Regulatory Genomics I: Regulatory Elements

Shamith Samarajiwa
email: ss861@mrc-cu.cam.ac.uk

Integrative Systems Biomedicine Group,
MRC Cancer Unit,
University of Cambridge.

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MRC Cancer Unit

Overview

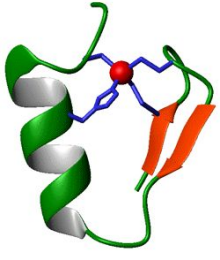
- Components involved in gene regulation
- What are regulatory elements and where are they located?
- Regulatory element classes
- DNA binding regulatory proteins
- Computational identification of regulatory elements
- What binds where and how to locate binding sites

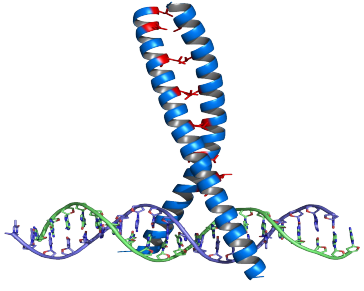
Introduction to gene regulation

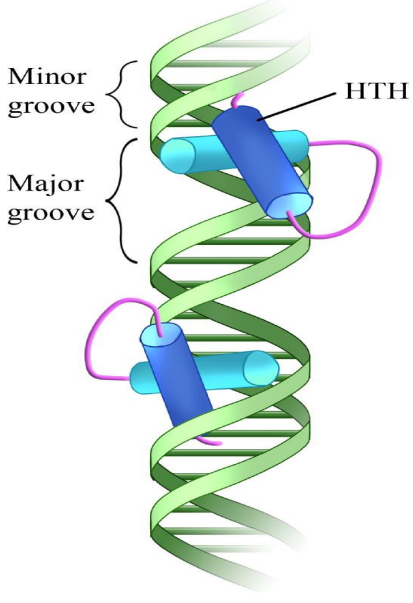
- **Transcription Factors** - DNA binding proteins that regulate transcription.
- **Histone modifications** - post-translational modifications of histone tails that modulate transcriptional activity of regulatory regions
- **Nucleosome positioning** - open chromatin
- **Chromatin domains** - Long distance interactions
- **Polycomb group (PcG) Proteins** - chromatin remodelling agents
- **DNA Methylation** - (usually) inhibition of transcription
- **Non coding RNA** - post-transcriptional modulation

Transcription Factors (TFs)

- More than 2000 in the human genome
- Direct binding to DNA or indirect binding via other DNA binding proteins
- Contain one or more **DNA binding** and **Transactivating** domains
- Acts as single molecules or homo or heteromeric complexes.
- Structural DNA recognition domains of TFs:
  - Helix-Turn-Helix
  - Zinc Finger
  - Leucine Zipper
  - Helix-Loop-Helix







- Transcriptional **Co-factors**
- **Polymerase** holoenzyme complex & **Mediator** complex

Specialised classes of TFs

- **Master Regulators** - Key transcription factors at the top of the regulatory hierarchy. They regulate multiple processes and phenotypes. (ex: TP53, NFkB, MYC etc.)
- **General Transcription Factors** -GTFs, RNA polymerase, and the mediator protein complex constitute the basic transcriptional apparatus that bind to the promoter, and starts transcription. GTF consists of TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH.
- **Pioneer factors**- Transcription factors that can directly bind condensed chromatin. They can have positive and negative effects on transcription and are important in recruiting other transcription factors and histone modification enzymes as well as controlling DNA methylation. (ex: PU.1, SP1, AP2, GATA, FOXA1, KLF4)
- **Tissue specific factors** - expressed in specific tissue or cell type (ex: HNF factors in liver)

DNA binding proteins

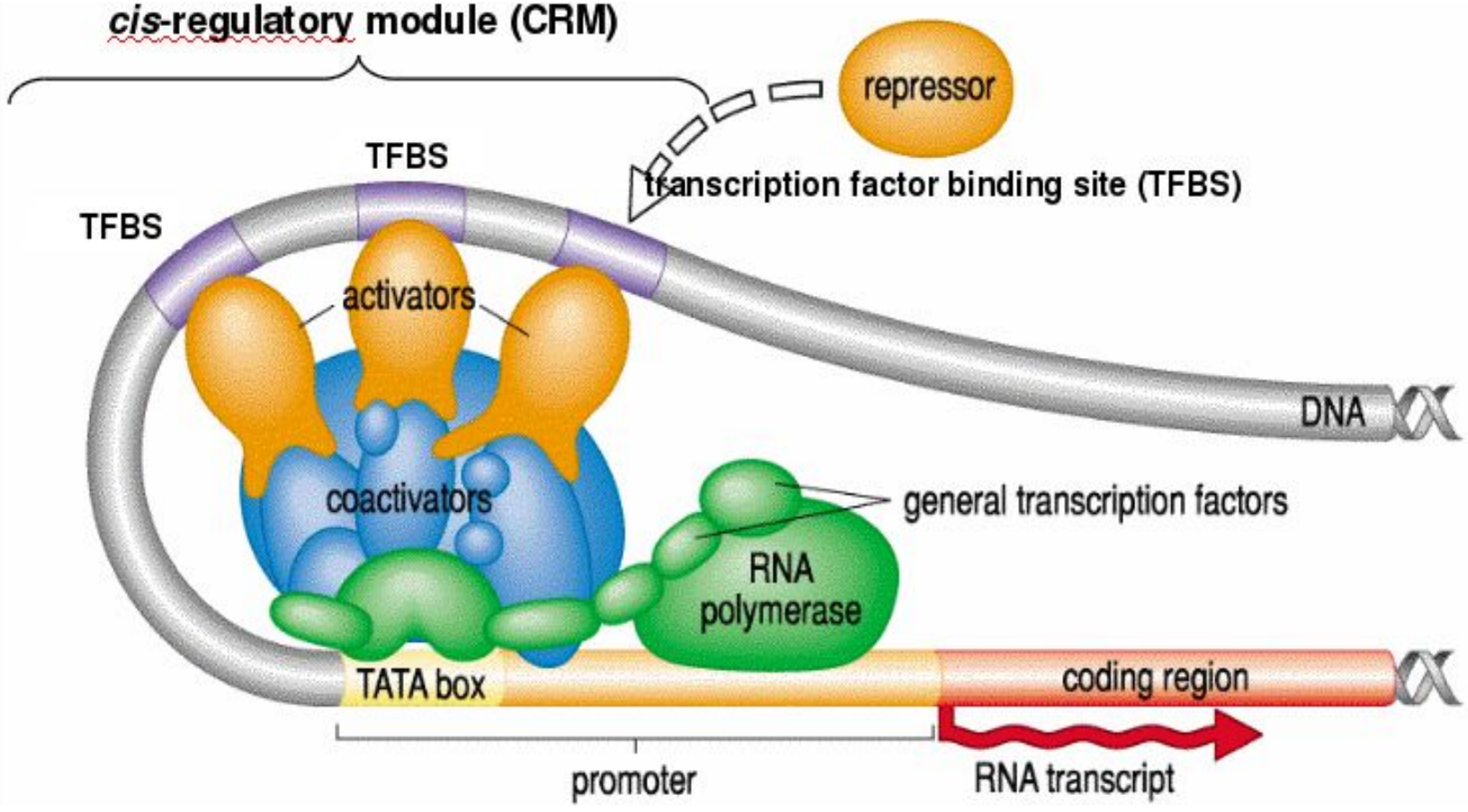


Image adapted from Wolpert, Principles of Development

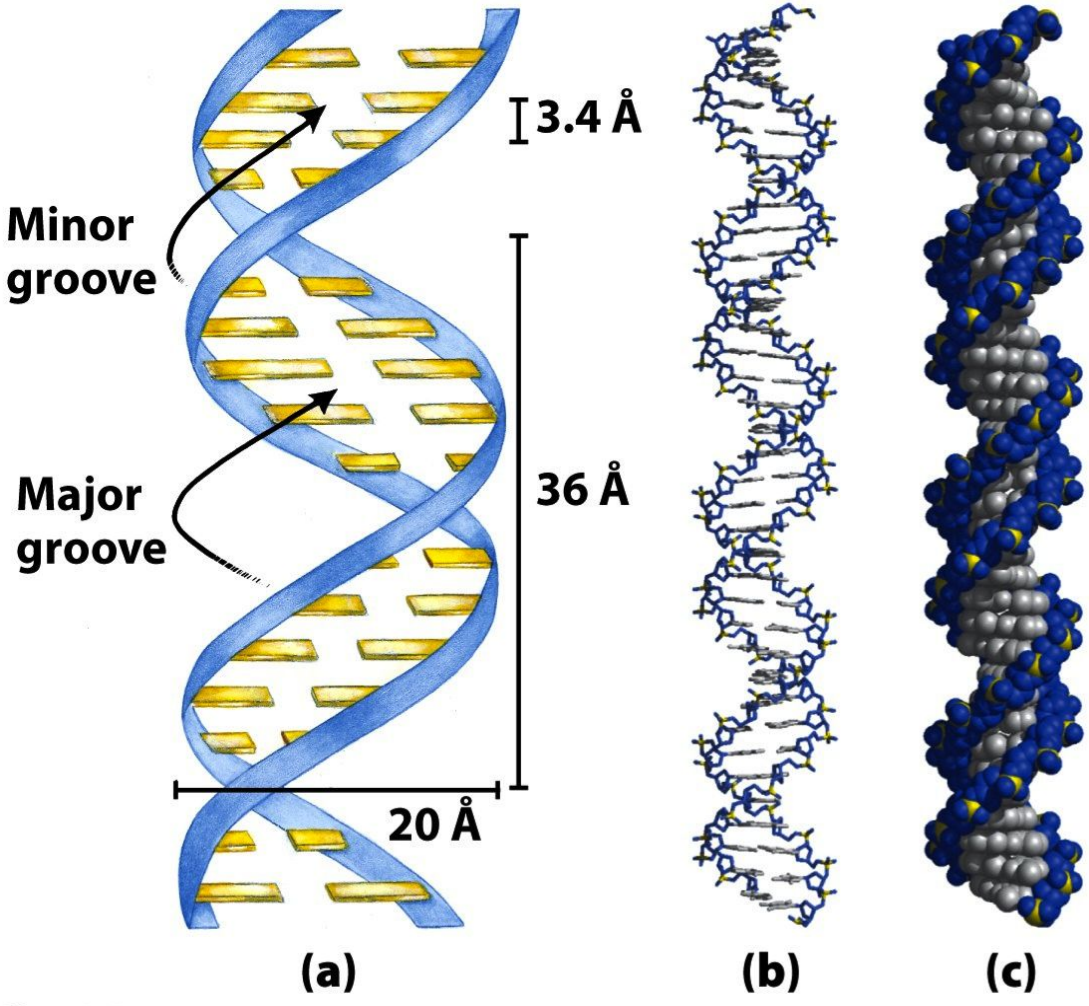
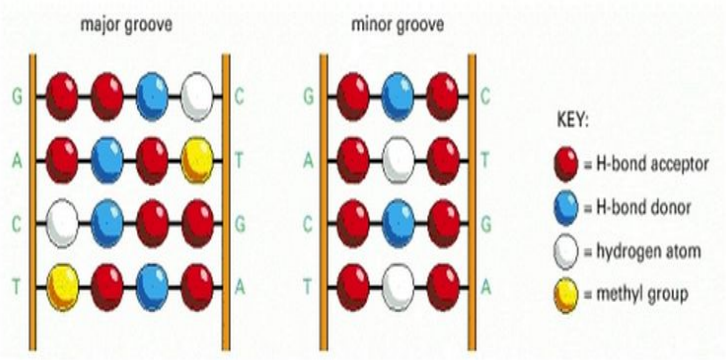
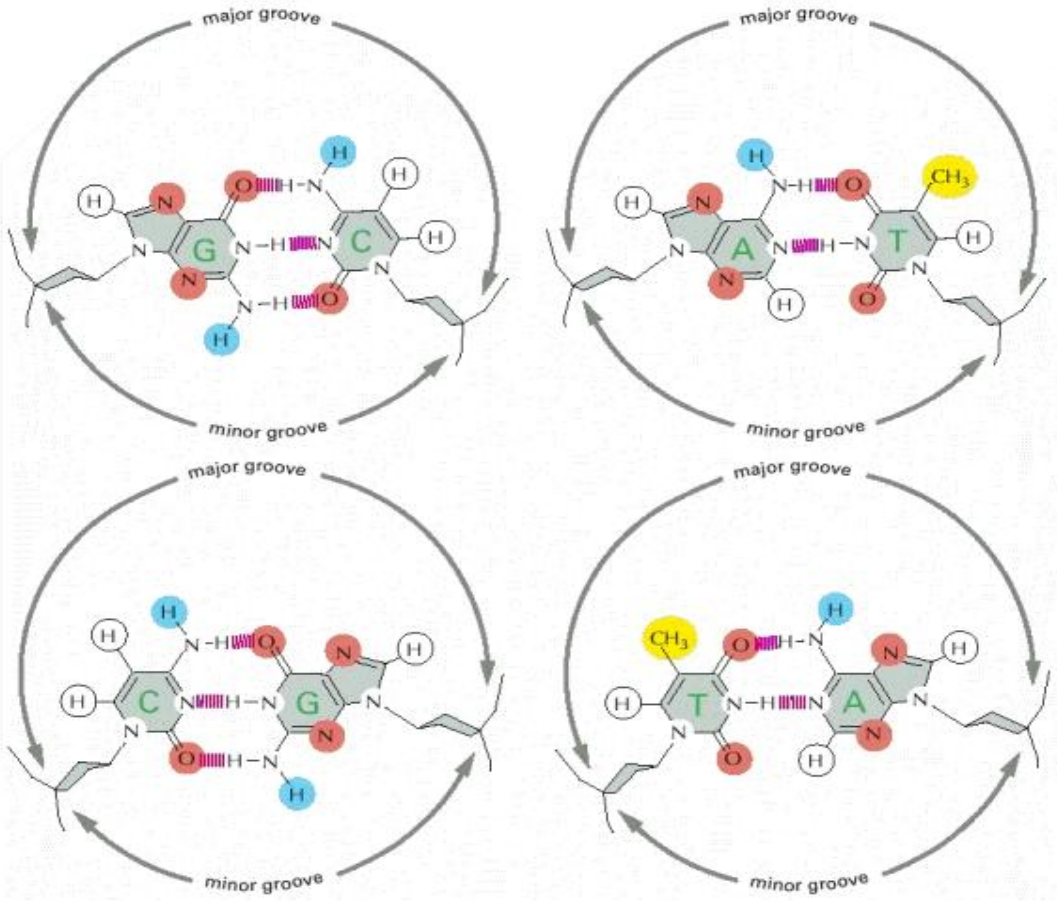


Figure 8-13
Lehninger Principles of Biochemistry, Fifth Edition

DNA recognition code

- The four possible configurations of base pairs are shown,
- potential ***hydrogen bond donors*** indicated in ***blue***
- potential ***hydrogen bond acceptors*** in ***red***
- hydrogen bonds of the base pairs are indicated as a series of short parallel ***pink*** lines.
- Methyl groups, which form ***hydrophobic protuberances***, are shown in ***yellow***
- Hydrogen atoms that are attached to carbons, and are therefore unavailable for hydrogen bonding, are *white*.



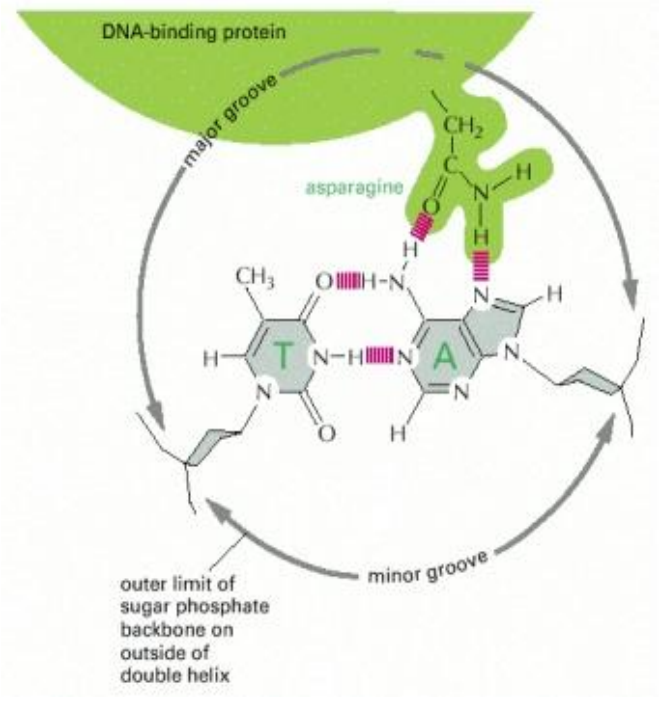


Alberts, "Molecular Biology of the Cell", 2002

Transcription Factor-DNA interactions

- Gene regulatory proteins recognise and bind to the major groove of DNA.

- Typically the protein-DNA interface would consist of 10-20 such amino acid contacts, each contributing to the binding energy of the protein-DNA interface.



Alberts, “Molecular Biology of the Cell”, 2002

Transcriptional Complexes: RNA Polymerases

- **RNAP** is an enzyme complex that uses a DNA template to produce primary RNA transcripts.
- **RNA polymerase I**: synthesizes a **pre-rRNA** 45S (35S in yeast), which matures into 28S, 18S and 5.8S rRNAs which will form the major RNA components of the ribosome.
- **RNA polymerase II**: synthesizes precursors of **mRNAs** and most **snRNA** and **miRNAs**.
- **RNA polymerase III**: synthesizes **tRNAs**, 5S **rRNA** and other small RNAs found in the nucleus and cytosol
- **RNA polymerase IV:** synthesizes **siRNA** in plants.
- **RNA polymerase V:** synthesizes RNAs involved in siRNA-directed heterochromatin formation in plants

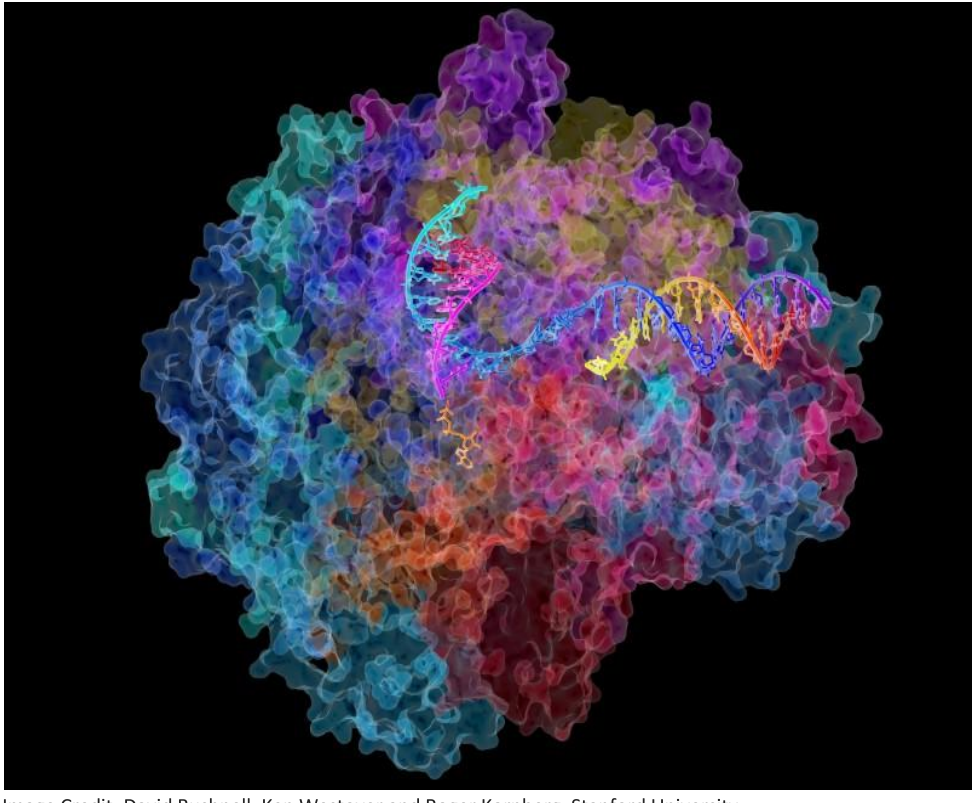
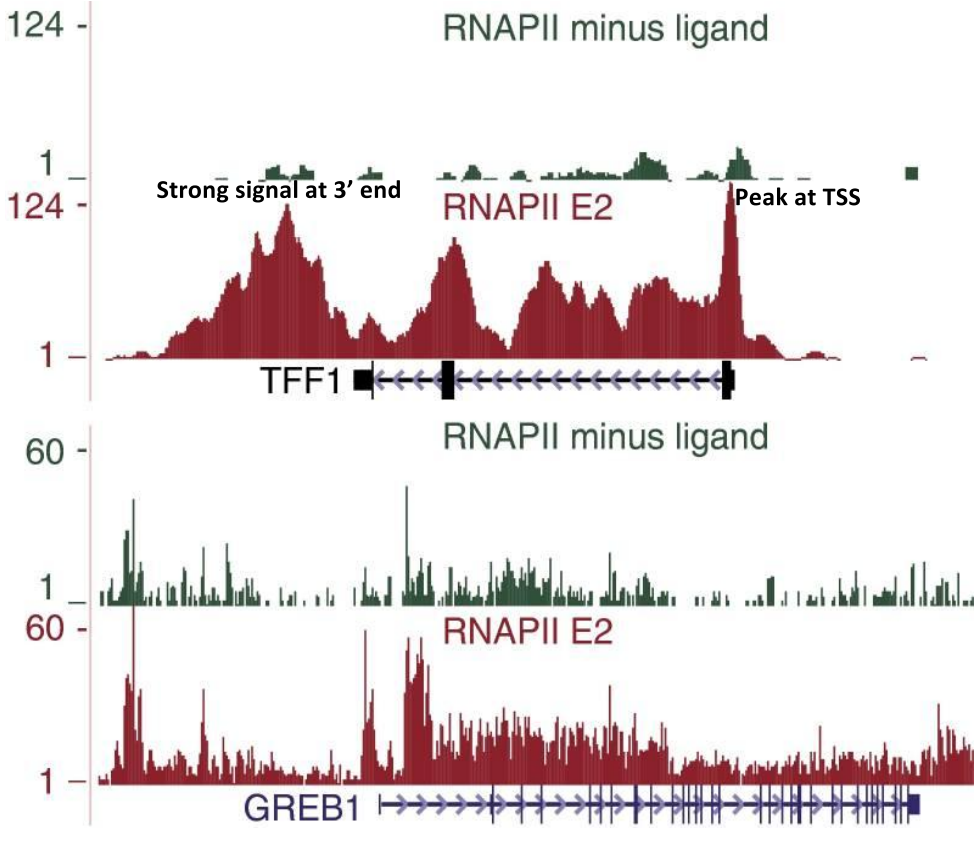


Image Credit: David Bushnell, Ken Westover and Roger Kornberg, Stanford University

Measuring RNAP-II occupancy

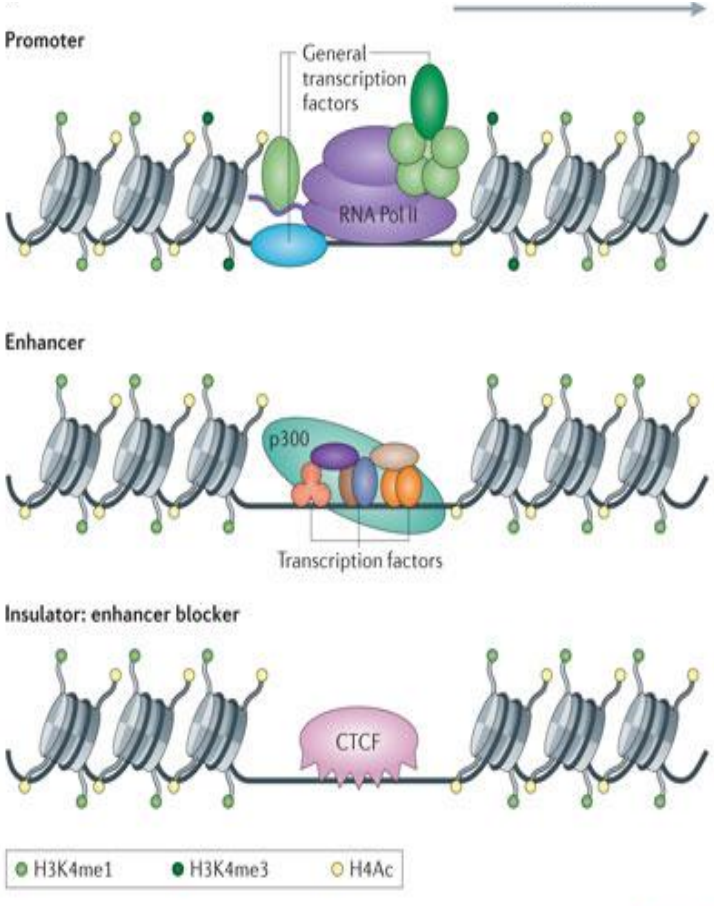
- Stages of RNAP-II activity can be experimentally determined by ChIP-seq:
  - Recruitment of the RNA Polymerase II complex
  - Transcription initiation
  - Clearance of RNAP-II
  - Pausing
  - Escape from pause
  - Elongation
  - Termination



Welboren et al., EMBO J. 2009

Classes of regulatory elements

- Core Promoter
  - CGI promoters
  - Non CpG island (CGI) promoters
- Enhancers & Super enhancers
- Locus Control Regions
- Silencers
- Insulators

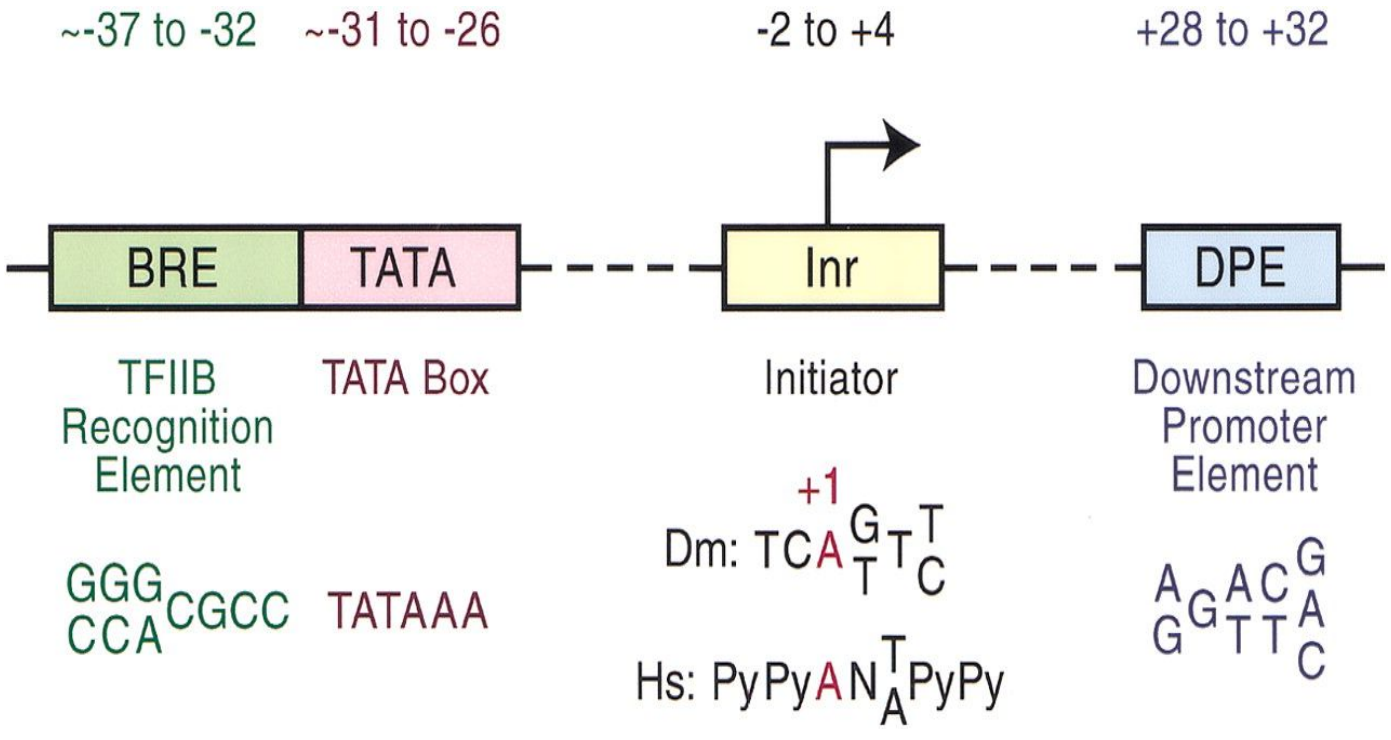


1. The Core Promoter

- **Core or minimal promoters** are regions present in **RNA PolII** transcribed genes and are sufficient to mediate gene expression in a reporter assay.
- **35-40 nt** up or downstream of TSS. May extend to 350nt
- Contains core promoter motifs that interact with basal transcription machinery:

    - **TATA** box - binds TBP (bound by TFIID complex)
    - **CAAT** box (binds NF-Y & CEBP family proteins)
    - **BRE** (B recognition element -bound by TFIIB complex)
    - **Inr** (Initiator element- bound by TFIID complex)
    - **DPE** (downstream promoter element- bound by TFIID complex)

Core promoter elements



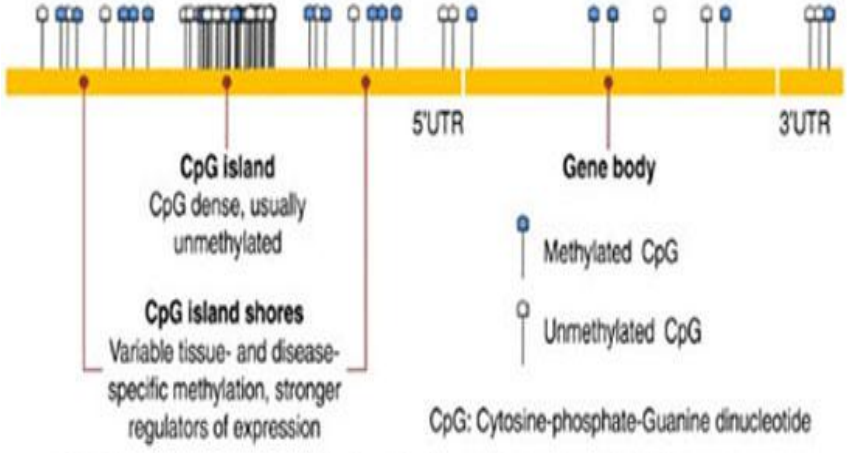
Butler J E , and Kadonaga J T Genes Dev. 2002;16:2583-2592

CpG Island promoters

- CpG islands -stretches of high GC content with over represented in CpG dinucleotides ranging between 0.5 -2 kb.

- Lacks TATA or DPE elements, contain multiple sites for GC box and SP1 .
- Transcription at CGI promoters initiates from multiple weak TSS.

- Promoter-CGI methylation can lead to transcriptional repression,
- Some CGI promoters are bidirectional.

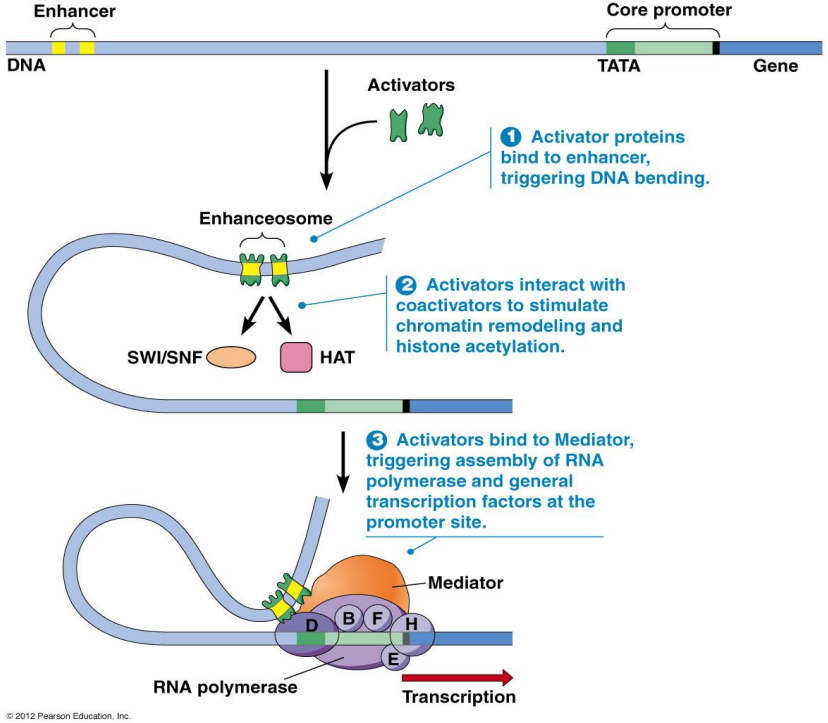


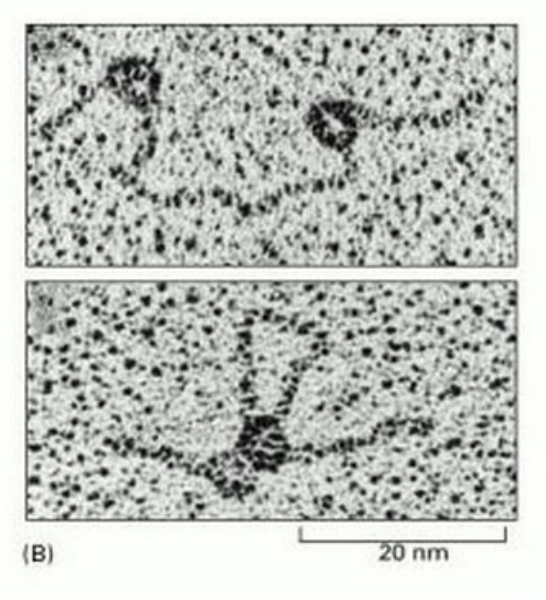
CpG Islands, shores, and methylation sites in relation to a hypothetical gene

2. Enhancers, Super Enhancers

Enhancers: Distal cis-regulatory modules located many Kb from the promoter. Enhancers interact with promoters by DNA looping.

Super Enhancers: Clusters of active enhancers bound by master transcription factors and the Mediator complex, termed super-enhancers, have been found to drive high-level expression of genes that control cell identity.





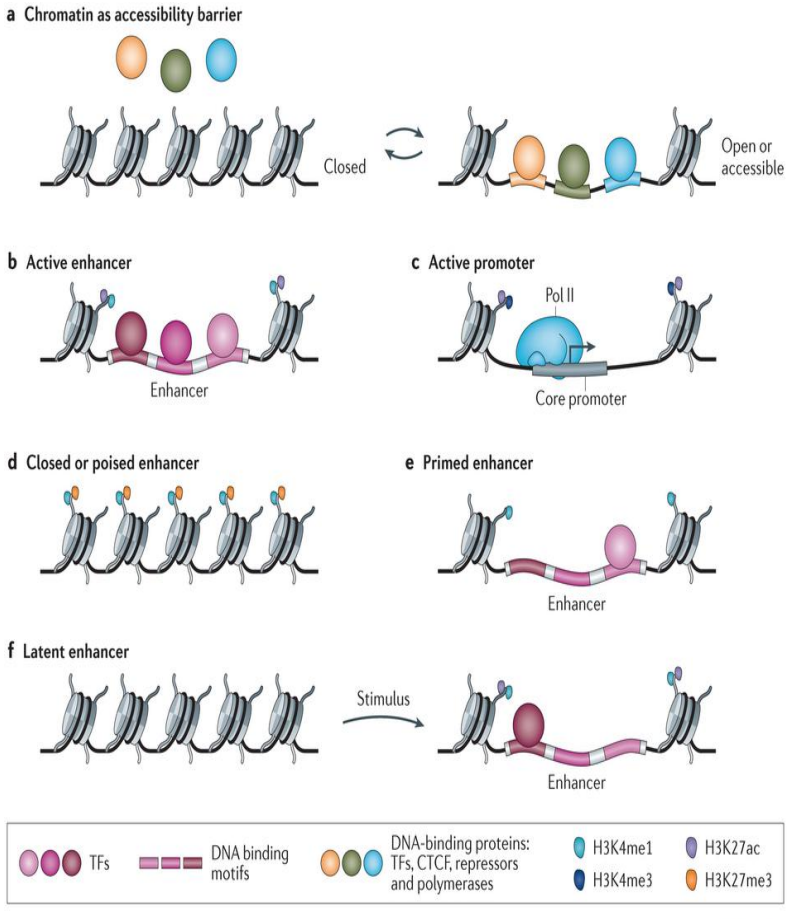
Alberts, "Molecular Biology of the Cell", 2002

Enhancers

- The **core promoter** alone allows a basal level of transcription to occur. When the core promoter is removed from the gene, no transcription occurs.

- An **enhancer** alone cannot substitute for the promoter region, but combining an enhancer with a core promoter results in a **significantly higher level** of transcription than occurs with the promoter alone.
- This increase in transcription is observed even when the enhancer is
  - moved farther upstream
  - inverted in orientation
  - moved to the 3 prime side of the structural gene.

- A number of histone marks (high H3K4me1 and H3K27Ac, low H3K4me3) are indicative of enhancers.



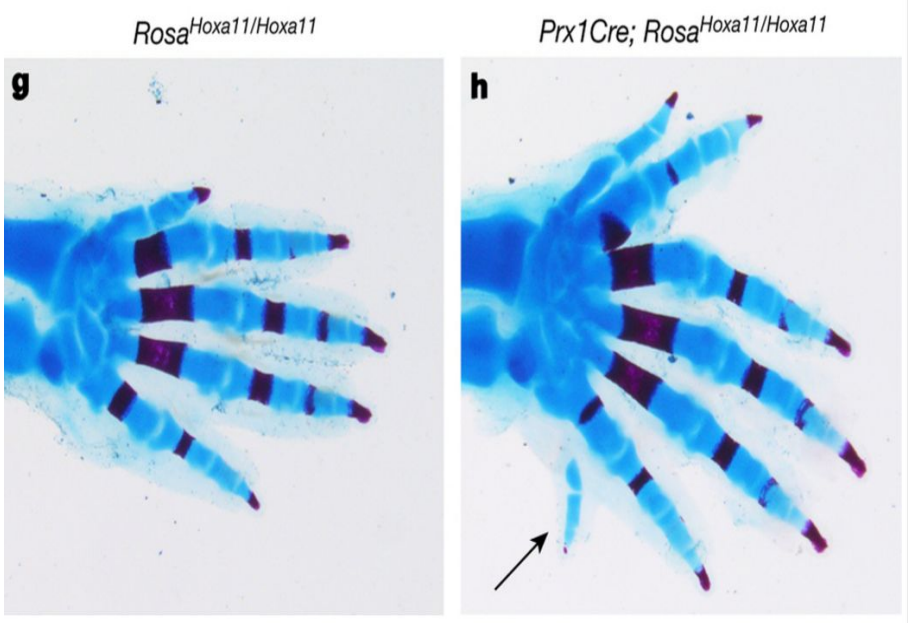
Shlyueva et al., Nat. Rev. Genetics 2014

Nature Reviews | Genetics

The hand skeleton of the *Rosa26*$^{Hoxa11/Hoxa11}$; prx1Cre mutant mouse. This mutation results in expression of the*Hoxa11* gene in distal limb buds, reminiscent of the expression in fin buds, and triggers the formation of extra-digits. Mosty living tetrapods — the four-legged land vertebrates — have five digits per limb.

If this number varies through mutation, it's always a reduction from the canonical five. But this state, called **pentadactyly**, wasn't always so hardwired. The earliest tetrapods had six, seven or even eight digits per limb — a polydactyly seen nowadays only in rare mutations. How did pentadactyly become established? Marie Kmita and colleagues show that mutually exclusive expression of *Hoxa11* and *Hoxa13* is required for five-digit limbs, that a transcriptional enhancer has evolved in the intron of the *Hoxa11* gene, and that its function is required to maintain the pentadactyl state.

The authors propose that the evolution of *Hoxa11* regulation has contributed to the transition from polydactyly in stem- group (extinct) tetrapods to pentadactyly in extant tetrapods.

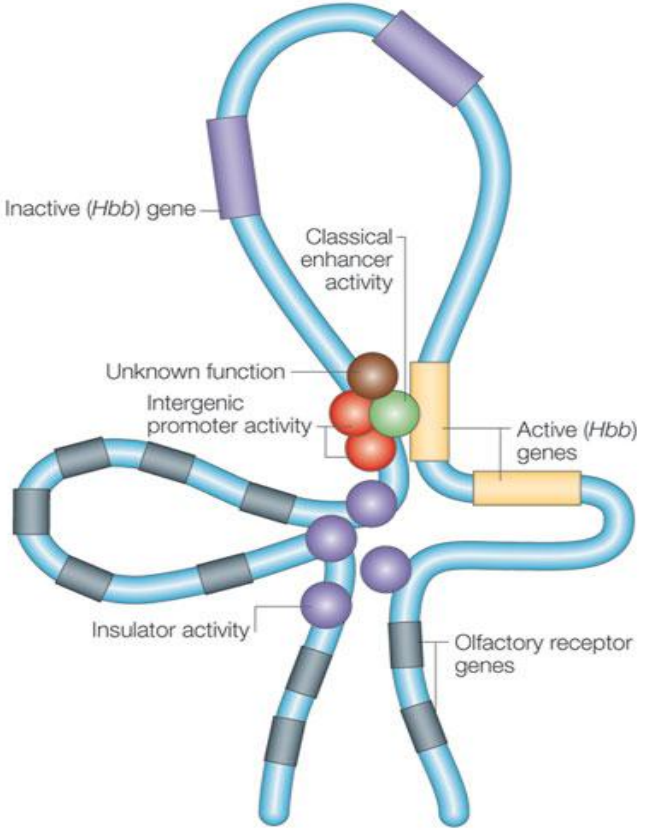


Kherdjemil et al., Nature 2016

3. Locus Control Regions

- Locus control regions (**LCRs**) are operationally defined by their ability to enhance the expression of **linked genes** to physiological levels in a tissue-specific and copy number-dependent manner at ectopic chromatin sites.

- Mouse globin gene cluster (*Hbb*) regulated by distal LCR (50kb away) by forming specific higher order looping structures



Chakalova et al., Nat. Rev. Genetics 2005

4. Silencers & Insulators

- Silencers are regulatory regions that repress gene expression when bound by repressor TFs.

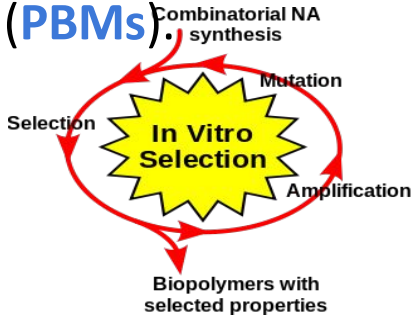
- Insulator elements prevent the activation of a promoter by an enhancer placed between them. In vertebrates, insulator function has primarily been attributed to 3D chromatin structures mediated by CTCCC-binding factor (CTCF).

Promoter resources

Gene Promoter Resources	URL
EPONINE	https://www.sanger.ac.uk/resources/software/eponine/
Eukaryotic Promoter Database	http://epd.vital-it.ch/
DBTSS	http://dbtss.hgc.jp/
FANTOM5	http://fantom.gsc.riken.jp/
FirstEF	http://rulai.cshl.org/tools/FirstEF/
MPromDb	http://mpromdb.wistar.upenn.edu/
Pleiades promoter project	http://www.pleiades.org/
Ensembl regulatory build	http://www.ensembl.org/info/genome/funcgen/regulatory_build.html
UCSC (ENCODE) regulation tracks	http://genome.ucsc.edu/

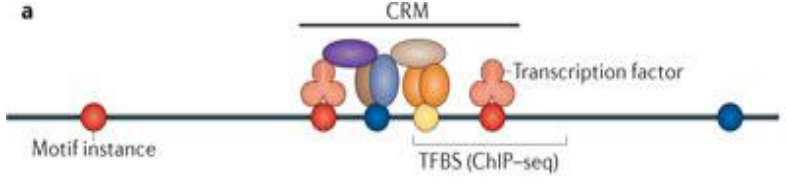
Computational identification of DNA regulatory elements

- Sequence **motifs**: short, recurring patterns in DNA or RNA associated with biological function.
- Sequence specific DNA binding sites for proteins (**nucleases, TFs**) or at the RNA level (**ribosome binding, mRNA processing and transcription termination**).
- Experimentally detected by DNase footprinting, EMSA or reporter constructs.
- Binding affinities are explored using **SELEX** (Systematic Evolution of Ligands by *in vitro* sElection) assays or Protein Binding microarrays (**PBMs**).



Cis Regulatory Modules (CRMs)

- Non-random clusters of TF binding sites that usually span a few 100bp.
- These dictate when, where and how genes are expressed.
- Can be **repressive** or **activating**, and output is the **combinatorial product** of multiple operations.
- Need to experimentally verify the function of CRMs to understand what the regulatory sequence does.
- Need robust computational methods to identify CRMs!



Hardison and Taylor, *Nature Reviews Genetics* 13, 469-483 (July 2012)

Scanning vs *de novo* motif identification

Don't scan a sequence with a motif model and expect all sites identified to be biologically active. Random matches will swamp the biologically relevant matches! This is a well known problem in motif searching, known as the **"Futility Theorem"** of motif finding. -(Wasserman and Sandelin, *Nat. Rev. Genet.* 2004;5:276-87)

1. PWM profile based **sequence scanning** methods. These methods uses prior information about TF binding sites and therefore can only be used to detect known Transcription Factor Binding Sites (**TFBS**).

2. ***De novo*** motif identification –Pattern discovery methods:

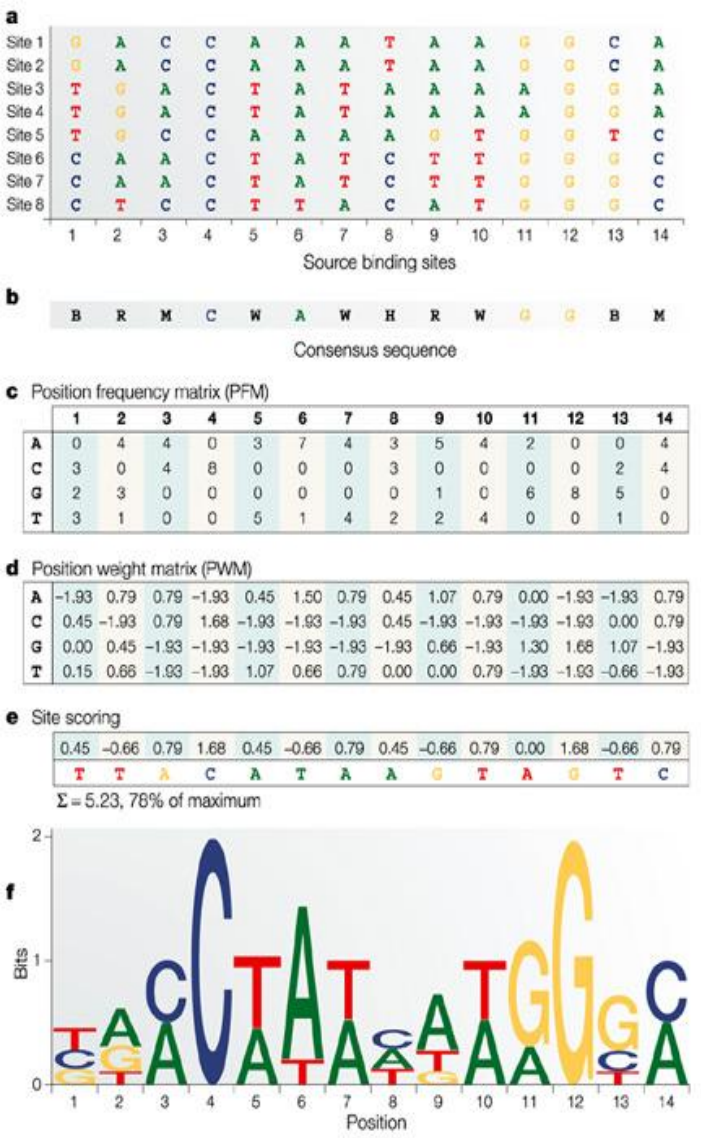
Word based – occurrence of each 'word' of nucleotides of a certain length is counted and compared to a background distribution.

Probabilistic- seek the most **overrepresented** pattern using algorithmic approaches like Gibbs sampling and Expectation Maximization. These iteratively evolve an initial random pattern until a more specific one is found.

Use *de novo* motif calling and alignment to build your own PWMs! Biostrings and Motiv Bioconductor packages have PFM to PWM conversion methods.

Representing binding sites: PFM and PWM

- Experimental binding data is collected and sequences are aligned.
- Consensus sequence: IUPAC codes that captures the sequence degeneracy of binding sites at each position.
- Position Frequency Matrix (PFM): Matrix encoding the nucleotide frequency at each position.
- Position Specific Scoring Matrix (PSSM) or Position Weight Matrix (PWM): Normalised frequency values of a PFM are converted into a log scale to get a PWM. For large and representative sequence collections the scores are proportional to binding energies.
- Sequence Logo: column specificity measured as information content for each nucleotide is scaled by the total bits of information multiplied by the relative occurrence of that nucleotide at that position.



Wasserman and Sandelin, Nat. Rev. Genet, 2004

Corrected probabilities of observing a given nucleotide can be calculated using equation 1.

Corrected probability calculation:

1
$$p(b,i) = \frac{f_{b,i} + s(b)}{N + \sum_{b' \in \{A,C,G,T\}} s(b')}$$

$f_{b,i}$ = counts of base b in position i; N = number of sites; $p(b,i)$ = corrected probability of base b in position i; $s(b)$ = pseudocount function

A position weight matrix (PWM) is constructed by dividing the nucleotide probabilities in (1) by expected background probabilities and converting the values to a log-scale (see equation 2).

PWM conversion:

2
$$W_{b,i} = \log_2 \frac{p(b,i)}{p(b)}$$

$p(b)$ = background probability of base b; $p(b,i)$ = corrected probability of base b in position i; $W_{b,i}$ = PWM vaue of base b in position i

The quantitative PWM score for a putative site is the sum of the PWM values for each nucleotide in the site (see equation 3).

Evaluation of sequences:

3
$$S = \sum_{i=1}^{w} W_{l_i,i}$$

l_i = the nucleotide in position i in an input sequence; S = PWM score of a sequence; w = width of the PWM

Probability values (1) can be used to determine the total information content (in bits) in each position (see equation 4).

Information content calculation:

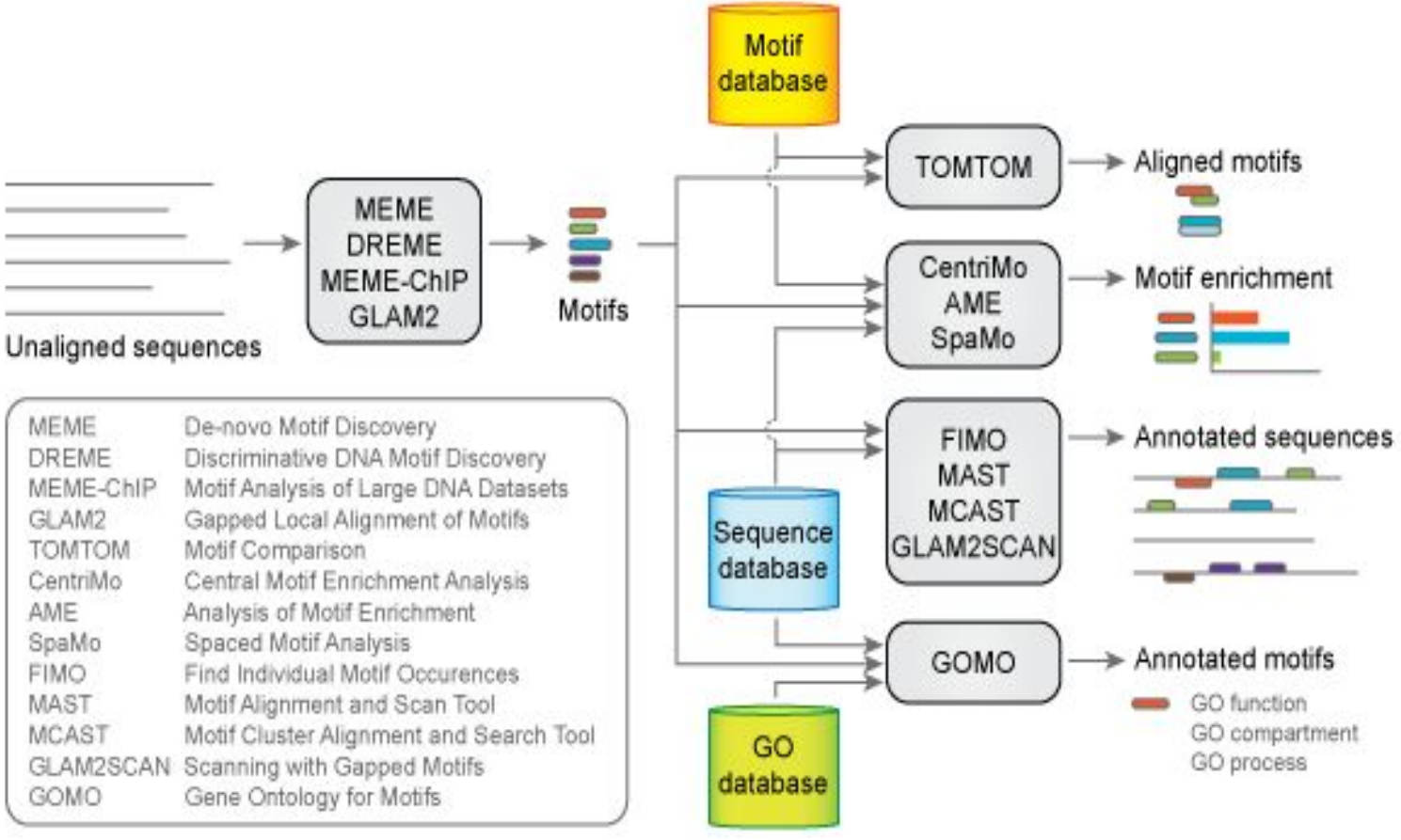
4
$$D_i = 2 + \sum_b p_{b,i} \log_2 p_{b,i}$$

D_i = information content in position i; $p(b,i)$ = corrected probability of base b in position i

Transcription factor binding databases

Transfac Professional	http://www.biobase-international.com/product/transcription-factor-binding-sites
Jaspar	http://jaspar.genereg.net/
UniProbe	http://the_brain.bwh.harvard.edu/uniprobe/index.php
Human Protein-DNA Interactome	http://bioinfo.wilmer.jhu.edu/PDI/FAQ.html
CistromeMap	http://cistrome.dfci.harvard.edu/pc/
OregAnno	http://www.oreganno.org/oregano/
FactorBook	http://www.factorbook.org/mediawiki/index.php/Welcome_to_factorbook
hmChIP	http://jilab.biostat.jhsph.edu/database/cgi-bin/hmChIP.pl
ChipBase	http://deepbase.sysu.edu.cn/chipbase/index.php
SwissRegulon	http://swissregulon.unibas.ch/fcgi/sr/swissregulon

Meme Suite



Bailey TL. et al., "MEME SUITE: tools for motif discovery and searching." Nucleic Acids Res. 2009

Meta-Motif Analyzers

URL: http://131.174.198.125/bioinfo/gimmemotifs/

GimmeMotifs: a *de novo* motif prediction pipeline, especially suited for ChIP-seq datasets. It incorporates several existing motif prediction algorithms in an ensemble method to predict motifs and clusters these motifs using the weighted information content (WIC) similarity scoring metric.

BioProspector http://motif.stanford.edu/distributions/bioprospector/

GADEM http://www.niehs.nih.gov/research/resources/software/gadem/index.cfm

Improbizer http://users.soe.ucsc.edu/~kent/

MDmodule (included in the MotifRegressor Package) http://www.math.umass.edu/~conlon/mr.html

MEME http://meme.sdsc.edu/

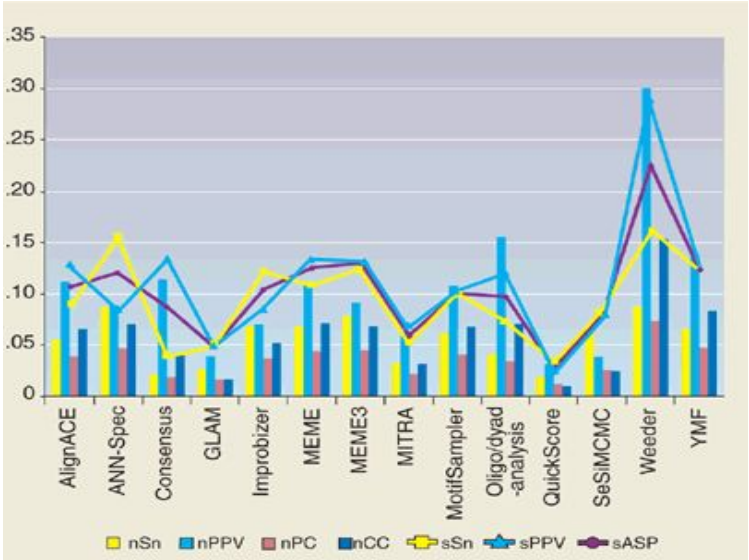
MoAn http://moan.binf.ku.dk/

MotifSampler http://homes.esat.kuleuven.be/~sistawww/bioi/thijs/download.html

Trawler http://ani.embl.de/trawler/

Weeder http://159.149.160.51/modtools/

Assessment of tools for TFBS discovery



Tompa et al., "Assessing computational tools for the discovery of transcription factor binding sites",Nature biotechnology 23 (1), 137-144

Table 1 Details about the operation principles, basic technical data and URLs of 13 analyzed tools

Program	Operating principle	Technical data	URL	Reference
AlignACE	Gibbs sampling algorithm that returns a series of motifs as weight matrices that are over-represented in the input set	Judges alignments sampled during the course of the algorithm using a maximum *a priori* log likelihood score, which gauges the degree of overrepresentation. Provides an adjunct measure (group specificity score) that takes into account the sequence of the entire genome and highlights those motifs found preferentially in association with the genes under consideration.	http://atlas.med.harvard.edu/	7
ANN-Spec	Models the DNA-binding specificity of a transcription factor using a weight matrix	Objective function based on log likelihood that transcription factor binds at least once in each sequence of the positive training data compared with the number of times it is estimated to bind in the background training data. Parameter fitting is accomplished with a gradient descent method, which includes Gibbs sampling of the positive training examples.	http://www.cbs.dtu.dk/~workman/ann-spec/	8
Consensus	Models motifs using weight matrices, searching for the matrix with maximum information content	Uses a greedy method, first finding the pair of sequences that share the motif with greatest information content, then finding the third sequence that can be added to the motif resulting in greatest information content, and so on.	http://bifrost.wustl.edu/consensus/	9
GLAM	Gibbs sampling-based algorithm that automatically optimizes the alignment width and evaluates the statistical significance of its output	Since the basic algorithm cannot find multiple motif instances per sequence, long sequences were fragmented into shorter ones, and the alignment was transformed into a weight matrix and used to scan the sequences to obtain the final site predictions.	http://zlab.bu.edu/glam/	10
The Improbizer	Uses expectation maximization to determine weight matrices of DNA motifs that occur improbably often in the input sequences	As a background (null) model it uses up to a second-order Markov model of background sequence. Optionally, Improbizer constructs a Gaussian model of motif placement, so that motifs that occur in similar positions in the input sequences are more likely to be found.	http://www.soe.ucsc.edu/~kent/improbizer	11
MEME	Optimizes the E-value of a statistic related to the information content of the motif	Rather than sum of information content of each motif column, statistic used is the product of the *P* values of column information contents. The motif search consists of performing expectation maximization from starting points derived from each subsequence occurring in the input sequences. MEME differs from MEME3 mainly in using a correction factor to improve the accuracy of the objective function.	http://meme.sdsc.edu/	12
MITRA	Uses an efficient data structure to traverse the space of IUPAC patterns.	For each pattern, MITRA computes the hypergeometric score of the occurrences in the target sequences relative to the background sequences and reports the highest scoring patterns.	http://www.calit2.net/compbio/mitra/	13
MotifSampler	Matrix-based, motif-finding algorithm that extends Gibbs sampling by modeling the background with a higher order Markov model	The probabilistic framework is further exploited to estimate the expected number of motif instances in the sequence.	http://www.esat.kuleuven.ac.be/~dna/BioI/Software.html	14

Table 1 continued on following page

Table 1 Continued

Program	Operating principle	Technical data	URL	Reference
Oligo/dyad-analysis	Detects overrepresented oligonucleotides with oligo-analysis[15] and spaced motifs with dyad-analysis[16]	These algorithms detect statistically significant motifs by counting the number of occurrences of each word or dyad and comparing these with expectation. Most crucial parameter is choice of appropriate probabilistic model for the estimation of occurrence significance. In this study, a negative binomial distribution on word distributions was obtained from 1,000 random promoter selections of the same size as the test sets	http://rsat.scmbb.ulb.ac.be/rsat/	15,16
QuickScore	Based on an exhaustive searching algorithm that estimates probabilities of rare or frequent words in genomic texts	Incorporates an extended consensus method allowing well-defined mismatches and uses mathematical expressions for efficiently computing *z*-scores and *P* values, depending on the statistical models used in their range of applicability. Special attention is paid to the drawbacks of numerical instability. The background model is Markovian, with order up to 3.	http://algo.inria.fr/dolley/QuickScore/	17
SeSiMCMC	Modification of Gibbs sampler algorithm that models the motif as a weight matrix, optionally with the symmetry of a palindrome or of a direct repeat, and optionally with spacers	Includes two alternating stages. The first one optimizes the weight matrix for a given motif and spacer length. The algorithm changes the positions of the motif occurrences in the sequences and infers the motif model from the current occurrences. These changes are used to optimize the likelihood of sequences as being segmented into the (Bernoulli) background and the motif occurrences. The optimization is organized via a Gibbs-like Markov chain, which samples positions in sequences one by one, until the Markov chain converges. The second stage looks for best motif and spacer lengths for obtained motif positions. It optimizes the common information content of motif and of distributions of motif occurrence positions.	http://favorov.hole.ru/gibbsIfm/	18
Weeder	Consensus-based method that enumerates exhaustively all the oligos up to a maximum length and collects their occurrences (with substitutions) from input sequences	Each motif evaluated according to number of sequences in which it appears and how well conserved it is in each sequence, with respect to expected values derived from the oligo frequency analysis of all the available upstream sequences of the same organism. Different combinations of 'canonical' motif parameters derived from the analysis of known instances of yeast transcription factor binding sites (length ranging from 6 to 12, number of substitutions from 1 to 4) are automatically tried by the algorithm in different runs. It also analyzes and compares the top-scoring motifs of each run with a simple clustering method to detect which ones could be more likely to correspond to transcription factor binding sites. Best instances of each motif are selected from sequences using a weight matrix built with sites found by consensus-based algorithm.	http://159.149.109.16/Tool/ind.php	19
YMF	Uses an exhaustive search algorithm to find motifs with the greatest *z*-scores	A *P* value for the z-score is used to assess significance of motif. Motifs themselves are short sequences over the IUPAC alphabet, with spacers ('N's) constrained to occur in the middle of the sequence.	http://bio.cs.washington.edu/software.html#ymf	20

Motif enrichment analysis (MEA)

- **MEA** determines which transcription factors control the transcription of a set of co-regulated genes by detecting enrichment of known binding motifs of TFs in the genes' regulatory regions.
- Is the **motif** ***over or underrepresented*** in the sample sequence set compared to a background sequence set?

- Identifying co-regulated gene sets is difficult. Use of Ontologies, pathways, GSEA etc is recommended.
- Picking the right background model will also contribute to the success of the motif enrichment analysis.

Background models

- All core-promoters from protein coding or non-coding genes etc.
- Window around TSS
- Higher order Markov model based backgrounds
- Open chromatin regions
- Shuffled sequence regions
- Known Regulatory regions - ENCODE regulatory modules
- Exonic or Intronic regions- ex: 3rd exon

- Selecting sequences
  - Similar number or genome wide
  - Defined or variable length

MEME-ChIP

url: http://meme.nbcr.net

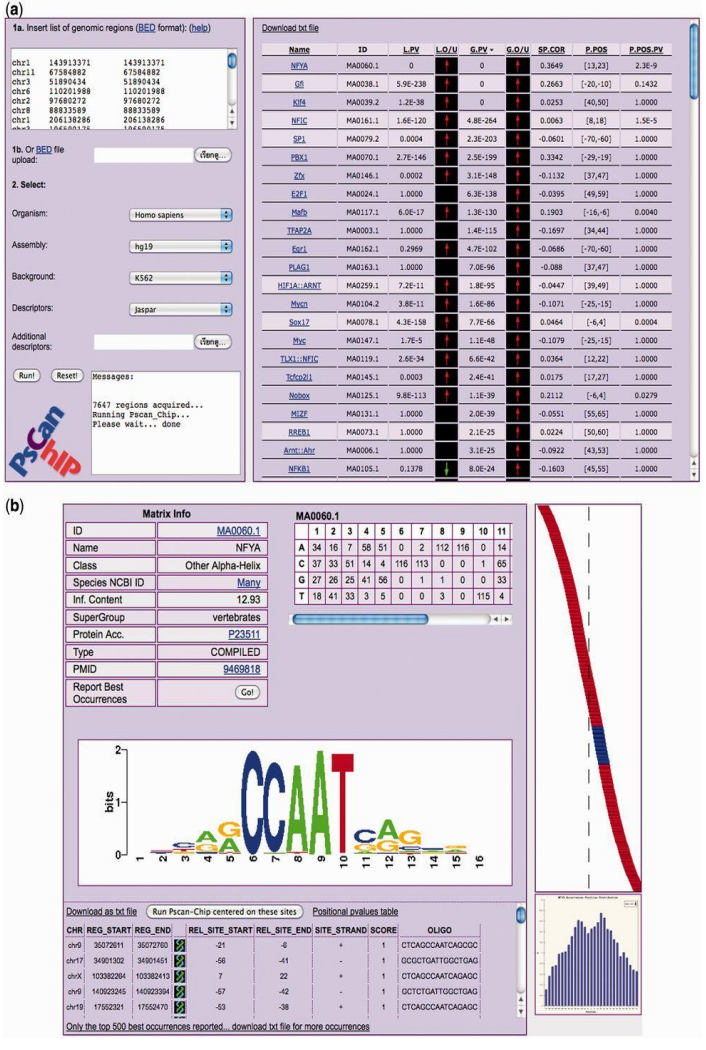
- Given a set of genomic regions, it performs
  - ab initio motif discovery -novel TF binding sites (MEME, DREME)
  - motif enrichment analysis -known TF enrichment (Centrimo/AME)
  - motif visualization (MAST and AMA)
  - binding affinity analysis
  - motif identification -compare to known motifs (TOMTOM)
- Uses two algorithms for motif discovery:
  - MEME -expectation maximization (EM) to discover probabilistic models of DNA-binding by single TFs or TF complexes.
  - DREME -simpler, non-probabilistic model (regular expressions) to describe the short binding motifs.

Machanick and Bailey, "MEME-ChIP: motif analysis of large DNA datasets." 2011 Bioinformatics

Pscan-ChIP

URL: http://159.149.160.51/pscan_chip_dev/

- Motif enrichment analysis using PWMs
- Profile databases and user defined background models.
- Optimized for ChIP-seq.
- Provides:
  - Ranked lists of enriched motifs.
  - Sequence logo's and motif enrichment distribution plots.



"PscanChIP: Finding over-represented transcription factor-binding site motifs and their correlations in sequences from ChIP-Seq experiments." Zambelli et al., 2013 Nucleic Acids Res.

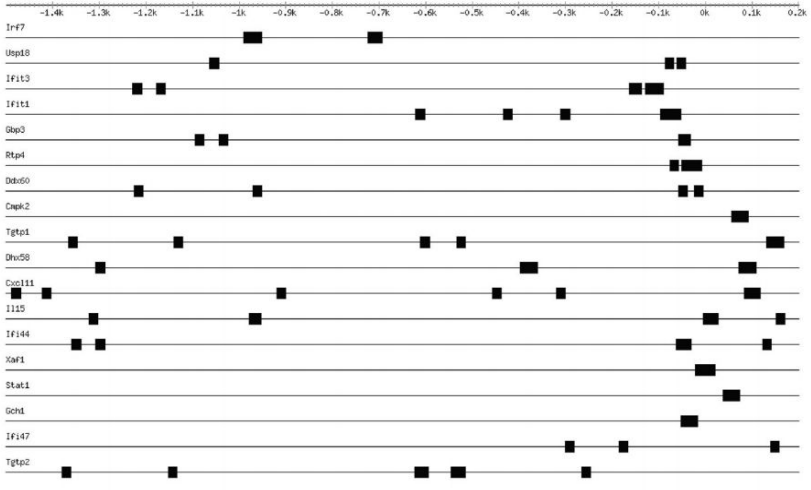
HOMER (Hypergeometric Optimization of Motif Enrichment)

URL: http://homer.salk.edu/homer/index.html

- Large number of (Perl and C++) tools for ChIP-seq motif and peak analysis.
- Provides both *de novo* and PWM scanning based motif identification and enrichment analysis.
- User can specify custom background. (Randomly selected, GC or CGI matched backgrounds.)
- Uses a collection of ChIP-seq derived PWMs or user can specify PWM.
- Peak annotation, GO enrichment analysis, Extract peak sequences, Visualization.

Real world applications of Motif Enrichment Analysis

- Breast cancer metastasis is a key determinant of long-term patient survival.
- By comparing the transcriptomes of primary and metastatic tumor cells in a mouse model of spontaneous bone metastasis, we identified that a substantial number of genes suppressed in bone metastases are targets of the interferon regulatory factor Irf7.
- Restoration of Irf7 in tumor cells or administration of interferon led to reduced bone metastases and prolonged survival time.



ARTICLES

nature medicine

Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis through immune escape

Bradley N Bidwell[1,2,9], Clare Y Slaney[1,3,9], Nimali P Withana[1,4], Sam Forster[5], Yuan Cao[1,2], Sherene Loi[6], Daniel Andrews[1–3], Thomas Mikeska[1,4], Niamh E Mangan[5], Shamith A Samarajiwa[5,7], Nicole A de Weerd[5], Jodee Gould[5], Pedram Argani[8], Andreas Möller[1–4], Mark J Smyth[1,3], Robin L Anderson[1,3,4], Paul J Hertzog[5] & Belinda S Parker[1–3]

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Jonathan Cairns

Rory Stark