Introduction to cis-regulation

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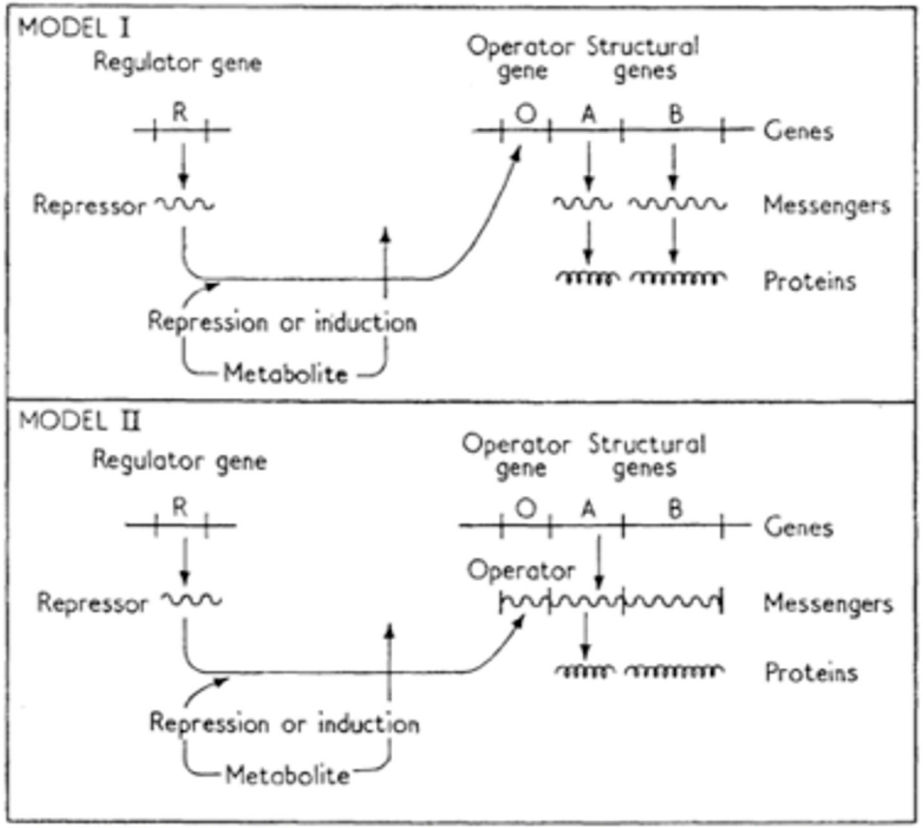
Back to history: the lac operon



Jacob, F. and Monod, J. (1961). Genetic regulatory mechanisms in the synthesis of proteins. J Mol Biol 3, 318-56.

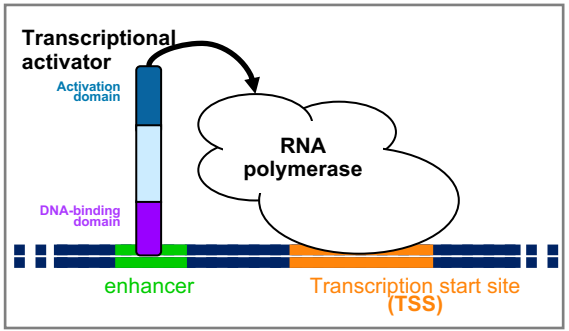
- 1960: François Jacob and Jacques Monod propose two alternative models for the regulation of the Lac operon
  - at the level of transcription,
  - at the level of mRNA
- The basic mechanism underlying their model is the negative control (repression) of gene expression.
- In both cases, they highlight the importance of feedback loops.

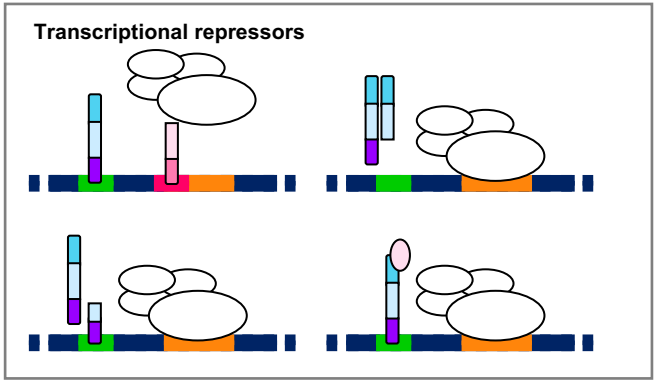
- Jacob, F., Perrin, D., Sanchez, C. and Monod, J. (1960). [Operon: a group of genes with the expression coordinated by an operator.]. C R Hebd Seances Acad Sci 250, 1727-9.
- Jacob, F. and Monod, J. (1961). Genetic regulatory mechanisms in the synthesis of proteins. J Mol Biol 3, 318-56.
- Jacob, F. (1997). L'opéron, 25 ans après. C. R. Acad. Sci. PAris 320, 199-206.

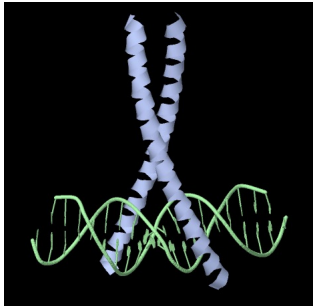
What is a transcription factor?

What is a transcription factor ?

- Protein affecting the level of transcription of a specific set of genes.
- Activity
  - A transcription factor is qualified as activator or repressor depending on whether it increases of represses the expression of its target gene(s).
  - It has to be noted that the activator/repressor qualifier applies to the interaction between the TF and a given gene rather than on the TF itself, since a factor can activate some genes and repress other ones.
- Specificity
  - Transcription factors are qualified of specific or global depending on whether they act on a restricted or a large number of genes (the boundary between specific and global factors is somewhat arbitrary).
- Mechanisms
  - DNA-binding transcription factors act by binding to specific genomic locations, called transcription factor binding sites. Transcription factor may also act indirectly on the expression of their target genes by interacting with other transcription factors. For example, the yeast repressor Gal80p does not bind DNA, but interacts with the DNA-binding transcription factor Gal4p and prevents it from activating its target genes.



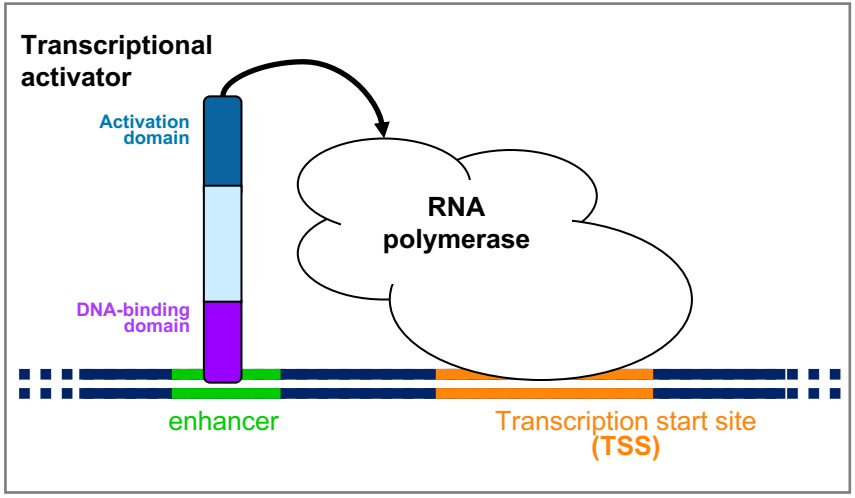




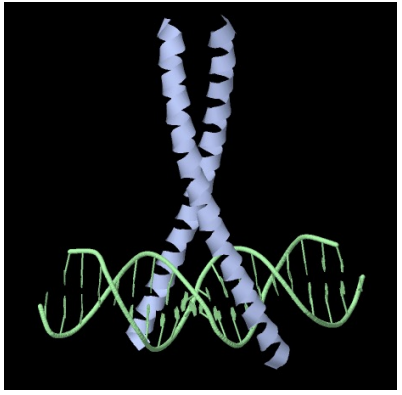
Gcn4p from Saccharomyces cerevisiae
PDB ***2DGC***
www.rcsb.org/pdb/explore.do?structureId=2DGC

- Jacques van Helden, in Concise Encyclopaedia of Bioinformatics and Computational Biology, 2nd Edition. John M. Hancock (Editor), Marketa J. Zvelebil (Editor). ISBN: 978-0-470-97871-9

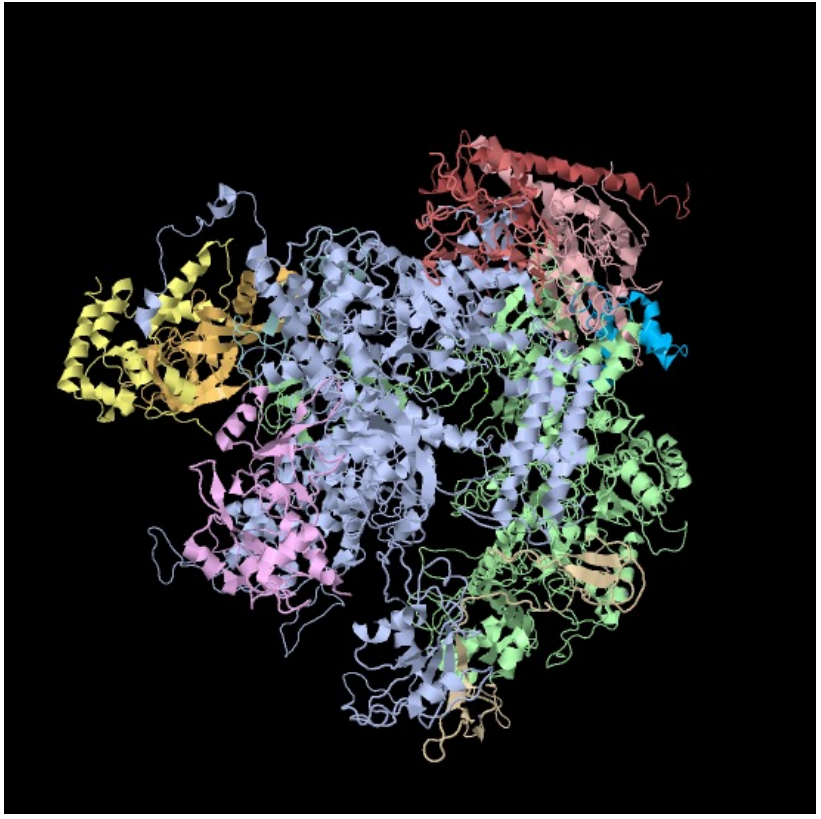
Transcriptional activation



Gcn4p from Saccharomyces cerevisiae
PDB 2DGC http://www.rcsb.org/pdb/explore.do?structureId=2DGC

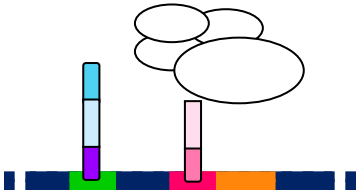


RNA polymerase II from Schizosaccharomyces pombe.
PDB 3H0G http://www.rcsb.org/pdb/explore.do?structureId=3H0G

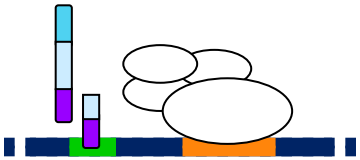


Transcriptional repression

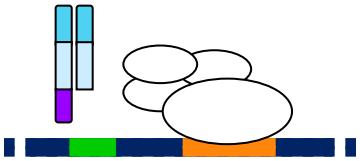
- The concept of transcriptional repression encompasses a variety of molecular mechanisms.



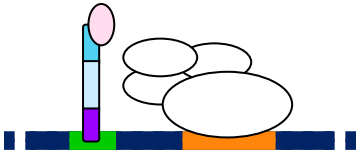
Promoter occupancy: prevent RNA polymerase from accessing DNA (e.g. many bacterial repressors)



Cis-regulatory element occupancy: competition for factor binding site (e.g. yeast GATA factors)



Titration of the activator: repressor forms dimer with activator, which prevents its binding to TFBS (e.g. Drosophila Helix-loop-helix)

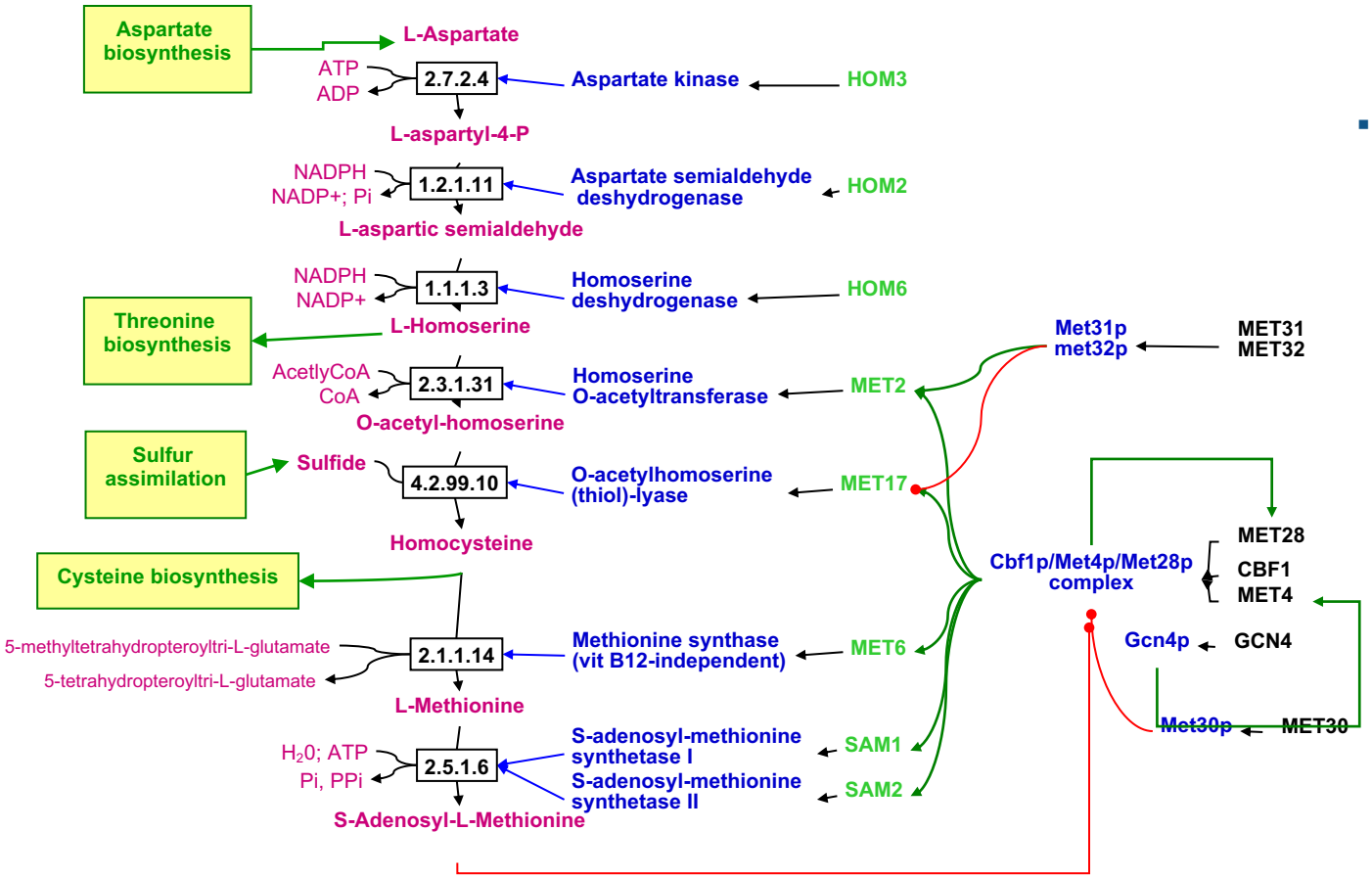


Allosteric regulation: repressor binds to activator, which alters activator conformation and prevents it from interacting with RNA-polymerase (e.g. yeast Gal80p)

What are transcription factors doing ?

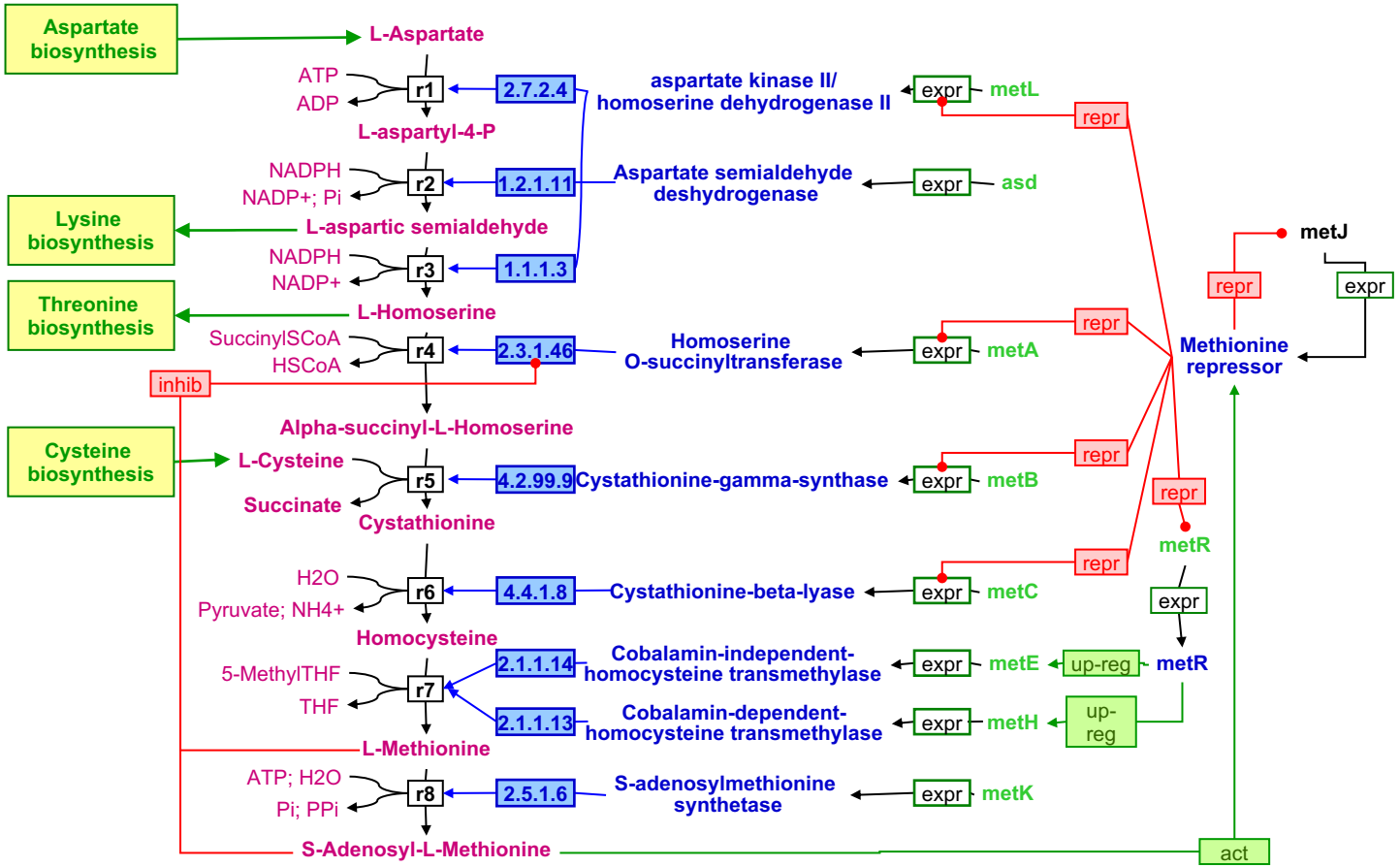
Regulation of biological processes: some examples

Methionine Biosynthesis in Saccharomcyes cerevisiae



- In the budding yeast, the enzymes involved in methionine biosynthesis are cis-regulated by various transcription factors.
- The main regulator (Cbf1/Met4p/Met28p complex) is itself trans-regulated by the end product (inhibition of the activator), thereby creating a negative feed-back loop that ensures homeostasis.

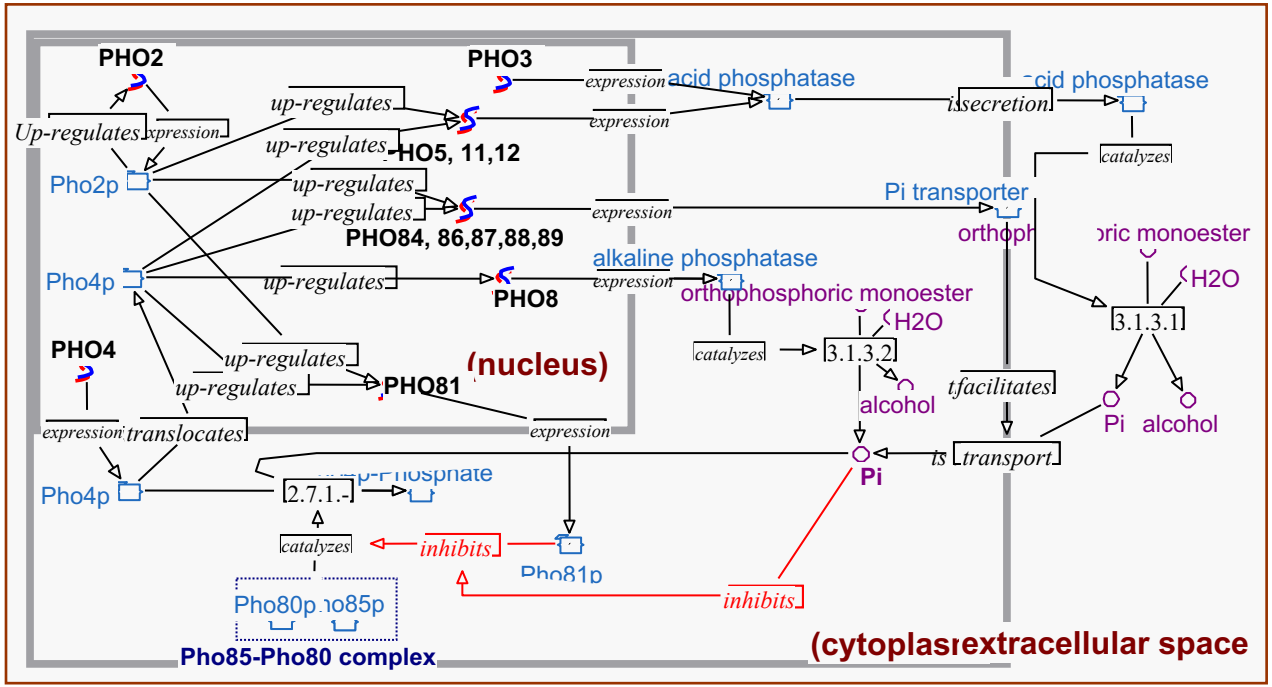
Methionine Biosynthesis in E.coli



- In the bacteria *Escherichia coli*, the enzymes involved in methionine biosynthesis are cis-regulated by the repressor metJ and the activator metR.
- The metR activator is repressed by the metJ repressor
- Those factors are themselves trans-regulated by the end product (activation of the repressor), thereby creating a negative feed-back loop that ensures homeostasis.

Phosphate utilization in Saccharomyces cerevisiae

- The budding yeast responds to a phosphate stress by expressing
  - Two types of phosphatases: alcaline (Pho8p) and acid (Pho5p, Pho11p, Pho12p).
  - Several phosphate transporters (Pho84p, Pho86p, Pho87p, Pho88p, Pho89p).
  - Regulatory proteins (Pho81p) ensuring a negative feedback loop)
- When Phosphate concentration is high, the transcriptional activator (Pho4p) is inactivated.



Where do transcription factors bind ?
(and how do we know)

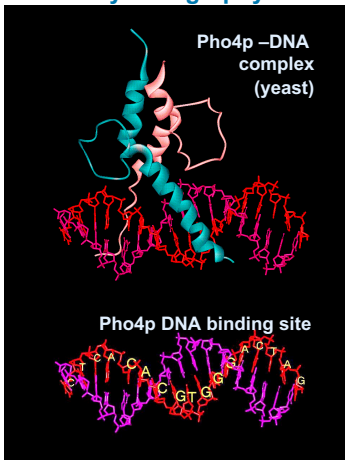
Transcription factor binding sites (TFBS)
Transcription factor binding regions (TFBR)

Definition: transcription factor binding site (TFBS)

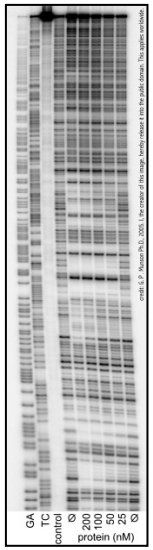
- Transcription factor binding site
  - Position on a DNA molecule where a transcription factor (TF) specifically binds.
  - By extension, the sequence of the bound DNA segment.
  - Note that there is a frequent confusion in the literature between the concepts of binding site and binding motif. We recommend to reserve the term “site” to denote the particular (genomic or artificial) where a factor binds, and the term “motif” for the generic description of the binding specificity, obtained by summarizing the information provided by a collection of sites.

How do we know ? (details in the next slides)

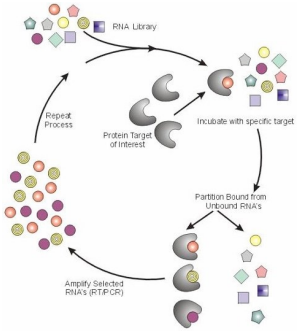
Crystallography



DNAse footprint

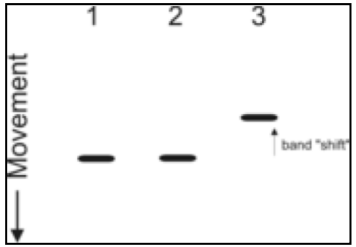


SELEX



http://www.molgen.mpg.de/~in-vitro/technology.html

Gel shift (EMSA)



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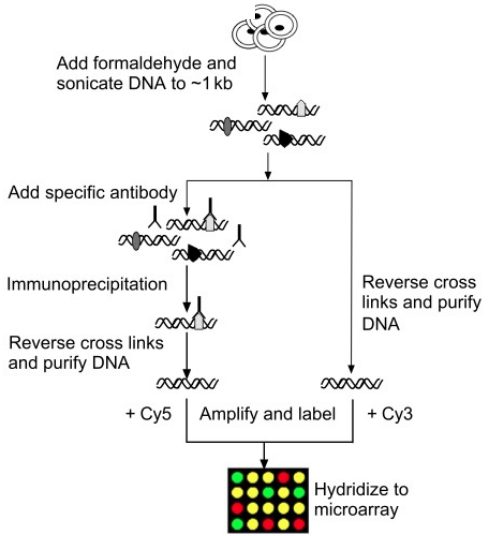
Definition: transcription factor binding region (TFBR)

TFBR: Transcription factor binding region

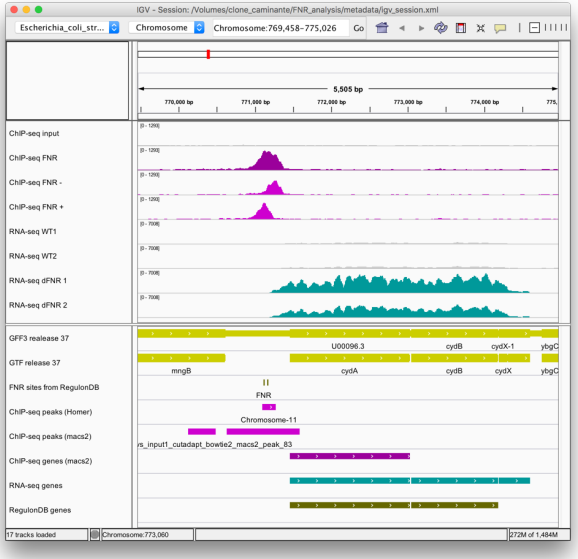
- A genomic region where a transcription factor (TF) specifically binds.
- Characterized by genome-wide location analysis methods (ChIP-on-chip, ChIP-seq).
- Note: avoid the confusion
  - TFBS: precise location covering a few nucleotides entering in direct contact with the transcription factor
  - TFBR: broader (a few tens or hundreds of base pairs) location, for which we have evidence that the TF bind somewhere therein.

How do we know ? *(details in the next slides)*

ChIP-on-chip



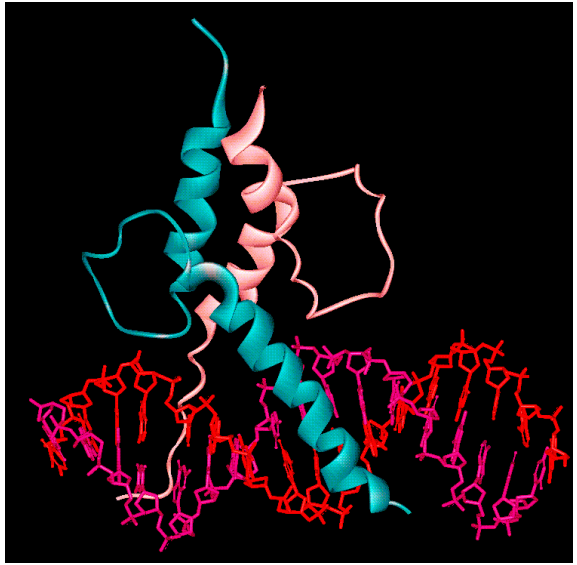
ChIP-seq



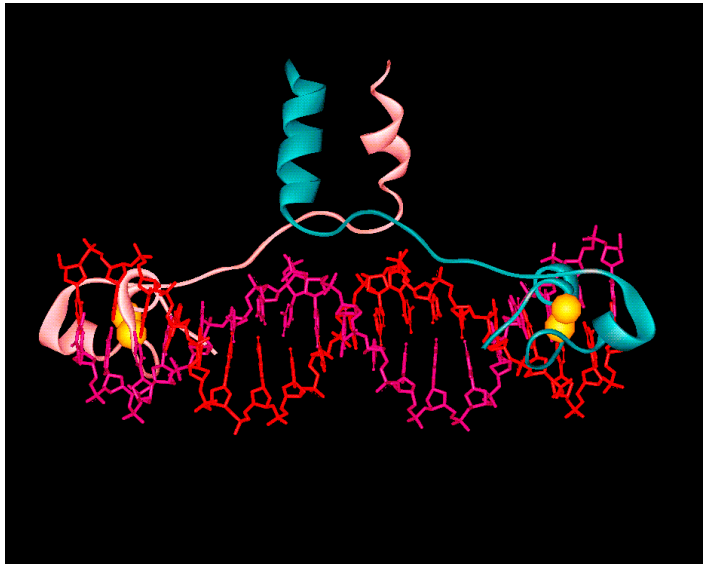
Experimental methods for characterizing transcription factor binding sites

Crystallography of TF-DNA complex

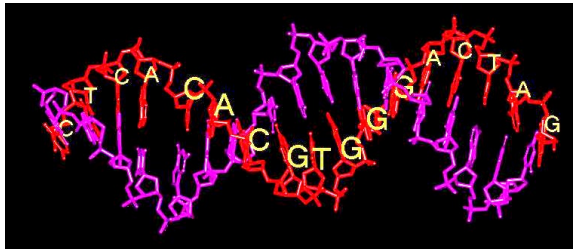
Pho4p (yeast)



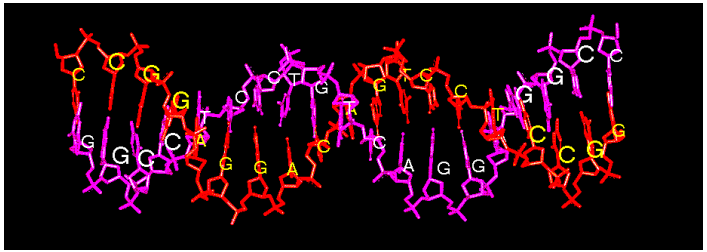
Gal4p (yeast)



Pho4p DNA binding site (oligonucleotide)



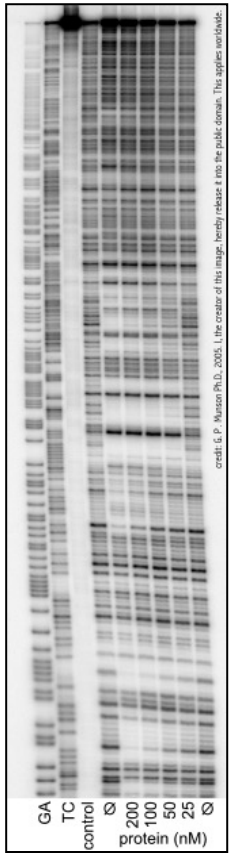
Gal4p DNA binding site (dyad)



DNAse footprinting

- DNAse footprint
  - Galas & Schmitz (1978). DNAse footprinting: a simple method for the detection of protein-DNA binding specificity. Nucleic Acids Res. 30: 1851-1858.
  - The residues participating in the DA-TF interface are protected from the DNAse.
  - Sites are characterized very precisely (typically 6-20bp)

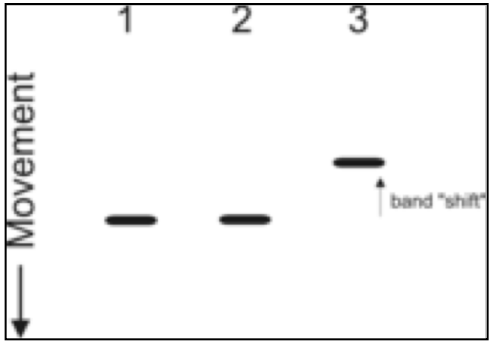
DNAse footprint



Electro-mobility shift assay (EMSA)

- EMSA
  - Garner & Revzin (1981). A gel electrophoretic method for quantifying the binding of proteins to specific DNA regions: applications to components of the Escherichia coli lactose operon regulatory system. Nucleic Acids Res. 5: 3157-3170.
  - Electrophoretic mobility shift assay (also called gel shift).
  - Larger fragments than footprints: sometimes 50bp or more.

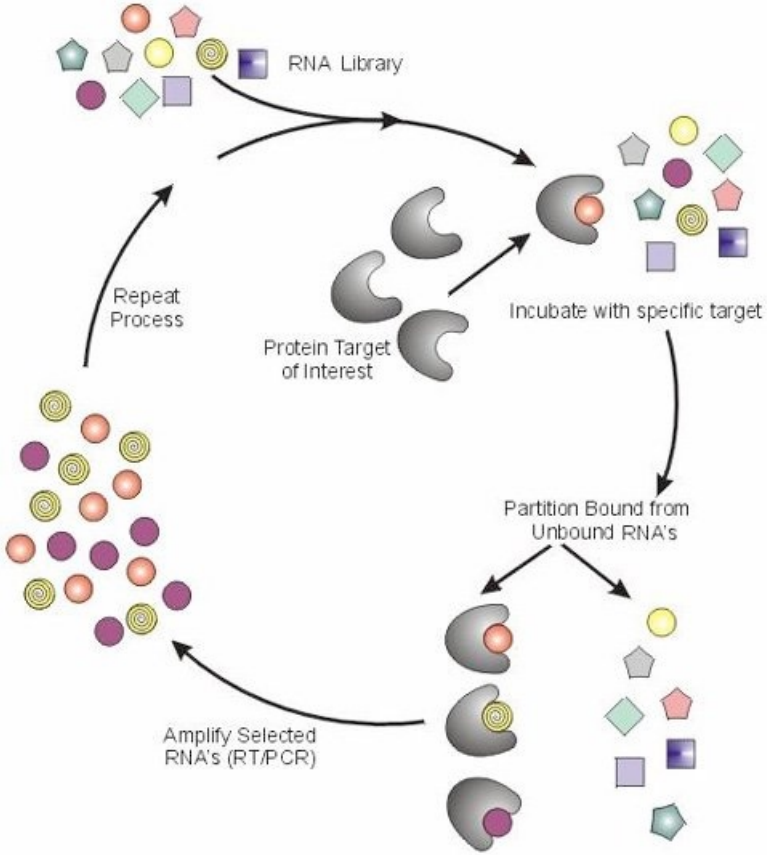
Gel shift (EMSA)



Lane 1 is a negative control, and contains only DNA. Lane 2 contains protein as well as a DNA fragment that, based on its sequence, does not interact. Lane 3 contains protein and a DNA fragment that does react; the resulting complex is larger, heavier, and slower-moving. The pattern shown in lane 3 is the one that would result if all the DNA were bound and no dissociation of complex occurred during electrophoresis. When these conditions are not met a second band might be seen in lane 3 reflecting the presence of free DNA or the dissociation of the DNA-protein complex.

Source: Wikipedia

SELEX



http://www.molgen.mpg.de/~in-vitro/technology.html

SELEX = Systematic Evolution of Ligands by EXponential enrichment.

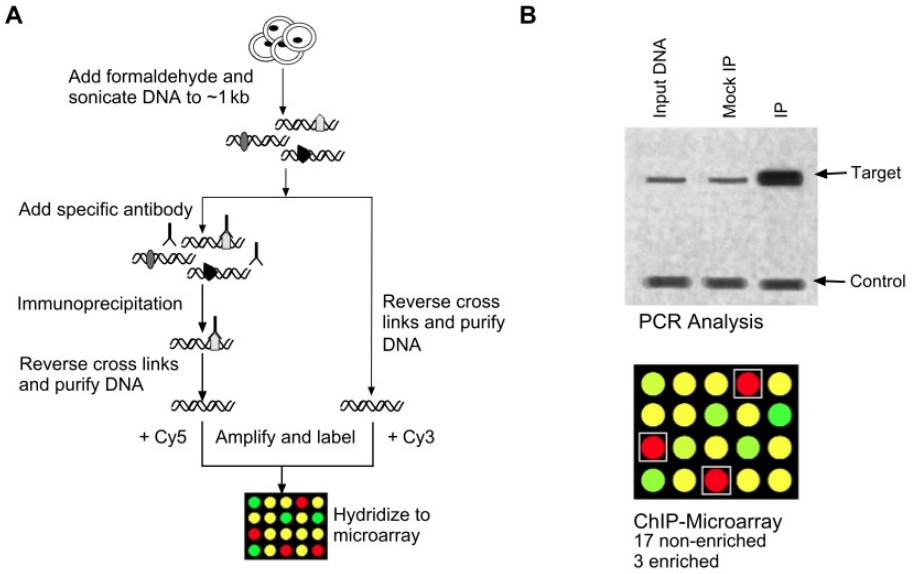
- Several rounds of selection – amplification.
- Each round selects (and then amplifies) oligonucleotides with increased specificity relative to previous round.
- Pros
  - All the usual advantages of *in silico* methods.
  - Easy to obtain hundreds of TFBS for a given TG
- Cons
  - All the usual weaknesses of *in silico* methods.
  - Provides TF binding sequences but no sites (no genomic location).
  - Motifs built from SELEX collections are usually over-selected, and thus too specific to reflect the in vivo binding specificity of the factor.

Variations on the theme

- High-throughput SELEX (HT-SELEX): use of next-generation sequencing → thousands of binding oligonucleotides
- Genomic SELEX: instead of random oligonucleotides, the process is seeded with genomic fragments → increased biological relevance (yet this is stil an *in vitro* method)

ChIP-on-chip

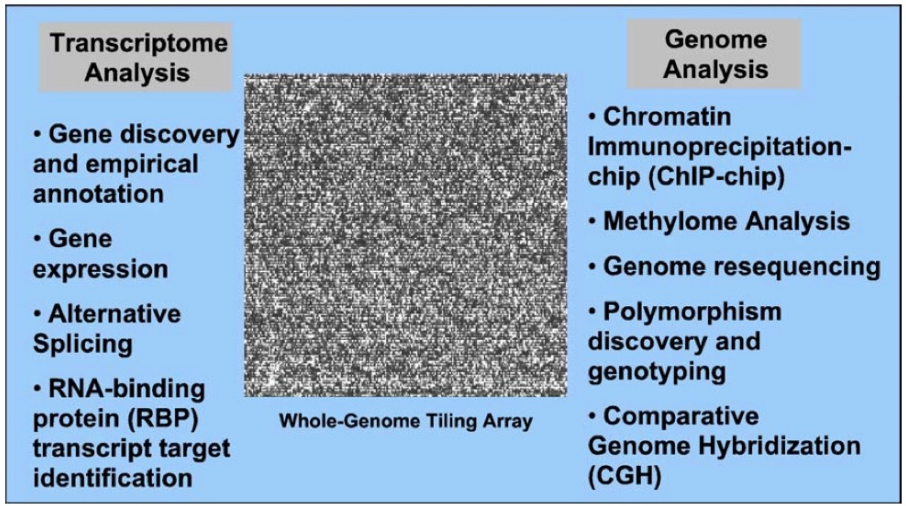
- Combines
  - Chromatin Immunoprecipitation (ChIP) to select genome fragments bound to a tagged transcription factor.
  - DNA microarrays (chip) spotted with several thousands of genome fragments (typically all the intergenic regions of agiven organism) are used to detect the relative enrichment: immuno-precipitated (IP) versus non-precipitated DNA (« mock » IP).
- Strength: genome-wide coverage
- Weakness: fragmentation by sonication -> large variations in DNA fragment sizes (from a few tens of bases to several kbs).

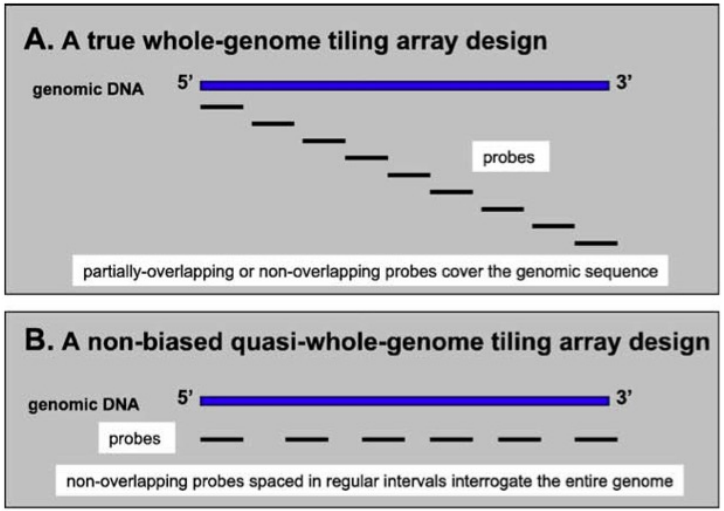


- Buck and Lieb. ChIP-chip: considerations for the design, analysis, and application of genome-wide chromatin immunoprecipitation experiments. Genomics (2004) vol. 83 (3) pp. 349-60

Tiling arrays

- Tiling arrays are high-resolution and full coverage ChIP-on-chip.
- High-resolution: spotted oligonucleotides (note: still limited by cDNA fragmentation).
- Tiling arrays cover the entirety of a genome, without pre-selection of any particular sequence type (intergenic, coding).
- Can be used to obtain high-coverage mapping of TF binding sites with the ChIP-chip method.
- Number of sequence fragments per array: between 10,000 and 6,000,000.





Mockler et al. Applications of DNA tiling arrays for whole-genome analysis. Genomics (2005) vol. 85 (1) pp. 1-15

“Universal” protein-binding microarrays (PBM)

- Microarrays containing each possible oligonucleotide of a given size (e.g. 12 nucleotides).
- Quantification of the binding for a given protein.
- Difficulties:
  - Some DNA binding protein domains (e.g. bacterial HTH, Fungal Zinc Cluster domains) recognize spaced motifs, much wider than 12 base pairs.
  - Choice of the algorithm strongly influences the motif derived from the bound oligonucleotides
  - Protein complexes

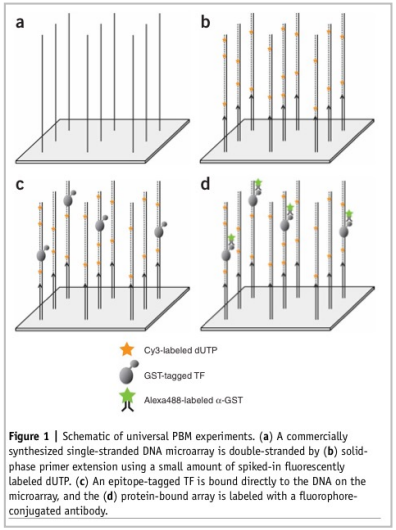
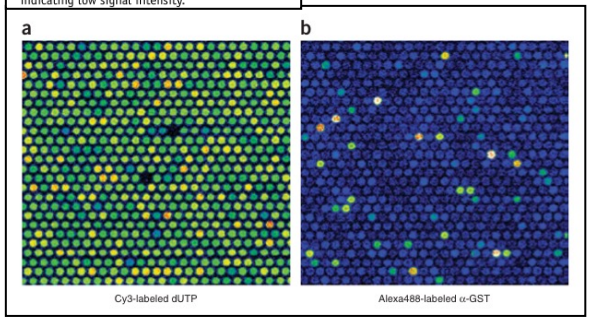


Figure 1 | Schematic of universal PBM experiments. (**a**) A commercially synthesized single-stranded DNA microarray is double-stranded by (**b**) solid-phase primer extension using a small amount of spiked-in fluorescently labeled dUTP. (**c**) An epitope-tagged TF is bound directly to the DNA on the microarray, and the (**d**) protein-bound array is labeled with a fluorophore-conjugated antibody.

Figure 3 | Zoom-in of a universal PBM scan. (**a**) Region of a single subgrid, consisting of just over 1% of the total slide area, scanned to detect relative DNA amounts, as indicated by Cy3-labeled dUTP. (**b**) The same region of the same microarray, scanned with a different laser to detect protein binding, as indicated by Alexa 488-labeled anti-GST antibody. Intensities are shown in false color, with white indicating saturated signal intensity, yellow indicating high signal intensity, green indicating moderate signal intensity and blue indicating low signal intensity.



- Berger and Bulyk. Universal protein-binding microarrays for the comprehensive characterization of the DNA-binding specificities of transcription factors. Nature Protocols (2009) vol. 4 (3) pp. 393-411

The “next generation sequencing” (NGS) era

- When sequencing costs followed Moore’s law
  - The cost of sequencing decreased exponentially since the end of the 1990s, due to the improvements and automation of sequencing, stimulated by the genome sequencing projects.
  - This decrease was more or less proportional to the exponential decrease of storage and computing costs (Moore’s law).
- Next Generation Sequencing
  - In 2007, several companies proposed new technologies enabling a much faster sequencing.
  - The cost of sequences now decreases much faster than the cost of computers.
  - We can foresee real problems for storing and analysing the massive amounts of sequences to be produced.

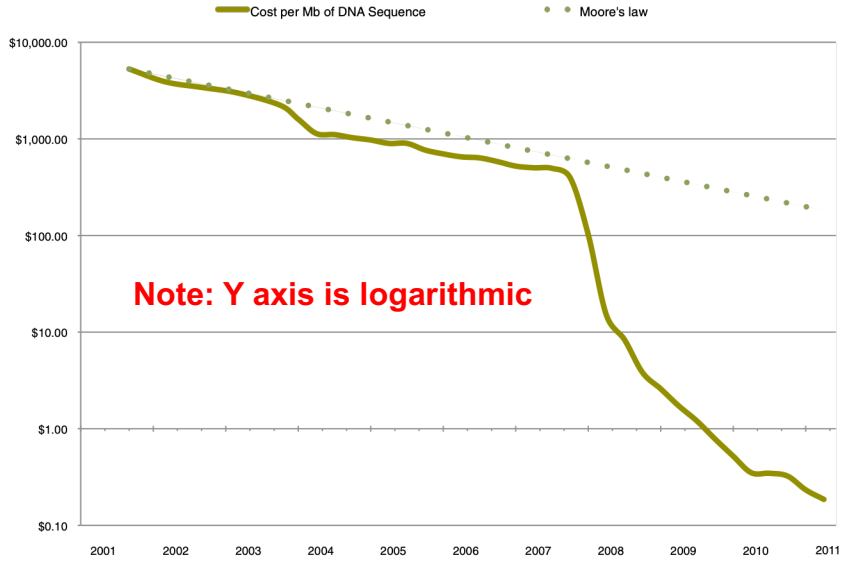


Figure 2. Cost of 1 MB of DNA sequencing. Decreasing cost of sequencing in the past 10 years compared with the expectation if it had followed Moore’s law. Adapted from [11]. Cost was calculated in January of each year. MB, megabyte.

Sboner et al. (2011) The real cost of sequencing: higher than you think!. Genome Biol 12: 125

Cost of sequencing projects

- The decrease of sequencing cost is accompanied by a drastic change in cost repartition, with a relative increase of the pre-processing (sample collection) and post-processing (bioinformatics analysis).
- There is thus an increasing need for bioinformatics know-how in all the laboratories treating next generation sequencing data.

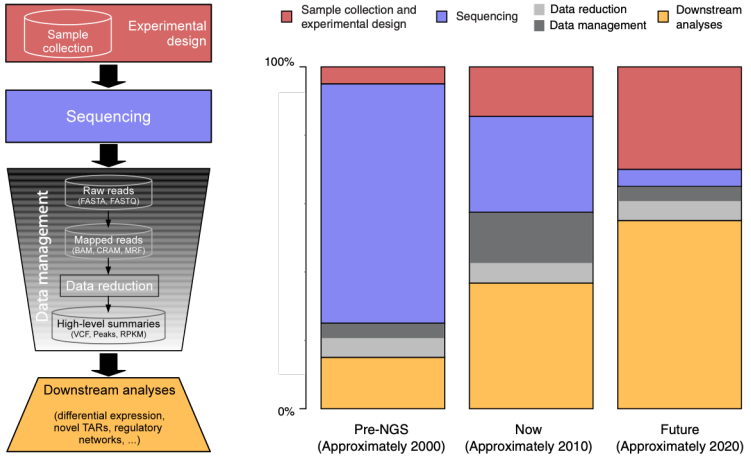
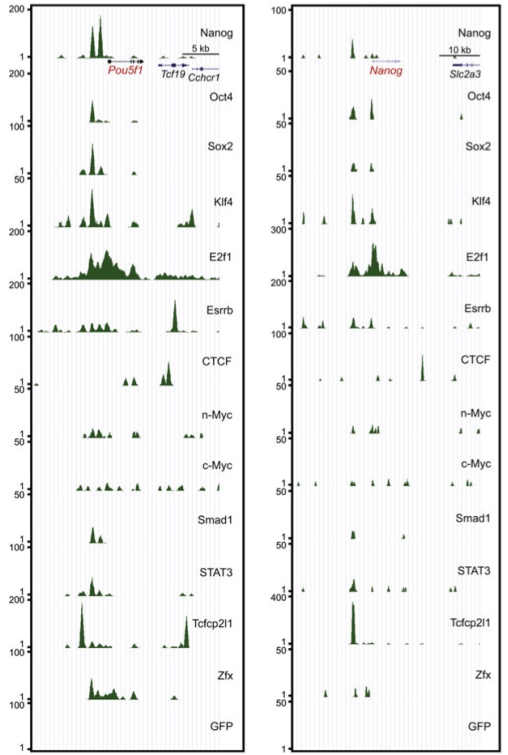


Figure 1. Contribution of different factors to the overall cost of a sequencing project across time. Left, the four-step process: (i) experimental design and sample collection, (ii) sequencing, (iii) data reduction and management, and (iv) downstream analysis. Right, the changes over time of relative impact of these four components of a sequencing experiment. BAM, Binary Sequence Alignment/Map; BED, Browser Extensible Data; CRAM, compression algorithm; MRF, Mapped Read Format; NGS, next-generation sequencing; TAR, transcriptionally active region; VCF, Variant Call Format.

- Sboner et al. (2011) The real cost of sequencing: higher than you think!. Genome Biol 12: 125

ChIP-seq

- Combination of
  - Chromatin Immunoprecipitation (ChIP), as for ChIP-chip.
  - Next Generation Sequencing (NGS) to characterize the immunoprecipitated DNA fragments.
- Strength:
  - No problem of imprecision due to the hybridation of large IP fragments to short spotted features.
  - Thanks to the « next » generation sequencing (NGC) methods, sequencing can be very efficient.
  - Does not require prior sequencing of the genome.
- Weaknesses
  - Variability of fragment sizes obtained by ultrasonication.
  - Detection of relevant peaks (peak calling) is not trivial.

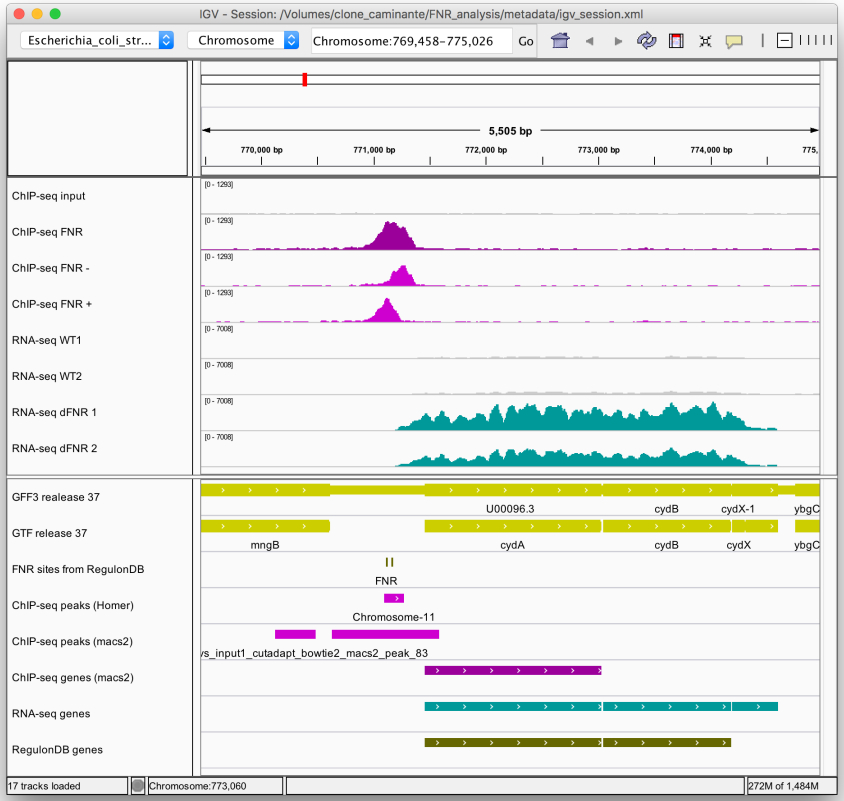


Source: Chen et al. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell (2008) vol. 133 (6) pp. 1106-17

A zoom on a ChIP-seq result

- IGV snapshots of E. coli FNR binding (ChIp-seq) and transcriptome (RNA-seq results for WT versus mutant FNR) in the region of the cydABX operon.

- Middle panel: genome coverage profiles for the two replicas of the wild-type (grey) and FNR mutant (jade).
- Lower panel: genome annotations for the genes (yellow), FNR binding sites from RegulonDB (grey), differentially expressed genes (jade) and FNR target genes annotated in RegulonDB (dark olive).
- Note the characteristic shift between reads on the + and – strands.



- Rioualen et al (2019). Integrating Bacterial ChIP-seq and RNA-seq data with SnakeChunks. Current Protocols in Bioinformatics. In press.

Notes about methods

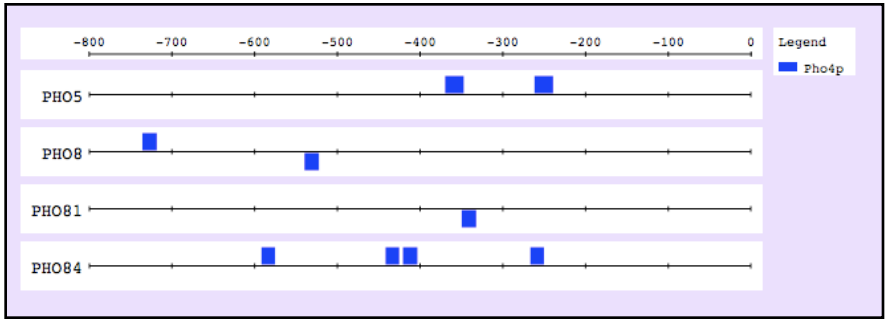
- Pros and cons of in vivo versus in vitro methods.
- Some methods characterize binding sites (i. e. the genomic locations) whereas others characterize binding sequences which might not even exist in the genome.
- The precision depends very much on the method
  - Crystallography: atomic level
  - Footprint: level of a base pair
  - EMSA: a few tens of base pairs
  - ChIP-chip: a few hundreds base pairs
  - First ChIP-on-chip in yeast: hundreds to thousands of base pairs.
  - Oligo tiling arrays: tens of bp
  - ChIP-seq : several tens of bp
- The concept of “binding site” itself can be questioned.
  - Transcription factors have a higher affinity for DNA than for the nucleoplasm.
  - According to some models, they can bind anywhere on DNA, but they spend more time on some sites than on other ones.
  - One could thus consider a continuum of binding affinities.

From binding sites to binding motifs

S.cerevisiae Pho4p binding sites (TFBS)

Gene	Ft_type	Factor	Strand	left	right	Sequence
PHO5	site	Pho4p	D	-370	-347	TAAATTAG**CACGTTTT**CGCATAGA
PHO5	site	Pho4p	D	-262	-239	TGGCACTCA**CACGTGGG**ACTAGCA
PHO8	site	Pho4p	R	-540	-522	ATCGCTG**CACGTGGCCCG**A
PHO8	site	Pho4p	D	-736	-718	ATATTAAGCGTGCGGGTAA
PHO81	site	Pho4p	R	-350	-332	TTATTCG**CACGTGCC**ATAA
PHO84	site	Pho4p	D	-592	-575	TTACG**CACGTT**GGTGCTG
PHO84	site	Pho4p	D	-421	-403	TTTCCAG**CACGTGGGGCGG**
PHO84	site	Pho4p	D	-442	-425	TAGTTC**CACGTGG**ACGTG
PHO84	site	Pho4p	DR	-879	-874	aaaagtg**tCACGTGa**taaaaat
PHO84	site	Pho4p	D	-267	-250	TAATACG**CACGTTTTT**AA

- A *transcription factor binding* ***site*** (***TFBS***) is a ***location*** within a sequence, where a transcription factor binds specifically.
- The site is characterized by
  - a position (start, end, strand) relative to some reference (chromosome start, gene TSS, …).
  - a sequence
- A site can be
  - experimentally proven(known site)
  - inferred by some algorithm (predicted site)
- Example
  - binding sites for the yeast transcription factor Pho4p. Coordinates are relative to the start codon.



Definition: transcription factor binding motif (TFBM)

- Transcription factor binding motif
  - Representation of the binding specificity of a transcription factor, generally obtained by summarizing the conserved and variable positions of a collection of binding sites. Several modes or representation can be used to describe TFBM: consensus, position-specific scoring matrices, Hidden Markov Models (HMM).
- We use the term motif (or pattern) in the sense of a model representing the specificity of binding for a transcription factor.
- A motif is generally built from a collection of transcription binding sites.
- A motif can be described using different formalisms.
  - Consensus string
    - nucleotide alphabet CACGTGGG
    - IUPAC alphabet CACGTGKK
    - regular expressions. CACGT[GT][GT][GT]
  - Position-specific scoring matrix (PSSM)
  - Logo representation (Schneider, 1986)
  - Hidden Markov Models (HMM)

- Jacques van Helden, in Concise Encyclopaedia of Bioinformatics and Computational Biology, 2nd Edition. John M. Hancock (Editor), Marketa J. Zvelebil (Editor). ISBN: 978-0-470-97871-9

Definition: consensus

- Consensus: string of letters (“word”) indicating the conserved residues in each column of a multiple alignment.
  - The consensus is obtained by retaining, at each position of the alignment, a single residue (strict consensus) or a combination of representative residues (degenerate consensus).
  - In the context of regulatory sequences, a consensus is typically used to synthesize the conserved residues of a transcription factor binding motif, built by aligning a collection of binding sites.
  - For motifs defined on nucleic sequences, the degenerate consensus is based on the IUPAC code for ambiguous nucleotides.
- The consensus provides a compact and intuitive representation of the binding specificity of a transcription factor, but suffers from several limitations.
  - Firstly, the rules for considering that a residue is over-represented or not vary between authors and transcription factor databases.
  - Secondly, in contrast with position-specific scoring matrices, the ambiguous code does not provide any information about the relative frequencies of the alternative residues found at a variable position of the aligned sites.

```
R06098    \TCACACGTGGGA\
R06099    \GGCCACGTGCAG\
R06100    \TGACACGTGGGT\
R06102    \CAGCACGTGGGG\
R06103    \TTCCACGTGCGA\
R06104    \ACGCACGTTGGT\
R06097    \CAGCACGTTTTC\
R06101    \TACCACGTTTTC\

Consensus   yvvCACGTkbkn
```

IUPAC ambiguous nucleotide code

A	**A**	**A**denine
C	**C**	**C**ytosine
G	**G**	**G**uanine
T	**T**	**T**hymine
R	**A** or **G**	pu**R**ine
Y	**C** or **T**	p**Y**rimidine
W	**A** or **T**	**W**eak hydrogen bonding
S	**G** or **C**	**S**trong hydrogen bonding
M	**A** or **C**	a**M**ino group at common position
K	**G** or **T**	**K**eto group at common position
H	**A**, **C** or **T**	not **G**
B	**G**, **C** or **T**	not **A**
V	**G**, **A**, **C**	not **T**
D	**G**, **A** or **T**	not **C**
N	**G**, **A**, **C** or **T**	a**N**y

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Binding specificity

- The binding specificity of Pho4p has been pretty well described (Source : Oshima et al. Gene 179, 1996; 171-177)
- High-affinity sites have the core CACGTG, followed by a few Gs or Cs
- Medium-affinity sites have the core CACGTT, followed by a few Ts.
- Some single-nucleotide mutations are sufficient to prevent the binding.

Gene	Site Name	Sequence	Affinity
PHO5	UASp2	---aCtCaCA**CACGTGGG**ACTAGC-	high
PHO84	Site D	---TTTCCA**GCACGTGGG**GCGGA--	high
PHO81	UAS	----TTATG**GCACGTGCG**AATAA--	high
PHO8	Proximal	GTGATCGCT**GCACGTGGC**CCGA---	high
group 1	consensus	---------**gCACGTGgg**-------	high
PHO5	UASp1	--TAAATTA**GCACGTTTT**CGC----	medium
PHO84	Site E	----AATA**CGCACGTTTT**TAATCTA	medium
group 2	consensus	--------**cgCACGTTt**-------	medium
Degenerate consensus		---------**GCACGTKKk**-------	high-med
Non-binding sites			
PHO5	UASp3	--TAATTTG**GCAT GTGCG**ATCTC--	No binding
PHO84	Site C	-----ACGTC**CACGTGG**AACTAT--	No binding
PHO84	Site A	-----TTTAT**CACGTG**ACACTTTTT	No binding
PHO84	Site B	-----TTAC**GCACGTT**GGTGCTG--	No binding
PHO8	Distal	---TTACCC**GCACG**C**TT**AATAT---	No binding

IUPAC ambiguous nucleotide code

A	**A**	**A**denine
C	**C**	**C**ytosine
G	**G**	**G**uanine
T	**T**	**T**hymine
R	**A** or **G**	pu**R**ine
Y	**C** or **T**	p**Y**rimidine
W	**A** or **T**	**W**eak hydrogen bonding
S	**G** or **C**	**S**trong hydrogen bonding
M	**A** or **C**	a**M**ino group at common position
K	**G** or **T**	**K**eto group at common position
H	**A**, **C** or **T**	not **G**
B	**G**, **C** or **T**	not **A**
V	**G**, **A**, **C**	not **T**
D	**G**, **A** or **T**	not **C**
N	**G**, **A**, **C** or **T**	a**N**y

Consensus representation

- The TRANSFAC database contains 8 binding sites for the yeast transcription factor Pho4p
  - 5/8 contain the core of high-affinity binding sites (CACGTG)
  - 3/8 contain the core of medium-affinity binding sites (CACGTT)
- The IUPAC ambiguous nucleotide code allows to represent variable residues.
- 15 letters to represent any possible combination between the 4 nucleotides (2^4 – 1 = 15).
- This representation however gives a poor idea of the relative importance of residues.

```
R06098   \TCACACGTGGGA\
R06099   \GGCCACGTGCAG\
R06100   \TGACACGTGGGT\
R06102   \CAGCACGTGGGG\
R06103   \TTCCACGTGCGA\
R06104   \ACGCACGTTGGT\
R06097   \CAGCACGTTTTC\
R06101   \TACCACGTTTTC\

Cons     yvvCACGTkbkn
```

IUPAC ambiguous nucleotide code

A	**A**	**A**denine
C	**C**	**C**ytosine
G	**G**	**G**uanine
T	**T**	**T**hymine
R	**A** or **G**	pu**R**ine
Y	**C** or **T**	p**Y**rimidine
W	**A** or **T**	**W**eak hydrogen bonding
S	**G** or **C**	**S**trong hydrogen bonding
M	**A** or **C**	a**M**ino group at common position
K	**G** or **T**	**K**eto group at common position
H	**A**, **C** or **T**	not **G**
B	**G**, **C** or **T**	not **A**
V	**G**, **A**, **C**	not **T**
D	**G**, **A** or **T**	not **C**
N	**G**, **A**, **C** or **T**	a**N**y

- TRANSFAC public version: http://www.gene-regulation.com/cgi-bin/pub/databases/transfac/search.cgi

Position-specific scoring matrix (PSSM)

- Position-specific scoring matrix (PSSM): matrix associating a score to each residue at each position of a set of aligned sequences (nucleic of peptidic).
- Sequence logo: graphical representation giving an intuitive perception of the importance of each residue at each position of a transcription factor binding motif. Each column contains a pile of letters whose relative sizes are proportional to the frequency of the corresponding residues. The total height of each column is proportional to the its information content.

Building a PSSM from aligned binding sites

Alignment of Pho4p binding sites (TRANSFAC annotations)

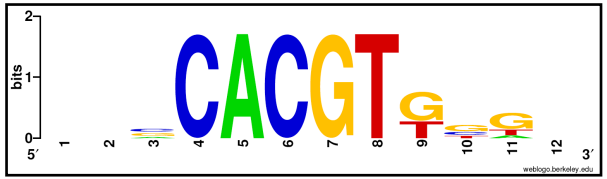
R06098	T	C	A	C	A	C	G	T	G	G	G	A
R06099	G	G	C	C	A	C	G	T	G	C	A	G
R06100	T	G	A	C	A	C	G	T	G	G	G	T
R06102	C	A	G	C	A	C	G	T	G	G	G	G
R06103	T	T	C	C	A	C	G	T	G	C	G	A
R06104	A	C	G	C	A	C	G	T	T	G	G	T
R06097	C	A	G	C	A	C	G	T	T	T	T	C
R06101	T	A	C	C	A	C	G	T	T	T	T	C

Count matrix (TRANSFAC matrix F$PHO4_01)

Residue\position	1	2	3	4	5	6	7	8	9	10	11	12
A	1	3	2	0	**8**	0	0	0	0	0	1	2
C	2	2	**3**	**8**	0	**8**	0	0	0	2	0	2
G	1	2	**3**	0	0	0	**8**	0	**5**	**4**	**5**	2
T	4	1	0	0	0	0	0	**8**	**3**	2	2	2
Sum	8	8	8	8	8	8	8	8	8	8	8	8

Tom Schneider's sequence logo

(generated with Web Logo http://weblogo.berkeley.edu/logo.cgi)



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TRANSFAC format

```
AC  M00064
XX
ID  F$PHO4_01
XX
DT  13.04.1995 (created); hiwi.
DT  18.07.2000 (updated); ewi.
CO  Copyright (C), Biobase GmbH.
XX
NA  PHO4
XX
DE  PHO4
XX
BF  T00690 PHO4; Species: yeast, Saccharomyces cerevisiae.
XX
PO     A     C     G     T
01     1     2     1     4     N
02     3     2     2     1     N
03     2     3     3     0     V
04     0     8     0     0     C
05     8     0     0     0     A
06     0     8     0     0     C
07     0     0     8     0     G
08     0     0     0     8     T
09     0     0     5     3     K
10     0     2     4     2     B
11     1     0     5     2     G
12     2     2     2     2     N
XX
BA  8 binding sites from 4 genes
XX
CC  compiled sequences
XX
RN  [1]; RE0002931.
RX  PUBMED: 1327757.
RA  Fisher F., Goding C. R.
RT  Single amino acid substitutions alter helix--loop--helix protein specificity for bases flanking the core
CANNTG motif
RL  EMBO J. 11:4103-4109 (1992).
XX
//
```

- The TRANSFAC database contains detailed information about each matrix.
  - Binding sites used to build it
  - References to the literature
  - Annotator name(s)
  - Comments
  - … many others
- Example: record for the yeast PHO4 matrix (ID M00064)
- **TRANSFAC format**
  - syntactically structured flat-file (field-value),
  - human-readable,
  - a bit tricky, but not complicated to handle computationally (flat file parsing)
- Notes
  - TRANSFAC database is now commercial (last available public version dates from 2008) but the TRANSFAC format is widely used.
  - Some tools use a TRANSFAC-like format but not fully compliant format (jargon).

Multiple transcription factors interact on cis-regulatory regions

Cis-regulatory modules (CRM), enhancers, silencers

Cis-regulatory modules (CRM)

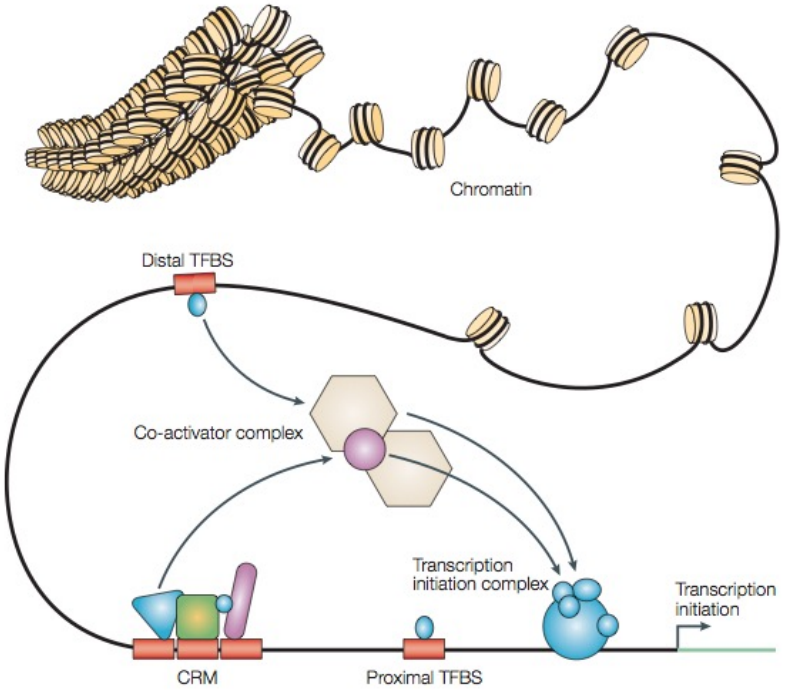


Figure 1 | **Components of transcriptional regulation.** Transcription factors (TFs) bind to specific sites (transcription-factor binding sites; TFBS) that are either proximal or distal to a transcription start site. Sets of TFs can operate in functional *cis*-regulatory modules (CRMs) to achieve specific regulatory properties. Interactions between bound TFs and cofactors stabilize the transcription-initiation machinery to enable gene expression. The regulation that is conferred by sequence-specific binding TFs is highly dependent on the three-dimensional structure of chromatin.

- In Metazoa, some non-coding regions (typically 100-200 bp) contain closely packed binding sites for distinct transcription factors.
- These regions are called **cis-regulatory modules** (**CRMs**)
- CRMs play the role of integrating devices.
- Depending on the combination of transcription factors present in the cell, they will activate or repress the expression of a target gene.
  - Activation -> enhancers
  - Repression -> silencers

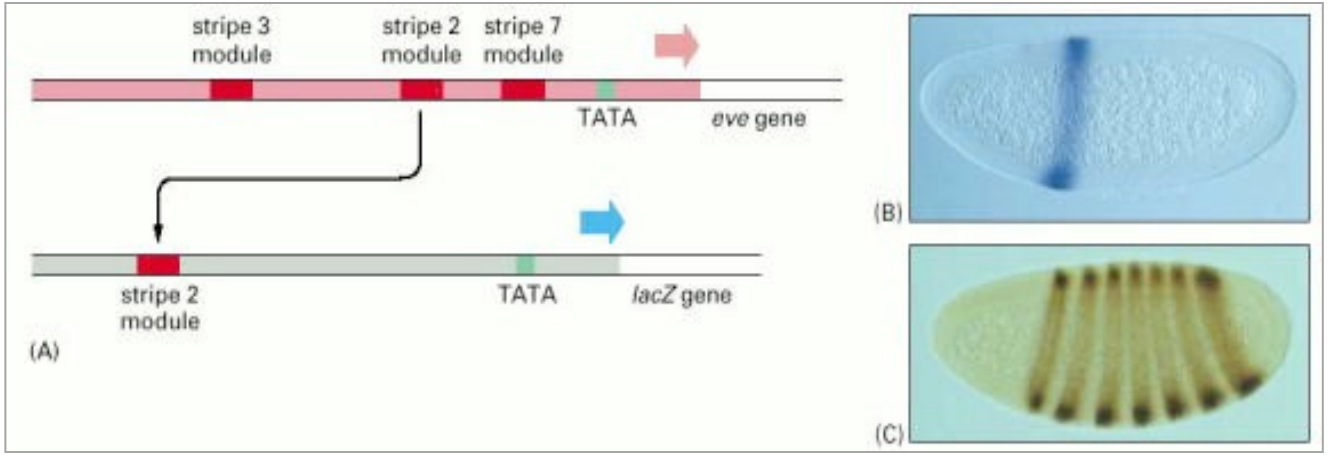
- Source: Wasserman and Sandelin. Applied bioinformatics for the identification of regulatory elements. Nat Rev Genet (2004) vol. 5 (4) pp. 276-87. PMID 15131651

Cis-regulatory module (CRM)

- A cis-regulatory module is a genomic region that combines multiple cis-regulatory elements, mediating the interaction between several transcription factors and a promoter.
- Homotypic / heterotypic
  - CRMs are qualified of homotypic when they are essentially composed of multiple binding sites for a single transcription factor, or heterotypic if they combine sites bound by several distinct transcription factors. A CRM typically covers a few tens to a few hundreds base pairs.
- Modularity
  - The modularity of a CRM (i.e. its ability to act separately from its native region) can be established experimentally by measuring its capability to drive the expression of a reporter gene.
- Enhancers / silencers
  - A CRM is qualified of enhancer or silencer depending on whether it increases or decreases the transcription level of the target gene.
  - The enhancing/silencing effect is typically measured by deletion analysis: a CRM will be qualified of enhancer (resp. silencer) if its deletion provokes a reduction (resp. increase) of the target gene. Enhancers can also be characterized by reporter gene experiments.
  - The distinction between enhancers and silencers seems somewhat simplistic, since the same cis-regulatory module can enhance the expression of a target gene in some conditions (tissue, developmental time), and silence it in other conditions.

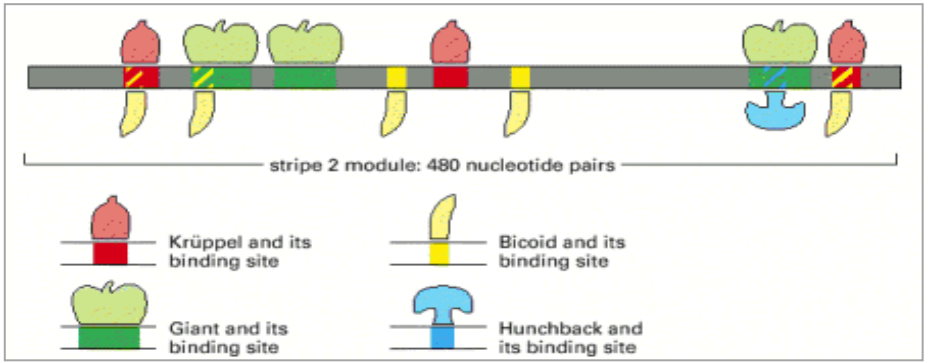
- Jacques van Helden, in Concise Encyclopaedia of Bioinformatics and Computational Biology, 2nd Edition. John M. Hancock (Editor), Marketa J. Zvelebil (Editor). ISBN: 978-0-470-97871-9

The stripe-specific enhancers of Drosophila even-skipped (eve)



Top: Each of the 7 stripes of even-skipped expression stripes is activated by a specific enhancer.

Right: The cis-regulatory module (enhancer) responsble for stripe 2 contains a density of sites for Kr, bcd, Hb and Gt.



- Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. Molecular Biology of the Cell (2002).

What is a cis-regulatory region ?

- Cis-regulatory region
  - Genomic region exerting a cis-regulatory effect on the transcription of a given gene.
  - In bacterial and fungal genomes, cis-regulatory elements are typically found in the non-coding sequences located upstream the regulated gene, and are restricted to a few hundreds base pairs per gene.
  - In metazoan genomes, cis-regulatory elements can be found in upstream regions, introns, downstream regions. They can be located in close proximity to the gene (proximal regions) or at larger distances (several kb away from the transcription start site).
  - In some cases, a cis-regulatory element can act on genes located further away than the nearest neighbour genes (e.g. cis-regulation of the *achaete-scute* complex in *Drosophila melanogaster*).

- Notes and questions
  - The term « region » is now commonly used to denote genomic intervals associated with chromatin modification marks (e.g. histone modifications) characterized by ChIp-seq and related technologies). This concept is also associated to a relatively wide region (also called « broad peak »).
  - Cis explicitly refers to the cis-trans test, a genetic experiments whereby the interaction between two loci is tested by assessing whether a phenotype differs when they are linked (cis) or separated (trans).
  - Cis-regulatory region should be defined by a cis-regulatory effect, which can be demonstrated by different types of experiments (deletion analyses, reporter assays).
  - How do we name the DNA regions that exert a regulatory effect in trans ?

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Genome sizes – some landmarks

- Genome sizes show strong variations between taxa
- The number of genes does not increase proportionally with genome size
- In multicellular organisms, a significant proportion of the genome is occupied by repetitive sequence elements
- The proportion of non-coding genome increases with complexity (phylla) and genome size

Species name	Common name	Genome completion	Genome size Mb	Number of genes	Average distance between genes Kb	Coding fraction %	Non-coding fracion %	Repeats %	Transcribed %	Remarks
Bacteria										
Mycoplasma genitalium	*Mycoplasma*	1995	0.6	481	1.2	90	10			Small igenome (intracellular parasite)
Haemophilus influenzae		1995	1.8	1 717	1.0	86	14			First sequenced bacterial genome
Escherichia coli	*Enterobacteria*	1997	4.6	4 289	1.1	87	13			
Yeasts										
Saccharomyces cerevisiae	*Budding yeast*	1996	12	6 286	1.9	72	28			First sequenced eukaryote genome
Animals										
Caenorhabditis elegans	*Nematod worm*	1998	97	19 000	5	27	73			First sequenced metzazoan genome
Drosophila melanogaster	*Fruit fly*	2000	165	16 000	10	15	85			
Ciona intestinalia			174	14 180	12					
Danio rerio	*Zebrafish*		1 527	18 957	81					
Xenopus laevis	*Xenopus (amphibian)*		1 511	18 023	84					
Gallus gallus	*Chicken*		2 961	16 736	177					
Ornithorhynchus anatinus	*Platypus*		1 918	17 951	107					
Mus musculus	*Mouse*	2002	3 421	23 493	146					
Pan troglodytes	*Chimp*		2 929	20 829	141					
Homo sapiens	*Human*	2001	3 200	21 528	149	2	98	46	28	(20001=draft version)
1000 génomes humains		> 2008								Project launched January 2008
Plants										
Arabidiopsis thaliana		2001	120	27 000	4	30	70			First plant genome
Oryza sativa	*Rice*		390	37 544	10					
Zea mais	*Maize*		2 500	50 000	50			50		Approximate number of genes
Triticum aestivum	*Wheat*		16 000							Hexaploid genome
Lilium	*Lilium*		120 000							
Psilotum nudum	*Fern-like plant*		250 000							

Cis-regulatory elements and their organization

- The localization of cis-regulatory regions varies depending on the type of organism.

organism	Bacteria	Fungi	Metazoa
location	upstream overlap. Initiation	upstream	upstream downstream intergenic regions within introns
distance range	-400 to +50 bp	-800 to -1 bp	from several Kbs to several Mb !
position effect	often essential	often irrelevant	often irrelevant
strand	sensitive or symmetric	insensitive	insensitive
most common core	spaced pair of 3nt	~5-8 conserved bp	~5-8 conserved bp
repeated sites	rare	occasional	frequent
cis-regulatory modules (CRMs)			frequent

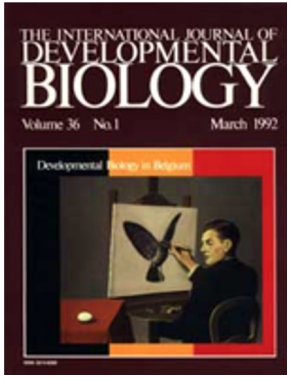
The broader picture: from cis-regulation to body shape

La clairvoyance

- It is these chromosomes, or probably only an axial skeleton fibre of what we actually see under the microscope as the chromosome, that contain in some kind of code-script the entire pattern of the individual's future development and of its functioning in the mature state.
- Every complete set of chromosomes contains the full code; so there are, as a rule, two copies of the latter in the fertilized egg cell, which forms the earliest stage of the future individual.
- In calling the structure of the chromosome fibres a code-script we mean that the all-penetrating mind, once conceived by Laplace, to which every causal connection lay immediately open, could tell from their structure whether the egg would develop, under suitable conditions, into a black cock or into a speckled hen, into a fly or a maize plant, a rhododendron, a beetle, a mouse or a woman.

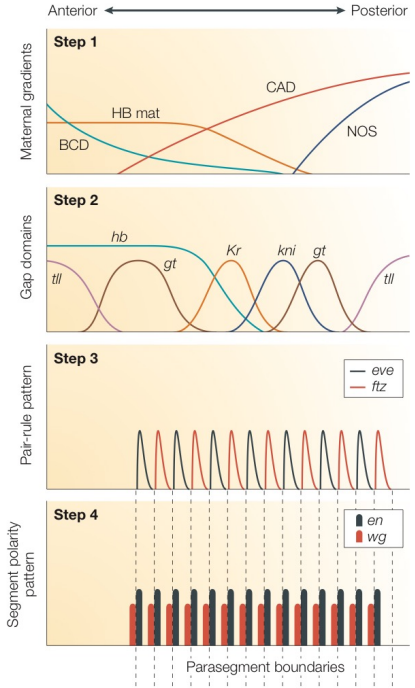
- Schrodinger (1944). What is life ?

René Magritte – "La Clairvoyance"



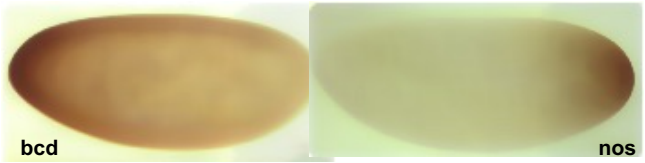
Drosophila Antero-Posterior (AP) segmentation – expression domains

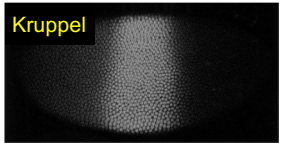
- Establishment of expression domains
  - Maternal genes: gradients of mRNAs coding for transcription factors.
  - Gap genes: broad domains.
  - Pair-rule genes: expressed every other segment (odd or even segments).
  - Segment polarity genes: expressed with a segmental periodicity, across 2-3 cells wide bands.



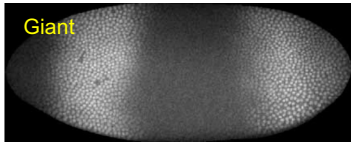
Peel et al. Arthropod segmentation: beyond the Drosophila paradigm. Nature Reviews Genetics (2005) vol. 6 (12) pp. 905-16. PMID 16341071

Adapted from Carroll, 2006

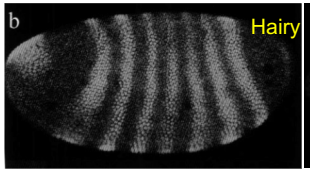




Source: Carroll, 2005.



Source: Thieffry and Sanchez (2003). http://flyex.ams.sunysb.edu/



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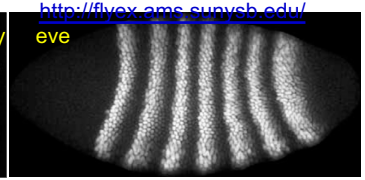
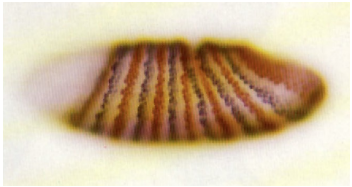


Source: Thieffry and Sanchez (2003). http://flyex.ams.sunysb.edu/ .



Source: Lawrence (1992). *The Making of a Fly: The Genetics of Animal Design. Blackwell Science Ltd. ISBN 0632030488*

Drosophila Antero-Posterior (AP) segmentation – expression domain

- The establishment of expression domains relies on a modular network of transcriptional regulations.
- Hierarchy: Maternal genes -> Gap -> Primary pair-rule -> Secondary pair rule -> Segment polarity.

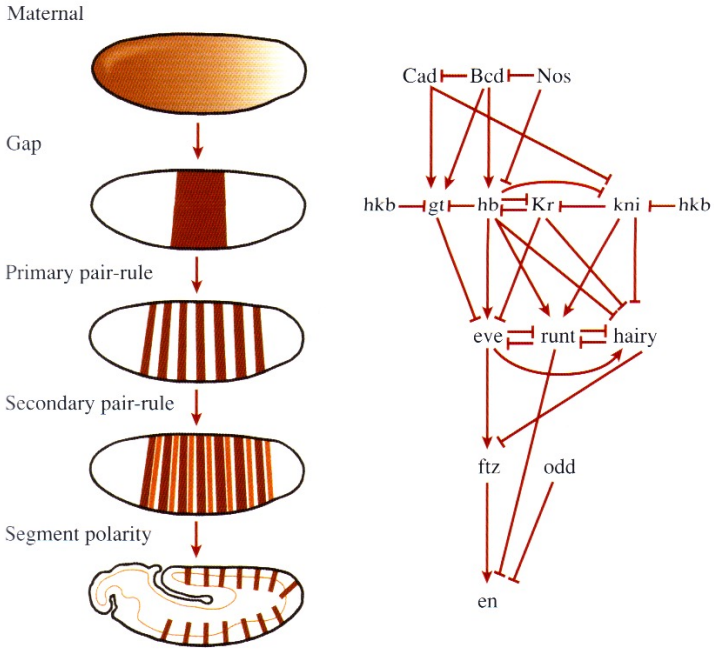
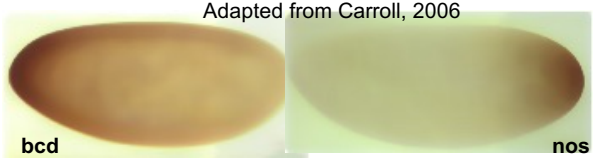
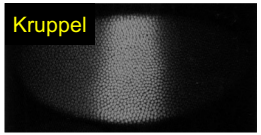


Figure 3.5
The segmentation genetic regulatory hierarchy
(**left**) The expression patterns of five classes of anteroposterior axis patterning genes are depicted in embryos at different stages. (**right**) Selected members of these classes are shown and the regulatory interactions between these genes are indicated. An arrow indicates a positive regulatory interaction; a line crossed at its end indicates a negative repressive regulatory relationship.

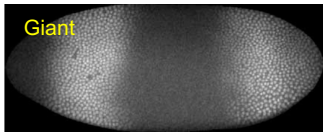
Source: Carroll, 2005. From DNA to diversity (2nd edition). Blackwell Publishing.

Adapted from Carroll, 2006

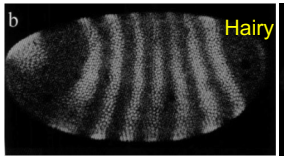




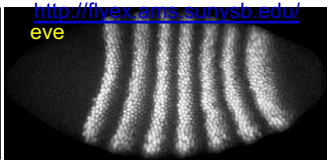
Source: Carroll, 2005.



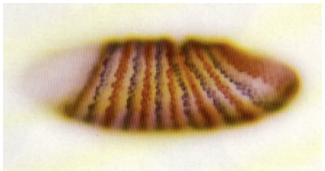
Source: Thieffry and Sanchez (2003). http://flyex.ams.sunysb.edu/



Source: Carroll, 2005.

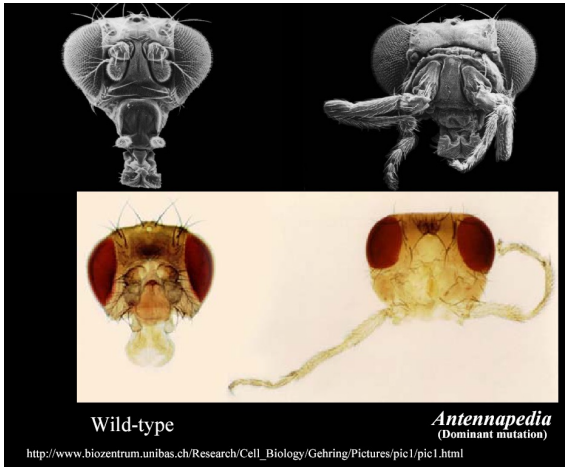


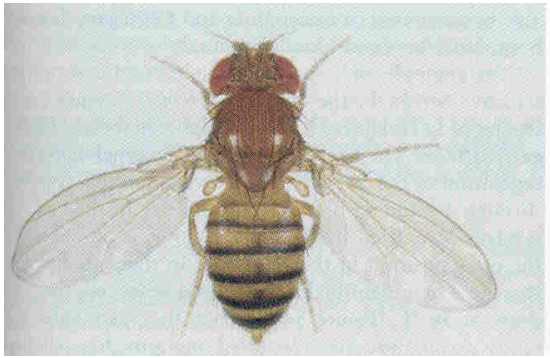
Source: Thieffry and Sanchez (2003). http://flyex.ams.sunysb.edu/ .

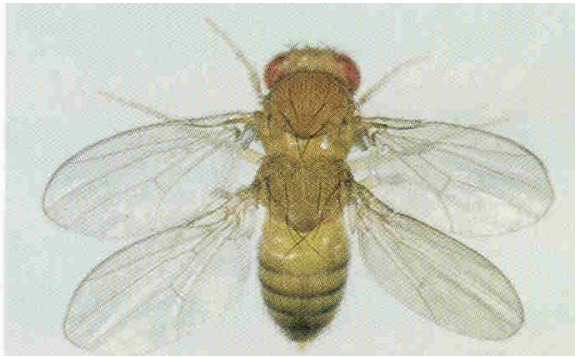


Source: Lawrence (1992). *The Making of a Fly: The Genetics of Animal Design. Blackwell Science Ltd. ISBN 0632030488*

Homeotic mutations





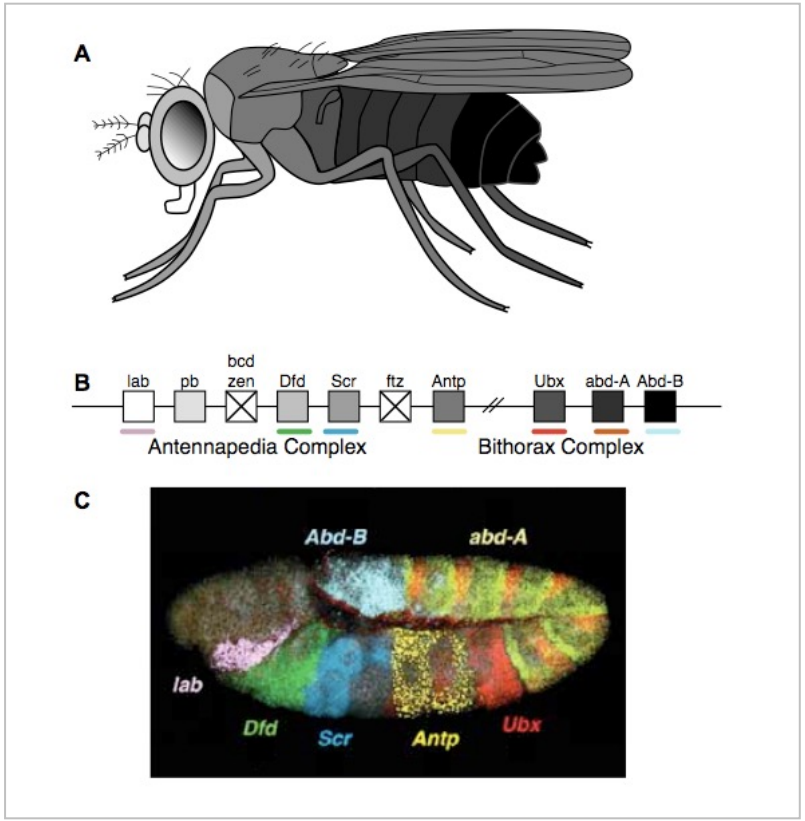


- Mutations of the Hox genes modify the segmental identity.
- Top
  - Antennapedia mutant fly: legs develop at the location of antennae.
- Bottom
  - Bithorax complex (triple mutant): the 3rd thoracic segment (metathorax) develops as a copy of the second segment (mesothorax), with wings instead of haltera.

http://www.cb.ku.ac.th/down_load/drsermsiri.ppt

Specification of segmental identity

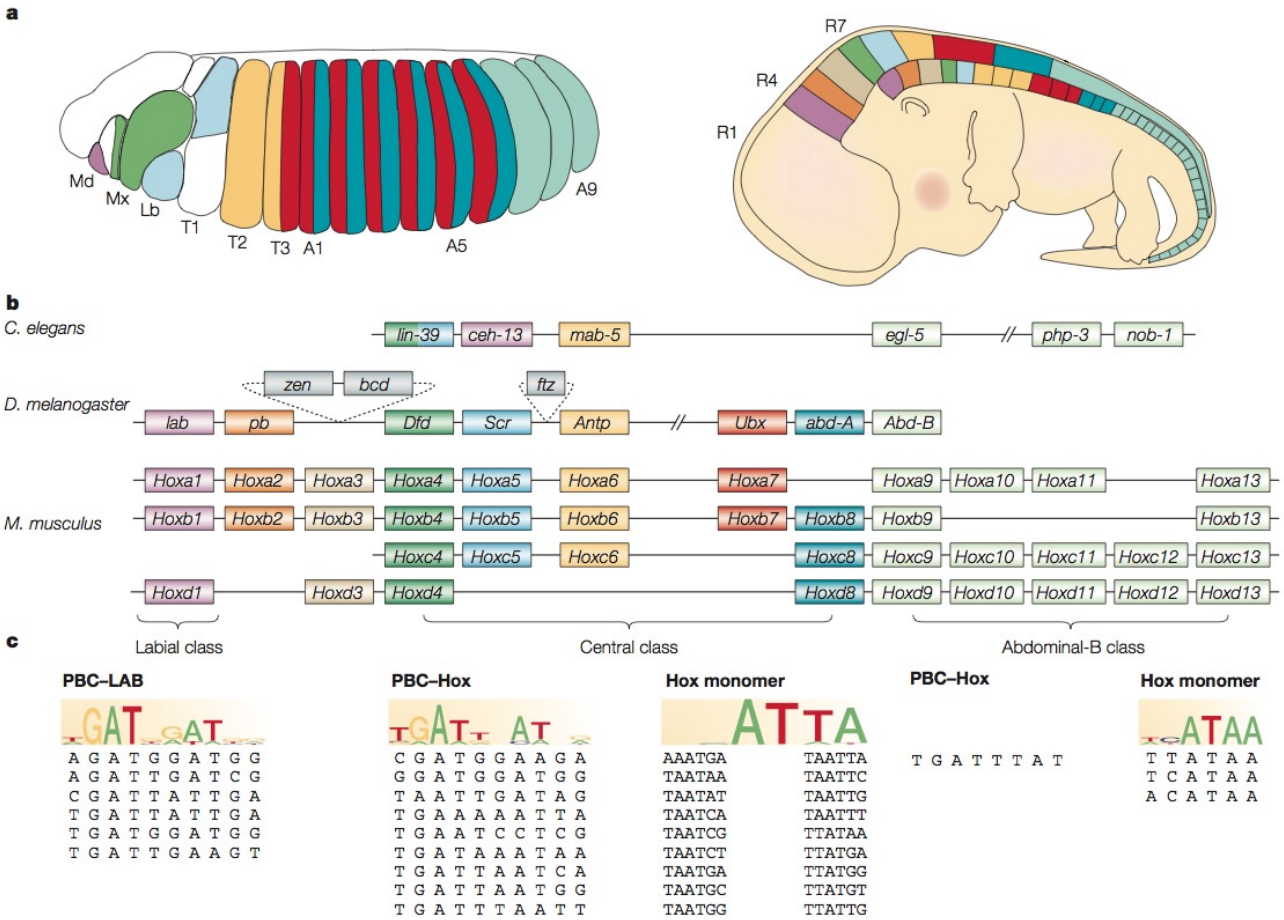
- After segmentation, each segment is commited to a particular « identity »: head, thorax, abdomen, ...
- This identify is specified by transcription factors belonging to the Hox family.
  - Bithorax complex
  - Antennapaedia complex
- Each factor is expressed in a specific antero-poserior domain.



Sources of the Figures:

- Morgane Thomas-Chollier (2008). PhD Thesis, ULB
- Lemons & McGinnis (2006).

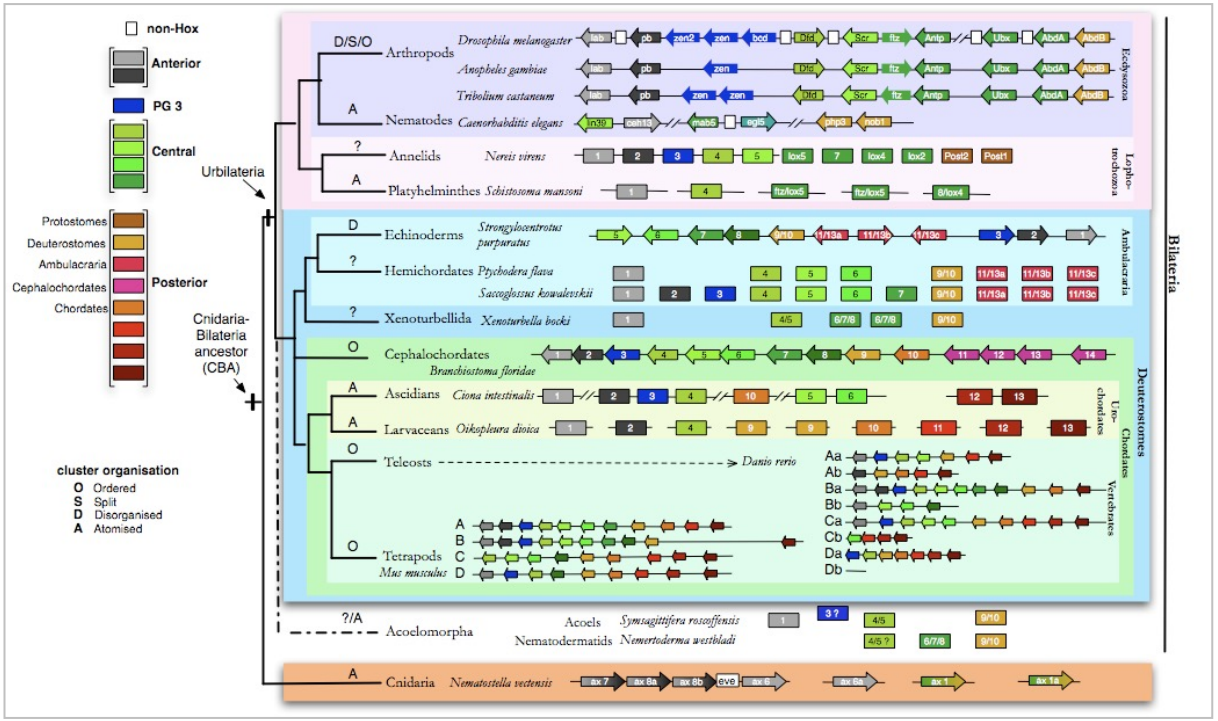
The Hox complex - from drosophila to mammals



- Morgane Thomas-Chollier (2008). PhD Thesis, ULB

Hox evolution: complexification by duplication/divergence

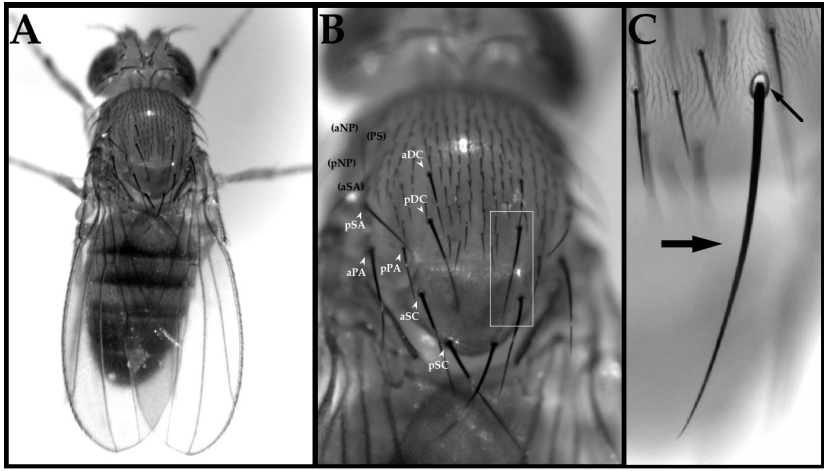
- Hox genes are found in all the Bilaterians, and they determine segmental identity.
- The topological organization of the complex has been partly conserved from invertebrate to vertebrate.
- The whole complex has been duplicated several times during evolution



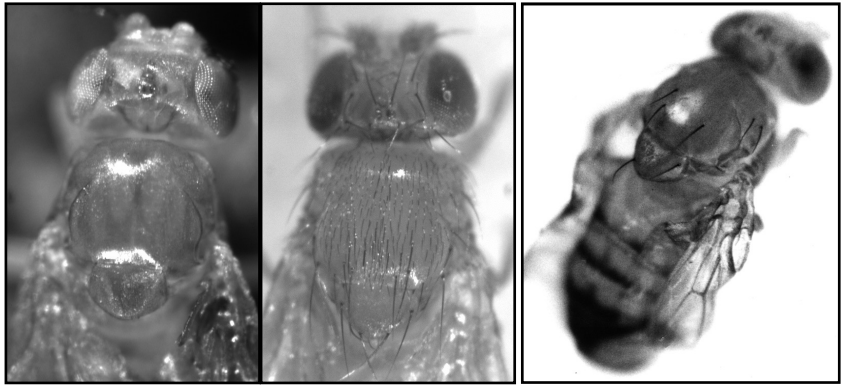
- Source: Morgane Thomas-Chollier (2008), thèse de doctorat ULB.

The proneural genes in Drosophila melanogaster

- In Drosophila, sensory organs are arranged in a species-specific way, identical between individuals of the same species.
- Sensry bristles are determined by the proneural genes achaete and scute.
- Loss of function: achate-scute double mutants (ac-sc-) are devoid of sensory bristles.
- Gain of function: an excess of achate-scute expression provokes the formation of ectopic bristle.
- Rescue: a time-controlled expression of scute partly rescues the achate-scute loss of function phenotype.



Wild type phenotype



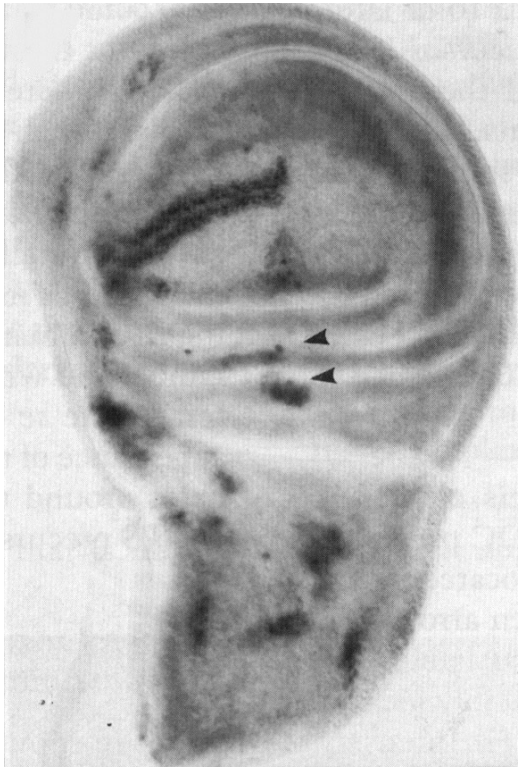
Loss of function ($sc^{10.1}$)

Gain of function (Hw)

Time-controlled expression rescues specific bristles ($sc^{10.1}$ + hs-sc)

- Figures: Jacques van Helden (1995). PhD Thesis. ULB.

Proneural genes: the achaete-scute complex

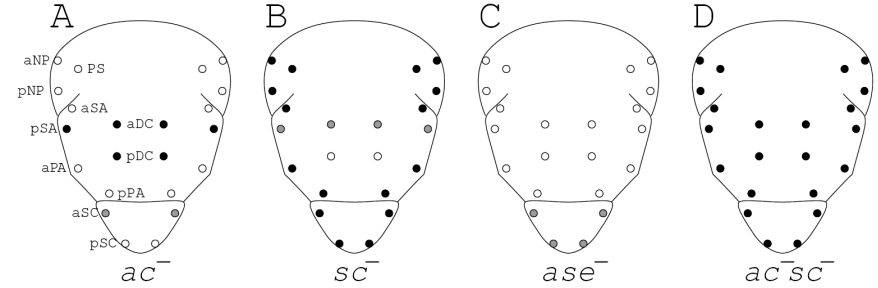


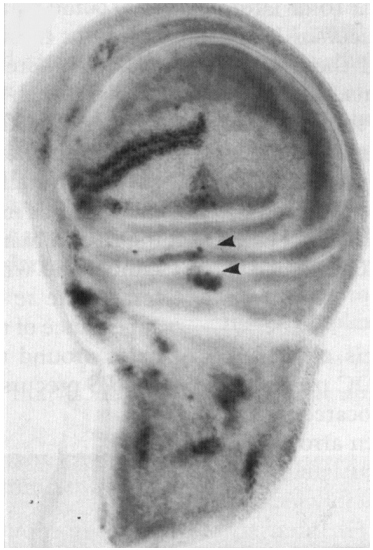
- Drosophila *achaete-scute* complex includes 4 paralogous genes coding for transcription factors.
- These genes are expressed in clusters of cells during embryonic and pupal development.

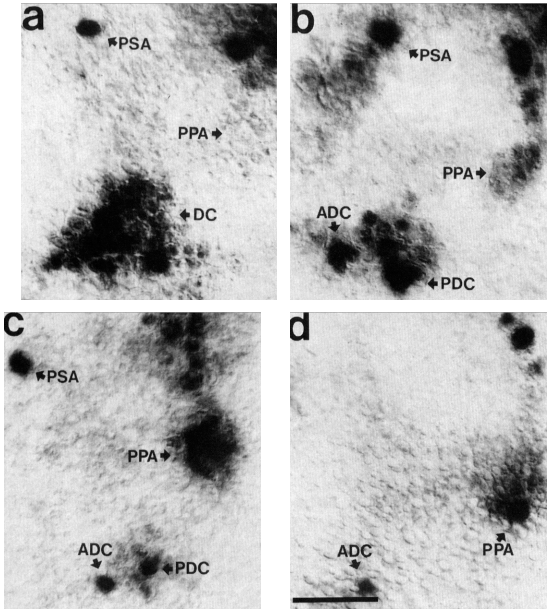
- Figures: Jacques van Helden (1995). PhD Thesis. ULB.

The expression of achaete-scute determines bristle development

- The deletion of each specific gene of the achaete-scute genes leads to the absence of a specific subsets of sensory bristles (black dots on the top schemas).
- The simultaneous deletion of both achaete and scute leads to the total absence of sensory bristles.
- In the wing imaginal disc, the specific deletions are characterized by the absence of the corresponding clusters of expression of achaete and scute.





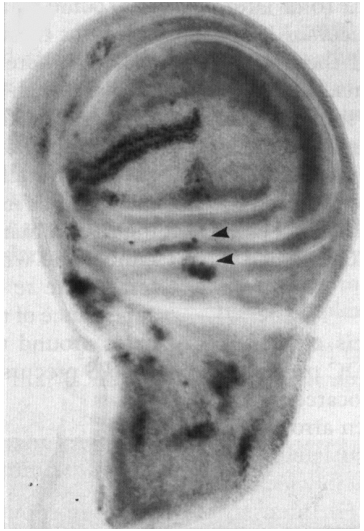


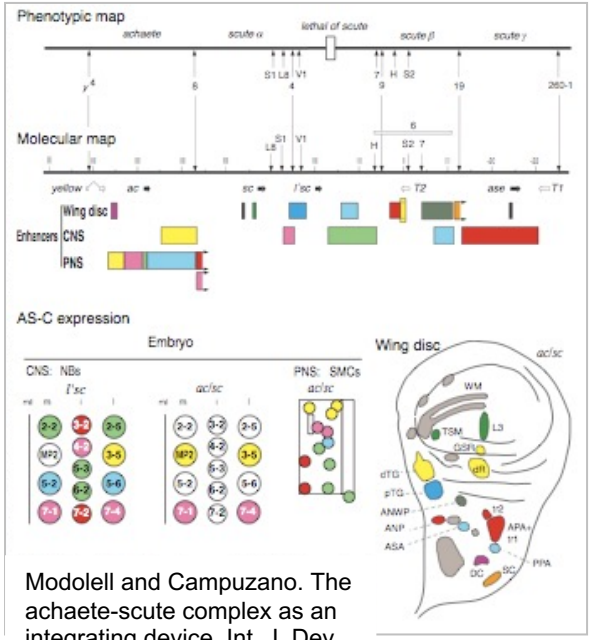
- Figures: Jacques van Helden (1995). PhD Thesis. ULB

Position-specific enhancers in the achaete-scute complex

- The ***achate-scute complex (ASC)*** contains 4 genes coding for paralogous transcription factors.
- Those genes are expressed in specific groups of cells (***proneural groups***) in the wing discs of the larva. A sensory organ mother cell emerges from each proneural cluster, and give rise to a bristle of the adult.
- This extremely complex, precise and reproducible expression pattern is determined by the action f specific cis-regulatory elements located in the 100kb region encompassing the 4 genes of the achaete-scute complex (ac, sc, l'sc and ase). Most of the region is made of non-coding sequences containing ***time- and position-specific enhancers***.

J.van Helden (1995). PhD thesis, ULB, 1995.

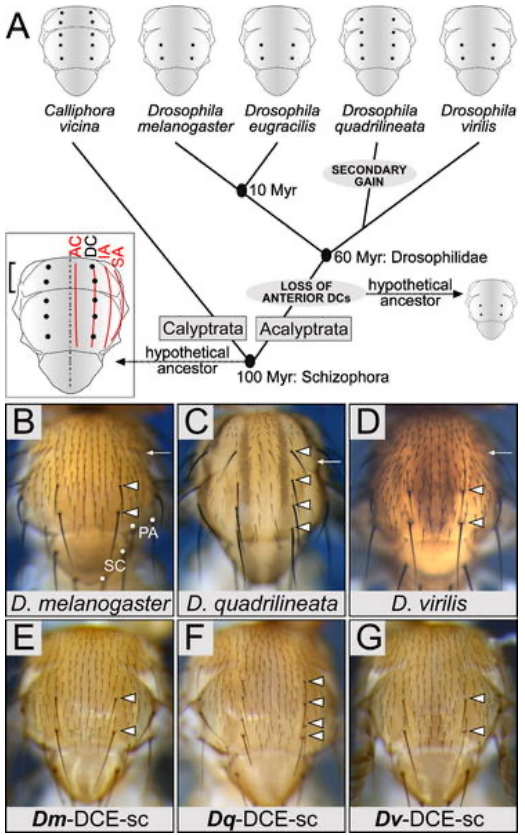




Modolell and Campuzano. The achaete-scute complex as an integrating device. Int. J. Dev. Biol (1998)

Species specificity of the developmental patterns

- Cis-regulation is a driving force for evolution.
- A substitution of aa single nucleotide can affect the binding of a TF and inactivate an existing TFBS or create a new one.
- Various species of the Drosophila genus are distinguished by the precise positioning of their dorsal macrochaetes (e.g. 2 or 4 dorsocentral macrochaetes, denoted by white arrows).
- These differences result from modifications of the cis-regulatory modules controlling the expression of the Acheta-Scute Complex during larval development.



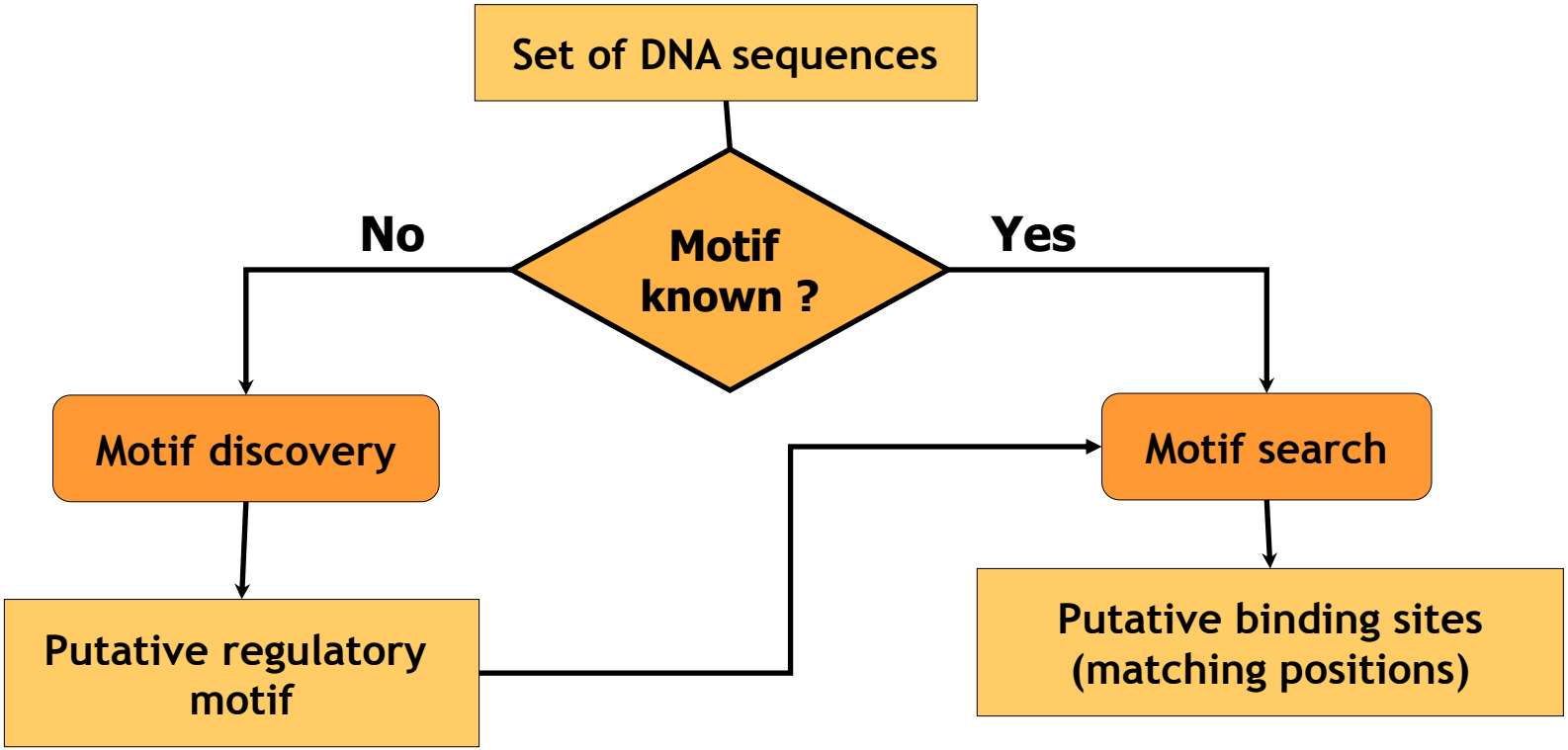
Marcellini et al. PLoS Biol (2006) vol. 4 (12) pp. e386

Questions and approaches

Questions and approaches

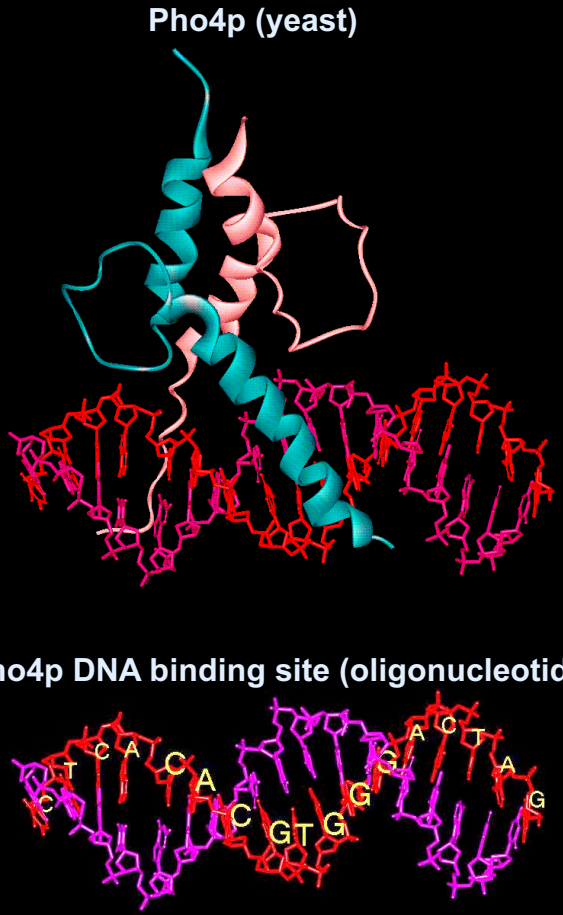
- Motif search (pattern matching)
  - Starting from a TFBM, scan DNA sequences to predict TFBS
- Motif discovery
  - Starting from a set of supposedly co-regulated genomic regions (promoters, ChIP-seq peaks), detect exceptional motifs (various criteria: over-representation, positional occupancy).
- Matching a library of patterns
  - Scan a sequence with all motifs of a given collection (database).
- Motif enrichment
  - Is my sequence set enriched in instances of a particular motifs (e.g. each motif from a database)?
- CRM prediction
  - Detect regions with a higher density of predicted sites than expected by chance (cis-regulatory enriched regions, CRERs).
- Phylogenetic footprinting
  - Detect regulatory signals by searching conserved elements in non-coding sequences of orthologous genes.
- Network inference
  - Infer networks of regulation (factor-gene) or co-regulation (gene-gene) from predicted cis-regulatory elements.
- TFBS-based sequence classification
  - Classify regulatory regions (promoters, ChIP-seq peaks, enhancers) based on TF binding sites (predicted or experimental).
  - Unsupervised (clustering): discover classes (clusters) without a priori knowledge of them.
  - Supervised: use a set of sequences belonging to predefined classes (training set) to train a program, and then assign new individuals (sequences) to these classes.

Motif matching vs motif search (= pattern matching)



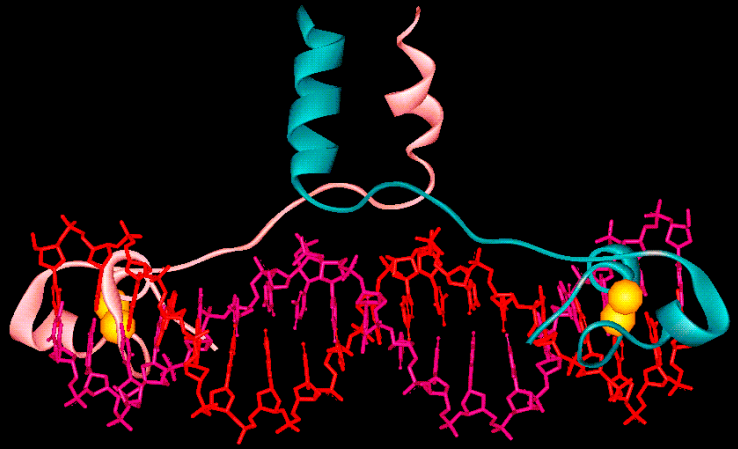
Supplementary material

Interface between the yeast Pho4p protein and one of its binding sites

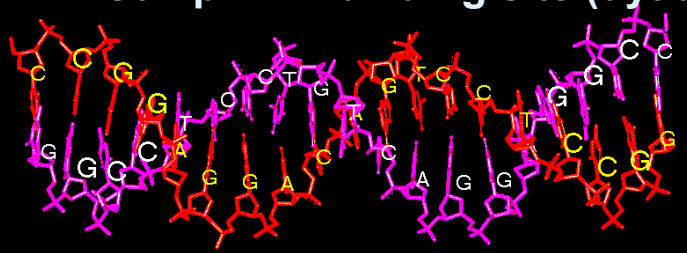


Interface between the yeast Gal4p protein and one of its binding sites

Gal4p (yeast)

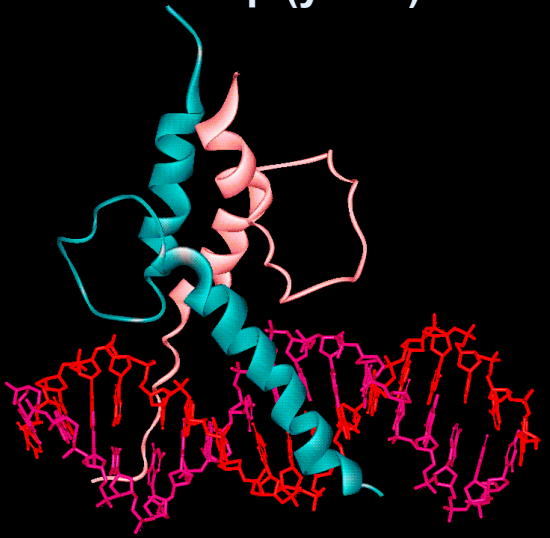


Gal4p DNA binding site (dyad)

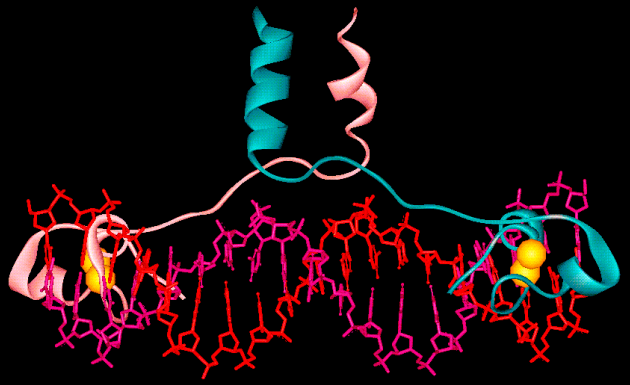


Transcription factor-DNA interfaces

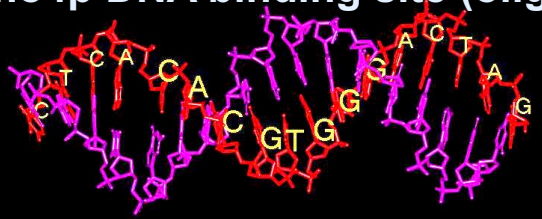
Pho4p (yeast)



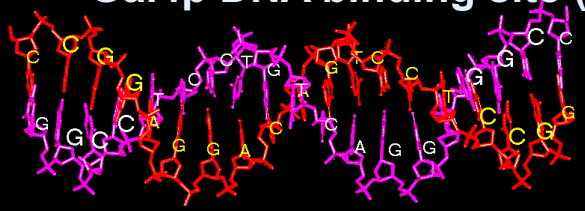
Gal4p (yeast)



Pho4p DNA binding site (oligonucleotide)

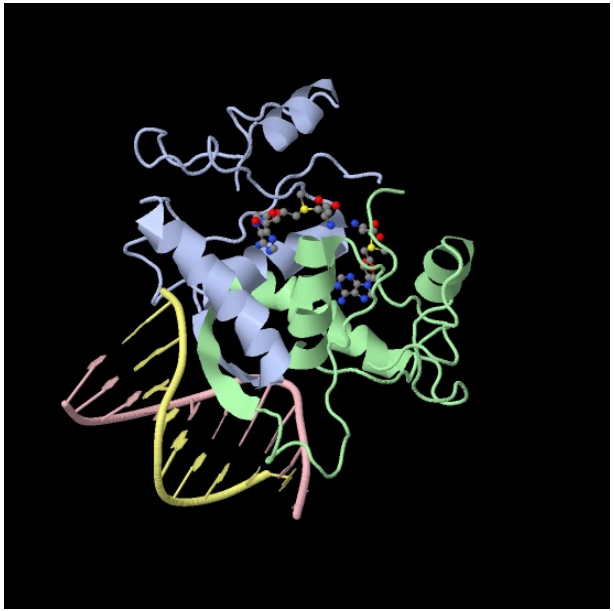


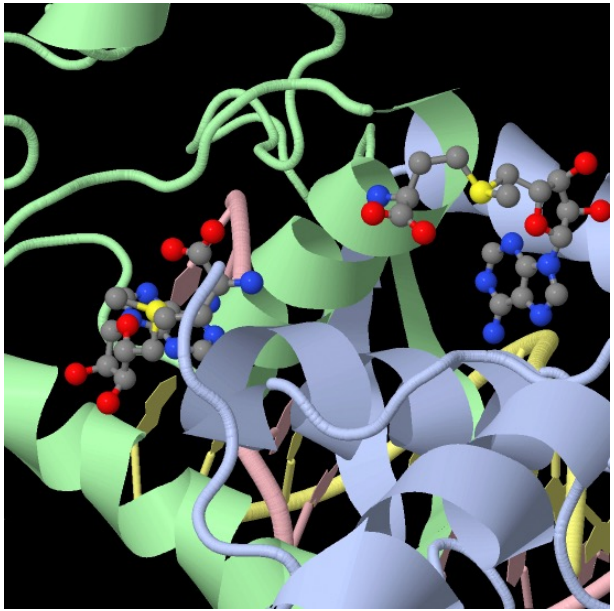
Gal4p DNA binding site (dyad)



Methionine repressor

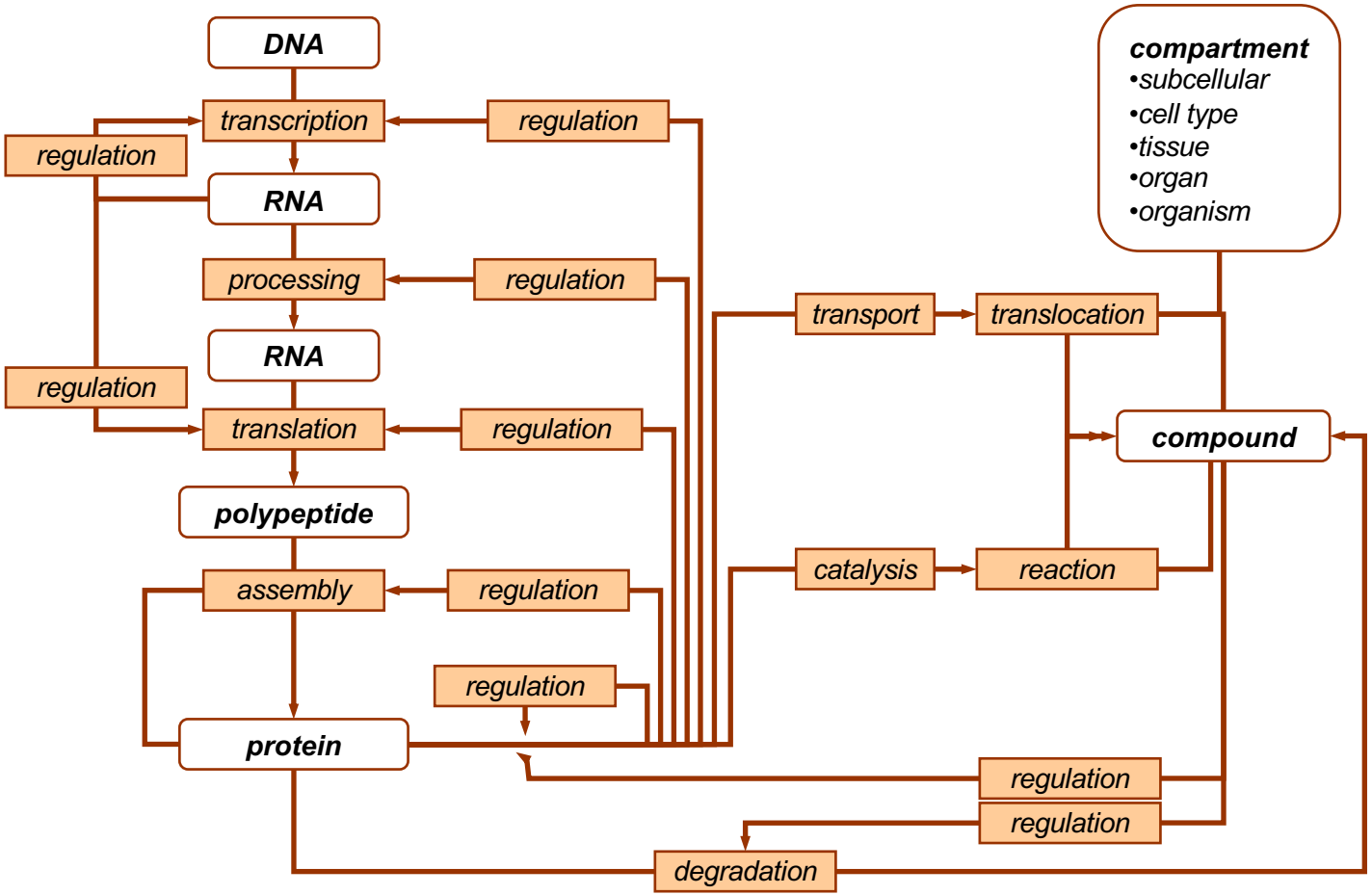
- Crystal structure : the methionine repressor of Escherichia coli.
- Green + violet: the MetJ protein forms a homodimer which is able to bind DNA.
- Pink + yellow: the two strands of the DNA binding site
- Detail: the repressor is activated by binding of methionine molecules





http://www.pdb.org/pdb/explore/jmol.do?structureId=1CMA

Molecular networks (shamefully simplified)



Met4p binding sites

gene	start	end	sequence
MET3	-367	-349	GAAAAG**TCACGTG**TAATTT
MET3	-384	-366	AAAAGG**TCACGTGA**CCAGA
MET14	-235	-217	CTAATT**TCACGTGA**TCAAT
MET16	-185	-167	ATCATT**TCACGTG**GCTAGT
ECM17	-311	-293	ATTTCA**TCACGTG**CGTATT
ECM17	-339	-321	.TTTGTC**CACGTGA**TATTTC
MET10	-255	-237	.CCACAC**CACGTGA**GCTTAT
MET10	-237	-219	.TAGAAG**CACGTGA**CCACAA
MET2	-360	-342	GTATTT**TCACGTGA**TGCGC
MET2	-554	-536	TAATAA**TCACGTGA**TATTT
MET17	-306	-288	.AAATGG**CACGTGA**AGCTGT
MET17	-332	-314	TTGAGG**TCAC**A**TGA**TCGCA
MET6	-540	-522	GCCACA**TCACGTGCA**CATT
MET6	-502	-484	AATATT**TCACGTGA**CTTAC
SAM2	-329	-311	.TCTACC**CACGTGA**CTATAA
SAM2	-381	-363	.TCTTCA**CA**T**GTGA**TTCATC

A	13	11	3	3	2	0	16	0	1	0	0	12
C	1	0	0	3	0	16	0	15	0	0	0	0
G	1	1	4	4	4	0	0	0	15	0	16	4
T	1	4	9	6	10	0	0	1	0	16	0	0

Met31p binding sites

gene	start	end	sequence
MET14	-202	-182	CCTC**AAAAA**A**TGTGG**CAATGG
MET2	-313	-293	TGC**AAAAAA**T**TGTGG**ATGCAC
MET17	-227	-207	TCATG**AAAACTGTG**TAACATA
MET6	-313	-293	GTCGC**AAAACTGTGG**TAGTCA
SAM2	-306	-286	GCTTG**AAAACTGTGG**CGTTTT
SAM1	-283	-263	ACAGG**AAAACTGTGG**TGGCGC
MET19	-173	-153	ATAAGC**AAACTGTGG**GTTCAT
MUP3	-188	-168	CGG**AAAAAACTGTGG**CGTCGC
MET8	-184	-164	GG**AAAAAAA**A**TGTG**AAAATCG
MET1	-232	-212	CATAAT**AAACTGTG**AACGGAC
MET3	-259	-239	ACAAAG**CCACAGTTTT**ACAAC
MET28	-159	-139	CTAACA**CCACAGTTTT**GGGCG
MET8	-434	-414	TCTTGT**CCGCAGTTTT**ATCTG
MET30	-168	-148	GGGAAG**CCACAGTTT**GCGCGG
MET6	-405	-385	CTATCGAA**CTCGTTT**AGTCGC

A	5	11	14	14	14	2	0	0	0	0	2	5
C	2	2	0	0	0	11	0	0	1	0	0	5
G	5	0	0	0	0	0	0	14	0	14	11	1
T	2	1	0	0	0	1	14	0	13	0	1	3

Pho4p binding sites

gene	start	end	sequence
PHO5	-260	-242	..GCACTCA**CACGTGGG**ACTA
PHO5	-260	-245	..GCACTCA**CACGTGGG**A
PHO5	-262	-239	TGGCACTCA**CACGTGGG**ACTAGCA
PHO8	-540	-522	...TCGGGC**CACGTGC**AGCGAT
PHO8	-736	-718	..ttacccg**CACGCTT**aatat
PHO81	-350	-332	...TTATGG**CACGTGCG**AATAA
PHO84	-421	-403	..TTTCCAG**CACGTGGG**GCGG
PHO84	-442	-425	...TAGTTC**CACGTGG**ACGTG
PHO84	-879	-874	.aaaagtgt**CACGTG**ataaaaat
PHO84	-267	-250	..taatacg**CACGTTTTT**aa
PHO84	-592	-575	....TTACG**CACGTT**GGTGCTG
PHO5	-368	-349	...AATTAG**CACGTTTT**CGCATA
PHO5	-369	-354	..AAATTAG**CACGTTT**CTC
PHO5	-370	-347	.TAAATTAG**CACGTTTT**CGCATAGA

IUPAC ambiguous nucleotide code

A	**A**	**A**denine
C	**C**	**C**ytosine
G	**G**	**G**uanine
T	**T**	**T**hymine
R	**A** or **G**	pu**R**ine
Y	**C** or **T**	p**Y**rimidine
W	**A** or **T**	**W**eak hydrogen bonding
S	**G** or **C**	**S**trong hydrogen bonding
M	**A** or **C**	a**M**ino group at common position
K	**G** or **T**	**K**eto group at common position
H	**A**, **C** or **T**	not **G**
B	**G**, **C** or **T**	not **A**
V	**G**, **A**, **C**	not **T**
D	**G**, **A** or **T**	not **C**
N	**G**, **A**, **C** or **T**	a**N**y

Pho4p binding specificity - matrix descriptions

C

Pho4p

A	14	0	5	7	6	0	26	0	0	0	0	3
C	2	8	5	16	6	26	0	26	0	1	0	4
G	4	2	1	1	12	0	0	0	26	0	16	12
T	6	16	15	2	2	0	0	0	0	25	10	7

D

Pho4p.cacgtg

A	2	17	0	0	0	0	2	1	8	5	5	13
C	16	0	18	0	0	0	6	3	4	5	0	1
G	0	1	0	18	0	18	9	12	2	5	2	1
T	0	0	0	0	18	0	1	2	4	3	11	3

E

Pho4p.cacgtt

A	7	0	2	5	1	0	8	0	0	0	0	1
C	0	1	1	3	3	8	0	8	0	0	0	0
G	0	0	0	0	4	0	0	0	8	0	0	2
T	1	7	5	0	0	0	0	0	0	8	8	5

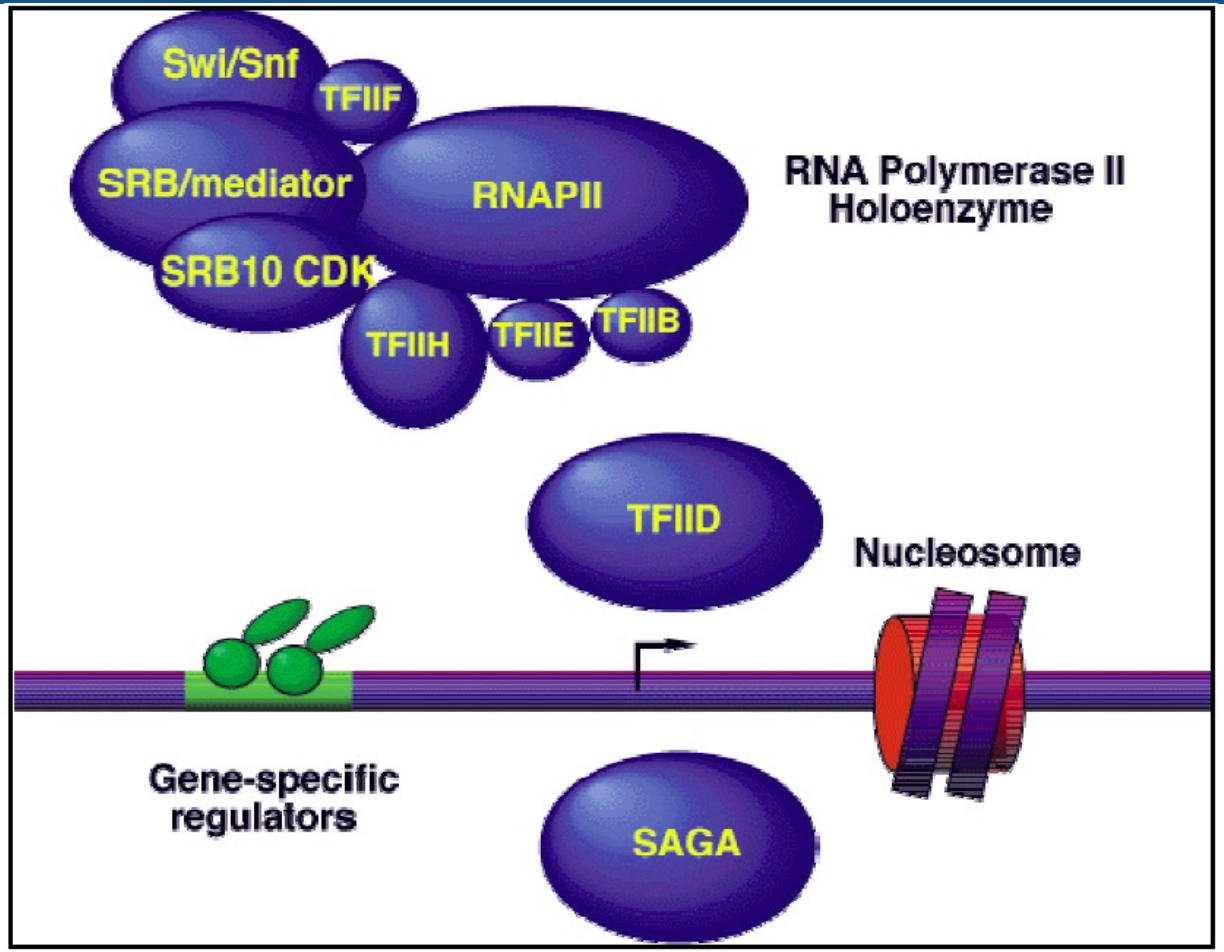
Position-specific scoring matrix (PSSM)

Pos	1	2	3	4	5	6	7	8	9	10
A	3	2	0	12	0	0	0	0	1	3
T	1	1	0	0	0	0	11	5	4	4
G	3	7	0	0	0	12	0	7	5	4
C	5	2	12	0	12	0	1	0	2	1

Binding motif for the yeast Pho4p transcription factor
Source : SCPD
http://rulai.cshl.edu/cgi-bin/SCPD/getfactor?PHO4

The genome challenge

RNA polymerase



Genomic sequences

- A genome G contains a set of n chromosomes.
  - G={S1,S2,...,Si,...,Sn}
- Each chromosome is a molecule of dexyribonucleic acid (DNA), a polymer of 4 nucleotides
  - A Adenosine
  - C Cytidine
  - G Guanosine
  - T Thymidine
- Each chromosome is represented as a sequence (Si) of a text written in a 4-letter alphabet (A)
  - A={A,C,G,T}
  - Si=(si1,si2,...,sij,...,SiLi)
  - Li is the length of the ith chromosome