Transcriptional regulation by antagonistic promoter binding of BCL-6 or STAT5 is modulated by Rac1 signalling

Patrícia Barros, Peter Jordan, Paulo Matos

RNA 2012
The control and flow of gene expression

Stimuli → Signalling → Transcription factor → DNA → RNA → Protein

DNA

Alternative Splicing

RNA

Stability

Translation efficiency

Protein
Rac1 signalling affects transcriptional control

Barros et al., 2009 Mol. Cell. Biol. 29(15), 4156-4166
Mock
Rac1-L61
PAK1-CA
α-GFP
α-tubulin
luc+ SV40 promoter

Fold increase in Luciferase activity

pGL3-5xBCL-6
Mock, Rac1-L61, PAK1-CA

BCL-6 reporter
5x TCGAATTCCTCGAAAG
BCL-6 and STAT5 recognise a similar core binding motif

Genes reported to be regulated by both factors:

cyclin D2, blimp-1, BCL-XL, Socs2, prolactin
Does Rac1 signalling coordinate the activity of BCL-6 and STAT5?

Rac1 = PAK1+STAT5

Fold increase in Luciferase activity

- Mock
- Rac1-L61
- PAK1-CA
- STAT5A
- PAK1-CA + STAT5A

GFP-STAT5A
GFP-PAK1
GFP-Rac1-L61

α-GFP
α-tubulin
Active Rac1 promotes a switch from BCL-6 to STAT5 at the promoter.
Does BCL-6/STAT5 transcription switching happen in cells?
Which endogenous genes may be targets of the transcription factor switch?
Cell cycle PCR array

1. Convert total RNA to cDNA.

2. Add cDNA to RT^2 qPCR Master Mix & Aliquot Mixture Across PCR Array.


4. Data Analysis.
NS IgG
αααα
-STAT5
αααα
-BCL-6
Input

DLD-1
SW480
HT29

OD
(abstract units)

SUMO1 promoter
−− −−
327 bp

CDKN2B promoter
−− −−
340 bp

CCND2 promoter
−− −−
344 bp

SW480
+++ Active Rac1
0   Active
PAK1
+   Active
STAT5

HT29
++ Active BCL-6
++ Active Rac1
++ Active PAK1
++ Active STAT5

DLD-1
++ Active BCL-6
+  Active Rac1
+  Active PAK1
+  Active STAT5
+  Active BCL-6
CCND2

Promoter occupancy (OD in absorbance units)

DLD-1

<table>
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<tr>
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<th>Bound BCL-6</th>
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<td>Mock</td>
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<td>500</td>
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<td>1000</td>
</tr>
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SW480

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HT29

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Transcript expression (Fold over mock)

phospho-STAT5

STAT5

GFP-PAK1

GFP-Rac1

α-GFP
Mock Rac1-L61 PAK1 PAK1-KD

Promoter occupancy (OD in abstract units)

GFP-Rac1

GFP-PAK1

phospho-STAT5

SUMO1

CDKN2B

CCND2

DLD-1

SW480

HT29

CDKN2B

Promoter occupancy (OD in abstract units)

CDKN2B

Promoter occupancy (OD in abstract units)

CDKN2B

Promoter occupancy (OD in abstract units)

CDKN2B

Promoter occupancy (OD in abstract units)

CDKN2B

Bound STAT5 Bound BCL-6

mRNA level

Bound STAT5 Bound BCL-6

mRNA level

Bound STAT5 Bound BCL-6

mRNA level

Bound STAT5 Bound BCL-6

mRNA level

Transcript expression (Fold over mock)

Transcript expression (Fold over mock)

Transcript expression (Fold over mock)

Transcript expression (Fold over mock)
Summary

Novel insights into the modulation of gene transcription by GTPase signalling.

- cyclin D2, CDKN2B and SUMO1 were identified to be inversely regulated by BCL-6 and STAT5 in colorectal cells.

- Rac1 signalling activates gene transcription by inducing a switch from repressor BCL-6 to activator STAT5 at their promoters.
Proposed model
Acknowledgements

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Eric Lam

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FCT
BioFig
EMBO
Cyclin D2: regulatory subunit of CDK4 or CDK6 required for cell cycle G1/S transition

The SUMO1 gene encodes a small ubiquitin-like protein that can be covalently attached to proteins as a monomer or a lysine-linked polymer. Unlike ubiquitin, sumoylation is not involved in proteolytic degradation of the attached protein but rather modulates nuclear transport or transcriptional regulation.

CDKN2B encodes the cyclin-dependent protein kinase inhibitor protein p15 encoded by the INK4b locus, which can form a complex with CDK4 or CDK6, and prevent the activation of the kinases by cyclin D, inducing cell cycle arrest.
Active Rac1 promotes a switch from BCL-6 to STAT5 at the promoter.
Acknowledgements

• FCT

• BioFig

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• Paulo Matos and Peter Jordan

• Sónia Moniz and Vânia Gonçalves

• Eric Lam