DNA nanotechnology

Chemical copying of connectivity

Three-dimensional DNA nanoscaffolds such as supramolecular tetrahedra can self-assemble from tris-oligonucleotidyls — synthetic three-armed building blocks in which three identical or non-identical short DNA sequences are connected by a tris-linking backbone[1,2]. Here we show that the connectivity information contained in these building blocks can be copied by using template-directed tris-linking. This finding is a crucial step towards the replication of nanoarchitectures that are based on tris-oligonucleotidyls and to the realization of artificially self-replicating systems on a nanometre scale.

The procedure is outlined in Fig. 1a. On the basis of the three template sequences of an asymmetric tris-15-nucleotide oligomer (ref. 2), we generated the three corresponding complementary linear sequences with a reactive group at their 5′ ends, and used these in different ligation reactions[3–5] with a suitable tris-linker molecule.

Template-directed tris-linking was rapid and robust — unhampered by hydrolysis — in the hydrazone-formation reaction shown in Fig. 1b. Three 5′-hydrazide-modified oligonucleotides, a 15-nucleotide molecule (15-mer; **A**), a 13-mer (**B**) and an 11-mer (**C**), the sequences of which are complementary to the respective arms of the tris-15-meric template **Y**, were synthesized by using standard solid-phase protocols. The 5′-hydrazide moieties were introduced by using a modifier amidite[6].

For a statistical, non-template-directed synthesis, ten 5′-tris-linked species would be expected, ranging from a branched 33-mer to a branched 45-mer, as well as six bis-linked species containing between 22 and 30 nucleotides. A template-directed synthesis should yield a single branched 39-mer, as well as three of the six possible bis-linked species, if the reaction has not reached completion. Figure 1c shows that the predicted 39-mer is formed as the principal product in the presence of the template (confirmed by MALDI mass spectroscopy); in the absence of the template, there are no tris-linked products detectable by polyacrylamide-gel electrophoretic analysis. There is also evidence that the template directs the formation of the intermediate bis-linked species, the distribution of which differs from that in the statistical synthesis.

The copies are stable, even without reductive amination in the presence of sodium cyanoborohydride, which has been used successfully in other imine-based, template-directed ligation reactions[7]. We also found that short-armed tris-9-mers can be used as templates to generate asymmetrical tris-30-mers, which are otherwise not easily accessible by chemical synthesis. Tris-linking was also possible with 3 + 1-armed templates[2], in which one arm is connected through its 5′ end. The 'Klenow' polymerase enzyme accepts the 3′ end of tris-15-mer copies hybridized to overhanging linear templates as extension sites.

A full replication cycle also requires reverse copying — that is, the tris-linking of 3′-modified oligonucleotides using 5′-tris-oligonucleotide templates. Once this step becomes feasible, surface-promoted replication and exponential amplification of DNA analogues (SPREAD)[8] may be adapted to connectivity templating to achieve multiple rounds of replication. For self-assembling, non-covalent nanostructures, including tetrahedral nanoscaffolds of modular functions[2], replication will depend only on the ability of the subunits to replicate. Non-covalent connectivity therefore constitutes transferable information that can instruct a subunit where to integrate during self-assembly. Covalent connectivity and/or connectivity by topological interweaving, as used in Seeman's DNA cube[9], cannot replicate but ensures ultimate stability. Switchable duplex crosslinkers, such as those based on disulphide bonds[10], may be suitable for stabilizing replicatable nano-objects.

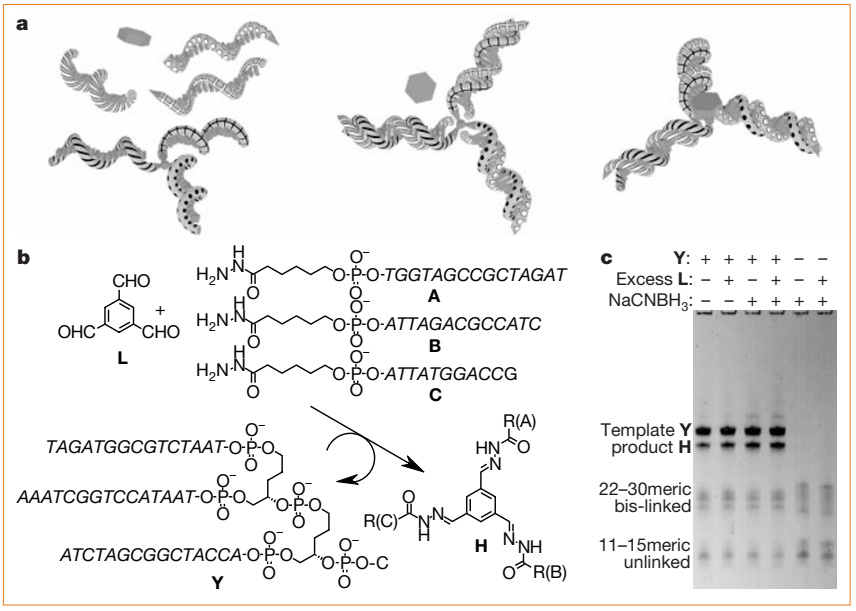

Figure 1 Chemical copying of connectivity by template-directed tris-linking. **a,** Left, chemical copying of connectivity involves a 3′-trisoligonucleotidyl template with three individually defined sequences, three linear complements with 5′ ends represented as blunt ends, and a tris-linker shown as a honeycomb cap. Centre, hybridization gives rise to a quartermolecular complex in which the blunt ends come into close spatial proximity. Right, the tris-linker has reacted to connect the 5′ ends in the copy. **b,** Chemical copying of connectivity on the basis of tris-hydrazone formation. Tris-linking of the 5′-hydrazide-modified oligonucleotides **A**, **B** and **C** with the tris-linker **L** in the presence of the 3′-tris-oligonucleotidyl template **Y** yields the tris-hydrazone **H**. **c,** Nucleic-acid staining (with SYBR gold), after denaturing polyacrylamide-gel electrophoresis, of an almost complete chemical copying of connectivity from a 10 μM solution of the components in 100 mM MOPS buffer (pH 5.6) after incubation for 2 h at room temperature in the presence or absence of template **Y**, an excess (2.5:1.0 equivalent) of tris-linker **L**, and sodium cyanoborohydride ($NaCNBH_3$; 100 equivalents).

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