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Review

DNA and nuclear aggregates of polyamines

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ABSTRACT

Polyamines (PAs) are linear polycations that are involved in many biological functions. Putrescine, spermidine and spermine are highly represented in the nucleus of eukaryotic cells and have been the subject of decades of extensive research. Nevertheless, their capability to modulate the structure and functions of DNA has not been fully elucidated. We found that polyamines self-assemble with phosphate ions in the cell nucleus and generate three forms of compounds referred to as Nuclear Aggregates of Polyamines (NAPs), which interact with genomic DNA. In an *in vitro* setting that mimics the nuclear environment, the assembly of PAs occurs within well-defined ratios, independent of the presence of the DNA template. Strict structural and functional analogies exist between the *in vitro* NAPs (*iv*NAPs) and their cellular homologues. Atomic force microscopy showed that *iv*NAPs, as theoretically predicted, have a cyclic structure, and in the presence of DNA, they form a tube-like arrangement around the double helix. Features of the interaction between *iv*NAPs and genomic DNA provide evidence for the decisive role of "natural" NAPs in regulating important aspects of DNA physiology, such as conformation, protection and packaging, thus suggesting a new vision of the functions that PAs accomplish in the cell nucleus.

1. Introduction

Putrescine, spermine, and spermidine are the most prevalent polyamines (PAs) in mammalian cells. They represent a class of small aliphatic polycations (Fig. 1) that are indispensable for the life of prokaryotes and eukaryotes [1–3]. Putrescine is synthesised by ornithine decarboxylase, while the other two PAs are derivatives of putrescine.

The PA content of cells is finely regulated by biosynthesis, degradation, uptake and excretion. Several enzymes, belonging to an evolutionarily ancient metabolic pathway [4], concur to the synthesis, interconversion and catabolism of these compounds, indicating their importance for cell metabolism and cell function [5,6] (Fig. 2). Accordingly, knocking out the genes encoding key enzymes in this pathway in mice is lethal, pointing to the vital role of PAs [5–8].

An additional exogenous supply of PAs is provided by dietary intake and by intestinal absorption of products of bacterial metabolism [9,10]. PAs from food significantly contribute to the total PA pool in the body [11], and the dietary intake of PAs exerts various direct and indirect trophic effects on the immature intestine, thus contributing to gut maturation [12]. In recent years, attention towards PAs has increased due to their possible implication in several diseases [5,13,14]. In particular, PA levels are increased in many pathological processes [14], such as carcinogenesis and chronic inflammatory bowel disease [13,15]. Consequently, PAs have been considered as a potential target for chemotherapeutic agents. Furthermore, a causative role has been established for PAs in longevity [14].

At the cellular level, PAs have long been shown to be involved in disparate biological processes, such as modulation of gene expression and enzyme activities, activation of DNA synthesis, transcriptional processes, and regulation of cell proliferation and differentiation [16–19]. Additionally, the basic functions of PAs have been related to cellular DNA protection against external agents and against radiation injury [20,21].

Under physiological pH and ionic strength conditions, PAs are fully protonated. Therefore, negatively charged macromolecules, such as DNA, RNA, ATP, phospholipids and certain proteins, are natural targets of their interaction [1,22].

A consequence of PA binding to DNA is the condensation that occurs with both naked and chromatin organised DNA [23,24]. Many studies indicate that this phenomenon is related to the modulation of several cellular functions *in vivo* [1]. In particular, spermidine and spermine have been shown to be associated with highly compacted mitotic chromosomes [25,26], thereby inducing more stabilising than regulating effects on the chromatin structure during cell cycle progression [27].

The stabilisation of duplex DNA by natural and synthetic PAs, independent of both their chemical structure and DNA sequence, remains a controversial issue [28]. However, several studies have revealed that PA structural specificity, in addition to DNA sequence selectivity, concurs with the electrostatic forces to rule the interaction [29–31].

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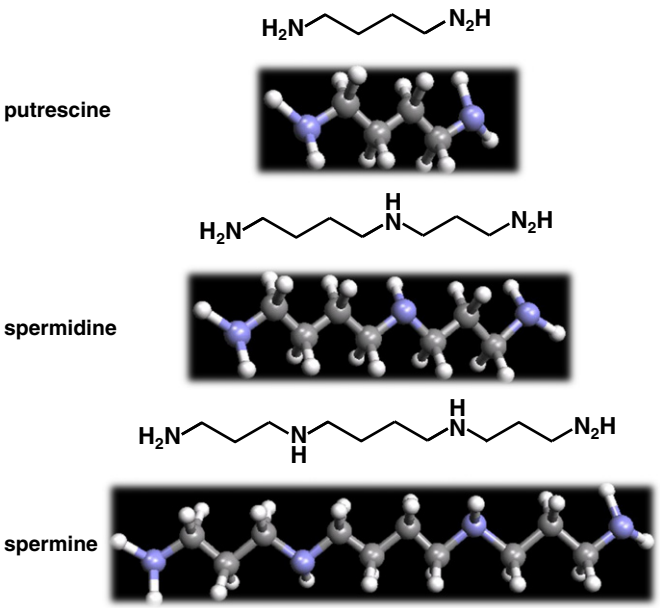

Fig. 1. Chemical structures of biogenic PAs.

Similarly, in spite of extensive investigation, the impact of the PA interaction on the functions of DNA has remained largely unanswered [2]. PAs induce DNA conformational transitions by promoting the conversion of right-handed DNA to either left-handed Z-DNA or to an alternative right-handed helix, A-DNA [32–35]. However, PAs can also bind and condense B-DNA, without inducing conformational transitions [36].

In brief, despite the large number of studies on the subject [2], many of the PA/DNA interaction traits have not been elucidated [37], most likely because the great part of the investigations made use of high PA concentrations and short DNA sequences, while only a few researchers have addressed the effects of low concentrations of single PAs on high molecular weight DNA [36,38,39]. In contrast, we approached the PAs/DNA interaction puzzle shifting the perspective of investigation toward the supramolecular chemistry and intermolecular cooperation that is fully supported by the evidence that PAs self-assemble and interact as aggregates with genomic DNA [40–43]. Therefore, we are persuaded that PA aggregation is the key to answer many open questions pertaining to the role of PAs in DNA physiology.

The aim of the present review is to summarise the current knowledge of Nuclear Aggregates of Polyamines (NAPs), with particular focus on their structure, the model of their interaction with DNA, and the expected structural and functional DNA modifications resulting from this interaction.

2. PAs spontaneously generate aggregates

We firstly discovered that in the cell nucleus, putrescine, spermidine and spermine self-assemble with phosphate ions to generate three forms of compounds, named nuclear aggregates of polyamines (NAPs). NAPs were isolated in 1999 in the course of experiments finalised to investigate the interaction of radiolabelled putrescine with nuclear peptides [44]. In that study, Gel Permeation Chromatography (GPC) assays of Caco-2 cell nuclear extracts led to the isolation of three compounds with an estimated molecular weight of about 1000, 5000 and 8000 Da which were at first erroneously assigned to polypeptide species (Fig. 3A). A panel of analytical results, including HPLC and NMR, showed that these compounds, present in the nuclei of eukaryotic cells [41], were indeed supramolecular structures formed by the interaction of natural PAs with phosphate groups through non-covalent bonds [41,42,45]. Even though the

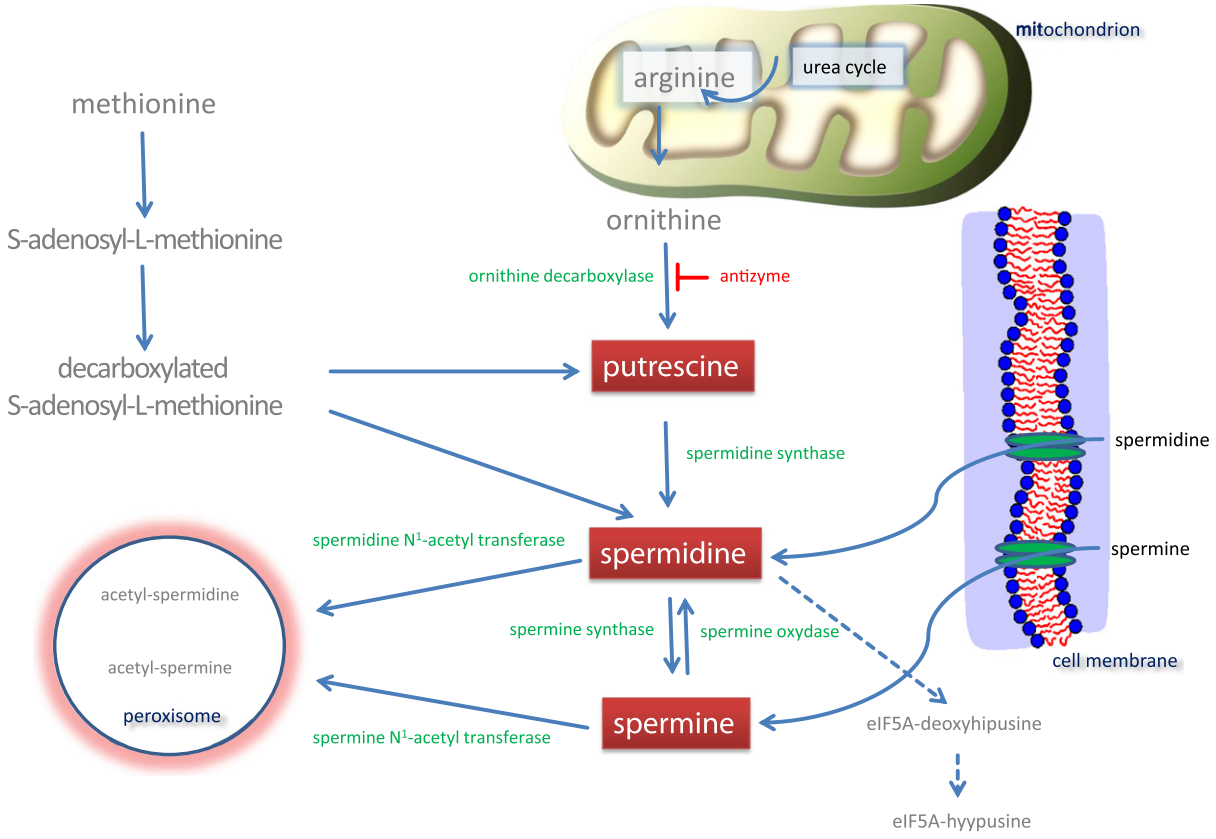

Fig. 2. Summary of PA metabolism. PA pools are finely controlled by synthesis, catabolism and uptake.

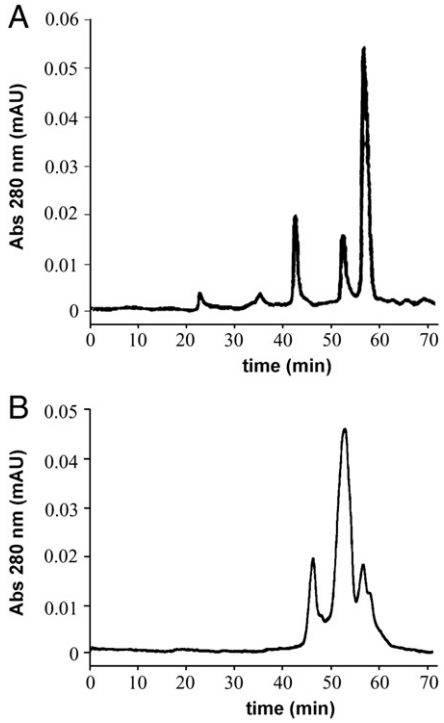

Fig. 3. Self-assembly of PAs in Caco-2 cell nuclear extracts (A) and in the *in vitro* model (B) assayed by GPC with detection at λ = 280 nm (Adapted from [41,43] with permission).

raw molecular formulas have been proposed for NAPs, their supramolecular structure should be considered as an extrapolation of a statistical distribution of a family of several co-eluted compounds. In other words, due to the non-covalent nature of the assembly, the molecular structure of NAPs remains undefined, although it is confined within a restricted range of possibilities [41,43].

The structural complexity of NAPs and their exclusive detection by GPC, which preserves the weak chemical interactions that are the basis of the assembly, is the reason for their delayed discovery in spite of the wide range of studies focused on PAs.

To confirm their structure and to overcome the operational drawbacks in the isolation of "natural" NAPs, we recently established the conditions required for the *in vitro* aggregation of PAs [43]. Under physiological conditions and in the presence of phosphates, PAs self-assemble into supramolecular structures, the *in vitro* Nuclear Aggregates of Polyamines (*iv*NAPs). The *iv*NAPs are characterised by a distinctive weak UV absorbance band centered at λ = 280 nm, suggestive of a certain degree of electronic delocalisation, which is completely absent in unassembled PAs. Attempts to assemble *iv*NAPs in phosphate-free buffers or at a non-physiological pH failed [43].

Analogous to the extractive NAPs, three molecular aggregates of the *iv*NAPs were separated by GPC analysis. They were named according to their molecular weights, l-*iv*NAP, m-*iv*NAP and s-*iv*NAP (*in vitro* large-, medium- and small-sized *iv*NAPs) [43]. Representative GPC profiles of the *iv*NAPs are shown in Fig. 3B.

The formation of cyclic compounds, which include both positive charges in the aliphatic chains and sites able to establish hydrogen bonds (H-bonds), is the result of the ionic interactions between the N-termini of each PA with the negative charges of the phosphate groups.

Synthetic macrocyclic PAs, named "super-polyamines", have been shown to promote several biological processes, such as actin polymerisation [46] and tRNA crystallisation [47], much more effectively than their linear counterparts do. This suggests that cyclised PAs might be particularly efficient in regulating specific biological processes.

Our studies demonstrate that nature is able to form a sort of macrocyclic PA that can rapidly assemble and disassemble in response to physiological requirements, without degradation of the basic components.

By modulating the concentration ratio of the three polyamines, it was possible to define the individual contribution of each PA to the *iv*NAP self-assembly process [43]. Spermine was the major component in both the l-*iv*NAP and m-*iv*NAP; spermidine was predominant in the s-*iv*NAP; and putrescine was dominant in the s-*iv*NAP and practically absent in the l-*iv*NAPs (Fig. 4).

However, the relative contribution of PAs to *iv*NAPs varies as a function of the initial concentration to some extent. For instance, it has been observed that an increase in the initial concentration of

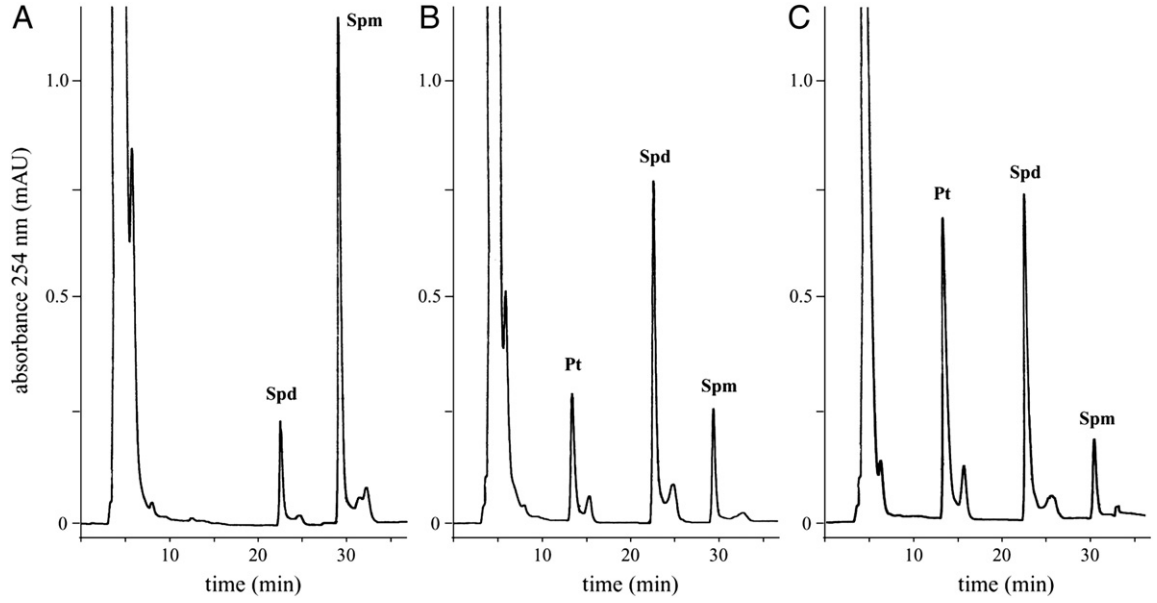

Fig. 4. Determination of the relative ratios of PA in *iv*NAPs by RP-HPLC analysis of dansyl chloride derivatives. Chromatograms of the derivatised PAs from (A) l-*iv*NAP, (B) m-*iv*NAP and (C) s-*iv*NAP (reprinted from [43] with permission).

spermine corresponds to a higher yield of the m-*iv*NAPs. It is worthwhile to note that *in vivo* a relative prevalence of the m-NAP has been detected in the nuclei of replicating cells [41]. In contrast, quiescent cells exhibit very low relative amounts of m-NAP. Therefore, it has been suggested that the well-established function of spermine as a growth factor [48] could be explained by the relative increase in m-NAP [41,45].

3. The cyclisation process in solution

Amines exhibit a natural aptitude for generating supramolecular assemblies with several anions, such as trifluoroacetate and nucleosides [49,50]. Furthermore, polyamine derivatised β-cyclodextrine has been shown to control the self-assembly of large protein constructs [51].

In the case of NAP assembly, the clustering of PAs and phosphate in alternate heterooligomers is triggered by the attraction between the opposite charges: each N-terminus of the PA interacts with a phosphate unit that N-terminally bridges another PA in turn, thereby configuring a linear arrangement [45]. The PA disposition in the chain is most likely random because it is ruled by forces equally calibrated for each of the compounds [52].

However, the elongation process cannot progress indefinitely. Coulombic repulsive interactions among the long, linear, charged chains [53] promotes the bending of PAs, whose flexibility is a function of their molecular length (putrescine<spermidine<spermine).

System stabilisation is achieved through cyclisation [43,45]. This theory is supported by the fact that arginine clusters, which aggregate via ionic interactions or salt bridges, possess exceptional stability when organised into cyclic arrays. Namely, arginine-based trimeric structures that lay in a planar arrangement are very stable in the gas phase [52].

The second step of the assembly process is the aggregation of the cyclic monomers into planar and polycyclic supramolecular structures (Fig. 5). Such a succession of events is promoted by a molecular recognition process consisting of the establishment of intercyclic H-bonds between phosphates as a consequence of a proper rotation of the circular elements in solution [45]. In theory, because H-bonds cannot exceed the distance of 3 Å, the inter-monomer matching should preferentially occur in the less convex part of the rings, i.e., where the phosphate ions are intercalated by a short and less flexible component, such as putrescine. Additionally, putrescine lacks the charges that exert inter-cyclic mutual repulsive forces. As for the linear PA–phosphate–PA sequence, the disposition of the linked rings should not be indefinitely linear, but might find stabilisation in a further circular arrangement. Therefore, we conceived a model in which m- and l-NAPs are organised as a combination of five rings in a rosette-like super-structure [45,54–57] (Fig. 5).

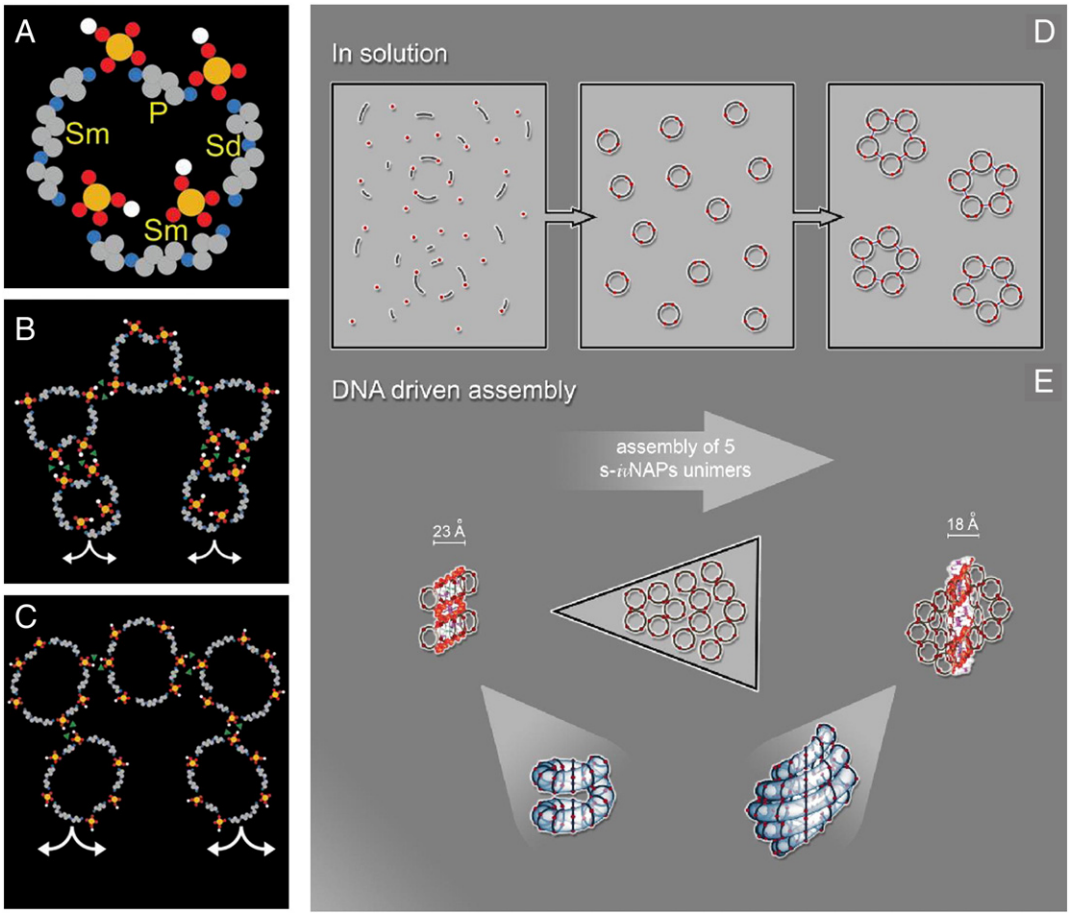

Fig. 5. NAPs structural models. (A) s-NAP. In accordance with the simplest formula indicating a spermine (Sm):spermidine (Spd):putrescine (P) ratio of 2:1:1, PA are represented as terminally linked by phosphate groups to form a single cyclic structure. (B) m-NAP. This NAP is represented as a supramolecular aggregate of five s-NAPs linked by hydrogen bonds (green triangles). The white arrows indicate the possible opening-closing movements, which allow the adaptation of NAPs to DNA grooves. The closure of the arch (resting state) may occur when the compound is in a phosphate buffer solution. (C) l-NAP. According to the simplest formula indicating a Sm:Sd:P ratio of 1:1:1, l-NAP is represented as a polymer of five 6-polyamine units, linked by hydrogen bonds. Compared to s- and m- NAP, a wider region of the external rings can interact with DNA. (D) A multistep process of supramolecular assembly occurs in solution. The electrostatic interactions between the amine termini of PA and the phosphate groups generate cyclic NAP unimers, which further aggregate to form disk-like supramolecular compounds. (E) The interaction of these compounds with the DNA and/or their *in loco* aggregation produces the DNA shielding and promotes and assists the DNA conformational changes. The ultimate result of the hierarchical self-assembly is the formation of organised PA–phosphate nanotubes that wrap but do not constrict the double helix (reprinted from [42,43] with permission).

We believe that the selective combination of circular elements that have similar—but not identical—polyamine sequences is a prerequisite for the formation of polycyclic and not mosaic-like supramolecular structures. In fact, a condition for the formation of a uniform polygonal sheet is the establishment of H-bonds among the identical components [58].

Furthermore, an aggregation process based on the molecular recognition provides a theoretical explanation for the coexistence in solution of three supramolecular aggregates, which is a peculiar behavior of NAPs/*iv*NAPs.

4. The *iv*NAPs and their hierarchical super-aggregation

Experimental evidence indicated that *iv*NAPs share structural and functional traits with their cellular counterparts [42,43]. The *iv*NAPs are generated in high yield and preserve adequate structural stability only when assembled at physiological ionic strength; however, they are less stable when the solution is deprived of NaCl [43]. Because of a relatively reduced stability in aqueous media, *iv*NAPs tends to hierarchically generate higher order aggregates. This process of supramolecular evolution initiates with the electrostatic interaction involving the terminal ends of PA and phosphate anions and ends with the formation of crystallites [43]. The development is time-dependent and, at a given time, there is a coexisting presence of different molecular entities. The system dynamically evolves toward a non-covalent supramolecular stacking of the cyclic building blocks, with the consequent production of monodisperse intermediate-sized super-aggregates as evidenced by Dynamic Light Scattering measures [43]. The process, completed in about a week, finally produces filamentous structures that are visible with the naked eye [40].

Macroscopic filamentous whirlpools rise from the bottom of the test tube upon a gentle agitation (Fig. 6). Vigorous shakes are destructive, provoking a complete disassembly of the filamentous structures, which then require an additional 5–6 days to reassemble. In accordance with the properties of supramolecular fibers—whose stability is temperature dependent [59,60]—the exposure of the filaments at 60 °C for a few minutes causes their disaggregation. It is interesting to note that tubes containing non-GPC separated *iv*NAPs remained free of visible precipitates for months, whereas the tubes containing the individual compounds did not. This indicates that a process of specific molecular recognition, lasting several days, is mandatory to trigger the hierarchical molecular super-assembly.

These observations promoted the development of a model based on H-bonds between the phosphates located in the superimposed circular modules wherein solvophobic and π–π—like interactions are established as the stabilising forces [40].

Therefore, the reversibility of the aggregation and the plasticity of the resulting super-superaggregates makes the *iv*NAP stacking an easily observable and excellent model of linear hierarchical self-assembly [61]. Several examples of supramolecular polymers generated by poly-association following functional recognition are known. These kinds of polymeric entities have been defined as "dynamers", pointing out their dynamic properties and the ability of easily reorganising and reshuffling their components [62,63].

Further characterisation of *iv*NAP crystallisation was, then, attempted by optical microscopy inspection of individual *iv*NAPs onto glass slides. This strategy revealed the formation of fractal structures corresponding to crystallites with dendritic morphologies [40,64] (Fig. 7A). With the last step, we completed the sequence of events characterising the classical structural evolution of the supramolecular compounds for *iv*NAPs: aggregation➔superaggregation➔crystallisation [65].

5. NAPs/*iv*NAPs influence the three-dimensional arrangement of DNA

Several experimental results indicate that NAPs/*iv*NAPs interact with DNA [40–43].

Early evidence of the interaction relied on electrophoretic studies that aimed to investigate the DNA protection exerted by NAPs. In fact, NAPs/*iv*NAPs shield DNA double strands against degradation by nucleases by reversibly modifying the DNA aggregation status in an unexpected way. A slight temperature variation within the physiological range led to striking modifications of the DNA migration patterns (Fig. 8). Specifically, the DNA migrated in a diffuse fashion in the presence of l-NAP and in a compact manner in the presence of m-NAP (or s-NAP) at 37 °C. In contrast, a thermal switch to 40 °C induced a change in the migration pattern to the other form. We concluded that the two patterns of migration corresponded to different condensation statuses, i.e. elongated and condensed respectively, and that the final condensation status of genomic DNA was affected by both the temperature and the interacting NAP sub-type. Therefore, within the context of an assured DNA protection, NAPs influence DNA folding in a temperature-dependent manner.

Spectrophotometric analysis further confirmed that NAPs affect DNA conformation [41]. We showed that at 37 °C, m-NAP induced

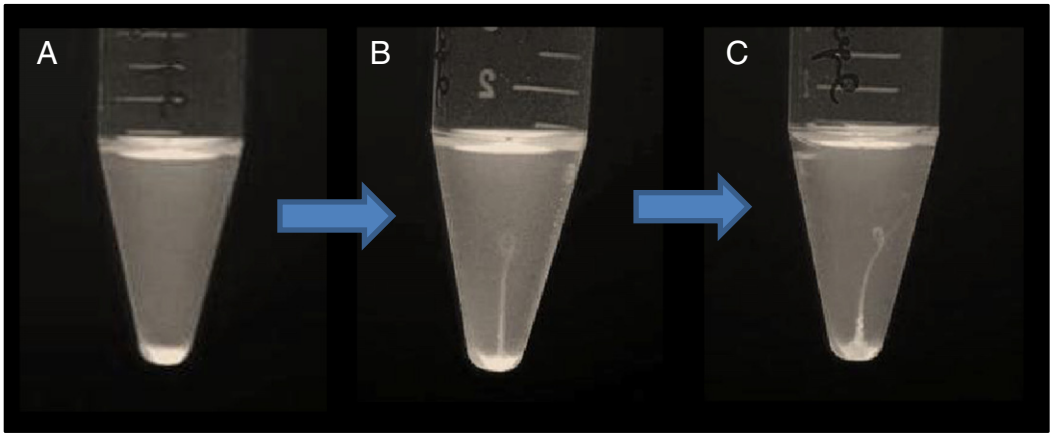

Fig. 6. The *iv*NAP supramolecular stacking. (A) The test tube containing *iv*NAPs (m- in the image) stored at 4 °C for at least 1 week show a milky sediment. (B–C) Gentle tube agitation instantly induced the emergence of a filament.

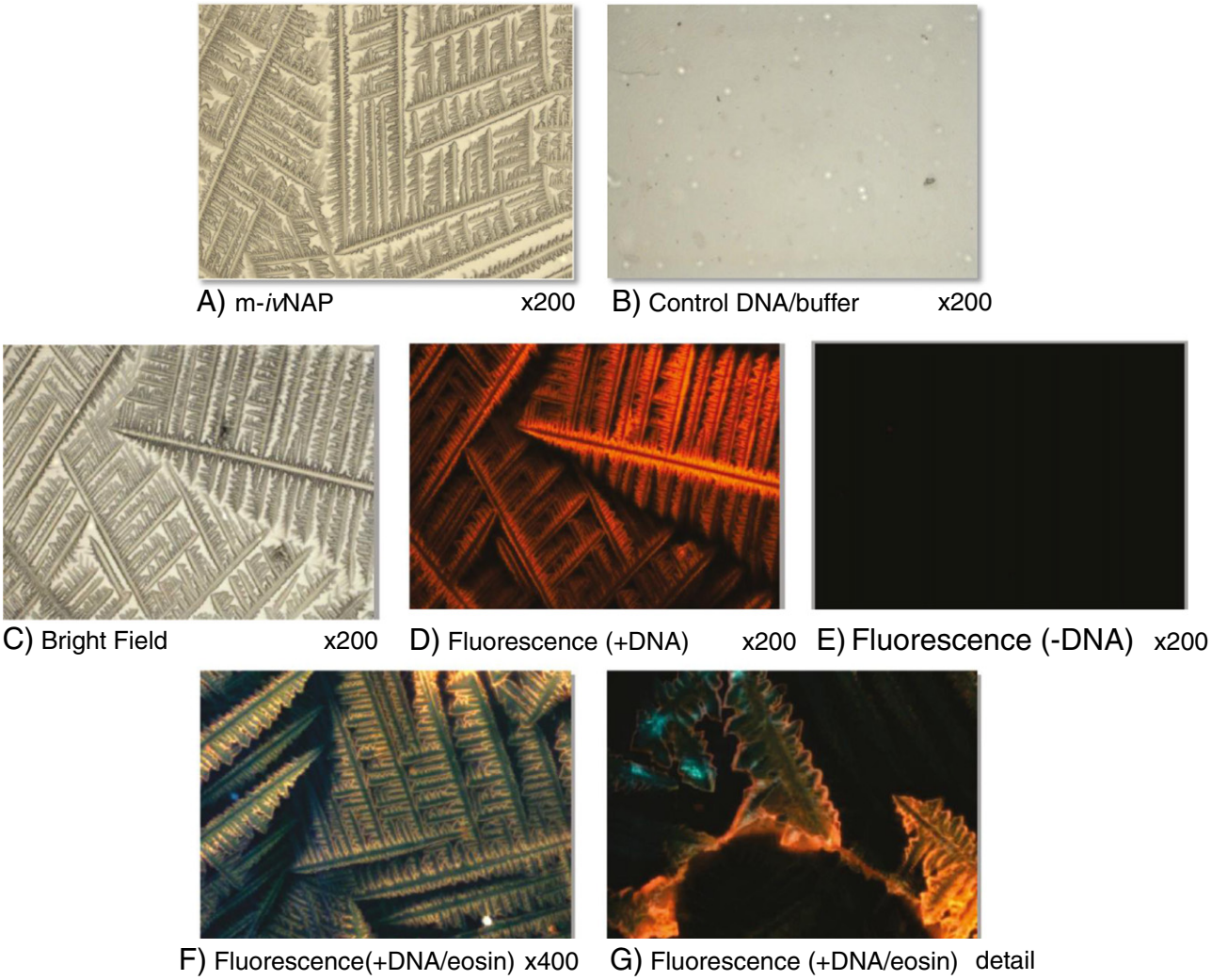

Fig. 7. Microscopy image of m-*iv*NAP assembly following dehydration on the microscopy glass slides. The *iv*NAPs assembly generates ordered and reproducible frost crystal-like geometries. The l-, m- and s-*iv*NAPs showed comparable profiles (data not shown), and the shapes obtained were independent of the presence of DNA. The image in panel A showing m-*iv*NAP was acquired by bright field illumination microscopy at x200 magnification. A control image of DNA/phosphate buffer dehydrated onto glass slides is shown in panel B. Images of DNA packed with the *iv*NAP scaffold were also documented. Dehydrated m-*iv*NAP/DNA images were acquired by both bright field light (panel C) and fluorescence (panel D) microscopy after EtBr staining; the fluorescent DNA-EtBr complex perfectly matched the *iv*NAP surface. No fluorescence was detected when the acquisition was performed in the absence of DNA (panel E). The m-*iv*NAP scaffold structure was highlighted with eosin, which produces green fluorescence (×400; panel F). Detailed view (software magnification) of the perfect and exclusive match between the EtBr-tagged DNA (orange) and the m-*iv*NAP frame (panel G). Analogous shapes were obtained with the other two *iv*NAPs (images not shown) (modified from [40] with permission).

a noticeable and rapid increase in DNA absorbance at 260 nm. The effect, exclusive of m-NAP, indicated that this compound influences the DNA conformation by inducing a structural rearrangement characterised by base extrusion. Conversely, the unaggregated polyamines induce only negligible effects on the secondary structure of genomic B-DNA [39]. Because base-extrusion is known to occur during the DNA transition to the left-handed conformation [66], we proposed a model in which m-NAP assists DNA in the transition to the Z-form [42,66].

When NAPs superassemble *in vivo* in the presence of the DNA template, s-NAPs function as the building blocks for the formation of m-NAP. According to our model (Fig. 5E), m-NAPs are assembled through the sequential aggregation of three s-NAP units to two other s-NAP units that are already bound to DNA. Furthermore, the progressive growth of the super-complexes, with an increasing number of monomers (up to five), induces a wedge-like progression that forces the DNA grooves to enlarge and triggers a transitional torsion towards the Z-form [66], which evolves along the two strands in a zipper-like fashion.

The possible role of l-NAP in affecting DNA conformation remains to be defined.

However, the relatively high abundance of l-NAP in the nuclei of non-replicating cells [44] suggests that it might be involved in the protection and stabilisation of B-DNA, the prevalent DNA form in quiescent cells [67].

It is well established that neutralisation of the DNA charges by PAs and other cations [68] induces DNA condensation by the compaction of the double helix. However, their action is exerted only on small DNA fragments and at high concentrations [28,69]. In contrast, NAPs play a protective and conformational role on genomic DNA at physiological concentrations and with a mechanism that includes more than just the electrostatic interaction, thereby assuring the preservation of DNA integrity without reducing the double helix's flexibility [41,42,45].

The protective effect of NAP/*iv*NAP on DNA against nucleases provided the first indirect proof of their interaction [41,42]. Direct evidence was obtained by means of microscopic studies that produced images at different scales. Morphological aspects of the interaction were clarified

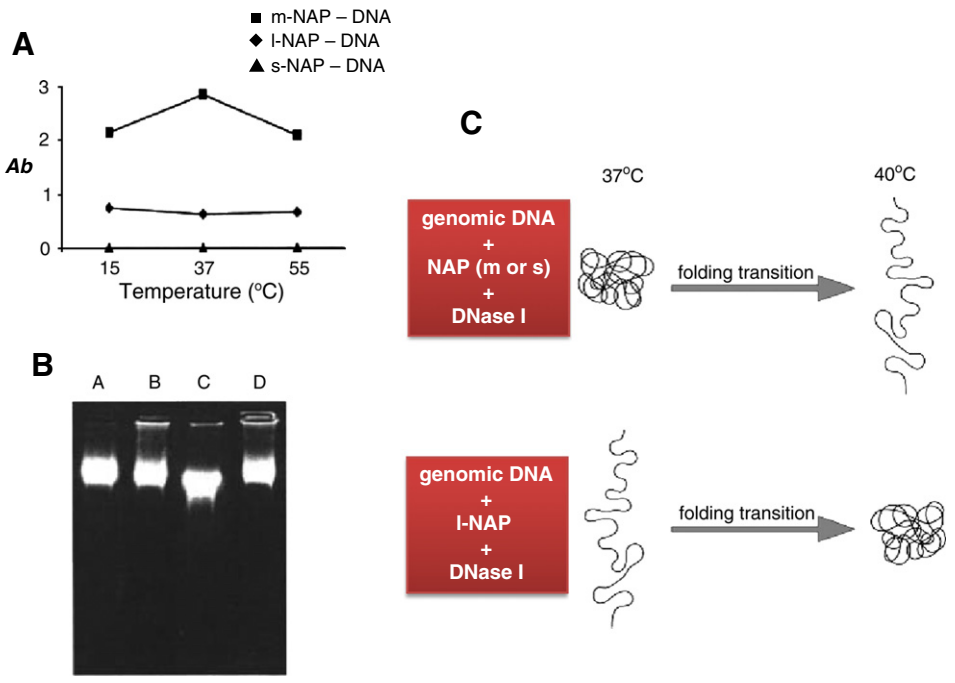

Fig. 8. (A) Differential absorbance (*Ab*) values and electrophoretic patterns of NAP–human genomic DNA solutions (l-NAP–DNA; m-NAP–DNA; s-NAP–DNA). The m-NAP–DNA solution showed the highest *Ab* values, which reached a maximum at 37 °C. Intermediate *Ab* values, unmodified by temperature variations, were recorded for the l-NAP–DNA solution. The s-NAP–DNA solution did not show any *Ab* value. *Ab* values were monitored for 6 min at 260 nm at different temperatures and calculated by subtracting the absorbance value of the DNA from those of NAP–DNA solutions. In the same experimental conditions, neither NAP solutions without genomic DNA nor single PAs (used at a concentration equivalent (1 μM) to that of the PAs composing the NAPs) showed any variation in *Ab* (data not shown). (B) Electrophoresis performed with a 1.5% agarose gel in Tris/borate/EDTA at 37 °C: Lane A corresponds to the migration of human genomic DNA. Lanes B, C and D show the same DNA pre-incubated for 6 min at 37 °C with l- and m- and s-NAPs, respectively. A faster migration of the DNA in the presence of m-NAP is evident. (C) Schematic representation of the temperature-dependent genomic DNA condensation patterns regulated by NAPs. The incubation with s-NAP, m-NAP or l-NAP and a successive exposure to DNAse I produced mirroring modifications of genomic DNA conformation. At 37 °C, the genomic DNA migrated in the 1.5% agarose gel in a compacted state in the presence of m-NAP or s-NAP and in a diffuse fashion (elongated state) in the presence of l-NAP. The thermal switch to 40 °C was sufficient to induce the reciprocal change in genomic DNA aggregation status. Either way, both protection from DNAse I and control of DNA conformation were simultaneously assured. The scheme was derived from electrophoretic results (adapted from [41,42,45] with permission).

by using a fluorescence microscopy strategy on mixtures of *iv*NAPs and genomic DNA dried onto glass slides [40]. In particular, the staining of DNA with ethidium bromide (EtBr) (Fig. 7D) and of *iv*NAPs with basophilic eosin (Fig. 7F, G) produced impressive images showing that the *iv*NAP fractal structures are perfectly surrounded by EtBr-tagged DNA. The microscopic pictures of ivNAP/DNA complexes are different from those obtained by Saminathan et al. that studied, with a similar crystallisation procedure, the liquid crystalline phase transitions of DNA in the presence of single natural and analogue PAs [38]. Furthermore, the patterns of genomic DNA alone (in absence of *iv*NAPs) lack the typical fractal shape Fig. 7B, indicating that, in dehydrated conditions, *iv*NAPs act as template for the DNA arrangement. Interestingly, macroscopic aggregation clusters between *iv*NAP and EtBr-tagged genomic DNA were observed in solution by UV light inspection [40].

The above morphological indications clearly suggest that *iv*NAPs, and by analogy their extractive counterparts, are able to regularly arrange into extremely long structures, like genomic DNA, thereby optimising both the occupancy of the available space in the nucleus and, presumably, the accessibility to the DNA strands. These observations encouraged a more detailed morphological investigation of both *iv*NAPs and *iv*NAP–genomic DNA complexes at the nanometre scale.

6. NAPs/*iv*NAPs form nanotubes that envelop the DNA

Atomic Force Microscopy (AFM) revealed for the *iv*NAPs a pattern that can be ascribed to a planar sedimentation on the mica of circular units that, when in solution, are dispersed in three dimensions [40]. Remarkably, an annular aggregation of five sub-units of m-*iv*NAP and l-*iv*NAP emerged on the mica surface, supporting our previous model.

AFM images of genomic dsDNA interacting with s-, m- and l-*iv*NAPs were also acquired, revealing a tubular disposition of circular elements enveloping the helices. The zoomed-in phase images (Fig. 9C, G, M, Q) showed round elements disposed with specific periodicities around the longitudinal axis of dsDNA. Furthermore, the coexistence in the same picture of both wrapped and naked DNA tracts proved that the images showing the enveloping of the double strands were authentic. More specifically, *iv*NAPs fill the DNA grooves in a spiral-like fashion that fits with the DNA helical motif (Fig. 10A). The impact of *iv*NAPs on the DNA morphology agrees with our previous theoretical models [41,42,45]. In fact, it was suggested that NAPs self-dispose along the longitudinal axis of DNA through the formation of hydrogen bonds between the phosphate groups of adjacent polycyclic units. As shown in Fig. 10B, DNA was envisioned to be wrapped by a nanotube formed by cyclic modules that have twelve hanging points per DNA helical turn (one for each phosphate pair per helix). However, this hypothesis does not meet the criteria of a continuous assembly because the distance between the two closest NAPs anchored to the DNA phosphates is too long to establish hydrogen bonds. The model was completed with accessory blocks flanking the DNA-anchored rings linked through additional hydrogen bonds established among the available phosphate groups [45]. This kind of self-assembly is determined exclusively by the interacting species and, therefore, is a valuable example of molecular recognition [70]. Among the non-covalent interactions, which maintain the structure and confer the same self-healing properties, hydrogen bonding plays a pivotal role [71–73].

Furthermore, AFM images were also compatible with a possible conformational switch from rosette-like to arch-like structures of both l- and m-*iv*NAPs because of their interacting with the DNA strands [45].

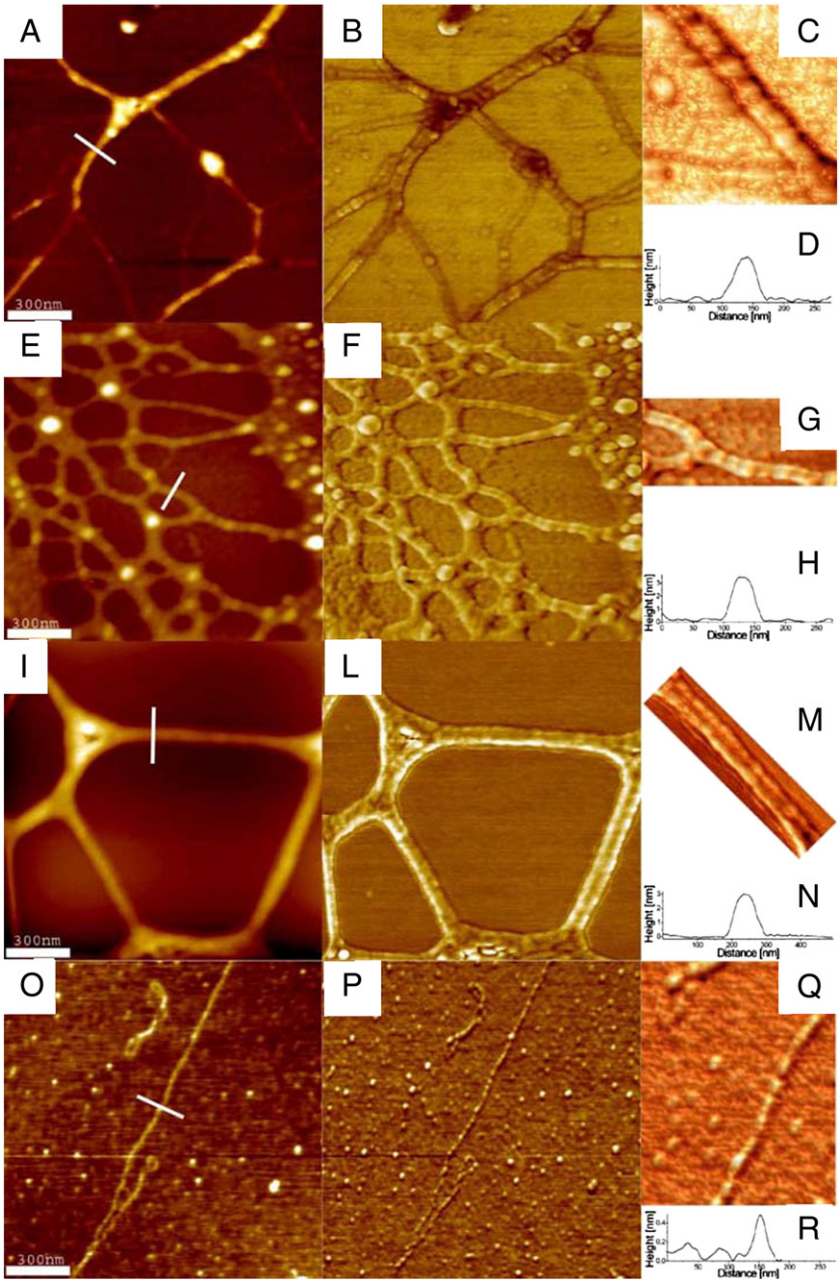

Fig. 9. AFM imaging of the *iv*NAP-DNA complexes. Representative topography and phase AFM images of s-*iv*NAP-DNA (A and B), m-*iv*NAP-DNA (E and F), l-*iv*NAP-DNA (I and L) and naked DNA (O and P) deposited on mica and imaged in air are shown. The images reveal tubular structures wrapping the DNA, noticeable in the digital zooms (C, G, M, and Q). The topography and phase images have sizes of 1.5 × 1.5 μm. The figure also shows the AFM height profiles evaluated along the white lines depicted in the topography images (D, H, N, and R) (reprinted from [40] with permission).

The ultimate result of this hierarchical self-aggregation process is the formation of a scaffold that both protects DNA and dynamically supports its conformational modifications.

7. *iv*NAPS as nanotools

Our data indicate that *iv*NAPs can be assembled by means of an easy, fast, reproducible, and inexpensive method [43], schematically described in the Supplementary information. The products are stable (if the GPC separation is made in the presence of NaCl), able to interact with the genomic DNA, and consequently, potentially utilisable in many fields of research in which polyamines are involved.

Biology is an attractive field for nanoscience applications [74,75]. Self-assembling nanomaterials [76,77] are increasingly proposed to produce geometrical motifs and spatial organisations on the nanometre scale using peptides, proteins, carbohydrates, and a variety of macromolecules [78–82]. Recently, an innovative approach toward nanoscale assembly utilised information-containing molecules, such as DNA, to control interactions and thereby minimise unwanted cross-talk [83].

In this sense, a DNA-guided assembly of small molecules like polyamine aggregates seems to be a unique opportunity.

For instance, it is possible to imagine that *iv*NAPs, complexed with DNA or alone, can act as templates for the production of silylated structures, such as what happens for the formation of diatom shells in nature [84–86]. In fact, the ability of long-chain

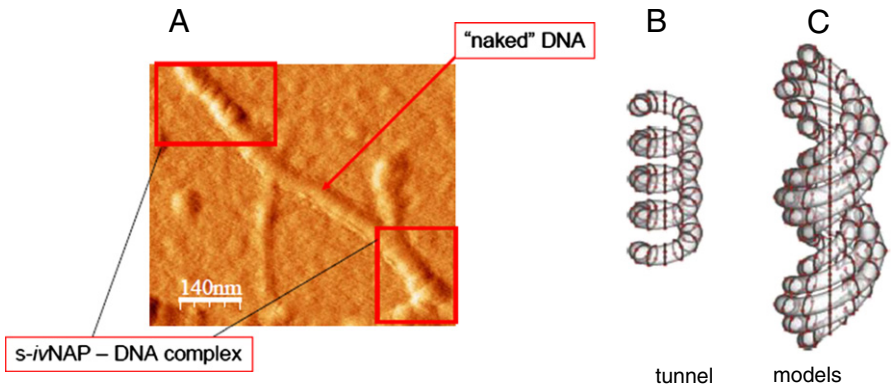

Fig. 10. (A) AFM image of s-*iv*NAP partly wrapping genomic DNA. Regions in which the s-*iv*NAP envelops the DNA tracts are boxed. Strands of "naked" DNA are indicated by an arrow. The impact of *iv*NAPs on the DNA morphology agrees with previous theoretical tunnel models. (B) Adjacent s-NAPs are envisioned to produce a tunnel-like structure enveloping the A-DNA. (C) Adjacent m-NAPs are imagined to produce a tunnel-like structure enveloping the Z-DNA. The NAPs depicted are those connected to the phosphates of the DNA (twelve per helix turn). Other NAPs can be envisioned in parallel along the transparent sections of the tunnels (Adapted from [40,41] with permission).

polyamines to prime biosilicification in diatoms has been found to depend on their capability of self-assembling with phosphate [87]: as a consequence of silicic acid condensation at the boundary of structured long-chain polyamines [86], astonishingly intricate patterns of the diatom valves are generated.

Due to the peculiarity of the *iv*NAP/DNA interaction, we also envision the application of *iv*NAPs as biotechnological molecular devices capable of modulating and assisting processes in which nucleic acids are involved [88–91]. For instance, polyamines have been shown to induce DNA condensation in nanoparticles, which have been suggested as pharmaceutical agents for gene therapy [92]. However, extended targeted investigations will be required to enable the employment of *iv*NAPs in these areas.

8. Concluding remarks

We demonstrated that the interaction of PAs and phosphates generates cyclic monomers, which hierarchically assemble into higher-order structures. Self-assembled PA aggregates represent an excellent example of the fundamental working strategy of nature: to achieve highly complex functional structures using the information encoded in simpler tools [93].

NAPs envelop the whole genome by a tubular structure that assures protection without spatially restraining the double helix. Furthermore, s-, m- and l-NAPs assist in the definite conformational changes of the DNA and thereby probably cooperate with other factors (proteins and ions) that were considered exclusive actors before the discovery of NAPs.

The interaction features between NAPs and genomic DNA suggest a novel perspective on the mechanisms operating in the cell nucleus and provide evidence useful for the development of more detailed structural models.

However, available data are adequate to indicate that these compounds are critical for the regulation of DNA physiology. This conviction finds recent support in reports indicating that inside a cell, the conformation of nuclear chromatin and the chromosome compartmentalisation are consistent with the model of a fractal globule, which is a knot-free conformation that enables maximally dense stuffing while maintaining the ability to easily fold/unfold any gene [94].

Therefore, we believe that NAPs can shed light on this matter, thereby providing some order to a research area in which the DNA is still in "disorder".

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamcr.2012.05.033.

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