Trends in Analytical Chemistry 65 (2015) 97–106

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Translating nucleic-acid hybridization into universal DNA-reporter sequences

Hadi Ravan *

Department of Biology, Faculty of Science, Shahid Bahonar University of Kerman, Kerman, Iran

ARTICLE INFO

Keywords:
DNA-hybridization translator
DNA nanostructure
DNA-reporter sequence
Nucleic-acid hybridization
Oligonucleotide
Recognition element
Sequence
Translator probe
Universal DNA reporter
Universal reporter element

ABSTRACT

Nucleic-acid hybridization occurs between an oligonucleotide with a known sequence (probe) and its complementary counterpart to form an organized structure. Generally, in hybridization-based detection, different labeled probes must be synthesized for various target sequences of interest. To avoid the trouble of having to label each individual probe for detecting each target sequence, the concept of translation of nucleic-acid hybridization was proposed. A hybridization translator is defined as a DNA sequence that can convert any hybridization reaction into a unique output sequence. The translation process is performed by applying a particular oligonucleotide probe consisting of two unrelated DNA fragments. One fragment is a recognition element and other is a universal reporter element, which comprises a sequence not present within the sample material. In this article, we summarize progress in the development of a DNA-hybridization translator for the detection of nucleic acids.

Contents

1. Introduction

Life uses striking processes in which some biological molecules are labeled with a universal tag in order to be recognized by a particular sub-cellular processing system. For example, the Shine-Dalgarno sequence is a ribosomal binding site in the mRNA molecules of the bacteria that locates eight bases upstream of the start codon [1]. The six-base consensus sequence of the Shine-Dalgarno binds to its complementary sequence at the 3' end of the 16S rRNA in the ribosome [1]. The hybridized mRNA molecules, regardless of their open reading frames, are used as a template for protein synthesis by the ribosome machinery. The discovery of such phenomena in living organisms inspires researchers to develop universal translators that are able to convert different molecular inputs into a unique output molecule. Particularly in the fields of molecular diagnostics [2,3], DNA computation [4–6], and programmed chemical synthesis [7–9], innovative efforts have been made to convert the nucleic-acid-hybridization reaction into a structurally related or unrelated output molecule [10].

* Tel.: +3413222082; Fax: +98 341 3222032.
E-mail address: ravan@uk.ac.ir (H. Ravan).

http://dx.doi.org/10.1016/j.trac.2014.09.012
0165-9936/

Nucleic-acid hybridization occurs between a probe molecule and its complementary counterpart (target) to form an organized structure [11,12]. Since nucleic-acid structures themselves possess no readily detectable functionality, probe molecules are normally labeled with an appropriate indicator to convert probe/target binding events into detectable signals [13]. Generally, in the hybridization-based detection of nucleic acids, different labeled probes must be designed, synthesized, and tested for various target sequences of interest. Preparation of different tagged probes is limited due to the high cost and the tedious procedures associated with probe labeling and optimizing. To avoid the trouble of having to label individual probes for detecting each target sequence, the concept of translation of nucleic-acid hybridization was proposed [14].

A nucleic-acid-hybridization translator is defined as a DNA sequence that can convert any nucleic-acid-hybridization reaction into a unique output sequence. The translation process is performed by applying particular probes that generally consist of two unrelated DNA fragments. One fragment is a recognition element and other part is a universal-reporter element, which comprises a sequence not present within the sample material [14]. Although the reporter sequences can be incorporated into all types of probe molecule, the principle behind the target detection can be varied from simple hybridization strategy to an advanced toehold-mediated DNA-strand-displacement mechanism [15]. In the present overview, we summarize the major advances in the development of new probe-based detection of nucleic acids, including those equipped with a universal DNA-reporter sequence for target detection.

2. Physicochemical properties of DNA-hybridization translators

Terminal unpaired nucleotides in partially double-stranded nucleic acids have been known as dangling ends [16]. It has been shown that terminal dangling residues affect the thermodynamic stability of a short duplex nucleic acid. Since the universal DNA-reporter probes contain several dangling residues, the free energy of duplex formation is influenced by contributions of dangling ends besides the Watson-Crick base pairing [16,17]. Recently, Di Michele and co-workers showed that a tail of dangling residues weakens the binding strength of a DNA duplex [18]. In their study, they showed that the electrostatic repulsion between dangling residues and the hybridized region is enough to raise the hybridization free energy of a core duplex by ~1 kcal/mol at physiological salt concentrations. Moreover, there is a difference between the instability resulting from 3′/3’ (or 5’/5’) and 5’/3’ dangling ends in nucleic-acid duplexes. The dangling residues, which are attached to the same side of the hybridized duplex (5’/3’dangling ends), due to tail-tail interactions, are energetically unfavored compared with the other dangling ends. In this situation, steric repulsion of the tails is added to total free energy distribution of the DNA-duplex formation. In this respect, accounting for dangling-end effects is crucial for designing a DNA-hybridization translator. In this way, an interpolating formula that developed by Di Michele [18] may be useful for evaluating the thermodynamic parameters of the universal DNA-reporter probes.

Based on a kinetic study, Zhang and Winfree showed that a probe with a dangling end slightly reduces the rate of DNA hybridization [19]. They designed a molecular circuit comprising DNA constructs S and C (Fig. 1A). Under toehold-mediated strand displacement, construct C is hybridized to construct S. When construct C changes to new construct B by adding new domain X at its 5′ end, the rate of DNA hybridization slightly reduces (Fig. 1B). This non-complementary domain (X) reduces the rate of nucleation step in the DNA-hybridization reaction.

3. Different formats of DNA-hybridization translators

3.1. Classical probes as the universal DNA translator

3.1.1. Classical linear probes

The conventional probe for detecting nucleic acids generally comprises a tagged oligonucleotide without any secondary structure. To optimize this probe molecule for use in the translation of nucleic-acid hybridization, a spurious oligonucleotide is tailored to the end of target-specific region of the probe molecule. Upon hybridization of the probe molecule, the tailored sequence is overhung and used as a binding site for a complementary labeled oligonucleotide.

Tani and co-workers developed a flexible real-time (RT) PCR method based on this translation strategy [20]. In their system, the probe molecule was designed to possess a cytosine residue at the 3′-end of the target-specific region of the probe molecule (Fig. 1C). The guanine residue complementary to the cytosine nucleotide is able to quench the fluorescence signal of a labeled linear oligonucleotide (LO). When the LO/3′-tailed probe complex hybridizes to the amplified target, the fluorescence of the dye is quenched by the guanine nucleotide in the target.

However, there are some difficulties with regard to the design of such sequence-specific probe molecules; the target should possess a particular guanine residue at the specific position. To overcome this limitation, the next generation of the universal probes was developed (see below) [21].

3.1.2. Molecular beacons

Molecular beacon (MB) is an oligonucleotide with a stem-loop structure that is generally labeled with a fluorophore and a quencher at its opposite ends [22]. Upon binding to the target, the MB switches to an elongated conformation and changes its fluorescence intensity. MBs distinguish mismatches over a wider temperature range than unstructured linear probes because the stem-loop structure stabilizes the dissociated state of the probe-analyte [23].

Several recent studies have used the MB as a platform for the translation of nucleic-acid hybridization into a universal output sequence. In this strategy, the probe molecule comprises a target-specific MB grafted onto an oligonucleotide motif that serves as the universal reporter element. Unlike LOs, MB-based universal probes are easily applicable for any target nucleic acid [21].

Tam-Chang and co-workers developed an MB-based universal probe for sensing unlabeled nucleic acids [24]. The MB used in this system is pre-hybridized to a fluorescently labeled oligonucleotide (FLO) via its universal reporter element (Fig. 1D). Fluorescent emission of the FLO is quenched by guanine residues in the stem region of the hairpin. Upon target binding, the stem region is opened in order that the guanine residues are taken apart from the fluorescent dye and the signal is restored. This turns off quenching, increases emission intensity, and indicates the presence of the target.

In another attempt, Zhou et al. developed a universal MB that worked based on a G-quadruplex quenching strategy [25]. In this system, the probe molecule is constructed from a continuous seven-guanine residue (G7s) tailed to a random oligonucleotide sequence (Fig. 1E). To sense, a fluorescein-labeled nucleotide is placed between the G7s and the oligonucleotide tail of the probe molecule (we call this structure a G7s-tail strand). Four G7s-tail strands form an intermolecular parallel G-quadruplex structure (IGQ) that quenches the fluorescent intensity of the dye molecule via photoinduced electron transfer. Moreover, to fabricate a functional probe, one oligonucleotide tail of the G7s-tail strand is hybridized to an unmodified MB (Fig. 1E). The stem portion of the MB is formed by hybridization of segments 1 and 2 that bear critical complementary cytosine and guanine residues, respectively. In the presence of the target DNA, segment 1 is released by loop-target hybridization and its cytosine residue is in turn hybridized to a