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Ordered intracellular assemblies: A last-resort survival strategy

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RecA-mediated DNA recombination and repair processes proceed through several sequential phases. A presynaptic filament in which RecA molecules coat a single-stranded DNA substrate is initially formed. The filament acts then as a sequence-specific DNA-binding entity, capable of searching and binding double-stranded DNA sites that are homologous to the RecA-coated segment. Within the resulting joint species, DNA strand exchange and heteroduplex extension processes are promoted. The mechanism that enables a rapid search for DNA homology in-vivo, within a highly crowded and complex genome, remains enigmatic.

In order to reach its target, any sequence-specific DNA-binding protein must overcome two general obstacles: a minute cellular concentration of the target, and a vast excess of non-target - yet still competitive - DNA sites. The search for a homologous DNA site conducted by the RecA-DNA presynaptic filament shares these hurdles, but is further encumbered by the uniquely adverse diffusion characteristics of its components. A DNA target corresponds to a segment that is part of, and embedded within, the chromosome. This, and the large structural asymmetry of DNA conspire to minimize the diffusion constant of DNA sites. In a homology search executed by the RecA-DNA filament, both the searching and the target entities are chromosomal DNA sites whose small diffusion constants drastically attenuate their encounter rate. How then does a RecA-mediated intracellular search evade the kinetic impediments that are intrinsic to the nature of its components?

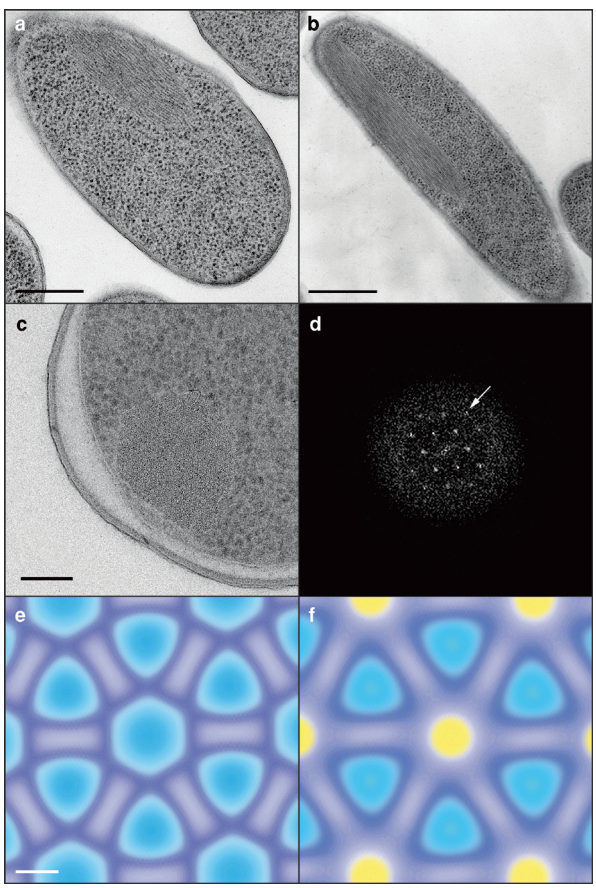

Fig. 1 *DNA-RecA crystals formed in SOS-induced bacteria.*
a, E. coli cells exhibiting a RecA-DNA assembly that is formed following short exposure of the bacteria to DNA-damaging agents which induce the SOS response. The lateral RecA-DNA assembly has been suggested to represent a repairosome, the actual site where RecA-mediated DNA repair activities are promoted. b, A DNA-RecA co-crystalline structure that is formed in wild-type E. coli cells following prolonged exposure to DNA-damaging agents. The tightly packed co-crystal allows for DNA protection by physical sequestration.
c, Cross-sectional view of the DNA-RecA co-crystal shown in panel b.
d, Calculated Fourier transform of the intracellular assembly shown in panel c. Arrow indicates reflection at 47x resolution.
e, Projection map of the RecA crystal obtained in-vitro in the absence of DNA, centered on the z axis of a RecA filament.
f, Density map of the intracellular crystal, derived from the crystalline assembly shown in panel c. The projections shown in e and f are practically identical, apart from one major disparity: at the site of the six-fold axis, a large buildup of density is detected in the intracellular crystal, whereas in the RecA crystal the corresponding region is empty space. The additional density (colored in yellow) is assigned to presence of DNA. Scale bars are 200 nm in a, 500 nm in b, 50 nm in c, and 30 nm in e and f.

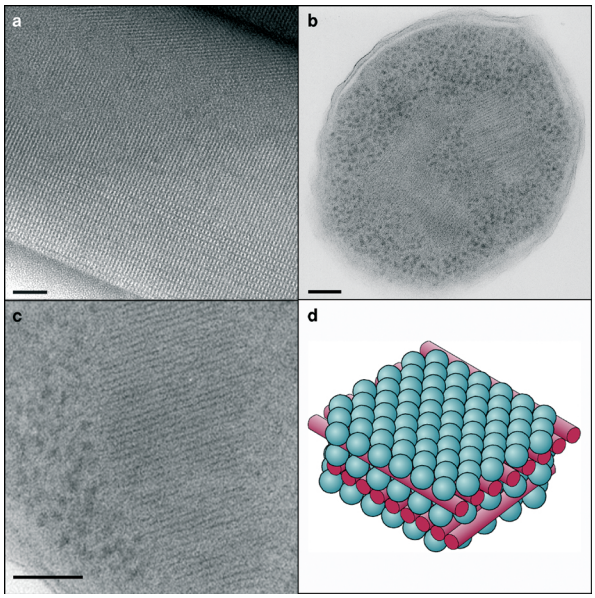

Fig. 2 *a, Electron micrograph of a section of a DNA-Dps co-crystal that is spontaneously obtained following interaction between purified DNA and and the DNA-binding protein Dps.*
b,c, Sections of starved E. coli cells that slightly over-produce the Dps protein. The layered organization are DNA-Dps co-crystals, and the dark particles surrounding the crystals are ribosomes. Starved wild-type bacteria reveal a dense assembly composed of DNA-Dps microcrystals. Scale bars are 40 nm in a, 100 nm in b, and 50 nm in c.
d, A proposed schematic model of the DNA-Dps co-crystal. The Dps dodecamers are depicted as blue spheres and DNA molecules are represented as red rods. The stacked layered structure is consistent with the co-crystal morphology detected within starved bacteria (panels b and c), as well as with the X-ray scattering patterns exhibited by the cells.

We show that damages inflicted upon bacterial DNA lead to a rapid formation of an ordered intracellular assembly that accommodates both RecA and DNA. We suggest that the striated morphology of this RecA-DNA assembly is capable of promoting in-vivo homology search by attenuating both the sampling volume and the dimensionality of the process. Moreover, RecA was shown to protect chromosomal DNA from degradation through unknown mechanisms. The tight crystalline packaging that is progressively assumed by the intracellular RecA-DNA assemblies as DNA damage accumulates, is proposed to confer efficient DNA protection through physical sequestration. An intriguing progression of a structure-function correlation is thus indicated. A dynamic RecA-DNA "repairosome" assembly whose loose longitudinal organization allows for translational motion and hence for repair processes is initially formed. Since these repair processes are heavily ATP-dependent, intracellular energy sources are rapidly and progressively depleted, resulting in an attenuated efficiency of "conventional", enzymatically-based repair. At this stage, the active "repairosome" is progressively transformed into a "real" crystal, in which DNA is structurally sequestered and physically protected.

Formation of intracellular crystalline assemblies in which protection of vital cellular components is provided through physical sequestration has been shown to occur in bacteria exposed to various severe and prolonged assaults. We have shown that when bacteria are starved, a starvation-induced DNA-binding protein called Dps is expressed. Upon binding to DNA, Dps-DNA co-crystals are formed within which DNA molecules are protected. Biocrystallization can therefore be considered as a generic "last-resort"survival strategy, which is deployed when cellular energy pools are depleted.

Selected Publications

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