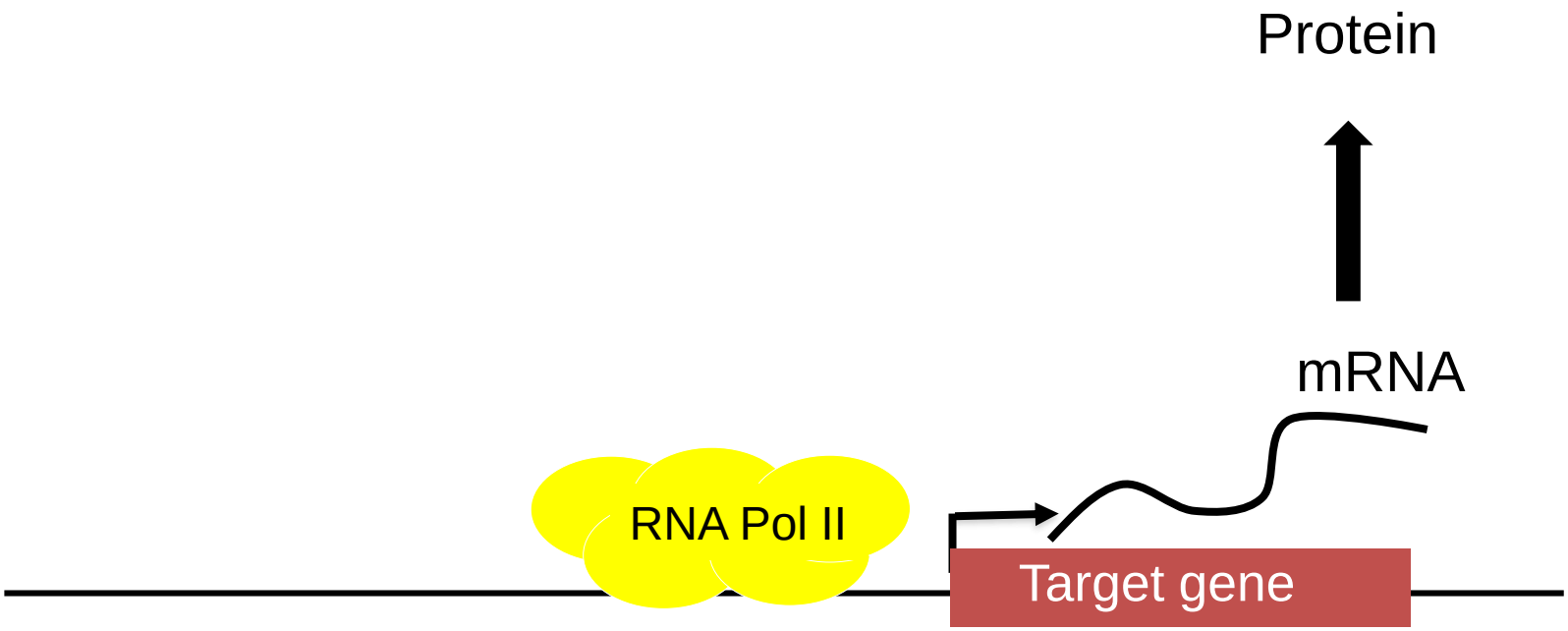
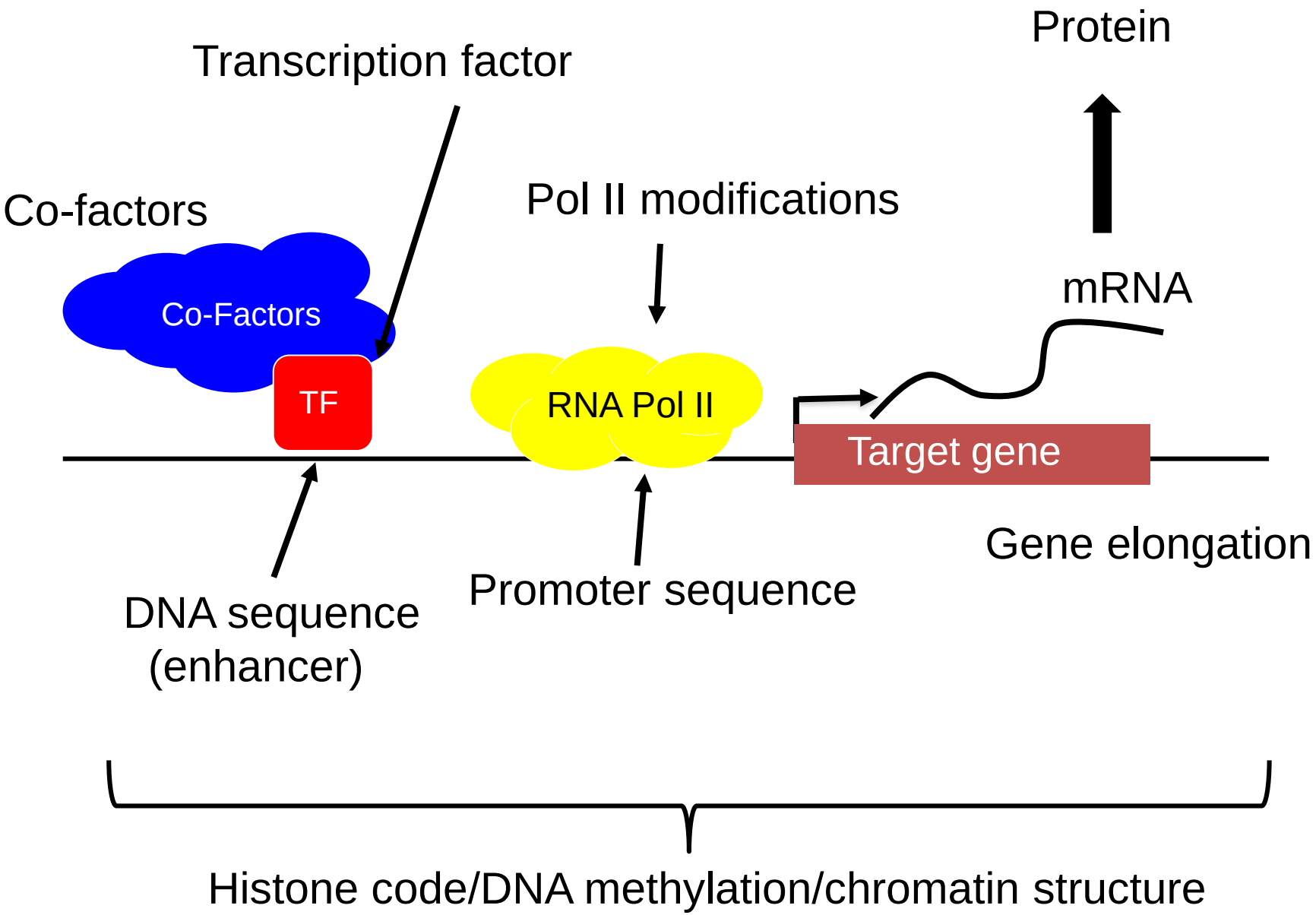
UNIVERSITY OF CAMBRIDGE

CANCER RESEARCH UK

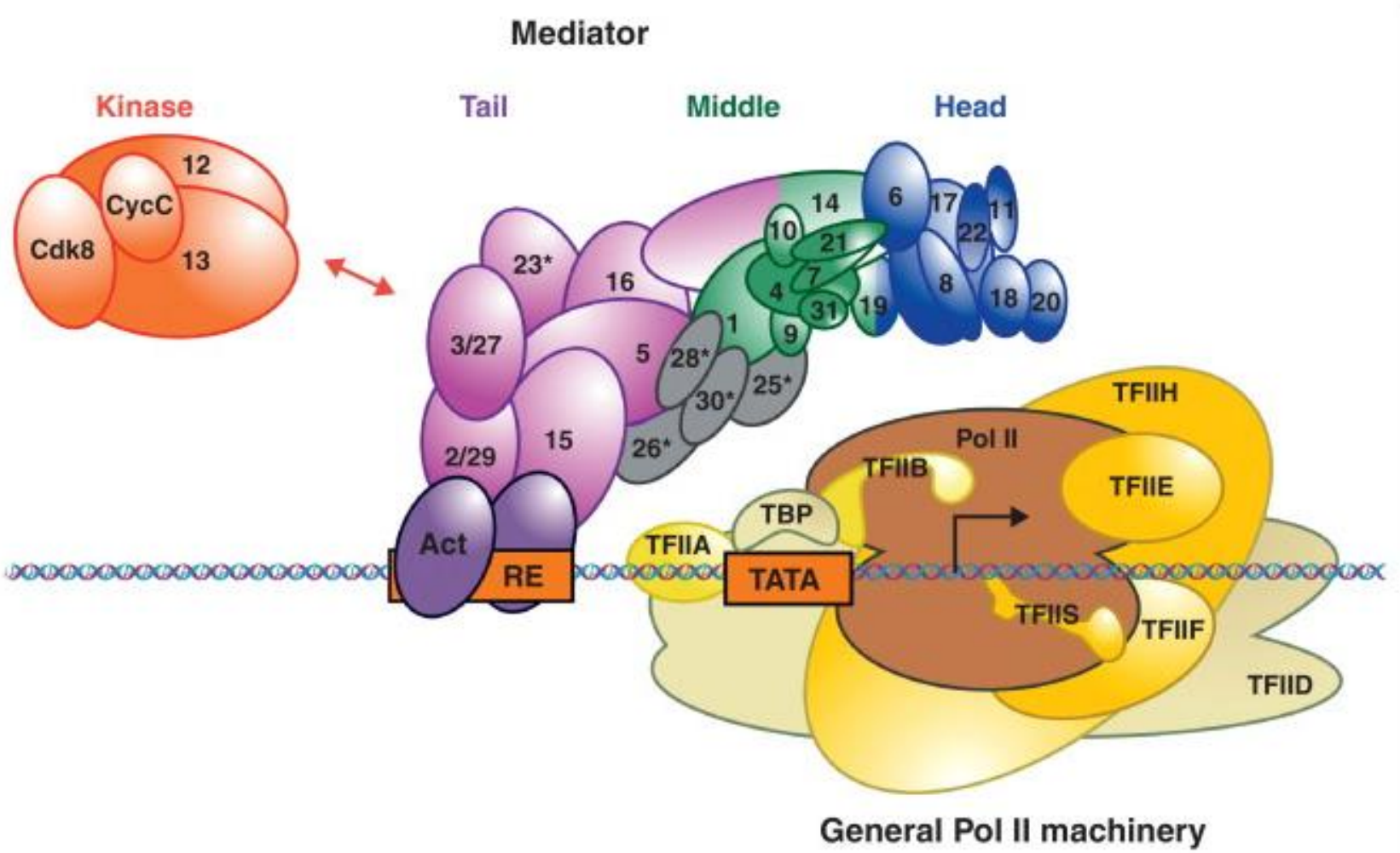
Transcription in cancer

Jason Carroll


Protein
mRNA
RNA Pol II
Target gene

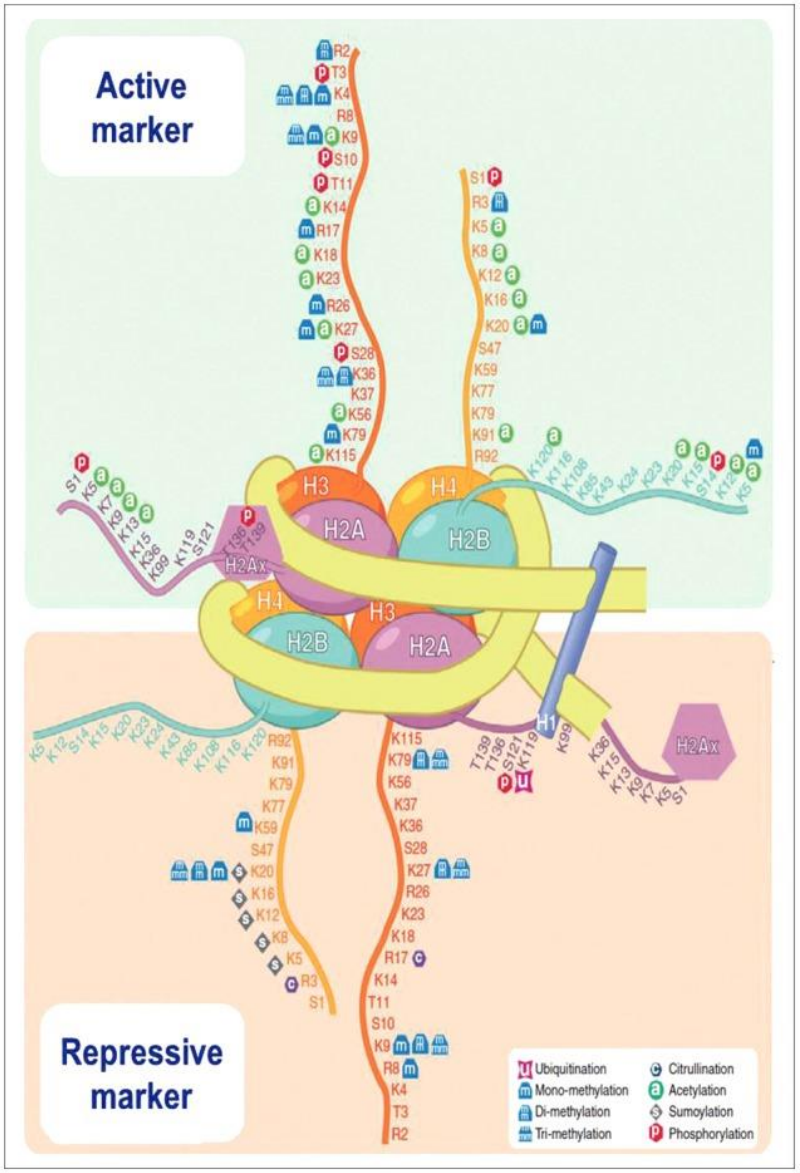

Protein
Transcription factor
Co-factors
Pol II modifications
mRNA
Co-Factors
TF
RNA Pol II
Target gene
Gene elongation
DNA sequence
(enhancer)
Promoter sequence
Histone code/DNA methylation/chromatin structure

Properties of transcription machinery



(44 core proteins)

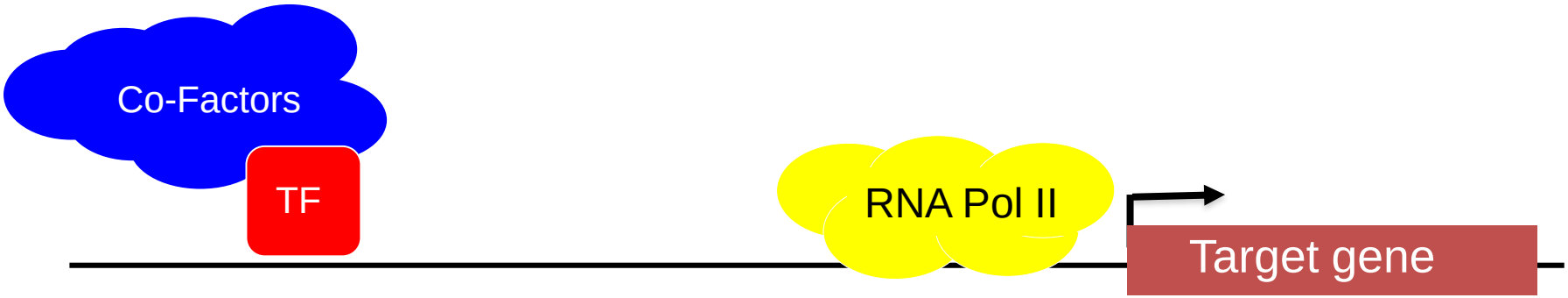
DNA: Histone modifications



DNA: Histone modifications

Enhancers
(TF binding sites)

Promoters



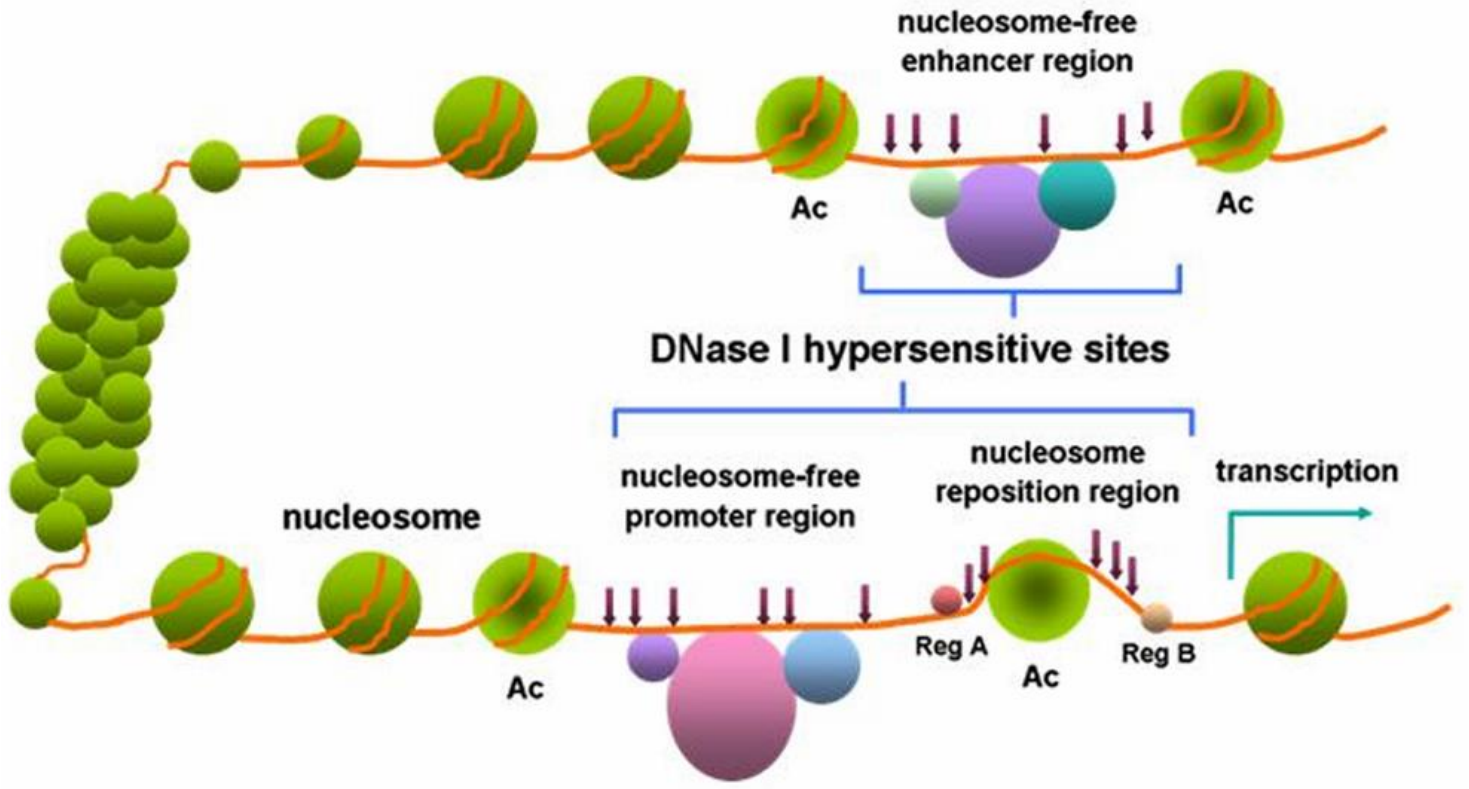
H3K4me1
H3K4me2

H3K4me3
H3K9Ac > H3K9Me3 is repressive
H3K27Ac > H3K27Me3 is repressive

Required for gene expression

Chromatin structure

- Enhancers (where TF bind) tend to be euchromatic



- Can identify using DNase I hypersensitivity (DHS)

- An enzyme that digests DNA at ‘open’ regions

A limited set of putative enhancers exist in the genome

Cell. 2013 August 15; 154(4): 888–903. doi:10.1016/j.cell.2013.07.020.

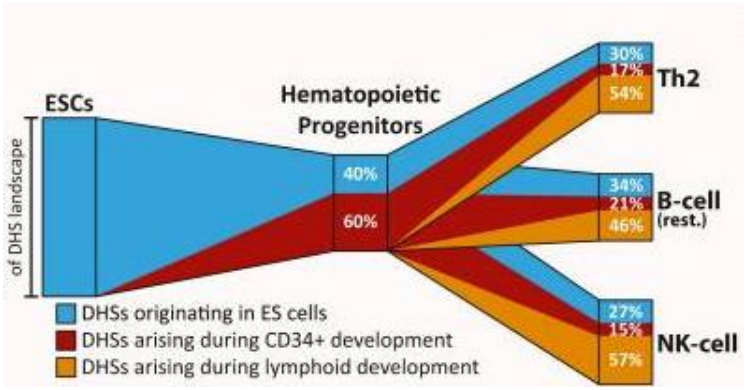
Developmental Fate and Cellular Maturity Encoded in Human Regulatory DNA Landscapes

Andrew B. Stergachis[#1], Shane Neph[#1], Alex Reynolds[1], Richard Humbert[1], Brady Miller[1,5], Sharon L. Paige[2,3], Benjamin Vernot[1], Jeffrey B. Cheng[9], Robert E. Thurman[1], Richard Sandstrom[1], Eric Haugen[1], Shelly Heimfeld[8], Charles E. Murry[2,3,4,6], Joshua M. Akey[1], and John A. Stamatoyannopoulos[1,7,*]

- Embryonic stem cells have a ‘universe’ of ~200,000 DHS regions

- ~3% of the genome can be an enhancer

- These close down as cells differentiate



- Enhancers recruit transcription factors

Nuclear receptors are important transcription factors

- Estrogen-Estrogen Receptor (ER) mediates cellular growth for mammary gland development
  - Two ER isoforms (ER-alpha and ER-beta)

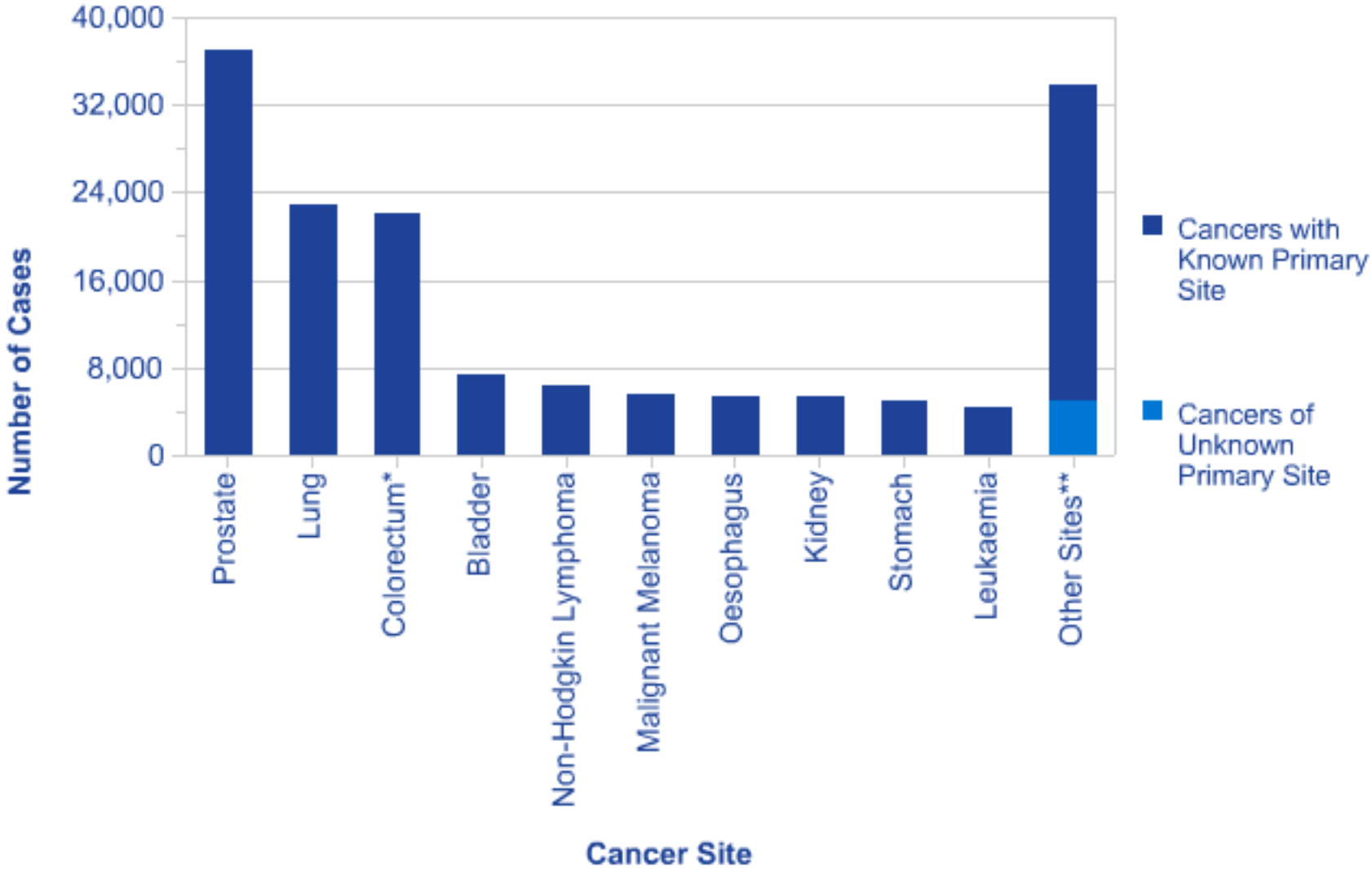
- Androgen-AR mediate cellular growth for prostate development

- Glucocorticoids, progesterone, prolactin, mineralocorticoids etc are all important for normal physiology

- But they're key drivers in cancer!
  - *75% of breast cancer: Estrogen Receptor (ER) driven*
  - *Almost all prostate cancer: Androgen Receptor (AR) driven*

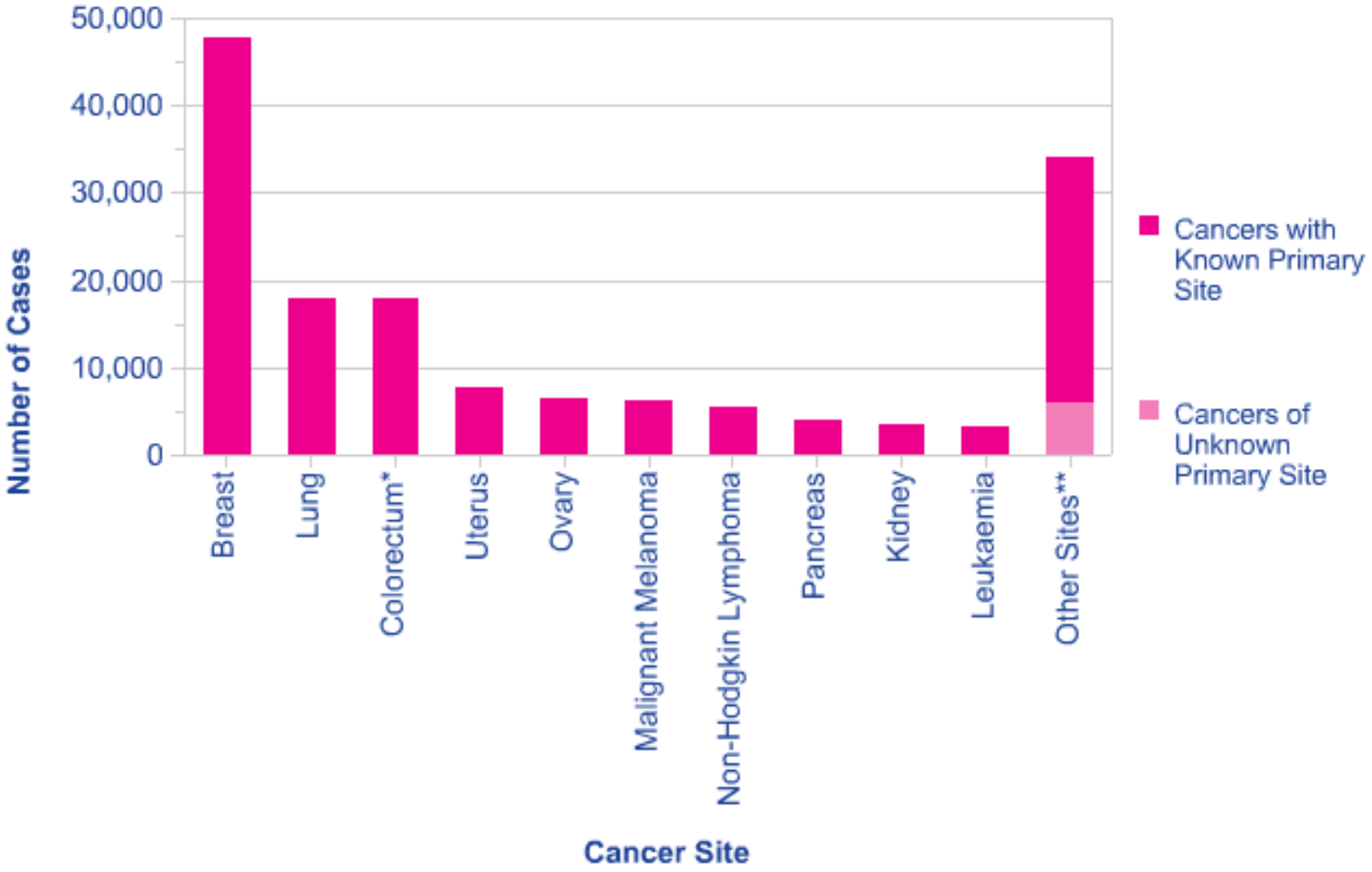
- ER/AR targeted drugs have changed survival rates

Major cancers in men in the UK

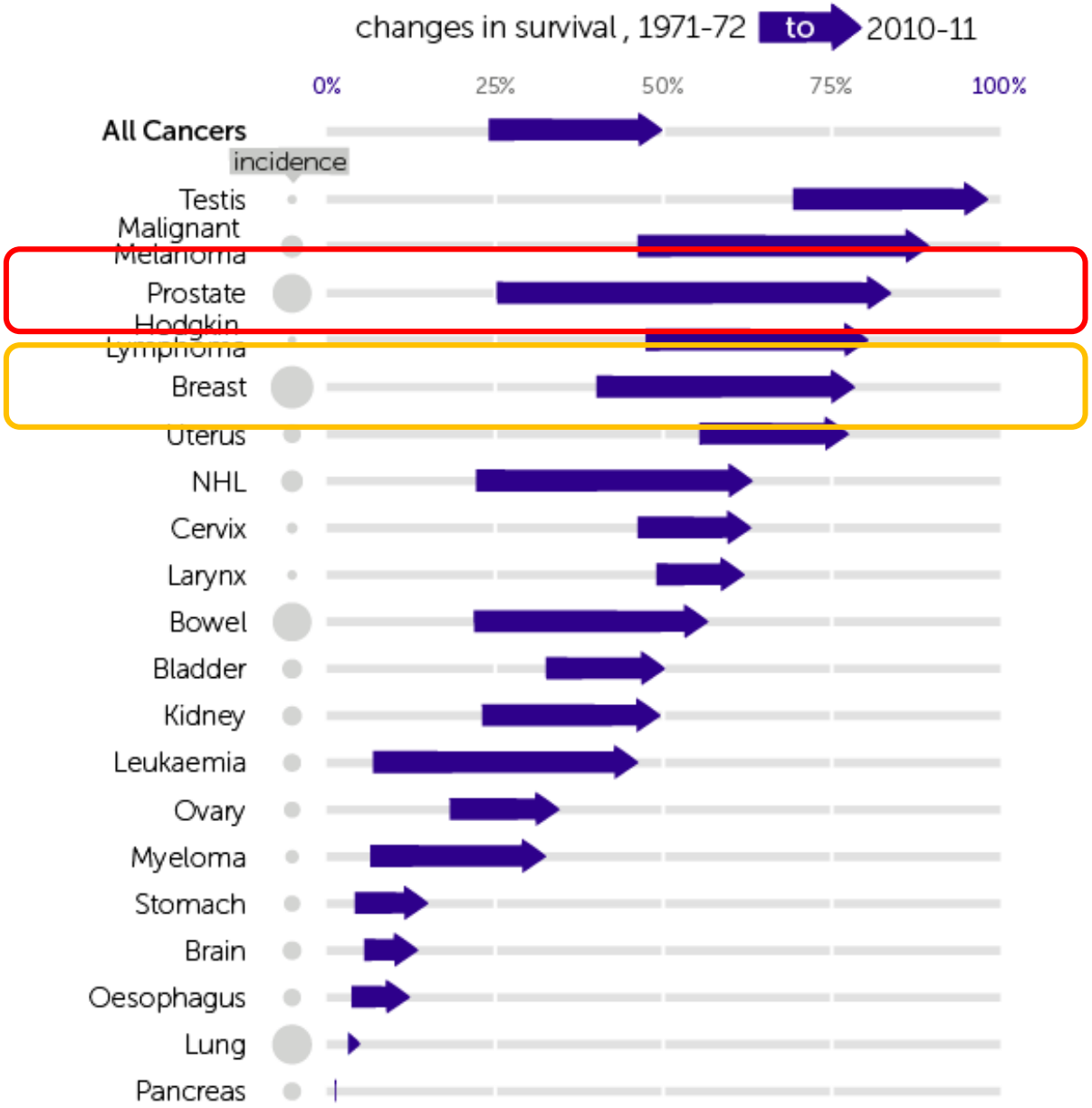


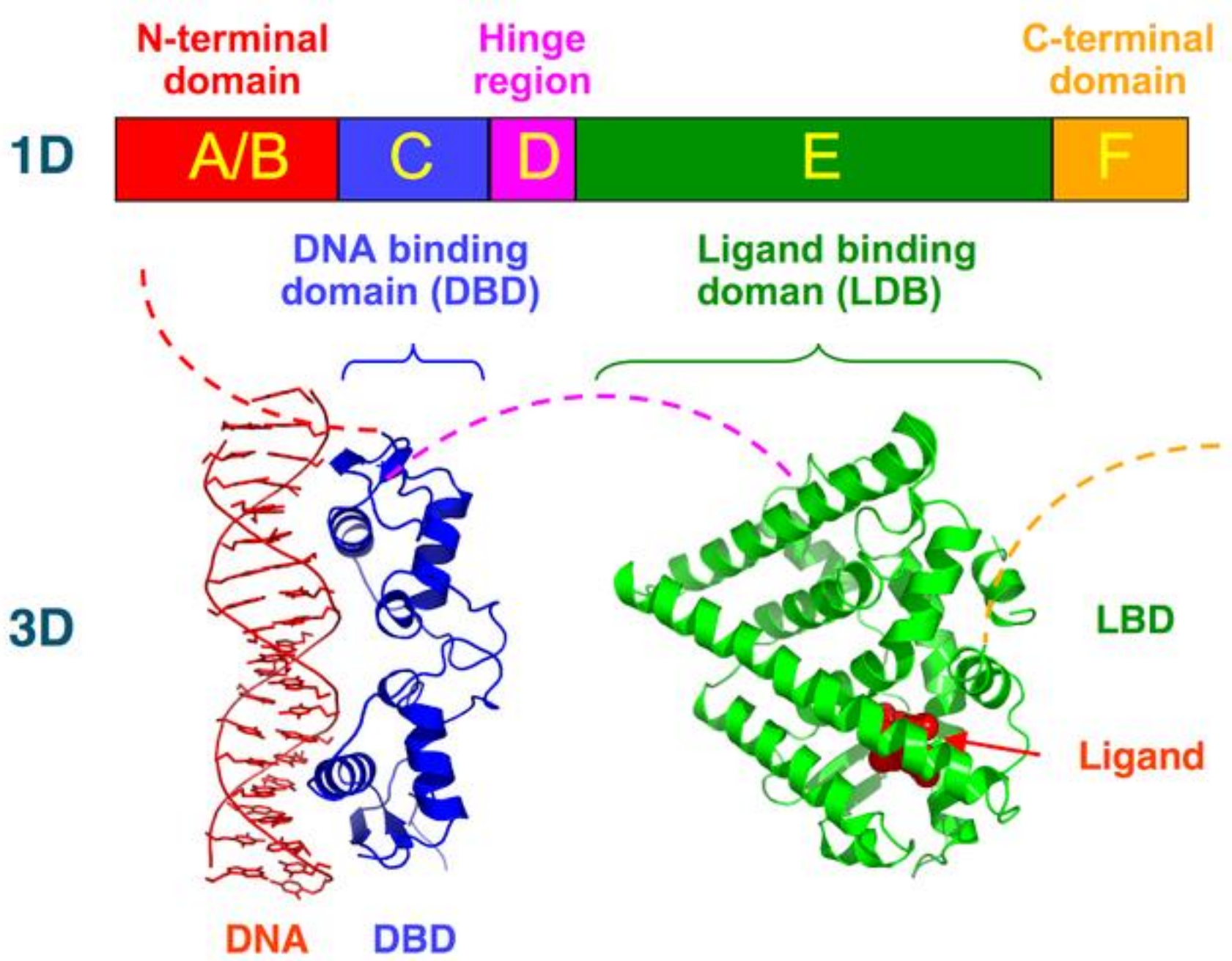
Data: Cancer Research UK

Major cancers in women in the UK

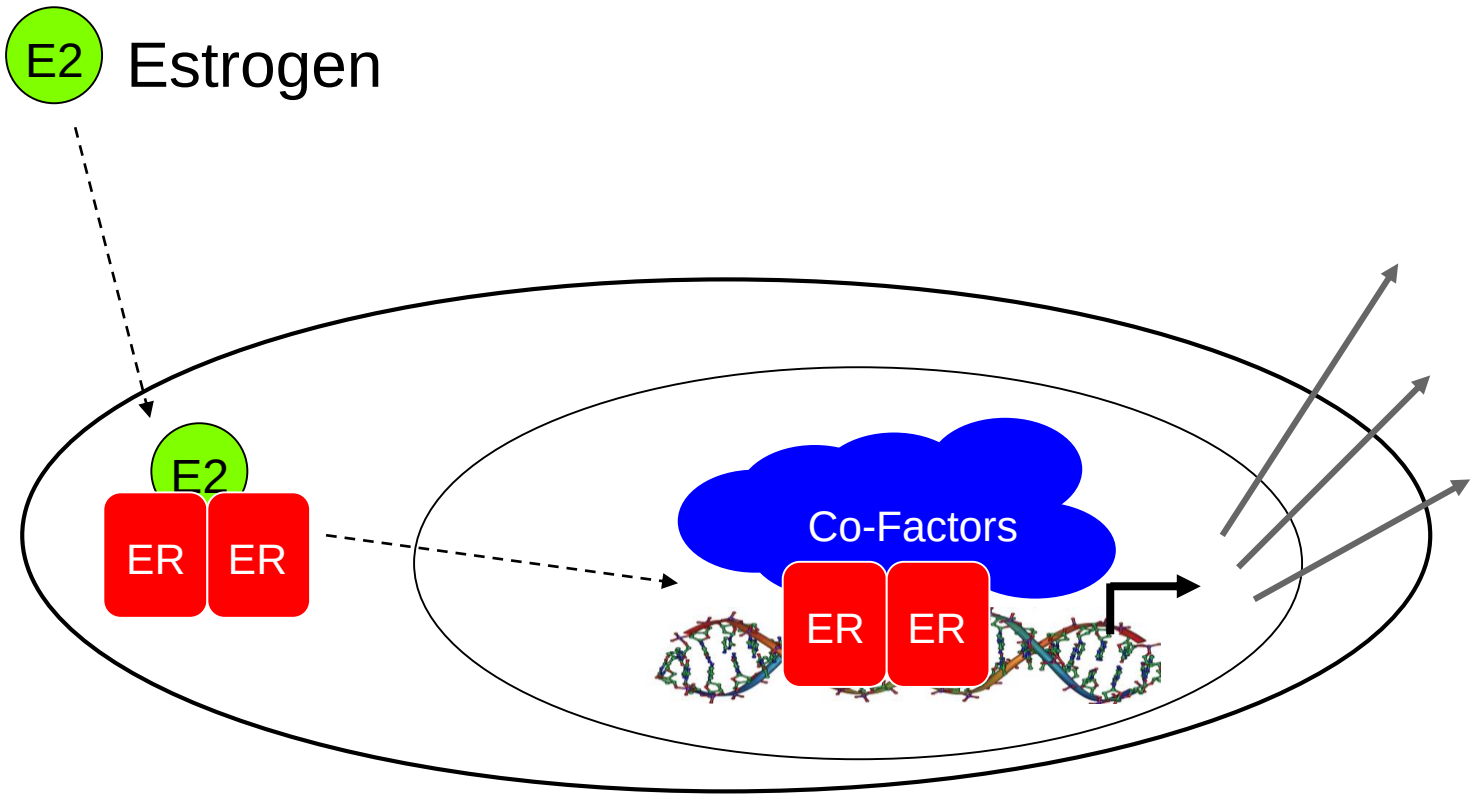


Data: Cancer Research UK


changes in survival , 1971-72 to 2010-11
0%
25%
50%
75%
100%
All Cancers
incidence
Testis
Malignant Melanoma
Prostate
Hodgkin Lymphoma
Breast
Uterus
NHL
Cervix
Larynx
Bowel
Bladder
Kidney
Leukaemia
Ovary
Myeloma
Stomach
Brain
Oesophagus
Lung
Pancreas

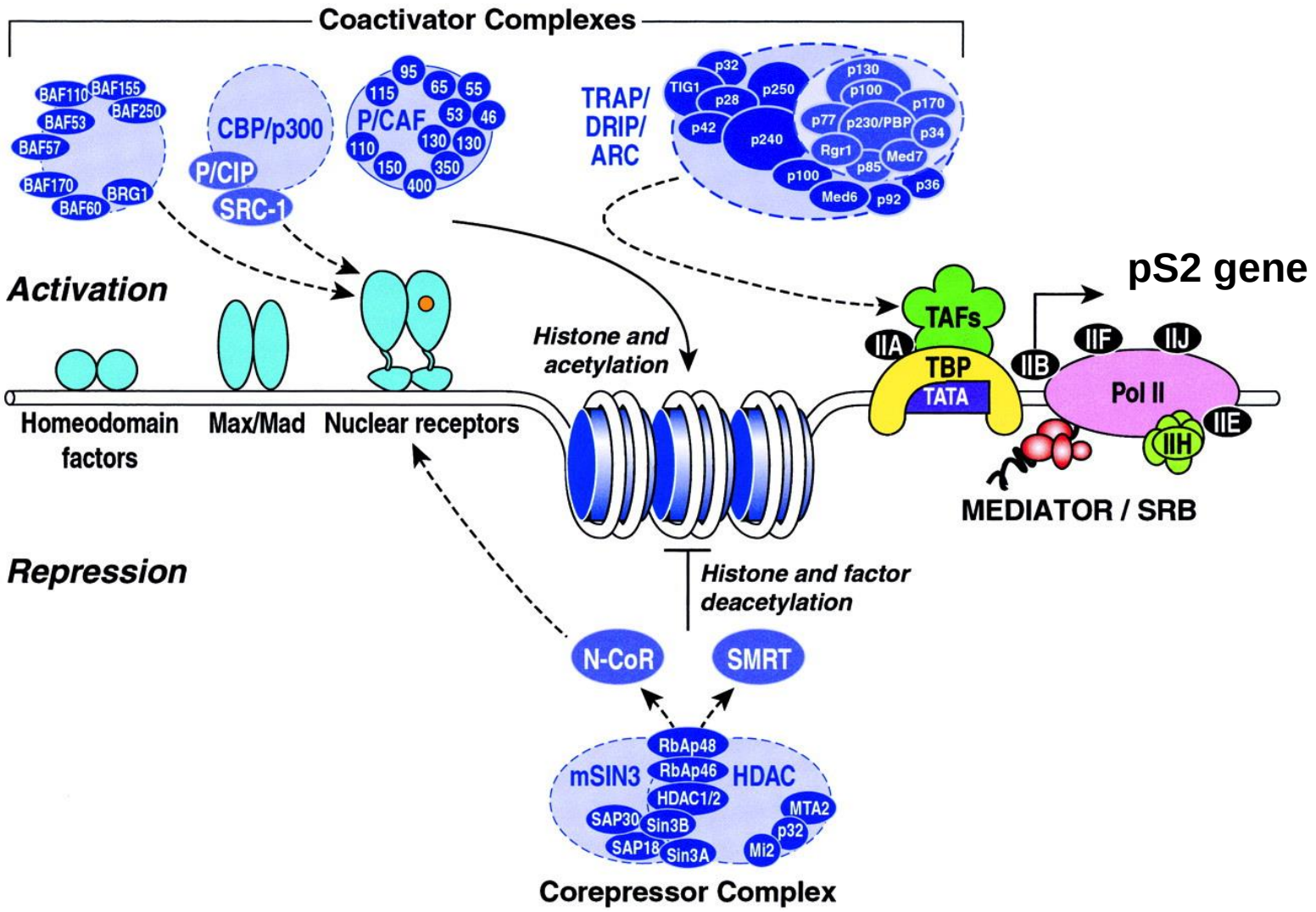

N-terminal domain
Hinge region
C-terminal domain
1D
A/B
C
D
E
F
DNA binding domain (DBD)
Ligand binding doman (LDB)
3D
LBD
Ligand
DNA
DBD

Ligand activation mediates ER (and AR) activity



- Drugs block ligand binding domain, or ligand (estrogen) levels

Known nuclear receptor (TF) co-factors



Glass and Rosenfeld, *Genes Dev*, 2000

Multiplatform Analysis of 12 Cancer Types Reveals Molecular Classification within and across Tissues of Origin

Katherine A. Hoadley,[1,20] Christina Yau,[2,20] Denise M. Wolf,[3,20] Andrew D. Cherniack,[4,20] David Tamborero,[5] Sam Ng,[6] Max D.M. Leiserson,[7] Beifang Niu,[8] Michael D. McLellan,[8] Vladislav Uzunangelov,[6] Jiashan Zhang,[9] Cyriac Kandoth,[8] Rehan Akbani,[10] Hui Shen,[11,22] Larsson Omberg,[12] Andy Chu,[13] Adam A. Margolin,[12,21] Laura J. van't Veer,[3] Nuria Lopez-Bigas,[5,14] Peter W. Laird,[11,22] Benjamin J. Raphael,[7] Li Ding,[8] A. Gordon Robertson,[13] Lauren A. Byers,[10] Gordon B. Mills,[10] John N. Weinstein,[10] Carter Van Waes,[18] Zhong Chen,[19] Eric A. Collisson,[15] The Cancer Genome Atlas Research Network, Christopher C. Benz,[2,*] Charles M. Perou,[1,16,17,*] and Joshua M. Stuart[6,*]

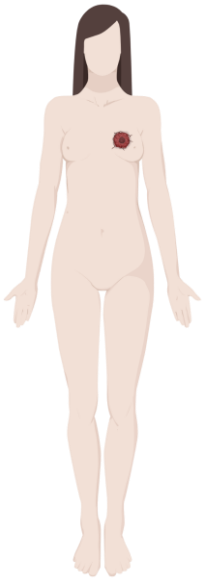
Transcription factors

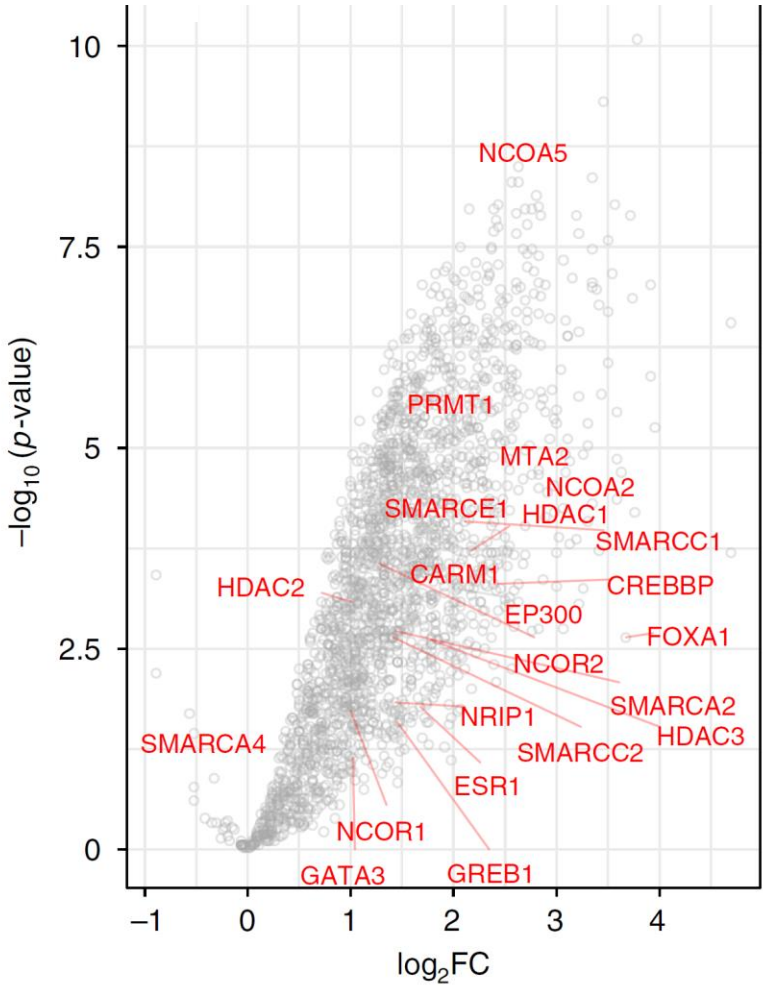
- Myc
- Sox2
- p53
- GATA3
- SMAD2/4
- CEBPA

Co-factors

- MLL2/3
- p300
- KDM5C
- KDM6A
- ARID1A
- AIB1

How many ER co-factors are there?





- 1,500 interactors

- Lots of subcomplexes

- They can't all be functional…
…….or can they?

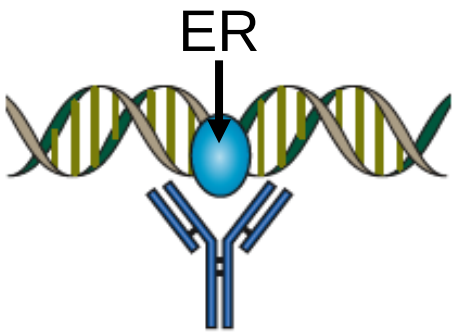
- Some are structural, some are enzymes

- They are co-factors, they don't touch the DNA directly

Papachristou et al. 2018

How do you study where a transcription factor sits on DNA (chromatin)

Chromatin IP (ChIP



Control (Input)

Can you identify all DNA (even unknown sites)?

High-throughput sequencing technologies

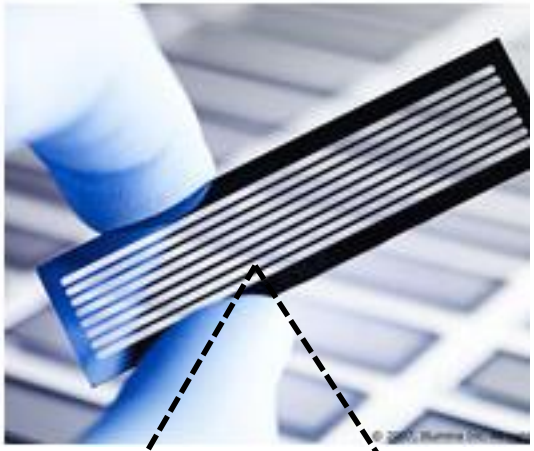
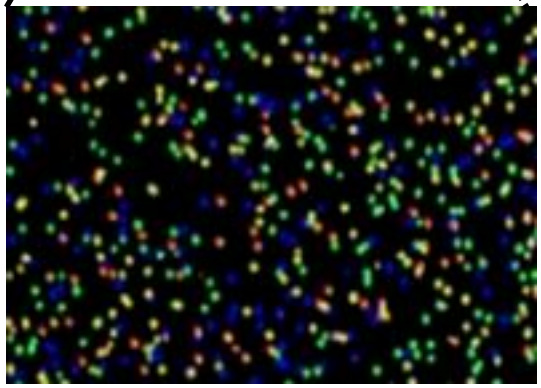
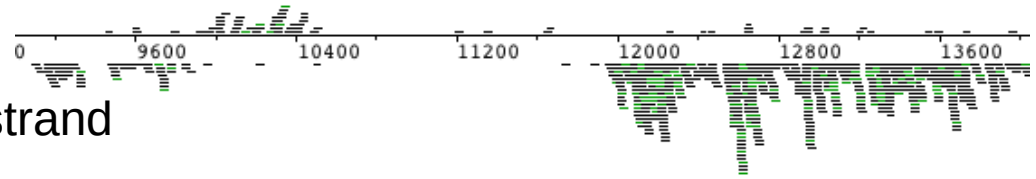


Image (cd-genomics.com)



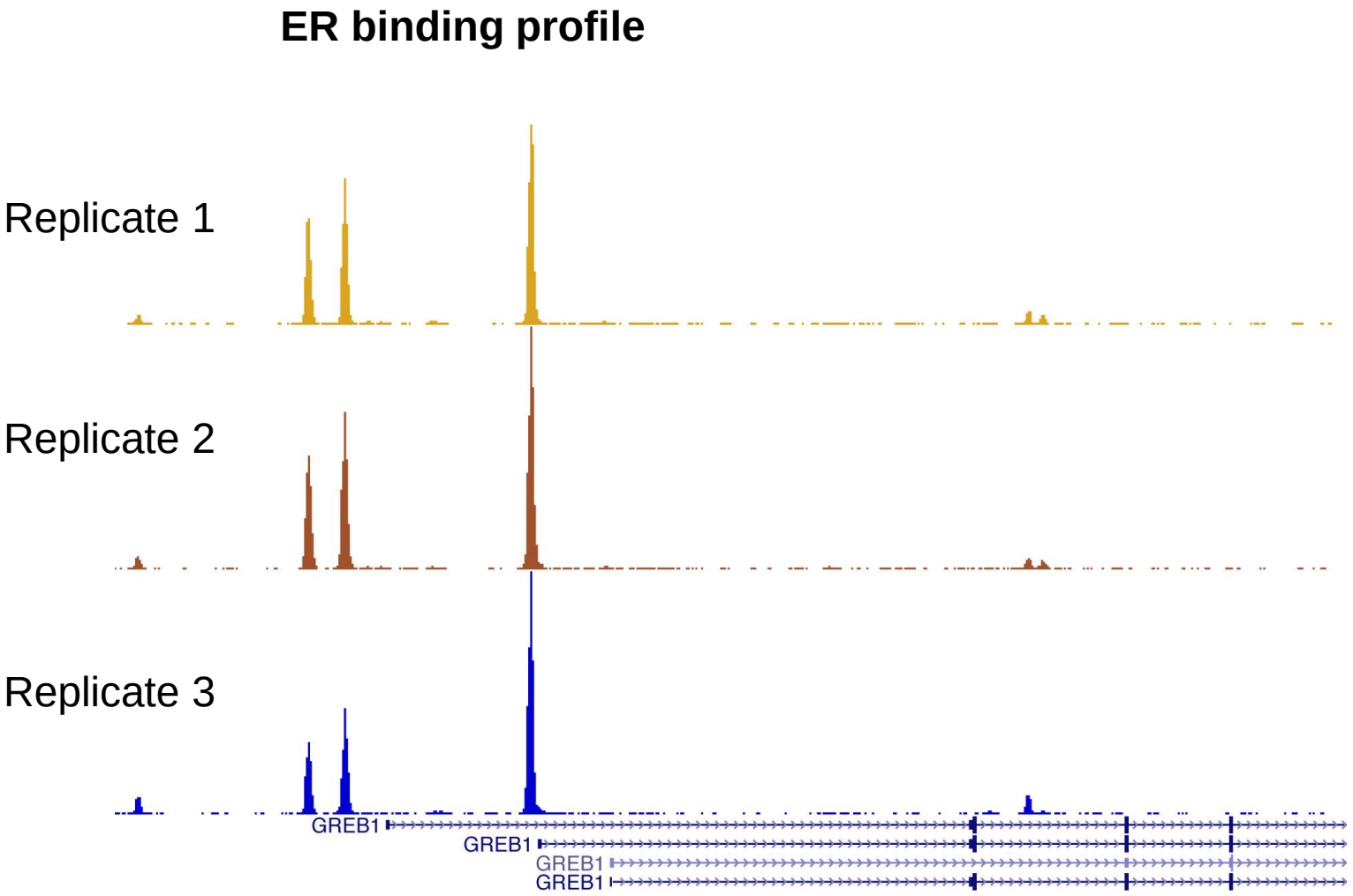
Counting small (36bp) DNA reads

+ strand



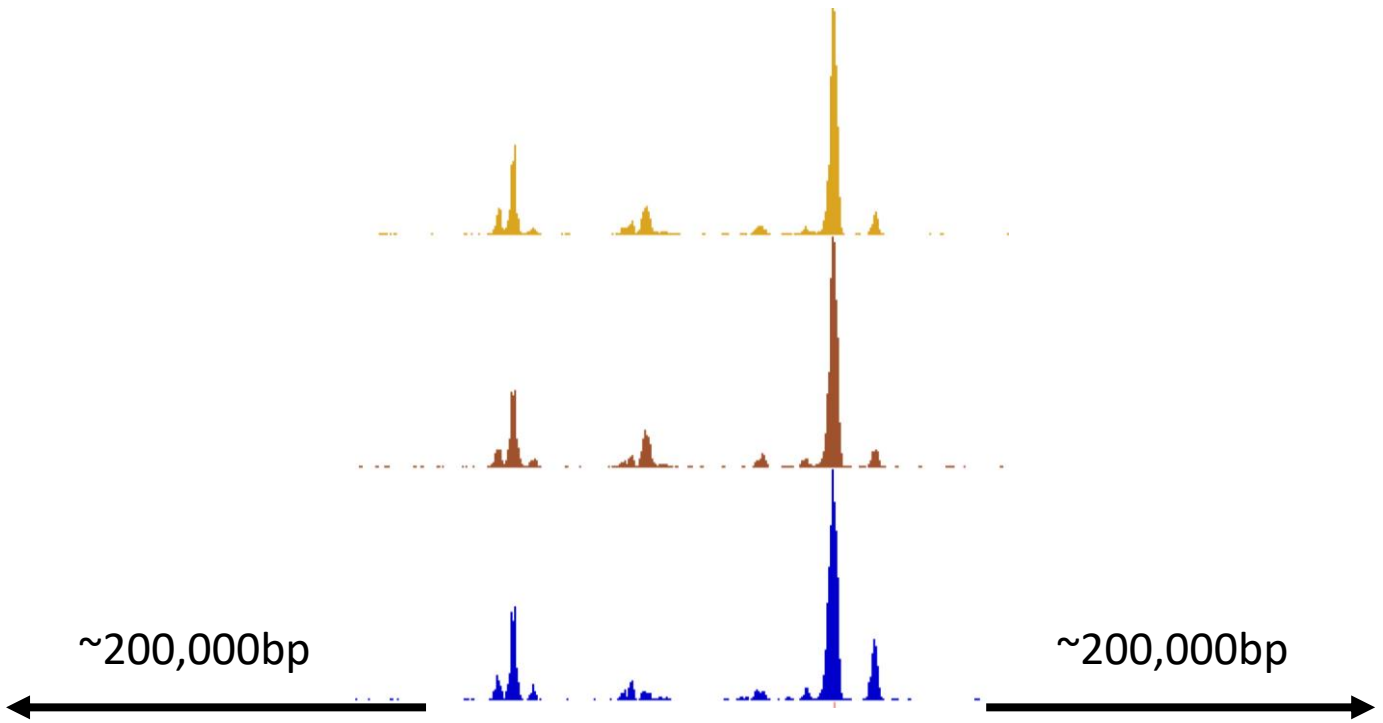
- strand

ER ChIP-sequencing (ChIP-seq) in breast cancer cells



ER: The first TF mapped genome-wide

In most cases, binding is in the middle of nowhere



- What gene is it switching on or off?

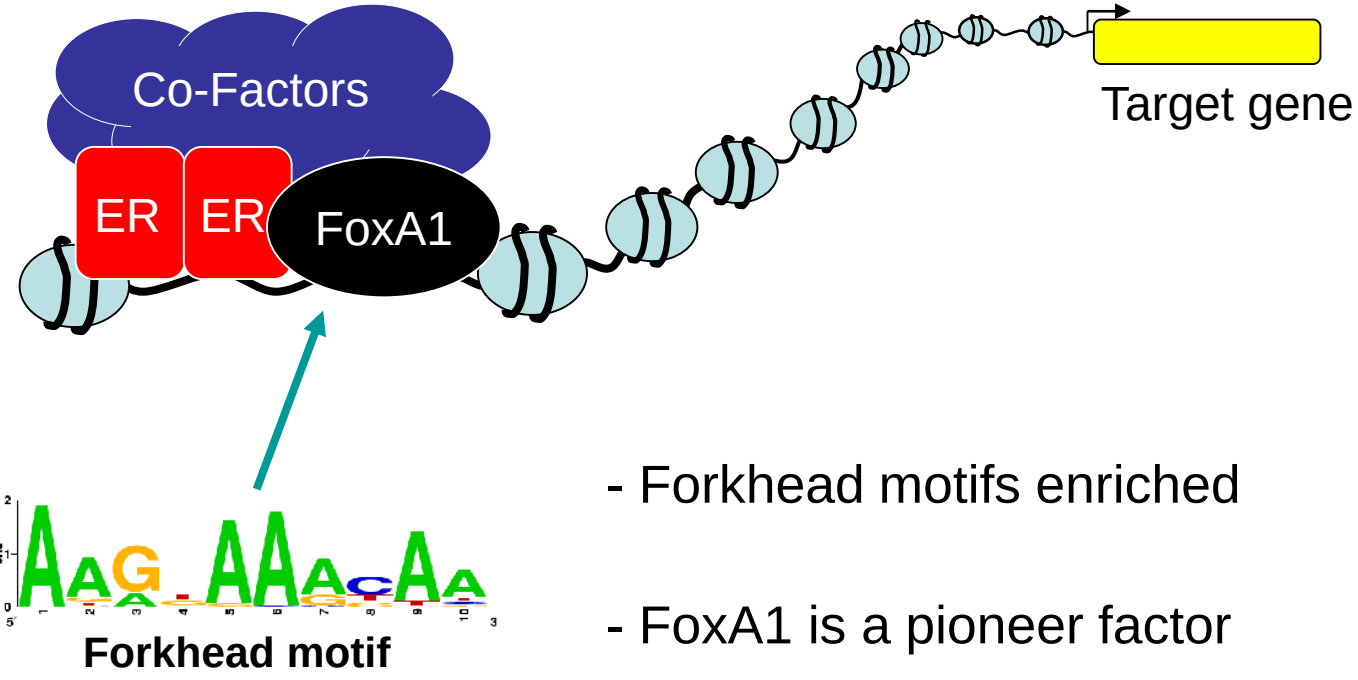
- Are they all doing something?

- We will come back to this in a few slides

What did we learn from mapping ER binding sites?

- Thousands of ER binding events

- Very few promoters bound



- Forkhead motifs enriched

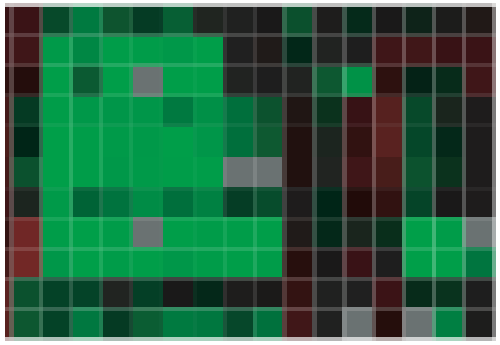
- FoxA1 is a pioneer factor

FoxA1 is a mandatory requirement for ER+ breast cancer

letters to nature

Molecular portraits of human breast tumours

Charles M. Perou*†, Therese Sørlie†‡, Michael B. Eisen*, Matt van de Rijn§, Stefanie S. Jeffrey‖, Christian A. Rees*, Jonathan R. Pollack¶, Douglas T. Ross¶, Hilde Johnsen‡, Lars A. Akslen#, Øystein Fluge☆, Alexander Pergamenschikov*, Cheryl Williams*, Shirley X. Zhu§, Per E. Lønning**, Anne-Lise Børresen-Dale‡, Patrick O. Brown¶†† & David Botstein*



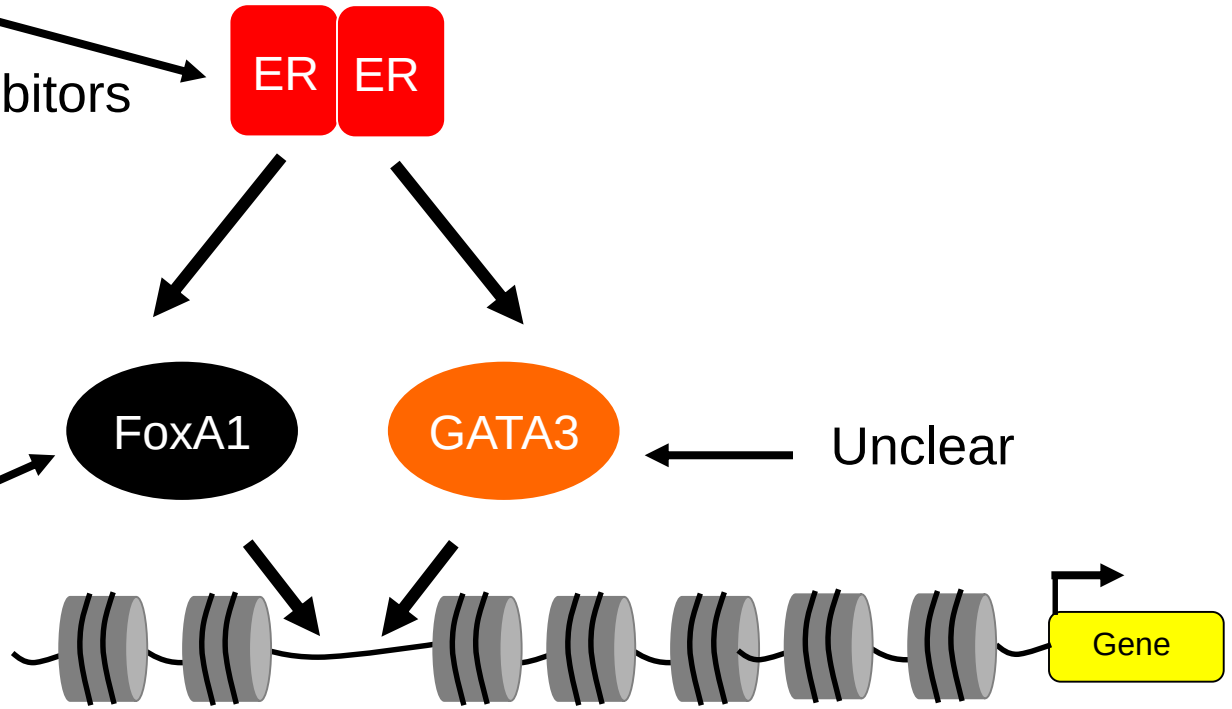
FoxA1
(aka. HNF3A)

FoxA1 is there in primary tumours, regardless of patient outcome

Perou, *Nature*, 2000

ER requires FoxA1 and GATA3 to function

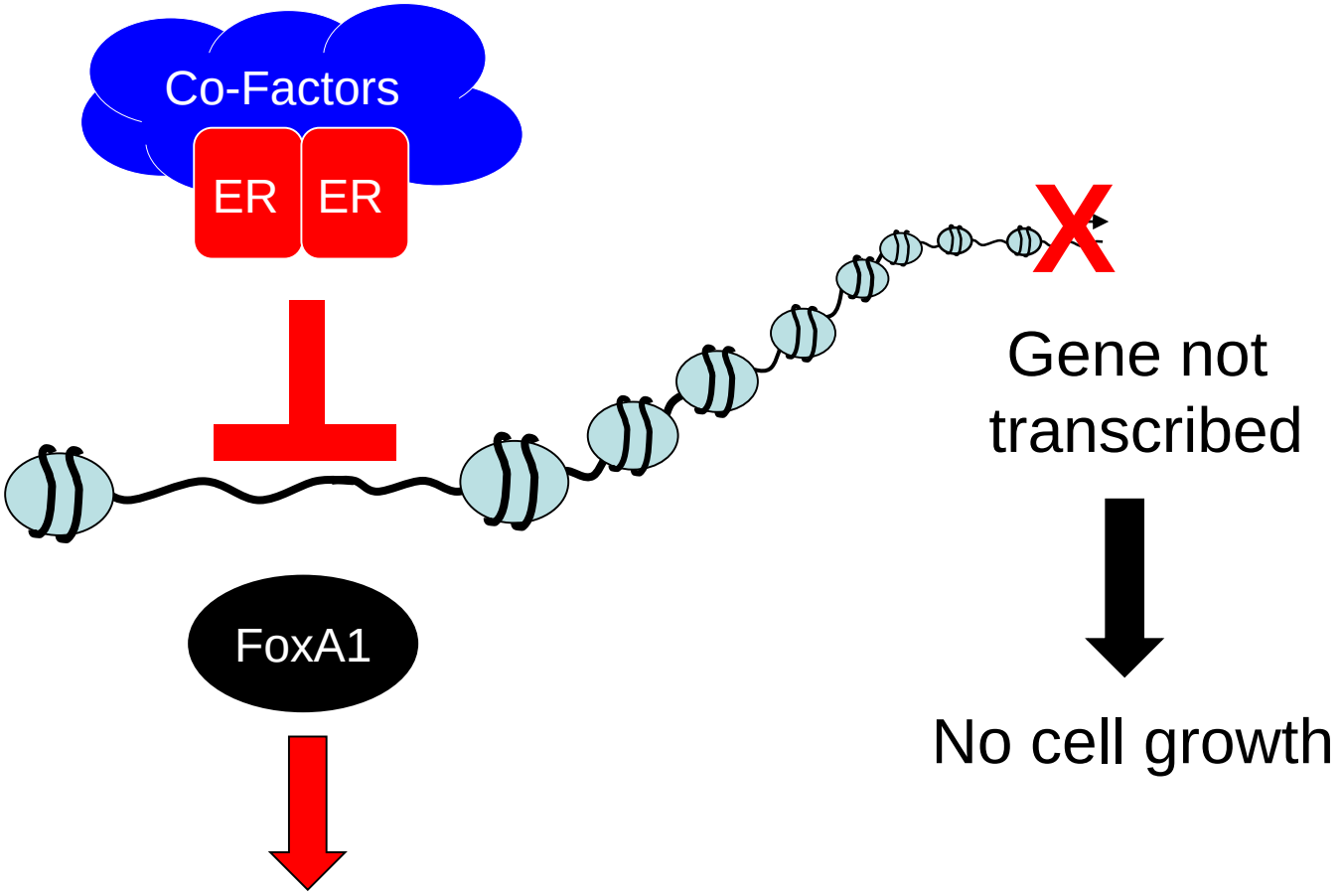
Drug target
- Tamoxifen
- Fulvestrant
- Aromatase Inhibitors



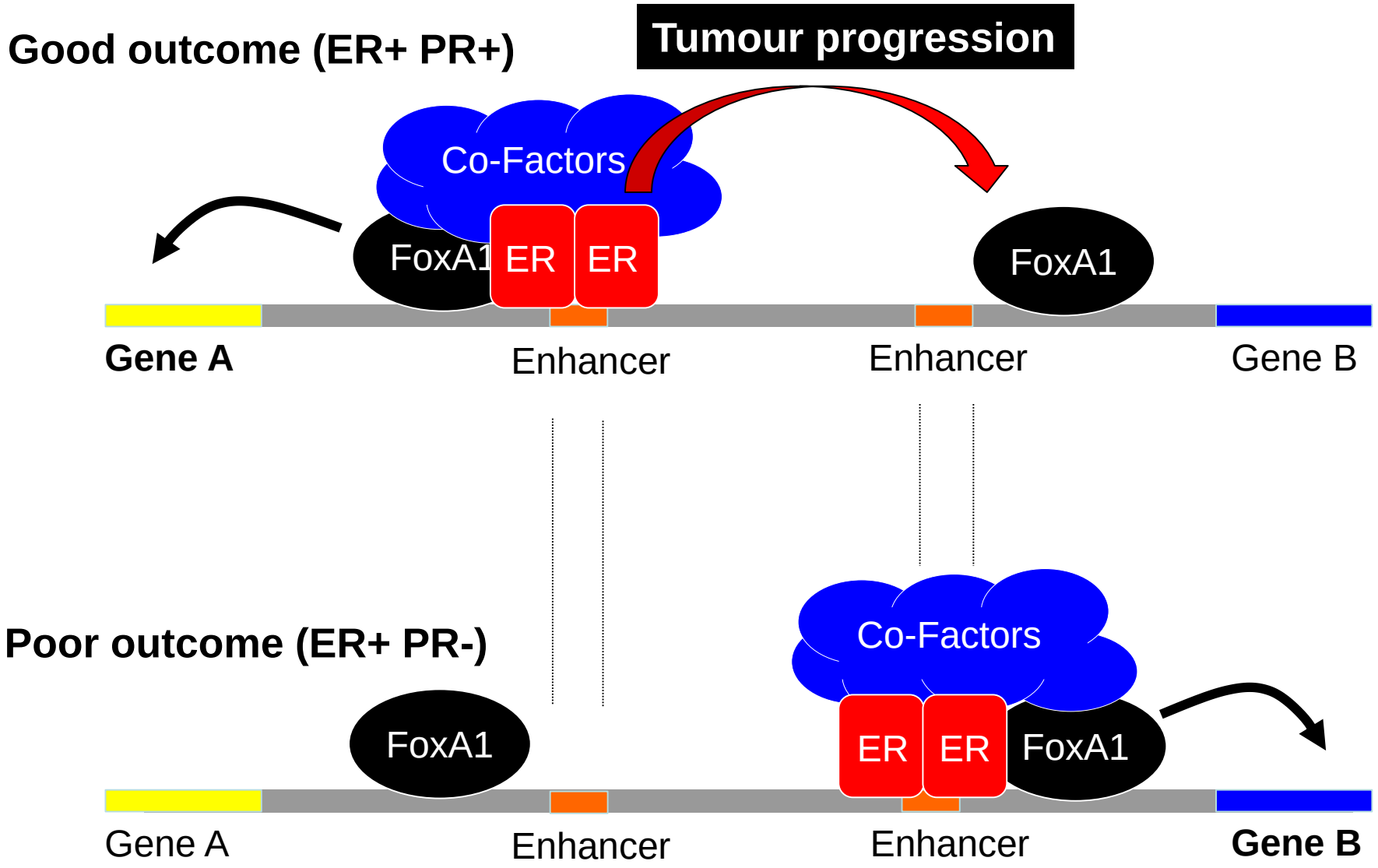
Is a pioneer factor
Invades ‘closed’
chromatin

ER binds to distal enhancers and needs FoxA1

Breast cancer cells

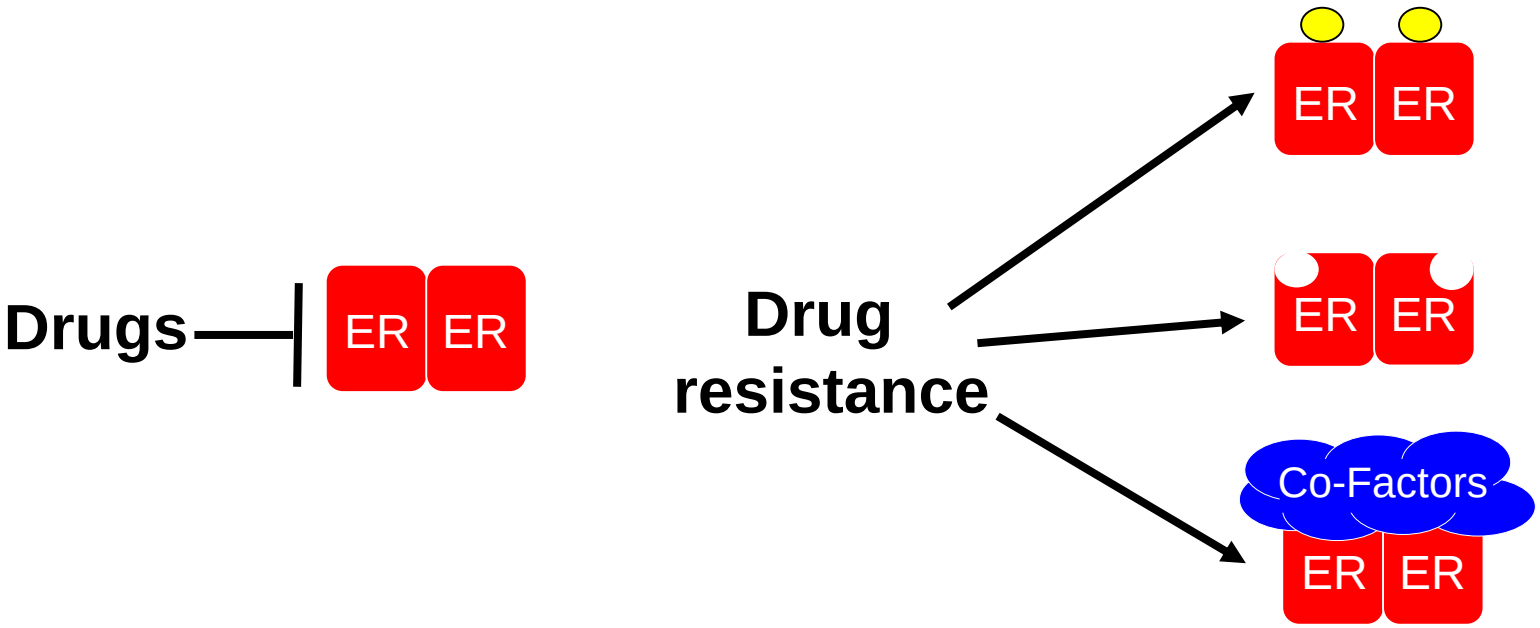


Transcription factors move around genome



Ross-Innes, *Nature*, 2012

There are multiple resistant mechanisms



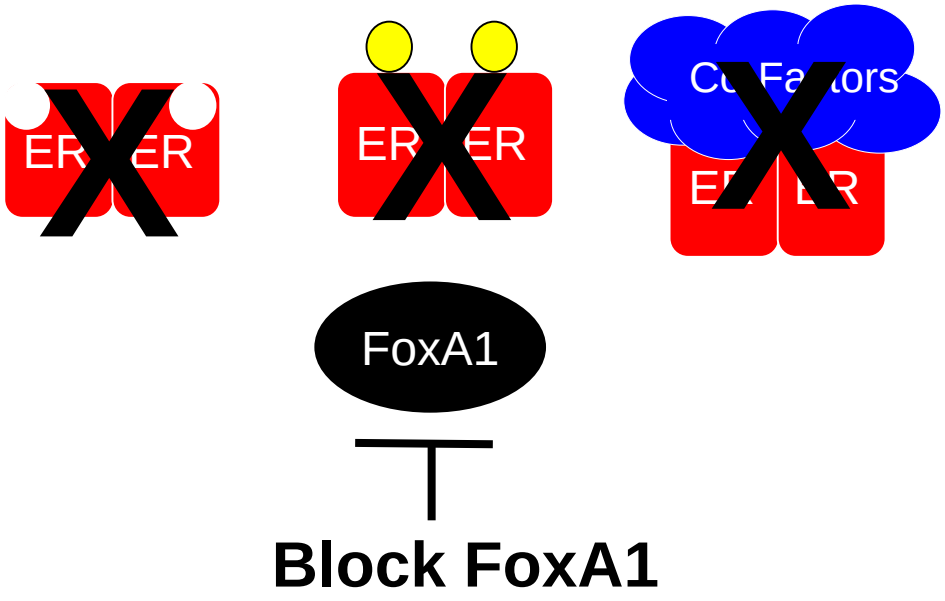
You need one cancer cell (out of many millions) with this altered ‘property’

Or, cancer cells can adapt and change

Can we drug FoxA1 instead of ER?

FoxA1 is needed, even in drug resistant cancer cells

Breast cancer



- A universal treatment for ER+ breast cancer?

Can we drug FoxA1?

AZERIA THERAPEUTICS

Azeria adds $41M to pioneer FOXA1 approach in breast cancer

November 21, 2019 By Nuala Moran No Comments

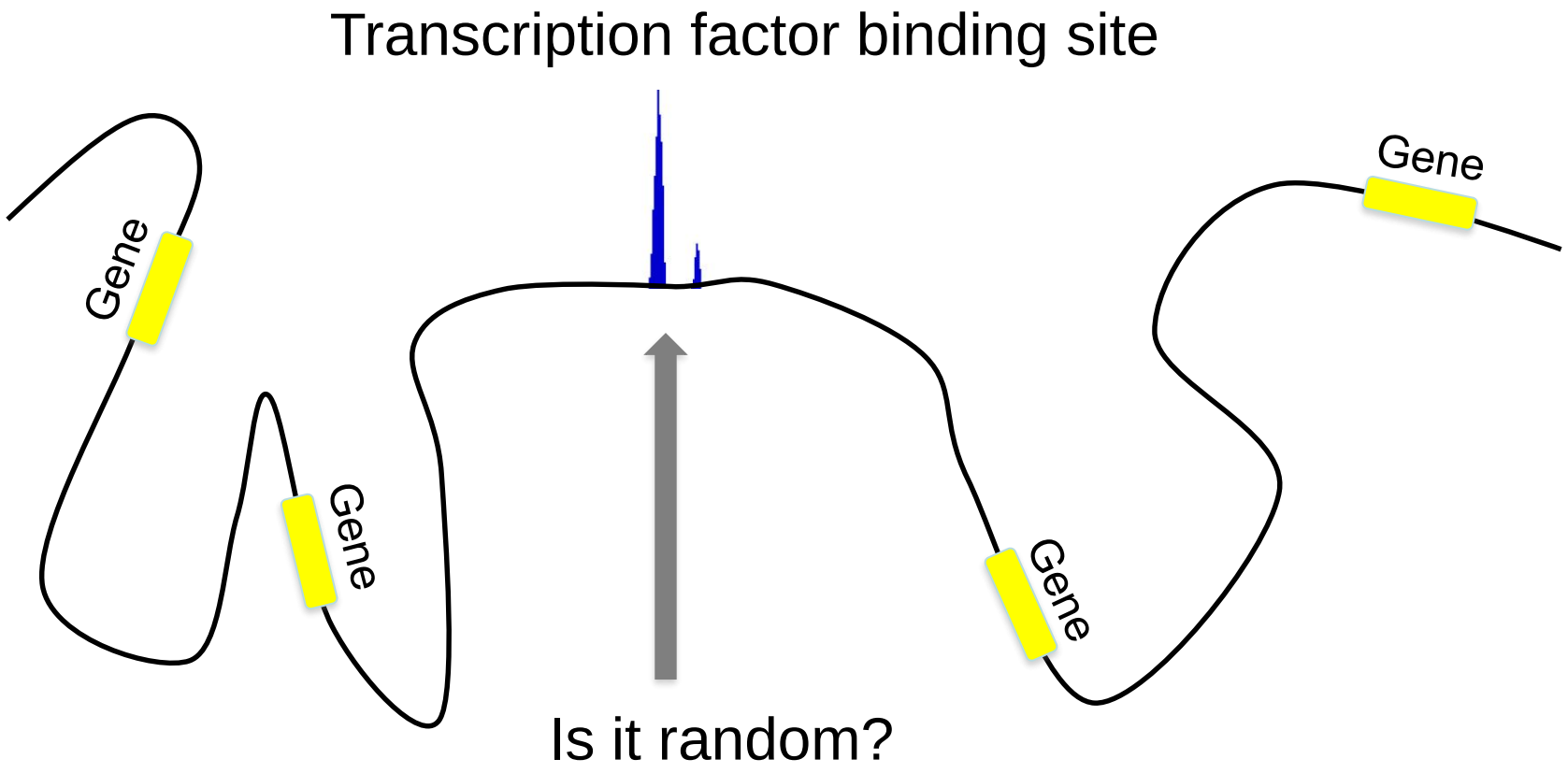
LONDON – Azeria Therapeutics Ltd. has raised £32 million (US$41.3 million) in a series B round to take forward small-molecule inhibitors of FOXA1, a transcription factor that is pivotal to the growth and progression of estrogen receptor (ER)-positive breast cancer.

- Can we make a new treatment?

- Why shouldn't Cambridge and CRUK benefit?

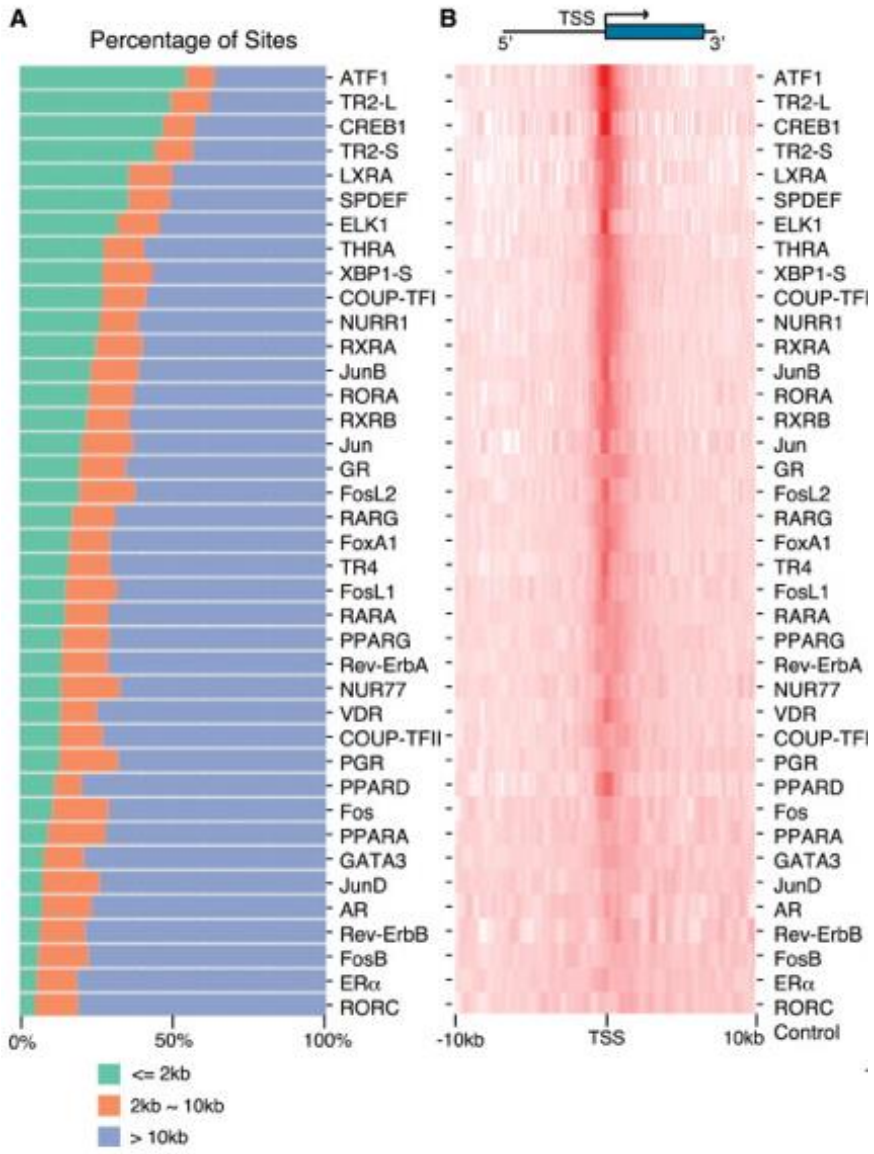
- Drugging FoxA1 requires insight into chromatin biology

In most cases, binding is in the middle of nowhere



- Transcription factors tend to cluster together
- DNase hypersensitivity: DNase digestion and sequencing
- Histone marks (H3K4me1/me2): ChIP-seq

Most transcription factors regulate from a distance

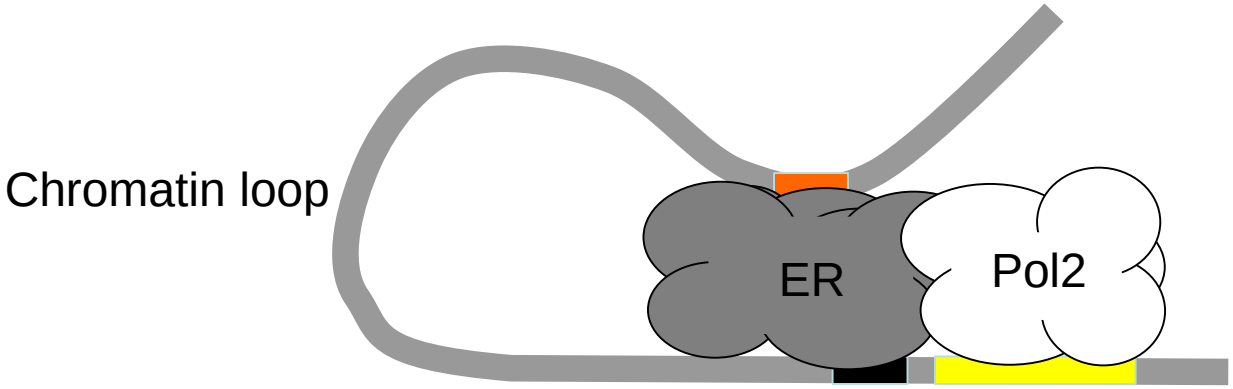


24 nuclear receptors

14 transcription factors

Kittler, *Cell Reports*, 2013

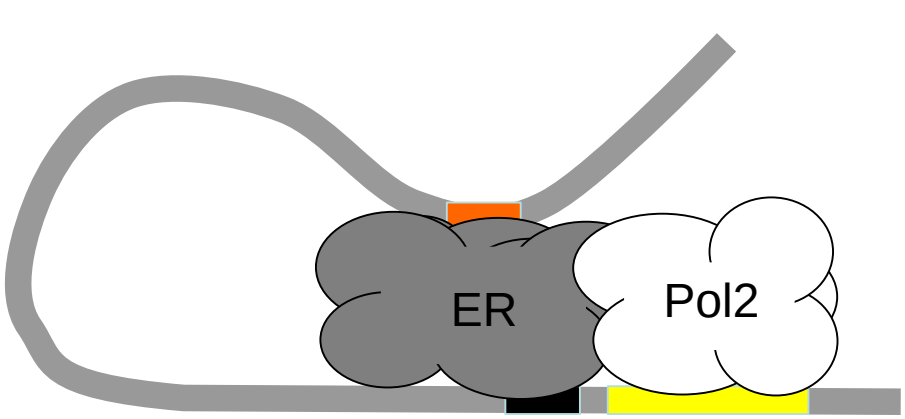
ER regulate genes from a distance. What gene?

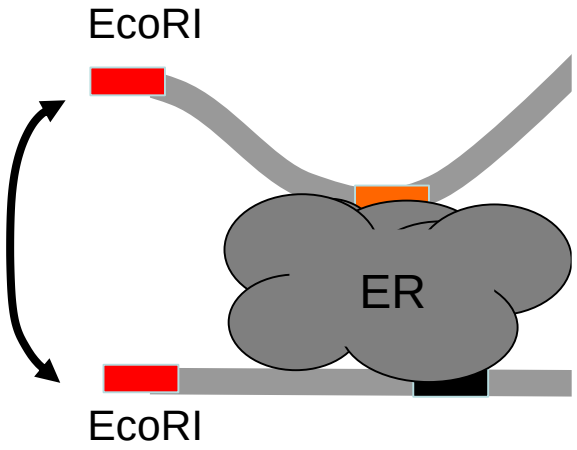


How to find what enhancer goes with what gene?

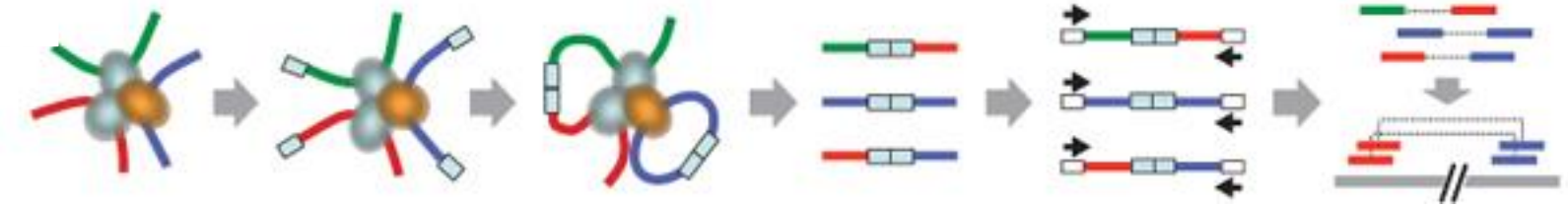
Chromosome Conformation Capture

ChIA-PET (Fullwood, *Nature*, 2009)

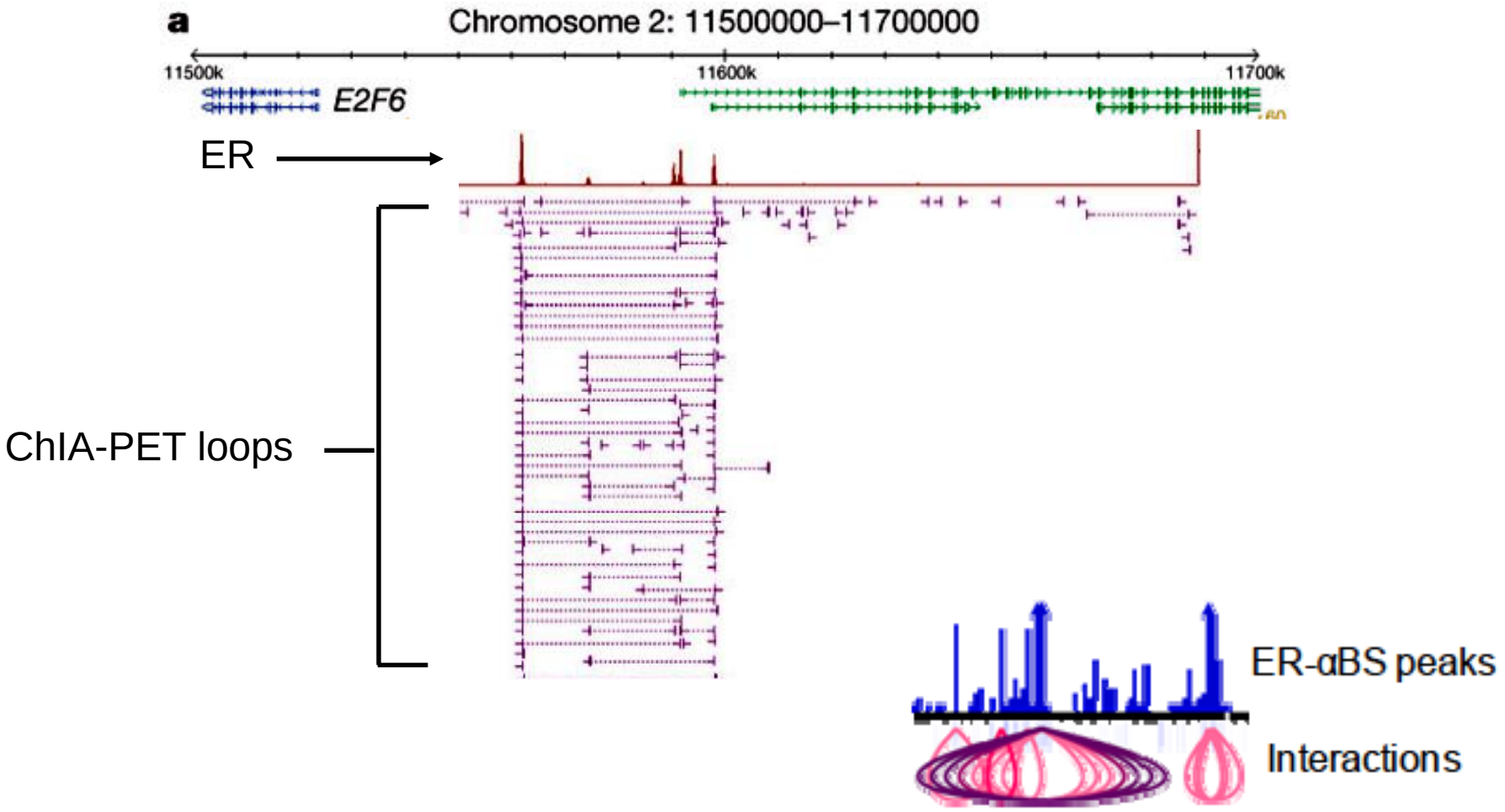




ChIP-Interaction Assay (ChIA) Sequencing (Paired End diTag: PET)



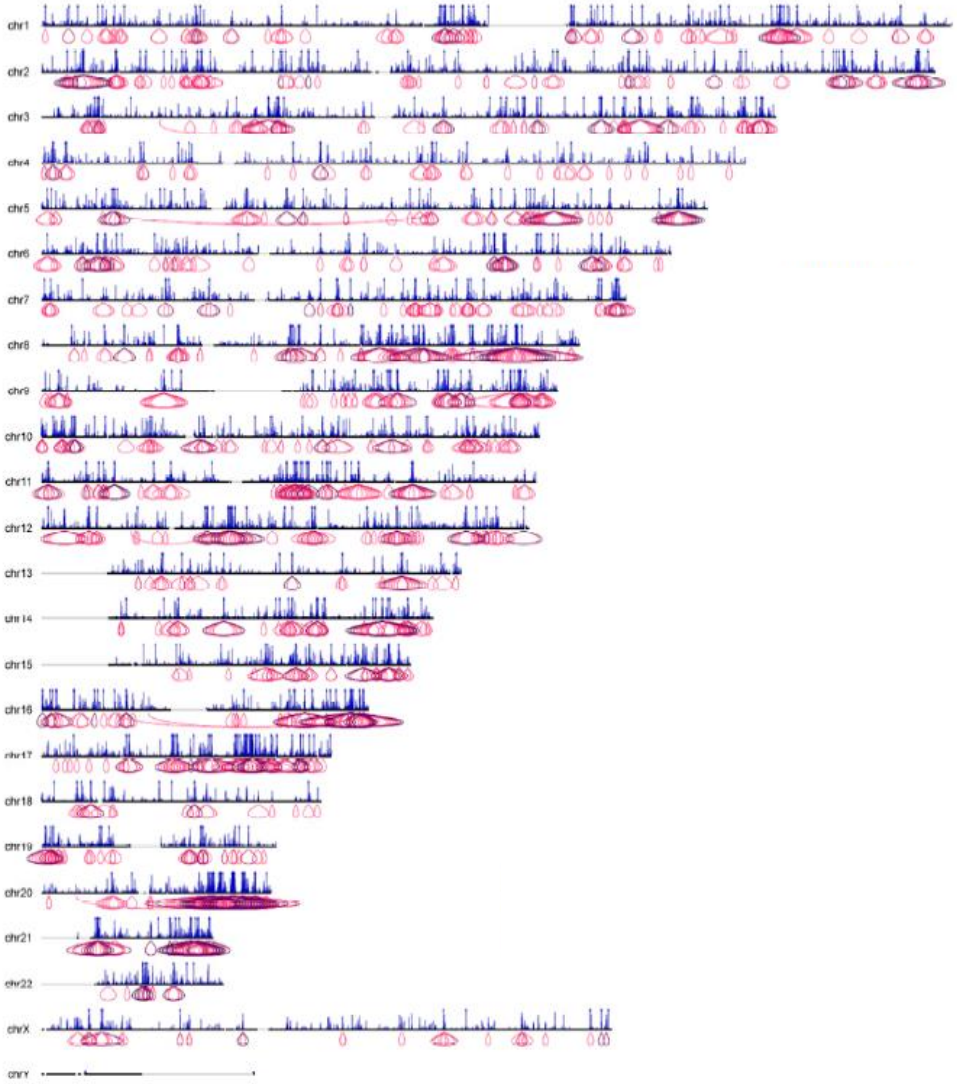
Discovery of what parts of the genome loop together



Fullwood, *Nature*, 2009

The ER-mediated interactome in breast cancer cells

Chromosome 1



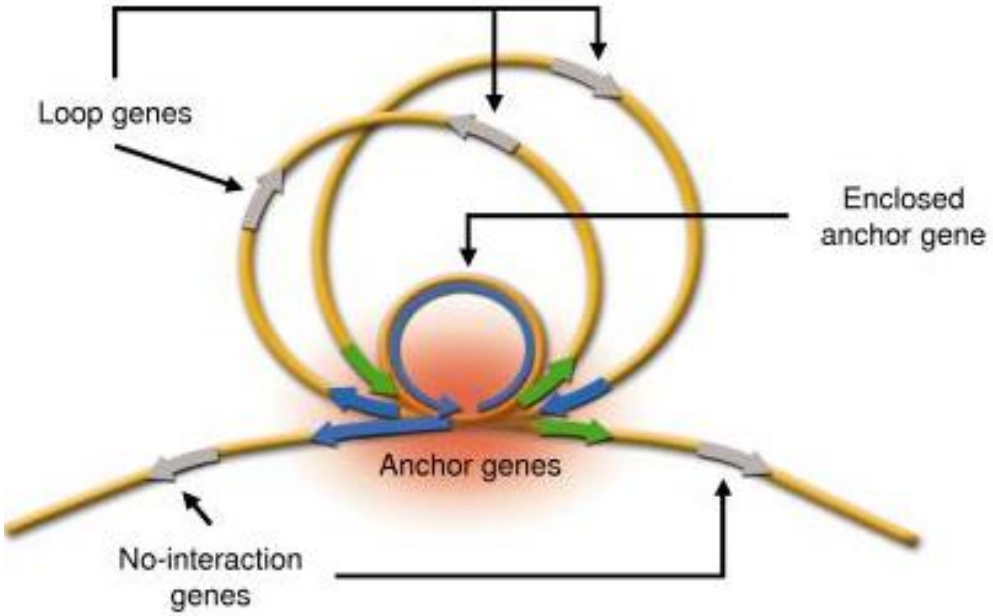
Chromosome X

Chromosome Y

Fullwood, *Nature*, 2009

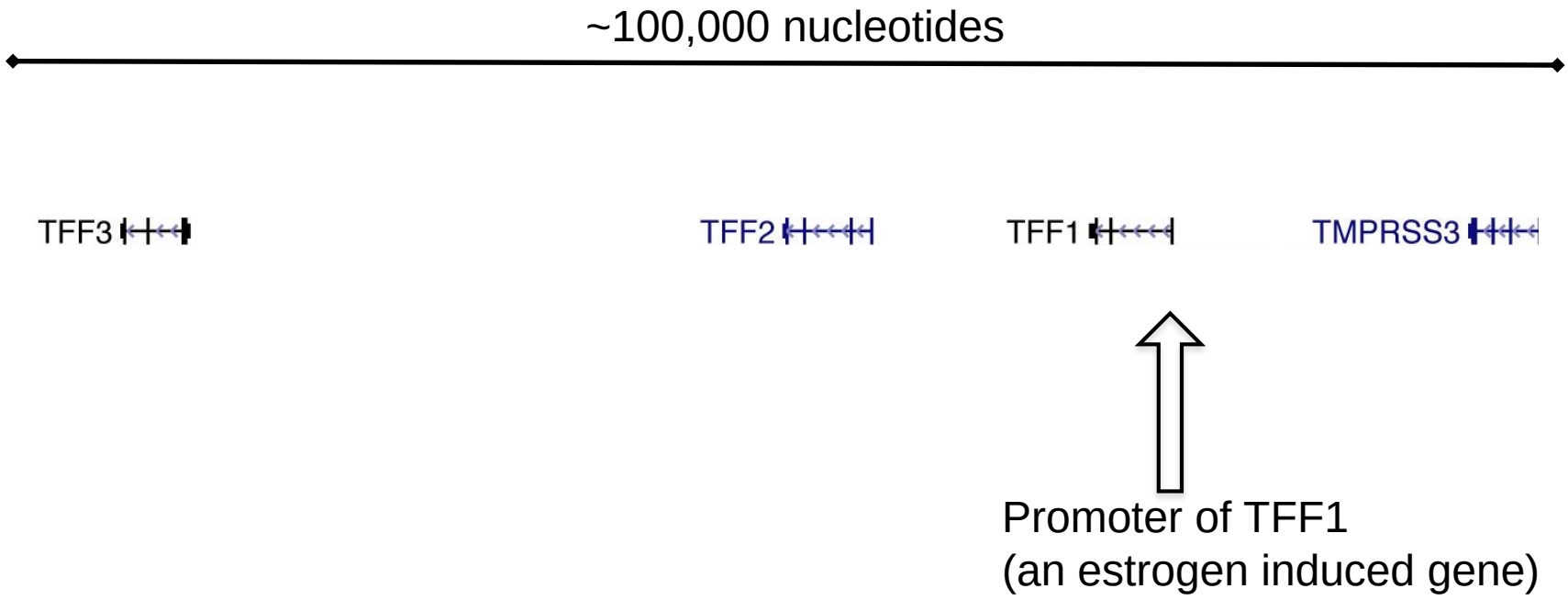
- No interchromosomal interactions!

ER binding events tend to work together in clusters



Fullwood, *Nature*, 2009

What is really being transcribed by ER?



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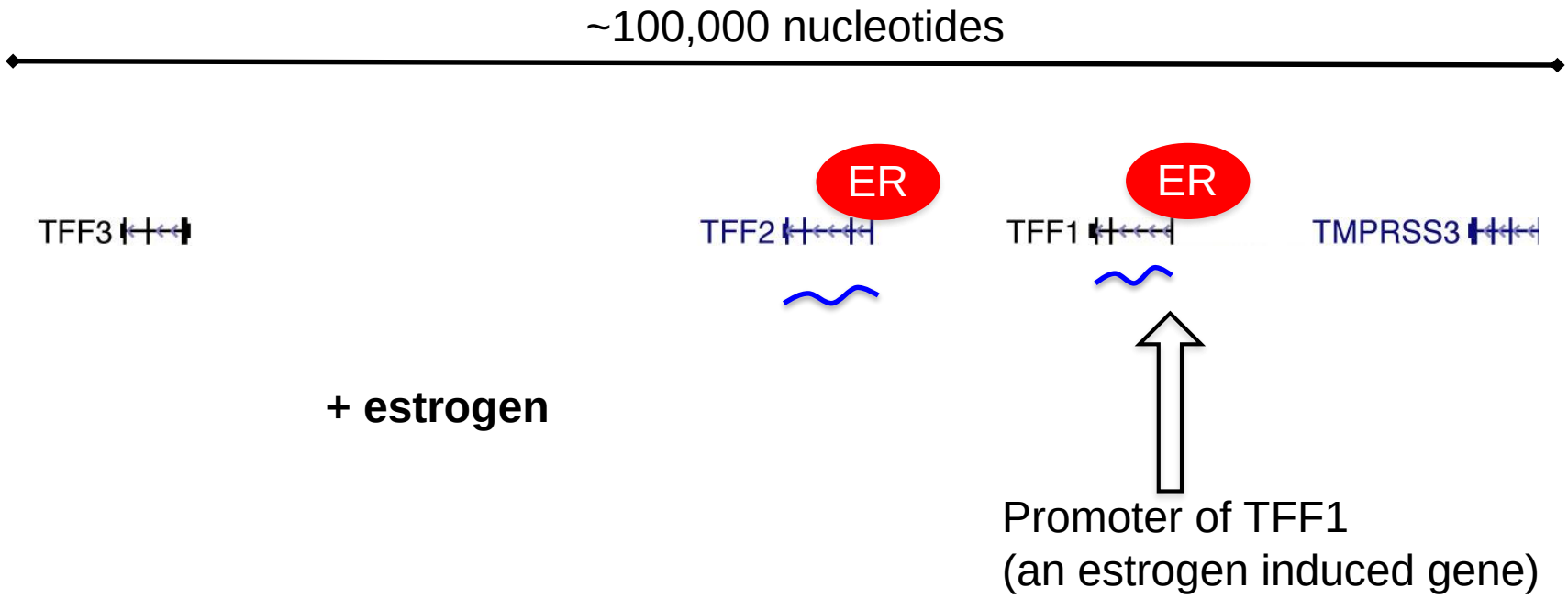
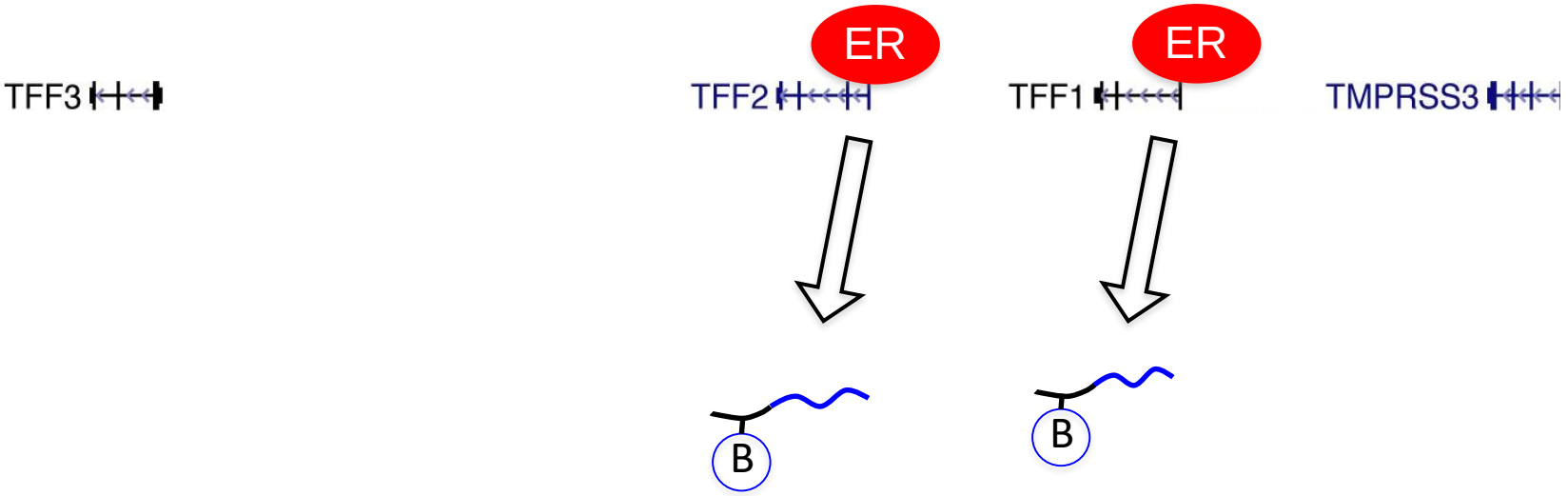


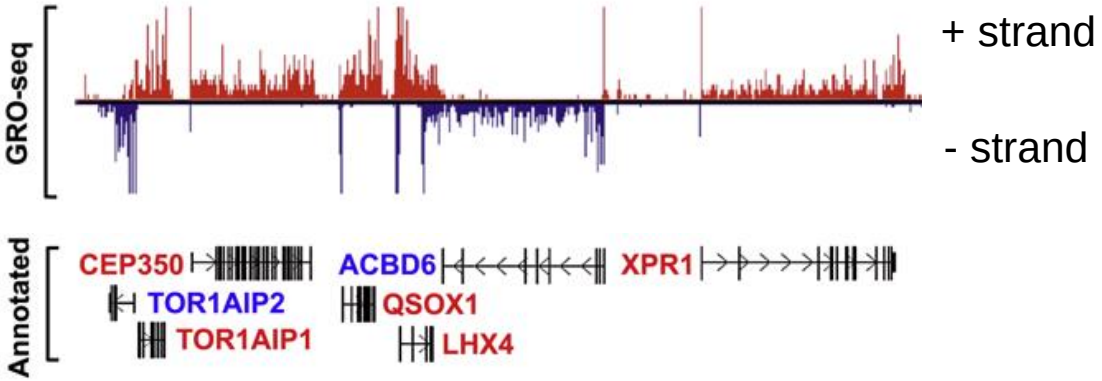
GRO-seq reveals massive global transcription



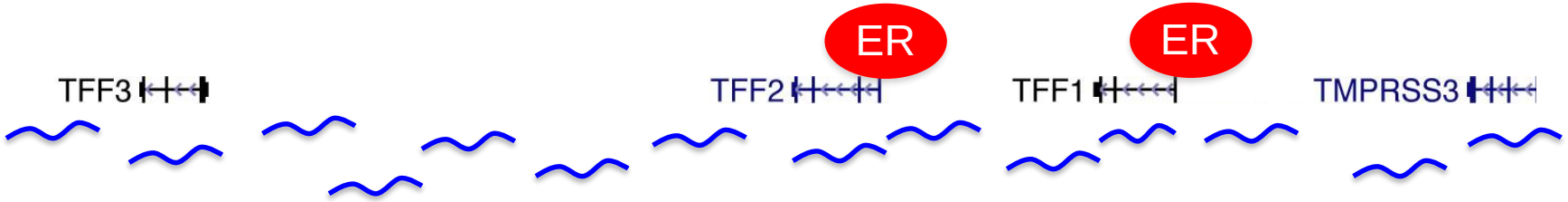
Nuclear run on
(incorporate biotin dNTP)

Purify biotin

Sequence everything



(Hah, *Cell*, 2011)

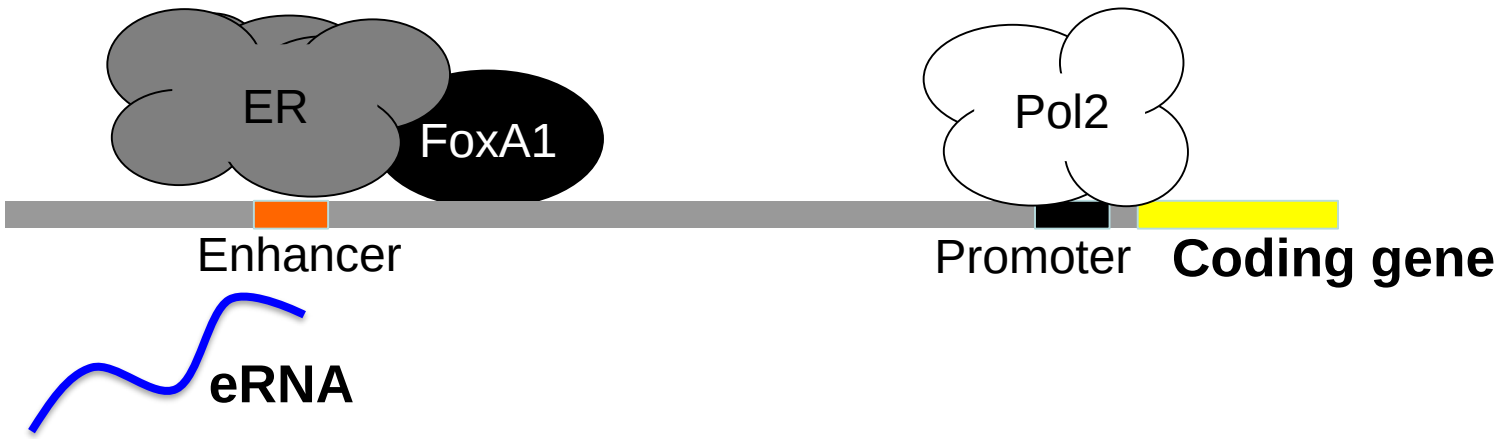


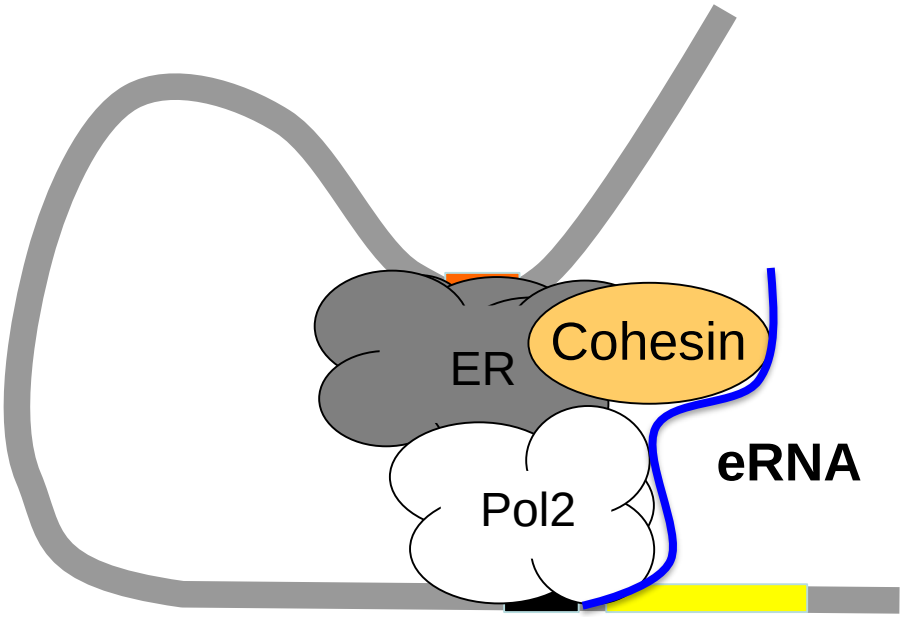
+ estrogen

- 27% of the genome is transcribed in response to estrogen

Of these
- 50% annotated or non-coding RNAs
- 5% to antisense transcripts
- 16% divergent transcripts
- 7% to ER enhancers (eRNAs)
- 12% un-annotated intergenic transcripts

Enhancer RNAs (eRNAs) play a role in transcription





- Required for coding gene
- Required for growth

Transcription factors as drug targets

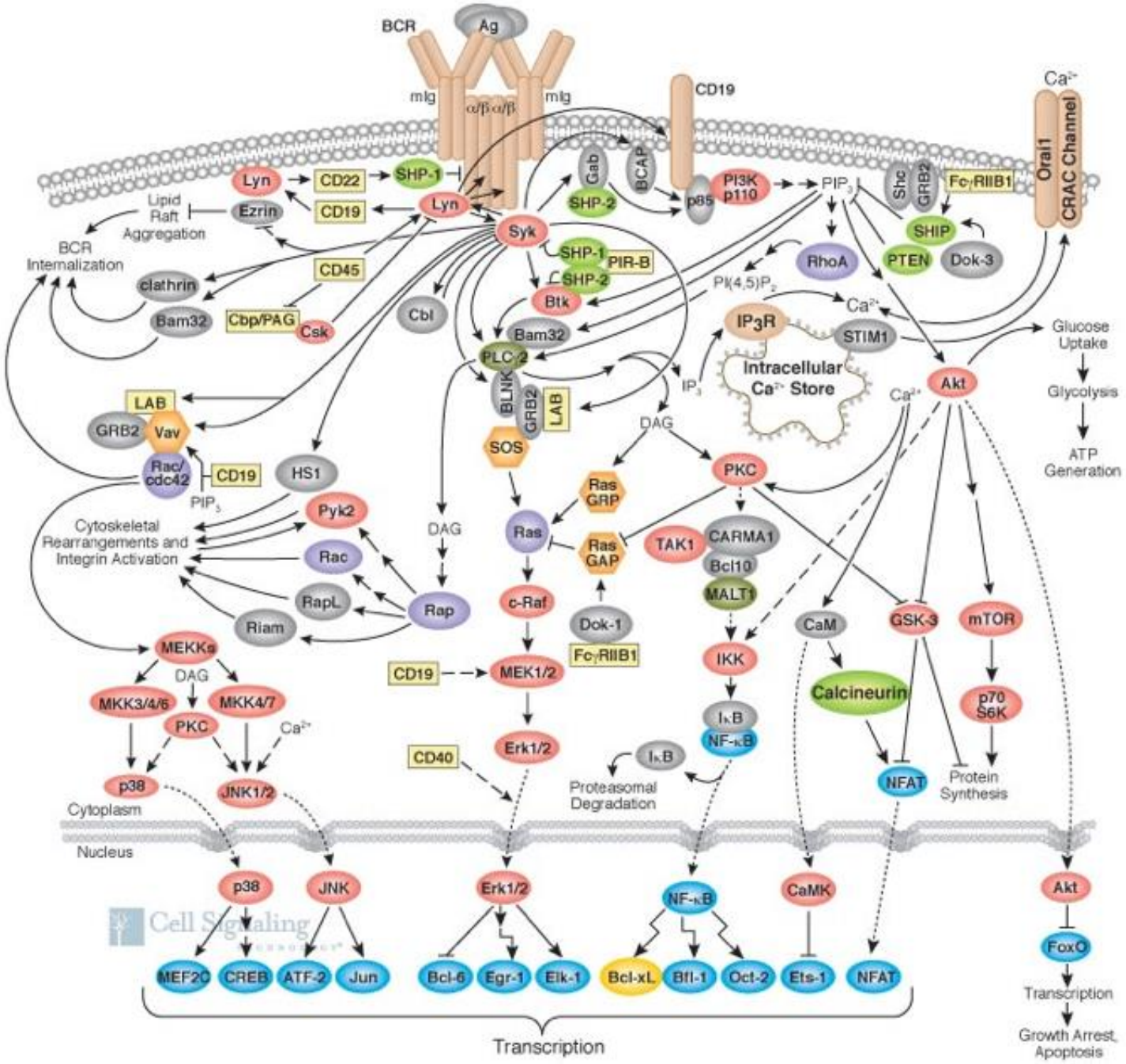
- Widely considered ‘undruggable’

- Most common treatment targets a TF (GR)

- In breast and prostate cancer: drugs target TFs (NR)

- Are nuclear receptors the exception for TFs?

- Novel approaches for drugging TFs
  - *Peptides/riptides*
  - *siRNA/knockdown*
  - *Regulatory enzymes*
  - *Degradation mechanisms (PROTACs)*


BCR
Ag
mIg
α/β α/β
mIg
CD19
Ca2+
Orai1
CRAC Channel
Lyn
CD22
SHP-1
Lyn
Gab
BCAP
SHP-2
p85
PI3K p110
PIP3
Shc
GRB2
FcγRIIB1
SHIP
PTEN
Dok-3
RhoA
Lipid Raft Aggregation
Ezrin
CD19
Syk
BCR Internalization
clathrin
Bam32
CD45
Cbp/PAG
Csk
SHP-1
SHP-2
PIR-B
Btk
Cbl
Bam32
PLCγ2
BLNK
GRB2
LAB
SOS
PI(4,5)P2
Ca2+
IP3R
STIM1
Intracellular Ca2+ Store
IP3
Ca2+
Akt
Glucose Uptake
Glycolysis
ATP Generation
LAB
GRB2
Vav
Rac/ cdc42
CD19
PIP3
HS1
Pyk2
Rac
RapL
Riam
Rap
Cytoskeletal Rearrangements and Integrin Activation
DAG
Ras GRP
Ras
Ras GAP
c-Raf
Dok-1
FcγRIIB1
MEK1/2
Erk1/2
CD19
CD40
DAG
PKC
TAK1
CARMA1
Bcl10
MALT1
IKK
IκB
NF-κB
IκB
Proteasomal Degradation
CaM
Calcineurin
NFAT
GSK-3
mTOR
p70 S6K
Protein Synthesis
MEKKs
DAG
MKK3/4/6
MKK4/7
PKC
Ca2+
p38
JNK1/2
Cytoplasm
Nucleus
p38
JNK
Erk1/2
NF-κB
CaMK
Akt
FoxO
Transcription
Growth Arrest, Apoptosis
MEF2C
CREB
ATF-2
Jun
Bcl-6
Egr-1
Elk-1
Bcl-xL
Bfl-1
Oct-2
Ets-1
NFAT
Transcription
Cell Signaling