Order in stress – Lessons from the inanimate world

Life can be considered as an outcome of complex molecular processes which result in the existence of a thermodynamically ordered, far from equilibrium state. The maintenance of this order in living systems is energy-dependent and follows the basic laws of physics and chemistry. However, at times of stress, when the energy pool of a cell goes down, maintaining dynamic order becomes difficult. In such a situation, how can the equilibrium imposed by the second law of thermodynamics be avoided? Can there be order in stress (Minsky *et al* 2002)?

Highly ordered intracellular assemblies have been found in cells subjected to prolonged stress. In this process, termed as biocrystallization, vital components of the cell, like DNA, are physically sequestered and protected. These intracellular assemblies are thermodynamically stable and are independent of energy, reminiscent of ordered states in the inanimate world such as crystals and liquid crystals, both of which correspond to free energy minima of the system.

The first report of biocrystallization of DNA appeared in 1999 (Wolf *et al* 1999). It was shown that DNA, along with a stress-induced protein (Dps) of *Escherichia coli*, can form biocrystals both *in vitro* and *in vivo* (figure 1). The functional relevance of this observation was shown two years later (Frenkiel-Krispin *et al* 2001) when it was found that DNA-Dps biocrystallization was a growth phase-dependent phenomenon, and was associated with prolonged starvation (2 day-old culture). Overexpression of Dps in the log phase did not result in its co-crystallization with DNA. Divalent cations were shown to trigger the process. Elevated concentrations of Mg^{2+} ions not only prevented co-crystallization, but also caused the pre-existing ordered complexes to fall apart, showing the reversible nature of this biological process. The protection of DNA in a DNA-Dps co-crystal was dependent on Mg^{2+} ions *in vitro*. It was proposed that extracellular divalent cations provided an on-off signal for intracellular DNA-Dps co-crystallization; the cations were known to be depleted upon prolonged starvation. The presence of Dps homologs like ferritin (Grant *et al* 1998) in other organisms suggests that this might be a conserved protective mechanism. Frenkiel-Krispin *et al* (2001) also showed that an alternative mechanism of DNA protection existed in *dps*$^{-}$ mutants. Upon prolonged stress (6 day-old culture), this mutant strain of *E. coli* showed DNA arranged in an ordered liquid-crystal phase known as the cholesteric phase (figure 2). Pure DNA is known to be in a highly ordered state, namely a

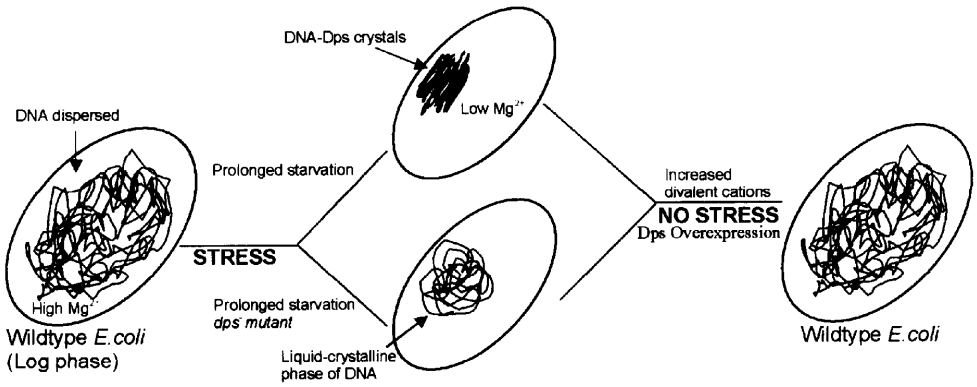

Figure 1. Model for DNA-Dps cocrystallization.

liquid-crystalline phase, at very high concentrations (Livolant 1991; Livolant and Leforestier 1996; Reich *et al* 1994). The liquid crystalline phase is known to reduce the accessibility of DNA to irradiation, radicals and nucleases (Newton *et al* 1996).

In mid-log phase *E. coli* cells treated with nalidixic acid (which effects double-strand breaks by stalling the activity of DNA gyrase), ordered RecA-DNA assemblies are seen (Levin-Zaidman *et al* 2000). The periodic order exhibited by RecA-DNA assemblies is high enough to allow crystallographic averaging (47 Å). It has been proposed that ordered assemblies accelerate RecA-mediated homologous repairing of DNA double-strand breaks by restricting the diffusion of DNA and reducing the dimensionality of the process (Adam and Delbruck 1968). It is hypothesized that RecA searches for the homologous template through one-dimensional motion that is enforced by parallel organization of the RecA-DNA assembly. Therefore, cocrystallization could protect DNA from further damage (i) by limiting its accessibility and (ii) by helping RecA to correct the damage more efficiently by reducing its search area for a homologous DNA template. Upon unstressed incubation, the *E. coli* cells revert back to the wild-type phenotype and the ordered assemblies disappear rapidly.

These reports suggest that bacteria survive harsh environmental conditions by sequestering DNA using minimum energy and subsequently reverse this process on return to favourable conditions. The DNA molecule is known to be less active (no duplication and little transcription) in the condensed form of chromatin. This is in accordance with the fact that compaction of DNA is in response to stress, which might lead to a reduction in the energy pool of the cell. Hence the cell minimizes the use of energy by reducing energy-consuming processes. The B form of DNA is maintained in the cholesteric liquid-crystalline phase *in vitro* as well as *in vivo* (Livolant 1991).

Are there examples of organisms that encounter harsh environmental conditions often and use this protection strategy over much of their lifetime? In a recent report, a similar protection mechanism was shown to be operating continuously in *Deinococcus radiodurans* (Levin-Zaidman *et al* 2003). This Gram-positive non-sporulating bacterium survives very high doses of ionizing radiations (~ 15000 Grays); much lesser doses (~ 10 Grays) are lethal to other organisms. The resistance to ionizing radiation is not attributed to highly efficient repair enzymes because the organism possesses a 'typical' bacterial complement of these enzymes. However, *D. radiodurans* has DNA arranged in an extremely ordered manner, shaped like a toroid (figure 3). Interestingly, *recA*-deficient *D. radiodurans* cells are capable of correcting a significant amount of damage. It was hypothesized that a non-RecA-dependent homologous recombination repair pathway is functional in the organism, aided by the precise alignment of templates in the toroidal form of DNA (Englander *et al* 2004). The presence of an X family DNA polymerase, $PolX_{Dr}$ being associated with the repair of double-stranded DNA breaks, suggests that non-RecA-dependent repair corrects lesions while being aided by the compact DNA structure (Englander *et al* 2004; Lecointe *et al* 2004).

Making spores is a strategy employed by various organisms to tide over unfavourable conditions. A toroidal DNA form was seen in *B. megaterium* spores (Ragkousi *et al* 2000). The DNA-SspC complex

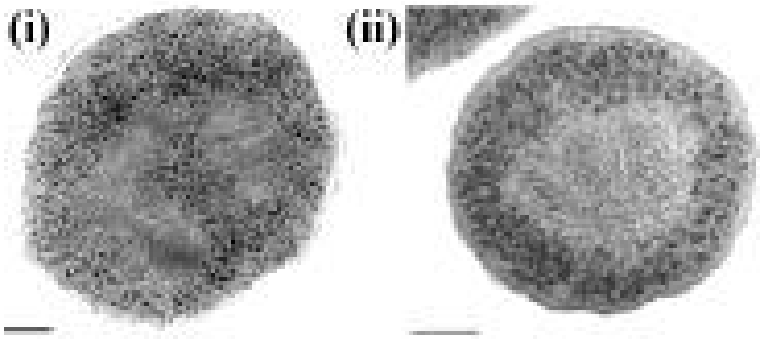

Figure 2. *In situ* DNA-Dps assemblies. (i) Crystalline assemblies in starved (48 h) wildtype *E. coli*. (ii) Liquid-crystalline phase of DNA seen in *dps*$^{-}$ mutant of *E. coli* after prolonged incubation (6 days). (Courtesy Minsky A).

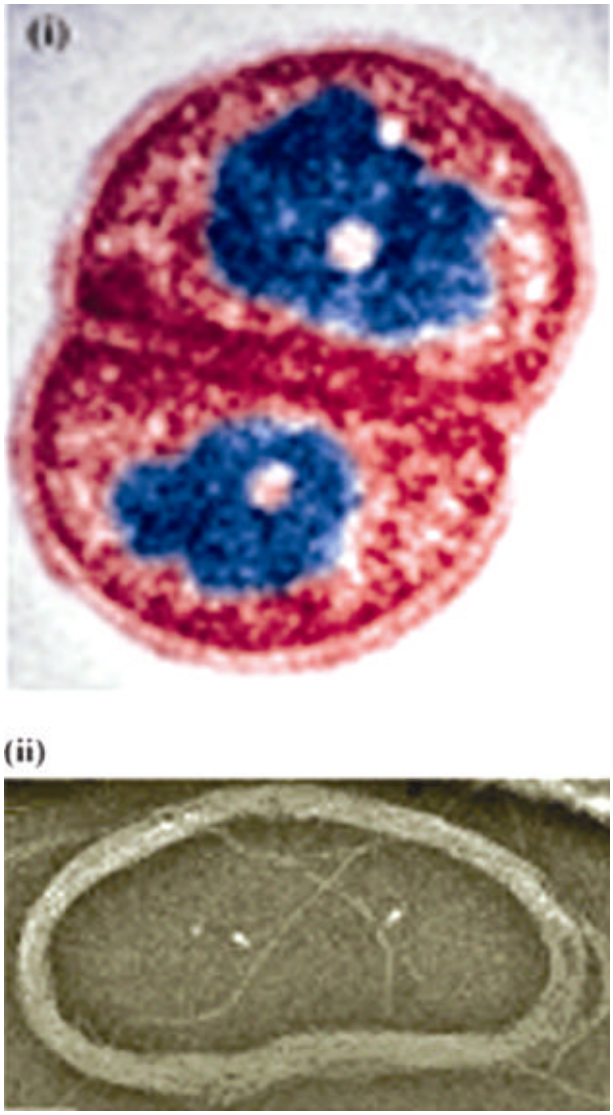

Figure 3. Toroidal DNA shape. (i) Colour-processed transmission electron micrograph of a cryofixed *D. radiopugnans* cell, depicting the toroid-like organization of its chromatin (blue). (ii) DNA-SspC complex seen in the form of a toroid. (Courtesy Minsky A).

(α/b type small, acid-soluble spore proteins) is said to assume a toroidal shape *in vitro* (figure 3); this, along with the *in vivo* data, could explain the robust nature of spores (Englander *et al* 2004; Frenkiel-Krispin *et al* 2004). Also, the nucleic acids in bacteriophage T7 has been shown to be tightly packaged in toroids (Cerritelli *et al* 1997).

Apart from DNA, ribosome crystals have been reported earlier. Large, crystalline assemblies of ribosomes are formed in mouse and lizard oocytes during winter hibernation (Barbieri *et al* 1995). The Hirano-bodies found in brain cells of a patient with Alzheimer's disease were found to be ribosome crystals (O'Brien *et al* 1980). These reports need to be further confirmed in order to conclusively prove that the compaction of vital cellular components is common (not only specific to DNA), and to make a strong case for biocrystallization as a general survival strategy used by different organisms. The adaptive value of such a mechanism is evident; given that crystallization is an inherent property of matter, it would be worth exploring whether natural selection has a role to play in the phenomenon.

The crystalline form, which was considered not long ago a property of the inanimate world, now seems to be an important and sometimes essential part of the animate world.

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