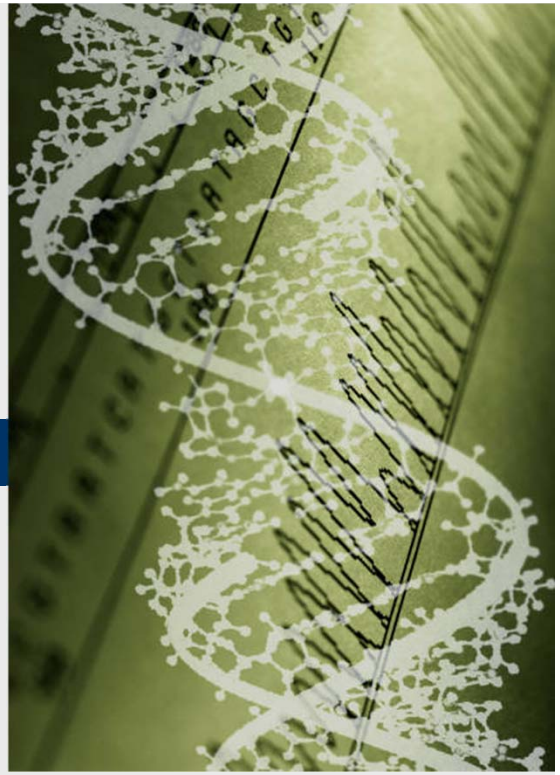
Center of Excellence in Chromosome Biology

Center for Cancer Research



Gordon L. Hager
Lab of Receptor Biology & Gene Expression

Dynamics of Cell-specific Nuclear Receptor Interactions with Regulatory Elements

National Cancer Institute

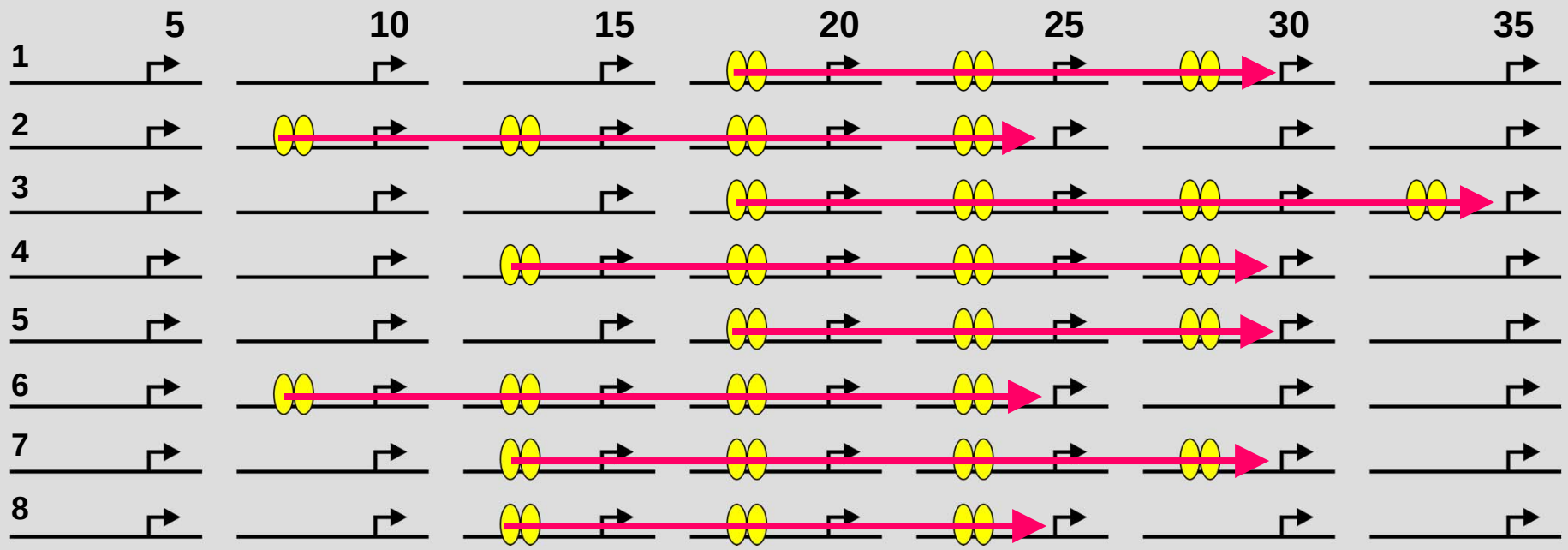
Three related topics

Dynamics of nuclear receptor interactions with regulatory elements in living cells

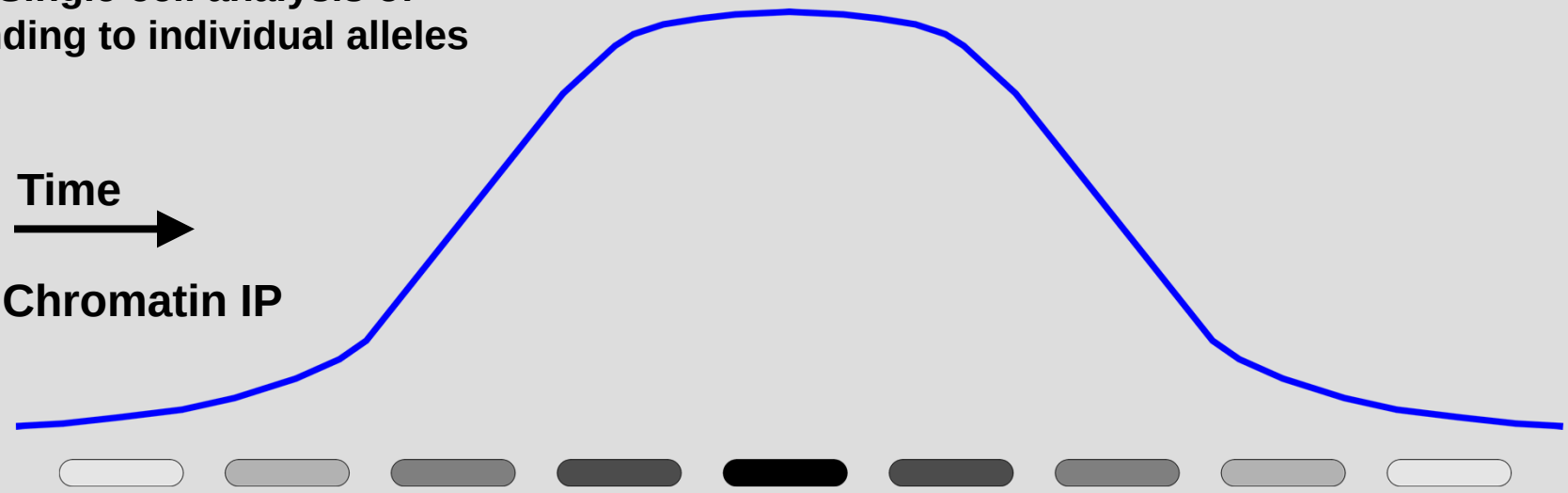
Importance of rapid GR dynamics for functional gene regulation in the physiological environment

Global interaction of nuclear receptors with chromatin

Conventional View of Regulatory Site Occupancy by a Transcription Factor

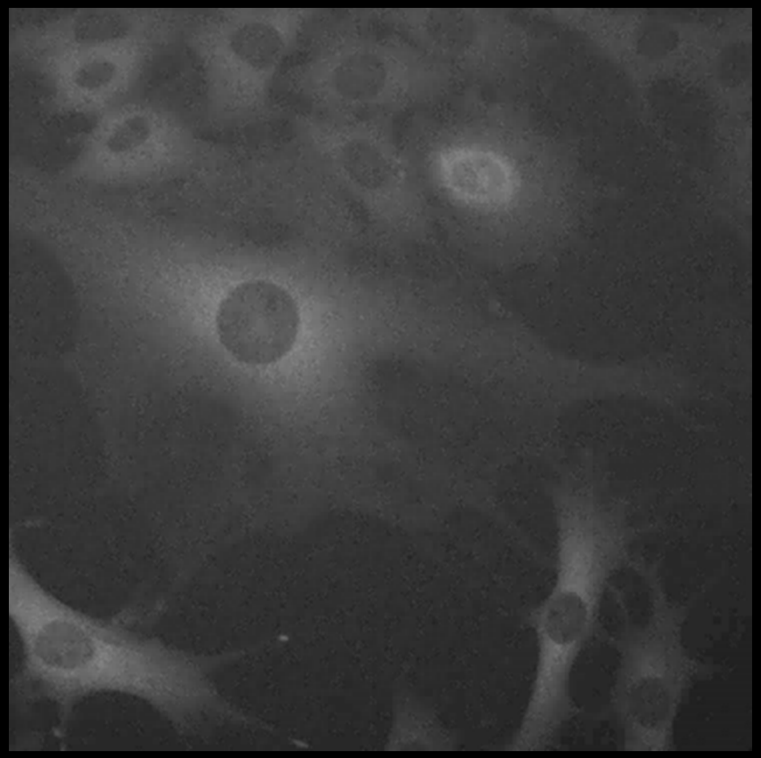


Single cell analysis of binding to individual alleles



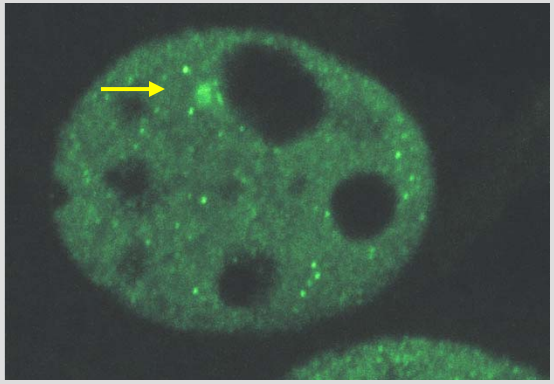
The “Green Revolution”

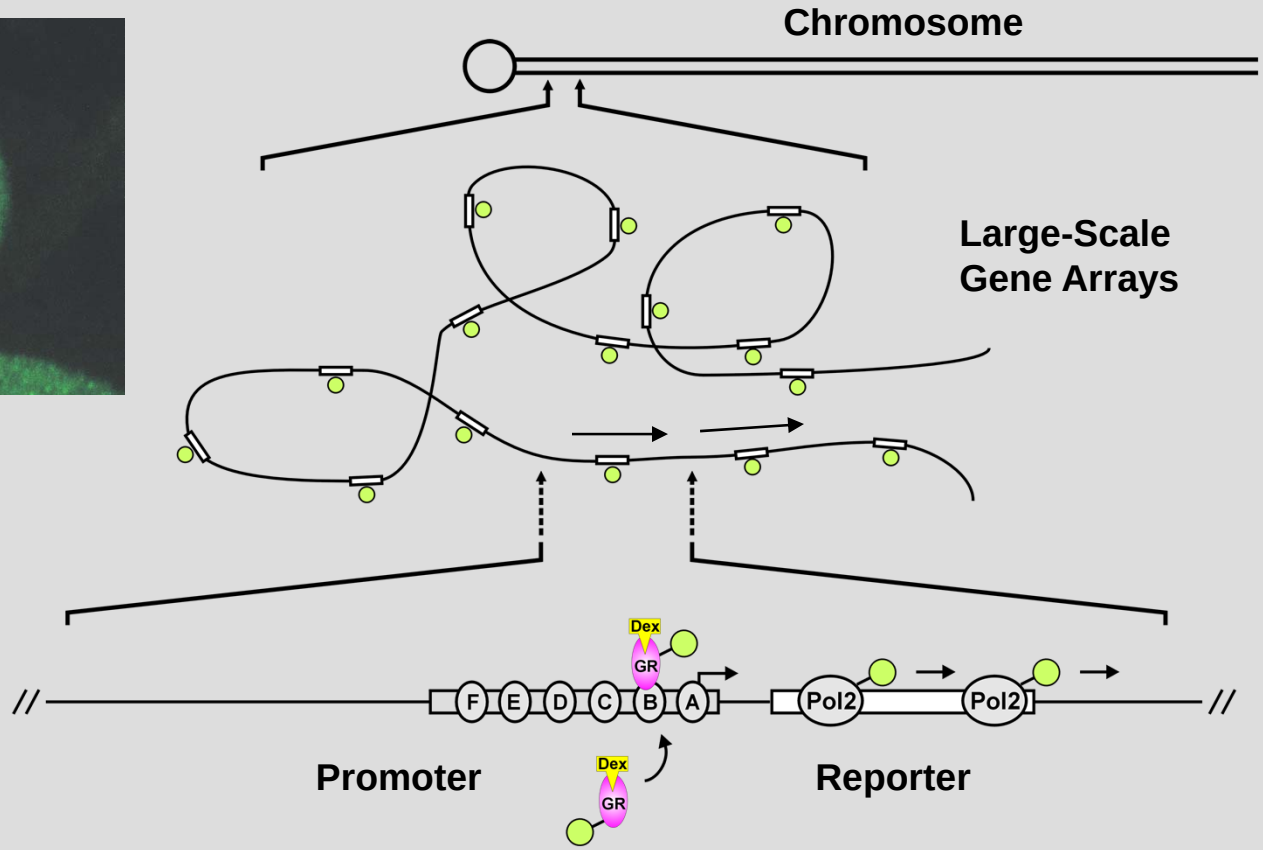
Visualize protein localization and movement with GFP tagged nuclear receptors in living cells



Glucocorticoid Receptor Translocation

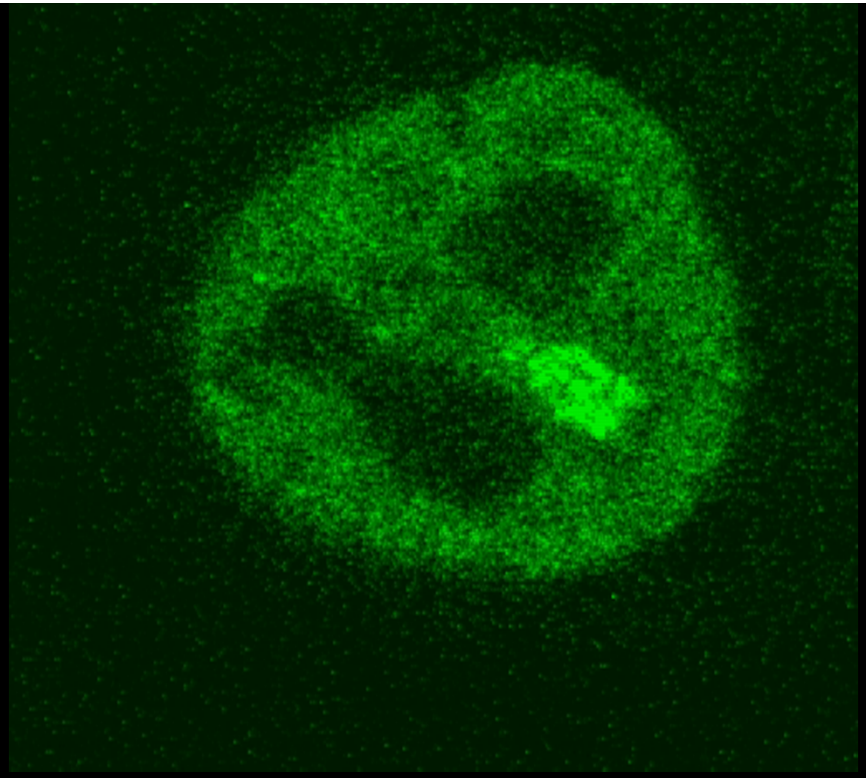
Methodology to Visualize Direct Interaction of Transcription Factors with Regulatory Elements in Real Time

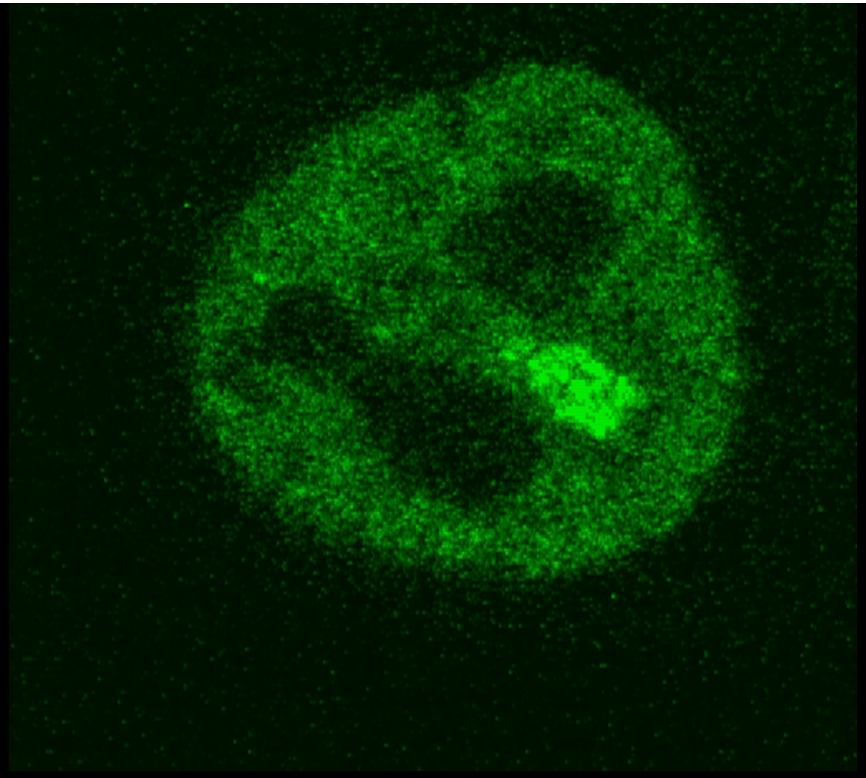




Science 287:1262-1265

J. Cell Biol. 154:33-48

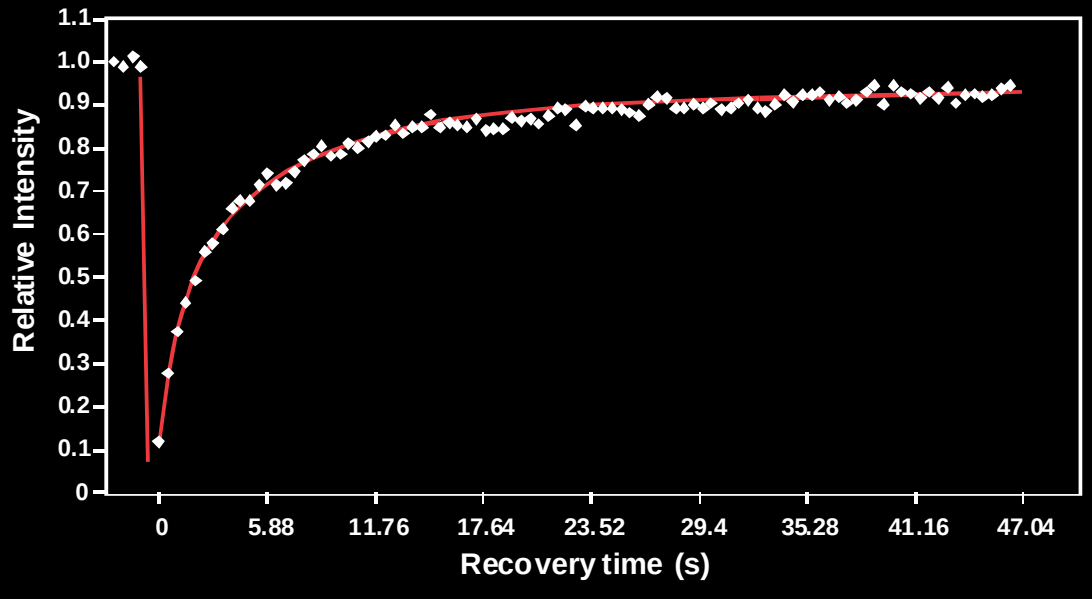


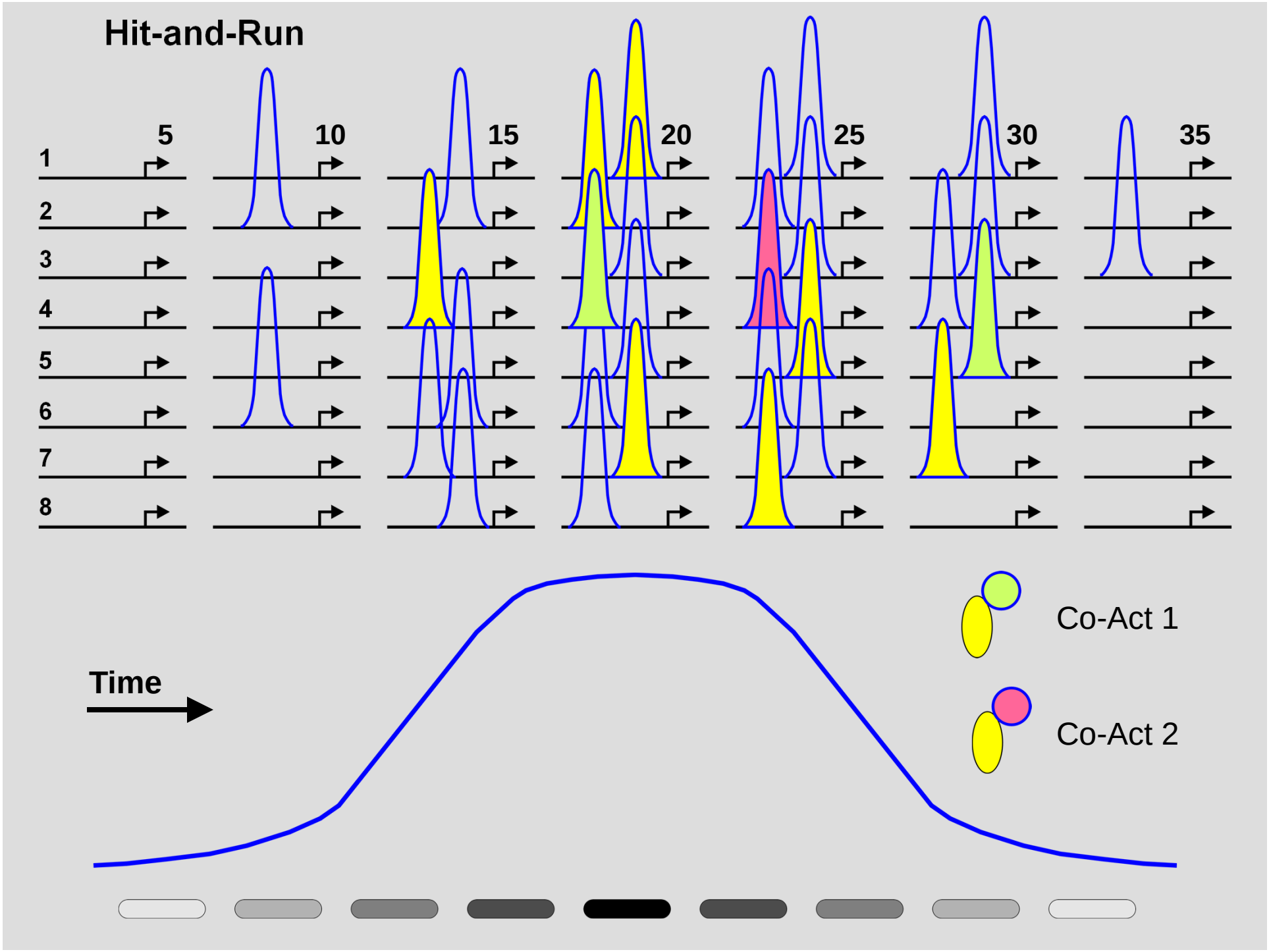


Photobleaching analysis shows rapid exchange of GR with hormone response elements

10 frames/sec
490 msec intervals

Running at 5x Real Time



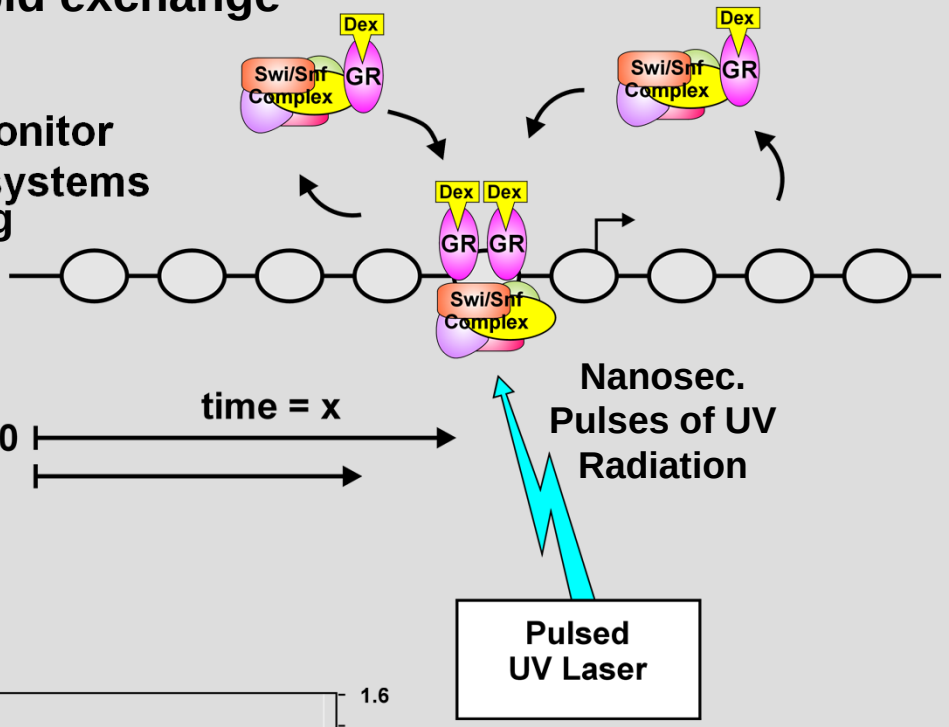

Hit-and-Run
5
10
15
20
25
30
35
1
2
3
4
5
6
7
8
Time
Co-Act 1
Co-Act 2

Mechanisms involved in rapid exchange

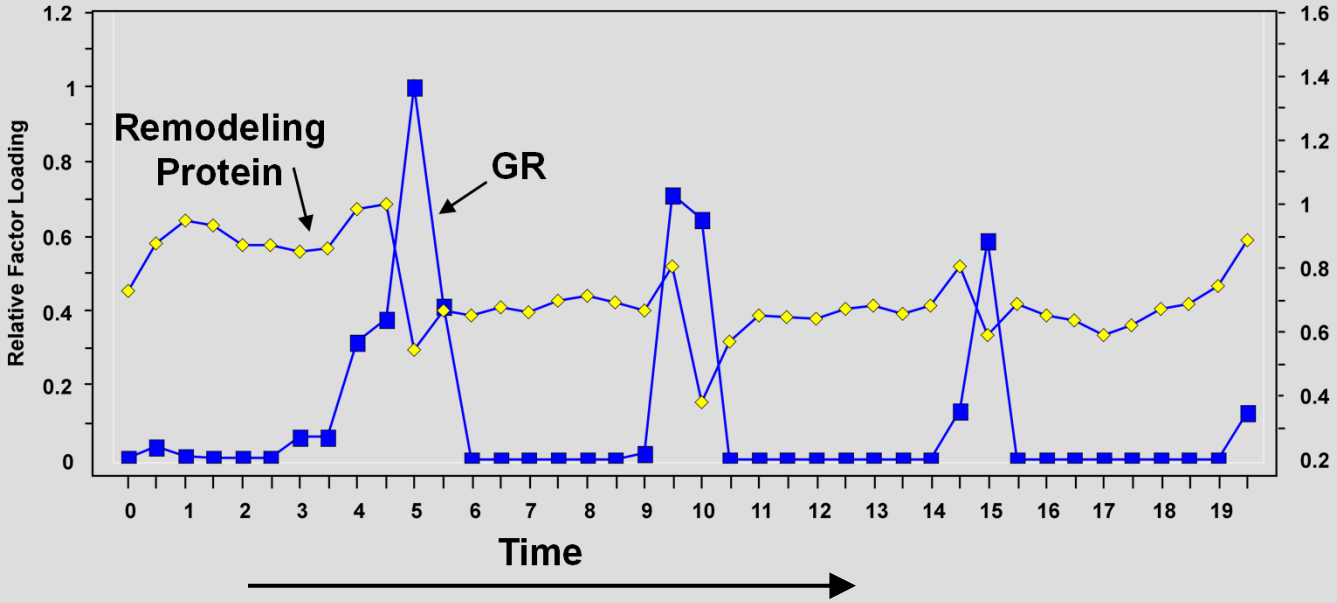
Conclude:

Used high speed UV laser crosslinking to monitor factor/chromatin interactions in defined systems

Nuclear receptors are highly mobile on 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

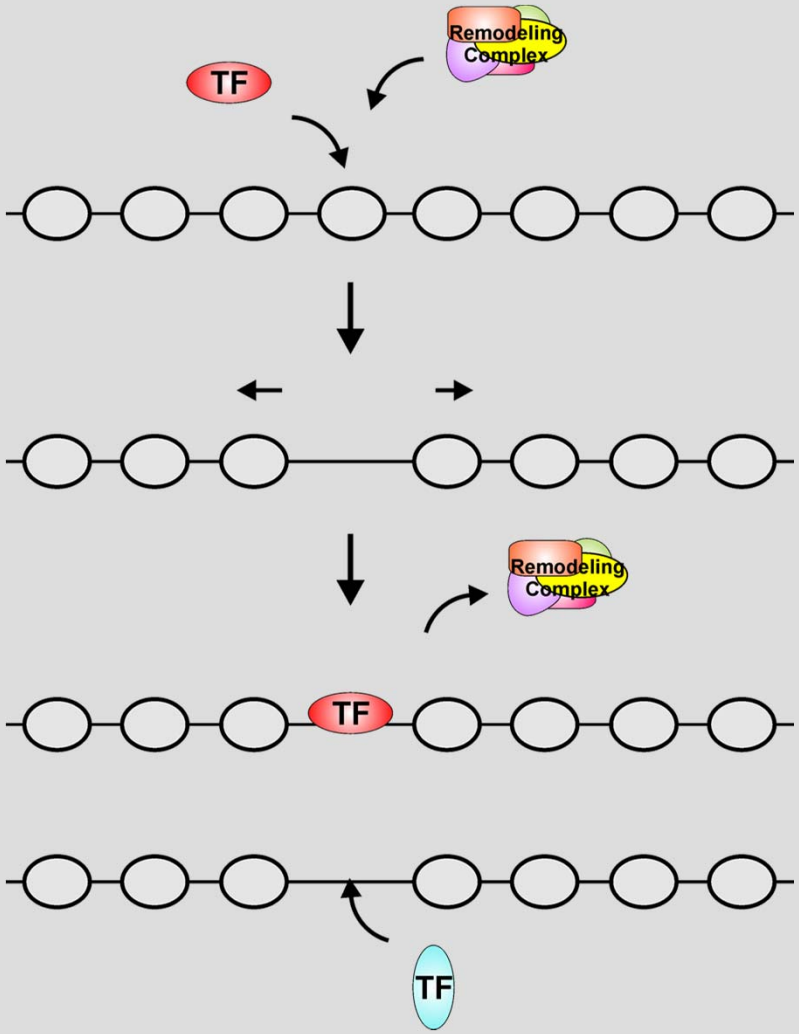


Molecular Cell 14:163

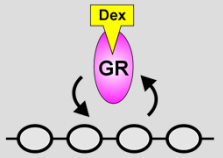
Science STKE 256:PL13

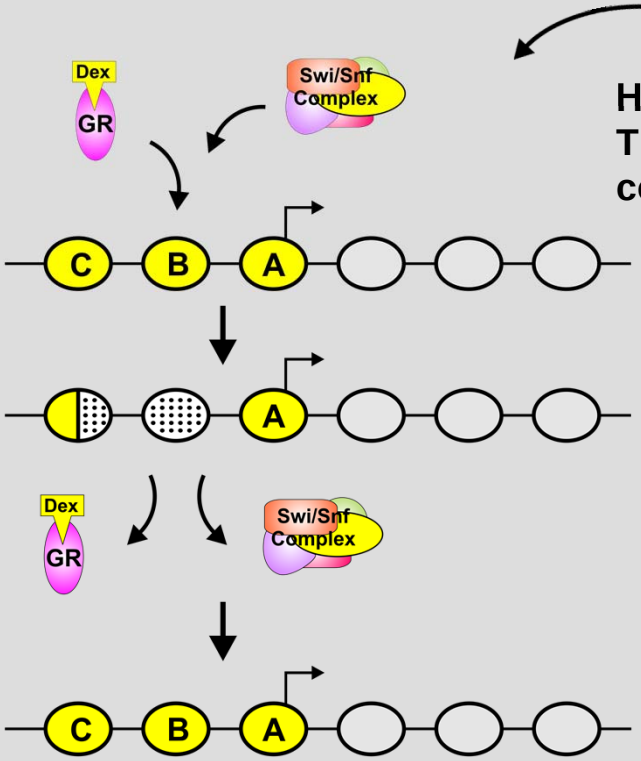
Annal NY Acad Sci 1024:213

Classic view, nucleosome remodeling



GR, PR, and AR all exchange rapidly with HREs in living cells





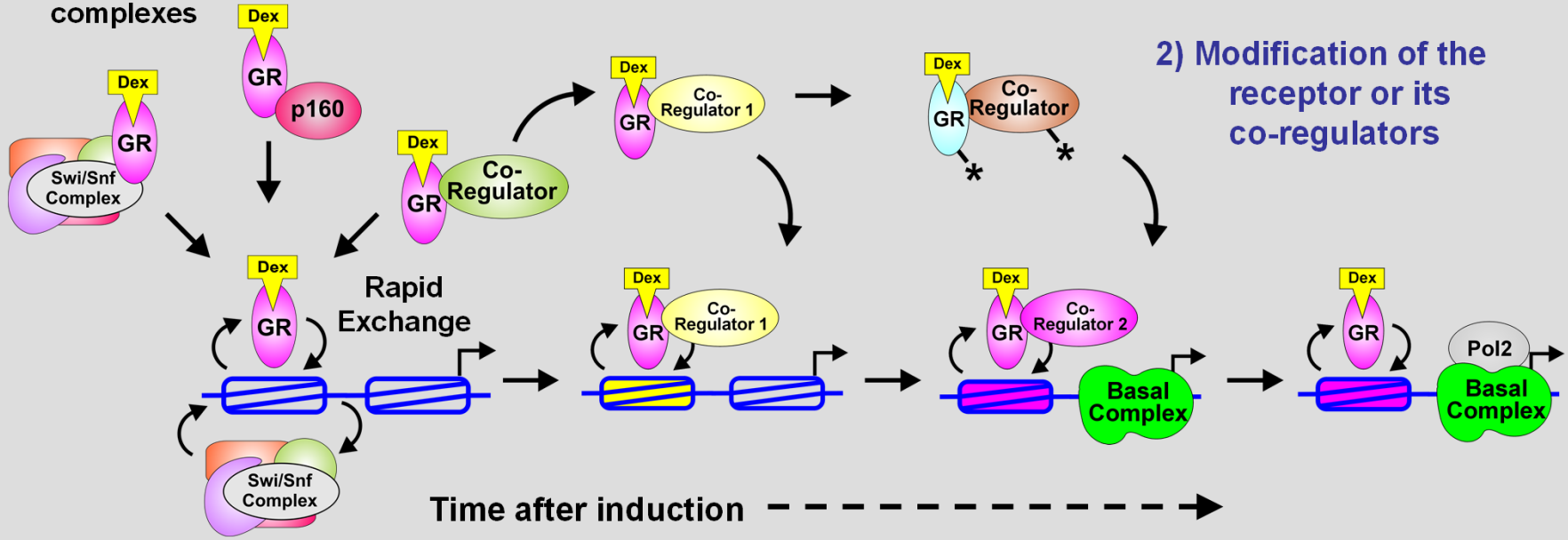
Hypothesis:
These dynamics are directly coupled to chromatin remodeling

Molecular Cell 22:669 (2006)
Science STKE 256:PL13 (2004)
Annal NY Acad Sci 1024:213 (2004)
Biophys J 87:1964 (2004)
Mol Cell Biol 27:1823 (2007)
Mol Cell Biol 27:2442 (2007)
J Cell Physiol 207:628 (2006)
FEBS Lett. 580:4757 (2006)
Mol Cell Biol 25:2406 (2005)

Dynamic Exchange and Promoter Progression

Receptors exist in many different complexes

During the continuous presence of ligand, multiple processes can modulate promoter activity:



2) Modification of the receptor or its co-regulators

These complexes interact randomly and transiently with a promoter

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

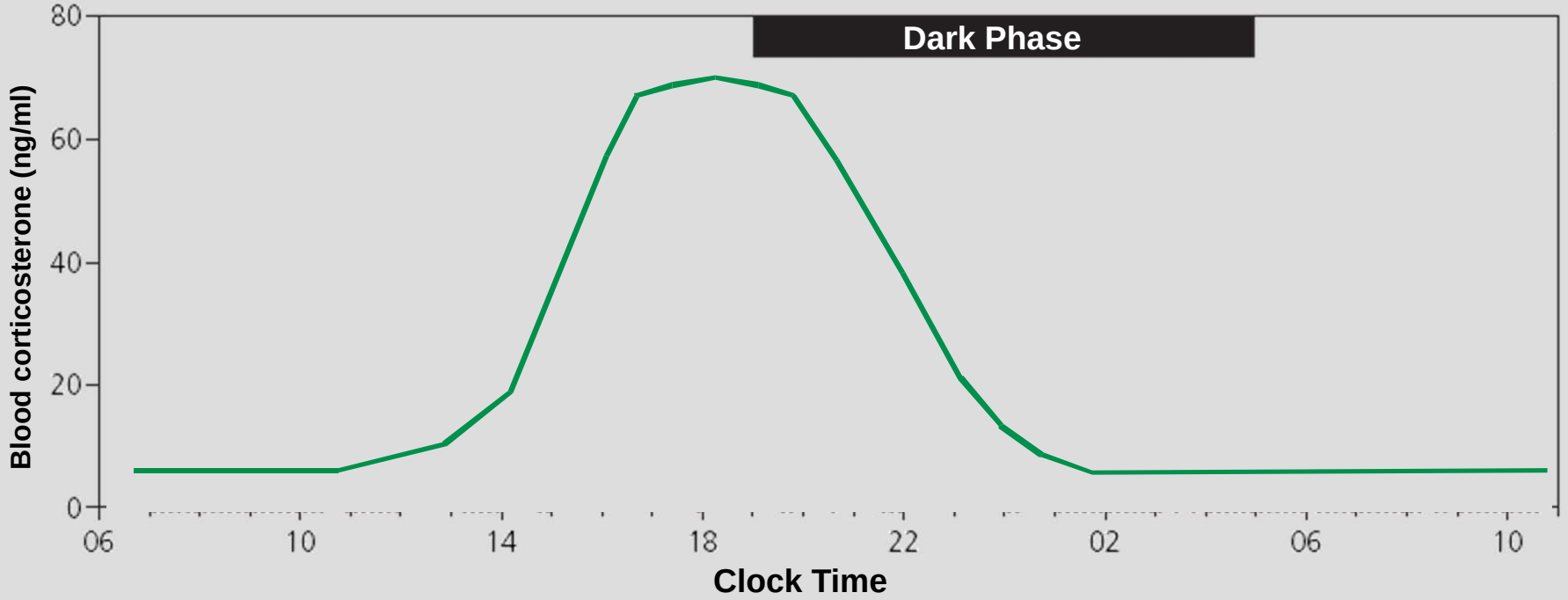
One example:

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

Circadian cycle for glucocorticoid secretion in mammals

Cortisol (in humans) and corticosterone (in rats) are secreted in a highly pulsatile manner

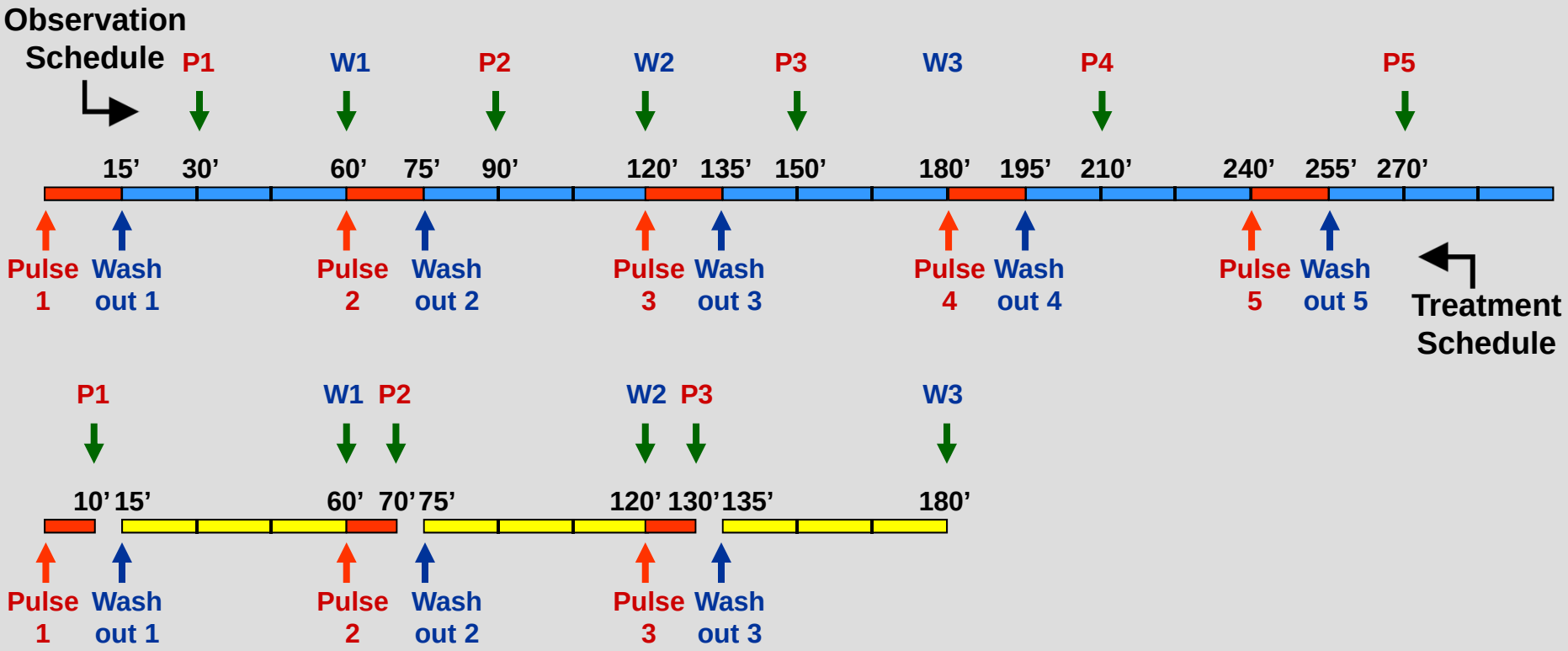
Atkinson et al. J. Neuroendocrinology 18:526 (2006)



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

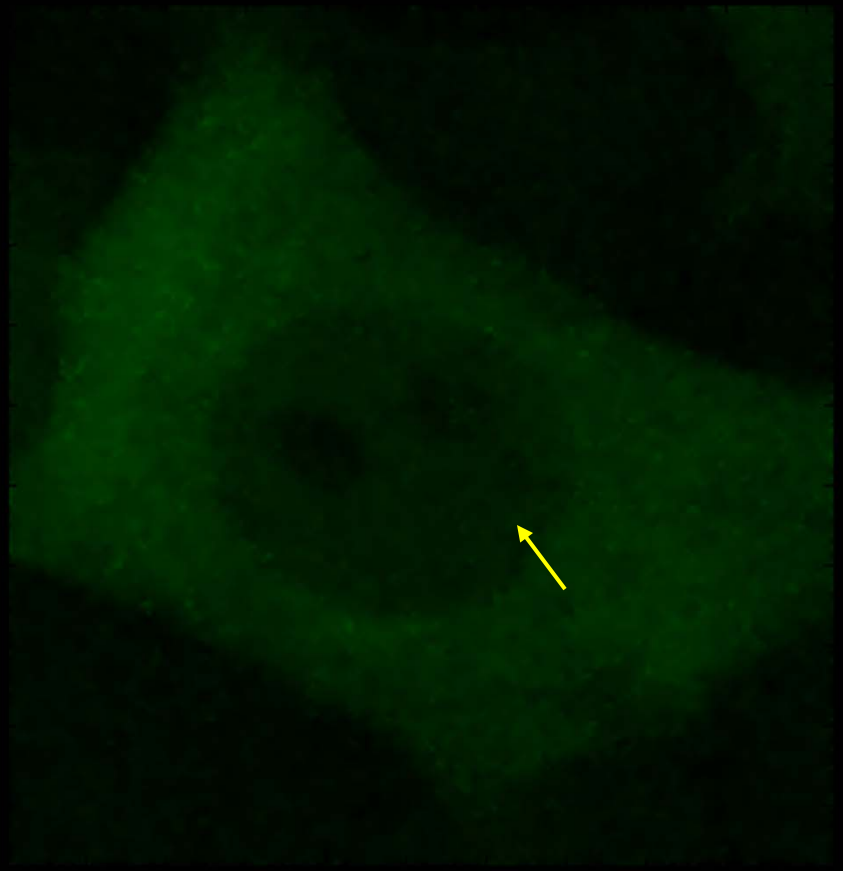


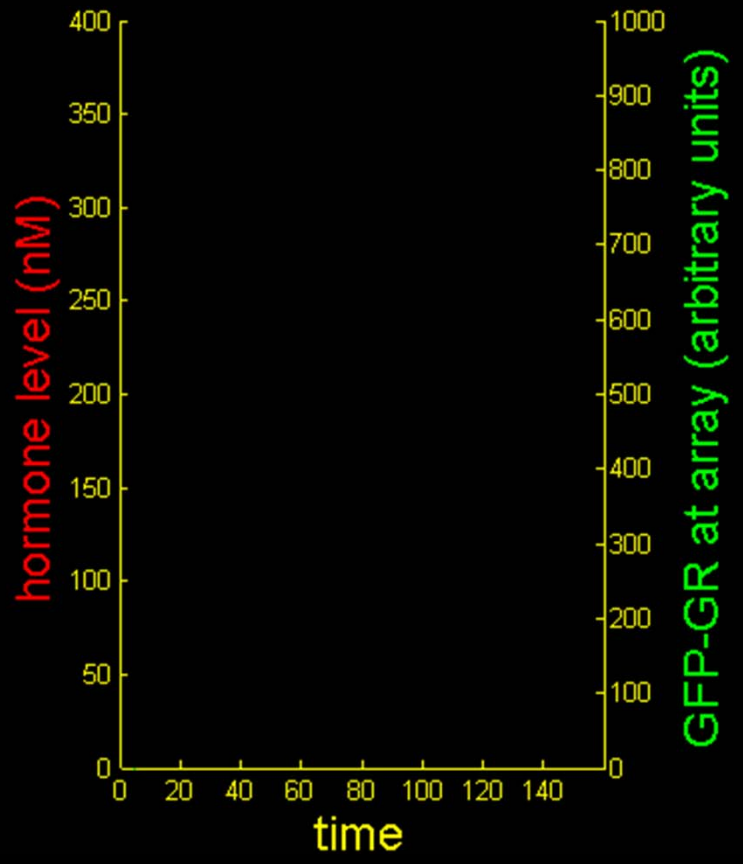
Consequences: GRE occupancy in living cells by Imaging

Transcriptional output at induced & repressed genes

Gene Pulsing

GR/Template Dynamics - Corticosterone

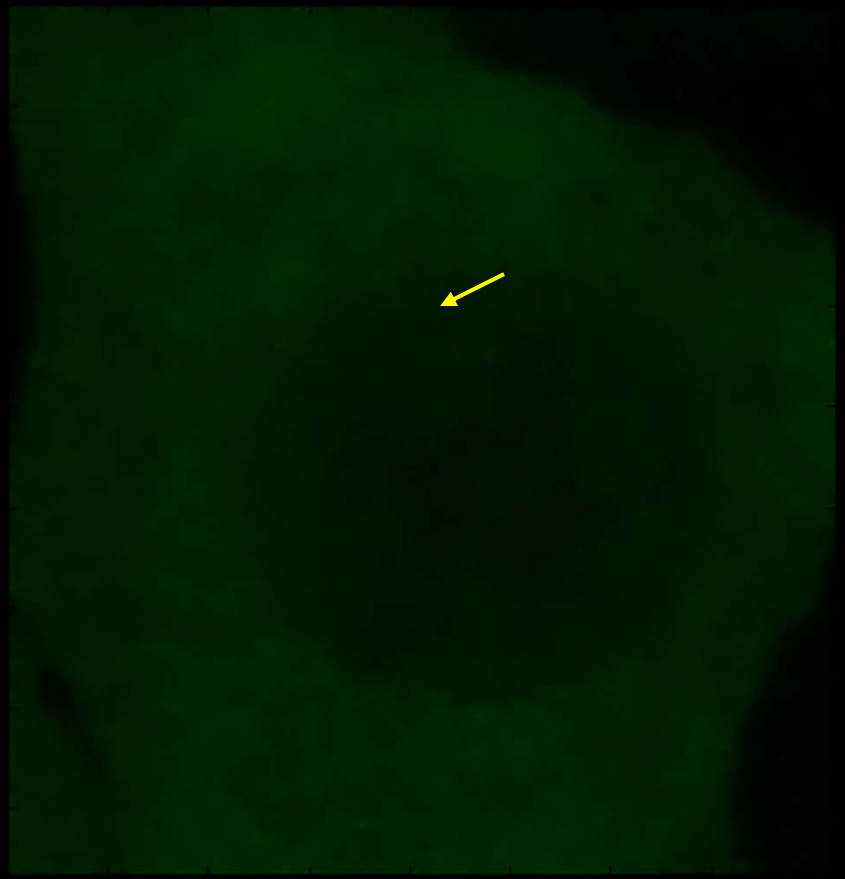


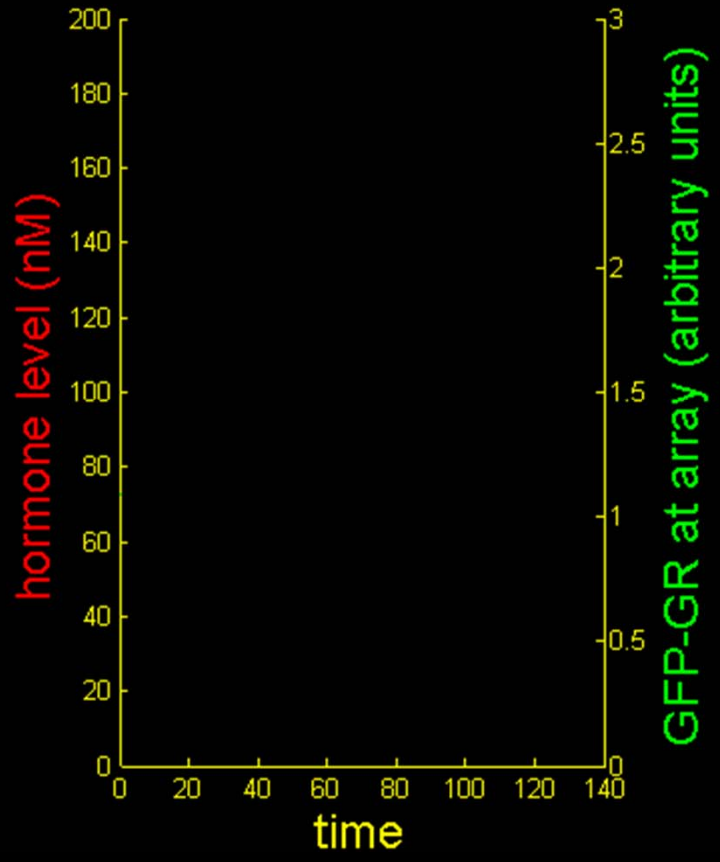


Nature Cell Biol. (*in review*)

Gene Pulsing

GR/Template Dynamics – Dexamethasone

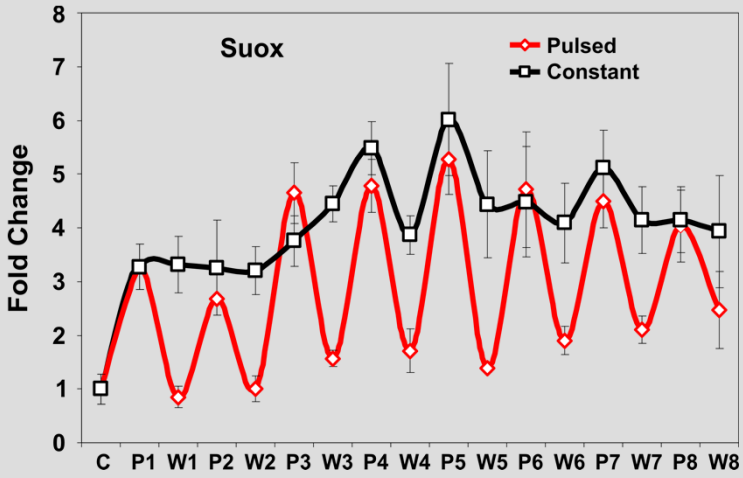


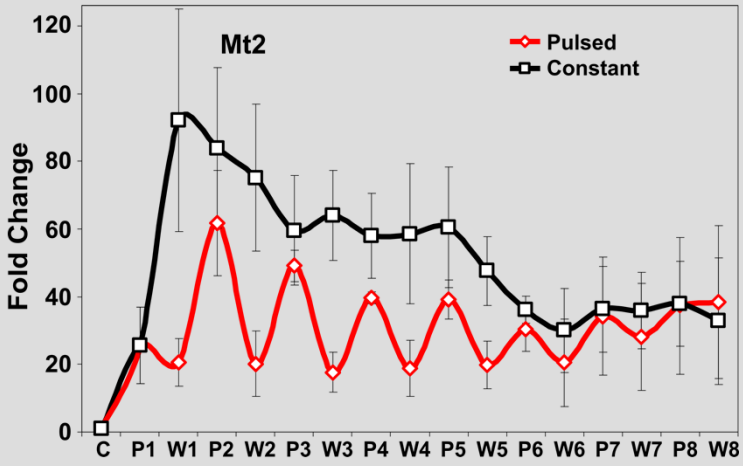


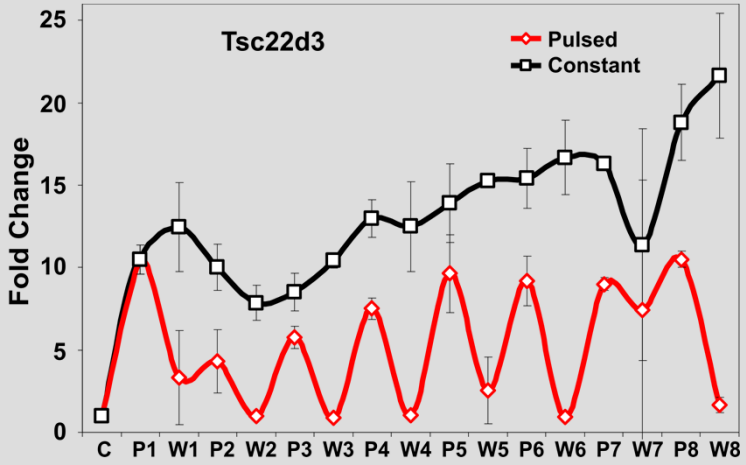
Nature Cell Biol. (*in review*)

Response to Ultradian Hormone Treatment
GR - Induced Genes

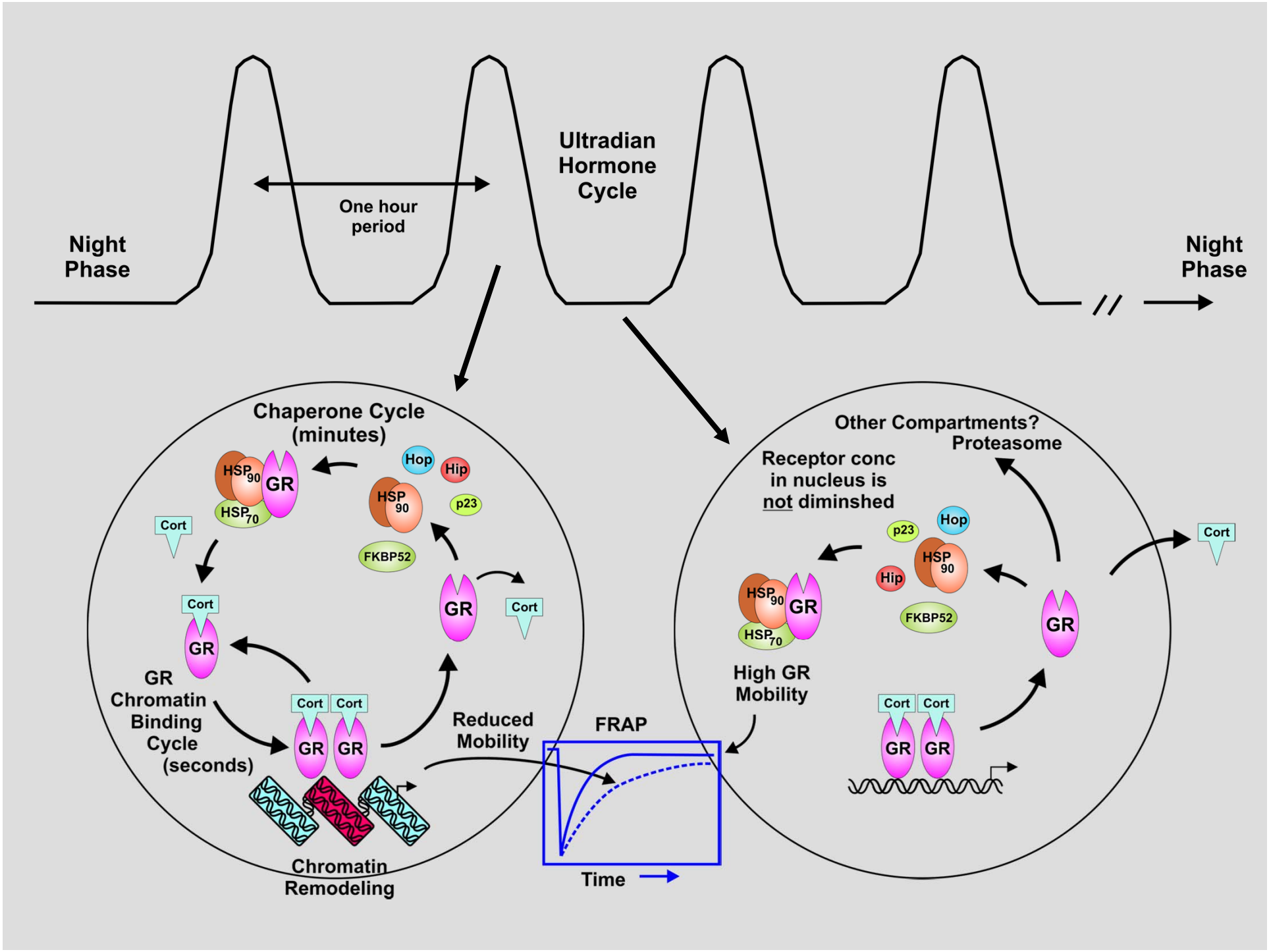
(nascent transcript analysis)







Nascent transcripts are repeatedly released in response to each hormone pulse


Ultradian
Hormone
Cycle
One hour
period
Night
Phase
Night
Phase
Chaperone Cycle
(minutes)
HSP90
GR
HSP70
Hop
Hip
p23
HSP90
FKBP52
Cort
Cort
GR
GR
Cort
GR
Chromatin
Binding
Cycle
(seconds)
Cort
Cort
GR
GR
Reduced
Mobility
Chromatin
Remodeling
FRAP
Time
Other Compartments?
Proteasome
Receptor conc
in nucleus is
not diminshed
HSP90
GR
HSP70
High GR
Mobility
p23
Hop
HSP90
Hip
FKBP52
GR
Cort
Cort
Cort
GR
GR

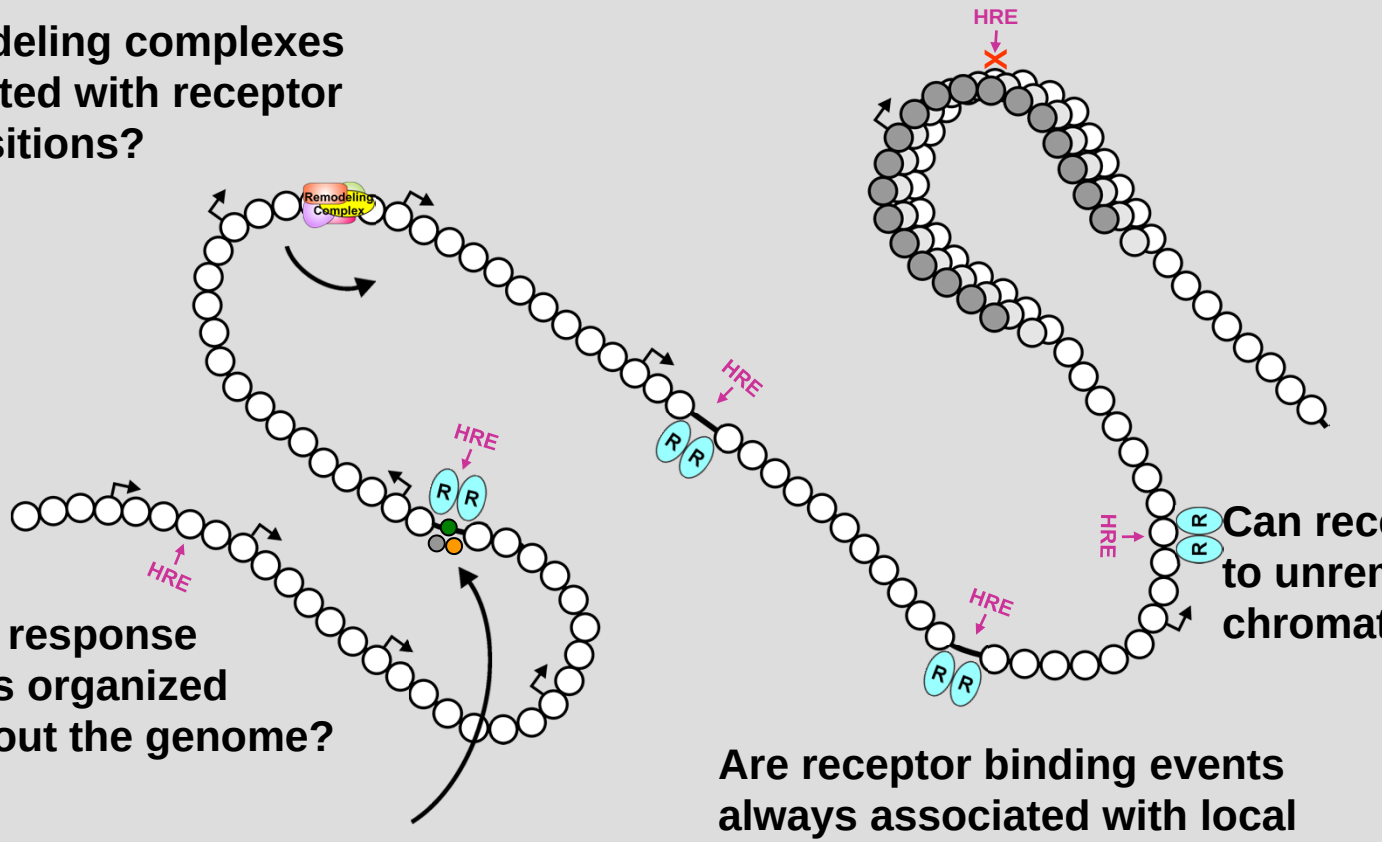
Global interaction of nuclear receptors with chromatin

Cell specific binding events?

Long range Interactions with regulatory elements?

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?

Are receptor binding events always associated with local remodeling?

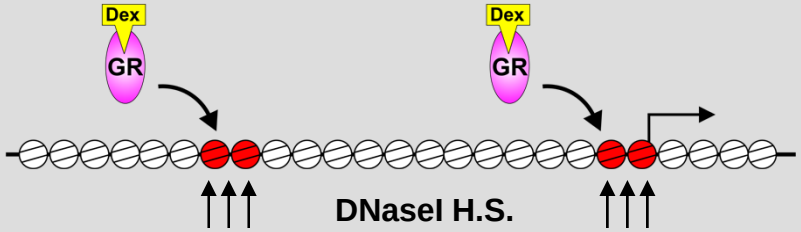
Unique composition, modification, of chromatin at binding sites?

Methodology

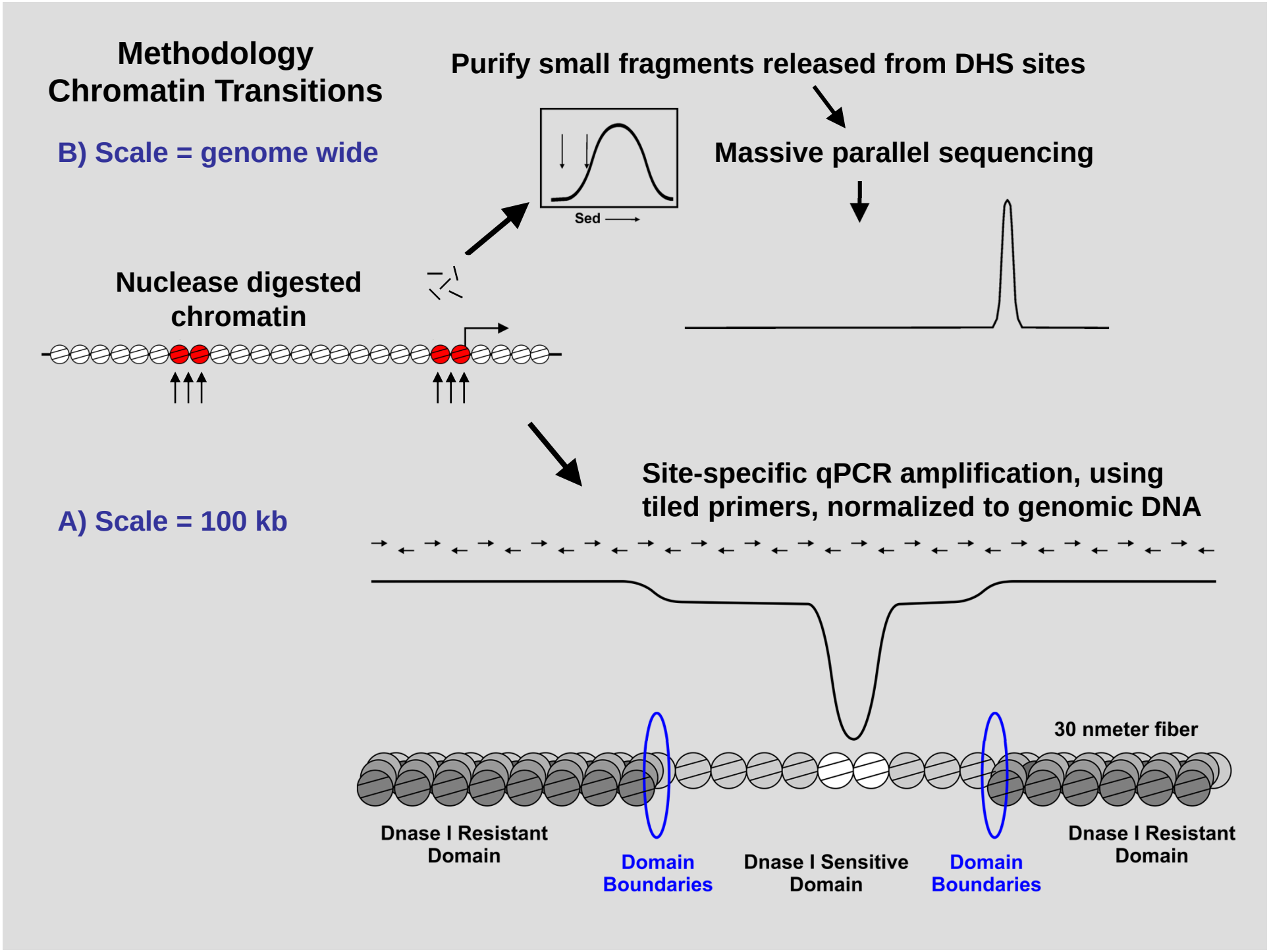
Transcription Factor Localization

ChIP - Seq **Solexa massively parallel sequencing**

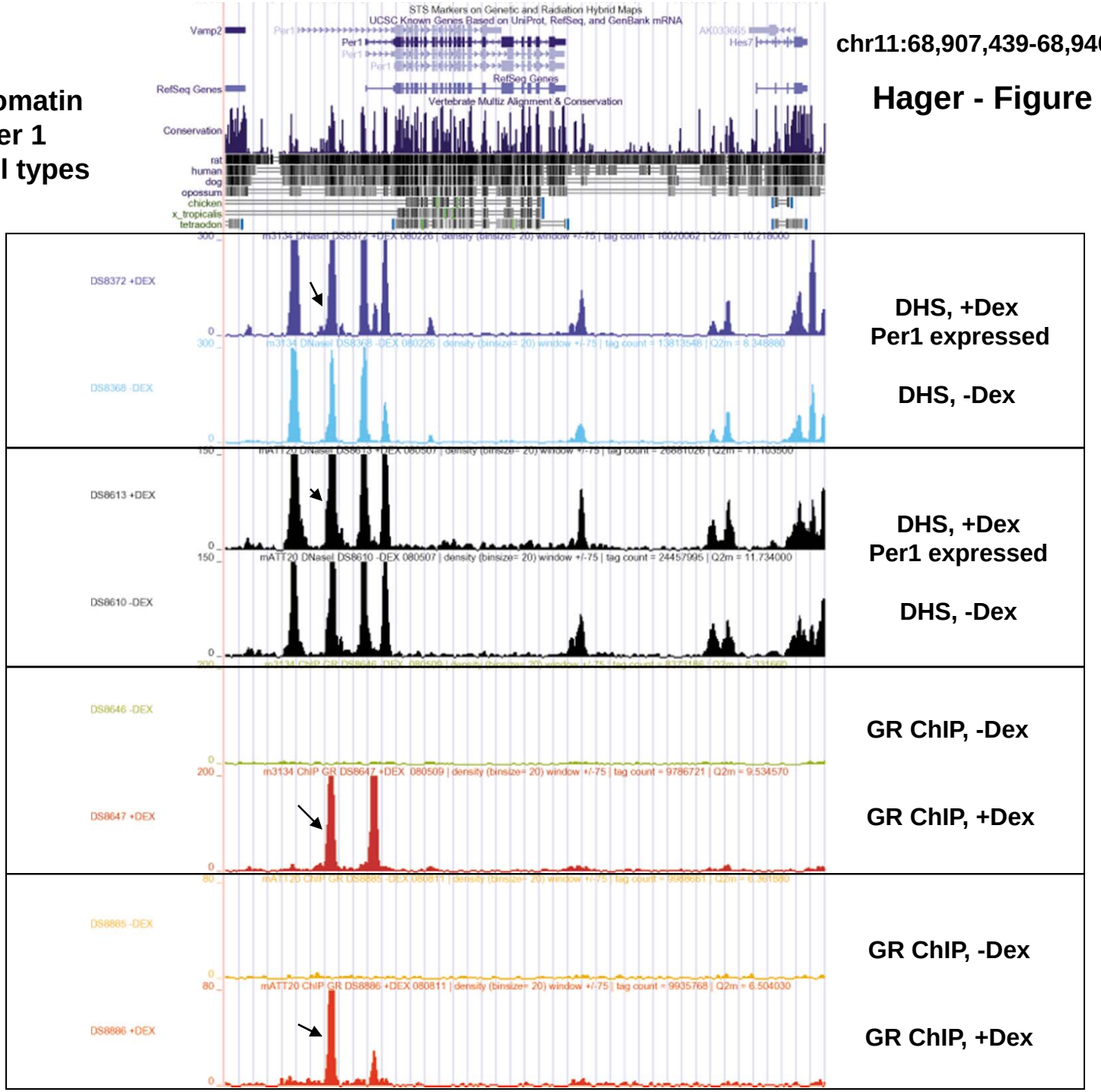
Chromatin Transitions



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


Methodology
Chromatin Transitions
B) Scale = genome wide
Purify small fragments released from DHS sites
Massive parallel sequencing
Sed
Nuclease digested
chromatin
A) Scale = 100 kb
Site-specific qPCR amplification, using
tiled primers, normalized to genomic DNA
30 nmeter fiber
Dnase I Resistant
Domain
Domain
Boundaries
Dnase I Sensitive
Domain
Domain
Boundaries
Dnase I Resistant
Domain

Cell Specific Chromatin Structures - Per 1 Active in both cell types



chr11:68,907,439-68,940,459

Hager - Figure 2

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

Example

Cell Specific Chromatin Structures

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

Hager - Figure 3

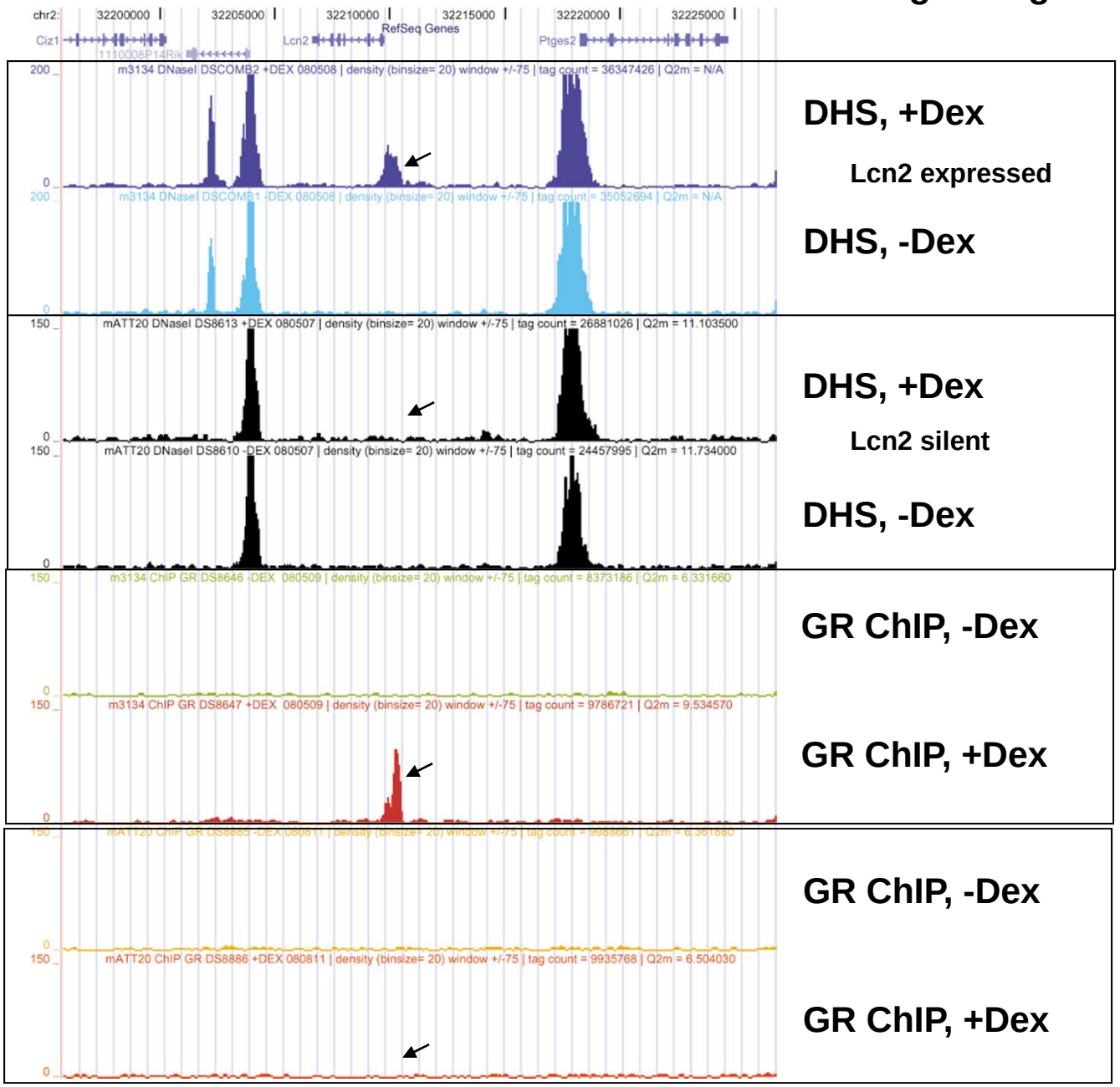
Cell Specific Chromatin Structures – Lcn2 Active only in mammary cell

3134 Mammary Cell Line

AtT-20 Pituitary Cell Line

3134 Mammary Cell Line

AtT-20 Pituitary Cell Line



Example

Cell Specific Chromatin Structures

chr10:117,530,397-117,571,145

Hager - Figure 4

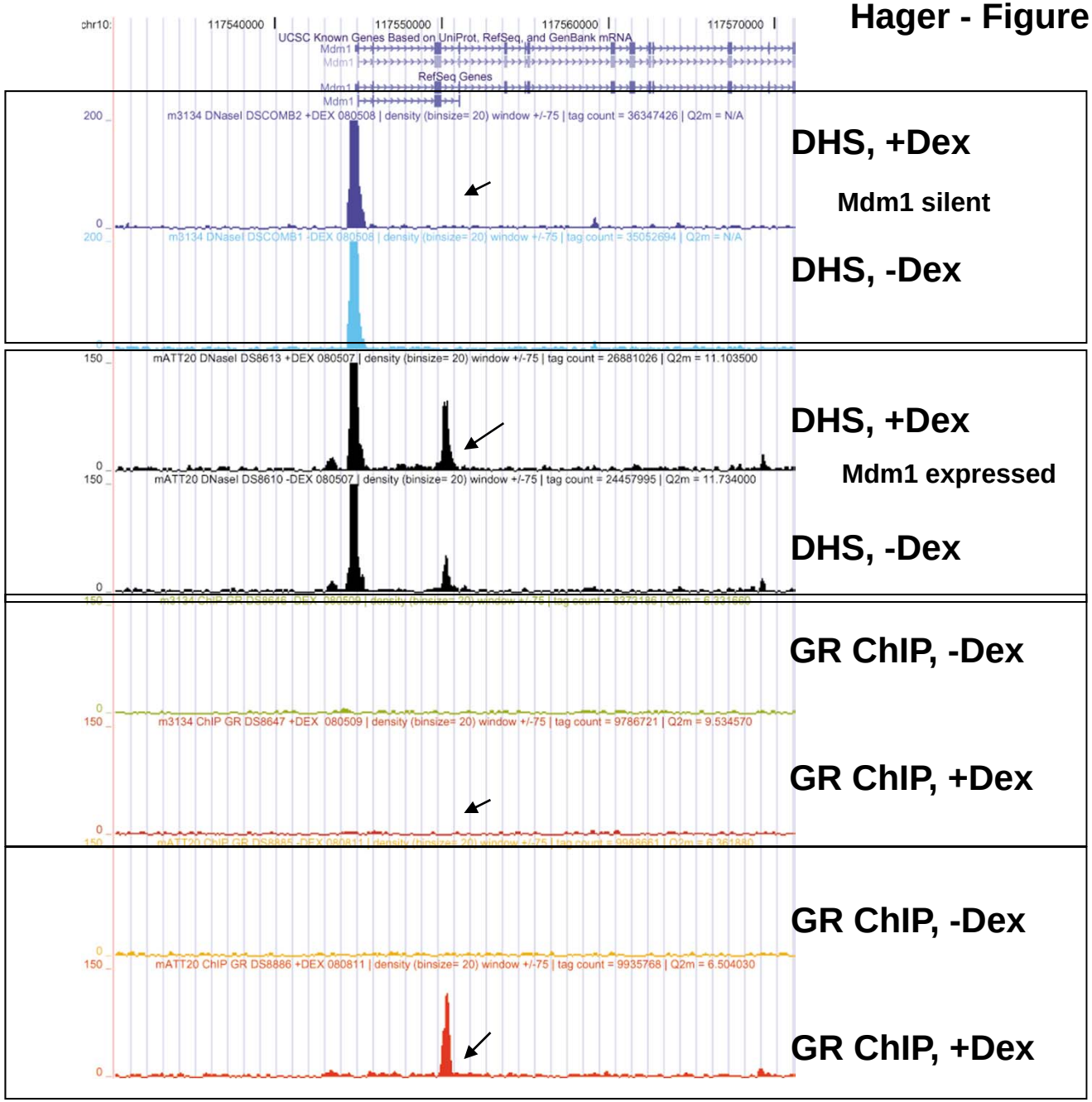
Cell Specific Chromatin Structures - Mdm1 Active only in pituitary cell

3134 Mammary Cell Line

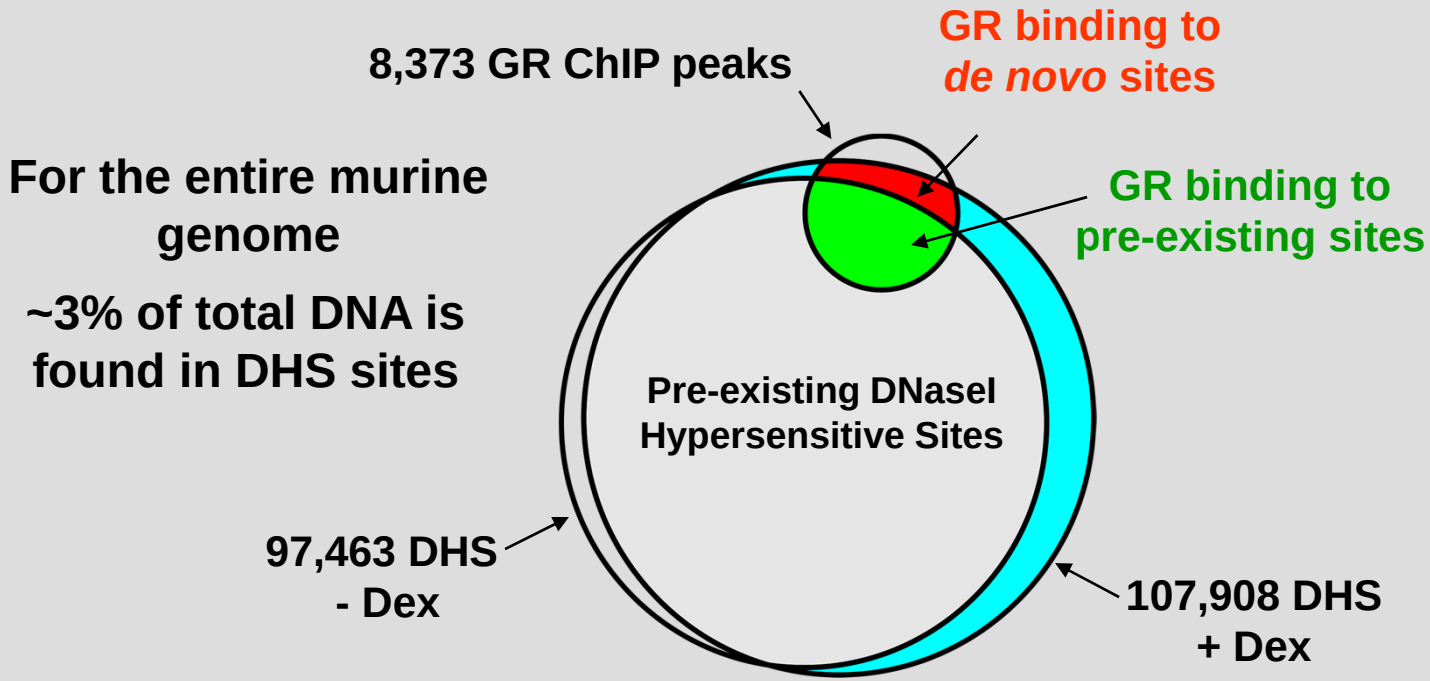
AtT-20 Pituitary Cell Line

3134 Mammary Cell Line

AtT-20 Pituitary Cell Line

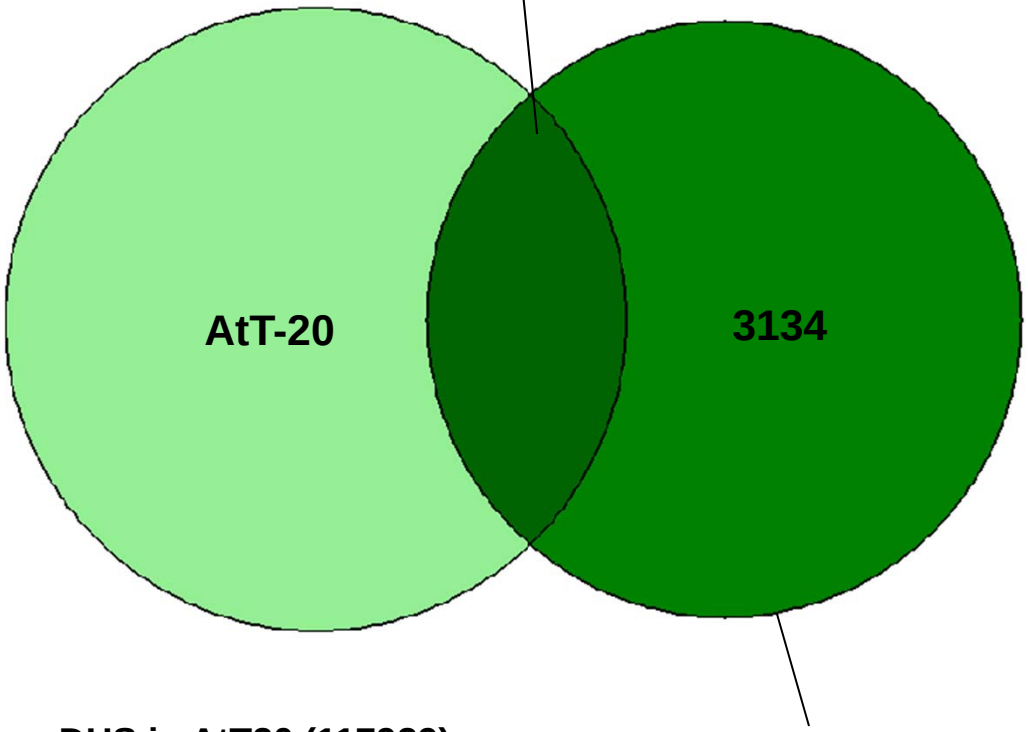


Global organization of GR binding elements in the murine mammary cell genome



Limited overlap in DHS or GR ChIP profiles between 3134 and AtT20 cell lines

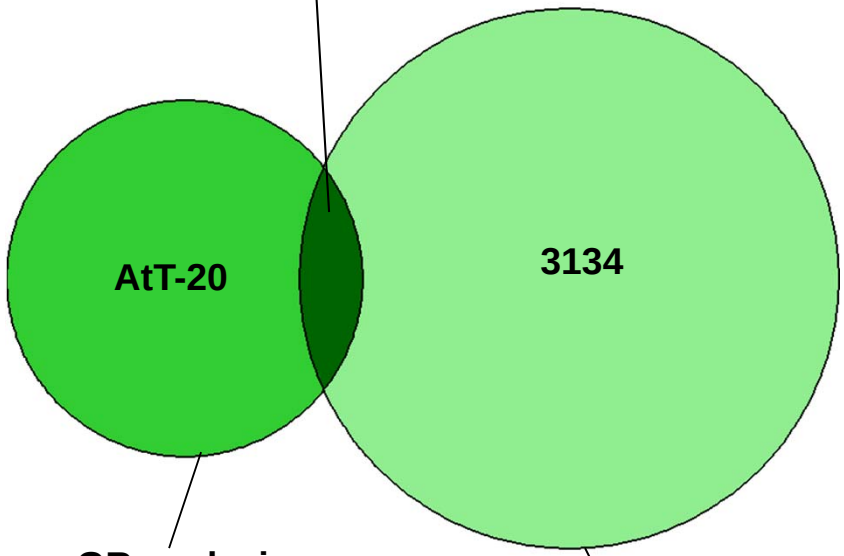
30715 DHS shared between
3134 (Mammary) and AtT20 (Pituitary)
(~30%) --- promoter regions



DHS in AtT20 (117929)

DHS in 3134 (107908)

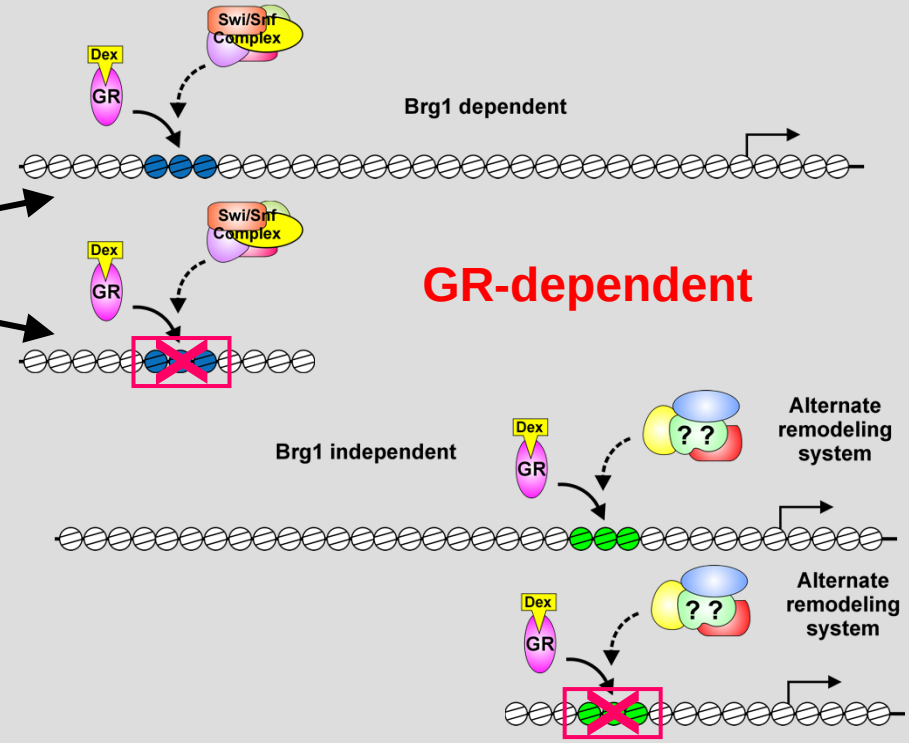
363 GR binding sites shared between
3134 (Mammary) and AtT20 (Pituitary)
(~5-10%) --- in promoter regions



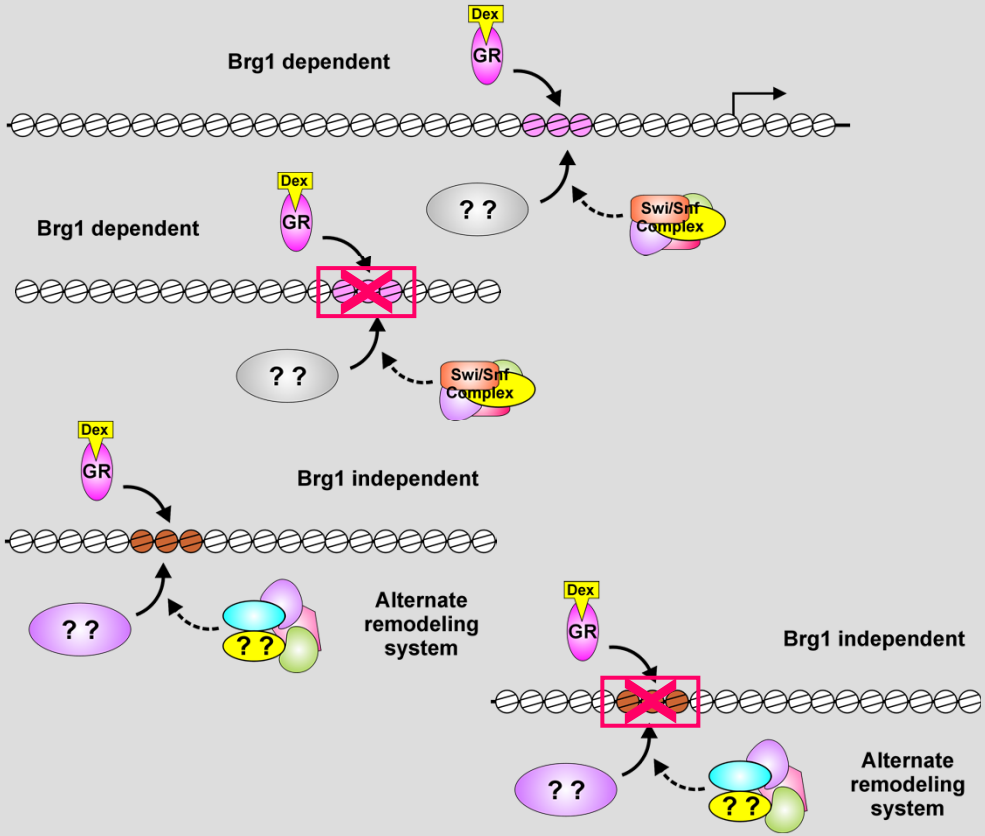
GR peaks in
AtT20 (3628)

GR peaks in
3134 (8373)

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 modificaitons


Sam John
Stephanie Morris
Kip Nalley
Simon Biddie
Lou Schiltz
Anindya Hendarwanto
Mia Sung
Diana Stavreva
Christine Koch-Paiz
Ty Voss
Ronit Salomon
Tom Johnson
Anuja George
Ofir Hakim
Gosia Wiench

Center for Cancer Research

Univ of Washington

John Stamatoyannopoulos
Pete Sabo
Bob Thurman