The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

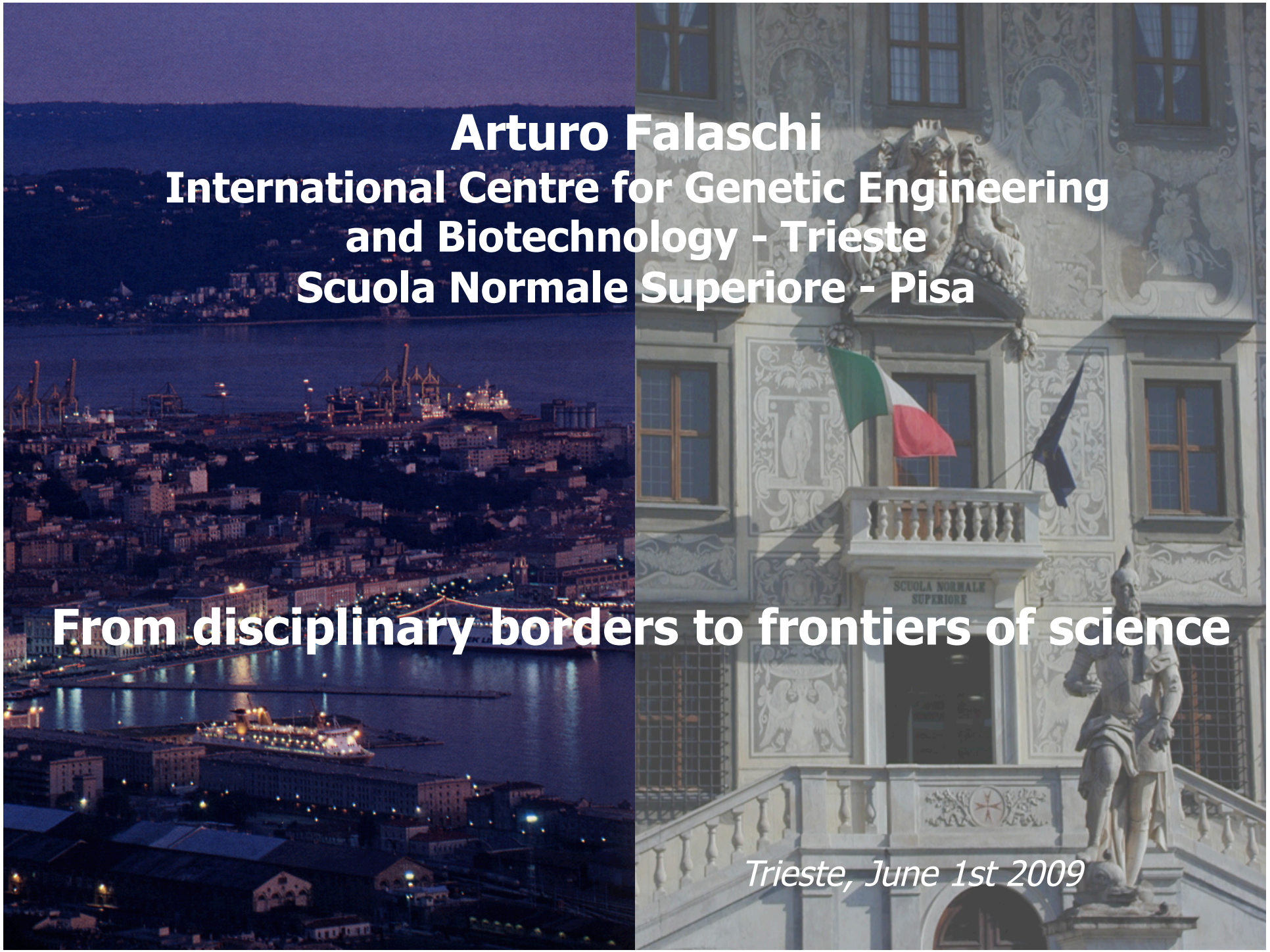
2038-18

Conference: From DNA-Inspired Physics to Physics-Inspired Biology

1 - 5 June 2009

From Disciplinary Borders to Frontiers of Science

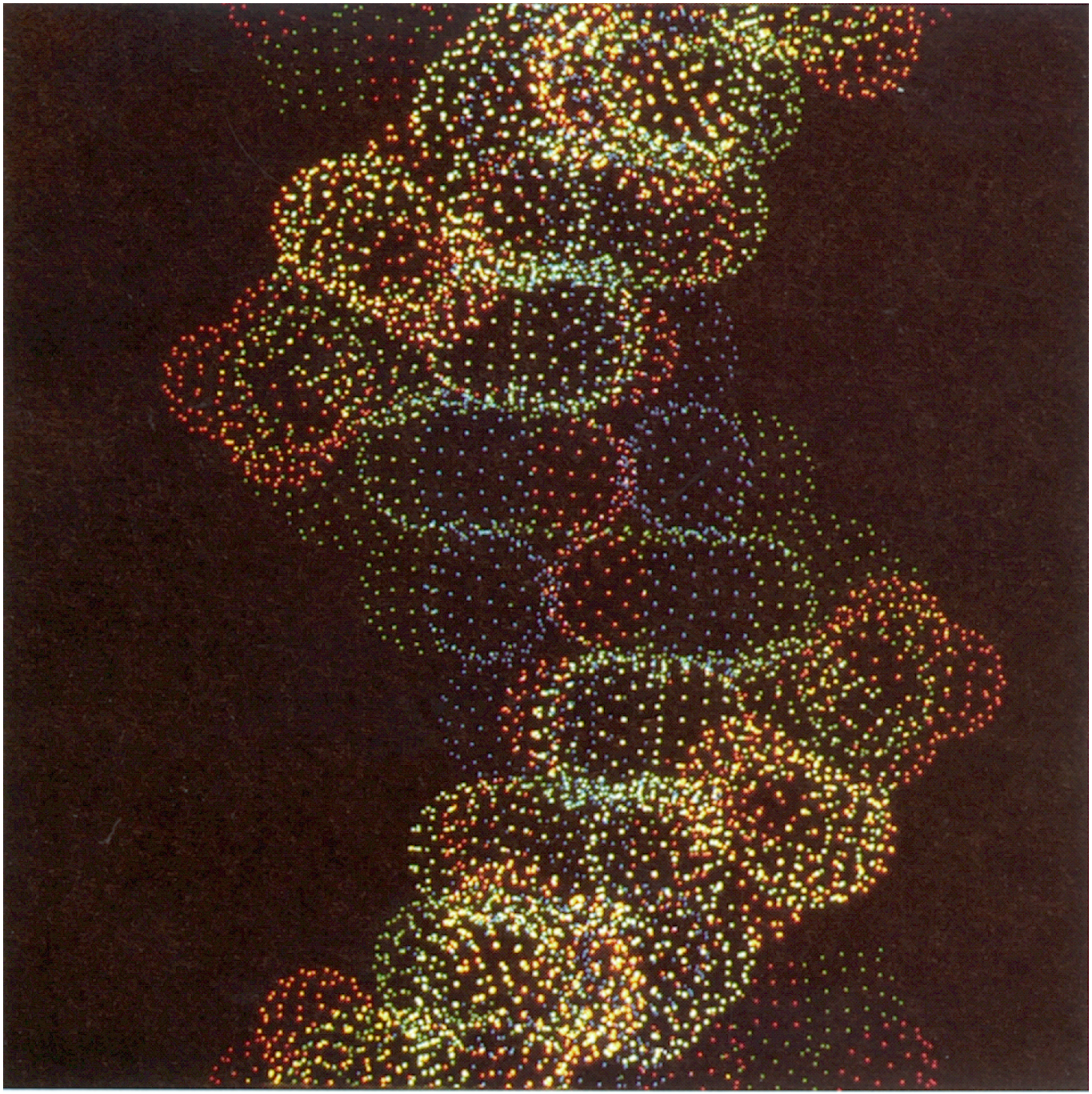
Arturo FALASCHI
ICGEB, Trieste
Italy
and
SNS, Pisa
Italy

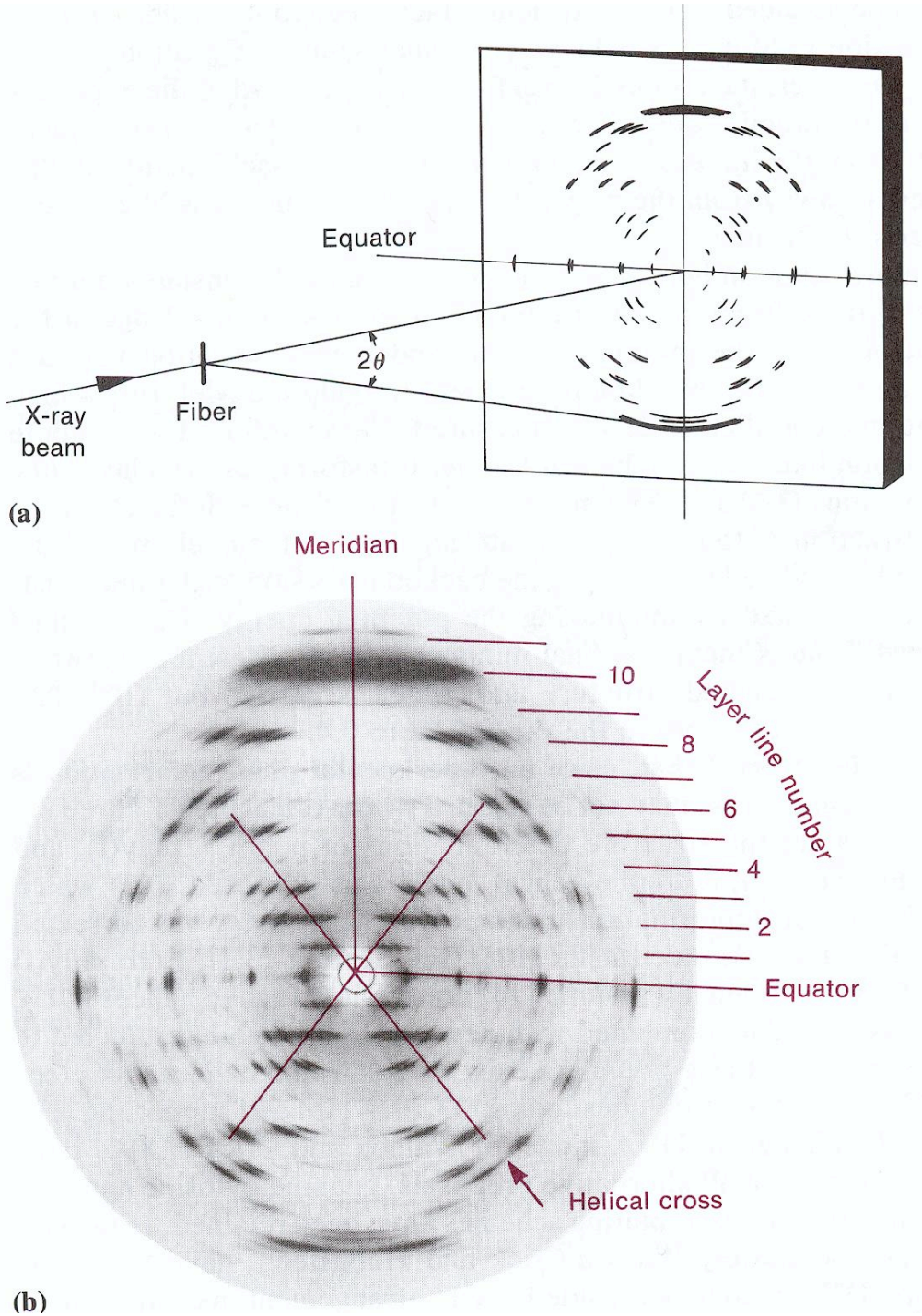


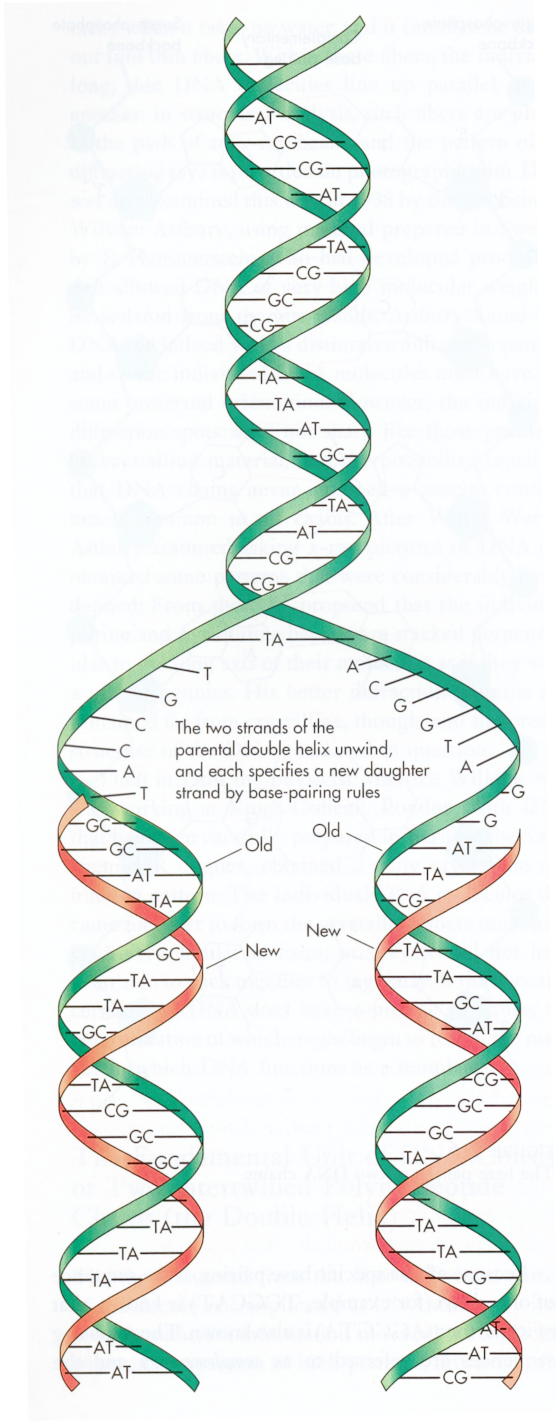
Arturo Falaschi
International Centre for Genetic Engineering and Biotechnology - Trieste
Scuola Normale Superiore - Pisa

From disciplinary borders to frontiers of science

Trieste, June 1st 2009




Equator
2θ
X-ray
beam
Fiber
(a)
Meridian
10
8
6
4
2
Layer line number
Equator
Helical cross
(b)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AT
CG
GC
GC
TA
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TA
CG
AT
AT
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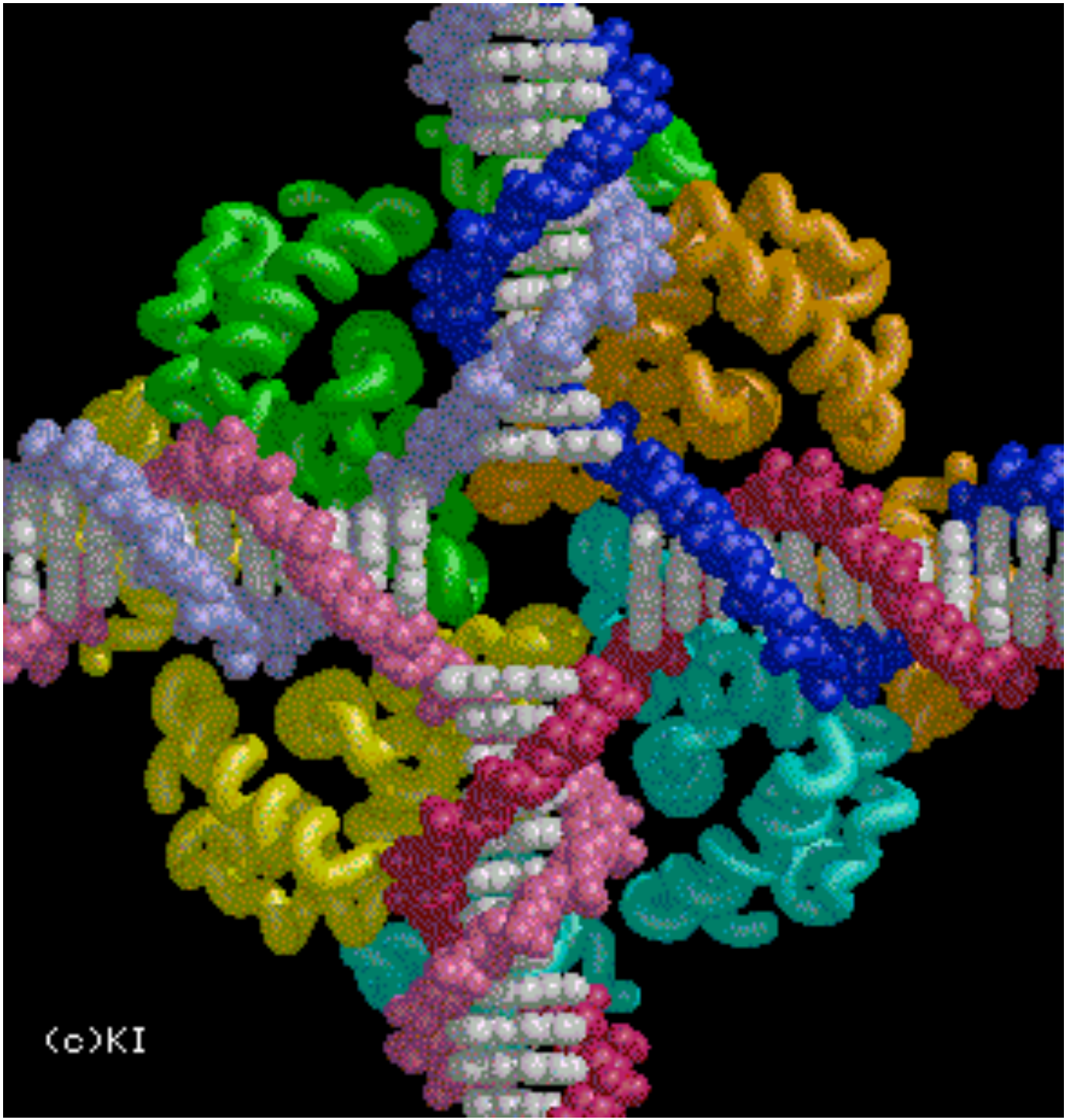
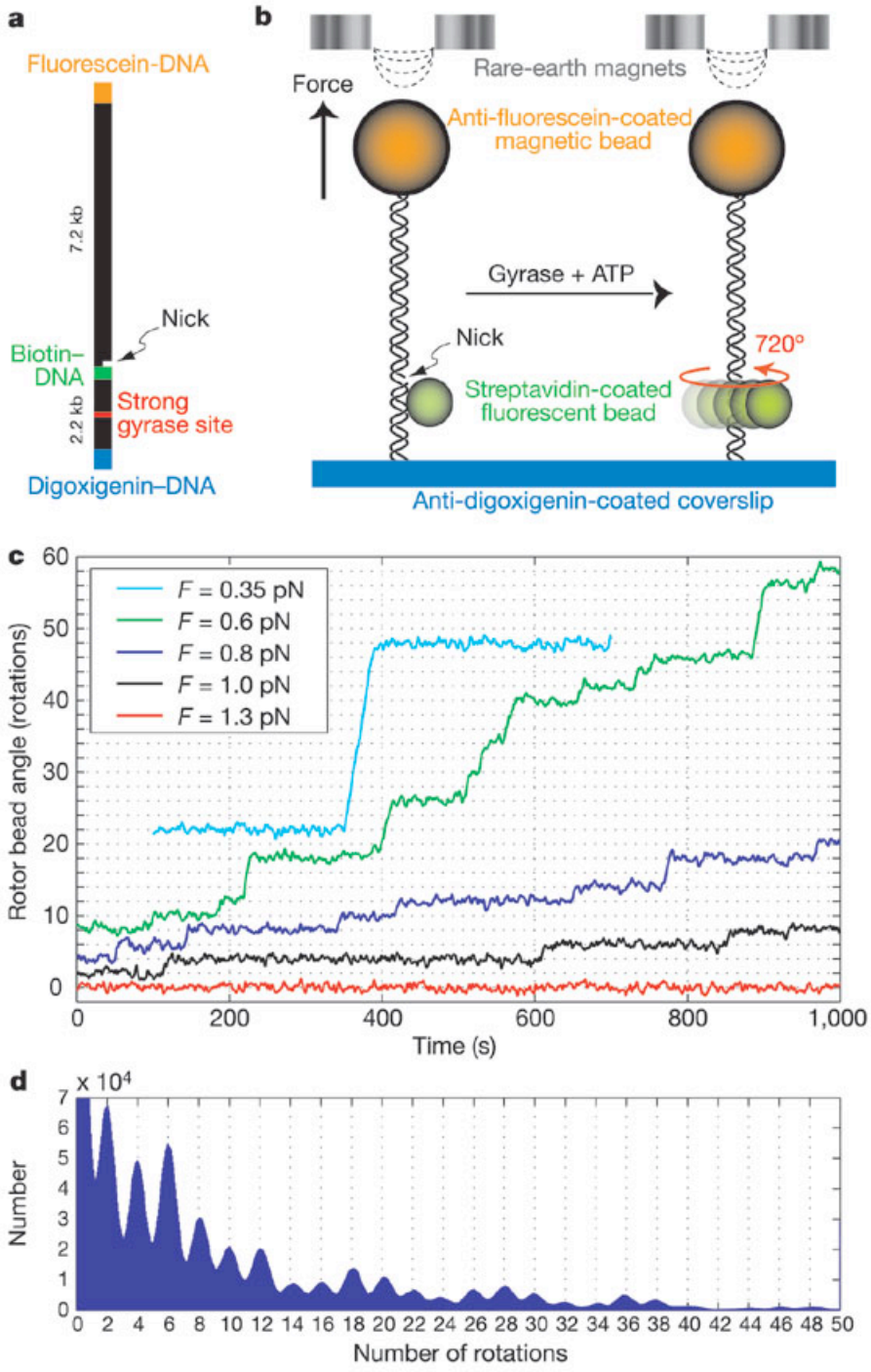
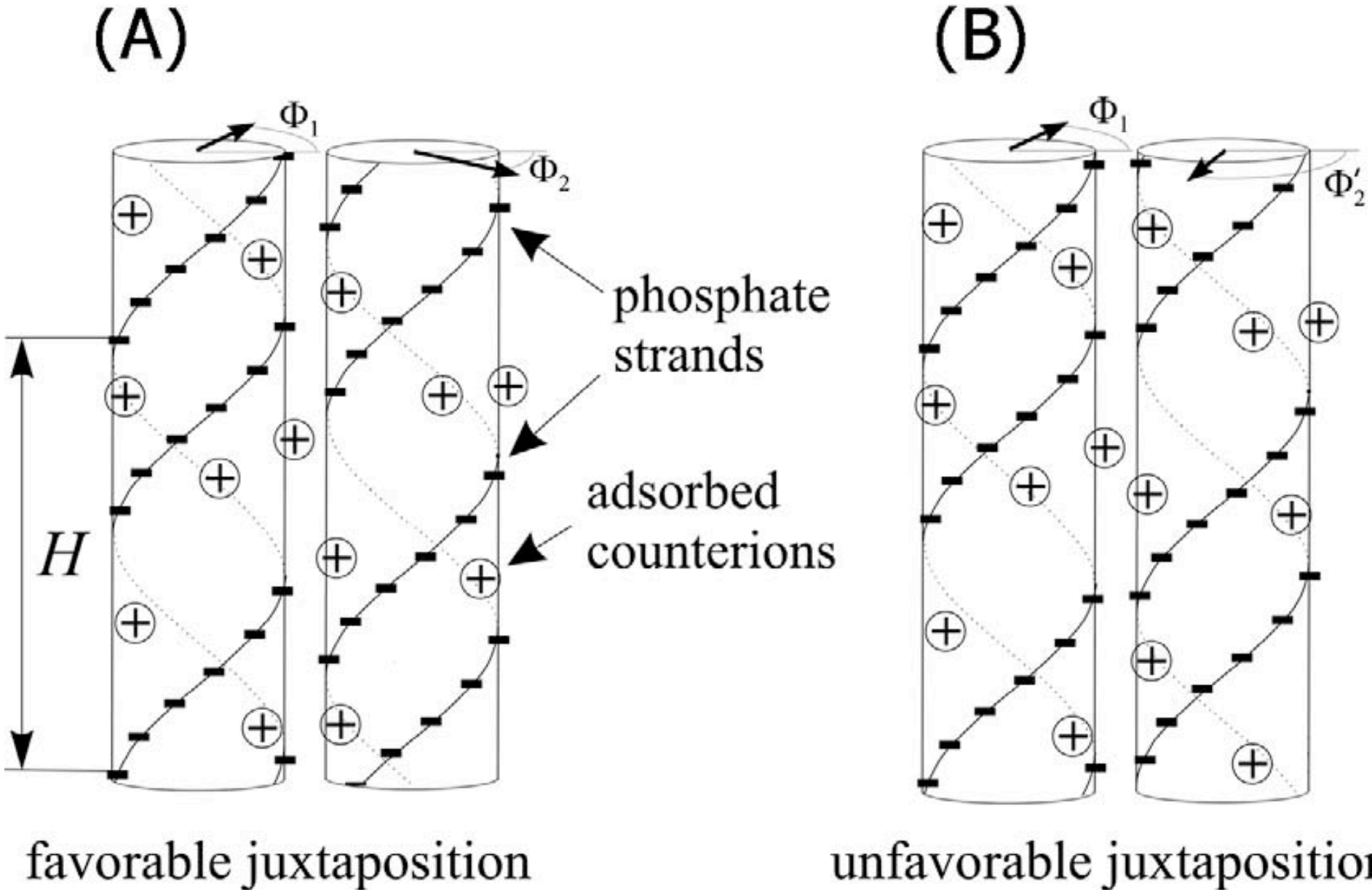
Old
New
Old
New
The two strands of the parental double helix unwind, and each specifies a new daughter strand by base-pairing rules



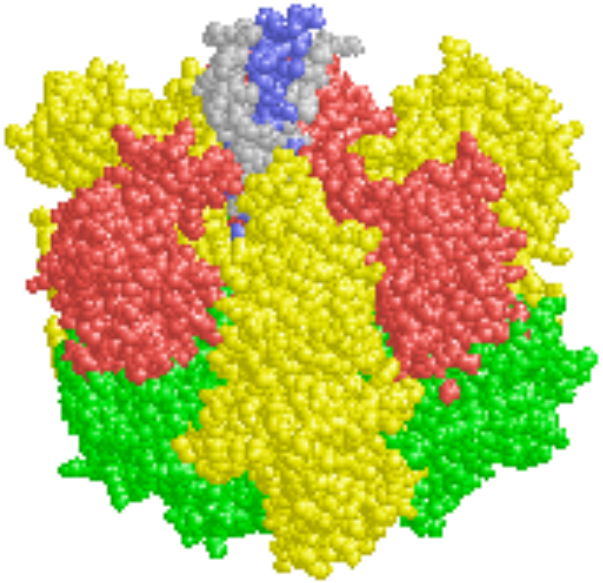
Holliday junction



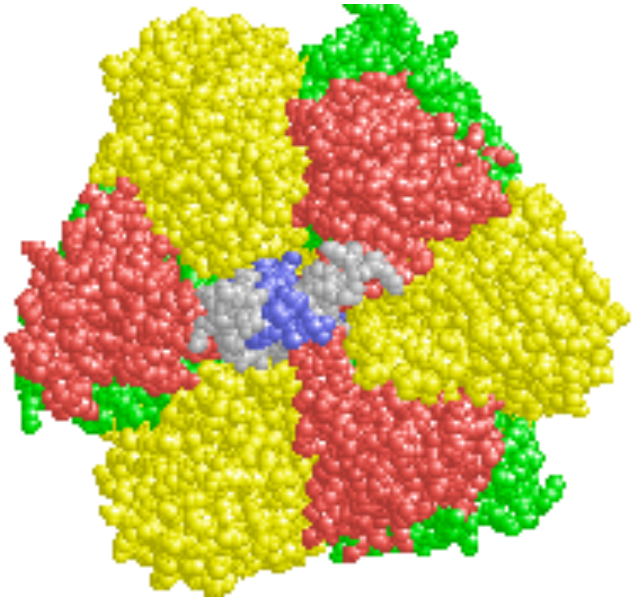
Gore et al., Nature 439, 100 (2006)


(A)
(B)
Φ_1
Φ_2
Φ_1
Φ'_2
phosphate
strands
adsorbed
counterions
H
favorable juxtaposition
unfavorable juxtaposition

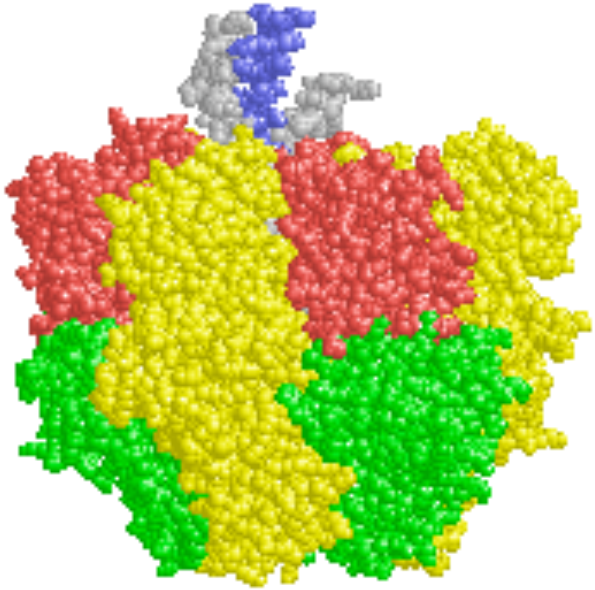
Perspective



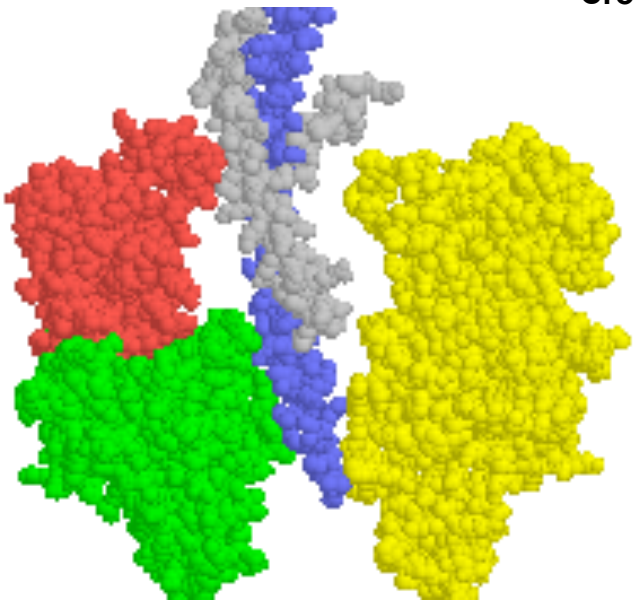
Top



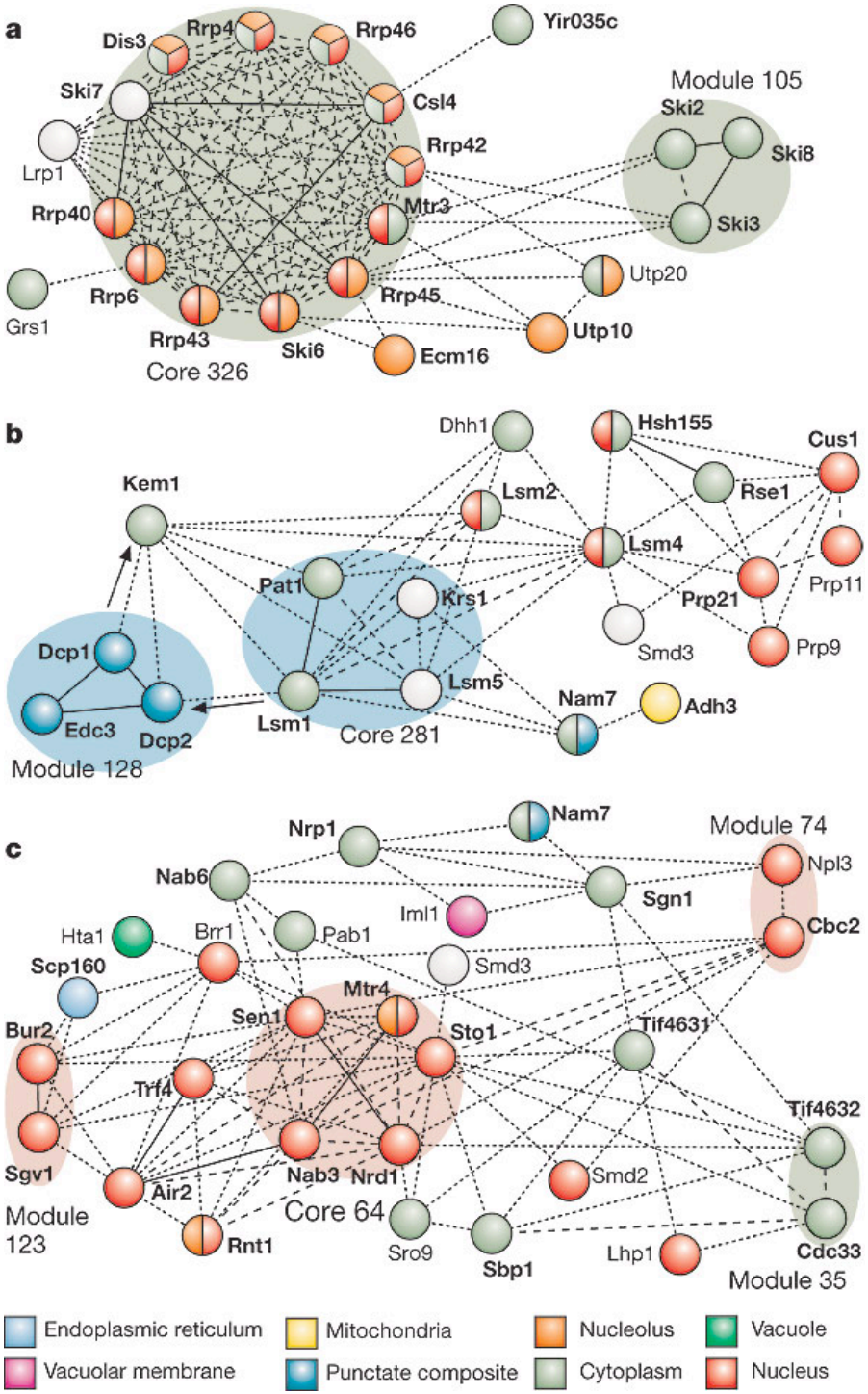
Side



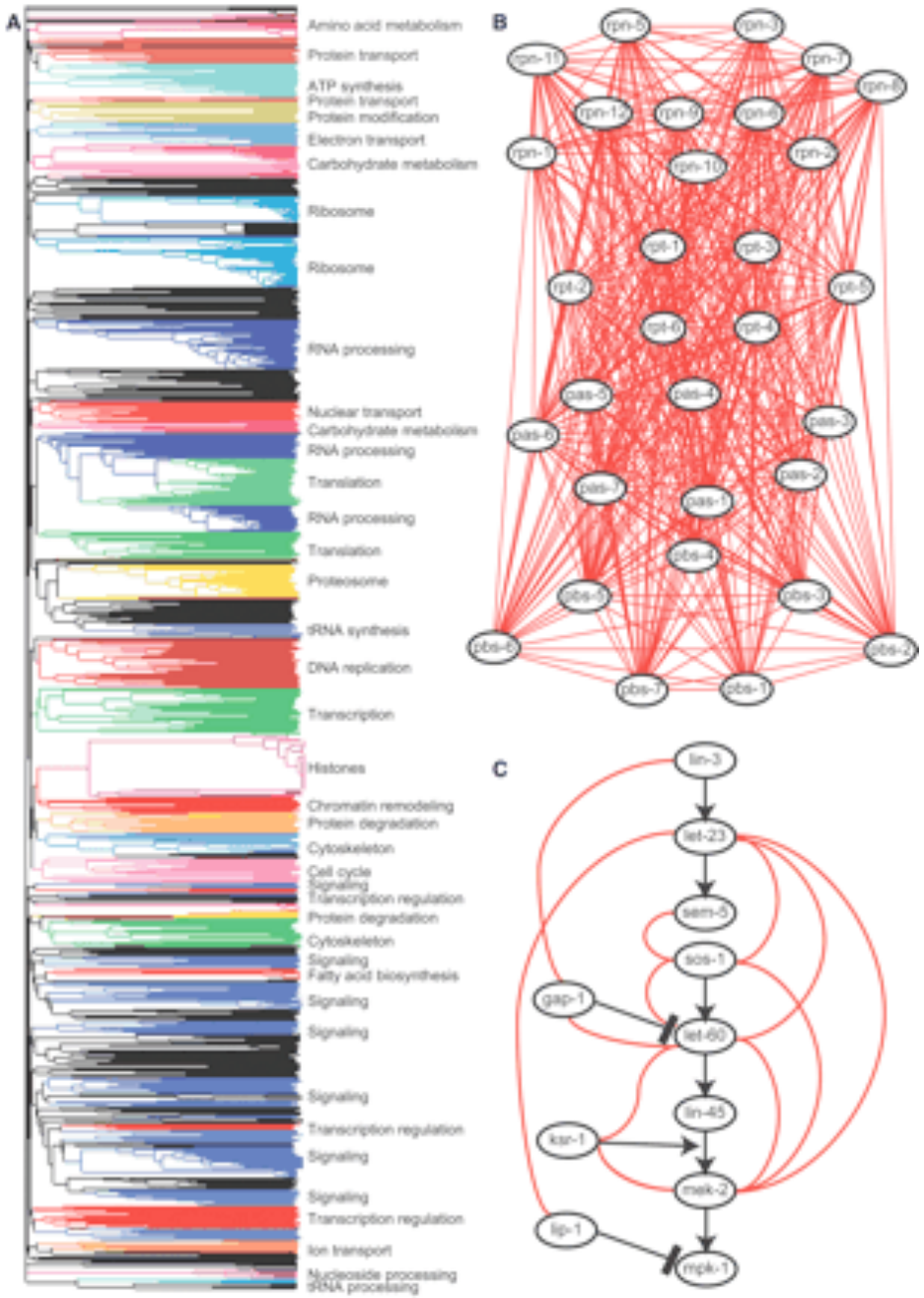
Cross section



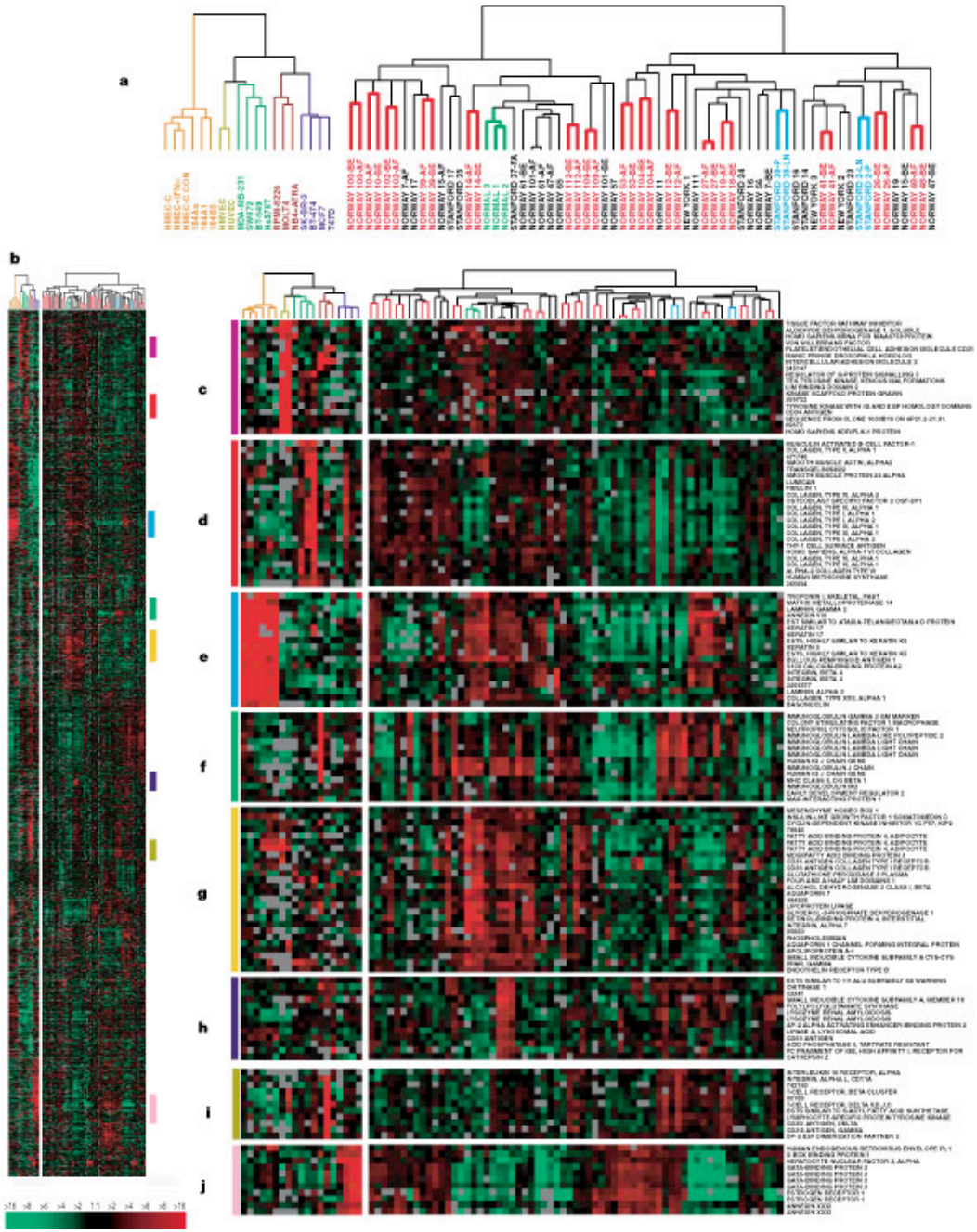
ATP Synthase structure (from www.nature.berkeley.edu)



Gavin et al., Nature 440, 631 (2006) - 1



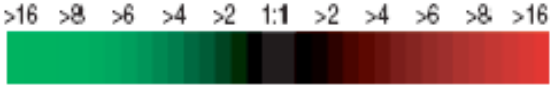
Zhong & Sternberg, Science 311, 1481 (2006)



Data are presented in a **matrix format**: each row represents a single gene, and each column an experimental sample. In each sample, the ratio of the abundance of transcripts of each gene to the median abundance of the gene's transcript among all the cell lines (left panel), or to its median abundance across all tissue samples (right panel), is represented by the colour of the corresponding cell in the matrix.

Green squares, transcript levels below the median; black squares, transcript levels equal to the median; red squares, transcript levels greater than the median; grey squares, technically inadequate or missing data.

Colour saturation reflects the magnitude of the ratio relative to the median for each set of samples.



Are we returning to stamp collection?

A Different Universe

REINVENTING PHYSICS
from the Bottom Down

Robert B. Laughlin

Laughlin, front page

It was a massive PowerPoint presentation in which levels of six thousand types of messenger RNA went up and down (or not) over the cell cycle of yeast. This was, in addition to interminable, thoroughly exasperating, for although they are supposedly a window on the cell's basic regulatory machinery, no one knows why these measurements take on the values they do, what the crude correlations of one signal with the next imply, or indeed whether there is any useful information in these measurements at all.

Laughlin, 159

We also know that while a simple and absolute law, such as hydrodynamics, can evolve from the deeper laws underneath, it is at the same time independent of them, in that it would be the same even if the deeper laws were changed.

Laughlin, 207

Much as I dislike the idea of ages, I think a good case can be made that science has now moved from an Age of Reductionism to an Age of Emergence, a time when the search for ultimate causes of things shifts from the behavior of parts to the behavior of the collective.

Emergence means complex organizational structure growing out of simple rules. Emergence means stable inevitability in the way certain things are. Emergence means unpredictability, in the sense of small events causing great and qualitative changes in larger ones. Emergence means the fundamental impossibility of control. Emergence is a law of nature to which humans are subservient.

Laughlin, 200

The messenger RNA experiment in yeast is an especially important kind of bad experiment, however, because it demonstrates clearly that geneticists do not know what they are doing. The screams of outrage and other indignant responses to this assertion will fall on deaf ears: I know a terrible experiment when I see one. The symptoms are always the same. The measurements do not reproduce, they do not lend themselves to commonsense analysis, and they cannot be quantified.

Laughlin, 162

One might subtitle this thesis the end of reductionism (the belief that things will necessarily be clarified when they are divided into smaller and smaller component parts), but that would not be quite accurate. All physicists are reductionists at heart, myself included. I do not wish to impugn reductionism so much as establish its proper place in the grand scheme of things.

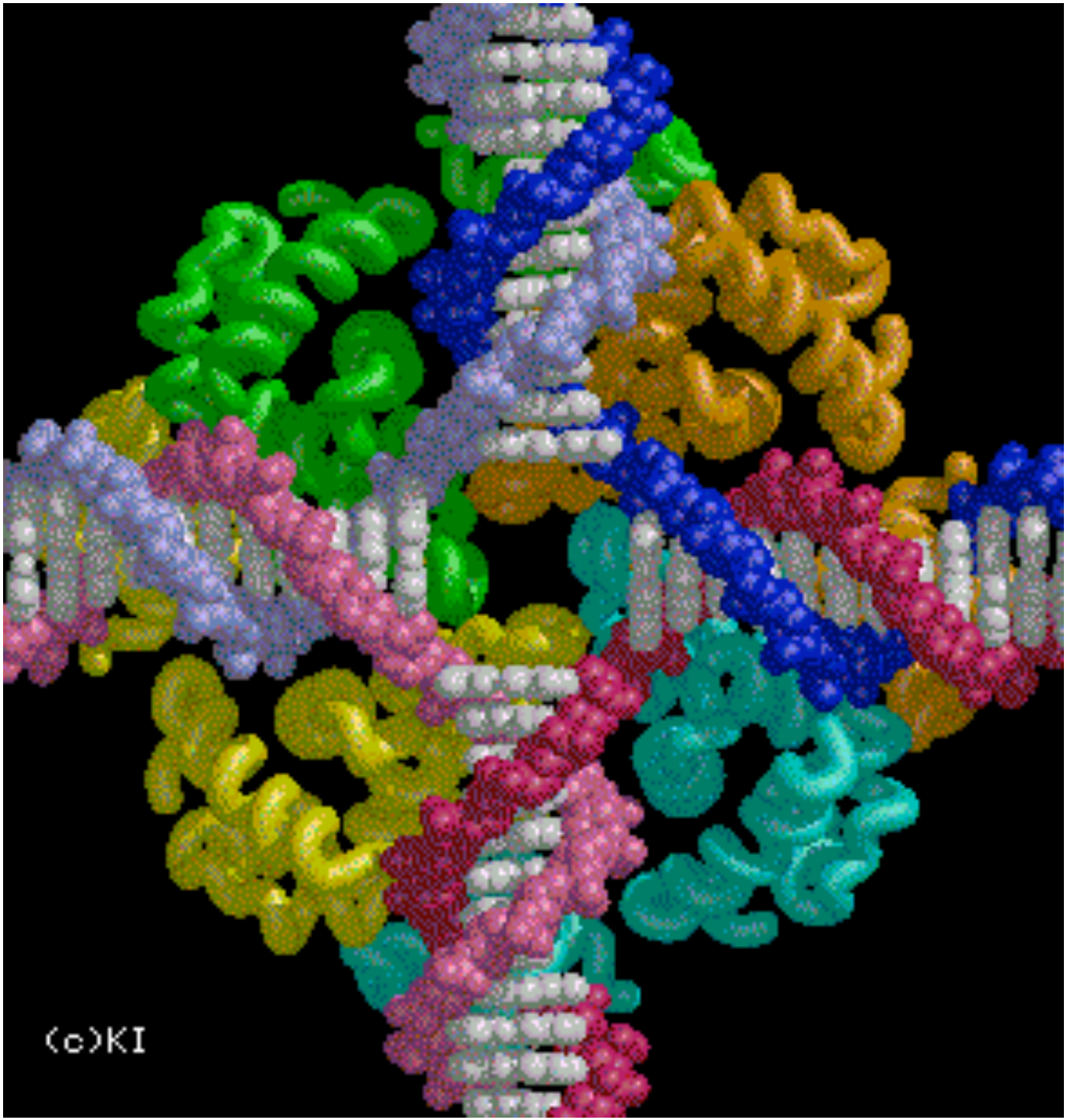
Laughlin, xv

This is not to say that microscopic law is wrong or has no purpose, but only that it is rendered irrelevant in many circumstances by its children and its children's children, the higher organizational laws of the world.

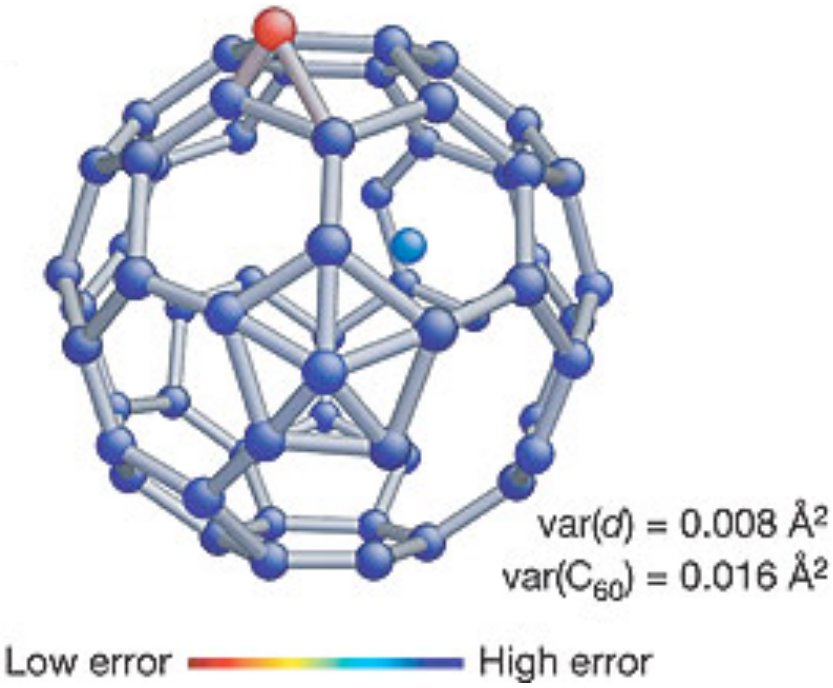
Laughlin, 221

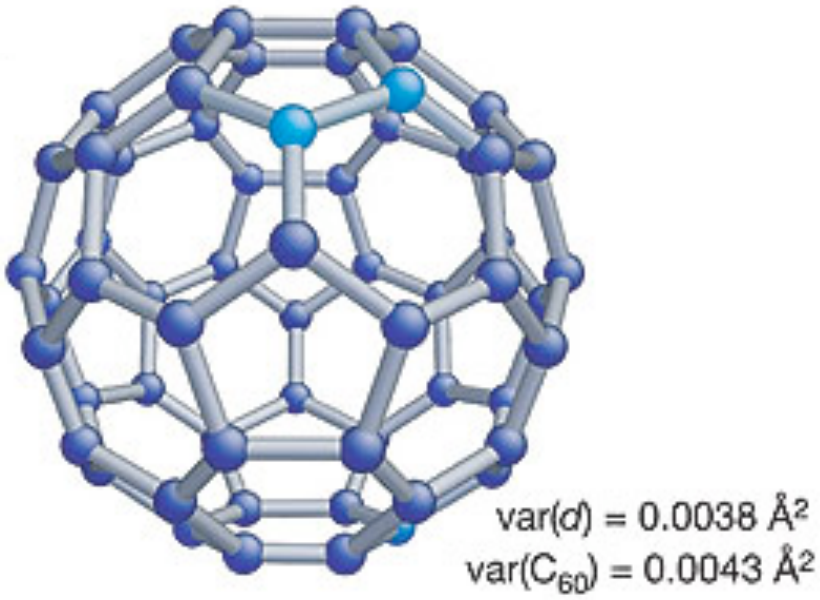
as-yet-undiscovered organizing principles might be at work at the mesoscopic scale, intermediate between atomic and macroscopic dimensions

Laughlin, Pines, Schmalian, Stojkovic and Wolynes, 2000; Proc. Natl. Acad. Sci., *97*,32

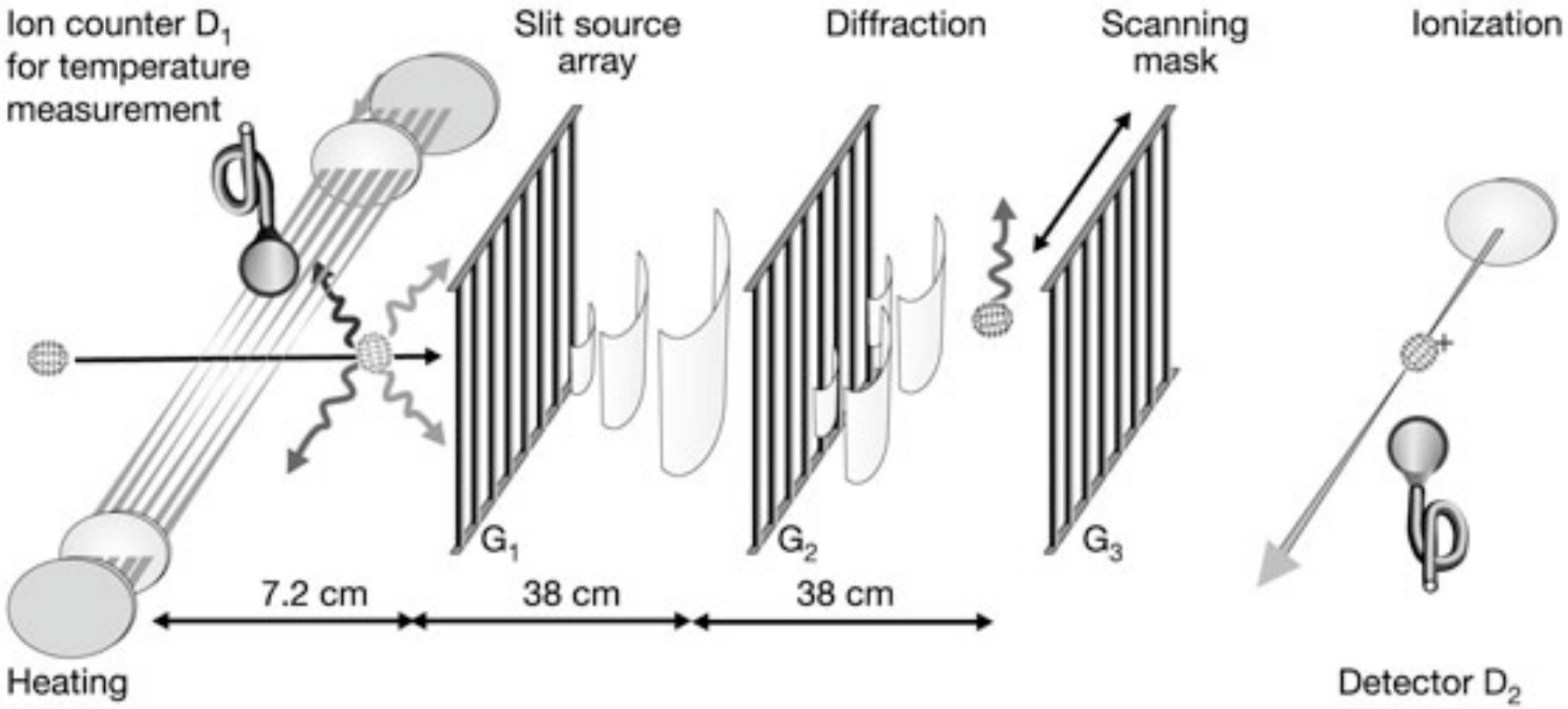


Holliday junction

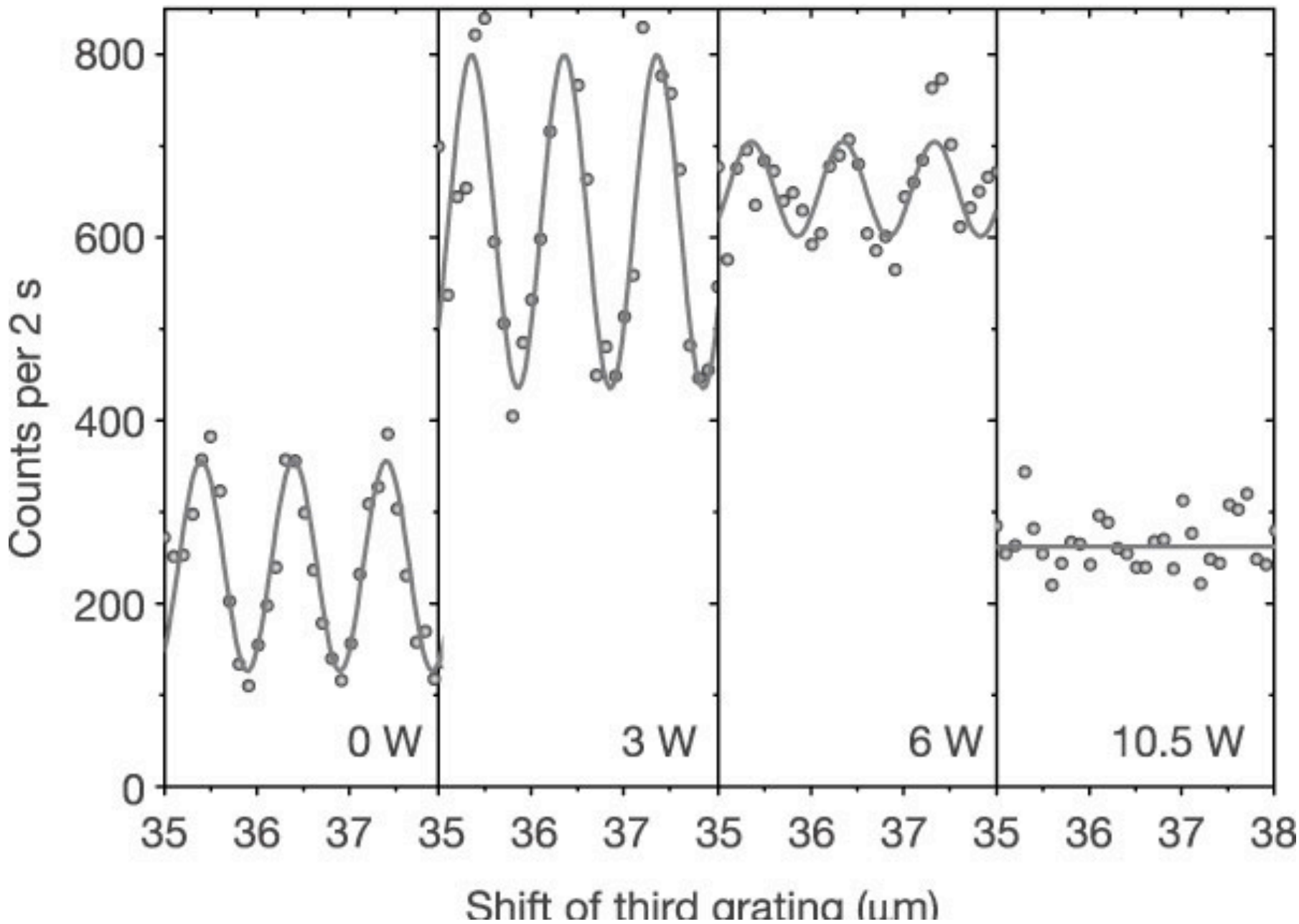




Juhás et al., Nature 440, 655 (2006)



Hackermüller, Nature 427, 711 (2004) - 1



Hackermüller, Nature 427, 711 (2004) - 2

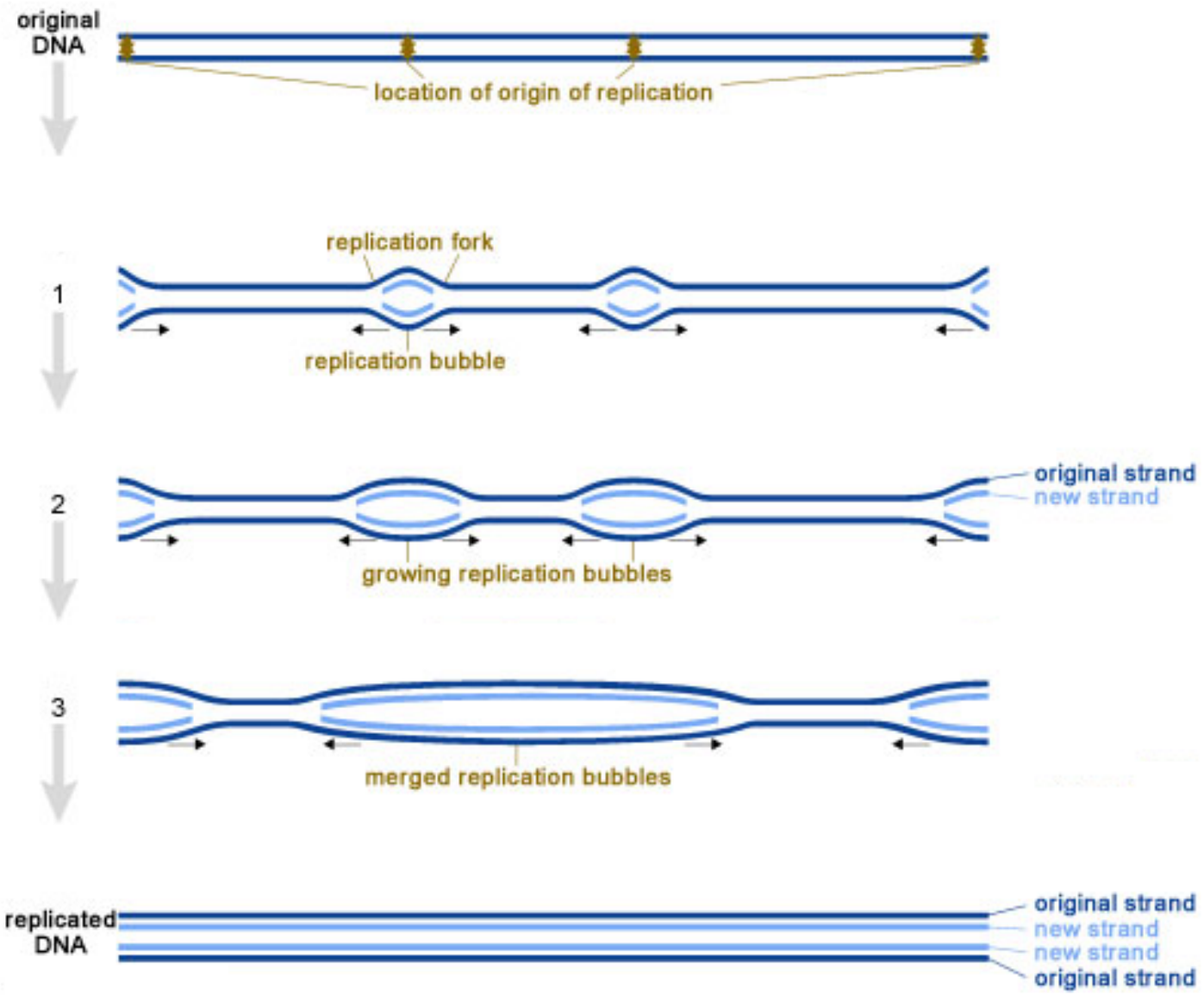
With relativity and quantum mechanics, physics moved from this accessible common sense world into a far more abstract one, much more difficult for the human mind to imagine and conceive. Perhaps a proper understanding of the complex regulatory networks making up cellular systems like the cell cycle will require a similar shift from common sense thinking. We might need to move into a strange more abstract world, more readily analyzable in terms of mathematics than our present imaginings of cells operating as a microcosm of our everyday world.

Paul Nurse, 2000; Cell, *100*, 71

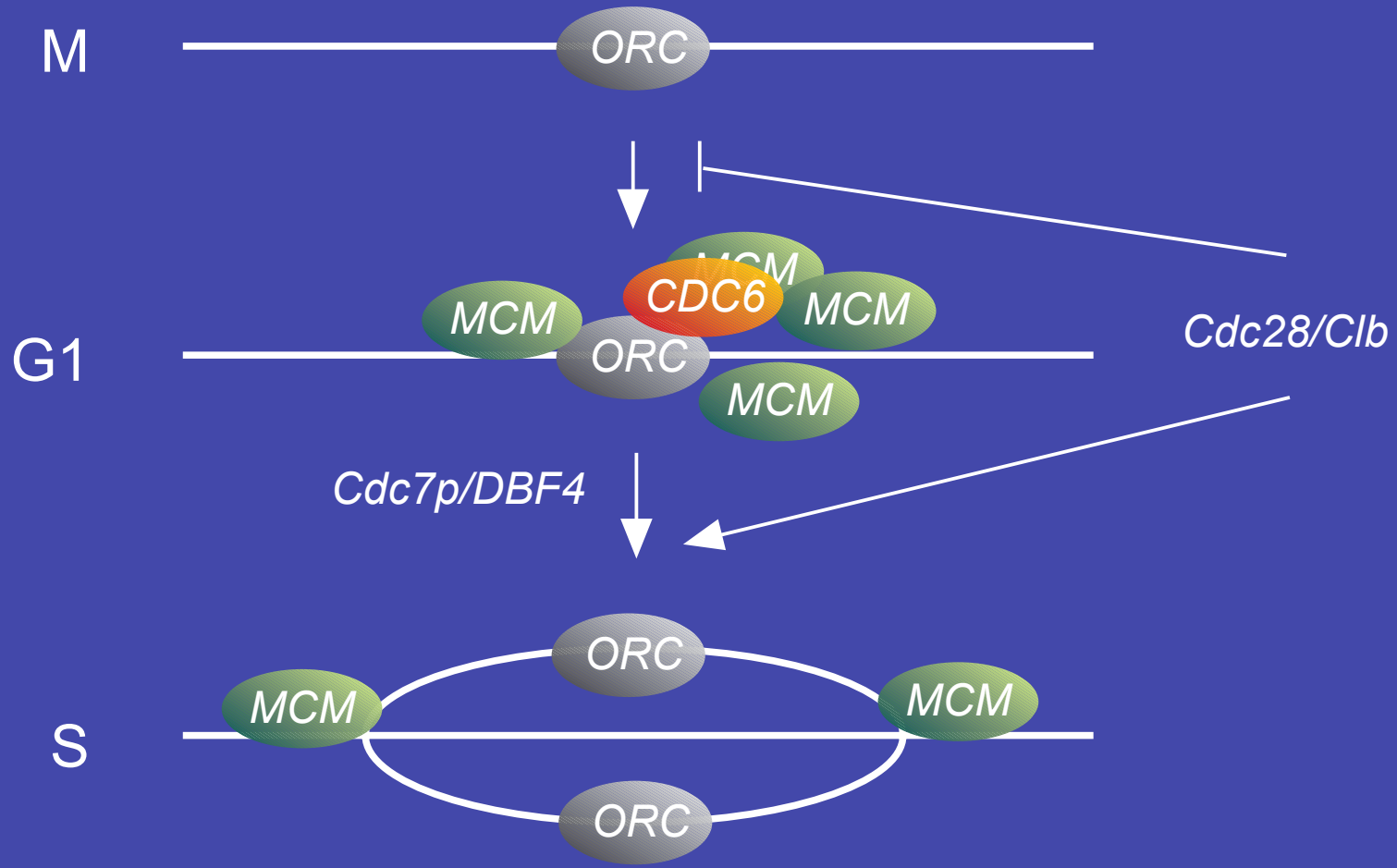
The *HFSP Journal* aims to publish high quality, innovative interdisciplinary basic research at the frontier of biology over a wide range of organizational levels (from the molecular level to population biology) using principles strategies or technologies from the more quantitative disciplines (e.g. physics, chemistry, mathematics, engineering, or informatics).

The goal of the *HFSP Journal* is to foster communication between the different disciplines of research, thus furthering the mission of the Human Frontier Science Program.

Human DNA Replication Origins



Protein-DNA interactions at S. cerevisiae ARS during the cell cycle



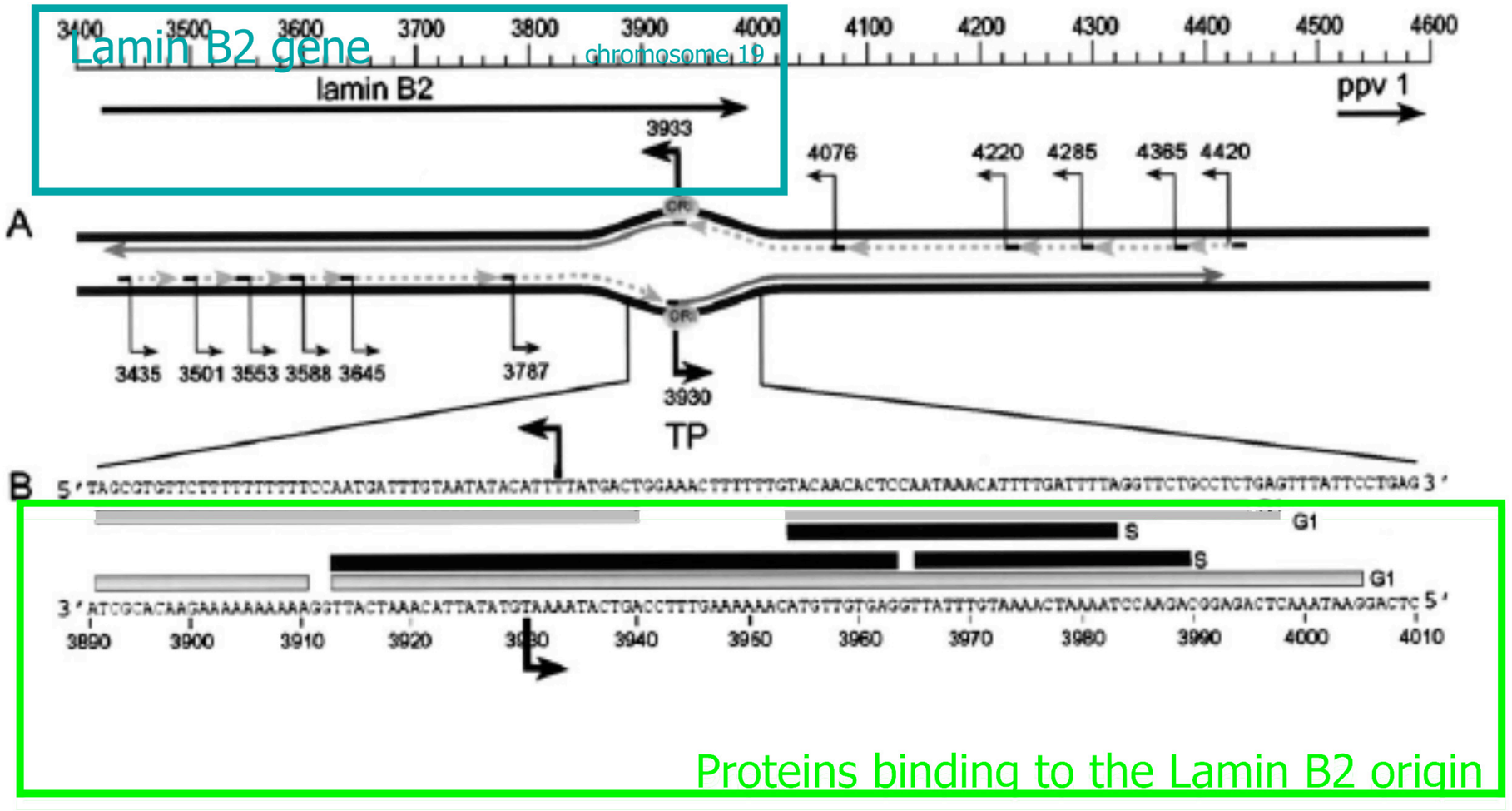


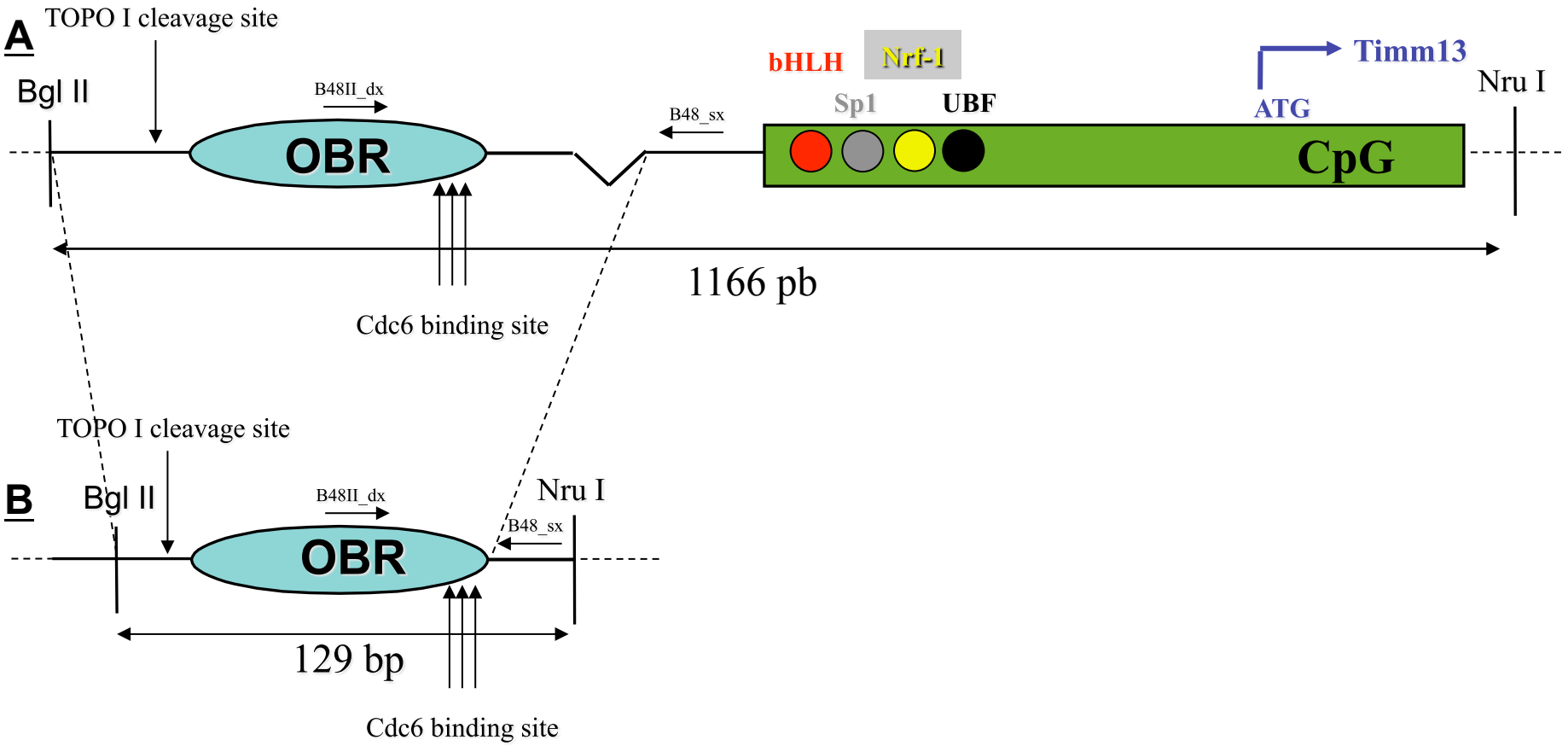
Fig. 4. Map of start sites of bidirectional DNA synthesis at the human lamin B2 ori. (A) Transition points (TP) from continuous to discontinuous DNA synthesis on the two strands are shown by thick arrows; 5′ ends of Okazaki fragments are indicated by thin arrows. Positions of the transcripts around ori area are shown. (B) Precise positions of the protein-DNA interactions occurring in G_1 and S phase (9) are shown by gray and black boxes, respectively.

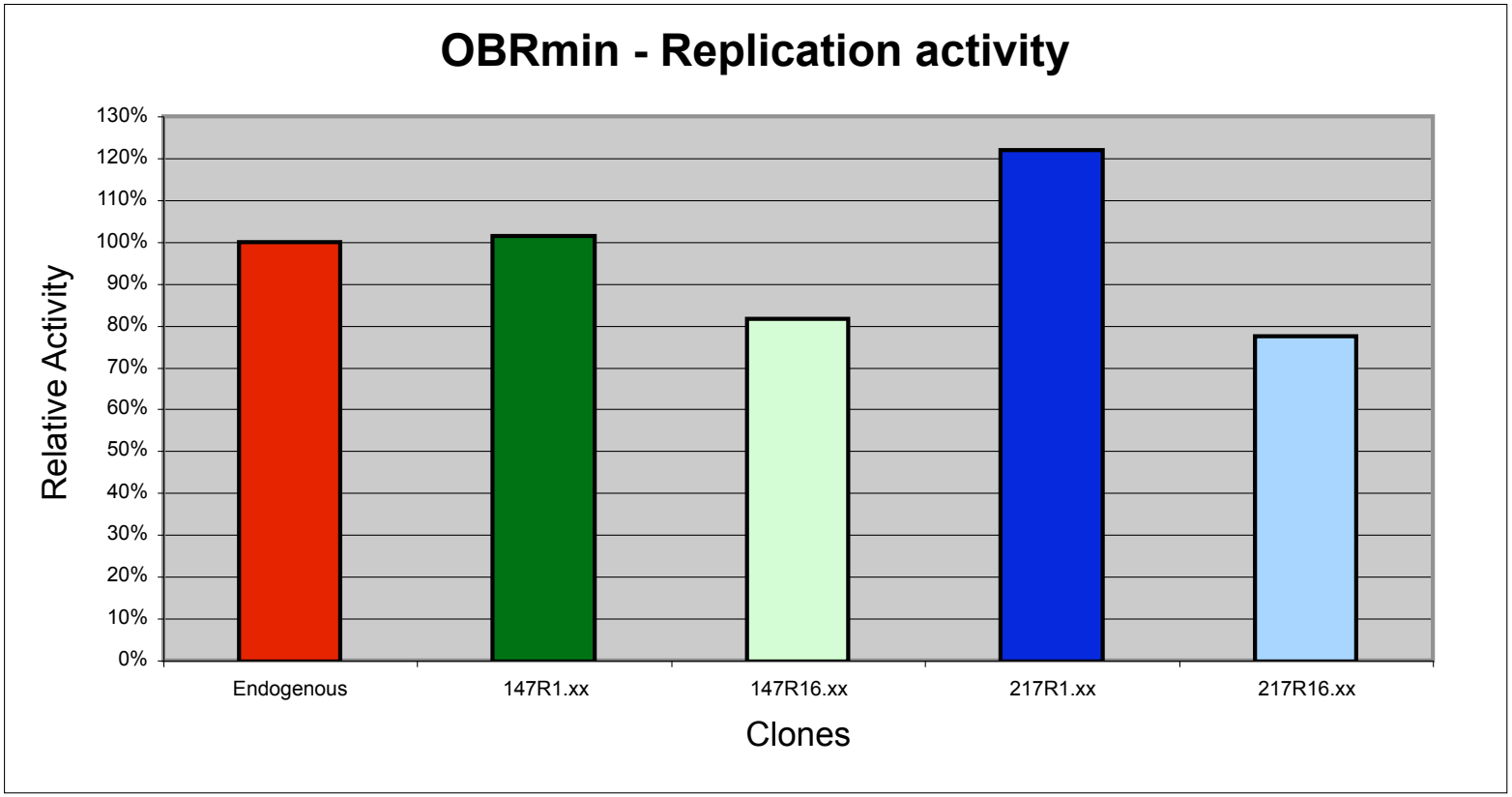
Abdurashidova G. et al., Science Vol 287, 17 March 2000

What is the minimal size sufficient for origin function?

Can we identify other members of the replicative complexes?

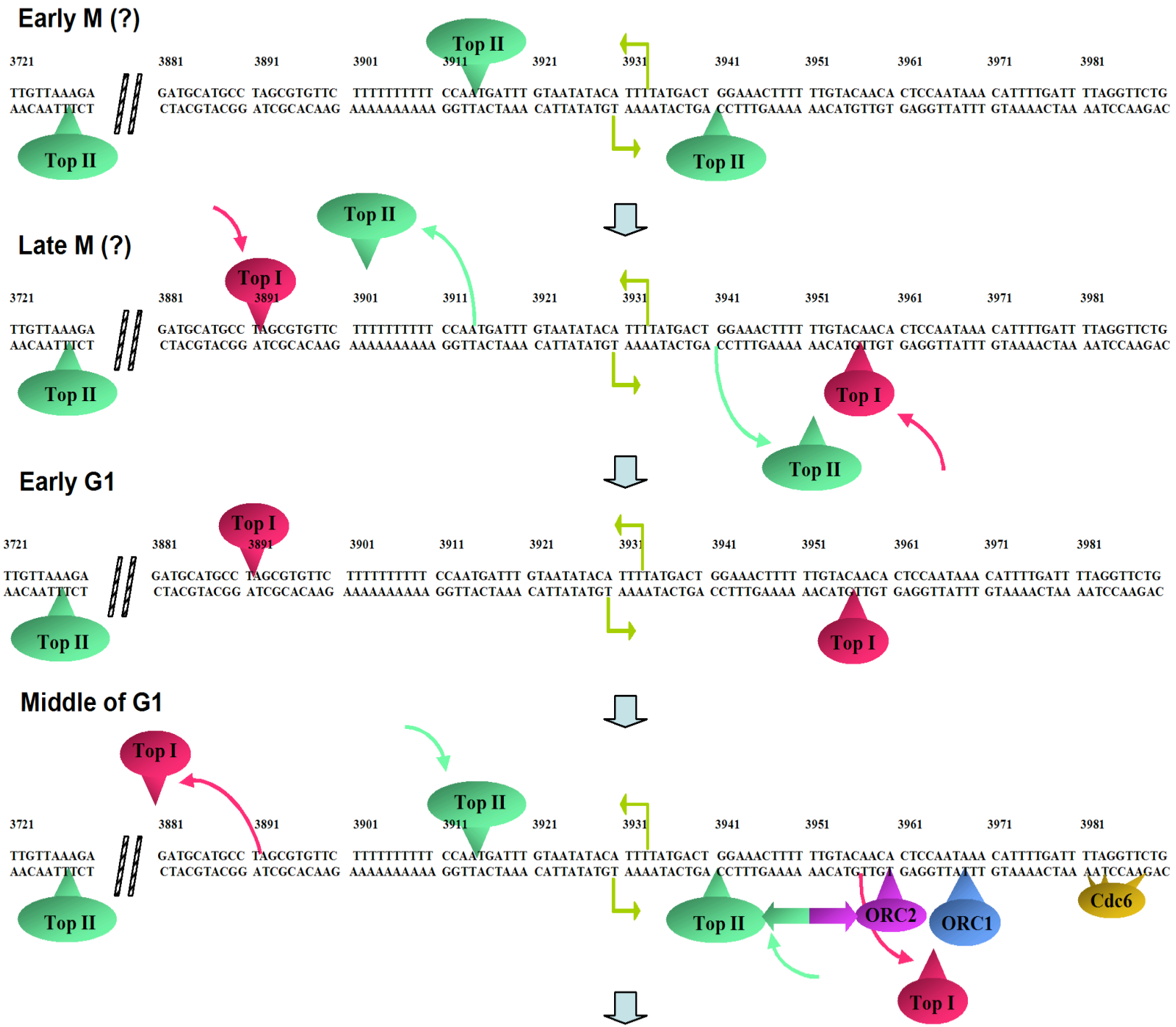
What is the minimal size sufficient for origin function?

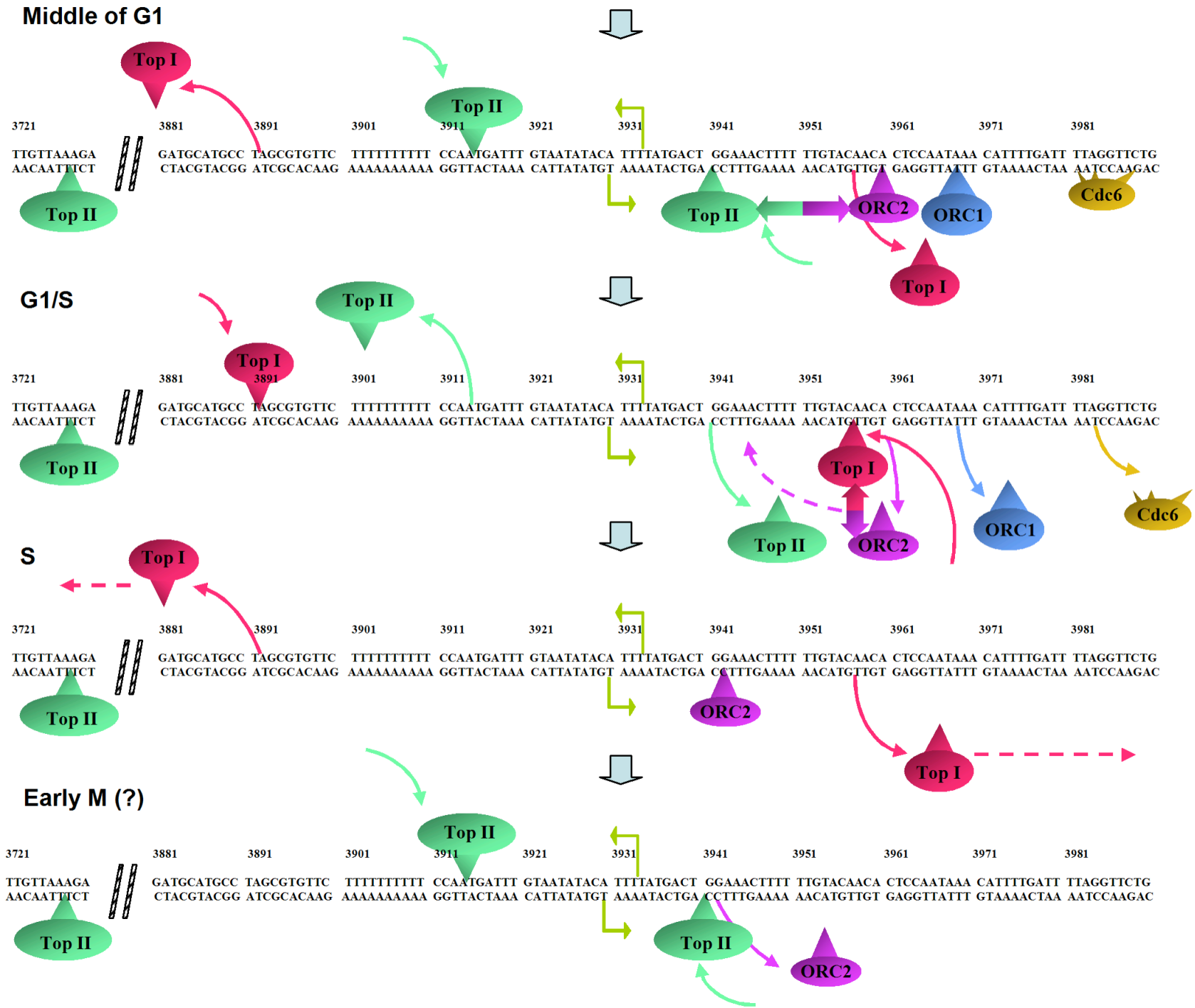




The minimum OBR displays a replicative activity comparable to Delta-CpG mutant.

129 bp containing the start sites and the sites of binding of the proteins of the replicative complexes are sufficient to confer origin function.

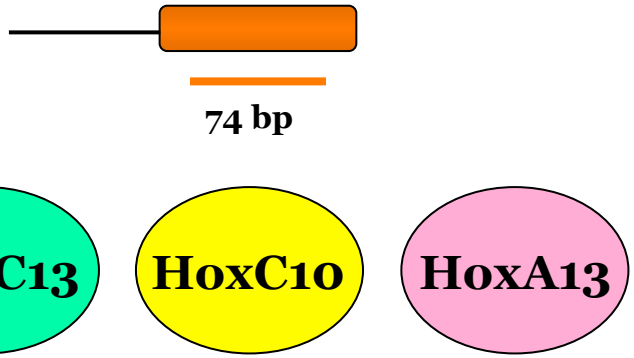

Early M (?)
3721
3881
3891
3901
3911
3921
3931
3941
3951
3961
3971
3981
TTGTTAAAGA GATGCATGCC TAGCGTGTTC TTTTTTTTTT CCAATGATTT GTAATATACA TTTTATGACT GGAAACTTTT TTGTACAACA CTCCAATAAA CATTTTGATT TTAGGTTCTG
AACAATTTCT CTACGTACGG ATCGCACAAG AAAAAAAAAA GGTTACTAAA CATTATATGT AAAATACTGA CCTTTGAAAA AACATGTTGT GAGGTTATTT GTAAAACTAA AATCCAAGAC
Top II
Top II
Top II
Late M (?)
Top I
Top II
Top II
Top I
Early G1
Top I
Top II
Top I
Middle of G1
Top I
Top II
Top II
ORC2
ORC1
Cdc6
Top I


Middle of G1
Top I
Top II
3721
3881
3891
3901
3911
3921
3931
3941
3951
3961
3971
3981
TTGTTAAAGA GATGCATGCC TAGCGTGTTC TTTTTTTTTT CCAATGATTT GTAATATACA TTTTATGACT GGAAACTTTT TTGTACAACA CTCCAATAAA CATTTTGATT TTAGGTTCTG
AACAATTTCT CTACGTACGG ATCGCACAAG AAAAAAAAAA GGTTACTAAA CATTATATGT AAAATACTGA CCTTTGAAAA AACATGTTGT GAGGTTATTT GTAAAACTAA AATCCAAGAC
Top II
ORC2
ORC1
Cdc6
Top I
G1/S
Top II
Top I
Top II
ORC2
Top I
ORC1
Cdc6
S
Top I
Top II
ORC2
Top I
Early M (?)
Top II
Top II
ORC2

Conclusions

1) DNA topology and related chromatin structure are important for origin function
2) Topoisomerases play essential roles
3) Topoisomerase II is probably involved in assembly of pre-replication complex
4) Topoisomerase I probably contributes to origin selection and is essential for origin firing

One-Hybrid assay

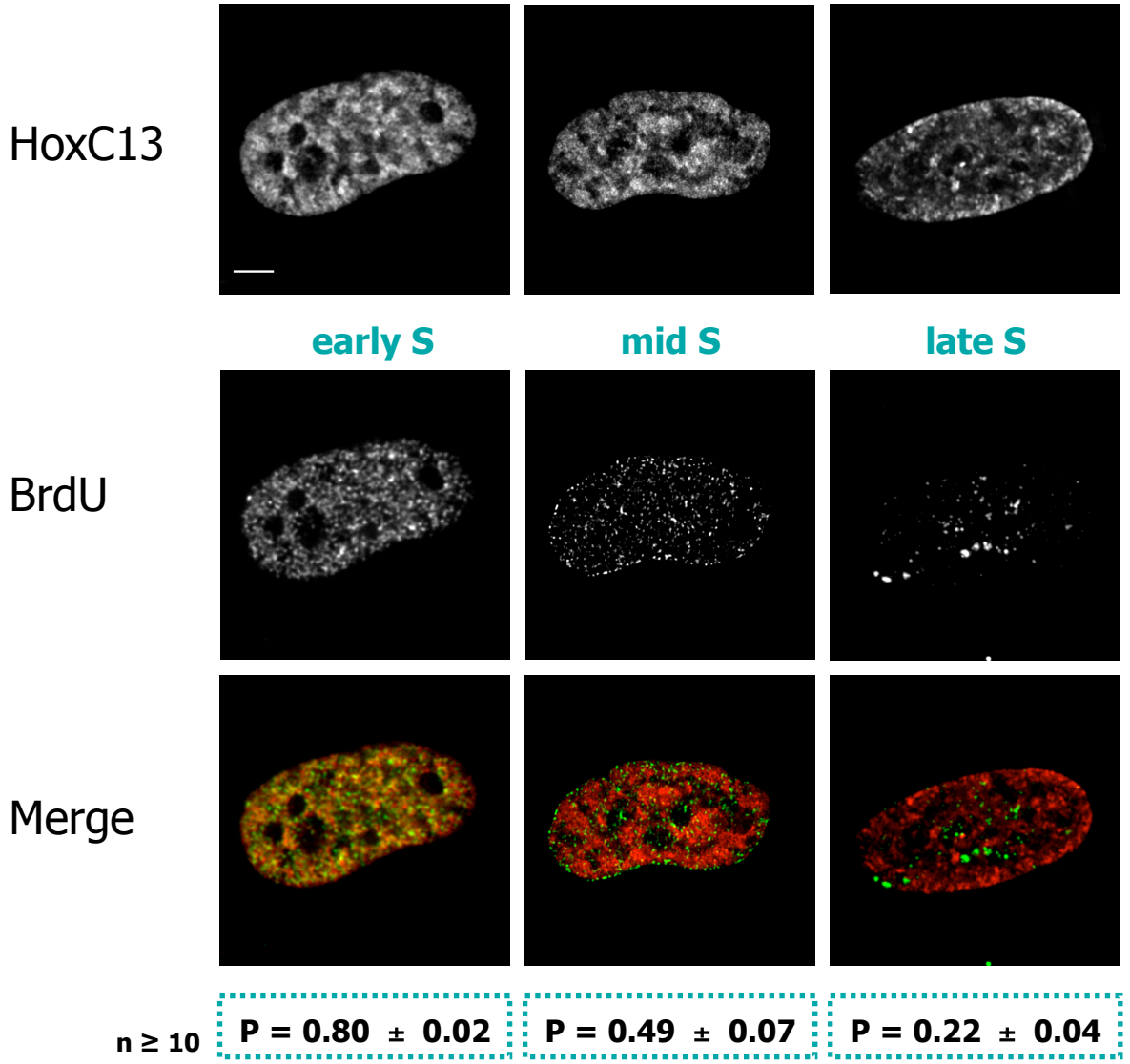


Analyses of the specificity of binding *in vitro* by EMSA assay and *in vivo* by CAT assay

- **Hox proteins are transcriptional factors assigning positional identities in the embryonic body**
- **Hox proteins are characterized by a conserved homeoDomain**

Does HOXC13 interact with members of the replicative complexes?

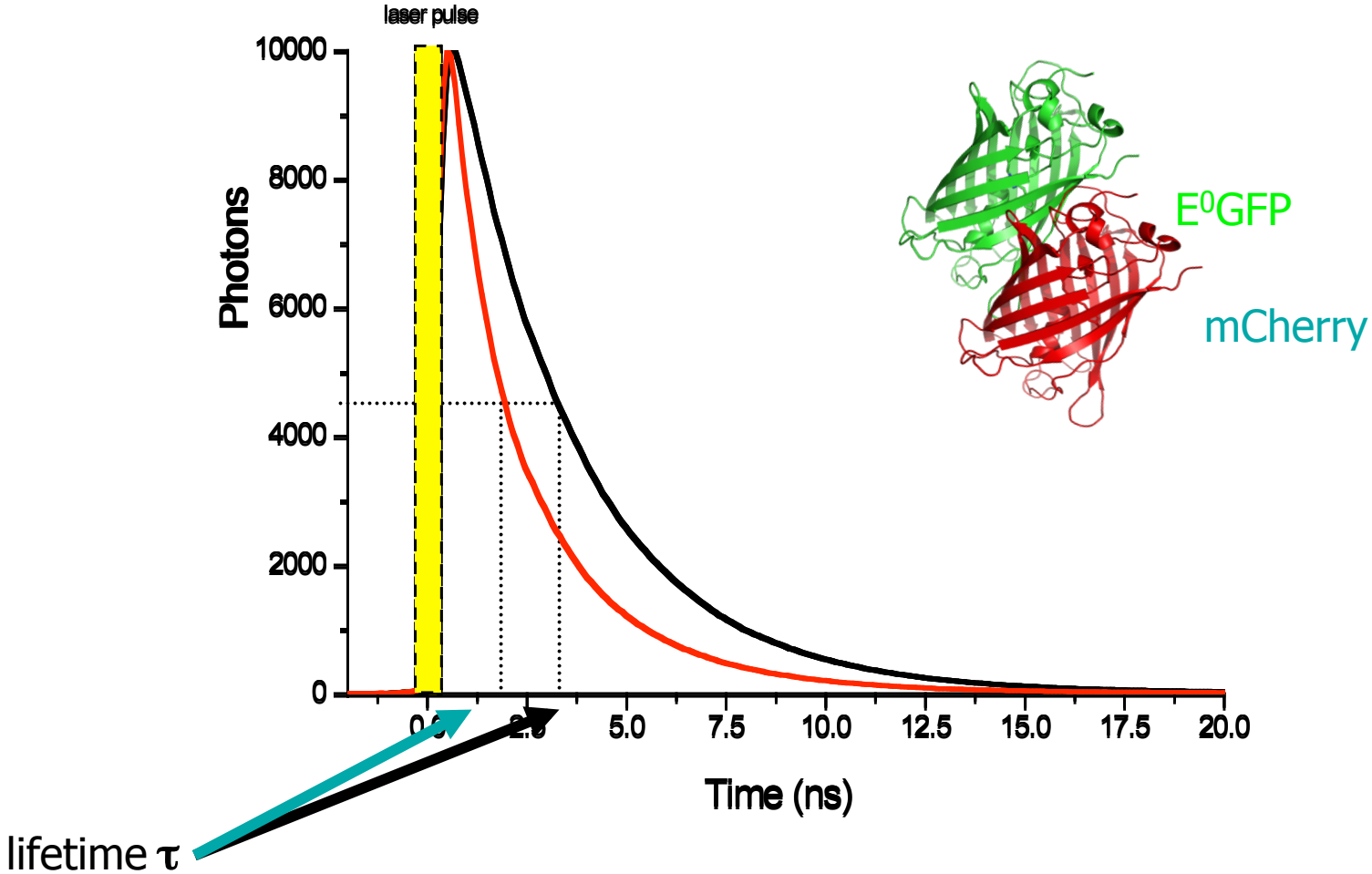
HoxC13 vs. replication foci



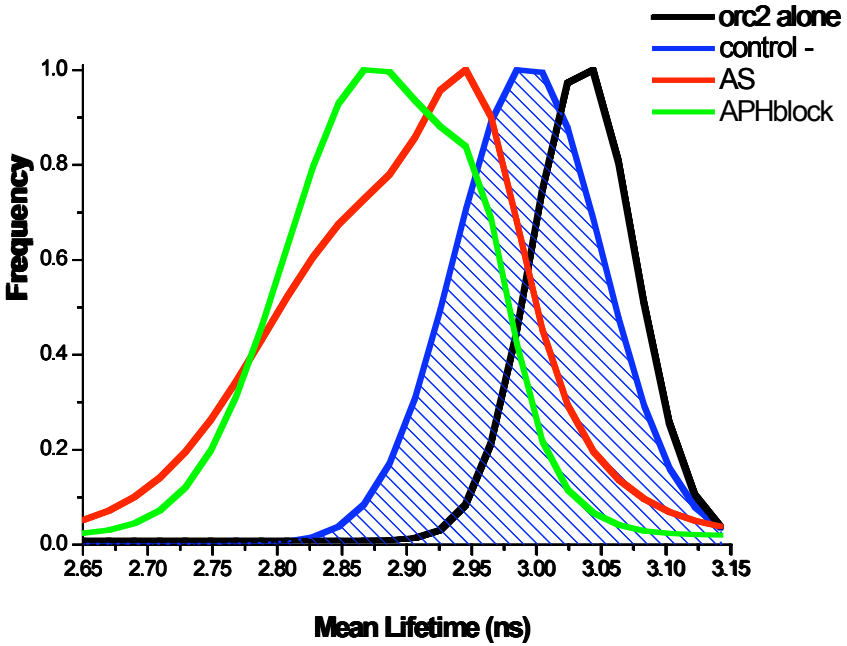
Scale bar 5μm

FLIM

Fluorescence Lifetime Imaging Microscopy



FLIM analysis of orc2-HoxC13 interaction



n≥50 nuclei

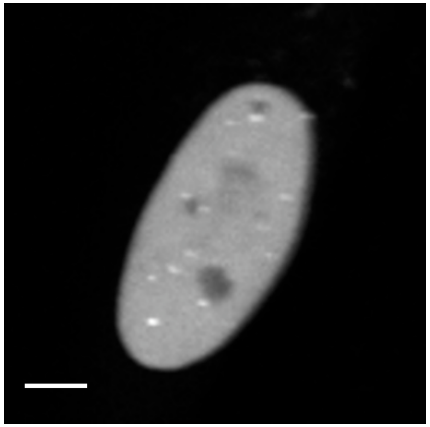
orc2 alone: 3.04 ± 0.004 ns

neg control: 2.99 ± 0.007 ns

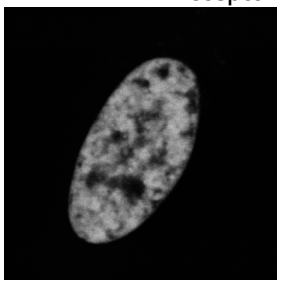
major peak 2.95 ± 0.006 ns

major peak 2.82 ± 0.014 ns

Donor

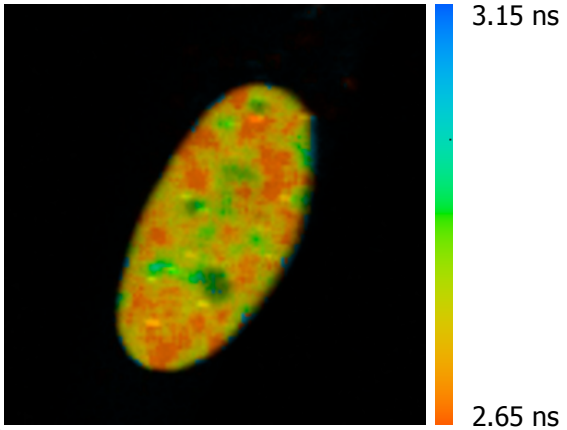


Acceptor



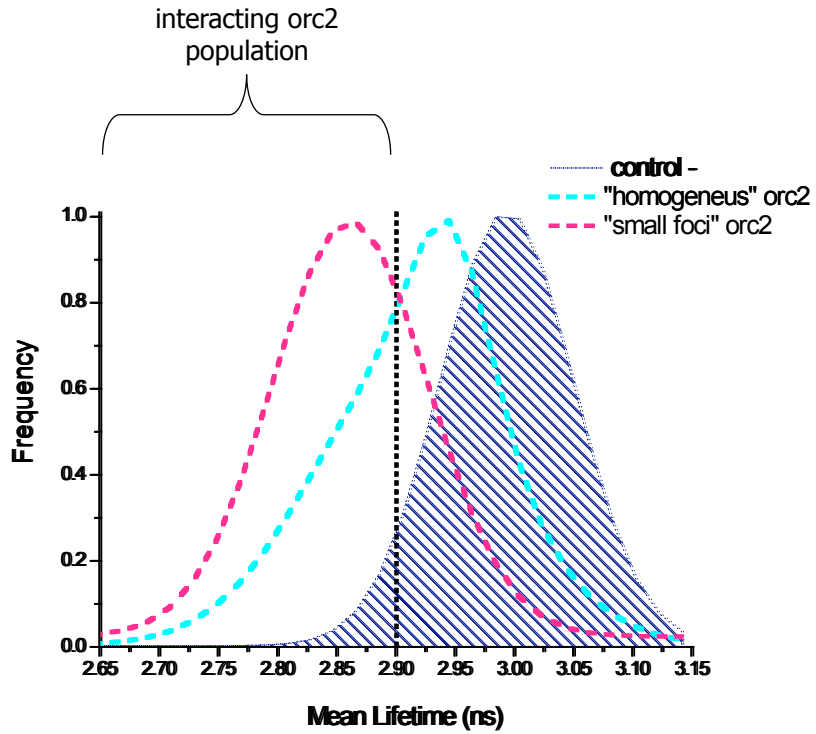
Cherry-HOX

Donor Lifetime Map



Scale bar 5μm

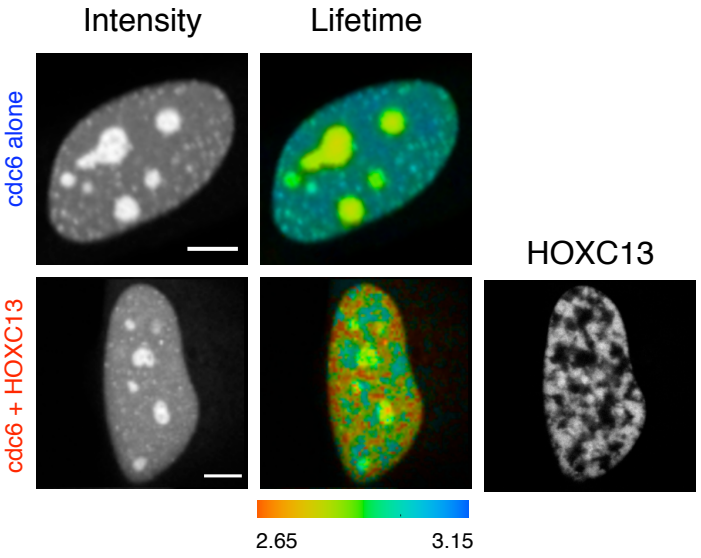
FLIM analysis of orc2-HoxC13 interaction



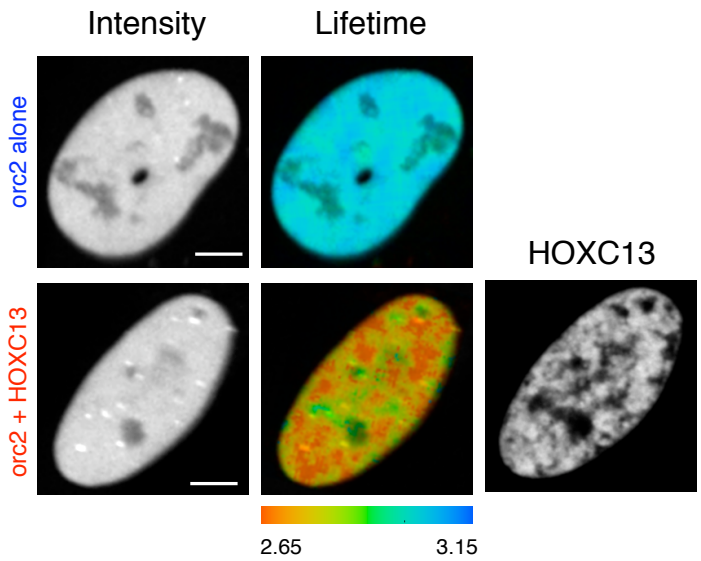
E_{fret}=17%

r = 6.6nm

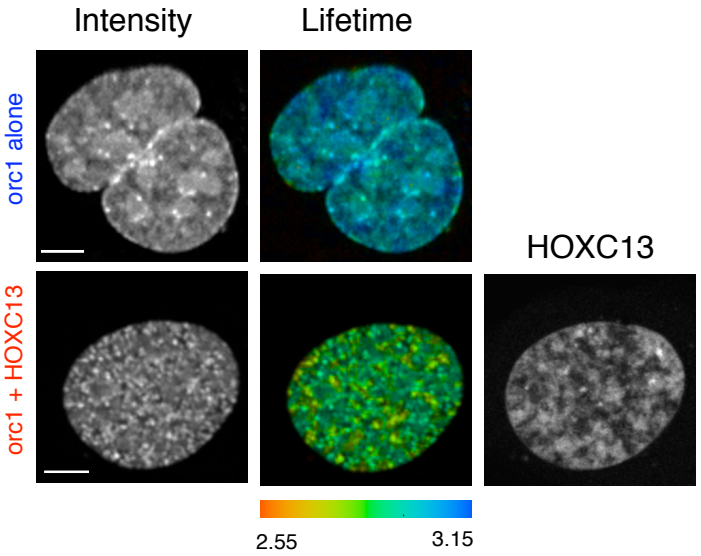
CDC6



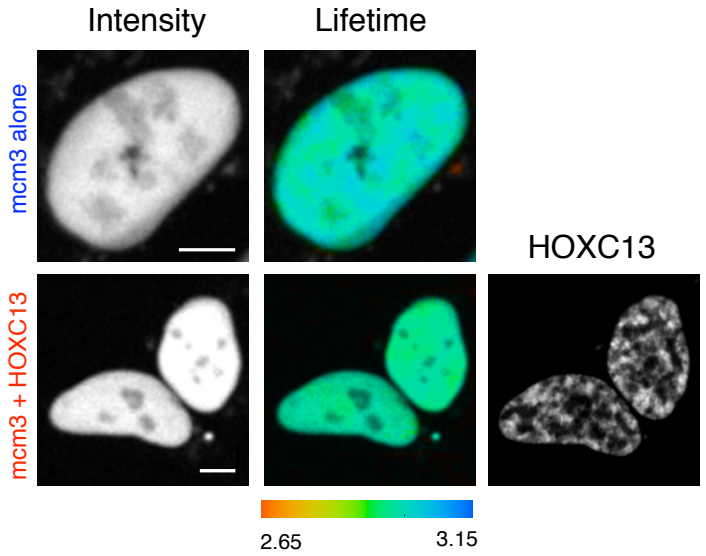
ORC2



ORC1

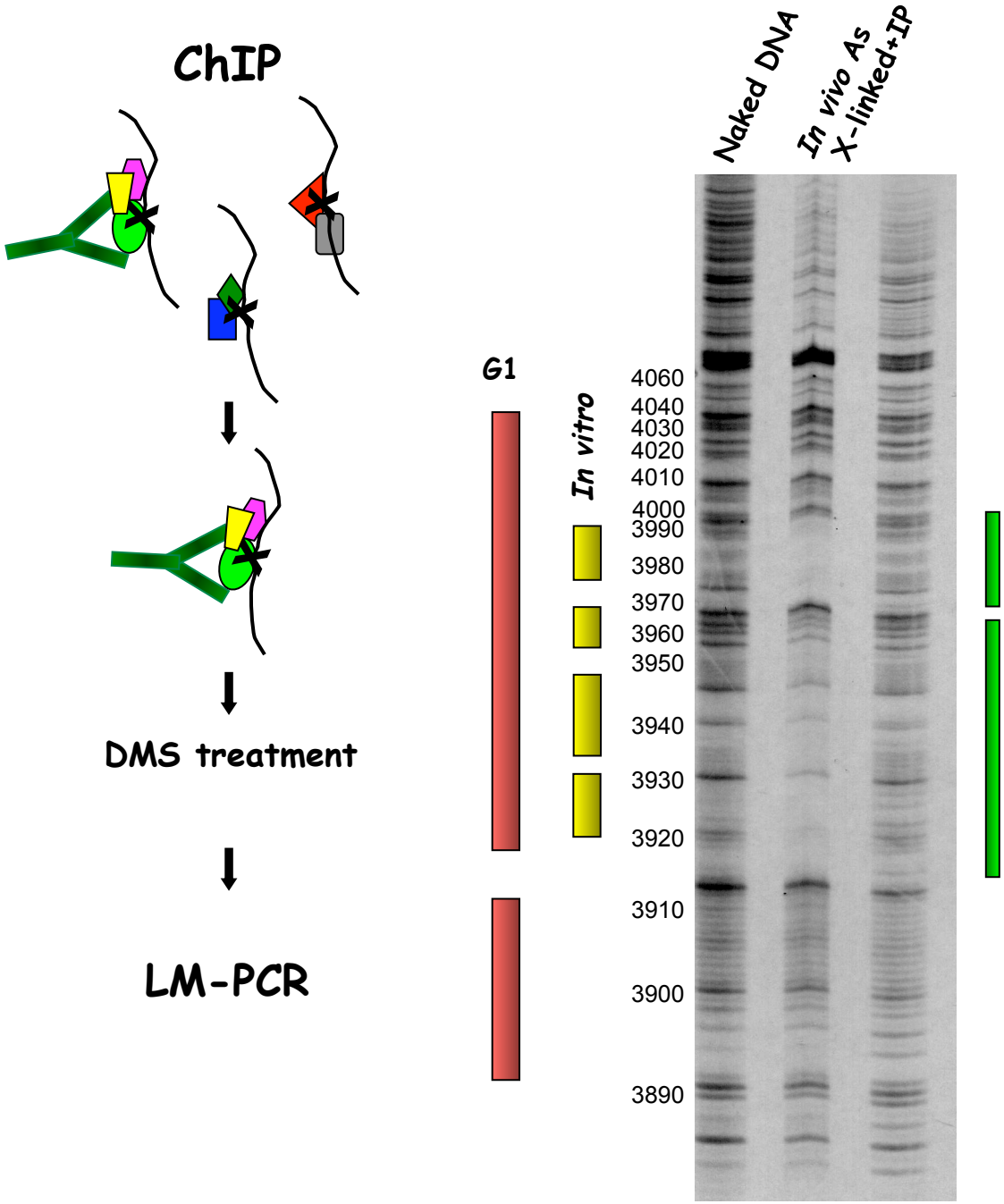


MCM3

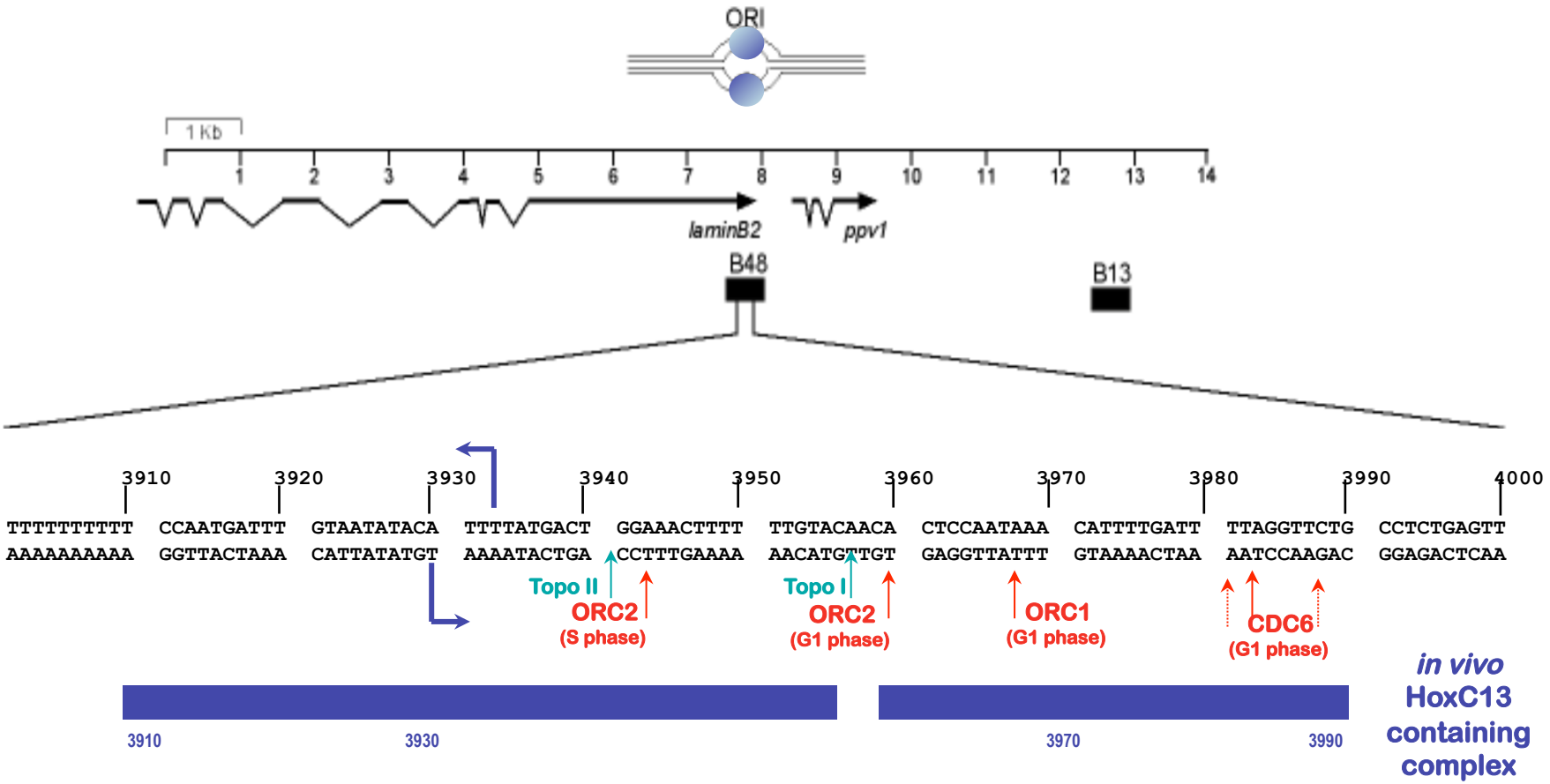


Scale bar 5μm

Is HOXC13 bound to the lamin B2 origin *in vivo*?


ChIP
DMS treatment
LM-PCR
G1
In vitro
Naked DNA
In vivo As
X-linked+IP
4060
4040
4030
4020
4010
4000
3990
3980
3970
3960
3950
3940
3930
3920
3910
3900
3890

Binding sites of different proteins around the origin sites



Conclusions - 3

HOXC13 colocalizes with early S replication foci

HOXC13 interacts with orc2 in living cells, with a maximum of interaction before S phase progression

HOXC13 colocalizes and interacts with cdc6 (probably through its homeodomain) before this is cytoplasm-exported in S phase

HOXC13 is bound *in vivo* at the lamin B2 origin at the G1/S border in the area of the replicative complex

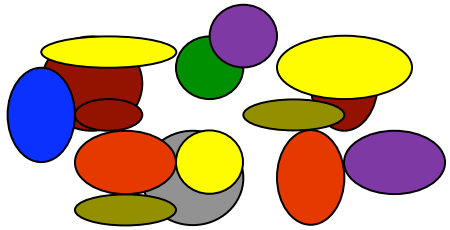
HOXC13 is bound *in vivo* at the TOP1 and MCM4 replication origins

Disruption of origin chromatin structure and topology affects origin function and binding of Cdc6, topoisomerases and HOXC13

HOXC13 is a new member of the replicative complexes

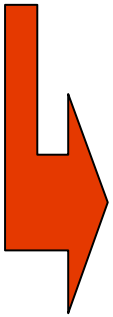
In vitro complex assembly

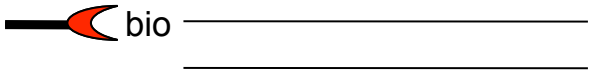
First incubation with:

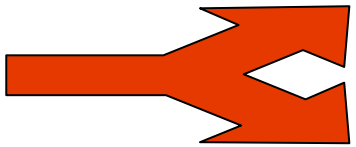


- HeLa nuclear extract;
- Competitor DNA (100 X molar excess over origin DNA).

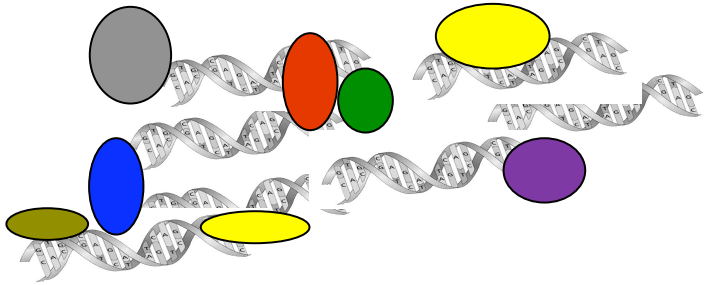
Then incubation with biotinylated origin DNA

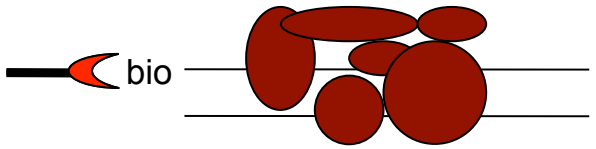




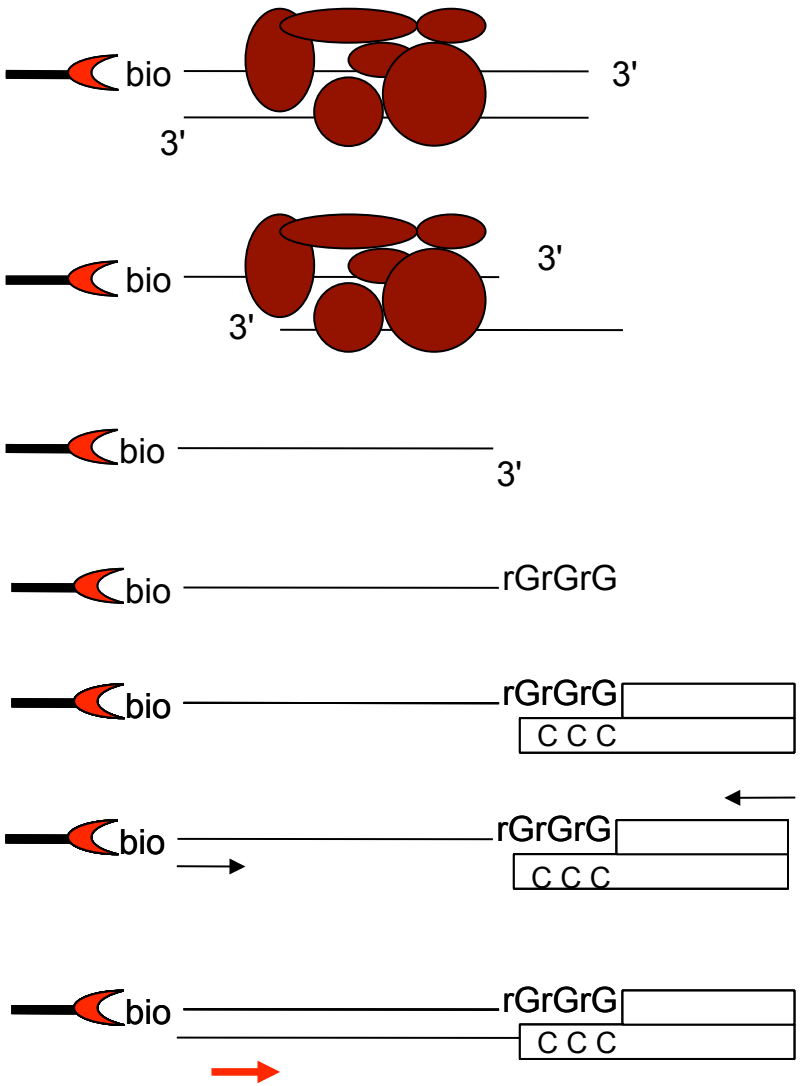


wash





TDPCR for *in vitro* footprinting



- Exo III digestion;
- Proteinase K treatment;
- NaOH treatment;
- Ribo-tailing;
- Linker annealing and ligation;
- PCR amplification;
- Extension with radioactive primer.

Members of the replicative complexes and oncogenesis

- Translocations of the gene for topoisomerase I to the gene for nucleoporin (Nup98) cause AML. (Iwase et al., Genes Chromosomes Cancer *38,* 102-105, 2003).
- Translocations of the gene for topoisomerase II to the gene for nucleoporin (Nup98) cause AML. (Nebral et al., Clin. Cancer Res., *11*, 6489-6494, 2005).
- Translocations of the gene for HOXC13 to the gene for nucleoporin (Nup98) cause AML. La Starza et al., Genes Chromosomes Cancer *36,* 420-423, 2003)
- The c-myc protein is bound *in vivo* to the lamin B2 origin (our data and Dominguez-Sola et al., Nature, *448*, 445-451, 2007)
- The c-fos protein is bound at the lamin b2 origin *in vitro* and *in vivo*

Overall conclusions

1. A 129 bp sequence containing the start site and the sites of binding of the proteins of the replicative complexes is sufficient to confer origin function
2. DNA topoisomerases play an essential dynamic role for origin function
3. Homeotic proteins participate in DNA replication regulation, perhaps coordinating it with the differentiation programme
4. A specific multi-protein complex can be assembled *in vitro* on the lamin B2 origin, resembling the pre-replicative complex of G1, containing ORC2, ORC4, CDC6, Topo II, C-fos,and c-jun
5. Regulation of origin activity involves at least five oncoproteins

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