Making bacteriophage DNA into a movie for panspermia

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0.0 Abstract

To satisfy the urge to communicate with another species, distant from our own in space or time, we explore the advantages of using the nucleic acid within a bacteriophage to encode a message and suggest how this might be achieved. We list some of the technical difficulties that need to be overcome and describe some of the advantages as a message-bearing medium that phage such as T5 possess. These advantages include those of stability in certain environments and DNA packed in a regular way within the capsid. We raise questions that would need to be answered and that would require close collaborations across the disciplines.

Keywords:

code, extraterrestrial, Kilroy, nucleic acid, virus, film

Bacteriophage DNA as a movie for panspermia

1.0 Introduction

The urge to communicate is hard-wired into many species. In humans, it is attested to by the outpourings of businessmen, scientists and artists and by the writings on the subway walls. This urge is heightened by awareness of the likely ephemeral nature of our tribal species which, in being selected for dominance is also selected for long-term self-destruction. To satisfy this urge, we have proposed a Kilroy project (Norris and Grondin, 2011) that would revisit the Pioneer space missions which contained information about our species on the surface of metallic plaques (Sagan *et al.*, 1972). There are, however, several differences with this earlier initiative. First, we propose devising a message that could be not just sent to an alien intelligence on some distant planet but also left here on our own planet for the terrestrial intelligence that succeeds Man. Second, we propose writing this message into the DNA of an organism so that it could benefit from the capacity of biological systems to survive and multiply. Third, we propose a strategy that would depend on rich interdisciplinary collaborations and that would help advance the diverse fields of synthetic biology, microbiology, biophysics and exobiology. In the previous proposal, we advocated constructing a bacterium, *Escherichia nuntius*, as the medium for the message. Here, we explore the advantages and disadvantages of writing the message into the nucleic acid sequence contained in the head of a bacteriophage.

2.0 The phage project

The idea is to encode a message into the DNA of a bacteriophage in a way that is sufficiently evident for it to be decoded. One possibility would be to exploit and, if necessary develop further, its periodic features (Figure 1). A periodic marker could be used to indicate, for example, the end of a line. If each base (or a specific sequence of bases such as GATC) were then encoded by a different colour a pattern could be generated (Figure 1). [Insert Figure 1 Here]

Finally, depending on the length of the DNA and its periodicities, yet another possibility would be to construct a 2-D matrix, which would make patterns easier to both detect and interpret. This might be taken still further by defining a series of separate frames and making a movie (see Figure 2, adapted from (Norris and Grondin, 2011)). [Insert Figure 2 Here]

3.0 Outstanding technical questions

There are many aspects to the interdisciplinary project that require our present understanding to be advanced before they can be exploited. Before beginning the process of encoding, a better understanding is needed of the principles and mechanisms of packing DNA (or indeed RNA) into the heads of phage. What controls the shape and nature of the toroid that this DNA is believed to adopt? Is it indeed largely determined by DNA-DNA interactions? What is the relative contribution of associations with polyamines such as spermine and spermidine, with proteins, and with ions? Is the transition via a cholesteric phase also important? What is the role of the capsid itself? Above all, what are the roles of DNA sequences? To what extent, do interactions between similar or identical sequences determine their association? Which proteins and polycations bind, and at what stage, in assembly and thereafter? Do these proteins associate with one another? How stable are phage in the wild and how might this

stability be improved? Would recombination necessarily scramble a message? In the following sections, we briefly summarise current understanding.

4.0 The advantages of using phage

4.1 Stability

In some environments, bacteriophage are very stable. They can be extremely heat resistant and survive pasteurization treatments such that, for example, 20 minutes at 88°C is needed to destroy 10^8 phage per ml in whey cream (for references see (Johnson *et al.*, 1997)) whilst others have survived over 2 hours in the bloodstream of mice (Cerveny et al., 2002). Their stability is one reason for the number of bacteriophage in the world (estimated at over 10^{30} bacteriophage in the world as against around 10^{30} bacteria (Clokie et al., 2011; Suttle, 2007; Whitman et al., 1998; Wommack and Colwell, 2000).

4.2 Regularity of DNA structure

An evident periodicity would be helpful in decoding certain types of message. Here we confine ourselves to discussion of the T5 dsDNA tailed phage, which belongs to the *Caudovirales* family, and about which much is known (note that similar phages are found in Archaea). In this phage, a 121750 bp dsDNA is packed into a 90 nm wide icosahedral capsid. The orientation of the toroid in the capsid is independent of the portal complex location and of the icosahedral symmetries of the protein capsid. In experiments in which varying lengths of DNA were packed into the head of this phage and then unpacked, it was found that the DNA bundle was twisted, and the toroid formed a series of constrained hexagonal domains

separated by twist walls (see Figure 3); each helix is correlated to the others and strongly curved in the toroid, which imposes variations on the DNA helical pitch (Leforestier and Livolant, 2009). [Insert Figure 3 Here]

Periodicity may be imposed by the constraint of packing the DNA into a container (the capsid) of fixed shape and size. Given the 40 nm internal radius of the T5 capsid, each line of the message could contain around 100 base pairs. It may, however, prove possible to increase this radius (see below).

4.3 Mutational inertia

An economical use of the code is needed to minimise the probability of mutation, recombination and rearrangement leading to viable phage. In general, a sequence that has more than one essential function is less likely to undergo alteration than one that only has one function as in the case of an enzyme that must interact with several partners (this is the case of ribosomal RNA and ribosomal proteins in which mutations risk of disrupting more than one vital interaction). Multi-functionality can also result from the overlapping of genes and in particular when this overlapping is substantial and on both strands (i.e., antiparallel overlapping) (Johnson and Chisholm, 2004). It has been argued that overlapping is maintained by the selection of having to pack a lot of information into an icosahedral head of limited size (Chirico *et al.*, 2010). Hence, an increased selective pressure to maintain overlapping might be achieved by incorporating functions into the phage DNA that are needed by – but absent from – their hosts, as in certain cyanophage, so that they complement one another (Alperovitch-Lavy et al., 2011; Monier et al., 2009; Thompson et al., 2011). In this case, the project might entail dispatching the phage and their bacterial hosts together (see below).

4.4 Phage can contain a lot of DNA

The quantity of DNA within the phage limits the information that can be sent. Contrary to what was once thought, it is now known that DNA viruses can contain even more DNA than some bacteria. The icosahedral *Mimivirus*, for example, contains nearly 1.2 Mb (Raoult *et al.*, 2004) whilst *Megavirus chilensis* contains still more (Arslan *et al.*, 2011).

4.5 Terraforming

It is conceivable that a terraforming motivation may lead to the dispatch of terrestrial organisms in a deliberate panspermia. Without wishing to question here the ethics of such an initiative, a promising strategy might involve sending both bacteria (possibly cyanobacteria) and their phage. This is based on the idea that a representative sample of the super-organism that constitutes the entire terrestrial population of interacting bacteria and phage (Mathieu and Sonea, 1995) may be particularly effective in starting life elsewhere as, we have proposed, it was here (Norris *et al.*, 2007b). In this context, it should be noted that the evidence for genetic exchange among phage and bacterial communities, which supports this proposal, continues to accumulate (Hambly and Suttle, 2005).

4.6 Likelihood of being sequenced

The message will not be read if the phage DNA is not sequenced. To draw attention to the phage, they might be included within some detectable evidently alien artefact such as a container inside a space probe or a metal object on the surface of a comet. Alternatively, if they are part of the seed sent in a terraforming initiative, it might be hoped they and their

message would both last and multiply to the extent that they would be revealed when the aliens reinvented Metagenomics.

5.0 Design questions

5.1 How can periodicity be enhanced and exploited?

Answers to this question depend on the nature of what is inside the capsid and what the interactions are. One might imagine that DNA-binding proteins in the space at the centre of the toroid (the jam in the centre of the doughnut) might be designed to bind to specific, regularly spaced, sequences in the DNA on the inner surface of the toroid. Alternatively, the capsid protein might be modified to bind to specific, regularly spaced, sequences in the DNA on the outer surface of the toroid. During the unpacking of DNA from the capsid *in vitro*, the DNA goes through a cholesteric phase. If this phase turns out to be important in the life of the phage (for example, in packing or infection), sequences might be introduced that are known to favour the formation of curved DNA and to influence this phase or indeed the dimensions of the toroid itself (Conwell *et al.*, 2003).

A different way to enhance periodicity would be to exploit the homologous pairing of two double-stranded DNA molecules that occurs *in vitro* especially since it has been proposed that direct recognition of homology between chemically intact B-DNA molecules should be possible *in vivo* (Baldwin et al., 2008; Danilowicz et al., 2009). The many possible explanations (Cortini et al., 2011; Wang et al., 2010), which invoke to some extent the primary nucleotide sequence, include, for example, sequence-dependent base-flipping (Inoue et al., 2007; Randall et al., 2009) and braiding. The latter refers to any winding of two DNA molecules around each other driven by chiral electrostatic interactions and may explain

homologous pairing (Cortini et al., 2011). Recent pairing experiments with DNA containing homologous and non-homologous regions are consistent with a sequence-dependent, long-range interaction and a sequence-independent, short-range interactions (Mara Prentiss and Nancy Kleckner, personal communication). Such mechanisms might help keep regions of the phage genome together when they are packed into the capsid and so generate a reproducible periodicity.

5.2 Could the host range be expanded?

It has been pointed out recently that 'we have little understanding of what the precise host range for any given phage is or whether there are universal patterns or principles governing the set of viruses able to infect a given bacterium and the set of bacteria that a given virus can infect. This deficit is unfortunate given that phage–bacterial interactions are important for both human health and ecosystem function' (Flores *et al.*, 2011). The evolution of interactions between phage and their hosts is rich and subtle (Hyman and Abedon, 2010; Poullain et al., 2008) and the task of creating a phage to last essentially unchanged over billions of years may be utopic. That said, the attempt itself should prove instructive; for example, is it possible for phage to deliver proteins during infection to help the survival of their hosts and, if so, which ones would be most useful?

5.3 Can the length of the DNA in a phage be increased indefinitely?

A minimum of 60 copies of the capsid protein is needed to make an icosahedral particle; bigger capsids can be made in which the total number of capsid proteins is a multiple of 60 (Caspar and Klug, 1962). What is the largest phage with (and possibly without) an icosahedral

head that might be made? Would the limitation on the maximum size of the phage actually be imposed by the capacity of the bacterium to make it? .

6.0 What goes into the message?

The message intentionally carried by Pioneer on its anodised aluminium plaques included information about the size and shape of a man and woman, the hyperfine transition of hydrogen, the binary code and positioning our Sun relative the centre of our galaxy and to 14 pulsars (Sagan et al., 1972). A future discussion of what information should be sent via phage might take into account the medium itself. For example, a set of different phage and bacteria might be sent containing information to allow self-assembly into some very different organism, or even a *bioputer*, assuming that synthetic biology ever advances far enough (Norris and Grondin, 2011; Norris et al., 2011).

7.0 Discussion

There is no consensus about the origins of life. We have hypothesized that networks of non-covalent assemblies (or composomes) of molecules, rather than individual protocells, evolved under the constraints of molecular complementarity (Hunding et al., 2006; Norris et al., 2007b; Norris and Root-Bernstein, 2009). These composomes then evolved into the hyperstructures of modern bacteria (Norris *et al.*, 2007a). Our 'ecosystems-first' hypothesis does not in itself exclude the possibility that life throughout the cosmos could also be spread by panspermia (Wickramasinghe, 2009) in a way analogous to the evolution of bacterial life on our planet which depends on both horizontal and vertical transmission. The project we have proposed here and elsewhere (Norris and Grondin, 2011) may be seen as intentional

panspermia whose probability of success may be subject to (or immune to) some of the criticisms levelled at panspermia itself. The project is, however, essentially about a one-way communication between our species and another *reader* species. This reader species may exist on our own planet in some distant future or it may encounter a space probe (or a comet) containing our message during its own exploration of space. In neither of these cases is panspermia really relevant. It would start to become relevant in the context of terraforming if we were to incorporate a message into the DNA of the material sent to seed life elsewhere. It would become fully relevant if a message were actually (and amazingly) discovered in some modern bacterium or phage.

A project to construct phage or bacteria (or both) would bring benefits in addition to creating the chance of communicating with other species distant in time and/or space. It would promote creativity and interdisciplinarity in many different fields including biotechnology, medicine, biophysics, cryptology, computer science, geology and astrophysics. For example, by bringing together synthetic biology and the physics of packing DNA into viruses and phage, the project could allow origins-of-life studies both to make a contribution to phage therapy, phage affinity and virology and, reciprocally, to benefit from them.

We have skirted here the question of what should go in any message as well as the related question of whether our species has anything worth saying to a reader species worth listening to it. Of course, a deliberate message of a scientific nature has already been sent on the Pioneer 10 and 11 missions. If the nature of the medium is to influence the nature of the message a DNA-based message might ultimately be devised to have a message that depends on biology, for example, in the patterns of colonies or even in the operation of a bioputer. Alternatively, biology might be married to art, as others have imagined (King and Angus,

1996; Ohno, 1987; Sanchez Sousa et al., 2005), and the message might encode music, in which case, we risk being spoilt for choice with candidates including the Beatles 'All you need is love', the Stones 'Sympathy for the devil', Nirvana's 'Come as you are', Jon Norris's 'The Crossing' and Mozart's Requiem.

7.0 References

Alperovitch-Lavy, A., Sharon, I., Rohwer, F., Aro, E.M., Glaser, F., Milo, R., Nelson, N., and Beja, O. (2011). Reconstructing a puzzle: existence of cyanophages containing both photosystem-I and photosystem-II gene suites inferred from oceanic metagenomic datasets. Environ Microbiol 13, 24-32.

Arslan, D., Legendre, M., Seltzer, V., Abergel, C., and Claverie, J.M. (2011). Distant Mimivirus relative with a larger genome highlights the fundamental features of Megaviridae. Proc Natl Acad Sci U S A 108, 17486-17491.

Baldwin, G.S., Brooks, N.J., Robson, R.E., Wynveen, A., Goldar, A., Leikin, S., Seddon, J.M., and Kornyshev, A.A. (2008). DNA double helices recognize mutual sequence homology in a protein free environment. J Phys Chem B 112, 1060-1064.

Caspar, D.L., and Klug, A. (1962). Physical principles in the construction of regular viruses. Cold Spring Harbor symposia on quantitative biology 27, 1-24.

Cerveny, K.E., DePaola, A., Duckworth, D.H., and Gulig, P.A. (2002). Phage therapy of local and systemic disease caused by Vibrio vulnificus in iron-dextran-treated mice. Infect Immun 70, 6251-6262.

Chirico, N., Vianelli, A., and Belshaw, R. (2010). Why genes overlap in viruses. Proc Biol Sci 277, 3809-3817.

Clokie, M.R., Millard, A.D., Letarov, A.V., and Heaphy, S. (2011). Phages in nature. Bacteriophage 1, 31-45.

Conwell, C.C., Vilfan, I.D., and Hud, N.V. (2003). Controlling the size of nanoscale toroidal DNA condensates with static curvature and ionic strength. Proc Natl Acad Sci U S A 100, 9296-9301.

Cortini, R., Kornyshev, A.A., Lee, D.J., and Leikin, S. (2011). Electrostatic braiding and homologous pairing of DNA double helices. Biophysical journal 101, 875-884.

Danilowicz, C., Lee, C.H., Kim, K., Hatch, K., Coljee, V.W., Kleckner, N., and Prentiss, M. (2009). Single molecule detection of direct, homologous, DNA/DNA pairing. Proc Natl Acad Sci U S A 106, 19824-19829.

Flores, C.O., Meyer, J.R., Valverde, S., Farr, L., and Weitz, J.S. (2011). Statistical structure of host-phage interactions. Proc Natl Acad Sci U S A 108, E288-297.

Hambly, E., and Suttle, C.A. (2005). The viriosphere, diversity, and genetic exchange within phage communities. Current opinion in microbiology 8, 444-450.

Hunding, A., Kepes, F., Lancet, D., Minsky, A., Norris, V., Raine, D., Sriram, K., and Root-Bernstein, R. (2006). Compositional complementarity and prebiotic ecology in the origin of life. Bioessays 28, 399-412.

Hyman, P., and Abedon, S.T. (2010). Bacteriophage host range and bacterial resistance. Adv Appl Microbiol 70, 217-248.

Inoue, S., Sugiyama, S., Travers, A.A., and Ohyama, T. (2007). Self-assembly of double-stranded DNA molecules at nanomolar concentrations. Biochemistry 46, 164-171.

Johnson, M.J., Bradley Jr., R.L., and Wendorff, W.L. (1997). Efficient use of whey cream in cheesemaking. In UW Diary Alert, U.o. Wisconsin, ed. (Madison, College of Agricultural and Life Sciences), pp. 1-7.

Johnson, Z.I., and Chisholm, S.W. (2004). Properties of overlapping genes are conserved across microbial genomes. Genome Res 14, 2268-2272.

King, R.D., and Angus, C.G. (1996). PM--protein music. Comput Appl Biosci 12, 251-252.

Leforestier, A., and Livolant, F. (2009). Structure of toroidal DNA collapsed inside the phage capsid. Proc Natl Acad Sci U S A 106, 9157-9162.

Mathieu, L.G., and Sonea, S. (1995). A powerful bacterial world. Endeavour 19, 112-117.

Monier, A., Pagarete, A., de Vargas, C., Allen, M.J., Read, B., Claverie, J.M., and Ogata, H. (2009). Horizontal gene transfer of an entire metabolic pathway between a eukaryotic alga and its DNA virus. Genome Res 19, 1441-1449.

Norris, V., Blaauwen, T.D., Doi, R.H., Harshey, R.M., Janniere, L., Jimenez-Sanchez, A., Jin, D.J., Levin, P.A., Mileykovskaya, E., Minsky, A., et al. (2007a). Toward a Hyperstructure Taxonomy. Annual review of microbiology 61, 309-329.

Norris, V., and Grondin, Y. (2011). DNA movies and panspermia. Life 1, 9-18.

Norris, V., Hunding, A., Kepes, F., Lancet, D., Minsky, A., Raine, D., Root-Bernstein, R., and Sriram, K. (2007b). Question 7: the first units of life were not simple cells. Orig Life Evol Biosph 37, 429-432.

Norris, V., and Root-Bernstein, R. (2009). The eukaryotic cell originated in the integration and redistribution of hyperstructures from communities of prokaryotic cells based on molecular complementarity. International journal of molecular sciences 10, 2611-2632.

Norris, V., Zemirline, A., Amar, P., Audinot, J.N., Ballet, P., Ben-Jacob, E., Bernot, G., Beslon, G., Cabin, A., Fanchon, E., et al. (2011). Computing with bacterial constituents, cells and populations: from bioputing to bactoputing. Theory Biosci 130, 211-228.

Ohno, S. (1987). Repetition as the essence of life on this earth: music and genes. Haematol Blood Transfus 31, 511-518.

Poullain, V., Gandon, S., Brockhurst, M.A., Buckling, A., and Hochberg, M.E. (2008). The evolution of specificity in evolving and coevolving antagonistic interactions between a bacteria and its phage. Evolution 62, 1-11.

Randall, G.L., Zechiedrich, L., and Pettitt, B.M. (2009). In the absence of writhe, DNA relieves torsional stress with localized, sequence-dependent structural failure to preserve B-form. Nucleic Acids Res 37, 5568-5577.

Raoult, D., Audic, S., Robert, C., Abergel, C., Renesto, P., Ogata, H., La Scola, B., Suzan, M., and Claverie, J.M. (2004). The 1.2-megabase genome sequence of Mimivirus. Science (New York, NY 306, 1344-1350.

Sagan, C., Sagan, L.S., and Drake, F. (1972). A message from Earth. Science (New York, NY 175, 881-884.

Sanchez Sousa, A., Baquero, F., and Nombela, C. (2005). The making of "The Genoma Music." Rev Iberoam Micol 22, 242-248.

Suttle, C.A. (2007). Marine viruses--major players in the global ecosystem. Nat Rev Microbiol 5, 801-812.

Thompson, L.R., Zeng, Q., Kelly, L., Huang, K.H., Singer, A.U., Stubbe, J., and Chisholm, S.W. (2011). Phage auxiliary metabolic genes and the redirection of cyanobacterial host carbon metabolism. Proc Natl Acad Sci U S A 108, E757-764.

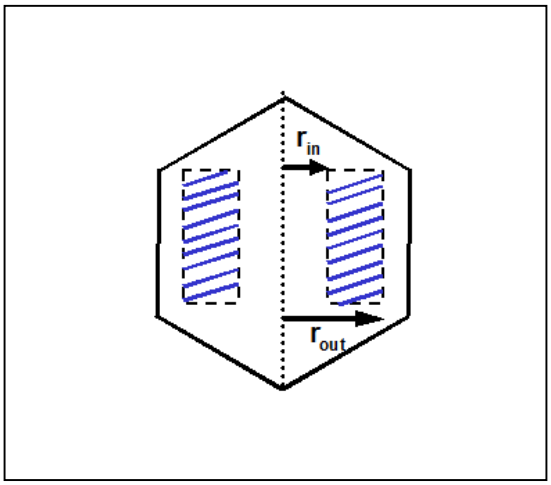
Wang, X., Zhang, X., Mao, C., and Seeman, N.C. (2010). Double-stranded DNA homology produces a physical signature. Proc Natl Acad Sci U S A 107, 12547-12552.

Whitman, W.B., Coleman, D.C., and Wiebe, W.J. (1998). Prokaryotes: the unseen majority. Proceedings of the National Academy of Science USA 95, 6578–6583.

Wickramasinghe, C. (2009). Life from space - astrobiology and panspermia. The Biochemist 31, 40-44.

Wommack, K.E., and Colwell, R.R. (2000). Virioplankton: viruses in aquatic ecosystems. Microbiology and Molecular Biology Reviews 64, 69-114.

Figure 1 Encoding a message in bacteriophage DNA. A. The length of each line of sequence corresponds to the length that the sequence would take within the toroidal structure of the DNA within the head of the phage (where the length is defined equal to the diameter of the toroid r_{out}-r_{in}, see text); DNA is blue. B. The message itself where the



GGATCGATCTGATCACGATCGATCGATCGGATCAGATCGATCGATCTTCACGATCCTTTGG
CGATCGATCGATCGGATCAGATCTGATCCGATCGATCGATCGATCGCCTTAGATCGCAGGT
TGATCGATCGATCCATATGCCATGTATGTGATCAGATCGATCGGTACCTCTTGATCTCGAT
AAGATCGATCGATCCGTTAATTTTTTAACCGATCGATCGATCACTTTTGGCTTGATCAAGT
ACGATCGATCGATCACCGCGGACTACCAGTGATCGATCGATCGCGGTTGTCGATCAGCCG
CGATCGATCGATCTTCGAAGTTCACCTTATTTGATCGATCTAACAGTTCGCAGATCCCACA
AGATCGATCGATCCTAAAGGCCTACGCCGCGATCGATCTGGGATDTATCAGGATCTAGAA
CGATCGATCCGATCGATCCGATCGATCGGATCGATCCGATCAACAACGTGCGATCTCTTTT
TGATCGATCGATCTGATCGATCTGATCGATCTGATCGATCGCCCTTACCAGTGATCTTGTT
CGATCGATCCGATCTGATCGATCAGATCTGATCGATCGGATCTTGGCTTCGCGATCCCGAC
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AAGATCGATCGATCCGCGTTTGGCCAGTGGTCGATCGATCCGATCCGAGCGGATCGTTCA
GGGATCAGATCGATCCATTAAAGGCTTCCGCTGATCGATCCGATCGCGAATGATCTTTTTT
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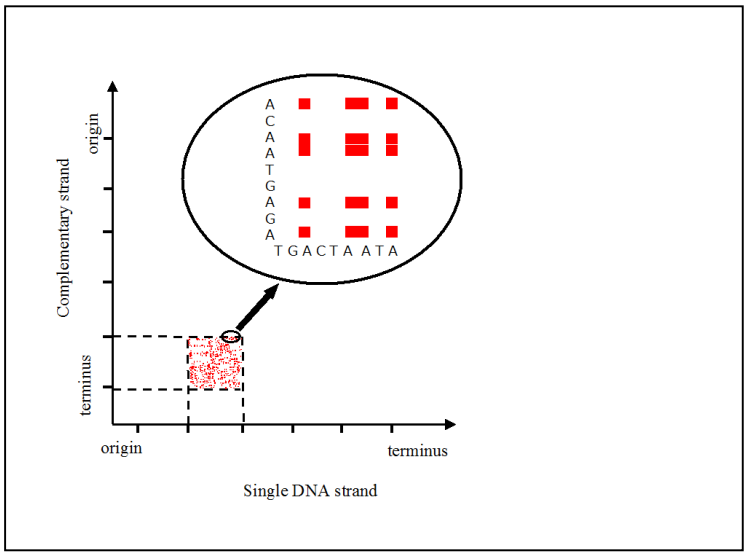
TTGATCGATCGATCTGATCCGATCGGATCAGATCGATCGATCCGATCGAATGATCGTTTCC
GCGATCGATCAGATCCGATCGATCGGATCGATCAGATCGATCGATCTCTCAGATCGGAAG
AGCCGTTTTCCTGATGAAAAAGGCCGACGAAAATGACATTCGTTTACCTGGCGAAGTGGC
GATTGCCAAGCGTCTACGATCTAACGTACGTGAGCTGGAAGGGGCGCTGAACCGCGTTAT
TGCTAACGCCAACTTTA

Figure 2 Making a DNA movie. A. Matrix of adenine-adenine (A,A) pairs. The DNA sequences of the two strands (5'-3') of a bacteriophage constitute the axes. A zoom of a region of one frame is shown in which each (A,A) pair is coloured red. B. Successive matrices or frames based on amino acids showing a man dancing. The DNA contains punctuation marks to define successive frames and is translated in both directions to give amino acids (only one reading frame per strand is shown). Within each frame, a grey block corresponds to the rectangle made by a sliding window along each axis in which a sequence of ten amino acids containing one or more glycines (G) is found in both strands.



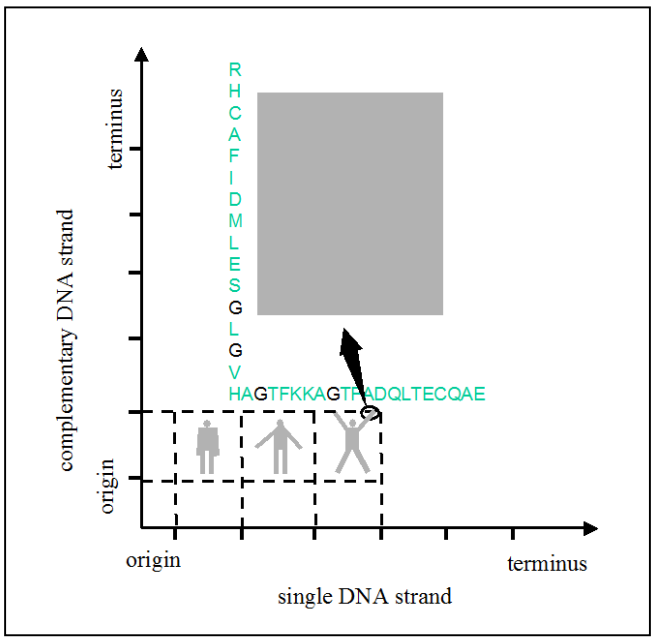


Figure 3 Schematic representations of side view toroids of different sizes: small (A), medium (B and C), and large (D). The outer radius rout of the toroid increases linearly with the DNA length up to the limit imposed by the internal radius of the capsid, i.e., 40 nm. This radius is reached when DNA is 25,000–27,000 bp long (8.5–9.2 μm). For longer DNA chains, the toroidal extension is limited by the capsid. Toroids are confined in the capsid and distorted according to their position and orientation relative to the icosahedral symmetries. Adapted from (Leforestier and Livolant, 2009).