Transcription Factors

ChIP-seq measures TF binding to DNA.

ChIP-seq also measures histone modification, cofactor, and RNA Polymerase genomic locations—however, their occupancy are a consequence of TF binding.

Mike Guertin

Broad lecture goals:

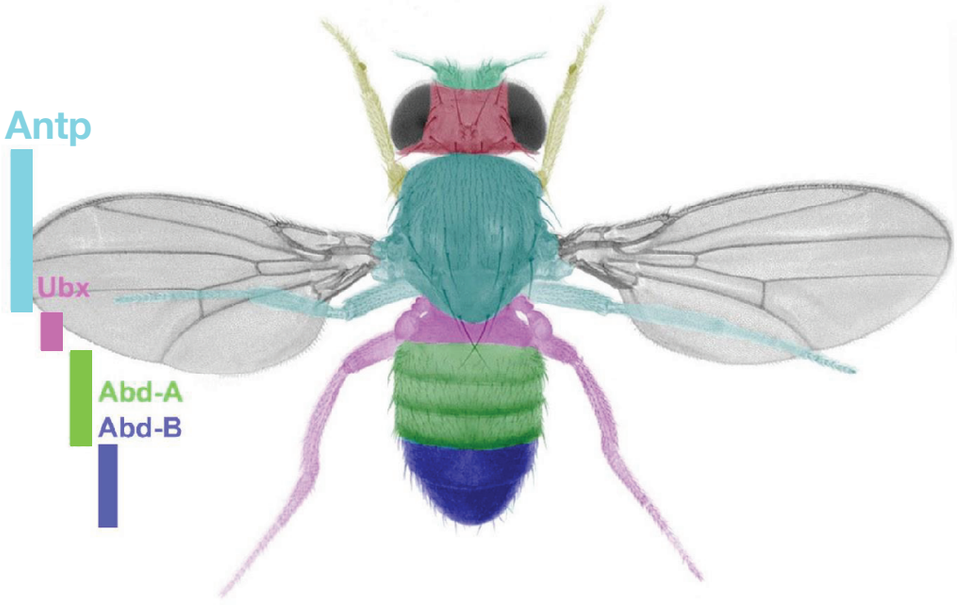
- Convince you of the importance of transcription factors in providing specificity in chromatin biology.

- Introduce classic experiments that defined principles of TF biology and provide references so one can follow up. Note that most molecular biology was interpreted through looking at bands on gels. **As a graduate student, you should aim to be able to take a well-written figure legend and figure and interpret the results.**

- Illustrate the point that biology is continuous, not discrete; relative quantification and controls are important.

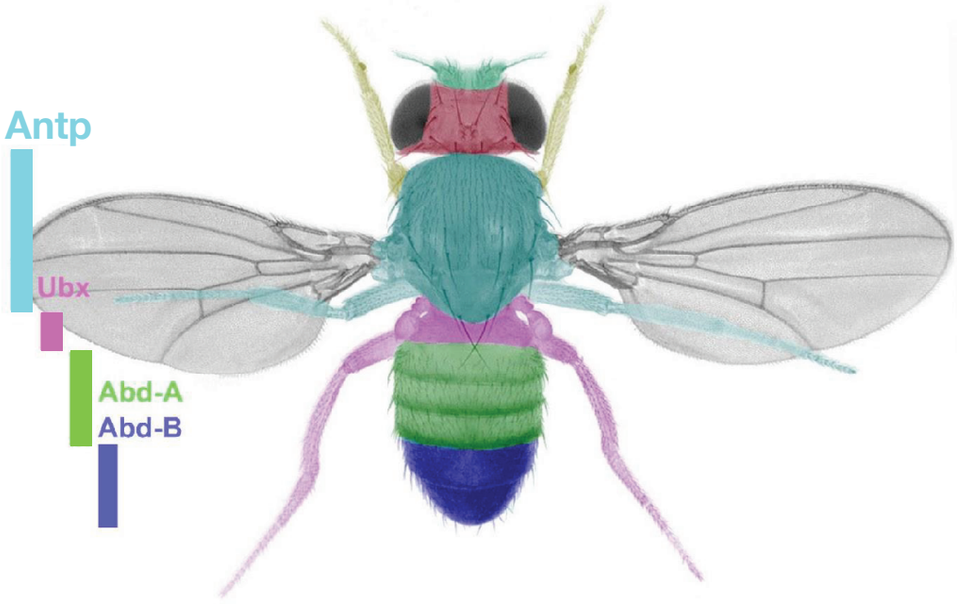
- Emphasize the role of question-driven exploratory experiments (screens, molecular genomics, unbiased proximity label transfers, solving structures, etcetera) in defining principles of transcription factor biology.

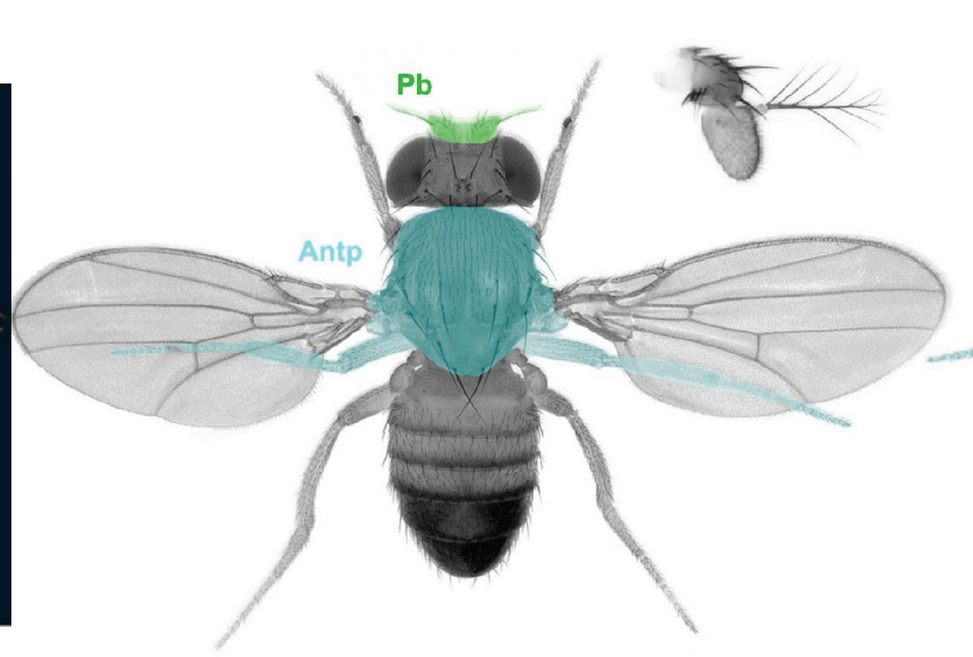
Transcription dysregulation alters developmental patterning



pseudocolored flies: Justin Crocker, Ed Lewis, Nicolas Gompel, and Welcome Bender

Transcription dysregulation alters developmental patterning





pseudocolored flies: Justin Crocker, Ed Lewis, Nicolas Gompel, and Welcome Bender

pseudocolored SEM heads: Jürgen Berger

Classic genetics (**perturb, observe, map**) found that Transcription Factors control developmental patterning

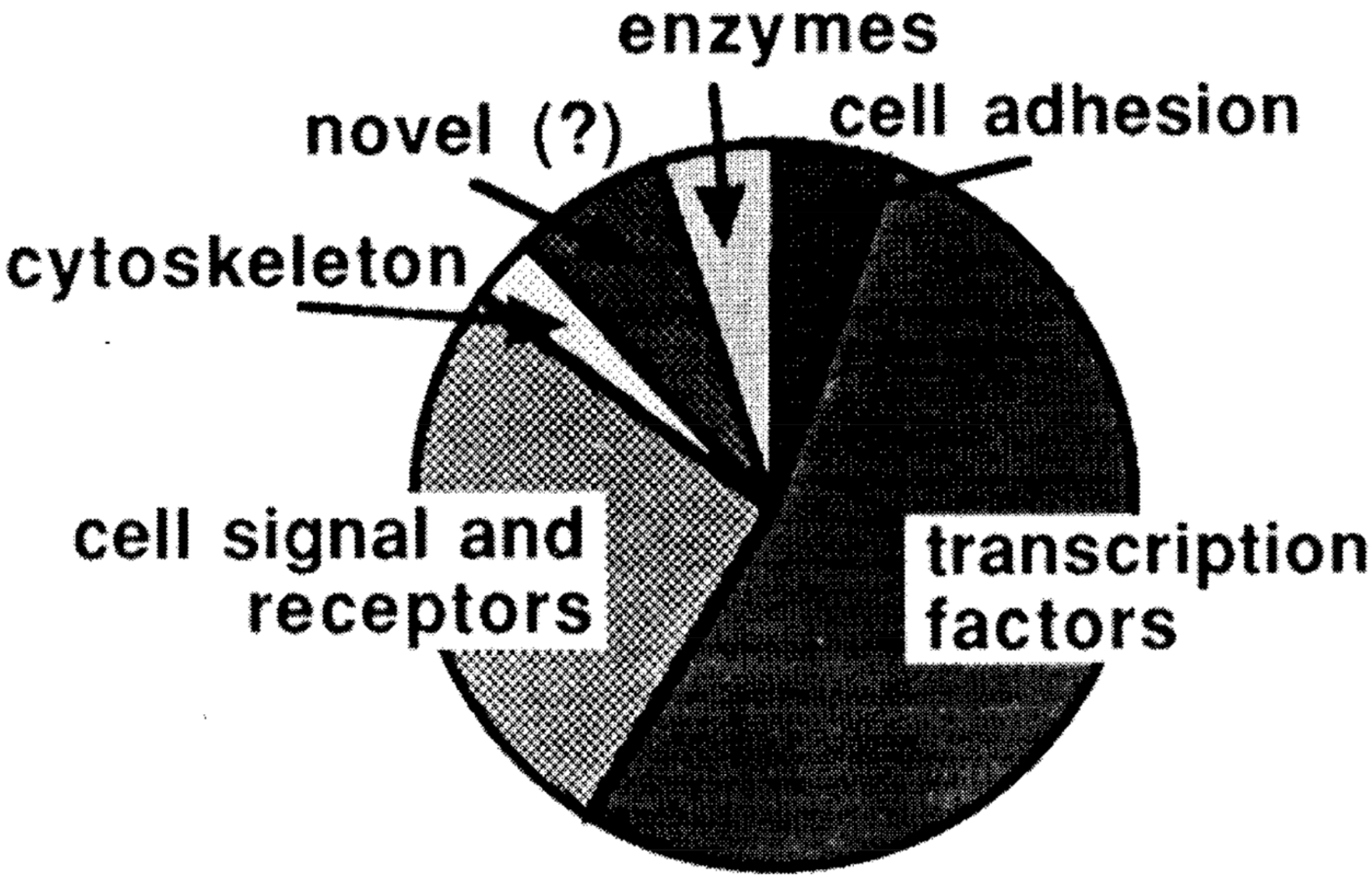
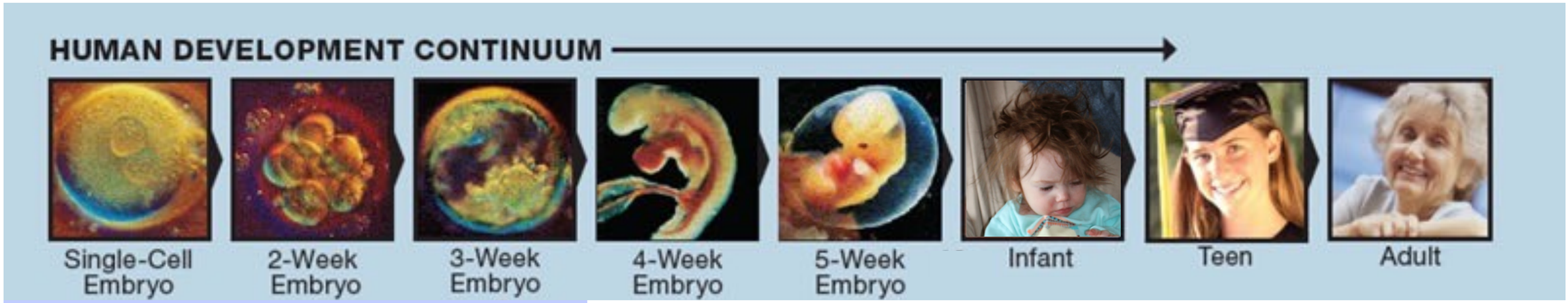
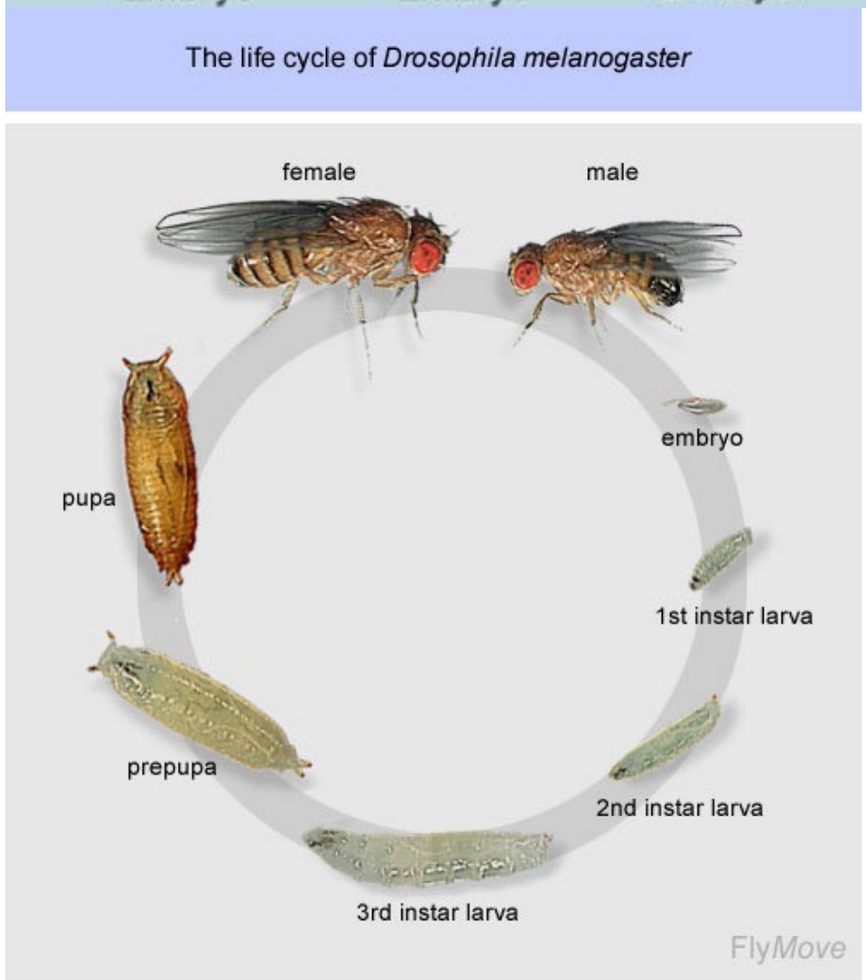


Figure 3. *Cellular Function of Heidelberg Mutations.* Based on the sequence of 75 cloned genes, most of the loci identified in Heidelberg encode transcription factors, or cell signals and receptors.

Eric Weischaus, Nobel Lecture 1995

Transcription control is key in development and homeostasis





Embryonic cells progress from totipotent to a spectrum of more specialized states.

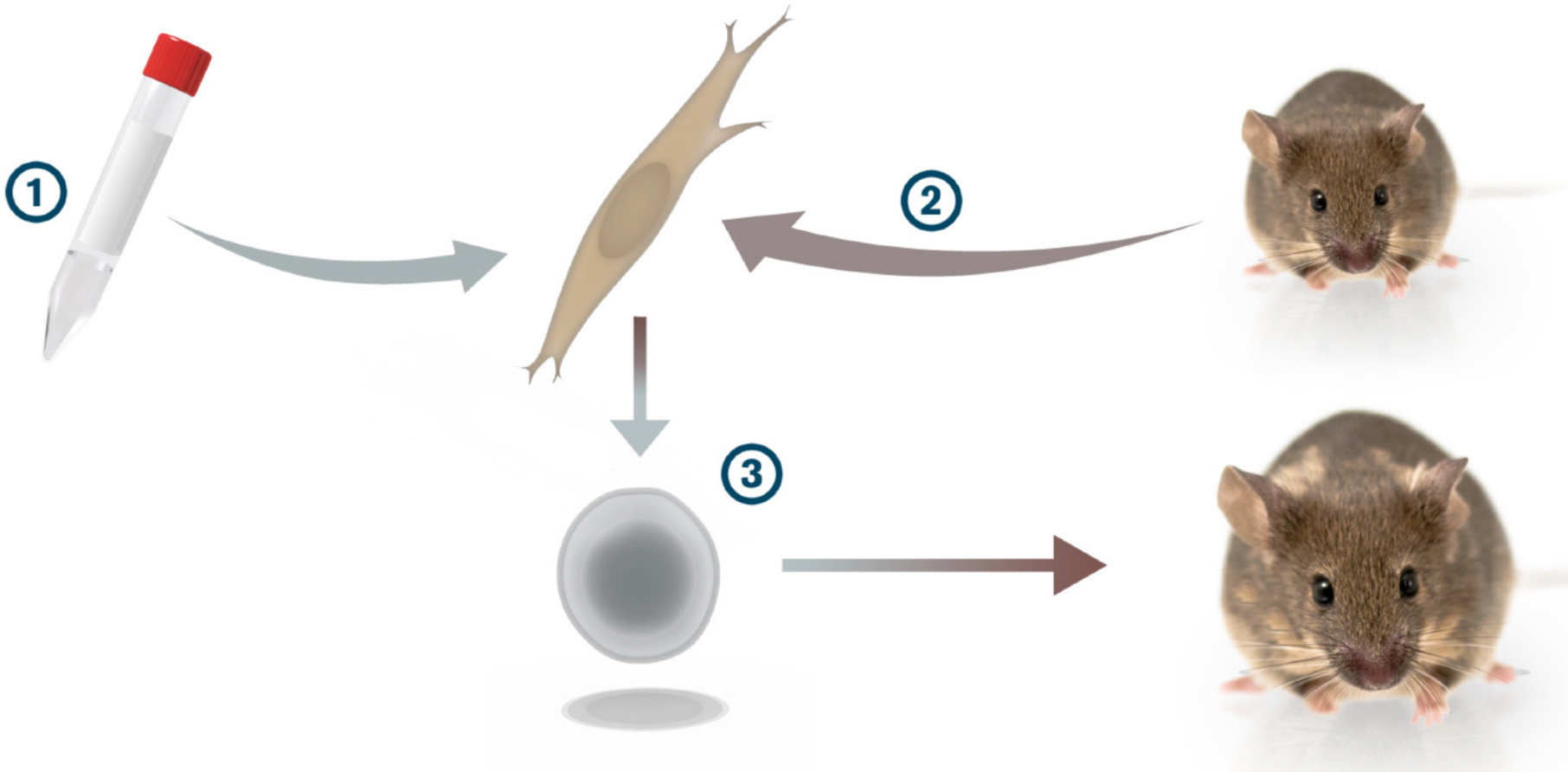
Much of this developmental regulation starts at transcription.

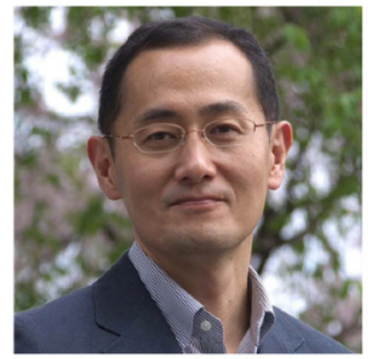
Cells need to respond to changing nutrients and environments.

Organisms have sophisticated programs of transcription regulation.

2012 Nobel in Physiology or Medicine:

"for the discovery that mature cells can be reprogrammed to become pluripotent"

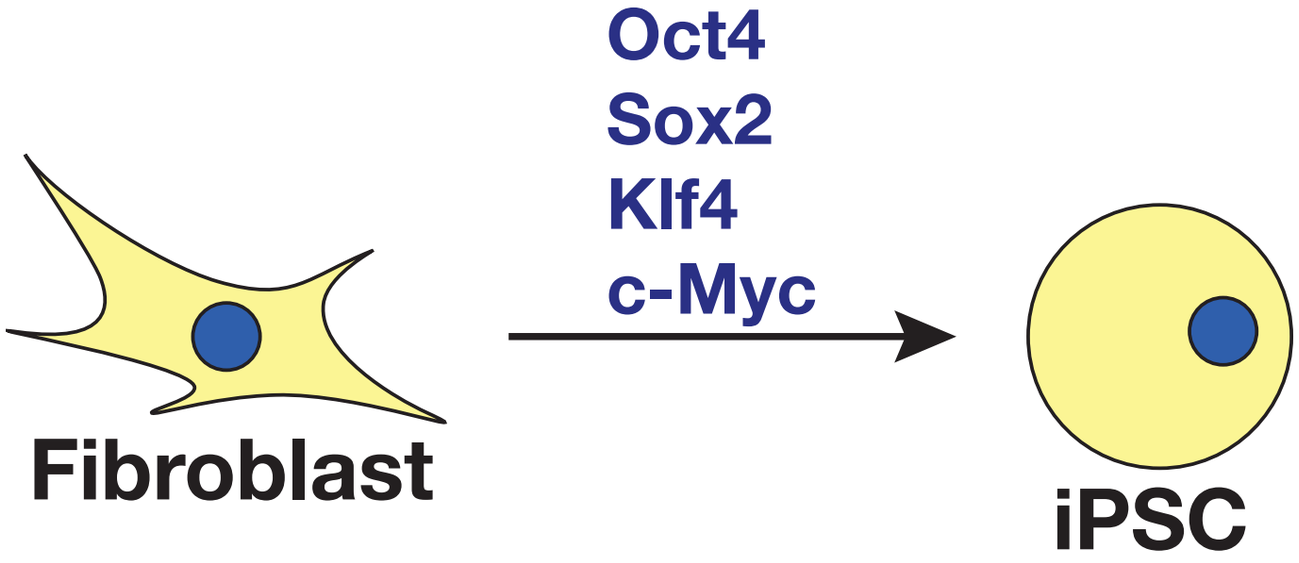




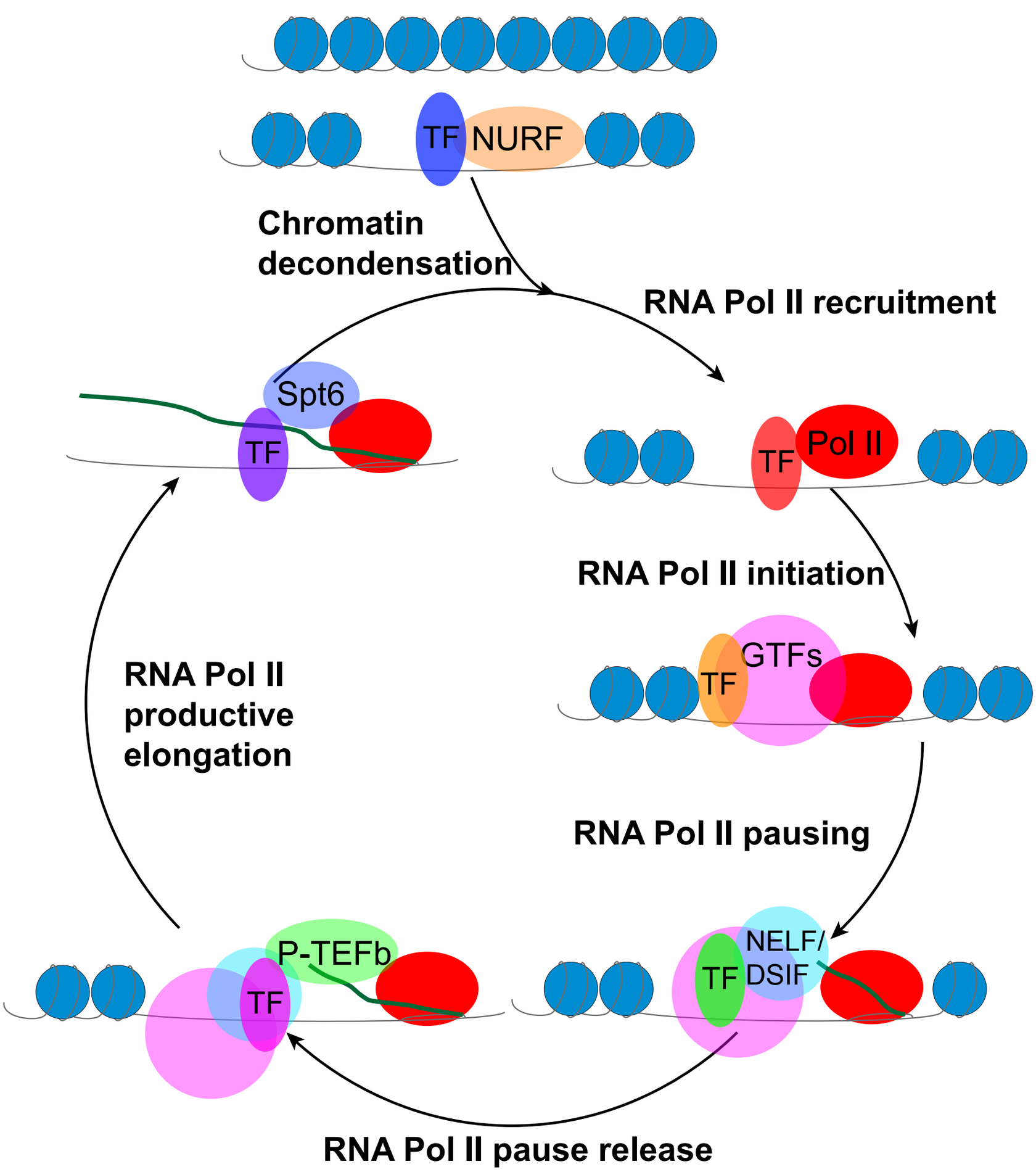
Shinya Yamanaka

Shinya Yamanaka studied genes that are important for stem cell function. When he transferred four such genes (1) into cells taken from the skin (2), they were reprogrammed into pluripotent stem cells (3) that could develop into all cell types of an adult mouse. He named these cells induced pluripotent stem (iPS) cells.

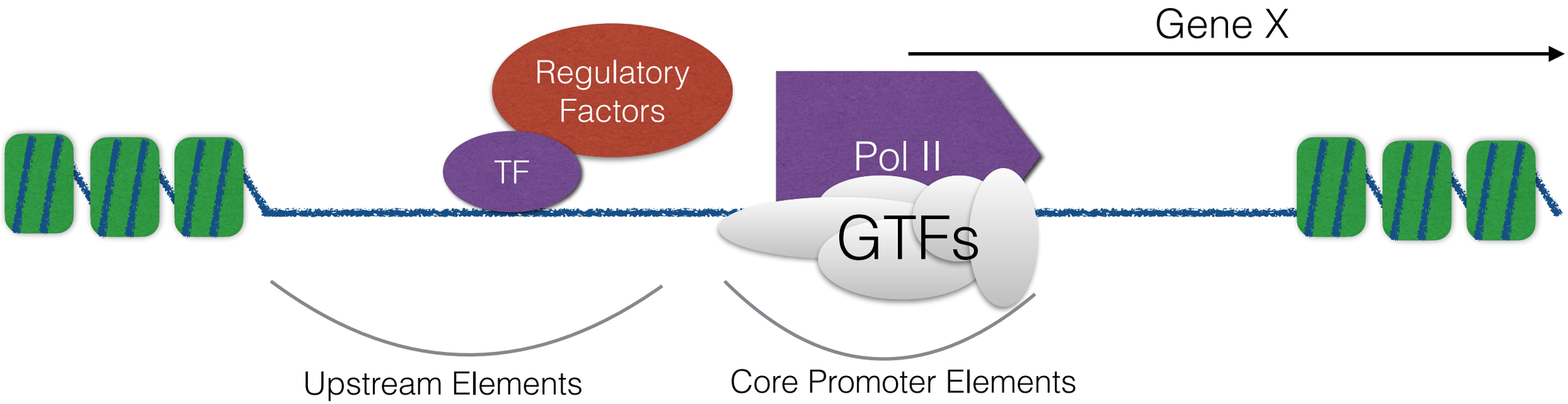
Activating transcription factors changes cell identity



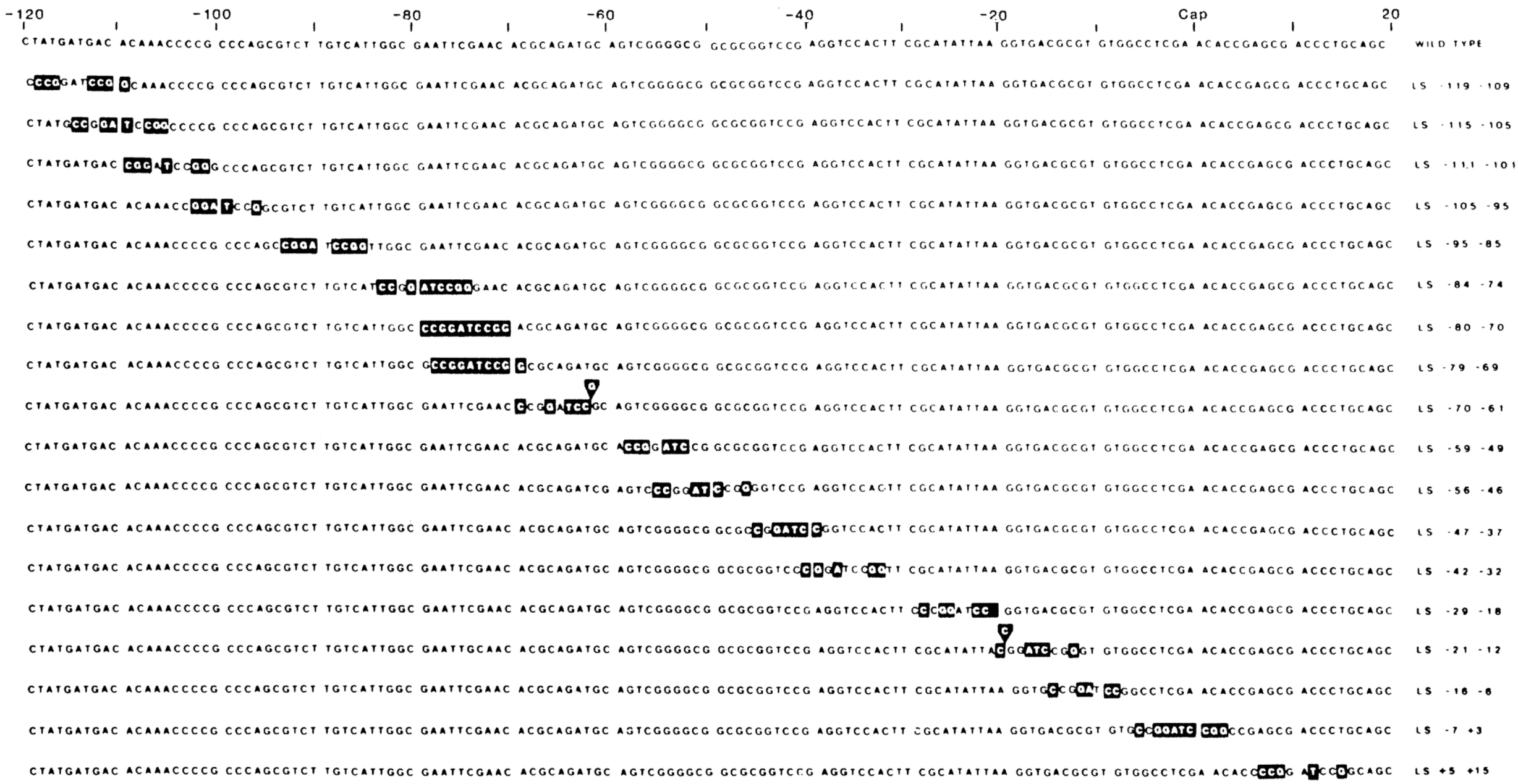
Takahashi & Yamanaka, Cell 2006


TF
NURF
Chromatin
decondensation
RNA Pol II recruitment
Spt6
TF
TF
Pol II
RNA Pol II initiation
GTFs
TF
RNA Pol II
productive
elongation
RNA Pol II pausing
P-TEFb
TF
NELF/
DSIF
TF
RNA Pol II pause release

Transcription Regulation by Transcription Factors (TFs) is determined by DNA sequence



Linker Scanning Mutations of the thymidine kinase gene of HSV



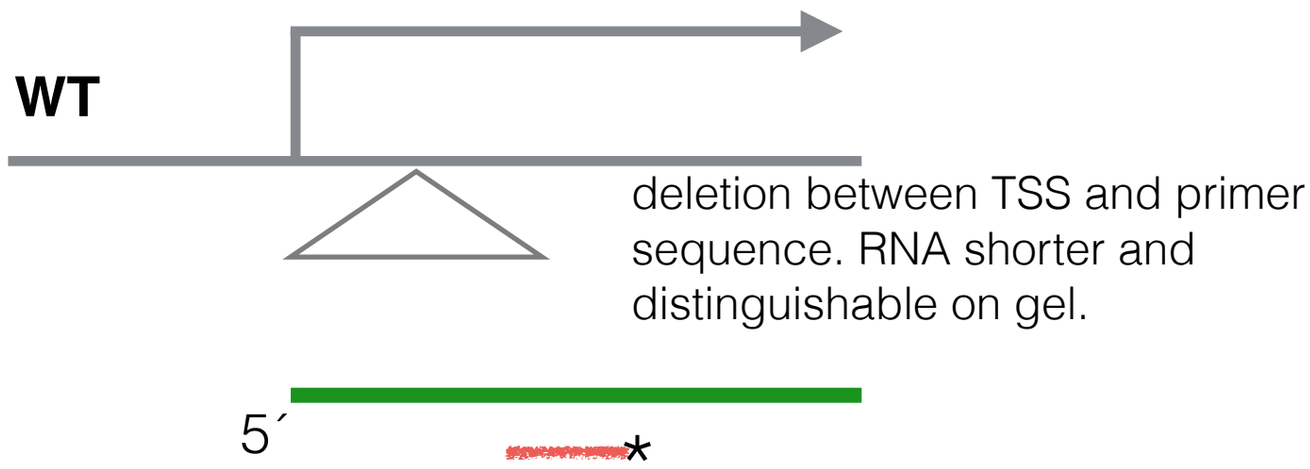
Clusters of point mutants are generated at the point of joining 5´ and 3´ deletions, where linker sequence substitutes for tk sequence.

Mcknight and Kingsbury, Science 1982

Assay expression of tk promoter mutants

Plasmid DNAs

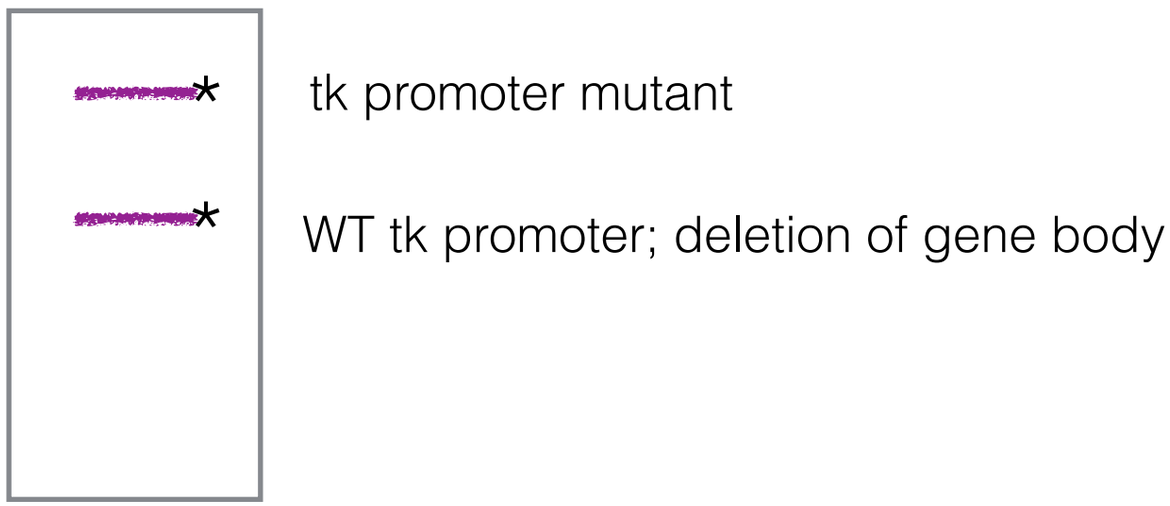
Inject mutant DNA into
frog oocyte nuclei, include **pseudo WT**
standard as internal control



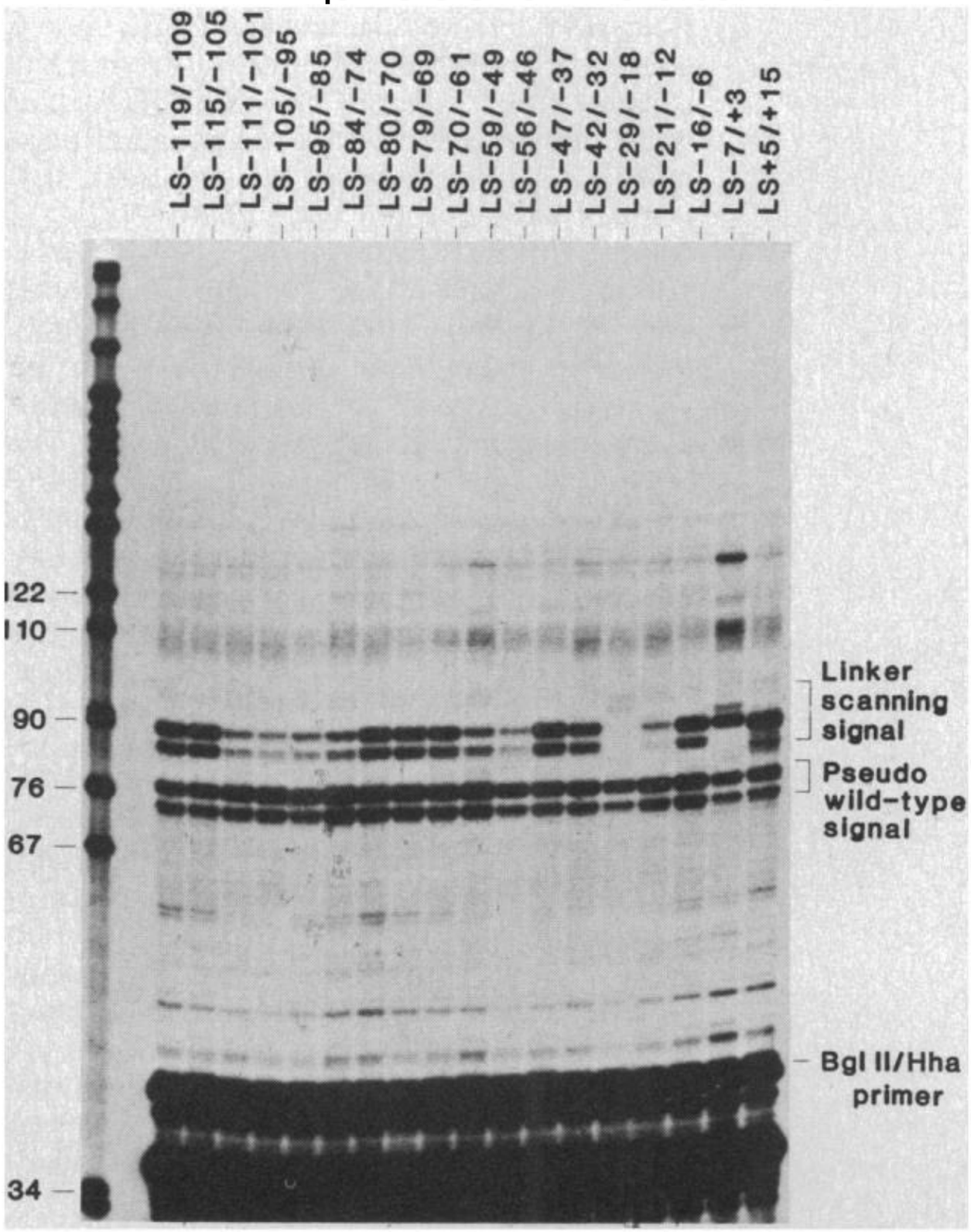
Isolate RNA

Primer Extension Assay

Gel Electrophoresis



Expression data from Linker Scanning Mutants

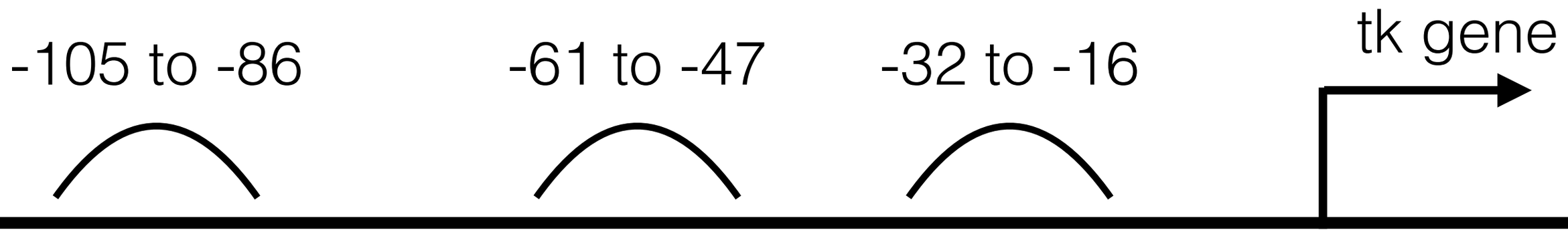


Short discontinuous regions of sequence are critical for basal expression.

Three promoter regions are critical for basal expression

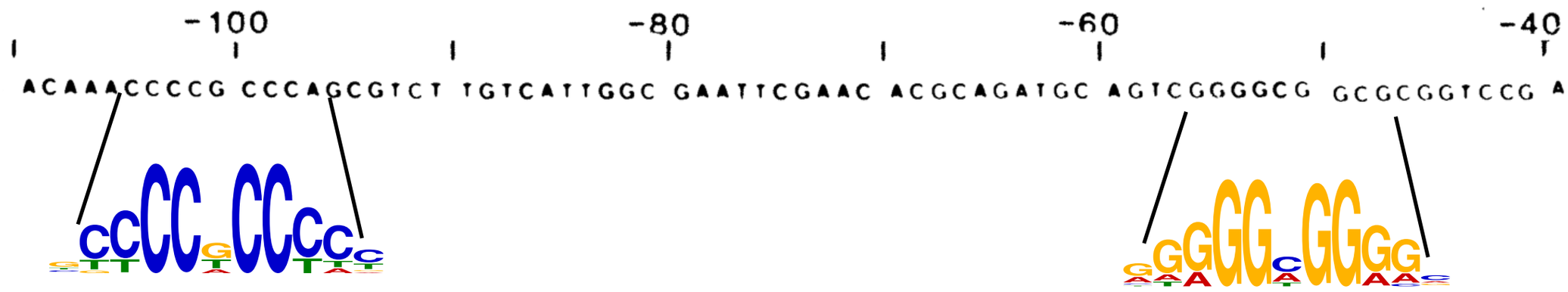
TATA element located approximately 30bp upstream of the TSS

Three promoter regions are critical for basal expression

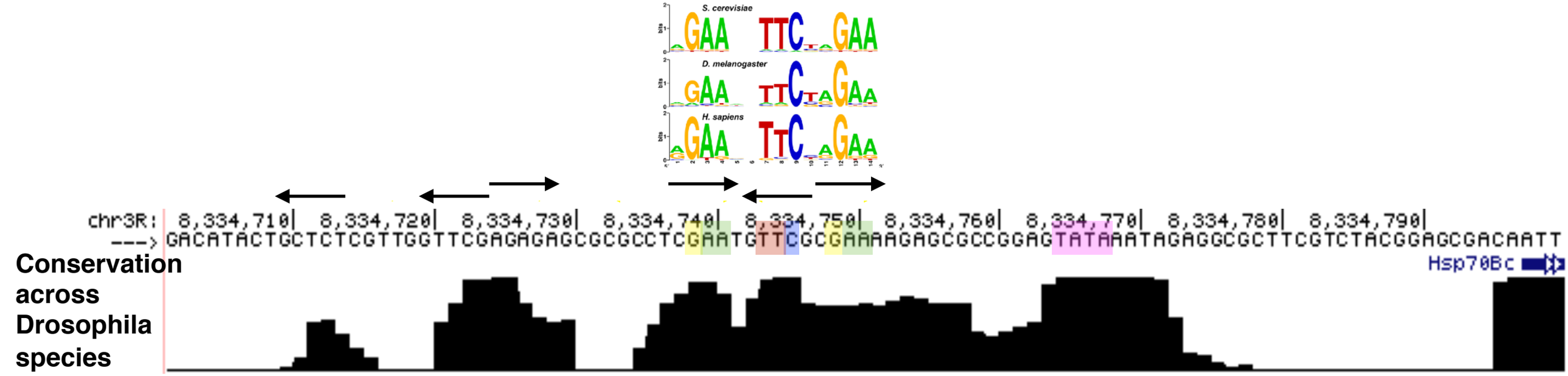


G/C-rich region

G/C-rich region



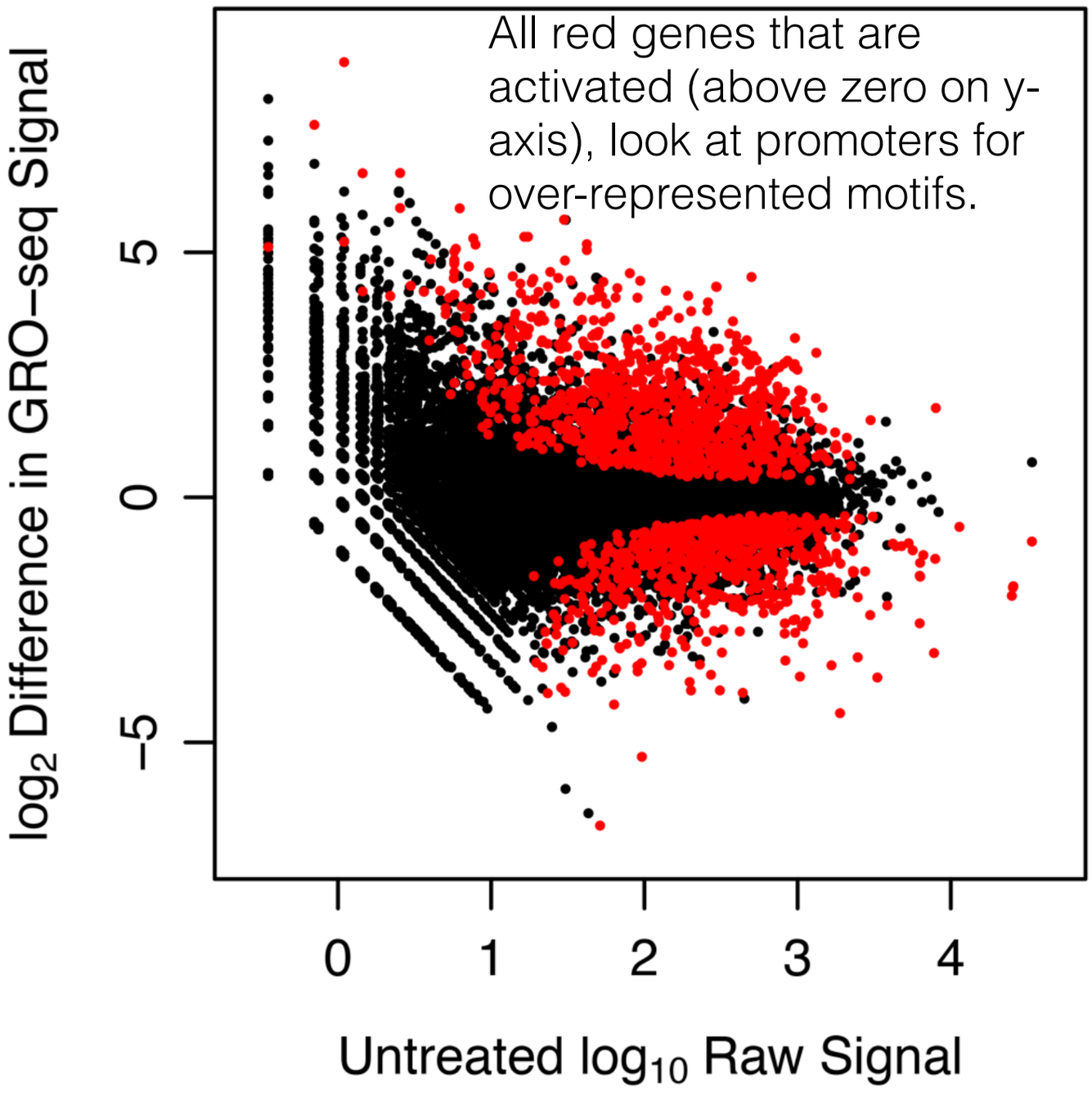
Evolutionary conservation and comparative genomics can identify crucial elements



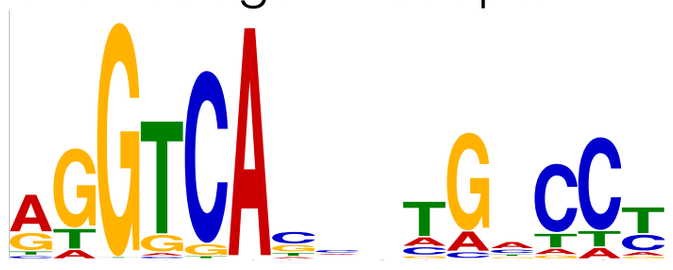
Significant changes in nascent transcription upon estrogen treatment in breast cancer cells

Collect the sequences of multiple (co-regulated) promoters within a species, search for common sequence motifs

Sustained Changes at both 10min and 40min



de novo motif analysis using MEME (or the alike) identifies the Estrogen Response Element, the known target of the Estrogen Receptor.

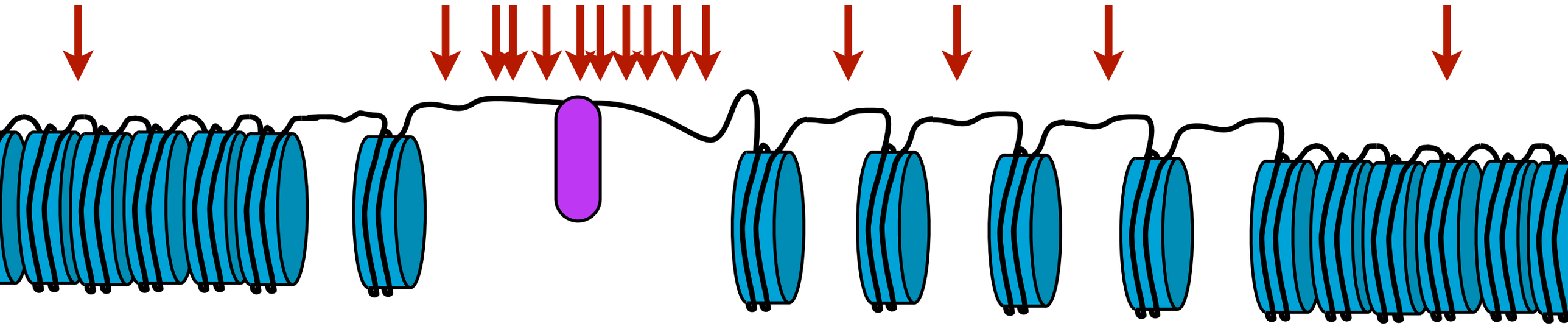


Note that not all regulatory elements bound by TFs are within promoters.

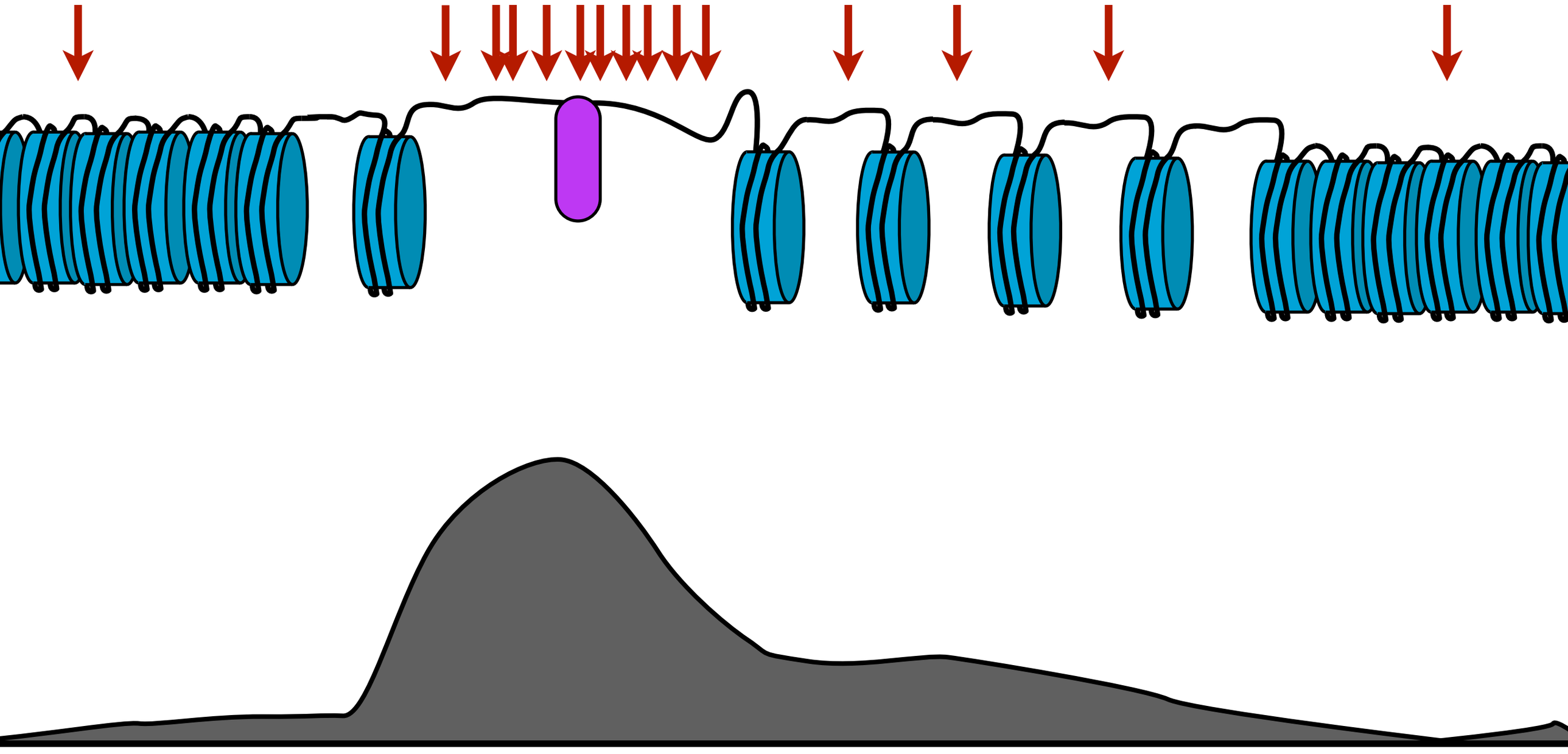
Identify All Active Regulatory Elements in a Cell Type: Enzyme Hypersensitivity (DNase-seq & ATAC-seq)

- A general measure of chromatin structure.
  - Factor/species-general
  - Changes in enzyme hypersensitivity landscape after drug treatment or throughout development can be used to identify novel regulatory elements and factors
  - Generally unbiased, but challenging to deconvolve
  - **TFs controlling chromatin landscape can be inferred from the data**

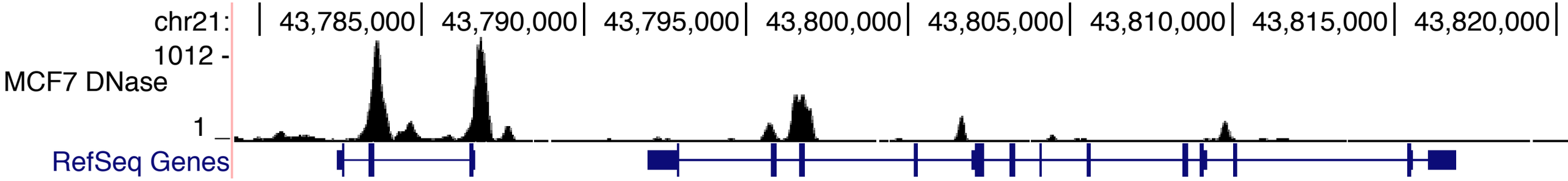
Enzyme Hypersensitivity



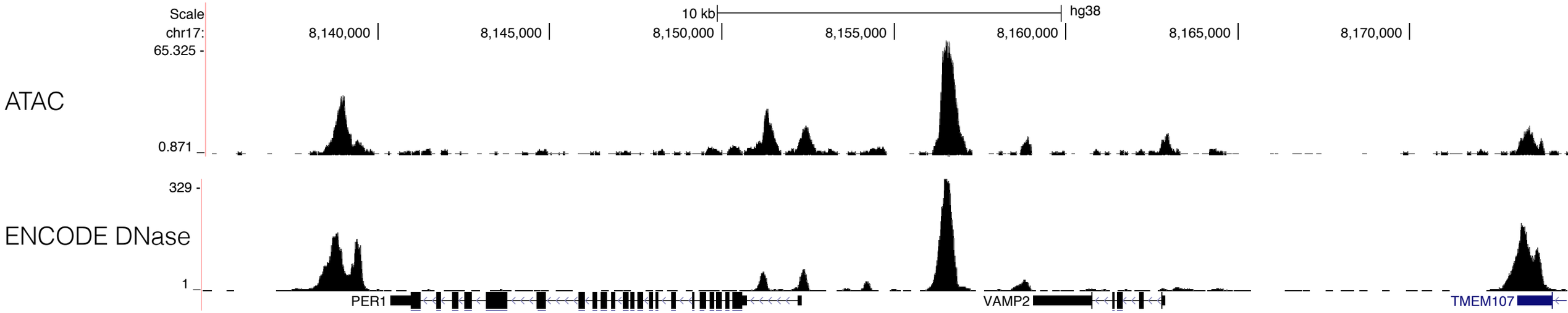
Enzyme Hypersensitivity



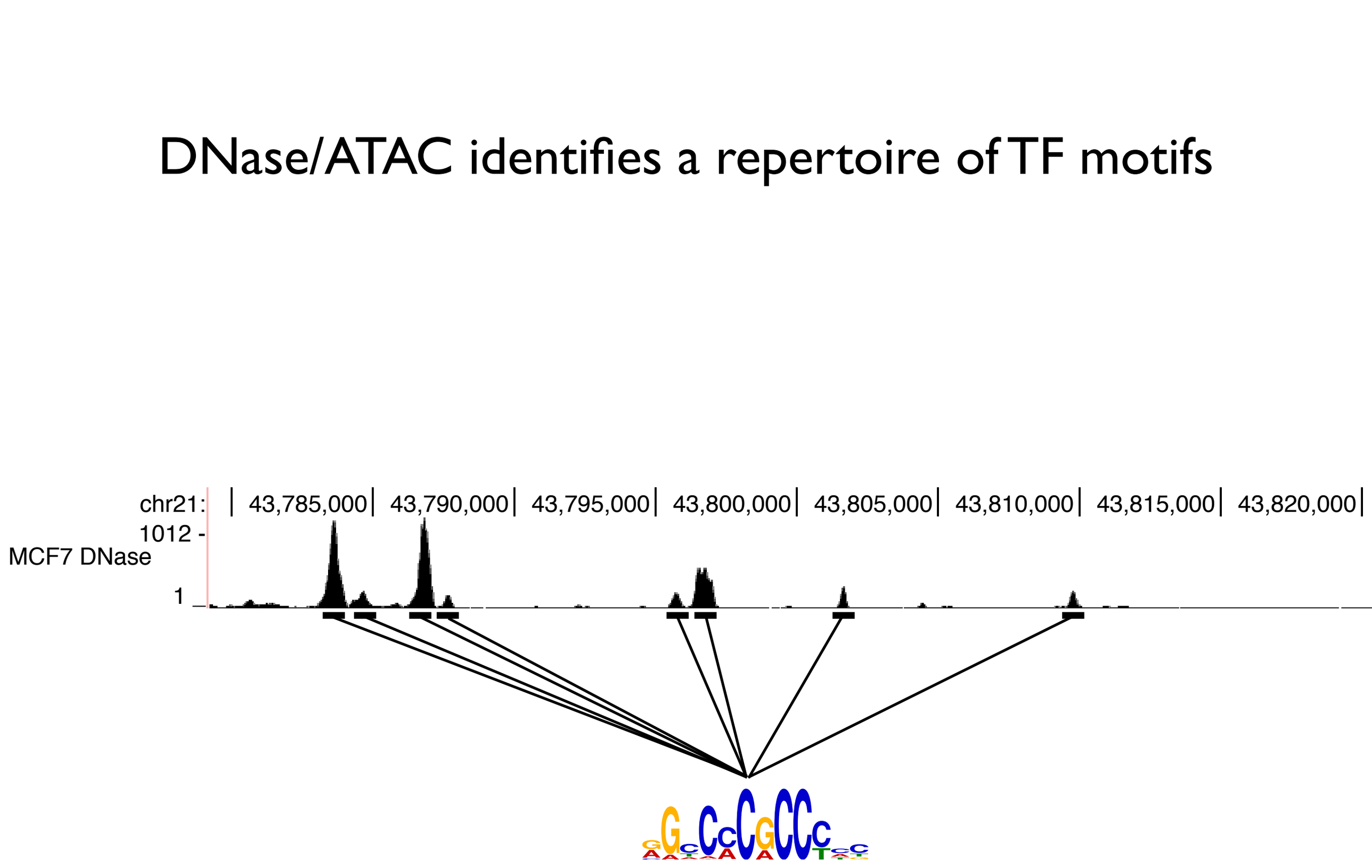
DNase-seq Data



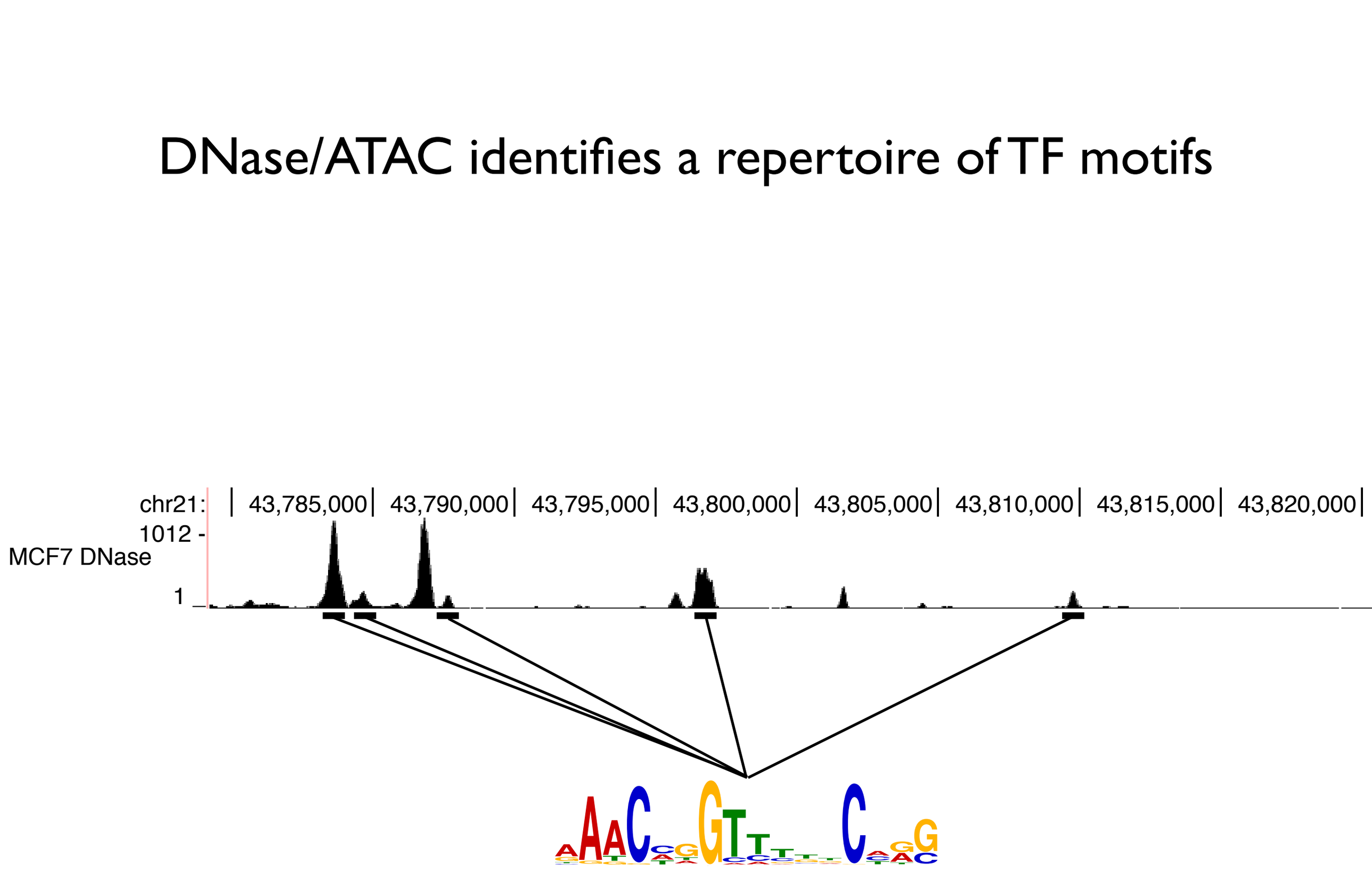
ATAC-seq vs. DNase-seq



DNase/ATAC identifies a repertoire of TF motifs

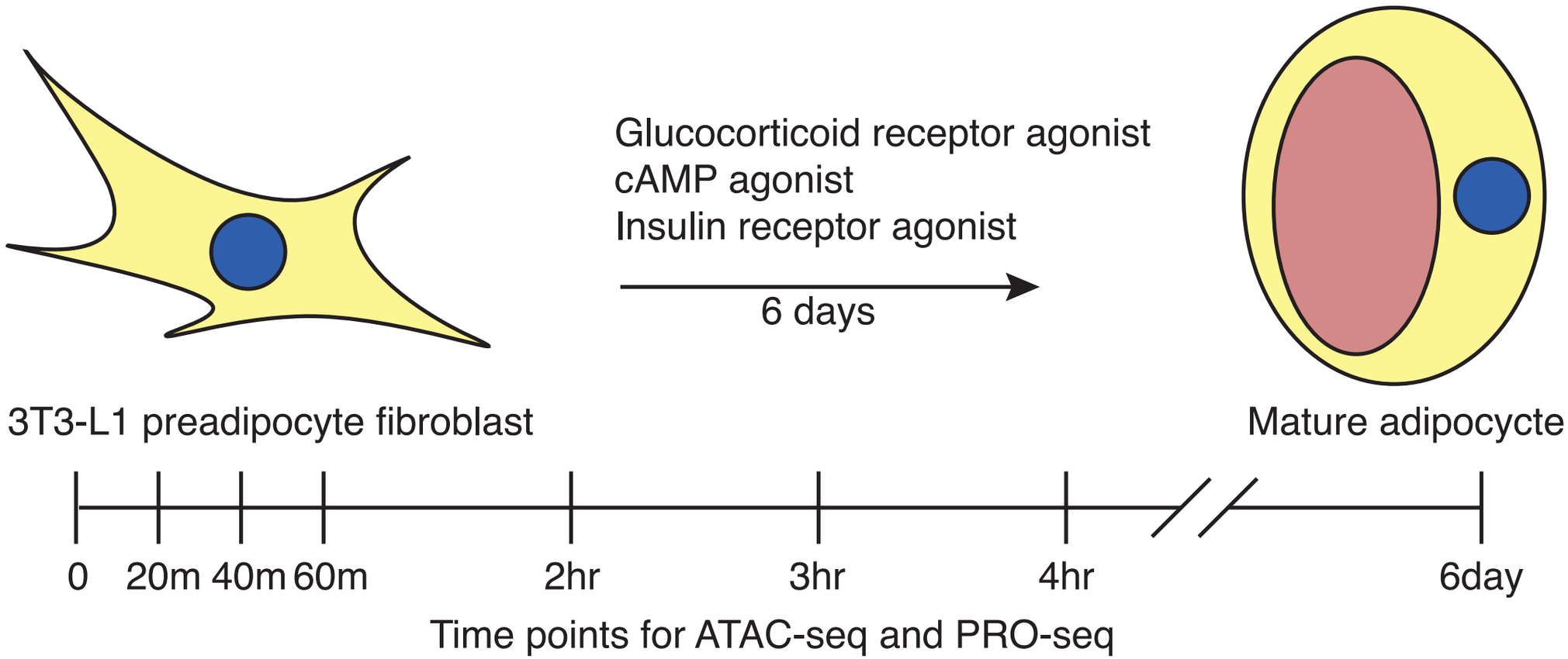


DNase/ATAC identifies a repertoire of TF motifs

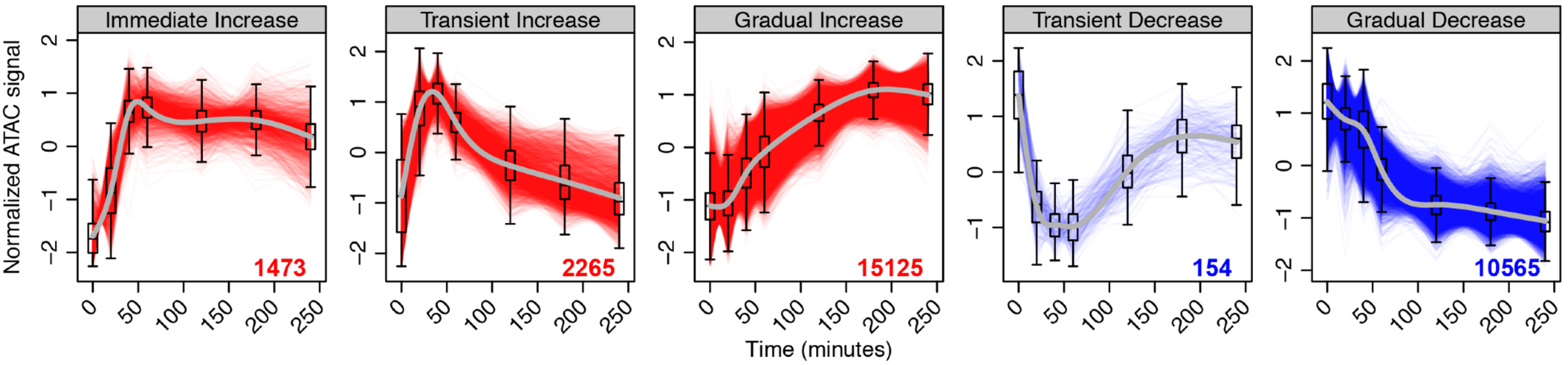


Identify sequence elements at hypersensitive site using iterative de novo motif analysis

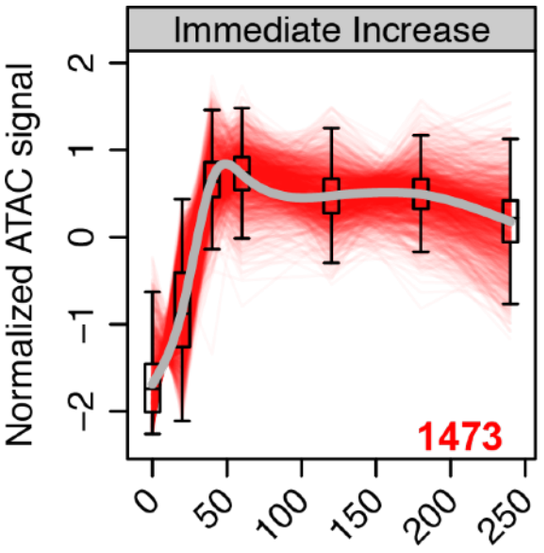
Experimental Design

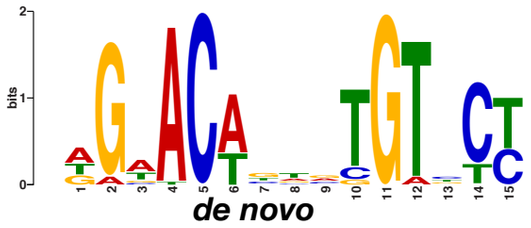


ATAC-seq peaks have distinct accessibility kinetics

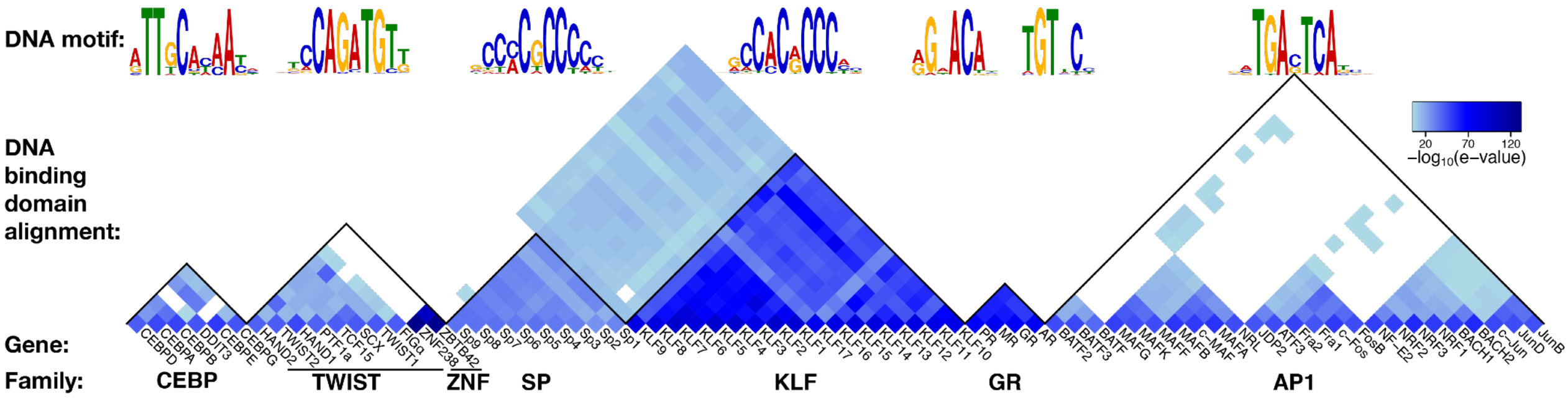


de novo motif analysis identifies enriched sequence elements within dynamic ATAC peaks



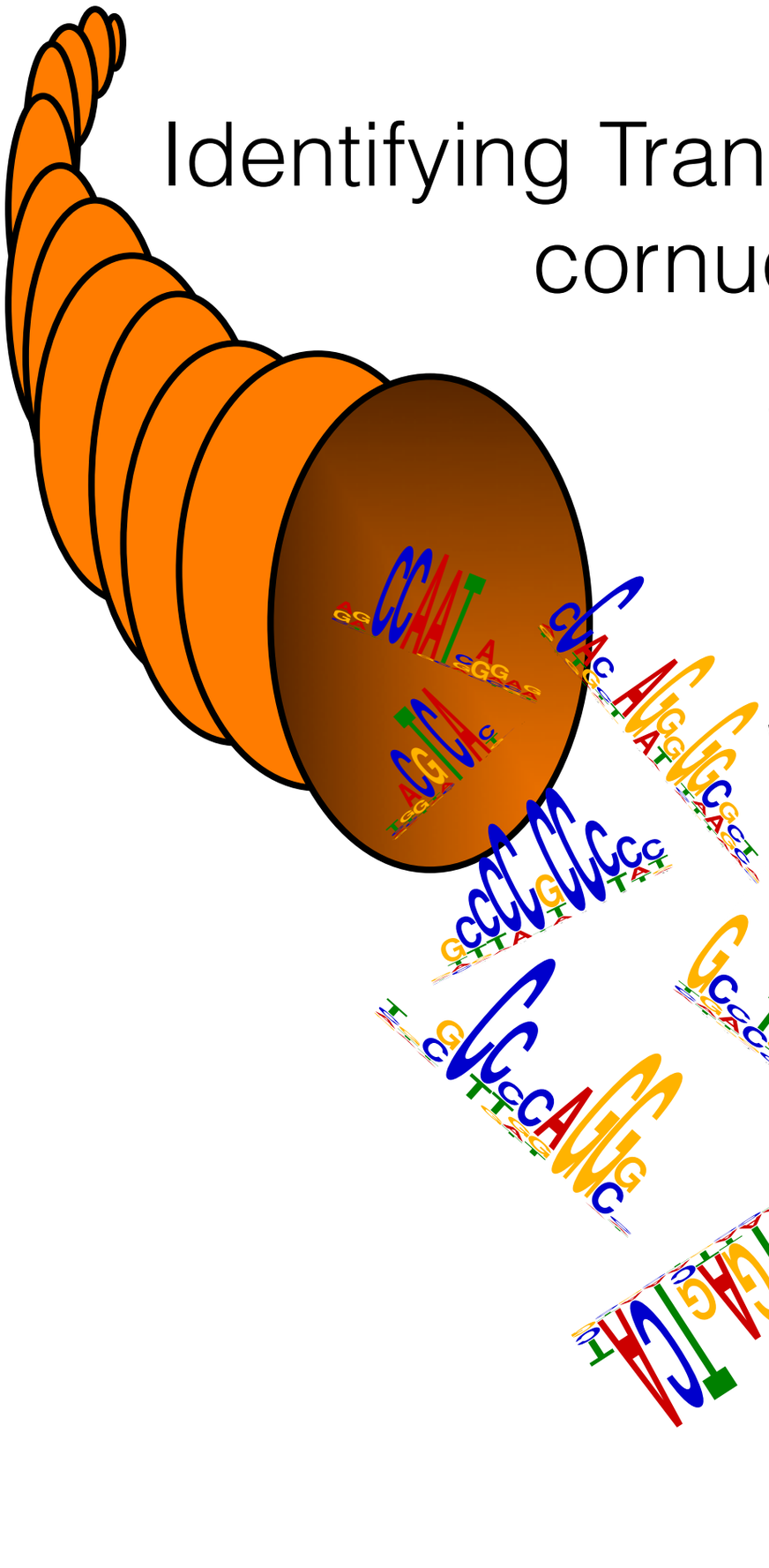


14 TF-family motifs (top 6 shown) drive early adipogenesis changes in accessibility



Paralogous TF DBD families that recognize each motif

Identifying Transcription Factors that bind to the cornucopia of DNA elements



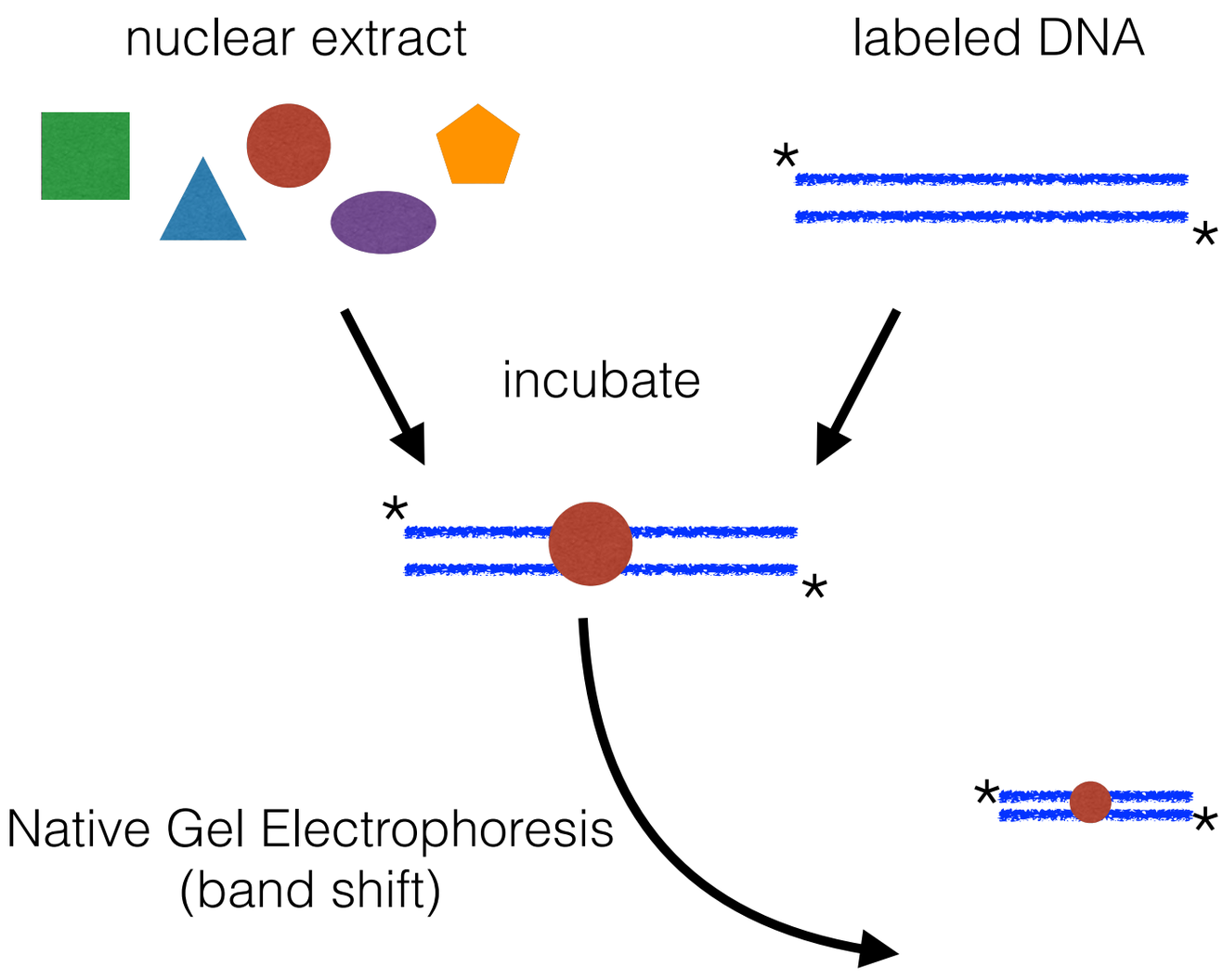
Specific proteins recognize and bind to these sequences.

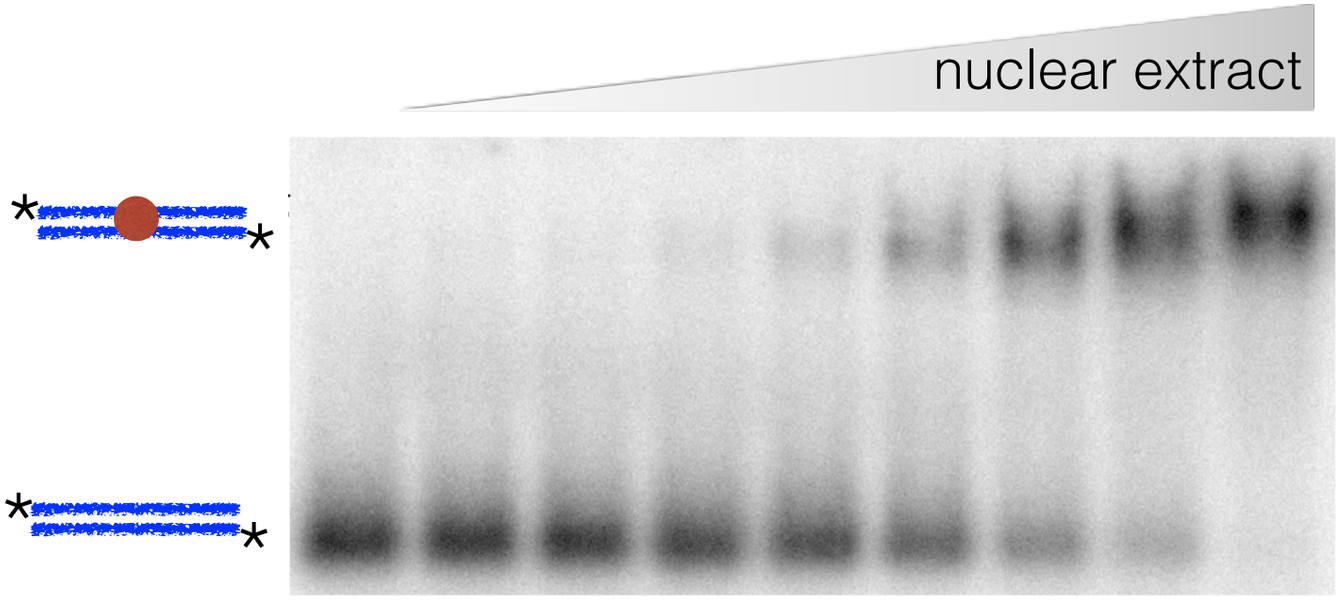
How can we identify them?

What is needed to purify such proteins?

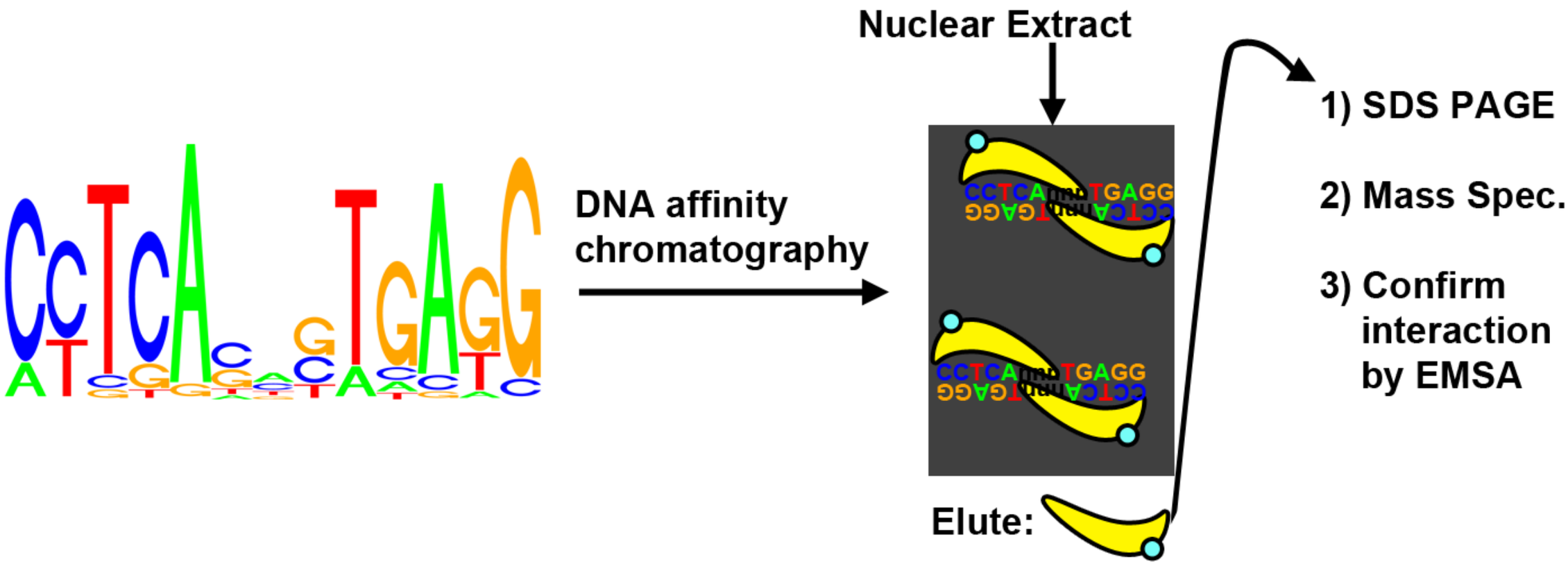
Knowing specific sequences helps create both an assay and tool for purifying.

Electrophoretic Mobility Shift Assay (EMSA) detect DNA binding factors





Purification of sequence-specific DNA-binding proteins



Order oligos with modest variants of your consensus sequence (include random flanking DNA). Biotinylate the ends of the duplexed DNA, bind to streptavidin beads/ column, elute, compare eluate to nuclear extract by PAGE, and mass spec.

Summary: Part I

- Transcription and its regulation is specified by short DNA sequence elements.
- These elements interact with particular transcription factors.
- See Lambert et. al., The Human Transcription Factors, Cell 2018 for a review of TF/DNA binding

"Next thing is how a cell's picking which GATs (stretches of nucleotides) get chosen, like Yogi in a picnic basket. Proteins and DNA? Some interesting chemistry. Cuz they getting jiggy with some different affinities."

–Tom McFadden

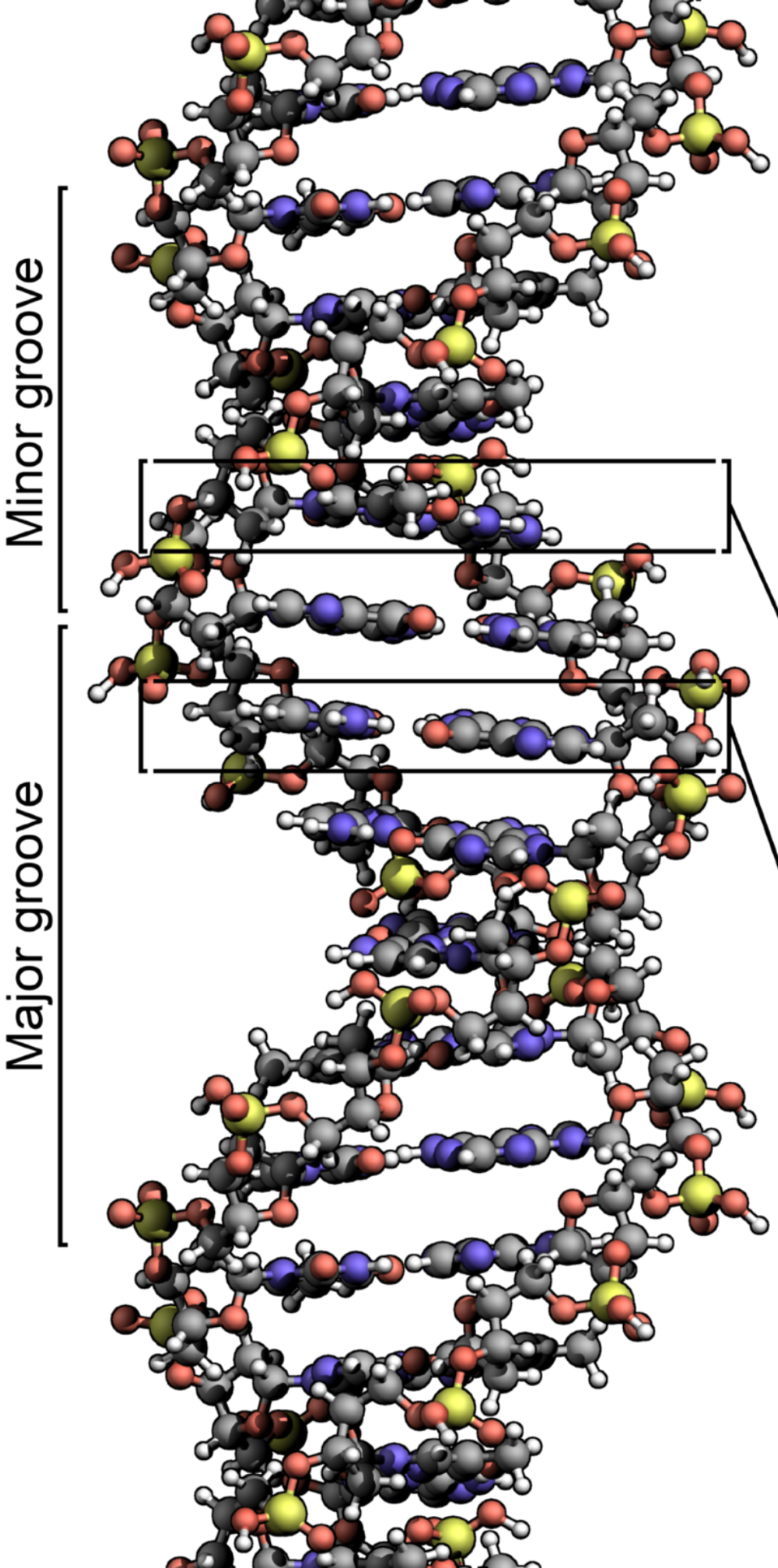
https://www.youtube.com/watch?v=9k_oKK4Teco&list=RD9k_oKK4Teco

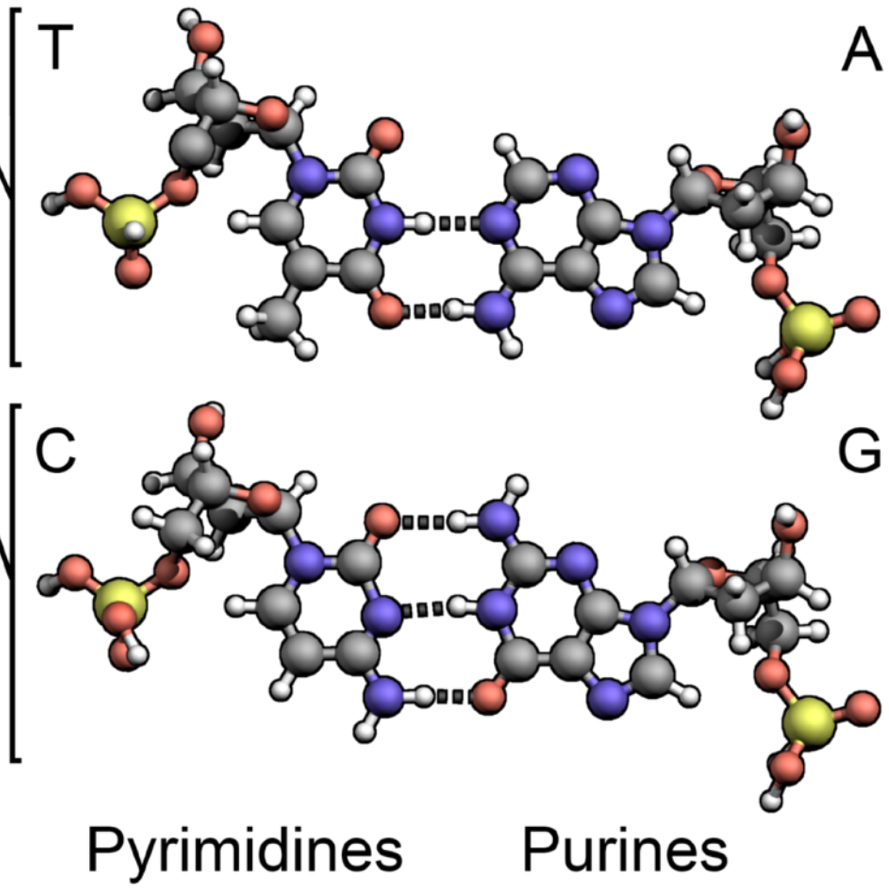
How do proteins interact with specific DNA sequences?

Hydrogen
Oxygen
Nitrogen
Carbon
Phosphorus

Minor groove

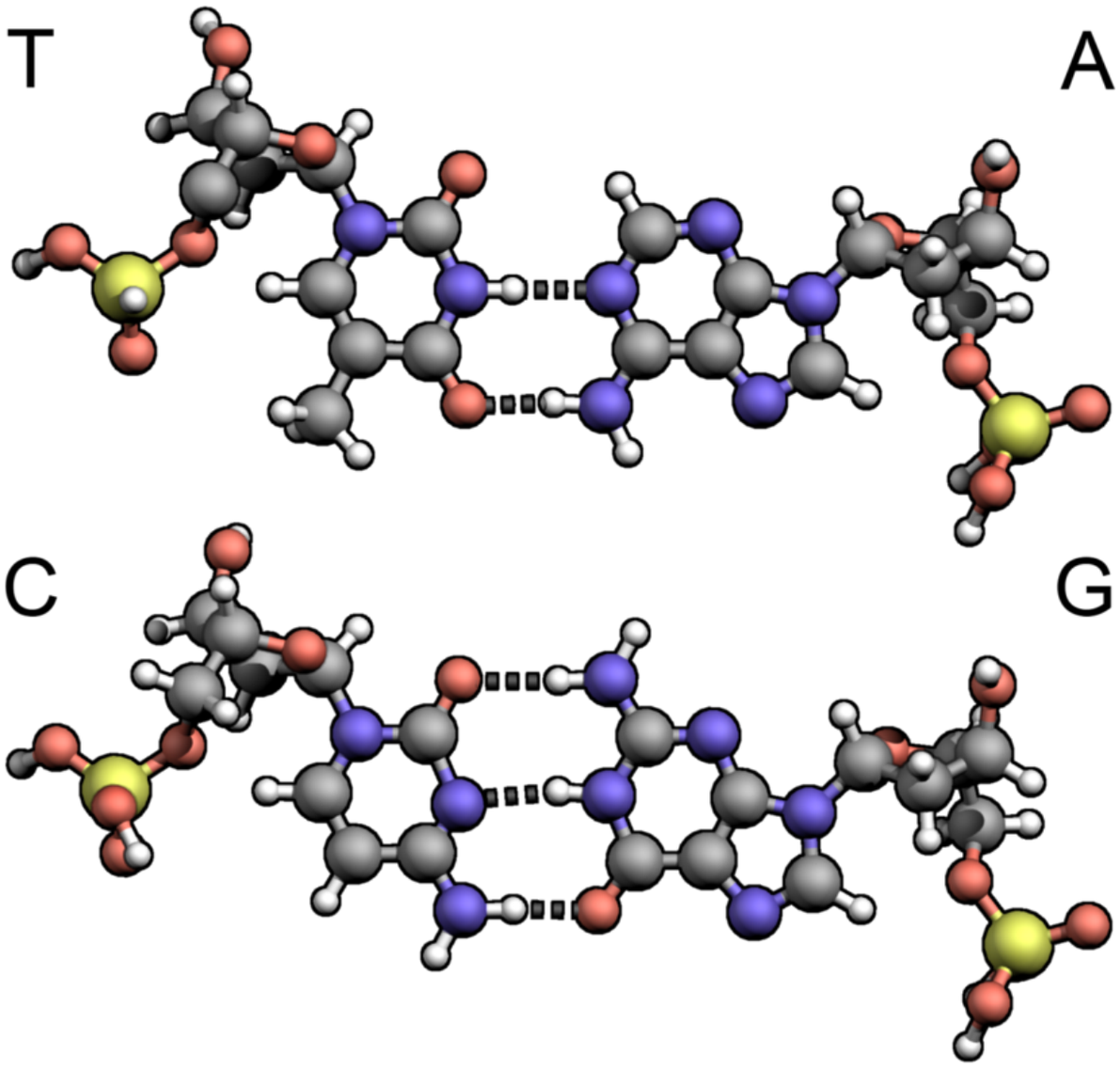
Major groove

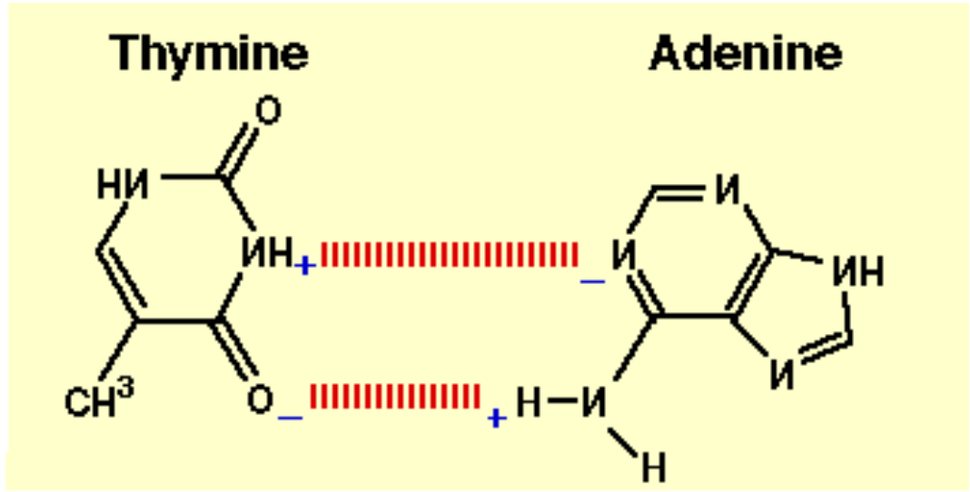


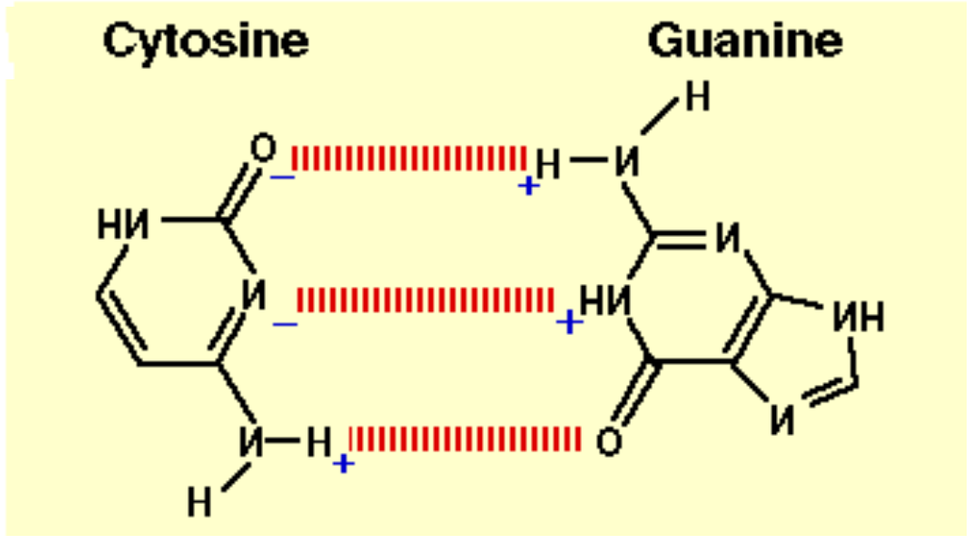


Pyrimidines Purines

Hydrogen bond is the electrostatic attraction between polar groups

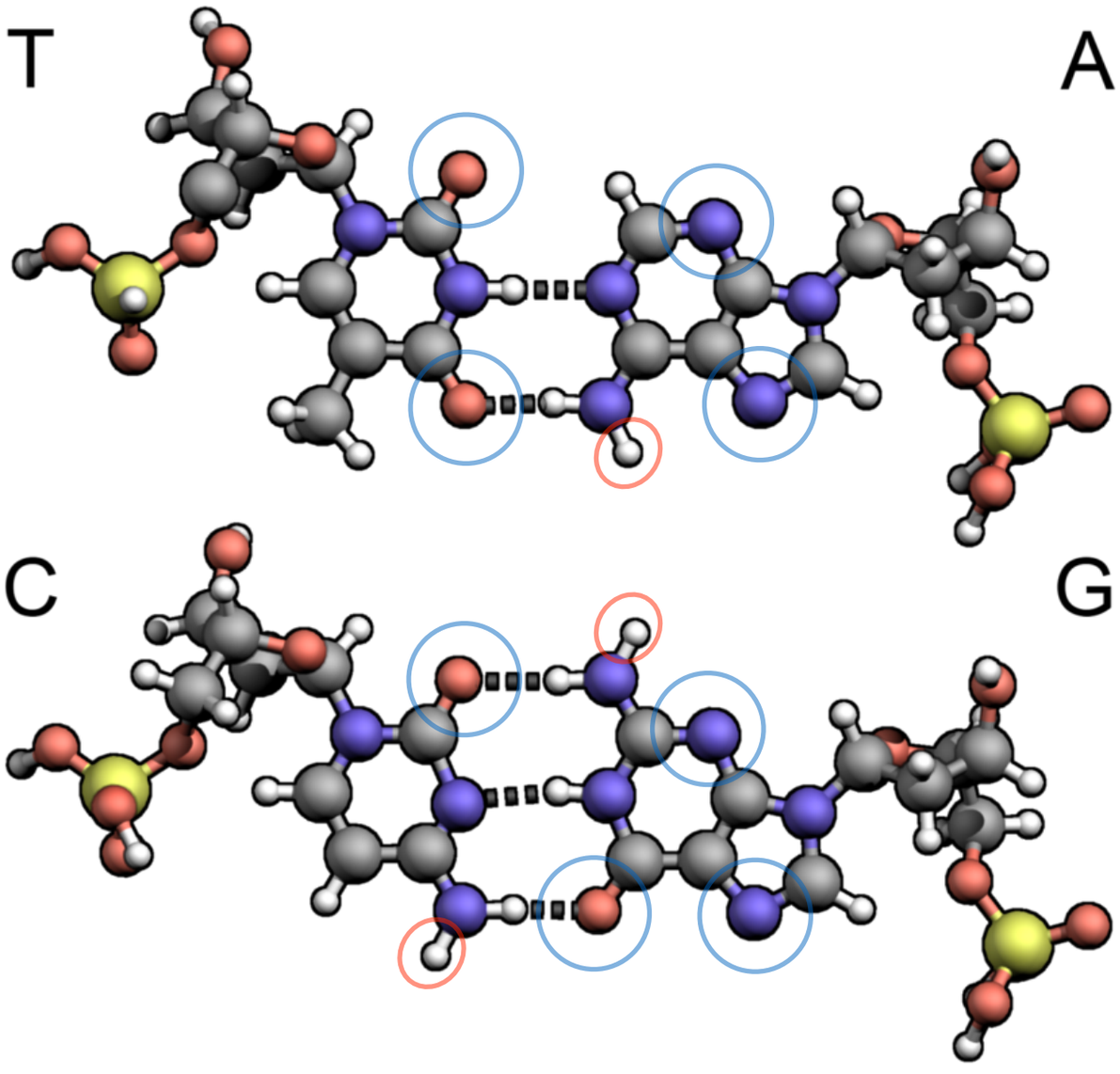


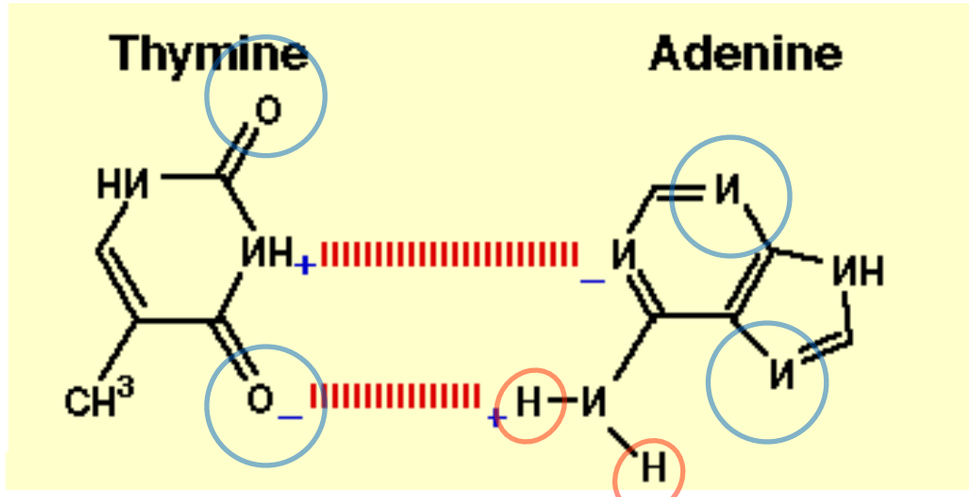


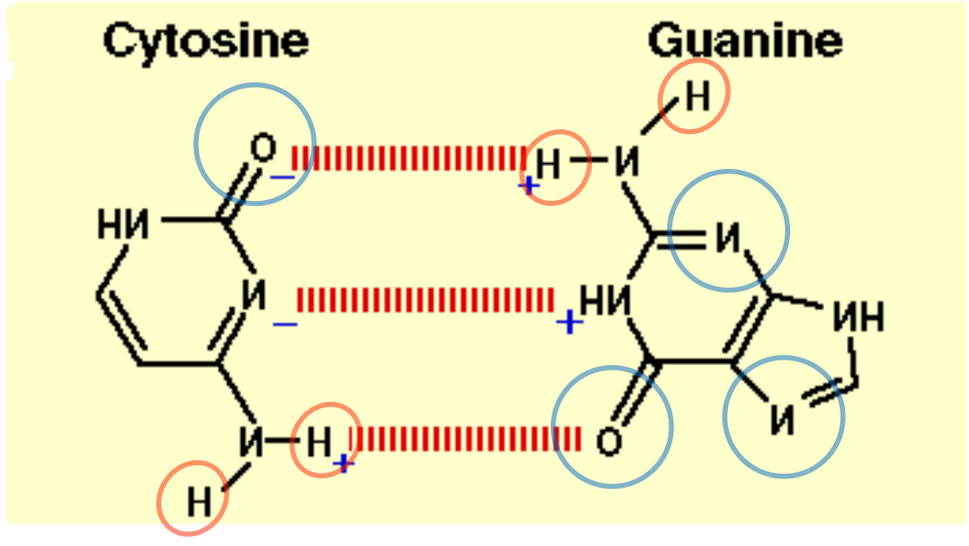


H-bond: a Hydrogen atom bound to a highly electronegative atom such as Nitrogen or Oxygen experiences attraction to another nearby highly electronegative atom.

The atoms shown below are available to mediate protein/DNA interactions via H-bonds







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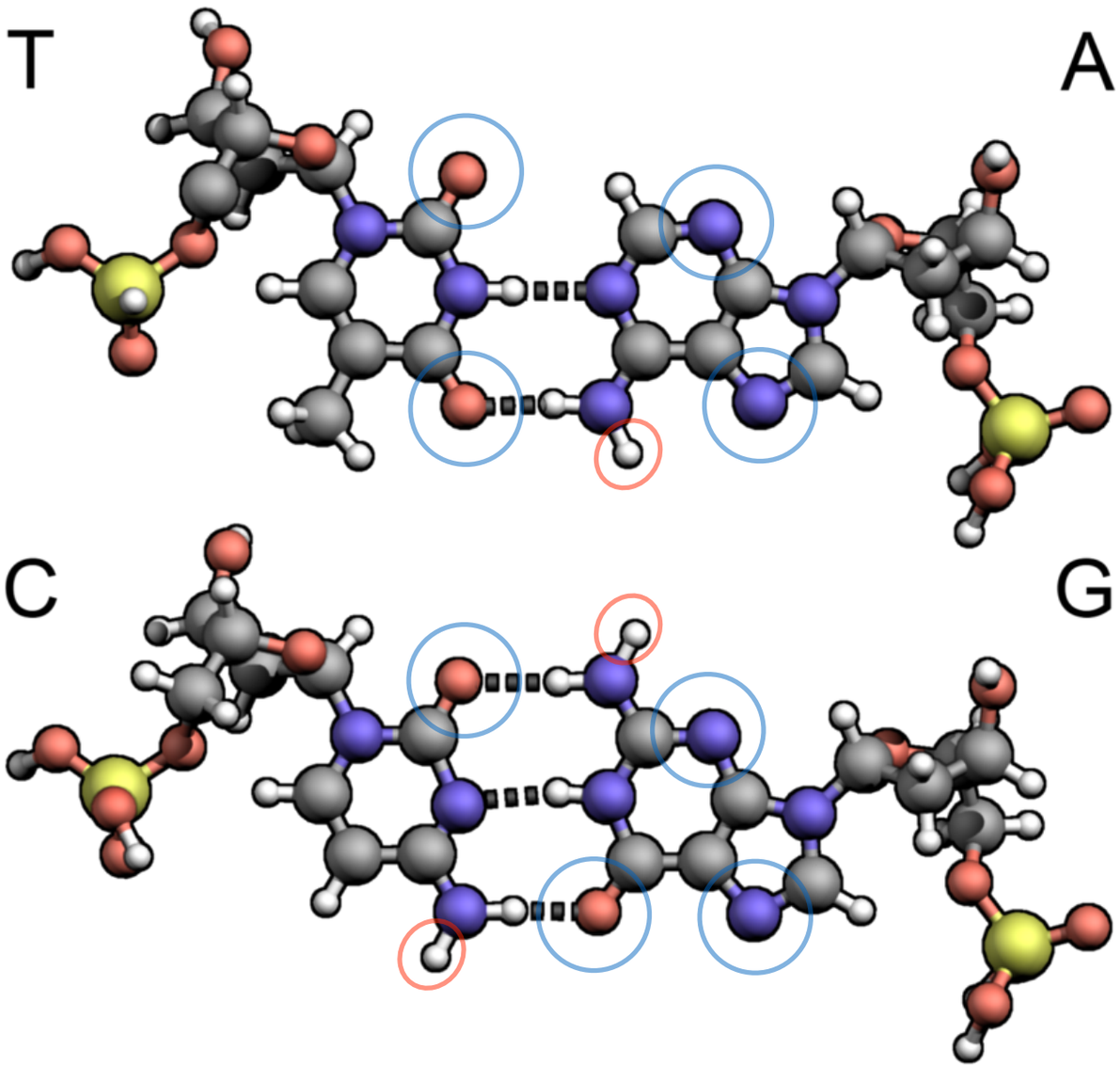
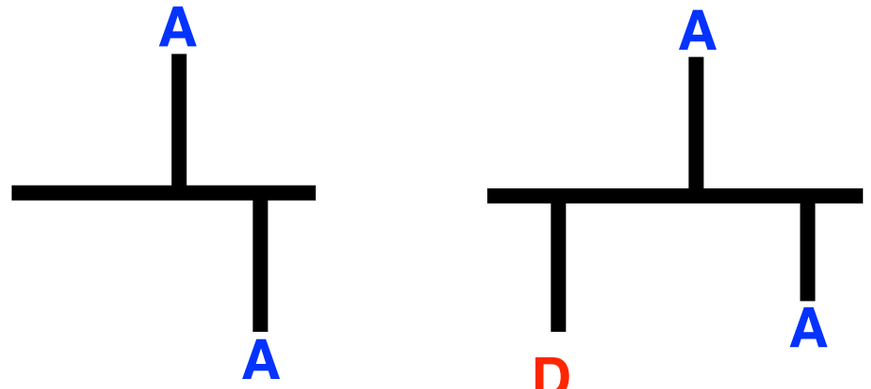


T-A

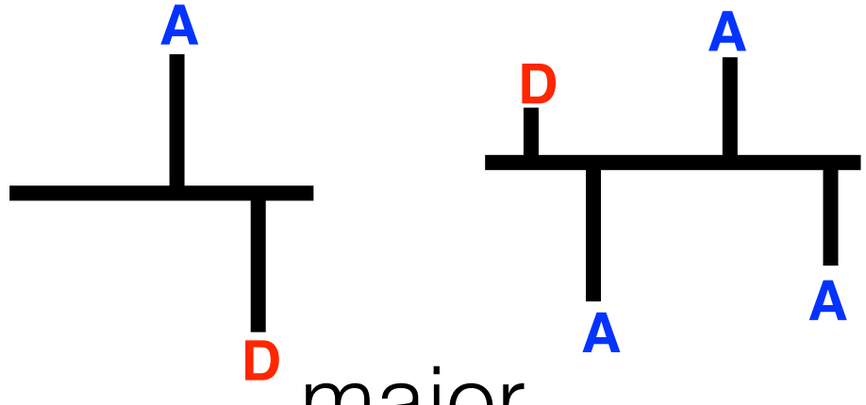
minor



major

C-G

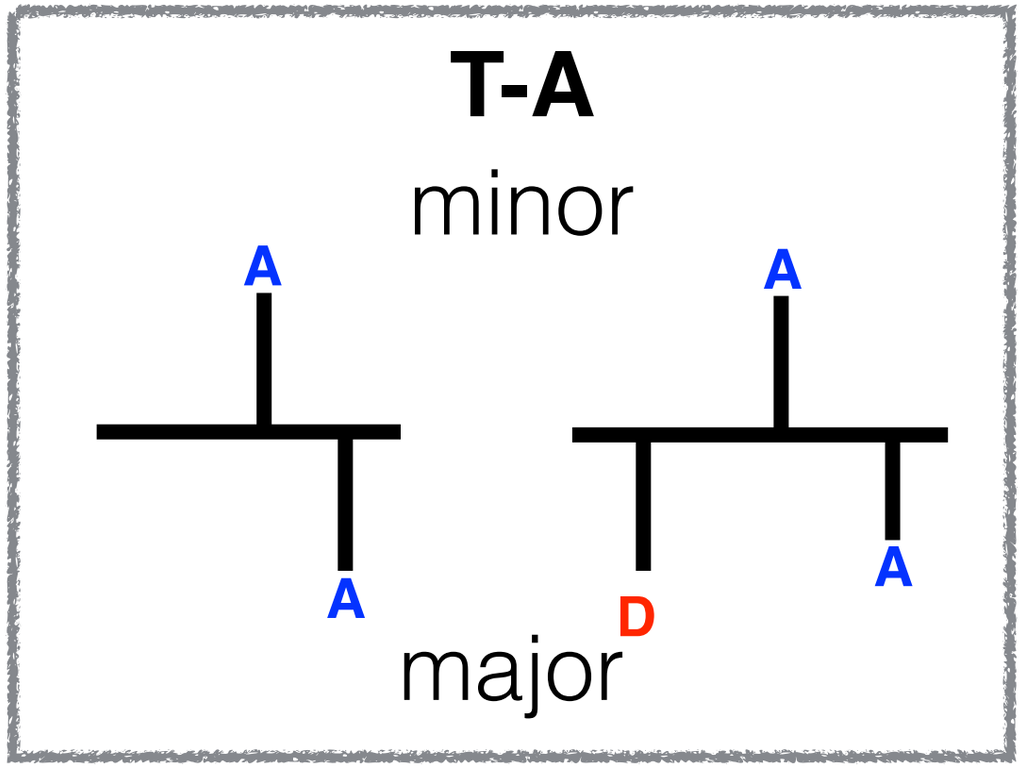
minor

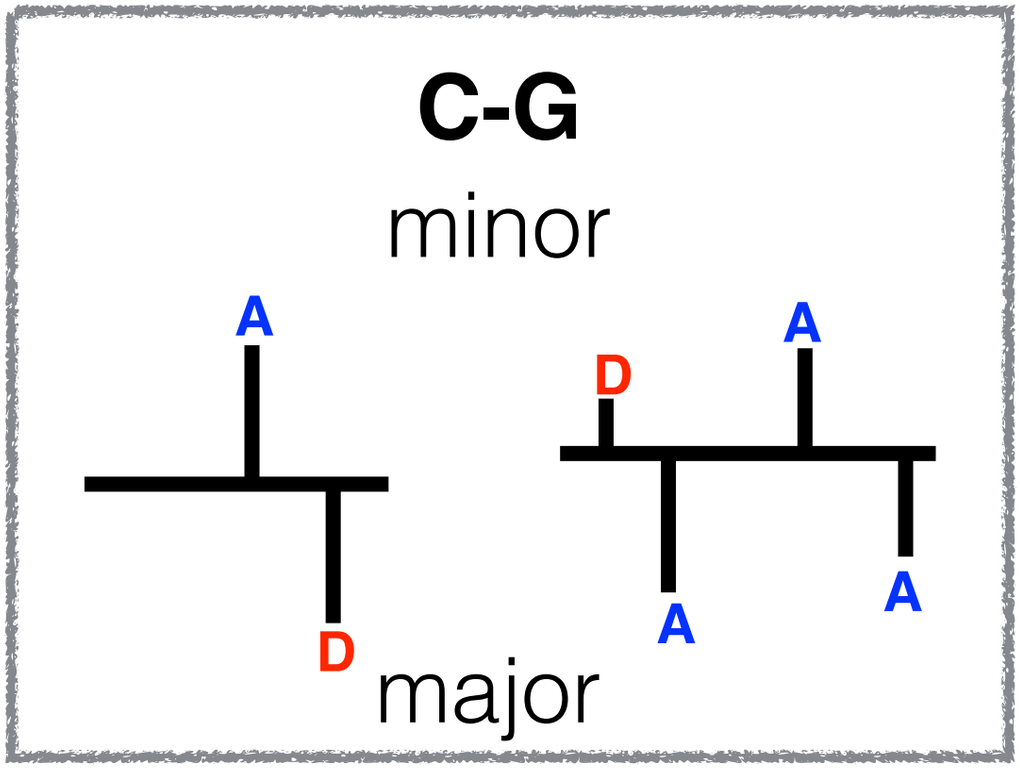


major

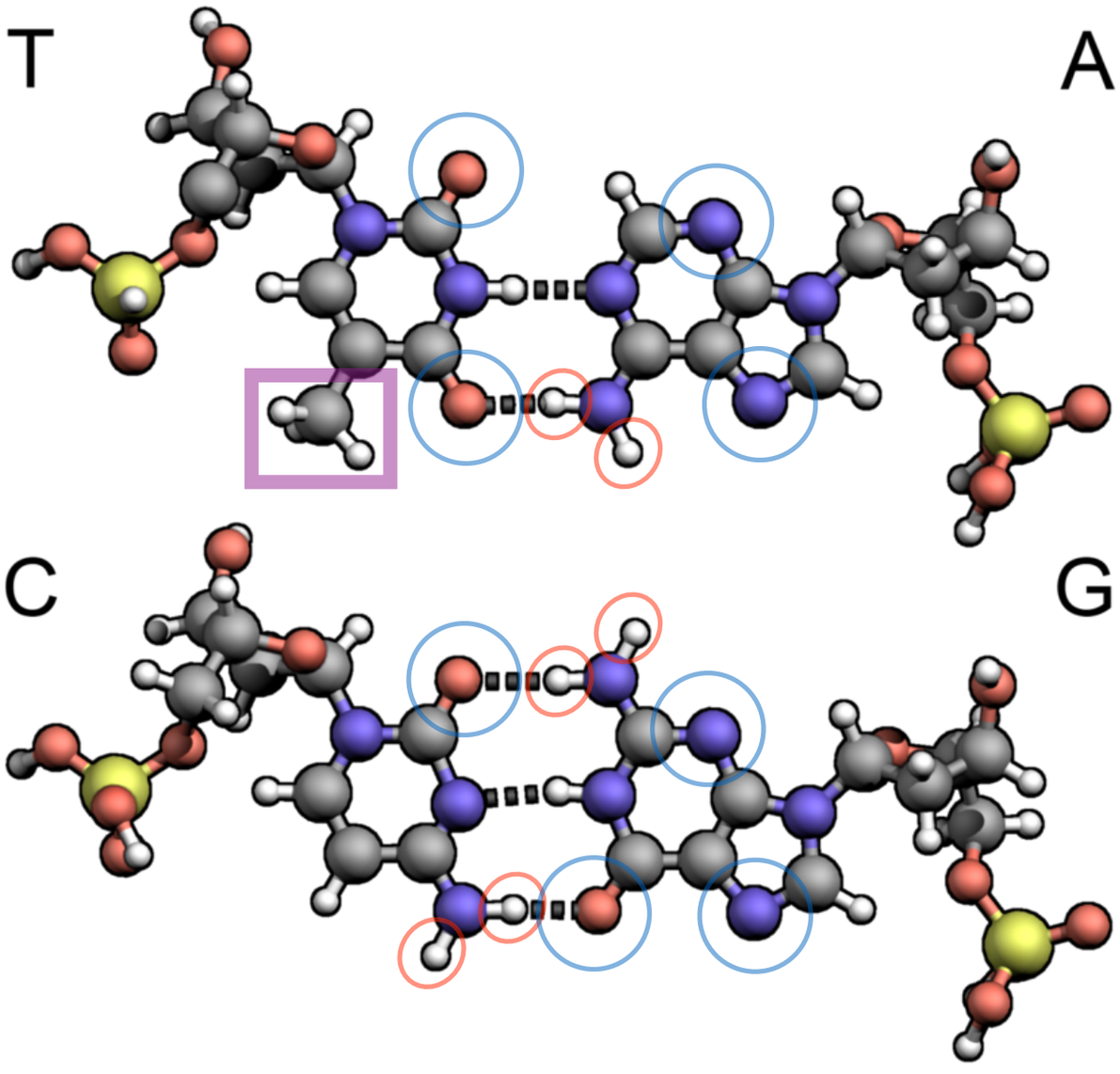
Hydrogen Bond Donors and Acceptors are exposed in the Major and Minor Grooves

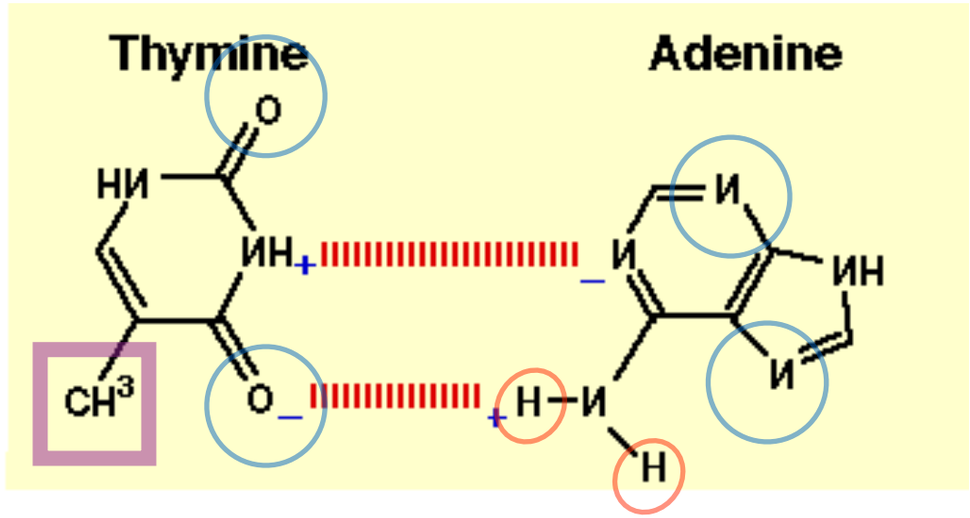
Base sequence is read an recognized by a protein probing the H-bonding possibilities in the Major and Minor Grooves

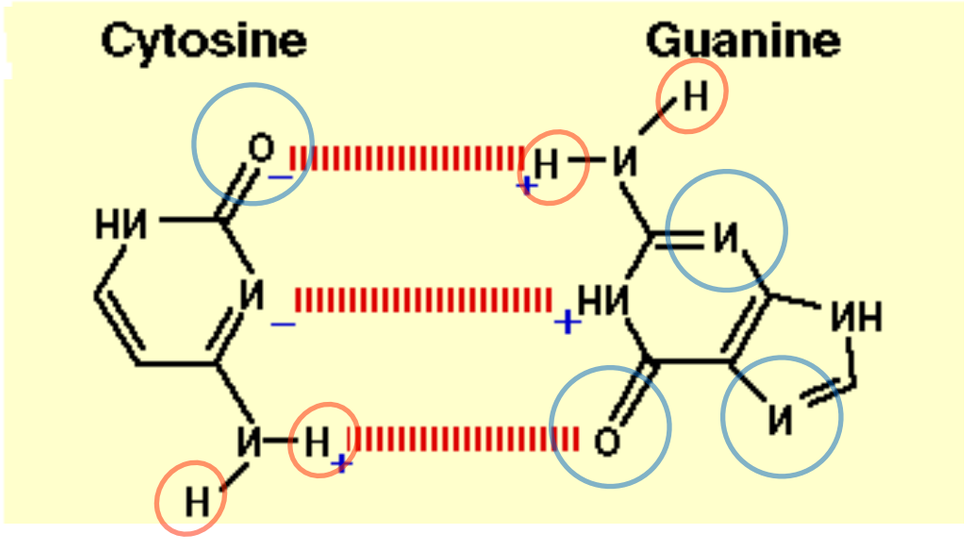




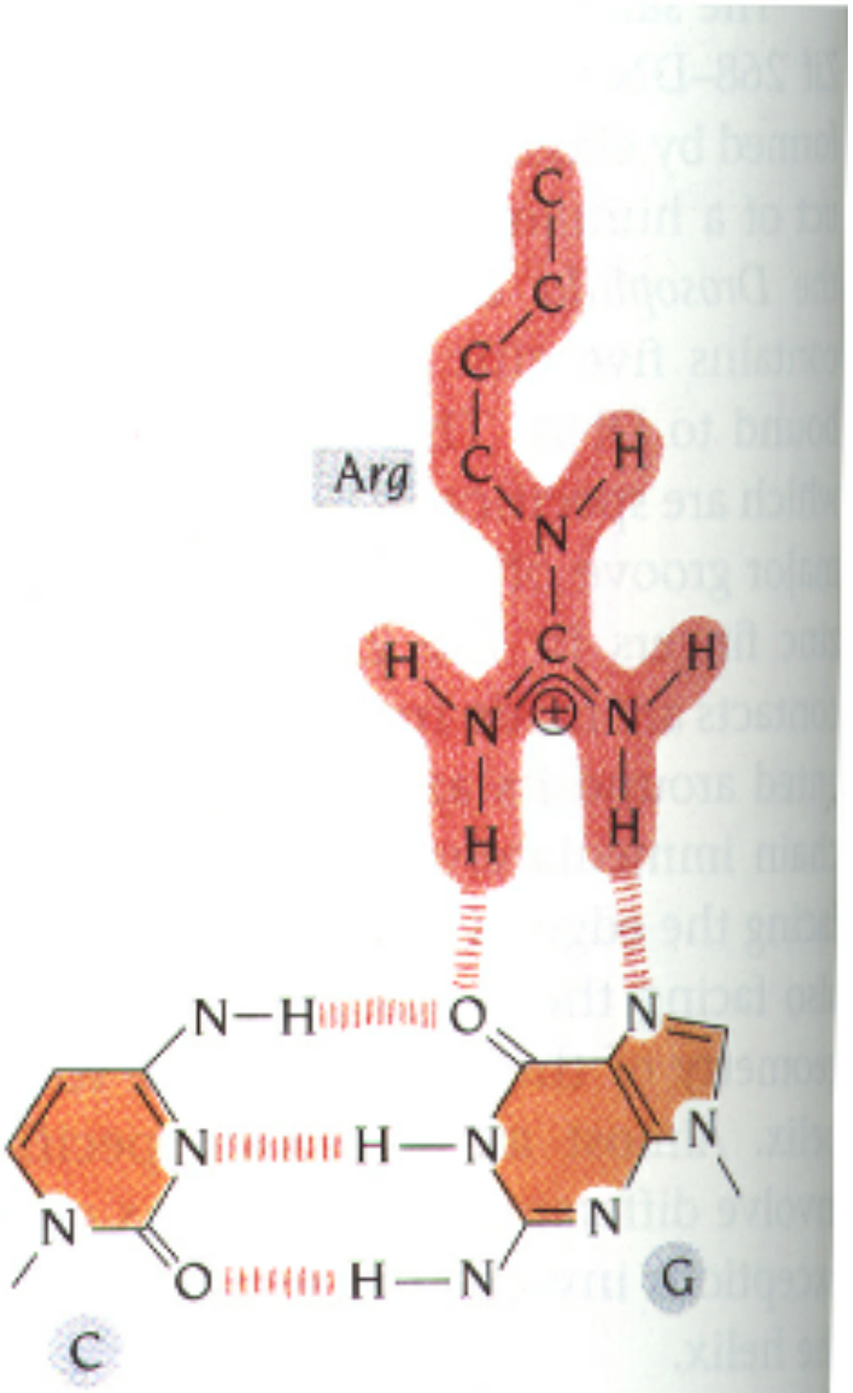
Thymine's methyl group provides an additional source of recognition/specificity







An example of an Amino acid/DNA base interaction



The interaction between arginine with its two hydrogen bond donors and a guanine base with its two acceptors in the major groove is an important component of many protein/DNA interactions.

How do proteins interact with specific DNA sequences?

Hydrogen
Oxygen
Nitrogen
Carbon
Phosphorus

Minor groove

Major groove

T

A

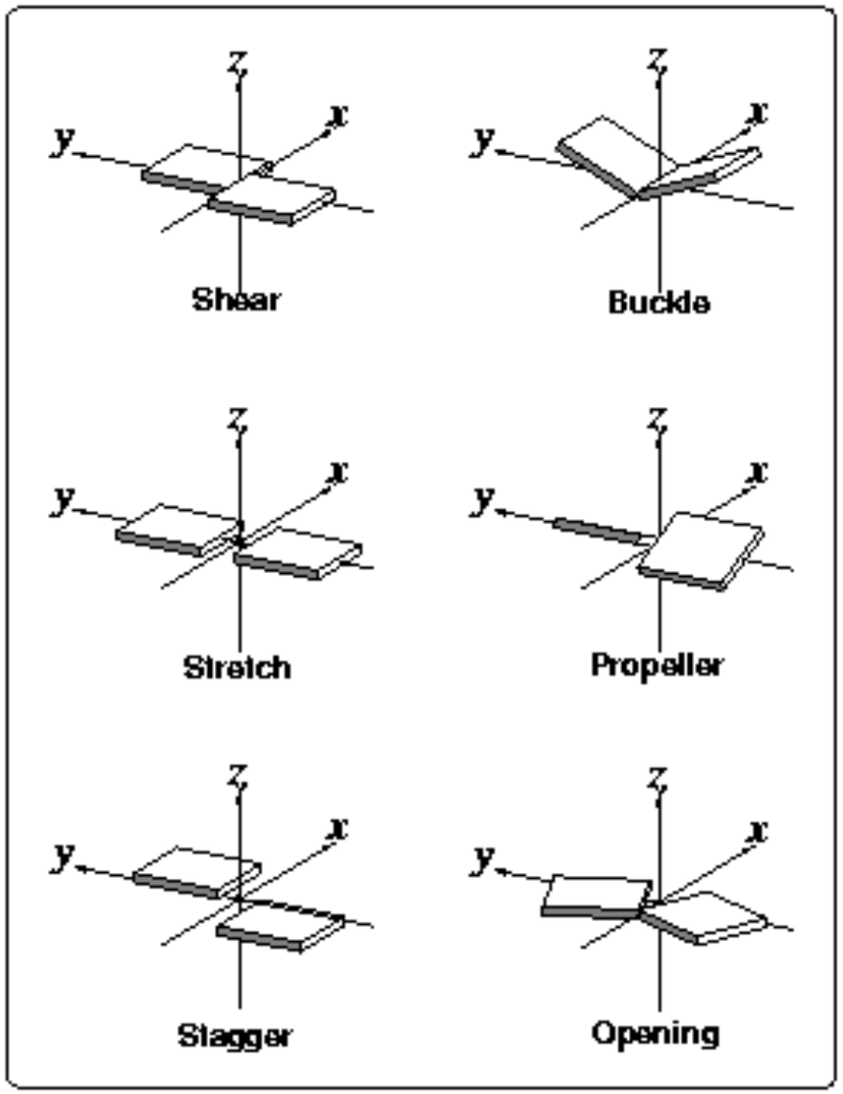
C

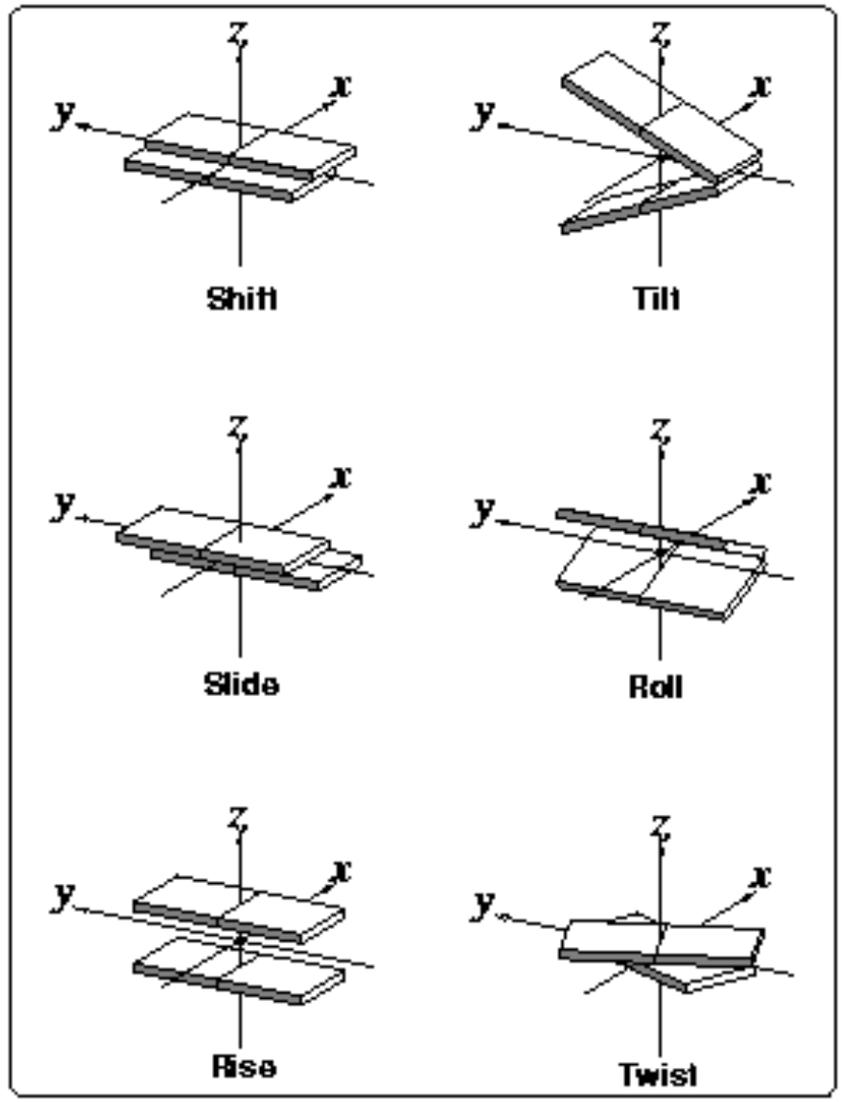
G

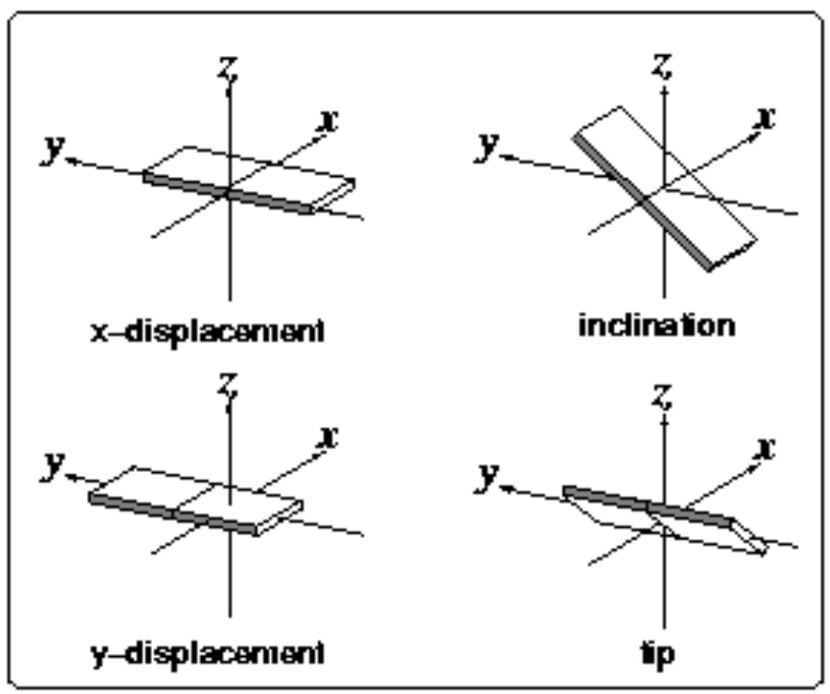
Pyrimidines

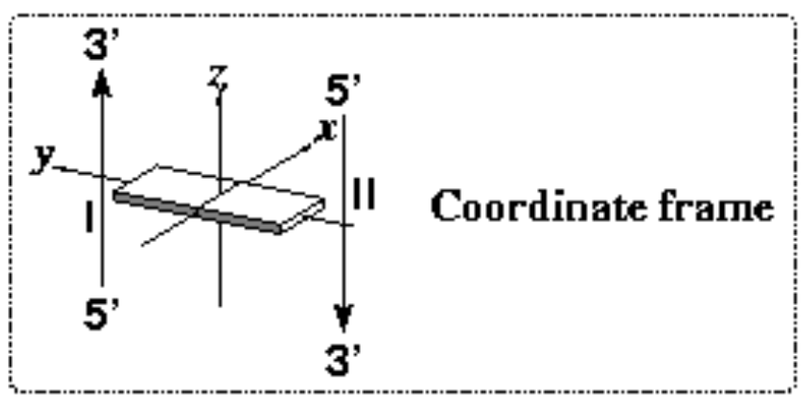
Purines

Base Pair Geometry



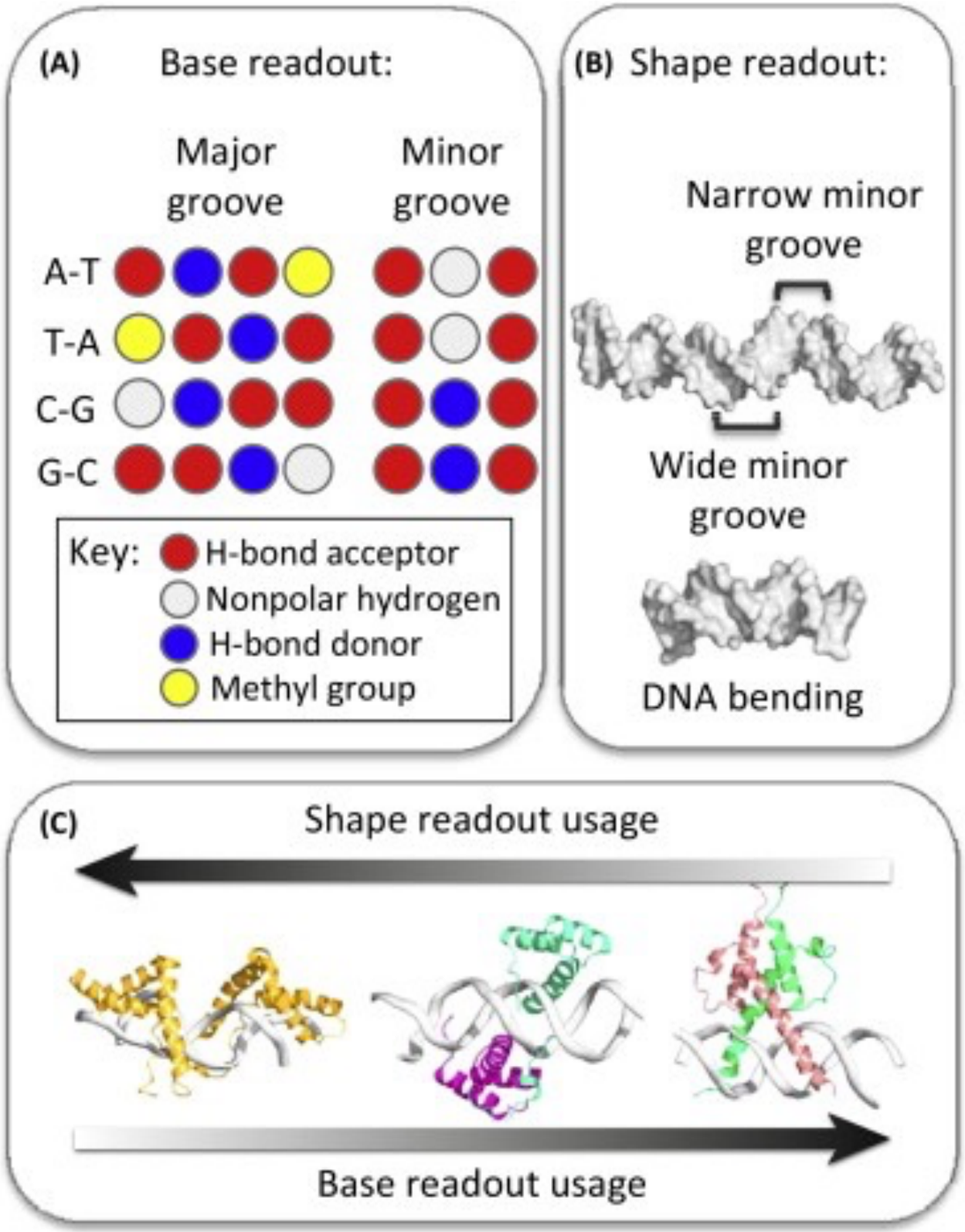






Backbone phosphates are differentially positioned based on the degree of tilt, buckle, twist, roll, etc. relative to the preceding base pair.

Base Composition and Shape Contribute to TF-DNA Specificity



- Base readouts are specific for bp in major groove but degenerate for minor groove.
- Shape dominates for a minor groove-binding high motility group (HMG) box protein
- Base readout is a major contribution in DNA recognition by the bHLH protein Pho4
- Both readout modes are ~equally present in the DNA binding of a Hox–Exd heterodimer

Slattery et al. (2014) Trends Biochem Sci. 39: 381-99