Packing of monodisperse DNA-RecA protein complexes

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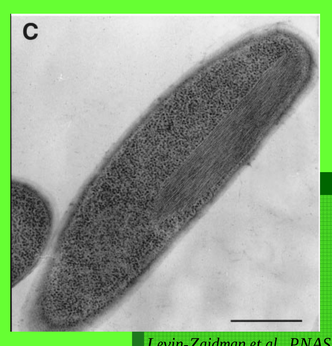
Motivation – Condensed phases are functional structures

• DNA in all living systems: highly condensed and tightly packed (mitotic chromosomes, sperm heads, virus capsids)

• DNA replication, transcription, protection, and repair in highly packed genetic material

• DNA packs by oppositely charged multivalent ions and proteins

Mangenot et al. BPJ2003
Raspaud et al. PRL 2000
Leforestier & Livolant, BPJ1993



Levin-Zaidman et al., PNAS 2000

A different packing: intracellular coaggregation of **RecA protein** and DNA into a macroscopic assembly

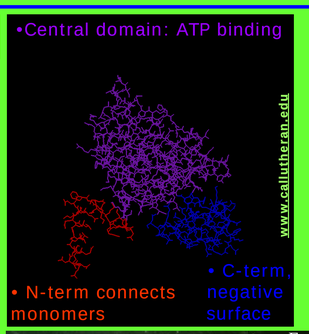
RecA protein overview

• E.coli RecA
• *MW* = 37,842
• 352 amino acid residues
• polymerizes in helices around DNA

• Structure → Function
• Archaea and Eukaryota have homologous proteins

• multirole:
- SOS response *LexA* repressor cleavage

- Promotes homologous genetic recombination *via* DNA strand exchange by forming nucleoprotein filament with free ssDNA ends



Samples & materials

• E. Coli RecA protein: overexpression plasmid pAIR79 in E. Coli strain STL327, described in *Lovett, PNAS1989*

• RecA purification according to *Cox et al. JBC81*

• 146bp DNA fragments of calf-thymus chromatin, enzymatically digested according to *Strzelecka & Rill JACS1987*

• ATP-γ-S, Roche

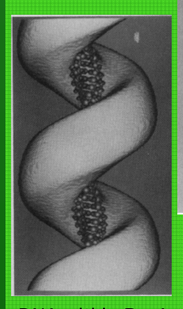
All concentrations determined by spectrophotometry

dsDNA within RecA filament

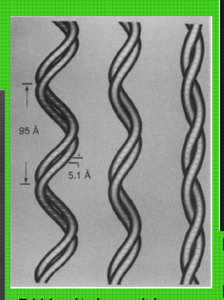
Egelman et al. PNAS'01

RecA + dsDNA
+ATPγS+Mg^{++}
in vivo & *in vitro*
active nucleoprotein filament
pitch=97Å
monomers/turn=6.20
stoichiometry=3bp/monomer

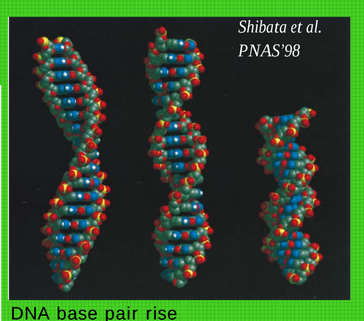
Egelman et al. Science'89



DNA within RecA



DNA pitch and base pair rise fixed

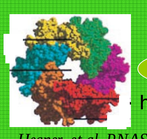


DNA base pair rise
5.1Å 5.1Å 3.4Å
DNA pitch
99 Å 67 Å 34 Å
stoichiometry
3bp/mer 2bp/mer ?!

• How the models correlate with known parameters of RecA+DNA complex, pitch, stoichiometry, monomers per turn?

RecA polymers

• RecA selfpolymers have similar structure as the complexes

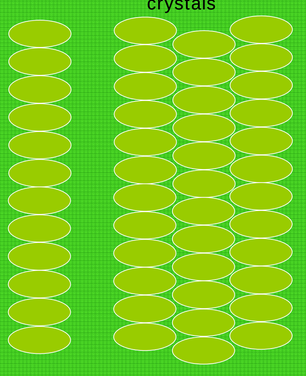


hexamer

Hegner et al. PNAS'99.

selfpolymers

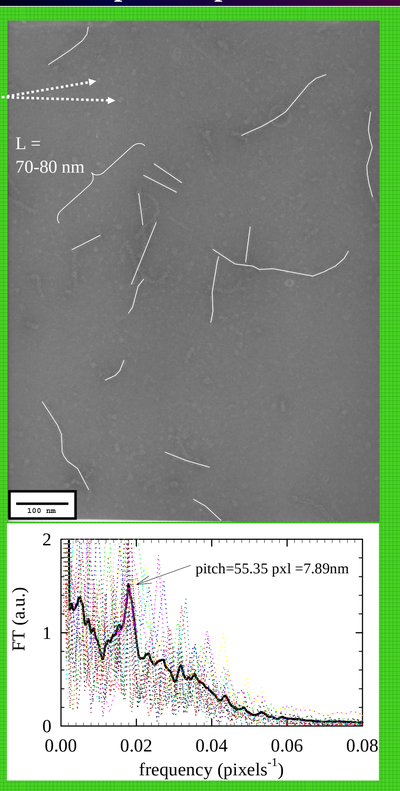
bundles, aggregates, crystals

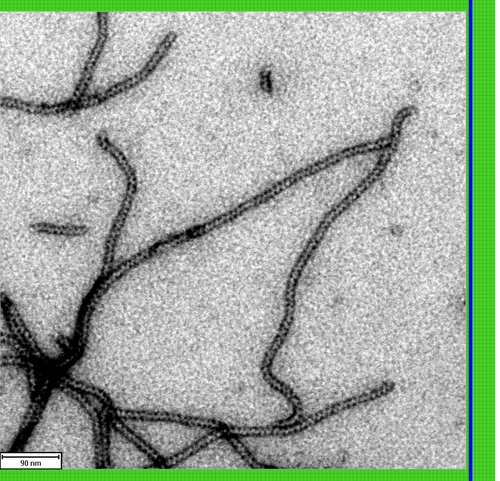


• RecA selfpolymers and aggregates are not intermediates *en route* to nucleoprotein filaments

Morrical&Cox Biochemistry 1985

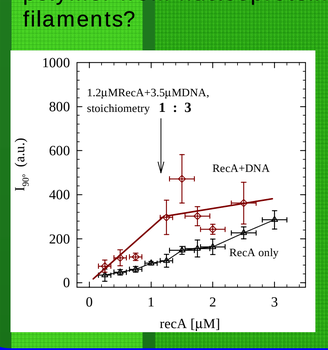
RecA complexation with 146 bp DNA fragments: rodlike nucleoprotein particles and some RecA polymers?



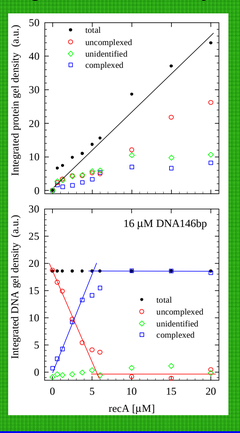


RecA/DNA stoichiometry: 3/1 or 2/1?

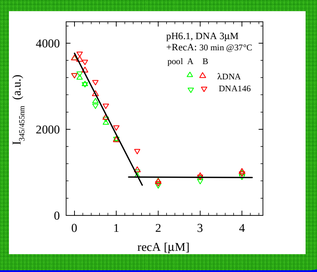
• **90° static light scattering**, is it distinguishing RecA polymer from nucleoprotein filaments?



• gel densitometry



• fluorometry – binding of DAPI fluorophore to uncomplexed DNA



RecA+DNA dense phase formation problem

• besides various liquid crystalline dense phases of DNA only, nucleoprotein dense phase is known, formed by nucleosome core particles and DNA *Mangenot et al . BPJ2003.*

• RecA-only filaments & RecA-DNA complexes: bundles and aggregates compete against liquid crystal *Egelman & Stasiak . JMB 1988. Di Capua et al. JMB 1982*

• a basic function of the system: DNA strand exchange is initiated by elevated Mg, spermidine or by crowding agents *Lavery & Kowalczykowsky . JBC 1992.*

• all these also induce aggregation/bundling of RecA based filaments

• DNA liquid crystals form more easily with monodisperse fragmented DNA

• RecA filaments/complexes have similar helical symmetry as DNA *Leforestier & Livolant BPJ 1993.*

• Short RecA complexes (formed on short DNA) might organize and not just aggregate

Summary

The structure of RecA-DNA complex is not solved, and the exact path of DNA within the nucleoprotein filament is not known, although it has been extensively studied by SANS, electron microscopy or NMR *DiCapua et al. JMB1990; Yu X. et al. PNAS 2001; Nishinaka et al. PNAS1998.*

We form RecA nucleoprotein filaments using very short, monodisperse, 146 bp long DNA. These 75 nm long filaments are shorter than their respective persistence length – i.e. they should behave as straight rods. A system of monodisperse rodlike particles of helicoidal simmetry is capable of forming cholesteric liquid crystalline phase. *Leforestier & Livolant, BPJ1993*

Such a dense phase of RecA-DNA filaments might be the most ordered possible preparation, facilitating structural studies.