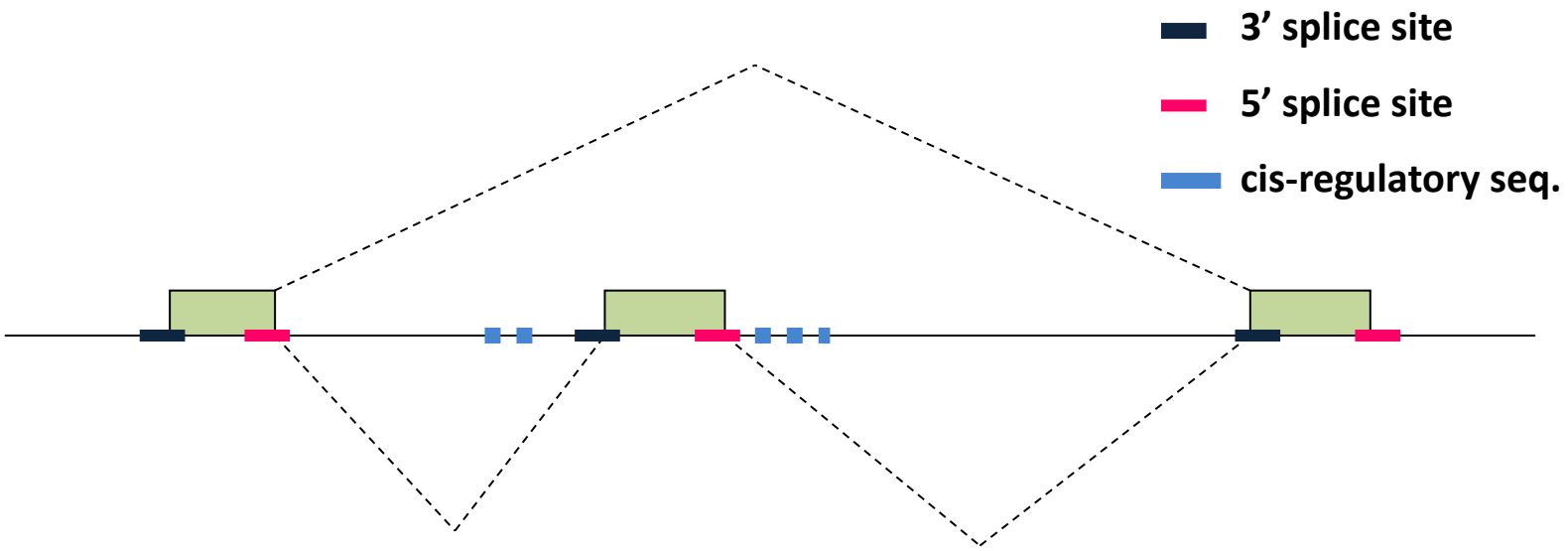
Ch4 - Lesson 2.3

- Mechanisms of alternative splicing

The mechanisms of alternative splicing regulation by trans-acting factors has been explained following the **competitive model**

We can consider the older competition model, as exemplified by the case of a «skipping exon»

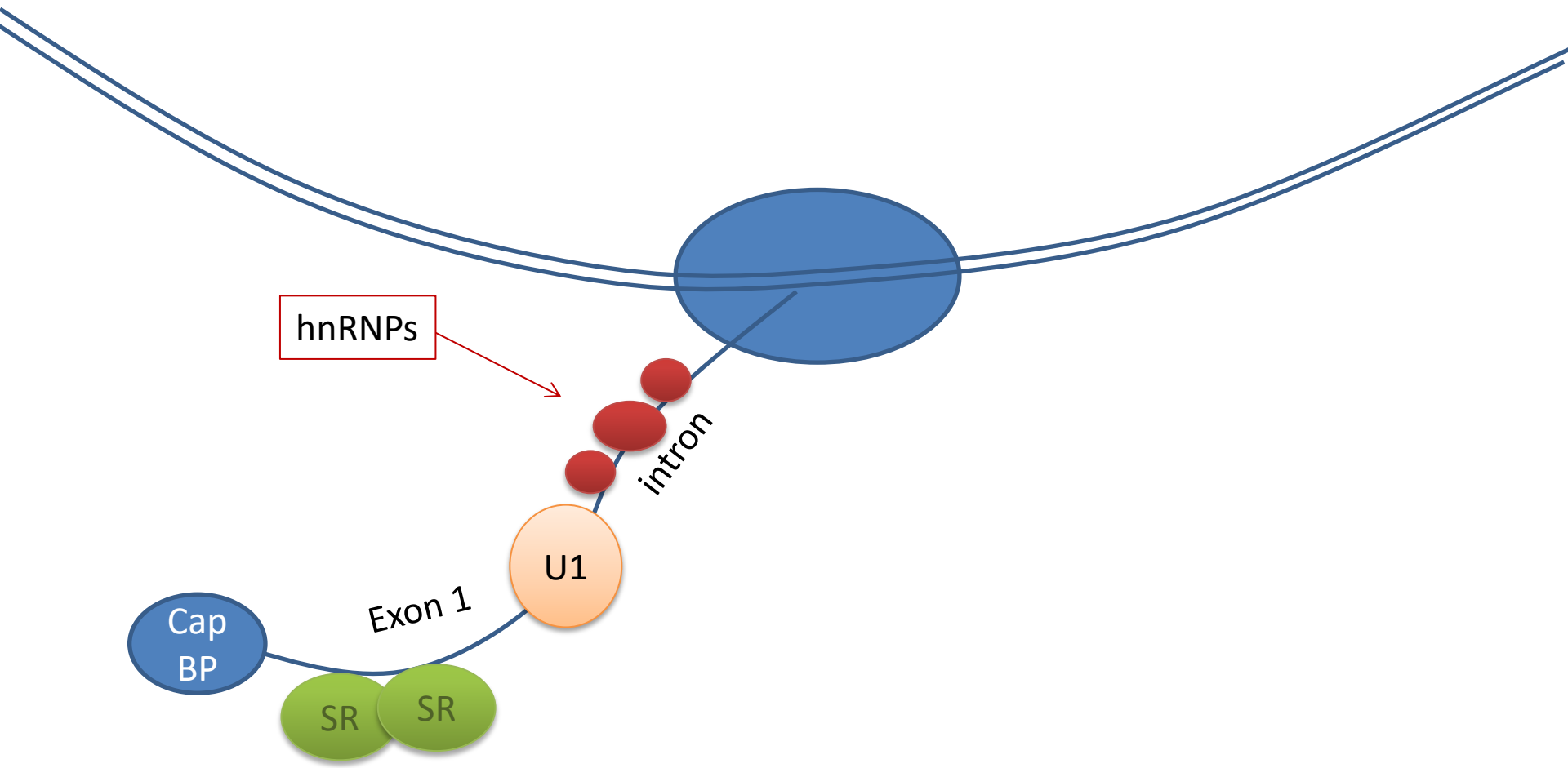


In absence of other regulatory elements (cis-regulatory), only one result is seen: the strongest splice sites will predominate.

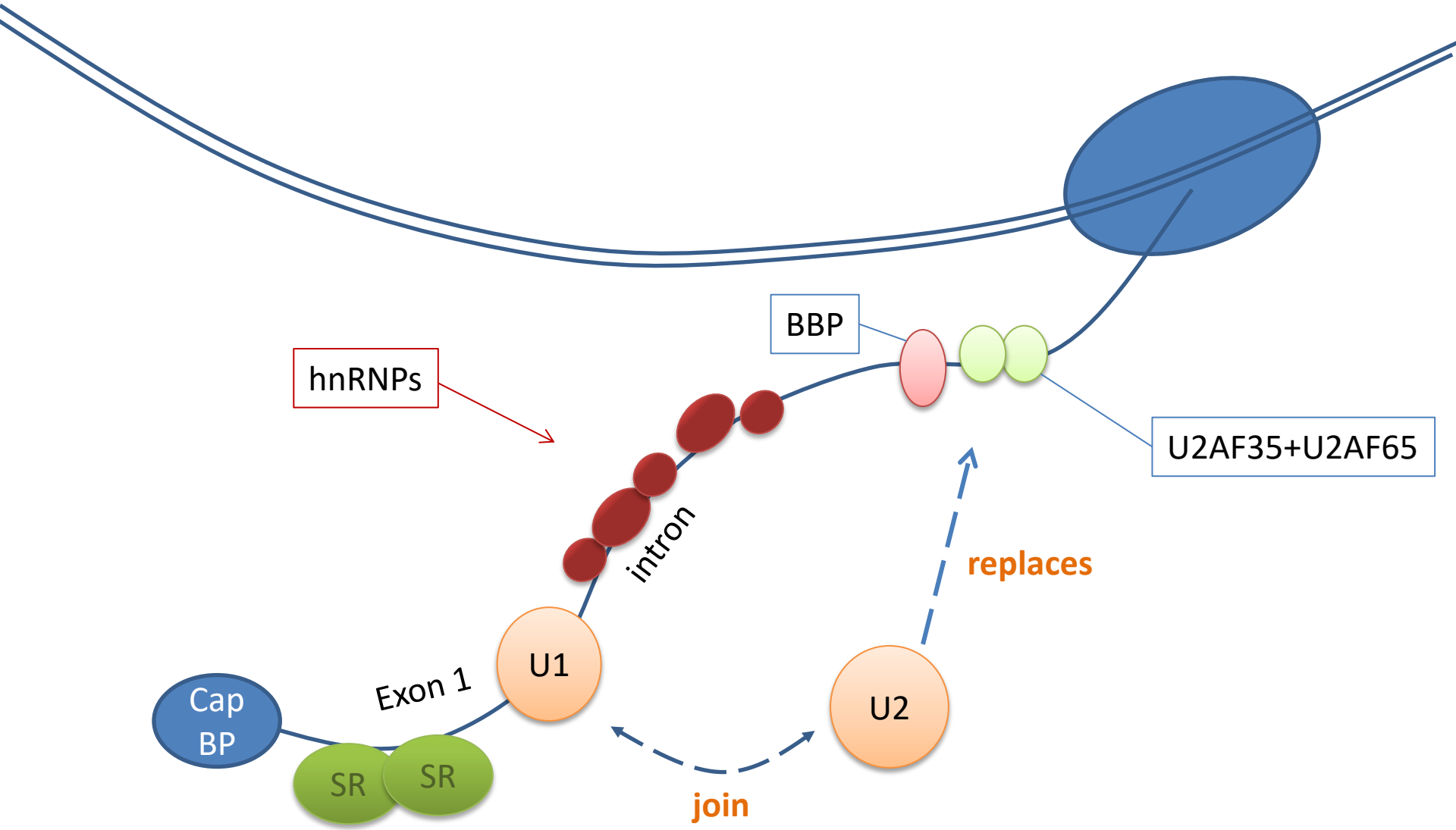
today replaced by

The kinetic competition model

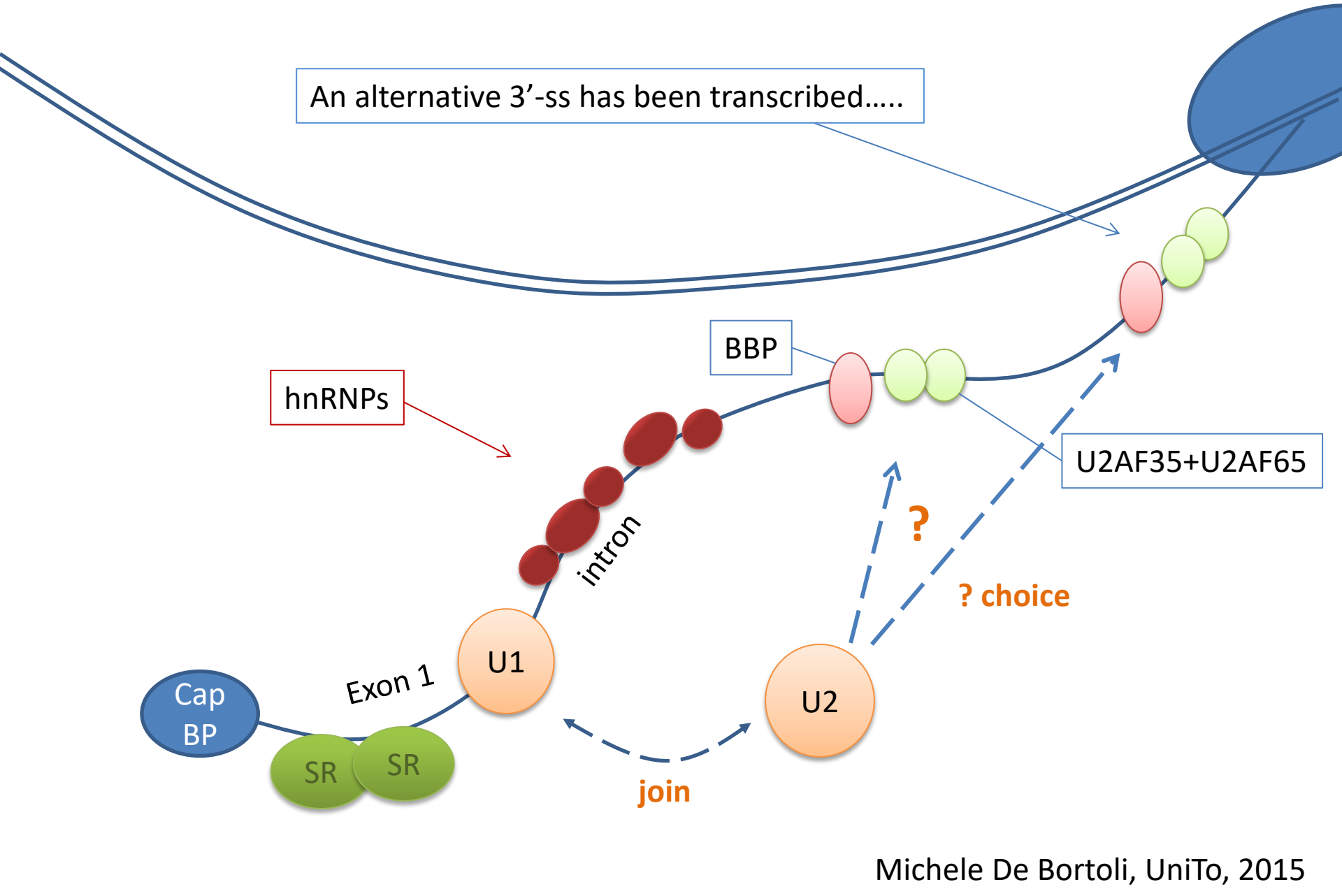
Exon definition



Intron definition



Alternative Splicing



Michele De Bortoli, UniTo, 2015

Based on 2 principles:

1 . The **« first arrived, first served »** model

Is compatible with the fact that, in general, splicing is well ordered and that Alternative Splicing normally (even though not always) concerns a choice between two consecutive splicing sites, only very rarely separated by multiple exons/introns

2 The RNA Polymerase **speed** effect

- ✓ the rate at which Pol II synthesizes RNA is variable and discontinuous, and may depend on elongation factors that are «charged» on RNA Pol II by different promoters/enhancers
- ✓ the «loading» of RNA Polymerase II CTD with specific Splicing Factors (interaction of several splicing factors with Pol II CTD identified by co-IP)

Regulation of alternative splicing by a transcriptional enhancer through RNA pol II elongation

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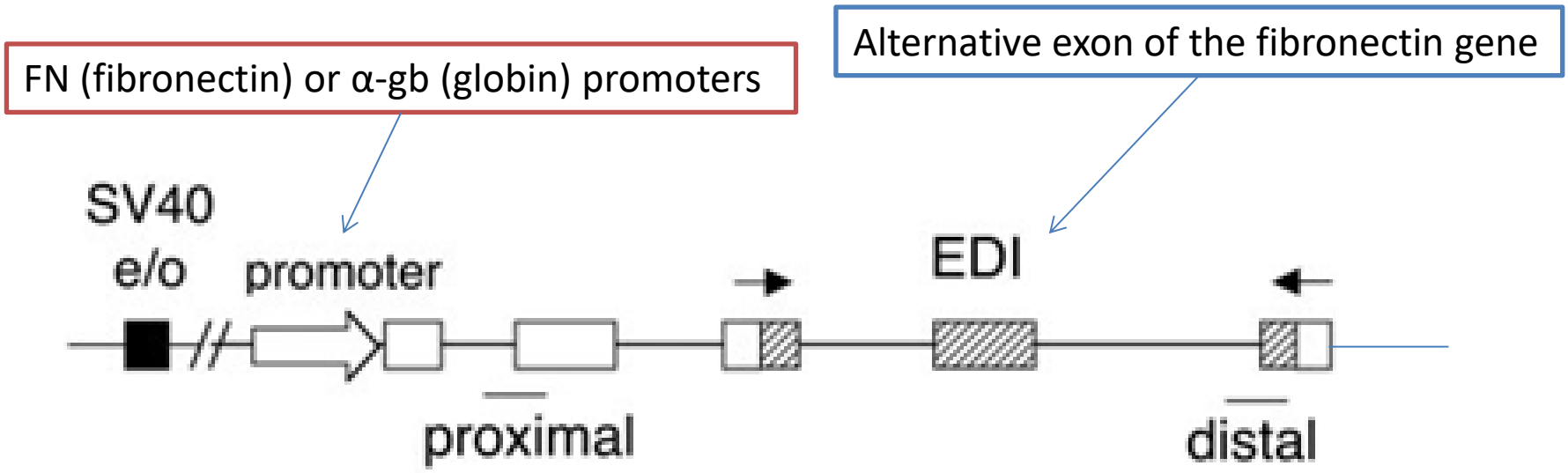
Communicated by César Milstein§, Medical Research Council, Cambridge, United Kingdom, April 24, 2002¶ (received for review January 18, 2002)

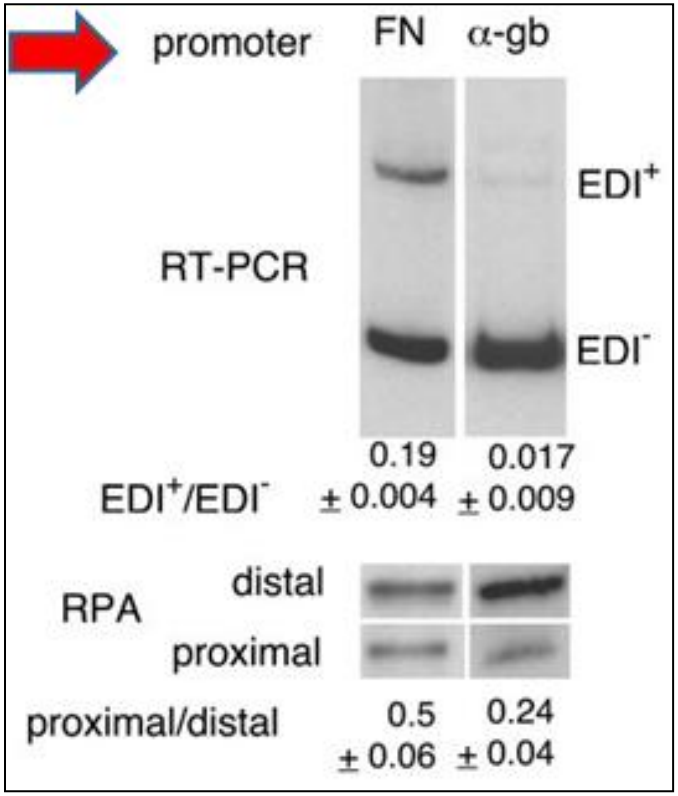
Promoters and enhancers are cis-acting elements that control gene transcription via complex networks of protein–DNA and protein–protein interactions. Whereas promoters deal with putting in place the RNA polymerase, both enhancers and promoters can control transcriptional initiation and elongation. We have previously shown that promoter structure modulates alternative splicing, strengthening the concept of a physical and functional coupling between transcription and splicing. Here we report that the promoter effect is due to the control of RNA pol II elongation. We found that the simian virus 40 (SV40) transcriptional enhancer, inserted in fibronectin (FN) minigene constructs transfected into mammalian cells, controls alternative splicing by inhibiting inclusion of the FN extra domain I (EDI) exon into mature mRNA. Deletion analysis of enhancer subdomains and competitions *in vivo* with excess of specific enhancer DNA subfragments demonstrate that the "minimal" enhancer, consisting of two 72-bp repeats, is responsible for the splicing effect. The 72-bp repeat region has been reported to promote RNA pol II elongation. When transcription is driven by the α-globin promoter linked to the SV40 enhancer, basal EDI inclusion and activation by the SR (Ser–Arg-rich) protein SF2/ASF are much lower than with other promoters. Deletion of only one of the two 72-bp repeats not only provokes higher EDI inclusion levels but allows responsiveness to SF2/ASF. These effects are the consequence of a decrease in RNA pol II elongation evidenced both by an increase in the proportions of shorter proximal over full length transcripts and by higher pol II densities upstream of the alternative exon detected by chromatin immunoprecipitation.

The Speed Bump model was born in Kornblihtt lab more than a decade ago.

Creating a splicing reporter with a weak exon



A) Scheme of the minigenes transfected to assess alternative splicing. Open exons, human-gb; dashed exons, human FN; black box, SV40 e/o; arrows, primers used to amplify the mRNA splicing variants by RT-PCR, and lines, proximal and distal probes used for RPA.



Hep3B cells were transfected with 600 ng of pSVEDAFN (FN promoter) or pSVEDATot (-gb promoter) plus 400 ng of pCMVgal. RNA splicing variants were detected by radioactive RT-PCR and analyzed in 6% native polyacrylamide gels. Ratios between radioactivity in EDI bands and radioactivity in EDI bands are shown under each lane. (*Lower*) RPA with proximal and distal probes shown in *A*, to measure levels of short and long transcripts of transfected Hep3B cells. RT-PCR and RPA ratios correspond to at least three independent transfection experiments.

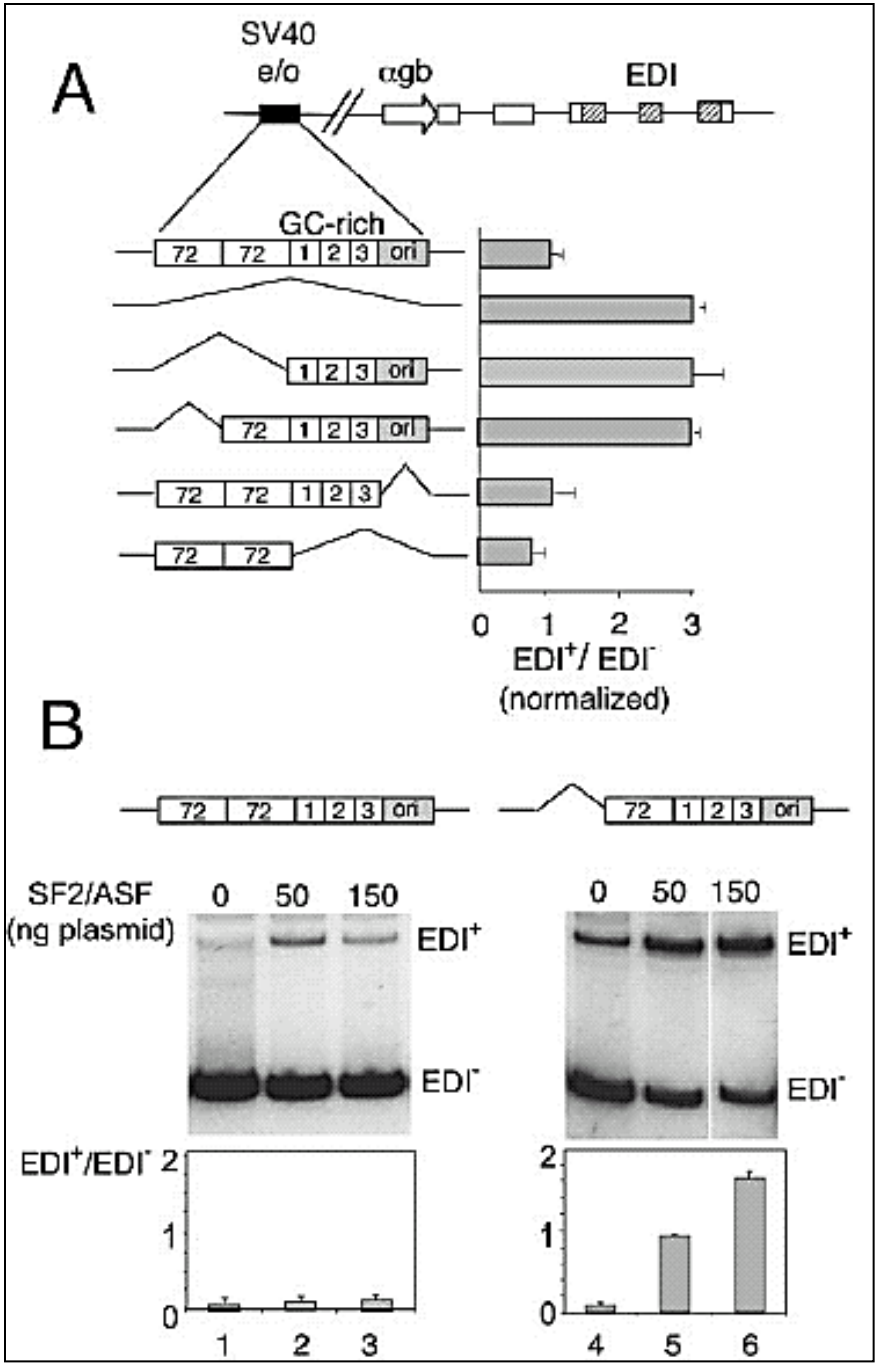
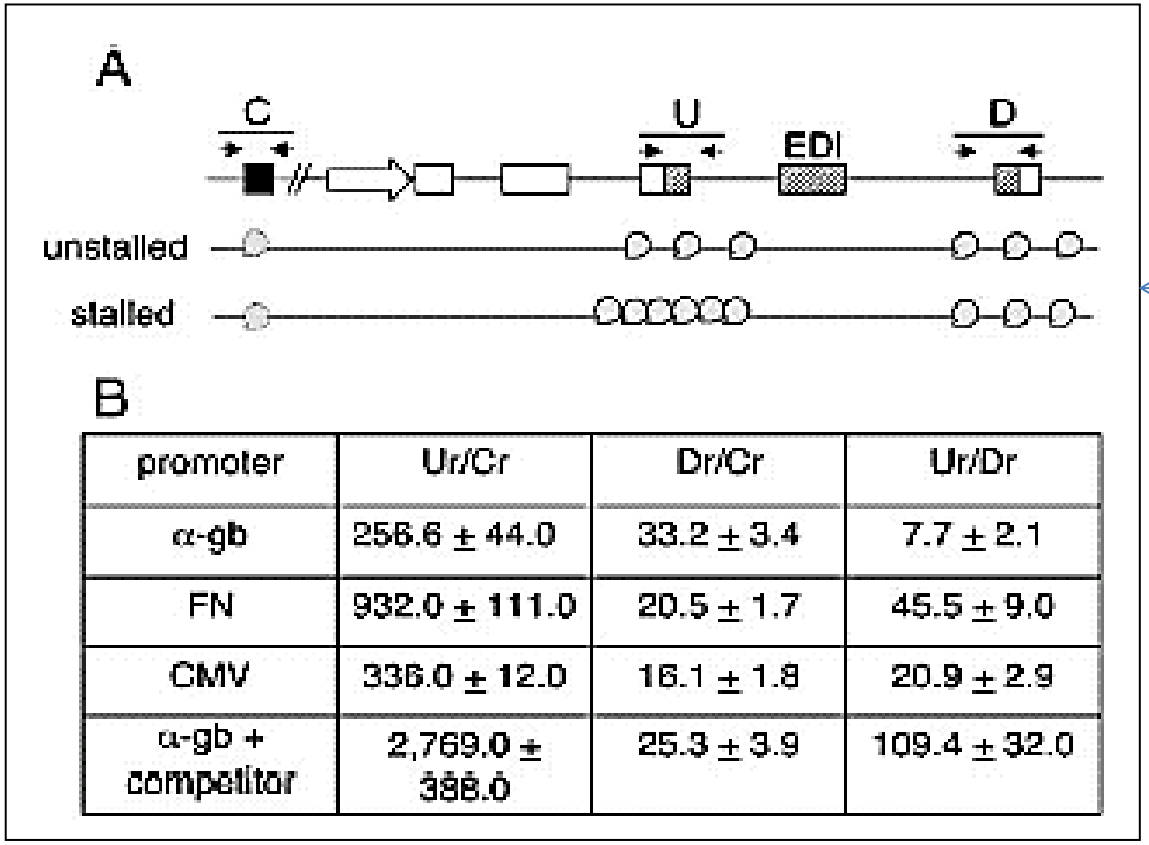


Fig. 3. (*A*) Deletion analysis of the SV40 eo with respect to alternative splicing of the EDI exon. Horizontal bars indicate normalized EDIEDI ratios of Hep3B cells transfected with a series of α–gb promoter constructs carrying different internal deletions of the SV40 eo. Results correspond to the mean SD of at least three independent transfection experiments.

(*B*) Deletion of only one 72-bp repeat confers responsiveness to SF2ASF to the α-gb promoter construct. Hep3B cells were transfected with pSVEDATot (lanes 1–3) or a variant lacking the distal 72-bp repeat of the SV40 enhancer (lanes 4–6) and cotransfected with the indicated amounts of a plasmid expressing SF2ASF (13). Transfections in lanes 1 and 4 contained 150 ng of empty DNA vector.

Similar results were obtained in Cos-7 and HeLa cells.



B

promoter	Ur/Cr	Dr/Cr	Ur/Dr
α-gb	256.6 ± 44.0	33.2 ± 3.4	7.7 ± 2.1
FN	932.0 ± 111.0	20.5 ± 1.7	45.5 ± 9.0
CMV	336.0 ± 12.0	16.1 ± 1.8	20.9 ± 2.9
α-gb + competitor	2,769.0 ± 388.0	25.3 ± 3.9	109.4 ± 32.0

Fig. 5. ChIP with an Ab to RNA pol II. (*A*) Scheme of the minigenes transfected to assess pol II densities. Arrows indicate the pairs of primers used in real time PCRs to quantitatively amplify DNA that is bound to the immunoprecipitated pol II, at two regions mapping U, D of the EDI alternative exon, and at a third C region outside of the transcription unit. (*B*) Cells were transfected with -gb, FN, or CMV promoter constructs and, where indicated, co-transfected with a 10-fold molar excess of a competitor plasmid carrying the SV40 e/o. After 48 h, cells were fixed with formaldehyde and treated for ChIP and real time PCR analysis as described in *Experimental Procedures*. UrUimUin; CrCimCin; DrDimDin where Uim, Cim, and Dim are the template DNA amounts recovered after *im*munoprecipitation by anti-pol II, and Uin, Cin, and Din are the *in*put DNA amounts, all estimated by real time PCR at regions U, C, and D, respectively. Results correspond to a representative transfection experiment of Cos-7 cells and show the mean SD of three real time PCR determinations.

This study demonstrated that:

1. Promoters/enhancers can influence the rate of inclusion of weak exons

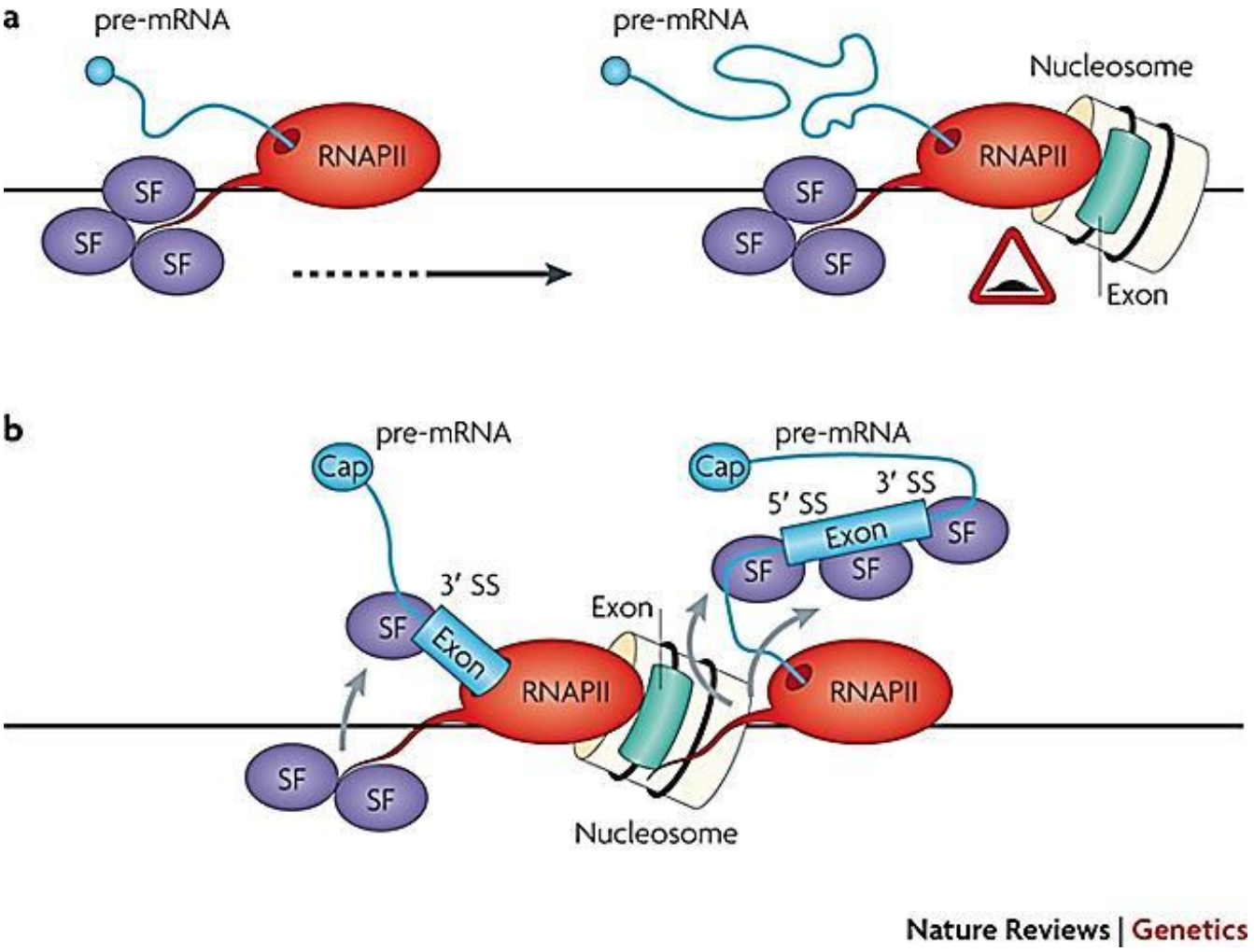
2. They do so in part by influencing the speed of RNA Pol II

3. When a weak exon is included, RNA PolII «slows down» in proximity of that exon

… and RNA Pol II CTD carries splicing factors.

the speed-bump model

the speed-bump model



Nature Reviews | Genetics

Nucleosome occupancy marks exons and is coupled to transcription.
a | RNA polymerase II (RNAPII), associated with different splicing factors (SFs), travels along the gene and transcribes it. When RNAPII reaches an area with high nucleosome occupancy and encounters specific histone modifications that mark an exon, it is slowed down.
b | This panel shows RNAPII and the nucleosome at the point at which their coupling marks the exon boundaries for the splicing machinery. RNAPII transcribes the exon and SFs detach from the carboxy-terminal domain of RNAPII and bind to the 3′ splice site (3′ SS) region of the precursor mRNA (pre-mRNA). During transcription elongation, additional SFs bind intronic and exonic splicing regulatory elements and the 5′ SS.

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Nucleosomes are best positioned on exons

Nucleosome positioning as a determinant of exon recognition

Hagen Tilgner[1,3], Christoforos Nikolaou[1,3], Sonja Althammer[1], Michael Sammeth[1], Miguel Beato[1], Juan Valcárcel[1,2] & Roderic Guigó[1]

Chromatin structure influences transcription, but its role in subsequent RNA processing is unclear. Here we present analyses of high-throughput data that imply a relationship between nucleosome positioning and exon definition. First, we have found stable nucleosome occupancy within human and *Caenorhabditis elegans* exons that is stronger in exons with weak splice sites. Conversely, we have found that pseudoexons—intronic sequences that are not included in mRNAs but are flanked by strong splice sites—show nucleosome depletion. Second, the ratio between nucleosome occupancy within and upstream from the exons correlates with exon-inclusion levels. Third, nucleosomes are positioned central to exons rather than proximal to splice sites. These exonic nucleosomal patterns are also observed in non-expressed genes, suggesting that nucleosome marking of exons exists in the absence of transcription. Our analysis provides a framework that contributes to the understanding of splicing on the basis of chromatin architecture.

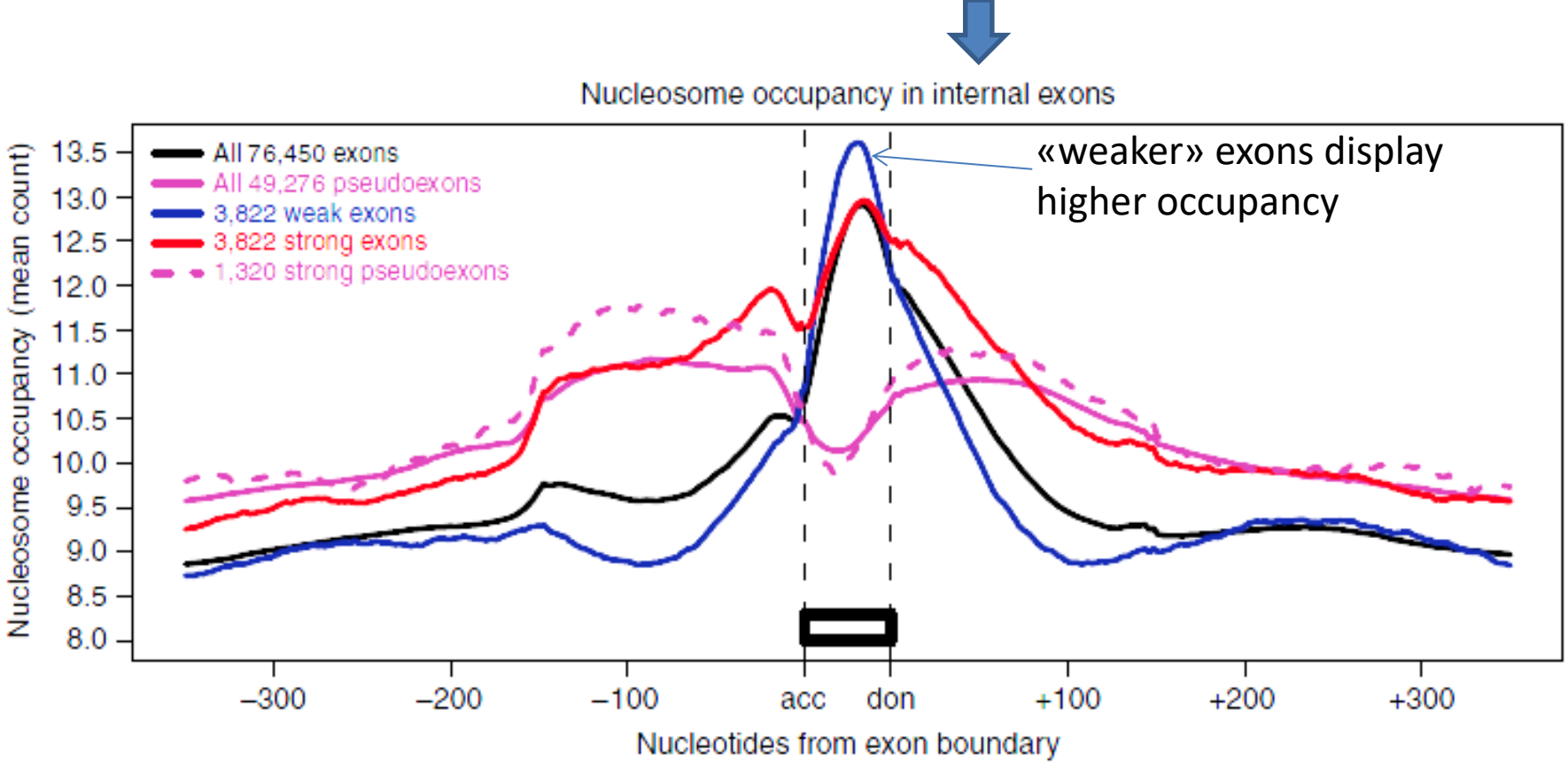

Figure 1 Observed and predicted nucleosome occupancy. (**a**) Nucleosome-occupancy profile across human internal constitutive exons in resting CD4+ T cells. We have computed the number of extended nucleosome reads overlapping each nucleotide. Upstream and downstream of an **idealized internal exon**, we plot the average number of nucleosome reads per nucleotide position, with negative positions relative to the acceptor (acc) site and positive positions relative the donor (don) site. Within the exon, reads have been mapped to 50 identically spaced intervals, irrespective of the length of the exon (see Online Methods). Strong exons are exons with a combined donor and acceptor score among the highest 5%; weak exons are the exons with a combined score among the lowset 5%; pseudoexons are intronic sequences bounded by splice sites; strong pseudoexons are exons with a combined score higher than the 90% percentile of real exons.

Tilgner et al., 2009

Reduction during trascription is less evident at weak exons

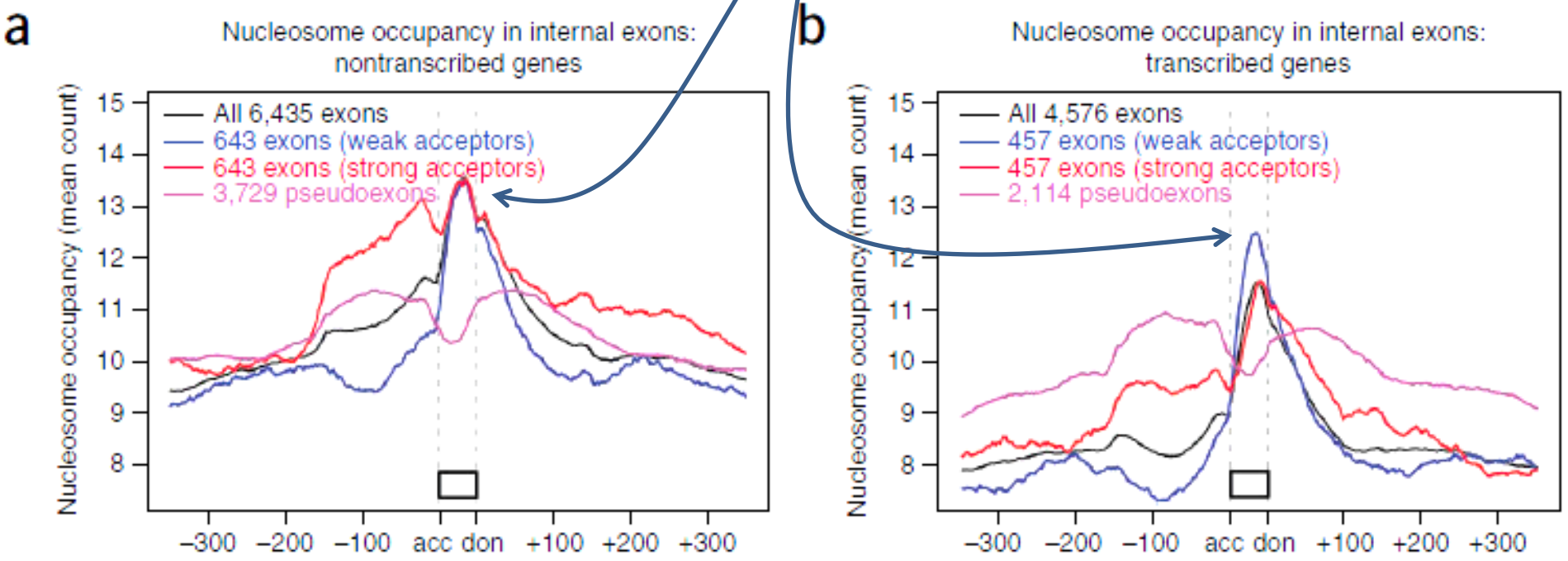

Figure 2 Nucleosome occupancy and expression of genes and exons. (**a**) Nucleosome-occupancy profile across internal acceptor sites from genes that are not expressed in resting CD4+ T cells. Gene expression has been determined using the Affymetrix platform. We plot the average number of nucleosome reads per position in all exons considered together (black), only in exons with strong (red) and weak (blue) acceptor sites, and in intronic pseudoexons. (**b**) Nucleosome-occupancy profile across internal acceptor sites from genes expressed in resting CD4+ T cells, shown as in **a.**

Tilgner et al., 2009