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Cause or effect: misregulation of microRNA pathways in neurodegeneration

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INTRODUCTION
Alzheimer’s disease (AD), frontotemporal dementia (FTD), Parkinson’s disease (PD), and other neurodegenerative disorders are a major health problem in both developed and developing countries (Hampel et al., 2011; Reitz et al., 2011; Wittchen et al., 2011). Since no effective treatments are available, it is unlikely that the adverse societal effects of these disorders will be substantially alleviated in the near future. These disorders are characterized by progressive neuronal dysfunction that initially affects selected groups of neurons in specialized neuronal circuits. A number of cellular and molecular mechanisms lead to neuronal demise. Among them, neurotoxicity induced by misfolding, mislocalization, or abnormally elevated concentrations of particular protein species seems to be a common theme (Jucker and Walker, 2011; Lee et al., 2012; Selkoe et al., 2012). Mutations in several genes that are apparently functionally unrelated can cause the same neurodegenerative disease. Multiple environmental factors (e.g., viral infections and exposure to certain toxins) might also contribute to the development of a neurodegenerative disease (Ahmed and Wicklund, 2011; Gao and Hong, 2011). Much remained to be learned about how the complex interactions of environmental and genetic factors initially lead to the misregulation of protein homeostasis and subsequently to neuronal dysfunction.

MicroRNAs (miRNAs) are small non-coding RNAs that post-transcriptionally regulate gene expression by degrading their target mRNAs or repressing their translation (Ambros et al., 2003; Bartel, 2004; Ghidiali and Zamore, 2009). Initially discovered in Caenorhabditis elegans (Lee et al., 1993), miRNAs have been found in plants, invertebrates, mammals, and humans (Bartel, 2009). Two major features of miRNAs indicate their potential contributions to neurodegenerative diseases. First, miRNAs can simultaneously regulate many target transcripts, and up to 50% of all coding genes may be regulated by miRNAs (Krol et al., 2010). Thus, miRNAs are central regulators of genetic networks. Second, miRNAs ensure stable protein levels under variable conditions and are therefore essential for the robustness of biological processes (Herranz and Cohen, 2010). Given the importance of protein homeostasis and the diversity of cellular pathways potentially leading to neurodegeneration, it has been hypothesized that miRNAs might contribute to neurodegenerative diseases (Eacker et al., 2009; Lau and de Strooper, 2010; Sonntag, 2010).

In this review, we briefly describe the biogenesis of miRNAs and their potential involvement in the evolution of the human brain. Then we will discuss accumulating evidence that miRNAs are important contributors to neurodegenerative diseases. Indeed, some observations suggest that miRNA alterations can disrupt protein homeostasis and may be at the root of neurodegenerative processes (Figure 2). Conversely, other data strongly suggest that altered miRNA networks are a consequence of abnormal neuronal physiology (Figure 3). Examples in specific neurodegenerative diseases will be presented.

miRNA BIOGENESIS
Although several alternative mechanisms also exist (Yang and Lai, 2011), the canonical pathway for miRNA biogenesis involves a primary transcript generated by RNA polymerase II (Lee et al., 2004). The primary miRNA is cleaved by a nuclear complex containing Drosha and DGCR8, giving rise to a hairpin precursor molecule (pre-miRNA) of 70–100 nt (Lee et al., 2003; Han et al., 2004). This pre-miRNA is exported to the cytoplasm, where Dicer, a RNA-III nuclease, catalyzes the final cleavage in the maturation process, resulting in an imperfect RNA duplex (Hutvagner et al., 2001). One strand (guide strand) is loaded into an RNA-induced silencing complex (RISC) to bind the target mRNA; the other strand (passenger strand) is usually destroyed (Chendrimada et al., 2005; Bartel, 2009). miRNAs control gene expression at
the post-transcriptional level through imperfect base pairing with specific sequences, located mostly in the 3’UTRs of mRNAs. After recognition, miRNA–target interactions often result in mRNA degradation or inhibition of mRNA translation (Krol et al., 2010; Figure 1).

ROLE OF miRNA IN BRAIN EVOLUTION

Neurodegenerative diseases are considered devastating disorders because they often impair cognitive and executive functions. Understanding how the human brain acquired such functions is a challenging task that might also provide important insights into the mechanisms of neurodegeneration. It was noted four decades ago that genetic differences among species do not account for brain divergence and that brain evolution could have been driven by changes in gene expression levels (King and Wilson, 1975). Early comparative transcriptome analyses confirmed this hypothesis and revealed more human-specific than chimpanzee-specific expression changes in the prefrontal cortex (PFC); no such changes were observed in blood, liver, or other tissues (Enard et al., 2002; Caceres et al., 2003). These findings supported the idea that brain-specific changes in gene expression levels shaped the evolution of the nervous system.

To determine whether miRNAs participated in this process, miRNA expression profiles in human and chimpanzee brains were compared (Berezikov et al., 2006). Many of the newly identified brain miRNAs were expressed only in humans, and many were restricted to primates. Another study reported that 10–35% of miRNAs were expressed in the human brain but not in chimpanzee or macaque brains (Hu et al., 2011). More importantly, developmental profiles of brain miRNAs and their target genes showed the fastest rates of human-specific evolutionary change (Somel et al., 2011). Although experimental evidence is still lacking, it is an attractive hypothesis that rapidly evolving miRNAs in the human brain are essential for neuronal function and maintenance.

GLOBAL LOSS OF miRNAs CAUSES NEURODEGENERATION

Genetic disruption of miRNAs biogenesis pathways has been used to probe the potential link between miRNAs and neurodegeneration. In mouse cerebellar Purkinje cells, conditional knockout of Dicer leads to age-dependent cerebellar degeneration and ataxia (Schaefer et al., 2007). Cell-type specific deletion of Dicer in striatal, retinal, spinal, and cortical neurons produced similar results (Cuellar et al., 2008; Damiani et al., 2008; Davis et al., 2008; Haramati et al., 2010). Dicer deletion also altered the phosphorylation pattern of tau before neuronal cell loss (Hebert et al., 2010), indicating that some mechanisms of neurodegeneration might be controlled through miRNAs. Of note, neurodegeneration in the absence of Dicer could also result from the toxic accumulation of pre-miRNAs or from the loss of other Dicer functions unrelated to miRNAs biogenesis. However, reduced production of a small proportion of miRNAs as a result of DGCR8 haploinsufficiency also leads to neuronal dysfunction (Stark et al., 2008; Fenelon et al., 2011; Schofield et al., 2011), supporting the notion that neurodegeneration could indeed arise from loss of miRNAs.

Glial cell defects may also profoundly influence neuronal survival (Ilieva et al., 2009; Prinz et al., 2011). Indeed, neurodegeneration ensues after targeted deletion of Dicer in astrocytes (Tao et al., 2011), oligodendrocytes (Shin et al., 2009), and Schwann cells (Pereira et al., 2010; Verrier et al., 2010; Wu et al., 2012). Thus, alteration of miRNA networks has the potential to disrupt neuronal function in a cell-autonomous or non-cell-autonomous manner and lead to neurodegenerative phenotypes.

LOSS OF INDIVIDUAL miRNAs LEADS TO NEURODEGENERATION

Genetic analyses have revealed essential roles for specific miRNAs in long-term neuronal survival, for example in mice lacking miR-124-1 (Sanuki et al., 2011), one of the most well studied miRNAs in neuronal development (Gao, 2010). The mouse genome has three miR-124 loci (miR-124-1, -2, and -3). Deletion of miR-124-1, the dominant source of this miRNA, increases apoptosis in the hippocampus and retina, causing a significant decrease in brain size. Furthermore, in the tail-suspension test, adult mutant mice exhibit a front and hind limb claspins response, a common phenotype in mouse models of neurodegenerative disorders. This effect seems to be mediated by regulation of the transcription factor Lhx2 (Sanuki et al., 2011). It is unclear whether the neurodegeneration also reflects the absence of miR-124’s well-documented developmental functions (Cao et al., 2007; Viswanathan et al., 2007; De Pietri Tonelli et al., 2008; Cheng et al., 2009; Maiorano and Mallamaci, 2009). This question could be answered by using a conditional knockout approach.

Another miRNA that might be involved in neuronal survival is miR-8 (Karres et al., 2007), which is not brain-specific and has a complex pattern of expression. Mutant flies lacking miR-8
have limb and wing defects and increased apoptosis in the central nervous system. Moreover, concomitant decrease of the transcriptional repressor atrophin in miR-8 mutant flies partially rescued the phenotypes (Karres et al., 2007). It remains to be determined whether miR-8 contributes to neurodegeneration in mammals.

### miRNAs IN NEURODEGENERATIVE DISEASES

Microarray studies have shown that the brain expresses a wide range of miRNAs, suggesting that these small RNA molecules participate in nervous system physiology (Lagos-Quintana et al., 2002; Miska et al., 2004; Lim et al., 2005; Manakov et al., 2009). More importantly, profiling studies revealed profound changes in several miRNAs (i.e., miR-9, miR-29 cluster, miR-107, miR-125b, and miR-128) in patient brains such as that of AD patients (Kim et al., 2007; Lukiw, 2007; Hebert et al., 2008; Johnson et al., 2008; Wang et al., 2008, 2011; Nunez-Iglesias et al., 2010). Although these global changes in miRNA expression under pathological conditions should be interpreted cautiously, they support the notion that dysregulation of miRNAs networks is a common theme in neurodegenerative diseases.

### ALZHEIMER’S DISEASE

A pathological hallmark of AD, the most prevalent neurodegenerative disease, is the accumulation of plaques formed by short β-amyloid (Aβ) peptides, commencing in the hippocampus, and spreading progressively throughout the brain (Ballard et al., 2011; Selkoe et al., 2012). It is likely the accumulation is caused by both increased production and impaired clearance of Aβ. Aβ peptides exert toxic effects and elicit an inflammatory response. Both may contribute to disrupted neuronal homeostasis and alter the integrity of neuronal networks involved in learning, memory, and other cognitive functions. Aβ peptides are generated through proteolytic cleavage of the amyloid precursor protein (APP) by γ-secretase and β-site APP-cleaving enzyme 1 (BACE1; O’Brien and Wong, 2011).

Amyloid precursor protein and BACE1 each contain several miRNA target sites in their 3’UTRs. Several miRNAs have been reported to repress APP expression, including miR-16, miR-101, miR-106a, and miR-520c (Patel et al., 2008; Liu et al., 2010; Long and Lahiri, 2011). miR-137 and miR-181c regulate serine palmitoyltransferase, which modulates the Aβ level (Geckiyanage and Chan, 2011). Interestingly, polymorphisms in miRNA binding sites in the 3’UTR of APP gene could influence the binding efficiency of these miRNAs. Thus, miRNAs might fine tune APP expression, which may enhance or limit the risk of AD (Delay et al., 2011).

A significant decrease in neuronal miR-107 expression and a parallel increase in BACE1 have been observed in AD patients (even those at the earliest stages of AD; Wang et al., 2008, 2011; Nelson and Wang, 2010). The 3’UTR of BACE1 miRNA has functional binding sites for miR-29 (Hebert et al., 2008), miR-107 (Wang et al., 2008), and miR-124 (Fang et al., 2012). Interestingly, miR-107 also controls the expression of other proteins relevant to AD pathology, such as coflin (Yao et al., 2010), an actin-binding protein that accumulates in cytoplasmic inclusions known as Hirano bodies (Hirano, 1994). Thus, a single miRNA deregulation could activate multiple potentially pathogenic cascades upstream of Aβ accumulation.

Another possibility is that alterations in miRNAs in AD brain are a consequence of amyloid deposits. For instance, miR-106b is aberrantly expressed in APPswe/PSE9 mice (Wang et al., 2010a), and miR-146a levels are increased in AD brains and in several mouse models of AD (Li et al., 2011). In vitro exposure of hippocampal neurons to Aβ peptides preferentially decreases the levels of mature miRNAs (only a small fraction of miRNAs were upregulated; Schonrock et al., 2010). miRNAs were similarly deregulated in the hippocampus of APP23 mice at the onset of plaque formation. Overall, these studies suggest that miRNA deregulation is an essential pathogenic mechanism that is induced by Aβ aggregation and contributes to the progression and severity of AD.

### POLYGLUTAMINE DISEASES

Polyglutamine (polyQ) diseases are a group of nine neurodegenerative disorders caused by an unstable CAG expansion in the coding region of their respective associated genes (Orr and Zoghbi, 2007). Apart from this common feature, polyQ diseases have distinct clinical presentations, and the proteins involved in these diseases have no structural-functional homology (Table 1). Huntington disease (HD), the most frequent polyQ disease, is characterized by the progressive loss of striatal neurons and motor impairment (typically resulting in the involuntary writhing movements called chorea) and is often associated with cognitive and behavioral deficits (Shoulson and Young, 2011).

The causal mutation in HD is an expanded repetition of the CAG trinucleotide in the first exon of the gene encoding huntingtin (HTT; Gilliam et al., 1987), a large protein (3300 amino acids) whose functions remains mostly unknown. HTT associates with Ago2 in P-bodies, and HTT deletion impairs miRNA-mediated gene silencing (Savas et al., 2008). Expanded HTT may sequester RNA processing factors in the cytoplasm (Jiang et al., 2011).

miRNAs were implicated in HD pathogenesis by two lines of evidence. First, the levels of repressor element 1 silencing transcription (REST) factor, a major pathogenic pathway in HD (Buckley et al., 2010), is elevated in HD neurons, resulting in repression of hundreds of key neuronal genes (Zuccato et al., 2003, 2007; Johnson et al., 2010b). Canonical and non-canonical REST binding motifs have been mapped in close proximity to 22 miRNA sites in the human genome, including several miRNAs that are abundant in neurons (Bruce et al., 2004; Jothi et al., 2008; Yu et al., 2011). Could abnormal REST deregulate miRNAs network in HD patients? REST and its cofactor coREST possess functional target sites for miR-9 and miR-9*, respectively (Packer et al., 2008), and miR-9 and miR-9* (together with miR-7, miR-124, miR-132, and other miRNAs) are downregulated in HD patients (Johnson et al., 2008; Packer et al., 2008; Marti et al., 2010). miRNA deregulation in HD was confirmed by profiling studies in animal models, although there was a high degree of variability among the models (Lee et al., 2011). These observations strongly suggest that altered miRNA transcription is a major event in HD pathogenesis.

Besides HD, there are eight other polyQ diseases: dentatorubral–pallidoluysian atrophy, spinal and bulbar muscular atrophy, and spinocerebellar ataxia (SCA) 1, 2, 3, 6, 7, and 17 (Gatchel and Zoghbi, 2005). In fly models of SCA3, reduction of
| miRNA | Disease | Type of evidence | Mechanism | Reference |
|-------|---------|------------------|-----------|-----------|
| miR-7 | PD      | In vitro reporter assay | Regulation of α-synuclein | Doxakis (2010), Junn et al. (2009) |
| miR-8 | PD      | Overexpression in vitro | | |
| miR-9/9* | HD | Profiling studies | | |
| miR-9/9* | ALS | Profiling in mouse model | Neurofilament expression | Haramati et al. (2010) |
| miR-16 | AD | Profiling in mouse model | Regulation of APP levels | Liu et al. (2010) |
| miR-19 | SCA1 | In vitro reporter assay | Regulation of ataxin-1 | Lee et al. (2008) |
| miR-29 | AD | Profiling in patients | Regulation of BACE1 levels | Hebert et al. (2008) |
| miR-29b | FTD | In vitro reporter assay | Regulation of progranulin | Jiao et al. (2010) |
| miR-34 | HD | Profiling in flies | Protective role | Liu et al. (2012) |
| miR-101 | AD | In vitro reporter assay | Regulation of APP levels | Long and Lahiri (2011) |
| miR-101 | SCA1 | In vitro reporter assay | Regulation of ataxin-1 | Lee et al. (2008) |
| miR-106a | AD | Profiling in patients | Regulation of APP levels | Patel et al. (2008) |
| miR-106b | AD | Expression in mouse model | TGFβ | Wang et al. (2010a) |
| miR-107 | AD | Profiling in patients | Regulation of BACE1 levels | Wang et al. (2008) |
| miR-107 | AD | In situ hybridization in patients | | |
| miR-107 | AD | Levels in mouse models | Regulation of cofillin | Yao et al. (2010) |
| miR-107 | FTD | In vitro overexpression | Regulation of progranulin | Wang et al. (2010b) |
| miR-124 | AD | miR-124-1 knockout mouse | Altered expression of Lhx2 | Sanuki et al. (2011) |
| miR-130 | SCA1 | Overexpression in vitro | Regulation of BACE1 levels | Fang et al. (2012) |
| miR-130 | SCA1 | In vitro reporter assays | Regulation of ataxin-1 | Lee et al. (2008) |
| miR-130 | SCA1 | In vitro gain of function | | |
| miR-133b | PD | Profiling in patients | ??? | Kim et al. (2007) |
| miR-137 | AD | Profiling in patients | Regulation of Aβ levels | Geekiyanage and Chan (2011) |
| miR-144 | SCA1 | Profiling in patients | Regulation of ataxin-1 | Persengiev et al. (2011) |
| miR-146a | AD | Profiling in patients | Downstream of Aβ | Li et al. (2011) |
| miR-153 | PD | In vitro reporter assay | Regulation of α-synuclein | Doxakis (2010) |
| miR-181c | AD | Profiling in patients | Regulation of Aβ levels | Geekiyanage and Chan (2011) |
| miR-520c | AD | Profiling in patients | Regulation of APP levels | Patel et al. (2008) |
| miR-659 | FTD-ALS | Human polymorphism | Regulation of progranulin | Rademakers et al. (2008) |

miRNA processing after knockout of Dicer1 markedly enhances the toxicity induced by mutant ataxin-3 (Bilen et al., 2006). In parallel genetic screens, a single miRNA, bantam, was identified as a potent downstream modulator of both polyQ and tau toxicity in...
flies (Bilen et al., 2006). In a recent paper, miR-34 was shown to be protective against expanded SCA3 (Liu et al., 2012). Subsequent work suggested that miR-19, miR-101, and miR-130 are important for the post-translational regulation of ataxin-1 (Lee et al., 2008). Inhibition of those miRNAs enhanced the cytotoxicity of polyQ-expanded ataxin-1 in human cells. Moreover, miR-144, a highly conserved miRNA, also regulated ataxin-1 expression and appeared to be associated with aging. Ataxin-1 levels are higher in the cerebellum and cortex of SCA1 patients than in healthy aged brains (Persengiev et al., 2011). On the other hand, ataxin-2 might be required for miRNA function (McCann et al., 2011), further supporting the intimate association between miRNAs and polyQ diseases.

PARKINSON’S DISEASE
Parkinson’s disease is a neurodegenerative disorder that primarily affects movement. Clinical symptoms include bradykinesia (decreased ability to start and continue movements), resting tremor, and rigidity. These symptoms are due to the relatively selective loss of dopaminergic neurons in the substantia nigra (Dauer and Przedborski, 2003) and reflect the impairment of neuronal networks important for regulating motor function. The past two decades have witnessed significant advances in the identification of distinct genetic loci at which pathogenic mutations are associated with parkinsonism (for review, see Lesage and Brice, 2009; Zimprich, 2011). Most research is focused on genes that have been conclusively linked to PD pathogenesis, including those encoding α-synuclein, leucine-rich repeat kinase 2, PTEN-induced putative kinase 1, parkin, and DJ-1.

Profiling studies of PD brains have revealed abnormalities in miRNA content (Kim et al., 2007; Minones-Moyano et al., 2011). One of the miRNAs found to be downregulated in these studies, miR-133b, plays a major role in the development of midbrain dopaminergic neurons by regulating the transcription factor Pitx3 (Kim et al., 2007). miR-7 and miR-153 control the expression of α-synuclein (Junn et al., 2009; Doxakis, 2010). Since intracellular levels of this protein appear to be critical in mediating its toxicity, deregulation of those miRNAs might lead to increased toxic levels of α-synuclein.

AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA
Amyotrophic lateral sclerosis (ALS) is a progressive, lethal, degenerative disorder characterized by the selective death of motor neurons in the brain and spinal cord (Pasinelli and Brown, 2006; Ferraiuolo et al., 2011). ALS shares many clinical, pathological, and molecular features with FTD, the second most common early-onset dementia (Ferrari et al., 2011). Clinically, FTD progresses from an insidious onset of behavioral changes, impaired frontal executive functions, and language deficits to more severe cognitive defects and, finally, to generalized dementia (Boxer and Miller, 2005). In familial FTD cases, the mutated locus has been identified in the genes encoding tau (Hong et al., 1998; Hutton et al., 1998), VCP (Watts et al., 2004), CHMP2B (Skibinski et al., 2005), progranulin (Baker et al., 2006; Cruts et al., 2006), and C9ORF72 (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Among them, CHMP2B (Parkinson et al., 2006; Cox et al., 2010), VCP (Johnson et al., 2010a), and C9ORF72 (DeJesus-Hernandez et al., 2011; Renton et al., 2011; Stewart et al., 2012) have also been implicated in ALS. Moreover, the RNA-binding proteins TDP-43 and FUS have been strongly implicated in both FTD and ALS (Arai et al., 2006; Neumann et al., 2006, 2009; Sreedharan et al., 2008; Vance et al., 2009).

As described above, Dicer deletion in spinal motor neurons mimics most of the clinical (e.g., progressive paralysis) and pathological (e.g., astrocytosis and signs of axonopathy) features of ALS (Haramati et al., 2010). Whether the miRNA pathway is involved in the molecular pathogenesis of FTD/ALS caused by C9ORF72 repeat expansion is unknown. However, a potential involvement for the miRNA pathway in other forms of FTD or ALS comes from limited studies on TDP-43. TDP-43 is mutated in a subset of ALS patients (Sreedharan et al., 2008), and the expression levels of some miRNAs are affected in TDP-43 mutant flies (Buratti et al., 2010). Biochemical interactions between TDP-43 and Drosha, a key miRNA processing enzyme (Han et al., 2004), have been observed (Gregory et al., 2004; Ling et al., 2010). These findings raise the possibility that TDP-43 may play a role in miRNA processing; however, the mechanism remains to be elucidated and whether endogenous Drosha and TDP-43 physically interact needs to be demonstrated.

Some miRNAs are emerging as important contributors to ALS pathogenesis. The muscle-specific miR-206 is upregulated upon nerve injury and is required for regeneration of neuromuscular synapses. Moreover, miR-206 deficiency accelerates disease progression in a mouse model of ALS (Williams et al., 2009). miR-9/9*, an evolutionarily conserved and multifunctional miRNA (Yuva-Aydemir et al., 2011), is also potentially involved in ALS. Profiling of miRNA expression in motor neurons harboring an SMN1mut allele found in pediatric spinal motor atrophy revealed decreases of more than 90% in miR-9 and miR-9* levels (Haramati et al., 2010). More importantly, changes in the expression levels of the neurofilament subunits likely contribute to the disease, and miR-9 is an upstream regulator of the neurofilament mRNAs.

Several other miRNAs might be linked to FTD–ALS through different mechanisms. For example, miR-29b and miR-107 regulate progranulin levels (Jiao et al., 2010; Wang et al., 2010b). Since progranulin haploinsufficiency can cause FTD(3,4,115), excessive levels of those miRNAs might decrease progranulin levels and be a risk factor for the disease. Consistent with these observations, a genetic polymorphism in the 3′UTR of the progranulin gene is associated with a higher risk of FTD–ALS, and multiple miRNAs are misregulated in FTD with TDP-43 pathology (Rade-makers et al., 2008; Kocerha et al., 2011). This genetic variant (rs5848) affects the miR-659 binding site, resulting in more efficient binding and, consequently, decreased progranulin levels. It is not known whether translational regulation by miRNAs is a common mechanism in FTD caused by progranulin deficiency or in other neurodegenerative diseases (Rollinson et al., 2011).

SUMMARY
Although much progress has been made in our understanding of how miRNAs control gene expression at the post-transcriptional level during development, their contributions...
to neurodegenerative disease remain poorly understood. Many fundamental questions need to be addressed. Is miRNA pathway disruption a downstream consequence or a cause of neurodegeneration (Figure 2 vs. Figure 3)? Are miRNAs essential for the proper regulation of aggregation-prone proteins or do they control additional pathogenic pathways? Are individual miRNAs especially important in particular neurodegenerative diseases? Which miRNA target or targets are relevant for the disease? To address these questions, it will be essential to generate novel experimental models, such as conditional knockouts in which developmental defects can be circumvented, allowing assessment of the functions of specific miRNAs in the adult brain. We expect many exciting findings will be made in years to come.

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