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Review

Intramers as promising new tools in functional proteomics

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Abstract

Aptamers are valuable tools for studying numerous aspects of biological processes, opening up new experimental opportunities to analyse the function of a wide range of cellular molecules. Functional RNA molecules can be rapidly selected in vitro from complex combinatorial mixtures of different sequences. Recently, it was shown that in vitro selection processes can be automated: the first generation selection robots will soon mean aptamers for several targets can be isolated in parallel within days rather than weeks. Aptamers not only exhibit highly specific molecular recognition properties but are also able to modulate the function of their cognate targets in a highly specific manner by agonistic or antagonistic mechanisms. These properties prompted the development of novel technologies to exploit the use of aptamers to modulate distinct functions of biological targets. Recent controlled expression of aptamers inside cells demonstrated their impressive potential as rapidly generated intracellular inhibitors of biomolecules. Intracellularly applied aptamers are also called 'intramers'. Here we discuss recent developments and strategies for intramer-based technologies that have the potential to greatly facilitate characterisation of unknown protein functions in the context of their natural expression status in vivo. Thus, intramer-based technologies offer many promising applications in functional genomics, proteomics and drug discovery.

Keywords: Aptamer; Functional proteomics; Intramer; Intracellular inhibitor; Protein function characterization

1. Introduction

In recent years, sequencing the genomes of individual organisms has provided invaluable insights into the genomic structure of assorted cells and organisms in different developmental or pathogenic states [1–6]. The efforts of the various genome-wide sequencing projects have already led to the accumulation of an immense flood of data, emphasising the urgent need to develop new strategies in drug target discovery and novel therapeutic concepts [7,8]. The challenge of functional genomics and proteomics, therefore, is to translate sequencing data into a precise understanding of how genes, proteins, metabolites and all the other molecules making up a cell are interconnected and function in normal and diseased states, and to develop techniques that facilitate rapid identification of agonistic and antagonistic inhibitors [9].

2. Functional analyses of intracellular proteins

Considerable progress has been achieved in identifying possible molecular causes for the onset of certain diseases. Nevertheless, it remains difficult to elucidate the function of a protein based solely on knowing the gene that encodes it. In classical reverse genetics specific genes are mutated or completely inactivated by so-called knock-out strategies, and the resulting phenotypes of the organism or cell can be characterised [10]. In higher eukaryotes, this may be facilitated by regulated expression techniques such as the Tet repressor system [11,12]. In its simplest form, fusion proteins comprising the bacterial tetracycline repressor (tetR) and transcriptional activator or repressor domains bind to tetR DNA binding site(s) (tetO) adjacent to a minimal promoter element following removal of tetracycline. This allows genes to be turned on or off in a targeted fashion. Alternatively, TetR mutants (rtT), which

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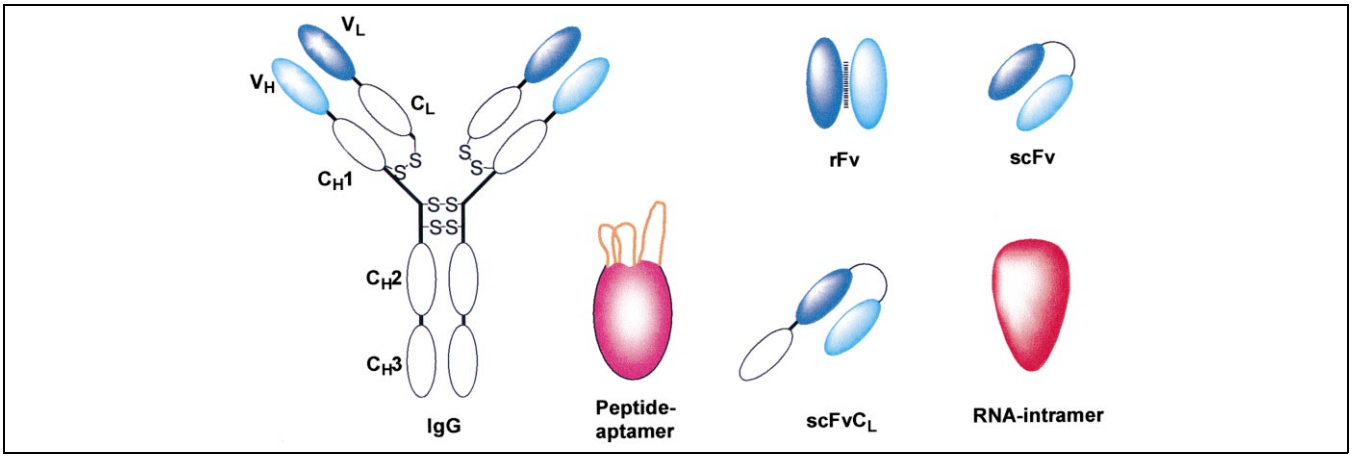

Fig. 1. Proteins engineered for target recognition. IgG: structure of an antibody of the IgG class. Immunoglobulins formed via the adaptive immune response consist of two light (V_L and C_L) and two heavy chains (V_H and C_H) which are covalently linked via disulphide bridges. The antigen binding paratope is formed by the amino-terminal V_L and V_H domains which present hypervariable peptide segments; rFv: Fv fragment stabilised by rational design; scFv: V_H and V_L domains covalently linked via peptides; $scFvC_L$: V_H, V_L and C_L domains covalently linked via peptides; peptide aptamer: variable peptides presented in the context of thioredoxin, lipocalin, or green fluorescent protein; RNA intramer: RNA aptamer in the context of RNA expression cassettes.

only bind to tetO in the presence of tetracycline, allow a controlled induction of genes or their mutated versions simply as a function of the tetracycline concentration [13].

Especially in diploid organisms, however, the generation and expression of mutated genes is often painstaking and identification of recessive phenotypes might be possible only after analysis of the F2 generation. Therefore, genetic knock-down technologies such as antisense, ribozymes and RNAi were developed to allow the inactivation of genes without directly manipulating the gene of interest [14,15]. While being extremely successful and increasingly used to assess the function of genes, all these approaches share the drawback that they alter the genetic information of an organism and, in most cases, the expression pattern of other genes in a somewhat unpredictable fashion. As a consequence, it can be difficult to interpret the biological data and to reach reliable conclusions about the function of the targeted molecule. For example, knocking out a gene says nothing about which part or domain of the protein is important for its function. Also these techniques do not normally indicate whether a target qualifies for inhibition by agonistic or antagonistic mechanisms. Therefore, approaches delivering alternative information about the function of a protein by inactivating it directly within its natural compartment are becoming increasingly important.

Most inhibitors or modulators currently used as tools in biomedical research, or indeed as pharmaceutical drugs, are based on membrane-permeable small organic molecules [16,17]. While being ideally suited for these purposes, their initial identification by screening compound libraries containing thousands of molecules is long and tedious. Considering the multitude of potential targets now arising from genome and proteome research, the capacity of high-throughput screens is still too limited to rapidly generate enough inhibitors for functional in vivo characterisation of the huge numbers of potential targets.

3. Protein-based intracellular effectors

Such limitations may be overcome by using antibody-, protein-, or peptide-based large molecular effectors to modulate intracellular processes. These highly specific antigen binding protein derivatives can be isolated relatively easily for large numbers of proteins by combinatorial methods such as phage [18] and ribosome display [19,20]. Intracellular application of antibodies, however, poses a major hurdle due to the paucity of molecules that function in eukaryotic cells, presumably because they fold or assemble incorrectly in the reductive intracellular environment: indeed, dimerisation of heavy and light chains and stabilisation by disulphide bonds often occur with low efficiency, if at all. To compensate for this limitation, stabilised forms of antigen binding proteins were developed (Fig. 1). Single chain fragment variable region (scFv) constructs can exist inside the cell as continuous polypeptides [21]. Recently, non-covalently associated Fv fragments (rFv) were functionally expressed inside cells [22].

Some of these protein derivatives, also called 'intrabodies', were used in eukaryotic cells to inactivate growth factor receptors, oncoproteins and viral polypeptides [23]. However, many of these in vitro-generated scFVs still lose their activity fairly rapidly inside the cell, or cannot be expressed at sufficient levels. To accommodate intracellular compartment conditions, strategies that use non-antibody-based structural frameworks to present the variable segments of the antigen binding site were developed. For example, peptides were embedded into the structural con-

text of *Escherichia coli* thioredoxin [24], green fluorescent protein [25], or members of the lipocalin protein family, such as the retinol binding protein or the bilin binding protein [26,27] ('peptide aptamers' and 'lipocalins', Fig. 1). Using yeast two-hybrid techniques, high affinity interactions for regulatory proteins such as cyclin-dependent kinases [24,28,29] could be selected. Expression under the control of the inducible Hsp70 promoter in transgenic flies resulted in a defective eye phenotype. These promising results show that specific protein inhibitors are potentially capable of modulating the biological function of a protein in vivo.

4. Intramers: intracellular nucleic acid aptamers

A potential alternative or supplement to these approaches are functional nucleic acids or aptamers. Aptamers are specific binders and potential inhibitors of proteins that can be routinely isolated in vitro from nucleic acid libraries of up to 10^{15} different molecules – the most complex combinatorial libraries currently available. It is becoming increasingly evident that aptamers can not only be expressed inside cells, but also retain their function and can alter the phenotype of a cell by modulating the biological function of the targeted protein. Because of their intracellular mode of action the term 'intramer' was coined for these types of nucleic acid molecules. Intramers can be easily modified and improved, for example, to specifically distinguish between proteins that are highly homologous. Therefore, intramers allow precise investigation of specific molecules without also having to consider closely related species. With respect to their functional characteristics, such as binding affinity or specificity, intramers are at least as efficient as monoclonal antibodies or their derivatives.

In this review we will summarise the current status of intramer research, focusing on intramers and their applications as novel tools for elucidating the function of proteins by modulating their biological effect within the context of the living cell. We also present our views on the potentials that may lie ahead for this promising new technology.

5. Intramers in HIV-1 research

Several groups have evolved aptamers against proteins essential for HIV-1 replication, such as HIV-1 reverse transcriptase [30–32], integrase [33], and the Rev protein [34,35]. Among them the *anti*-HIV-1 Rev aptamers, which were inserted into the full-length Rev-responsive element in place of the Rev binding element (RBE) were found to be functionally equivalent to the wild-type RBE in their ability to promote Rev function in vivo [36]. Within the viral genome Rev interacts with the RBE and is involved in transport regulation of the viral RNA from the nucleus to the cytoplasm. When expressed via RNA polymerase III-dependent expression vectors, intracellular *anti*-Rev aptamers effectively inhibited HIV-1 production in cell culture models [37]. Similar studies were carried out in HeLa cells co-transfected with HIV-1 provirus (HXBΔBgl) and *anti*-Rev aptamer DNA. Here, too, virus formation was specifically inhibited [38]. Furthermore, no toxic effects of the intracellular aptamers were found. Considering possible lifelong gene therapy treatment, strategies that are solely based on functional RNAs may be superior to proteins because of their considerably lower immunogenic potential.

6. Intramers targeting nuclear proteins

While these examples showed that it is possible to block viral proteins expressed in the nucleus, the following studies demonstrated that aptamers can also inhibit endogenous nuclear targets in vivo, thus acting as effective antagonists for nucleic acid binding proteins. For example, Thomas et al. selected RNA aptamers against yeast RNA polymerase II [39]. The isolated RNA aptamers exhibit binding specificity for RNA polymerase II and do not interact with RNA polymerases I or III. Interaction studies showed that the aptamers preferentially bind the two largest subunits of RNA polymerase II, B220 and B150. Constitutive intracellular expression of the anti-polymerase II aptamer under the control of the RNA polymerase III promoter in yeast cells with an artificially reduced level of endogenous RNA polymerase II, resulted in a cell growth defect that was not observed with non-binding negative control RNAs [39].

More recently, a transgenic intramer-expressing animal model characterised the effect of intramer expression on the phenotype of *Drosophila melanogaster* [40]. The target for the in vitro aptamer selection was protein B52, a member of the *Drosophila* SR protein family [41]. B52 participates in the splicing of the pre-mRNA and in the selection of alternative splice sites. For in vivo expression, a pentameric aptamer construct was engineered which co-localised with the B52 protein at its site of insertion in the polytene chromosome. Although similar to the lethal phenotype in B52 knock-out flies, expression of the multimeric anti-B52 aptamer led only to a 50% reduction in the development of adult transgenic flies. The difference in survival rates compared to the genetic knock-out was explained by incomplete inhibition of the splice factor B52 by the intramer under equilibrium conditions in vivo.

While the absence of B52 is lethal, over-expression of B52 can result in morphological defects such as absence of salivary glands or missing bristles in adult animals. Intramers expressed under the control of inducible promoters with different RNA expression levels allowed a dose-dependent investigation of intramer-mediated B52 counter-

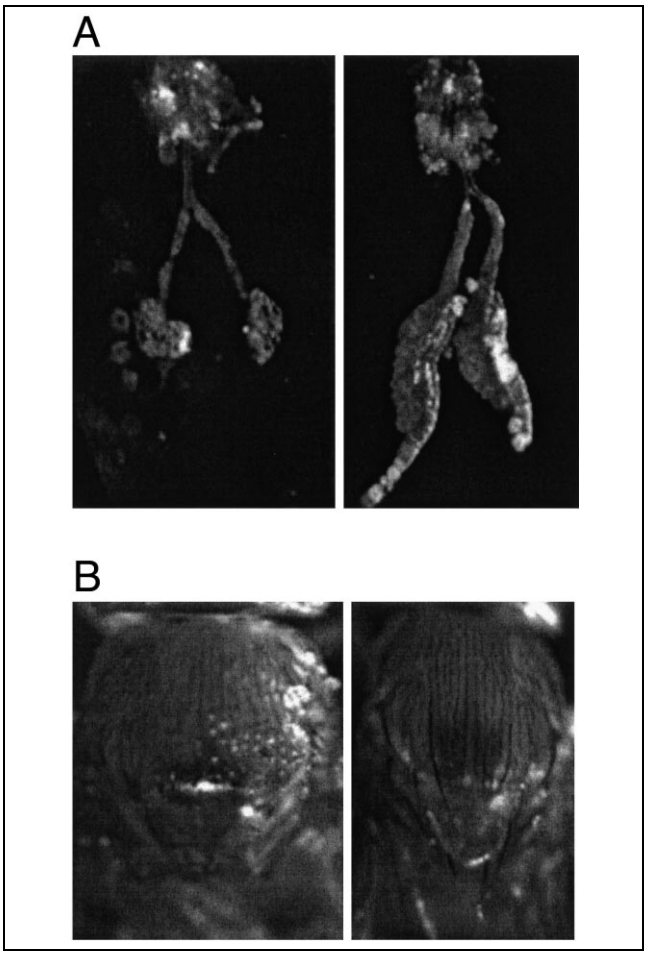

Fig. 2. In vivo expression of aptamers suppresses the phenotypes caused by B52 over-expression. (A) The larval salivary gland phenotype. Left: over-expression of B52 results in 100% undeveloped larval salivary glands. Right: co-expression of the anti-B52 aptamer construct rescues the phenotype resulting from B52 over-expression – salivary glands are 100% developed with near normal morphology. (B) The morphological marker of bristle development. Left: nearly no bristles are observed when B52 is overproduced. Right: bristles develop with nearly normal density in the presence of the anti-B52 pentameric intramer.

inhibition by monitoring these morphological markers in adult animals. The phenotypic effects resulting from over-expression of B52 could be reversed in the presence of certain levels of intramer, indicating that the intramers were able to inhibit B52 function in vivo (Fig. 2).

Another interesting nuclear target is the transcription factor NF-κB. This DNA binding heterodimeric protein is an important activator of genes with functions related to immunity, such as inflammation and the synthesis of chemokines, interferons, MHC proteins, growth factors, and cell adhesion molecules [42–45]. A small RNA aptamer that recognised the NF-κB p50 subunit with nanomolar affinities and effectively competed with DNA for binding to the transcription factor was identified by in vitro selection [46]. This aptamer was also recently shown to bind NF-κB p50 in yeast cells [47] by using a yeast three-hybrid system [48].

7. Intramers that inhibit membrane and regulatory proteins in the cytoplasm

The examples described above demonstrate that in vitro selected RNA aptamers that target nuclear factors can be functionally expressed in vivo. A significant advance in intramer technology is the expression of intramers that target non-nucleic acid binding proteins located in the cytoplasm.

As a first step in this direction, Blind et al. [49] selected RNA aptamers that recognise the cytoplasmic domain of the β_2 subunit of the human $\alpha_L\beta_2$-integrin. The β_2-integrins are a family of heterodimeric transmembrane proteins whose extracellular domains, by binding to extracellular ligands, mediate the adhesion of leukocytes activated by extracellular stimuli, such as MHC antigen complexes, in immune and inflammatory responses [50]. 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' signalling [51]. Fig. 3 shows a model for the activation of $\alpha_L\beta_2$-integrin through the cytoplasmic β_2-integrin domain [51,52]. PI 3-kinases are activated via signalling events originating from cell surface receptors, such as the T-cell receptor (TCR) or chemokine receptors. These enzymes then phosphorylate the phospholipid phosphatidyl inositol 4,5-bisphosphate to yield phosphatidylinositol 3,4,5-trisphosphate. This event recruits the cytoplasmic regulator protein cytohesin-1 (cyh1) to the cell membrane via its C-terminal plextrin homology (PH) domain. Here, the Sec7 domain of cyh1 can interact with the cytoplasmic domain of the β_2-integrin chain, which in turn activates the extracellular domain of the $\alpha_L\beta_2$-integrin and induces binding to intercellular adhesion molecule 1 (ICAM-1).

To achieve T7 RNA polymerase-controlled expression of aptamers in the cytoplasm of leukocytes we used an intramer expression system based on the infection of T-cells with recombinant vaccinia viruses [49]. Since the complete life cycle of these poxviruses, from DNA replication to protein translation, occurs in the cytoplasm of the host cells [53], the transcription of the intramers will take place exclusively in the same compartment as the location of the target protein. Furthermore, vaccinia viruses exhibit a broad host spectrum and thus are ideal vectors to infect $\alpha_L\beta_2$-integrin-bearing T-cells. The parental aptamer sequences were cloned into a T7 RNA polymerase-dependent RNA expression cassette based on a modified version of a highly effective transcription system, in a vector called vTR [54]. Double infection with two recombinant vaccinia viruses, one (vTR) carrying the intramer-encoding DNA under the control of the T7 promoter, the other (vT7) encoding the T7 RNA polymerase, led to high levels of intramers in the cytoplasm. In the expression constructs, the aptamer RNA is flanked by additional stem-loop structures at the 5′- and 3′-ends.

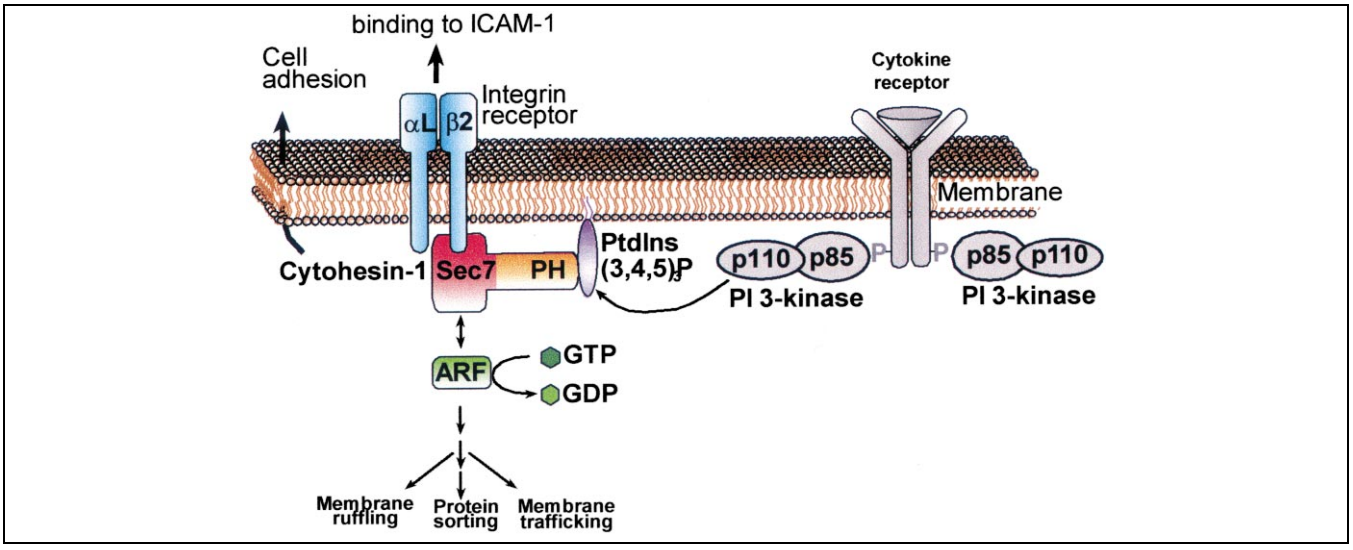

Fig. 3. Model for the activation of $\alpha_L\beta_2$-integrins and the activation of ADP-ribosylation factors (ARF) by the cytoplasmic regulator protein cytohesin-1. Stimulation of leukocytes by cell surface receptors (e.g. cytokine receptors or the T-cell receptor, TCR) leads to the activation of phosphatidylinositol phospholipase C (PI-PLC), which catalyses the phosphorylation of phosphatidylinositol bisphosphate. The regulatory protein cytohesin-1 is recruited to the cytoplasmic membrane via binding to phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3). Interaction of the Sec7 domain with the β2-integrin subunit is thought to activate the LFA-1 integrin, which subsequently binds to its extracellular ligand. This process is also known as inside-out signalling. A second activity of the Sec7 domain is stimulation of the GDP/GTP exchange, which activates small GTPases. In vitro data suggest that cytohesin-1 is a GDP/GTP exchange factor of the ARF family of GTPases, which are involved in the control of membrane-associated events or the remodelling of the cytoskeleton.

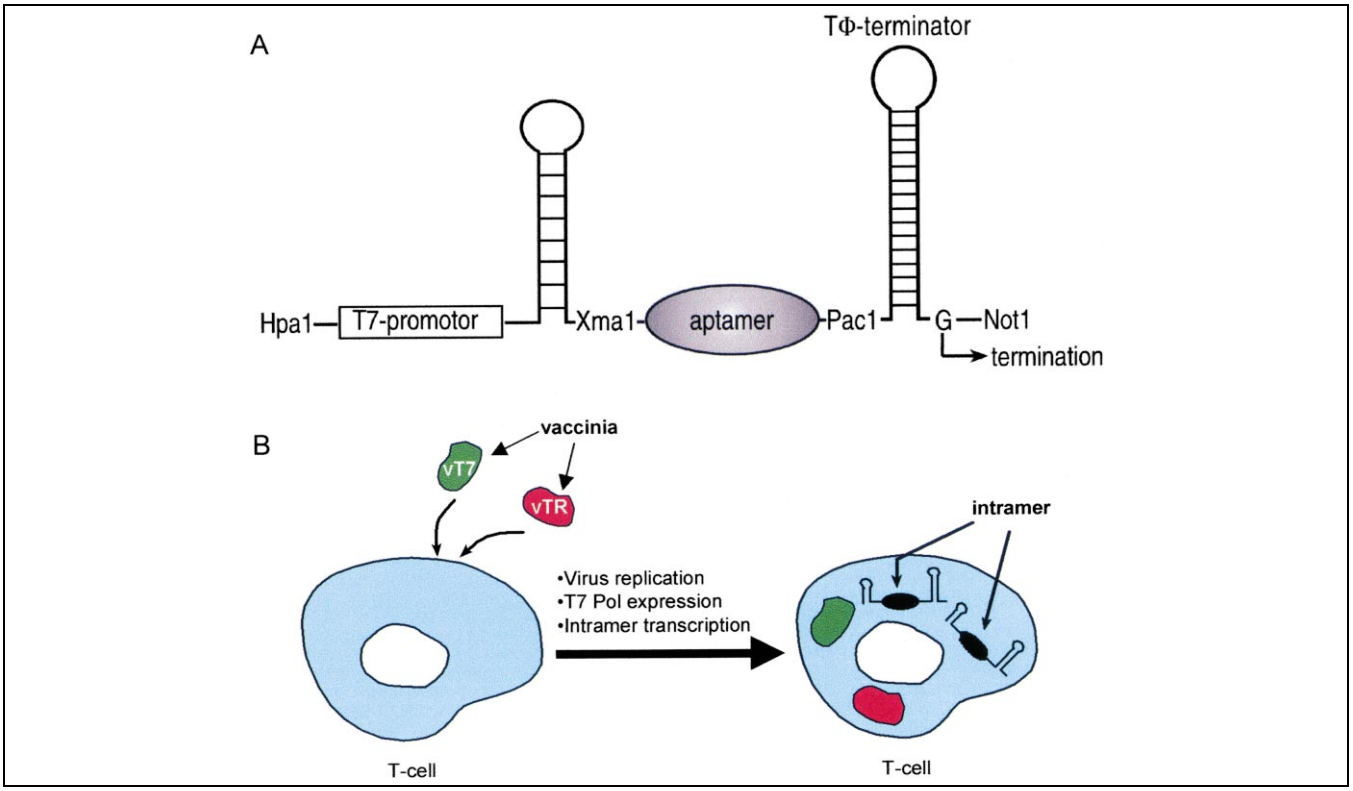

Fig. 4. Scheme for the aptamer expression cassette TR and the vaccinia virus-based cytoplasmic RNA expression system. (A) The aptamer-encoding DNA was inserted between the stabilising 5′-stem loop and the 3′-hairpin that acts as the TΦ terminator signal for T7 RNA polymerase. Both sequences serve to stabilise the intramer transcripts. The binding behaviour of the aptamer is maintained when inserted into this new sequence context. (B) Scheme for the double infection of Jurkat E6 cells with two recombinant vaccinia viruses, one encoding T7 RNA polymerase (vT7), the other encoding the intramer (vTR). After virus entry, excretion and replication of viral DNA, T7 RNA polymerase is expressed. The enzyme then synthesises intramer RNA in the cytoplasm (blue) from the transcription units controlled by the T7 promoter integrated into the genome of the virus vTR.

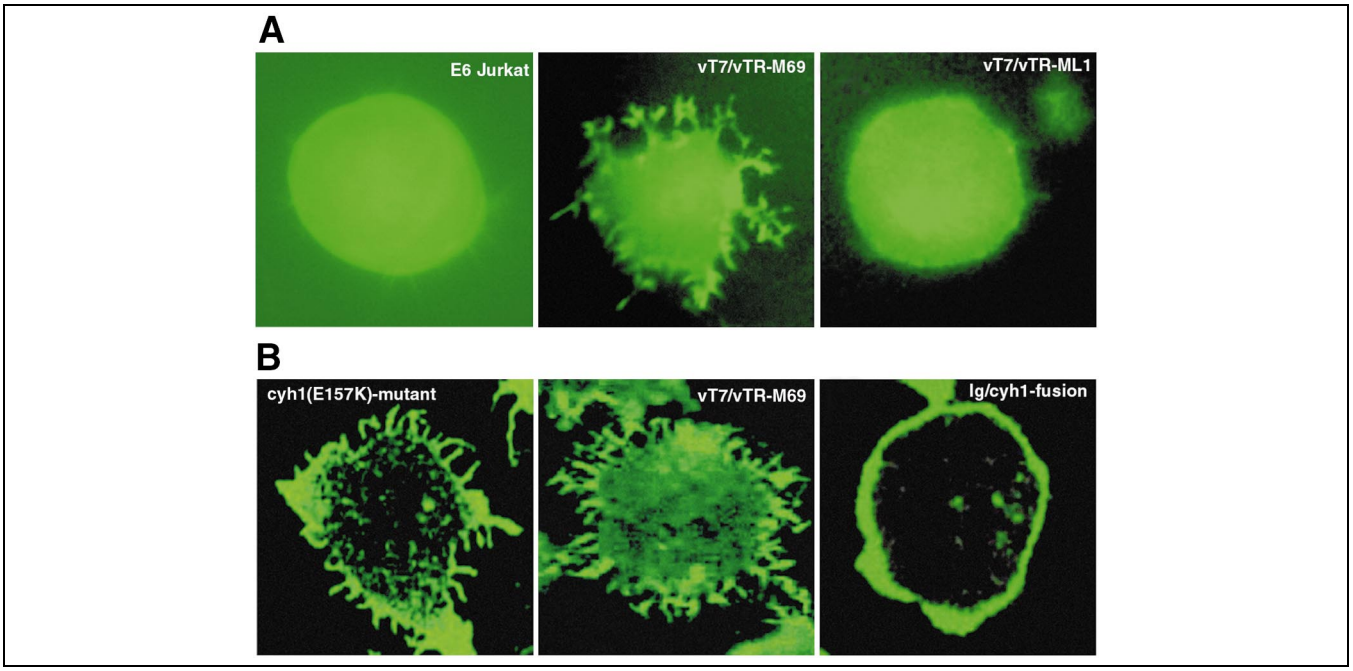

Fig. 5. Cytoplasmic expression of an aptamer that inhibits the GEF function exerted by the Sec7 domain of cyh1, a member of the class of small (< 50 kDa) GEFs. For this class of GEFs, no inhibitors have been previously identified. (A) Intramer expression specifically induces considerable reorganisation of F-actin distribution (vT7/vTR-M69, middle) when the human T-cells were attached to fibronectin. No significant difference to wild-type cells (E6 Jurkat, left) was found when a non-cognate intramer was expressed at similar levels (vT7/vTR-ML1, right). Actin distribution was visualised by TRITC phalloidin that recognises F-actin. (B) Dominant negative over-expression of a GEF-deficient mutant of cyh1 (cyh1(E157K) mutant; left) leads to a phenotype indistinguishable from that obtained by expressing the GEF inhibitory intramer M69 (vT7/vM69; middle). The right panel shows the wild-type phenotype obtained after expression of cyh1 fused to an immunoglobulin tag. Visualisation was by confocal microscopy.

These motifs serve as stabilising elements, ensure maintenance of aptamer functionality and lead to correct termination of the RNA transcripts. The intramer RNA should not undergo significant further RNA processing or association with the ribosomal translation machinery [54] (Fig. 4). Expression in T-lymphocytes revealed that anti-β_2 intramers were able to specifically inhibit $\alpha_L\beta_2$-integrin-mediated phorbol ester-stimulated cell adhesion to immobilised ICAM-1 [49]. This study established intramers as powerful tools for modulating the biological function of cytoplasmic membrane protein domains, leading to highly specific cellular effects.

In a more recent investigation, Mayer et al. applied the same technology to gain new biological information [55]. This time, the target for an in vitro aptamer selection was cyh1 itself. The goal was to probe the function of the Sec7 domain of cyh1, which is thought to act as a small guanine nucleotide exchange factor (GEF) on ARF GTPases (Fig. 3). ARF GTPases and their regulatory proteins have been implicated in the control of vesicle transport and cell adhesion. Two main classes of positive regulatory elements for ARF have been discovered so far, namely the large Sec7/Gea, and the small cytohesin/ARNO families. These proteins harbour GEF activity exerted by the common Sec7 domain. The availability of a specific inhibitor, the fungal metabolite brefeldin A, enabled the involvement of the large GEFs in vesicle transport to be documented. However, the biological roles of the small GEFs have remained controversial due to the lack of equivalent tools [56,57]. Using cyh1 immobilised on a Sepharose support, Mayer et al. obtained an aptamer, called M69, that binds the Sec7 domain of cyh1 and inhibits its GEF activity, thus preventing ARF activation in vitro. In contrast, the Sec7 domain of Gea2, a member of the large GEF family, is not inhibited. This in vitro function of M69 correlated with effects in vivo: when expressed in the cytoplasm of T-cells, M69 aptamers specifically led to a cell-spreading deficiency accompanied by dramatic reorganisation of F-actin distribution when the cells adhered to fibronectin (Fig. 5). Important proof that this in vivo effect clearly resulted from the aptamer's inhibitory activity characterised in vitro came from the same effect observed after dominant negative expression of a GEF-deficient cyh1(E157K) mutant [55]. Such a clear correlation between in vitro and cytoplasmic functions of an inhibitory intramer had never been demonstrated before. Nor was it known before that a previously observed cell-spreading deficiency [58] can be linked to rearrangement of the actin cytoskeleton resulting directly from inhibition of the GEF activity of the cyh1 Sec7 domain.

This work added an entirely new quality to studying the biological functions of proteins or protein domains in vivo: the intramer approach has the capacity to inhibit a function without over-expressing a normal or aberrant

protein, thereby avoiding possible perturbance of a system by elevated expression levels. The efficient combinatorial selection of an inhibitor, combined with the ability to express it readily without loss of function in the context of the living cell, and in the relevant cellular compartment, represents a new paradigm for developing novel approaches to gaining information about individual proteins or sub-domains in the context of their natural expression and location.

Several very promising aptameric inhibitors targeting intracellular regulatory proteins with impressive specificity are now available. The most efficient aptamers are capable of reliably distinguishing polypeptides with 96% homology [59]. Seiwert et al. [60] recently described RNA aptamers that can selectively recognise the extracellularly regulated kinase 2 (ERK2), a member of the class of mitogen-activated protein kinases (MAPK), with high picomolar affinity. In vitro, the aptamers inhibit the phosphorylation activity of ERK2, presumably by competing with the ATP cofactor. The specificity of these aptamers is high: only phosphorylation by ERK1 and ERK2, but not by the related Jun N-terminal kinase or p38 MAPK, is inhibited.

Taken together, all these examples show that intramers provide a potential means to analyse the function of closely related proteins in a very focused manner. Furthermore, it was recently shown that the very robust in vitro selection process could also be carried out in an automated fashion [61]. This advancement allows complete in vitro selections to be performed within a couple of days, instead of several weeks as required by current manual protocols. Thus, it will be possible in the near future not only to generate aptamers for a huge number of targets considerably faster, but to use them directly in cellular systems.

8. Targeting specific cellular compartments

The vaccinia virus-based intramer expression system was used as an initial approach to ensure intramer production directly in the relevant cellular compartment. However, for general, more widely applicable use this expression system may have certain limitations. For example, it would be highly desirable to have expression systems that use inducible endogenous promoters producing intramers containing RNA signals designed to automatically target the intramer into the cellular compartment harbouring its cognate ligand. For approaches that employ ribozymes to control gene expression, intracellular RNA expression systems have been developed. In most cases these transcription systems use retroviral or polymerase III-controlled promoters [15,62,63]. The *anti*-Rev aptamers discussed above were expressed via U6 and tRNA constructs in cell culture [37]. The problem one may have to face when using these approaches is that transcription yields of 10^3–10^4 molecules per cell may be too low to exert detectable effects. In some cases the addition of sequences from natural genes of small RNA molecules, such as U6 snRNA or $tRNA^{met}$, may lead to 10-fold higher expression levels and sometimes also to increased stability [37].

One can imagine a number of approaches that may be used to direct intramers to the cytoplasm or facilitate their localisation to other cellular compartments. Hamm et al. [64] used antibodies specific for the nuclear export signal (NES) of the HIV-1 Rev protein to select RNA mimics of the NES by in vitro selection. When injected into the nucleus of *Xenopus* oocytes, the RNA mimics were either exported actively, or blocked Rev-dependent export of a reporter RNA. In addition, they inhibited the cap-dependent U1 snRNA export in *Xenopus* oocytes in a manner similar to NES–peptide conjugates. Recently it was shown that the inhibition of Rev-mediated export results from the RNA mimics binding to the NES recognition domain of the exportin CRM1, the export receptor for leucine-rich NES sequences [65]. When fused to an intramer sequence that targets a cytoplasmic protein, such 'export aptamers' may serve as nucleic acid-based exportin ligands for delivering an intramer to its cytoplasmic target through the nuclear pore complex.

Grimm et al. [66] also isolated RNAs from combinatorial RNA libraries that are exported from nuclei of *Xenopus* oocytes. When fused to non-selected RNAs, the selected sequences acted like a NES in promoting efficient export of RNAs that are otherwise not exported. However, these chimeric RNAs, unlike the selected RNAs, were not exported in the presence of matrix protein indicating that the non-selected sequences can cause retention of the selected RNA sequences under certain conditions. RNAs that are actively imported into the nucleus have also been isolated [67]. It was shown that import of a class of in vivo selected RNAs was distinct from that of U6 RNA.

9. Conclusion

To develop highly specific drugs with minimal side effects it will become increasingly important to know the cellular and molecular mechanisms that lead to a particular dysfunction. Molecular engineering based on combinatorial methods has led to a wide range of functionally active protein, peptide, or RNA molecules that can be used to elucidate the biological activity of intracellular proteins. Methodologies that apply intracellular inhibitors may also be a highly efficient means of validating potential pharmaceutical targets as well as rapidly finding out whether agonistic or antagonistic mechanisms can affect a protein. The advantage of inhibitors based on biopolymers is that most of them can be easily and rapidly identified by in vitro techniques. Due to their cellular compatibility RNA intramers may be particularly suited for

acting as efficient intracellular antagonists for a large number of potential targets.

Intramers can easily be tailored for their use in vivo by adding additional functions, such as enhanced stability, and signals for cellular localisation. Where appropriate, intramers may be inserted into appropriate vectors where intramer expression simply requires the cell's own transcription machinery. When expressed in human cells, intramers can specifically bind and potentially inhibit the targeted molecule, making them highly effective tools for dissecting an intracellular protein, protein domain or subdomain. Because of their high affinities and specificities, intramers are excellently suited to specifically inhibit key events in signal transduction, cell growth, transcription, and many other intracellular processes.

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