

Lezione XI-XII

giovedì 27-X-2011

corso di genomica

laurea magistrale Biotecnologia
Industriale

aula 8

orario : Martedì ore 14.00 - 16.00

Giovedì ore 13.00 - 15.00

non ci sarà lezione martedì 1 e giovedì 3 Novembre
ci si rivede martedì 8 Novembre

D. Frezza

da chi è diretto il movimento della cromatina

cercare di capire come si muove (attiva o passiva)

analisi in vivo con microscopia

i movimenti fuori dal territorio cromosomico controllati da actina-miosina in topi transgenici [Chuang CH et al. Curr Biol. 2006 Apr 18;16(8):825-31.]

effetto “looping out” dal territorio cromosomico dipendente dal tipo cellulare: Hoxd di topo ha il “looping” sull’asse antero-posteriore ma non negli abbozzi degli arti,

effetto di ricollocamento nel territorio nucleare del crms X dopo inattivazione di Xist, ma i territori crms non sono barriere per la trascrizione da parte della pol. II

looping chromatine

Chromatin looping and the probability of transcription.

Trends Genet. 2006 Apr;22(4):197-202. Epub 2006 Feb 21.

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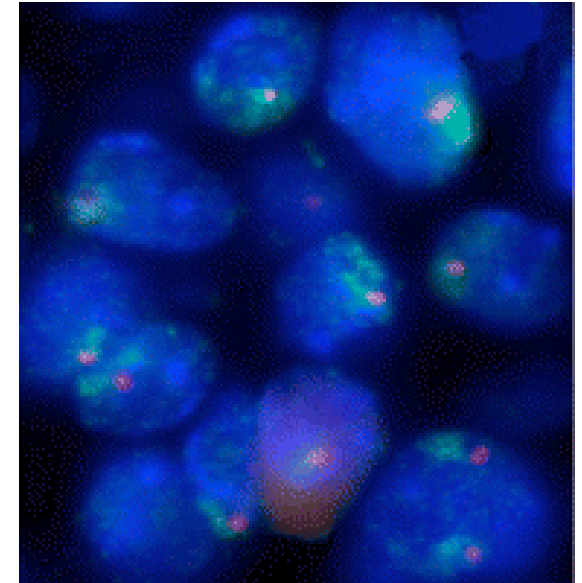
Recent studies of several multigene clusters have shown that gene activation by a remote enhancer is associated with chromatin loop formation. It is not fully understood how a chromatin loop forms in a nucleus or how it is involved in gene regulation. In this article, we propose that the major feature that determines loop formation is the flexibility of chromatin, and that this flexibility is modulated by histone acetylation (and other modifications). Thus, histone modifications will modulate distribution of the preferential looping site in chromatin, which, in turn, determines the probability of interaction between a remote enhancer and the cognate genes. This model can explain gene expression changes in the Hoxd gene cluster and the beta-globin locus.

looping out the chrms territory

Looping the loop with Hoxd

the relocation of gene loci outside chromosome territories and chromatin decondensation are generally regarded as linked facets of gene activation. Now, however, Wendy Bickmore and co-workers reveal that these processes can occur separately during activation of the Hoxd gene cluster (see p. 909). The researchers analysed the nuclear organisation of the mouse Hoxd cluster in differentiating embryonic stem (ES) cells and embryonic day 9.5 mouse embryos. The Hoxd cluster, they report, like the Hoxb cluster, looped out from its chromosome territory and unfolded after its activation in ES cells and in the embryonic tailbud. But, in the limb bud (another site of Hoxd activation), the chromatin unfolded without looping out. Furthermore, during ES cell differentiation, parts of the Hoxd cluster looped out before their chromatin visibly decondensed. Thus, although the chromosome remodelling mechanisms that underlie Hox gene activation predate the duplication of mammalian Hox loci, the exact mode of regulation of the Hoxd cluster depends on its developmental context.

Development 2007 134(5):909-19. Morey C, Da Silva NR, Perry P, Bickmore WA



Il modello del cluster Hoxd

Analisi intorno al cluster di ~ 1 Mb

su cellule ES ed embrioni stadio (E) 9.5 days

- sulla base di analogia del cluster Hoxb che fa “looping out” dal territorio cromosomico
- per l'esistenza di regioni ancestrali del cluster conservate e con meccanismi di regolazione dei loci Hox basati sulla struttura della cromatina
- Cellule OS25 ES mantenute a stadio indifferenziato con selezione Oct4- hyg, differenziate ganciclovir/Oct4 expressing
- Embrioni di topo E9.5 da incrocio Dct-LacZ omozigoti x CD1xCD1 in formaldeide
- 3D FISH multicolor e RT-PCR; BAC e fosmidi per i probes x ibridazione

Nuclear reorganization of the *Hoxd* regulatory domain in the embryo

The conservation of Hox clusters has been attributed to enhancers located within a systematic search for conserved noncoding sequences (CNS) in the human Hox cluster conserved noncoding sequences named GCR*: global control region. Although non-coding sequences generally lack sequence conservation between highly divergent species there are a number of striking examples. (guardare figura su pdf)

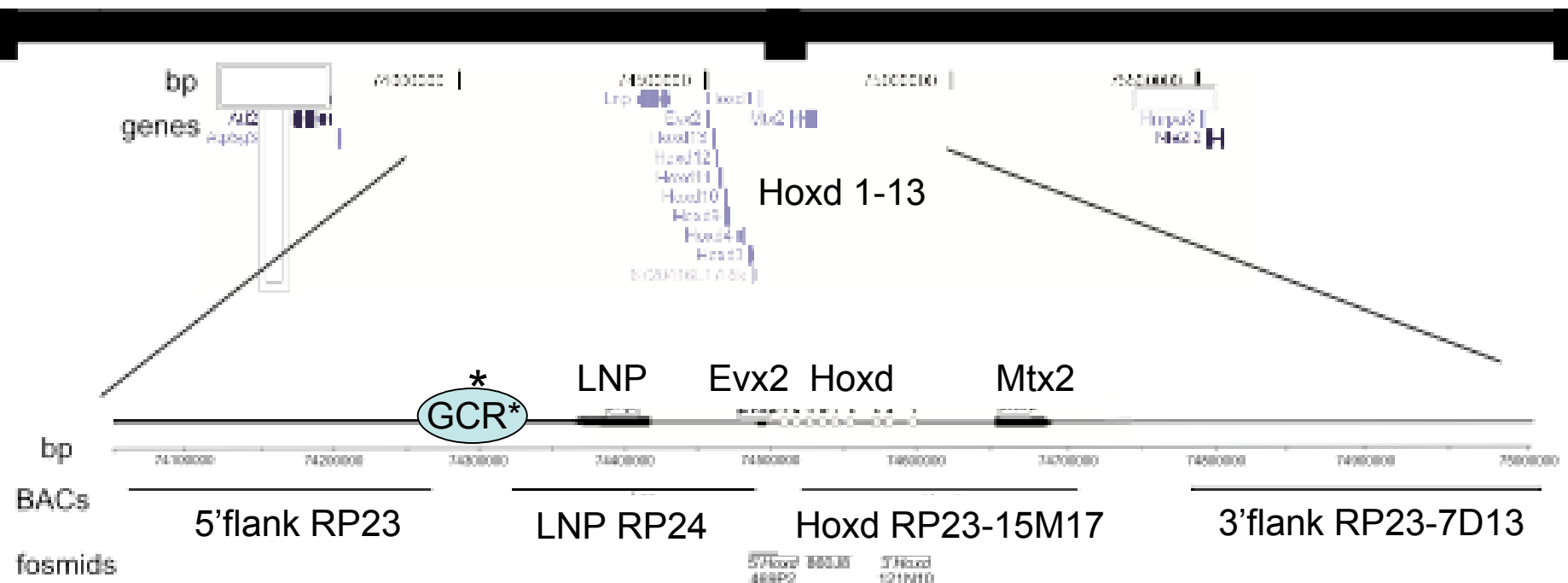
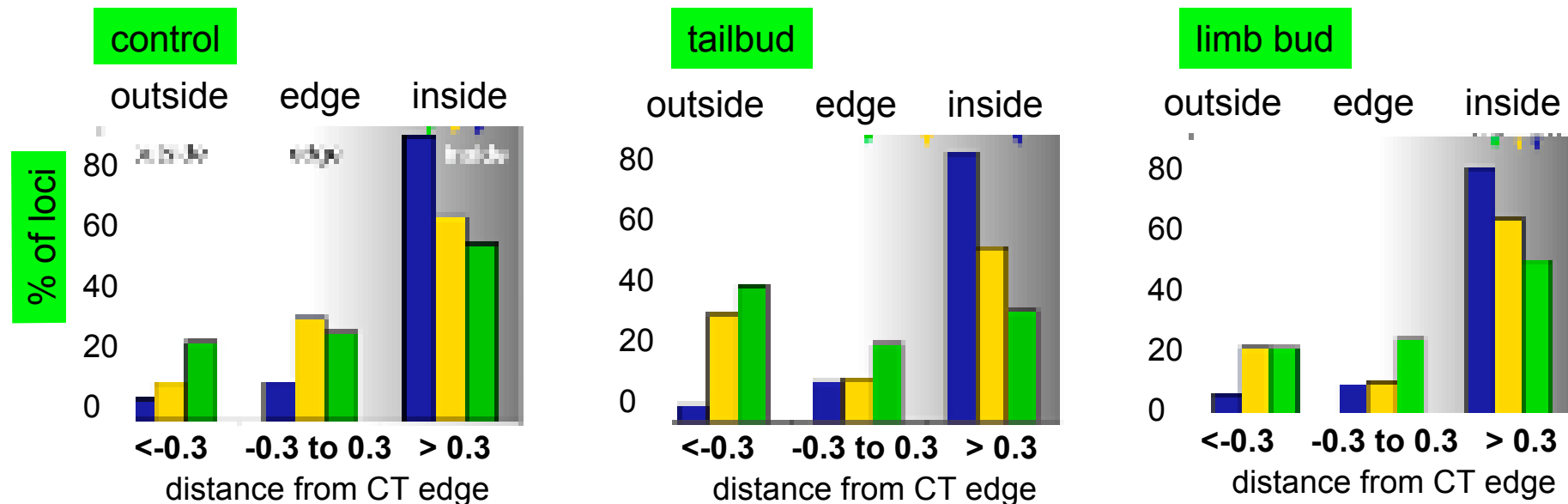


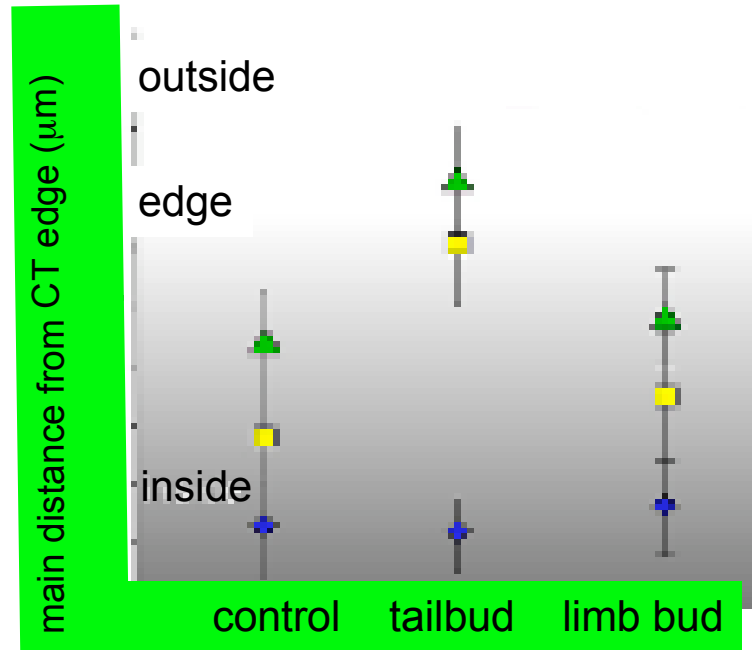
Fig nuclear reorganisation



Histograms showing the 3D position of hybridization signals for a BAC lying within the 5' flanking region (blue), BAC covering the Lnp gene (yellow), BAC covering Hoxd genes (green) position relative to MMU2 CT edge in nuclei of E9.5 control tissues, tail bud or limb bud. Coloured arrowheads indicate the medium location for each respective probe. n>100 loci obtained from 3 embryos.

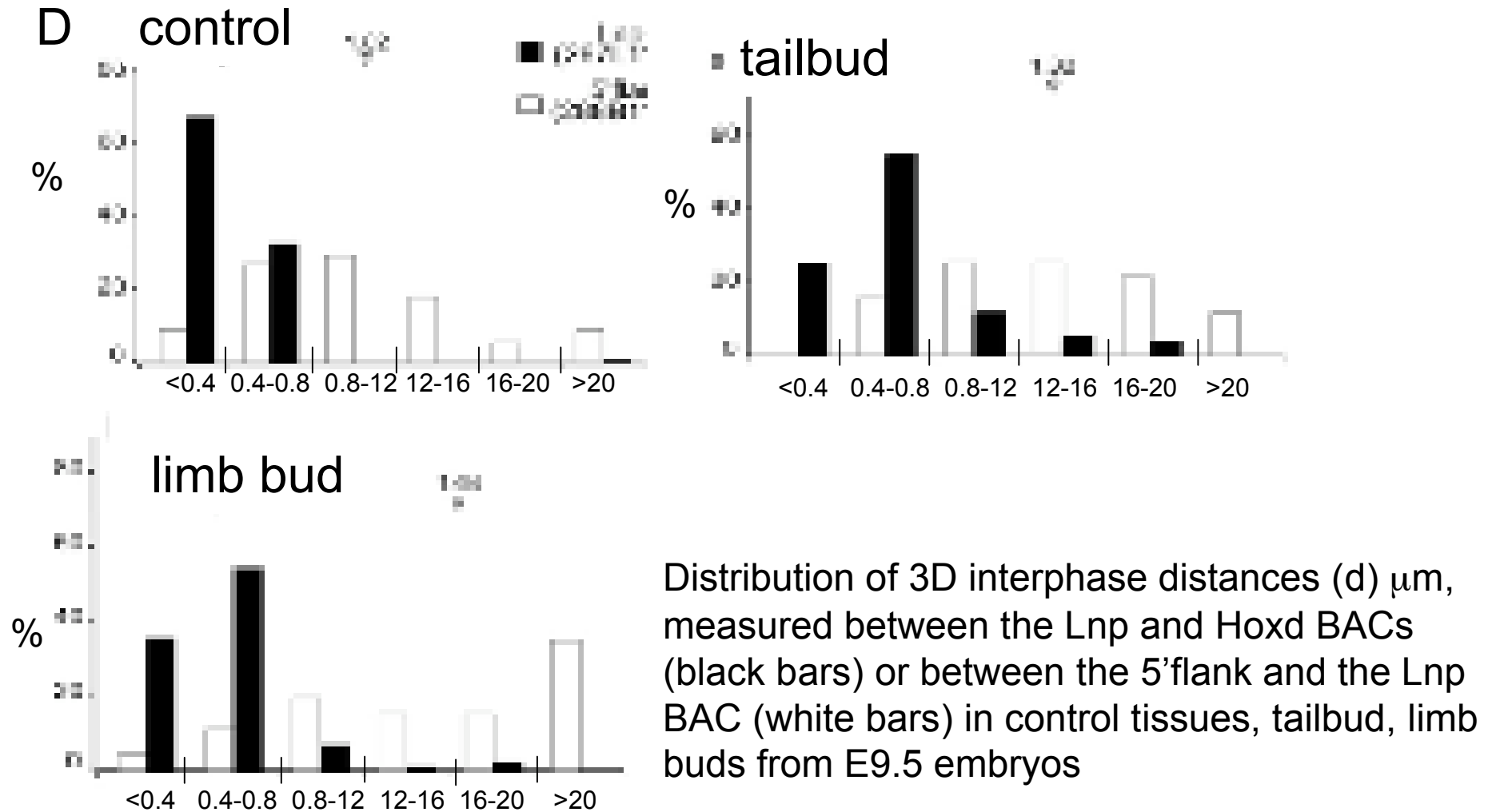
Figura posizioni medie

5' flank 
Lnp 
Hoxd 



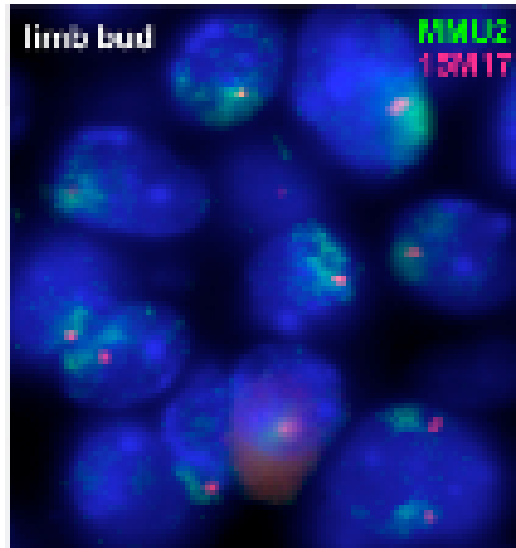
Mean position + sem (μm), relative to the edge of the MMU2 CT (chrms territory) for the 5' flank (blue), Lnp (yellow), Hoxd (green) BACs (bacterial artificial chromosome)

Fig nuclear reorganisation

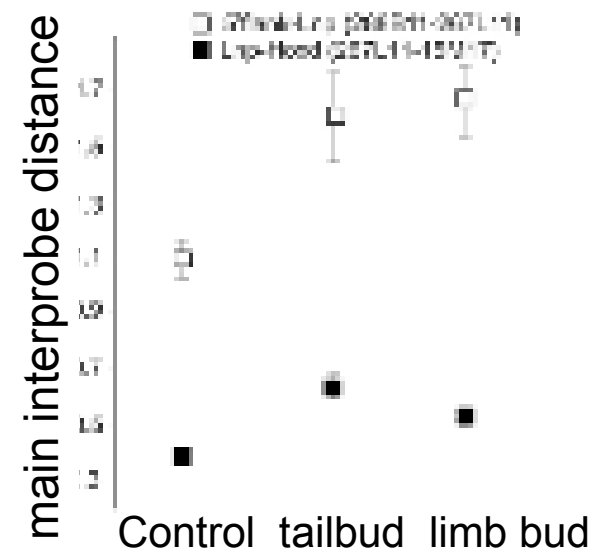


Nuclear reorganization

C



E



C) three dimensional DNA fish using the Hoxd probe (red) hybridised together with a MMU2 chromosome paint (green) on DAPI counterstained nuclei of a 4μm limb bud section from E9.5 embryos. Raw image before deconvolution.

E) means $\pm$ s.e.m. interphase separation μm measured between Lnp and Hoxd BACs (black square), between 5'flank and Lnp BAC (white square)