Nucleic Acid Aptamers—From Selection in Vitro to Applications in Vivo

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ABSTRACT

Aptamers are nucleic acid ligands which are isolated from combinatorial oligonucleotide libraries by in vitro selection. They exhibit highly complex and sophisticated molecular recognition properties and are capable of binding tightly and specifically to targets ranging from small molecules to complex multimeric structures. Besides their promising application as molecular sensors, many aptamers targeted against proteins are also able to interfere with the proteins' biological function. Recently developed techniques facilitate the intracellular application of aptamers and their use as in vivo modulators of cellular physiology. Using these approaches, one can quickly obtain highly specific research reagents that act on defined intracellular targets in the context of the living cell.

The synthesis and functional screening of large libraries of compounds is commonly known as "combinatorial chemistry". In recent years, a number of methods have been developed to isolate molecules with desired functions from libraries of small organic molecules, nucleic acids, proteins, peptides, antibodies or single-chain antibody fragments (scFv), or other polymers (for comprehensive reviews on these topics, see ref 1). The identification of active compounds from composite mixtures proceeds in iterative cycles of selection and amplification. This not only permits screening of libraries with high complexity but also facilitates further increases in the library diversity by mutating the pool during amplification steps. These types of selection consequently require encoding strategies that allow unambiguous resolution of the composition and sequence of any active molecules,

information required for their subsequent amplification or optimization. With peptide, protein, or antibody fragment libraries, this is most easily and logically achieved by approaches such as displaying peptides, proteins, or antibody fragments on phage and cell surfaces, which convert the amino acid sequence into genetic information that can be amplified in vivo. Alternatively, two encoding strategies have been developed recently that facilitate entirely in vitro synthesis and selection of proteins: mRNA-protein fusions[2] and ribosome display.[3]

Nucleic acids are particularly suited for combinatorial selection approaches because they can fold into well-defined secondary, tertiary, and quaternary structures and they are easily amplified by the polymerase chain reaction (PCR) or in vitro transcription. By their very nature, nucleic acids provide the blueprint for their own replication and, by that same token, for their improvement and optimization. Consequently, the sequence space that can be successfully screened in parallel is the most extensive among all the combinatorial chemistry techniques: far more than 10^{15} different molecules can be screened simultaneously for a particular function. In vitro selection of combinatorial oligonucleotide libraries can lead to the isolation of nucleic acids such as RNA, ssDNA, modified RNA, or modified ssDNA that bind a wide variety of targets with high specificity and affinity[4,5] (for a recent review, see ref 6). But it is another feature that makes them enormously flexible and powerful: possessing highly selective molecular recognition properties, nucleic acids can target key molecules inside or outside a diseased cell, or may be used like antibodies for diagnostic purposes. Therefore, we have chosen this unique class of "dual mode" molecules, called aptamers, to form the basis of a simple yet highly versatile molecular toolbox.

Isolation and Application of Aptamers Binding Small Molecules

Aptamer structures have been comprehensively reviewed and discussed in a number of commentaries and reviews.[7–9] Comparisons of various ligand-binding aptamer structures with proteins that bind related molecules have shown that nucleic acids and proteins use strikingly similar strategies for the formation of well-defined binding pockets.[10] Structural studies performed with aptamer/ligand complexes have revealed insights into principles of folding, shape, and surfaces, as well as the molecular diversity associated with nucleic acid architecture, molecular recognition, and adaptive binding.[11]

Our first steps into combinatorial nucleic acid selection led to the isolation of aptamers which bind to small molecules such as amino acids and biological cofactors[12–16] (for a recent review, see ref 17; the first aptamer selected for a biological cofactor was an ATP-binding RNA sequence,[18] the solution structure of which was also elucidated by NMR spectroscopy[19–21]). Like others in this field,

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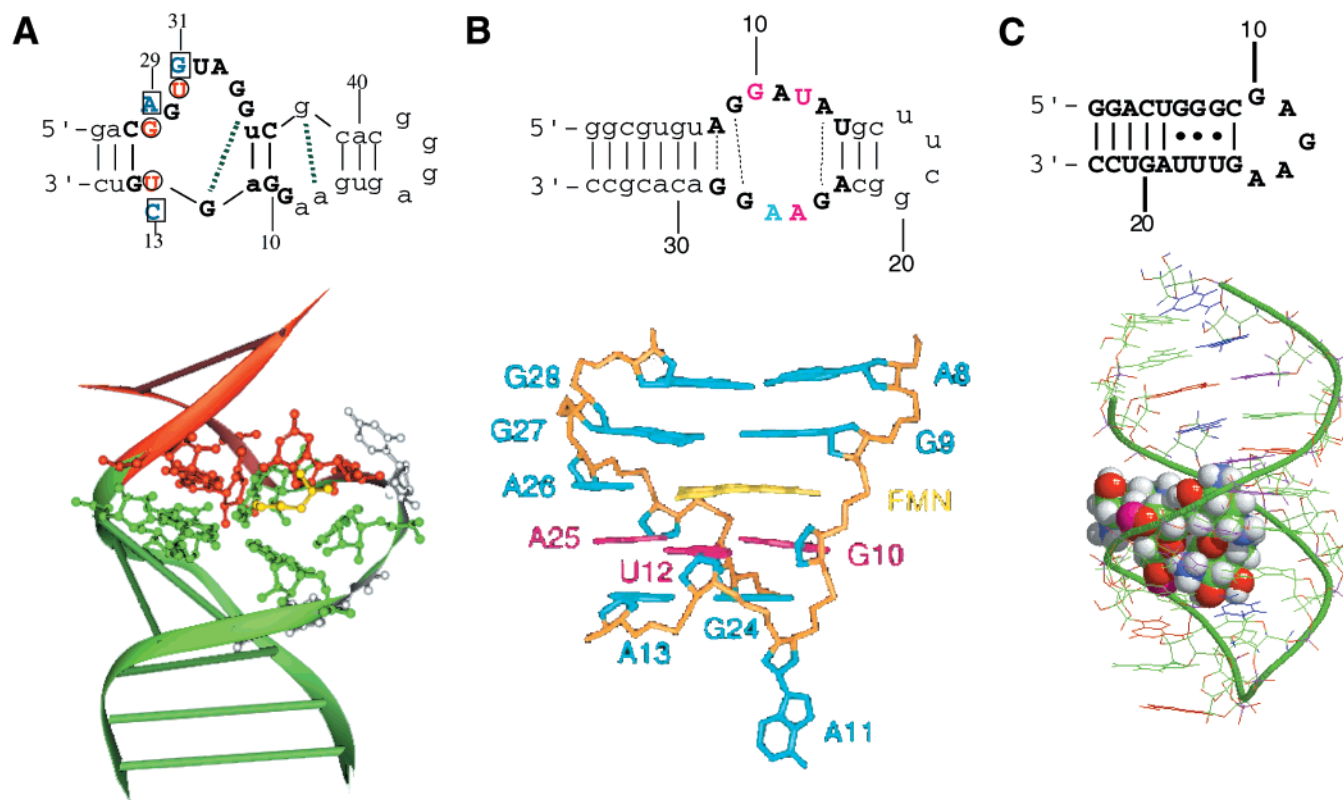
FIGURE 1. Structures of some aptamers from our group. (A) Secondary structure proposed previously for the citrulline- and arginine-specific aptamers, based on covariations of selected sequences and on the chemical footprinting pattern obtained in the presence of the cognate amino acid, as well as in damage selection experiments. The bases which were conserved among different isolates are shown in uppercase, while variant bases are in lowercase. The three nucleotides critical for amino acid specificity (13, 29, and 31) are indicated by circles (for citrulline) and boxes (for arginine). The tertiary structure is shown for the citrulline-binding aptamer.[23] (B) Secondary structure of the FMN aptamer and tertiary structure of the FMN-binding region in this aptamer as determined by NMR spectroscopy.[30] (C) Secondary structure of the neomycin B aptamer[15,27] and tertiary structure of this aptamer as determined by NMR spectroscopy.[29]

we wanted to investigate certain principles of molecular evolution and recognition of specific ligand-binding RNA molecules. For example, an RNA aptamer specifically recognizing the amino acid L-arginine was "evolved" from an in vitro-selected L-citrulline-binding parent sequence.[12] The two aptamers differ by only three mutations, yet each exhibits very high specificity for its cognate ligand. The three-dimensional fold defined by chemical probing analysis and NMR spectroscopy showed how the three mutations within the amino acid binding site of these RNAs determine which of the two amino acids is specifically recognized (Figure 1A).[22,23]

The structures of the citrulline and arginine aptamers, together with those of several other arginine aptamers for which structural data exist, were used as an experimental starting point to analyze a hypothesis that the genetic code might have evolved via mechanisms of molecular recognition between amino acids and short RNA motifs.[24] Statistical evidence suggests that arginine aptamers, together with several other arginine aptamers for which structural data exist, appear to have a significant bias in favor of arginine codons at their binding sites.[25] These data support the hypothesis that amino acids can specifically interact with RNA sequences that contain their cognate codons. In the meantime, similar evidence has also been

obtained for other aptamer/amino acid complexes (M. Yarus, personal communication).

The degree of molecular discrimination achieved by aptamer/small molecule complexes can match or even surpass that of antibodies. An aptamer specific for theophyllin distinguishes it from caffeine, which differs by only one methyl group, at least 10-fold more efficiently than an antibody isolated for the same purpose.[26] An aptamer selected for specific binding to L-arginine shows a 12 000-fold reduced affinity to the D-arginine enantiomer.[14]

Aptamers for small molecules,[17] such as neomycin[15,27] and FMN,[13] have been used in surface plasmon resonance technology to generate target-specific biosensors.[28] The three-dimensional structures of the FMN and neomycin B aptamers[29] have also been solved by Patel and colleagues[30] (Figure 1B,C). This and other analyses[31,32] have revealed insights into the mode and dynamics of molecular recognition of a dimethylisoalloxazine moiety by an RNA aptamer.

One of the remarkable characteristics of in vitro-selected RNA aptamers is that ligand binding is always accompanied by significant structural changes in the binding RNA molecule.[11] Ligands seem to become an integral part of the RNA aptamer structure once they are bound.[8,33] This property of aptamers might have inspired

Table 1. Selected Targets for Aptamers That Recognize Extracellular Proteins

target	K_d (nM)	nucleic acid	ref
acetylcholine receptor	2.0	RNA	70
L-Selectin	3.0	RNA	71
basic fibroblast growth factor (bFGF)	0.35	RNA	72
platelet-derived growth factor (PDGF)	0.1	ssDNA	73
keratinocyte growth factor (KGF)	0.0003	2′-modified RNA	43
vascular endothelial growth factor (VEGF)	0.14	2′-modified RNA	44
interferon-γ (IFN-γ)	6.8	2′-modified RNA	74
cellular prion protein (PrP^c)	nd	RNA	40
anti-acetylcholine autoantibodies	60	2′-amino-RNA	42

the idea of fusing aptamer sequences with known catalytic RNAs, incorporating the principle of allosteric regulation into ribozyme catalysis. None of the existing natural ribozymes were known to operate as allosteric enzymes in vitro or in vivo. Breaker and his colleagues[34,35] and Araki et al.[36] integrated sequences of the ATP,[18] theophylline,[26] or FMN aptamers[13] into the hammerhead ribozyme (HHR) to rationally engineer allosteric HHRs, which carry out the phosphodiester cleavage—or inhibit it—only after the relevant ligands have been added to the cleavage buffer.[34,35] In the case of the FMN aptamer, modular rational design and in vitro selection techniques were combined to generate precision molecular switches comprising ribozyme/aptamer chimeras.[35] Intracellular ribozymes which target and inactivate certain mRNAs are currently under investigation for their use to control the expression of proteins. Allosteric ribozymes which are activated or inhibited by membrane-permeable, nontoxic, low-molecular-weight molecules may provide extremely powerful tools for conditional gene expression.[37] It is now possible to directly select for aptameric regulatory RNA motifs that activate a "silenced" ribozyme[38] or that inhibit an active ribozyme (Piganeau, N.; Jenne, A.; Thuillier, V.; Famulok, M., manuscript submitted) in the presence of small organic molecules. A more direct but nevertheless efficient route to use aptamers inside cells for the control of protein expression was recently described.[39] Placing an aptamer specific for the organic dye Hoechst 33528 in the 5′-untranslated region (5′-UTR) of a reporter gene mRNA prevented its translation in the presence of the cognate ligand, whereas gene expression proceeded normally in its absence.

Functional Aptamers for Proteins and Their Application in Biotechnology, Molecular Medicine, and Diagnostics

The specificity of molecular recognition combined with the ease by which protein-binding aptamers can be isolated, engineered, evolved, and modified chemically—exclusively ex vivo—makes these molecules very attractive as tools in molecular medicine, biotechnology, and diagnostics. Consequently, the vast majority of aptamers that have been isolated so far are for specific binding to protein targets. Table 1 summarizes some examples of aptamers

that recognize proteins that are expressed on cell surfaces or are localized extracellularly. Among them are aptamers composed of ssDNA, RNA, or chemically modified nucleic acids, where a repertoire of chemically introduced functional groups increases oligonucleotide stability and functional group diversity.

We have recently applied in vitro selection to isolate RNA aptamers that are directed against the Syrian golden hamster cellular prion protein PrP23-231 (PrP^c). A recombinant PrP/glutathion-S-transferase (GST) fusion protein immobilized on glutathion agarose was used for the selection.[40] Sequence comparisons suggested that all aptamers isolated are likely to contain a three-layered G-quartet as a structural element critical for PrP recognition. Mapping experiments with GST-PrP peptides revealed that the region of PrP^c critical for aptamer binding spanned the N-terminal amino acids 23−52. Individual radiolabeled aptamers specifically recognized authentic prion protein in brain homogenates from various species, such as wild-type mice, hamster, and cattle, as demonstrated by supershifts obtained in the presence of PrP-specific antibodies, but no interaction was observed with brain homogenates from PrP knock-out mice. This study showed that aptamers are able to recognize their specific target among the hundreds of different proteins present in tissue homogenates.

Nuclease-Resistant Functional Aptamers

The PrP-binding aptamers probably possessed sufficient stability in crude brain homogenates due to the stable G-quartet scaffold protecting them from exonuclease degradation. Small protein-binding RNA aptamers, however, usually do not contain stabilizing structural scaffolds of this kind. Therefore, for them to be widely applicable as potential therapeutics, diagnostics, or assay components, the capacity of aptamers to evade nuclease degradation has to be increased. A number of studies have established that functional oligonucleotides can be made to be not only strikingly small but also resistant to degradation in biological materials.[41] One possible way to circumvent the vulnerability of RNA to nuclease degradation is indirect: in a first step, an aptamer that binds the enantiomer of the target is selected, then, in a second step, the enantiomer of the aptamer is synthesized (from L-phosphoramidites) as a nuclease-insensitive ligand of the natural target. This mirror-image approach has been applied to L-arginine, D-adenosine, and the peptide hormone vasopressin (for review, see refs 6 and 41).

An alternative approach is the direct selection of an aptamer from libraries of modified RNAs. Modifications must be chosen that are compatible with nucleic acid replicating enzymes such as reverse transcriptase or DNA and RNA polymerases. The modifications most commonly used are those where the 2′-OH group of pyrimidines is substituted by a 2′-fluoro or 2′-amino group. 2′-Amino-modified nuclease-resistant aptamers have been selected which bind to autoantibodies of patients affected by the muscular disease *myasthenia gravis*. Such aptamers inhibit

the binding of these autoantibodies to acetylcholine receptors on human cells, blocking the associated pathogenic consequences.[42] Similarly, 2′-fluoro-modified nuclease-resistant aptamers directed against the human keratinocyte growth factor block its activity with a K_i of 34 pM.[43]

An aptamer with 2′-aminopyrimidine modifications selected for binding to vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) was minimized to a modified 24-mer. The 2′-OH groups of defined purine residues were subsequently modified by 2′-methoxy groups in a damage selection experiment where variants of this aptamer chemically synthesized with a mixture of 2′-OH- and 2′-OCH$_3$-purines were screened for enhanced binding to VPF/VEGF.[44] 2′-Methoxy-substituted purine residues in this aptamer were identified by protection from alkaline hydrolysis. Of the 13 purines in the 24-mer, nine could be substituted by 2′-methoxy purine, while the other four could not be changed without significant loss of binding affinity. The end result was a modified aptamer which bound to the target protein with a K_d of 0.14 nM and specifically blocked the binding of ^{123}I-labeled VPF/VEGF to cell surface receptors expressed on human umbilical vein endothelial cells.

These and many other examples show that aptamers can routinely be obtained against almost any desired target. Most of the targets summarized in Table 1 are either located on the cell surface or otherwise easily accessible to a nucleic acid aptamer. Because most aptamers are also capable of very specifically modulating the biological function of their target, these molecules are potentially excellent candidates for drugs or drug leads.

Functional Aptamers in Vivo

Recently, our interest turned to using aptamers for another fascinating purpose. As RNA molecules, aptamers can be synthesized directly by the cell's own transcription machinery. By being expressed within the cell, aptamers can be used to target a certain protein in its natural environment. Due to their high affinities and specificities, such intracellular aptamers (for which the term "intramers" was coined [45]) may provide excellent tools to specifically inhibit signal transduction, cell growth, transcription, translation, or other intracellular processes or to study RNA−protein interactions in vivo.

Intramers in Procaryotes and in the Nucleus

Several studies already existed where RNA aptamers isolated in vitro were expressed in vivo to study their biological function within cells. In most cases the question was whether aptamers are capable of functionally replacing a natural RNA sequence in vivo. Among those tested were anti-HIV-1 Rev aptamers, which were inserted into the full-length Rev-responsive element (RRE) in place of the Rev-binding element (RBE). They were found to be functionally equivalent to the wild-type RBE in their ability to mediate Rev function in vivo.[46] Our laboratory took a different initial approach to investigate in vivo-expressed

aptamers in procaryotes. We had selected a series of aptamers that bind the special elongation factor SelB[47,48] (for a commentary, see ref 49) In *Escherichia coli*, SelB is required for the co-translational incorporation of the unusual amino acid selenocysteine into proteins such as the formiate dehydrogenases. To do this, SelB binds simultaneously to selenocysteyl-tRNA^{Sec} and to an RNA hairpin structure located directly 3′ of the selenocysteine opal (UGA) codon in the mRNA of formiate dehydrogenases (*fdhF*) (Figure 2).

Using a doped pool of this mRNA hairpin, we were able to select a mutated version of this motif which also had the ability to bind tightly and specifically to SelB. To dissect SelB binding to the *fdhF* mRNA hairpin from the overall biological activity of this complex, four selected aptamers were analyzed in vivo for UGA readthrough in a *lacZ* fusion construct. Of these, only one promoted UGA readthrough in vivo. The other three aptamers, despite their secondary structures and binding affinities being similar to those of the wild-type motif, were severely impaired or unable to replace the *fdhF* mRNA hairpin function in vivo. This finding implies that the functions of the *fdhF* hairpin go beyond the mere tethering of selenocysteyl-tRNA^{Sec} to the UGA codon via SelB.

RNA aptamers against yeast polymerase II were selected in a collaborative study with the laboratory of A. Sentenac, Gif-sur-Yvette, France. These oligonucleotides revealed specificity for polymerase II but do not inhibit polymerase I or polymerase III. The aptamers were found to interact preferentially with the two largest subunits of pol II, B220 and then B150. In vivo inhibition studies showed that, in yeast cells with an artificially reduced level of endogenous polymerase II, pol III-dependent expression of binding aptamers caused a cell growth defect, whereas expressing control RNAs did not[50] (Figure 3).

Shi et al. selected RNA aptamers that bind the RNA-binding protein B52.[51] B52 is a member of the SR protein family,[52] a class of nuclear proteins that play an essential role during pre-mRNA splicing in *Drosophila melanogaster*. To elucidate the function of selected RNA aptamers in vivo, a pentameric aptamer was constructed and expressed in transgenic flies under the control of an inducible promotor. It was demonstrated that B52 colocalizes with the pentameric aptamer at its sites of insertion in the polytene chromosome, indicating that the aptamer interacts with B52 in vivo. Since the level of B52 is critical for *Drosophila* development, deletion or overexpression of B52 leads to enhanced lethality and morphological defects. Shi et al. demonstrated that induced expression of the RNA aptamer in transgenic flies resulted in 50% higher lethality, presumably due to a reduced amount of available B52, the rest being sequestered by bound RNA aptamer. A second transgenic fly model that can overexpress both B52 and the RNA aptamer was used to further characterize the effects of the aptamer in vivo. In this model the authors showed that all effects caused by B52 overexpression could be reversed by induced expression of the RNA aptamer.
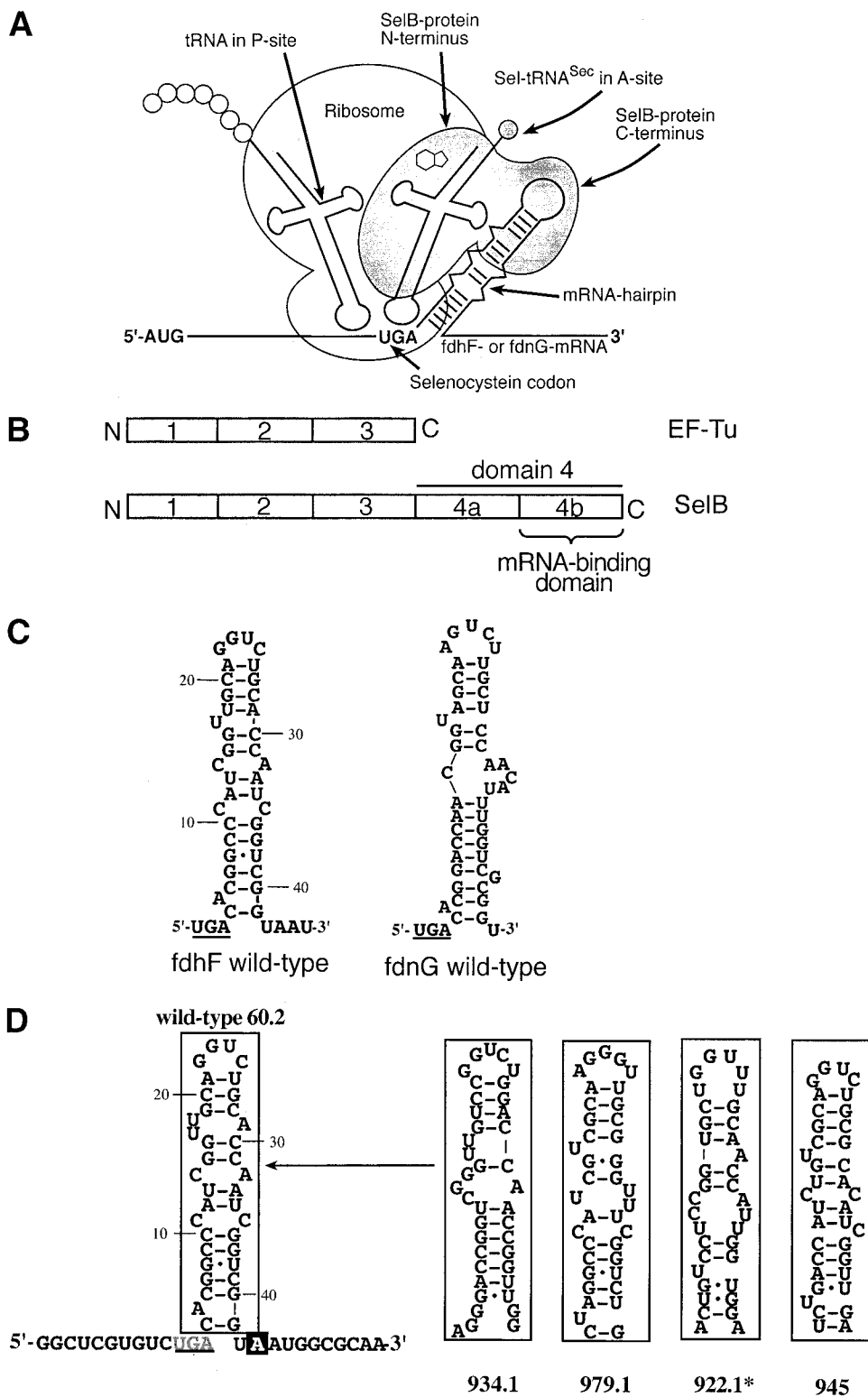
FIGURE 2. SelB protein and the *fdhF* and *fdnG* mRNA hairpins. (A) Schematic representation of the processes occurring during the cotranslational incorporation of selenocysteine at the ribosome. The quaternary complex between SelB, GTP (hexagon), selenocysteyl-tRNA[Sec], and the *fdhF* or *fdnG* mRNA hairpin is attached to the ribosome. (B) Schematic representation of SelB and its derivatives. Bottom: the full-length SelB protein (aa 1−614). The protein domain 4b corresponds to the ultimate C-terminus of SelB (aa 472−614). In the truncated version SelB1−474, the C-terminal domain 4b was deleted. This derivative shows extensive homologies to EF-Tu (top). (C) Secondary structures of the *fdhF* and *fdnG* mRNA hairpin motifs located immediately 3′ of the UGA selenocysteine codon. (D) Constructs for testing in vivo activity of SelB-binding aptamers. Aptamer sequences shown in the boxes (right) were substituted for the boxed region in the wild-type sequence (left). These constructs were used in a UGA stop codon readthrough assay using *lacZ* as the reporter gene.[47]
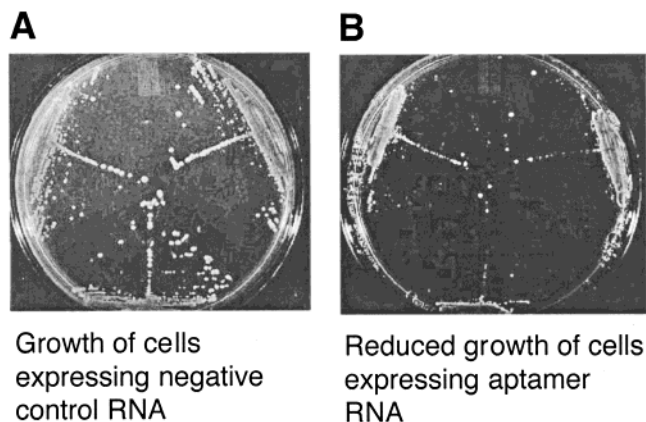
A

B

Growth of cells expressing negative control RNA

Reduced growth of cells expressing aptamer RNA

FIGURE 3. Growth of yeast strain YF1971 cells expressing Pol II-specific aptamer RNA (B) compared to nonbinding RNAs from the unselected pool (A). Growing these cells in a leucine-rich medium leads to a reduction in the endogenous amount of Pol II. Aptamer-expressing cells clearly showed reduced growth under conditions of reduced Pol II expression.

Intramers as Tools in Functional Genomics

So far, successful in vivo expression of aptamers had been restricted to target proteins with a natural affinity for nucleic acids (Rev, SelB, Pol II, B52) and, in the case of eukaryotes, to a limited cellular compartment, the nucleus. We therefore decided to devote further research efforts to the question of whether techniques for in vivo expression of aptamers could be expanded to cover a much broader scope, i.e., to target proteins with no intrinsic affinity for nucleic acids and to include other cellular compartments. As a start in this direction, we were interested in ablating the protein function of cytoplasmic targets with intacellular aptamers or intramers, since we believe this technology could have considerable impact on disease treatment and functional genomics. The emerging field of functional genomics is dedicated to unmasking the physiological role of a gene product, particularly within the dynamic context of the interactive network of a cell where any information received from the environment is processed and results in a cellular response. In other words, functional genomics is an approach which links gene sequences obtained by various genome sequencing projects[53-57] to the function of the proteins they encode, including a huge number of gene products about which nothing is known.[58,59]

Similar to intracellular antibodies (intrabodies[60-62]), intracellularly expressed chemokines (intrakines), or peptide aptamers,[63,64] intramers promise to be an important tool in the attempts to inactivate a gene product without altering the genetic material. Like other intracellular modulators of protein function, intramers provide a conceptually simple approach to phenotypically knocking out protein function without altering the actual proteome status of a cell. Modulating a protein target within the context of its actual expression status inside a living cell would allow simpler and more focused analyses of its biological function and of its value as a potential pharmaceutical target. In contrast to genetic knock-out strate-

gies, these molecules can be used to recognize different protein domains, subdomains, catalytic centers, or post-translational modifications within the same protein, allowing a direct investigation of functional epitopes at the molecular level. Intracellularly expressed modulators of protein function could provide a high-resolution picture of the function of a protein in the context of a living cell. Why is this important?

Most approaches to inhibiting a cellular component rely on investigating phenotypes of a cell or organism that differ from the wild-type as a result of altering the genetic information either by knock-out technologies or by expression/overexpression of a protein or one of its mutant derivatives. As the target protein is probably involved in continuous cross-talk with other cellular components participating in the various regulatory pathways, its absence or overrepresentation is likely to alter the cellular composition, activity, or distribution of other molecules. Despite the power of these techniques, such effects can complicate the interpretation of an observed altered phenotype: it could be due to specifically targeting a key player that regulates the observed phenomenon, or alternatively to unspecific deregulation of the regulatory system responsible. A good example of the complex effects caused by depleting a protein are the studies of the genome-wide transcriptional circuitry in yeast. Knock-out of the cyclin-dependent kinase Srb10, which is implicated in transcriptional regulation, resulted in the derepression of over 170 genes as well as the repression of a few others.[65,66] In addition to the problems associated with such complex effects, these approaches in functional genomics hardly ever distinguish between different domains or posttranslationally modified forms of the same protein. Thus, it is highly desirable to develop and apply methods that permit functional investigations of gene products in the natural context of a cell's proteome. We reasoned that intramers should be perfectly suited to act in various cellular compartments within the context of a living cell and thus help to fulfill the demand for specific intracellular inhibitors or modulators.

Cytoplasmic Intramers

As initial proof that this concept is valid, we recently developed a system that allows the expression of cytoplasmic intramers.[45] As a first target, we chose the cytoplasmic domain of the human αLβ2 integrin subunit (CD18cyt), a transmembrane protein involved in various regulatory steps in immunology and cell adhesion, found exclusively on leukocytes. The β2 integrins are a family of heterodimeric transmembrane proteins whose extracellular domains mediate the adhesion of leukocytes in immune and inflammatory responses by binding to the intercellular adhesion molecule-1 (ICAM-1) expressed on the surface of endothelial cells. The cytoplasmic domains of the integrin α and β chains are thought to be involved in the transmission of signals from inside the cell across the plasma membrane to the surface—a process that is also referred to as "inside-out" signaling.[67,68]
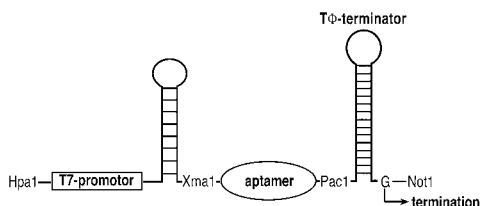
FIGURE 4. Design of the T7 RNA expression cassette. In the TR vector the T7 promoter is located upstream of aptamer-encoding DNA, which is inserted between 5′- and 3′-stem-loop structures. These serve as both RNA-stabilizing motifs and a termination signal for the T7 transcript. Aptamers retain full binding activity when inserted between the stabilizing stem-loops.
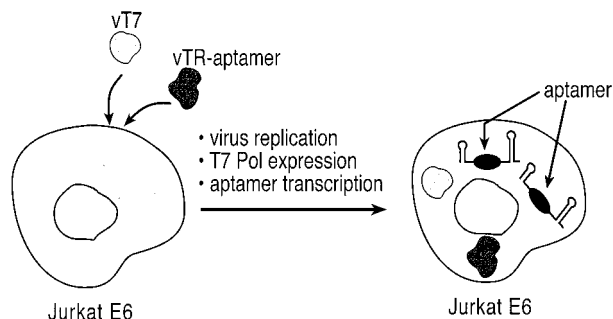

FIGURE 5. Schematic representation of the vaccinia virus-based RNA expression system.

The RNA aptamers used for this approach were isolated by in vitro selection against a synthetic, agarose-immobilized peptide of 46 amino acids that comprised the cytoplasmic domain of the β_2 integrin LFA-1, or CD18. To allow endogenous expression and investigation into their biological function in vivo, aptamer sequences were cloned into an RNA expression cassette, the TR vector. In this vector, the T7 promotor is located upstream of aptamer-encoding DNA, which is in turn inserted between 5′- and 3′-stem-loop structures that serve as RNA-stabilizing motifs and are required for correct termination of the T7 transcripts (Figure 4).

By native gel-shift assays it was shown that aptamers retain full binding activity, even when located between the stabilizing stem-loops. We then infected Jurkat E6 and peripheral blood mononuclear cells (PBMCs) with vaccinia viruses that encode the intramer sequences. Infection with a second vaccinia virus encoding for the T7 RNA polymerase was required for endogenous intramer expression (Figure 5).

Adhesion assays demonstrated that the intramers specific for the cytoplasmic domain of β_2 integrin can block phorbolester-stimulated cell adhesion to immobilized ICAM-1 in Jurkat E6 cells and PBMCs.

This study answered a number of questions that arose before we considered using intramers as modulators of cytoplasmic regulatory pathways; it showed that (i) aptamers can be inserted between different sequence motifs without loss of function, (ii) functional intramers can be expressed at high levels in the cytoplasm, and (iii) they can selectively alter the phenotype of cells in which they are expressed. In the case of the β_2 integrin-binding

intramers, it is likely that they do this by localizing and binding their target at the plasma membrane.

Future Prospects
Intracellular aptamers provide diverse and versatile instruments that allow the targeted manipulation of cellular physiology. They can be used to control the intracellular function of proteins either by blocking ribosomal translation or by modulating the gene product directly. The strategy of using intramers as specific inhibitors of endogenous proteins has significant advantages over the most frequently applied current approaches in functional genomics. Because nucleic acids are naturally located inside cells, intramers are pre-adapted to function in the intracellular context. Furthermore, intramers can be applied in diverse cellular compartments, including the cytoplasm, where most antibodies or scFv would lose their function, presumably due to incorrect folding in the reductive intracellular environment. As the in vitro selection approach is highly robust and requires no intermediate amplification steps in living cells, it has been possible to adapt the process to fully automated selection protocols which might be further optimized to screen for hundreds of aptamer effectors in a few days.[69] In principle, the powerful methods which are established for the high-throughput decryption of the genetic information could now be used to analyze the encoded functionalities in a similarly efficient manner. Intramers selected either in vitro or in vivo could offer a convenient and time-saving method to quickly gain insights into the biological role of the numerous, so far poorly characterized intracellular proteins.

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