

lezione 11-12

martedì 17 Novembre 2009

corso di genomica

a.a. 2009/10

aula 6a ore 14.00-16.00

corso di laurea specialistica
magistrale Biotecnologia

lezione 11 Dicembre sequenziamento shot-gun metodo
pyrofosfato 454 e 480 Roche. Dr.Rodriguez

lezione 15 Dicembre Programmi informatici per confronti
genomici. Dr.P. Daddabbo

compartimentazione nucleare

il citoplasma è altamente compartimentato $\times \neq$ funzioni

anche il nucleo lo è (forse Golgi non ci pensò)

chromosome territories

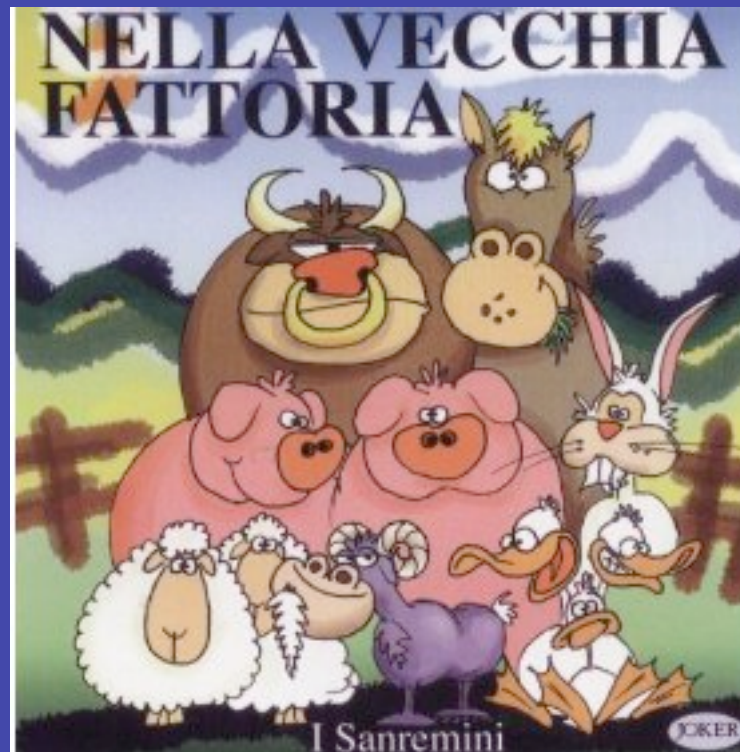
transcription factories

nella vecchia fattoria ja ja oh

transcription factories

factory = fabbrica

fattoria = farm



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 [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” in cellule 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

colocalizzazione di geni attivi

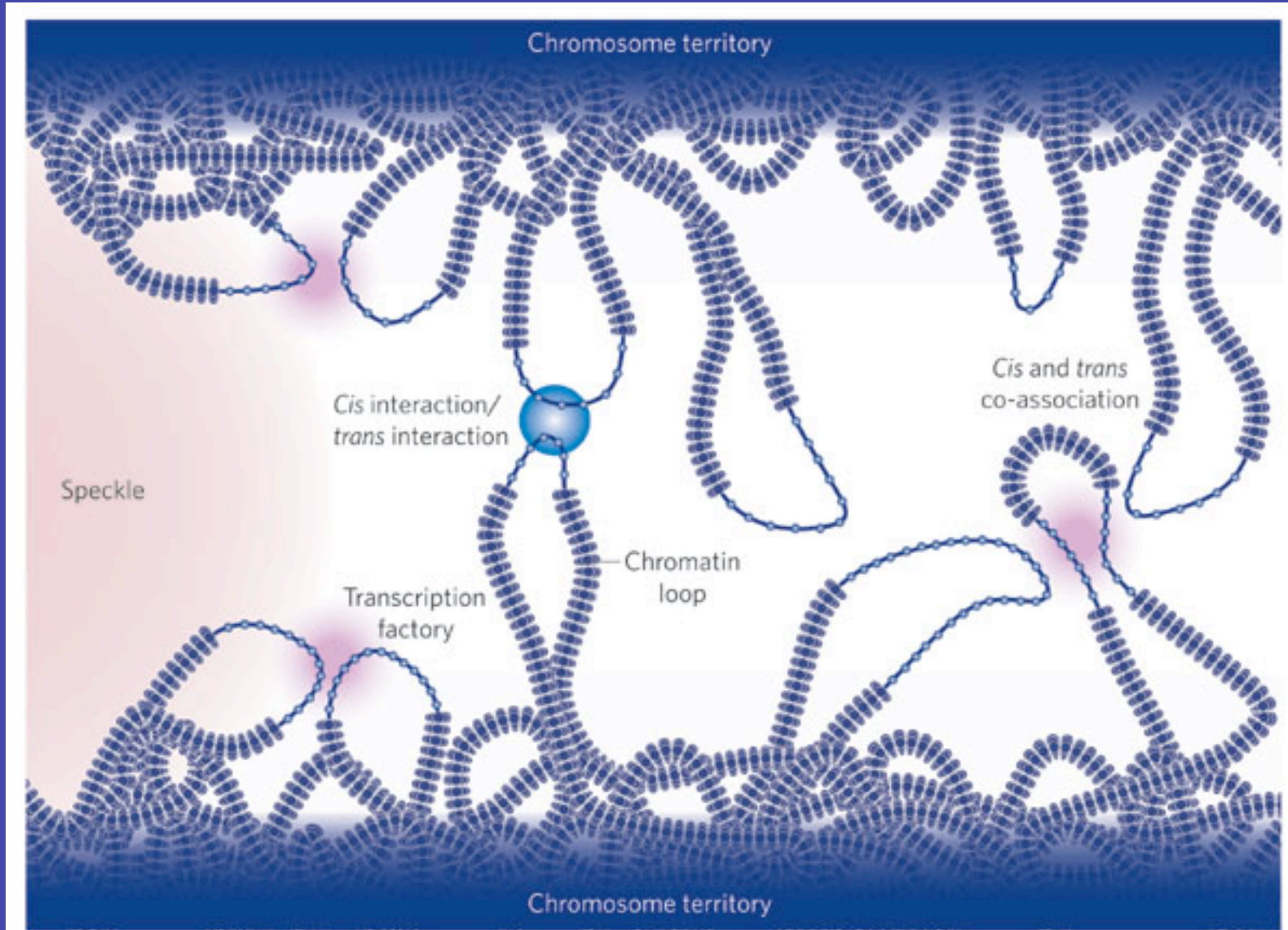
movimenti dei geni fuori dai territori cromosomici

come tradurreste “transcription factories”?
(fabbriche di trascrizione?)

normalmente nelle transcription factories (pol II attiva)

- il numero di fattorie è minore del n. di geni attivi
- i.e. molti geni condividono le stesse fattorie
- RNA FISH e 3C confermano presenza geni lontani
megabasi o su # crms nella stesse fattorie
- geni attivi a fase alterna stanno dentro o fuori (FISH),
- la mobilità è un fattore di controllo di attività genica

chromosome interactions I



colocalization of genes in nucleus

Active genes on decondensed chromatin loops that extend outside chromosome territories can colocalize both in cis and in trans at sites in the nucleus with local concentrations of Pol II (namely transcription factories; dark pink) and adjacent to splicing-factor-enriched speckles (pale pink). Interactions can also occur between regulatory elements and/or gene loci and lead to coregulation in trans (blue circle).

colocalizzazione di geni e RR

Hbb non espresso nel SNC in distretti con altri geni inespressi

paradigmatico 1 solo gene dei 1300 geni dei recet.
olfatt. espresso per ogni cellula nervosa

presenza di un enhancer in cis vista col 4C

analisi 4C su pool di cellule

analisi probabilistica, due possibilità (analisi su pool di cellule):

- a) interazioni diverse in cellule indipendenti
- b) interazioni multiple dinamiche (variabili) transienti

Hbb (non espresso nel SNC) come esca ha catturato geni
silenti recettori dell'olfatto in cis

recettori dell'olfatto hanno regolazione particolare

sistema opposto ad HLA (coespressi, codominanti)

1 cellula x 1 recettore con esclusione allelica (come TCR o Ig)

1 gene alla volta su ~ 1300 è espresso in singolo neurone

ogni gene per essere espresso necessita l'enhancer H

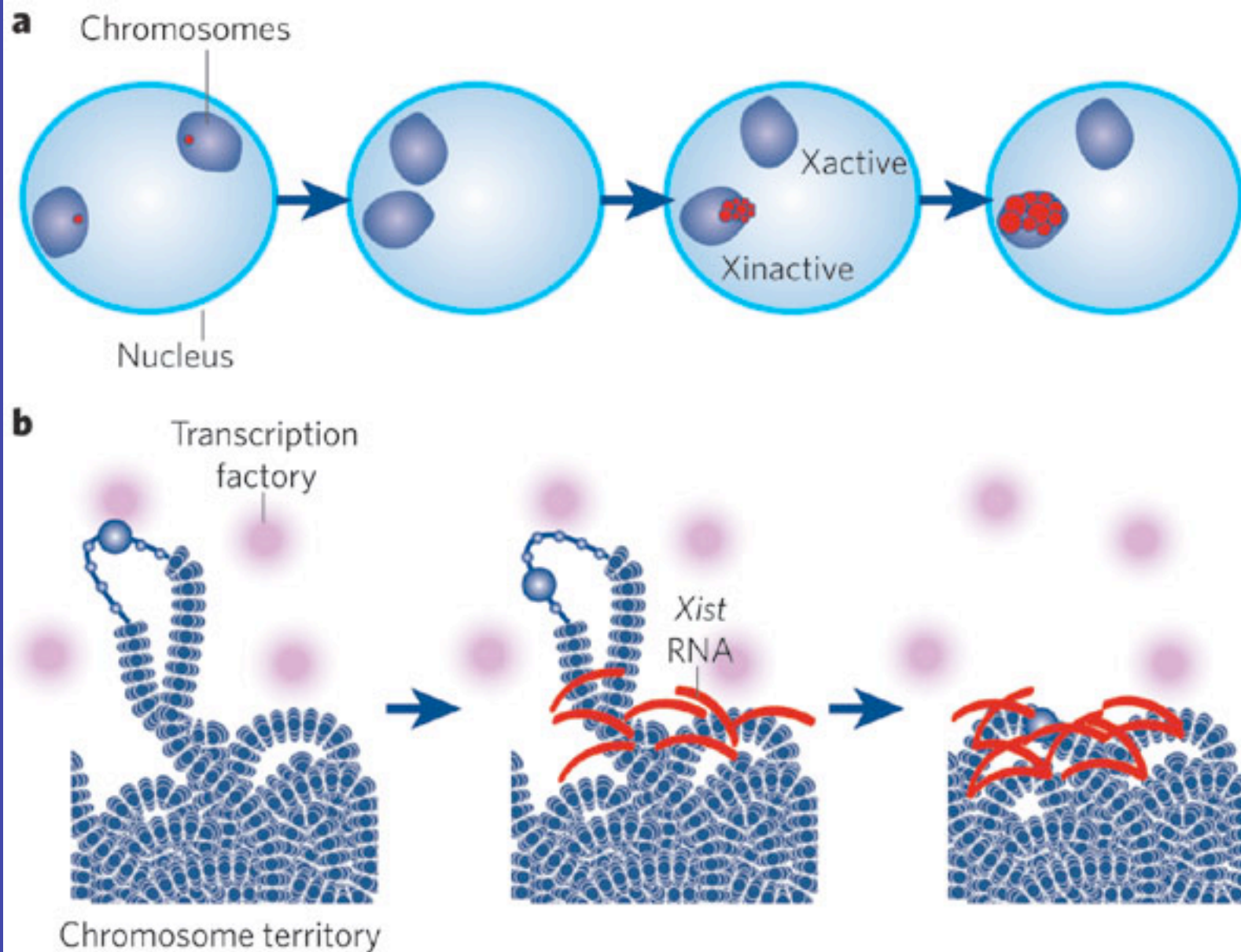
quanto influenza l'epigenetica

il sistema modello è l'inattivazione ed imprinting del crms X

colocalizzano transientemente,
i centri di inattivazione interagiscono durante differenziamento
delle cellule staminali femminili

il tempo di interazione corrisponde all'inizio della inattivazione di
quel cromosoma

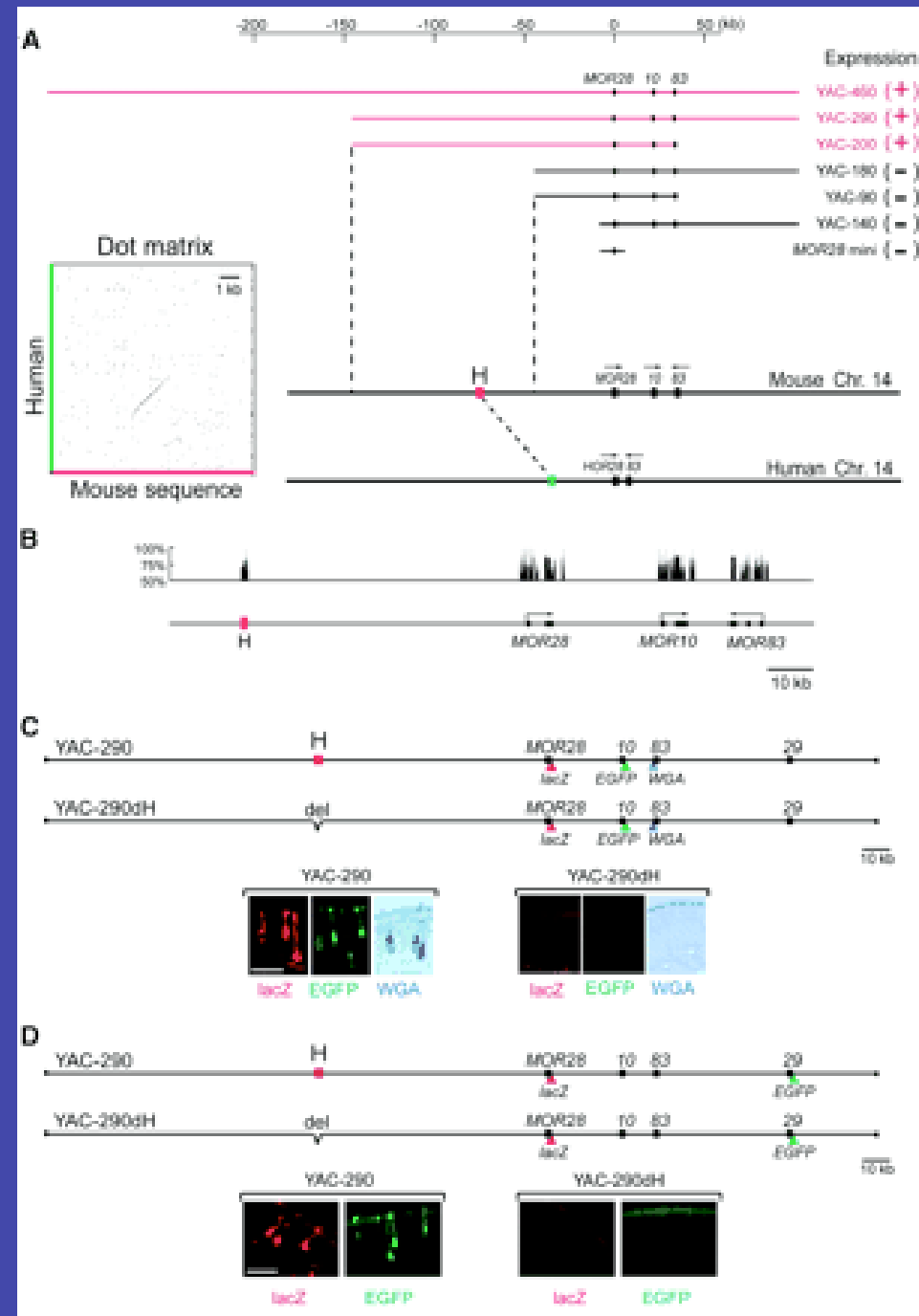
chromosome interactions Xist



X chromosome inactivation

Soon after female embryonic stem cells start to differentiate, the two X chromosomes (purple) come together in the nucleus, and the X-inactivation centres, which initiate X-chromosome inactivation, interact. These events occur concomitantly with the process of X-chromosome counting and choice and lead to upregulation of Xist transcription (red) from the future inactive X chromosome (Xinactive). b, The coating of the inactive X chromosome by Xist RNA molecules excludes Pol II and the transcriptional machinery (pink) from the inactive X-chromosome territory. Genes initially located outside the domain (purple circles) coated by Xist RNA are retracted back inside the Xist compartment as they become silenced through a mechanism dependent on the A repeats of Xist RNA.

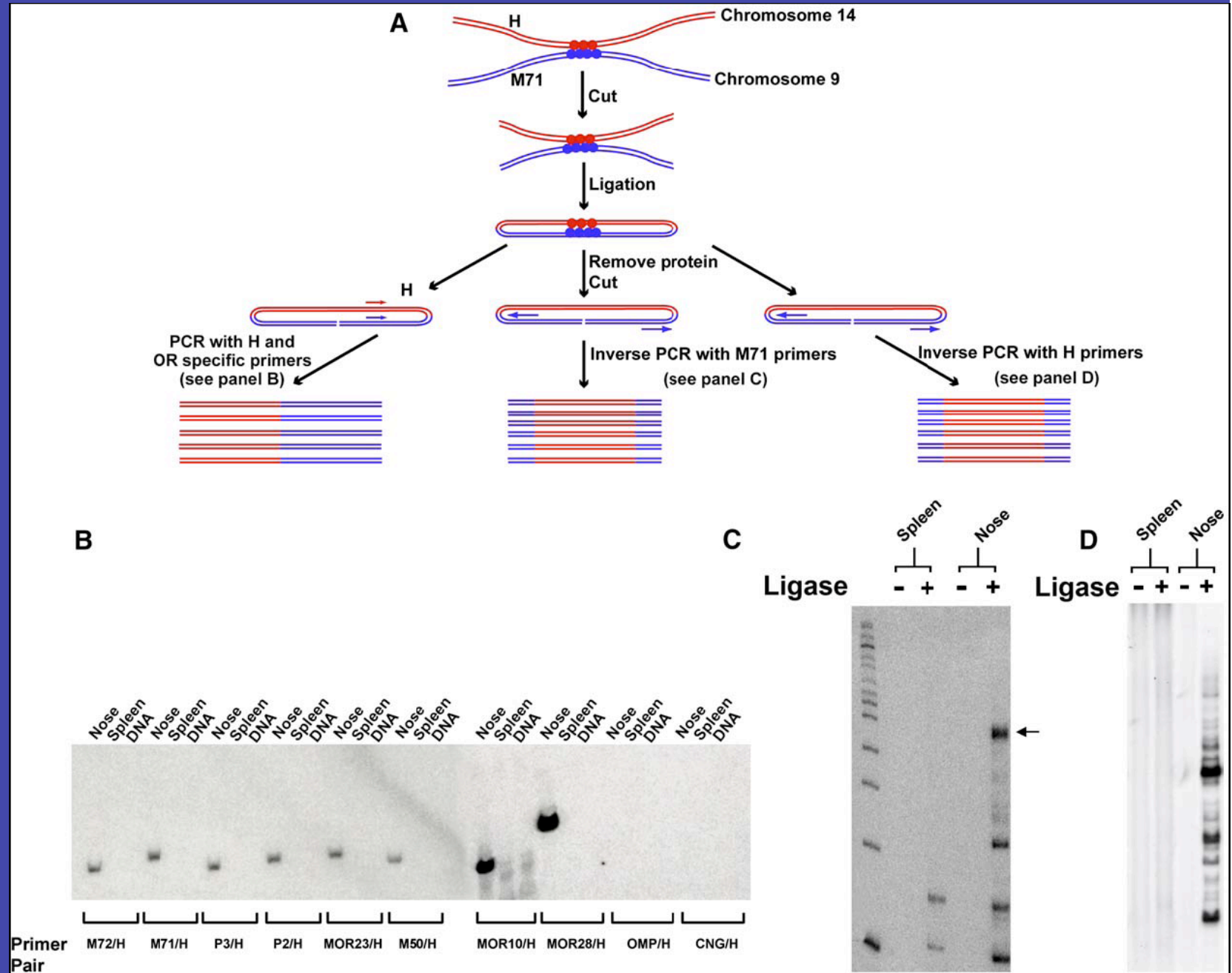
conserved olfactory enhancer region



legend to figure olfactory enhancer

The homology (H) region that activates the MOR28 cluster. (A) Structures of seven transgenic constructs are compared. MOR28 in each construct is shown as expressed (+) or not (–). Nucleotide sequences were compared between mouse and human for the upstream regulatory region. A 2-kb homology (H) was identified (dot matrix) 75 kb upstream of MOR28. (B) Percent identity plot for the mouse MOR28 cluster against the human homolog. Repetitive sequences were masked with RepeatMasker. Similarities higher than 50% are shown as vertical bars. (C) Deletion of the H region abolishes the expression of the MOR28 cluster. The H region was deleted from the YAC-290 construct, generating YAC-290dH. Three zone 4–specific OR genes in the constructs were separately tagged. OE sections of the transgenic mice were examined for the expression of the transgenes by fluorescence (EGFP) or by immunostaining (lacZ and WGA). The same results were obtained from six transgenic lines for YAC-290 and five lines for YAC-290dH. (D) Expression of the zone 1–specific MOR29. OE sections were analyzed by fluorescence (EGFP for MOR29) and by immunostaining (lacZ for MOR28). The same results were obtained from four transgenic lines for YAC-290 and five lines for YAC-290dH. Scale bars, 20 μm .

H enhancer olfactor 3C



legend to 3D olfactor H enhancer

Figure 1. Chromosome Conformation Capture Reveals the Association of the H Enhancer with Olfactory Receptor Genes

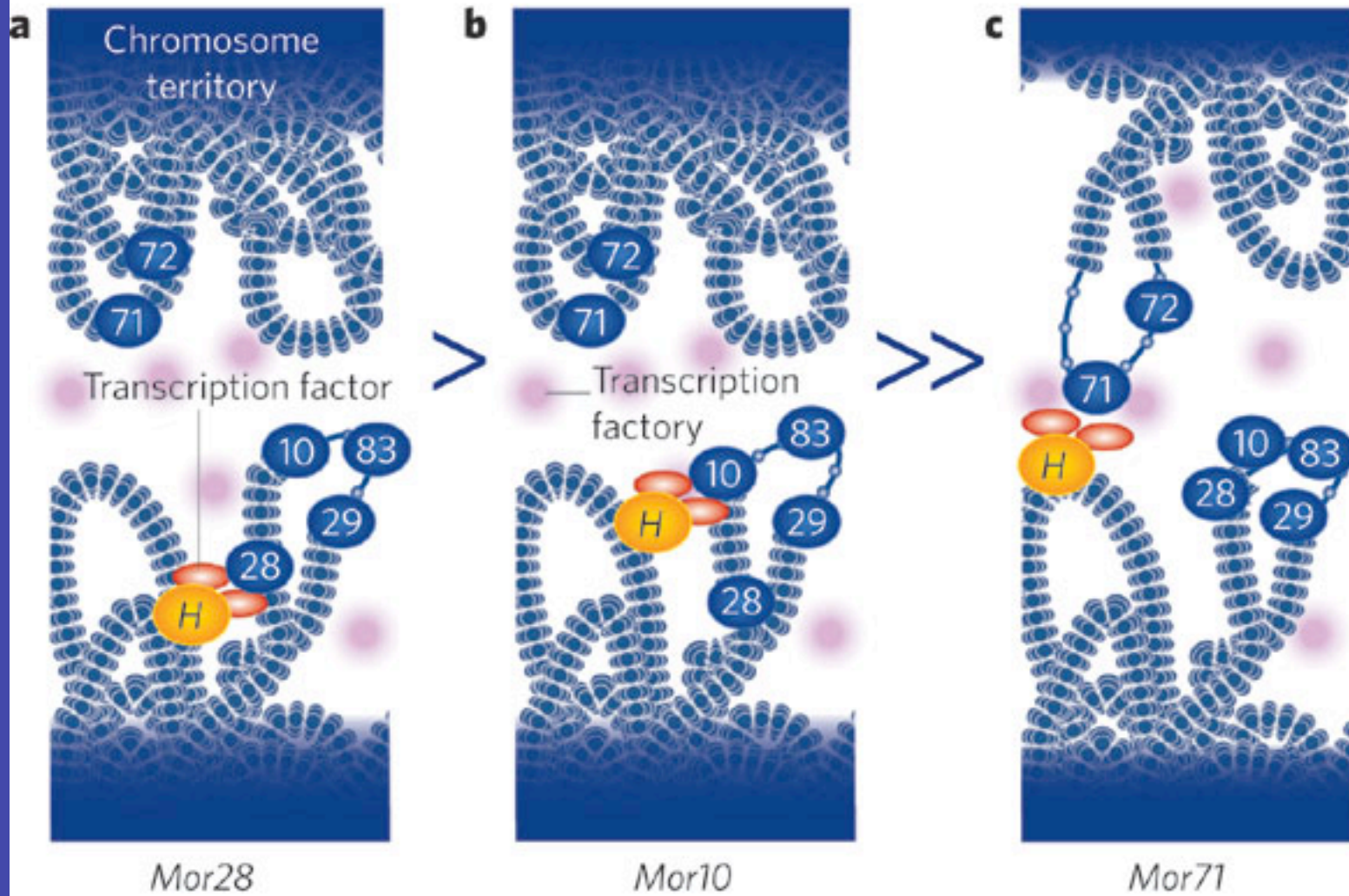
(A) A schematic representation of three different 3C strategies to reveal the association of the H element with OR promoters. On the left, a PCR primer specific to H is used with the second primer specific to OR gene promoters. In the center, a pair of inverse primers specific to M71 is used to isolate sequences associated with the M71 gene. On the right, a pair of inverse PCR primers specific to H is used to detect DNA sequences that colocalize with the H element.

(B) Acrylamide gel electrophoresis of the PCR products using a primer specific to H together with primers specific to 50 flanking DNA from genes that are expressed in the olfactory epithelium. PCR was performed on chromatin from olfactory epithelium (Nose), spleen (Spleen), or naked DNA from olfactory epithelium (DNA).

(C) Agarose gel electrophoresis of the PCR products using inverse primers specific to the M71 promoter following 3C. Either spleen or olfactory epithelium chromatin were subjected to 3C in the presence or absence of ligase. The arrow points to the 2.3 kb band that was amplified from nose chromatin and corresponds to the ligation product of H with M71 sequences.

(D) Acrylamide gel electrophoresis of PCR products using inverse primers specific to the H enhancer. 3C was performed in the presence or absence of ligase and subjected to PCR with inverse primers specific to the H element. The products were then cloned and analyzed by DNA sequencing.

chromosome interactions II



olfactory receptors

A quarter of 4C products captured in the nuclei of sensory neurons with the olfactory-receptor H enhancer (orange) are olfactory-receptor genes themselves (numbered circles)³⁶. More than half of these are the promoter of Mor28, which is adjacent to H on mouse chromosome 14 (a). The next most frequent interaction found was with the promoter of Mor10, which is also in cis with H (b). Much less frequent were interactions with olfactory-receptor genes (for example, Mor71) located on other mouse chromosomes, and so in trans with H (c). Each olfactory neuron expresses only one allele of one olfactory-receptor gene, and the frequencies of 4C interactions with H reflect the relative frequencies of expression of different olfactory-receptor genes in the population of sensory neurons. The H enhancer might function by recruiting transcription factors (red) and high concentrations of Pol II (present in transcription factories) to the expressed gene.

75% olfactory genes + H in cis (4C)

25% geni upstream in trans

75% dei geni catturati erano in cis

rispecchia la frequenza con cui H enhancer lega i geni dell'olfatto (gli si colloca vicino)

analisi FISH: colocalizzazione nel 30% dei casi tra H ed olfactory gene,
l'altro allele H è metilato in CpA

85% mRNA olfactory gene + FISH H e olf. gene colocalizzano durante il pulse di trascrizione, nella fase di riposo si separerebbero

interazioni transienti

durante i pulse di attività ci sono avvicinamenti transienti

- fenomeno associato a regolazione coordinata

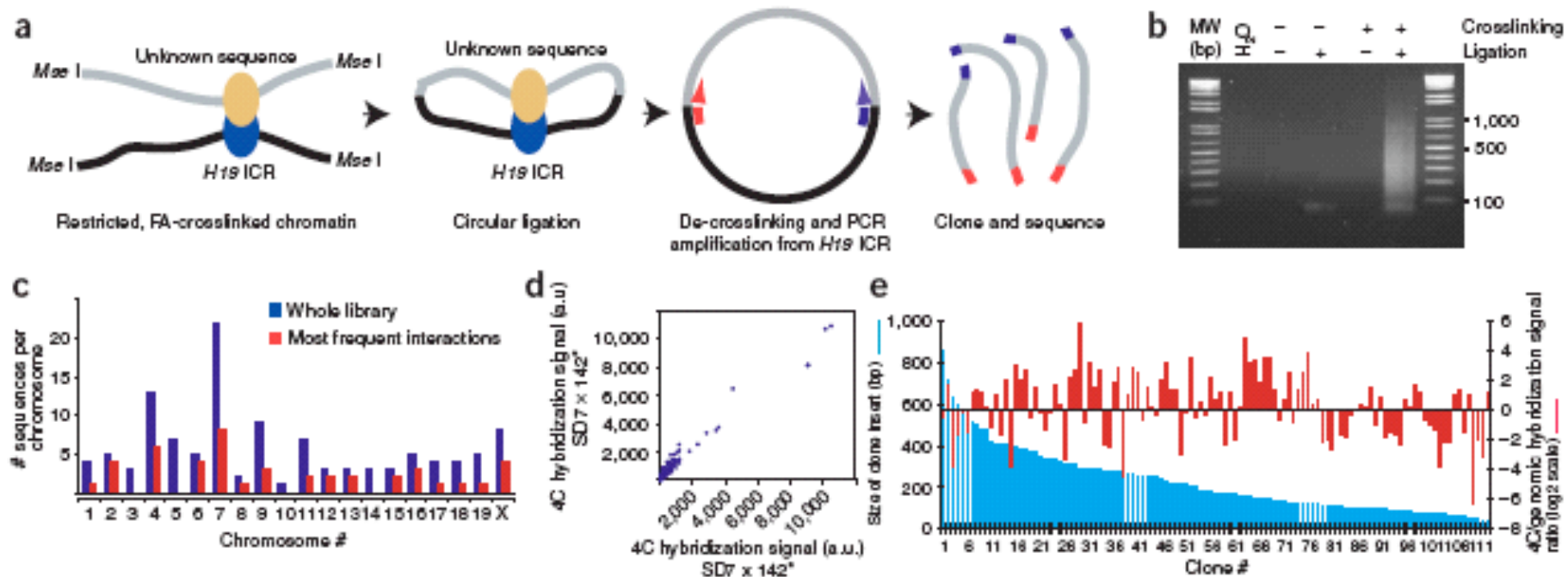
- interferon γ + LCR del T-helper 2 cytokine locus colocalizza con 3C e FISH assay, (espressione minima)

- dopo attivazione - differenziamento in Th1 o Th2
delocalizzazione e alta trascrizione da posizione
intercromosoma passa a intracromosoma

- resta da determinare l'analisi con il 4 C per vedere le
frequenze dei due eventi

effetti dell'epigenetica

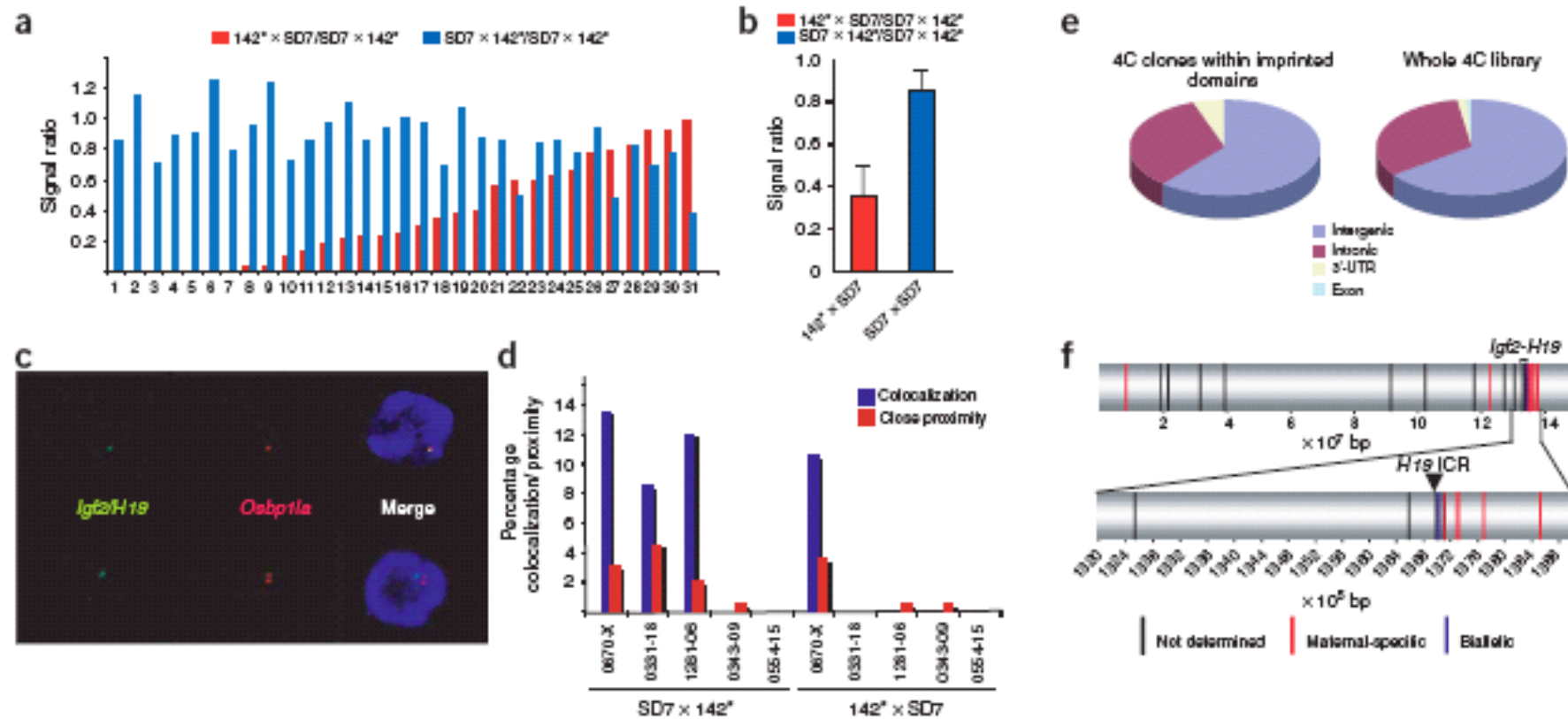
Topo, fegato neonatale, studi 4C,
Imprinting control region (ICR) del gene H19



didascalica 4C H19

Figure 1 The circular chromosomal conformation capture (4C) assay of intra- and interchromosomal interactions. (a) Schematic representation of the 4C assay. The red and blue arrows and rectangles indicate the nested primers within the H19 ICR, which is common to all 4C products. The gray line indicates any sequence interacting with the H19 ICR and captured by the 4C approach. (b) Nested polymerase chain reaction (PCR) amplification of 4C samples. After amplification, only the crosslinked, ligated chromatin sample generated amplified sequences of a wide range of sizes. (c) Chromosomal annotation of 4C library sequences. The entire 4C library (blue bars) is distributed on all autosomes and the X chromosomes, indicating that H19 ICR can interact both in cis and in trans. Red bars show the most frequent interactions as determined by the semiquantitative hybridization to microarrays. (d) Scatter plot analysis of hybridization signals to dedicated microarrays covering the entire 4C library. The probes compared were two independently pooled 4C samples (each containing three independent 4C samples of neonatal liver). (e) The 4C assay does not introduce clear inserted size bias. Blue bars show the size of each unique sequence from the 4C library (left axis). Red bars (right axis) show the ratio of the hybridization signal from d to the signal from the genomic DNA input.

H19 4C screening



■ colocalization
■ close proximity

didascalia fig screening H19 4C

Figure 2 Epigenetic control of intra- and interchromosomal networks. (a) Semiquantitative microarray hybridization analysis of the most frequently occurring interactions. Blue bars compare the signal ratios between two independent pools (three independent samples in each pool) of 4C samples derived from neonatal liver of SD7 $\times$ 142* crosses. Red bars compare the signal ratios between two pools (three independent samples in each pool) of 4C samples from 142* $\times$ SD7 versus SD7 $\times$ 142* crosses. (b) Summary of the data in a. (c) Serial confocal images of a neonatal nucleus of an SD7 $\times$ 142* cross showing superimposed hybridization signals to the Igf2-H19 domain (green) and Osbpl1a on chromosome 18 (red). Counterstain was Topro 3. (See Supplementary Figure 2 for light microscopy analysis of hybridization signals.) (d) Frequency of hybridization signals from other chromosomal regions either overlapping or in close physical proximity of the Igf2-H19 domain. Clone 0331-18 is situated within Osbpl1a, and clone 1281-06 is within Abcg2 (Supplementary Table 1). (e) Relative representation of various sequence categories of the entire 4C library and the subset of sequences belonging to imprinted domains. (f) Schematic maps depicting 4C library sequences distributed on the entire chromosome 7 (based on build 35). The mono- or biallelic interactions are color-coded.

Imprinting, epigenetica e compartimenti genomici

esempio per le interazioni nucleari transienti

- topo, fegato neonatale, localizzazione di H19:

interazioni trans e cis

sequenze multiple di molti cromosomi catturati negli stessi cloni 4C dopo ligasi intramolecolare

dimostrazione di associazioni multiple simultanee nello stesso nucleo

topi con H19 distinguibili (paterno da materno) 75% di derivazione materna

conclusione: H19 ICR metilato su allele paterno è meno attivo

le regioni che hanno avuto imprinting non sono metilate

Fraser P. Bickmore W. Nature 2007, 447: 413-417