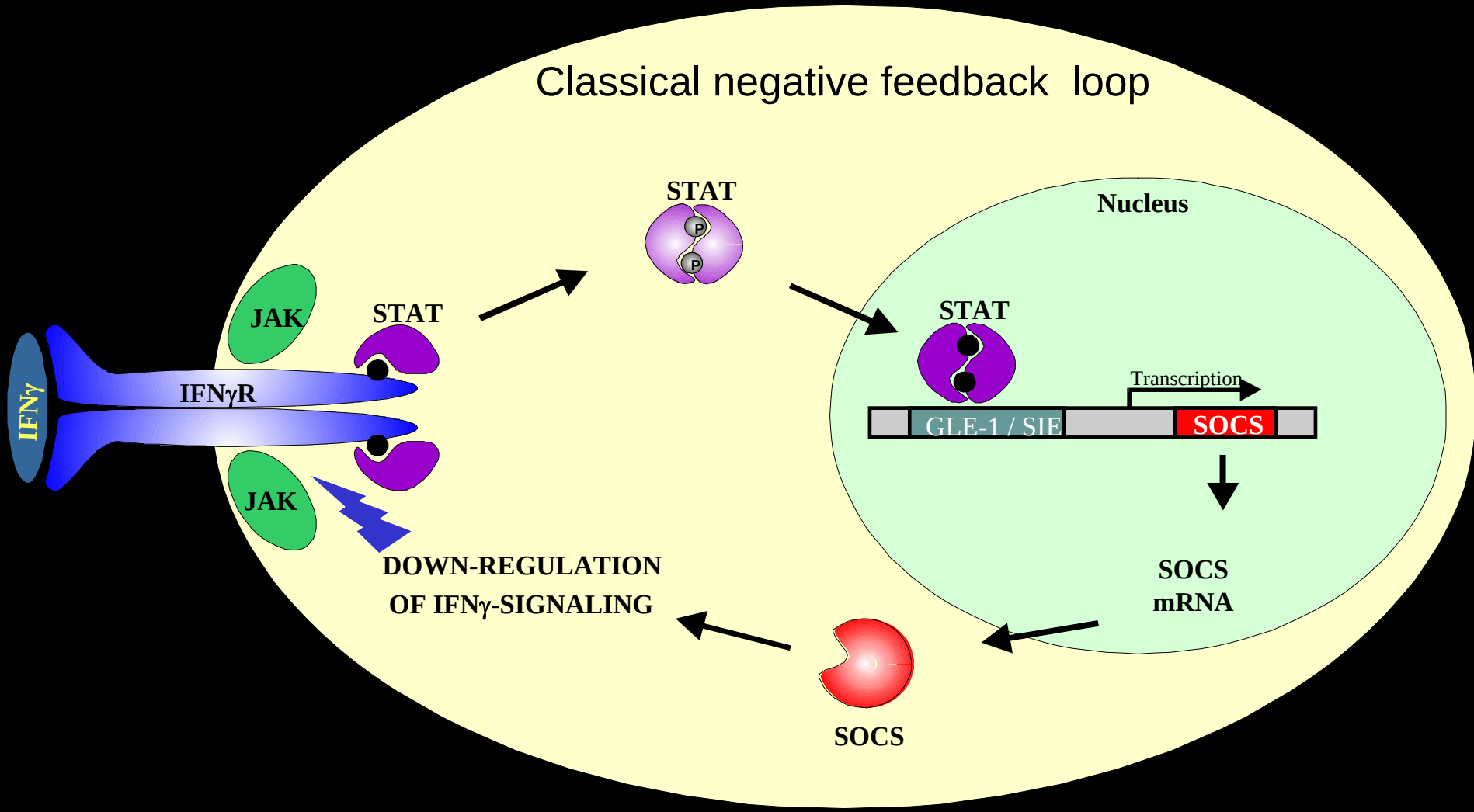
- Finally the relevance in diabetes needs to be determined

An example: SOCS-3, Suppressor of cytokine signaling



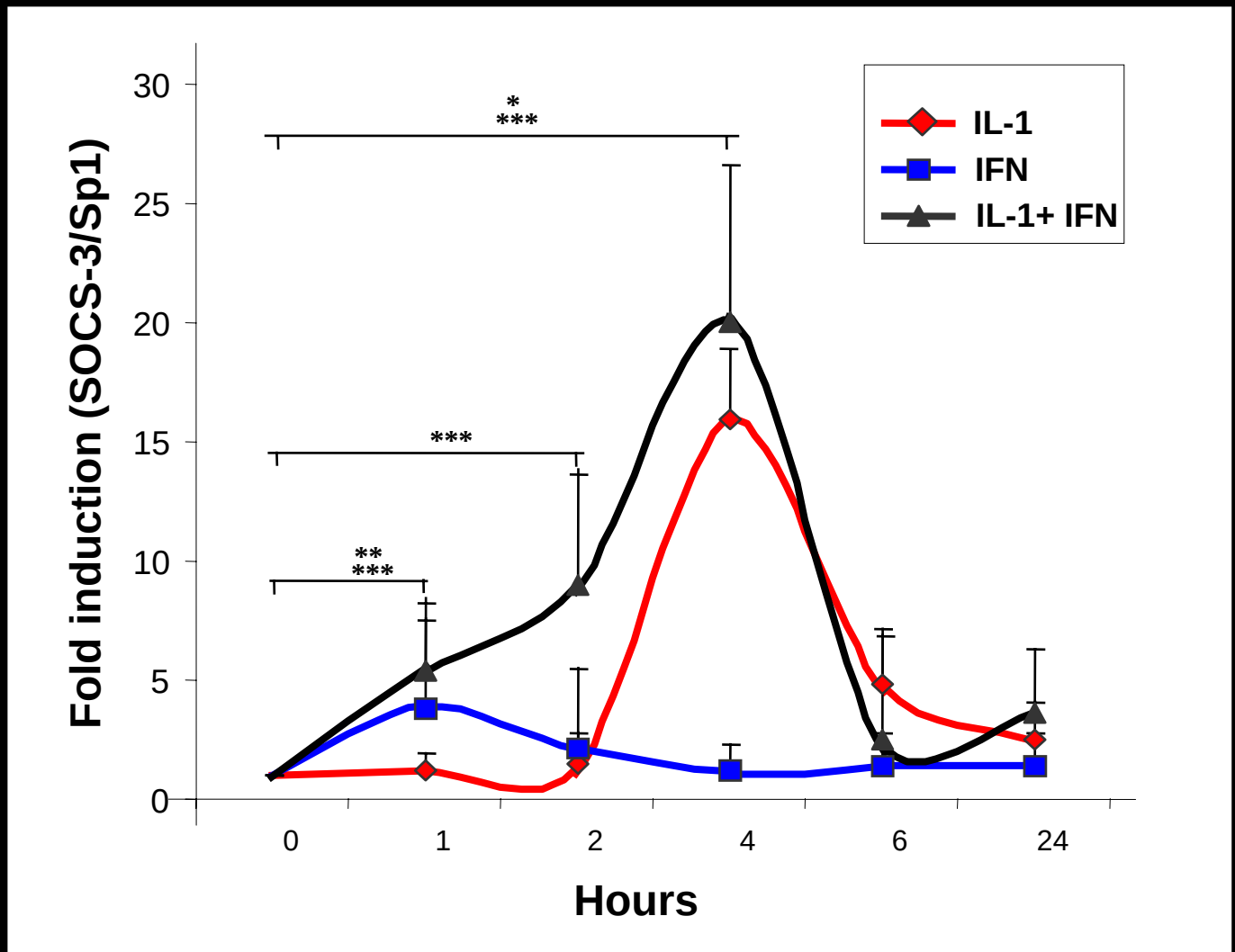
J. Biol. Chem. 276:48879, 2001

Suppression Of Cytokine Signaling (SOCS)



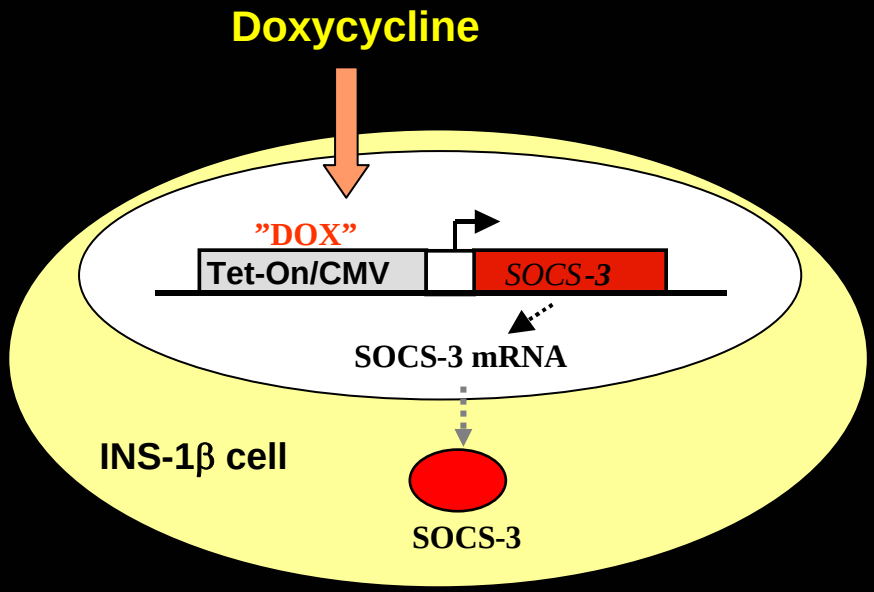
Level 2: SOCS-3 identified as up-regulated by IL-1 in β-cells

Cytokine induced SOCS-3 mRNA expression in rat islets



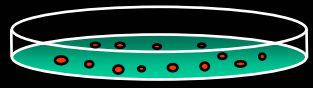
Mean ± SD,
n=3

Level 3: SOCS-3 inhibits IL-1β induced β-cell death



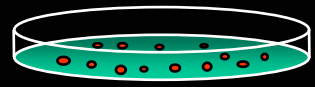
Functional assay

- DOX, +/- cytokines



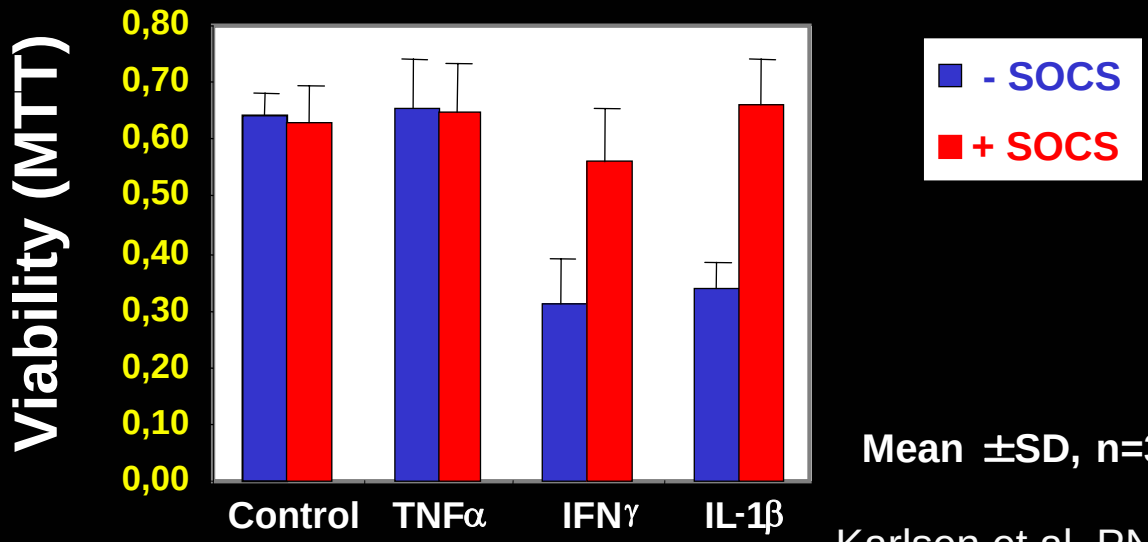
- SOCS

+ DOX, +/- cytokines



+ SOCS

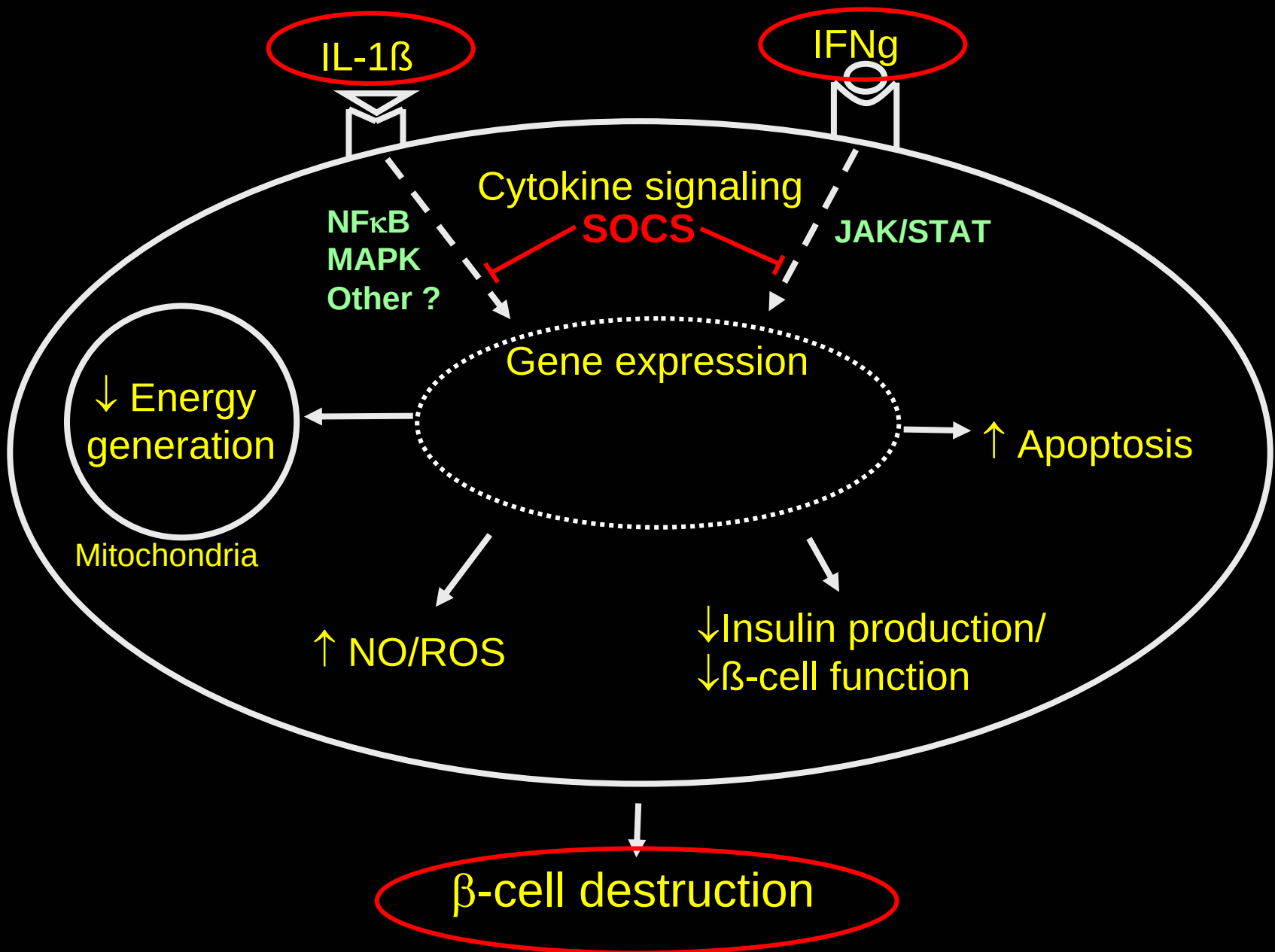
Measurement of viability etc.



Mean ±SD, n=3

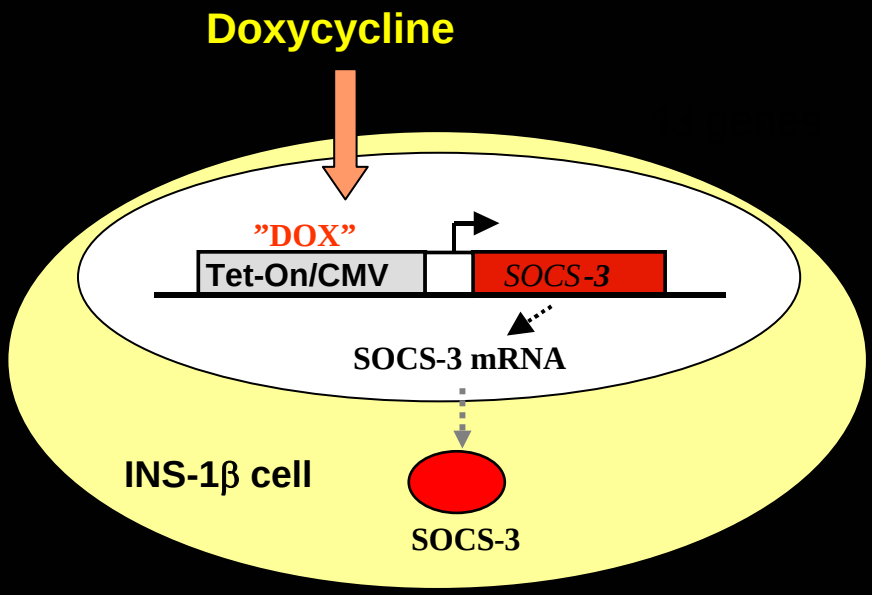
Karlsen et al, PNAS, 98; 2001

SOCS-3 inhibits cytokine induced β-cell death



Level 3b: Identify mechanism of action

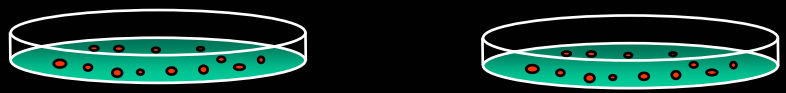
- gene profiling



- First pre-culture with or without dox
- Then 6 or 24 hr additional culture in the absence or presence of IL-1
- Score of ≥2.5-fold-changes.

Profiling assay

- DOX, +/- cytokines **+ DOX, +/- cytokines**



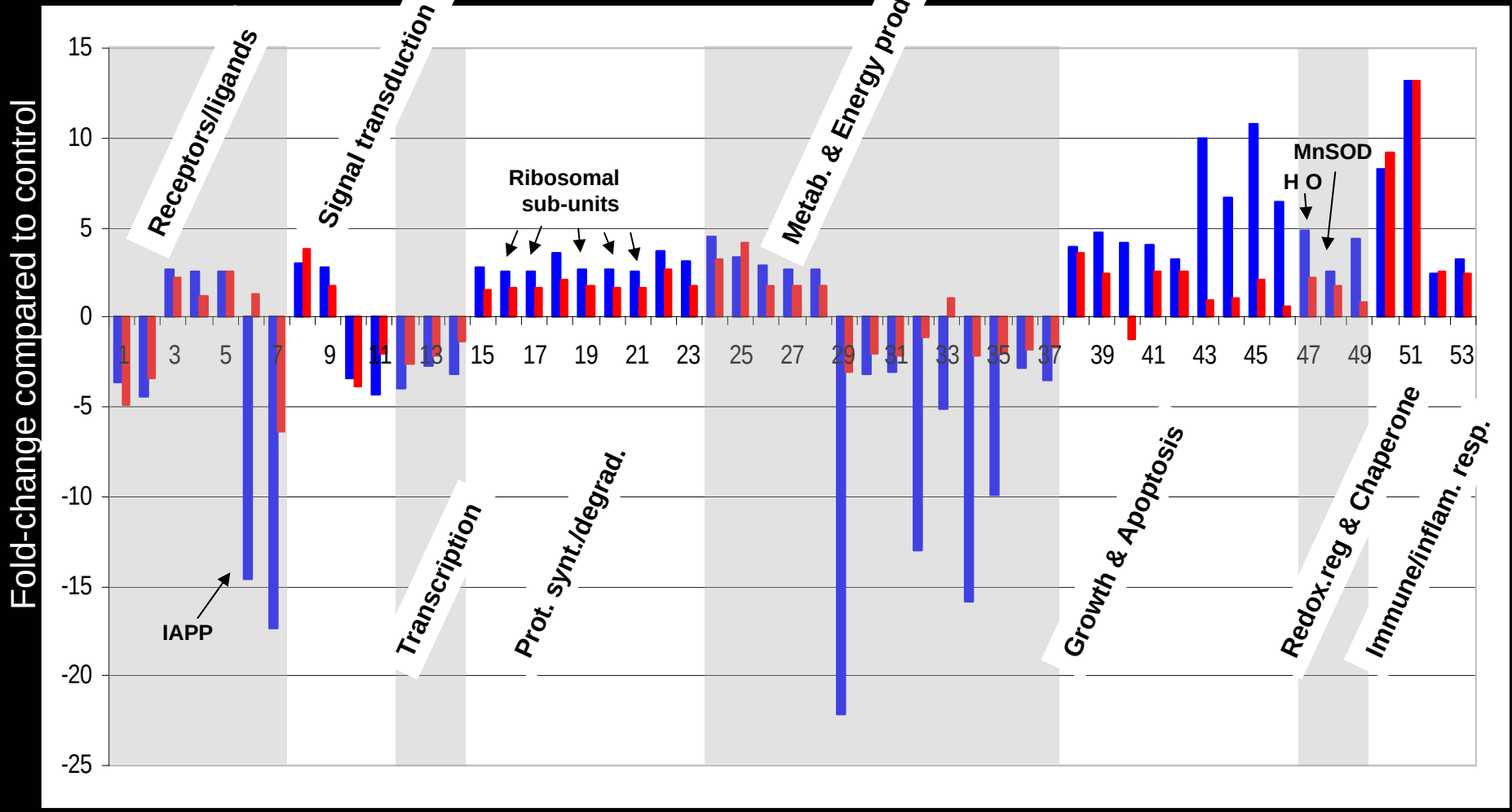
- SOCS + SOCS

Array analysis, Affymetrix U34A chips

Karlsen et al., Diabetologia, 2004, 47:2004

SOCS-3 expression “normalizes” gene-expression

24 hrs IL-1 exposure: without SOCS-3 with SOCS-3

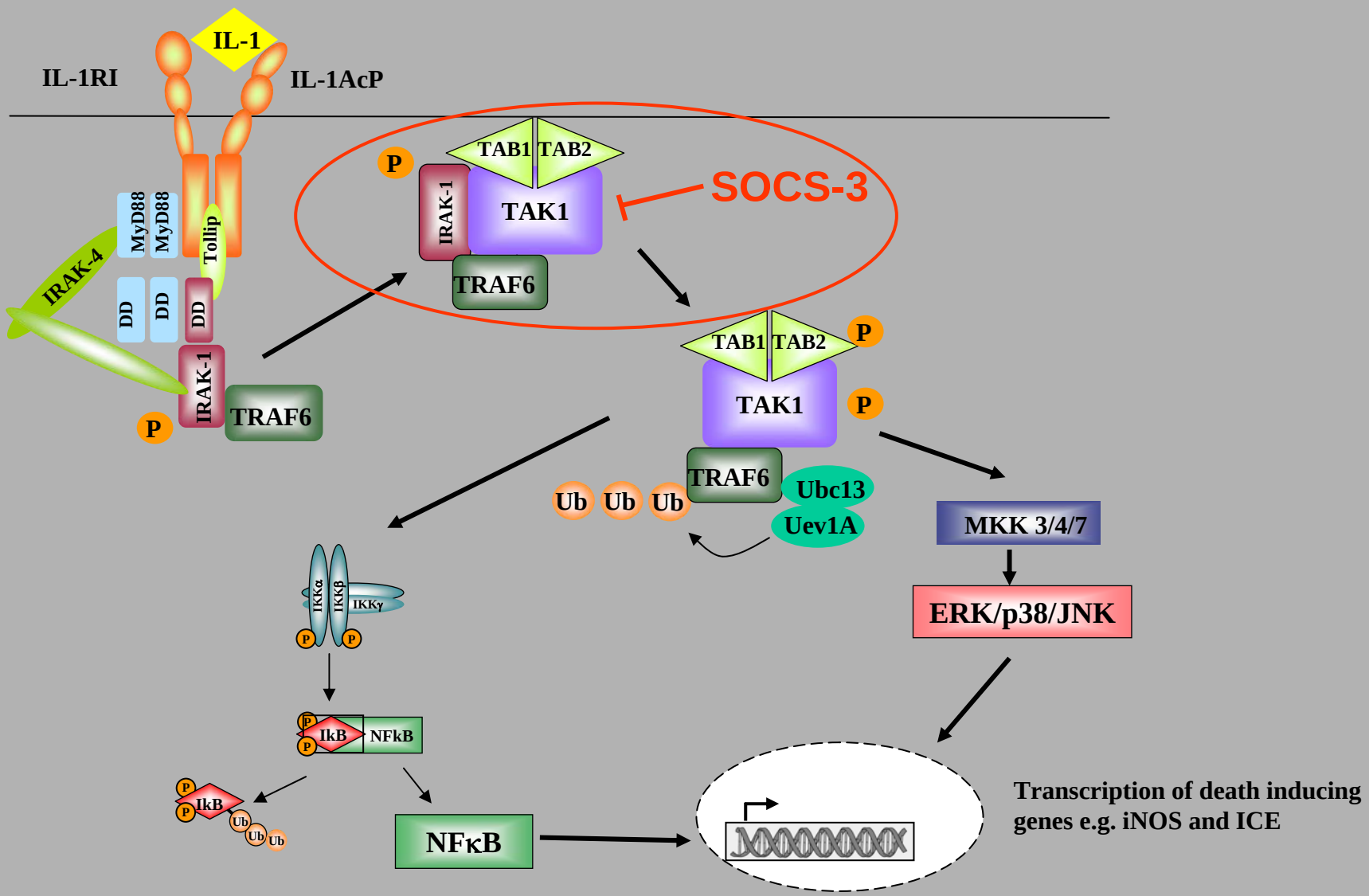


Summary of array study – 6-hr data

- 23 transcripts were up-regulated in response to IL-1
- 16 of these were inhibited by SOCS-3
- 8 of these encoded proteins related to immune/inflammatory responses, e.g. chemokines and receptors
- 9 of the SOCS-3 inhibited transcripts encoded pro-apoptotic transcripts previously shown to be NF-κB dependent early response genes (iNOS, ICAM, complement C3, Mob-1, MIP-1, CX3C, NFκB-p105, IRF-1, Fibrinogen-γ) suggesting that SOCS-3 inhibits NFκB mediated signal transduction

SOCS-3 inhibits IL-1 signaling at the TAK1 level

- biochemical analysis



Perspective
- 4rd level, involvement in diabetes?

- SOCS-3 inhibits signaling through both IL-1β and IFNγ at proximate steps in the β-cell (cellular level)

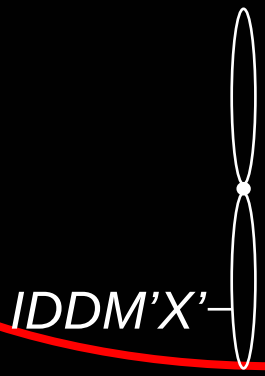
- Thus, SOCS-3 may represents a potential target in pharmacological and/or generic engineering strategies to protects the β-cells against the toxic effects of the immune system in T1D (organ level)

- SOCS-1 and SOCS-3 causes insulin resistance by inhibition of IRS1/2 phosphorylation.
  Increased SOCS expression in liver, muscle and fat associated with obesity (low grade inflammation) has a negative effect on insulin signaling (individual)

  Ueki, Kondo and Kahn; *Mol. Cell. Biol*. 24, 2004
  Ueki, Kadowaki and Kahn; *Hepatolgy Res*. 33 (2005)

Systems Biology- Population level

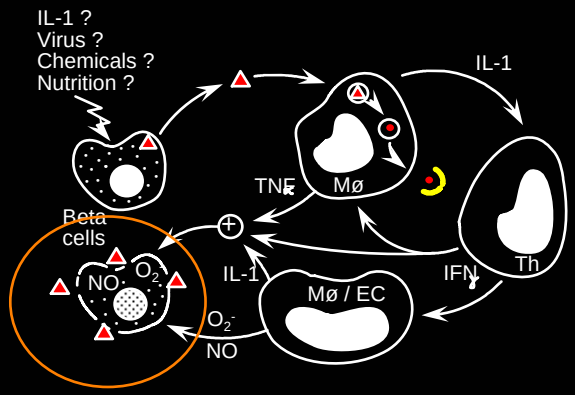
Genome approach



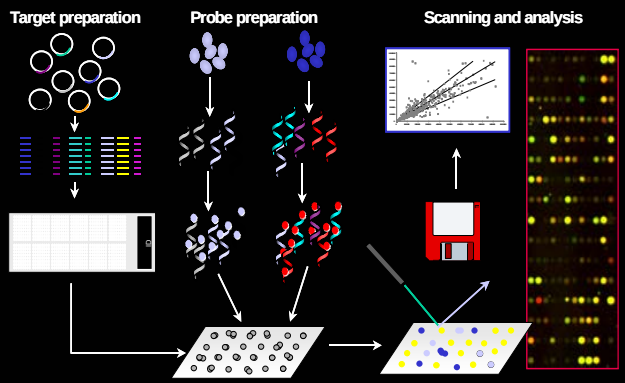
Diabetes associated SOCS-3 polymorphisms

Molecular mechanisms in β-cell destruction in T1DM

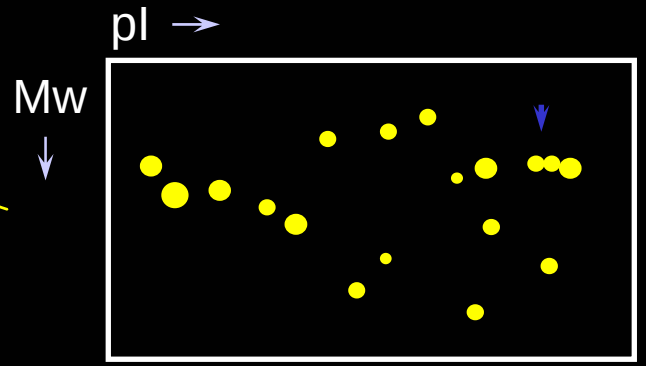
Candidate approach



Transcriptome approach

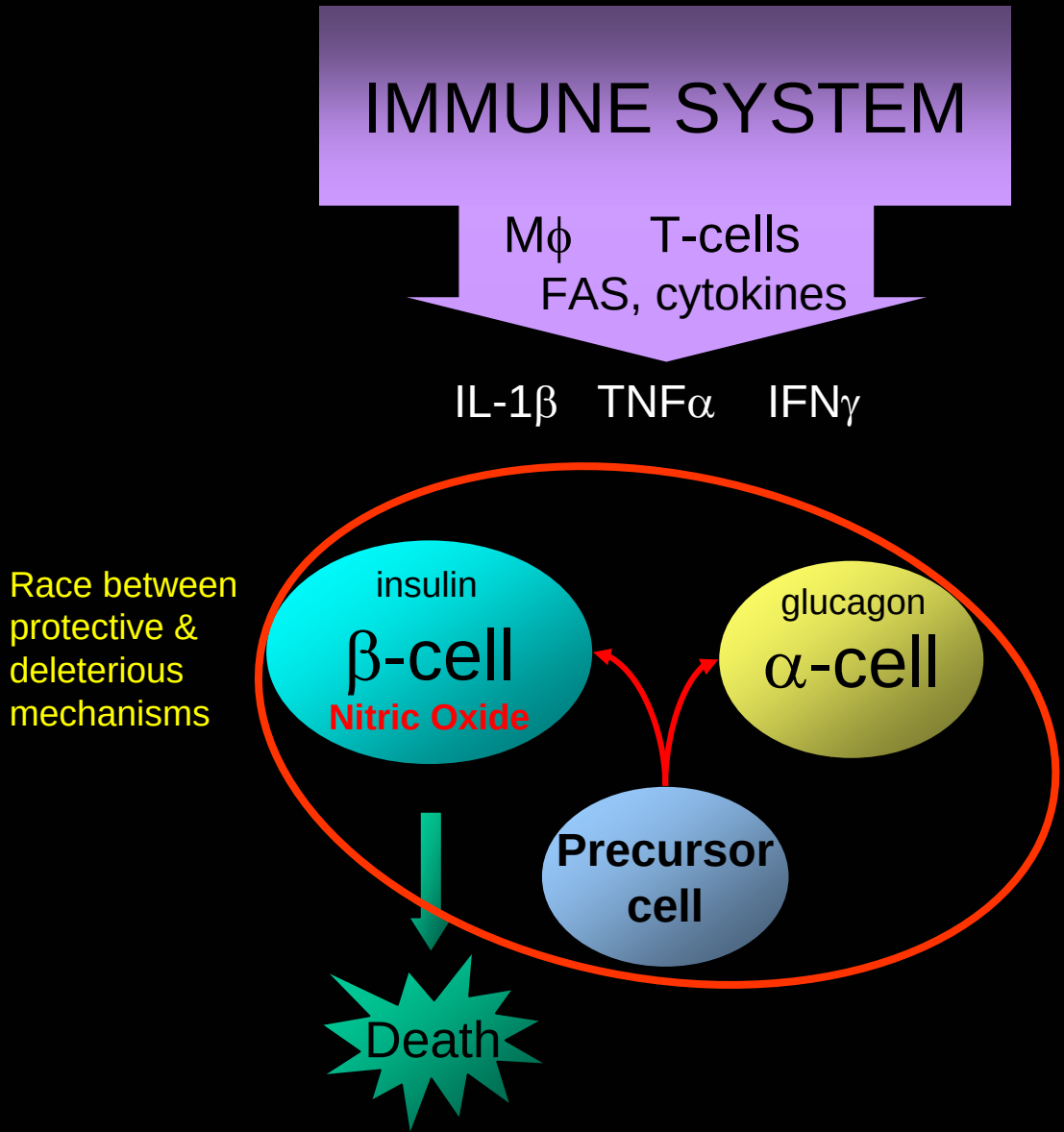


Proteome approach

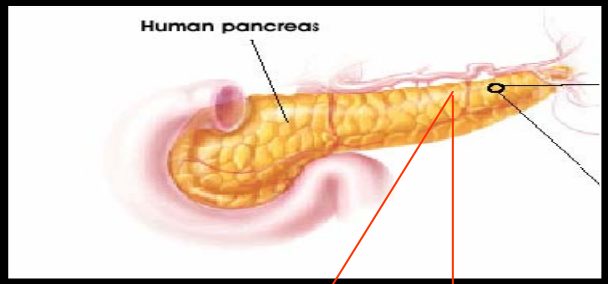


2-D gel

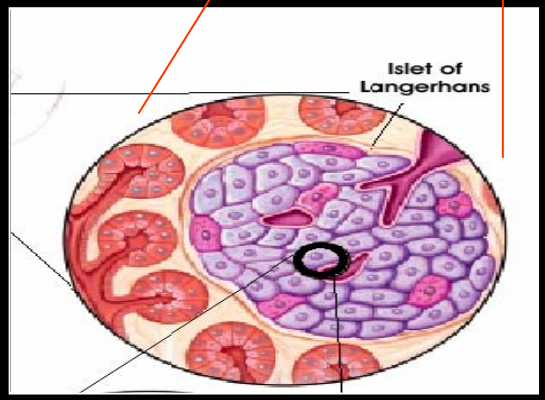
What are the mechanisms responsible for the acquired cytokine sensitivity during β-cell maturation?



Development of the 4 endocrine cell-types



Pancreas



Islet of Langerhans

Mesoderm — Endoderm — Ectoderm

↓ TF

Lung Stomach Pancreas Liver Interstine

↓ TF

Exocrine Endocrine Duct

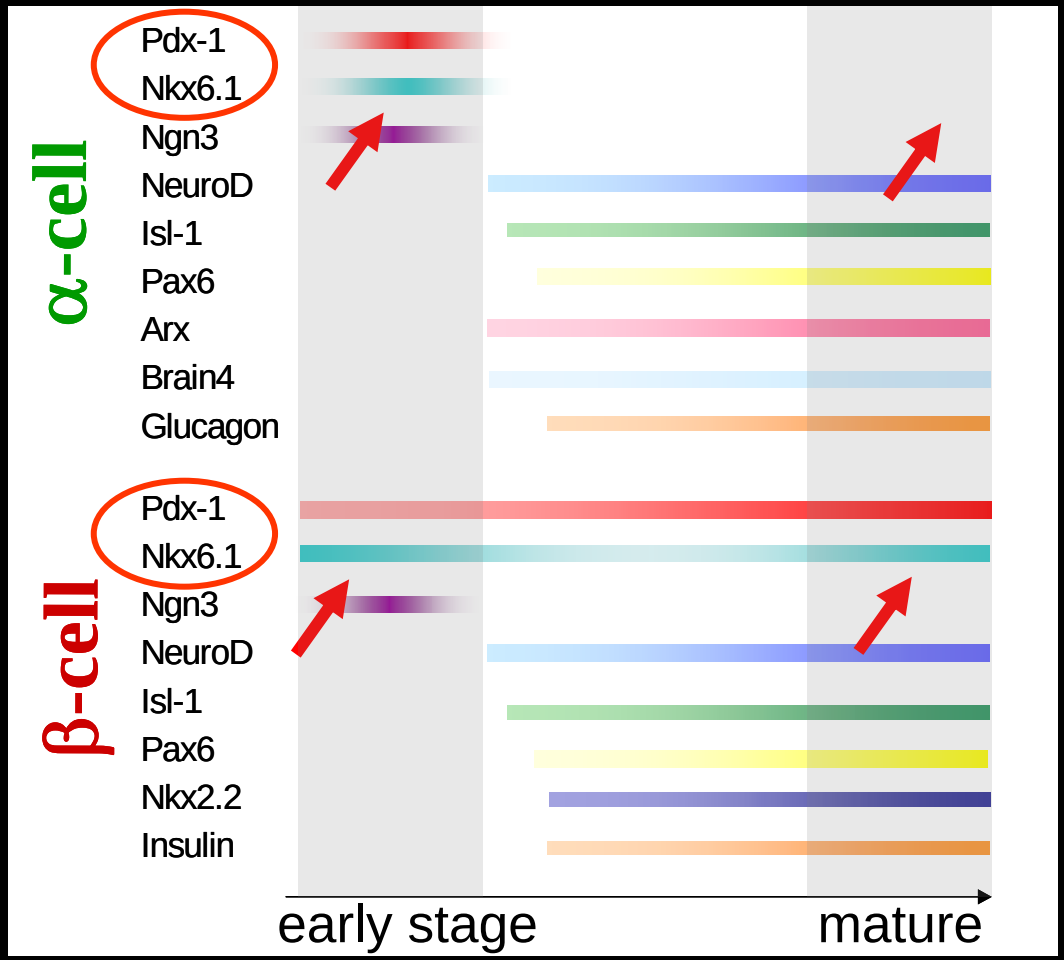
TF → α Glucagon-producing

TF → β Insulin-producing

TF → γ Somatostatin-producing

TF → PP Polypeptide-producing

Expression pattern of some of the transcription factors during maturation towards mature α- and β-cells



K. Nielsen et al. Diabetes 48, 1999.

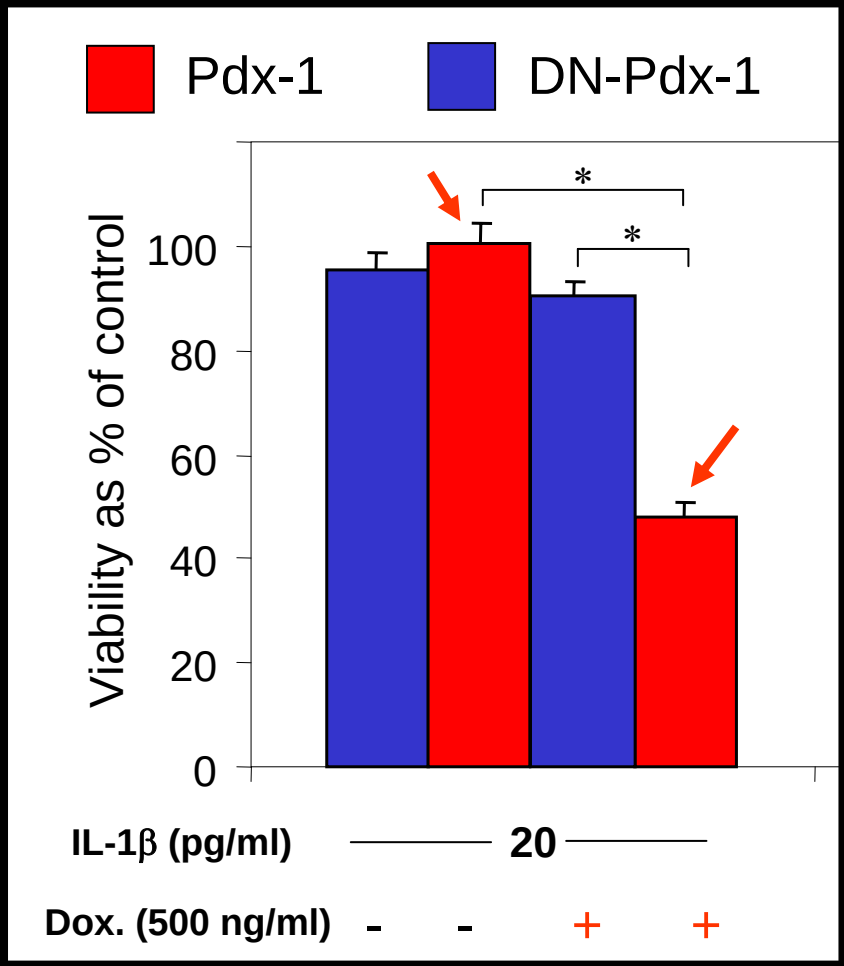
K. Nielsen et al, Diabetologia, 47;62-74; 2004

K. Nielsen et al, Diabetologia, 47:2185-2199 , 2004

Pdx-1 expression in β-cells is reduced by IL-1 exposure

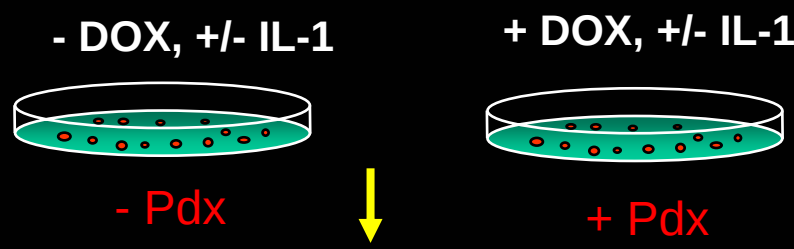
Modified from Jensen et al., Diabetes, 49, 2000

- Is Pdx-1 expression important for the acquired β-cell sensitivity to IL-1?
- If so how is that reflected in GEPs and PEPs?



K. Nielsen et al, Diabetologia, 47:2185-2199 , 2004

Functional and Profiling assay

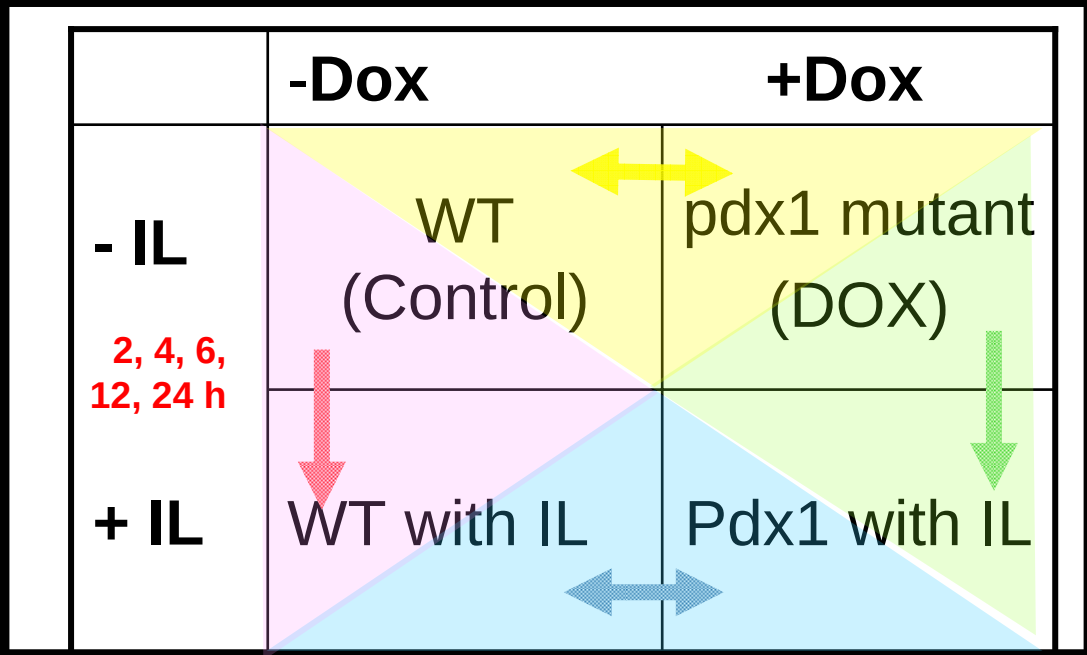


Functional assays (viability etc.)

Array analysis, Affymetrix U34A chips

3 days culture, n=3
Mean ± SD, *p<0.001

Effect of IL treatment



n=4

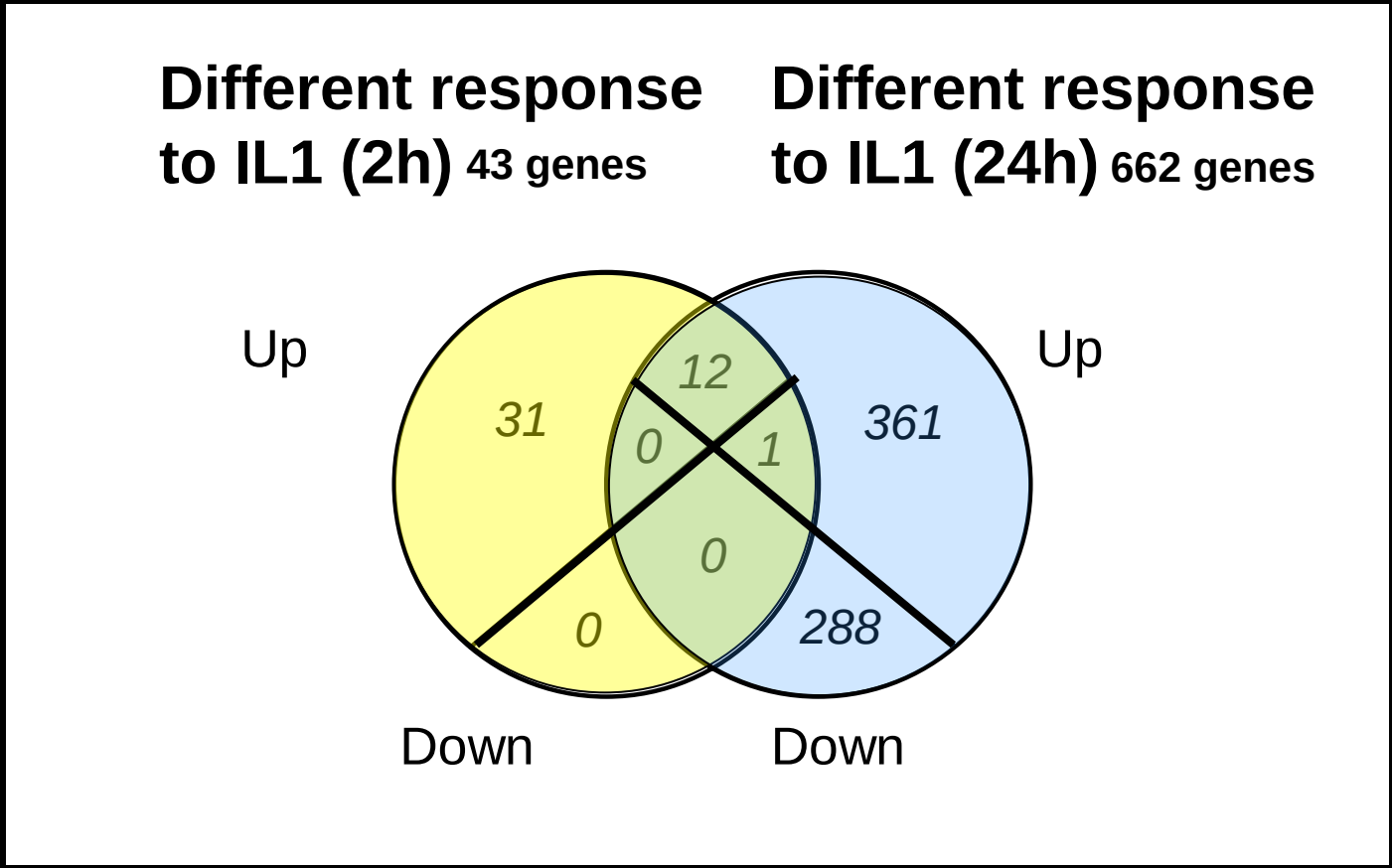
What is the effect of over-expressing pdx?

What is the effect of IL-1 treatment in pdx-negative cells?

What is the effect of IL-1 in pdx expressing cells?

What is the effect of pdx-1om IL-1 exposure? Does pdx change the way beta cells respond to IL-1?

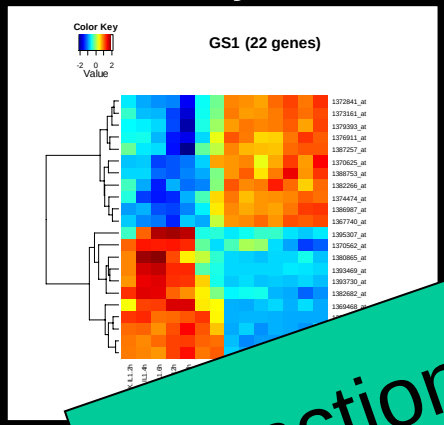
Venn diagram – Time perspective



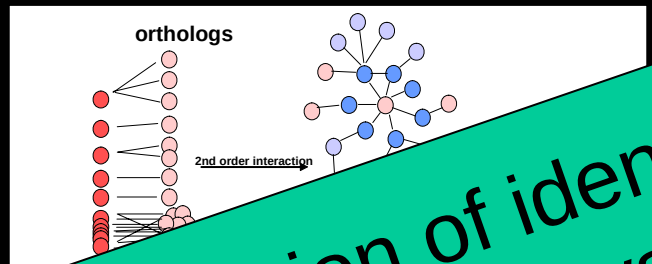
693 genes differentially expressed

Systems biology analysis

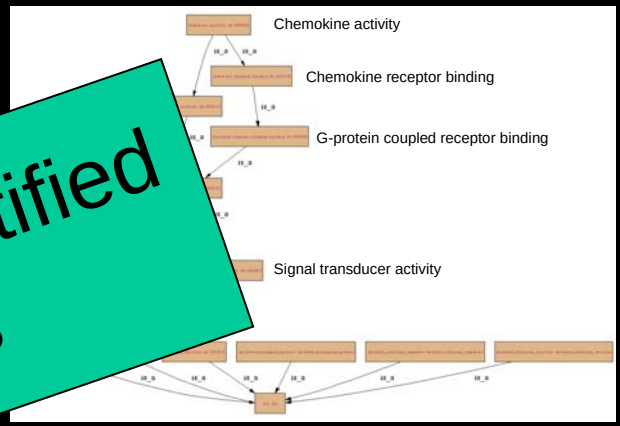
Geneshaving and SuperParamagnetic cluster analysis



Protein interaction network



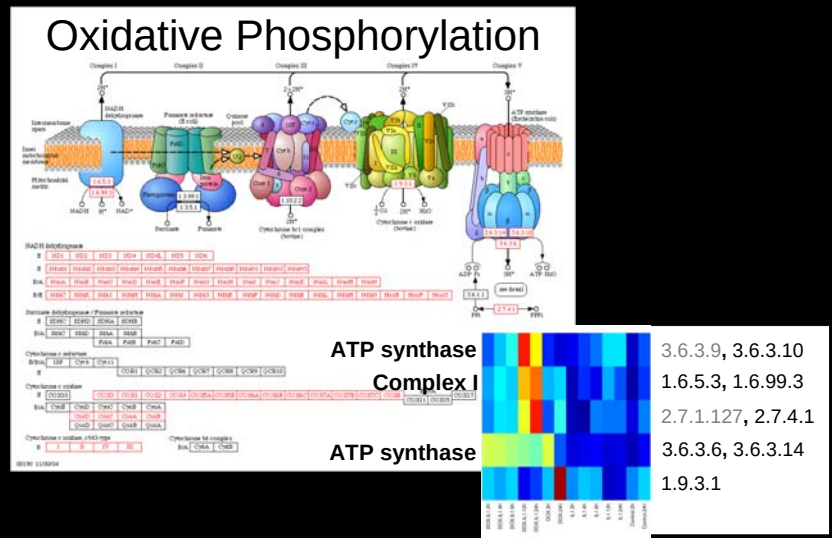
Gene Ontology analysis



Functional evaluation of identified mechanisms/pathways

ProbeSet ID	Lo	ame	Alone	Diabetes	Interleukin	pdx
1368527_at	29527	Ptgs2	10372	109	1157	1
1369200_at	58813	Nt5	4604	34	39	0
1367877_at	25715	Slc11a2	408	6	6	0
1387343_at	25695	Cebpd	212	1	47	0
1367679_at	25599	Cd74	108	0	6	0
1370562_at	171519	Calcb	3	0	0	0

Pathway analysis



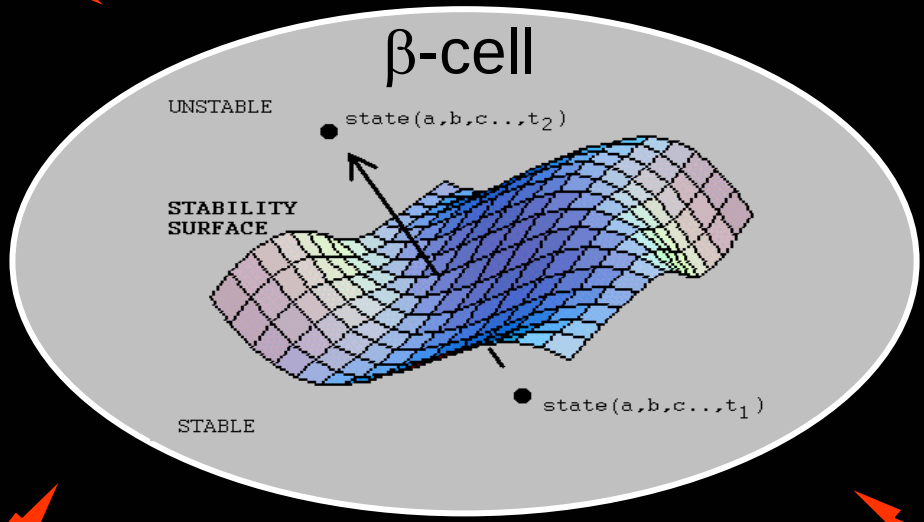
The β-cell
- a “sophisticated” and brittle target in T1D & T2D

Specialized phenotype

Pdx-1, Nkx6.1
Insulin; GLUT-2
iNOS
IL-1β
IL-1Ra
Fas/FasL
SOCS-3
scavengers
etc.

Genetics

MnSOD
HFN
Pdx-1
GK



Freisleben et al, Diabetes 48 (1999)

Autoimmunity

T-cells
FAS
auto antibodies

Environment

Cytokines
High Glucose
High FFA
Virus
GH, EGF, GLP-1
Drugs

Perspective in diabetes treatment

Knowledge about the β-cell response to cytokines may allow the development of new therapies to prevent β-cell destruction in diabetes.

Knowledge about regulation of β-cell neogenesis and proliferation may allow the development of new therapies to increase β-cell mass in diabetes.

Acknowledgement

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Claes Wollheim

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Brussels, Belgium
Martine Darville
Decio L Eizirik

Aarhus Uni. Hospital
Skejby, Denmark
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Søren Brunak

NeuroTech
Copenhagen, Denmark
Peter Hagedorn
Mogens Aalund

Sponsors:

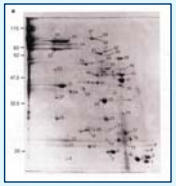
Juvenile Diabetes Research Foundation
E.U. Framework Programme
EASD/ JDRF /Novo Nordisk partnership
Danish Diabetes Association
Danish Medical Research Council

Steno Diabetes Center

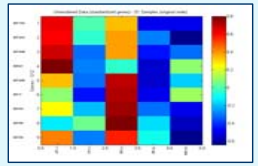
A Systems Biology Approach to Understand Type 1 Diabetes Pathogenesis

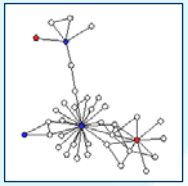
Steno Diabetes Centre (SDC) is a centre that specializes in treating and managing diabetes. SDC has a long research tradition.

It was researchers at SDC who first identified Type 1 diabetes (T1D) as an autoimmune disease with a strong genetic component located in the HLA-region



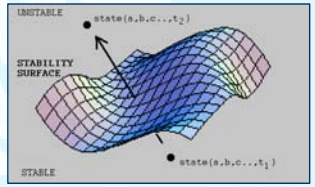
We were the first to use proteomics to address the pathogenesis of T1D. These studies showed that complex protein expression patterns were associated with progressive decline in beta-cell function leading to T1D.

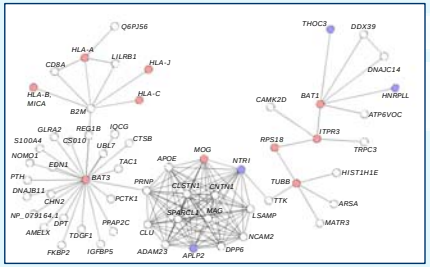




Extensive transcriptional profiling of several T1D model systems have been performed. In combination these demonstrate that genes involved in cytokine signaling, oxidative phosphorylation, defense responses, and apoptosis play crucial roles.

We have attempted to describe the earliest pathogenetic processes leading to T1D by mathematical language. This led to the suggestion that disease arises when a virtual surface is broken and the system changes from dynamic stability to instability.





Most recently we have integrated this information in a ‘systems biology approach’, where e.g. genetic, functional genomics and proteomics are combined in modeling T1D pathogenesis.

Selected references: Nerup et al (1971); Nerup et al (1974) Lancet ii:864; Pociot et al (1993) Eur J Immunol 23: 224; Andersen et al (1995) Diabetes 44: 400; Freiesleben et al (1999) Diabetes 48: 1677; Larsen et al (2001) Diabetes 50: 1056; Sparre et al (2002) Diabetologia 45: 1550; Sparre et al (2003) Diabetologia 46: 1497; Nielsen et al (2004) 47: 62; Nielsen et al (2004) Diabetologia 48: 2185; Sparre et al (2004) Diabetologia 47:982; Pociot et al (2004) Am J Hum Genet 74: 647; Karlsen et al (2004) Diabetologia 47: 1998; Sparre et al (2005) Mol Cell Proteomics 4: 441; Concannon et al (2005) Diabetes 54: 2995; Karlsen et al (2006) Biochem Bioph Res Co 344: 406;

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