

O-Glycosylation of Transcription Factor BCL11b: Truth or Myth?



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T-cell Leukemia



- T-cell Leukemia: Cancer of the white blood cells, or T-cells.
 - Affects approximately 50,000 people per year
 - About 4,000 people die from it annually
- Recovery Rate: ~90% in children
- Relapse Rate: ~50% in adults
- Looking for potential drug targets

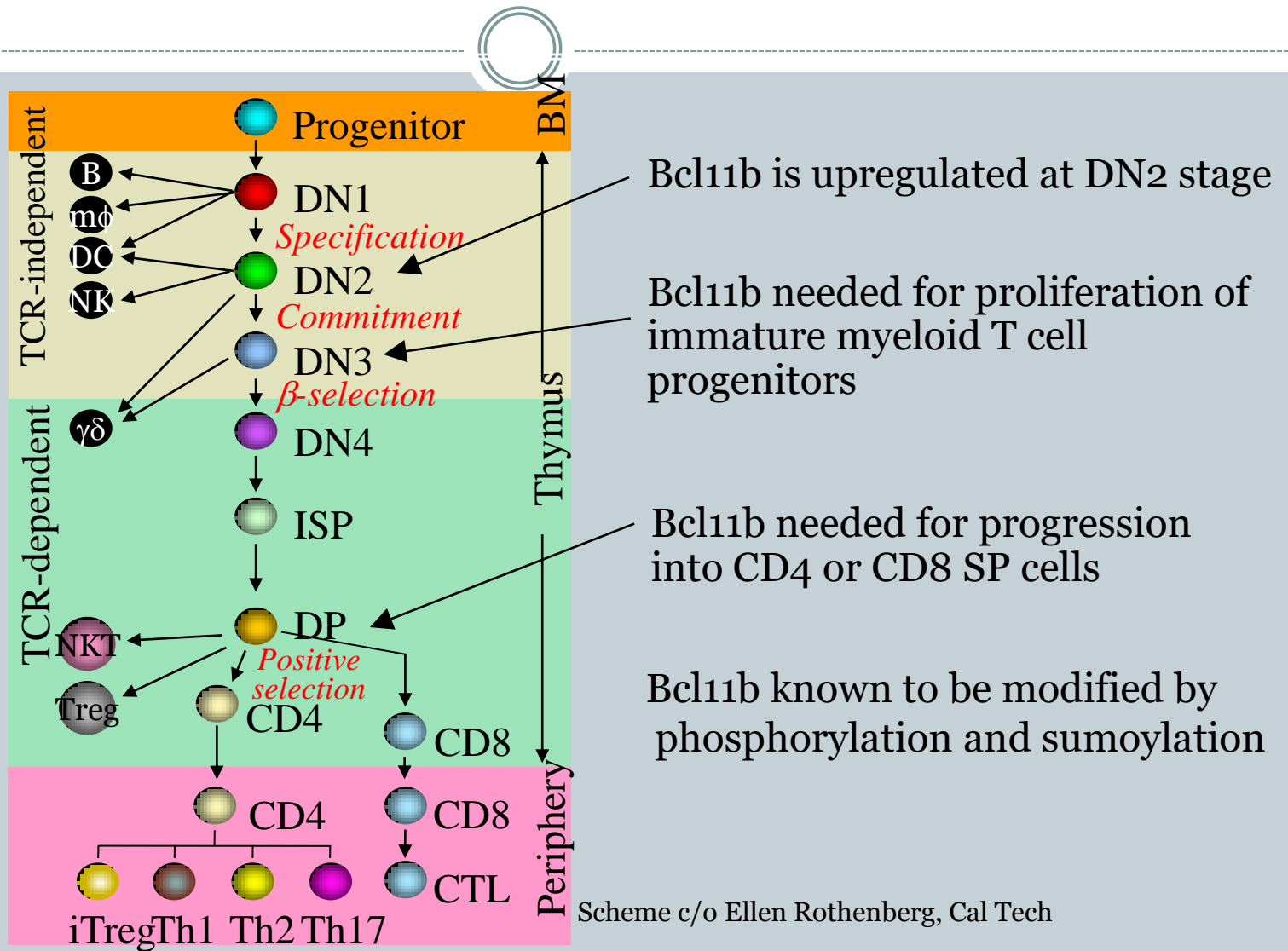


BCL11b: a haploinsufficient tumor suppressor



- Loss or mutation of BCL11b results in T-cell leukemia
- BCL11b mutation is associated with ~15% of T-cell leukemia cases

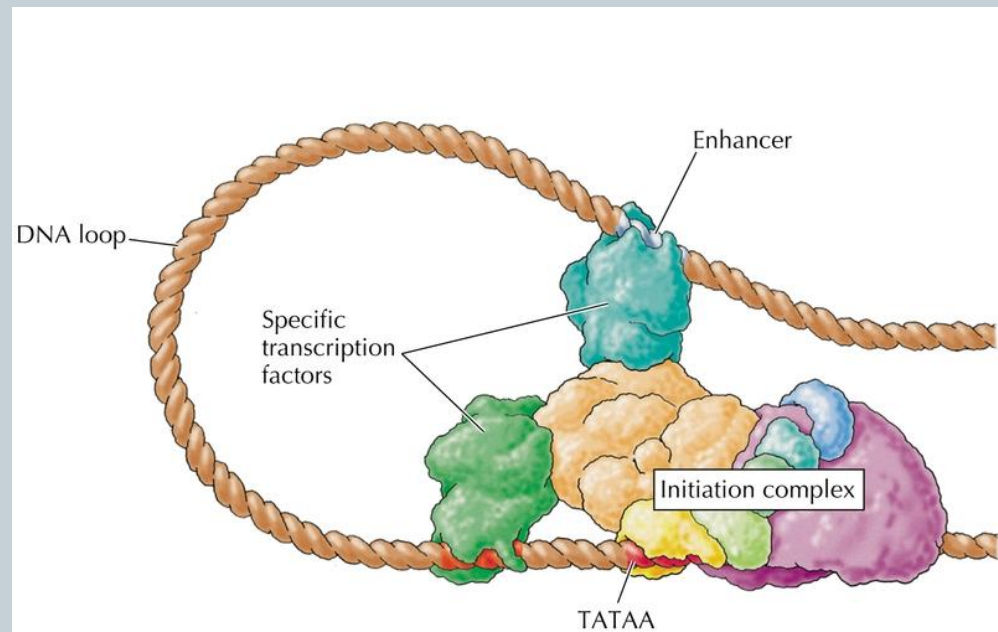
BCL11b: Essential for Thymocyte Development



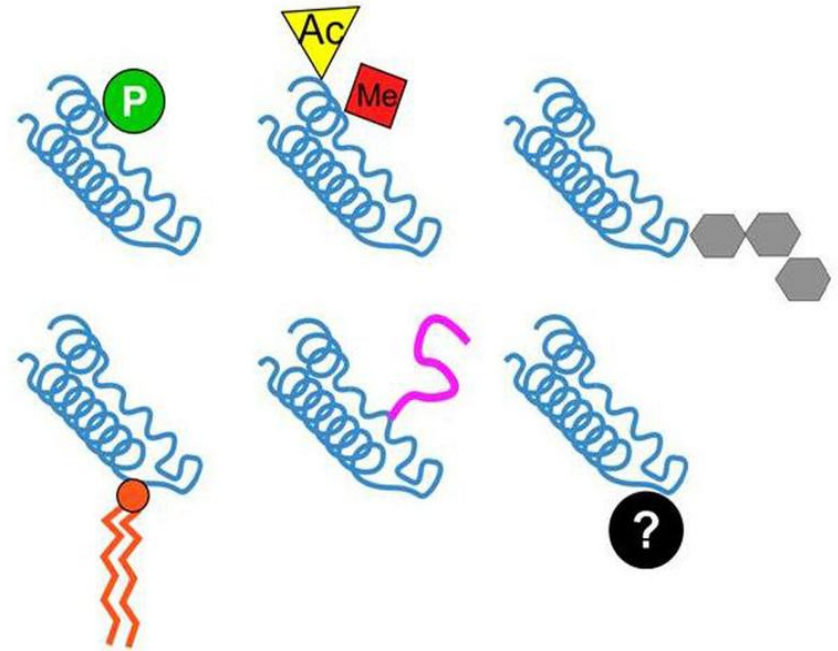
Genetic Regulation

Transcription Factors – Control gene expression by activating or inhibiting transcription of DNA sequences

Regulated by localization, synthesis/degradation, and **post-translational modifications.**



Post Translational Modification



PTM	Major Targeted Amino Acids	Modifies BCL11b
Phosphorylation	Ser, Thr, Tyr	Yes
Sumoylation	Lys	Yes
O-Glycosylation	Ser, Thr	?

Methods for Detecting O-Glycosylation



- *Antibodies* targeted to O-glycosylation residues
- *Beta-N-Acetylhexosaminidase* to remove glycosylated residues
- *Wheat germ agglutinin* to precipitate glycosylated proteins
- *Mass Spectrometry* to characterize location on protein

PUGNAc

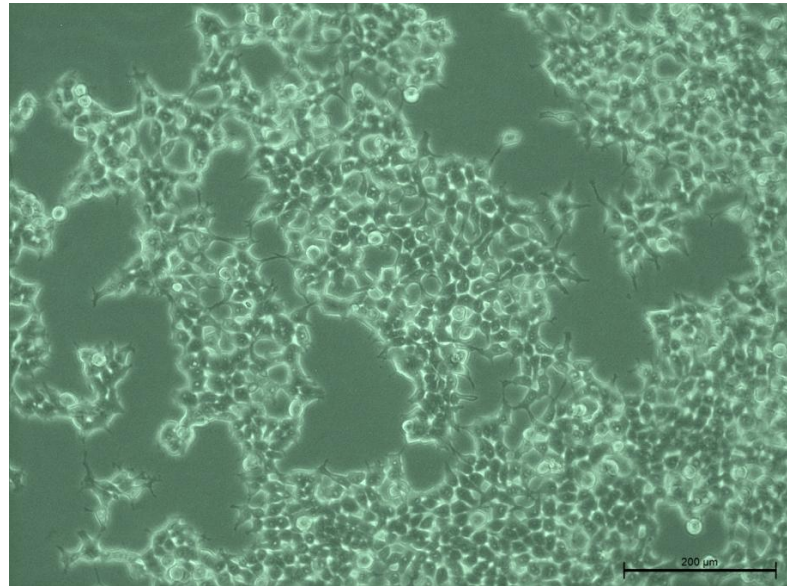


- Inhibits *O*-GlcNAc- β -N-acetylglucosaminidase (the enzyme for deglycosylation) in the cells
- Hypothesis: If BCL11b is *O*-glycosylated, PUGNAc treatment will cause a significant increase in *O*-glycosylation signal.

HEK293T Cells

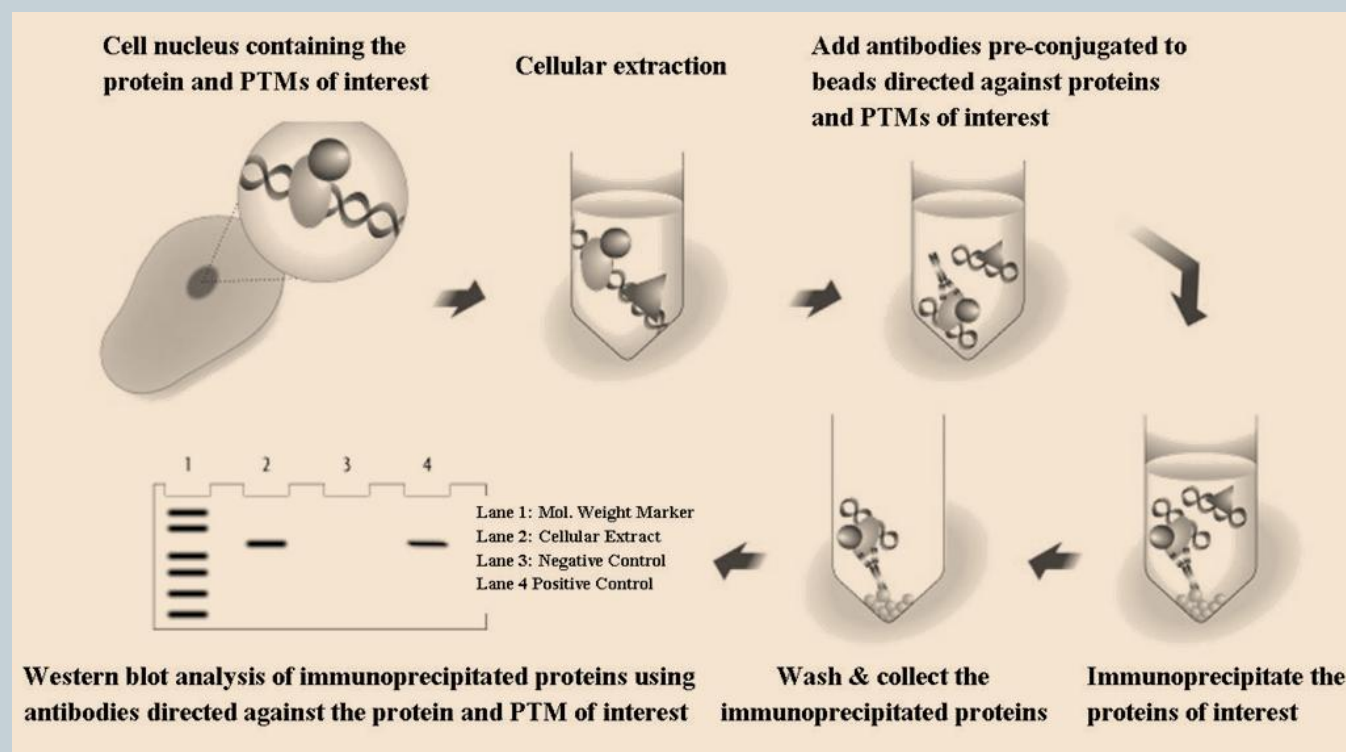
Human Embryonic
Kidney Cells
Transformed

- Can easily be transfected to express BCL11b
- Can also easily be treated with PUGNAc



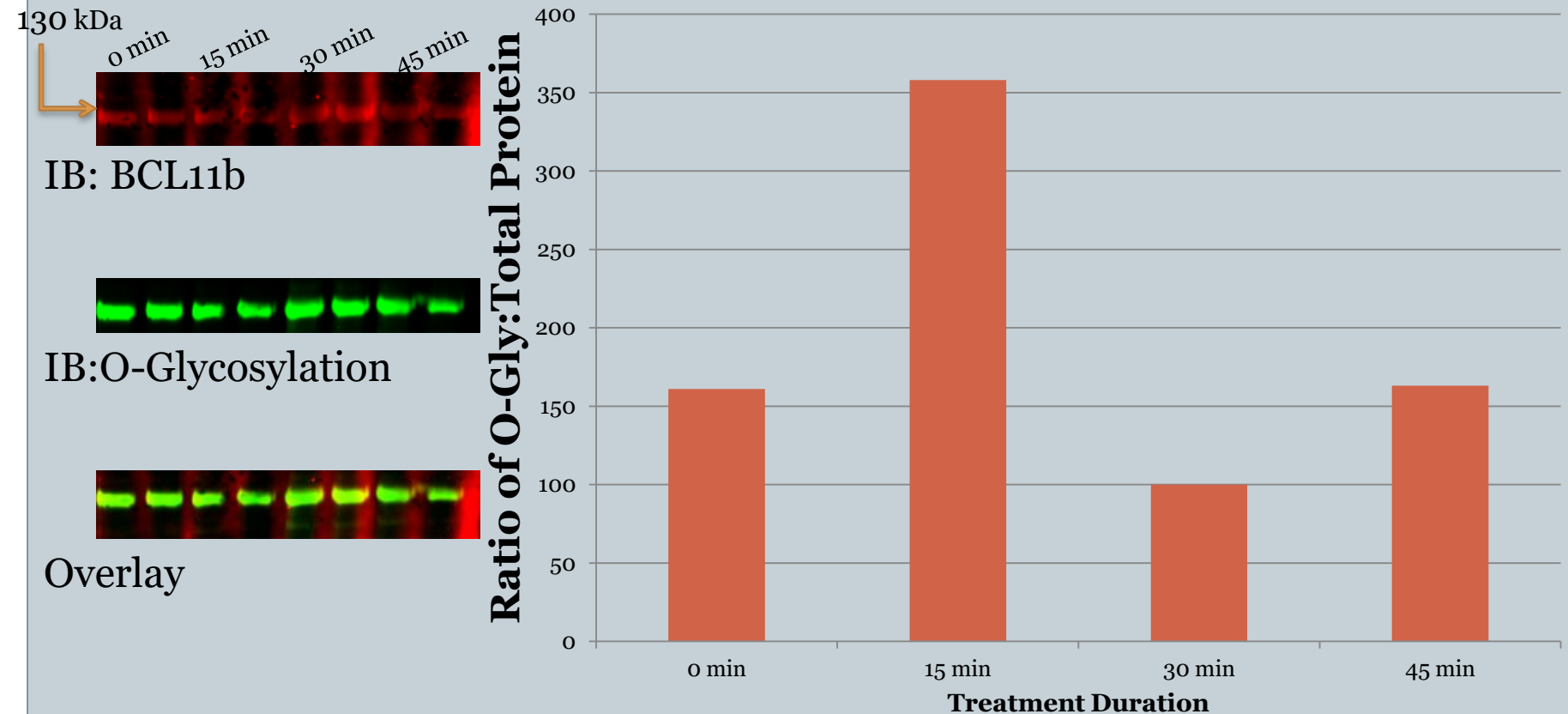
Experimental Methods

- Cell Culture
- Immunoprecipitation
- Western Blot



Changes in O-Glycosylation caused by PugNAc In BCL11b-Transfected HEK cells

HEK Cells Treated with PUGNAc

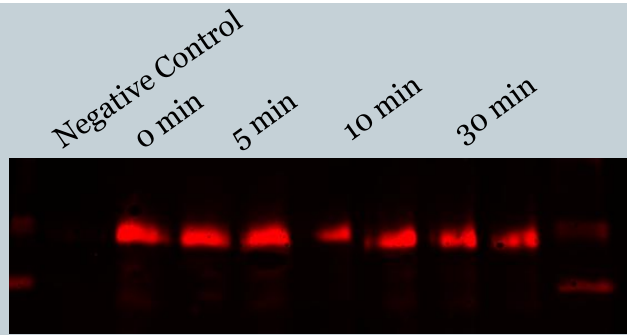


Beta-N-acetylhexosaminidase

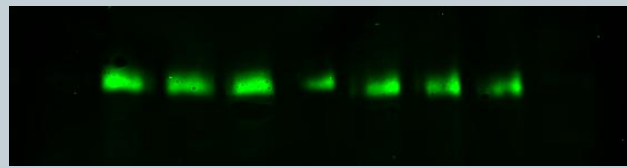


- An enzyme that removes an O-linked oligosaccharide from a glycoprotein
- Designing a negative control for the O-glycosylation antibodies

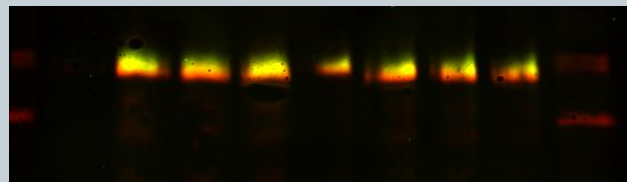
Beta-N-acetylhexosaminidase



IB: BCL11b

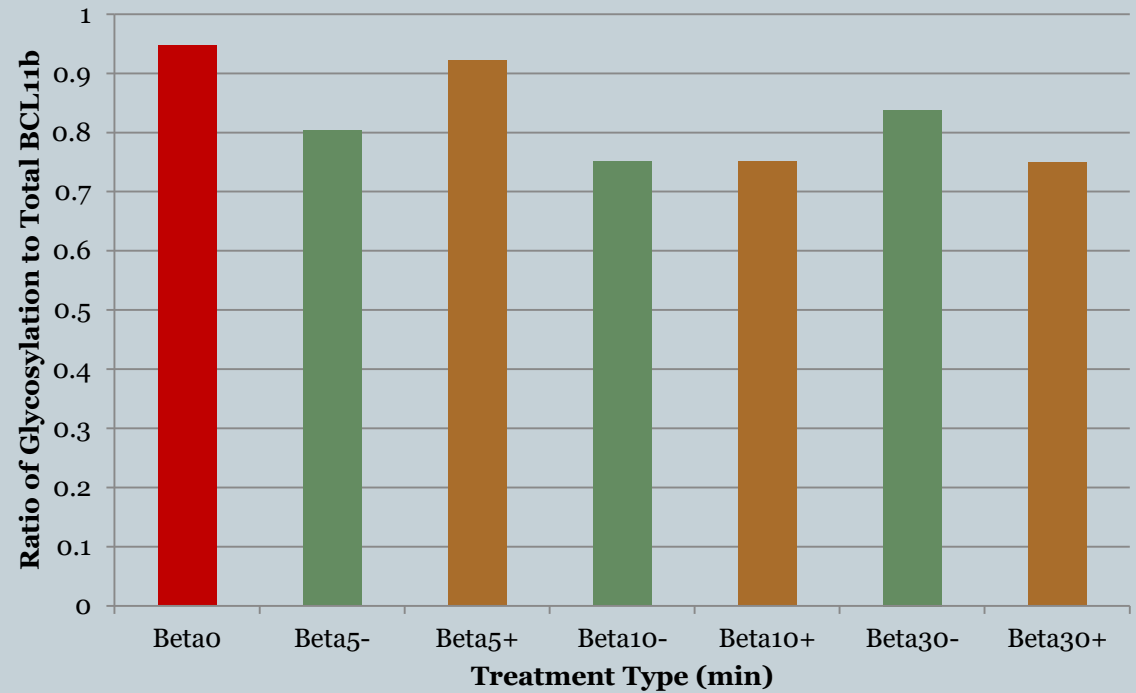


IB: O-Glycosylation



Overlay

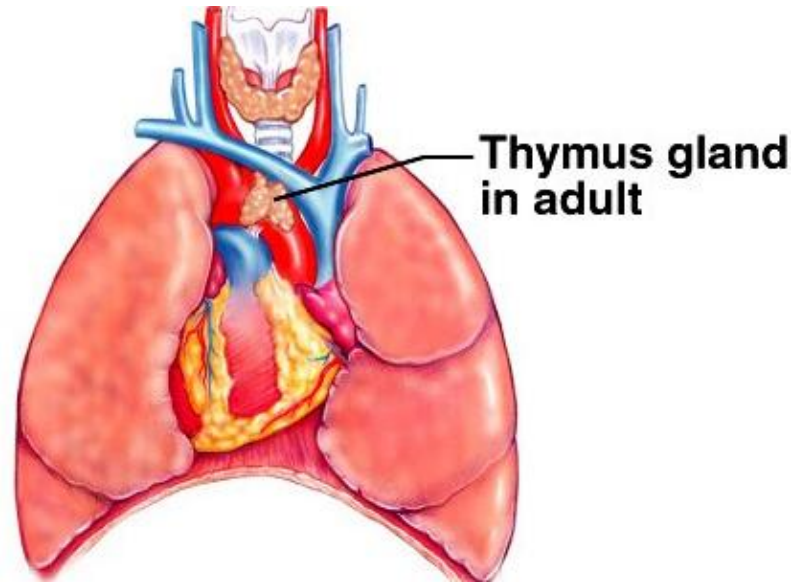
**HEK 203T cells Treated with
Beta-N-acetylhexosaminidase over time**





Mouse Thymocytes

Why are they a
better model?



Natively express BCL11b

Phosphorylation and sumoylation
change dynamically in thymocytes upon
stimulation

Is BCL11b glycosylated?

Does it also change?

P/A (Phorbol Ester and A23187)

- Mimics T-cell receptor activated signaling and phosphorylation cascades
 - MAPK pathway stimulated by Phorbol ester
 - Ca²⁺ pathway stimulated by A23187

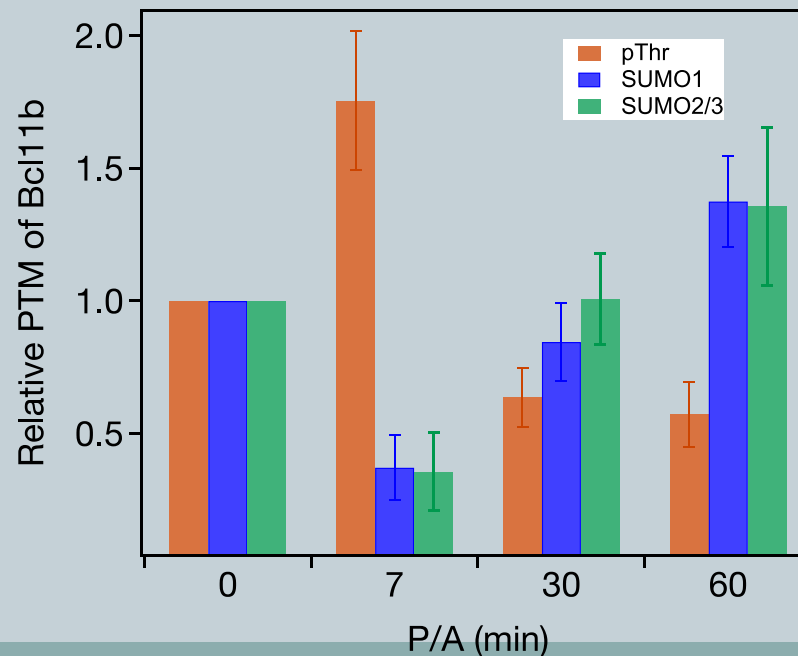
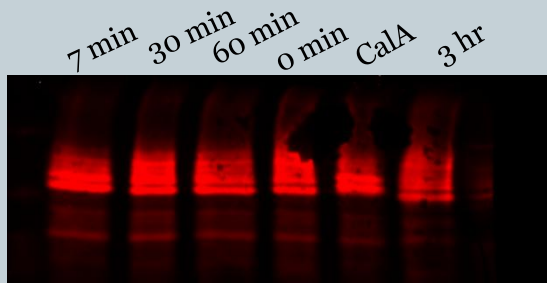
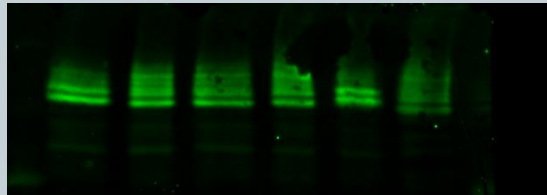


Image from Walter Vogel;
Data from JBC vol 287:26971

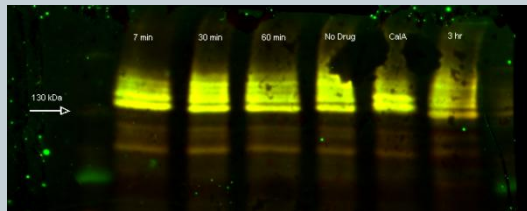
Changes in Glycosylation (P/A) On BCL11b in Thymocytes



IB:BCL11b

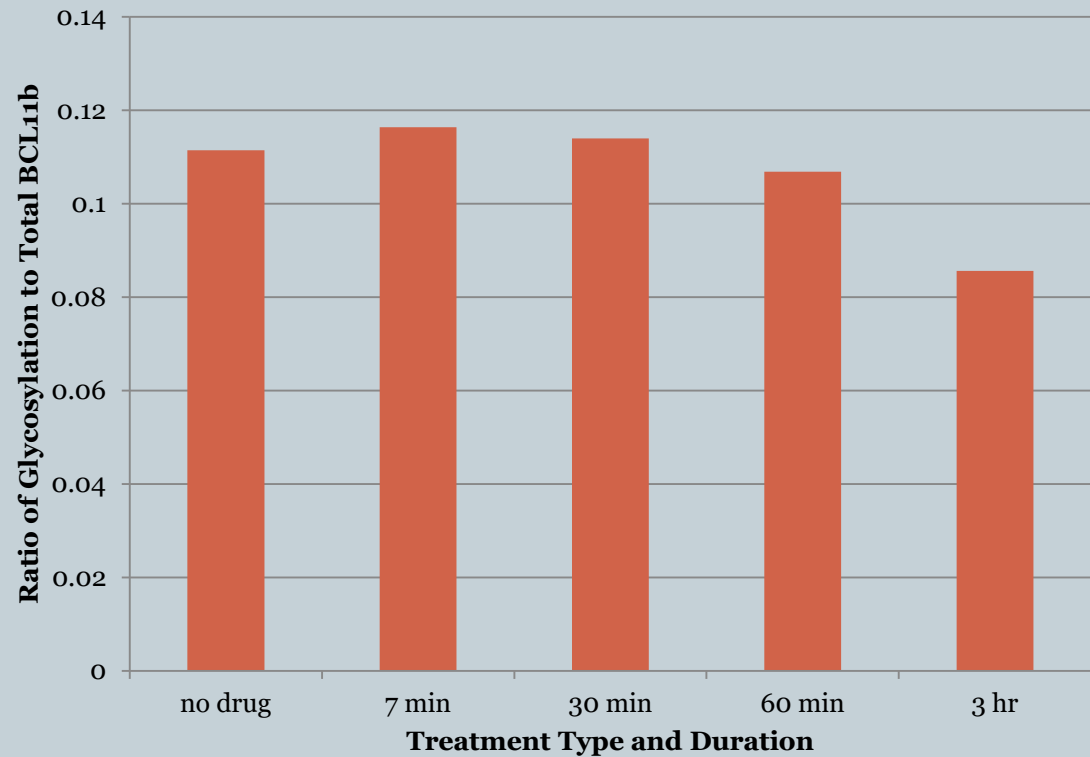


IB:O-Glycosylation



Overlay

Thymocytes Stimulated with P/A



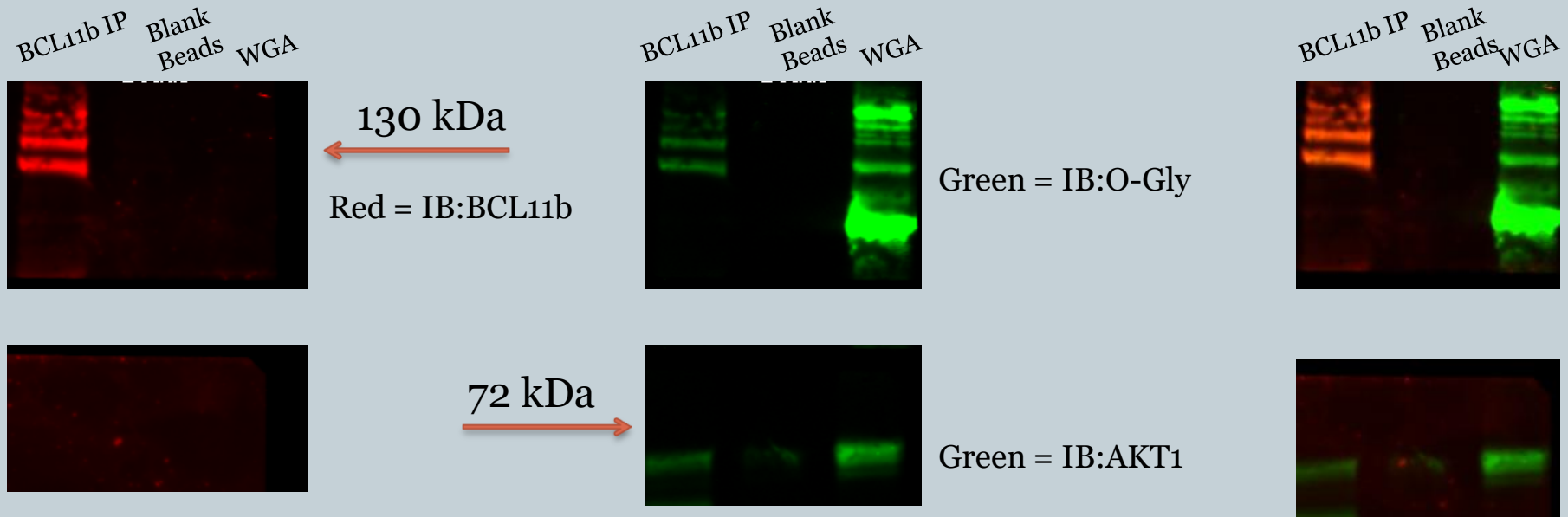
-Non-specific binding of the antibody

Wheat Germ Agglutinin (WGA)



- Binds to N-acetyl-D-glucosamine
- Procedure is identical to the immunoprecipitation described previously
- Pulls down all glycosylated proteins
- Gel is then scanned for specific proteins
- AKT1 is used as a positive control, as it is known to be glycosylated in mouse thymocytes

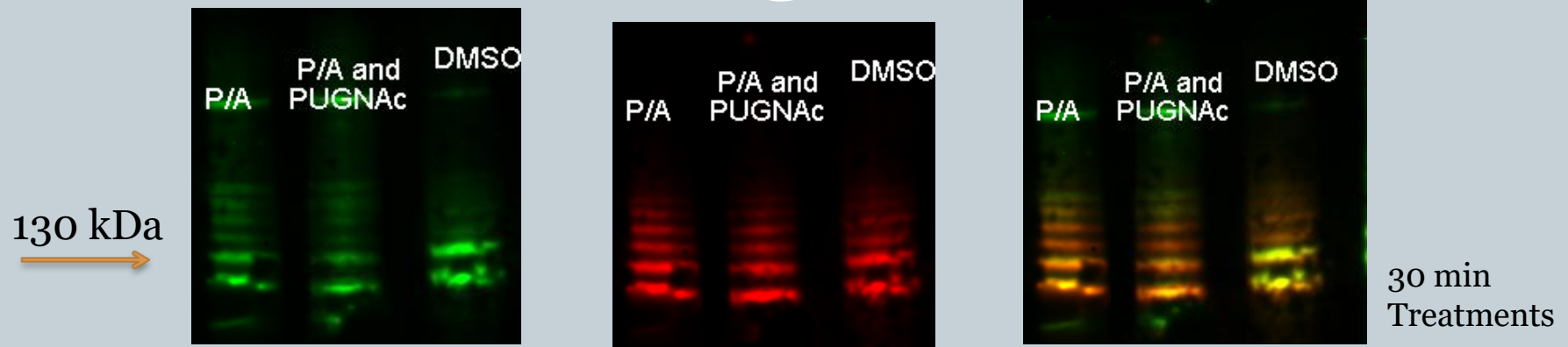
Wheat Germ Agglutinin Blot



Result: WGA did not bind BCL11b
AKT1 was present in the WGA lane

Conclusion: BCL11b is not directly glycosylated

Thymocytes Scanned for Phosphorylation

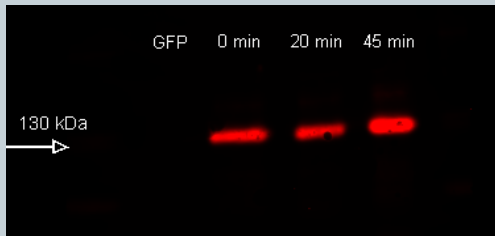


Result: Phosphorylation levels of BCL11b decreased when PUGNAc was added

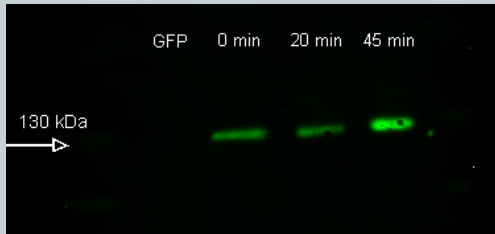
Conclusion: Glycosylation levels affect the activity of BCL11b, but not directly.

Changes in Phosphorylation caused by PUGNAc In BCL11b-Transfected HEK293T cells

IB: BCL11b Signal



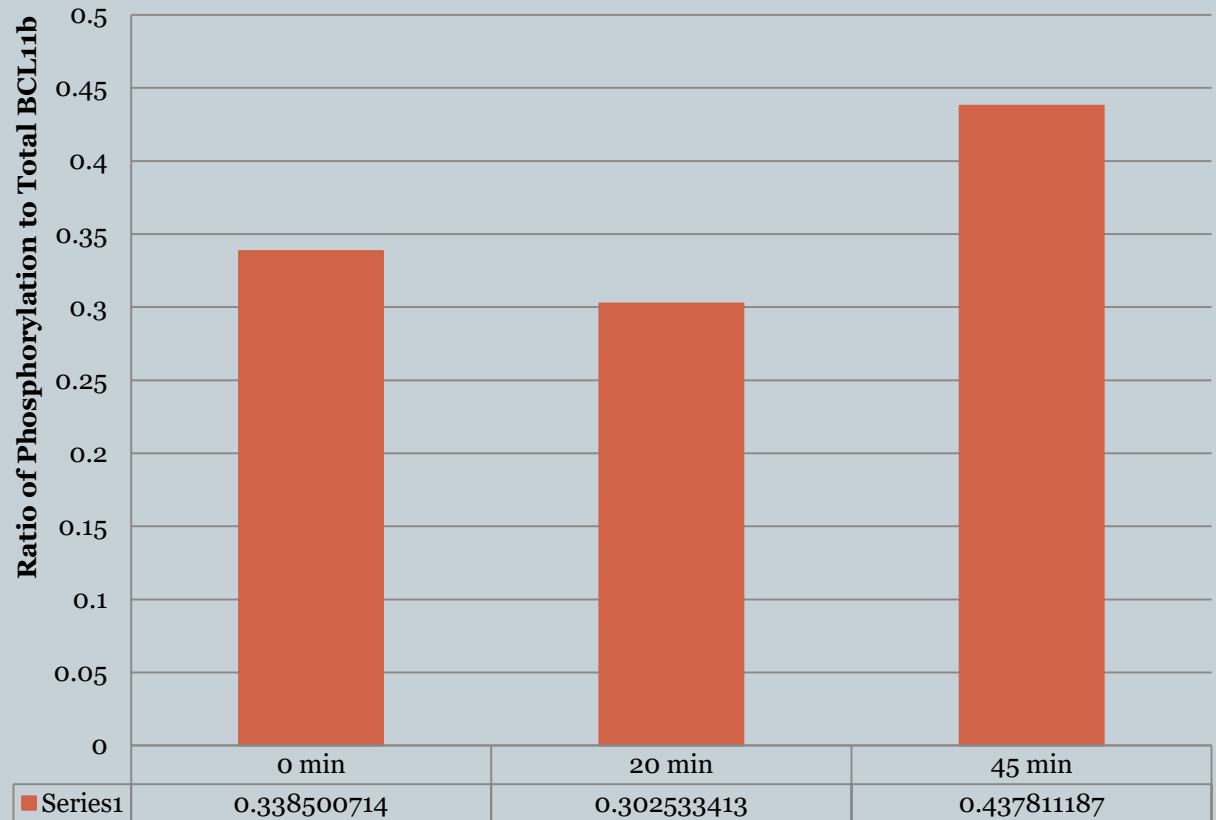
Phosphorylation Signal



Overlay



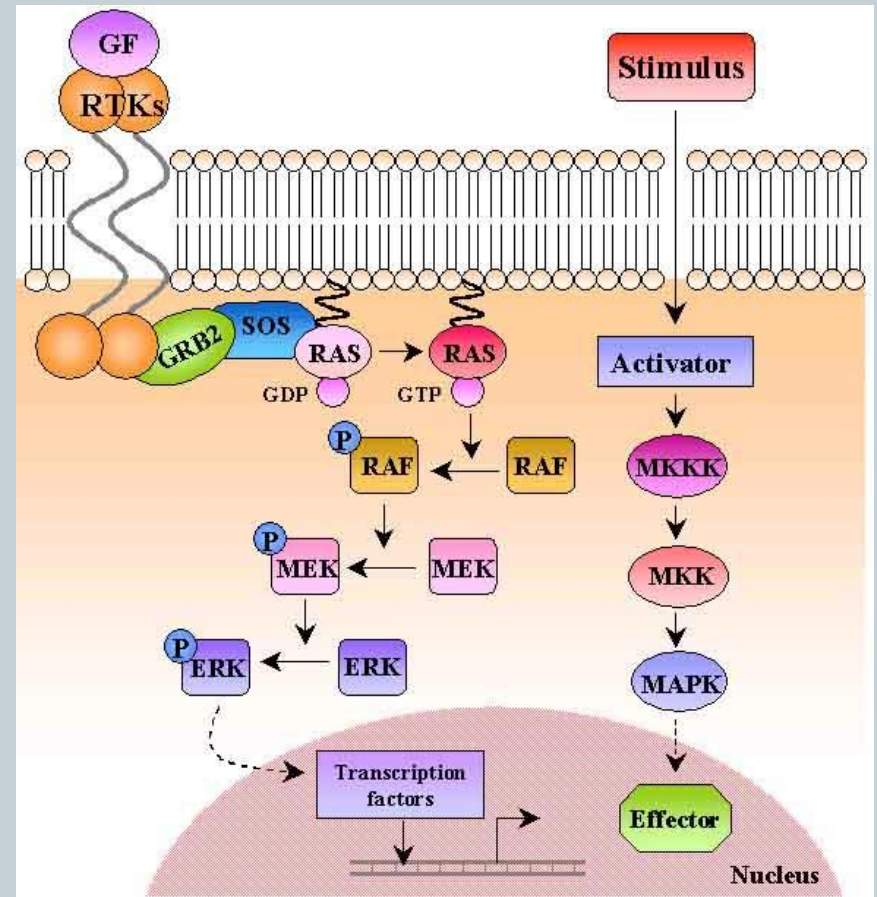
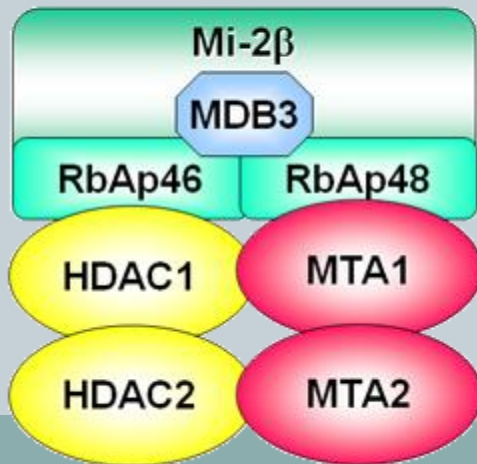
Phosphorylation of HEK cells Treated with PUGNAc



Next Up:

- Determining the effects of glycosylation on other proteins in the NuRD complex and along the MAPK and Ca²⁺ dependent pathways.

NuRD Complex



Special Thanks



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