Involvement of PP6 in Dephosphorylation of Bcl11b (an Anti-Tumorigenic Transcription Factor)

University Honors College Thesis

by

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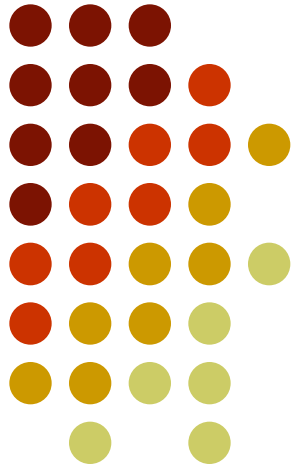


T-cell acute lymphoblastic leukemia (T-ALL)

- Leukemia is a cancer characterized by the uncontrolled accumulation of blood cells
- In 16% of T-ALL cases Bcl11b has been made incorrectly or is absent
- Defects contribute to progression to leukemia because Bcl11b is a crucial component of several stages of T-cell development
- Findings could be used to develop better treatments for aggressive childhood and blood cell cancers

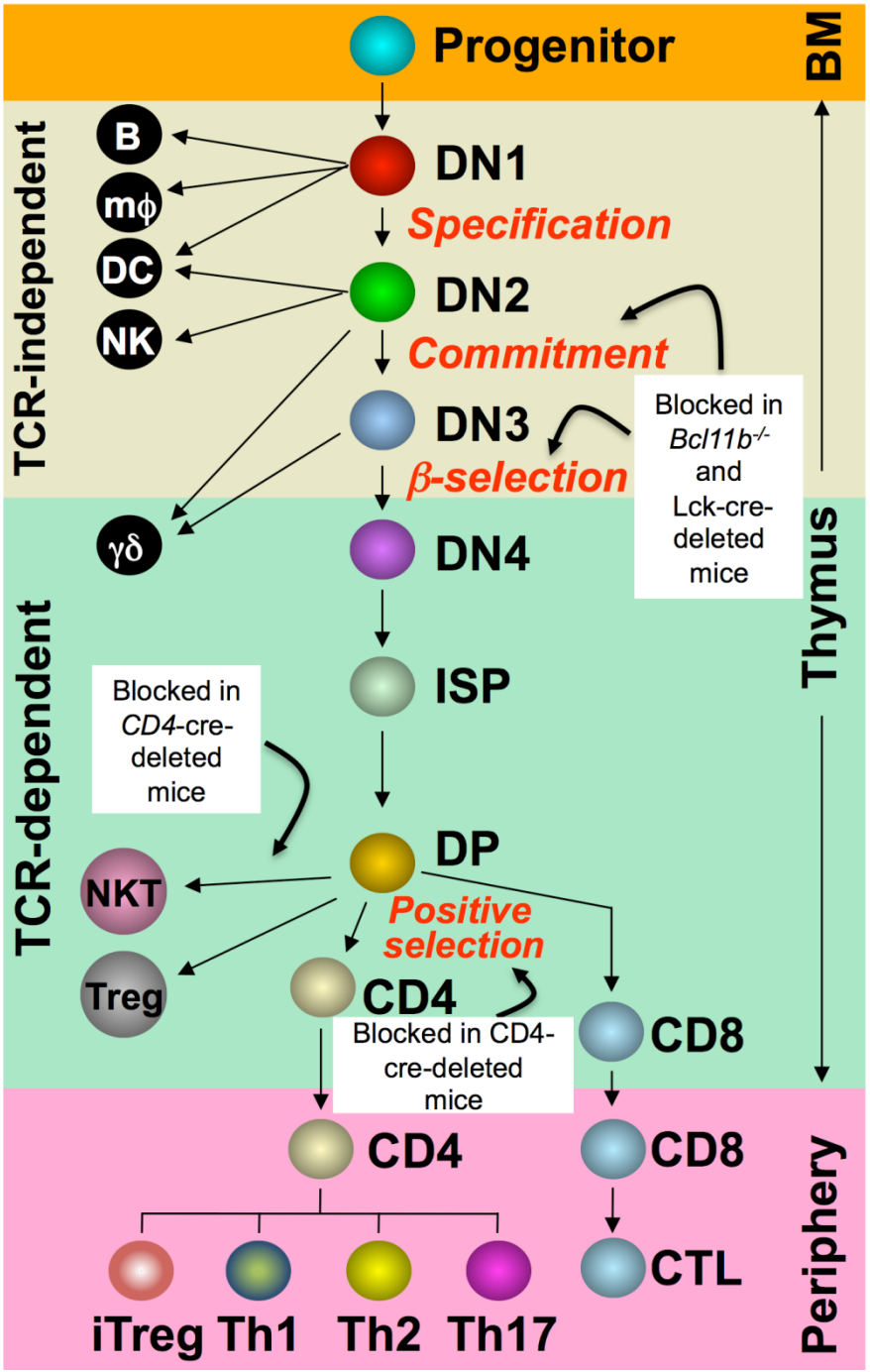


Image courtesy of Rothenberg and Taghon, modified by Leid and Filtz

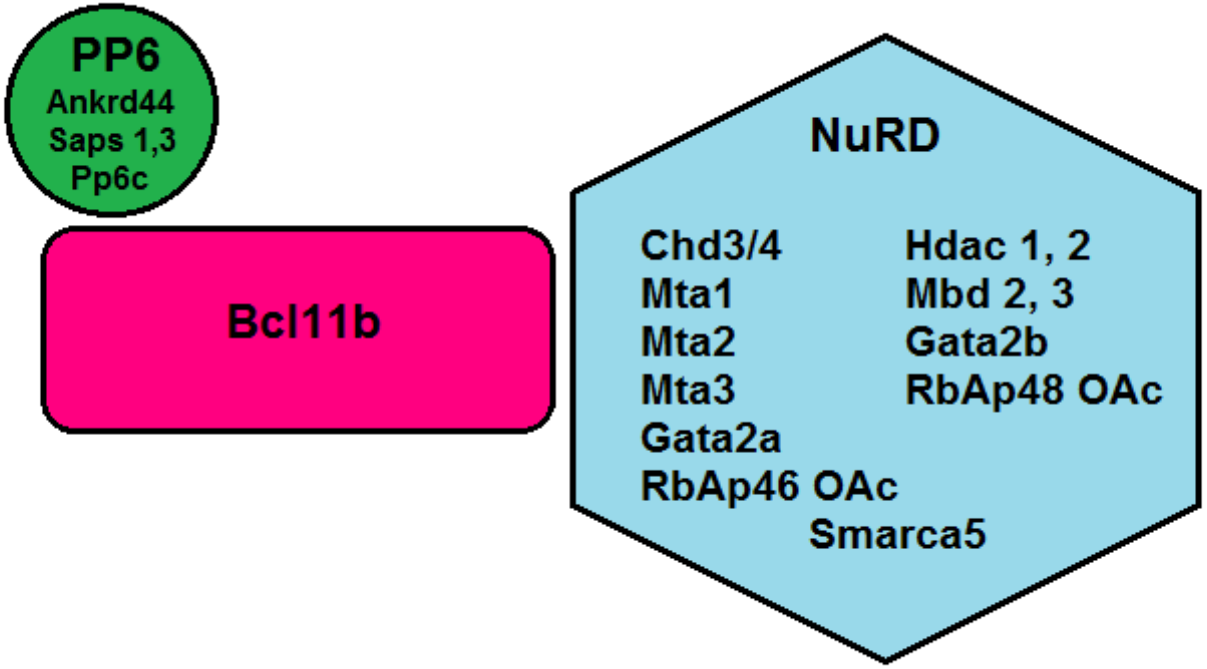
Bcl11b Transcription Factor

- Also known as CTIP2
- Runs at 100-150kDa
- In thymocytes:
  - Acts as a tumor suppressor against Id2 gene
  - Undergoes a cycle of phosphorylation followed by dephosphorylation
  - Dephosphorylation of Bcl11b happens concurrently with derepression of the Id2 gene

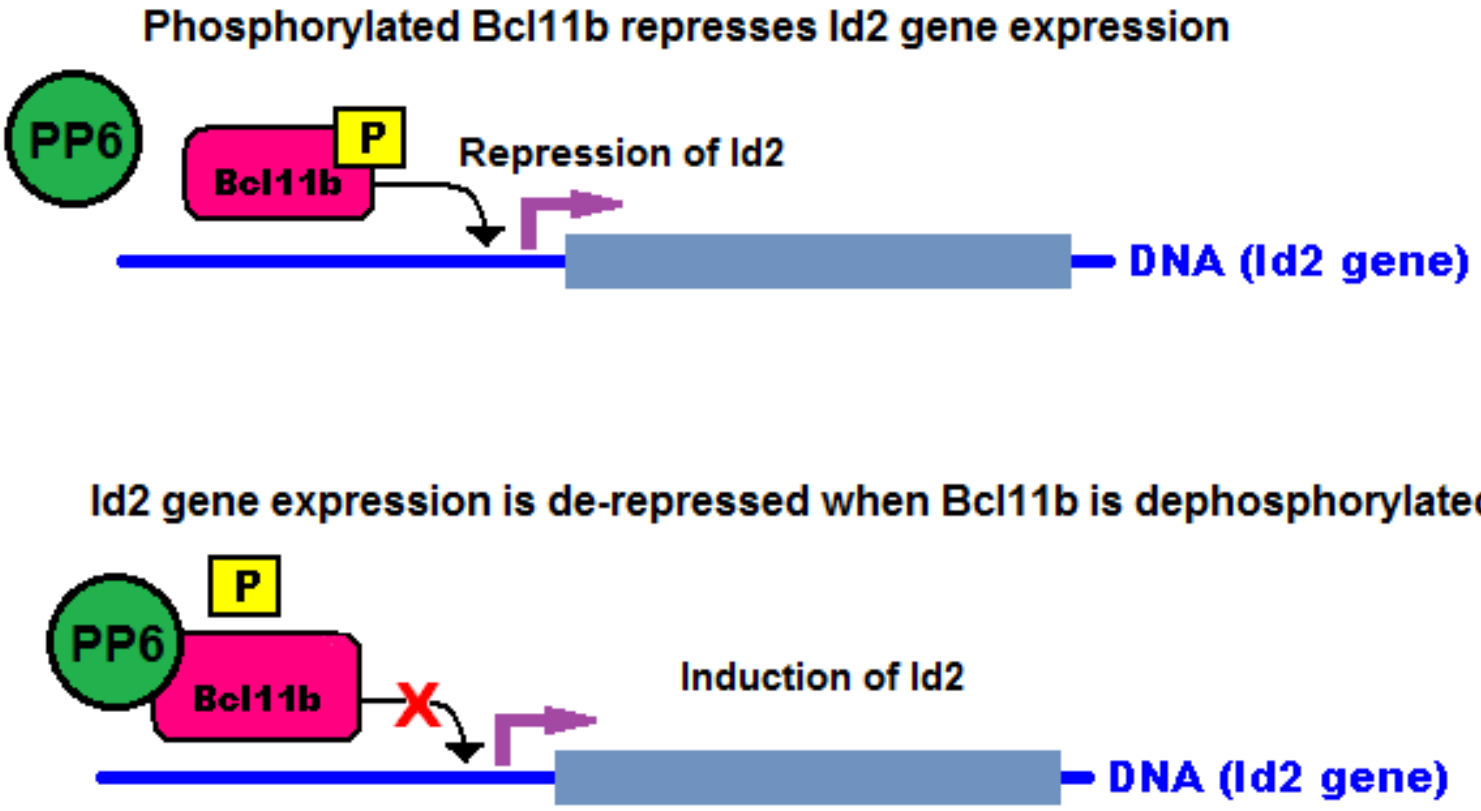
- Which phosphatase dephosphorylates Bcl11b?

Potential Phosphatase

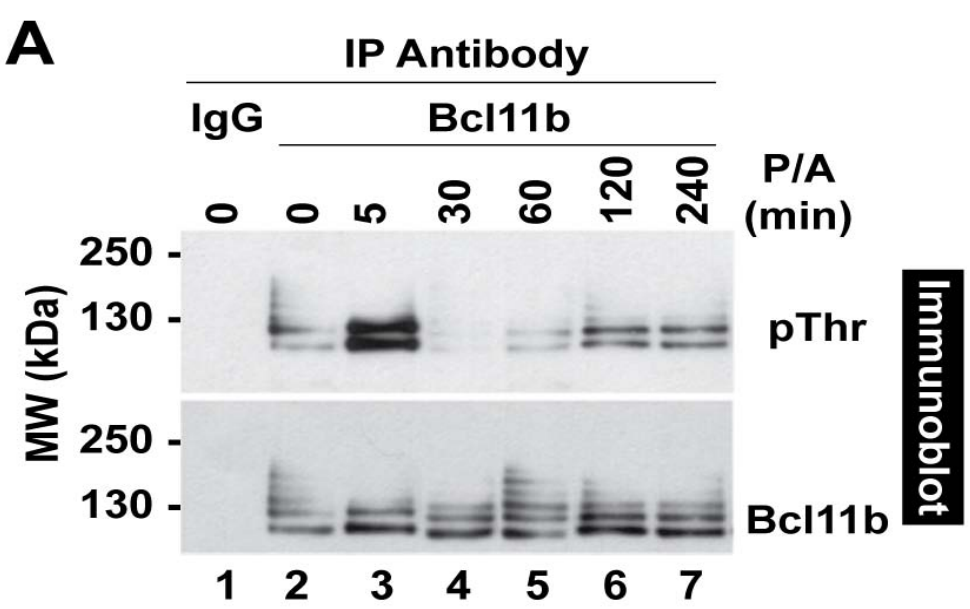
- Bcl11b is associated with the NuRD complex
- Co-precipitation of Bcl11b pulls down PP6



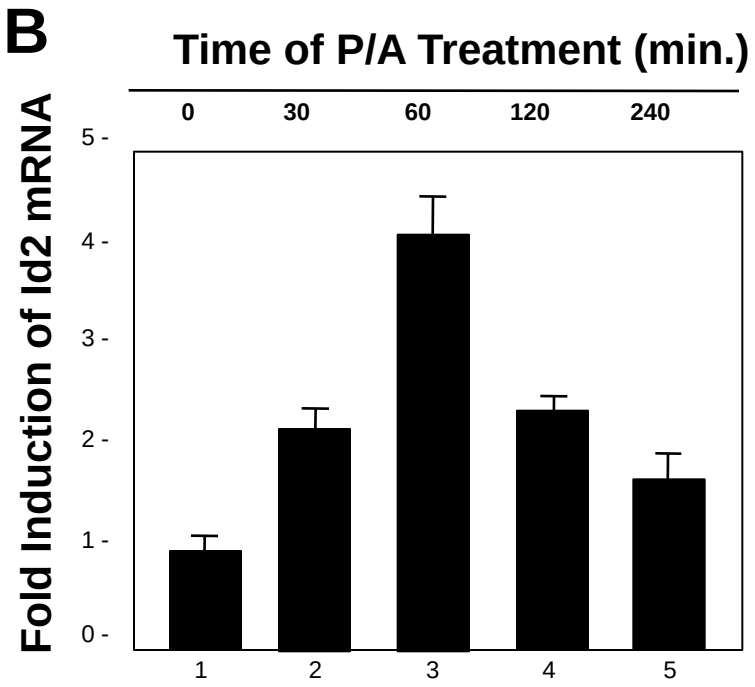
Id2 Regulation (Hypothesized)



Background Research

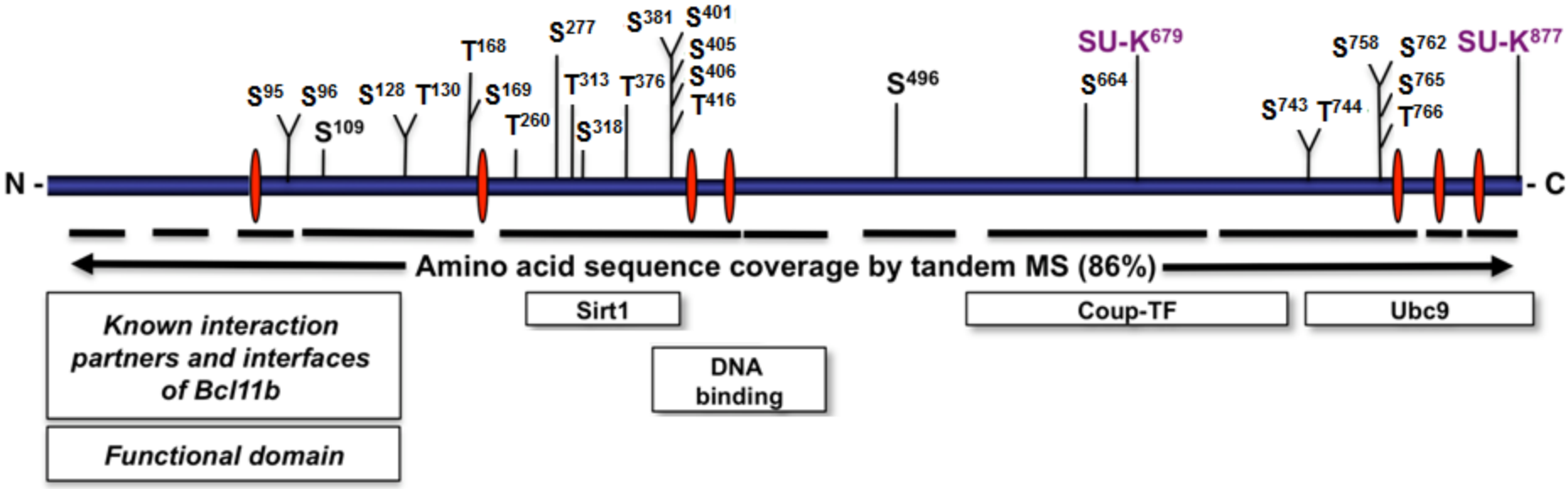


P/A-induced phosphorylation-dephosphorylation of Bcl11b in thymocytes



Courtesy of Drs. Ling Zhang and Mark Leid, Dept of Pharmaceutical Sciences, OSU

Bcl11b Phosphorylation Sites



Preliminary data from Walter Vogel

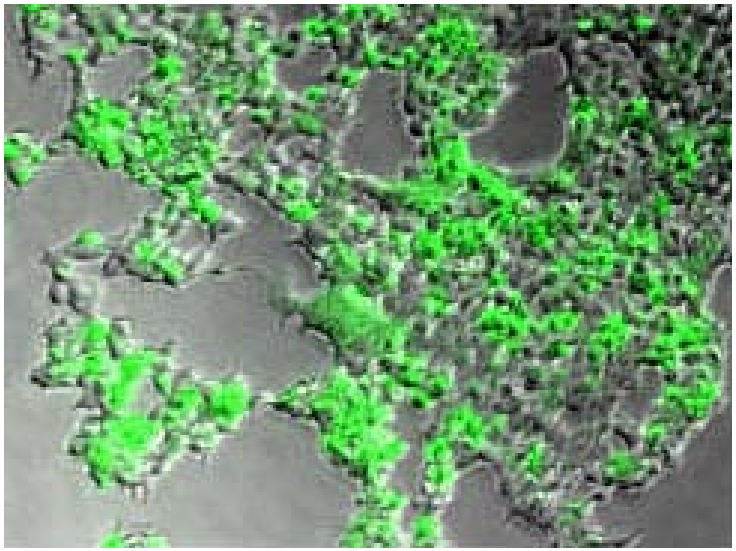
Hypothesis

- The Bcl11b transcription factor is dephosphorylated by PP6
- Co-expression of PP6 and Bcl11b will result in de-repression/increased expression of the reporter gene controlled by the Id2 promoter

Predictions

- HEK-293T cells will show less phosphorylation of Bcl11b in samples where PP6 is also present, with or without PMA stimulation
- HEK-293T cells will exhibit changes in levels of expression of the Id2 gene in CAT reporter assays

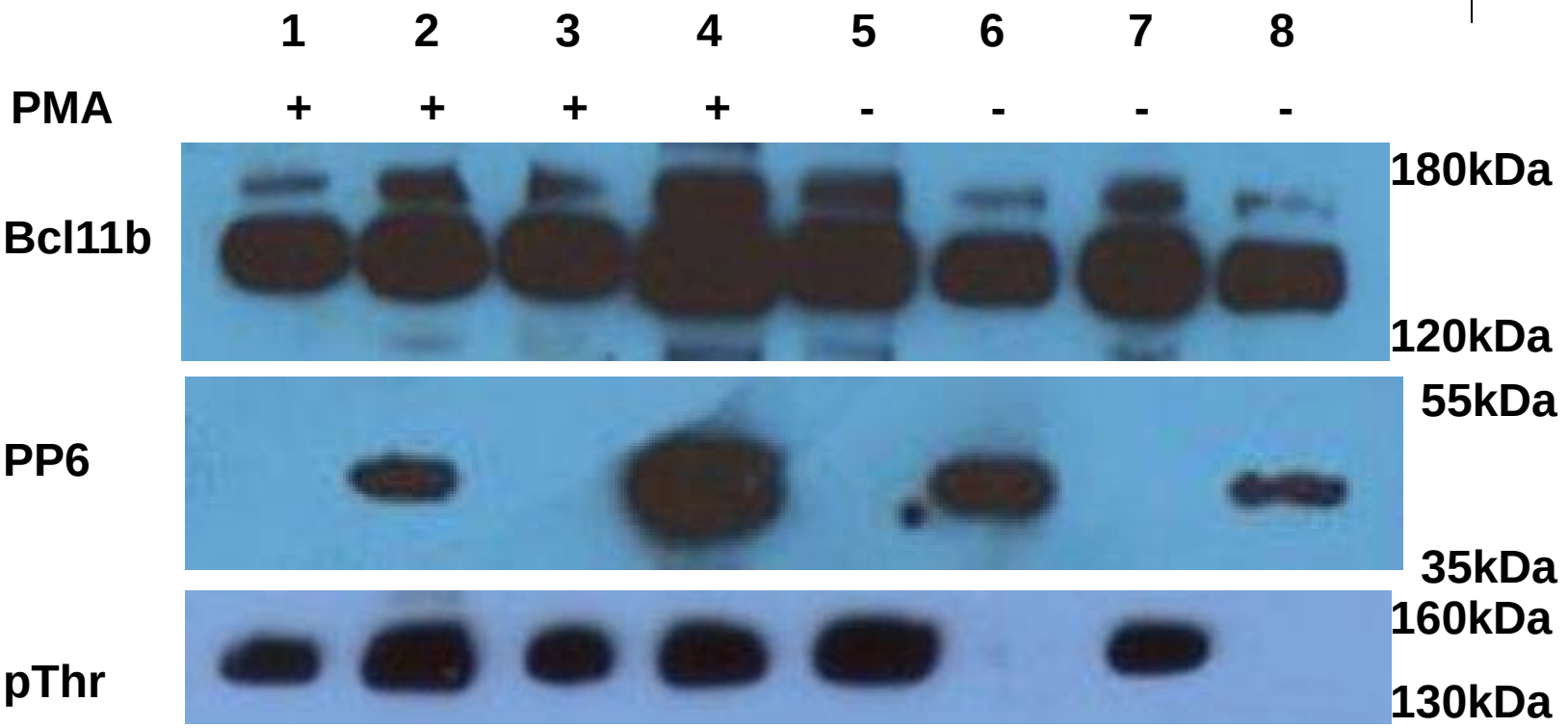
HEK-293T Cells



HEK293T cells
http://www.sigmaaldrich.com

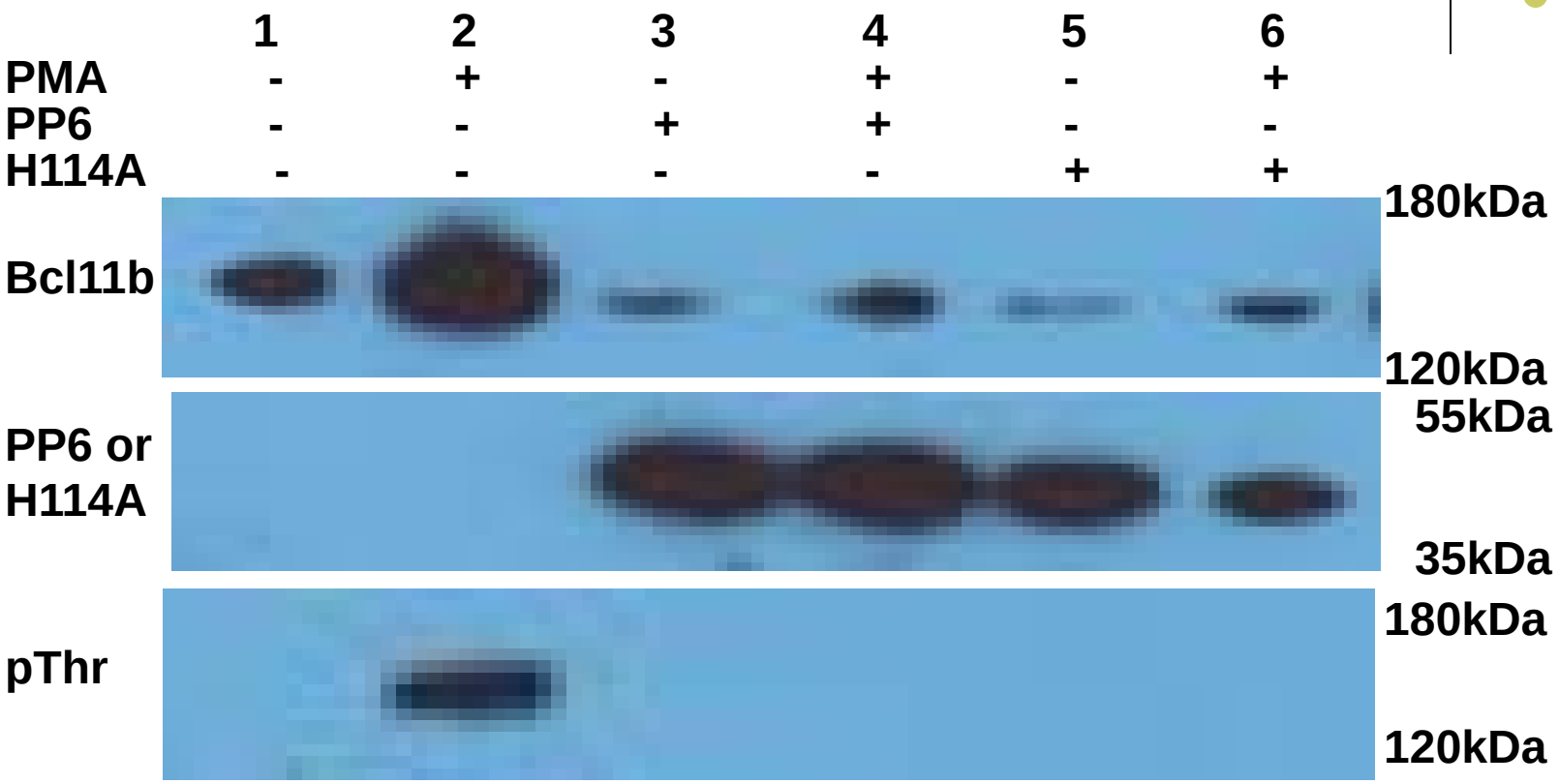
- Human Embryonic Kidney cell line
- Easily transfected
- No endogenous Bcl11b
- Previous research indicated it is a useful model as Bcl11b, when exogenously expressed, functioned appropriately as a repressor of Id2 reporter gene

Results



PP6 co-expression reduced basal phosphorylation of Bcl11b, but not after 4hrs of PMA stimulation. Cells were treated with PMA (1.0uM) or DMSO as indicated. Samples were harvested after 4 hours of incubation and immunoprecipitated with anti-Bcl11b antibodies prior to separation by SDS-PAGE and immunoblotting with antibodies as indicated.

Results

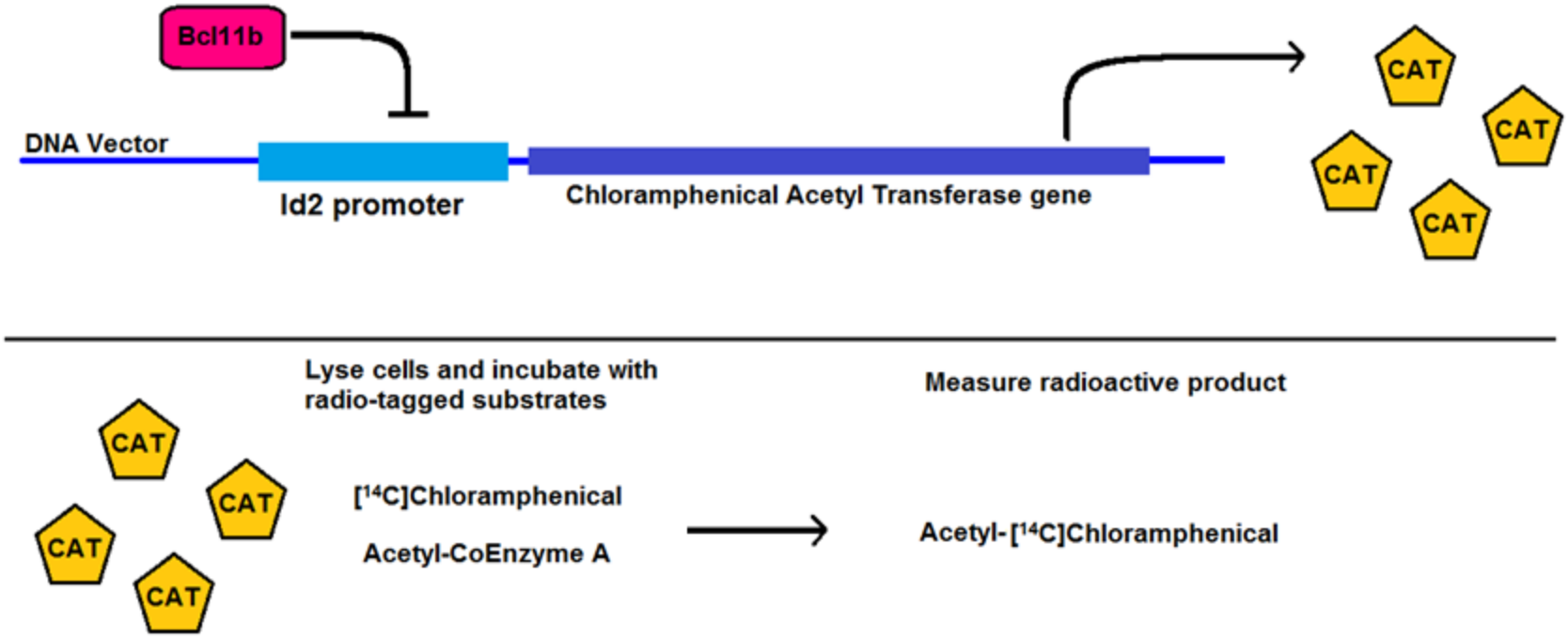


Dephosphorylation of Bcl11b is observed when co-expressed with PP6 or H114A, with and without PMA stimulation for 30 min. Cells were treated with PMA or DMSO as indicated for 30 min, and then harvested and immunoprecipitated with anti-Bcl11b antibodies prior to separation by SDS-PAGE and immunoblotting with antibodies as indicated.

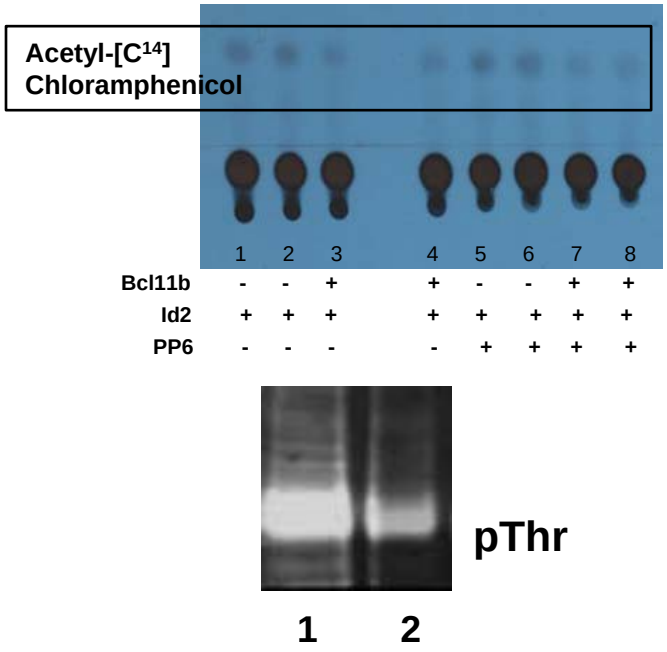
Reporter Gene Assay

- Chloramphenical acetyl transferase (CAT) assay can be used to measure expression of the Id2 gene with and without co-transfection of Bcl11b and PP6
- Id2-promoter sequence (-2.9 to +.152 kb fragment of mouse Id2 locus) subcloned into pCR2.1 vector in front of chloramphenical acetyl transferase gene
- β-galactosidase expression vectors used as an internal control for normalization of transfection efficiency and protein expression
- All cells are transfected with plasmids containing reporter construct and β-galactosidase, some receive constructs for Bcl11b and PP6
- Radioactive C^{14} is used to tag and quantify regulation of the Id2 promoter by Bcl11b in the absence and presence of PP6

CAT Assay


Bcl11b
DNA Vector
Id2 promoter
Chloramphenical Acetyl Transferase gene
CAT
CAT
CAT
CAT
CAT
CAT
CAT
Lyse cells and incubate with
radio-tagged substrates
[14C]Chloramphenical
Acetyl-CoEnzyme A
Measure radioactive product
Acetyl-[14C]Chloramphenical

Results



pThr

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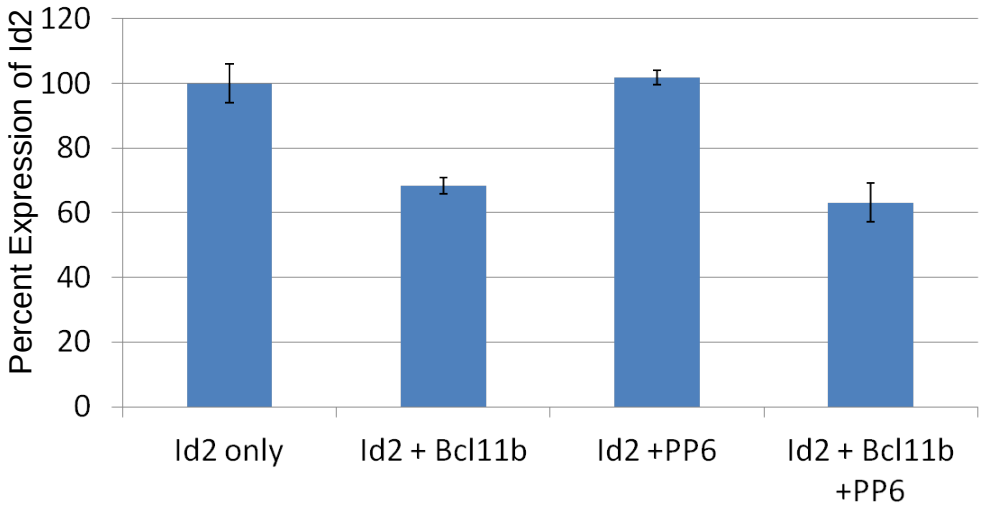

CAT Reporter Assay shows PP6 co-expression with Bcl11b has no effect on Id2 repression. Column 1 shows basal levels of Id2 promoter-CAT reporter expression without co-expression of Bcl11b (100%). Expression levels decrease by ~32% when co-expressed with Bcl11b (Column 2). Columns 3 and 4 show no repression of the reporter construct when co-expressed with PP6, or with PP6 and Bcl11b together.

Conclusion

- PP6 co-expression is associated with dephosphorylation of Bcl11b in HEK293T.
- H114A is not a good negative control for PP6
  - PP6 may be a nucleating factor? PP2A?
  - May require regulatory subunits R1 and R3?
- PP6 has Bcl11b-independent action in reporter gene assays
- HEK293T cells are not the best model for these experiments
- Next steps: Bcl11b phosphosite mutants, PP6 knockdown experiments

Special Thanks

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