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RNA Self-Assembly and RNA Nanotechnology

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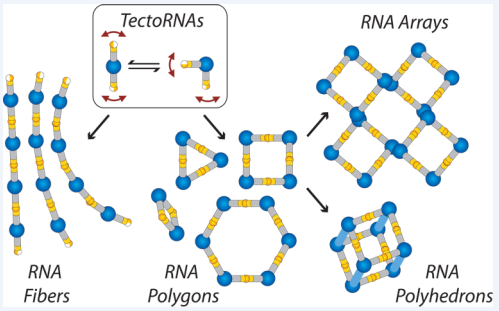
CONSPECTUS: Nanotechnology's central goal involves the direct control of matter at the molecular nanometer scale to build nanofactories, nanomachines, and other devices for potential applications including electronics, alternative fuels, and medicine. In this regard, the nascent use of nucleic acids as a material to coordinate the precise arrangements of specific molecules marked an important milestone in the relatively recent history of nanotechnology.



While DNA served as the pioneer building material in nucleic acid nanotechnology, RNA continues to emerge as viable alternative material with its own distinct advantages for nanoconstruction. Several complementary assembly strategies have been used to build a diverse set of RNA nanostructures having unique structural attributes and the ability to self-assemble in a highly programmable and controlled manner. Of the different strategies, the architectonics approach uniquely endeavors to understand integrated structural RNA architectures through the arrangement of their characteristic structural building blocks. Viewed through this lens, it becomes apparent that nature routinely uses thermodynamically stable, recurrent modular motifs from natural RNA molecules to generate unique and more complex programmable structures. With the design principles found in natural structures, a number of synthetic RNAs have been constructed. The synthetic nanostructures constructed to date have provided, in addition to affording essential insights into RNA design, important platforms to characterize and validate the structural self-folding and assembly properties of RNA modules or building blocks. Furthermore, RNA nanoparticles have shown great promise for applications in nanomedicine and RNA-based therapeutics.

Nevertheless, the synthetic RNA architectures achieved thus far consist largely of static, rigid particles that are still far from matching the structural and functional complexity of natural responsive structural elements such as the ribosome, large ribozymes, and riboswitches. Thus, the next step in synthetic RNA design will involve new ways to implement these same types of dynamic and responsive architectures into nanostructures functioning as real nanomachines in and outside the cell. RNA nanotechnology will likely garner broader utility and influence with a greater focus on the interplay between thermodynamic and kinetic influences on RNA self-assembly and using natural RNAs as guiding principles.

INTRODUCTION

Modern biology continues to reveal the astonishing complexity by which cellular processes are elegantly orchestrated, interconnected, and regulated. Protein expression in eukaryotes, once thought to exist as a simple linear informational pathway from gene to protein through a ribonucleic acid (RNA) intermediate, involves an extensive array of cellular infrastructure. In this regard, RNA exists as a central cellular processor responsible for reproduction and replication.[1] The ongoing list of discovered classes of noncoding RNAs (ncRNAs), such as, riboswitches, ribozymes, short-interfering (siRNAs), small nucleolar (snoRNAs), and long noncoding (lncRNAs), suggests that RNA has exceedingly specialized cellular functions, working as the "dark matter" in eukaryotic cells.[2,3] This new reality is supported by the fact that while less than 2% of the information stored in the entire human genome is designated for protein coding, more than 80% is transcribed into RNAs with still unspecified functions.[4]

RNA's diverse functions, structural adaptations, and assorted spatial and temporal choreography in the cell offer distinct advantages for a host of cell-based applications. RNA is easily produced in multiple copies via the transcription machinery and unlike DNA and proteins, which are largely confined to the nucleus and cytoplasm, respectively, RNA commonly transverses the nuclear membrane to direct biological pathways or regulate gene expression. Furthermore, RNA's ability to self-assemble and interact with itself and other key biological molecules and its favorable therapeutic properties make it a promising material for a variety of applications in nanomedicine and synthetic biology in and outside of cellular contexts that are not directly available to DNA or proteins.[5−9]

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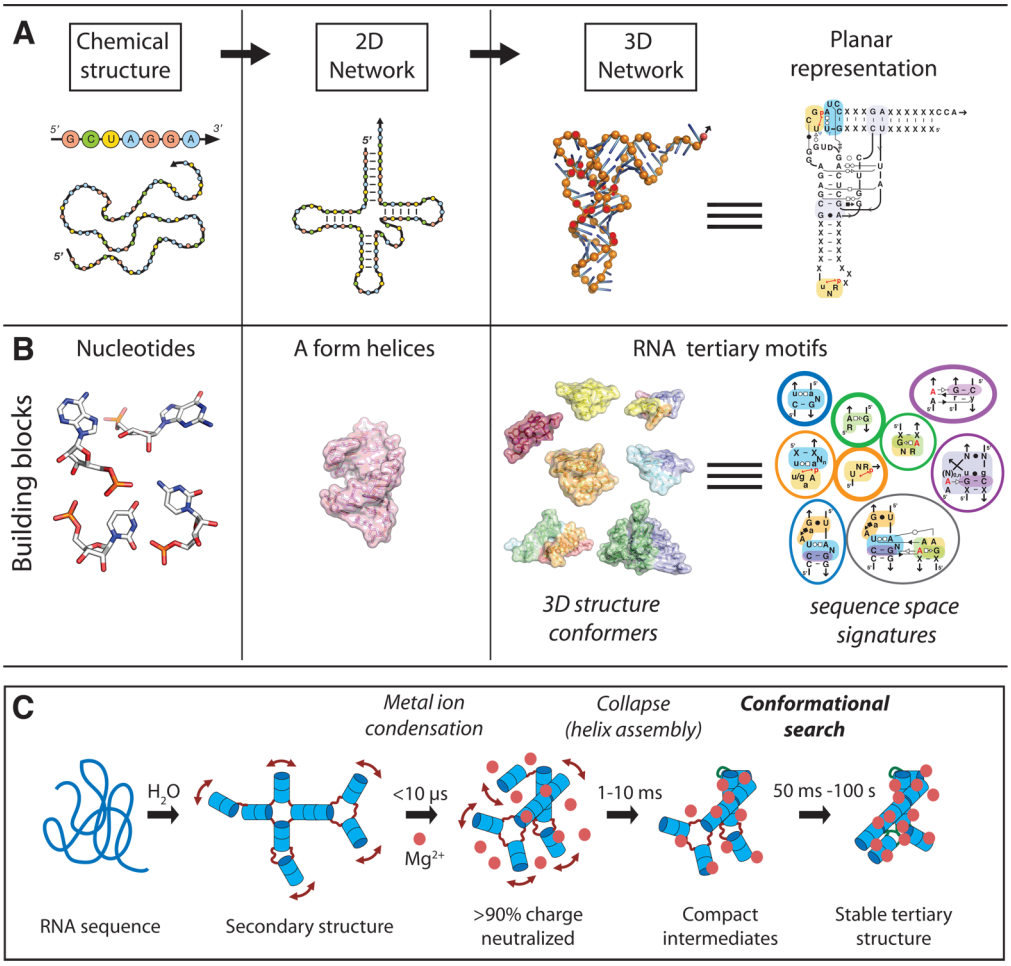

Figure 1. Structural modularity and hierarchy of RNA. (A) The information encoded within a specific primary RNA sequence guides RNA folding to form a secondary structure network consisting of A-form RNA helices (B, middle) defined by canonical WC bps. A-form RNA helices (with C3′ sugar pucker) are thermodynamically more stable than B-form DNA helices (with C2′ sugar pucker). In the context of a stable secondary structure RNA nucleotides (B, left) can also form stable noncanonical bps, which contribute to the formation of RNA tertiary motifs, each motif being specified by a sequence space signature usually coding for a well-defined 3D structure conformer (B, right). (C) While RNA folding into a stable tertiary structure requires a conformational search that is mostly sequence dependent, it is also aided by the presence of salts.

In recent years, a variety of nanoparticles, relying on the self-assembly of an assortment of multimeric RNA units, have been designed to synthesize 2D and 3D polygons, arrays, and filaments.[10−20] In the same way that many of these RNA-based nanostructures have been inspired by naturally occurring structural RNA components, their continued development and advancement is contingent upon the further consideration, investigation, and integration of natural RNAs and the principles by which they self-assemble into functional three-dimensional architectures.[6,21] While comprehensive reviews of tertiary motifs in particular offer useful structural insight,[22,23] less has been written about the specific and fundamental aspects of RNA structure, folding, and self-assembly that make RNA highly advantageous for nanotechnology, which is the focus of this Account.

RNA SELF-ASSEMBLY, STRUCTURE, AND MODULARITY

RNA's broad range of cellular functions results from the structurally diverse adaptations that individual RNA strands can form. RNA self-assembly generally refers to the spontaneous process by which a pre-existing sequence of nucleotides forms an organized structure consisting of a specific network of local noncovalent interactions (i.e., hydrogen bonding and stacking between distant nucleotide sites (or nodes) (Figure 1A).[24] Properly understood, RNA structure, folding, and self-assembly are hierarchical phenomena resulting in modular structural components. As an informational molecule, the linear sequence of nucleotides (which are themselves modular) codes for modular secondary structures consisting of canonical Watson−Crick (WC) base pairs (bps), which influence tertiary folds consisting of both WC bps and noncanonical hydrogen-bonding interactions. Analysis of natural RNA biomolecules, surveyed across an array of organismal contexts, has shown that recurrent structural modules (or motifs) specify localized arrangements of conserved and semiconserved nucleotides. These same structural motifs are routinely used as modules in a variety of combinations to code for distinctive and specialized local architectures (or native folds) able to perform specific operations including intermolecular recognition, catalytic, or mechanical functions (Figure 1B).[25,26]

While the ability to self-assemble is by no means exclusive to RNA, RNA self-assembly is unique in its own right and offers other marked advantages as a building material. For example, ionic salts have important (although often underemphasized) influences on RNA self-assembly and tertiary structure.

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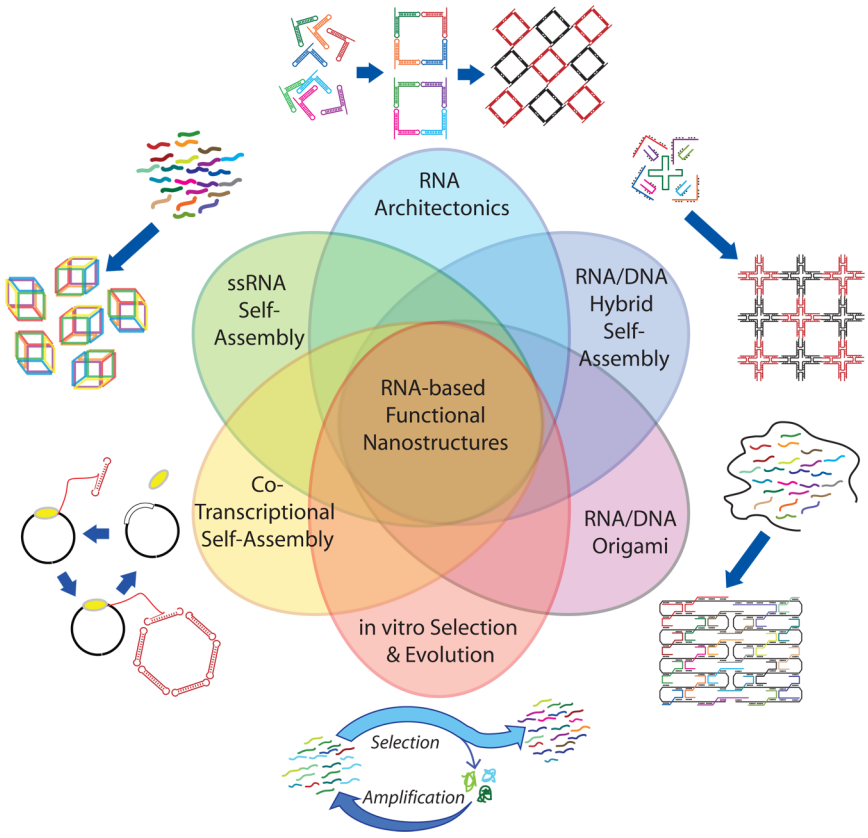

Figure 2. Self-assembly strategies and other principles governing the design of RNA-based functional nanostructures.

Divalent cations, like magnesium, screen the negative charges associated with the ribose phosphate backbone allowing for the formation of RNA helices and tertiary contacts (Figure 1C).[27−29] As a result, tertiary structures are quite dependent on salt concentration. This feature can be used to manipulate dynamic RNA molecules, which can alternate between different unique 3D shapes having small variations in free energy of formation.[27,29]

RNA SELF-ASSEMBLY STRATEGIES

Structural investigation of complex cellular RNA machineries, like the ribosome,[30,31] RNase P RNAs,[32,33] group I and group II introns,[34−37] and the spliceosome,[38] besides suggesting that RNA is a choice material for constructing complex multifunctional self-assembling nanoparticles, offer important information regarding the design of artificial nanostructures.[26] Using natural systems as inspiration, at least four RNA self-assembly strategies have been applied to the design of synthetic RNA nanoarchitectures: (1) RNA architectonics, (2) single-strand RNA assembly, (3) RNA/DNA hybrid self-assembly, and (4) cotranscriptional assembly (Figure 2). While distinct, these strategies are complementary and can be used in concert with one another to direct RNA self-assembly.

RNA Architectonics Self-Assembly

RNA architectonics is defined as the scientific study of RNA architecture.[21] Fundamentally, this strategy seeks to understand the structural components associated with the 3D shape of a molecule in terms of the essential intermolecular interactions that define a particular geometry. The process of reverse engineering is used to deconstruct and identify the modular structural components of which a particular natural RNA is comprised. Once properly characterized, these structural motifs (or RNA tectons), extracted from known X-ray and NMR solution structures of natural RNA molecules, can be cataloged for use as suitable "parts" for the computer assisted 3D design of self-assembling RNA units (or tectoRNAs) forming synthetic RNA nanoarchitectures.[8,10,11,13,21] Functioning as independently stable modular structures, tectoRNAs can generate a limitless number of supramolecular assemblies.[13,21,39]

By this strategy, thermodynamically stable motifs, taking advantage of both canonical WC and noncanonical bps, can be interchanged or grafted onto one another to build predefined artificial RNA nanostructures. Some "parts" take a leading role in determining the shape of the overall particle as in the case of the hexagonal nanoring,[16] which is built on the characteristic 120° of the kissing loop (Figure 3B). Other "parts" may play more supporting roles in the makeup of a structure (like the HIV kissing loop, which promotes coaxially stacking of helices in the assembly of the RNA tectosquares).[11,13,14] The versatility of this approach has been demonstrated by the rational design of various nanostructures including fibers,[12,15,16] triangles,[17−19] squares,[11,13,40] hexagons,[16,41] polyhedrons,[13,14] and 2D arrays[11,13,18] (Figure 4).

From the design standpoint, the sequence constraints encoded into a particular tectoRNA are essentially localized at the level of sequence signature corresponding to the particular tertiary motifs on which the tectoRNA is composed.[21] Thus, the helical struts used to connect or "glue" together the desired motifs can be any combination of complementary nucleotides coding for the desired helical structure.[42,43] The most important feature of the helix itself pertains to its length. Because any helix used to connect two

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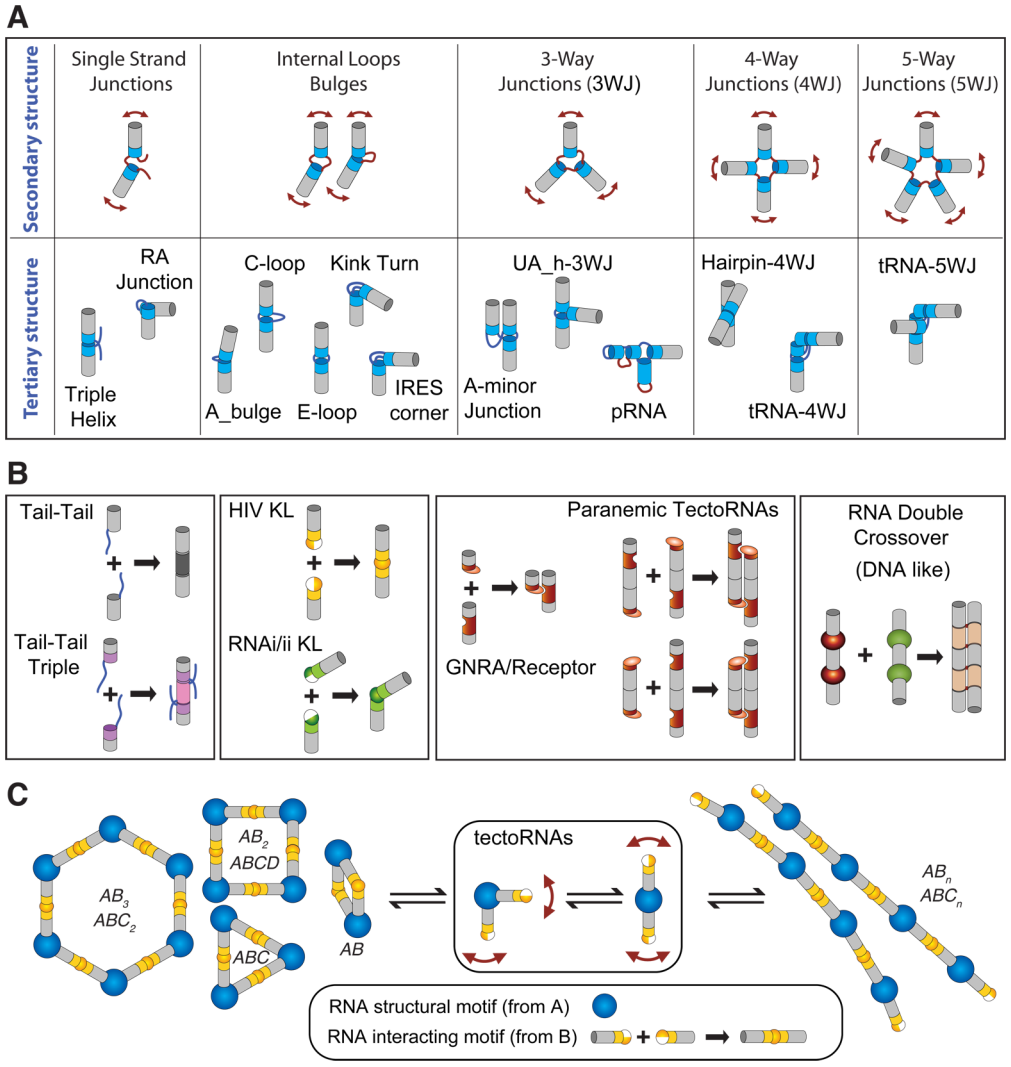

Figure 3. Representative "parts" useful for RNA architectonics. (A) Junctions involve points of contact where multiple helices converge and can be used to direct the orientation and packing of conjoined helices. Representative families of secondary structure motifs with corresponding examples of tertiary motifs are presented. (B) Tail−tail interactions,[11,13,14,17,49] kissing loops (KL),[11,13−16,40] loop receptors,[10,12,69,70] and double crossovers[47] provide means to direct the programmed long-range self-assembly of RNA units. (C) Structural motifs (from panel A) and interacting motifs (from panel B) involve points of contact where multiple helices converge and can be used to direct the orientation and packing of conjoined helices.

adjoining motifs spirals with each additional base pair, its length dictates the orientation of a long-range interaction.[42,44]

In the future, sequence design of RNA tertiary structures will beneficiate from the recent progress in the design of RNA secondary structures.[45] Additional design considerations are addressed elsewhere in this issue.[46]

While the RNA architectonics strategy offers the possibility to design structurally complex architectures mimicking large naturally occurring RNA nanomachines, several other strategies have been used to successfully build synthetic RNA-based nanostructures.

Single Strand RNA Self-Assembly

In contrast to the architectonics strategy, single strand RNA assembly (ssRNA) relies on RNA strands that are essentially unstructured by themselves but when mixed together are able to assemble through classic WC bps (including the formation of WC bps between G−U and G−A, which are quite common in natural RNAs). This strategy finds much inspiration in those developed for DNA nanotechnology reviewed elsewhere in this issue. DNA is particularly well-suited for forming and maintaining high-fidelity secondary structures formed by canonical WC bps, a useful quality for building chromosomes in cells. In the same way that DNA has been used to form few elementary secondary motifs including crossover Holliday junctions, RNA can also form nanostructures using base pair hybridization (Figure 4E).[17,43,47−50] Additionally, ssRNA assembly is a reliable method that can be used in conjunction with the other strategies to promote the programmed assembly of RNA units through complementary tail−tail interactions.[11,13,14,17,49,51]

One defining benefit of the ssRNA assembly strategy is that it can simplify the process of sequence design associated with the programmed assembly. The nucleotide sequences themselves are not constrained by the sequence signature of tertiary motifs but only by the ability to form WC bps. Due to its increased thermodynamic stability, however, RNA shows a greater tendency to encounter and tolerate mismatches between bases. Thus, the ssRNA strategy has been limited to rather short RNA fragments, whose sequence has been optimized computationally.[43,48] Even so, new techniques including short

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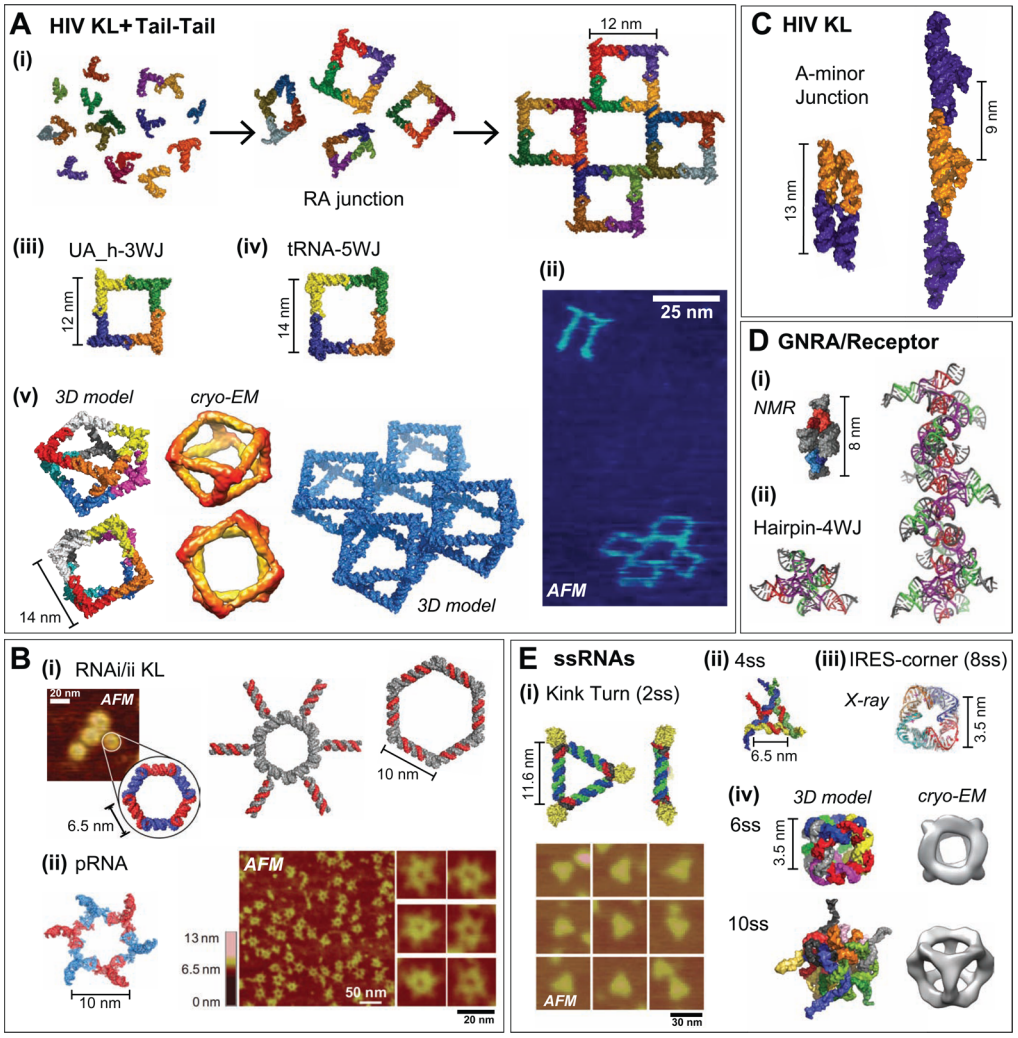

Figure 4. RNA nanostructures constructed using RNA self-assembly. (A) Several tertiary interactions directing a 90° bend between adjoining helices have been used to generate (i–iv) tectosquares[11,13] and (v) antiprism shaped polyhedrons[14] including (i, ii) the right angle (RA),[11] (iii) UA_h-3WJ,[11] and (iv, v) tRNA-5WJ[11,14] motifs [parts i, ii reproduced from ref 11, Copyright 2004 American Association for the Advancement of Science; parts iii, iv reproduced from ref 13, Copyright 2009 American Chemical Society; part v reproduced from ref 14, Copyright 2010 Nature Publishing Group]. (B) Hexagonal nanoparticles[16,41] built from the (i) RNAI/IIi kissing loop[16] and (ii) the pRNA[41] [part i reproduced from ref 16, Copyright 2011 American Chemical Society; part ii reproduced from ref 41, Copyright 2013 Cold Spring Harbor Laboratory Press]. (C) RNA particles and fibers incorporating the HIV KL and A-minor Junction [Reproduced from ref 15, Copyright 2011 Oxford University Press]. (D) Particles (i) and fibers (ii) using GNRA loop-receptor tectoRNAs [Reproduced from refs 12 and 70, Copyright 2006 and 2008 Oxford University Press].[10,12,69,70] (E) RNA nanoparticles built using the ssRNA strategy: (i) Kink Turn triangle based on two single strands (ss) assembling with a protein [Reproduced from ref 17, Copyright 2011 Nature Publishing Group]; (ii) 4ss triangle [Reproduced from ref 43, Copyright 2011 American Chemical Society]; (iii) IRES nanosquare based on 8ss [Reproduced from ref 49, Copyright 2011 National Academy of Sciences, USA]; (iv) RNA nanocubes based on 6ss and 10ss [Reproduced from ref 48, Copyright 2010 Nature Publishing Group]. Nanostructures have been characterized by atomic force microscopy (AFM), cryo-electron microscopy (cryo-EM), NMR, or crystallography (X-ray) as indicated.

self-complementarity or systematic repeats, which can be extended to form larger arrays in cells (like what is thought to occur in the naturally occurring nanostructures like DsrA and GcvB)[52,53] have been used to circumvent some of these same issues.[54] Alternatively, the DNA self-assembly strategy using hundreds of short (32-nt) modular "bricks" is another approach that could be amenable to ssRNA self-assembly.[55]

RNA–DNA Hybrid Self-Assembly and Origami

The RNA–DNA hybrid strategy involves a simultaneous attempt to leverage the self-assembly and functional properties uniquely intrinsic to both RNA and DNA. RNA and DNA sequences readily form complementary WC bps, adopting helices that are more A-form than B-form. Examples of RNA–DNA hybrids equipped with toehold strands have been used to enable selective strand displacement allowing the formation of functional RNA moieties.[56,57] In other cases, the formation of nucleic acid nanoparticles relying on RNA–DNA hybrids or DNA self-assembly have been functionalized with double-stranded RNAs (pre-siRNAs) through complementary sticky tails.[48,58] Alternatively, RNA–DNA hybrid self-assembly,[59] drawing on inspiration from strategies used in DNA origami, has been used to create large nucleic acid architectures using hundreds of DNA staples to fold long RNA templates.[59–62] These examples demonstrate that RNA, like DNA, can be used as a template or scaffolding agent to create higher ordered structures.

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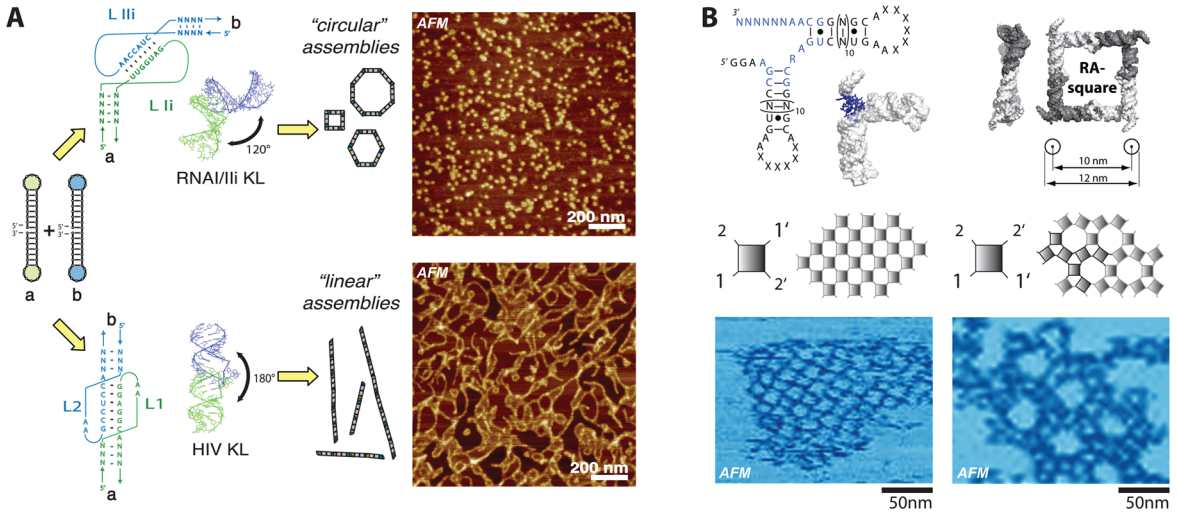

Figure 5. Design and characterization of RNA nanostructures. (A) The RNAI/II inverse and HIV kissing loops dramatically impact the formation of nanorings (top) versus fibers (bottom) as characterized by AFM [Reproduced from ref 16, Copyright 2011 ACS]. (B) The RA motif in conjunction with HIV-like kissing loops and tail−tail interactions provide a variety of different geometric arrays [Reproduced from ref 11, Copyright 2004 AAAS].

Cotranscriptional Self-Assembly: Coupling of RNA Synthesis with Self-Assembly

Cotranscriptional self-assembly is unique in that it attempts to take advantage of RNA's ability to be produced inside cells using endogenous transcription machinery, thus coupling RNA synthesis with RNA self-assembly *in vitro* or *in vivo*.[46,48,54,63] One of the key factors associated with this strategy pertains to the need to kinetically control the formation of self-assembly. As a complementary strategy to the previous three, cotranscriptional assembly has been applied to RNA nanostructures formed by the other self-assembly strategies. For example, the RNA nanoring uses RNA architectonics cotranscriptional self-assembly,[46,63] while the nanocube[48] and the organelle-like RNA scaffolds[54] take advantage of ssRNA cotranscriptional assembly.

Finally a unique example of this cotranscriptional strategy involves rolling circle transcription to form long RNAs with periodic repeats.[64] The resulting RNA microsponges are able to form lamellar-like structures indicative of other types of polymers produced in bulk. As a relatively new approach, less is known about its precise assembly process, rules, and associated constraints. For example, it is unclear how the nature of the repeat units and relative spacing between these units affects the ability to form lamellar-like structures assembling further into nanoparticles. However, we generally postulate that the sequence and overall degree of constraints falls between those found in the WC hybridization and architectonics strategies.

RNA ARCHITECTONICS AND THE CHARACTERIZATION OF RNA STRUCTURAL MOTIFS

The identification of natural, recurrent structural motifs demonstrates that nature uses modular tertiary motifs programmed into the primary sequences of various RNAs to aid the self-assembly of RNA structures.[28] Thus, the development of RNA as a viable medium for nanoscale self-assembly has been facilitated by the atomic scale crystallographic structures associated with large stable RNAs like the ribosome[30,31,65] and ribozymes,[33,35−37,66] providing a valuable source of "parts" that can be used to construct more complex and interesting nanostructures with dynamic, catalytic, and molecular recognition properties.

Generally speaking, these "parts" (or structural RNA motifs) fall into one of two categories: tertiary interactions that are localized about a central region (most often junctions; Figure 3A) and those that involve long-range interactions (i.e., kissing loops, pseudoknot-like interactions, and loop receptors; Figure 3B). As the result of being localized around a specific set of nearby or neighboring nucleotides, junctions are responsible for directing the local intramolecular folding of RNA, that is, the directional arrangement of adjoining helices. By and large, junctions can promote either the coaxial stacking or bending of helices. In contrast, motifs that coordinate long-range contacts such as kissing loops and loop receptor interactions can include applications that are more intermolecular in nature. Regardless of the type, each "part" must be properly validated.

The RNA architectonics strategy uses synthetic constructs as a controlled context in which recurrent RNA tertiary motifs can be studied and characterized, revealing empirically and quantitatively ascertained details related to their salt dependencies, thermodynamic stabilities, and folding and assembling tendencies (Figure 3C). With this methodology, an extensive study of RNA nanoparticles, stabilized by the tertiary folding of multihelix junctions[15] and that assemble via kissing-loop interactions,[11,13,14,16] has provided important experimental insights into some of the constraints that influence RNA folding and assembly.[25] For example, the inverse *ColE1* plasmid-encoded RNA I and RNA II transcripts (RNA I/IIi) demonstrate very different RNA assembly properties (Figure 5A). The HIV kissing loop provides a characteristic 180° angle between the complementary terminal loops to create a nearly perfectly stacked helical interface. This structural feature has been exploited in the controlled assembly of a variety of RNA architectures including RNA tectosquare,[11,13,40] 2D arrays and nanogrids,[11,13,40] fibers,[15] and polyhedrons[14] (Figures 4 and 5B). In contrast, the RNA I/IIi kissing loop directs the assembly of an approximate 120° interior angle between adjoining loops to program the formation of hexagonal RNA nanorings.[16,67] Subsequent experimental characterization of the RNAI/IIi kissing loop in the context of the nanoring not only

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confirmed initial models but provided a platform to elucidate the sequence signature of the seven nucleotides responsible for the interaction's specific geometry, enabling the creation of a fully programmable nanoring.[16]

With respect to junctions that promote bends, the right angle (RA)[26,40] represents a well-characterized and commonly used motif to generate a 90° angle between adjacent helices in 2D (Figures 3, 4A, and 5B). Based on this characteristic geometry, the RA motif was originally placed in the context of square-shaped architectures.[11,13] In this context, the sequence signature of the RA motif was characterized, allowing for its predicted presence in group IC1 and ID introns.[40] Similarly, the structural characterization of the pRNA,[18,41,68] A-minor junction,[15] GNRA tetraloop/receptor,[10,12,69,70] and kink-turn motifs,[17,26,71] in the context of artificial RNA nanoparticles, have led to their further refinement and in some cases have led to their subsequent incorporation into next generation nanostructures.[14,20,50]

CREATING NEXT-GENERATION RNA NANOSTRUCTURES

While RNA has also been used to build higher order assemblies *in vitro*[10−13,15,16,18,53] and more recently *in vivo*,[54] RNA nanotechnology is still in its early infancy, and many challenges remain.[6,7] For example, the ability to stabilize RNA, which is more chemically labile than DNA, is an important area of current development to expand the potential of RNA nanostructures for nanotechnology applications.[5,63,72] Furthermore, while RNA ribozymes and riboswitches demonstrate RNA's natural ability to dynamically switch between multiple structural conformations, the proficiency to design and construct artificial 3D RNA architectures able to respond conditionally to particular environmental cellular cues, such as proteins and small ligands, is an area that is still largely unexplored.[9,73,74] And even though the architectonics strategy is predicated on the use of thermodynamically stable motifs, the current knowledge of RNA tertiary motif sequence signatures (e.g., refs 25 and 26) suggests that the ability to construct complex RNA assemblies through modular networks of recurrent RNA motifs and submotifs is well founded. Thus, the identification and characterization of more dynamic motifs remains a necessity.

It is worth pointing out that structural motifs are not just confined to naturally occurring ones. The inception and development of more sophisticated selection and evolution techniques (or SELEX) have spawned and isolated a plethora of artificial RNAs with novel binding or catalytic properties.[70,75−80] In fact, the ability to select for new structural and functional elements currently outpaces the ability to characterize these same new moieties. Thus, the future of RNA nanotechnology depends, at least in part, on the careful characterization of these new components in a variety of different contexts in order to assess their respective usefulness for their incorporation into and development of more and more complex nanostructures for in vivo and in vitro purposes. Along these same lines, the continued development of new directed evolution strategies to generate more complex and robust RNAs that operate in predictable and controllable manners whether acting *in vivo* or *in vitro* is sure to pay dividends.[73,80]

The ability to create larger and more complex structures poses unique constraints on the kinetics and thermodynamics associated with RNA folding. In the case of the relatively simple nanoring, reliance on the wild-type sequence signature of the RNAI/IIi KL alone produced a distribution of nanoparticles due to formation of moderately stable kinetic traps ranging from tetramers to octamers.[16] In terms of RNA structure and its relation to thermodynamics, nature seems to prefer suboptimal solutions that favor cooperativity.[40,81] Selection of thermodynamically stable (but not too stable) structural motifs provides a delicate balance between thermodynamic and kinetic influences on folding and assembly. Thus, structural motifs that provide just enough thermodynamic stability to direct certain folding pathways[28] without prohibiting other desired structural rearrangements are preferred. In general, as the size of an RNA increases, kinetic factors associated with folding tend to increase disproportionately compared with thermodynamics. This suggests that a more complex RNA has a much, much greater potential to become kinetically trapped in undesired conformations than a smaller or shorter RNA.

CONCLUSIONS

As a relatively new technology, aspects of nanotechnology (particularly with respect to *in vivo* applications) continue to raise public concerns with respect to toxicity and environmental impact.[82,83] In this regard, the use of nucleic acids as a nanomaterial has the potential to provide a fundamental advantage over exotic synthetic materials in terms of concerns related to toxicity, biocompatibility, and biodegradability. As building blocks fundamentally involved in the makeup and existence of living organisms, nucleic acids represent an exemplar material that is relatively inexpensive and easy to synthesize in the research laboratory. Of course, many challenges remain as RNA nanotechnology transitions into viable therapeutic and biotechnology applications where matters of scale, purity, and delivery (among other things) are paramount.

As an emerging field in its own right, RNA self-assembly provides distinct advantages and methodologies. Rather than mimicking DNA nanotechnology, in terms of both applications and assembly strategies, RNA nanotechnology is best served by taking advantage of the intrinsic strengths that RNA possesses as a material capable of folding and assembling like those found in nature.[6,9,46] The ability to couple cotranscriptional synthesis with RNA self-assembly is one of these important characteristics. Thus, the continued development of RNA nanotechnology must focus on balancing the kinetic aspects associated with RNA folding with the thermodynamic control provided by modular motifs. This may include combining an increased number of motifs (a majority of the particles created to date only integrate two or three motifs at most) as well as finding new ways to integrate the complementary self-assembly strategies highlighted above. Along these same lines RNA nanotechnology will benefit from increased efforts to coordinate the rational design with new strategies in directed evolution, for example, using more advanced structures as scaffolds to provide a platform for further evolutionary development or devising selection schemes with larger more complex RNAs as the goal.[6,80]

It remains to be seen the new ways in which researchers can take advantage of RNA self-assembly to impact and develop RNA-based applications for new fields like nanotechnology, synthetic biology, and therapeutics.[5−7,9,46] Nevertheless, RNA appears well-suited to provide new and exciting advancements, the likes of which it has already and continues to make.

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The authors declare no competing financial interest.

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DEDICATION

Luc Jaeger wishes to dedicate this paper to saint Giovanni Leonardi, patron saint of pharmacists.

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