Dynamics of Cell-specific Nuclear Receptor Interactions with Regulatory Elements
Three related topics

Dynamics of nuclear receptor interactions with regulatory elements in living cells

Importance of rapid GR dynamics for functional gene regulation in the physiological environment

Global interaction of nuclear receptors with chromatin
Conventional View of Regulatory Site Occupancy by a Transcription Factor

Single cell analysis of binding to individual alleles

Time

Chromatin IP
The “Green Revolution”

Visualize protein localization and movement with GFP tagged nuclear receptors in living cells.
Methodology to Visualize Direct Interaction of Transcription Factors with Regulatory Elements in Real Time
Photobleaching analysis shows rapid exchange of GR with hormone response elements.

10 frames/sec
490 msec intervals
Running at 5x Real Time
Used high-speed UV laser crosslinking to monitor factor/chromatin interactions in defined systems gene targets during chromatin remodeling.

Receptor interaction with the template during chromatin remodeling is transient and periodic.

Receptor is actively displaced during the remodeling reaction.

Conclude: Nucellar receptors are highly mobile on gene targets during chromatin remodeling.

Mechanisms involved in rapid exchange

Molecular Cell 14:163
Science STKE 256:PL13
Annal NY Acad Sci 1024:213
Classic view, nucleosome remodeling
GR, PR, and AR all exchange rapidly with HREs in living cells

Hypothesis:
These dynamics are directly coupled to chromatin remodeling

Dynamic Exchange and Promoter Progression

Receptors exist in many different complexes that interact randomly and transiently with a promoter. During the continuous presence of ligand, multiple processes can modulate promoter activity:

1) Progressive modulation of promoter architecture
   histone modifications
   nucleosome movement
   higher order interactions

2) Modification of the receptor or its co-regulators

These complexes interact randomly and transiently with a promoter.
One example:

Importance of rapid GR dynamics for functional gene regulation in the physiological environment
Cortisol (in humans) and corticosterone (in rats) are secreted in a highly pulsatile manner.


Circadian cycle for glucocorticoid secretion in mammals

What are the mechanistic implications for this pulsatile variation (ultradian rhythm) in circulating cortisol levels?
We have simulated the in vivo ultradian rhythm in cultured cells

Hormone Treatment and Withdrawal Schedules

Observation Schedule

Pulse 1 Wash out 1 P1 15’ 60’
Wash out 2 P2 60’ 75’
Pulse 3 Wash out 3 W2 90’ 120’
Pulse 4 Wash out 4 P3 135’ 150’
Pulse 5 Wash out 5 W3 180’ 195’
P5 210’ 240’
P4 255’ 270’

Consequences:
GRE occupancy in living cells by Imaging

Transcriptional output at induced & repressed genes
Gene Pulsing

GR/Template Dynamics - Corticosterone

Nature Cell Biol. (in review)
Gene Pulsing

GR/Template Dynamics – Dexamethasone

Nature Cell Biol. (in review)
Response to Ultradian Hormone Treatment
GR - Induced Genes
(nascent transcript analysis)

Nascent transcripts are repeatedly released in response to each hormone pulse
Global interaction of nuclear receptors with chromatin

- Are receptor binding events always associated with local remodeling?
- Cell specific binding events?
- Are some receptor sites masked by chromatin organization?
- What remodeling complexes are associated with receptor based transitions?
- Can receptors bind to unremodeled chromatin?
- How are response elements organized throughout the genome?
- Unique composition, modification, of chromatin at binding sites?
- Are receptor binding events always associated with local remodeling?
- Can receptors bind to unremodeled chromatin?
- Long range Interactions with regulatory elements?
- Are some receptor sites masked by chromatin organization?
- What remodeling complexes are associated with receptor based transitions?
Methodology

Transcription Factor Localization

ChIP - Seq     Solexa massively parallel sequencing

Chromatin Transitions

Breaks in the chromatin fiber serve as a straightforward and unbiased method for identification of regulatory elements
Methodology
Chromatin Transitions

A) Scale = 100 kb

B) Scale = genome wide

Nuclease digested chromatin

Purify small fragments released from DHS sites
Massive parallel sequencing

Site-specific qPCR amplification, using tiled primers, normalized to genomic DNA
Cell Specific Chromatin Structures - Per 1
Active in both cell types

3134 Mammary Cell Line
- DHS, +Dex
- Per1 expressed
- DHS, -Dex

AtT-20 Pituitary Cell Line
- DHS, +Dex
- Per1 expressed
- DHS, -Dex

3134 Mammary Cell Line
- GR ChIP
- -Dex
- GR ChIP
- +Dex

AtT-20 Pituitary Cell Line
- GR ChIP
- -Dex
- GR ChIP
- +Dex

chr11:68,907,439-68,940,459
Hager - Figure 2
## Cell Specific Chromatin Structures

**chr2:32,195,785-32,226,814**

Hager - Figure 3

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Treatment</th>
<th>Lcn2 Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>3134 Mammary Cell Line</td>
<td>DHS, +Dex</td>
<td>Lcn2 expressed</td>
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<tr>
<td></td>
<td>DHS, -Dex</td>
<td>Lcn2 silent</td>
</tr>
<tr>
<td>AtT-20 Pituitary Cell Line</td>
<td>DHS, +Dex</td>
<td>Lcn2 silent</td>
</tr>
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<td></td>
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<td>GR ChIP, -Dex</td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>
Cell Specific Chromatin Structures - Mdm1
Active only in pituitary cell

3134 Mammary Cell Line

DHS, +Dex
Mdm1 silent
DHS, -Dex

AtT-20 Pituitary Cell Line

DHS, +Dex
Mdm1 expressed
DHS, -Dex

3134 Mammary Cell Line

GR ChIP, -Dex
GR ChIP, +Dex

AtT-20 Pituitary Cell Line

GR ChIP, -Dex
GR ChIP, +Dex
Global organization of GR binding elements in the murine mammary cell genome

8,373 GR ChIP peaks for de novo sites

For the entire murine genome, ~3% of total DNA is found in DHS sites

97,463 DHS - Dex

107,908 DHS + Dex

Pre-existing DNaseI Hypersensitive Sites

GR binding to pre-existing sites

GR binding to de novo sites
Limited overlap in DHS or GR ChIP profiles between 3134 and AtT20 cell lines

- 30,715 DHS shared between 3134 (Mammary) and AtT20 (Pituitary) (~30%) --- promoter regions
- 363 GR binding sites shared between 3134 (Mammary) and AtT20 (Pituitary) (~5-10%) --- in promoter regions
Each class of GR related chromatin transition can be open, or closed, in specific cell types.

Constitutive

Cell selective
GR action at specific genes

Absence/presence of specific remodeling systems
Absence/presence of factors that recruit the remodeling systems
Epigenetic chromatin modifications