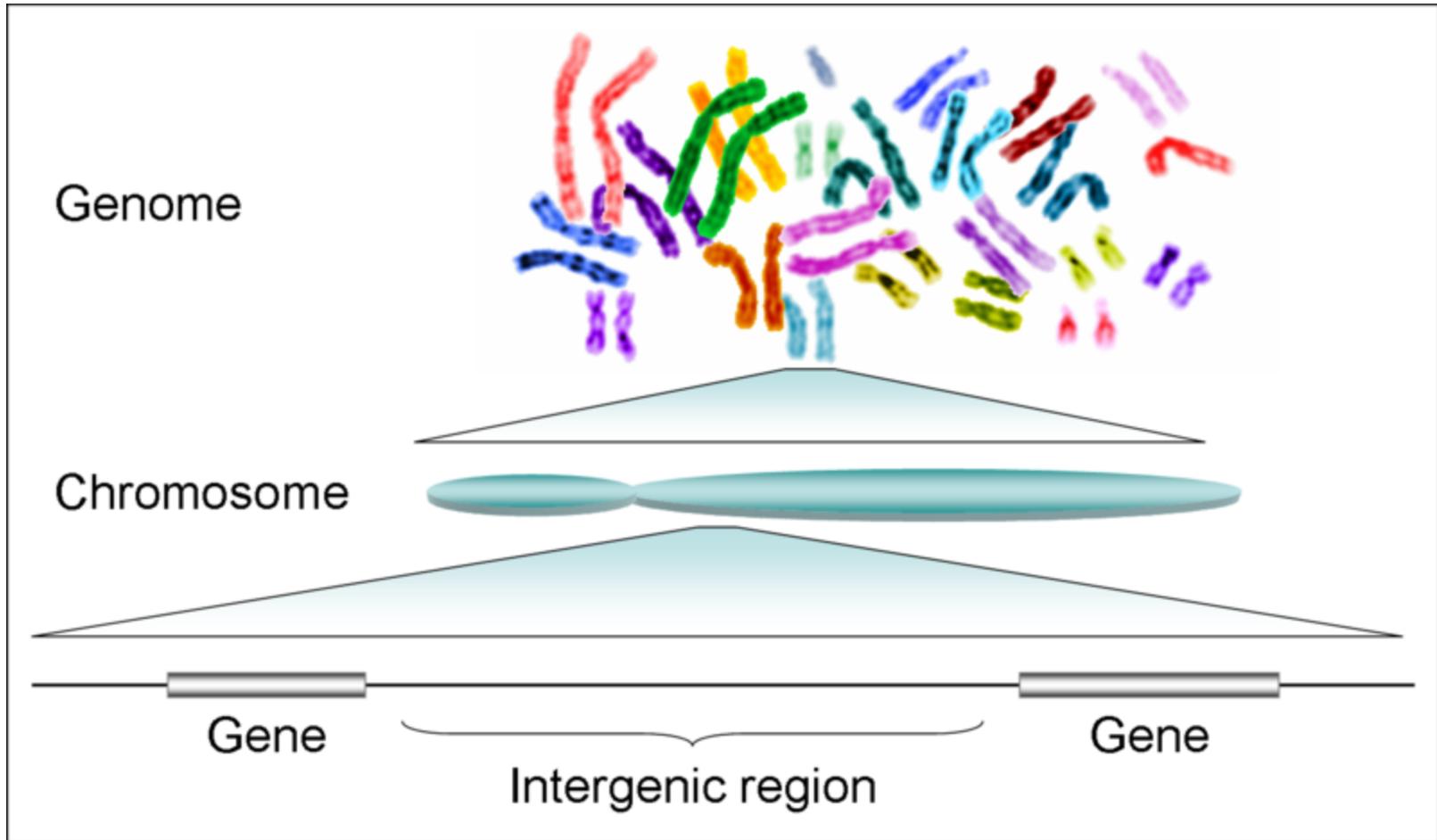
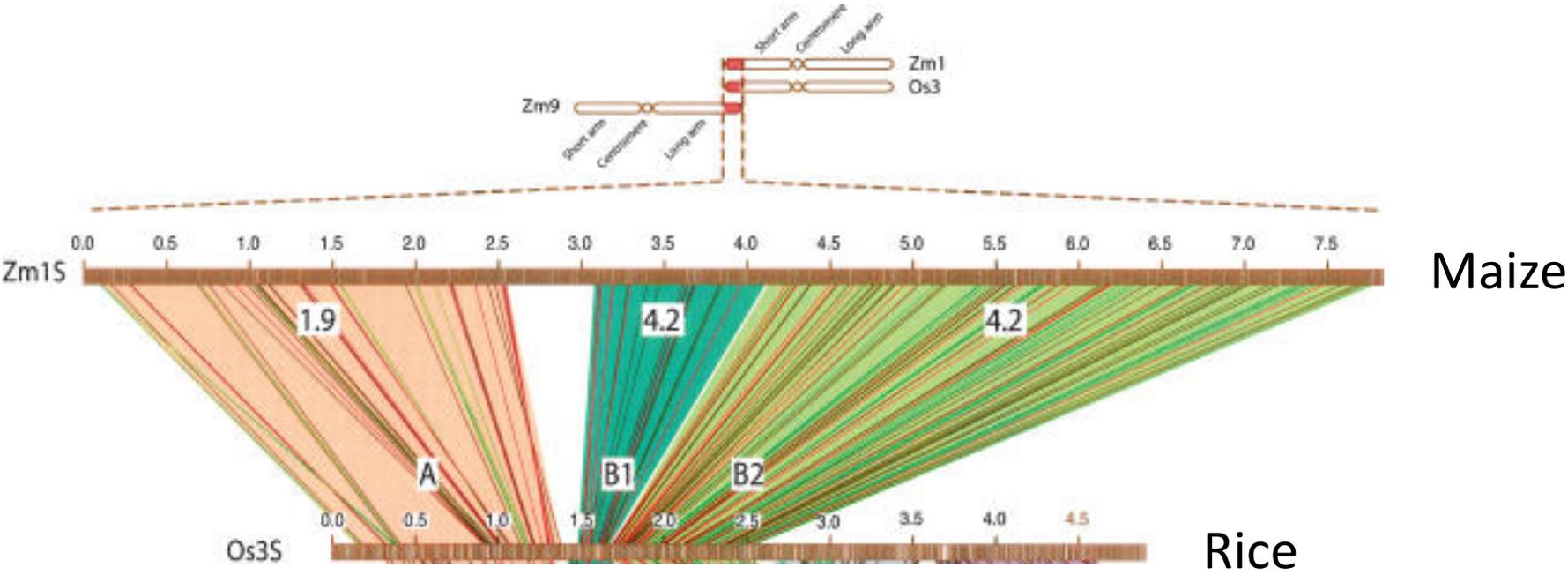


Gene expression and its regulation

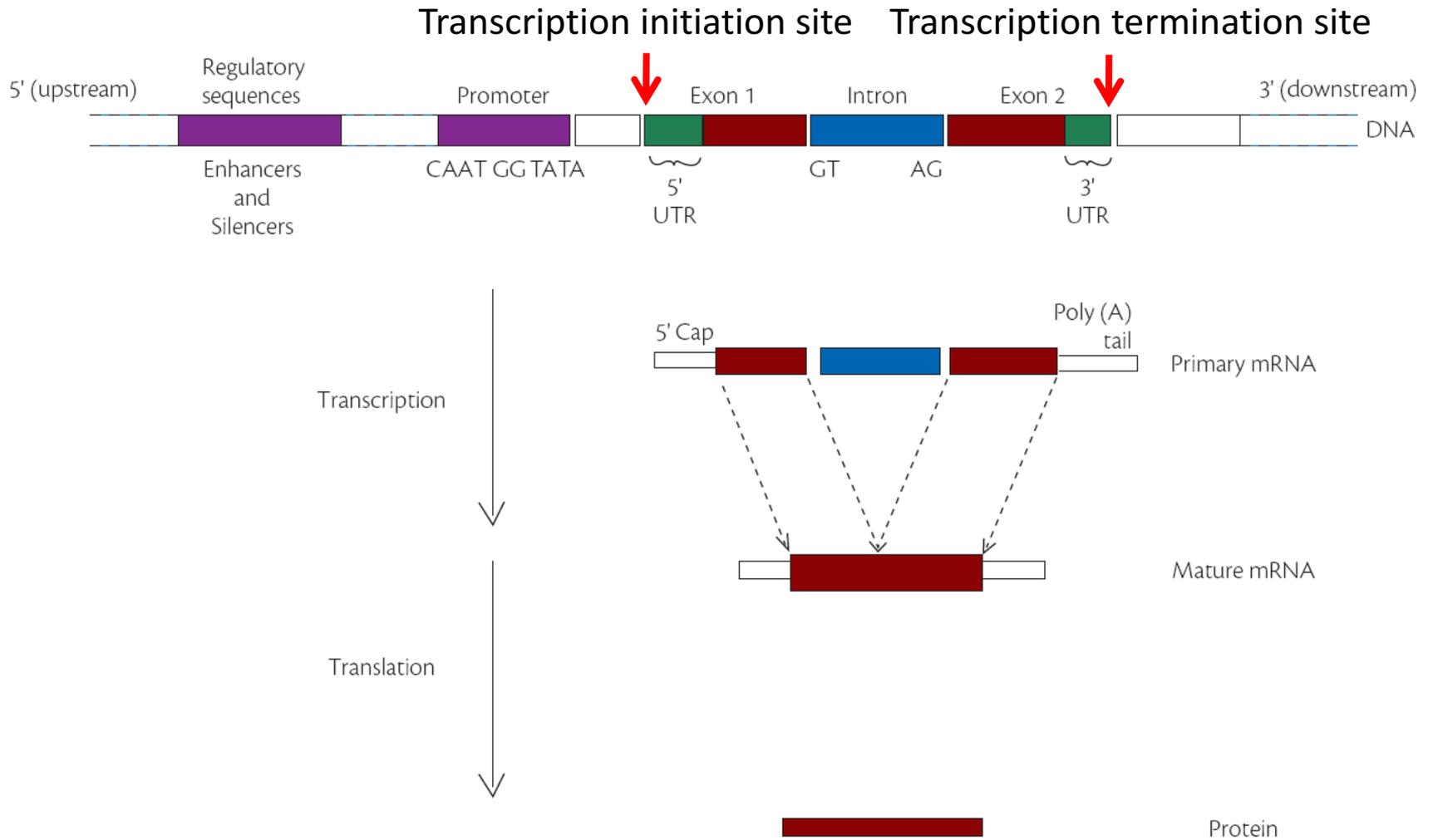
Genome organization



Rice and maize genomes have different size but about the same gene content



Anatomy of a gene



Anatomy of a gene

Intron - A DNA sequence that interrupts the sequences coding for a gene product (exon). Most genes in eukaryotes contain introns.

Exon - A DNA sequence in a gene that codes for a gene product

5' -UTR and 3' -UTR - Untranslated regions of the first and last exons

Transcription initiation site - The first nucleotide of a transcribed DNA sequence where RNA polymerase II begins synthesizing mRNA molecule.

Transcription termination site - The last nucleotide of a transcribed DNA sequence after which RNA polymerase stops mRNA synthesis

Promoter – segment of DNA that initiates transcription of a gene.

Other regulatory elements:

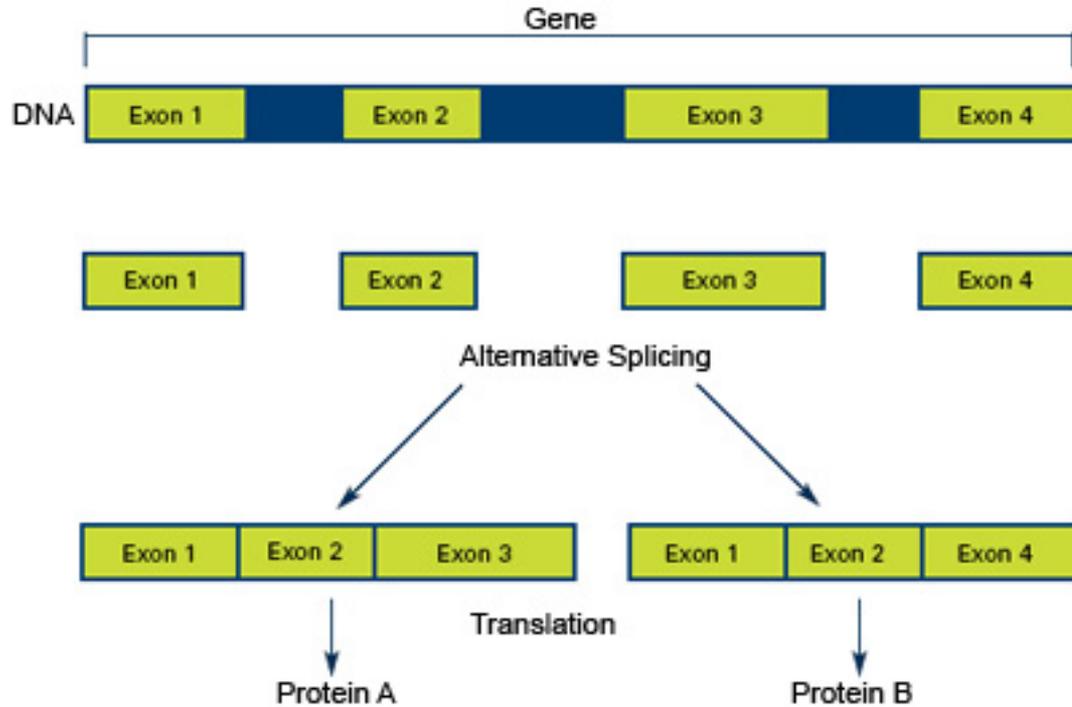
Enhancer – region of DNA located that bind transcription factors increasing gene expression;

Silencers - regions of DNA that bind transcription factors decreasing gene expression;

Poly-A tail - A string of adenine nucleotides added to the 3' end of eukaryotic mRNA after transcription

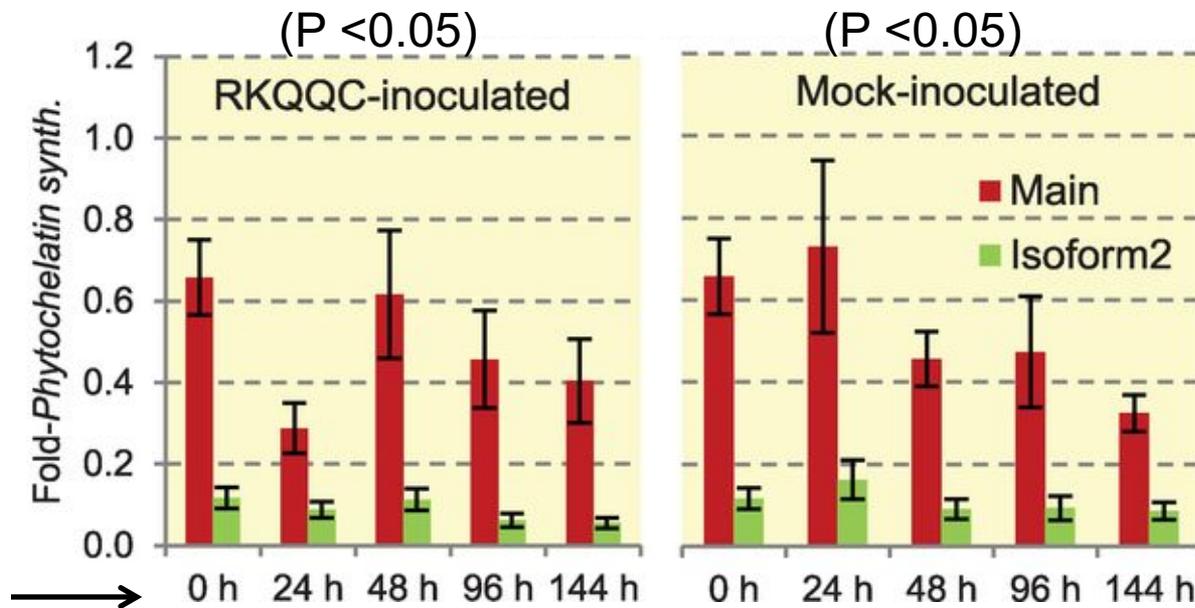
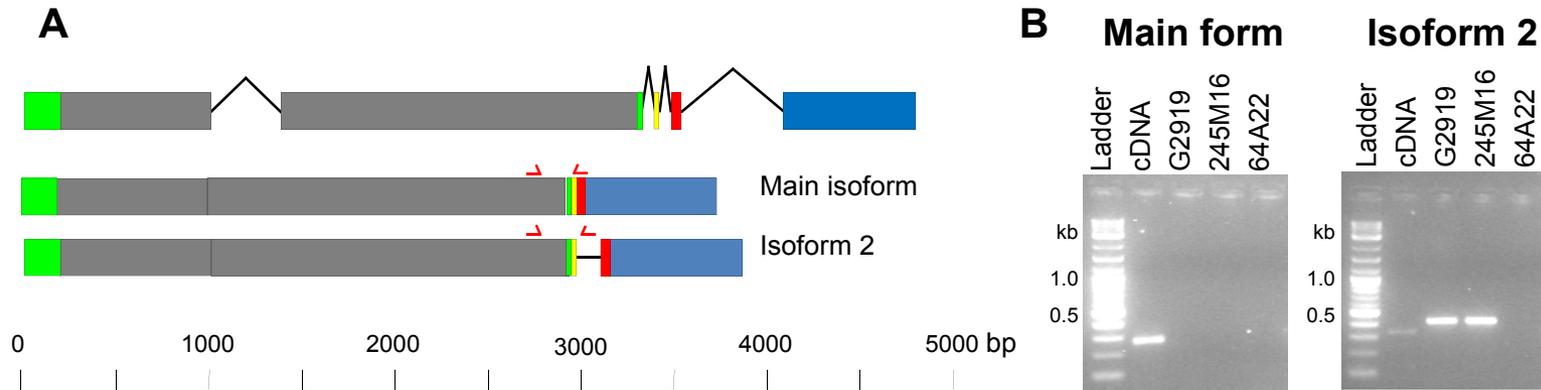
5' -cap - A methylated guanine residue added in reverse polarity to the 5' end of eukaryotic mRNA during transcription initiation. The cap binds a cap binding protein and acts as an initial binding site for ribosomes during translation.

Alternative splicing

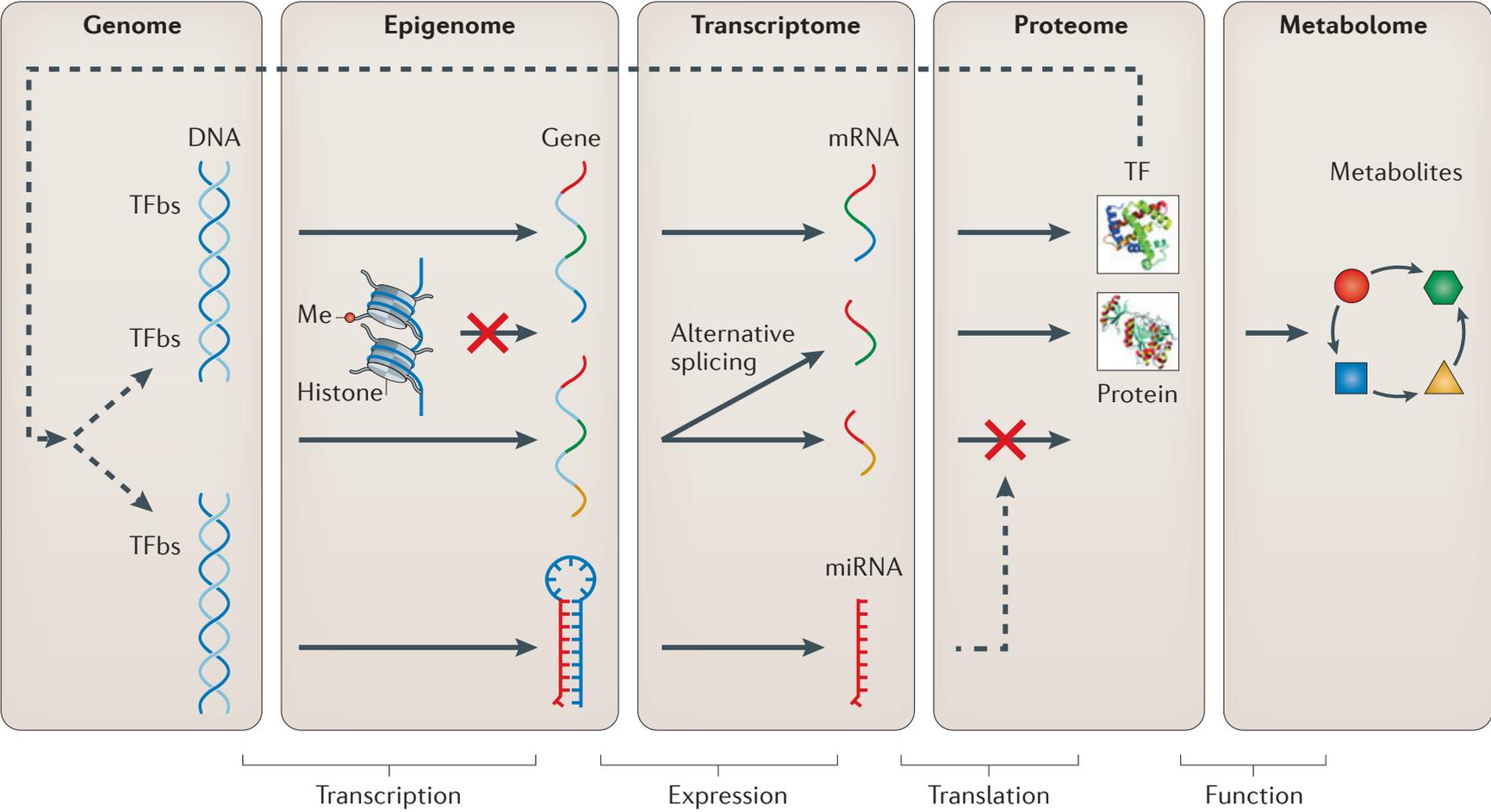


Arranging exons in different patterns, called alternative splicing, enables cells to make different proteins from a single gene. In plants about 45% of genes showed evidence of alternative splicing.

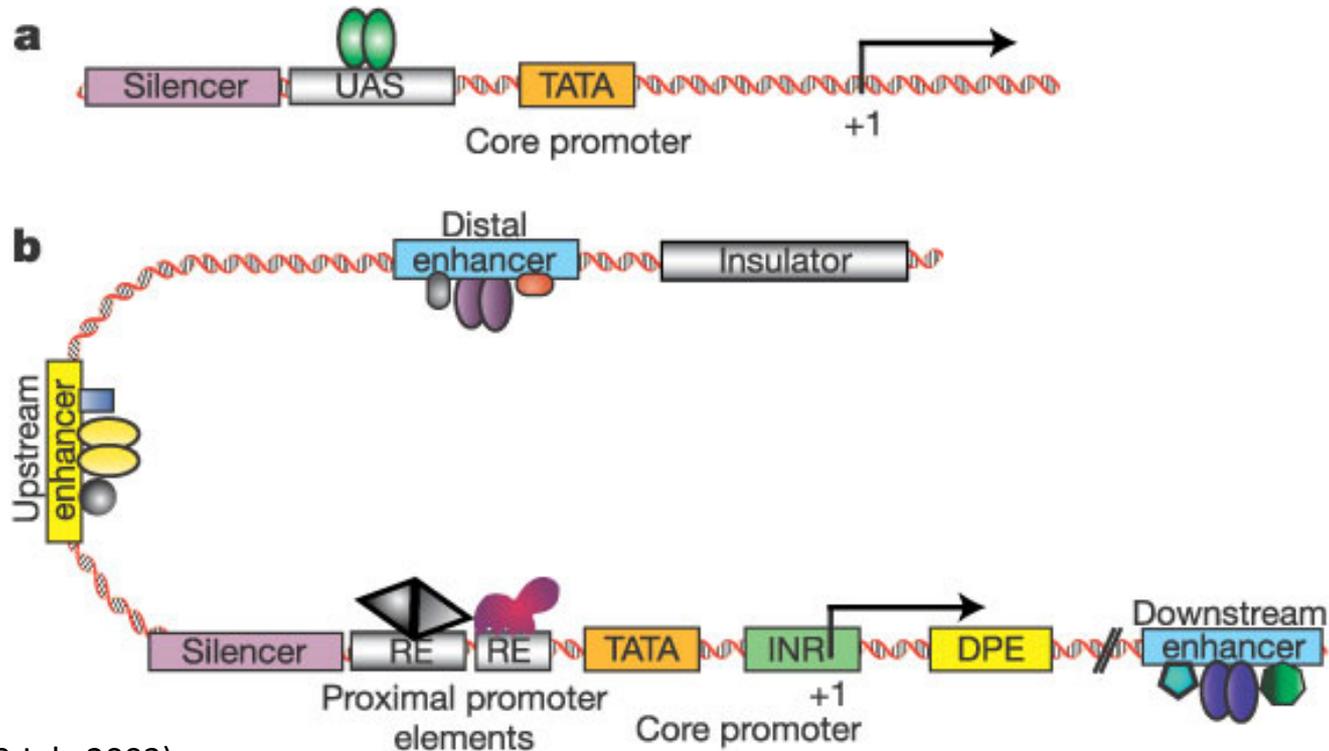
Wheat stem rust resistance gene Sr35 has two isoforms



Information flow in biological systems



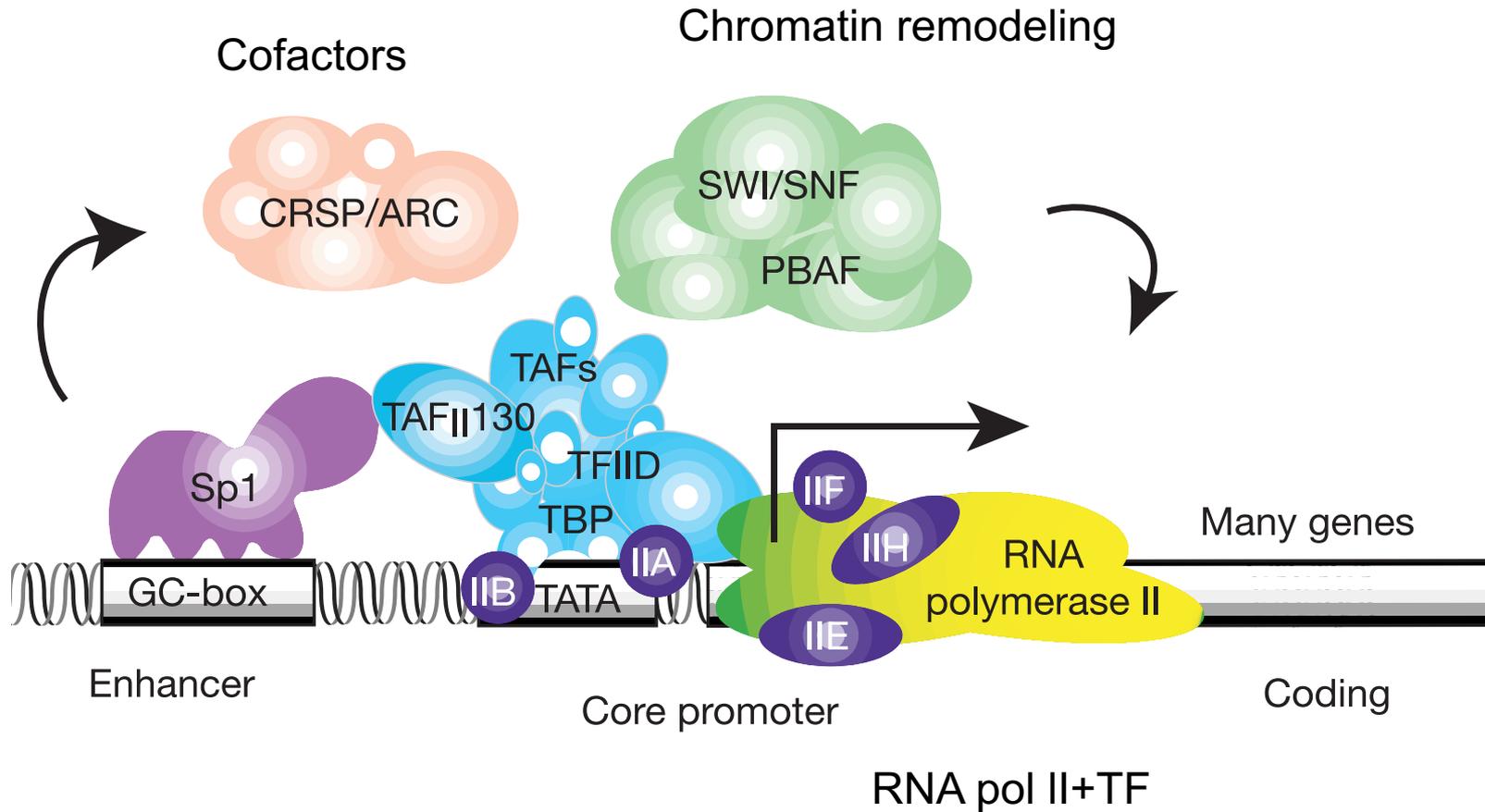
Regulatory sequences: promoters and terminators



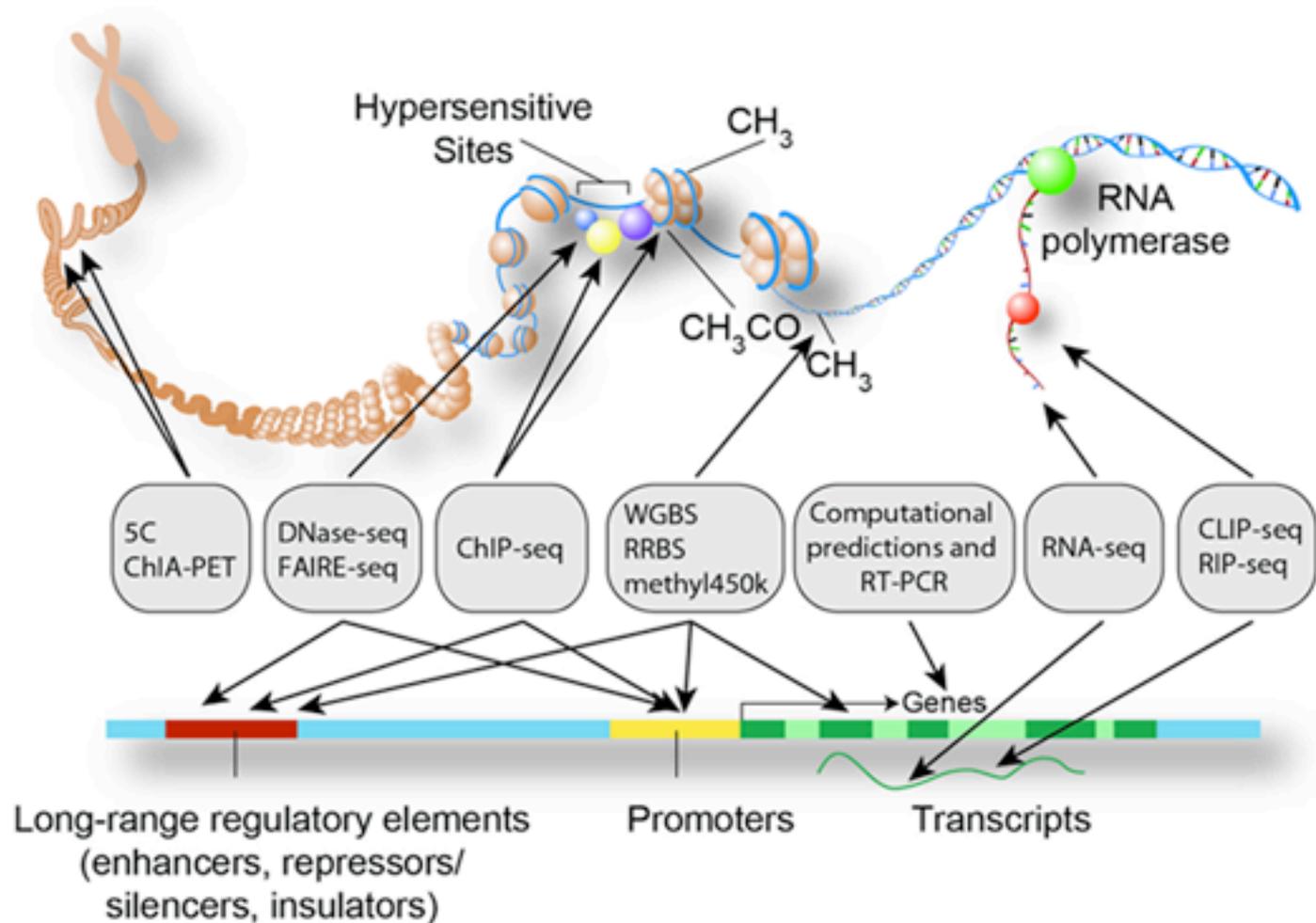
Nature **424**, 147-151 (10 July 2003)

A. Simple eukaryotic transcriptional unit typically found in unicellular eukaryotes (upstream activator sequence - UAS). **B.** Complex metazoan transcriptional control modules (initiator sequences (INR) and downstream promoter elements (DPE)).

Multi-subunit transcription apparatus

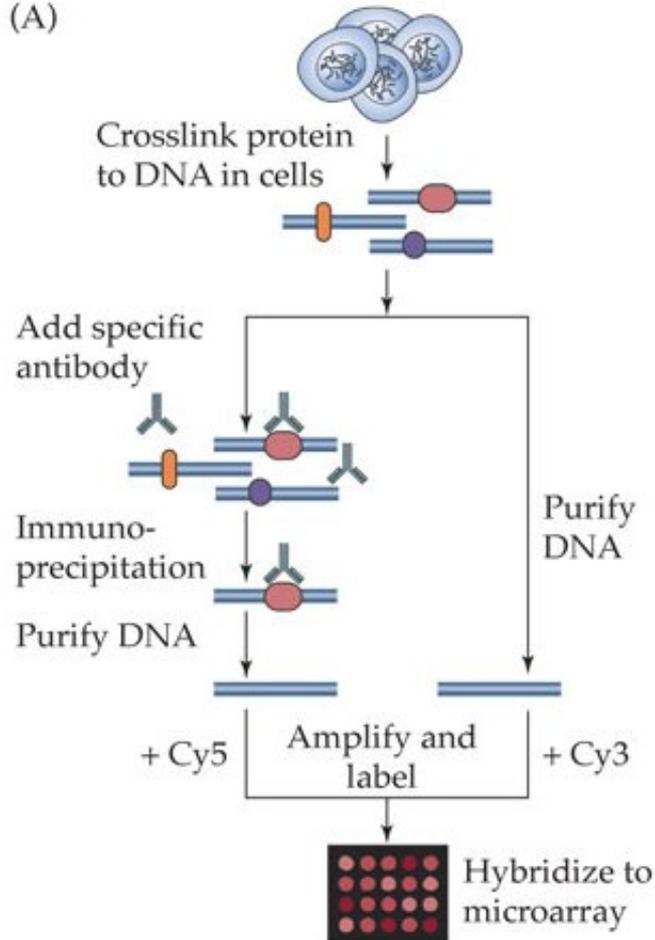


Encyclopedia of DNA elements (ENCODE)



ChIP chip / ChIP seq

(A)



ChIP – chromatin immunoprecipitation
Used to study of gene regulation and finding the transcription factor binding sites

A Global Map of p53 Transcription-Factor Binding Sites in the Human Genome

Chia-Lin Wei,¹ Qiang Wu,¹ Vinsensius B. Vega,¹ Kuo Ping Chiu,¹ Patrick Ng,¹ Tao Zhang,¹ Atif Shahab,² How Choong Yong,² YuTao Fu,³ Zhiping Weng,^{3,4} JianJun Liu,¹ Xiao Dong Zhao,¹ Joon-Lin Chew,^{1,6} Yen Ling Lee,¹ Vladimir A. Kuznetsov,¹ Wing-Kin Sung,¹ Lance D. Miller,¹ Bing Lim,^{1,5} Edison T. Liu,¹ Qiang Yu,¹ Huck-Hui Ng,^{1,6,*} and Yijun Ruan^{1,*}

¹Genome Institute of Singapore, Singapore 138672

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⁵Harvard Institutes of Medicine, Harvard Medical School, Boston, MA 02115, USA

⁶Department of Biological Sciences, National University of Singapore, Singapore 117543

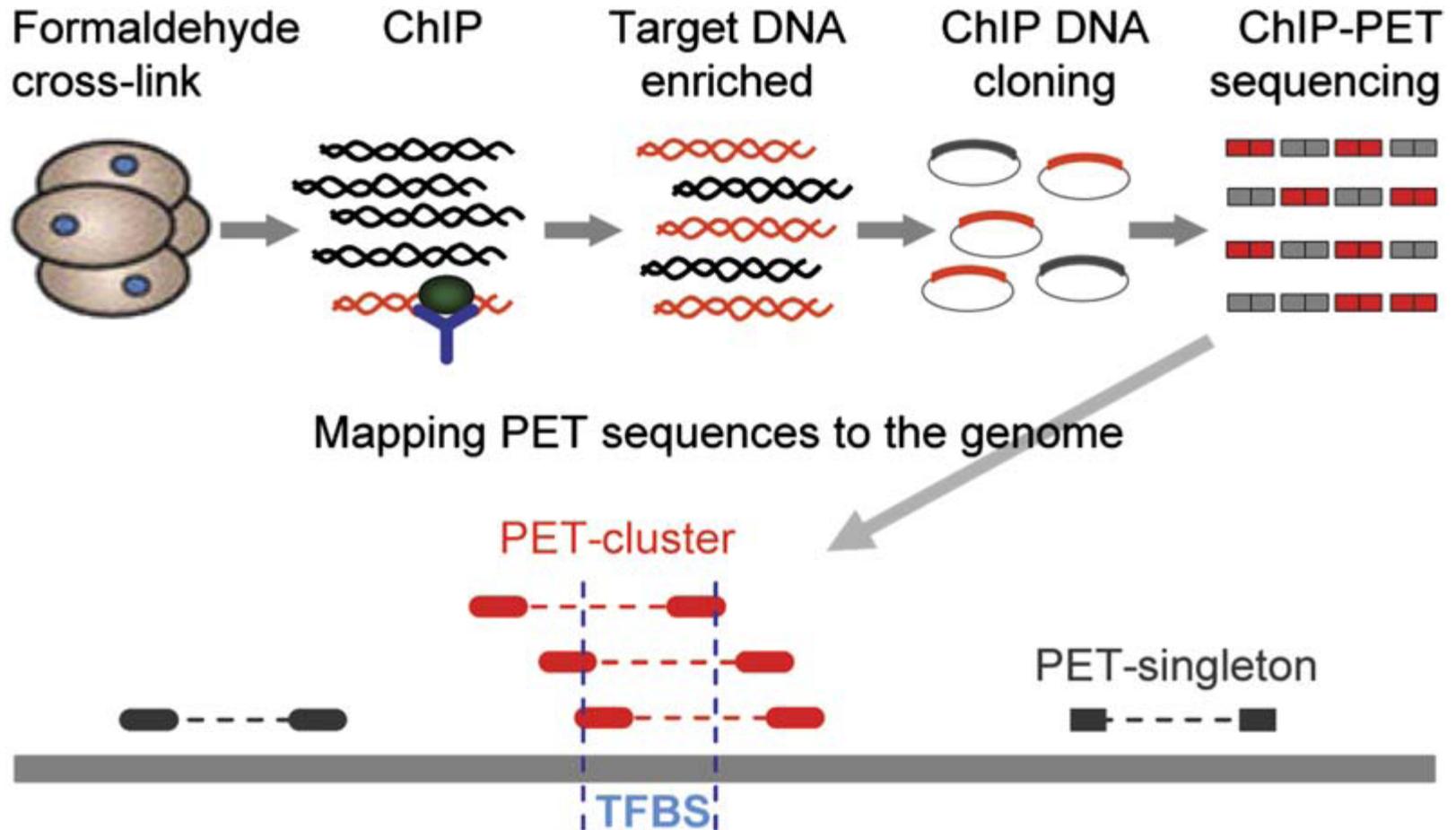
*Contact: nghh@gis.a-star.edu.sg (H.-H.N.); ruanyj@gis.a-star.edu.sg (Y.R.)

DOI 10.1016/j.cell.2005.10.043

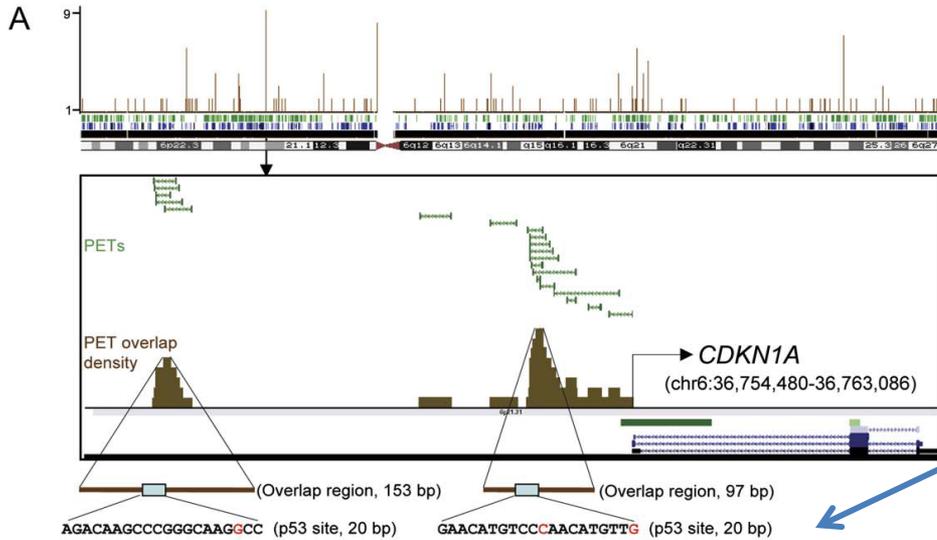
As a transcription factor, p53 regulates the expression of genes involved in a variety of cellular functions, including cell-cycle arrest, DNA repair, and apoptosis

The ability to derive a whole-genome map of transcription-factor binding sites (TFBS) is crucial for elucidating gene regulatory networks. Herein, we describe a robust approach that couples chromatin immunoprecipitation (ChIP) with the paired-end ditag (PET) sequencing strategy for unbiased and precise global localization of TFBS. We have applied this strategy to map p53 targets in the human genome. From a saturated sampling of over half a million PET sequences, we characterized 65,572 unique p53 ChIP DNA fragments and established overlapping PET clusters as a readout to define p53 binding loci with remarkable specificity. Based on this information, we refined the consensus p53 binding motif, identified at least 542 binding loci with high confidence, discovered 98 previously unidentified p53 target genes that were implicated in novel aspects of p53 functions, and showed their clinical relevance to p53-dependent tumorigenesis in primary cancer samples.

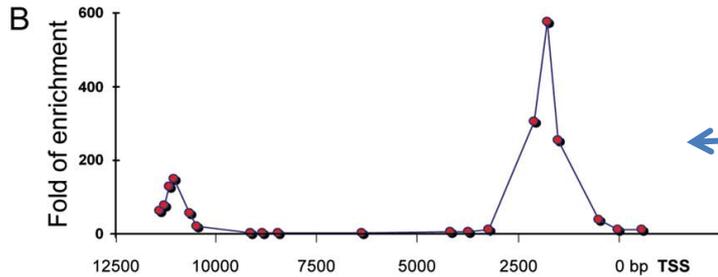
Experimental design



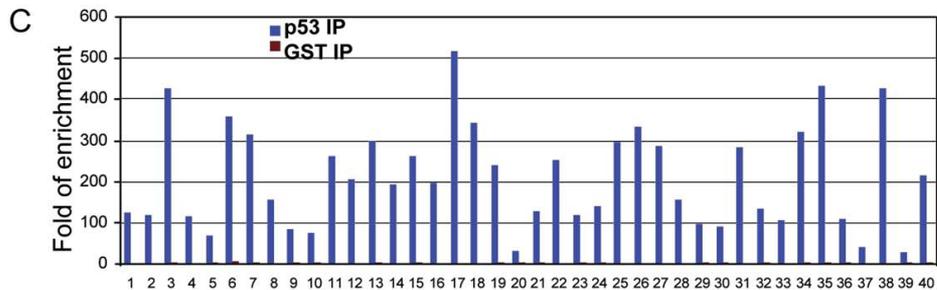
Validation of PET-Cluster-Identified p53 Binding Loci



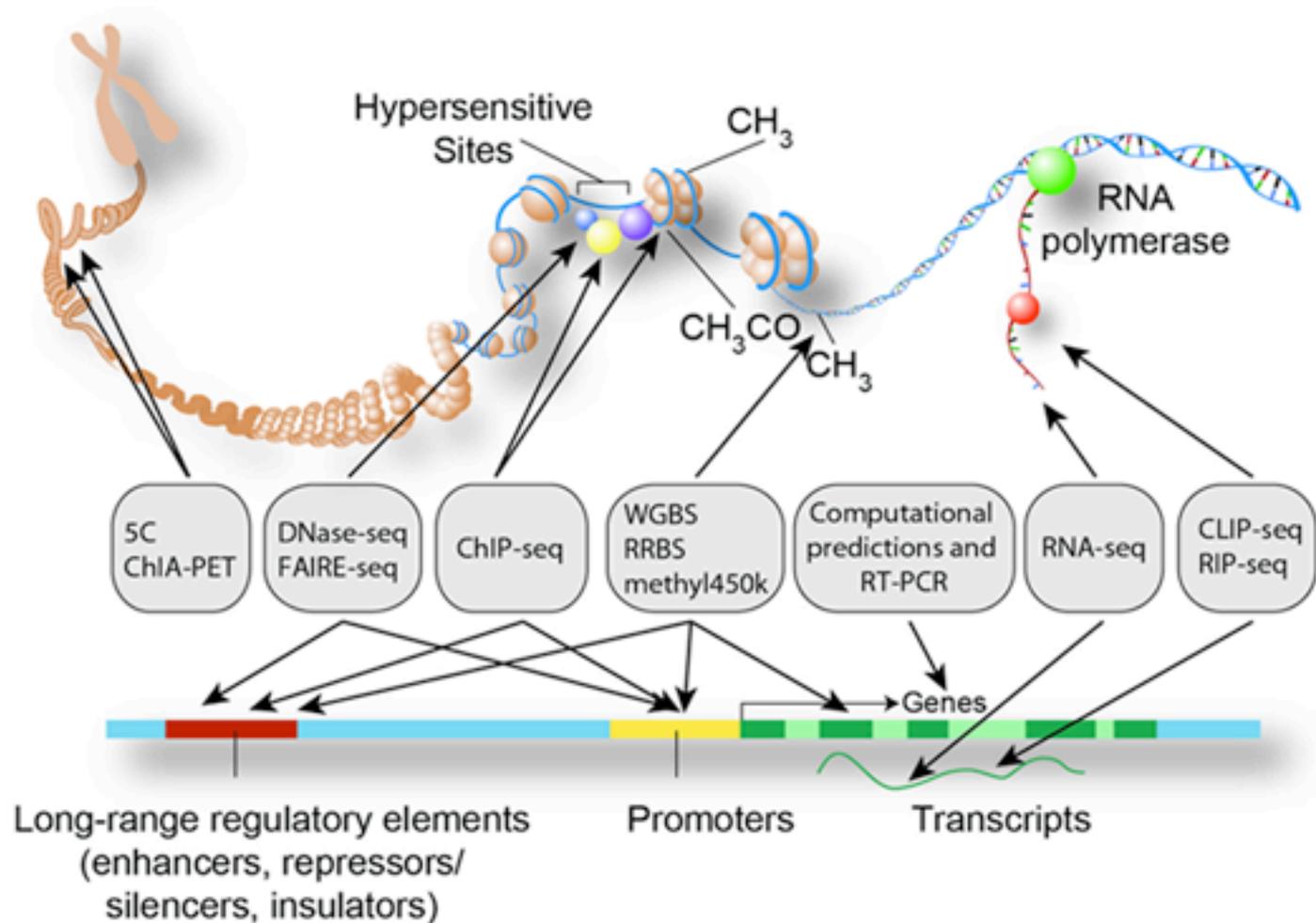
Enriched motif



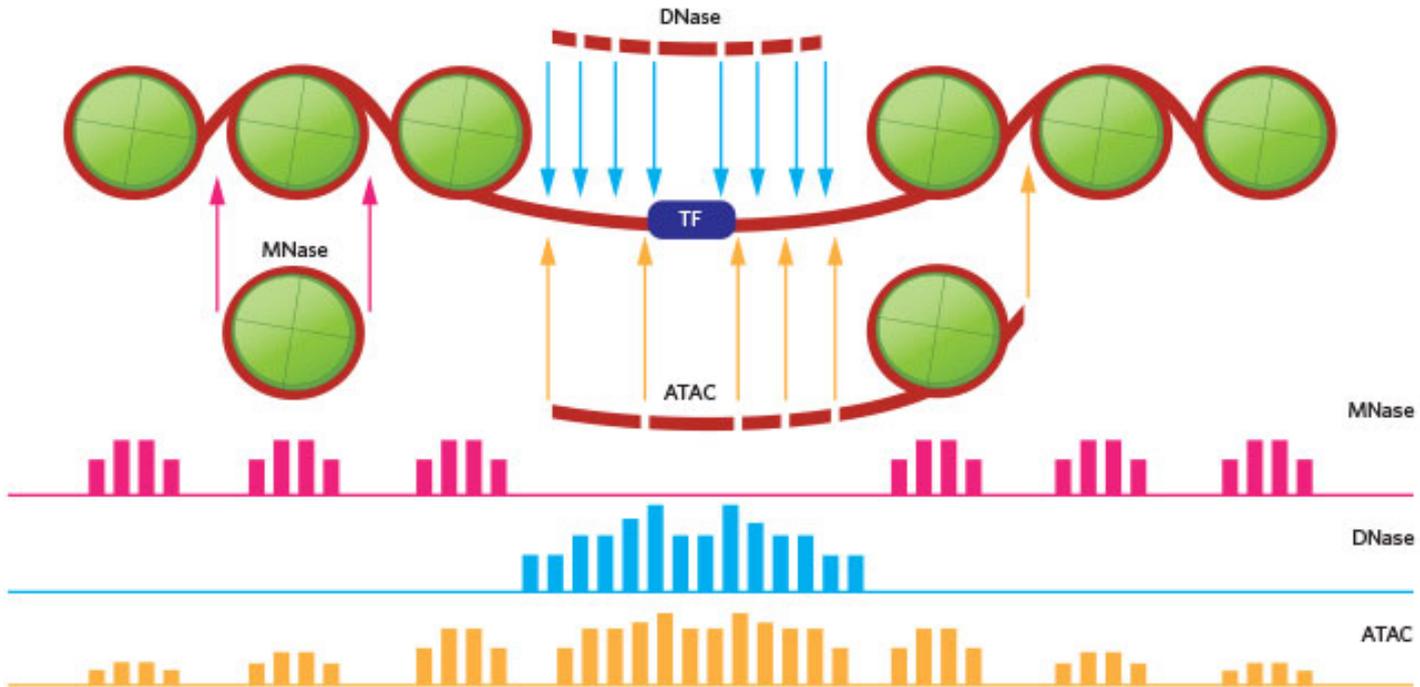
ChIP-qPCR validation in the 50 upstream regions of CDKN1A



Encyclopedia of DNA elements (ENCODE)



How to study chromatin structure?



What we learn?

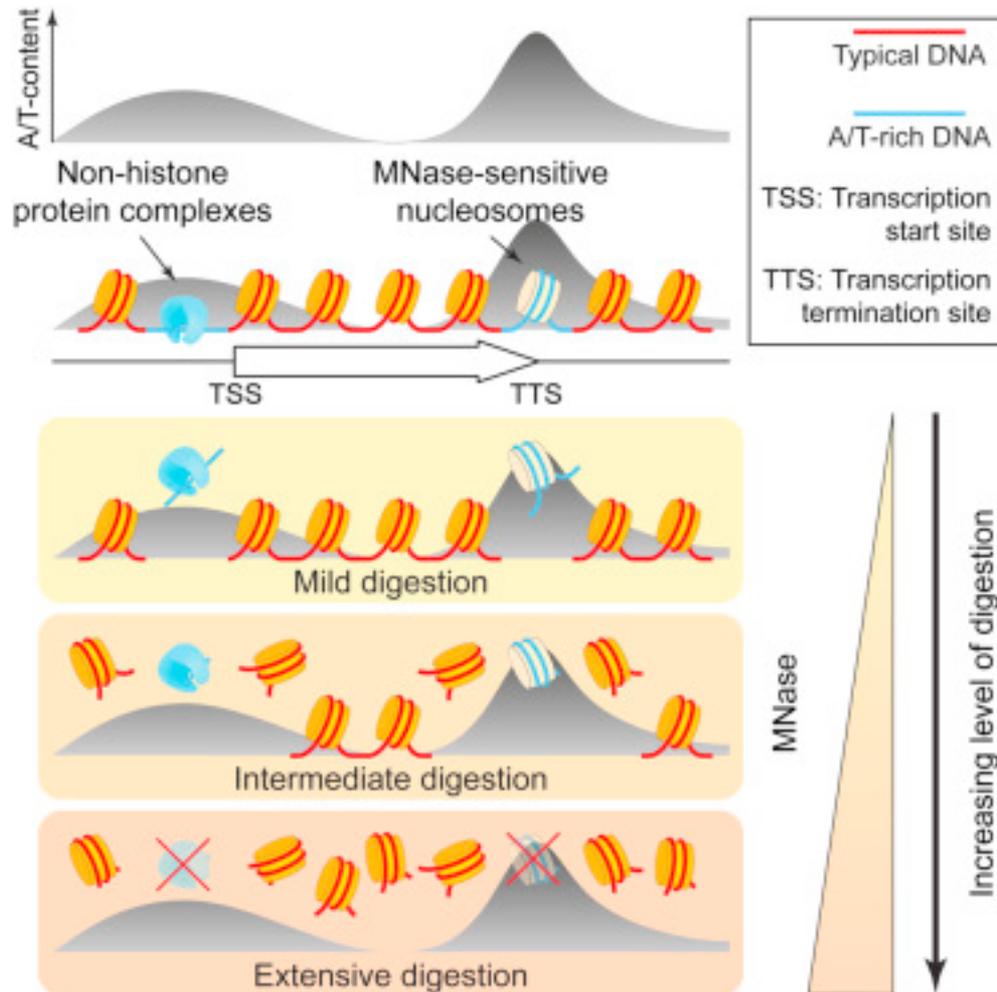
Open-closed chromatin

Location of TFs

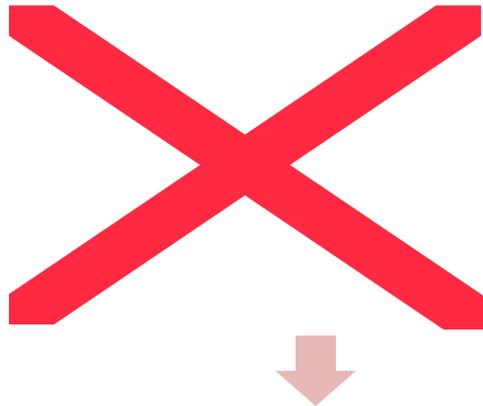
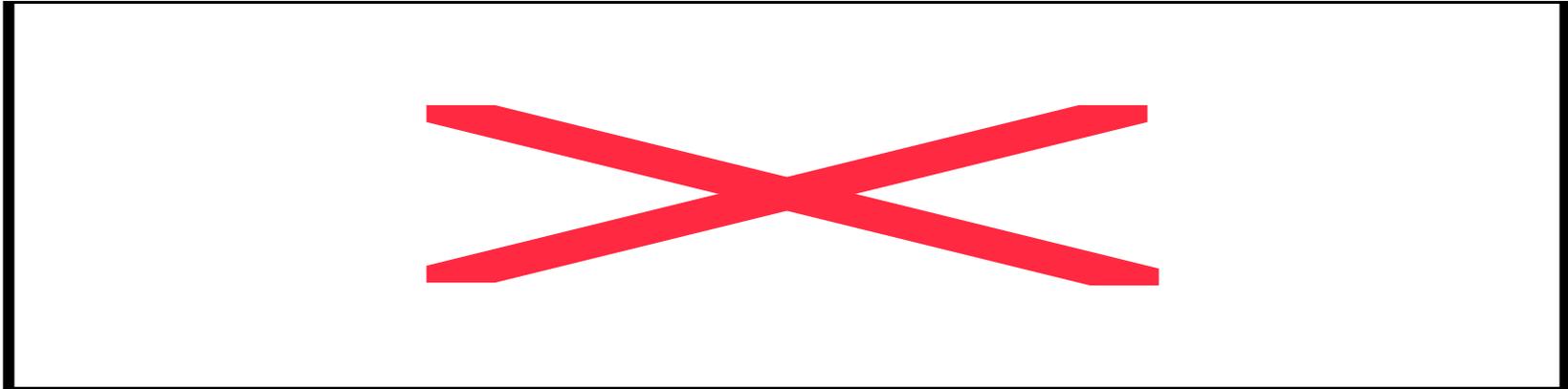
Open chromatin structure assays for wheat

- Genome function is affected by the distribution of nucleosomes and DNA-associated proteins.
- **Assays for wheat were optimized for:**
 - **MNase** - Micrococcal nuclease
 - **ATAC** - assay for transposase-accessible chromatin
- **Chinese Spring genome was analyzed using MNase-Seq approach:**
 - Two digests with HIGH and LOW concentration of Mnase
 - differential nuclease sensitivity = subtract the mean normalized depth (in reads per million) of the heavy digest replicates from those of the light digest replicates

Open chromatin structure assays for wheat



Functionally active hypersensitive chromatin regions
were found associated with regions upstream of
transcription initiation sites



Functional open chromatin regions explain most of the phenotypic variation in wheat

