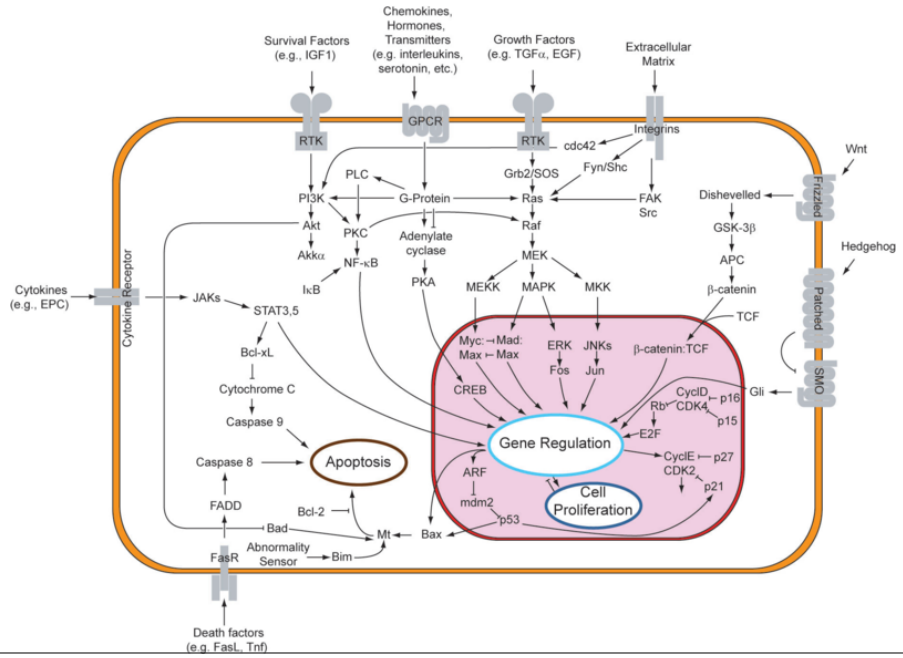
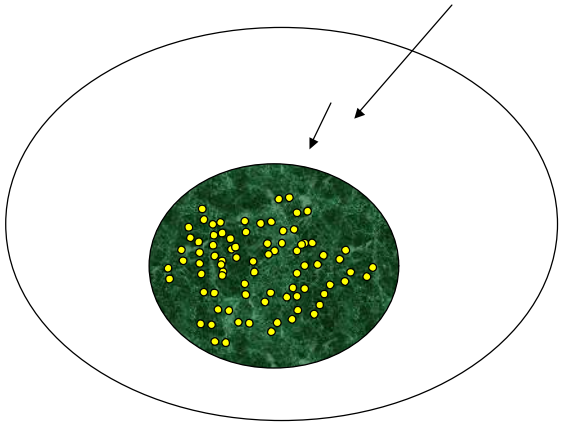
Signal transduction in the nucleus and chromosome organization



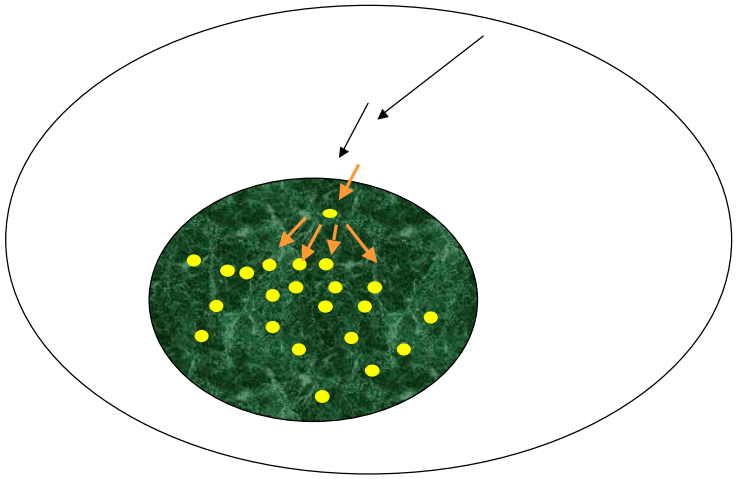
Understanding genome regulation

Several open questions

- How is a signal propagated ?
- How does a signal reach target genes ?
- Is the genome spatially “organized” in the nucleus in order to expose activable genes first?



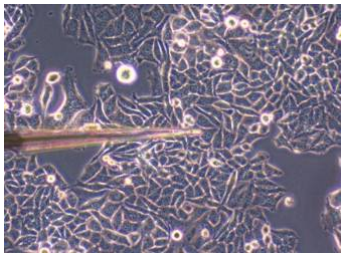
All targets are reached at the same time through equivalent physical pathways

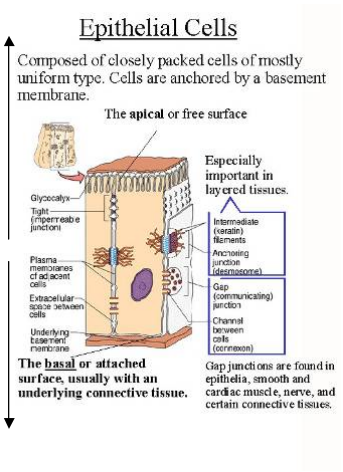


Targets are reached hierarchically through consecutive physical pathways

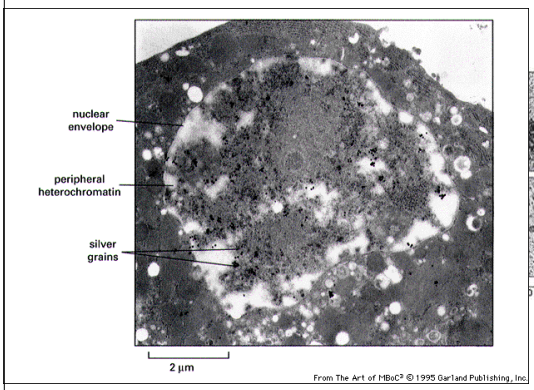
How the same transduction pathway may activate or repress different genes in different cell types ?

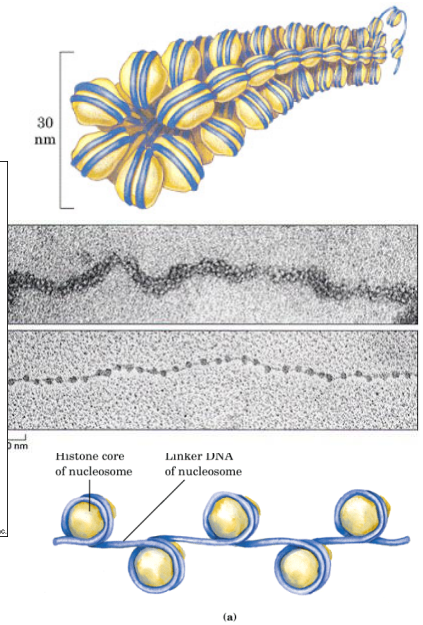
Cell culture model systems *versus* real life



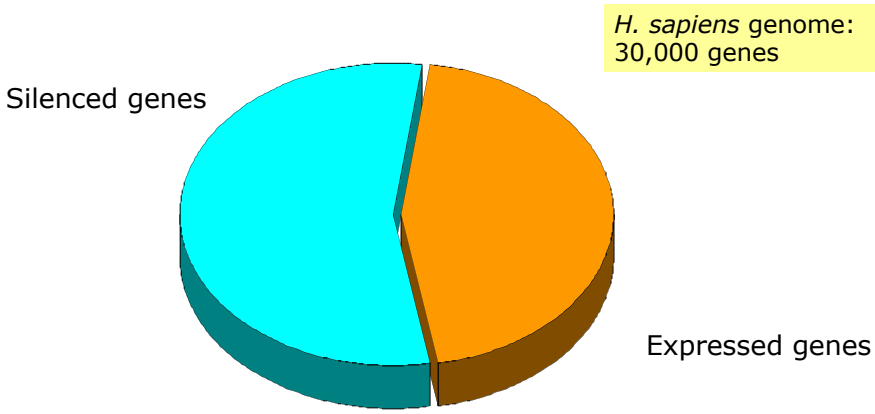


First studies on chromatin structure by E.M. in middle '70. The obtained images made the authors hypotesizing that the compacted form was typical of nonexpressed genes.





In any cell type, a large part of the genes are kept inactive (not expressed) by gene silencing



genes

- Always active or activable — Housekeepers, essential, CpG-rich nonTATA promoters, multiple TSS, unmethylated
  - euchromatic
- "On-off" type — Strictly dependent on corepressor-coactivator exachange, usually TATA-dep. promoters, one sharp TSS, cell-type specific
  - Euchromatic-like ⇄ Heterochromatic-like
- Silenced — Cell-type specific, fully heterochromatic, condensed, hypermethylated
  - heterochromatic

La cromatina in nuclei di cellule di diversi tipi e tessuti è organizzata in modo visibilmente diverso e tipico.

The nuclear envelope and transcriptional control

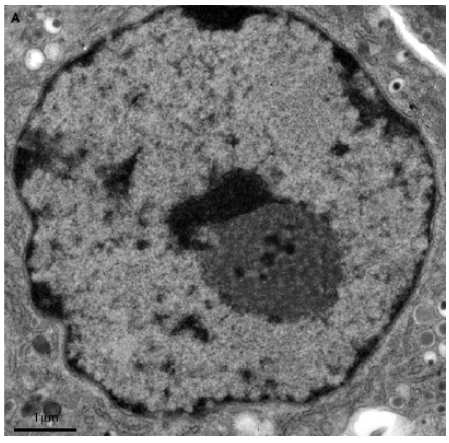
2007

Asifa Akhtar and Susan M. Gasser‡*

Abstract | Cells have evolved sophisticated multi-protein complexes that can regulate gene activity at various steps of the transcription process. Recent advances highlight the role of nuclear positioning in the control of gene expression and have put nuclear envelope components at centre stage. On the inner face of the nuclear envelope, active genes localize to nuclear-pore structures whereas silent chromatin localizes to non-pore sites. Nuclear-pore components seem to not only recruit the RNA-processing and RNA-export machinery, but contribute a level of regulation that might enhance gene expression in a heritable manner.

NATURE REVIEWS | GENETICS VOLUME 8 | JULY 2007 | 507

A



B

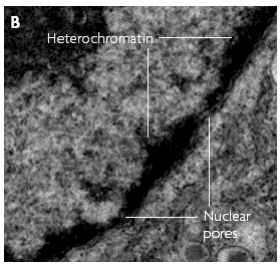


Figure 1 | **Heterochromatin in mammalian and yeast cells is distinct from nuclear pores. A** | An electron micrograph of the mammalian liver nucleus (with an enlarged section shown in part **B**), showing dense-staining heterochromatin located around the nucleolus and against the nuclear envelope. Nuclear pores open onto lighterstaining open chromatin.

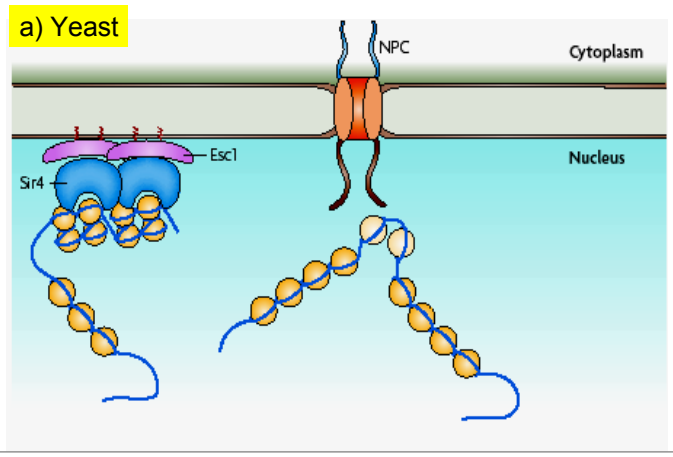
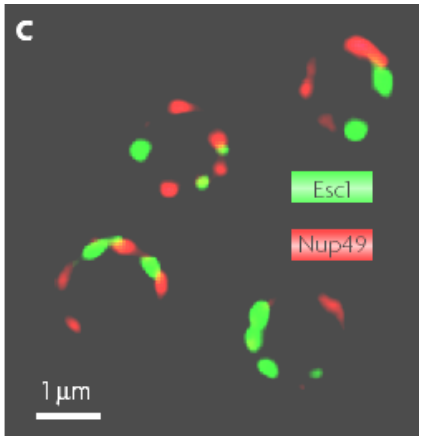


Figure 2 | **The nuclear periphery in metazoans and yeast.** In eukaryotic cells, the nuclear compartment is separated from the cytoplasm by the inner and outer nuclear membranes. This membrane bilayer is perforated by nuclear pores, which are constituted by a large multiprotein complex (the nuclear pore complex (NPC)) that is composed of about 30 proteins. This nuclear membrane, together with the pores, is commonly referred as the 'nuclear envelope' (NE).
a | In **yeast** nuclei, envelope-associated proteins such as Esc1 (enhancer of silent chromatin 1) are present in foci at the periphery; however, they do not coincide with the pores. Esc1 binds Sir4 (silent information regulator 4), which is an integral component of repressed heterochromatin in yeast.



C | In budding yeast, heterochromatin binds the nuclear envelope through Esc1 (**enhancer of silent chromatin 1**; labelled green), which forms distinct foci alternating with nuclear pores (visualized in red through labelling of Nup49 (**nucleoporin 49**).

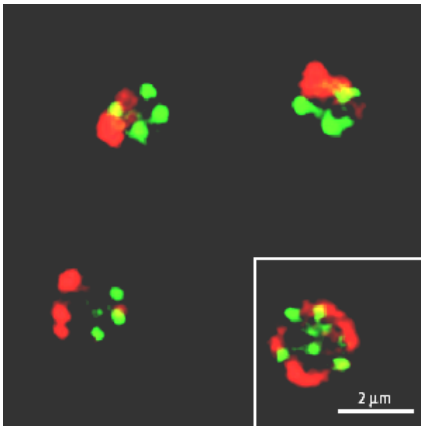
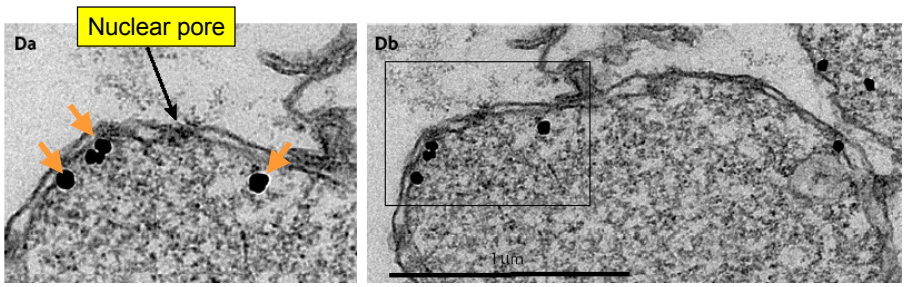


Figure 3 | **Telomere position through Sir4–Esc1 is independent of nuclear-pore positioning.** Mutation of **nup133** shifts nuclear the pore proteins Nup116 and Nup96 to one side of the nucleus. This does not affect the position of silent telomeres (**Rap1**)



D | An electron micrograph showing **Esc1** at non-pore sites along the yeast inner nuclear envelope. An arrow indicates the nuclear pore, and black dots represent the labelling of **Myc-epitope-tagged Esc1** using fluoronanogold Alexa488 anti-mouse antibody18.

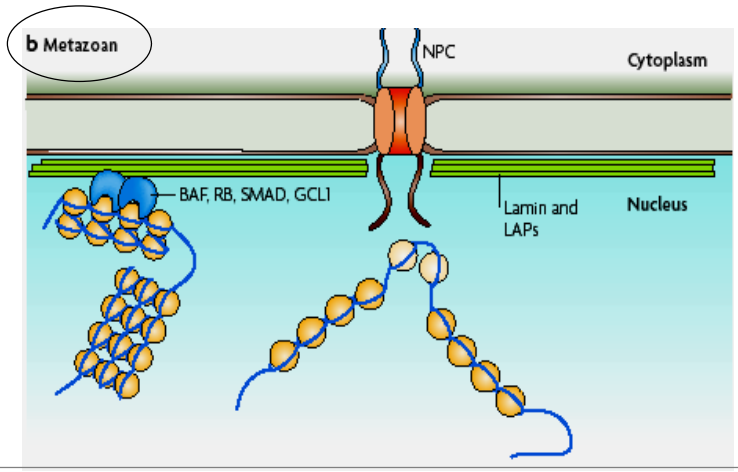
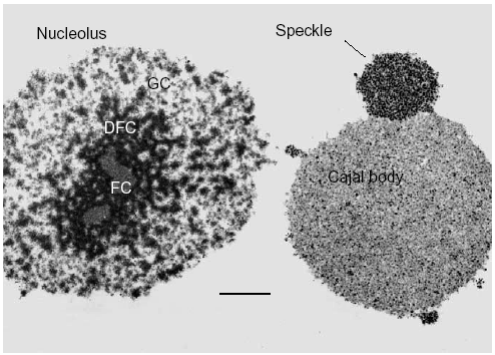
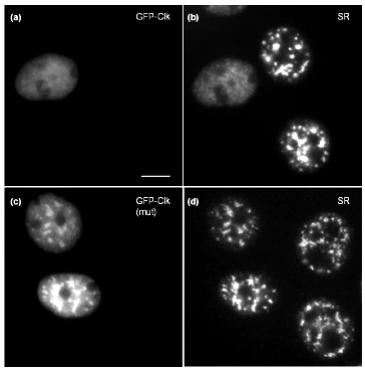


Figure 2 | **The nuclear periphery in metazoans and yeast.** In eukaryotic cells, the nuclear compartment is separated from the cytoplasm by the inner and outer nuclear membranes. This membrane bilayer is perforated by nuclear pores, which are constituted by a large multiprotein complex (the nuclear pore complex (NPC)) that is composed of about 30 proteins. This nuclear membrane, together with the pores, is commonly referred as the 'nuclear envelope' (NE).
b | In metazoan nuclei, the nuclear envelope is underlaid by a continuous meshwork of lamins and lamin-associated proteins (LAPs), which preferentially associate with inactive chromatin regions. Increasing evidence implicates interactions of chromatin with various nuclear-envelope components in gene repression as well as gene activation. BAF, barrier to autointegration factor; GCL1, germ-cell-less homologue; RB, retinoblastoma 1.

Nuclear substructures



Nucleolus, Cajal bodies and speckles



Phosphorylation redistribute splicing factors

Handwerger 2005, TRENDS in Cell Biology, 16:19-26.

Conclusion

- The nuclear space is highly organized
- Constitutive heterochromatin is placed outside roads going to pores
- Splicing factors occupy discrete speckles in the nucleus

Open Access

Research

Tissue-specific spatial organization of genomes

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Genome Biology 2004, 5:R44

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2004/5/7/R44

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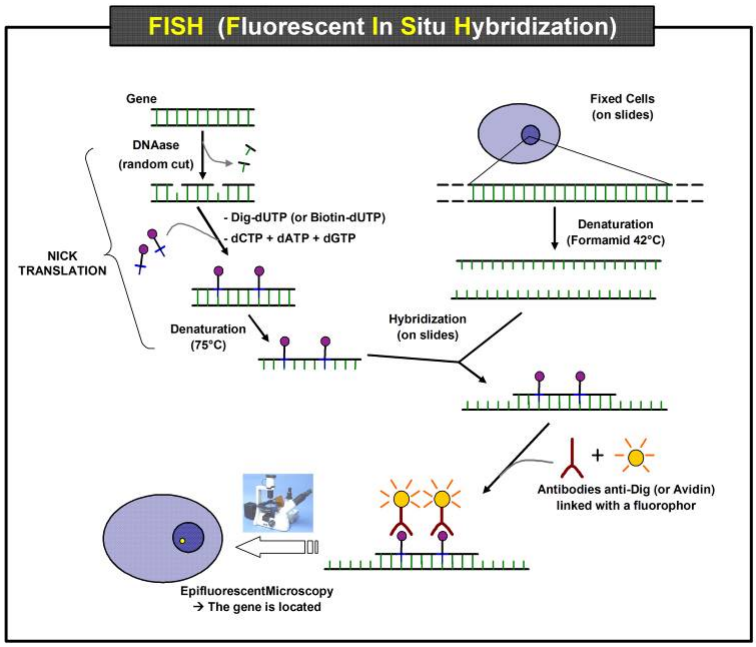
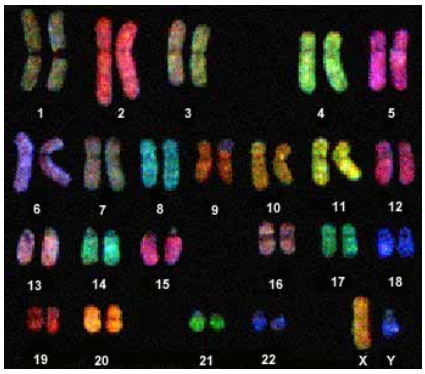
Revised: 24 May 2004
Accepted: 25 May 2004

This paper is thoroughly discussed in the next slides, students are expected to download and read it



Chromosome painting



metafasici

Conclusions:

- Interphase chromosomes occupy discrete “territories” within the nucleus
- Position of interphase chromosomes is cell-specific
- Relative positioning is also cell type-specific

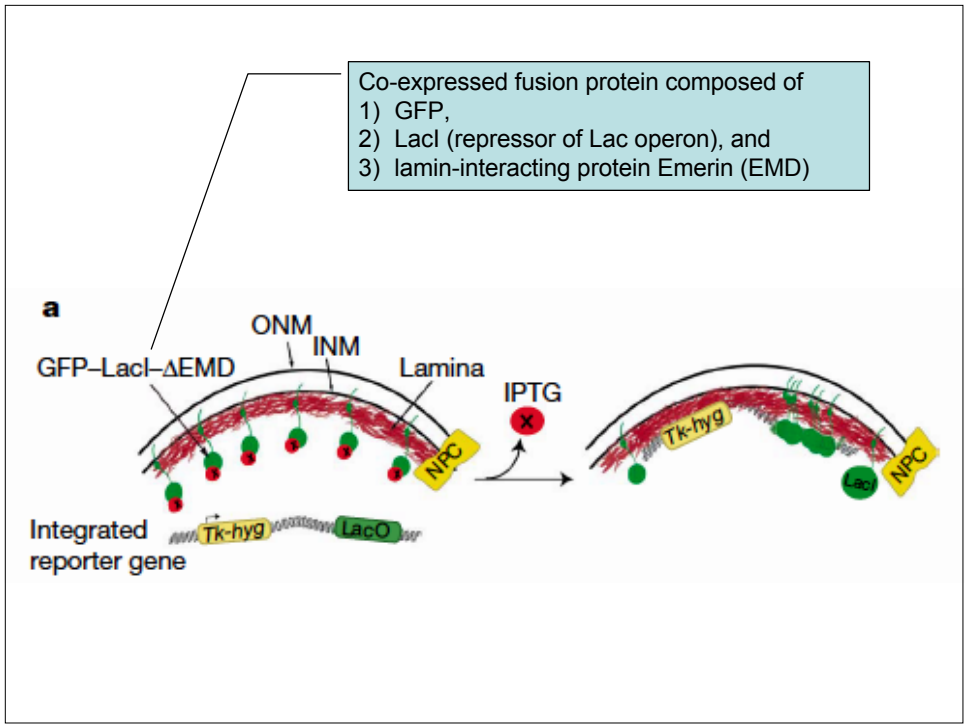
Vol 452|13 March 2008|doi:10.1038/nature06727 nature

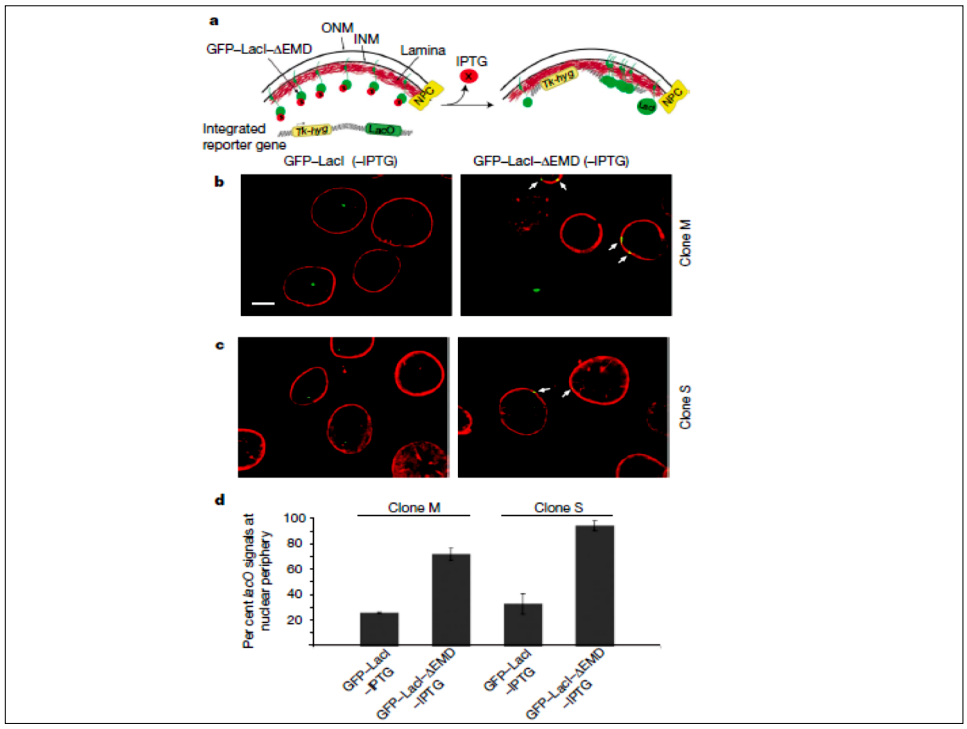
LETTERS

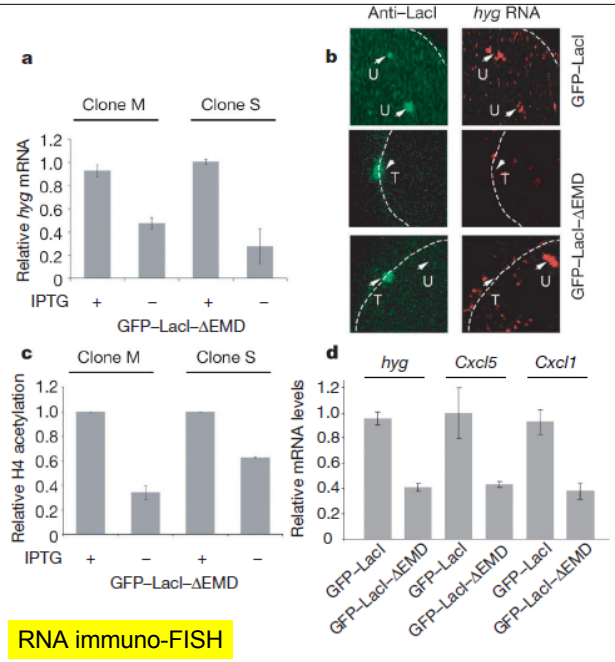
Transcriptional repression mediated by repositioning of genes to the nuclear lamina

K. L. Reddy[1,2], J. M. Zullo[1,2], E. Bertolino[2] & H. Singh[1,2]

Does sub-laminar positioning lead to repression of genes
or
are repressed genes repositioned to nuclear periphery
?


Co-expressed fusion protein composed of
1) GFP,
2) LacI (repressor of Lac operon), and
3) lamin-interacting protein Emerin (EMD)
a
ONM
INM
GFP–LacI–ΔEMD
Lamina
IPTG
NPC
Tk-hyg
LacI
Integrated
reporter gene
LacO


a
ONM
INM
GFP–LacI–ΔEMD
Lamina
IPTG
NPC
Tk-hyg
Integrated
reporter gene
LacO
GFP–LacI (–IPTG)
GFP–LacI–ΔEMD (–IPTG)
b
Clone M
c
Clone S
d
Clone M
Clone S
Per cent lacO signals at nuclear periphery
100
80
60
40
20
GFP–LacI –IPTG
GFP–LacI–ΔEMD –IPTG
GFP–LacI –IPTG
GFP–LacI–ΔEMD –IPTG



RNA immuno-FISH

RNA immunofish shows that the re-located (-IPTG) reporter gene is transcriptionally repressed (a).

Genes neighboring reporter integration site are also repressed (d)

Conclusions

relocation of genes to the inner nuclear membrane may lead to repression