# Role of Circular RNA in neurological disease

By

Labiba Salsabil
19346055

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

School of Pharmacy
BRAC University
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## Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing my degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

**Student's Full Name & Signature:**

**Labiba Salsabil**

19346055

# Approval

The thesis/project titled “Role of circular RNA in neurological disease” submitted by Labiba Salsabil (19346055) of Summer, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on September 2023.

**Supervised By:**

Namara Mariam Chowdhury
Senior Lecturer, School of Pharmacy
BRAC University

**Approved By:**

Program Director:

Professor Dr. Hasina Yasmin
Program Director and Assistant Dean
School of Pharmacy
BRAC University

Dean:

Professor Dr. Eva Rahman Kabir
Dean
School of Pharmacy
BRAC University

## Ethics Statement

This study does not involve any kind of animal and human trial.

## Abstract

Neurological disorders are increasingly recognized as major causes of death and disability worldwide. Among different types of treatment options, circRNAs might play vital roles in Alzheimer's disease, Parkinson's disease, ischemic brain injury, and neurotoxicity. However, the mechanisms of action of circRNAs have not been fully characterized. Based on current research, this review article outlines the involvement of various circRNAs in brain development and CNS diseases. A better understanding of the circRNA function will help to develop novel therapeutic strategies to treat CNS complications.



**Keywords:** Neurological disease; Circular RNA; Exonic and intronic circRNA; Therapy; Delivery process.

# Dedication

*Dedicated to those who have been suffering from neurological disease.*

## Acknowledgment

First of all, I want to give thanks and appreciation to God, the Almighty Allah, for granting me good health as well as the patience, dedication, and knowledge I needed to finish the thesis.

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## List of Acronyms

| | |
|---|---|
| CNS | Central nervous system |
| PNS | Peripheral nervous system |
| PD | Parkinson’s disease |
| HD | Huntington’s disease |
| αS-pathy | alpha-synucleinopathies |
| NFTs | neurofibrillary tangles |
| GABA | gamma-aminobutyric acid |
| MSNs | medium spiny neurons |
| tPA | tissue plasminogen activator |
| OGD | oxygen-glucose deprivation |
| mTOR | mammalian target of rapamycin |
| JAK | janus kinase |
| STAT | signal transducers and activators of transcription |
| MT | mechanical Thrombectomy |
| AVM | arteriovenous malformation |
| AChE | acetylcholinesterase |
| BPSD | behavioral and psychological symptoms of dementia |
| miRNA | micro RNA |
| AD | Alzheimer’s disease |
| tRNA | transfer ribonucleic acid |
| mRNA | messenger ribonucleic acid |
| rRNA | ribosomal ribonucleic acid |

| | |
|---|---|
| bpA | branch point adenosine |
| exoG | exogenous guanosine |
| DBRI | debranching enzyme |
| ADAR | adenosine deaminase acting on RNA |
| RBPs | RNA binding proteins |
| GU | genitourinary |
| IRESs | internal ribosome entry sites |
| ORFs | open reading frame |
| VSMCs | vascular smooth muscle cells |
| CDR1 | complementarity-determining regions |
| tMCAO | transient middle cerebral artery occlusion |
| TIPARP | TCDD-inducible poly [ADP-ribose] polymerase |
| AIS | androgen insensitivity syndrome |
| siRNA | small interfering RNA |
| VEGFB | vascular endothelial growth factor |
| NLRP3 | nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 |
| shRNAs | short hairpin RNAs |
| RPE65 | retinal pigment epithelium-specific 65 kDa protein |

# Chapter 1: Introduction

## 1.1 Neurological Disease:

Neurological diseases are the pathological conditions that affects the nervous system i.e. central nervous system (CNS) and peripheral nervous system (PNS) in both adults and children. More precisely, the affectation of nervous system involves spinal cord and brain included in central nervous system and; nerve roots, peripheral nerves, neuromuscular junction, autonomic nervous system included in peripheral nervous system. These are hampered by causing structural, motorized or biochemical dysfunction, infections, degeneration, disruption in blood flow thus obstruct the potentiality of self-regulate neural activity (*Neurological Disorders - Physiopedia*, n.d.).

## 1.2 Prevalence

Neurological diseases have perceived as a huge public health challenge worldwide due to the tremendous rate of death and disability causing from these diseases. According to *Burden of Neurological Conditions - PAHO/WHO | Pan American Health Organization*, n.d. , the data of neurological burden of the region of America shows that 533,172 people died in 2019 where 60% of the people were women and 40% were men respectively 3,20,043 and 2,13,219. As per population of 1,00,000, deaths are 32.9. Considering worldwide, (*Nearly 1 in 6 of World's Population Suffer from Neurological Disorders – UN Report | UN News*, n.d.) reveals one billion of people suffer from neurological disease globally every year where 50 million of people from epilepsy, 326 million of people are from migraine, 62 million of people from cerebrovascular disorder and 24 million of people from Alzheimer disease including other dementias. Wistfully, 6.8 million of people die in every year due to neurological disorder. Dr. Margaret Chan, WHO

Director-General said, 90% of the people go untreated in Africa due to the high cost treatment (*Neurological Disorders Affect Millions Globally: WHO Report*, n.d.).

## 1.3 Symptoms

Emotional and physical symptoms result depending on the type of disorder and its specificity leading to dysfunction in nervous system e.g. an unexpected headache, loss of balance, short-term memory, impaired intellectual power, loss of muscular strength, tingling, tremors, inabilities in concentrating, muscle rigidity, unresponsiveness, difficulties in learning, slow speech etc. These are the common symptom shows in neuro patients (*Overview of Nervous System Disorders | Johns Hopkins Medicine*, n.d.).

## 1.4 Pathology of Common Neurological Diseases

As a result, some leading neurological disease that are impacting mental as well as physical health such as Alzheimer's disease, Parkinson's disease, Huntington's disease, ischemic brain injury, multiple sclerosis, epilepsy, brain tumors, neurotoxicity.

Central nervous system injuries causing from trauma or stroke affects the interconnection of neurons in brain thus resulting in severe functional default. As a result, this functional default requires to reconstruct but the brain and spinal cord in central nervous system have very limited adequacy to do so (Ueno, n.d.). Moreover, the molecular and structural dysfunctions result from protein aggregation. The general proteinopathies of neurodegenerative disorders are alpha-synucleinopathies, tauopathies, myloidosis, and transactivation response DNA-binding protein 43 proteinopathies. These show some common features e.g., clinical, molecular, histopathological. However, their mechanism results genetic alteration, protein accumulation, neural inflammation,

oxidative stress, mitochondrial abnormalities, microglial stimulation, axonal transport disruptions (Mallhi et al., 2021).

### 1.4.1 Cerebral Ischemia

Inadequate delivery of oxygen and blood flow cause cerebral ischemia resulting the development of neural infraction. More precisely, the blood supply in the central nervous system can be stopped or reduced and that results in acute brain injury which can be initiated by cardiac arrest.

The common pathological mechanism involved in cerebral ischemia are lack of electrolytes in cell homeostasis, intracellular calcium abundance, acidosis, cell toxicity due to free radical, impaired energy production (Béjot & Garnier, 2022).

However, it is thought that the relative contributions of each activity differ greatly, particularly in terms of the amount of the blood supply of cerebral. Necrosis or apoptosis may cause neuronal death. Energy failure and fast necrotic cell death are the major processes at the center of an infarct, where blood flow is extremely low. Abundance of oxygen-derived free radicals may result from the inflow of inflammatory cells and oxygen that occurs after reperfusion of ischemic tissue (Macdonald & Stoodley, 1998).

### 1.4.2 Alzheimer Disease

In case of Alzheimer disease or other dementias are caused by involuntary movement abnormalities which shows the primary symptoms affecting the basal ganglia and associated systems (Nani, 2021).

The pathophysiology of Alzheimer disease is characterized by cortical atrophy, generally mostly enriched in hippocampus embedded in the medial temporal lobe of the brain. It is microscopically

seen that the damaged area in the brain results in granulovascular degeneration, inflammation, amyloid plaques as well as neurofibrillary tangles (NFTs).

NFTs, found in brain cell bodies, appear to be connected with the clinical presentation based on their density and distribution. These are the collection of aggregated tau protein and neurofilaments. Neurofibrillary changes in Alzheimer's disease include neuropil threads and neuritic plaques. Moreover, amyloid plaques are abnormal configuration of proteins that builds in the expanse between nerve cells. These may be either neuritic or diffuse. Neuritic comprises of A*β,* and so thus in diffuse. These including neurofibrillary tangles are seen in autopsy in a noticeable amount in clinically unaffected elderly people (Gijsen & Bischoff, 2012).

### 1.4.3 Parkinson Disease

Additionally, inadequate nigrostriatal dopaminergic innervation and disruption of neural network of cells in CNS pathologically leads to parkinson disease (Kouli et al., 2018).

More precisely, the mechanism of pathophysiology of Parkinson disease is largely idiopathic, although research shows the mechanism of this are generally linked to genetic factor as well as environmental factors. However, the symptoms that are shown due to Parkinson disease generally caused by the deprivation of neurotransmitter particularly dopamine.

Symptoms are seen to be shown more over time since the number of affected cells are increasing. The evidence has shown that the disease first affects the dorsal motor nucleus situated in vagus nerve and also olfactory bulbs and nucleus and then it affects the locus coeruleus and then substantia nigra in the basal nigra. As a result, comprehensive pathophysiological changes occurs due to the damage of these neural systems lead to affectation in moto system as well as cognitive

and physiological psychology system (*2. Pathophysiology of Parkinson's Disease | ATrain Education*, n.d.).

### 1.4.4 Huntington's Disease

Huntington's disease is pathologically characterized by the loss of gamma-aminobutyric acid (GABA), substance P, and also lack of caudate nucleus atrophies and medium spiny neurons which is a GABAergic inhibitory cell in basal ganglia structure of corpus striatum(*Huntington's Disease - Neurologic Disorders - MSD Manual Professional Edition*, n.d.).

The striatum, which includes the caudate nucleus and putamen, corrupts in Huntington's and loses efferent medium spiny neurons (MSNs). Huntington's disease patients have a specific region locating in the cell body thinning of the cortical ribbon, despite the fact that the striatum appears to be the region of the brain that is most affected. However, this cortical mass loss starts early in the pathophysiology of Huntington's disease and expands with the illness from the posterior to the anterior cortical regions. This spatially localized cortical degeneration may help to explain the range of clinical symptoms associated with Huntington's disease. Weight loss, skeletal muscle wasting, and heart failure are among the additional symptoms that Huntington's disease patients typically experience. (Jimenez-Sanchez et al., 2017).

## 1.5 Existing Treatments

However, due to distinct etiology e.g. traumatic, genetic, infectious, vascular, environmental, high prevalence of neurological disorders causes huge economic burden worldwide although getting effective therapeutic response to existing therapies (Thakur et al., 2016). Spinal cord stimulation, microsurgery, medication therapy, physiotherapy, spinal surgery, stereotactic radiosurgery,

medicine with the patient with severe involuntary muscular contraction are the used and established neurophysiology till now (*Neurosciences: MedlinePlus Medical Encyclopedia*, n.d.).

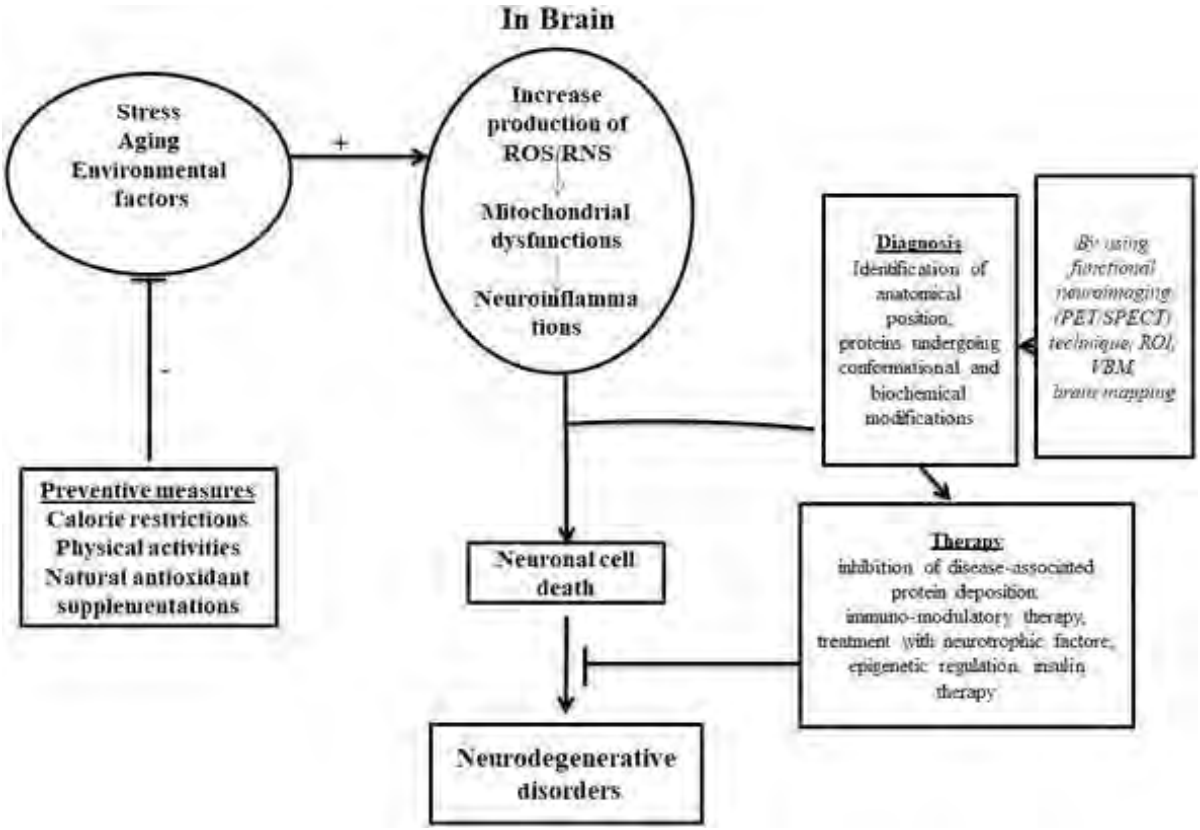


*Figure 1: Schematic representation of overall causes, diagnosis, prevention and therapy of neurological disease*

### 1.5.1 For Cerebral Ischemia:

Currently, there are only two methods of treatment for ischemic stroke. The first is mechanical thrombectomy, and the second is tissue plasminogen activator (tPA) are widely used.

- From the study of National Institute of Neurological Disorders and Stroke (NINDS) in 1995 demonstrated the efficacy of tPA in treating acute ischemic stroke upon replacing the previous thrombolytic drug streptokinase. Later trials confirmed that tPA is effective up to

4.5 hours after the onset of symptoms of an ischemic stroke. Basically, tPA consists of 527 amino acid chain with three glycosylation sites and 17 disulfide bridges that can break down fibrin in a way that is plasminogen-dependent, breaking up blood clots and triggering reperfusion after an ischemic stroke. Numerous studies have demonstrated that tPA also causes neuroprotective impaction. For instance, tPA can reduce the cell death caused by zinc, shield neurons from oxygen-glucose deprivation (OGD), and shield cortical neurons by activating signaling pathways related to neuroprotection like mTOR and JAK/STAT (Hurd et al., 2021).

- The second extensively used therapy is Mechanical Thrombectomy (MT), which received prompt notice in 2015 with the release of five clinical trials proving its efficacy within 6 to 8 hours of the onset of an ischemic stroke. Effectiveness has been shown in recent trials to last as long as 24 hours after starting. MT uses stent-retriever tools that physically eliminate the clot rather than chemically dissolving it. MT is an excellent option over thrombolysis in cases of substantial proximal circulatory occlusions, particularly those involving the carotid artery (where it is no more than 10 percent effective). If done correctly, it has fewer adverse effects and offers a longer window of time than thrombolysis after the onset of stroke.
- Endovascular emergency procedures: In some cases, doctors will treat ischemic strokes inside the blood capillary that is blocked. Endovascular therapy has been shown to produce noticeably better outcomes and reduce long-term disability following an ischemic stroke. This procedure conduct by directly delivering of drug into the brain and extract the clot from blocked vessels by using a stent retriever.

- There is other existing procedure are i.e. carotid endarterectomy, angioplasty, stereotactic radiosurgery, surgical AVM removal. If an AVM is discovered in an area of the brain that is simple to access, a smaller piece may be surgically removed. This eliminates the chance of rupture and lowers the danger of hemorrhagic stroke. An AVM may not always be able to be removed if it is large, deeply positioned in the brain, or if doing so would have a negative impact on brain function (Hasan et al., 2018).

### 1.5.2 For Alzheimer Disease

Research shows that there is not any permanently cure to treat Alzheimer disease though a number of medicines are preferred by the physicians which are shown to reduce the symptoms. More precisely the behavioral and psychological behaviors relating dementia are treated using those medicines. However, the medicines i.e. acetylcholinesterase inhibitors, memantine (*Alzheimer's Disease - Treatment - NHS*, n.d.).

- These drugs increase the production of acetylcholine, an amino acid that aids in the signaling of nerve cells in the brain. Doctors may suggest donepezil, galantamine, and rivastigmine for patients with early- to mid-stage Alzheimer's disease. According to the most recent recommendations, these drugs should be proceeded all the way through the later, more severe stages of the disease. All three of the AChE inhibitors are identically effective, but some people respond better to one than the other or experience less adverse reactions, which might include feeling nauseous, throwing up, and appetite loss. Usually, side effects of medication fade off after two weeks.
- Memantine is also advised since it works to block the effects of too much glutamate in the brain. It is also suitable for people with severe Alzheimer's disease who are taking an AChE

inhibitor. Side effects like headaches, dizziness, and constipation are possible, however they usually only last a short while.

- Risperidone should only be used temporarily and at a dose as low as possible due to its serious adverse consequences. Haloperidol should only be used when other treatments have failed. Antidepressants can occasionally be recommended if depression is suspected to be the cause of the anxiety.
- Occasionally, other medications may be advised for treating specific BPSD symptoms, although they will be used "off-label" (not specifically approved for BPSD).
- Cognitive stimulation therapy includes to participate in group activities and exercises meant to boost memory and problem-solving abilities.
- A personal goal, such as acquiring the use of a mobile phone or other everyday tasks, can be achieved through cognitive rehabilitation by working with a family member, friend, or licensed professional, such as an occupational therapist. Utilizing the patient's healthy brain to support the damaged brain is how this kind of therapy succeeds.

### 1.5.3 For Parkinson Disease

In order to treat Parkinson disease, there three existing possible ways i.e. physiotherapy, medications and surgery in some specified cases.

- According to *Parkinson's - Physiotherapy Management and Interventions - Physiopedia*, n.d., Parkinson's patients usually struggle to perform two tasks at once, such as walking and talking. Motor-cognitive dual-task training has the potential for enhancing cognition, gait, and balance in addition to dual-task capacity. However, a 2021 study concluded that dual task training can improve gait performance, notably by increasing gait velocity, step

length, and decreasing freezing of gait in senior people. This study found that dual task training benefited the participants' overall well-being.

- A physiotherapist assists the patient to aid in movement (manipulate) and exercise away stiffening of the muscles and joint pain. The goal of this form of exercise is to make moving easier and more flexible, both of which serve to improve muscular endurance. (*Parkinson's Disease - Treatment - NHS*, n.d.).
- The majority of Parkinson's disease patients eventually need the medication levodopa. It functions by using dopamine, which is produced when the nerve cells in the brain receive levodopa, to transfer signals between the regions of the brain and the nerves that control movement. When dopamine levels are increased with levodopa, movement problems often improve. It is normally provided as a pill or liquid, and is frequently taken alongside different drugs like benserazide or carbidopa. Levodopa is unable to reach the brain as a result of these medications' ability to stop it from being broken down in the bloodstream.

### 1.5.4 For Huntington's Disease

The neurotransmitters i.e. dopamine, glutamate, and γ-aminobutyric acid which are degenerated in Huntington's disease are theoretically the targets of pharmacotherapies, few well-conducted trials for symptomatic interventions have produced promising outcomes, and current treatments have concentrated on the motor elements of Huntington's disease (Frank, 2014).

- Tetrabenazine serves as a dopamine-depleting medication that, despite the potential for serious side effects, may be one of the most successful chorea treatments. Some newer neuroleptic medications, including olanzapine and aripiprazole, may be just as effective as previous neuroleptic medications for the treatment of chorea and psychosis while having a superior adverse effect profile. The course of HD cannot currently be altered, but doctors

can help patients and families affected by HD by providing education and symptomatic therapy.

## 1.6 New Potential Discovery

Though current research shows a new perspective to treat neurological disorder using circular RNA which works in modulating normal neural functions thus showing great clinical potential following non-coding RNA based diagnostic and therapeutic strategies (Lu et al., 2019).

## 1.7 Circular RNAs

Circular RNAs (CirRNAs) are single-stranded RNAs named based on their circular structure, covalently closed continuous loop that are currently categorized as non-coding RNA generated by non-canonical splicing sites and its function plays a role in miRNA sponges in both pathological and physiological processes and also have the ability to code proteins. (Kristensen et al., 2019).

Although, cirRNAs are produced in two ways, either from lariat introns or by back-splicing exons. Greene et al., 2017 demonstrated the difference between linear RNA and circular RNA. The 3′ and 5′ ends of an RNA molecule, which are typically present, have been connected by covalent bonds to form circular RNA, as opposed to linear RNA.

Intriguingly, it has been discovered that they are widespread, evolutionarily sustained, and comparatively stable in the cytoplasm than other forms of RNAs and also express in the immunological identity of antigen in tissues particularly enriched in nervous system. Recent studies and researches show that thousands of these non-coding RNAs are present in the nervous system and using deep sequencing technology advancement leads potential clinical characteristics which are differ from other types of RNAs (Ma et al., 2020). These specific identical characteristics give cirRNAs a wide range of potential roles including treating in neurological disorder (Miao et

al., 2021). Few research have now examined the roles and mechanisms of circRNAs in neurological disorders; instead, the majority mainly focus on circRNAs' roles as miRNA regulators. According to one study, circRNAs are incredibly abundant in the brain, particularly in neuropils and dendrites, and may help to control synapse function and neuronal development. These traits imply that circRNAs might be crucial in disorders of the neurological system such epilepsy, Parkinson's disease (PD), and Alzheimer's disease (AD), Hungtinton disease (HD) (Shao & Chen, 2016).

## 1.8 Aims

In this review, the aim is to summarize the central roles of cirRNA regulation in CNS development and diseases by demonstrating relevant translation potential of cirRNAs.

## 1.9 Objectives

The objective of this research is to discuss the biogenesis as a therapeutic strategy to overexpress circRNAs in order to determine the future strategies for the creation of circRNA-based therapies for neurological disorders.

# Chapter 2: Methodology

For conducting this review study, relevant data were gathered by a comprehensive literature search. A number of reliable sources, including peer-reviewed journals and an online scholarly database, were used to compile the information. Here is a list of a few of the several databases that have been searched for this study.

- Journal Database
- Newspaper Database
- Professional website
- Library Catalogue

In order to assemble as much essential information as possible regarding the use of metformin in cancer treatment, and associated clinical trial data, a thorough search of several journals, review articles and research papers from official websites and research databases was performed. Utilizing well-known and reliable sources including PubMed, SCOPUS, Google Scholar, and ScienceDirect, clinicaltrials.gov the data for this review study was collected. Relevant papers were gathered using appropriate important keywords, such as neurodegenerative disease, cirRNAs, and its biogenesis and treatment of CNS disorder via cirRNAs. Around 122 articles have been assessed based on the title and keyword content. Then, 49 papers were reduced after reading the abstracts. The 72 papers that made up this review research were carefully selected and examined. Mendeley software was used for accurate and fair referencing in order to show respect for the writer's original works.

# Chapter 3: Formation and delivery process of CirRNA

## 3.1 Formation of Circular RNAs

Circular RNAs are typically created during splicing processes in vivo, either as exonic circRNAs from the circularization of exons or as intronic circRNAs, for instances circular tRNA and rRNA introns created during archaeal splicing. However, circular RNAs can be found in viroids, viruses, and satellite RNAs that resemble viroids.

### 3.1.1 Formation of Exonic Circular RNAs

The spliceosome functions in eukaryotic cells in a two-step method in order to withdraw introns from primary transcripts. A free 3′-OH group is produced at the site of 5′-exon and the lariat intermediate in the initial step and as a result of the branch point adenosine's 2′-OH group attacking the 5′-splice sites in the intron. In the next step, the end products are an excised lariat intron and a linear RNA made up of the two joined exons, which are produced by the nucleophilic assault of the created 3′-OH group onto the 3′-splice site (Petkovic & Müller, 2015).

i. In the above figure shown the basic linear splicing along with two models of the exonic cirRNAs formation. When the branch point adenosine (bpA) folds, it attacks the 5′-splice site and releases the 5′-exon with a free 3′-OH group and the lariat intermediate, where it still has the intron connected to the 3′-exon. The two exons are ligated together and hence the intron is released as a lariat as a result of the 3′-OH group of the 5′- exon's nucleophilic attack on the 3′-splice sites.

ii. The mechanism is shown below:

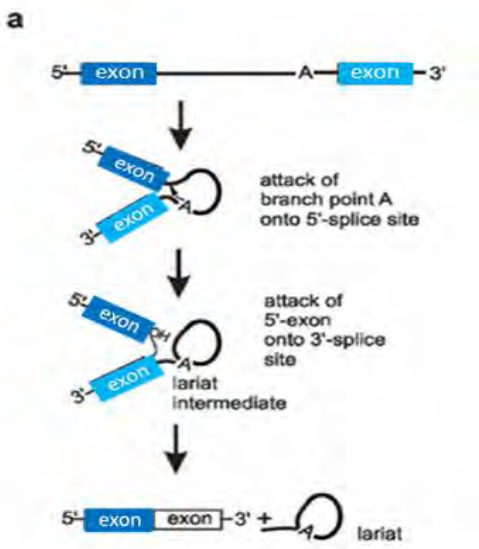


*Figure 2: Basic linear splicing*

iii. Backsplicing directly- In order to juxtapose the branch point of the 5′-intron and the 3′-intron-exon junction (3′-splice donor) for nucleophilic attack and cleavage, two unspliced introns engage in complementary base pairing. The circularized exon is then released when the 3′-splice donor attacks the 5′-intron-exon junction (5′-splice acceptor) connecting the two introns.

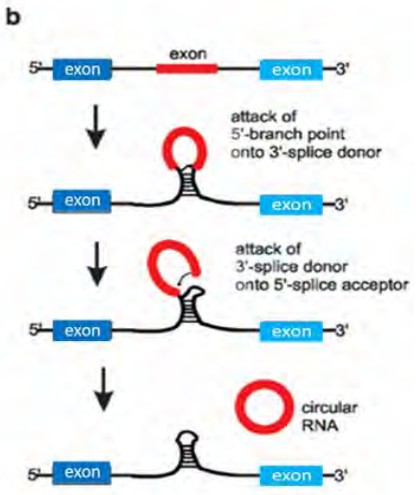


*Figure 3: Backsplicing directly*

iv. Skipping exons- Lastly, by skipping a single exon, an exon containing lariat is produced using the standard splicing method. The same process of backsplicing then takes place, just inside the lariat. The intron lariat is consequently freed, and circular RNA is created.

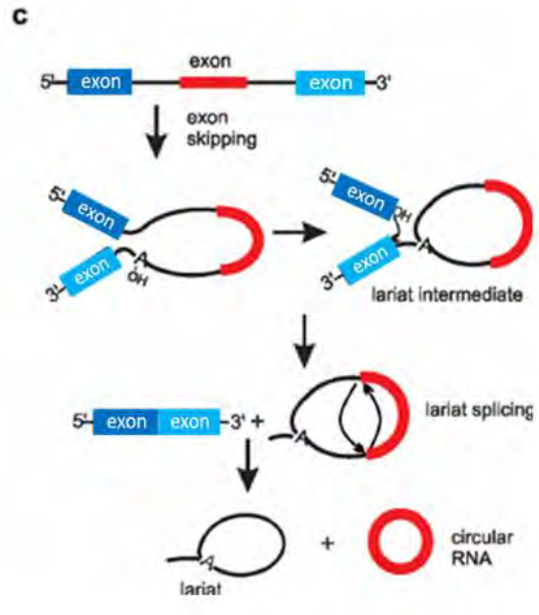


*Figure 4: Skipping exons*

Exonic circRNAs may also be produced via spliceosomal activity. In 1991, they were first noticed. Since then, tens of thousands of endogenous circRNAs have been found in mammalian cells, some of which are extremely numerous and conserved across evolutionary time. The precise mechanism underlying circRNA biogenesis is still unknown. Direct backsplicing and exon skipping are currently the two main techniques involving the canonical spliceosome that are being discussed.

Direct backsplicing, when exons are spliced in a non-canonical order, is now referred to as "mis-splicing" by exon shuffling or exon scrambling. However, 'backsplicing' is a more appropriate moniker given that circRNAs may be produced on purpose rather than as a result of mis-splicing events. The 3′-tail of an expected downstream exon is joined to the 5′-head of an exon that is typically upstream in the process known as "direct backsplicing." The upstream splice acceptor and downstream splice donor pair up during splicing. The exon therefore circularizes. The second approach entails making a lariat with an exon that was obtained through exon skipping. The intron is then removed by internal splicing, creating a circRNA from this lariat. Although direct backsplicing is preferred as the more frequent approach, both mechanisms are conceivable in vivo. Additionally, the possibility that several pathways contribute to the creation of exonic circRNA cannot be discounted. Exon circularization is dependent on flanking intronic sequences, according to recent discoveries. The efficiency of exon circularization is determined by the competition between pairing within individual introns and RNA-RNA interactions across adjacent introns. Additionally, it was shown that circularization and linear splicing are in competition with one another, giving circRNAs a functional purpose in gene regulation (*Formation of Three Types of CircRNAs. a Exonic Circular RNA (EcircRNA)... | Download Scientific Diagram*, n.d.).

Overexpression vectors that comprise the exon to be circularized and partial sequences of the adjacent introns to create pairing, but lack additional upstream and downstream exonic sequences, have been developed in order to produce a desired circRNA in vivo. These vectors were demonstrated to successfully create circRNAs after delivery in mammalian cells.

### 3.1.2 Formation of Intronic Circular RNAs

The chemistry of the spliceosome is used by group II self-splicing introns to produce a branching lariat-intermediate and a lariat-intron. Additionally, there is evidence for a method in which circularized RNA forms from group II introns, even though circularization is caused by the development of a 2′, 5′-phosphodiester bond. Prior to circle formation, the 3′-exon must be released, perhaps by a trans-splicing process. The circularized intron and the 5′-exon are created when the terminal 2′-OH group of the intron hits the 5′-exon-intron junction (5′-splice site) (Laroche-Johnston et al., 2021).

The above figure shown the formation of intronic cirRNAs.

i. CircRNA production is mediated by group II introns. The 3′-exon must first be released for circle formation. By attacking the 5′-splice site with its terminal 2′-OH group, the intron produces a circular RNA by forming a 2′,5′-phosphodiester.

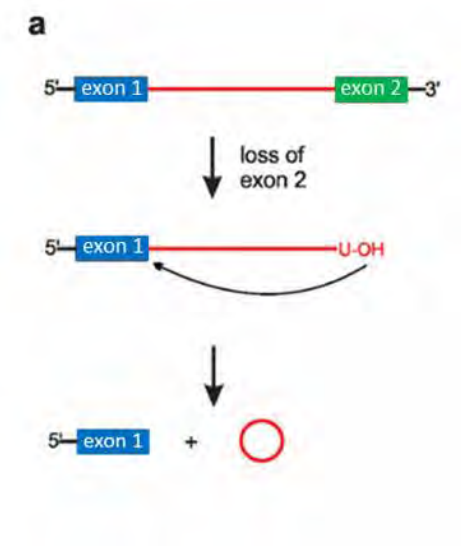


*Figure 5: Intronic circRNA formation*

ii. Regular splicing was facilitated by Group I intron. The intron structure contains an exogenous guanosine (exoG), which acts as a nucleophile to assault the 5′-splice site. The 5′-exon is removed during the first transesterification, and exoG joins the intron. The ligated exons and a linear intron are then released as a result of the terminal 3′-OH group of the 5′-exon attacking the 3′-splice site. The terminal guanosine (G) 2′-OH group eventually nucleophilically attacks a phosphodiester bond close to the 3′ end of the linear intron, circularizing it and releasing a brief 3′ tail. In this instance, the circle is closed by a 2′–5′-phosphodiester bridge.

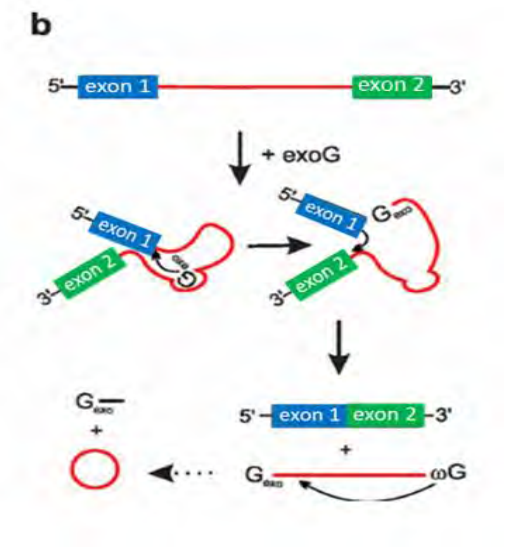


*Figure 6: Regular splicing*

iii. Circle creation by direct nucleophilic attack of G onto the 5′-splice site is made possible by exon 2's prior hydrolysis.

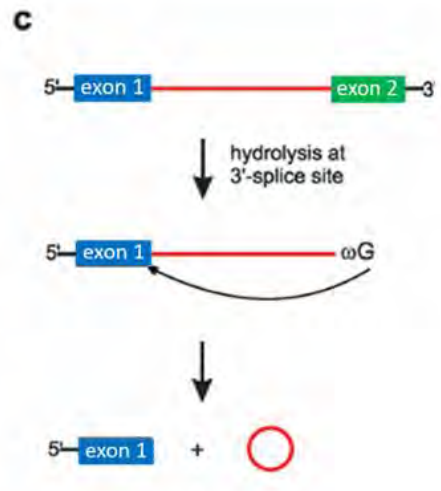


*Figure 7: Circle reaction*

As opposed to the spliceosome and group II introns, group I introns self-splice by first enlisting guanosine (exoG) as an external nucleophile that attacks the 5′-splice site to begin splicing and

then attaches to the intron's 5′ end. Exons are tied together during second transesterification, releasing a linear catalytic intron.

A phosphodiester bond close to the intron's 5′ end can be attacked nucleophilically by the 3′-terminal guanosine to cause the excised linear intron to undergo circularization. The intron is circularized and the 5′-terminal sequence is liberated. The three nucleotides that come before the cleaved phosphate must pair with a particular binding site within the intron in order to define the phosphate that will be attacked and determine the circularization site. As a result, several truncated intron circles are created, however they seem to vanish quickly in living organisms (Nielsen et al., 2003).

Full-length intron circles were also seen to occur in addition to truncated circles. This route is started by hydrolytic cleavage at the 3′-splice site, then G attacks the 5′-splice site nucleophilically. The end products are non-ligated exons and a circular, full-length intron. Full-length intron circles for the Tetrahymena intron were reported to be small and hardly noticeable in vivo (Nielsen & Johansen, 2009). Full-length circular introns, which are likewise highly detectable in vivo, are generated as the primary product at splicing circumstances in vitro for more complex nuclear group I introns, such as the one from Didymium iridis. Truncated circular introns were not found in this instance.

All nuclear group I intron types appear to share the capacity to create full-length intron rings. Although its purpose is unknown, full-length and truncated circular introns were thought to be involved in intron mobility.

## 3.2 Delivery Process of CirRNAs

The spliceosomal system or group I and II ribozymes are two of the unique mechanisms that the circRNA biogenesis follows as explained above. The debranching enzyme (DBR1) will hydrolyze a lariat with a unique 2',5'-phosphodiester bond linkage to remove introns sequentially during the canonical splicing of pre-RNAs to create mature mRNAs. These stages are also involved in exon-derived circRNA creation, but back-splicing results in lariat-driven and intron-pairing-driven circularization (Bai et al., 2018). In contrast to canonical splicing, back-splicing events happen less frequently and are less effective. Due to the close proximity of the exon-donor site and the exon's acceptor site on the same locus, incomplete splicing of pre-mRNA during lariat-driven circularization causes one or more exons to be skipped. It results in the creation of exon- and intron-containing circRNA intermediates, which canonical splicing machinery will then process to create circRNAs generated from exons. In the model of intron-pairing-driven circularization, cis-element-containing introns, such as inverted Alu repeat sequences, pair with one another to bring the downstream donor and upstream acceptor sites into close proximity, which causes the circularization of exons. Importantly, introns surrounding an exon will be circularized if they contain a lot of inverted Alu repeats (Sun et al., 2022). Recent research reveals that not all sequences on each side of exons and introns that are complementary to one another necessarily induce circularization. Adenosine deaminase acting on RNA (ADAR), muscleblind (Mbl), quaking, and the nuclear helicase DHX9 are other RNA binding proteins (RBPs) that influence circRNA synthesis. By interacting with the surrounding introns, Mbl encourages RNA circularization at a second exon of the original RNA transcript. By attaching to its consensus sequences in the nearby introns of the pre-RNA, quaking promote circularization. While Mbl and quaking encourage circularization, ADAR prevents it by switching the adenine to inosine. By

pairing inosine with guanosine, it limits the possibility of RNA complementarity between flanking introns. This pairing prevents the production of circRNA by repelling the upstream acceptor site and the downstream donor site. Similarly, DHX9 exclusively binds to inverted-repeat Alu sites to adversely control circRNA synthesis. Overall, stable 3' ends of pre-mRNAs rather than nascent RNA transcripts are typically exposed to back-splicing (Aktaş et al., 2017).

Introns are eliminated by complementary binding of consensus sequences, such as GU-rich sections and Cregions close to the branching points at the 5' and 3' ends of the introns, during conventional splicing of precursor RNA to create mRNA. It is possible for a circular lariat with a 2', 5'-phosphodiester bond linkage to arise as a result of two rounds of trans-esterification between the exon and intron branch locations. This circular lariat will be linearized by the debranching enzyme and destroyed (Mehta et al., 2020a). The lariat avoids the action of the debranching enzyme, resulting in the creation of an intron-derived circRNA. It comprises a conserved 7-nucleotide GU-rich motif at the 5′ splicing site and an 11-nucleotide C-rich motif at the 3′ branch site. RBPs maintain the stability of circRNAs produced from introns by binding close to the unique 2'5' link (Ashwal-Fluss et al., 2014).

Unspliced introns can occasionally be maintained during the creation of exon-derived circRNAs and continue to exist as such. Following splice-site mutagenesis of the DBR1, yeast exon-consisting lariat precursors were also discovered, indicating that exon-intron-derived circRNAs are widespread.

Exon-derived circRNAs, which are highly conserved, make up the majority of them. Based on orthologous coordinates and having splice sites within two nucleotides, it has been demonstrated that circRNAs display great conservation between mammals. A recent study also found that 70,186 of the circRNAs in the human, macaque, and mouse, which totaled 104,388, 96,675, and 82,321

correspondingly, were evolutionarily conserved. Notably, it has been found that the DNA sections that encode exon-derived circRNAs are more conserved than the exon-flanking DNA (Chandran et al., 2017). Despite being translated from their coding sections, most genes do not produce any translated proteins because they lack internal ribosome entry sites (IRESs) and open reading frames (ORFs).

## 3.3 Clinical Use and Advantages

In particular, malignancies, cardiovascular illnesses, and neurological problems have all been linked to the deregulation of circRNA expression. Numerous studies have demonstrated the functional roles that circRNAs play in either causing or preventing various disorders.

i. High-throughput exome capture RNA sequencing was used in a recent pan-cancer investigation on more than 2000 patient samples to gain a worldwide view on the expression of circRNA in malignancies (Yu & Kuo, 2019). Results revealed that the expression profile of circRNAs in various malignancies is diverse, and the expression of a particular circRNA varies dramatically between cancer types. These results, along with circRNAs' stability, point to the possibility of using them as cancer biomarkers.

ii. The human heart has a large number of circRNAs, many of which are cardiac-specific. CircRNAs have been found to play a variety of functional roles in either escalating cardiovascular illnesses or exerting cardio-protective benefits (Kristensen et al., 2019).

iii. CircRNAs can function as miRNA sponges in endothelial cells and vascular smooth muscle cells (VSMCs) in the same manner as they do in cardiomyocytes. For instance, hsa_circ_0010729 controls the miR-186 and HIF-1 axis to control the proliferation and death of vascular endothelial cells.105 Another illustration is the discovery that circ_Lrp6

is more abundant in VSMCs and functions as a miRNA sponge for miR-145 (Santer et al., 2019).

## Chapter 4: Successful Use of CirRNA in Neurological Disease

The brain has a high level of CDR1as expression, which has been linked to neurodegenerative illnesses like Alzheimer's disease and Parkinson's disease. Previous research revealed that CDR1as was downregulated in AD patients' brains. It decreased the levels of APP and BACE1 in a way that was NF-B dependent. CDR1as overexpression had a neuroprotective effect by reducing the generation of amyloid- (A) peptides. Additionally, a circRNA in the brain has been found to contain the APP gene's A-coding region. It was shown that this circRNA effectively translated in vitro and in the human brain into a new protein that could then be converted into A peptide. As a result, this circRNA is a possible therapeutic target for AD (Santer et al., 2019).

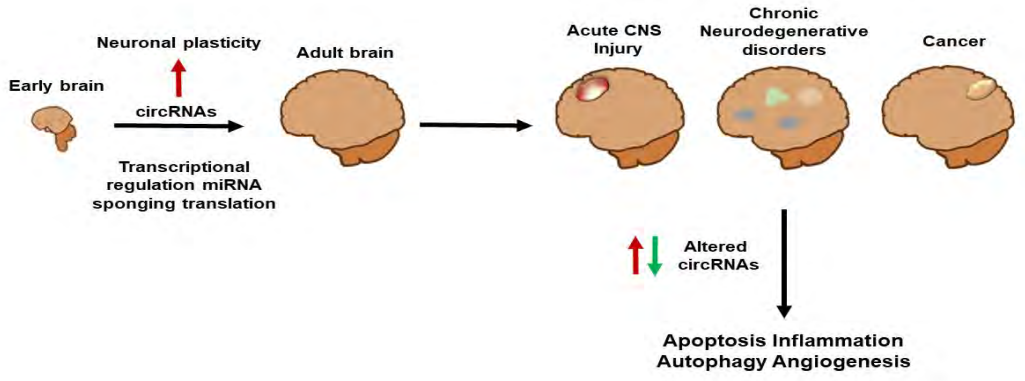


*Figure 8: Numerous illnesses are hypothesized to be mediated by altered circRNA expression*

A transient middle cerebral artery occlusion (tMCAO) mice stroke model and the plasma of acute ischemic stroke (AIS) patients both had considerably higher levels of CircHECTD1. Through autophagy, CircHECTD1 suppressed astrocyte activation and sponged miR-142 to upregulate TIPARP. In vivo, circHECTD1-targeting siRNA decreased astrocyte activation and infarction. Similar to AIS patients, tMCAO mice, and humans, circTLK1 was elevated. It likewise controlled

TIPARP, but it did so via sponging miR-335-3p. In vivo, infarction and neurological impairments were decreased by shRNA targeting circTLK1. However, in the plasma of AIS patients and photothrombotic stroke mice, circSCMH1 expression was downregulated. The MeCP2 transcription factor was discovered to bind to it, derepressing its target genes. Intriguingly, circSCMH1 overexpression in mice and rhesus monkeys following stroke was aided by intravenous injection of rabies virus glycoprotein-circSCMH1-extracellular vesicles (RVG-circSCMH1-EVs) (Mehta et al., 2020b).

In the dorsal horn of rats after spinal nerve ligation (SNL), CircAnks1a was increased. It was discovered to upregulate VEGFB through a number of pathways, leading to an increase in dorsal horn neuron excitability and pain-like behavior after SNL. CircaAnks1a siRNA injection into the spine reduced the pain-like behavior. Additionally, it was shown that circHIPK3 targets miR-124 and was elevated in the serum of diabetics with neuropathic pain as well as in the dorsal root of diabetic rats. In diabetic rats, circHIPK3 shRNA dramatically reduced neuropathic pain. On the other hand, circRNA.2837 was downregulated in a rat model of sciatic nerve damage. It affected autophagy negatively by targeting miR-34a. By triggering autophagy, the delivery of the circRNA.2837 inhibitor to the damaged nerve had protective effects.

CircHIPK2 stimulated miR-124-2hg and increased SIGMAR1 to aid in astrocyte activation. By controlling ER stress and autophagy, delivery of circHIPK2 siRNA into the mouse hippocampus dramatically reduced astrocyte activation. A more recent investigation on the function of circHIPK2 in depression found that inhibiting circHIPK2 with gut microbiota transplanted from NLRP3 KO mice reduced astrocyte dysfunction and depressive-like behavior brought on by chronic unpredictable stress (Lu et al., 2019).

# Chapter 5: Future Perspectives

It is yet unknown how age-related circRNAs build up in the brain, most likely because of their circular form. Because of this property, they are resistant to RNase R and challenging to break down, which finally causes them to accumulate with age (Rahmani-Kukia & Abbasi, 2022). Recent research has discovered sex-biased accumulation, suggesting that a number of factors may be at play in the occurrence of this phenomena.

However, in order to produce exact ratios of the two in particular cell types, the splicing pattern of genes to create circRNAs and linear RNAs is carefully managed. Therefore, it is essential to ascertain the transcriptome complexity of individual cells in order to synthesize a circRNA and a linear RNA from a gene locus in order to construct cell-specific gene therapy (Miao et al., 2021). Numerous single-cell RNA-seq techniques, such as Smart-seq, CEL-Seq, Quartz-Seq, and single-cell universal poly(A)-independent RNA sequencing (SUPeR-seq), have been developed to address this problem. Verboom et al. recently discovered circRNAs using this method in addition to linear RNA biotypes. Although the field is relatively young, mounting data suggests that it may one day be possible to create lentiviral vectors that can modify circRNAs in a cell-specific way (He et al., 2021).

In addition to that, with the use of siRNAs and shRNAs encoded by adenoviruses or lentiviruses, certain cirRNAs can be shut down. The capacity to specifically target backsplice junction locations in order to prevent the nonspecific targeting of linear host RNAs is one of the difficulties of siRNA/shRNA-mediated cirRNA suppression. The development of techniques to stop the synthesis of a particular cirRNA in addition to knocking down a mature cirRNA that has already developed represents an intriguing and urgently required future approach in cirRNA-based therapeutics. In a recent study, CRISPR/Cas9 genome editing was utilized to remove the cirRNA

CDR1as region in order to better understand the biological and functional significance of CDR1as in mammalian brains. By removing the intronic complement sequence of the introns that surround circGCN1L1, expression of circGCN1L1 was reduced without impacting the transcription of the linear GCN1L1mRNA (Miao et al., 2021).

Recent approvals by the US FDA include LUXTURNA (voretigene neparvovec-rzyl) to deliver a normal copy of the RPE65 gene to correct a biallelic RPE65 mutation in patients with retinal dystrophy and Zolgensma, an adenovirus-based gene therapy to correct bi-allelic mutations in the survival motor neuron 1 (SMN1) gene. Therefore, further therapeutic success can be expected from gene-therapy based efforts to modify cirRNAs.

Lastly, another potential future strategy is to deliver a synthetic functional RNA circle to a cell in an effort to raise particular cirRNA levels.

# Chapter 6: Conclusions

CirRNAs are developing non-coding RNAs that may have regulatory abilities. All tissues express cirRNAs, although the CNS has the highest concentration. However, recent research suggests that some neuronal-specific genes create circRNAs, suggesting that these molecules may play a role in synaptic plasticity and brain development. CirRNA disruption may be related to subsequent brain damage after acute CNS trauma and neurodegenerative disorders. Finding novel therapeutic approaches to treat central nervous system problems will benefit from a deeper comprehension of the roles played by cirRNAs, their interactions with other classes of non-coding RNAs, and their effects on transcriptional and translational pathways (He et al., 2021).

# References

*2. Pathophysiology of Parkinson's Disease | ATrain Education*. (n.d.). Retrieved April 6, 2023, from https://www.atrainceu.com/content/2-pathophysiology-parkinson's-disease

Aktaş, T., Ilik, I. A., Maticzka, D., Bhardwaj, V., Pessoa Rodrigues, C., Mittler, G., Manke, T., Backofen, R., & Akhtar, A. (2017). DHX9 suppresses RNA processing defects originating from the Alu invasion of the human genome. *Nature*, *544*(7648), 115–119. https://doi.org/10.1038/NATURE21715

*Alzheimer's disease - Treatment - NHS*. (n.d.). Retrieved April 14, 2023, from https://www.nhs.uk/conditions/alzheimers-disease/treatment/

Ashwal-Fluss, R., Meyer, M., Pamudurti, N. R., Ivanov, A., Bartok, O., Hanan, M., Evantal, N., Memczak, S., Rajewsky, N., & Kadener, S. (2014). circRNA biogenesis competes with pre-mRNA splicing. *Molecular Cell*, *56*(1), 55–66. https://doi.org/10.1016/J.MOLCEL.2014.08.019

Bai, Y., Zhang, Y., Han, B., Yang, L., Chen, X., Huang, R., Wu, F., Chao, J., Liu, P., Hu, G., Zhang, J. H., & Yao, H. (2018). Circular RNA DLGAP4 Ameliorates Ischemic Stroke Outcomes by Targeting miR-143 to Regulate Endothelial-Mesenchymal Transition Associated with Blood-Brain Barrier Integrity. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *38*(1), 32–50. https://doi.org/10.1523/JNEUROSCI.1348-17.2017

Béjot, Y., & Garnier, P. (2022). Cerebral Ischemia. *Hormesis in Health and Disease*, 185–200. https://doi.org/10.1201/b17042

*Burden of Neurological Conditions - PAHO/WHO | Pan American Health Organization*. (n.d.). Retrieved April 4, 2023, from https://www.paho.org/en/enlace/burden-neurological-conditions

Chandran, R., Mehta, S. L., & Vemuganti, R. (2017). Non-coding RNAs and neuroprotection after acute CNS injuries. *Neurochemistry International*, *111*, 12–22. https://doi.org/10.1016/J.NEUINT.2017.01.015

*Formation of three types of circRNAs. a Exonic circular RNA (ecircRNA)... | Download Scientific Diagram*. (n.d.). Retrieved July 9, 2023, from https://www.researchgate.net/figure/Formation-of-three-types-of-circRNAs-a-Exonic-circular-RNA-ecircRNA-is-formed-through_fig3_332513344

Frank, S. (2014). Treatment of Huntington's Disease. *Neurotherapeutics*, *11*(1), 153. https://doi.org/10.1007/S13311-013-0244-Z

Gijsen, H. J. M., & Bischoff, F. P. (2012). Secretase Inhibitors and Modulators as a Disease-Modifying Approach Against Alzheimer's Disease. *Annual Reports in Medicinal Chemistry*, *47*, 55–69. https://doi.org/10.1016/B978-0-12-396492-2.00005-9

Greene, J., Baird, A. M., Brady, L., Lim, M., Gray, S. G., McDermott, R., & Finn, S. P. (2017). Circular RNAs: Biogenesis, function and role in human diseases. *Frontiers in Molecular Biosciences*, *4*(JUN), 38. https://doi.org/10.3389/FMOLB.2017.00038/BIBTEX

Hasan, T. F., Rabinstein, A. A., Middlebrooks, E. H., Haranhalli, N., Silliman, S. L., Meschia, J. F., & Tawk, R. G. (2018). Diagnosis and Management of Acute Ischemic Stroke. *Mayo Clinic Proceedings*, *93*(4), 523–538. https://doi.org/10.1016/J.MAYOCP.2018.02.013

He, A. T., Liu, J., Li, F., & Yang, B. B. (2021). Targeting circular RNAs as a therapeutic approach: current strategies and challenges. *Signal Transduction and Targeted Therapy 2021 6:1*, *6*(1), 1–14. https://doi.org/10.1038/s41392-021-00569-5

*Huntington's Disease - Neurologic Disorders - MSD Manual Professional Edition*. (n.d.). Retrieved April 5, 2023, from https://www.msdmanuals.com/professional/neurologic-disorders/movement-and-cerebellar-disorders/huntington-disease

Hurd, M. D., Goel, I., Sakai, Y., & Teramura, Y. (2021). Current status of ischemic stroke treatment: From thrombolysis to potential regenerative medicine. *Regenerative Therapy*, *18*, 408. https://doi.org/10.1016/J.RETH.2021.09.009

Jimenez-Sanchez, M., Licitra, F., Underwood, B. R., & Rubinsztein, D. C. (2017). Huntington's Disease: Mechanisms of Pathogenesis and Therapeutic Strategies. *Cold Spring Harbor Perspectives in Medicine*, *7*(7), 1–22. https://doi.org/10.1101/CSHPERSPECT.A024240

Kouli, A., Torsney, K. M., & Kuan, W.-L. (2018). Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. *Parkinson's Disease: Pathogenesis and Clinical Aspects*, 3–26. https://doi.org/10.15586/CODONPUBLICATIONS.PARKINSONSDISEASE.2018.CH1

Kristensen, L. S., Andersen, M. S., Stagsted, L. V. W., Ebbesen, K. K., Hansen, T. B., & Kjems, J. (2019). The biogenesis, biology and characterization of circular RNAs. *Nature Reviews Genetics 2019 20:11*, *20*(11), 675–691. https://doi.org/10.1038/s41576-019-0158-7

Laroche-Johnston, F., Monat, C., Verreault, E., & Cousineau, B. (2021). Molecular characterization of both transesterification reactions of the group II intron circularization pathway. *Nucleic Acids Research*, *49*(12), 6996–7010. https://doi.org/10.1093/nar/gkab537

Lu, S., Yang, X., Wang, C., Chen, S., Lu, S., Yan, W., Xiong, K., Liu, F., & Yan, J. (2019). Current status and potential role of circular RNAs in neurological disorders. *Journal of Neurochemistry*, *150*(3), 237–248. https://doi.org/10.1111/JNC.14724

Ma, Y., Liu, Y., & Jiang, Z. (2020). CircRNAs: A new perspective of biomarkers in the nervous system. *Biomedicine & Pharmacotherapy*, *128*, 110251. https://doi.org/10.1016/J.BIOPHA.2020.110251

Macdonald, R. L., & Stoodley, M. (1998). Pathophysiology of cerebral ischemia. *Neurologia Medico-Chirurgica*, *38*(1), 1–11. https://doi.org/10.2176/NMC.38.1

Mallhi, T. H., Saifullah, A., Khan, Y. H., Khan, A., Alotaibi, N. H., & Alzarea, A. I. (2021). *Pathogenesis of Neurodegeneration and Associated Neurological Disorders*. 1–30. https://doi.org/10.1007/978-3-030-66376-6_1

Mehta, S. L., Dempsey, R. J., & Vemuganti, R. (2020a). Role of Circular RNAs in Brain Development and CNS Diseases. *Progress in Neurobiology*, *186*, 101746. https://doi.org/10.1016/J.PNEUROBIO.2020.101746

Mehta, S. L., Dempsey, R. J., & Vemuganti, R. (2020b). Role of Circular RNAs in Brain Development and CNS Diseases. *Progress in Neurobiology*, *186*, 101746. https://doi.org/10.1016/J.PNEUROBIO.2020.101746

Miao, Q., Ni, B., & Tang, J. (2021). Coding potential of circRNAs: new discoveries and challenges. *PeerJ*, *9*. https://doi.org/10.7717/PEERJ.10718

Nani, S. (2021). Patterns of Tissue Pathology in Neurological Diseases. *Journal of Multiple Sclerosis*, *8*(5), 1–1. https://doi.org/10.35248/2376-0389.21.8.247

*Nearly 1 in 6 of world's population suffer from neurological disorders – UN report | UN News*. (n.d.). Retrieved April 4, 2023, from https://news.un.org/en/story/2007/02/210312

*Neurological Disorders - Physiopedia*. (n.d.). Retrieved March 28, 2023, from https://www.physio-pedia.com/Neurological_Disorders

*Neurological disorders affect millions globally: WHO report*. (n.d.). Retrieved April 4, 2023, from https://www.who.int/news/item/27-02-2007-neurological-disorders-affect-millions-globally-who-report

*Neurosciences: MedlinePlus Medical Encyclopedia*. (n.d.). Retrieved March 29, 2023, from https://medlineplus.gov/ency/article/007456.htm

Nielsen, H., Fiskaa, T., Birgisdottir, Å. B., Haugen, P., Einvik, C., & Johansen, S. (2003). The ability to form full-length intron RNA circles is a general property of nuclear group I introns. *RNA (New York, N.Y.)*, *9*(12), 1464–1475. https://doi.org/10.1261/RNA.5290903

Nielsen, H., & Johansen, S. D. (2009). Group I introns: Moving in new directions. *RNA Biology*, *6*(4). https://doi.org/10.4161/RNA.6.4.9334

*Overview of Nervous System Disorders | Johns Hopkins Medicine*. (n.d.). Retrieved March 28, 2023, from https://www.hopkinsmedicine.org/health/conditions-and-diseases/overview-of-nervous-system-disorders

*Parkinson's - Physiotherapy Management and Interventions - Physiopedia*. (n.d.). Retrieved April 14, 2023, from https://www.physio-pedia.com/Parkinson%27s_-_Physiotherapy_Management_and_Interventions

*Parkinson's disease - Treatment - NHS*. (n.d.). Retrieved April 14, 2023, from

https://www.nhs.uk/conditions/parkinsons-disease/treatment/

Petkovic, S., & Müller, S. (2015). RNA circularization strategies in vivo and in vitro. *Nucleic Acids Research*, *43*(4), 2454. https://doi.org/10.1093/NAR/GKV045

Rahmani-Kukia, N., & Abbasi, A. (2022). New insights on circular RNAs and their potential applications as biomarkers, therapeutic agents, and preventive vaccines in viral infections: with a glance at SARS-CoV-2. *Molecular Therapy - Nucleic Acids*, *29*, 705–717. https://doi.org/10.1016/J.OMTN.2022.08.012

Santer, L., Bär, C., & Thum, T. (2019). Circular RNAs: A Novel Class of Functional RNA Molecules with a Therapeutic Perspective. *Molecular Therapy*, *27*(8), 1350–1363. https://doi.org/10.1016/J.YMTHE.2019.07.001

Shao, Y., & Chen, Y. (2016). Roles of circular RNAs in neurologic disease. *Frontiers in Molecular Neuroscience*, *9*(APR), 25. https://doi.org/10.3389/FNMOL.2016.00025/BIBTEX

Sun, P., Hamblin, M. H., & Yin, K. J. (2022). Non-coding RNAs in the regulation of blood–brain barrier functions in central nervous system disorders. *Fluids and Barriers of the CNS*, *19*(1). https://doi.org/10.1186/s12987-022-00317-z

Thakur, K. T., Albanese, E., Giannakopoulos, P., Jette, N., Linde, M., Prince, M. J., Steiner, T. J., & Dua, T. (2016). Neurological Disorders. *Disease Control Priorities, Third Edition (Volume 4): Mental, Neurological, and Substance Use Disorders*, 87–107. https://doi.org/10.1596/978-1-4648-0426-7_CH5

Ueno, D. M. V. M. (n.d.). *System Pathology for Neurological Disorders*. Retrieved April 4, 2023, from http://www.bri.niigata-u.ac.jp/~system_neurodis/ueno/home-e.html

Yu, C. Y., & Kuo, H. C. (2019). The emerging roles and functions of circular RNAs and their generation. *Journal of Biomedical Science 2019 26:1*, *26*(1), 1–12. https://doi.org/10.1186/S12929-019-0523-Z