Title: AGENTS FOR THE REGULATION OF TRANSCRIPTION VIA ZINC-FINGER PROTEINS

Abstract: Novel nucleic acid molecules or ligands with binding affinities for zinc-finger protein targets, and uses of such nucleic acids as therapeutic or screening or diagnostic agents, or as potential lead compounds for rationalised drug design.
Agents for the Regulation of Transcription via Zinc-finger Proteins

The present invention relates to novel nucleic acid molecules or ligands with affinities for specific zinc-finger target molecules or proteins, and uses of such molecules, especially but not particularly as therapeutic or screening or diagnostic agents, or as potential lead compounds for rationalised drug design.

Background to the Invention

Zinc-finger DNA-binding proteins (ZFPs) are the dominant class of naturally occurring transcription factors in organisms from yeast to humans. They are transcription factors, found in the nucleus of every cell, where they bind to specific DNA sequences leading to regulated gene expression. Zinc-fingers are the name given to a particular polypeptide fold containing cysteine and/or histidine amino acid side chains so arranged that they can form a planar tetra-co-ordinate interaction with a zinc ion, Zn\(^{2+}\). The polypeptide chain encompassing these metal ion ligands folds into a pair of β-strands and an alpha helix. Zinc-finger motifs are heavily involved as the interaction motifs between macromolecules. This can include protein-protein interactions but primarily it includes sequence-specific DNA interactions in which the zinc-finger motif inserts its alpha helix into the major groove of largely B-form DNA duplexes. Each zinc-finger domain interacts sequence-specifically with ~3 bp of the DNA. In order to ensure that zinc-finger proteins interact with unique sequences within the genome of an organism, they generally contain multiple, up to 35, zinc-finger domains. The amino acid sequences of these domains vary in the region that contacts the DNA duplex and thus alters the sequence specificity in that region.

Novel synthetic DNA/RNA ligands, known as aptamers, have been defined as artificial nucleic acid ligands that can be generated against amino acids, drugs, proteins and other molecules. They are isolated from complex libraries of synthetic nucleic acids by an iterative process of adsorption, recovery and re-amplification.
RNA aptamers are nucleic acid molecules with affinities for specific target molecules. They have been likened to antibodies because of their ligand binding properties. They may be considered as advantageous agents over antibodies for a variety of reasons. Specifically, they are soluble in a wide variety of solution conditions and concentrations, and their binding specificities are largely undisturbed by reagents that have significant effects on antibody reagents, e.g. detergents and other mild denaturants. Moreover, they are relatively cheap to isolate and produce compared to antibodies. They may also readily be modified to generate species with improved properties whereas antibodies cannot always be adapted easily. Extensive studies show that nucleic acids are largely non-toxic and non-immunogenic and aptamers have already found clinical application, whereas antibodies being proteins are strongly immunogenic and require extensive and expensive manipulation to be used in humans. Furthermore, it is known how to modulate the activities of aptamers in biological samples by the production of inactive dsRNA molecules in the presence of complementary RNA single strands (Rusconi et al., 2002).

However, a serious disadvantage associated with RNA aptamers is that natural RNAs are unstable in biological fluids. It is known in the prior art how to improve stability by chemically modifying RNAs so as to block nuclease action at 5' and 3' ends, and throughout the length of the molecule. However, such chemical modification can ultimately detrimentally alter the binding properties of the RNA and hence render them ineffective.

It is known from the prior art how to isolate aptamers from degenerate sequence pools by repeated cycles of binding, sieving and amplification. Such methods are described in US 5,475,096, US 5,270,163 and EP0533 38 and typically are referred to as SELEX (Systematic Evolution of Ligands by EX-ponential Enrichment). The basic SELEX system has been modified for example by using Photo-SELEX where aptamers contain photo-reactive groups capable of binding and/or photo cross-linking to and/or photo-activating or inactivating a target molecule. Other modifications include Chimeric-SELEX, Blended-SELEX, Counter-SELEX, Solution-SELEX,
Chemi-SELEX, Tissue-SELEX and Transcription-free SELEX which describes a method for ligating random fragments of RNA bound to a DNA template to form the oligonucleotide library. However, these methods even though producing enriched ligand-binding nucleic acid molecules, still produce unstable products. In order to overcome the problem of stability it is known to create enantiomeric “spiegelmers” (WO 01/92566). The process involves initially creating a chemical mirror image of the target, then selecting aptamers to this mirror image and finally creating a chemical mirror image of the SELEX selected aptamer. By selecting natural RNAs, based on D-ribose sugar units, against the non-natural enantiomer of the eventual target molecule, for example a peptide made of D-amino acids, a spiegelmer directed against the natural L-amino acid target can be created. Once tight binding aptamers to the non-natural enantiomer target are isolated and sequenced, the Laws of Molecular Symmetry mean that RNAs synthesised chemically based on L-ribose sugars will bind the natural target, that is to say the mirror image of the selection target. This process is conveniently referred to as reflection-selection or mirror selection and the L-ribose species produced are significantly more stable in biological environments because they are less susceptible to normal enzymatic cleavage, i.e. they are nuclease resistant.

Statement of the Invention

The present invention reveals novel compounds in the form of aptamers which selectively and specifically are able to block active zinc-finger proteins binding to DNA and thus alter gene expression.

Reference herein to an aptamer is intended to include nucleic acid molecules with binding affinities for specific target molecules, especially but not exclusively RNA nucleic acid molecules.

According to a first aspect of the invention there is provided an isolated nucleic acid ligand capable of binding to an aptope of a zinc finger protein (ZFP), the ligand
being capable of preventing active ZFP protein binding to DNA so as to alter expression of said DNA.

Preferably, the nucleic acid ligand is non-naturally occurring.

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Preferably, the ZFP comprises of the amino acid sequence as set forth in SEQ ID NO:46.

Preferably, the ZFP amino acid sequence is independently D or L configuration or a mixture thereof.

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Preferably the 3ZFP construct comprises a first domain as set forth in SEQ ID NO:46, a second domain as set forth in SEQ ID NO:47 and a third domain as set forth in SEQ ID NO:48.

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Preferably, the ZFP construct comprises fragments of those polynucleotides as set forth in any one of SEQ ID NOS: 46-48 that retain the ZFP apatope and function as ZFP by altering DNA expression, and variants of those polynucleotides with sufficient similarity to the non-coding strand of those polynucleotides to hybridise thereto under stringent conditions.

The term "a ZFP domain" includes fragments or analogues thereof which have the properties of an apatope of a ZFP as herein defined. Polynucleotides of the invention such as those described above, fragments of those polynucleotides, and variants of those polynucleotides with sufficient similarity to the non-coding strand of those polynucleotides to hybridise thereto under stringent conditions are all aspects of the invention. Exemplary stringent hybridisation conditions are as follows: hybridisation at 42 DEG C in 5X SSC, 20 mM NaPO4, pH 6.8, 50% formamide; and washing at 42 DEG C in 0.2X SSC. Those skilled in the art understand that it is desirable to vary these conditions empirically based on the length and the GC nucleotide base content of the sequences to be hybridised, and that formulae for determining such variation

\[ T_m = 81.5^\circ C + 16.6 \log [Na^+] + 0.41(\% G + C) - 0.63 (\% formamide) \]

Preferably, the nucleic acid ligand is capable of binding to at least 1, 2 or 3 domains of the 3ZFP construct.

Preferably, the isolated nucleic acid comprising a nucleotide sequence is selected from the group consisting the nucleic acids depicted in any one of SEQ ID NOS: 1 to 42 or having a corresponding DNA sequence and nucleic acids having complementary sequences thereto and L-ribose analogues of the foregoing.

Preferably, the nucleic acid ligand or aptamer is substantially homologous to and has substantially the same ability to bind said ZFP as a ligand and is selected from the group comprising the nucleic acids as set forth in any one of SEQ ID NOS: 1 to 42.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

According to a further aspect of the invention there is provided an isolated non-naturally occurring nucleic acid ligand that modulates expression of a nucleic acid molecule under regulation of a ZFP transcription factor.

According to a further aspect of the invention there is provided an isolated nucleic acid ligand to a ZFP or biologically active fragment thereof, wherein said ligand is an RNA ligand selected from the group comprising:

(i) nucleic acids depicted in any one of SEQ ID NOS: 1 to 42;
(ii) nucleic acids having a corresponding DNA of any one of SEQ ID NOS: 1 to 42, nucleic acids having a complementary sequences thereto or L-ribose analogues of any of the foregoing; or

(iii) nucleic acids analogous to the sequence depicted in any one of SEQ ID NOS: 1 to 42 having at least about 60%, 70%, 80% or 90%, sequence identity to any one of the nucleotide sequences, and which have a binding affinity to ZFP.

Accordingly the nucleic acids of the present invention may be RNAs or their L-ribose derivatives or may be the DNAs encoding the RNAs or their L-ribose derivatives.

Our results have shown the remarkably surprisingly and unexpected outcome that both L and D forms of a particular aptamer can inhibit ZFP DNA binding constructs, i.e. there appear to be protein aptapes that do not have a handedness to them. Accordingly, given this unexpected observation the present invention may also include L-ribose aptamers that were not selected against non-natural enantiomers.

Sequence identity is the similarity between two nucleic acid sequences, or two amino acid sequences, and is expressed in terms of the percentage similarity between the sequences. The higher the percentage, the more similar the two sequences are. Homologues or orthologues of the protein, and the corresponding cDNA or gene sequence, will possess a relatively high degree of sequence identity when aligned using standard methods. This homology will be more significant when the orthologous proteins or genes or cDNAs are derived from species that are more closely related (e.g., human and chimpanzee sequences), compared to species more distantly related (e.g. human and C. elegans sequences).

Reference herein to binding affinity is intended to include binding affinities expressed as equilibrium dissociation constants, Kd, in the millimolar to picomolar range.
Accordingly, one aspect of the invention pertains to isolated nucleic acid molecules (e.g., RNAs) comprising a nucleotide sequence which has a binding affinity for a ZFP, or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection or amplification of nucleic acid ligands for a ZFP. In particularly preferred embodiments, the isolated nucleic acid molecule comprises any one of the nucleotide sequences set forth in SEQ ID NO: 1 to 42 or a complement thereof of one of these nucleotide sequences or L-ribose versions thereof. In other particularly preferred embodiments, the isolated nucleic acid molecule of the invention comprises a nucleotide sequence which hybridizes to or shows at least about 50%, preferably at least about 60%, more preferably at least about 70%, 80% or 90%, and even more preferably at least about 95%, 96%, 97%, 98%, 99% or more sequence similarity to a nucleotide sequence as in SEQ ID NOS: 1 to 42, or a functionally equivalent portion thereof.

Preferably, the aptamers of the present invention further include any one or more of the following features as herein recited such as a fluorescent label, an imaging label or a flanking region.

Reference herein to a flanking region includes that the sequences maybe terminal sequences.

The core for the aptamer is the random RNA oligonucleotide sequence, which is flanked by 5' and 3' constant sequences (SEQ ID NOS: 43 and 44) that provide primer hybridisation sites for Klenow extension, cDNA synthesis, polymerase chain reaction (PCR) amplification and T7 RNA polymerase transcription, all of which are involved in the SELEX protocol. It should be appreciated that the selection of the constant flanking region is important to ensure optimum efficacy of SELEX. The 3' flanking region acts as the attachment site for the primer for the AMV reverse transcriptase that converts the RNA aptamers to DNA. The 5' flanking sequence acts as the point of attachment for PCR primers that initiate amplification of the selected
sequence and also contains the T7 promoter sequence required for in vitro transcription.

The aptamers or nucleic acids of the present invention share some commonality of sequence. For example multiple consecutive cytosine bases are common to SEQ ID NOs: 5, 7, 8, 13, 16, 17, 18, 21, 23, 24, 25, 27, 33, 34, 35, 37 and 40. In addition the sequence CCCG is common to SEQ ID NOs: 13, 16, 23, 25, 33, 34 and 35 and multiple consecutive thyamines are common to SEQ ID NOs: 1, 3, 4, 6, 14, 15, 17, 21, 28, 29, 30, 33, 34, 36, 38 and 42. Other noted commonalities include multiple consecutive guanine residues in SEQ ID NOs: 2, 9, 10, 14, 19, 20 and 42 and the sequence GGT in SEQ ID NOs 2, 9, 19, 20 and 42. The sequence GCGAT in SEQ ID NOs: 3, 4 and 7, sequence GGXTTYCATCTT (where X and Y = 0 or 1) in SEQ ID NOs: 3 and 38. Also SEQ ID NO:24 differs from SEQ ID NO:27 by only the second base in the sequence of 30 and SEQ ID NO:10 and 38 share the sequence TAAGGT.

According to a yet further aspect of the invention there is provided a vector comprising or encompassing at least one, or more, aptamer of the present invention.

This aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid having a binding affinity for a ZFP (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors
are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

According to a yet further aspect of the invention there is provided a host cell including at least one aptamer or a vector comprising at least one aptamer of the present invention.

According to a yet further aspect of the invention, there is provided a pharmaceutical composition comprising at least one aptamer of the present invention.

Preferably, the pharmaceutical composition includes a physiologically acceptable excipient, diluent or carrier.

Preferably, the aptamer or pharmaceutical composition comprising an aptamer is administered directly to an adipose site or it may be administered by an intra-venous, intra-muscular, intra-peritoneal route and preferably may be administered on more than one occasion. It may also be delivered as part of a larger formulation in a drug delivery vehicle, such as a liposome or a virus-like particle (Wu et al., 1995; Brown et al., 2002).

It will be appreciated that the aptamers may be modified with fluorescent labels by simple inclusion of, for example, fluorescein-labelled UTP in *in vitro* transcription reactions. The fluorescence properties of these molecules are sensitive to their bound state and may be the basis of simple diagnostic screening and imaging reagents. For instance, the physiological state of a tissue may be judged by staining/screening with differently labelled aptamers directed against a ZFP and therefore preferably the
aptamers and pharmaceutical compositions comprising one or more aptamers will have utility not only in treating disease states but also in assaying for disease prevention and progression.

According to a further aspect of the invention there is provided a method for the isolation of nucleic acid ligands to a ZFP target comprising:

(i) contacting a candidate mixture of nucleic acids with a polypeptide selected from a ZFP or fragment thereof; and

(ii) identifying nucleic acids which bind to the polypeptide.

Preferably, the method may optionally include:

(i) preparing a candidate mixture of nucleic acids;

(ii) contacting the candidate mixture of nucleic acids with a biotinylated immobilised protein, wherein nucleic acids having an increased affinity to a ZFP relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

(iii) partitioning the increased-affinity nucleic acids from the remainder of the candidate mixture;

(iv) amplifying the increased-affinity nucleic acids to yield a mixture of nucleic acids with relatively higher affinity and specificity for binding to ZFP, whereby a nucleic acid ligand of the protein may be identified.

Preferably, the candidate mixture comprises single stranded nucleic acids and more preferably the single stranded nucleic acids comprise ribonucleic acids.

Preferably, the target comprises at least one biotin molecule.

Preferably, the ZFP target is isolated and immobilised.

Preferably, the ZFP target comprises a ZFP construct.
Preferably, the method further includes the step of modifying the nucleic acid ligands with a fluorescent label and/or an imaging reagent. A non-limiting example of a suitable fluorescent labels is fluorescein-labelled UTP, and non-limiting example of imaging agents are uranyl acetate, and radioactive technetium and indium-labelled species, for both in vitro and in vivo applications.

Preferably, the method further includes the step of flanking the aptamer of the present invention with at least one further nucleic acid sequence comprising the nucleic acid as set forth in SEQ ID NO:43 and optionally the aptamer is flanked by a further nucleic acid sequence as set forth in SEQ ID NO:44. It will be appreciated that other constant flanking regions may be used and the composition of the flanking regions is not intended to limit the scope of the invention.

The method of the present invention advantageously allows for rapid selection and characterisation and is accomplished in vitro without recourse to animal work. It will be appreciated that aptamers of the present invention will have commercial application in many areas currently making use of antibodies, for example and without limitation, as diagnostic and screening tools and as therapeutic agents in a variety of different disease conditions.

Preferably, the product may be further modified as hereinbefore described.

According to a further aspect of the invention there is provided use of a 3ZFP construct in the identification of aptamers capable of binding to at least one domain of the construct.

**Brief Description of the Figures**

Figure 1 shows a schematic diagram of the interaction of a synthetic ZFP encompassing three zinc-finger domains (ribbon representation) interacting with a dsDNA target, shown as blocks representing the base pairs. The zinc ion ligands are shown as ball and stick models. [Kim, C. A. & Berg, J. M. (1996) A 2.2 Resolution
Crystal Structure of a Designed Zinc Finger Protein Bound to DNA Nat. Struct. Biol. 3, 940-945].

Figure 2 shows the three dimensional structure of domain 1 from the ZFP in Figure 1, which was the natural enantiomeric selection target used here (corresponding to SEQ ID NO:46). Figure conventions as in legend to Figure 1.

Figure 3 shows Circular Dichroism (CD) spectra of zinc-finger peptides, encompassing domain 1 (SEQ ID 46), synthesised from D- and L- amino acids, in the presence and absence of 1.5x molar excess of ZnCl₂. Upon addition of ZnCl₂, the peptides fold from a largely random coil conformation (red and green traces) into more ordered structures capable of forming the zinc-finger DNA-binding domain (blue and yellow traces). The rough mirror symmetry of the CD spectra confirms the enantiomeric identity of the selection targets used here.

Figure 4 shows an Electrophoretic Mobility Shift Assay (EMSA) of the binding of a synthetic ZFP construct encompassing the three zinc-fingers shown in Figure 1 to a double-stranded DNA oligonucleotide encompassing its target sequence.

Figure 5 shows the results of an aptamer competition-EMSA assay. The position of the retarded protein-DNA complex [lanes 2-9] is indicated by the arrow. Lane 1 in each panel contains 5 pmoles of FAM-labelled DNA alone, as in Figure 4. Lanes 2-9 also contain 30 pmoles of the ZFP construct (approximately equivalent to lane 7 of Fig. 4). Lanes 3 – 9 show the effects of adding 0.1, 0.2, 0.5, 1, 2, 5 & 10 times the protein concentration of either Anti-1ZnF aptamer 2 (SEQ ID NO: 3 6), left hand panel, or the unselected naïve starting pool, right hand panel.

Figure 6 shows an aptamer-EMSA competition assay run under conditions similar to those of the assay shown in Fig. 5 for aptamers corresponding to SEQ ID NOS: 41 (left-hand panel) and 21 (right-hand panel), selected against the tZF peptide.
Figure 7 shows an aptamer-EMSA competition assay run under conditions similar to those of the assay shown in Fig. 5 for aptamers corresponding to SEQ ID NOS: 16 (left-hand panel) and 3 (right-hand panel), selected against the $\beta$ZF peptide.

Figure 8 shows an RNA EMSA. 5 μM fluorescein-UTP labelled Anti-$\beta$ZnF aptamer 15a (SEQ ID NO: 3) was incubated with increasing concentrations of the 3ZFP protein construct, i.e. the non-cognate target. Lanes 1 – aptamer RNA alone [5μM]; lanes 2-10 contained increasing ZFP protein [10, 50, 100, 200, 300, 400, 500, 600, 650 μM].

Material and Methods

Preparation of the 3ZFP construct
The 3ZFP peptide was over-expressed, extracted and purified following standard procedures (Derek Jantz, pers. comm). Purification was carried out using an ÄKTA Explorer (Amersham Pharmacia Biotech) with a HiTrap Heparin Sepharose column.

Magnetic Bead Derivatisation
6 mg streptavidin microspheres were rinsed in 1 ml bead preparation solution (20 mM Tris HCl pH 7, 200 mM NaCl) to remove preservatives. The prepared beads were then suspended in 200 μl bead binding solution (20 mM Tris HCl pH 7, 1 mM EDTA, 1 M NaCl) containing 2.4 nmoles of the folded, biotinylated ZFP peptide. The beads were incubated for 30 min at room temperature on an orbital shaker (200 rpm) to prevent the beads settling out. After incubation, the beads were separated, rinsed in 1 ml working buffer (binding buffer for SELEX) and resuspended in working buffer to 10 mg ml$^{-1}$ final concentration.

SELEX
All rounds of in vitro selection were carried out on a Biomek 2000 automated workstation (Beckman Coulter) using methods adapted from those described by Cox et al,. The Biomek has an integrated PTC-200 thermocycler with heated power
bonnet (MJ Research), a multiscrn filtration system & vacuum manifold (Beckman Coulter) and a Thermal Exchange Unit (Beckman Coulter) with a Thermal 48 cooling block (Acme-Automation). The Biomek and all integrated components are controlled using Bioworks 3.1c (Beckman Coulter).

To produce the initial DNA pool; 20 µl 10x PCR buffer[200 mM Tris HCl pH 8.4, 500 mM KCl], 1.6 µl 10 mM dNTP's, 10 µl 10 µM primer 1 [SEQ ID 43], 10 µl 10 µM primer 2 [SEQ ID 44], 20 µl 0.1 µM 30N template DNA consisting of (5'-3') SEQ ID 43-30N-complement which lacks the T7 site of P1, 6 µl 50 mM MgCl2, 2 µl Taq DNA polymerase and 124.4 µl R H2O are thoroughly mixed and divided into 50 µl aliquots. These are then placed into a Techne Progene thermocycler and cycled as follow; 94°C for 90 sec (Hot start), 8 cycles of: 94 °C for 45 sec (Denature); 50°C for 60 sec (Anneal) and 72°C for 90 sec (Extend)

As this thermocycler has a heated lid to prevent evaporation, the addition of mineral oil to the sample is unnecessary The efficiency of PCR was assessed by polyacrylamide gel electrophoresis (PAGE).

**RNA Pool**

Transcription reactions were carried out by adding 5 µl 10x transcription buffer (to give final concentrations of 40 mM Tris-HCl pH7.9, 26 mM MgCl2, 2.5 mM spermidine, 5 mM DTT, 0.01% (v/v) Triton X-100), 16 µl R H2O, 16 µl 25 mM NTP mix, 10 µl template DNA. 40 U RNAsin and 100 U T7 RNA polymerase were then added to give a final volume of 50 µl. After mixing by rapid aspiration and dispensing, the transcription mix was incubated at 37°C for 120 min in the thermocycler.

**Selection**

20 µl derivatised Dynabeads (~ 6.6 x 10^6 beads) are added to 80 µl binding buffer and mixed by rapid aspiration and dispensing. The binding buffer used was 10 mM HEPES, pH7.4, 100 mM NaCl. The entire transcription reaction is mixed into this.
Mixing is repeated after a 5 min. room temperature incubation, to ensure the beads do not settle out. Partitioning of bound from unbound species is carried out by filtration through a multiscreen 96-well plate (PVDF membrane) supplied by Millipore. The Dynabeads are then rinsed by resuspending in binding buffer and repeating the filtration. Bound RNA species are eluted by resuspending the Dynabeads in 53 μl R' H2O and incubating at 95°C for 15 min.

**RT-PCR Amplification**

The following are added to the eluted RNA; 5 μl 10 mM primer 1, 5 μl 10 mM primer 2, 2 μl 10 mM dNTPs and 33 μl RT-PCR buffer to give final concentrations of 10 mM Tris HCl pH8.4, 50 mM KCl, 5% (w/v) acetamide, 0.05% (v/v) Nonidet P40. This mixture is heated to 65°C for 10 min. After reducing the temperature to 50°C, 200 U Superscript II™ reverse transcriptase and 5U Taq DNA polymerase are added. This is thermocycled as follows: 42°C for 60 min (Reverse transcribe), 8 cycles of: 94°C for 45 sec (Denature); 50°C for 60 sec (Anneal) and 72°C for 90 sec (Extend).

10 μl of the RT-PCR product was used as a template in the transcription reaction for the next round. After 10 successful rounds of SELEX, samples of the RT-PCR products are cloned and sequenced.

The zinc-finger peptides were synthesised, in both D & L forms, using commercially available f-moc amino acids. In both cases, the peptide was derivatised at the N-terminus with a biotin tag through which it was attached to the streptavidin beads. Selections were carried out in 10 mM HEPES buffer pH7.4, 100mM NaCl. RNA was incubated with the beads for 10 min before partitioning. The nucleic acid library used contained an N30 random region flanked by fixed primer sequences (shown below as SEQ ID NOS: 43 and 44). RNA resulting from this pool starts at the underlined GGG sequence.
GATAATACGACTCACTATA<br>GGGATGGATCCACATCTACGAAATTC (SEQ ID NO: 43)<br><br>TTCACGTGCCAGACTTGACGAAGCTT (SEQ ID NO: 44)<br><br>EXAMPLE 1

With reference to Figure 4, it is shown the fluorescently (FAM)-labelled dsDNA forms a protein-DNA complex (indicated by an arrow) upon addition of the protein construct. This complex has a higher molecular mass than that of free DNA and therefore its migration through the acrylamide gel is retarded. 5 pmoles of labelled DNA was loaded onto each lane. Protein concentration increases from left to right; Lane 1 - no protein; lanes 2-13- increasing in 5 pmoles steps)

Figure 5 shows the results of an aptamer competition-EMSA assay. The position of the retarded protein-DNA complex [lanes 2-9] is indicated by the arrow. Lane 1 in each panel contains 5 pmoles of FAM-labelled DNA alone, as in Figure 4. Lanes 2-9 also contain 30 pmoles of the ZFP construct (approximately equivalent to lane 7 of Fig. 4). Lanes 3 – 9 show the effects of adding 0.1, 0.2, 0.5, 1, 2, 5 & 10 times the protein concentration of either Anti-κZNF aptamer 2 (SEQ ID NO: 36), left hand panel, or the unselected naïve starting pool, right hand panel. Under these conditions the unselected RNA has little or no effect on DNA-ZFP construct binding. However, the Anti-κZNF aptamer has an inhibitory effect, completely preventing binding at the higher concentrations.

EXAMPLE 2

Figure 6 shows an aptamer-EMSA competition assay run under conditions similar to those of the assay shown in Fig. 5 for aptamers corresponding to SEQ ID NOS: 41 (left-hand panel) and 21 (right-hand panel), selected against the κZF peptide. Figure 7 shows an aptamer-EMSA competition assay run under conditions similar to those of the assay shown in Fig. 5 for aptamers corresponding to SEQ ID NOS: 16 (left-
hand panel) and 3 (right-hand panel), selected against the βZF peptide. These results show that some aptamers appear to compete better than others, as they completely inhibit DNA-construct binding at lower aptamer concentrations. The results also show that interestingly, aptamers selected against the β zinc finger have the ability to bind to the γ zinc finger construct and hence inhibit DNA-construct binding.

**EXAMPLE 3**

Figure 8 shows an RNA EMSA. 5 μM fluorescein-UTP labelled Anti-βZnF aptamer 15a (SEQ ID NO: 3) was incubated with increasing concentrations of the 3ZFP protein construct, i.e. the non-cognate target. Lanes 1 – aptamer RNA alone [5μM]; lanes 2-10 contained increasing ZFP protein [10, 50, 100, 200, 300, 400, 500, 600, 650 μM]. These results confirm the ability of the non-natural enantiomeric RNA aptamers to bind to natural peptide sequences. Multiple retarded species (red arrows) appear present as the protein concentration increases, suggesting that the aptamers can bind all the ZF domains in the construct, although they were selected against just domain 1 (Figure 1).
Claims

1. An isolated nucleic acid ligand capable of binding to an aptope of a zinc finger protein (ZFP), the ligand being capable of preventing active ZFP protein binding to DNA so as to alter expression of said DNA.

2. A nucleic acid ligand according to claim 1 wherein the ZFP comprises the amino acid sequence as set forth in SEQ ID NO:46.

3. A nucleic acid ligand according to either claim 1 or 2 wherein the ZFP amino acid sequence is independently D or L configuration or a mixture thereof.

4. A nucleic acid ligand according to any preceding claim wherein the ZFP amino acid sequence comprises a 3ZFP construct which comprises a first domain as set forth in SEQ ID NO:46, a second domain as set forth in SEQ ID NO:47 and a third domain as set forth in SEQ ID NO:48.

5. A nucleic acid ligand according to any preceding claim wherein the ZFP construct comprises fragments of those polynucleotides as set forth in any one of SEQ ID NOS: 46-48 that retain the ZFP aptope and function as ZFP by altering DNA expression, and variants of those polynucleotides with sufficient similarity to the non-coding strand of those polynucleotides to hybridise thereto under stringent conditions.

6. A nucleic acid according to claim 5 wherein the stringent hybridisation conditions are hybridisation at 42 °C in 5X SSC, 20 mM NaPO4, pH 6.8, 50% formamide; and washing at 42 °C in 0.2X SSC.

7. A nucleic acid ligand according to any preceding claim that is capable of binding to at least 1, 2 or 3 domains of the 3ZFP construct.
8. An isolated nucleic acid comprising a nucleotide sequence selected from the group consisting the nucleic acids depicted in any one of SEQ ID NOS: 1 to 42 or having a corresponding DNA sequence and nucleic acids having complementary sequences thereto and L-ribose analogues of the foregoing.

9. A nucleic acid ligand according to any preceding claim further including a fluorescent label and/or an imaging label and/or a flanking region.

10. A nucleic acid ligand according to any preceding claim having 5' and 3' flanking or terminal sequences of SEQ ID NOS: 43 and 44 respectively.

11. Use of a nucleic acid ligand according to any preceding claim as a primer or hybridization probe for the detection or amplification of nucleic acid ligands for a ZFP.

12. A vector comprising at least one or more nucleic acid ligand as defined in any of claims 1 to 10.

13. A host cell including at least one nucleic acid ligand as defined in any of claims 1 to 10 or the vector of claim 12.

14. A pharmaceutical composition comprising at least one nucleic acid ligand as defined in any of claims 1 to 10.

15. A pharmaceutical composition according to claim 14 further including a pharmaceutically acceptable excipient, diluent or carrier.

16. Use of a nucleic acid ligand as defined in any of claims 1 to 10 or the vector of claim 12 in assaying for disease prevention and progression.

17. An isolated nucleic acid ligand to a ZFP or a biologically active fragment thereof, wherein said ligand is an RNA ligand selected from the group consisting of:
(i) nucleic acids depicted in any one of SEQ ID NOS: 1 to 42;
(ii) nucleic acids having a corresponding DNA sequence of any one of SEQ ID NOS: 1 to 42, and nucleic acids having a complementary sequences thereto and L-ribose analogues of the foregoing; or
(iii) nucleic acids analogous to a sequence depicted in any one of SEQ ID NOS: 1 to 42 having at least about 60%, 70%, 80% or 90%, sequence identity to any one of the nucleotide sequences and having a binding affinity to ZFP.

18. A method for the isolation of nucleic acid ligands to a ZFP target comprising:
(iii) contacting a candidate mixture of nucleic acids with a polypeptide selected from a ZFP or fragment thereof; and
(iv) identifying nucleic acids which bind to the polypeptide.

19. A method according to claim 18 wherein nucleic acids having an increased binding affinity to the ZFP target relative to the candidate mixture are partitioned from the remainder of the candidate mixture.

20. A method according to either claim 18 or 19 wherein the candidate mixture comprises single stranded nucleic acids or ribonucleic acids.

21. A method according to any one of claims 18 to 20 wherein the ZFP target is isolated and immobilised.

22. A method according to any of claims 18 to 21 wherein the ZFP target comprises at least one biotin molecule.

23. A method according to any of claims 18 to 22 wherein the nucleic acids are bound to a fluorescent label and/or an imaging reagent.
24. A method according to any one of claims 18 to 23 wherein the nucleic acids include a flanking or terminal sequence as set forth in SEQ ID NOS: 43 and 44.

25. A method according to any one of claims 18 to 24 wherein the ZFP target is a 3ZFP construct.

26. A method according to claim 25 wherein the 3ZPP construct comprises fragments of those polynucleotides as set forth in any one of SEQ ID NOS: 46-48 that retain the ZFP apatope and function as ZFP by altering DNA expression, and variants of those polynucleotides with sufficient similarity to the non-coding strand of those polynucleotides to hybridise thereto under stringent conditions.
SEQ ID NO: 1
TGTCGGATTGCATTCGAATTTCCTTGATT

SEQ ID NO: 2
5 TCGGCTACAACACATCGGAACCTTGCGCGGAT

SEQ ID NO: 3
ACTGGTTACATCTTTGTGTGCAGATTTCGG

10 SEQ ID NO: 4
ATTGCATAAGTCGGAGTTAGCGATT

SEQ ID NO: 5
AGCCGAGACCCCTCGGTTACTATAACAGGT

15 SEQ ID NO: 6
AGGTCTCGTTTTANAGGACCATTGGTCTCCTCNCTGCAGACTTGACGAAGCTTG

SEQ ID NO: 7
20 GATCGCTCCAGACCCTGCGATGCCTAC

SEQ ID NO: 8
CGCCGCAACCCACTCAGCGCTCAACCATAA

25 SEQ ID NO: 9
TGGGTGACTGCTAATGGTGAGCTAATGGTC

SEQ ID NO: 10
GTGCTGAATATGTAAGGTCAAGCCAGGGGC

30 SEQ ID NO: 11
CTAAGACGAAATGCGCAACTGATAGGACCN
SEQ ID NO:12
ACGTTCATAAGTGAACGTCTCCAAAGCCGN

SEQ ID NO:13
GAGTAACCCGATGATGTCGCCGCTATGTAAGC

SEQ ID NO:14
ATAGGTTTAAGGGAAAGGGGCTTTAGTT

SEQ ID NO:15
GAGCAGGTGTGTTAGGCCGTCTAAAAAGTTAG

SEQ ID NO:16
TCAGGTGATCGATTATGTGATGTCGCCCGGA

SEQ ID NO:17
ACGCATAAGGAGCTTCCCCTTGGTTGTTA

SEQ ID NO:18
TCGGACGACCCCCCTGCAAGGCA

SEQ ID NO:19
GGGGTGATTGAAAGGAGGTTGCAGCCGACA

SEQ ID NO:20
GAAGGGGGTTAAACGCCGTACGGATAAG

SEQ ID NO:21
AGTTTCGGACCTCTTAGTCACCCCCAGAA

SEQ ID NO:22
ACAGTCTCCTNTAGAACAACACGGCAAGTA
SEQ ID NO:23
ATTNTGGATAACGCAAGCACAATACCCGACCCGCA

SEQ ID NO:24
5 TCTGCCAAGGTAACATGTGTTCAACGTCAA

SEQ ID NO:25
CTTCATCATTAACCCGGTCCAGC

10 SEQ ID NO:26
TGGATAACGCAAGCACAATANCGNCCGCAT

SEQ ID NO:27
TNTGCCAgGTAACATGTGTTCAACGTCAA

15 SEQ ID NO:28
GTATCATCTGTGTTGTTTCTTCTTTTGGTGG

SEQ ID NO:29
20 AAGTGTAGATATTTAGTGCGTGTGCGC

SEQ ID NO:30
AAGTGTAGATATTTAGTGCGTGTGCGC

25 SEQ ID NO:31
ATGAAACTCGTTCAGC

SEQ ID NO:32
TGCCGTTCTCTCCTACGTGCTCTTGAG

30 SEQ ID NO:33
TAACCGTCAACGCTTGGAACCACCACCGTG
SEQ ID NO:34
ACACAAATGCCTTTACGTTCGCCGACCCGCA

SEQ ID NO:35
5 TGAGACGACAAGCTCCCGATATGCTCCGGTGC

SEQ ID NO:36
GTGTTTGCAGTAGCTTTTAGCAACTTCAC

SEQ ID NO:37
10 ACCTGACGCGCGAGTGTCCTGGGTCCTC

SEQ ID NO:38
15 GTCTCATAAGGTTCACATCTTCCTCAAT

SEQ ID NO:39
20 GACACGACCACCTCCGGTTCGAC

SEQ ID NO:40
25 ACTGAGCTCGATAAACCCCGCCGCTACG

SEQ ID NO:41
30 TGCGGTTTGTAGTTCATAGTGTTGCTG

SEQ ID NO:42
35 ATGAGTTGGAGGTTGTCATAGTCTT

SEQ ID NO:43
40 5 prime end

SEQ ID NO:44
45 GATAATACGACTCACCATAGGGAATGGGATCCACATCTACGAATTC

SEQ ID NO:45
50 3 prime end

SEQ ID NO:46
55 TTCACACTCGAGACTTGACAAAGCTT