The present invention provides polynucleotides encoding human RNase III and polypeptides encoded thereby. Methods of using said polynucleotides and polypeptides are also provided.
**Figure 1**
HUMAN RNASE III AND COMPOSITIONS AND USES THEREOF

[0001] The present application is a continuation-in-part of U.S. application Ser. No. 09/900,425, filed Jul. 6, 2001, which is a continuation-in-part of U.S. application Ser. No. 09/479,783, filed Jan. 7, 2000, which is a divisional of U.S. application Ser. No. 08/870,608, filed Jun. 6, 1997, U.S. Pat. No. 6,107,004, which is a continuation in part of U.S. application Ser. No. 08/659,440, filed Jun. 6, 1996, U.S. Pat. No. 5,898,031. All of the above are assigned to the assignee of the present invention and are incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to a human RNase III, the gene for which has now been cloned and characterized, and compositions and uses thereof. Antisense inhibitors of human RNase III are also described.

BACKGROUND OF THE INVENTION

[0003] Ribonuclease III (RNase III) is an endoribonuclease that cleaves double stranded RNA. The enzyme is expressed in many organisms and is highly conserved. I. S. Mian, Nucleic Acids Res., 1997, 25, 3187-95. All RNase III species cloned to date contain an RNase III signature sequence and vary in size from 25 to 50 kDa. Multiple functions have been ascribed to RNase III. In both E. coli and S. cerevisiae, RNase III has been reported to be involved in the processing of pre-ribosomal RNA (pre-rRNA). Elela et al., Cell, 1996, 85, 115-24. RNase III has also been reported to be involved in the processing of small molecular weight nuclear RNAs (snRNAs) and small molecular weight nuclear RNAs (snRNAs) in S. cerevisiae. Chanfreau et al., Gene Dev, 1996, 11, 2741-51; Gu et al., Mol. Cell. Biol. 1996, 19, 1144-58. In E. coli, RNase III has also been reported to be involved in the degradation of some mRNA species. D. Court, in Control of messenger RNA stability, 1993, Academic Press, Inc, pp. 71-116.

[0004] A human double strand RNA (dsRNA) activity has been described. Wu et al., J. Biol. Chem., 1998, 273, 2532-2542; Crooke, U.S. Pat. No. 5,898,031; U.S. Pat. No. 6,017,094. By the rational design and testing of chemically modified antisense oligonucleotides that contained oligoribonucleotide stretches of varying length, a dsRNA activity was demonstrated in human T24 bladder carcinoma cells which produced 5'-phosphate and 3'-hydroxyl termini upon cleavage of the complementary cellular RNA target. This pattern of cleavage products is a feature of E. coli RNase III. The cleavage activity in human cells required the formation of a dsRNA region in the oligonucleotide. This human dsRNA activity is believed to be useful as an alternative terminating mechanism to RNase H for antisense therapeutics. Because it relies on “RNA-like” oligonucleotides, which generally have higher potency than the “DNA-like” oligonucleotides required for RNase H activity, it may prove an attractive alternative to RNase H-based antisense approaches.

[0005] RNA interference (RNAi) is a form of sequence-specific, post-transcriptional gene silencing in animals and plants, elicited by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. Elbashir et al., Nature, 2001, 411, 494-498. dsRNA triggers the specific degradation of homologous RNAs, only within the region of homology. The dsRNA is processed to 21- to 23-nucleotide fragments, sometimes called short interfering RNAs (siRNAs) which are believed to be the guide fragments for sequence-specific mRNA degradation. The processing of longer dsRNA to these short siRNA fragments is believed to be accomplished by RNase III. Elbashir et al., ibid., Elbashir et al., Genes and Devet., 2001, 15, 188-200. Thus it is believed that the human RNase III of the present invention may be useful in further understanding and exploiting the gene silencing mechanism, particularly in human cells.

[0006] Despite the substantial information about members of the RNase III family and the cloning of genes encoding proteins with RNase III activity from a number of lower organisms (E. coli, yeast, and others), no human RNase III has previously been cloned. This has hampered efforts to understand the structure of the enzyme(s), its distribution and the functions it may serve. The present application describes the cloning and characterization of a cDNA that expresses a human RNase III. Cloning and sequencing of the cDNA encoding human RNase III allowed characterization of this nucleic acid as well as of the location and function of the RNase III protein itself.

SUMMARY OF THE INVENTION

[0007] The present invention provides a polynucleotide sequence (set forth herein as SEQ ID NO: 1) which has been identified as encoding human RNase III by the homology of the calculated expressed polypeptide (provided herein as SEQ ID NO: 2) with known amino acid sequences of yeast and worm RNase III as well as by functional analysis.

[0008] The present invention provides polynucleotides that encode human RNase III, the human RNase III polypeptide, vectors comprising nucleic acids encoding human RNase III, host cells containing such vectors, antibodies targeted to human RNase III, nucleic acid probes capable of hybridizing to a nucleic acid encoding a human RNase III polypeptide, and antisense inhibitors of RNase III expression. Methods of inhibiting RNase III expression or activity are also provided, as are pharmaceutical compositions which include a human RNase III polypeptide, an antisense inhibitor of RNase III expression, or a vector containing a nucleic acid encoding human RNase III.

[0009] Methods for identifying agents which modulate activity and/or levels of human RNase III are also provided. Methods of promoting inhibition of expression of a selected protein via antisense, methods of screening oligonucleotides to identify active antisense oligonucleotides against a particular target, methods of prognosticating efficacy of antisense therapy, methods of promoting RNA interference (RNAi) or other forms of gene silencing in a cell and methods of eliciting cleavage or modification of a selected cellular RNA target are also provided. As all of these methods exploit the RNA-binding and cleaving activity of RNase III polypeptides. In preferred embodiments the polynucleotides used in these methods are RNA-like oligonucleotides. Also provided are methods of identifying agents which increase or decrease activity or levels of human RNase III.

[0010] The compositions and methods of the present invention are useful for research, biological and clinical purposes. For example, the polynucleotides and antisense oligonucleotides are useful in defining the roles of RNase III and the interaction of human RNase III and cellular RNA (including pre-mRNA or pre-rRNA).
BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. 1 shows the amino acid sequence of human RNase III (SEQ ID NO: 2) and a comparison of the sequence of the RNase III domain of the human RNase III to RNase III domains of C. elegans (Worm; SEQ ID NO: 3), S. pombe (PAC; SEQ ID NO: 4) and S. cerevisiae (RNT; SEQ ID NO: 5) and E. coli (RNC; SEQ ID NO: 6). Bold letters: identical amino acids of human RNase III to other species. @@@: putative catalytic center; BHHI: alpha helix; BBB: beta sheet (dsRNA binding region at C-terminus). Amino acid identity of human RNase III to Worm (41%), PAC (17%), RNT (15%) and RNC (16%): *: Potential phosphorylation sites analyzed using OMIGA (Oxford Molecular Ltd.).

DETAILED DESCRIPTION OF THE INVENTION

[0012] A cDNA encoding human RNase III has now been cloned and characterized. The cloned sequence is provided herein as SEQ ID NO: 1. This cDNA encodes a protein of 160 kDa which is ubiquitously expressed in human cell and tissue types, and is involved in processing of preribosomal RNA (pre-rRNA).

[0013] Thus, in accordance with one aspect of the present invention, there are provided isolated polynucleotides which encode human RNase III polypeptides. By “polynucleotides” it is meant to include any form of RNA or DNA such as mRNA, pre-mRNA or cDNA or genomic DNA, respectively, obtained by cloning or produced synthetically by well known chemical techniques. The term “polynucleotide” is also meant to include oligonucleotides, e.g. synthetic antisense oligonucleotides. DNA or RNA polynucleotides may be double- or single-stranded. Single-stranded DNA or RNA polynucleotides may comprise the coding or sense strand or the non-coding or antisense strand.

[0014] Methods of isolating a polynucleotide of the present invention via cloning techniques are well known. For example, to obtain the polynucleotide sequence of SEQ ID NO: 1, a similarity search of the yeast Rnt1 gene (RNase III, Genbank accession no. AAB04172; SEQ ID NO: 5) and the Caenorhabditis elegans RNase III gene (Genbank accession no. 001326; SEQ ID NO: 3) with the XREF database (National Center for Biotechnology Information, NIH, Rockville Md.) was performed. A 393 base pair (bp) human EST clone (GenBank AA083886) was identified.

[0015] Using primers based on this EST sequence, a clone (U4) corresponding to the COOH-terminal portion of the protein (molecular 3569-4764 of full length cDNA) was cloned by 3’ RACE. Eight positive clones were isolated by screening a liver cDNA library with this clone. With primers based on one of these clones, 5’ RACE was performed to clone a cDNA of approximately 1 kb, which corresponds to the middle part of the full length cDNA. In the same way, a cDNA of the NH2-terminal portion was cloned. Primers based on the NH2-terminal-most clone were used to perform additional 5’-RACE to obtain the NH2-terminal portion of the cDNA. The overlapping clones were sequenced and assembled to a full length human RNase III cDNA with a total of 4764 nucleotides. This human RNase III polynucleotide sequence is provided herein as SEQ ID NO: 1 and has been deposited as GenBank accession no. AF189011. The cDNA contained a coding sequence of 4125 nucleotides (from 246-4370 of SEQ ID NO:1) that was calculated to encode a 1374 amino acid protein. This polypeptide sequence is provided herein as SEQ ID NO: 2, shown in FIG. 1. The calculated molecular weight of the protein is 160 kDa based on the prediction of the first translated methionine as the translation initiation site. Northern hybridization analysis demonstrated that the human RNase III mRNA was approximately 5 kb in size. It was found to be ubiquitously expressed in human tissues and cell lines. Compared to C. elegans, yeast and bacterial RNase III, human RNase III is substantially larger and contains multiple domains. The RNase III domain (amino acids 949-1374) is located at the carboxy terminus of the protein and is homologous to C. elegans, yeast and bacterial RNase III. The human RNase III also contains a proline rich (amino acids 1-220) and serine-arginine rich (amino acids 221-470) domains near the amino terminus. The SR and RNase III domains are separated by 478 amino acids.

[0016] The RNase III domain of human RNase III is conserved with other species and is most homologous with C. elegans RNase III (41% identity). Both the human RNase III domain and C. elegans RNase III contain two RNase III signature sequences (HNERLEFLGDS; SEQ ID NO 7). Sequence identity was also compared with the yeasts S. pombe (PAC gene) (17% homology) and S. cerevisiae (RNT gene) (15% homology) and with E. coli RNase III (RNC gene) (16% homology). Human RNase III also contains multiple phosphorylation sites. The SR domain is usually present in SR or SR related proteins that play crucial roles in mRNA splicing. The fusion of SR and RNase III domains into a single protein suggests that human RNase III may be involved in a member of RNA metabolic events. The presence of multiple potential phosphorylation sites suggests that the enzyme is regulated by phosphorylation.

[0017] As used herein, the phrase “homologous nucleotide sequence,” or “homologous amino acid sequence,” or variations thereof, refers to sequences characterized by a homology, at the nucleotide level or amino acid level, of at least the specified percentage. Homologous nucleotide sequences include those sequences coding for isoforms of proteins. Such isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. Homologous nucleotide sequences include nucleotide sequences encoding for a protein of a species other than humans, including, but not limited to, mammals. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the nucleotide sequence encoding other known RNase III. Homologous amino acid sequences include those amino acid sequences which contain conservative amino acid substitutions and which polypeptides have the same binding and/or activity. A homologous amino acid sequence does not, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison WIs.), using the default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489, which is incorporated herein by reference in its entirety).
In a preferred embodiment, the polynucleotide of the present invention comprises the nucleic acid sequence of SEQ ID NO: 1. However, as will be obvious to those of skill in the art upon this disclosure, due to the degeneracy of the genetic code, polynucleotides of the present invention may comprise other nucleic acid sequences encoding the polypeptide of SEQ ID NO: 2 and derivatives, variants or active fragments thereof.

The invention further provides homologs of the human RNase III DNA. Such homologs, in general, share at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% homology with the human RNase III DNA of the invention. Species homologs, sometimes referred to as “orthologs,” in general, share at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% homology with the human RNase III DNA of the invention. Generally, percent sequence “homology” with respect to polynucleotides of the invention can be calculated as the percentage of nucleotide bases in the candidate sequence that are identical to nucleotides in the human RNase III sequence set forth in the appended Sequence Listing, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity.

Another aspect of the present invention relates to the polypeptides encoded by the polynucleotides of the present invention. In a preferred embodiment, a polypeptide of the present invention comprises the deduced amino acid sequence of human RNase III provided in SEQ ID NO: 2. However, by “polypeptide” it is also meant to include fragments, derivatives and analogs of SEQ ID NO: 2 which retain essentially the same biological activity and/or function as human RNase III. Alternatively, polypeptides of the present invention may retain their ability to bind to double stranded RNA even though they do not function as active RNase III enzymes in other capacities. Thus an enzyme may “modify” its RNA substrate, e.g., bind and interfere with the function of the RNA but not cleave it, or may bind and cleave. In some embodiments cleavage is a preferred form of modification. In another embodiment, polypeptides of the present invention may retain nuclease activity but without specificity for an RNA/RNA duplex. Polypeptides of the present invention include recombinant polypeptides, isolated natural polypeptides and synthetic polypeptides, and fragments thereof which retain one or more of the activities described above.

The invention further provides homologs of the human RNase III polypeptide. Such homologs, in general, share at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% homology with the RNase III polypeptides of the invention. Generally, percent sequence “homology” with respect to polypeptides of the invention can be calculated as the percentage of amino acid residues in the candidate sequence that are identical to amino acid residues in the RNase III sequences set forth in the appended Sequence Listing, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity.

In some embodiments the present invention provides recombinant polypeptides that comprise RNase III domains from one or more organisms. Domains of RNase III that exhibit certain functions can be replaced with RNase III domains from other organisms that exhibit similar functions, while maintaining the overall function of the polypeptide. As a non-limiting example, a hybrid RNase III may comprise one or more E. coli RNase III domain, one or more C. elegans RNase III domain, and one or more human RNase III domain. As a non-limiting example, such hybrid RNase III polypeptides can be produced by first designing and producing recombinant DNA molecules encoding such polypeptides. Such recombinant DNA molecules are produced, for example, by replacing DNA sequences that encode individual domains in a polynucleotide encoding RNase III with DNA sequences from other organisms that encode RNase III domains that exhibit similar functions. The recombinant DNA construct thus produced can then be expressed and purified using means familiar to one of ordinary skill in the art.

To confirm the expression of the human RNase III protein, two anti-peptide antibodies were produced. The “anti-III” peptide antibody was derived from a peptide corresponding to amino acids 1356-1374 within the RNase III domain present in the C-terminal portion of the putative protein. The “anti-SR” peptide antibody was derived from a peptide corresponding to amino acids 266-284 within the SR-domain of the putative protein. Using these antibodies, Western blot analyses were performed to determine the size and localization of human RNase III. The anti-SR peptide antibody recognized a band in HeLa whole cell lysate with a molecular weight of approximately 160 kDa which is near the calculated protein size confirming that the full coding region is expressed in HeLa cells. Similar experiments were performed using different human cell lines e.g. A549, T24 and III.60 with equivalent results. To determine the localization of the protein, nuclear and non-nuclear fractions from HeLa cells and other human cell lines were prepared and equal amounts of proteins were analyzed by Western blots. RNase III was present primarily in the nuclear fractions. Non-nuclear fractions contained only trace amounts of protein, possibly due to the contamination during sample preparation. The anti-III peptide antibody gave results equivalent to those obtained with the anti-SR peptide antibody. To better understand the localization of human RNase III, the protein was identified in cells by indirect immunofluorescence microscopy. The nuclei of HeLa cells were stained by both anti-SR and anti-III antibodies, confirming that human RNase III is present in the nucleus. RNase III is localized extensively in nuclei and occasionally observed in nucleoli. This localization suggests possible involvement in both pre-mRNA and pre-rRNA processing. In E. coli, RNase III is associated with ribosomes in the cytoplasm. Robertson et al., J. Biol. Chem., 1968, 243, 82-91. Eukaryotic RNase III has not previously been shown to be localized in the nucleus.

The localization of human RNase III to nucleoli was found to be cell cycle regulated. Double thymidine treatment was used to synchronize HeLa cells to early-S phase. Two to four hours after releasing the thymidine block, HeLa cells entered S phase as determined by fluorescence activated cell sorting (FACS). Six to eight hours after release, HeLa cells entered the G2/M phase. There were no significant changes in the MRNA or protein levels of the
RNase III during pre-S, S or G2/M phases. However, the subcellular localization of the protein changed during the cell cycle. When the cells were treated with thymidine and synchronized in early S phase, RNase III protein was present only in the nucleus and not the nucleoli, as determined by immunofluorescent labeling. After releasing from thymidine block, RNase III was translocated to nucleoli, reaching a peak at 4 hours when cells were in S phase. At that time, RNase III was present both in the nucleolus and the nucleus. The protein was present in the nucleoli for approximately 8 hours, and then disappeared from nucleoli as cells entered M phase. Localization of RNase III in the nucleoli was confirmed by double staining with an anti-nucleolin monoclonal antibody (MBL, Watertown, Mass.).

[0025] In human cells, nucleoli undergo phases of condensation and dissociation as a function of the cell cycle. Nucleoli dissociate upon entering prophase and disappear entirely during the late prophase and metaphase periods of mitosis, then begin to reappear during telophase and form dense organelles during the G1 phase. Human RNase III was only translocated to and remained in the nucleoli during S phase suggesting that RNase III may serve one or more specific functions in nucleoli during S phase.

[0026] The present invention also provides antisense inhibitors of RNase III expression, which may be used, for example, therapeutically, prophylactically or as research reagents. The modulation of function of a target nucleic acid (in this case a nucleic acid encoding RNase III) by compounds which specifically hybridize to it is generally referred to as “antisense”. The functions of DNA to be interfered with include replication and transcription. The functions of RNA to be interfered with include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of the target. In the context of the present invention, “modulation” means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene. In the context of the present invention, inhibition is the preferred form of modulation of gene expression and mRNA is a preferred target. In some embodiments gene silencing is a preferred form of inhibition of gene expression and refers to a decrease in gene expression mediated by a double-stranded RNA polynucleotide, one strand of which is homologous to the RNA to be silenced.

[0027] It is preferred to target specific nucleic acids for antisense. “Targeting” an antisense compound to a particular nucleic acid, in the context of this invention, is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. The targeting process also includes determination of a site or sites within this gene for the antisense interaction to occur such that the desired effect, e.g., detection or modulation of expression of the protein, will result. Within the context of the present invention, a preferred intragenic site is the region encompassing the translation initiation or termination codon of the open reading frame (ORF) of the gene. Since, as is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the “AUG codon,” the “start codon” or the “AUG start codon”. A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UGG or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in vivo. Thus, the terms “translation initiation codon” and “start codon” can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, “start codon” and “translation initiation codon” refer to the codon or codons that are used in vivo to initiate translation of the target, regardless of the sequence(s) of such codons.

[0028] It is also known in the art that a translation termination codon (or “stop codon”) of a gene may have one of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively). The terms “start codon region” and “translation initiation codon region” refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms “stop codon region” and “translation termination codon region” refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon.

[0029] The open reading frame (ORF) or “coding region,” which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Other target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA or corresponding nucleotides on the gene, and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA or corresponding nucleotides on the gene. The 5' cap of an mRNA comprises an N7-methylated guanosine residue joined to the 5' most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap. The 5' cap region may also be a preferred target region.

[0030] Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as “introns,” which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as “exons” and are spliced together to form a continuous mRNA sequence. mRNA splice sites, i.e., intron-exon junctions, may also be preferred target
regions, and are particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular mRNA splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also preferred targets. It has also been found that introns can also be effective, and therefore preferred, target regions for antisense compounds targeted, for example, to DNA or pre-mRNA.

[0031] Once one or more target sites have been identified, oligonucleotides are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect.

[0032] In the context of this invention, “hybridization” means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases. For example, adenine and thymine are complementary nucleobases which pair through the formation of hydrogen bonds. “Complementary,” as used herein, refers to the capacity for precise pairing between two nucleotides. For example, if a nucleotide at a certain position of an oligonucleotide is capable of hydrogen bonding with a nucleotide at the same position of a DNA or RNA molecule, then the oligonucleotide and the DNA or RNA are considered to be complementary to each other at that position. The oligonucleotide and the DNA or RNA are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides which can hydrogen bond with each other. Thus, “specifically hybridizable” and “complementary” are terms which are used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the oligonucleotide and the DNA or RNA target. It is understood in the art that the sequence of an antisense compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. An antisense compound is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are performed.

[0033] Antisense and other compounds of the invention which hybridize to the target and inhibit expression of the target are identified through experimentation, and the sequences of these compounds are hereinbelow identified as preferred embodiments of the invention. The target sites to which these preferred sequences are complementary are hereinbelow referred to as “active sites” and are therefore preferred sites for targeting. Therefore another embodiment of the invention encompasses compounds, including primers, probe siRNAs, other double stranded RNAs including RNAi or gene silencing agents, ribozymes, external guide sequence (EGS) oligonucleotides (oligozymes), and other short catalytic RNAs or catalytic oligonucleotides which hybridize to these active sites.

[0034] Antisense compounds are commonly used as research reagents and diagnostics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes. Antisense compounds are also used, for example, to distinguish between functions of various members of a biological pathway. Antisense modulation has, therefore, been harnessed for research use.

[0035] The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotide drugs, including ribozymes, have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans.

[0036] In the context of this invention, the term “poly-nucleotide”, which includes oligonucleotides, refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nuclease.

[0037] In general, nucleic acids or polynucleotides (including oligonucleotides) may be described as “DNA-like” (i.e., having 2-deoxy sugars and, generally, T rather than U bases) or “RNA-like” (i.e., having 2'-hydroxyl or 2'-modified sugars and, generally U rather than T bases). Nucleic acid helices can adopt more than one type of structure, most commonly the A- and B-forms. It is believed that, in general, oligonucleotides which have B-form-like structure are “DNA-like” and those which have A-form-like structure are “RNA-like”.

[0038] While antisense oligonucleotides are a preferred form of antisense compound, the present invention comprehends other oligomeric antisense compounds, including but not limited to oligonucleotide mimetics such as are described below. The antisense compounds in accordance with this invention preferably comprise from about 8 to about 50 nucleobases (i.e. from about 8 to about 50 linked nucleosides). Particularly preferred antisense compounds are antisense oligonucleotides, even more preferably those comprising from about 12 to about 30 nucleobases. Antisense compounds include ribozymes, external guide sequence (EGS) oligonucleotides (oligozymes), and other short catalytic RNAs or catalytic oligonucleotides which hybridize to the target nucleic acid and modulate its expression.

[0039] As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines.
Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn, the respective ends of this linear polymeric structure can be further joined to form a circular structure, however, open linear structures are generally preferred. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

**[0040]** Specific examples of preferred antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

**[0041]** Preferred modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphoriesters, amioalkylphosphoristriesters, methyl and other alkyl phosphates including 3'-alkylen phosphates, 5'-alkylene phosphates and chiral phosphates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and amioalkylphosphoramidates, thionophosphoramidates, thioalkylphosphonates, thionoalkylphosphoristriesters, selenophosphates and borophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage. Preferred oligonucleotides having inverted polarity comprise a single 3' to 3' linkage at the 3'-most internucleotide linkage i.e., a single inverted nucleoside residue which may be abasic (the nucleobase is missing or has a hydroxyl group in place thereof). Various salts, mixed salts and free acid forms are also included.

**[0042]** Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S.: U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,266,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,194,599; 5,565,555; 5,527,899; 5,721,218; 5,672,697 and 5,625,050, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

**[0043]** Preferred modified oligonucleotide backbones that do not include a phosphorus atom in their backbone that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfoxine backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; riboacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneiminio and methylenehydradizino backbones; sulfonyl and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

**[0044]** Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S.: U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; 5,792,608; 5,646,269 and 5,677,439, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

**[0045]** In other preferred oligonucleic mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligonucleic compound, an oligonucleotide mimic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to az氮 nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S.: U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., Science, 1991, 254, 1497-1500.

**[0046]** Most preferred embodiments of the invention are oligonucleotides with phosphorothioate backbones and oligonucleotides with heteroatom backbones, and in particular —CH₆—NH—O—CH₂—, —CH₆—N(CH₃)₂—O—CH₂— [known as a methylene (methylimino) or MMI backbone], —CH₂—O—N(CH₃)₂—CH₂—, —CH₂—N(CH₃)₂—N(CH₃)₂—CH₂— and —O—N(CH₃)₂—CH₂—CH₂— [wherein the native phosphodiester backbone is represented as —O—P—O—CH₂—] of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

**[0047]** Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S-, or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C₂ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Particularly preferred are O(CH₃)₂O, O(CH₂)₄O, O(CH₂)₄N(CH₃)₂, O(CH₂)₄CH₂, O(CH₂)₄OCH₂, O(CH₂)₄NH₂, O(CH₂)₄CH₂, O(CH₂)₄ON(CH₂)₂CH₂, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C₂ to
C₁₀ lower alkyl, substituted lower alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₂, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocyclovarkyl, aminokallymamo, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2-methoxyethoxy (2-O—CH₂CH₂O CH₃, also known as 2-0-(2-methoxyethoxyl) or 2-MOE) (Martin et al., *Hepid. Clin. Acta*, 1995, 78, 486-504) i.e., an alkoxalkoxy group. A further preferred modification includes 2'-dimethoxyminoethoxy, i.e., a O(CH₂)₂ON(CH₂)₂ group, also known as 2'-DMAEO, as described in examples hereinbelow, and 2'-0-dimethoxyminoethoxy (also known in the art as 2'-0-dimethoxyminoethoxy or 2'-DMAEO), i.e., 2'-O—CH₂—O—CH₂—N(CH₂)₂, also described in examples hereinbelow.

[0048] A further preferred modification includes Locked Nucleic Acids (LNAs) in which the 2'-hydroxyl group is linked to the 3' or 4' carbon atom of the sugar ring thereby forming a bicyclic sugar moiety. The linkage is preferably a methylene (—CH₂—), group bridging the 2'-oxygen atom and the 3' or 4' carbon atom wherein n is 1 or 2. LNAs and preparation thereof are described in WO 98/39352 and WO 99/14226.

[0049] Other preferred modifications include 2'-methoxy (2-O—CH₃), 2'-aminoproxoy (2'-OCH₂CH₂CH₂NH₂), 2'-allyl (2'-CH══CH═CH₂), 2'-O-allyl (2'-O—CH═CH₂), and 2'-fluoro (2-F). The 2'-modification may be in the arabino (up) position or ribo (down) position. A further preferred modification is 2'F. Smaller modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Represented United States patents that teach the preparation of such modified sugar structures are present, but are not limited to, U.S.: U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,792,747; and 5,700,920, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

[0050] Oligonucleotides may also include nucleobase (often referred to in the art simply as “base”) modifications or substitutions. As used herein, “unmodified” or “natural” nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoo adenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiobutyrimide and 2-thiouracil, 5-halouracil and cytosine, 5-propynyl (—C═C—CH₃) uracil and cytosine and other alkyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thio- alkyl, 8-hydroxy and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-aza- guanine and 8-azaadenine, 7-deazaquinine and 7-deaza- adenine and 3-deazaquinine and 3-deazaadenine. Further modified nucleobases include tricyclic pyrimidines such as phenoxazine cytidine (1H-pyrindol[5,4-b][1,4]benzoazepin-2(3H)-one), phenothiazine cytidine (1H-pyrindol[5,4-b][1,4]benzoazepin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (e.g. 9-(2-aminoethoxy)-H-pyrimido[5,4-b][1,4]benzoazepin-2(3H)-one), carbazole cytosine (2H-pyrido[3,2,4-j]pyrido[2,3-d]pyrimidin-2-one). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaquinine, 2-aminopyridine and 2-pyridine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in *The Concise Encyclopedia of Polymer Science And Engineering*, pages 885-899, Kroschwitz, J. I., ed. John Wiley & Sons, 1990, those disclosed by Ennschel et al., *Angewandte Chemie*, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y. S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Crooke, S. T. and Lebleu, B., eds., *Antisense Research and Applications*, CRC Press, Boca Raton, 1993, pp. 276-278 and are presently referenced hereinbelow, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

[0051] Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S.: U.S. Pat. Nos. 4,845,205; 5,130,302; 5,134, 066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459, 255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587, 469; 5,594,121; 5,596,001; 5,614,617; 5,645,985; 5,830, 653; 5,763,588; 6,005,096, and 5,658,541, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference, and U.S. Pat. No. 5,750,692, which is commonly owned with the instant application and also herein incorporated by reference.

[0052] Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. The compounds of the invention can include conjugate groups covalently bound to functional groups such as primary or secondary hydroxyl groups. Conjugate groups of the invention include intercalators, reporter molecules, polymers, polyamides, polyethylene glycols, polyethers, groups that enhance the pharmacodynamic properties of oligomers, and groups that enhance the pharmacokinetic properties of oligomers. Typical conjugates groups include...

[0054] It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide. The present invention preferably includes antisense compounds which are chimeric compounds. “Chimeric” antisense compounds or “chimeras,” in the context of this invention, are antisense compounds, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids.

[0055] By way of example, RNase H cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Oligonucleotides, particularly chimeric oligonucleotides, designed to elicit target cleavage by RNase H, thus are generally more potent than oligonucleotides of the same base sequence which are not so optimized. Cleavage of the RNA target can be routinely detected by, for example, gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

[0056] Chimeric oligonucleotides may have one or more modifications of the internucleoside (backbone) linkage, the sugar or the base. In a preferred embodiment, the oligonucleotide is a chimeric oligonucleotide having a modification at the 2'-position of at least one sugar moiety. Presently believed to be particularly preferred are chimeric oligonucleotides which have approximately four or more deoxy nucleotides in a row, which provide an RNase H cleavage site, flanked on one or both sides by a region of 2'-modified oligonucleotides.

[0057] Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S.: U.S. Pat. Nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

[0058] The antisense compounds used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City,
Antisense inhibition of human RNase III expression was used to further evaluate the role(s) of RNase III. To identify optimal sites in RNase III mRNA for antisense effects, 2′-O-methoxymethyl chimeric antisense oligonucleotides targeted to 10 sites in the MRNA were designed and screened for inhibition of RNase III. These are shown in Table 1. These chimeric or “gapped” oligonucleotides are designed to serve as substrates for RNase H when bound to RNA resulting in degradation of the target RNA and oligonucleotides of this type have been shown to be highly specific when used under the described conditions.

<table>
<thead>
<tr>
<th>ISIS #</th>
<th>Sequence (5′→→3′)</th>
<th>Target sites % Inhib’n SEQ ID NO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>25690</td>
<td>ATCCCTTCCCTCCGCAATGTG</td>
<td>3051-3070 79 8</td>
</tr>
<tr>
<td>25691</td>
<td>GCCAGGCTGCAAGGATAT</td>
<td>3085-4004 96 9</td>
</tr>
<tr>
<td>25692</td>
<td>CGGACTCTAAAGAGGAGACG</td>
<td>3442-3461 78 10</td>
</tr>
<tr>
<td>25693</td>
<td>TATCAACCAAGAGGTTAGCG</td>
<td>3776-3795 49 11</td>
</tr>
<tr>
<td>25694</td>
<td>CAATTGAGGAAGGAGAGCA</td>
<td>3973-3992 50 12</td>
</tr>
<tr>
<td>25695</td>
<td>GTCCCACTTGGGCTGTTG</td>
<td>4197-4216 81 13</td>
</tr>
<tr>
<td>25696</td>
<td>ATGCTCCCTTCCTCCACCTCA</td>
<td>4308-4327 70 14</td>
</tr>
<tr>
<td>25697</td>
<td>AATACCTCCACACCTGTACG</td>
<td>4378-4397 79 15</td>
</tr>
<tr>
<td>25698</td>
<td>TGCCATTTGGCAAGATGCA</td>
<td>4420-4439 44 16</td>
</tr>
<tr>
<td>25699</td>
<td>AGCTAGGCTTACAAATCGTG</td>
<td>4688-4707 31 17</td>
</tr>
<tr>
<td>27110</td>
<td>TCCAGTGTATGGTCCGACG</td>
<td>3-mismatch of 25691</td>
</tr>
</tbody>
</table>

All oligonucleotides in Table 1 have phosphorothioate (P=S or PS) backbones and 2′-methoxymethoxy (2′MOE) “wings” flanking a 2′deoxy gap. 2′MOE nucleotides are shown in bold. All cytosines are 5-methyl cytosines (5mC). Target site refers to nucleotide numbers on the cloned RNase III cDNA (SEQ ID NO: 1) to which the oligonucleotide binds. Oligonucleotide concentration was 200 nM. Table 1 shows that ISIS 25690, 25691, 25692, 25693, 25694, 25695, 25696 and 25697 (SEQ ID NO: 8, 9, 10, 11, 12, 13, 14 and 15) inhibited human RNase III expression by about 50% or more. These compounds are therefore preferred. The most effective agent was ISIS 25691 (SEQ ID NO: 9), targeted to nucleotides 3085-4004 in the coding region of the mRNA. This compound was selected for further studies.

Increasing concentrations of ISIS 25691 caused increasing loss of RNase III mRNA, with 300 nM resulting in loss of more than 90% of the RNase III mRNA. The mismatch control oligonucleotide, ISIS 27110 (SEQ ID NO: 18), at 300 nM had no effect on the RNase III mRNA level. ISIS 25691 at 300 nM suppressed RNase III mRNA levels in HeLa cells from 2 to 72 hours after a single treatment. After treatment with ISIS 25691 at 100, 150 or 200 nM for 24 hours, RNase III protein was reduced to 67%, 44% or 19% of control respectively. The level of RNase II protein was slightly reduced at 5 hours after treatment and reached a maximum reduction of about 70% at 18 hours. Immunofluorescence staining showed that after treatment with ISIS 25691 (150 nM, 24 hours), RNase III was dramatically reduced or absent in the nucleus and nucleoli. After treatment of HeLa cells with ISIS 25691 at 300 nM for 18 hours, the morphology of HeLa cells changed from fusiform to oval. After 24 hours of treatment, approximately 5-10% of the cells detached from the plate and could be stained with trypan blue indicating cell death. The cells that remained attached to the solid substrate grew much more slowly than untreated cells and appeared unable to enter mitosis (data not shown). After 48 hours, 40-50% of the cells treated with 300 nM ISIS 25691 were dead. These results were highly reproducible and indicate that RNase III is required for HeLa cell survival. The control oligonucleotide had no effect at any time or at any concentration on cell morphology, RNase III mRNA or protein levels demonstrating the antisense effect was highly specific.

One function that has been attributed to RNase III in lower species is pre-ribosomal RNA (pre-rRNA) processing. Human pre-rRNA processing is thought to involve cleavage of 45S pre-rRNA into 30S and 32S fragments. The 32S RNA product of the cleavage of 45S pre-rRNA contains 5.8S rRNA, 5.8S rRNA and 28S rRNA. Cleavage of the 32S RNA results in 12S pre-rRNA and 28S rRNA products. The 12S pre-rRNA is further cleaved to 5.8S rRNA. Because ribosomes are made in the nucleolus, and the human RNase III protein appeared to be translocated to and from the nucleolus during the cell cycle, its potential role(s) in human pre-rRNA processing was evaluated. Two hybridization probes for human pre-rRNA were synthesized, 5′ETS-1 (5′-GAA GGC AGC CCT CTC AGA TCG CTA GAG AAG GCT TTT CTC A-3; SEQ ID NO: 19), designed to bind to the 5′ external transcribed spacer (5ETS) of human pre-rRNA and 5.8S-1 (5′-CAT TAA TTC TCG CAY CAG CAC GCT GCT CTC TCT TCA TCG ACG C-3; SEQ ID NO: 20),
designed to bind to 5.8S rRNA. When total cellular RNA (15 μg) from untreated HeLa cells was fractionated by agarose gel electrophoresis, transferred to a nylon membrane and probed with 32P-5'ETS-1, a band corresponding to 45S pre-rRNA and a very faint band corresponding in mobility to 30S (5'ETS-18S-ITS1) pre-rRNA were observed. When 32P-5.8S-1 was used, bands corresponding to 45S, 32S (5.8S-ITS2-28S) and 12S (5.8S-ITS2) pre-rRNA and 5.8S rRNA were observed. At concentrations at which the antise... the RNase III level, no effect on the 45S pre-rRNA level was observed. In contrast, the 5.8S-1 probe demonstrated that antisense inhibition of RNase III increased the levels of 32S and 12S pre-rRNAs.

[0063] To provide further confirmation that human RNase III is involved in preribosomal RNA processing, the effects of ten antisense oligonucleotides on RNase III mRNA levels were compared to the effects of these oligonucleotides on accumulation of the two pre-rRNA species (32S and 12S) that accumulated after treatment with the most potent of the antisense inhibitors, ISIS 25691. The potency of antisense inhibitors designed to bind to different sites in RNase III mRNA varied. The correlation between the reduction of RNase III RNA levels and the accumulation of both 32S and 12S pre-rRNAs was excellent, thus confirming the conclusion derived from the Northern blot analysis.

[0064] Antisense inhibition of RNase III resulted in substantial accumulation of 12S pre-rRNA, less pronounced accumulation of 32S pre-rRNA and no accumulation of 45S pre-rRNA. Thus this human RNase III appears to be required for the processing of 12S pre-rRNA. It may also be involved in the processing of 32S pre-rRNA. The principal site of cleavage induced by human RNase III described here is in the 5.8S-ITS2 region of pre-rRNA.

[0065] RNase III enzymes are double-strand RNA (dsRNA) endoribonucleases. To test whether the human RNase III domain can specifically cleave dsRNA, the RNase III domain-coding region was subcloned into a glutathione S-transferase (GST) expression vector. The GST-RNase III fusion protein and GST alone were expressed, purified using glutathione agarose and analyzed by coomassie blue staining of the SDS-PAGE and Western blot analysis with an antibody that reacts with human RNase III peptide antibody. These studies showed that human RNase III was expressed in E. coli. Ribonucleases V, dsRNases, and T, and A (srRNases) were used as controls to confirm that the cleavage observed was dsRNA cleavage.

[0066] RNase III is a double-strand RNA endonuclease, specifically cleaving double-helical structures in cellular and viral RNAs. It is believed that this cleavage can be exploited to promote cleavage of a cellular RNA target, by providing “RNA-like” antisense oligonucleotides which hybridize to the cellular RNA target to form an RNA duplex, thus eliciting RNase III cleavage. Methods of promoting inhibition of expression by antisense oligonucleotides, and methods for screening oligonucleotides are thus provided. In the context of this invention, “promoting antisense inhibition” or “promoting inhibition of expression” of a selected RNA target, or of its protein product, means inhibiting expression of the target or enhancing the inhibition of expression of the target. In some embodiments of these methods, the RNase III is present in an enriched amount. In the context of this invention, “enriched” means an amount greater than would naturally be found. RNase III may be present in an enriched amount through, for example, addition of exogenous RNase III, through selection of cells which overexpress RNase III or through manipulation of cells to cause overexpression of RNase III. The exogenously added RNase III may be added in the form of, for example, a cellular or tissue extract, a biochemically purified or partially purified preparation of RNase III, or a cloned and expressed RNase III polypeptide.

[0067] The expression of large quantities of a cloned human RNase III of the present invention has been shown to be useful in characterizing the activities of this enzyme. In addition, the polynucleotides and polypeptides of the present invention provide a means for identifying agents, such as the antisense compounds described herein, which modulate the function of this enzyme in human cells and tissues. For example, a host cell can be genetically engineered to incorporate polynucleotides and express polypeptides of the present invention. Polynucleotides can be introduced into a host cell using any number of well known techniques such as infection, transduction, transfection or transformation. The polynucleotide can be introduced alone or in conjunction with a second polynucleotide encoding a selectable marker. In a preferred embodiment, the host comprises a mammalian cell. Such host cells can then be used not only for production of human RNase III, but also to identify agents which increase or decrease levels of expression or activity of human RNase III in the cell. In these assays, the host cell would be exposed to an agent suspected of altering levels of expression or activity of human RNase III in the cells. The level or activity of human RNase III in the cell would then be determined in the presence and absence of the agent. Assays to determine levels of protein in a cell are well known to those of skill in the art and include, but are not limited to, radioimmunoassays, competitive binding assays, Western blot analysis and enzyme linked immunosorbent assays (ELISAs). Methods of determining increased activity of the enzyme, and in particular increased cleavage of dsRNA substrate can be performed in accordance with the teachings of the examples of the present application. Agents identified as modulators of the level or activity of this enzyme may be useful.

[0068] Antisense modulators of human RNase III are provided herein and may be used diagnostically, therapeutically and for research purposes.

[0069] The following nonlimiting examples are provided to further illustrate the present invention.

EXAMPLES

Example 1

[0070] cDNA Cloning

[0071] An internet search of the XREF database in the National Center of Biotechnology Information (NCBI) yielded a 393 base pair (bp) human expressed sequenced tag (EST, GenBank accession AA083868), homologous to the yeast RNase III (RNT1) gene (GenBank accession #AB041722; SEQ ID NO: 5) and the C. elegans RNase III gene (GenBank accession 001326; SEQ ID NO: 3). Three
sets of oligonucleotide primers encoding the human RNase H EST sequence were synthesized. Sequence-specific primer sets listed in Table 2 were designed based on the human expressed tag sequence or early cloned cDNA fragments. These are shown in Table 2. These primers were used in polymerase chain reaction for 3'- and 5'-RACE and/or for detection on Southern blots.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Position in full length</th>
<th>Primer</th>
<th>cDNA Source</th>
<th>Sequence</th>
<th>SEQ ID</th>
<th>HE</th>
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<tr>
<td>NIII-2 EST AAT 830888</td>
<td>3516-3550 CCAATATCTGACTGACAATTTAGTGAACCTTCCC</td>
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<tr>
<td>NIII-4 EST AAT 830888</td>
<td>3569-3606 GAAATGAGAAAGGATGGGAATTTTCTACTAG</td>
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<tr>
<td>NIII-6 EST AAT 830888</td>
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<tr>
<td>3RACE1 Clone #3-4</td>
<td>2708-2863 CAGCTGAGCAGCTTTTTCAG</td>
<td>24</td>
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<tr>
<td>3RACE2 Clone #3-4</td>
<td>2868-2943 TCTGAGTGTGAGTGTATGCA</td>
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<tr>
<td>3RACE1 Clone #3-4</td>
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<tr>
<td>RACE4 Clone #L40</td>
<td>1923-1894 GCTGAGATGGCCCTCGTCGAGAATAGCC</td>
<td>27</td>
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<tr>
<td>RACE5 Clone #L40</td>
<td>1898-1869 AATGCTTGCGATTCTCTGCTTGAGC</td>
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<tr>
<td>RACE Det Clone #L40</td>
<td>1723-1676 CAGCTGAGCAGCTTTTTCAG</td>
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**Example 2**

- **Screening of the cDNA Libraries, DNA Sequencing and Sequence Analysis**
- **A human liver cDNA lambda phage Uni-ZAP library (Stratagene, La Jolla, Calif.) was screened using the RACE products as specific probes. Several positive clones** were isolated. The two longest clones, 3-1 and 3-4, correspond to the COOH-terminal region, nucleotides 2636-3912 and 3350-4764, respectively, of the full-length cDNA. With primers (3RACE1, 3RACE2 and 3RACE3) based on the NH2-terminal portion of the clone 3-4, 5' RACE was performed to clone a cDNA (clone L40) of approximately 1 kb, which encodes the middle part (nucleotides 1661-2088) of the full-length cDNA. In the same way, a cDNA (clone 25) of the NH2-terminal portion (nucleotides 645-1898) was cloned. Using clone 25 to screen the liver library again, several clones were isolated, but none included additional NH2-terminal sequence. The most NH2-terminal clone (328) corresponded to nucleotides 799-2191. The last 5' RACE was performed with primers 3G, 3S and 33D, based on clone 25, and the NH2-terminal portion of the cDNA (clone 81, corresponding to nucleotides 1-802) was generated.

**Example 5**

- The positive cDNA clones were excised into pBluescript phagemid from lambda phage and subjected to DNA sequencing. Sequencing of the positive clones was performed with an automatic DNA sequencer by Retrogen Inc. (San Diego, Calif.). The overlapping sequences were aligned and combined by the assembling program of MacDNASISv3.0 (Hitachi Software Engineering Co., America, Ltd.) to give the full length (4764 nucleotides) polynucleotide sequence. (SEQ ID NO: 1). Protein structure and analysis were performed by the program MacVector v6.0 (Oxford Molecular Group, UK). A homology search was performed on the NCBI database.
Example 3

[0076] Antisense Treatment

[0077] HeLa cells were transfected with oligonucleotide mixed with Lipofectin ( Gibco BRL, Gaithersburg, Md.) at a concentration of 37.5-300 μM for 5 hours in Opti-MEM ( Gibco BRL). After removal of the medium containing oligonucleotide, cells were cultured in DMEM for times indicated and harvested for analysis. Inhibition by antisense oligonucleotides is expressed compared to control (without oligonucleotide treatment).

Example 4

[0078] Northern Hybridization

[0079] Total RNA was isolated from HeLa cells consisting of guanidine isothiocyanate method (R. E. Kingston, in Current protocols in molecular biology, F. M. Ausubel, et al., Eds., John Wiley & Sons Inc., New York, 1997, vol. 1, pp. 4.2.3-4.2.5.). Fifteen μg of total RNA was separated on a 1% agarose/formaldehyde gel and transferred to Hybond-N+ (Amersham, Arlington Heights, Ill.) followed by washing using UV crosslinker (Stratagene, La Jolla, Calif.). To detect RNase III mRNA, hybridization was performed by using 32P-labeled human RNase III cDNA in Quik-Hyb buffer (Stratagene, La Jolla, Calif.) at 68°C for 2 hours. After hybridization, membranes were washed in a final stringency of 0.1x SSC/0.1% SDS at 60°C for 30 minutes. Membranes were analyzed using a PhosphorImager Storm 860 (Molecular Dynamics, Sunnyvale, Calif.). The level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used to normalize the amount of total RNA loaded.

[0080] For Northern hybridization of pre-rRNAs, HeLa cells were treated with ISIS 25691 and ISIS 27110 for 24 hours using 32P-end labeled ribose probes 5′-CAT TGA CTC AGA TCG ATG CAA ACG GCT TTT CTC A-3′; SEQ ID NO: 33), corresponding to 5ETS and 5.8S-(15′-CAT TAA TTC TCG CAG CTA GGC TGA CGT CCT TCA TCG ACC G-3′; SEQ ID NO: 34), corresponding to 5.8S RNA. Hybridizations were performed at 40°C for 2 hours and washed in 2× SSC/0.1% SDS at 40°C for 1 hour. All others were as described above. Data were mean ± SD of triplicate determination of representative experiment.

Example 5

[0081] Western Blot Analysis of Human RNase III

[0082] Nuclear and non-nuclear fractions from HeLa cells were prepared as described (Dignam et al., Nucleic Acids Res 1983, 11, 1475-89. Whole cell, non-nuclear and nuclear fractions were boiled in SDS-sample buffer. The samples were separated by SDS-PAGE using 4-20% Tris-glycine gels (NOVEX, San Diego, Calif.) under reducing conditions. Molecular weight prestained markers were used (NOVEX) to determine the protein sizes. The proteins were electrophoretically transferred to a PVDF-membrane and processed for immunoblotting using affinity purified anti-SR peptide antibody at 5µg/ml. The immunoreactive bands were visualized using the enhanced chemiluminescence method (Amersham, Arlington Heights, Ill.) and analyzed using a PhosphorImager Storm 860 (Molecular Dynamics, Sunnyvale, Calif.).

Example 6

[0083] Antibody Production

[0084] Antibodies were prepared to peptides synthesized having amino acid sequences contained within the SR domain and the III domain of human RNase III. The SR domain peptide (H-CRSYDGRGRPSHRSYERS-OH), amino acids 226 to 284; SEQ ID NO: 35) and the III region peptide (H-CRWERHIQEPEDTEDIKK-OH), amino acids 1356 to 1374; SEQ ID NO: 36) were synthesized, coupled to diphtheria toxoid through maleimidocaproyl-N-hydroxysuccinimide (MCS), mixed with Freund’s adjuvant (complete for first immunization, incomplete for remaining immunizations) and injected intramuscularly into New Zealand White rabbits. Serum was collected after the second immunization. Antibody titer was measured by ELISA. Anti-SR and anti-III peptide IgGs were affinity purified with SR and III peptides coupled to thiopropyl-Sepharose 6B, respectively.

Example 7

[0085] Indirect Immunofluorescence Staining of Human RNase III

[0086] HeLa cells were cultured in chamber slides for immunostaining. Cells were washed once with Dulbecco’s Phosphate Buffered Saline (D-PBS, pH 7.0), and then fixed in 10% neutral-buffered formalin for 10 minutes following washing three times with D-PBS. Fixed cells were then blocked for 30 minutes with 20% fetal bovine serum plus 0.5% Tween 20. Cells were then stained with anti-SR antibody (10 µg/ml) for 1 hour at 37°C, washed three times with D-PBS plus 0.1% NP-40, and incubated for 1 hour at 37°C with the FITC goat anti-rabbit IgG (Jackson ImmunoResearch Laboratory, Inc. West Grove, Pa.). The cells were washed with D-PBS three times and mounted in mounting medium (Vector, Burlingame, Calif.) for examination under a fluorescence microscope. NR IgG: normal rabbit IgG was used as control.

Example 8

[0087] Indirect Immunofluorescence Staining of Human RNase III in HeLa Cells in Different Phases of the Cell Cycle.

[0088] HeLa cells were synchronized at early-S phase using the double thymidine method (Johnson et al., in The Cell Cycle: A Practical Approach P. Fantus, R. Brooks, Eds., IRL Press, 1993, pp. 1-24). Briefly, cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM, 10% fetal calf serum) containing 2 mM of thymidine for 17 hours. After washing twice with D-PBS, cells were cultured in DMEM for 9 hours followed by second thymidine treatment for 15 hours. Synchronized cells were then washed twice with D-PBS, cultured and harvested at 0, 2, 4, 6, 8 and 24 hours for immunofluorescence staining and FACS analysis.

[0089] HeLa cells were detached from culture flasks with trypsin-EDTA and washed once with D-PBS containing 5 mM of EDTA. Cells were then fixed in 70% ethanol for 1 to 24 hours at 4°C followed by propidium iodide (PI, 50 µg/ml) staining for 1 hour at room temperature. Cell counts (Y axis) and PI content (X axis) were determined by FACS analysis (Becton Dickinson Co., San Jose, Calif.).
Example 9

[0090] Expression of GST-RNase III Domain Fusion Protein

[0091] A cDNA fragment encoding the human RNase III-like domain (C-terminal-most 466 amino acids) was amplified by PCR and introduced into a BamHI I site upstream and Not I site downstream. This fragment was further subcloned into the sites of the expression vector pGEX-4T-1 (Pharmac Biotech, Piscataway, N.J.) to produce the RNase III fusion protein with Glutathione S-transferase (GST) at its N-terminus. The identity of the construct was proven by DNA sequencing. The GST-RNase III fusion protein was expressed in E. coli strain BL21 and purified using glutathione agarose (Pharmac Biotech, Piscataway, N.J.) under native conditions with B-PER bacterial protein extraction reagent (Pierce, Rockford, III). Control GST protein was also prepared in parallel from the pGEX-4T-1 plasmid. The purified products were identified by Coomassie staining after 12% SDS-polyacrylamide gel electrophoresis and Western blot analyses with anti-RNase III peptide antibody (see examples above).

Example 10

[0092] In Vitro Cleavage of dsRNA

[0093] The dsRNA substrate was generated by hybridization of two complementary strands of RNA produced with T7 and T3 polymerase transcription of the polylinker region of the pBluescript KS(−) plasmid (Stratagene, San Diego, Calif.). The plasmid was digested with either Sst I or Kpn I and further purified with phenol/chloroform extraction and ethanol precipitation. The Sst I or Kpn I-digested plasmids were then transcribed using T7 or T3 RNA polymerase respectively (Stratagene, San Diego, Calif.) with or without 32P-AUTP. The resulting transcribed RNAs (about 100 nt) were purified by electrophoresis on 6% denaturing polyacrylamide gel. The 32P radiolabeled T7 transcript and unlabeled T3 transcript fragments were mixed and heated for 5 min at 90°C in a buffer containing 20 mM KCl, 50 mM Tris-HCl (pH 7.5), 0.1 mM EDTA. MgCl2, BSA and RNase inhibitor were added to the mixture after heating (final concentrations were 10 mM, 100 ng/ml and 10 unit/ml respectively). The mixture was incubated at 37°C for 2 hr and the duplex RNA was purified on 6% non-denaturing gels. The 32P-labeled T7 transcript was also used as the ssRNA control substrate. To evaluate cleavage, 0.4 μg of GST protein or GST-RNase III (approximately 5-10 pmole of purified GST-RNase III) fusion protein was incubated with labeled dsRNA (250,000 cpm) (approximately 5-10 fmole) and ssRNA (250,000 cpm) at 37°C in a buffer containing 20 mM KCl, 50 mM Tris-HCl (pH 7.5), 5 mM MgCl2, 50 mM NaCl, 0.1 mM DTT, 0.1 mg/ml yeast RNA and 10 unit/ml RNase inhibitor in the total volume of 60 μl. The digested samples were quenched at specific times and analyzed using non-denaturing polyacrylamide gel electrophoresis and PhosphorImager analysis.

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Cys Lys Gly Met Ile Val Thr Asn Pro Gly Thr Lys Pro Ser Ser Val 740 745 750
Arg Ile Asp Glu Leu Asp Arg Glu Glu Asp Ala Arg Ala Glu Ser Thr 755 760 765
Phe Pro Ile Ile Val His Phe Gly Ile Arg Pro Ala Glu Leu Ser Tyr 770 775 780
Ala Gly Asp Pro Glu Tyr Glu Leu Trp Lys Ser Tyr Val Lys Leu 785 790 795 800
Arg His Leu Leu Ala Asn Ser Pro Lys Val Lys Glu Thr Asp Lys Glu 805 810 815
Lys Leu Ala Glu Arg Glu Ala Glu Ala Lys Ile Arg Lys Asn 820 825 830
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Gly Leu Ala Thr Tyr Arg Thr Ala Leu Glu Asn Arg Asn Leu Ala
50  55  60
Thr Leu Ala Lys Asn Cys Arg Ile Asp Glu Met Leu Glu Tyr Ser His
65  70  75  80
Gly Ala Asp Leu Ile Asn Val Ala Glu Phe Lys His Ala Leu Ala Asn
85  90
95
Ala Phe Glu Ala Val Met Ala Ile Tyr Leu Asp Gly Glu Leu Ala
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Pro Cys Asp Val Ile Phe Ser Lys Ala Met Tyr Gly His Gin Pro Val
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Leu Glu Leu Ile Val Ser Asp Phe Leu Tyr Arg Arg Phe Pro Tyr His
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Gln Val Phe Met His Ile Ser Arg Ala Tyr Glu Ile Tyr Pro Asn Glu
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165 170 175
Leu Gly Asp Ser Phe Phe Asn Leu Phe Thr Thr Arg Ile Ile Phe Ser 180 185 190
Lys Phe Pro Gln Met Asp G1y Ser Leu Ser Lys Leu Arg Ala Lys 195 200 205
Phe Val Gly Asn Glu Ser Ala Asp Lys Phe Ala Arg Leu Tyr Gly Phe 210 215 220
Asp Lys Thr Leu Val Leu Ser Tyr Ser Ala Glu Lys Asp Gln Leu Arg 225 230 235 240
Lys Ser Gln Lys Val Ile Ala Asp Thr Phe Glu Ala Tyr Leu Gly Ala 245 250 255
Leu Ile Leu Asp Gly Gln Glu Thr Ala Phe Gln Trp Val Ser Arg 260 265 270
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Lys Leu Ala Lys Ser Lys Leu Phe His Lys Tyr Ser Thr Leu Gly His 290 295 300
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Glu Gly Tyr Val Ile Ala Cys Ile Phe Asn Gly Lys Val Ala Arg 325 330 335
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Val Ile Gln Leu Glu His Ala Val Thr Lys Leu Val Gln Ser Tyr Asn 50 55 60
Lys Ile Ile Glu Leu Ser Pro Asn Leu Val Ala Tyr Asn Glu Ala Val 65 70 75 80
Asn Asn Gln Asp Arg Val Pro Gln Ile Leu Pro Ser Leu Ser Arg 85 90 95
Tyr Gln Leu Lys Leu Ala Ala Glu Leu Lys Thr Leu His Asp Leu Lys 100 105 110
Lys Asp Ala Ile Leu Thr Glu Ile Thr Asp Tyr Glu Asn Phe Asp 115 120 125
Thr Glu Gln Gly Gln Pro Ile Leu Glu Ile Ser Lys Ala Asp Met 130 135 140
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What is claimed is:

1. A method for eliciting modification of a selected RNA target in a cell comprising:
   (a) providing an RNA-like polynucleotide hybridizable with said RNA target;
   (b) hybridizing the RNA-like polynucleotide and the RNA to form a polynucleotide-target duplex; and
   (c) contacting the duplex with a polypeptide comprising an RNase III domain, under conditions selected to effect modification of the duplex by the polypeptide, and modification of the RNA target thereby.

2. The method of claim 1 wherein said modification of the RNA target occurs in the cell's nucleus.

3. The method of claim 1 wherein the polypeptide comprising an RNase III domain is an RNase III polypeptide.

4. The method of claim 1 wherein the RNase III polypeptide is a human RNase III polypeptide.

5. The method of claim 1 wherein modification of the selected RNA target is cleavage of the RNA target.

6. The method of claim 1 wherein the polypeptide comprising an RNase III domain is present in enriched amounts.

7. The method of claim 6 wherein the polypeptide comprising an RNase III domain present in enriched amounts is overexpressed or exogenously added.

8. The method of claim 1 wherein the polypeptide comprising an RNase III domain is a purified RNase III polypeptide.

9. The method of claim 1 wherein the RNA-like polynucleotide has a modification at the 2' position of at least one sugar.

10. The method of claim 1 wherein step (c) is performed within a cell.

11. The method of claim 1 wherein step (b) is performed within a cell.

12. The method of claim 1 wherein step (b) is performed outside a cell.

13. The method of claim 1 wherein at least one furanosyl moiety of the RNA-like polynucleotide is a ribofuranosyl moiety.

14. The method of claim 13 wherein a majority of the furanosyl moieties of the RNA-like polynucleotide are ribofuranosyl moieties.

15. A method for promoting gene silencing in a cell comprising providing to the cell, in an amount effective to promote said gene silencing, a polypeptide comprising an RNase III domain.

16. The method of claim 15 wherein said promotion of gene silencing occurs in the cell's nucleus.

17. The method of claim 15 wherein the polypeptide comprising an RNase III domain is an RNase III polypeptide.

18. The method of claim 15 wherein the RNase III polypeptide is a human RNase III polypeptide.

19. The method of claim 15 wherein the RNase III polypeptide is exogenously added.

20. The method of claim 15 wherein the RNase III polypeptide is provided through upregulation of endogenous production of the polypeptide.

21. The method of claim 15 wherein said RNase III polypeptide is a purified RNase III polypeptide.

22. The method of claim 15 wherein said RNase III polypeptide is expressed by an exogenously added vector encoding said RNase III polypeptide.

23. The method of claim 15 wherein said cell is a mammalian cell.

24. The method of claim 15 wherein said cell is a human cell.

25. A method for promoting gene silencing in a cell comprising enriching the amount or activity of RNase III polypeptide in said cell to a level effective to promote said gene silencing.

26. The method of claim 25 wherein said enriching is by exogenous addition of RNase III polypeptide.

27. The method of claim 25 wherein said enriching is by exogenous addition of RNase III polypeptide is a purified RNase III polypeptide.

28. The method of claim 27 wherein said RNase III polypeptide is provided through upregulation of endogenous production of the polypeptide.

29. The method of claim 25 wherein said enriching is by addition of a vector encoding the RNase III polypeptide.

30. The method of claim 25 wherein said cell is a mammalian cell.

31. The method of claim 25 wherein said cell is a human cell.
33. A method for promoting gene silencing of a gene in a cell comprising:
   (a) providing to said cell a polynucleotide hybridizable with a target RNA encoded by a selected gene whose expression is to be silenced;
   (b) hybridizing said polynucleotide and said target RNA to form a polynucleotide-target duplex; and
   (c) contacting said duplex with a polypeptide comprising an RNase III domain, under conditions selected to effect cleavage or modification of the target RNA strand of the polynucleotide-target RNA duplex by the polypeptide comprising an RNase III domain, and silencing of the gene thereby.
34. The method of claim 33 wherein said promotion of gene silencing occurs in the cell's nucleus.
35. The method of claim 33 wherein the polypeptide comprising an RNase III domain is an RNase III polypeptide.
36. The method of claim 33 wherein the RNase III polypeptide is a human RNase III polypeptide.
37. The method of claim 36 wherein the human RNase III polypeptide comprises an amino acid sequence with at least 90% homology to SEQ ID NO: 2.
38. The method of claim 33 wherein the polynucleotide is provided as a single stranded polynucleotide.
39. The method of claim 33 wherein the polynucleotide is provided as part of a double stranded nucleic acid structure.
40. The method of claim 33 wherein the polynucleotide is an antisense oligonucleotide.
41. The method of claim 33 wherein the polynucleotide is an RNA-like polynucleotide.
42. The method of claim 33 wherein at least one sugar moiety of the polynucleotide is a ribofuranosyl sugar moiety.
43. The method of claim 42 wherein at least 50% of the sugar moieties of the polynucleotide are ribofuranosyl sugar moieties.
44. The method of claim 33 wherein the polynucleotide has at least one modification of the base, sugar or internucleoside linkage.
45. The method of claim 44 wherein the polynucleotide has a modification at the 2' position of at least one sugar.
46. The method of claim 33 wherein the RNase III polypeptide is present in enriched amounts.
47. The method of claim 46 wherein the RNase III polypeptide present in enriched amounts is overexpressed or exogenously added.
48. The method of claim 46 wherein the RNase III polypeptide is a purified RNase III polypeptide.
49. The method of claim 46 wherein enriching is by addition of a vector encoding said RNase III polypeptide.
50. The method of claim 46 wherein the RNase III polypeptide is provided through upregulation of endogenous production of the polypeptide.
51. The method of claim 33 wherein said cell is a mammalian cell.
52. The method of claim 33 wherein said cell is a human cell.
53. The method of claim 33 wherein said polynucleotide-target RNA duplex forms inside the cell.
54. The method of claim 33 wherein said polynucleotide-target RNA duplex forms outside the cell.
55. A method for inhibiting the expression of a gene in a cell comprising providing to said cell an agent effective to elicit RNase III modification of double-stranded RNA in a cell.
56. The method of claim 55 wherein said inhibition of gene expression occurs in the cell's nucleus.
57. The method of claim 55 wherein said agent is a nucleic acid which is hybridizable with an RNA encoded by the gene whose expression is to be inhibited.
58. The method of claim 55 wherein said RNase III modification is RNase III cleavage.
59. The method of claim 55 wherein the polynucleotide is provided as a single stranded polynucleotide.
60. The method of claim 55 wherein the polynucleotide is provided as part of a double stranded nucleic acid structure.
61. The method of claim 55 wherein the polynucleotide is an antisense oligonucleotide.
62. The method of claim 55 wherein the polynucleotide is an RNA-like polynucleotide.
63. The method of claim 55 wherein at least one sugar moiety of the polynucleotide is a ribofuranosyl sugar moiety.
64. The method of claim 63 wherein at least 50% of the sugar moieties of the polynucleotide are ribofuranosyl sugar moieties.
65. The method of claim 55 wherein the polynucleotide has at least one modification of the base, sugar or internucleoside linkage.
66. The method of claim 65 wherein the polynucleotide has a modification at the 2' position of at least one sugar.
67. A method for promoting inhibition of expression of a gene in a cell comprising:
   (a) providing to said cell a polynucleotide hybridizable with a target RNA encoded by the gene whose expression is to be inhibited;
   (b) hybridizing the polynucleotide and the target RNA to form a polynucleotide-target duplex; and
   (c) contacting the duplex with a polypeptide comprising an RNase III domain, under conditions effective to effect cleavage or modification of the target RNA strand of the polynucleotide-target RNA duplex by the RNase III polypeptide, and inhibition of expression of the gene thereby.
68. The method of claim 67 wherein said promotion of inhibition of gene expression occurs in the cell's nucleus.
69. The method of claim 67 wherein the polypeptide comprising an RNase III domain is an RNase III polypeptide.
70. The method of claim 69 wherein the RNase III polypeptide is a human RNase III polypeptide.
71. The method of claim 70 wherein the human RNase III polypeptide comprises an amino acid sequence with at least 90% sequence identity to SEQ ID NO: 2.
72. The method of claim 67 wherein the polynucleotide is provided as a single stranded polynucleotide.
73. The method of claim 67 wherein the polynucleotide is provided as part of a double stranded nucleic acid structure.
74. The method of claim 67 wherein the polynucleotide is an antisense oligonucleotide.
75. The method of claim 67 wherein the polynucleotide is an RNA-like polynucleotide.
76. The method of claim 67 wherein at least one sugar moiety of the polynucleotide is a ribofuranosyl sugar moiety.
77. The method of claim 76 wherein at least 50% of the sugar moieties of the polynucleotide are ribofuranosyl sugar moieties.

78. The method of claim 67 wherein the polynucleotide has at least one modification of the base, sugar or internucleoside linkage.

79. The method of claim 78 wherein the polynucleotide has a modification at the 2′ position of at least one sugar.

80. The method of claim 67 wherein the polypeptide comprising an RNase III domain is present in enriched amounts.

81. The method of claim 80 wherein the polypeptide comprising an RNase III domain and present in enriched amounts is overexpressed or exogenously added.

82. The method of claim 81 wherein the polypeptide comprising an RNase III domain and present in enriched amounts is a purified RNase III polypeptide.

83. The method of claim 81 wherein enriching is by addition of a vector encoding said polypeptide comprising an RNase III domain.

84. The method of claim 67 wherein said cell is a human cell.

85. The method of claim 67 wherein step (c) is performed within a cell.

86. The method of claim 67 wherein step (b) is performed within a cell.

87. The method of claim 67 wherein step (b) is performed outside a cell.

88. A cell having enhanced RNase III activity over an activity exhibited by a second cell, said second cell not enriched with respect to the amount or activity of RNase III polypeptide.

89. The cell of claim 88 wherein said enhanced RNase III activity is detectable in the cell’s nucleus.

90. The cell of claim 88 wherein said enhanced RNase III activity is due to overexpression of RNase III.

91. The cell of claim 88 wherein the RNase III polypeptide is provided through upregulation of endogenous production of the RNase III polypeptide.

92. The cell of claim 88 wherein said enhanced RNase III activity is due to exogenously added RNase III.

93. A method for eliciting modification of an RNA target in a cell comprising:

(a) providing an RNA-like polynucleotide hybridizable with said RNA target;

(b) hybridizing the RNA-like polynucleotide and the RNA to form a polynucleotide-target duplex; and

(c) contacting the duplex with a polypeptide comprising an RNase III domain, under conditions selected to effect modification of the duplex by the polypeptide, and modification of the RNA target thereby.

94. A hybrid RNase III comprising at least one domain from a human RNase III and at least one domain from an RNase III of an organism other than human.

95. The hybrid RNase III of claim 94 wherein the non-human RNase III domain is derived from an organism selected from the group consisting of E. coli, S. pombe, C. elegans and S. cerevisiae.