snoRNA, COMPOSITIONS AND USES

The present invention concerns the use of particular RNA sequences as a medicament. More precisely, it concerns the use of small nucleolar RNAs (snoRNAs) which inventors have shown to be involved in the mechanisms of aging. The snoRNAs of the invention can be used in particular to increase the stress resistance of a subject, to fight against the harmful effects of aging, typically for preventing or treating a degenerative disease, in particular neurodegenerative, a laminopathy, in particular Hutchinson–Gilford syndrome (HGPS) (also known as progeria), diabetes, obesity or a cancer and, more generally, to prolong the lifespan of a subject. The snoRNAs of the invention can also be used in the treatment of infertility.

The invention further relates to vectors, cells, transgenic animals and compositions capable of expressing a snoRNA of the invention, and methods using any of the above products of the invention as a tool for identification of a molecule active in the prevention or treatment of a pathology, abnormality, disorder or an apparent or functional deterioration linked to a mechanism of aging.

TECHNOLOGICAL BACKGROUND

The mechanisms of aging (i.e., progressive decline in the capacity to survive and reproduce with age) have perplexed society and the scientific community for centuries. There are two currently prevailing theories, one that aging results from a genetically preprogrammed evolutionary path and the other that aging is a normal consequence of existence during which cellular and molecular damage accumulates. This damage would include oxidative damages induced by free radicals, defective mitochondria, somatic mutations, progressive shortening of telomers, programmed cell death and proliferation of damaged cells, etc. (Semsei I. (2000) On the nature of aging. Mech Aging Dev 117:93–108).

It has been shown, in organisms like yeast and mice, that caloric reduction exerts a decisive positive impact on lifespan extension (Sohal, R S, Weindruch, R (1998) Oxidative stress, caloric restriction, and aging. Science 273:59–63; Finch, C E, Revkun, G. (2001) The genetics of aging. Annu. Rev. Genom. Hum. Genet. 2:435–462). Recent studies have shown that caloric restriction would also be effective in primates, including humans (Roth, G S, Lasnikov, V, Lesnikov, M, Ingram, D K, Land, M A (2001) Dietary caloric restriction prevents the age–related decline in plasma melatonin levels of rhesus monkeys. J Clin Endocrinol Metab. 86: 3292–5; Roth G S, Lane M A, Ingramn D K, Mattison J A, Elahi D, Tobin J D, Muller D, Metter E J (2002) Biomarkers of caloric restriction may predict longevity in humans. Science. 297: 811–813; Walford R L, Mock D,

Verdery R, MacCallum T. (2002) Calorie restriction in biosphere 2: alterations in physiologic, hematologic, hormonal, and biochemical parameters in humane restricted for a 2–year period. J Gerontol A Biol Sci Med Sci 57: 211–24). Unfortunately, it is probable that the majority of humans are not capable of following the strict diet required to benefit from this discovery.

For several years, many research groups have been dedicated to identifying the genes and signalling pathways involved in the aging process. The studies reported here are conducted in numerous organisms, including yeast *Saccharomyces cerevisiae*, worm *Caenorhabditis elegans* and fly *Drosophila melanogaster* (Fontana et al., 2010), and permitted identifying a growing list of genes. Many of them are involved in hormone signalling and are conserved in a large variety of eukaryotic organisms. It has become clear that, at least for lower species, the pathways responsible for development and growth at the start of life retain a lifelong influence and even partially condition its duration. These studies have also indirectly shown the importance of metabolic capacity and stress resistance for evaluating the lifespan of a subject.

For example, mutants of the clk–1 gene of *Caenorhabditis elegans* were involved in reducing the extra–mitochondrial production of reactive oxygen species (Hekimi, S, Guarente, L. (2003) Genetics and the specificity of the aging process. Science 299:1351–1354). Patent WO 98/17823 describes the function of the clk–1 gene in the processes of development and longevity. It particularly claims a method to increase an individual's lifespan by regulation of clk–1 gene expression.

A mutation of the Methuselah gene (which codes for a G–protein coupled receptor) has also been described as capable of increasing the lifespan of Drosophila by around 35% (US 6,303,768).

Telomer shortening is also known as a mechanism responsible for cell aging. In vertebrates, telomerase is a ribonucleoprotein (RNP) reverse transcriptase whose role is to maintain the length of telomers by addition of telomeric DNA to the end of chromosomes in dividing eukaryotic cells (Kiss, 2002). In humans, a mutation in the H/ACA box of telomerase snoRNA causes a pleiotropic genetic disease, dyskeratosis congenita, whose patients present shorter telomers (Mitchell et al., 1999; Vulliamy et al., 2001). Despite the efforts of the scientific community to decode the mechanisms of aging and identify new therapeutic targets to delay this process and/or combat its harmful effects, the tools available remain insufficient. The need for tools that permit "aging well", that is without the diseases and discomforts generally associated with old age, is felt all the more now that medical progress combined with general improvement in life conditions have already permitted the human population, in just a few decades, to increase its life expectancy in a very significant manner.

SUMMARY OF THE INVENTION

The present disclosure relates to the prophylactic or therapeutic use of the RNA sequences of interest for which the inventors have demonstrated particular involvement in the mechanisms of aging. They have especially shown the ability of these sequences to spectacularly increase the lifespan of a subject by stopping the mechanisms of aging and fighting the harmful effects associated therewith. The sequences disclosed herein also fight diseases associated with aging such as degenerative diseases, laminopathies and cancers, as well as diabetes and obesity. They have proven especially effective in the treatment of infertility or sterility (i.e., difficulty conceiving or inability to conceive) as well as increasing the resistance of subjects, in particular subjects who have reached sexual maturity, preferably elderly subjects, to stress.

One aspect of the invention relates to an isolated or synthetic RNA sequence comprising sequence SEQ ID NO: 1, a SEQ ID NO:1 orthologous sequence, or a SEQ ID NO:1 functionally analogous sequence, for use as a medicament.

The inventors have demonstrated that the RNA sequence SEQ ID NO: 1 discovered in Drosophila corresponds to orthologous sequences in mammals such as primates and human being, and in rodents such as mice and rats. The disclosure also covers the use, in one or the other of the applications described in this text, of an isolated or synthetic RNA sequence preferably chosen from among sequence SEQ ID NO: 2 of human origin and sequences SEQ ID NO: 3 and SEQ ID NO: 4 of mouse origin. Sequences SEQ ID NO: 2, 3, 4, 9, 11, 13, 15, 17, 19, 21, 22, 24, 26, and 28 identified in Table 1 of this text are examples of sequences orthologous to SEQ ID NO: 1.

Another aspect is a DNA sequence coding for a RNA sequence of interest as disclosed herein.

The disclosure also concerns a vector permitting *in vitro*, *ex vivo* or *in vivo* expression of a RNA sequence disclosed herein. In one aspect, there is provided a vector allowing the expression of an RNA sequence as disclosed herein for use as a medicament, wherein the vector is chosen, for example, from among a plasmid, a cosmid, a viral vector and a phage, wherein the vector preferably comprises a promotor and/or regulator elements promoting the expression of the RNA sequence in intestinal or ovarian cells.

Another aspect concerns a cell comprising a RNA sequence as disclosed herein or a cell transformed by a vector as disclosed herein, as well as a composition comprising a RNA sequence, vector or cell as disclosed herein and a dietarily- or pharmaceutically–acceptable support for use as a medicament.

Another aspect concerns the use of a RNA sequence, vector, cell or a composition according to the invention, continuously or sequentially, to restore or modulate the expression of said RNA sequence *in vivo*, *in vitro* or *ex vivo*.

Another aspect relates to a transgenic mouse whose genome has been genetically modified to prevent or modify, preferably alter, the expression of a RNA sequence of interest as disclosed herein, typically one and/or the other of sequences SEQ ID NO: 3 and SEQ ID NO: 4.

The disclosure also covers a method for evaluating the cosmetic or therapeutic efficacy of a test compound to fight the harmful effects of aging or stress, or to fight infertility, said method comprising i) exposure of a cell or population of cells not expressing the RNA sequence disclosed herein or expressing an abnormal version of said RNA sequence; or a transgenic mouse as disclosed herein, ii) evaluation of the effects, if any, of the test compound on the phenotype of said cell(s) or said transgenic mouse, and iii) determination of the cosmetic or therapeutic efficacy of said test compound, a restoration of the activity and/or expression of said RNA sequence being correlated with efficacy of said test compound.

The disclosure also covers a method for evaluating the cosmetic or therapeutic efficacy of a test compound to fight the harmful effects of aging or stress, or to fight infertility, said method comprising i) exposure of a cell comprising an RNA sequence as disclosed herein or a population of cells comprising a cell as disclosed herein; ii) evaluation of the effects, if any, of the test compound on the phenotype of said cell or population of cells, and iii) determination of the cosmetic or therapeutic efficacy of said test compound, an increase of the activity and/or expression of said RNA sequence being correlated with efficacy of said test compound.

Also disclosed is a method for evaluating the cosmetic efficacy of a test compound, said method comprising i) exposure to a test compound of a cell, or a population of cells, not expressing the RNA sequence as disclosed herein or expressing an abnormal version of said RNA sequence; or a transgenic mouse as disclosed herein, ii) evaluation of the effects, if any, of the test compound on the phenotype of said cell(s) or said transgenic mouse, and iii) determination of the cosmetic efficacy of said test compound, restoration of the activity and/or expression of said RNA sequence being correlated with efficacy of said test compound.

Also disclosed is a method for evaluating the therapeutic efficacy of a test compound to fight the harmful effects of aging or a stress, a laminopathy, diabetes, obesity or to fight infertility, said method comprising i) exposure to a test compound of a cell or population of cells not expressing the RNA sequence as disclosed herein or expressing an abnormal version of said RNA sequence; ii) evaluation of the effects, if any, of the test compound on the phenotype of said cell(s), and iii) determination of the therapeutic efficacy of said test compound, a restoration of the activity and/or expression of said RNA sequence being correlated with efficacy of said test compound.

Also disclosed is a method for evaluating the cosmetic efficacy of a test compound, said method comprising i) exposure to a test compound of a cell as disclosed herein comprising a RNA sequence as disclosed herein or of a population of cells comprising a cell as disclosed herein, ii) evaluation of the effects, if any, of the test compound on the phenotype of said cell or said population of cells, and iii) determination of the cosmetic efficacy of said test compound, increase of the activity and/or expression of said RNA sequence being correlated with efficacy of said test compound.

Also disclosed is a method for evaluating the cosmetic efficacy of a test compound to fight the harmful effects of aging or a stress, a laminopathy, diabetes, obesity or to fight infertility, said method comprising i) exposure to a test compound of a cell as disclosed herein comprising an RNA sequence as disclosed herein or of a population of cells comprising a cell as disclosed herein, ii) evaluation of the effects, if any, of the test compound on the phenotype of said cell or said population of cells, and iii) determination of the therapeutic efficacy of said test compound, increase of the activity and/or expression of said RNA sequence being correlated with efficacy of said test compound.

Another aspect relates to the use of the RNA sequence as disclosed herein in the preparation of a medicament for extending the lifespan of a subject, increasing stress resistance, or fighting the harmful effects of aging in a subject.

Another aspect relates to the use of the RNA sequence as disclosed herein in the preparation of a medicament for the prevention or treatment of a neurodegenerative disease induced by mutation of the fah2 gene, a laminopathy, diabetes, obesity or cancer.

Another aspect relates to the use of the RNA sequence as disclosed herein in the preparation of a medicament for the treatment of infertility or stimulation of fertility in a subject.

The invention also concerns kits comprising nucleic acids, vectors, cassettes and/or cells such as described previously.

DETAILED DESCRIPTION

The invention results from the demonstration by the inventors of the influence of a small sequence of nucleolar DNA (snoRNA:Ψ28S–1153 called *"youth"*, identified in this text as SEQ ID NO: 1) on the lifespan of Drosophila. This snoRNA has been identified as part of a systematic screening of snoRNAs associated with the Drosophila genome (Huang et al., 2005) without any function being ascribed to it to date.

The inventors have characterized this sequence and notably shown that a genomic deletion of 632 base pairs of the F4 region of the Drosophila genome containing the snoRNA:Ψ28S–1153, shortens the lifespan of Drosophila by around 30%. They then demonstrated that the over–expression of this RNA sequence, for example using a transgene containing genomic DNA coding for this snoRNA, spectacularly increases the lifespan of Drosophila by around 100%. Flies overexpressing the RNA sequence of the invention live up to twice as long. Furthermore, the inventors have identified and detected the expression of orthologous *"youth"* sequences in mice and humans.

Characteristics of the RNA sequences and other products of the invention

As explained previously, the invention concerns an isolated or synthetic ribonucleotide sequence (RNA sequence) with the functional characteristics of sequence SEQ ID NO: 1 identified originally in Drosophila (*Drosophila melanogaster*), for use as a medicament in a subject who can benefit from it, typically an animal or insect, for example a mammal or a rodent, preferably a human being.

"*Medicament*" means a substance possessing preventive or curative properties. In the context of the invention, a medicament is intended to cure, promote cure, relieve or prevent, in a subject as defined previously, a pathology, abnormality or impairment related to an abnormal or simply undesirable aging process.

In Drosophila, the RNA sequence of interest is comprised in the genomic region identified as region F4, itself located on chromosome 2R. This nucleotide sequence is, for the first species of Drosophila (*Drosophila melanogaster*) studied by the inventors, constituted of 148 base pairs. It is identified in this text as SEQ ID NO: 1.

Homologs/orthologs as well as functional analogs and variants of sequence SEQ ID NO: 1 are, likewise, sequences of interest that are objects of the present invention.

The term "*homolog*" is used in the context of the present invention to designate a structure whose nucleotide sequence is identical or sufficiently close to that of sequence SEQ ID NO: 1, identified in this first species of Drosophila, to be considered as its equivalent in another species (Drosophila, insect, or any other animal). A sequence is considered as a homolog of sequence SEQ ID NO: 1 if it has therewith, or with a fragment thereof of at least 50 consecutive nucleotides, a sequence identity of at least 70%, 80% or 85%, preferably at least 90 or 95 %, more preferably at least 96, 97, 98 or 99% obtained, for example, by the blastN sequence alignment program (Altschul *et al.*, 1990), or by the INFERNAL program for "INFERence of RNA ALignment", which identifies DNA sequences from RNA structure and their sequence similarity. Two homologous genetic sequences of two different species are considered orthologs if they descend from a unique sequence present in the last common ancestor of the two species.

The percentage of identify is determined by comparing two sequences for which an optimal alignment has been done. The percentage of identity is calculated by determining the number of positions for which an identical residue appears over both sequences at the same position divided by the total number of positions and multiplied by 100. The optimal alignment of the two

sequences can be obtained, for example, with a local homology search algorithm (Smith & Watherman, 1981) or equivalent systems known to the person skilled in the art.

The term "*analog*" designates any mimetic that is a chemical compound existing in nature and isolated therefrom, or produced artificially, and which has at least one of the endogenous functions of the nucleotide sequence that it imitates (functionally–equivalent RNA sequence), for example all the endogenous functions of the nucleotide sequence which it imitates. An example of an analog compound consists, for example, in a sequence permitting splicing the same genes. A particular object of the invention therefore concerns a sequence analogous to SEQ ID NO: 1 that permits splicing one or more genes chosen from among Ir56d, buttonhead, klarsicht, CG3262, CG30502 (fatty acid 2–hydroylase - *fa2h*), CG11125, CG9339, and CG40006 of *Drosophila melanogaster*. Experiments conducted by the inventors have demonstrated, for example, that gene CG9339 (called *skywalker*) (Uytterhoeven *et al.*, 2011) is not correctly spliced in the F4 mutant, indicating that the *youth* snoRNA is involved in the alternative splicing of this gene (see Figure 17). Similar results have also been obtained for a transcript of the *klarsicht* gene, for which less spliced RNA is observed in the F4 mutant than in the Canton–S wildtype control flies (Figure 18A), as well as for gene CG30502, coding for gene *fa2h* (Figure 18B), the latter having been involved in humans in various forms of demyelination, such as neurodegeneration related to the accumulation of iron, leukodystrophies (typically those related to fa2h gene mutations) and hereditary spastic paraplegia (typically SPG35 hereditary spastic paraplegia) (Dick et al., 2008; 2010; Pierson et al., 2012).

The term "*functional variant*" means any nucleic acid having one or more modifications or mutation (i.e., for example, a deletion, substitution or addition of one or more bases) relative to parent sequences described in this application, and permitting the applications described in the context of the present invention.

A sequence considered to be a homolog, preferably an ortholog, of a reference sequence in the sense of the invention is an example of a functional variant in the sense of the invention of said reference sequence. Such a sequence can be natural, recombinant or synthetic.

Sequence SEQ ID NO: 1 is a highly conserved sequence throughout evolution and present in many animal species. The inventors have therefore notably shown that this sequence is present in the 12 other species of Drosophila whose genome is known. Examples of sequences homologous/orthologous to RNA sequence SEQ ID NO: 1 therefore also comprise RNA sequences SEQ ID NO: 9, 11, 13, 15, 17, 19, 21, 22, 24, 26, and 28 identified in Table 1 of this text (see Figure 9).

The inventors have also identified an orthologous RNA sequence in the human being, located on chromosome 11, in position 12822722–12822811. It is the sequence identified in this text as SEQ ID NO: 2. The inventors have also identified two orthologous RNA sequences in mice (these are the sequences identified, respectively, as SEQ ID NO: 3 and 4 in this text), located on chromosome 15, in position 30336889–30337017 (SEQ ID NO: 7) and on chromosome 18, in position 6495012–6495091 (SEQ ID NO: 8).

Nucleotide sequences of interest identified in Drosophila, humans and mice are listed in Table 1 below.

Table 1:

Species	RNA sequences (from 5' to 3')	SEQ ID NO:
Drosophila melanogaster	AAAGCGUUAGAUAUUAAACUGUGGUUGAAUUCACAAA AUAGGCCACAGUUAUGCAAUAAACGCUAGAAAAAAAAC GGUAGUAUUUAAUAACGUGUUGACUAACAUCUGCGGAU AAGAAGCUUUGCGUUUUGAGGUACUAACCACAGUA	1
Human	GUAAGUGUAGCCUAGAAAUUGGGGCUGGAUUUGAAAA UUAGCCCCAAUUCUGCAAUUUUCACCGCAAUAAAAGCU UCUCCAGUUAUACAUGGUGAUUGGUCUUGAUGGGCUAU UGUGGACAGAGGAGGGUGCUAGGUUGGGGUGGACGGG GCCACAGCU	2
Mouse–1	AAAGGGGUUGAAGAAUGGUAUGGAUUCAGAAUGAACU AUCAGAGAAACUCCAGAGCCAGCAGGAAACAUUAUAGA GCCUUUGCUACAAUGUCCUGUUUCUUUCUUGGCUUUUA GUUCUGUUCCACAGAU	3
Mouse–2	GAAAGCAUUUAAUAUUUACCAAUAGUUUAUUCCGAGCU AGGGUAAAGCAGUCAGUGCUAGAAAAAUGAGAAAACAC AAUACAUAAGACCUCUCAAGGGGAGAUGCUGUUACUGU AUAUACUG	4
Drosophila simulans	AAAGCGUUAGGUAUAAACCUGUGGUUGAAUUCACAAAA UAGGCCACAGUUAUGCAAUAAACGCUAGAAAAAAAACG GUAGUAUUUAAUAACGUGUUGACUAACAUCUGCGGAUA AAACAGCUUUGCGUUUUGAGGUACUAACCACAGUA	9
Drosophila sechellia	AAAGCGUUAGGUAUUAACCUGUGGUUUAAUUCACAAAA UAGGCCACAGUUAUGCAAUAAACGCUAGAAAAAAAACG GUAGUAUUUAAUAACGUGUUGACUAACAUCUGCGGAUA AAAUAGCUUUGCGUUUUGAGGUACUAACCACAGUA	11

Drosophila yakuba	CAAGCGUUGUAUAAUAUUAAACUGUGGAAACUUCACAA AUAGGCCACAGUUAUGCAAGAAACGCUAGAAAAACUUG GUAGUAUUUAAUAACGUGCUGACUAACGUCUGCGGAUA AAAGCUUUGCGUUUUGAGGUACUAACCACAUUA	13
Drosophila erecta	CAUGCGUUGAAUAAUAUUAAACUGUGGACGAAUUCCCA AAUAAGCCACAGUUAUGCAAGAAACGCUAGAAAAAAUA GGUAGUAUUUAAUAACGUGCUGACUAACAUCUGCGGAU AAGAGCUUUGCGUUUUGAGGUACUAACCACAAUA	15
Drosophila ananassae	UUAGGCGUUCAAAACAUUAAACGUUGGCUGAUUUAUGA UUAGGCUAGUGUUAUGCACGCAACGCUAGAGAAAAUUG GUAGUAUUUAAUAAUGCGUUGGUGGCAAAUCUAUCGA UUUUGAUCUUCGCAUUUUGAGGUACUAGCACAGUU	17
Drosophila pseudoobscura	CAUGGCGCUCAAACAUUAAUCGGUGGCCUGAUCCACAC GUCGGCCACAUUUAUGCACGCAGCGCCAGAUAAUAGUA GGCAGCAUUUAAUAAUGUAUUGGUUCCAAAUGACUCAG ACUUUUGCAUUUUGAGGUGUUAGCCACAACG	19
Drosophila persimilis	CAUGGCGCUCAAACAUUAAUCGGUGGCCUGAUCCACAC GUCGGCCACAUUUAUGCACGCAGCGCCAGAUAAUAGUA GGCAGCAUUUAAUAAUGUAUUGGUUCCAAAUGACUCAG ACUUUUGCAUUUUGAGGUGUUAGCCACAAAG	21
Drosophila willistoni	AGAGGCGUUCAACAUUUAACUUUAGCCGUUCUUUUAAA CUAAGCUAUUUGUUAUGCAAGUAACGCUAGAAUAUAAU UUCUGCAACAUUUAAUAAUGCUUUGGUCGUAUACUAAC AAAAAUCUCAGCAUUUUGAGGUGUCUGCUACAAUU	22
Drosophila mojavensis	CCCUGCGUUGAGCAGUAAAUUGUCGCUGAUUGAUAAUU AGGCGACGAUAAUGCAAACAACGGUAGAAAAAACUAAC GACAUUUAAUAAUACAUUGACCACUAAAUCCCAUGAAC AUUGCAUUUUGAGGUGUCGCGACAUGU	24
Drosophila virilis	UUAUGCGUUCAACAGUAAAUUGUCGCCGAUAGAUUACU AGGCGACAGUUAUGCAAGGCAACGCUAGAAUAAGCAGA CGACAUUUAAUAAUGCAUUGGCCGACAAACUCCACGGC CUUUGCGUUUUGAGGUGUCGCCACAUGU	26
Drosophila grimshawi	UUGUGCGUUCGACAGUAAAUUUUCGAUGACUGUAGAUA GGCGAAAAUUAUGCAAGGCAACGCUAGACCAAUUAGAU GACGACAUUUAAUAAUGCAUUGGCCGAUUAACUCAGAU CUUGCAUUUUGAGGUGUCGCCACAUGU	28
	Corresponding DNA sequences	
Drosophila melanogaster	AAAGCGTTAGATATTAAACTGTGGTTGAATTCACAAAAT AGGCCACAGTTATGCAATAAACGCTAGAAAAAAACGGTA GTATTTAATAACGTGTTGACTAACATCTGCGGATAAGAA GCTTTGCGTTTTGAGGTACTAACCACAGTA	5

human	GTAAGTGTAGCCTAGAAATTGGGGCTGGATTTGAAAATT AGCCCCAATTCTGCAATTTTCACCGCAATAAAAGCTTCTC CAGTTATACATGGTGATTGGTCTTGATGGGCTATTGTGGA CAGAGGAGGGTGCTAGGTTGGGGTGGACGGGGCCACAG CT	6
mouse–1	AAAGGGGTTGAAGAATGGTATGGATTCAGAATGAACTAT CAGAGAAACTCCAGAGCCAGCAGGAAACATTATAGAGCC TTTGCTACAATGTCCTGTTTCTTTCTTGGCTTTTAGTTCTG TTCCACAGAT	7
Mouse–2	GAAAGCATTTAATATTTACCAATAGTTTATTCCGAGCTAG GGTAAAGCAGTCAGTGCTAGAAAAATGAGAAAACACAA TACATAAGACCTCTCAAGGGGAGATGCTGTTACTGTATAT ACTG	8
Drosophila simulans	AAAGCGTTAGGTATAAACCTGTGGTTGAATTCACAAAAT AGGCCACAGTTATGCAATAAACGCTAGAAAAAAAACGGT AGTATTTAATAACGTGTTGACTAACATCTGCGGATAAAA CAGCTTTGCGTTTTGAGGTACTAACCACAGTA	10
Drosophila sechellia	AAAGCGTTAGGTATTAACCTGTGGTTTAATTCACAAAATA GGCCACAGTTATGCAATAAACGCTAGAAAAAAAACGGTA GTATTTAATAACGTGTTGACTAACATCTGCGGATAAAATA GCTTTGCGTTTTGAGGTACTAACCACAGTA	12
Drosophila yakuba	CAAGCGTTGTATAATATTAAACTGTGGAAACTTCACAAA TAGGCCACAGTTATGCAAGAAACGCTAGAAAAACTTGGT AGTATTTAATAACGTGCTGACTAACGTCTGCGGATAAAA GCTTTGCGTTTTGAGGTACTAACCACATTA	14
Drosophila erecta	CATGCGTTGAATAATATTAAACTGTGGACGAATTCCCAA ATAAGCCACAGTTATGCAAGAAACGCTAGAAAAAATAGG TAGTATTTAATAACGTGCTGACTAACATCTGCGGATAAG AGCTTTGCGTTTTGAGGTACTAACCACAATA	16
Drosophila ananassae	TTAGGCGTTCAAAACATTAAACGTTGGCTGATTTATGATT AGGCTAGTGTTATGCACGCAACGCTAGAGAAAATTGGTA GTATTTAATAATGCGTTGGTGGCAAATCTATCGATTTTGA TCTTCGCATTTTGAGGTACTAGCACAGTT	18
Drosophila pseudoobscura	CATGGCGCTCAAACATTAATCGGTGGCCTGATCCACACG TCGGCCACATTTATGCACGCAGCGCCAGATAATAGTAGG CAGCATTTAATAATGTATTGGTTCCAAATGACTCAGACTT TTGCATTTTGAGGTGTTAGCCACAACG	20
Drosophila persimilis	CATGGCGCTCAAACATTAATCGGTGGCCTGATCCACACG TCGGCCACATTTATGCACGCAGCGCCAGATAATAGTAGG CAGCATTTAATAATGTATTGGTTCCAAATGACTCAGACTT TTGCATTTTGAGGTGTTAGCCACAAAG	22

Drosophila willistoni	AGAGGCGTTCAACATTTAACTTTAGCCGTTCTTTTAAACT AAGCTATTTGTTATGCAAGTAACGCTAGAATATAATTTCT GCAACATTTAATAATGCTTTGGTCGTATACTAACAAAAAT CTCAGCATTTTGAGGTGTCTGCTACAATT	24
Drosophila mojavensis	CCCTGCGTTGAGCAGTAAATTGTCGCTGATTGATAATTAG GCGACGATAATGCAAACAACGGTAGAAAAAACTAACGA CATTTAATAATACATTGACCACTAAATCCCATGAACATTG CATTTTGAGGTGTCGCGACATGT	26
Drosophila virilis	TTATGCGTTCAACAGTAAATTGTCGCCGATAGATTACTAG GCGACAGTTATGCAAGGCAACGCTAGAATAAGCAGACGA CATTTAATAATGCATTGGCCGACAAACTCCACGGCCTTTG CGTTTTGAGGTGTCGCCACATGT	28
Drosophila grimshawi	TTGTGCGTTCGACAGTAAATTTTCGATGACTGTAGATAGG CGAAAATTATGCAAGGCAACGCTAGACCAATTAGATGAC GACATTTAATAATGCATTGGCCGATTAACTCAGATCTTGC ATTTTGAGGTGTCGCCACATGT	30

One particular subject of the invention concerns the use of RNA sequence SEQ ID NO: 1 to identify a target gene, characterized in that said sequence SEQ ID NO: 1 is capable of recognizing a target sequence within a *Drosophila melanogaster* gene preferably chosen from among Ir56d, buttonhead, klarsicht, CG3262, CG30502 (*fatty acid 2–hydroylase*: *fa2h*) and CG11125, CG9339, CG40006, said target sequence being preferably chosen from among SEQ ID NO: 31 (TGGTTGAATTCACAAAA), SEQ ID NO: 32 (TTGAATTCACAAAATA), SEQ ID NO: 33 (AATTCACAAAATAGGC), SEQ ID NO: 34 (AAGCGTTAGATATTAA), SEQ ID NO: 35 (ACATCTGCGGATAAGA), SEQ ID NO: 36 (AAGCTTTGCGTTTTGA), and SEQ ID NO: 37 (AGAAGCTTTGCGTTTT), or an analogous sequence thereof.

In the context of the present invention, the RNA sequences of interest are preferably in the form of snoRNAs. In the context of the invention, the term "*snoRNAs*" encompasses a group of non–coding RNAs present in all eukaryotic cells. They are located in the nucleus and more particularly in the nucleolus where they are associated with proteins with which they form small nucleolar ribonucleoproteins or snoRNPs. They are generally produced from pre–mRNA introns. On the evolutionary scale, these non–coding RNAs are present from archaea to mammals. snoRNAs can have several functions, such as modification of ribosomal RNA, like 2'–O–ribose methylation or pseudouridylation of various classes of RNA. They have been involved in the nucleolytic process of ribosomal RNAs or even in the synthesis of telomeric DNA (Kiss, 2002; Kiss et al., 2010; Ye, 2007). There are two main classes of snoRNAs: the one comprising a C/D box and the one comprising an H/ACA box (Figure 2A). C/D boxes serve as a guide for 2'–O–ribose methylation

on specific sites, while H/ACA boxes direct the conversion of uridine into pseudouridine (Kiss, 2002; Gardner et al., 2010; Huang et al., 2005) (Figure 2B).

The snoRNA:Ψ28S–1153 (*youth*) of interest, identified in a first species of Drosophila, belongs to the H/ACA class, that is, its structure comprises an H/ACA box. It would therefore be involved in pseudouridylation (i.e., the conversion of uridine into pseudouridine) during the maturation of ribosomal RNA, and would regulate the protein synthesis of certain genes.

The snoRNAs according to the invention can be chemically modified, typically to increase the resistance of the snoRNAs to nucleases, to confer a better affinity/selectivity and therefore a better functionality of the snoRNAs. The modifications typically concern a nucleoside or internucleoside link. They can concern the sugar, for example (typically the 2' position of the sugar), the nitrogenous base of the nucleoside (which typically comprises a substituent in position 2, 4 or 6) or one (or more) phosphate groups.

The modification may consist, for example, in an amination, halogenation, for example fluoridation, or alkylation, for example methylation. A modified sugar group can therefore be chosen, for example, from among the 2'–O–methyl and 2'–O–methoxyethyl groups.

A modified nucleotide can be a nucleotide comprising a heterocyclic base incapable of creating hydrogen bonds with heterocyclic DNA or RNA bases.

The modification of the internucleoside link is, for example, chosen from among a phosphorothioate, methylphosphonate, phosphotriester, phosphorodithioate and phosphoselenate bond.

The present invention also concerns the deoxyribonucleotide sequences (DNA) responsible for the expression of the RNA sequences of interest of the invention. The DNA sequence permitting the expression of RNA sequence SEQ ID NO: 1 is the sequence identified in the present invention as SEQ ID NO: 5, the one permitting the expression of sequence SEQ ID NO: 2 is the sequence identified in the present invention as SEQ ID NO: 6, the one permitting the expression of sequence SEQ ID NO: 3 is the sequence identified in the present invention as SEQ ID NO: 7, and the one permitting the expression of sequence SEQ ID NO: 4 is the sequence identified in the present invention as SEQ ID NO: 8.

One object of the invention therefore also concerns an isolated or synthetic DNA sequence selected in the group comprising SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, and SEQ ID NO: 30, and

characterized in that it permits the expression of an RNA sequence of interest according to the invention.

Like the snoRNAs, the DNAs according to the invention can be modified chemically (see the chemical modifications possible above).

The invention also concerns any recombinant expression cassette, characterized in that it comprises a nucleic acid sequence of interest according to the invention such as defined in this text. The term expression cassette designates a nucleic acid construct/construction comprising a nucleic acid sequence permitting the expression of an RNA sequence of interest according to the invention and a regulator region, operably linked. The expression "operably linked" indicates that the components are combined so that the expression of the nucleic acid sequence (responsible for the expression of the RNA sequence of interest) and/or the targeting of the RNA sequence of interest are under control of the transcriptional promoter. Typically, the promotor sequence is placed upstream of the nucleic acid sequence, at a distance from the latter compatible with expression control. Spacer sequences can be present, between the regulator elements and the coding sequence, as long as they do not impede the expression and/or targeting of the RNA sequence of interest.

Another object of the invention concerns any (expression) vector comprising a nucleic acid or a cassette such as previously defined permitting the expression of a RNA sequence of interest according to the invention in a host cell or a host organism. The vector can be a DNA or RNA, circular or otherwise, single or double strand. It is typically a plasmid, phage, viral vector (chosen, for example, from among an adenoviral vector, a retroviral vector, an adenovirus–associated vector, a lentiviral vector, a poxvirus vector, a herpetic vector, etc.), a cosmid or an artificial chromosome. Advantageously, it is a vector capable of transforming a eukaryotic cell, preferably an animal cell, typically a human cell, preferably a cell of intestinal or ovarian origin. Such vectors are well known to the person skilled in the art and are particularly described in patent application WO 06/085016 or in the articles by Barton and Medzhitov, 2002; Tiscornia et al., 2004; Xia et al., 2002 et Shen et al., 2003.

One preferred vector permitting the expression of a RNA sequence of interest according to the invention can be chosen from among, for example, a plasmid, a cosmid, a viral vector or a phage. One preferred vector usable in particular in Drosophila is the pChs–Gal4 vector, in which is inserted, upstream of the Gal4 transcription factor, a specific DNA sequence, known to regulate the expression of a given gene in a given tissue, such as for example Myo1A–Gal4, snail–Gal4, Su (H)–GBE–Gal4, Delta–Gal4. Simultaneously, a second vector (pUAs–snoRNA) comprising the effector gene of interest, here the snoRNA, is preferably positioned downstream of the UAS

(Upstream Activating Sequence) regulatory sequences, these latter being recognized by the P[GAL4] transcription factor. Each of these two vectors is then introduced by transgenesis into an animal. These two animal lines are then crossed. This system is called: P[GAL4] binary expression system (Brand and Perrimon, 1993; Elliott and Brand, 2008). Therefore, more specifically, the Myo1A–Gal4, snail–Gal4, Su(H)–GBE–Gal4 and Delta–Gal4 vectors comprise a promotor and regulator elements promoting the expression of said RNA sequence in intestinal cells, while the nanos–Gal4, or MTD–(maternal–Tubulin)–Gal4 vectors comprise a promotor and regulator elements promoting expression of said RNA sequence in the ovaries.

The vectors of the invention can also comprise an origin of replication, a selection gene, a reporter gene and/or a recombination sequence, etc. The vectors can be constructed by standard molecular biology techniques, well known to the person skilled in the art, using, for example, restriction, ligation, cloning, replication, etc. enzymes. Specific examples of vectors in the sense of the invention are provided in the experimental part. These vectors may include, for example, the P[GAL4] binary system (mentioned above) (Brand and Perrimon, 1993; Elliott and Brand, 2008).
The invention also concerns a cell comprising an RNA sequence according to the invention or transformed by means of a construct or vector according to the invention. The cell is preferably an intestinal or ovarian cell. Even more preferred, it is an intestinal stem cell, an enteroblast, an enterocyte, an entero–endocrine cell, a nurse cell or an oocyte.

The invention also comprises the use of a product according to the invention, typically a RNA sequence, a DNA sequence, a vector or a cell according to the invention, continuously or sequentially, to restore or modulate, typically amplify, *in vitro, ex vivo* or *in vivo*, the expression of the RNA sequence of interest according to the invention.

The invention also concerns a transgenic mouse whose genome (typically SEQ ID NO: 7 or SEQ ID NO: 8) has been genetically modified, using techniques well known to the skilled person, to prevent or modify, for example increase or decrease, expression, preferably alter expression of one or the other of SEQ ID NO: 3 and SEQ ID NO: 4.
The mouse is advantageously genetically modified by homologous recombination (knock–in, knock–out, knock–down) techniques known to the skilled person, including, for example, the use of the CRE/LOX system.

The invention also concerns compositions comprising an RNA sequence, a DNA sequence, a construct, a vector or a cell according to the invention.

The RNA sequences of the invention are very stable and very resistant *in vitro*. However, in the case of an administration to a subject to be treated, the compositions according to the invention may also advantageously comprise a dietarily- or pharmaceutically–acceptable support.

The expression "*dietarily–acceptable support*" designates a carrier permitting the subject to ingest and digest without risk the composition comprising a RNA sequence, a construct, a vector or a cell according to the invention comprising such a sequence, and capable of protecting said sequence from any attack, in particular related to food digestion, that could alter it before it produces its therapeutic action.

The expression "*pharmaceutically–acceptable support*" designates a carrier permitting risk–free administration of the composition comprising a RNA sequence, a construct, a vector or a cell according to the invention comprising such a sequence, according to one of the possible administration routes described below.

Advantageously, the carrier of the present composition facilitates penetration of the RNA of interest, the construct or the expression vector according to the invention into cells, ideally into particular cells as identified in this text, of the subject being treated, and/or protects the RNA, the construct or the expression vector from any damage that may impair its efficacy.

The choice of carrier as well as the content of active substance in the carrier are generally determined relative to the solubility and chemical properties of the active substance, the mode of administration and the characteristics of the subject to be treated.

Usable carriers or supports, acceptable pharmaceutically, include natural cationic polymers such as chitosan or atelocollagen, or synthetic polymers, such as poly(L–lysine), polyethyleneimine (PEI) or dendrimers, which form complexes with the nucleic acids of the invention; liposomes; cationic liposomes; galactosylated liposomes; liposomes coated with a ligand allowing them to target a cell type such as immunoliposomes coated with an antibody specific for the target cell (Zheng et al., 2009); liposomes positioned within a nanoparticle formed by polymers (Carmona et al., 2009) or even multilayer films of polycations and polyanions.

Excipients such as lactose, sodium citrate, calcium carbonate, dicalcium phosphate and disintegrating agents such as starch, alginic acids and certain complex silicates combined with lubricants such as magnesium stearate, sodium lauryl sulfate and talc can be used to prepare tablets. To prepare a lozenge, it is advantageous to use lactose and high molecular weight polyethylene glycols. Aqueous suspensions contain emulsifiers or agents that facilitate suspension. Diluents such as sucrose, ethanol, polyethylene glycol, propylene glycol, glycerol and chloroform or mixtures of these are also usable.

Adjuvants such as aluminum salts (for example aluminum hydroxide, aluminum phosphate, aluminum sulfate), surfactants (such as lysolecithin, pluronic polyols, polyanions, peptides and

emulsions), complete and incomplete Freund's adjuvant, MPL–TDM adjuvant (monophosphoryl lipid A, synthetic trehalose dicorynomycolate), tyrosine, aluminum, saponins such as Stimulon™, and cytokines can also be added to improve the efficiency of the composition.

The RNA sequences of the invention can be prepared with an agent, such as injectable microspheres, bioerodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes that may provide controlled or sustained release of the product.

Examples of dietarily–acceptable supports comprise, for example, sugars, saponins, etc.

The composition may also include one or more other active ingredients chosen preferably among an agent usable in the treatment of a laminopathy, in particular in treating Hutchinson–Gilford syndrome or HGPS (also known as progeria and which consists in an early and accelerated aging in humans), obesity, diabetes, cancer, degenerative diseases, in particular neurodegenerative (e.g. leukodystrophy or hereditary spastic paraplegia still identified as SPG35), a stress or infertility.

An active principle in the treatment of obesity may be, for example, leptin or one of its derivatives.

An active principle in the treatment of diabetes may be, for example, insulin or one of its derivatives.

An active principle in the treatment of cancer may be, for example, a conventional cytotoxic chemotherapeutic agent selected from an inhibitor of DNA replication (such as DNA binding agents, in particular intercalating or alkylating compounds), an anti–metabolite agent (such as DNA polymerase inhibitors or a topoisomerase I or II inhibitor), an anti–mitogenic agent (e.g., an alkaloid), and an agent blocking the growth of a cancerous tumor (such as a tyrosinase inhibitor or a monoclonal antibody).

The RNA according to the invention (possibly comprised or expressed using another product according to the invention described in this text) and the other active compound or compounds, can be administered simultaneously, if applicable in a same composition, or sequentially.

The products of the invention (nucleic acid sequences, vectors, cells, compositions) can be adapted for intravenous, oral, sublingual, parenteral, rectal, topical, transdermal, subcutaneous, mucosal, intramuscular, intrapulmonary, intranasal, vaginal, etc., administration according to standard protocols well known to the person skilled in the art.

Preferably, the product is adapted for oral administration and presented in a solid or liquid form. The product can be in the form of a food, tablet, capsule, pill, dragee, lozenge, granules, or oral solution. In the case of parenteral administration, it is preferably in the form of a liquid solution, an emulsion or a suspension. In the case of intravenous, intradermal or subcutaneous administration, it is typically in the form of a solution for injection.

The nucleotide sequence of interest can also be administered by electroporation, into the muscles or through the skin of the subject.

The dosage (or therapeutically–effective quantity) is easily determined by the skilled person so that the desired effects (i.e., extending the lifespan, fighting the harmful effects of aging, stress and/or infertility) are attained. The specific dose of the product should be adjusted to the subject concerned and the desired application. It depends on several factors, including the weight, health status, sex and diet of the subject, the administration route, absorption and excretion rates, and possible combination with one or more other active molecules.

The total daily dose of the product administered to a human subject in a single dose or in several doses may be, for example, comprised between 1 µg and 20 mg per kilo, preferably between 100 µg and 5 mg. The administrations can be weekly or daily or even repeated several times a day. The RNAs or compositions according to the invention comprising them can be administrated in the form of a unit dose comprising 0.05 to 20 mg of RNA, preferentially 0.1 to 5 mg.

In one particular embodiment, a RNA sequence of interest of the invention is used in a therapeutic composition, for example a vaccine.

In another particular embodiment, a RNA sequence of interest of the invention is used in a cosmetic composition.

Applications of the products of the invention

The present invention concerns in particular a product such as described in this text consisting in, or comprising, a RNA sequence or a DNA sequence of interest according to the invention for use as a medicament.

It also concerns a product or composition according to the invention for use i) to prevent or treat a pathology, an abnormality, a disorder or an apparent or functional deterioration related to a mechanism of aging, ii) to extend the lifespan of a subject, or iii) to increase the resistance of a subject to a stress.

In one particular aspect, the present invention relates to the use of such a product in a therapeutically–effective quantity for the preparation of a pharmaceutical composition intended i) to prevent or treat a pathology, an abnormality, a disorder or an apparent or functional deterioration related to a mechanism of aging, ii) to extend the lifespan of a subject, or iii) to increase the resistance of a subject to a stress.

In still another particular aspect, the present invention concerns the use of such a product for the preparation of a cosmetic composition.

The term "treatment" as used in this document refers to improvement or resolution of symptoms, slowing the progression of the disease or aging process, stopping the progression of the disease or resolution of the disease.

The inventors have demonstrated the surprising efficacy of the RNA sequences according to the invention in delaying aging process(es) and fighting pathologies and harmful effects associated with these process(es).

The inventors have notably demonstrated that the overexpression in Drosophila of snoRNA:Ψ28S–1153 of sequence SEQ ID NO: 1 spectacularly doubles the lifespan of said Drosophila. In particular, they demonstrated that mutant flies for snoRNA:Ψ28S–1153 have more neurodegenerative lesions than wildtype flies at 40 days old (Figure 12). In general, flies overexpressing snoRNA:Ψ28S–1153 have fewer, or even no brain lesions, which shows the protective nature of the snoRNA according to the invention relative to neurodegenerative pathologies. Furthermore, the inventors have shown that 40–day–old flies overexpressing snoRNA:Ψ28S–1153 have better sensorimotor performances (represented here by the distance travelled during a test quantifying locomotor activity) than wildtype flies and are therefore protected from the harmful effects associated with mechanisms of aging (Figure 13).

The inventors have demonstrated that the overexpression of a RNA sequence of interest according to the invention permits extending the life of a subject, delaying the mechanisms of aging and fighting the harmful effects associated with these mechanisms. In particular, they showed that this overexpression is neuroprotective and serves in particular to prevent or treat a degenerative disease, in particular neurodegenerative, typically neurodegenerative diseases associated with accumulation of iron which result in demyelination in the brain such as, for example, neurodegenerative diseases caused by one or more mutations of the fa2h gene. The invention also particularly permits treating leukodystrophies (typically those related to mutations of the fa2h gene) or hereditary spastic paraplegia (typically SPG35 hereditary spastic paraplegia). It also permits preventing or treating diabetes, a cancer, or a fertility or sterility problem (Figure 14), and also favors maintenance of the locomotor activity of the treated subject.

"*Subject*" means any living being that could benefit from such a treatment, regardless of its sex or age. Preferably, the subject is an adult subject. The subject may be an insect such as a Drosophila,

or an animal, for example a mammal (preferably a primate or a human being) or a rodent (preferably a mouse or rat).

Preferably, the subject i) does not express the RNA sequence of interest or expresses an abnormal version of said RNA sequence, ii) is a subject experiencing conditions of stress (particularly a subject having reached sexual maturity, preferably an elderly subject) such as fasting, heat shock, oxidative stress (such as paraquat exposure) or a stress (in the intestine) responsible for a detectable cellular proliferation or deregulation of the Delta/Notch pathway, and/or iii) is a subject suffering from a disease related to or promoted by the aging, typically a degenerative disease, in particular neurodegenerative, a laminopathy such as progeria, obesity, diabetes, cancer or fertility problems (particularly including infertility or sterility).

In a particular embodiment, the genome of the subject likely to benefit from the invention i) does not express a RNA of interest sequence as described in this text, ii) expresses an abnormal version of said RNA sequence of interest, or iii) contains a DNA sequence comprising a mutation responsible for the non–expression or abnormal (i.e. non–functional) expression of said RNA sequence of interest.

The normal (wildtype) version of the DNA sequence can be chosen, for example, from among sequences SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28 and SEQ ID NO: 30, identified previously. A mutation likely to affect the normal version of the DNA sequence, i.e., to impede the expression of a RNA sequence of interest according to the invention, is typically chosen from among a deletion, an addition and/or a substitution of one or more nucleotides.

The term "*longevity*" or "*lifespan*" refers to the lifespan for which a subject is programmed as a biological species, living under ideal conditions and in the absence of disease or accident. "*Maximum longevity*" or "*maximum lifespan*" is the maximum lifespan the individual of a given species can attain. For practical purposes, the maximum longevity of a subject is estimated by the maximum age reached by one of the members of a population. "*Mean longevity*" or "*mean lifespan*" is the age at which 50% of a given population survives.

A widely–recognized method to measure the effect of a treatment on *"extension of lifespan"* is by reference to maximum longevity or mean longevity. If a treatment significantly increases the maximum or mean longevity of a group of subjects relative to a group of control subjects, then there is considered to be an extension of lifespan and delay of aging.

In Drosophila, sexual maturity is reached, on average, at the age of one day. The mean lifespan is around 30 days. The maximum lifespan is comprised between around 60 and 80 days depending on

the Drosophila species and line and the living or rearing conditions. In mice, sexual maturity is reached, on average, 42 days after birth. The mean lifespan is around 832 days. In human beings, sexual maturity is reached between 9 and 14 years of age. The mean lifespan is 73 years for men and 79 years for women. The maximum lifespan is currently 122 years, 5 months and 14 days.

The term "*aging*" in the context of this invention, is a process of gradual and spontaneous changes ranging from maturation, through childhood and puberty, up to functional decline of the subject. Aging therefore comprises the positive component of development and maturation and the negative component of decline.

The RNA sequences of interest of the invention increase the maximum or mean longevity (or lifespan) of a subject. Preferably, these sequences extend lifespan and delay aging of a subject or a subpopulation of adult subjects, defined, for example, by sex and/or age (i.e. by a minimum age or a maximum age or by an age window).

Using the present invention, extending the lifespan of Drosophila as well as mammals, including mice and, in particular, human beings, by around 50% or even 100%, while improving conditions of aging is typically considered.

Using the RNA sequences of the present invention, it is also possible to fight "the harmful effects of aging". In the context of the present invention, this expression means the negative component of aging, i.e., decline of the subject. Progressive decline, with age, of physiological capacities varies from one organ to another and from one subject to another. The physiological decline of a subject leads to a reduced ability to respond to an environmental stimulus and increase in the susceptibility and vulnerability to diseases and impairments. The harmful effects of aging encompass the accumulation of undesirable changes that have an impact on weakening a given subject and that can precipitate its death. These changes may be attributed to development, to innate aging processes, to possible genetic abnormalities, to the environment and to diseases that affect or did affect the subject.

Moreover, using the present invention, it is possible to prevent or treat degenerative diseases, in particular neurodegenerative, typically degenerative diseases affecting the central and/or peripheral nervous system.

In a preferred embodiment, the invention permits preventing or treating neurodegenerative diseases associated with the accumulation of iron leading to demyelinization in the brain, in particular neurodegenerative diseases induced by one or more mutations of the fah2 gene. The invention

therefore permits treating leukodystrophies (typically those linked to mutations of the fa2h gene) or hereditary spastic paraplegia (typically SPG35 hereditary spastic paraplegia)
In other embodiments, the invention will prevent or treat a pathology selected from among myopathy, Hunter syndrome, dyskeratosis congenita, Prader–Willi Syndrome, Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and amyotrophic lateral sclerosis (ALS).

In another embodiment, the present invention will prevent or treat laminopathies such as Hutchinson–Gilford syndrome (or progeria), mandibuloacral dysplasia (MAD), Emery–Dreifuss muscular dystrophy, atypical Werner syndrome, restrictive dermatopathy, lethal fetal akinesia, and LIRLLC (Generalized lipoatrophy, insulin–resistant diabetes, leukomelanodermic papules, liver steatosis, and hypertrophic cardiomyopathy), preferably Hutchinson–Gilford syndrome.

In yet another embodiment, the present invention will prevent or treat diabetes or obesity.

It may also participate in the prevention or treatment of cancer or neoplastic process. The cancer is typically a cancer selected from among carcinoma, sarcoma, lymphoma, melanoma, pediatric tumor or leukemia. It may, for example, be breast cancer, ovarian cancer, endometrial cancer, prostate cancer, esophageal cancer, colon cancer, rectal cancer, kidney cancer, lung cancer, thyroid cancer, osteosarcoma, melanoma, leukemia, neuroblastoma, etc.
In one particular embodiment, this invention is particularly useful to fight cancer induced by free radicals such as colon cancer and stomach cancer.

It is also possible to fight problems of infertility or sterility using the product according to the invention. "*Infertility*" or "*sterility*" means difficulty conceiving or inability to conceive. In the context of this use, the subject is typically female, sexually mature and having difficulty, for example premature, reproducing. Typically the subject is sterile, infertile or has had an early or premature reduction in fertility. The RNA sequences of interest according to the invention allow treating infertility or restoring, increasing, enhancing or stimulating the fertility of the treated subject.

The present invention also concerns a method for treating a pathology, abnormality, disorder, harmful effect or apparent or functional deterioration related to a mechanism of aging or to a stress, such as described in this text.

Methods permitting evaluating the preventative or therapeutic efficacy of a test compound

The invention also provides a method for evaluating the cosmetic or therapeutic efficacy of a test compound to fight the harmful effects of aging or a stress as well as against the pathologies associated with such a stress or such a mechanism of aging or for fighting infertility.

"*Test compound*" means any compound potentially involved in the fight against the harmful effects of aging, against a stress or against infertility. For example, it can be a natural or chemical molecule, a metal, an irradiating agent, etc.

A compound can be tested for its potential therapeutic efficacy, i.e., its ability to prevent, treat or enhance the treatment of diseases and harmful effects of aging or a stress, e.g. laminopathy, a degenerative disease or a cancer as described previously, to prevent, treat or enhance the treatment of diabetes or obesity, or to prevent, treat or enhance the treatment of infertility.

A compound can also be tested for its potential cosmetic efficacy, i.e., its ability to improve the physical appearance of the subject and better fight an apparent deterioration of the subject related to a mechanism of aging. The test compound can prove effective, for example, in the cosmetic treatment of the epidermis, body hair and/or capillary system, nails, lips, external genital organs, teeth or mucosa.

In a first embodiment, the method described in the invention comprises the following steps:

i) exposure of a cell, a population of cells or a tissue, not expressing the RNA sequence according to the invention or expressing an abnormal version of said RNA sequence; or a transgenic mouse according to the invention,

ii) evaluation of the effects, if any, of the test compound on the phenotype of said cell(s) or said tissue or said transgenic mouse, and

iii) determination of the cosmetic or therapeutic efficacy of said test compound, a restoration of the activity and/or expression of said RNA sequence being correlated with an efficacy of said test compound.

The cosmetic or therapeutic efficacy of said test compound can be evaluated or determined in reference to a control value. This control value can be established from the level of activity and/or expression of said RNA sequence in a cell, a population of cells or a tissue not expressing, or expressing an abnormal version of said RNA sequence or in a transgenic mouse according to the invention.

If the activity and/or expression of said RNA sequence present in the cell, population of cells, tissue or transgenic mouse exposed to the test compound is greater than the activity and/or expression of the RNA sequence in the cell, population of cells or tissue not expressing, or expressing an abnormal version of said RNA sequence or in said transgenic mouse of the invention, then the test compound has cosmetic or therapeutic efficacy.

In a second embodiment, the method described in the invention comprises the following steps:

i) exposure of a cell according to the invention comprising a RNA sequence according to the invention, a population of cells or a tissue comprising a cell according to the invention,

ii) evaluation of the effects, if any, of the test compound on the phenotype of said cell or population of cells or said tissue, and

iii) determination of the cosmetic or therapeutic efficacy of said test compound, an increase of the activity and/or expression of said RNA sequence being correlated with an efficacy of said test compound.

According to this aspect of the invention, a control value can be established from the level of activity and/or expression of said RNA sequence in a cell or a population of cells expressing said RNA sequence.

In this case, if the activity and/or expression of said RNA sequence present in the cell or population of cells exposed to the test compound are greater than the activity and/or expression of the RNA sequence in the cell or population of cells expressing said RNA sequence, then the test compound has cosmetic or therapeutic efficacy.

The level of activity and/or expression of the RNA sequence can be quantified using techniques known to the skilled person, typically by quantitative PCR or immunohistochemistry (staining by antibodies directed against the genes/proteins regulated by the snoRNA of interest).

The therapeutic effect can also be evaluated, for example, by determining the lifespan of the cells, by measuring the number of cell divisions, etc.

Preferably, the cell or population of cells exposed to the test compound is of intestinal or ovarian origin. Preferred cells can be chosen from among the cells mentioned in this text, typically from among enteroblasts, enterocytes, entero–endocrine cells, intestinal stem cells, nurse cells, typically nurse cells of the ovarian chamber, oocytes, ooblasts, etc.

Other aspects and advantages of the present invention will appear upon reading the figures and examples that follow, which should be considered as illustrative and non–limiting.

LEGENDS OF THE FIGURES

Figure 1: map of the genomic region of locus P[Gal4]4C, position of the *youth* snoRNA and the F4 deletion.

Figure 2: A) H/ACA box motif, B) Pseudouridylation diagram. (Extract from: Kiss et al., (2010) *Molecular Cell,* **37,** 597–606).

Figure 3: Longevity of female flies. Cumulative decrease (in %) of the number of living flies as a function of the number of days. Cont–CS = wildtype control flies. F4= F4 mutant, G5= flies bearing the G5 genomic snoRNA transgene, F4;G5= flies bearing the G5 snoRNA transgene in the F4 mutant genetic background, to restore the wildtype phenotype (in parentheses: the number of flies).

Figure 4: stress resistance test (heat shock, fasting, paraquat) performed on three–day–old mice.

A) Heat Shock: The F4 mutants are more resistant than the controls (females only). Flies overexpressing the snoRNA (G5), as well as F4 mutants provided with the transgene (F4;G5) are more resistant than the controls (males and females).

B) Fasting: The F4 mutants are more resistant than the controls (males and females). Flies over–expressing the snoRNA (G5) are more resistant (females only). F4 mutant female flies provided with the transgene (F4;G5) are similar to the control flies; therefore the wildtype is restored by the transgene.

C) Paraquat: The F4 mutants are more resistant than the controls (males and females). Flies over–expressing the snoRNA (G5) are more resistant (females only). F4 mutant flies provided with the transgene (F4;G5) are similar to the control flies, therefore the wildtype is restored by the transgene.

Figure 5: *In situ* hybridization in the intestine and ovaries.

A) in the wildtype control mice (Canton–S), the snoRNA is expressed in the epithelial cells of the intestine. Blue: DNA staining in the nucleus by DAPI. Red: snoRNA staining. Note that the snoRNA staining (red points) is distinct and complementary to that of the nucleus (blue), demonstrating that this latter is localized in the nucleolus.

B) In the F4 mutant (deletion of the snoRNA), the blue staining is seen (DAPI) but no red staining, because the snoRNA is deleted and therefore not expressed.

C) control flies, wildtype (Canton–S). The snoRNA is expressed in ovarian nurse cells.

D) F4 mutant flies. The snoRNA is not expressed in the ovaries.

Figure 6: Targeted expression of the snoRNA in the epithelial cells of the intestine, via four separate Gal4 driver lines, driving the expression of a reporter gene GFP (UAS–GFP). Detection of the expression of the snoRNA by *in–situ* hybridization (red and/or orange) (left column: a1,b1,c1,d1,e1), staining of the nucleus with DAPI (blue) (second column: a2,b2,c2,d2,e2), expression of GFP by immunostaining (anti–GFP antibody revealed by FITC (green) (third column: a3,b3,c3,d3,e3). Right column (a4,b4,c4,d4,e4) superimposition of the previous three images showing co–localization. Note that there is double staining only at (A) (Myo1A–Gal4), showing that the snoRNA is only expressed in the enterocytes. A) Targeted expression of the snoRNA in enterocytes, under the control of Myo1A–Gal4 (Myo1A–Gal4;UAS–mCD8–GFP). B) Targeted expression in enterocytes by Myo1A–Gal4, in the F4 mutant genetic background (Myo1A–Gal4, F4/F4;UAS–snoRNA–8M/+). C) Targeted expression in the intestine under the control of esg–Gal4 (esg–Gal4,UAS–GFP) staining intestinal stem cells (ISCs). D) Targeted expression in the intestine under the control of Su(H)–Gal4 (Su(H)–GBE–Gal4;UAS–mCDB–GFP), staining enteroblasts. E) Targeted expression in the intestine under the control of Delta–Gal4 (Dl–Gal4,UAS–GFP) staining intestinal stem cells (ISCs).

Figure 7: Targeted expression of the snoRNA in other cell types of the intestinal epithelium via three other separate Gal4 driver lines, driving the expression of the snoRNA (UAS–8M), demonstrating that the snoRNA can be expressed ectopically in other types of cells.

A) Targeted expression under the control of esg–Gal4 (esg–Gal4, F4/F4;UAS–8M/+)

B) Targeted expression under the control of Su(H)–Gal4 (Su(H)–GBE–Gal4, F4/F4;UAS–8M/+).

C) Targeted expression under the control of Delta–Gal4 (Delta–Gal4,F4/F4;UAS–8M/+).

Figure 8: Resistance to various stresses following targeted expression (re–expression of the snoRNA in the F4 mutant genetic background) of the snoRNA in the intestine of three–day old flies.

A) Fasting: Targeted expression of the snoRNA (UAS–8M) under the control of Myo1A–Gal4. There is a clear increase in resistance in flies expressing the snoRNA (Myo,F4/F4; 8M/+) relative to the two control lines.

B) Fasting: Targeted expression of the snoRNA (UAS–8M) under the control of esg–Gal4. There is a clear increase in resistance in flies expressing the snoRNA (esg,F4/F4; 8M) relative to the two control lines.

C) Fasting: Targeted expression of the snoRNA (UAS–8M) under the control of Su(H)–Gal4. There is a clear increase in resistance in flies expressing the snoRNA (Su(H),F4/F4; 8M) relative to the two control lines.

D) Heat shock at 36°C: Targeted expression of the snoRNA (UAS–8M) under the control of Su(H)–Gal4. There is a clear increase in resistance in flies expressing the snoRNA (Su(H),F4/F4; 8M) relative to the two control lines.

Figure 9: A) Homologies in the twelve species of Drosophila whose genome is known; B) consensus structure of the 12 species of Drosophila.

Figure 10: Human homolog on chromosome 11.

Figure 11: 2 homologous genes in *Mus musculus* (mouse–1 and mouse–2).

Figure 12: Brain histology: visible neurodegeneration in the brain of 40–day–old flies. Control flies (Canton–S wildtype) (a1) present neurodegeneration lesions (vacuoles/gaps) while F4 mutant flies (a2) present more lesions. Flies expressing the snoRNA in the mutant genetic background (rescued: F4;G5) (b1) present fewer lesions than the CS and F4, as do flies overexpressing the snoRNA (G5) (b2). F4 mutant flies re–expressing the snoRNA specifically in enterocytes (Myo,F4/F4;UAS–snoRNA) (c2) also have fewer lesions than those not expressing it (Myo,F4/F4) (c1). In (d), quantification of lesions in young flies (4 days old) and old flies (40 days old) for these various genotypes. All these results show that the expression of the snoRNA protects against neurodegenerative lesions in old flies (0, +, ++, +++ = degree of severity of lesions based on their number and area)

Figure 13: A sensorimotor parameter (locomotor activity) quantified by video tracking, represented here by the distance travelled during 7 hours of recording. Forty–day–old flies are compared to young 4–day–old flies. A) Distance travelled by females. B) Distance travelled by males.

Figure 14: Fly fertility (number of eggs laid A) per female/day and B) per female in 17 days). In all, the F4 mutants lay a few more eggs than the controls (CS), but with a slight delay. The expression of the transgene (snoRNA) in the F4 mutant (F4;G4 - also called F4;4M) reduces fertility. Overexpression of the transgene (G4 - also called 4M) increases fertility (number of eggs laid).

Figure 15: Diagram of the intestinal epithelium (Extract from: Jiang and Edgar, *Exp. Cell. Res.*, 2011).

A) Model of intestinal epithelium regeneration in the adult Drosophila.

B) Model of the Notch function in entero–endocrine cells in the Drosophila (embryo, larva, pupa, adult) and in mammals. Dl= Delta, N= Notch, NO = no Notch signal. This diagram clearly shows the perfect similarity between the intestinal epithelium of Drosophila and that of humans.

Figure 16: Retro–control mechanism regulating intestine homeostasis and regeneration (midgut) in the Drosophila (Extract from: Jiang and Edgar, Exp. *Cell. Res.*, 2011). (same abbreviation as Figure 15).

Figure 17: The *youth* snoRNA is involved in the alternative splicing of gene CG9339 (*skywalker*). Comparative RT–PCR was done in some potential target genes in order to see if, in the F4 mutant, some of these genes were not spliced correctly relative to wildtype flies (Control–CS). Among others, 4 transcripts of 345–400–432–462 bp of gene CG9339 (*skywalker*) were analyzed. The rp49 gene (300–bp fragment) is used as an internal control, as well as a control with RNA (with no RT beforehand) in order to demonstrate that the RNA used is not contaminated by traces of DNA. Note the absence of the small 345–bp transcription of gene CG9339 (*skywalker*) in the F4 mutant (arrow).

Figure 18: The *youth* snoRNA is involved in RNA level regulation of two other genes: *klarsicht* and gene CG30502 (*fa2h*). A) For *klarsicht*, amplification of a 464–bp fragment (comprised in an exon) shows that there is less product transcribed in the F4 mutant than in wildtype control flies (Canton–S). B) For *fa2h*, amplification of a 429–bp fragment (comprised in an exon) shows that there is less transcribed in the F4 mutant than in wildtype control flies (Canton–S).

Figure 19: Model describing the role of *klarsicht*. A) *klarsicht*, via its KASH domain, interacts with the lamina, located on the internal surface of the nuclear membrane (according to: Patterson et al., 2004). B) Schematic view showing the structure and function of nuclear lamins. The lamins are located on the internal surface of the nuclear membrane and serve to maintain the stability of the nucleus, organize chromatin and bind nuclear pores (NPC). Several proteins interacting with the lamins are also diagrammed (according to: Coutinho et al., Immunity & Ageing, 2009).

Figure 20: Orthologous mammal sequences of the *youth* snoRNA are expressed in mice and humans (Detection by RT–PCR of mice and human homologs). The expression of these snoRNAs indicates that they are very likely to be functional. In humans, the 159–bp snoRNA (SEQ ID NO: 2) is expressed in the intestine and brain and slightly in the ovaries and kidneys (marked with a red asterisk for more precision). In mice, the 129–bp snoRNA–1 (SEQ ID NO: 3) is expressed only in the brain. In contrast, the 122–bp snoRNA–2 (SEQ ID NO: 4) is expressed in the intestine, brain and ovaries, but not in the kidneys.

Figure 21: Flies mutated for the *youth* snoRNA (F4 mutant) show fat body hypertrophy. Significant alterations thereof are observed in 40–day old elderly flies compared to their respective controls, also 40 days old. These lesions are shown by activated anti–caspase–3 antibody labeling, which labels cells undergoing apoptosis. A) "CS" control flies. The fat body, surrounded by a white dotted line, is fairly homogenous and smooth. B) F4 mutant flies: note large cell aggregates (white arrow). C) In flies bearing the snoRNA transgene (G5): the fat body, without aggregate, resembles the fat body of the "CS" control. D) In flies with the G5 transgene in the F4 mutant genetic background (F4;G5), these flies only show a few aggregates, and these aggregates are smaller,

which indicates that the transgene partially rescues the fat body lesions due to the mutation. These results strongly suggest a disruption in the metabolism of carbohydrates and fats.

EXPERIMENTAL PART

1) Genetic and molecular characterization of snoRNA:Ψ28S–1153 (*youth*) located at locus P[GAL4]4C.

This experiment is part of the search for the neural basis involved in locomotor behavior in Drosophila and the study of the relationship between structure and function of the central complex and especially the ellipsoid body. In this context, screening a library of P[GAL4] enhancer trap lines permitted identifying the P[GAL4]4C line that is specifically expressed in the ellipsoid body (Figure 1). Different genetic approaches, notably the targeted expression of tetanus toxin, have shown that blocking these neurons generates defects in locomotor activity (Martin et al., 2002). In a second step, in order to further characterize these neurons in detail and better determine their function within the neural network involved in locomotor activity, the insertion locus of the P[GAL4]4C line was characterized genetically and molecularly. PCR–rescue was done and permitted showing the insertion point of the P–element of P[GAL4]4C on chromosome 2R (Right: i.e., on the right arm), in position 50A, between two genes: CG13333 and CG13334 (Figure 1). Then, in order to be able to show the respective phenotype and function of these two genes, mutations were generated in them by re–excision of the P–element (by a genetic approach called: jump–out, or revertant). A small deletion of 632 base pairs (bp) named F4 was thus obtained (Figure 1).

snoRNA:Ψ28S–1153 was identified at this locus in position 67331 (Figure 1) as part of the systematic screening done by Huang et al. (2005) of all the potential small nucleolar RNA (snoRNA) in the Drosophila genome. The corresponding article, however, does not distinguish any particular snoRNA and does not associate any function with snoRNA:Ψ28S–1153. In the context of the present invention, this snoRNA, absent the small F4 deletion, has been precisely positioned and characterized structurally and functionally. This snoRNA is thus made up of 148 base pairs (bp) and its structure comprises an H/ACA box.

2) Phenotypic characterization of the F4 deletion: reduction of lifespan.

At the same time as the study done to quantify the locomotor activity of the flies via the targeted expression of tetanus toxin in labelled ring neurons of the P[GAL4] 4C line, the inventor observed that these flies had a very short lifespan and decided to precisely quantify said life span of the P[GAL4]4C/UAS–tetanus–toxin flies, as well as that of the F4 flies (mutated at locus 4C). Thus the

F4 flies have a short lifespan (of around 30% relative to wildtype control flies), suggesting that the snoRNA deletion could affect lifespan (longevity) (Figure 3). The inventors also observed that this effect is different according to the sex of the flies, the effect being much more pronounced in females than in males.

3) Genesis of a transgenic line containing the genomic region of the snoRNA (*youth)* for purposes of restoring the wildtype phenotype ("rescue").

In order to demonstrate that the phenotype of the F4 mutant is actually due to the deletion of the snoRNA, a line of transgenic Drosophila bearing a genomic DNA sequence of 1723 bp of the region (from 66377 to 68100), comprising the snoRNA (Figure 1), and its regulator sequences, was generated. More precisely, a genomic fragment from the 1723–bp region was amplified by PCR and inserted via the Xba1 restriction site into the pCaSper–4 vector (this vector not containing any promotor/regulator sequence). Transgenic flies were then generated according to a standard technique and lines of flies expressing the same transgene, but inserted in distinct places of the genome, were obtained (independent insertions: G4, G5).

Next, in order to verify that the transgene is functional and can rescue the F4 mutation (i.e., restore the wildtype phenotype, that is restore a lifespan equivalent to that observed with no F4 deletion), the inventor introduced these transgenic lines in the context of the F4 genetic mutant, by standard genetic crosses (F4;G4 and F4;G5) It could thus be demonstrated that the transgene could rescue the phenotype due to the mutation responsible for the reduction in lifespan (Figure 3) (see, for example: F4 versus F4;G5). Moreover, this transgene placed in a wildtype genome (normal) (which corresponds to an overexpression of this snoRNA, since there are now 4 copies of the snoRNA instead of the two endogenous copies) (G5) increases the lifespan (even doubles the lifespan in the case of F4;G5 or increases it by around 30% for G5) (Figure 3). This experiment thus clearly shows that the lifespan of the animal can be increased by overexpressing this snoRNA (or by modifying its expression level). In summary, a small genomic deletion in the region (632 base pairs, called F4) (Figure 1), corresponding or equivalent to mutation of this gene, shortened lifespan, while overexpression (via a transgene containing genomic DNA of this snoRNA) by gene therapy not only rescues the same mutation but dramatically extends (doubles) the lifespan of the subject treated (Figure 3).

4) Stress resistance tests

It is now generally acknowledged that genes acting on longevity generally increase stress resistance. The inventor has verified and obtained confirmation that overexpression of this snoRNA

is effectively able to increase the lifespan of the subject concerned under stress conditions, such as fasting, heat shock and oxidative stress (induced by paraquat) (Figure 4).

A) heat resistance test (heat–shock test).

Males and females are raised together in standard tubes containing feed for 3 days. At age 3 days, the males and females are distributed separately by group of 20 in a standard tube containing feed. To subject the flies to heat shock, tubes containing the flies are placed in an incubator at 36°C. The number of dead flies is counted every 6 hours. For heat shock (36°C), in Figure 4A, note that F4 mutant flies (females only) are more resistant than the control flies. However, the G5 flies (male and female) are more resistant than the controls and the F4 mutant, while as a result of the expression of the G5 transgene in the F4 mutant (F4;G5 flies) these flies are also much more resistant than the control flies and the F4 mutant flies.

B) fasting test

Like the heat resistance test, males and females are raised together in standard tubes containing feed for 3 days. At age 3 days, the males and females are distributed separately by group of 20 into a tube containing a filter paper and 400 µL of water in order to prevent desiccation. The flies are kept in a humid room at 24°C, and the number of dead flies is counted every 6 hours. For fasting, in Figure 4B, note that the F4 mutant flies (males and females) are more resistant than the control flies. Moreover, the G5 flies (female) are still more resistant, while the expression of the transgene in the F4 mutant (F4;G5) restores normal survival (in females).

C) resistance to oxidative stress (paraquat)

Paraquat (1,1'–dimethyl–4,4'–bipyridinium dichloride) reduces NADH, which generates stable paraquat radicals, which react with oxygen to generate ROS (reactive oxygen species). Consequently, the ROS cause cell damage (Rzezniczak et al., 2011). Like for the previous tests, males and females are raised together in standard tubes containing feed for 3 days. At age 3 days, the males and females are distributed separately by group of 20 into an empty standard tube in order to fast them for 6 hours. Next, the flies are transferred into a tube containing a filter paper and 450 µl of 20 mM paraquat diluted in 1% sucrose to promote dietary intake. The flies are kept in a humid room at 24°C, and the number of dead flies is counted every 6 hours.

For oxidative stress, in Figure 4C, note that the F4 mutant flies (females only, although there is also a strong trend for males) are more resistant than the control flies. Similarly, the female G5 flies are more resistant than the controls and similar to the F4 mutants, while the expression of the transgene in the F4 mutant (F4;G5) restores survival of the flies (equivalent to the control flies).

In summary, the F4 mutant flies are more resistant than the control flies, while overexpression (G5) further increases this resistance (an effect that is more marked and consistent in females than in males). Moreover, in both tests (fasting and oxidative stress) the expression of the transgene in the

F4 mutant (F4;G5) restores fly survival, especially in females. Thus, contrary to longevity where the F4 mutation reduces lifespan, the F4 mutation increases resistance in the three stress tests done in young three–day–old flies, and this effect can be restored by the genomic transgene of the snoRNA (in two tests). In conclusion, modulation of expression (suppression, reduction or increase) of the *"youth"* snoRNA changes the lifespan of the subjects tested.

5) Determining the space–time expression profile of the snoRNA (*in situ* hybridization)

In order to determine in what cells and/or tissues the snoRNA of interest is expressed and acts, the space–time expression profile of this snoRNA was determined in the context of the invention, in the adult fly, by *in situ* hybridization (HIS), using an anti–sense snoRNA. Tyramide was used to amplify labelling. In both males and females, the *youth* snoRNA is expressed in the intestine wall (epithelium) (Figure 5A), while, as expected, it is absent in the F4 mutant (Figure 5B). More specifically, the use of P[Gal4] lines specific for various cell types of the intestine, combined with double labelling, demonstrated that the *youth* snoRNA is expressed specifically in the nucleolus of enterocytes, the main and majority cells forming the intestinal epithelium (Figure 6A), while it is not expressed in other cell types (Figure 6B, C, D, E). Furthermore, in females, it is also expressed in the ovaries, and more particularly in nurse cells (Figures 5C and 5D).

6) Targeted expression of snoRNA in cell types other than intestine epithelium.

Targeted expression (ectopic) of snoRNA (UAS–8M) in types of cells other than intestine epithelium via the use of three other distinct Gal4 driver lines was done: esg–Gal4 (esg–Gal4,F4/F4;UAS–8M/+) and Delta–Gal4 (Delta–Gal4,F4/F4;UAS–8M/+) targeting intestinal stem cells (ISCs), while Su(H)–Gal4 (Su(H)–GBE–Gal4, F4/F4; UAS–8M/+) target enteroblasts (Figure 7). These results demonstrate: 1) that the snoRNA can be expressed ectopically in other cell types, 2) that this ectopic expression also increases the resistance to certain stress (Figure 8). In summary, these results show that the snoRNA can also confer protection when it is expressed in cell types other than intestine epithelium.

7) Targeted (selective) expression of the snoRNA leading to rescue of stress resistance

The inventor has demonstrated, by two independent approaches, that a targeted expression in intestine cells is sufficient to restore the stress resistance phenotype. It could be demonstrated, by the *in situ* hybridization technique, done from genomic transgenic lines (those increasing longevity: G5, as well with another independent insertion (G4) that the snoRNA is expressed in intestinal cells and that, for regulation of longevity, the expression of the snoRNA in intestinal cells is sufficient.

In order to confirm this first result, the snoRNA was targeted only in intestinal cells, by using the P[Gal4] binary expression system. A plasmid vector (p[UAS–snoRNA]) was constructed in which the snoRNA of interest (only 148 bp) is placed under the control of regulator elements (Upstream Activating Sequence: UAS) of Gal4. Transgenic fly lines (UAS–snoRNA: 4M, 5M and 8M) were then generated. For targeted expression, so–called "driver" lines of transgenic flies containing a transgene (pChs–Gal4) (plasmid vector lines Myo1A–Gal4, esg–Gal4 (escargot–Gal4), Su(H)GBE–Gal4, and Dl–Gal4 (Delta–Gal4)), known to be expressed in particular in intestinal cells were used (Jiang and Edgar, 2011; Takashima et al., 2011). Next, these various transgenes were placed in the F4 mutant genetic background, and it was demonstrated, by *in situ* hybridization, that the snoRNA is actually expressed in these various types of intestinal cells (Figures 6 and 7) after its targeted expression (esg–Gal4,F4/F4;UAS–snoRNA–8M) (Myo1A–Gal4,F4/F4;UAS–snoRNA–8M) (Su(H)–GBE–Gal4,F4/F4;UAS–snoRNA–8M) (F4/F4; Delta–Gal4,/UAS–snoRNA–8M). The results show that this targeted expression (in the F4 mutant) substantially restores the stress resistance phenotype (fasting and heat shock) (Figure 8).

These experiments clearly show that manipulating these snoRNAs in intestinal cells, and more particularly in enterocytes, is necessary and sufficient to restore stress resistance. These experiments, although conducted in Drosophila, permit suggesting that re–expression (restoration of expression or rescue) or overexpression of the snoRNA in epithelial cells of the intestine is able to lead to an increase in lifespan in mammals and in particular in humans.

8) Identification of homologs of the *youth* sequence in other species of Drosophila and in mammals, including mice and humans.

Sequence homology searching showed that these snoRNA also exist in the 11 other species of Drosophila whose genome is available (Figure 9A) (see also the consensus structure of 12 species of Drosophila: Figure 9B).

Moreover, a homology search and especially via the RNA structure (using INFERNAL software) allowed identifying a homolog in humans, located on chromosome 11, at position: 12822722–12822811 (Figure 10). In mice, two homologs have also been identified: on chromosome 15, position: 30336889–30337017; and on chromosome 18, position: 6495012–6495091 (Figures 11A and 11B).

Like in Drosophila, it is very probable that the overexpression of this gene and/or a synthetic analog (either genetically or by oral administration, or by injection) will extend the human lifespan. Such expression is also able to protect against the harmful effects of various degenerative diseases.

9) Role of the snoRNA in neuro–degeneration and neuro–protection

Since F4 flies have a very short lifespan, while flies overexpressing the snoRNA (G5) live longer, the old flies (40 days old) were studied in order to check for the presence of any neurodegenerative lesions (more or fewer holes of larger or smaller size present in the brain). Overall, in the control flies (Canton–S), around 60% of flies present lesions (but note that 50% of the CS flies are already dead at 40 days) (Figure 12) [for further detail: see Figure 12d for a semi–quantitative analysis]. In F4 mutants, all the flies (100%) have lesions, and they are generally more severe than those of controls (size and number of holes), while F4;G5 flies have significantly fewer holes (only about 10% of the flies), these holes being also of smaller size. Finally, in the G5 flies, around the same percentage of flies as the CS controls present lesions (around 40%) but these lesions are clearly less severe (Figure 12d). In summary, the genomic transgene (F4;G5) partially rescues the neurodegeneration phenotype, while overexpression of the snoRNA (G5) protects against neurodegeneration.

10) Role of the snoRNA in protection of sensorimotor parameters.

Similarly, in order to ascertain whether the neuroprotection provided by the snoRNA has effects on physiological parameters such as sensorimotor parameters, the locomotor activity of flies older than 40 days was quantified (by video–tracking) (Martin, 2004) and compared to those of young flies, aged 4 days (Figure 13). Firstly, an enormous difference between the 4–day–old flies and the 40–day–old ones is observed; the 40–day–old flies travel a third of the distance of that travelled by the 4–day–old flies. However, in the elderly 40–day–old flies, the flies that overexpress the snoRNA (G5) walk more than the control flies (Canton–S), F4 mutants, and those expressing the snoRNA in the genetic mutant background (F4;G5). This effect is more marked in males than in females. In summary, in elderly 40–day old flies, we see that the flies overexpressing the snoRNA have better sensorimotor performance than control flies. These results demonstrate the protective nature of the snoRNA relative to the harmful effects associated with mechanisms of aging.

11) Testing the function of the snoRNA in the ovaries: role in fertility (reproduction)

In females, the snoRNA is also expressed in the ovaries and more precisely in nurse cells (Figures 5C,D). The inventors have also shown that mutant flies (F4) also have fertility changes (reproducer phenotype). The F4 mutants lay a few more eggs than the control flies (CS) (Figure 14). The flies bearing the snoRNA in the mutant genetic background (F4;G4) lay many fewer eggs (reduced fertility) while flies overexpressing the snoRNA (G4) lay many more eggs than the control flies (Figure 14). These results undeniably demonstrate the important effect exerted by the snoRNA in the ovaries and its influence on fertility in females.

12) Treatment with the snoRNA (administration by oral route or injection).

The snoRNA can be administered either orally (*per os*) or by injection. Other administration routes such as, for example, inhalation and local/cutaneous application can also be used depending on the type of vector used.

As the experiments conducted as part of the invention show, oral administration of the snoRNA is possible given its stability and *in vitro* resistance as well as its ability to act locally on the cells of the intestinal wall (enteroblasts, enterocytes, ISCs). As explained previously, the administration of the snoRNA of interest is therefore able to permit expression that would otherwise be absent (or, in other words, to rescue an existing mutation) or, if necessary, permit overexpression, in order to protect the intestinal wall from damage while maintaining a better hormonal and metabolic equilibrium.

13) Cancer treatment.

In both mammals and Drosophila, it was shown that the *delta/notch* genes regulate the differentiation of intestine (gut) progenitor cells, while the Wnt signalling pathway participates in maintenance and proliferation of ISCs. Likewise, the cytokine signalling pathways (Upd/Jak/Stat) and the EGFR (Epidermal Growth Factor Receptor) pathway regulate the proliferation of ISCs (Figures 15–16) (Jiang and Edgar, 2011; Takashima et al., 2011).

Our experiments show that the snoRNA of interest according to the invention is able to regulate certain genes, such as *notch*, *delta, JNK, EGFR,* etc. (Figure 15) whose deregulation could lead to hyperproliferation of the intestinal epithelium and therefore to cancer (Jiang and Edgar, 2011; Takashima et al., 2011). The SnoRNA of interest of the invention is particularly able to prevent or treat cancer through its ability to regulate the expression of EGFR, the EGFR signaling pathway in the regulation of proliferation of ISCs in the intestine of Drosophila and mammals being, in fact, particularly well conserved (Figure 16) [several therapies are currently under clinical trial for the treatment of colon cancer, including two anti–EGFR monoclonal antibodies (*cetuximab* and *panitumumab*) (Amado et al., 2008; Di Nicolantonio et al, 2008)].

14) Involvement of the snoRNA of interest ("*youth*") in Hutchinson–Gilford syndrome (HGPS), also known as progeria, which consists in early and accelerated aging in humans.

We have demonstrated that the *youth* snoRNA regulates splicing (quantity of spliced RNA) of the *klarsicht* gene (Figure 18A). It has been shown that this gene interacts with the lamina (Patterson et al., 2004), a protein located in the inner membrane of the nucleus and involved in progeria (disease causing early and accelerated aging in humans) (Figure 18B). Progeria is a rare disease affecting 1

in 8 million individuals (Scaffidi and Misteli, 2006; Broers et al., 2006; Cohen et al., 2001; Cau et al., 2014; Coutinho et al., 2009). The *youth* snoRNA provides, through its phenotype (involvement in longevity) and its involvement in the regulation of the *klarsicht* gene, a novel prospect for treating laminopathies such as Hutchinson–Gilford syndrome or progeria, mandibuloacral dysplasia (MAD), Emery–Dreifuss muscular dystrophy, atypical Werner syndrome, restrictive dermatopathy, lethal fetal akinesia, and LIRLLC (Generalized lipoatrophy, insulin–resistant diabetes, leukomelanodermic papules, liver steatosis, and hypertrophic cardiomyopathy) (Hutchison, 2002; Broers et al, 2006), in particular Hutchinson–Gilford syndrome (progeria).

15) Involvement of the snoRNA of interest (*"youth"*) in the regulation of fatty acid 2–hydroylase (*fa2h*) gene splicing, and consequently with the various forms of neurodegeneration associated with this gene.

We have demonstrated that the *youth* snoRNA regulates splicing (quantity of RNA spliced) of gene CG30502 coding for fatty acid 2–hydroxylase (*fa2h*) (Figure 18B). This gene is involved in the process of fatty acid biosynthesis and more particularly in the metabolic processes of complex lipids such as sphingolipids and ceramides (Carvalho et al., 2010). In humans, this *fa2h* has been associated with various forms of neurodegeneration (demyelinization) such as those associated with iron accumulation in the brain, certain leukodystrophies, and finally hereditary spastic paraplegia (SPG35) (Kruer et al., 2010; Pierson et al., 2012; Schneider and Bhatia, 2010). Due to its phenotype (involvement in longevity and/or fat body hypertrophy observed in the F4 mutant) and its involvement in the regulation of the *fa2h* gene, the *youth* snoRNA offers a novel prospect for treatment of the neurodegenerative diseases mentioned above.

16) Metabolic and neuro–endocrinal relationship between the intestine and the brain (brain–gut axis)

As mentioned previously, snoRNA mutants (F4) present fat body hypertrophy, visible both at the abdomen and around the brain in the head capsule (Figure 21). Furthermore, triglyceride quantification confirmed this hypertrophy (hence the "obese" qualifier of the flies). This phenotype suggests a metabolic and/or neuroendocrine relationship between the expression of the snoRNA in the intestine, neurodegenerative lesions and increased lifespan. More specifically, this metabolic disruption suggests an involvement of the insulin signalling pathway, this pathway being described many times as involved in Drosophila longevity (Tatar et al., 2001; Bai et al., 2012; Partridge et al., 2011; Fontana et al., 2010). Immunohistochemical markers directed against activated anti–caspase–3 have shown, in adult Drosophila, that the fat located around the perimeter of the brain is greatly disrupted (i.e. hypertrophied) in F4 mutants, while is protected by overexpression of the snoRNA

(transgenes G5) as well as in the rescue (restoration of expression) (F4; G5) (Figure 21). These results demonstrate that the snoRNA protects the organism that expresses it from changes in the fat body, and therefore, by extension, from obesity. Furthermore, genetic interaction experiments (double mutants) involving insulin receptor (InR) mutations and the F4 mutant show that mutation of the snoRNA can at least partially rescue the phenotype of the insulin receptor mutation (see Figure 21 and its caption). These results show the existence of links (whether direct or indirect) between the insulin signalling pathway, the snoRNA and longevity. The preceding results show that mutation and/or deregulation of the snoRNA disrupts carbohydrate and lipid metabolism. The *youth* snoRNA therefore offers a novel prospect for treating both diabetes and obesity.

17) Expression of the snoRNA of interest ("youth") in humans and mice.

By a RT–PCR approach, we have demonstrated the existence of homologous sequences, more precisely orthologous sequences, to the *youth* snoRNA (first identified in the Drosophila) concretely expressed in mammals (Figure 20). In humans, the 159–bp snoRNA (SEQ ID NO: 2) is expressed in the intestine and brain and slightly in the ovaries and kidneys. In mice, the 129–bp snoRNA–1 (SEQ ID NO: 3) is expressed only in the brain and the 122–bp snoRNA–2 (SEQ ID NO: 4) is expressed in the intestine, brain and ovaries, but not in the kidneys. These data support the expression, very likely to be functional, of these snoRNAs in both humans and mice.

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CLAIMS

1. Isolated or synthetic RNA sequence comprising sequence SEQ ID NO: 1 or a SEQ ID NO:1 orthologous sequence for use as a medicament.

2. RNA sequence for use according to claim 1, characterized in that said orthologous sequence is of human origin and consists in sequence SEQ ID NO: 2 or is of mouse origin and consists in a sequence chosen from among SEQ ID NO: 3 and SEQ ID NO: 4.

3. RNA sequence for use according to claim 1 or 2, characterized in that said sequence is a small nucleolar RNA (snoRNA).

4. RNA sequence for use according to claim 1, characterized in that said functionally–analogous sequence permits splicing a *Drosophila melanogaster* gene chosen from among Ir56d, buttonhead, klarsicht, CG3262, CG30502 (*fa2h*), CG11125, CG9339 and CG40006.

5. DNA sequence coding for an RNA sequence according to one of claims 1 to 4 for use as a medicament.

6. Vector allowing the expression of an RNA sequence according to one of claims 1 to 4 for use as a medicament, wherein the vector is chosen, for example, from among a plasmid, a cosmid, a viral vector and a phage, and wherein the vector preferably comprises a promotor and/or regulator elements promoting the expression of said RNA sequence in intestinal or ovarian cells.

7. Cell comprising a RNA sequence according to one of claims 1 to 4 for use as a medicament or cell transformed using a vector as described in claim 6 for use as a medicament.

8. Composition comprising a RNA sequence for use according to one of claims 1 to 4, a vector for use according to claim 6 or a cell for use according to claim 7 and a dietarily- or pharmaceutically–acceptable support.

9. Use of a RNA sequence according to one of claims 1 to 4, of a vector according to claim 6, of a cell according to claim 7 or of a composition according to claim 8, continuously or sequentially, to restore or modulate the expression of said RNA sequence *in vitro* or *ex vivo*.

10. Transgenic mouse in the genome of which one and/or the other of sequences SEQ ID NO: 3 and SEQ ID NO: 4 have been modified to prevent or modify the expression of one and/or the other of said sequences.

11. Use of the RNA sequence according to one of claims 1 to 4 in the preparation of a medicament for the prevention or treatment of a neurodegenerative disease induced by mutation of the fah2 gene, a laminopathy, diabetes, obesity or cancer in a subject.

12. The use according to claim 11 wherein the cancer is a cancer induced by free radicals.

13. Use of the RNA sequence according to one of claims 1 to 3 in the preparation of a medicament for the treatment of sterility or infertility in a subject.

14. The use according to one of claims 11 to 13, characterized in that the subject is an animal or insect, typically a mammal, preferably a human, even more preferably a subject not expressing the RNA sequence according to one of claims 1 to 3 or expressing an abnormal version of said RNA sequence, and/or a subject suffering from a neurodegenerative disease induced by mutation of the fah2 gene, a laminopathy, diabetes, obesity, a cancer or a fertility problem.

15. The use according to claim 14, characterized in that the genome of the subject, not expressing the RNA according to one of claims 1 to 3 or expressing an abnormal version of said RNA sequence, contains a DNA sequence comprising a mutation, said sequence being in the wild state chosen from among SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8; and said mutation being preferably chosen from among a deletion, substitution or addition of one or more base pairs.