Genome-Wide Mapping of Estrogen Receptor Binding Sites and Activity

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Estrogen receptor and breast cancer

Sorlie, PNAS, 2003

Estrogen-ER action in breast cancer cells
In vivo mapping of binding events:
Chromatin Immunoprecipitation (ChIP)

- Deprive of hormones
- MCF-7 cells
- Imunoprecipitate ER
- Sonicate chromatin
- Elute pure DNA
- PCR suspected target

Chromatin Immunoprecipitation (ChIP)
on pS2/TFF1 gene promoter

Estrogen Time (min)
0 15 30 45 60 75 90 105 120 135 150 165
ChIP
ER
AIB1
p300
CBP
p/CAF
AcH
PolII
Input

Shang, Cell, 2000

Limitations with this approach
- Assumes ER binds to promoter regions
- Ignores the rest of the genome
- pS2/TFF1 is a marker gene

Can ER bind to non-promoter regions?
- Globin gene uses distant enhancers
- ER does not bind to promoters of other estrogen regulated
genes such as c-Myc or IGF-I

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Model of ChIP-chip assay

MCF-7 cells
Dephrase of hormones
Immunoprecipitate ER
Sonicate chromatin
Amplify and label DNA

Gene expression
Tiling microarrays

Characteristics of Affymetrix chromosome 21 and 22 arrays
- 25 bp probes every 35 bp
- Tiled probes along all of the non-repetitive sequence (approx. 50%) of chromosomes 21 and 22
- 1 million probes covering 35 million bp
**Processing of ER ChIP-chip data**

- Perfect Match minus Mismatch probe
- Non-parametric analysis
- Weighted value based on neighboring probes
- Eliminate peaks that do not reproduce

**Summary of data from Chromosome 21 and 22**

- 57 ER binding sites
- Almost all are distant from promoter regions
- Majority on chromosome 21

**ER binding sites on chromosome 21**

Carroll et al., Cell, 2005
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ER binding sites on chromosome 22

Directed ChIP against novel ER binding sites

Chromosome conformation capture (with ChIP)
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TFF-1 and NRIP-1 enhancers interact with promoters

TFF-1- promoter and enhancer (10.5kb)  NRIP-1- promoter and enhancer 3 (144kb)

E2: + + + + + + + + +
Ligase: - - - - - - - - -
Undigested  AflII digested

Conservation of binding sites in T-47D cells

Fold enrichment versus vehicle

Human-mouse conservation of ER binding sites

Average peak size
What defines a genuine ER binding site?

- ~5% of predicted EREs function as binding sites

<table>
<thead>
<tr>
<th>Predicted binding site</th>
<th>Real binding sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERE</td>
<td>Forkhead</td>
</tr>
<tr>
<td>p=1.33×10^-15</td>
<td>p=1.23×10^-8</td>
</tr>
</tbody>
</table>

Carroll et al., Cell, 2005

Recruitment of FoxA1 to ER binding sites

What FoxA1 does?

- HNF-3α/FoxA1 can bind pS2/TFF-1 promoter
- FoxA1 important for GR activity
- FoxA1 correlates with ER in breast tumors
- FoxA1 functions as a pioneer factor

FoxA1 ChIP

- Vehicle
- Estrogen

FoxA1 ChIP on 57 ER binding sites

- Vehicle
- Estrogen

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Targeted knockdown of FoxA1 inhibits ER binding

siFoxA1 inhibits estrogen-mediated transcription

Model of ER action
Genome-wide mapping of ER and RNA PolII binding

**ChiP-on-chip**
- Affymetrix 7 array set
- Covers 1.5 billion bp of non-repetitive DNA at 35 bp resolution
- Data analyzed using MAT (Johnson et al., PNAS, 2006)
- ER and RNA PolII ChiP-chip performed at 45 minutes estrogen treatment
- All ChiP-on-chip experiments performed in triplicate

**Gene expression (estrogen treatment)**
- 0hr Control
- 3hr Direct transcriptional targets
- 6hr potential direct targets
- 12 hr Indirect transcriptional targets

**ER and PolII binding relative to transcription start sites**

**ER and RNA PolII binding conservation**

Carroll et al., Nature Genetics, 2006
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ER and RNA PolII on specific target genes

Validation of ER binding to regions defined by ChIP-on-chip

Enrichment of motifs at ER binding sites
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Validation of co-operating factor binding

Distribution of motifs in ER binding sites

Pairwise analysis of motif clustering
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Estrogen regulated gene expression changes

![Pie charts showing gene expression changes at 3, 6, and 12 hours.]

- 3hr genes: Total 294
- 6hr genes: Total 625
- 12hr genes: Total 968

ER and RNA PolII binding relative to transcription start sites of differentially regulated genes

- Late down genes have ER binding sites with a bias of AP-1 motifs (p < 0.01)
- Late up genes have ER binding sites with a bias of EREs

What defines the direct ER binding near late down-regulated genes?

- These ER binding sites have an enrichment of AP-1 motifs
- Likely to involve an early (3hr) estrogen induced gene
- Can bind to ER as a repressor via AP-1 motifs

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**Does siRNA to NRIP-1 influence down regulation?**

NRIP-1 Western blot

NRIP-1 mRNA levels

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**siRNA to NRIP-1 influences late down-regulated genes**

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**NRIP-1 mRNA NRIP-1 protein**

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Conclusions

- ER rarely binds to promoter regions
- ER requires FoxA1 to get to the chromatin
- Every gene has a unique ER regulatory pattern
- Other transcription factors are involved
- Only a subset of ER sites are active at any one time

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Carroll JB et al., Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1, Cell, 2005, 122(1):33-43