Epigenetic Control of Effector T cell Development

Robin D. Hatton, PhD
University of Alabama at Birmingham
Diversity of T Helper Cell (Th) Responses

Th1
- Intracellular pathogens including viruses
- Autoimmune diseases
- IFN-γ
- STAT4
- STAT1
- T-bet
- Runx3

Th17
- Extracellular bacteria
- Fungi
- Autoimmune diseases
- IL-17
- STAT3
- RORyt
- RORα
- IRF4

Th2
- Worms
- Helminthes
- Asthma
- IL-4
- STAT6
- Gata3
- c-Maf

Th9
- IL-9
- STAT6
- PU.1

Th1-like
- IL-10
- Regulatory function
- Loss of immune-regulation

Naïve CD4+ T cells
- IL-12
- IFN-γ
- IL-23
- IL-6
- TGF-β
- IL-21

Tfh
- IL-21
- STAT3
- Bcl6

iTreg
- FoxP3
- Runx1
- c-Rel
- IL-10

Regulatory function
- Loss of immune-regulation
~ 140 kb encompasses the Ifng locus

All Ifng CNSs interact with the promoter although functions of individual CNSs yet to be established
High-throughput Analyses: ChIP-chip and DNase-chip

Crawford et al. Nat. Methods 2006
The Extended *Ifng* Locus: Th1 Specific Accessibility

Balasubramani et al. *Immunity* 2010
Ifng CNS-22 Deletion

CNS: -54 -34 -22 -6 P Ifng +17 +19 +30 +40 +46 +54

Naive
Th1
Th2
Th17
CTCF

9126bp

391bp

CNS-22 deletion
Impairment in Cytokine Driven *Ifng* Gene Transcription in CD4⁺ T Cells

**Resting**

**IL-12 + IL-18**

**anti-CD3 + anti-CD28**

**anti-CD3 + anti-CD28**

**OTII/Ifng-CNS-22 +/-**

**OT-II/Ifng-CNS-22 -/-**

**day5**

**day3**

Balasubramani, A.
CNS-22 Partially Controls Ifng Locus Remodeling

**DNase I**
- Naïve CNS-22 wt
- Naïve -CNS-22 KO
- Th1 CNS-22 wt
- Th1 CNS-22 KO

**H3K4me1**
- Th1 CNS-22 wt
- Th1 CNS-22 KO
Diminished Hyperacetylation in the Absence of CNS-22

The diagram illustrates the levels of hyperacetylation at different regions of the genome. The regions are labeled as follows:

- **DNase**
- **H4K12 ac**

The y-axis represents different conditions:

- **Resting WT**
- **Resting CNS-22−/−**
- **IL-12 + IL-18 WT**
- **IL-12 + IL-18 CNS-22−/−**

The x-axis indicates genomic positions relative to the Ifng gene. The positions are labeled as follows:

- From -70 to +66
- Specific regions marked with numbers (-70, -54, -34, -22, -6, P, +17-19, +30, +40, +46, +54, +66)

The diagram shows a comparison of hyperacetylation levels across these conditions, highlighting diminished hyperacetylation in the absence of CNS-22.
Summary

- CNS-22 resides in an area of open chromatin early in T cell development and in all T cell lineages analyzed

- CNS-22 recruits factors involved in the optimal expression of IFN$\gamma$

- Deletion of CNS-22
  - Impacts IL-12 and IL-18 driven induction of Ifng in both T cells and NK cells
  - Impacts local chromatin structure
Acknowledgements

Casey Weaver

Weaver Lab
Anand Balasubramani
Henrietta Turner
Karen Janowski
James Oliver
Craig Maynard
Benjamin Weaver
Rita Luther

Collaborators
Greg Crawford (Duke)
Yoichiro Shibata (Duke)
* From the first set of data for med1 and med12
IL-12 + IL-18

Resting

IL-12 + IL-18

αCD3 + αCD28

WT

CNS-22-/-

ChIP-chip datasets normalized against respective resting H4K12ac levels..

IFN-γ

day 5

-70 -55 -34 -22 -6 Ifng +17-19 +30 +40 +46 +54 +66

IL-12 + IL-18

WT

CNS-22-/-

Th1 DNase

TCR

WT

CNS-22-/-
IL-12 + IL-18
Th1 DNase
IL-12 + IL-18
TCR
WT
CNS-22/-
WT
CNS-22/-
T-bet-Dependent Rel A Recruitment to Ifng Locus in Th1 Cells

**Th1 cells**

<table>
<thead>
<tr>
<th></th>
<th>Resting</th>
<th>P + I</th>
<th>IL-12 + IL-18</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>3.9</td>
<td>0.0</td>
<td>41.1</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Tbx21</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>1.0</td>
<td>0.1</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td></td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Tc1 cells**

<table>
<thead>
<tr>
<th></th>
<th>Resting</th>
<th>P + I</th>
<th>IL-12 + IL-18</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>3.2</td>
<td>0.1</td>
<td>74.3</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Tbx21</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>2.8</td>
<td>0.0</td>
<td>69.5</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td></td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Relative RelA binding**

- **IL-4**
  - WT: PMA + Ionomycin
  - Tbx21**: PMA + Ionomycin

- **IL-12 + IL-18**
  - WT: PMA + Ionomycin
  - Tbx21**: PMA + Ionomycin
T-bet Dependent Remodeling of the *Ifng* Locus
Diminished STAT4 Recruitment to \textit{Ifng} Locus in the Absence of T-bet

Th1 cells

\begin{tabular}{c c c}
 IL-12 + IL-18 & WT & \textit{Tbx21}^{-/-} \\
 RelA & - & + \\
 T-bet & - & + \\
 Cyclophilin B & - & + \\
\end{tabular}

Th1 cells

\begin{center}
\includegraphics[width=0.5\textwidth]{Th1.png}
\end{center}

Tc1 cells

\begin{center}
\includegraphics[width=0.5\textwidth]{Tc1.png}
\end{center}

T-bet

\begin{center}
\includegraphics[width=0.5\textwidth]{T-bet.png}
\end{center}

Relative STAT4 binding

\begin{center}
\includegraphics[width=0.5\textwidth]{Relative.png}
\end{center}

Resting

\begin{center}
\includegraphics[width=0.5\textwidth]{Resting.png}
\end{center}
Enhancers: How Do They Drive RNA Pol II Dependent Transcription

- **Pol II dynamics**
  - Abortive transcription
  - Productive elongation

- **Enhancers**
  - Permissive epigenetic remodeling
  - Push Pol II past the promoter i.e. facilitate transition from initiation to elongation: Thought to impact elongation, but dispensable for Pol II recruitment and initiation
  - Pol II has been shown to be recruited to other enhancers: even involved in generation of enhancer associated transcripts (eRNAs)

Margaritis et al. Cell 2008
CNS-22: Transcript Initiation or Elongation

Initiated transcripts

Spliced transcripts

IL-12+IL-18

Initiated transcripts

Spliced transcripts

Ifng-CNS-22 +/+  
Ifng-CNS-22 −/−
Recruitment of p300 and RNA Pol II to Distal Enhancers

Margaritis et al. *Cell* 2008
H4 Association Remains Unperturbed

Th1 cells

\[ H4 \text{ acetylation} \]

- Resting
- IL-12 + IL-18

NCS-24

NCS-20
CNS-22 Dependent Local Permissive Remodeling
Development of a Novel System for Site-Specific, Single-Copy, Directional Targeting of BAC Transgenes: The HAT-BAC System

- ES cell based
- Targeting to hprt1 locus:
  - X-linked (single copy in ES cells)
  - ubiquitously expressed, provides a favorable chromatin environment for transgene expression
  - prior success in targeting and expressing BAC transgenes
    - mice derived from independent ES cell clones containing transgene expressed from the same promoter exhibit comparable levels of expression
    - reconstitution of hprt1 expression permits HAT selection of correctly targeted clones
- Implementation of a novel DNA recombinase for efficient targeting via exchange reaction
Cre and Flp vs $\phi$C31 Integrase

• Cre or Flp
  – insertion of a circular DNA into the genome (trans event), two cis-positioned recognition sites are created
  – intramolecular interactions are kinetically favored over intermolecular interactions; these recombinases favor deletion rather than integration of DNA
  – transgene integration occurs at low efficiency because the reaction equilibrium is shifted in favor of excision

• phiC31 integrase
  – can be optimized to work well in mammalian cells (NLS, codon usage); no other phage or bacterially-encoded proteins or factors required
  – catalyzes only the attB x attP reaction and not the reverse reaction (lack of excisionase)
  – better suited for cassette exchange reactions due to its unidirectionality
Targeting of hprt1 Locus in ES Cells

Murine hprt1 locus with φC31 Integrase docking site

Select with puromycin
Engineering BACs for Site-Specific Recombination

- Insert 5′ attB site
- Insert the cassette: [human hprt promoter/exon 1+ second attB site]

BAC transgene of interest
Targeting of BAC Transgene to the Docking Site in HAT-BAC ES Cells

HAT-BAC ES hprt1 locus containing docking site

Ifng/Thy1.1/EGFP BAC-In transgene

Restored hprt1 locus containing BAC transgene

Select with HAT
Histone Modifying Enzymes

Image created by Kosi Gramatikoff
p300 Binding Maps to CNS Elements Across Ifng Locus

DNase

Th1, p300

Resting

IL-12 + IL-18

Tc1, p300

Resting

IL-12 + IL-18

Tc1 : IL-12 + IL-18

9126bp

p300

WT

H4K12ac

WT

H4K12ac

CNS-22 -/-

391bp

CNS-22
Activation-induced Hyperacetylation of CNS-22 Precedes \( \text{Ifng} \) Transcript Induction

- Graph showing H4 acetylation relative to H4 abundance over time (Resting vs. IL-12 + IL-18).
- Graph showing spliced \( \text{Ifng} \) transcripts over time (Resting vs. 1h, 2h, 4h).

Legend:
- Resting
- IL-12 + IL-18 4h
- IL-12 + IL-18 2h
- IL-12 + IL-18 1h
Histone Acetylation as a Measure of Transcriptional Activity

The image shows a graphical representation of histone acetylation as a measure of transcriptional activity. It features a DNA sequence with various transcription factor binding sites and acetylation marks. The acetylation patterns are depicted for different transcription factors and cell types, highlighting the dynamic nature of gene regulation through histone modifications.
## Acetylation of Lineage-specifying Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression pattern</th>
<th>Levels of H4K12 acetylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Th1</td>
</tr>
<tr>
<td>Tbx21</td>
<td>Th1</td>
<td>++++</td>
</tr>
<tr>
<td>Gata3</td>
<td>Th2</td>
<td>+</td>
</tr>
<tr>
<td>Rora</td>
<td>Th17</td>
<td>-</td>
</tr>
<tr>
<td>Rorc</td>
<td>Th17</td>
<td>-</td>
</tr>
<tr>
<td>Il21</td>
<td>Th17 &gt; Th1/Th2</td>
<td>++</td>
</tr>
<tr>
<td>Il10</td>
<td>Th2 &gt; Th1/Th17</td>
<td>++</td>
</tr>
<tr>
<td>Ccr6</td>
<td>Th17</td>
<td>-</td>
</tr>
<tr>
<td>Fasl</td>
<td>Th1</td>
<td>++++</td>
</tr>
</tbody>
</table>
Summary II

• **Deletion of CNS-22 impacts *Ifng* gene transcription**
  - 391 bp deletion in a locus that is approximately 140 kb in length.
  - First element in the *Ifng* locus whose function has been directly examined *in vivo*.

• **Original Hypothesis: CNS-22 plays an essential role in long-range remodeling of the *Ifng* locus**
  - Several differences between the BAC-transgenic and endogenous deletion of CNS-22.
  - So what is the function of CNS-22?
CNS-22 Initiates Local Changes in Remodeling

Relative H4 acetylation

Th1 cells

activated: IFN-γ secreting cell

antigen