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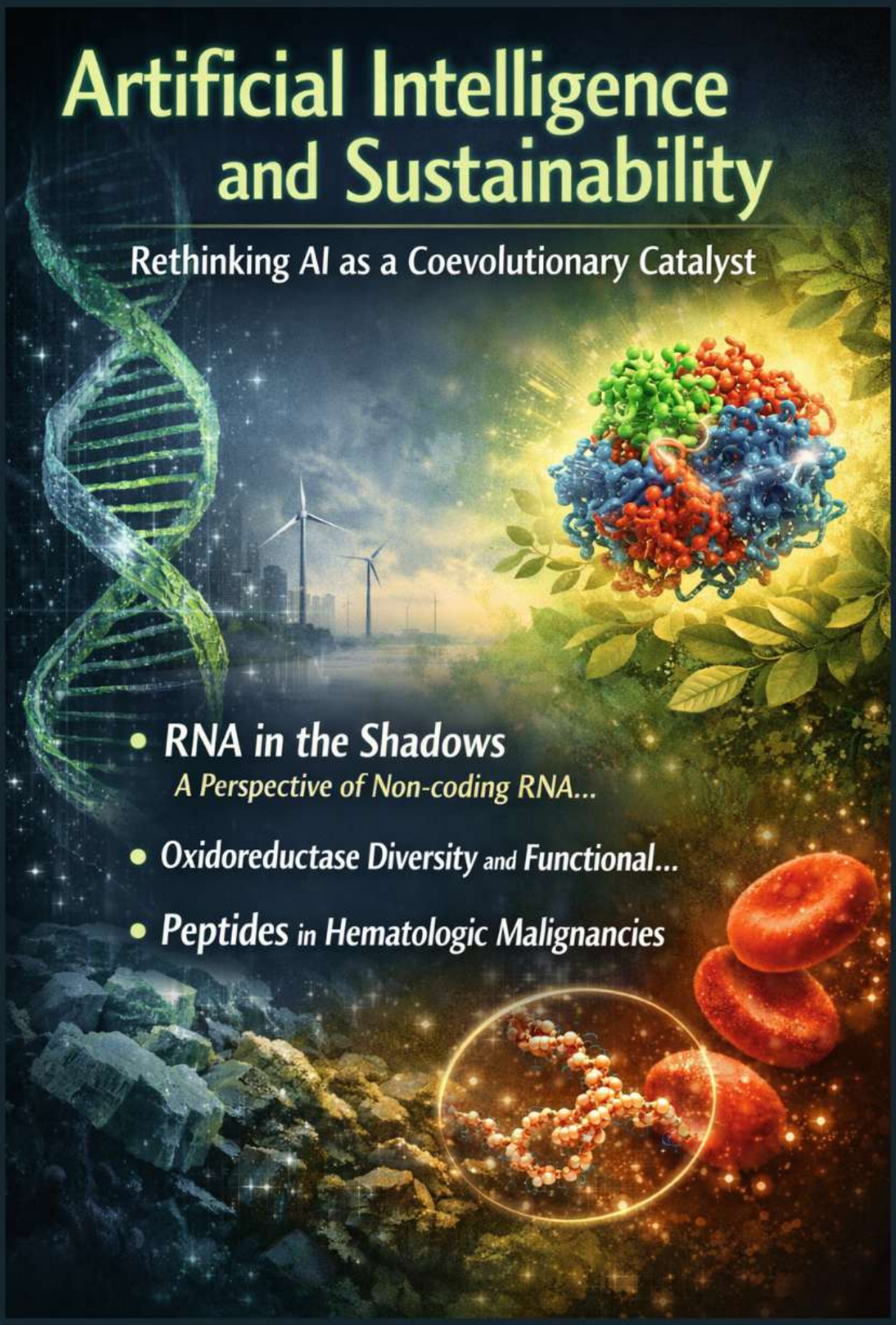

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Short Commentary Article

Artificial Intelligence and Sustainability: Rethinking AI as a Coevolutionary Catalyst

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For decades, Artificial Intelligence (AI) was cast as a background tool—an efficient processor of data, a passive observer assisting scientists in making sense of complexity [1]. Sustainability, by contrast, was championed as a fundamentally human pursuit, tied to ethical choices and political will. Yet this dichotomy is now dissolving. Emerging applications suggest that AI is not simply supporting sustainability but coevolving with it, offering dynamic, context-sensitive strategies for navigating the environmental and biological crises of our time.

Artificial Intelligence (AI) plays a major role in the field of sustainability. The interplay between AI and sustainability can be captured through a metaphor: AI as a coevolutionary catalyst. Much like the mutualistic relationships observed in ecosystems, AI and the life sciences are learning from one another, adapting in tandem, and driving forward solutions that neither could achieve in isolation. This reframing invites us to reconsider AI not merely as a computational instrument but as an active partner shaping ecological resilience, agricultural innovation, and planetary health.

From Data to Foresight: AI in Environmental Stewardship

In the early 40”s (1943) the term AI was invented by McCulloch & Pitts and since then AI has reached in this stage developing day by day by the passage of time. Between 1950s- 1970s the foundational theories, logics, between 1980-90s symbolic reasoning like things were happening and from 2000s onwards "experienced rapid advances” and “better algorithm” have been continuing till today. [1]

Traditionally, conservation biology relied on painstaking field observations, often limited in scale and scope. Today, AI extends human perception by processing vast, multimodal datasets—satellite imagery, acoustic recordings, drone footage—and translating them into actionable insights. Machine learning models now detect illegal logging, identify species from vocalizations, and predict shifts in biodiversity hotspots under climate change [2,3]. This transition resembles moving from a static map to a dynamic navigation system. While maps depict what is, navigation systems anticipate what will be, continuously recalibrating based on real-time data. Similarly, AI transforms ecological monitoring from retrospective documentation to prospective guidance, empowering conservationists to act before irreversible damage occurs. Yet, as with navigation systems, overreliance on AI carries risks—blind spots in data or biased training sets may misguide decisions, underscoring the need for interpretive human oversight [4].

Precision Agriculture: AI as the "Microscope of the Field"

Drones and sensors feed real-time data into algorithms that recommend targeted irrigation, nutrient management, or pest control [5]. The result is not brute-force intensification but smarter, more efficient farming—yielding more with less environmental cost. These technologies are closely connected to life sciences, linking plant physiology, genetics, and ecology. AI helps predict crop yields by combining genetic and climate data, which could improve how we grow and breed plants. But like a microscope, AI only shows details—it doesn't explain them. Scientists and farmers still need to understand and interpret the data carefully, keeping nature and ethics in mind.

Smart Cities and Public Health: AI as an Urban Immune System

Urban ecosystems, much like biological organisms, require regulation to maintain homeostasis. Here, AI functions as an urban immune system, monitoring flows of traffic, energy, waste, and pollutants. By adjusting these processes, AI reduces metabolic strain on cities—cutting emissions, conserving energy, and improving air quality [6]. Cleaner air helps reduce breathing problems, better waste management stops the spread of harmful germs, and smart infrastructure makes communities stronger against climate-related challenges. These changes can greatly improve public health. This immunological metaphor also highlights vulnerabilities. Just as autoimmune disorders arise when regulation falters, AI-driven cities risk inequity or dysfunction if algorithms are biased or opaque. In low-resource settings, where infrastructure is fragile, AI could either be a shield that enhances resilience or a stressor that exacerbates disparities [7].

Industry and Manufacturing: AI as the Metabolic Regulator

Industrial production has long been criticized as a driver of ecological imbalance, consuming energy and producing waste with little regard for planetary boundaries. AI introduces the possibility of metabolic regulation within manufacturing systems. Predictive maintenance reduces material waste, defect detection enhances product quality, and supply chain optimization curtails unnecessary resource consumption [8]. AI is like enzymes in a living system—it speeds things up, cuts waste, and keeps balance. But just like enzymes need the right conditions to work, AI also needs clear rules, fair data, and goals that support sustainability. Without these, its power might be used to increase consumption instead of helping the environment.

Ethical Crossroads: The Paradox of AI's Carbon Footprint

While AI promises sustainability, its own ecological cost cannot be ignored. Training large models consumes vast amounts of energy, generating a carbon footprint that contradicts its green promise [9]. This paradox mirrors the classic dilemma in medicine: treatments that alleviate one condition may inadvertently cause side effects elsewhere in the body. To resolve this tension, the AI community must prioritize "green AI"—models optimized for efficiency without sacrificing performance. Similarly, ethical safeguards must ensure that AI does not entrench inequalities or obscure accountability. In sustainability, as in medicine, interventions must be evaluated holistically, balancing benefits against unintended harms [10].

Toward a Coevolutionary Framework

Taken together, these perspectives suggest that AI in sustainability should not be imagined as a one-way transfer of tools but as a coevolutionary partnership. AI learns from the biological and ecological systems it monitors, while these systems, in turn, are shaped by the decisions AI informs. This reciprocity mirrors coevolutionary dynamics in nature—pollinators and flowers, predators and prey—where both partners adapt in response to one another. Such a framework shifts the question from "How can AI help sustainability?" to "How can AI and the life sciences co-adapt to achieve sustainability?" This reframing acknowledges that AI is not neutral; it encodes human choices,

societal priorities, and ecological assumptions. Recognizing this, researchers and policymakers must cultivate AI systems that are transparent, equitable, and energy conscious.

Conclusion

The narrative of AI in sustainability is still unfolding. Early portrayals cast AI as a passive observer, but emerging evidence suggests a more dynamic role: a coevolutionary catalyst capable of reshaping life sciences research and sustainability practices. By conceptualizing AI as a microscope of the field, an urban immune system, or a metabolic regulator, we capture its ability to illuminate hidden patterns, maintain balance, and accelerate change. However, responsible use is crucial to tackle challenges like data privacy and environmental costs of AI itself. When applied ethically, AI accelerates innovation and supports achieving global sustainability goals by enhancing operational efficiency and lowering emissions across sectors.

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Review Article

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RNA in the Shadows: A Perspective of non-coding RNA on gene regulation in plants

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Summary

Advances in next-generation sequencing (NGS) technologies have revolutionized our knowledge of the transcriptome, leading to the discovery of multiple classes of non-coding RNAs (ncRNAs) across all kingdoms of life. While coding RNA mainly serves as a template for synthesis of protein, the ncRNAs carry out diverse regulatory functions and modulate gene expression at multiple stages mainly at epigenetic, transcriptional, post-transcriptional, and translational levels. Recent advances in plant research have revealed the potential of ncRNAs in various cellular processes, which includes growth and developmental aspects, vegetative to floral meristem transition, gametogenesis and response to unescapable environment factors like biotic and abiotic stresses. Hence, studying the ncRNA biology, their mode of action and its interaction with the binding proteins, greatly enhance our understanding and help in crop improvement programs. Characterizing and leveraging the potential ncRNAs to specifically modulate plant gene expression, provides a significant scope for improving and enhancing desirable traits. This review highlights foundational aspects of ncRNA biology, including their biogenesis and the diverse regulatory role of several ncRNA classes and discusses the recent discoveries that emphasize their essential roles in plant development and stress resilience, giving insight into their applicability in modern agriculture.

Keywords

Non-coding RNA, circular RNA, gene regulation, RNAi, epigenetic modification, crop improvement

Introduction

The central dogma of molecular biology illustrates the sequential movement of genetic information through the key processes of replication, transcription, and translation, in which deoxy ribonucleic acid (DNA) is copied, transcribed into messenger ribonucleic acid (mRNA), and subsequently translated into protein. During transcription, RNA acts as a vital intermediary between DNA and protein synthesis. The RNA world hypothesis suggests that the life likely originated with a relatively unstable molecule, the RNA. Later, DNA which is a more stable molecule, took over as the principal carrier of genetic information and RNA was left with the role of a messenger [1]. Eventually, scientific advancement unravelled that RNA was also involved in dynamic modulation of gene expression pattern. Thus, RNA mainly contributes to establish cellular homeostasis, adaptation to stress conditions and overall functioning of organism through its unique catalytic activity [2].

Aside from mRNA, numerous forms of RNA have been identified and become prominent with the advent of modern techniques like high-throughput sequencing, microarray analysis, and transcriptomics. Since these RNAs are not directly involved in protein synthesis, they are classified as “non-coding RNAs” (ncRNAs).

These ncRNAs were previously dismissed as transcriptional noise or cellular by-products, but now they have recognized as crucial regulators in cellular functions through experimental and computational studies [3]. The regulatory power of ncRNAs may be attributed to their distinct structural and functional properties, such as catalytic activity, reduced stability, and their capacity for precise interactions with DNA, RNA, and proteins [4].

Several evidences prove that ncRNAs play essential roles in regulating the gene expressions by operating at genomic, transcriptional, post-transcriptional, translational levels ultimately influencing the biological processes such as growth, cellular differentiation, and stress adaptation[5]. Moreover, ncRNAs are now emerging as promising biomarkers in disease diagnosis and also been used as therapeutics of human diseases [6]. Typically, ncRNAs constitute a diverse family of RNA molecules that are transcribed by various RNA polymerases, and these ncRNAs can be broadly classified as structural/ housekeeping/ constitutive and regulatory class. Constitutive ncRNAs are expressed constantly at high levels in all cells, which mainly participate in basic cellular processes such as translation and RNA processing [7]. This class of ncRNAs comprises ribosomal RNA (rRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), and small nucleolar RNA (snoRNA). Whereas the regulatory classes are usually expressed in a cell-type, stage-specific, or condition-dependent manner, functioning mainly to regulate gene expression [7] and are classified into small ncRNA (sncRNA), long ncRNA (lncRNA), circular RNAs (circRNA) and derived ncRNAs, the detailed classifications is presented in Figure 1.

Generally, the ncRNAs follow well-defined pathways that begin with activation by specific signals, followed by their biogenesis and are subsequently subjected to various modifications or processing steps. The mature ncRNAs execute their precise regulatory role in transcriptional and post-transcriptional gene silencing, translational repression, RNA stability, chromatin remodelling [5]. Additionally, to maintain homeostasis and prevent nonspecific regulations, ncRNAs are degraded once they are no longer required by the cell through cellular RNA turnover pathways [8,9] Although cells may generate a wide range of ncRNAs, not all of them are functional; hence may have no regulatory roles [3]. Therefore, differentiating the functional ncRNAs from the total RNA pool and elucidating their regulatory roles in developmental, physiological, and stress-responsive processes is highly important. Such comprehensive studies on ncRNAs provide better opportunities to exploit the functional ncRNAs as valuable tools for crop improvement. The present review basically describes the diverse regulatory classes of ncRNA, with an emphasis on their biogenesis and the molecular mechanisms underlying their regulatory functions in plants.

In addition, we highlight representative examples which illustrate the functional significance of ncRNAs along with the recent discoveries that enhance our understanding of their roles in plant development, stress adaptation, and crop improvement.

Historical significance

The discovery of ncRNAs started in 1939, when Torbjörn Caspersson and Jean Brachet independently showed that the cytoplasm is very rich in RNA and its amount increases during protein synthesis [10]. This provided the first hint about the requirement of RNA during protein synthesis, more importantly, acting as a link between DNA and proteins [1]. Later, in 1955, the first noncoding RNA (rRNA) was discovered by Georges Palade, which is part of the very abundant cytoplasmic ribonucleoprotein (RNP) complex: the ribosome [11]. Two years later, in 1957, the second class of ncRNAs was discovered by Mahlon Hoagland and Paul Zamecnik: the tRNA, which is an “adapter” molecule for the translation of

information from RNA to amino acid synthesis [12]. Further, in the late 1960s, other RNA groups in structural class, such as heterogeneous nuclear RNA (hnRNA), snRNAs, as well as snoRNAs were discovered [1].

The first regulatory non-coding RNA, micF, was discovered in *Escherichia coli* in 1984 and became notable for its role in suppressing translation of the outer membrane protein F (ompF) mRNA by directly pairing with its ribosome-binding site through sense–antisense base interactions [13]. In the late 1980s, H19 RNA was identified as the first regulatory ncRNA discovered in eukaryotes. Initially, it was misclassified as mRNA due to the small open reading frame (ORF) present in the gene, but subsequent research revealed the absence of translation, establishing H19 as a non-coding, regulatory RNA [14]. The function of H19 as an RNA molecule remained a mystery until the functional characterization of another lncRNA, X-inactive specific transcript (Xist) [15]. This discovery revealed that both H19 and Xist are involved in dosage compensation in mammals, a process that maintains balanced levels of X-linked gene products between sexes which is critical for cellular equilibrium [1].

During the early 1990s, researchers documented a molecular phenomenon in different kingdoms, for instance, “co-suppression” in plants, “posttranscriptional gene silencing” in nematodes, and “quelling” in fungi, all characterized by the inhibition of protein production through RNA-mediated silencing pathways. However, none suspected the RNA to be a key actor until the identification of the first micro RNA (miRNA) lin-4, in the nematode *Caenorhabditis elegans* in the year 1993[16]. Later in 1998, Fire *et al.* (17) reported that exogenous double-stranded RNA (dsRNA) can explicitly silence genes by RNA interference (RNAi) mechanism. These discoveries led to the characterization of numerous small ncRNAs, which led to the establishment of two major categories: miRNAs, which regulate endogenous gene expression, and small interfering RNAs (siRNA), which protect genome integrity against foreign or invasive elements such as transposons, viruses and transgenes [18].

During the genomic era, with the onset of advanced technologies and robust NGS, together with extensive international consortiums such as the Functional Annotation of the Mammalian Genome (FANTOM) (https://fantom.gsc.riken.jp/) and the Encyclopaedia of DNA Elements (ENCODE) (https://www.encodeproject.org/) it was concluded that 80 per cent of the DNA is transcribed into RNA yet only a meagre 1.5 per cent of that RNA is actually translated into protein in humans [1]. Extensive transcription activity fundamentally transformed scientific understanding of the transcriptome and sparked a growing interest within the research community, leading to the identification and characterization of numerous non-coding RNAs.

Small noncoding RNA

The sncRNAs are typically short molecules, ranging from 20 to 30 nucleotides in length. These small RNAs generally act as gene expression inhibitors and are mainly involved in RNA silencing processes. In these mechanisms, mature sncRNAs serve as specificity factor, directing effector proteins to their complementary nucleic acid targets via base-pairing interactions [18]. Among the various classes of small RNAs, three major types hold key regulatory roles: miRNAs, siRNAs, and PIWI-interacting RNAs (piRNAs). Notably, siRNAs and miRNAs are widely present across many species and physiological contexts, both originating from dsRNA precursors. In contrast, piRNAs are found primarily in animals, function mostly in the germline, and are derived from single-stranded precursors that are presently not very well understood.

Biogenesis of sncRNAs

miRNAs

Plant miRNAs are endogenous ncRNAs that typically consist of approximately 20–24 nucleotides that play a key role in post-transcriptional gene regulation. The biogenesis of miRNAs begins with the transcription of single-stranded primary miRNA (pri-miRNA) transcripts from MIR genes by RNA polymerase II (Pol II). These transcripts fold into imperfectly paired stem-loop structures known as precursor miRNAs (pre-miRNAs) [19]. The hairpin-shaped precursor is then processed into a miRNA–miRNA* duplex by the coordinated action of DICER-LIKE 1 (DCL1), the double-stranded RNA-binding protein HYPONASTIC LEAVES 1 (HYL1), and the zinc-finger protein SERRATE (SE) [20]. The mature miRNAs are initially methylated by HUA ENHANCER 1 (HEN1) [21] and then exported from the nucleus to the cytoplasm by the HASTY(HST) export protein [22]. Finally, mature miRNAs associate with the Argonaute (AGO) protein and result in formation of the RNA-induced silencing complex (RISC) [20]. Only one strand of the duplex is stably associated with an miRISC complex; usually, the miRNA strand is more strongly favoured than the miRNA* strand, guiding it to complementary target transcripts (mRNA) [18]. Typically, they bind to the 3' untranslated region (UTR) of target mRNAs and can either degrade the mRNA or inhibit its translation, thereby controlling the expression of specific genes (Figure 2B). (Note: * in miRNA represents the antisense strand of duplex form)

siRNAs

The siRNA can originate either exogenously from viral RNA and transgenes or endogenously from repeat-rich genomic regions, transposable, and retro-elements [23]. In plants, depending on their origin and processing enzyme involved, siRNAs can be grouped into 7 subclasses; the detailed description of each class is given in Table 1 [7; 24]

The generation of siRNA is primarily dependent on one of six RNA-dependent RNA polymerases (RDR1–6) that copy single-stranded RNA (ssRNA) to generate dsRNA. The dsRNA is then processed by DCL1–4 into sRNA duplexes: DCL1 mainly generates 18–21 nt sRNAs, while DCL2, DCL3, and DCL4 produce 22-, 24-, and 21-nt sRNAs, respectively. Following processing, these duplexes are either retained in the nucleus to regulate chromatin or exported to the cytoplasm, where they assemble with AGO proteins within the RISC to mediate post-transcriptional gene silencing (PTGS) (25; 26). Similar to the miRNA processing, only one strand of the siRNA duplex, the guide strand, is selectively retained in the siRISC and the 'passenger' strand is discarded (Figure 2).

Regulatory role of sncRNAs

In plants, sncRNAs play a central role in regulating gene expression, primarily through the RNAi mechanism, which silences the target gene at transcriptional level. The mechanism involves the loading of mature siRNA or miRNA into the AGO protein, resulting in the formation of the RISC complex. The guide strand specifically base pairs with the target mRNA by complementarity and leading to the initiation of mRNA degradation or inhibition of translation. Another important role of

small RNAs, particularly siRNAs, is their involvement in the RNA-directed DNA methylation (RdDM) pathway, where they guide epigenetic modifications at specific genomic location.

Table 1: Classification of major siRNAs and their features

siRNA Type	Size (nt)	Origin/Description	Processing Enzyme(s)
cis-acting siRNA (ca-siRNA)	24	Derived from the same locus as their target RNA	DCL3

trans-acting siRNA (ta-siRNA)	21	Produced from non-coding TAS gene loci; act in trans	DCL4, RDR6, AGO1
heterochromatic siRNA (hc-siRNA)	24	Originates from heterochromatic/repetitive regions	DCL3, Pol IV, RDR2
repeat-associated siRNA (ra-siRNA)	24	Produced from repetitive sequences (TEs, repeats)	DCL3, Pol IV, RDR2
long siRNA (lsiRNA)	30–40	Found under stress; derived from long dsRNA	DCLs (exact type may vary)
phased siRNA (phasiRNA)	21 or 24	Produced in a “phased” manner from precursor transcripts	DCL4 or DCL5, RDR6, (miRNA trigger)
natural antisense siRNA (nat-siRNA)	21–24	Generated from overlapping sense–antisense transcripts	DCL1/2/3/4 (varies), RDR6

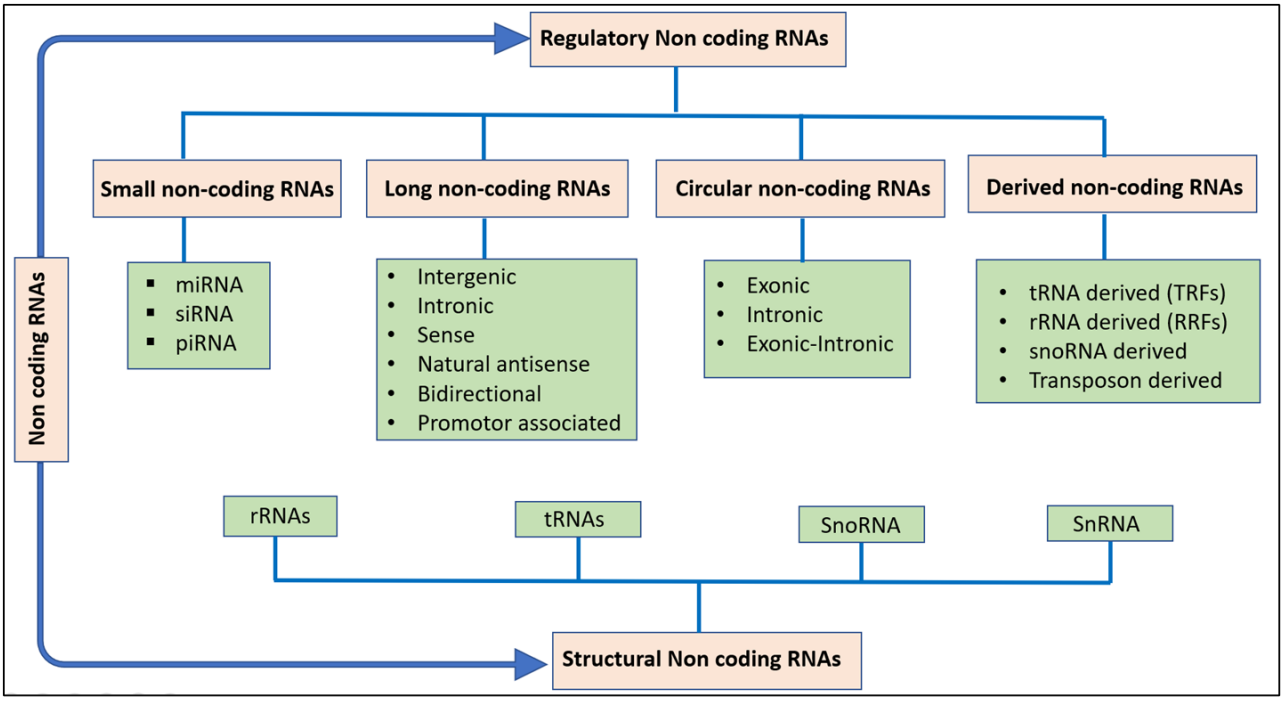

Figure 1. Classifications of non-coding RNA

In RdDM pathway, the Pol IV and RDR2 help in generating the double-stranded precursor siRNA and upon processing by DCL 3, the precursor siRNA is cleaved into specific 24-nucleotide (nt) siRNA and loaded into AGO 4 (or AGO6/9). Simultaneously, Pol V produces scaffold lncRNA, and together they serve as a platform to recruit the AGO4–siRNA complex to trigger DNA methylation by DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) [27]. Thus, 24nt siRNA guides the DRM2 to the specific location where methylation is necessary. This process plays an important role in regulating developmental programs,

facilitating stress adaptation, and ultimately contributing to genome evolution.

sncRNA in biotic stress

RNAi primarily regulates plant development by modulating gene expression at various developmental stages such as flowering, vernalization, fruit development, formation of seeds, and other processes. RNAi plays a pivotal role in plant immunity against pathogen attack, specifically in case of viruses. Naturally, plants produce several siRNAs and degrade the viral genome. However, many viruses evolved to synthesize the silencing suppressor proteins that help in counter-attack the host RNA silencing machinery. To overcome these challenges, transgenic methods have been deployed in several crops, where viral genes are expressed in the form of antisense RNA, hairpin RNA (hpRNA) or intron hairpin RNA (ihpRNA). Upon expression, these constructs activate RNAi, leading to enhanced resistance against viruses [28]. Notable successes include the development of the virus-resistant 'HoneySweet' plum targeting *plum pox virus* genes [29], transgenic tomato lines producing short hairpin RNA (shRNA) against the silencing suppressor (*NSS*) gene of *tomato spotted wilt orthotospovirus* (TSWV) [30], and *Nicotiana benthamiana* plants expressing inverted hairpin RNA targeting the *helper component proteinase* gene for resistance to *papaya ringspot virus* [31]. Collectively, the above reports demonstrate that expressing small RNA precursor constructs effectively induces RNAi, conferring increased viral resistance in plants.

In pest management, RNAi technology offers a novel and environmentally friendly method by selectively silencing critical genes in target pests [32]. This is achieved by delivering dsRNA into insect tissues, either by developing transgenic plants expressing specific dsRNA molecules or through external applications like dsRNA sprays. RNAi-based strategies have been successfully implemented against various insect pests, including *Helicoverpa armigera, Diabrotica virgifera,* and *Leptinotarsa decemlineata*, resulting in decreased pest survival and reduced crop damage. For instance, transgenic tomato plants expressing dsRNA constructs were developed, targeting crucial genes, *Acetylcholinesterase* 1 (*AChE1*) and *SEC23*, in *Phthorimaea absoluta,* which resulted in enhanced resistance against infestation [33]. Significant progress has also been achieved in improving dsRNA delivery methods to overcome degradation and enhance their uptake in insect system using several approaches. These include coating or complexing dsRNA with polymers, nanoparticle-mediated encapsulation and employing interpolyelectrolyte complexes (IPECs) or paperclip dsRNA; these strategies together improve the protection, delivery, and persistence of dsRNA in the insect system, thereby significantly increasing the efficacy of dsRNA sprays [34]. For example, the oral administration of an artificial diet consisting of dsRNA, which targets the *Acetylcholinesterase-like protein* (*AchELP*) and *SWItch/Sucrose Non-Fermentable 7* (*SNF7*) genes in *Bemisia tabaci* [35] and *Leucinodes orbonalis* [36], respectively, induced significant larval mortality, suggesting the potential role of dsRNA-based strategies as effective biopesticides.

RNAi has been successfully applied in managing fungal pathogens by targeting the essential fungal genes using two major strategies they are host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS). In HIGS, plants are genetically engineered to express dsRNA molecules against fungal virulence genes, which are subsequently taken up by invading pathogens during infection. SIGS, on the other hand, involves direct application of dsRNA sprays on plant surfaces, allowing uptake by fungal cells and silencing of target genes without the need for transgenic plants [37]. A few examples include the use of HIGS strategy to manage *Magnaporthe oryzae* by targeting pathogenicity and development genes to control rice blast disease [38], and using SIGS for the topical

application of *BcTRE1*-targeting dsRNA (BcTRE1-dsRNA), which exerted a strong inhibitory effect against *Botrytis cinerea*, that was evidenced by significantly reduced fungal growth and lesion formation [39]. Although HIGS and SIGS approaches are effective they have certain limitations and challenges associated with commercial use. HIGS strategy requires stable genetic transformation of the host plant, which is technically challenging for many crop species, time-consuming and is classified under Genetically Modified Organism (GMO) technology, making it subject to strict, complex, and costly authorisation processes [40]. HIGS may not be considered as effective against certain types of pathogens, such as necrotrophic fungi that feed on dead host tissue, which cannot provide a sufficient, continuous supply of silencing RNAs [41]. Pathogens can potentially develop resistance to HIGS over time, for example, by evolving mechanisms to evade or suppress the hosts RNAi machinery. The complete mechanism by which silencing RNAs are secreted from plant cells and taken up by pathogen or pest cells is still unclear, making it difficult to optimize the process [42]. Meanwhile, SIGS bypasses the stringent regulatory process and is generally considered a non-GMO approach, as the dsRNA is an externally applied product that does not alter the host genome, and is regulated as a conventional pesticide or biopesticide [43]. Several limitations of SIGS include the instability of dsRNA in the environment and the variable efficiency of dsRNA uptake by target pathogens [44].

sncRNA in abiotic stress

Several evidence suggested that the dynamic regulation of small RNAs occurs during various abiotic stresses (Table 2). Examples include the salinity-responsive miRNAs identified in *Arabidopsis thaliana* [45], *Zea mays* [46], the miRNA expression profiles in response to drought are documented in *Sorghum bicolor* [47], *Gossypium hirsutum* [48] and chilling-responsive miRNAs have been characterised in *Glycine max* [49] and *Zea mays* [50]. Leveraging this knowledge and modifying the expression profiles of target genes holds great potential for developing crop varieties with enhanced resilience to adverse climatic conditions.

The advances in high-throughput sequencing and bioinformatics allow the discovery and functional analysis of novel sncRNAs, which guide targeted genetic improvement [57]. Current strategies, include use of tissue-specific promoters and genome editing tools to precisely modulate miRNA expression, which mainly assist to avoiding negative phenotypic effects. Recently, the new RNAi design, Loop ended RNA (ledRNA) exhibited stronger RNAi activity than traditional RNAi, and ledRNA-based gene expression regulation has been proven in diverse kingdoms of life such as plants, fungi and aphids [58]. Similarly, researchers identified the most effective small interfering RNAs (esiRNAs) and combined them into "effective dsRNAs." This innovative approach targets multiple viral strains simultaneously, offering broader and more potent protection against several viruses [59].

Presently, the regulatory status of exogenous dsRNA-biopesticides is not well established. There is a wide difference in perception on the application of exogenous dsRNA-biopesticides. For example, New Zealand has adopted a liberal stance, the USA and Australia have a moderate approach and the EU is stringent on regulatory approach [60]. The dsRNA in EU and Australia is considered as chemical pesticide. In Australia, it is regulated through APVMA and the OGTR [43], whereas in the EU, approval involves a two-step process: EFSA evaluates the active substance, followed by zonal assessment by Member States [61]. In USA, it is considered as a biochemical pesticide which requires EPA approval under FIFRA and FFDCA [62]. The regulatory or safety concerns associated with the RNAi mechanism, should be subjected to a robust safety assessment before commercial use. In plants, dsRNA can stimulate pattern-triggered immunity (PTI) independent of RNAi [63], and RNAi can trigger epigenetic

changes such as RdDM. Hence, long-term risk assessments are essential, as RNAi products may show delayed efficacy or non-lethal phenotypes [64]. The regulatory framework must address environmental fate, non-target effects, and biosafety issues in order to declare RNAi as a secure, eco- friendly, and targeted alternative approach for crop protection [65]. However, the regulatory aspects related to the use of transgenic research will continue to fall under the GMO regulatory framework in different countries.

Long Non-coding RNAs

RNA molecules longer than 200 nucleotides which are involved in a regulatory role are categorized under lncRNA and found ubiquitously in plants, animals, fungi, and prokaryotes. Although most lncRNAs are primarily located in the nucleus and associate with chromatin, they can function in both nuclear and cytoplasmic compartments. Many lncRNA functions are based on the capacity to fold into secondary structures, which allows them to interact with other types of RNA, DNA, and proteins [66].

Biogenesis of lncRNA

Most plant lncRNAs are transcribed by RNA Pol II, which produces capped, polyadenylated transcripts. Additionally, in plants, certain lncRNAs are produced by two unique RNA polymerases, Pol IV and Pol V, especially those linked to RdDM pathways [67]. Relative to the genomic location, lncRNAs are divided into six subclasses: sense, antisense, long intergenic (lincRNA), bidirectional, promoter-associated and intronic (Table 3) [68]. Many lncRNAs undergo typical co-transcriptional RNA processing like splicing. Some lncRNAs are polyadenylated; however, non-polyadenylated lncRNAs also exist, especially those related to RdDM, which may be synthesized by Pol IV or Pol V and often lack poly(A) tails (Figure 2C) [67].

Regulatory role of lncRNA

The important functions of lncRNA is to regulate gene expression mainly at epigenetic, transcriptional, post-transcriptional, translational, and post-translational levels through diverse mechanisms. The main mechanism includes, association with chromatin remodelling, activation of transcription, transcriptional interference, processing of RNA, and inhibition of translation process.

lncRNAs in epigenetic regulation

In case of epigenetic mediated gene regulation, the lncRNAs perform its function either by directly associating with histone- modifying complexes, or acting as a scaffold molecule to regulate the histone modifications. The well-known plant lncRNA: COLD ASSISTED INTRONIC NONCODING RNA (COLDAIR), was first to be discovered in plants, which are found to regulate methylation of histone proteins in the chromatin region of *FLOWERING LOCUS C (FLC)*. Under cold conditions, the COLDAIR helps in the recruitment of PRC2 complex to the *FLC* locus, this leads to accumulation of H3K27me3 and consequently, it accounts for silencing of *FLC* gene during vernalization. The other mechanisms of lncRNAs are acting as molecular scaffolds in which they bind two or more protein molecules in order to perform specific biological functions [69]. The lncRNA named AUXIN REGULATED PROMOTER LOOP RNA (APOLO) is a lincRNA, transcribed by RNA Pol II, and modulate the *PINOID (PID)* gene expression by interacting with the PRC1 and PRC2

Table 2: List of some examples of sRNA which are involved in abiotic stress

Sl No	sRNA (family)	Main target(s)	Species (example)	Role under abiotic stress	Source
1.	**miR169 (miR169z)**	NF-YA (NF-YA5)	Rice / Arabidopsis	Improves drought tolerance via NF-YA regulation and downstream metabolic adjustments.	[51]
2.	**miR395**	ATP sulfurylases (APS/ATPS), SULTR transporters	Arabidopsis / Tomato / Rice	Regulates sulfate assimilation and root development during sulfate deficiency; modulates stress responses linked to sulfur metabolism.	[52]
3.	**ta-siRNAs (TAS1/2/3/4)**	Auxin Response Factor (ARF), MYBs, other TFs (via ta-siRNA action)	Arabidopsis and crops	ta-siRNAs generated from TAS transcripts alter expression of TFs (e.g., ARFs) and change in response to various abiotic cues (nutrient, heat, hypoxia).	[53]
4.	**miR156**	SPL transcription factors	Wheat, Alfalfa, Apple, Arabidopsis	Overexpression or induction of miR156 improves tolerance to drought and heat (via SPL repression, increased flavonoids/ROS scavenging).	[54]
5.	**miR398**	CSD1, CSD2 (Cu/Zn SODs), CCS1	Arabidopsis, Tomato, other crops	miR398 is temperature/ROS responsive; modulates antioxidant system (dynamic regulation under heat/oxidative stress) to alter stress tolerance.	[55]
6.	**miR399**	PHO2 (ubiquitin E2 related)	Arabidopsis, Banana	Classic phosphate-starvation miRNA but also responsive to heat in some species (regulatory movement shoot→root; affects Pi homeostasis under stress).	[56]

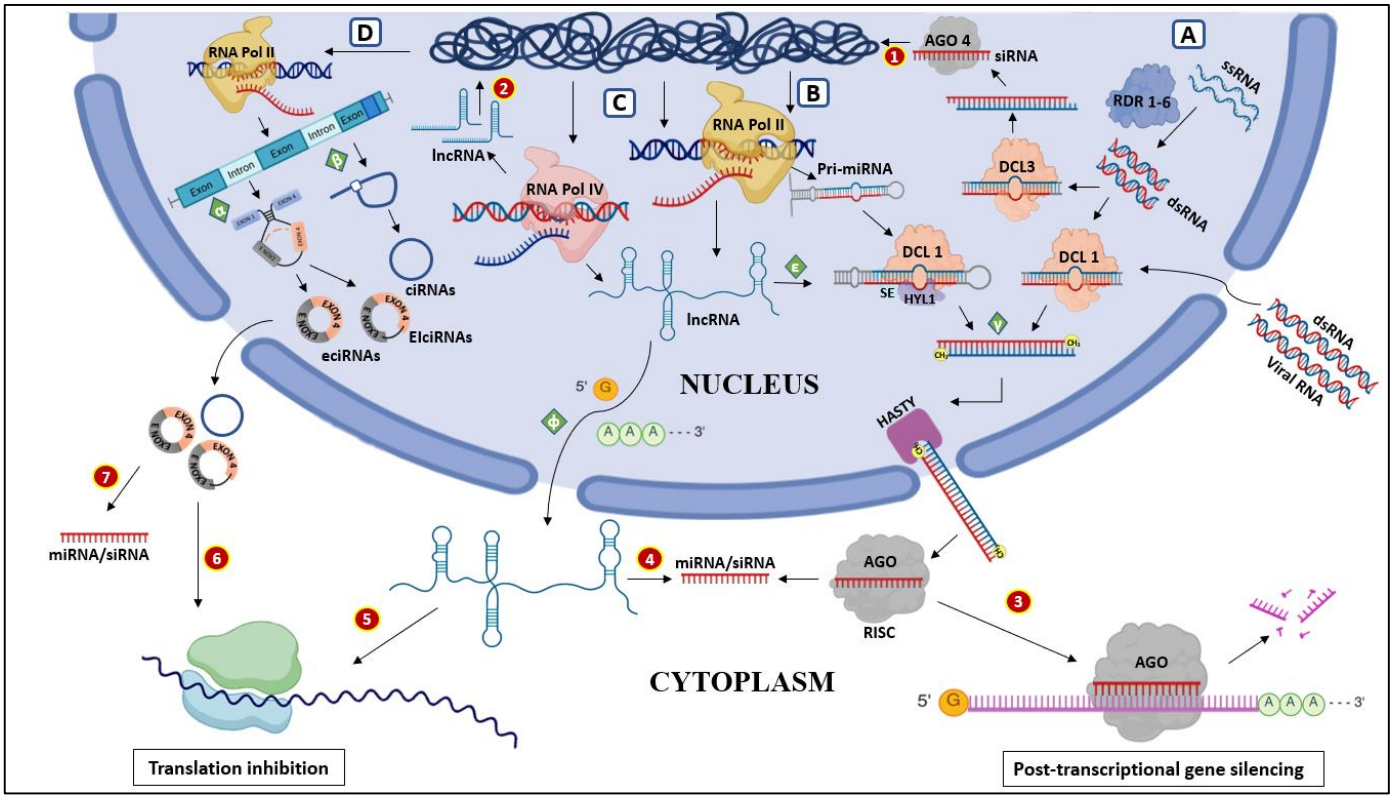

Figure 2. Overview of the biogenesis and important functions of ncRNA. (Illustration was created using BioRender App (www.biorender.com))

A: Biogenesis pathway of siRNA, B: miRNA, C: lncRNA, D: circRNA; α: Generation of exonic circRNA and Exon-intron circRNA by backsplicing, β: Generation of intron lariat, which act as a precursor for circular intronic RNA, γ: Formation of miRNA-miRNA* duplex or siRNA-siRNA* duplex through cleavage by dicer enzyme. ε: lncRNA acting as a precursor for miRNA, φ: Upon transcription from RNA polymerase II, lncRNA either get capped and polyadenylated like mRNA or remains as it is without any modification; The important functions of ncRNAs. 1: The AGO-bound siRNA 4 participates in RNA-directed DNA methylation (RdDM) pathway, 2: The lncRNA generated from pol IV or V, functioning in RdDM, 3: The AGO-bound-miRNA or -siRNA complementarily base pair with its target mRNA and activation of PTGS mechanisms, 4: lncRNA acting as miRNA sponge, represses the miRNA activity, 5: lncRNA participating in translation inhibition, 6: circRNA functioning in translation inhibition, 7: circRNA acting as miRNA sponge. (ssRNA – single-stranded RNA, RDR-RNA-dependent RNA polymerase, DCL- Dicer-like enzyme, HYL1-Hyponastic leaves 1, SE: Serrate, AGO-sArgonaute, RISC-RNA-induced silencing complex, RNA pol II and IV- RNA polymerase II, and IV, ciRNA-circular intronic RNA, EIciRNA- Exon-Intron circular RNA, EciRNA- Exonic circular RNA)

Table 3: Classification of lncRNA based on genomic location and their biogenesis

Type	Genomic Location	Key Features	Biogenesis
Intergenic lncRNAs	Between protein-coding genes	No overlap with known gene	Transcribed by RNA Pol II; Capped, polyadenylated, spliced
Intronic lncRNAs	Within the introns of coding genes	Derived from intron regions	Produced from intronic transcripts; Often processed post-splicing
Sense lncRNAs	Same direction as coding gene	Overlap exons or full gene sequence	Synthesized from sense strand; May share promoter; Canonical splicing
Antisense lncRNAs	Opposite direction to coding gene	Overlap exons, introns, or full gene	Transcribed from antisense strand; Epigenetically regulated
Bidirectional lncRNAs	Adjacent and opposite to a coding gene	Share promoter; transcribe in opposite direction	Initiated near gene promoter; Divergent transcription
Promoter-associated	Near gene promoters	Regulate transcriptional activity	Start at or near promoter; Similar biogenesis to mRNAs

components. This leads to the redeposition of H3K27me3 repressive marks at the promoters of *APOLO* and *PID* genes. Simultaneously, *APOLO* transcript synthesized by Pol V produces a 24 nt siRNA, and help in recruiting the AGO4-siRNA complex to the chromatin region, subsequently the chromatin modifiers attach to these complexes leading to the establishment of DNA methylation. Thus, chromatin loop changes occur in the promoter of *PID,* which dynamically regulate the *PID* gene expression and further, modulate the auxin response pathway [70].In another study, the same lncRNA APOLO regulates shade avoidance syndrome by dynamically modulating the three-dimensional chromatin structure of key genes such as *BRC1, YUCCA2, PID,* and *WAG2* [74].

Similarly, a lncRNA LAIR, derived from the antisense transcript of *leucine-rich repeat receptor kinase (LRK)* gene clusters has been studied [71]. The overexpression of LAIR remarkably increases the H3K4me3 and H3K16ac at *LRK1* locus, which helps in open chromatin structure. Further, LAIR was shown to associate with the chromatin-modifying complexes: OsMOF and OsWDR5. LAIR helps to co-localize these chromatin modifiers at the *LRK1* genomic locus, accordingly increases the transcription of *LRK1* and thus accounts for increased grain yield in rice [71]. In another study, the cold-induced lncRNA, MAS which is derived from antisense transcript (NAT) shown to activate the *MADS AFFECTING FLOWERING4 (MAF4)* by interacting with WDR5a. This leads to an increase in H3K4me3 histone marks on *MAF4*, which in turn suppresses premature flowering in Arabidopsis [72]. In rice, the lncRNA, RICE FLOWERING ASSOCIATED (RIFLA) which is transcribed from the first intron of the *OsMADS56* gene found to specifically associate with H3K27 methyltransferase, OsiEZ1. Overexpressing RIFLA reduces the expression of *OsMADS56* (a floral repressor), indicating that RIFLA and OsiEZ1 cooperatively suppress *OsMADS56* expression by epigenetic pathway to promote early flowering in rice [73].

Transcriptional regulation

lncRNAs can directly target the DNA sequences and represses the transcription process. Additionally, they can associate with proteins, mainly transcription factors and inhibit or

activate the gene expression. Recently, a long intergenic noncoding RNA (lincRNA) ELF18-INDUCED LONG-NONCODING RNA1 (ELENA1) was shown to activate transcription. The ELENA1 acts as a positive factor in increasing the resistance against *Pseudomonas syringae* pv tomato DC3000. Knockout and overexpression studies revealed that ELENA1 affects the expression of *PATHOGENESIS-RELATED GENE 1 (PR1)* gene by directly interacting with the mediator subunit 19a (MED19a). The level of MED19a increases at the *PR1* promoter region through ELENA1 interaction, impels the *PR1* expression [75]. The inhibitory activity of lncRNAs have also reported, for instance, lncRNA named SVALKA (SVK), which originated from antisense strand between *C-repeat binding factor* (*CBF*) 3 and *CBF1,* participates in modulating the *CBF1* expression level in cold stress. Mutant studies reported that cold-induced *CBF1* expression was repressed by SVK and this has biological link during cold acclimation and cold tolerance in Arabidopsis [76].

lncRNAs in post-transcriptional regulation

The role of lncRNA has been reported in alternative splicing processes. In Arabidopsis, lncRNAs: ENOD40 and lnc351 were shown to interact with nuclear specific splicing regulators known as nuclear speckle RNA-binding proteins (NSRs), which control alternative splicing. lnc351 competes with mRNA for binding to NSRs, thereby influencing the alternative splicing of auxin-responsive genes regulated by NSRs, which affects lateral root development [77]. Moreover, lncRNAs also modulate the gene expression patterns through involvement of siRNAs and miRNAs. Some lncRNAs function as endogenous target mimics (eTMs), competing with miRNAs in a process called “miRNA sponging,” and are referred to as competitive endogenous RNAs (ceRNAs) [78]. For instance, the IPS1: a lncRNA acts as a ceRNA during phosphate starvation in Arabidopsis, where 23-nucleotide conserved region of IPS1 mimics miR399 targets and binds to miR399 without degradation. This leads to inhibition of miR399 activity, as a consequence the expression of the *PHOSPHATE 2 (PHO2)* gene increases to support normal growth under phosphate deficiency [79]. Similarly, in maize, the lncRNA PILNCR1 operates by the same mechanism to respond to low phosphate stress [80]. In strawberry fruit ripening, the lncRNA FRILAIR regulates *LAC11a* expression by acting as a noncanonical target mimic of miR397 [81]. The miR858 in *Malus spectabilis* inhibits MsMYB62-like, an anthocyanin repressor transcription factor. Under normal conditions, lncRNAs, eTM858-1 and eTM858-2 serve as endogenous target mimics of miR858 and prevents the cleavage of MsMYB62-like mRNA. The expression of these lncRNAs reduce significantly under low-nitrogen conditions, thus allowing miR858 to suppress the MsMYB62-like more effectively, which ultimately enhances the anthocyanin production [82].

Additionally, some lncRNAs serve as precursors for miRNAs; for example, npc83 and npc521 in Arabidopsis produce mature miRNAs: miR869a and miR160c, respectively, while lncRNAs such as npc34, npc351, npc375, npc520, and npc523 are identified as precursors of 24-nucleotide siRNAs [83]. In addition, lncRNA can mediate decay of RNA, for example, a lncRNA 23468, which functions as a decoy for miR482b, reducing miR482b levels and thereby upregulating NBS-LRR resistance genes, enhancing resistance to *Phytophthora infestans* in tomato [84]. The lncRNA67 is shown to sequester miR3367 and prevent the interaction of miR3367 with *GhCYP724B* gene in fertile cotton (*Gossypium hirsutum*) line 2074B. In the cytoplasmic male sterile line 2074A, the absence of or reduced levels of lncRNA67 allow miR3367 to interact with the target *GhCYP724B* mRNA, which suppresses the expression and thereby reduces the GhCYP724B protein. This reduces the Brassinosteroids (BR) biosynthesis, resulting in male sterility [85].

lncRNAs in translational regulation

During the process of translation, lncRNAs are known to be recruited selectively to polysomes with the help of complementary base pairing, which enhances or inhibits protein synthesis. In addition, lncRNAs can increase the translation process indirectly by sequestering miRNAs [84]. In rice, the *PHOSPHATE1;2 (PHO1;2)* gene plays a crucial role in exporting phosphate into the apoplastic space of xylem vessels. Under phosphate deficiency, the levels of cis-natural antisense transcript (cis-NAT) PHO1;2 a lncRNA, and the PHO1;2 protein increase; although the mRNA levels of *PHO1;2* remains unchanged. Modulating the expression of the lncRNA cis-NAT PHO1;2 either by downregulation or constitutive overexpression, results in a corresponding decrease or significant increase in PHO1;2 protein levels without altering the expression or nuclear export of PHO1;2 mRNA. This indicates that cis-NAT PHO1;2 facilitates PHO1;2 translation by promoting its recruitment to polysomes, thereby helping to regulate phosphate homeostasis [86]. Additionally, global analyses of polysome-associated RNAs and ribosome footprints in Arabidopsis have identified five cis-NAT lncRNAs, including those associated with ATP BINDING CASSETTE SUBFAMILY G transporters: ABCG2 and ABCG20 and a POLLEN-SPECIFIC RECEPTOR-LIKE KINASE 7 (PRK7) family member, which are linked to nutrient uptake, lateral root development, and root cell elongation, respectively [87].

Currently, the databases such as PlantNATsDB—a comprehensive database of plant NATs, lncRNAdb—a reference database for lncRNAs, NONCODE—integrative annotation of lncRNAs, EVLncRNAs, PLNlncRbase, CANTATAdb, GreeNC—Green non-coding Database, RNAcentral — non-coding RNA sequences and PLncDB—plant lncRNA database, are used to deposit the lncRNA sequences. These databases serve as an important platform for the plant lncRNA community and provide a comprehensive resource for data-driven discoveries and functional investigations in plants [88].

Circular RNAs

CircRNAs are a unique group of single-stranded RNA molecules characterised by a covalently closed continuous loop, in which the 3′ and 5′ ends are joined together. The circRNAs were initially discovered in the 1970s within plant viroids, like the potato spindle tuber viroid, where they appeared as covalently closed circular RNA molecules. In eukaryotic cells, circRNAs were first identified in the 1990s in animal cells, but their widespread abundance and regulatory functions have only been elucidated recently due to advances in high-throughput RNA sequencing technologies [89]. The size of circRNAs varies in organisms, ranging from 100 nucleotides or less to over 4 kilobases. While traditionally classified as noncoding RNAs, recent evidence has shown that some circRNAs can encode proteins and act as regulator of gene expression through influencing transcription and microRNA activities [90]. Predominantly localised in the cytoplasm, circRNAs can be present at levels up to ten times higher than the associated linear RNAs from the same locus of a gene. Their circular structure, lacking the free 5' and 3' ends found in linear RNAs, makes them resistant to exonuclease degradation, resulting in greater stability within cells. circRNAs expression was known to be tissue-specific and cell-specific and can be largely independent of the corresponding linear host gene expression. This suggests that the regulation of expression might be crucial concerning control of its function [91].

Depending on the splice junction location in the genome, circRNAs are classified into three basic types they are exonic–intronic, exonic and intronic. However, studies have recently summarised 10 different types of circRNA [92]. Earlier reports suggest, the presence of antisense circRNA, overlapping circRNA, and sense overlapping circRNA in *Triticum aestivum* [93].

Biogenesis of circRNA

In general, circRNAs originate from exons closer to the 5' end of a protein-coding gene and may consist of multiple or only a single exon. Despite the fact that, the majority of circRNAs consisting of exons from protein-coding genes, and can also arise from introns or intergenic regions, Untranslated regions (UTR) and ncRNA loci, including those from the locations antisense to known transcripts [94]. The formation of circRNAs happens generally through the backsplicing mechanism and alternate splicing (Figure 2D). Current research has established that the canonical spliceosomal machinery is essential for back-splicing, with the process being supported by specific protein factors and complementary sequence elements. The spliceosome catalyzes the typical eukaryotic pre-mRNA splicing by removing introns and joining exons. However, the generation of circRNAs via back-splicing differs significantly from the conventional splicing of linear RNAs. It is also distinct from other types of circular RNA formation, such as those produced by direct single-strand RNA ligation, circularized introns, or intermediates from processed rRNAs [95].

In back-splicing, the downstream splice donor site is linked to an upstream splice acceptor site, in contrast to canonical splicing, which joins an upstream (5') donor site to a downstream (3') acceptor site. This unique mode of splicing results in a covalently closed circRNA and an alternatively spliced linear RNA missing some exons. Despite these differences, both canonical splice signals and the spliceosomal machinery are required for back-splicing. Most highly expressed circRNAs originate from internal exons of precursor mRNAs and often consist of multiple exons, suggesting that back-splicing generally occurs alongside canonical splicing [96]. Two main models describe the mechanism behind back-splicing, mainly differing in which splicing event occurs first. The "exon skipping" or "lariat intermediate" model proposes that canonical splicing initially skips certain exons, producing a linear RNA and a long intron lariat containing the skipped exons, which then forms a circRNA via back-splicing. Alternatively, the "direct back-splicing" model suggests that circRNAs are generated directly by back-splicing, producing an exon-intron(s)-exon intermediate that either degrades or is processed into a linear RNA with skipped exons. While further biochemical studies are needed to fully clarify these mechanisms, it seems both models can operate in living cells [97].

Regulatory role of circRNA

The most captivating feature of circRNA is its stability. The circular nature of 5′-3′ back spliced or 2′-5′ linked RNA is stable for more than 48h, as evidenced by its resistance to exonuclease activity when compared to linear RNA which possesses a half-life of less than 10h. Even though circRNAs constitute only 1 per cent of total RNA in the cell, it can be detected due to its longer stability [98]. Analyses of multiple RNA-seq datasets reveal that circRNAs are conserved across various species in both animals and plants [99]. Moreover, the expression patterns of circRNAs are specific to particular tissues, isoforms, and developmental stages, as observed in rice [100]. The interaction between circRNAs and RNA-binding proteins (RBPs) can either sequester RBPs away from their typical functions or allow circRNAs to act as RBP sponges. Exonic circRNAs predominantly reside in the cytosol, while intron-retaining circRNAs, such as exon–intron circRNAs and intronic circRNAs, are mainly found in the nucleus. Similarly, circRNAs containing retained introns, like intron–intergenic circRNAs, are believed to localize in the nucleus as well [101].

Increasing evidence highlights the potential involvement of circRNAs in responses to stress in plants. Their expression varies in reaction to different biotic and abiotic stresses, including nutrient deficiency, intense light, heat, cold, drought, and salinity. However, the specific regulatory mechanisms and biological functions of circRNAs under these stress conditions

remain incompletely understood. For example, stress-induced circRNA expression has been documented in rice during copper tolerance [102], in wheat under drought stress [93], and in grapevine during cold tolerance [103]. CircRNAs were initially reported under biotic stress in Arabidopsis during pathogen interactions [104] and have since been found in other crops, for instance, the circRNAs are differentially expressed in kiwifruit in response to pathogen invasion [105]. Specifically, 584 circRNAs showed defined expression patterns during *Pseudomonas syringae* pv. actinidiae infection, correlating with different infection stages. Network analyses have further identified circRNAs linked to plant defense responses [105]. Additional studies demonstrated that circRNAs act as negative regulators in response to *tomato yellow leaf curl virus* in tomato [106], play a crucial role in cotton's defense against *Verticillium wilt* [107], and contribute to maize's response to *maize iranian mosaic virus* infection [108]. circRNAs are also recognized as important regulatory molecules in plant developmental processes [109]. To support this, miRNA target mimicry was validated by overexpressing the circRNA Os08circ16564 in a transgenic rice line. This circRNA was predicted to act as a target mimic for canonical miRNAs from the miR172 and miR810 families, which play key roles in the development of rice spikelets and floral organs [110]. In Arabidopsis, an increase in circRNA expression linked to porphyrin and chlorophyll metabolism, as well as hormone signal transduction, has been observed during leaf senescence [111]. Another study showed that a circRNA derived from the sixth exon of the *SEPALLATA3 (SEP3)* gene acts in cis by binding to its own DNA locus to form an R-loop, which causes transcriptional pausing and elevates levels of alternatively spliced SEP3 transcript variants, leading to pronounced floral homeotic phenotypes [112]. Further detailed research into the regulatory roles of circRNAs in plants will enhance our understanding of their functions and foster their potential application in crop improvement.

Transfer RNA-derived fragments (tRFs)

The tRFs are generated either from the processing of precursor tRNAs or from the cleavage of mature tRNAs by specific endonucleases. These fragments, also known as tRNA-derived small RNAs (tsRNAs), tRNA-derived RNA (tDRs), or stress-induced RNAs (tiRNAs). Typically, tRFs are 13 to 40 nucleotides in length and have been recognized as important regulators in cellular processes. Initially, tRFs were thought to be mere degradation by-products; however, their accumulation is regulated since defective tRNAs are targeted for degradation through adenylation signals [113].

Based on the length and the position of cleavage on the mature tRNA or pre-tRNA, the tRFs are classified as type I, type II and tRNA halves. Type I and II tsRNAs, ranging from 18 to 30 nucleotides in length, originate from cleavage on mature tRNA and pre-tRNA, respectively. The type I tsRNAs are further divided into two subgroups: 5′tsRNA (tRF-5 or 5′tRF) and 3′tsRNA (tRF-3 or 3′tRF), which are derived from the 5′ and 3′ ends of mature tRNA, respectively [114]. Type II tsRNAs (tRF-1 or 3′U tRF) 16–48 nt in length are processed from a 3′ trailer sequence of pre-tRNA that begins 1 to 2 nt downstream of the 3′ end of the tRNA genomic sequence [115]. tsRNAs of length 30–40 nt are called "tRNA halves" because their lengths are almost half that of mature tRNA. They are often referred to as "tiRNA" due to their stress-induced characteristic. Additionally, other types of tsRNAs that are not included in the classes described above, are i-tRF, referred to as tRF-2, with a variable length and is derived from the internal region of mature tRNA straddling the anti-codon region. Another type begins at the 5′ end of the leader sequences in pre-tRNA and ends in the 3′ terminus of the 5′ exon in the anti-codon loop after removal of introns [116].

Biogenesis of tsRNA

The different classes of tsRNAs are synthesized by different processing enzymes. Growing

evidences suggest that type 1, 18- to 30-nt tsRNAs are processed by endonucleases, for instance, LysTTT3′tsRNA is processed by angiogenin (ribonucleases) in mammals [117] and Rny1p, a RNase known to process tsRNA in yeast [118], whereas in case of plants, RNase T2 is responsible for the production of tsRNAs [119]. Type II tsRNAs are processed by different endonucleases. During processing of tRNA, the ribonucleases RNase P and RNase Z remove the 5′ leader and 3′ trailer portions from the pre-tRNA sequence in the nucleus, respectively. Consequently, the released 3′ trailer sequence in the processing of tRNA becomes a type II tsRNA [114]. The biogenesis of tRNA halves (30–40 nt) initially found in *E. coli* and was generated by PrrC nuclease in response to bacteriophage infection [120]. Later, this was reported in fungi and mammals during various stress conditions, including amino acid or glucose starvation, heat shock, hypoxia, UV irradiation, or heavy metal exposure [116]. In plants, tRNA halves are generated primarily by the activity of RNase T2 family enzymes, such as S-LIKE RIBONUCLEASE 1 (RNS1), in response to various stress conditions [119].

Regulatory role of tRFs

The tRFs have various biological functions and participate in several cellular activities, most of which have been reported in mammalian and yeast systems. In initial studies, the researchers found that the plant tRFs are associated with various stress responses by quantifying the tRF levels. For instance, oxidative stress induces the accumulation of tRNA halves in plants. Thompson et al. [121] found that, the abundance of tRNA halves from $tRNA^{Trp}CCA$, $tRNA^{Arg}CCT$ and $tRNA^{His}GTG$ peaked upon 4 h treatment with 5–10 mmol/L H2O2. Similarly, Cognat et al. [122] reported the higher amounts of tRF-5s from $tRNA^{Val}AAC$, $tRNA^{Gly}TCC$, $tRNA^{Gly}GCC$ and $tRNA^{Pro}TGG$, accumulated in UV-stressed plants and also noticed that plastid tRF-5 populations fluctuated in drought, salinity and cold conditions [122]. Various reports represent the roles of plant tRFs in phosphate (Pi)-limited conditions. The research by Hsieh et al. [123] showed that 19 nt tRF-5s accumulated at higher levels in Pi-starved Arabidopsis roots. The probable reason is that Pi deprivation could induce the level of RNS1 and an RNase T2, leading to the jumble of specific tRFs [119]. Above studies suggest that tRFs can participate in numerous stress responses in plants.

The functional studies related to molecular mechanisms of plant tRFs are relatively limited, recent research supports that the plant tRFs regulate gene expression by mechanisms such as translation inhibition and RNA silencing. tRFs have been shown to participate in AGO-dependent gene silencing; for instance, in Arabidopsis, 19 nt tRFs were enriched in AGO1-IP sRNA populations, and the Long Terminal Repeat Gypsy retrotransposons are the major targets of tRFs [124]. Further, 19-nucleotide tRF-5 fragments have been demonstrated to cleave transposable element (TE) RNAs. This was supported by degradome/PARE sequencing and validated through an *in vivo* reporter assay, providing evidence for their role in targeting and slicing TE transcripts [124]. These results clearly suggest that plant tRFs regulate mobility of transposon by TE silencing. Ren et al. [125] recently reported that *Bradyrhizobium japonicum* (rhizobial symbiont) delivers tRFs to root cells of soybean (*Glycine max*). Further, the study highlighted that two rhizobium tRF-3s from $tRNA^{Val}$ and $tRNA^{Gly}$, and one tRF-5 from $tRNA^{Gln}$, are loaded into soybean AGO1, to cleave three essential genes responsible for root hair development in soybean by hijacking the host RNA silencing machinery. Thus, rhizobium-derived tRFs help in inducing the nodulation in soybean and participate in symbiotic interactions [125]. tRF also participate in plant pathogen interaction, for example, the 5′ tsR-Ala negatively regulates *cytochrome P450 71A13(CYP71A13)* expression and camalexin biosynthesis which repress the anti-fungal defense [126]. Further, they observed that upon fungal infection the expression of 5′tsR-Ala is downregulated as a plant defense strategy against fungal disease

[126]. In *Triticum aestivum*, the expression of four out of nine wheat ribonuclease T2 family members is strongly induced by challenge with *Fusarium graminearum*. Further, the levels of three 5′-tRFs (tRF^{Glu}-CUC, tRF^{Lys}-CUU, and tRF^{Thr}-CGU) are significantly higher in a *Fusarium*-susceptible than in a *Fusarium*-resistant cultivar, suggesting a potential role of these tRFs in *Fusarium* infections [127]. More recently, three specific tRFs (5′-tRF^{Gln}-UUG, 5′-tRF^{Gln}-CUG, and i-tRF^{Glu}-UUC) were detected as highly abundant in the mycelium and other parts of the barley powdery mildew pathogen, *Blumeria hordei.* Their presence suggests a possible role in modulating host defense responses through cross-kingdom regulation [128].

Presently there are few reports on the role of plant tRFs in regulating translation. Studies have demonstrated that RNA molecules present in phloem sap can inhibit protein translation *in vitro*. It has also been reported that synthetic tRNA-derived fragments within the phloem sap may interfere with ribosomal function [129]. Similarly, using a green fluorescent protein reporter system in Arabidopsis, it was demonstrated that a subset of tRNA halves including other tRFs repress translation *in vitro* [130]. Combined together, the above evidences support the viewpoint that plant tRFs are engaged in several cellular activities, mainly in response to stress, transposon silencing and host-pathogen interactions by utilizing the translation inhibition mechanism and gene silencing.

Currently, NGS technologies have greatly hastened the quantitative analysis of tsRNAs. The sequencing methods, such as tRNA-seq [131], AQRNA-seq (absolute quantification RNA sequencing) [132] and 2′,3′-cyclic phosphate RNA sequencing (cP-RNA-seq) (133;134), are shown to sequence tRFs more efficiently, and databases such as tRFdb will help in the application of these identified tRFs in crop improvement. Thus, the identification of tRFs and their binding proteins are necessary to elucidate overall tRFs regulatory pathway and advance studies in this field is essential for uncovering the regulatory roles of these tRFs in plant cellular functions.

Conclusion and perspective

The regulatory landscape of ncRNAs in plants is vast and intricately complex, with these molecules functioning as key modulators of diverse cellular and developmental processes. The expression and biogenesis of ncRNAs are highly dynamic and strongly controlled, enabling plants to fine-tune the gene expression in response to developmental programs and ever-changing environmental stimuli. Upon perception of internal or external cues, specific ncRNAs are rapidly induced and orchestrate precise molecular interactions that regulate downstream pathways. This highlights the necessity of systematically identifying all regulatory factors, particularly ncRNAs, and deducing their functional significance for improving crop productivity, resilience, and quality.

Although notable progress has been achieved in elucidating the roles of small ncRNAs, particularly miRNAs and siRNAs, a large proportion of these molecules remain uncharacterized. Moreover, our understanding about ncRNA classes, such as lncRNAs, circRNAs, and ncRNA-derived fragments remains relatively limited. These unravelled categories of ncRNAs may hold crucial regulatory functions which are yet to be identified. High throughput sequencing technologies like NGS platform and integrative bioinformatics approaches now provide powerful tools to accelerate the identification, annotation, and functional characterization of ncRNAs. Meanwhile there is a need for development of robust, user-friendly databases that can curate and integrate the information on non-coding genetic elements to enhance the accessibility and utility for researchers worldwide. Altogether, these advances in technologies will aid in the discovery of novel

ncRNAs and greatly improve the dissection of their mode of action in regulating major cellular processes. Ultimately, such knowledge will not only enhance our understanding about plant gene regulation but also provide innovative strategies for exploiting the information gained in crop improvement, enabling sustainable agricultural practices in current global challenges.

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Conflict of Interest

The authors declare no conflict of interest

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Vita Scientia

Review Article

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Oxidoreductase Diversity and Functional Versatility in *Paracoccus denitrificans* (denitrifying bacterium): Insights into Flavin, Iron, Quinone, and Chromate Reductases

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Summary

Paracoccus denitrificans is a metabolically adaptable prokaryote equipped with diverse oxidoreductase enzymes that enable persistence across soil, marine, and industrial environments. This study reviews key reductase enzyme families, including flavin, iron, quinone, and chromate/chromate-related reductases, emphasizing their biochemical roles and biotechnological potential. Flavin reductases catalyze coupled electron transfer, reducing both NAD(P)H to its active nicotinamide form and FAD to $FADH_2$. These reactions support essential pathways such as DNA biosynthesis, quinone detoxification, and light-associated microbial functions including bioluminescence. Iron reductases convert ferric iron (Fe^{3+}) into bioavailable ferrous iron (Fe^{2+}), a process critical for iron acquisition in nutrient-restricted habitats, where iron bioavailability dictates microbial competition and survival. Quinone reductases further strengthen stress tolerance by performing two-electron reductions that suppress harmful redox cycling, thereby preventing excess reactive oxygen species formation and improving oxidative stress resistance. Chromate reductases reduce toxic Cr(VI) to the stable, less soluble Cr(III) state, offering promising applications for chromium detoxification and water bioremediation. The broad substrate range and structural diversity of these enzymes highlight the unique capacity of microbial metabolism to sustain elemental cycling and chemical transformations distinct from higher organisms. Understanding these *oxidoreductases* advances microbial biochemistry while guiding innovative strategies in bioremediation, industrial biocatalysis, and environmental biotechnology.

Keywords

Electron transfer enzymes; Redox metabolism; Flavoenzymes; Environmental detoxification; Cofactor interaction.

Introduction

Microorganisms can inhabit a wide range of environments due to their remarkable metabolic capabilities [1]. *Paracoccus denitrificans* is a free-living coccoid bacterium commonly found in soil and water [2]. It is highly metabolically versatile and has long served as a model organism for studying diverse biochemical pathways [2]. *P. denitrificans* contains various enzymes and proteins, including several belongings to the flavoenzyme superfamily with NAD(P)H:FMN oxidoreductase activity. Until recently, FerA and FerB were the only well-characterized members of this group [3].

FerA and FerB are flavoenzymes with distinct physiological roles. FerA functions primarily as an iron and flavin reductase, enabling the bacterium to extract iron from extracellular sources—an essential adaptation for survival in iron-limited environments [4]. FerB, a quinone reductase, contributes to the detoxification of reactive species such as quinones and plays a protective role against oxidative stress. Together, these enzymes highlight the diverse strategies P. denitrificans employs to adapt to environmental pressures [5–7].

Flavin reductases constitute a major class of oxidoreductases that use NAD(P)H to reduce FMN and FAD cofactors [7]. These enzymes are essential for maintaining intracellular redox balance and participate in processes such as hydroxylation reactions, detoxification, and DNA synthesis [8]. They are classified into two types based on their flavin-binding properties, and their ability to act on substrates with varied structures reflects their versatility and significance in microbial metabolism [9].

Iron reductases are key enzymes involved in microbial iron metabolism, reducing ferric iron (Fe^{3+}) to its more bioavailable ferrous form (Fe^{2+}) [10]. This reduction is crucial in siderophore-mediated iron uptake, particularly under iron-limiting conditions. These enzymes also contribute to metal detoxification and redox homeostasis. FRE1 and FRE2 in Saccharomyces cerevisiae perform similar functions, demonstrating the evolutionary conservation of iron reductase activity across species. *P. denitrificans* and other relatives also possess quinone and chromate reductases that contribute to the environmental detoxification and xenobiotic degradation. While quinone reductases are related to protecting cells from oxidative damage by minimizing redox cycling of quinones, chromate reductase is involved in the process of converting toxic Cr(VI) to its nontoxic form Cr(III). These enzymes are structurally related to and may have the same substrate specificity as flavin reductases. The metabolic versatility of *Paracoccus denitrificans* is driven by oxidoreductase enzymes that mediate essential redox transformations, such as:

(1) $NAD(P)H + FAD \rightarrow NAD(P)+ + FADH_2$

(2) $Fe^{3+} + e^- \rightarrow Fe^{2+}$

(3) $Quinone + 2H^+ + 2e^- \rightarrow Hydroquinone$.

Such reactions demonstrate the organism's ability to maintain redox balance, acquire important nutrients, and detoxify harmful compounds.

Flavin Reductases

Flavin reductases are oxidoreductase enzymes (EC 1.5.1.x) that helps in catalysing the reduction of flavin cofactors, specifically flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD), to their reduced forms $FMNH_2$ or $FADH_2$ [15]. The reducing equivalents come from either NADH or NADPH, which are nicotinamide cofactors [16].

$Flavin\ (oxidized) + NAD(P)H + H^+ \rightarrow Flavin\ (reduced) + NAD(P)^+$ [16]

The products, $FMNH_2$ or $FADH_2$, act as electron donors for various downstream biochemical processes, they play key roles in maintaining cellular redox balance and in allowing oxidative biochemical changes [17][18].

Class I Flavin Reductases

The active site of these enzymes contains flavin cofactors that are tightly bound, sometimes covalently bound [19]. They usually act according to a ping-pong (double displacement) mechanism, in which the flavin cofactor is reduced after accepting electrons from NAD(P)H [19][23]. Electrons are then transferred to an external electron acceptor by the reduced flavin. In a case study of Escherichia coli's Fre (flavin reductase), which catalyses the reduction of substrates and has a tightly bound FMN [20]. In Class I enzymes, the tight binding of flavin allows for rapid cycling between oxidized and reduced forms, enabling high turnover rates [21].

Class II Flavin Reductases

These enzymes do not have bound flavin in their active site. Rather, they reduce free flavin molecules (FMN or FAD) present in the medium [22]. They work by sequential kinetic mechanism in which a ternary complex (enzyme–NAD(P)H–flavin) develops rapidly during catalysis. Vibrio fischeri's NADPH-flavin reductase converts free FMN to $FMNH_2$ for the bacterial luciferase reaction [22]. Class II flavin reductases are necessary in pathways where reduced flavin is required as a diffusible intermediate for other enzymes [23]. They are small to medium-sized proteins (~20–35 kDa), some multi-domain enzymes are larger.[24]. Many flavin reductases share a Rossmann fold for binding NAD(P)H [25]. Some of them prefer NADPH, while others accept both NADH and NADPH [26]. Many flavin reductases can be identified by their substrate promiscuity, which allows them to reduce several electron acceptors apart from flavins, including [12]: Quinones: Flavin reductases convert quinones to hydroquinone, reducing oxidative stress by inhibiting redox cycling and ROS production [27]. Nitroaromatic compounds: Fre in E. coli can reduce nitroaromatic contaminants such as nitrobenzene, contributing to detoxification processes [10] (table 1). Azo dyes: Flavin reductases reduce azo bonds (–N=N–) to decolorize dye, which is relevant in bioremediation [11]. Chromate (Cr(VI)): Bacterial flavin reductases can reduce chromate Cr(VI) to less harmful Cr(III), potentially contributing to environmental maintenance [9]. (Physiological functions of Flavin reductase *is illustrated in Figure 1*).

Physiological Functions Iron Reductases

Flavin reductase activity is involved in several important cellular processes: DNA synthesis – Provides reduced flavins essential for ribonucleotide reductase, enabling deoxyribonucleotide production crucial for DNA replication [28]. Monooxygenase reactions – Supplies $FMNH_2$ for monooxygenases, facilitating the oxygenation of aromatic and xenobiotic compounds [29]. Bioluminescence in marine bacteria – Generates $FMNH_2$ for bacterial luciferase, supporting light production for communication and survival [30]. Iron acquisition and metabolism – Reduces Fe^{3+} in siderophore complexes, enhancing iron uptake under limiting conditions [31], (*The mechanism is illustrated in Figure 1*).

Iron Reductases

Iron reductases are a class of oxidoreductase enzymes that catalyze the conversion of ferric iron (Fe^{3+}) to its bioavailable ferrous form (Fe^{2+}) [36]. This reduction represents a critical step for microorganisms, plants, and some animal systems to acquire and utilize iron efficiently under iron-limited conditions [36].

$$Fe3+ + e- \rightarrow Fe2+ \text{ [36]}$$

Fe^{3+} is poorly soluble and can't be used by cell under normal conditions, hence it needs to be converted to Fe^{2+} is necessary for adsorption and intracellular utilization [37][38]. Integral membrane proteins play important role in transmembrane electron transfer. Example: FRE family in Saccharomyces cerevisiae [39]. Soluble (cytoplasmic or extracellular) iron reductases are located in the cytosol, periplasm, or released into the extracellular medium [36]. Help in reduction of Fe^{3+} outside the plasma membrane. Many iron reductases use flavin cofactors such as FMN or FAD as electron carriers [40]. In these enzymes, flavins mediate electron transfer from NAD(P)H or reduced cytochromes to Fe^{3+}, promoting its reduction [41][42]. Molecular weight is ~20–40 kDa to >100 kDa for soluble enzymes for membrane-bound complexes [42][43]. Active site

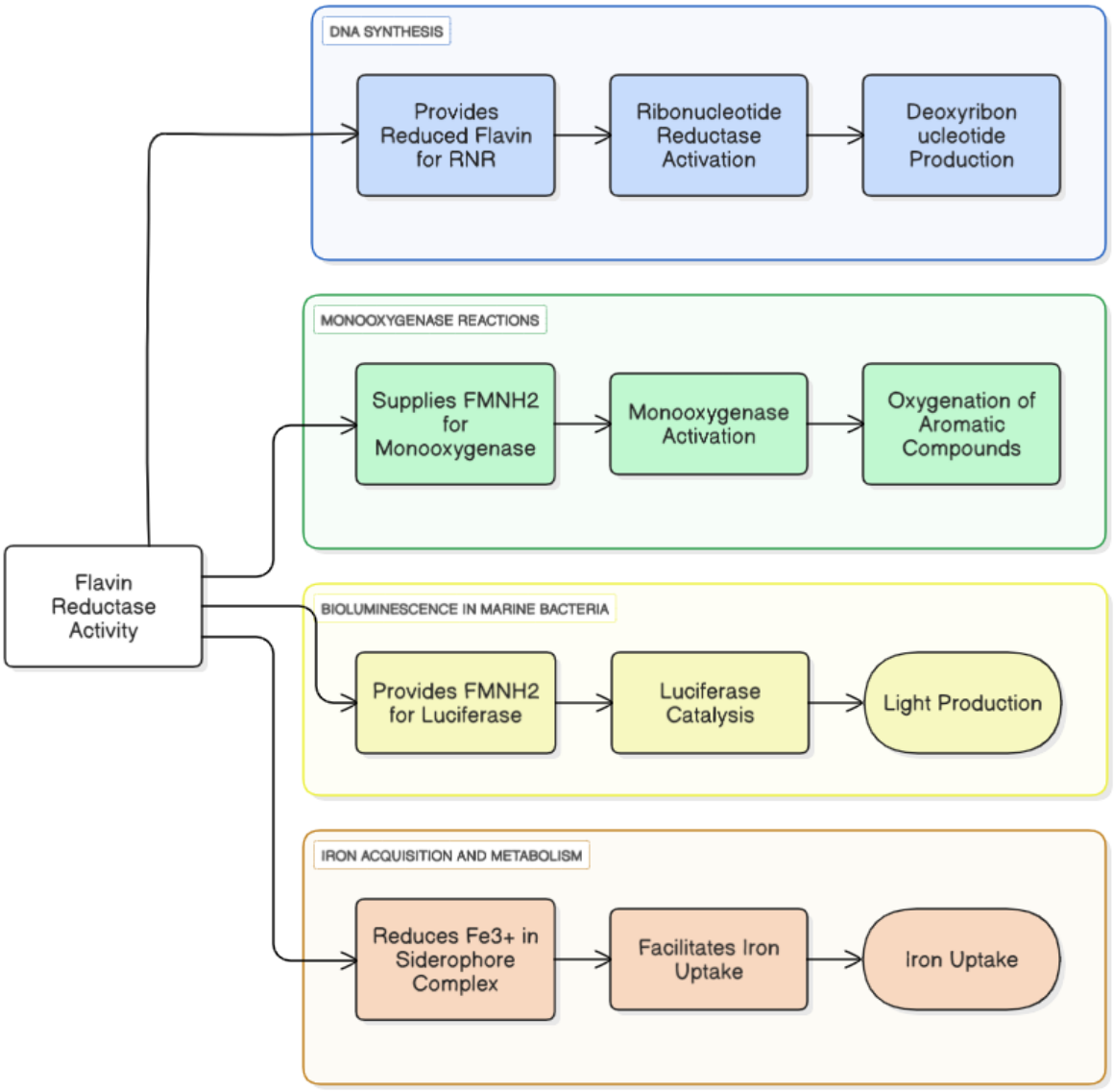

Figure 1. Physiological functions of Flavin reductase.
Flavin reductases generate reduced flavins ($FMNH_2$ /$FADH_2$) that support multiple cellular processes. These include activation of ribonucleotide reductase for DNA synthesis, provision of $FMNH_2$ to monooxygenases for substrate oxygenation, fueling luciferase-driven bioluminescence, and reduction of Fe^{3+} in siderophore complexes to facilitate iron uptake. (Source: Authors' own work)

Enzyme	Organism	Cofactor	Function
Fre	*E. coli*	FMN-bound	Reduction of quinones, nitroaromatics [32][33]
LuxG (flavin reductase)	*V. fischeri*	FMN (free)	Generates $FMNH_2$ for bioluminescence [34]
ChrR	*Pseudomonas putida*	FMN-bound	Cr(VI) reduction [35]

NfsA/B	*E. coli*	FMN-bound	Nitroaromatic reduction [32]

Table 1: Representative examples of Flavin reductase

contains redox cofactors such as flavins, iron-sulfur clusters, or heme groups [41][44]. Many exhibits high affinity for Fe^{3+}, crucial under iron-limited conditions [44][45].

Mechanism of Action

Cells require Fe^{2+} for uptake. They obtain it by reducing extracellular Fe^{3+} through three main pathways: Transmembrane Electron Transport: Electrons from cytosolic donors like NADPH are transferred across the membrane, reducing Fe^{3+} outside the cell [40]. Direct Reduction in Solution: Soluble reductases bind Fe^{3+} (or Fe^{3+} chelators) and convert it to Fe^{2+} [40]. Reduction of Ferric Siderophore Complexes: Iron reductases reduce Fe^{3+} bound in siderophores, releasing Fe^{2+} for uptake. All pathways ensure Fe^{2+} is available for cellular needs [50]. *(The mechanism of Flavin reductase is illustrated in Figure 2)*

Quinone Reductases

Quinone reductases are a subgroup of oxidoreductase enzymes that catalyse the reduction of quinones to hydroquinone [53][54]. This reaction is biologically crucial because it prevents quinones from engaging in redox cycling, a process that generates reactive oxygen species (ROS) and contributes to oxidative stress and cellular damage [55]. The general reaction catalysed by quinone reductases can be represented as:

$$\text{Quinone} + 2e^- + 2H^+ \rightarrow \text{Hydroquinone}$$ [54]

These enzymes are often NADH- or NADPH-dependent and frequently contain flavin cofactors, particularly FMN or FAD, which mediate electron transfer during catalysis [56]. Quinone reductases are widely distributed across bacteria, fungi, plants, and animals, underscoring their conserved and essential protective roles in diverse biological systems [56].

Structural and Biochemical Features

Quinone reductases tend to be relatively small, with molecular weights of around 20-40 kDa [57]. Most multimeric complexes, such as dimers or tetramers, are formed from these proteins [57]. These enzymes often carry out two-electron reductions. This strategy not only avoids producing semiquinone radicals but also minimizes the generation of reactive oxygen species ('ROS') fcc and so helps to protect cells [58]. This two-electron reduction stands in stark contrast with one-electron pathways. The latter could easily produce short-lived intermediate radical semi-quinone prone to undergo harmful redox cycling processes [58].

Physiological Roles of Quinone Reductases

Roles of Quinone Reductase Activity in Cellular Protection and Therapy

Quinone reductase activity contributes to detoxification and protection through xenobiotic metabolism, antioxidant defense, redox balance, and reactive oxygen species (ROS) detoxification [58][59]. These functions support cellular protection and have therapeutic relevance, particularly in cancer therapy [60]. *(Physiological functions of Quinone reductase is illustrated in Figure 3*).

Chromate Reductases Discussion

Chromate [Cr(VI)] is a toxic and mutagenic environmental contaminant [65]. Chromate reductases play a crucial role in reducing Cr(VI) to Cr(III), a much less toxic and insoluble form, using NAD(P)H as an electron donor [66]. These enzymes are also attracting interest for their potential application in bioremediation [67]. Many have the same structure as flavin reductases, and the same dependency on

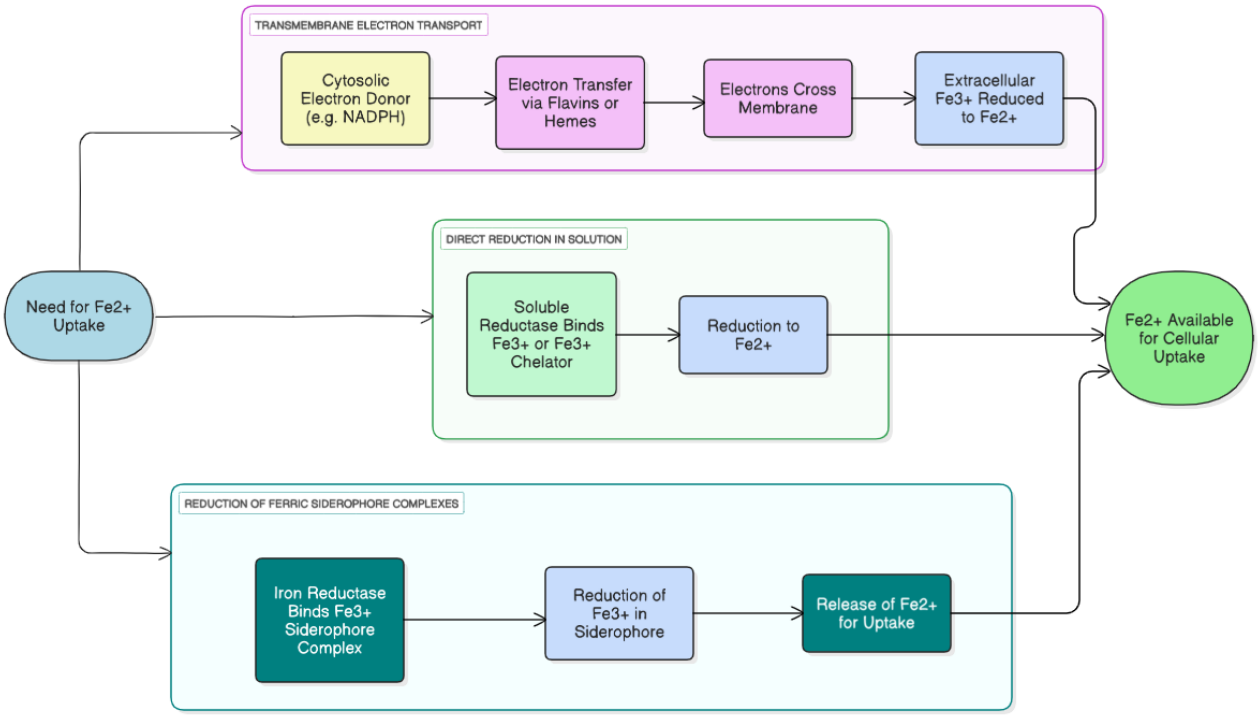

Figure 2. Mechanism of Action of Iron Reductase.
The figure illustrates three mechanistic routes for converting Fe^{3+} to Fe^{2+}: transmembrane electron transfer from cytosolic donors to extracellular Fe^{3+}, direct reduction of soluble Fe^{3+} or Fe^{3+}–chelator complexes, and reduction of ferric siderophore complexes. All pathways converge on generating Fe^{2+} in a form accessible for cellular uptake. (Source: Authors' own work)

Enzyme	Organism	Location	Function
FRE1	*S. cerevisiae*	Plasma membrane	Reduces extracellular Fe^{3+} for uptake [51].
FRE2	*S. cerevisiae*	Plasma membrane	Similar function as FRE1, with broader substrate range [51].
Ferric reductase	*E. coli*	Periplasmic/cytoplasmic	Reduction of ferric-siderophore complexes [49].
FRO2	*Arabidopsis thaliana*	Root epidermis	Reduces soil Fe^{3+} for uptake under iron deficiency [52].

Table 2: Representative examples of Iron reductase

cofactors [68]. Some of the best-characterized chromate reductases are: ChrR from Pseudomonas putida [69], NAD(P)H:quinone reductase (NQR) from Arabidopsis thaliana, which exhibits action against chromium [70]. These enzymes usually act on a broad spectrum of non-polar substrates. Cr(VI) reduction alleviates its toxic effects and precludes damage to DNA by stopping ROS formation [71]. The homology between these enzymes and FerC paralogs indicates that FerC might also have chromate reductase-like activity [72].

Physiological Roles of Chromate Reductases

Roles of Chromate Reductase Activity in Cellular and Environmental Protection Chromate reductase activity aids in reducing toxic chromium species, maintaining redox balance, and detoxifying contaminated environments [73]. It overlaps with flavin reductases, protects cells from oxidative and genotoxic stress, and reduces other toxic compounds like quinones and azo dyes, contributing to overall cellular and environmental protection [74]. *(Physiological functions of Chromate reductase is illustrated in Figure 4)*

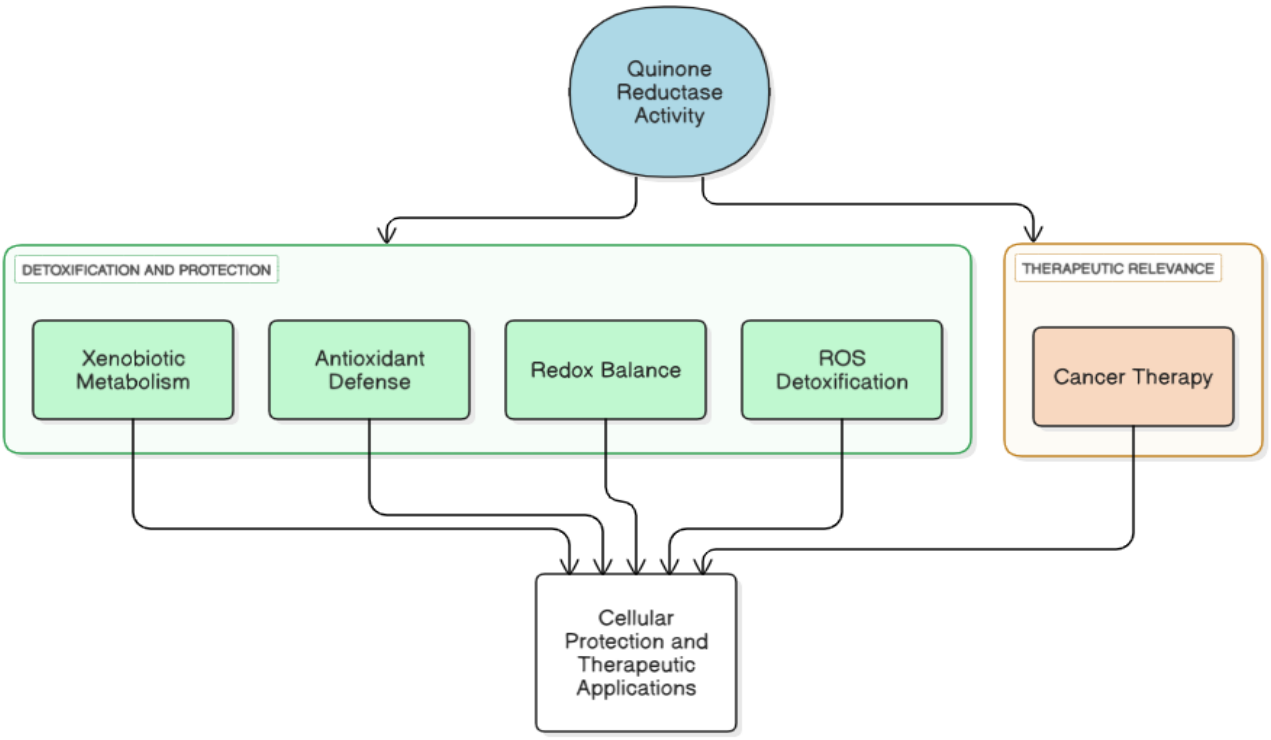

Figure 3. Physiological Roles of Quinone Reductase.
The figure summarizes the roles of quinone reductase activity in detoxification and cellular protection through xenobiotic metabolism, antioxidant defense, redox balance, and reactive oxygen species (ROS) detoxification. These processes collectively contribute to cellular protection and underpin therapeutic relevance, including applications in cancer therapy. (Source: Authors' own work)

Enzyme	Organism	Cofactor	Function
NQO1	Humans	FAD	Detoxifies quinones, antioxidant defense [61].
ChrR	*E. coli*	FMN	Quinone and chromate reduction [62].
YhdA	*B. subtilis*	FMN	Reduction of quinones, azo dyes [63].
FerB	*P. denitrificans*	FMN	Quinone and iron reduction [64].

Table 3: Representative examples of Quinone reductase

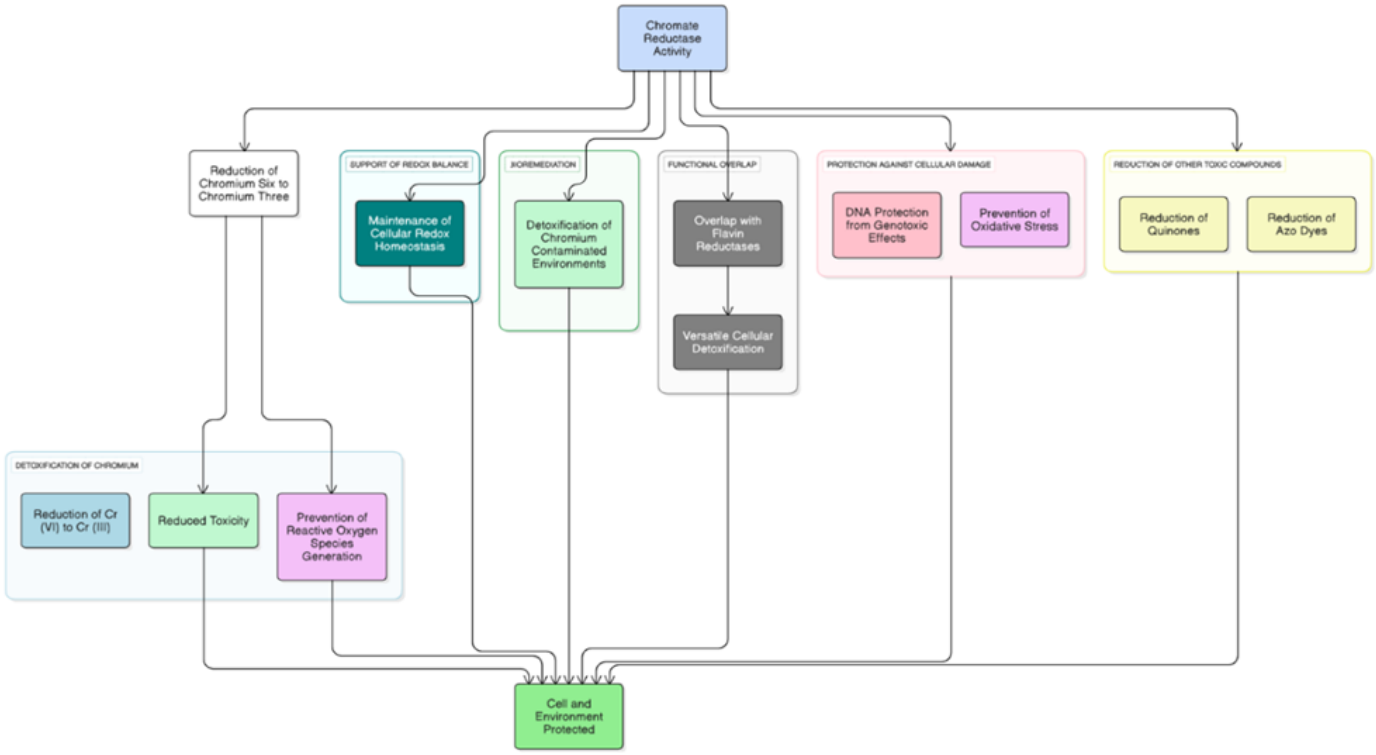

Figure 4. Physiological Roles of Chromate Reductase. (Source: Authors' own work)

Enzyme	Organism	Cofactor	Substrates Reduced
ChrR	*P. putida*	FMN	Cr(VI), quinones, azo dyes [69].
ChrR	*E. coli*	FMN	Cr(VI), quinones, azo dyes [70]
NQR	*A. thaliana*	FAD	Cr(VI), quinones [76]

Table 4: Representative examples of Chromate reductase

Discussion

The enzyme system of water nitrate and similar microorganisms, specifically Paracoccus denitrificans, is reported to be versatile in its metabolism [1, 2, 3]. NAD(P)H can hydroxymethylamine, while a flavoenzyme (such as ferA or ferB) stimulates the reaction by accepting two electrons and one proton [15,16,17,18]. The reduced forms of flavins such as $FMNH_2$ or $FADH_2$ are important electron donors for post reduction processes. They are used to entangled injury, DNA is synthesized through ribonucleotide reductase, monooxygenase oxygenates alien compounds onto the planet [29,30]; while light transformation occurs in living bioluminescent marine bacteria like Vibrio fischer [20].

Iron reductases also illustrate a general strategy of adaptation among micro-organisms. Under iron-limiting conditions, by reducing ferric iron (Fe^3) to ferrous iron (Fe^2), they are able efficiently obtain this side product [10,36,39,40].

That such iron reductive systems are used by quite different types of living beings (such as the FRE family in Saccharomyces cerevisiae) is proof that they are basic to life. Reducing ferric ion to ferrous is something without which it would no longer be subsistable [39,51]. PdN1FerB is a typical example of the quinone reductase. When an organism's environment is full of metal ions, this enzyme plays an important role in quenching ROS and makes them harmless and transportable by reducing quinones to hydroquinones [55,58,64]. Enzymes such as ChrR in P. putida also display enzymatic and structural similarities with reductase, so evolutionarily there are similarities between these two types. The enzymes of this group possess a wide substrate promiscuity and ecological significance, having the capability to reduce such varied substances as quinones, azo dyes, and Cr(VI) [68,69,70,72].

Conclusion

This work highlights the biochemical diversity and significance of oxidoreductase enzymes in *Paracoccus denitrificans* and other bacteria. Flavin, iron, quinone, and chromate reductases collectively contribute to cellular redox balance, detoxification, nutrient acquisition, and ecological adaptation. Their ability to catalyze electron transfer reactions across a wide range of substrates demonstrates remarkable metabolic flexibility and environmental importance. Beyond their physiological roles, these enzymes hold considerable promise for biotechnological applications such as the degradation of hazardous pollutants and the synthesis of valuable biochemicals. Their substrate versatility and catalytic efficiency make them strong candidates for future structural and mechanistic studies, which will deepen our understanding of microbial metabolism and support the development of innovative approaches in environmental and industrial biotechnology.

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Conflict of Interest

The authors declare no conflict of interest.

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Review Article

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Peptides in Hematologic Malignancies: A Review

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Summary

Cancer continues to pose a significant global health challenge, prompting continuous research and innovation in therapeutic modalities. Among the evolving methods and approaches, therapies derived from peptides have emerged as an upcoming frontier, within the bounds of anticancer treatments. Peptides play a crucial role in pathophysiology, diagnosis, and treatment of hematologic malignancies. These short amino acid chains influence tumour growth, immune response, and cellular signaling in leukemia, lymphoma, and multiple myeloma. Therapeutic peptides, including peptide-based vaccines and receptor-specific targeted therapies such as those interfering with tumour-specific antigens or overexpressed surface proteins are emerging as promising treatment modalities. Over the last two decades, the advent of anticancer peptides (ACPs) has brought about transformative changes in the pharmaceutical landscape, offering novel avenues for combating malignancies. This comprehensive review analyzes the implementation of peptide-based treatments concerning blood malignancies, uncovering the mechanisms behind the effectiveness of anticancer peptides (ACPs), including interactions with negatively charged cellular surfaces, pore formation, and immune responses, it also states their targeted toxicity towards cancer cells. Additionally, peptide biomarkers aid in early diagnosis and disease monitoring. This review also emphasizes the role of peptides in pharmaceutical applications, investigating various drug delivery methods such as oral, nasal, ocular, and blood-brain barrier routes.

Keywords

Anticancer Peptides, Peptide Biomarkers and Peptide-based vaccines.

Introduction

Cancer being the second leading cause of death, stands as a formidable public health issue globally. As it is the leading cause of illness and death rates worldwide, it requires continuous research and exploration for the development of treatment methods and advancement in strategies [1]. It's crucial to address difficulties and obstacles in cancer treatment, such as the emergence of polydrug resistance and the restraint to neoplasm targeted treatments.

Cancer is medically diagnosed as an uncontrolled and abnormal growth of cells within the living body, leading to their amplification and propagation to other tissues and organs. It can be classified into various types based on their characteristics, risk element and treatment methods and models. Usually, they are categories by the organ location or prevalence basis such as bladder cancer, breast cancer, colon cancer, kidney cancer, liver or lung cancer, melanoma, non-Hodgkin lymphoma etc. For understanding, cancer is classified as solid cancer and non-solid cancer (cancers of the

blood, such as leukemias) also called hematologic malignancies. Solid cancer is a neoplasm that doesn't contain a liquid region or cyst. It can be cancerous (malignant) or non-cancerous (benign). Whereas nonsolid cancer such as leukemias is the result of production of large numbers of abnormal cells that enter the bloodstream.

While significant steps have been taken in cancer treatments and therapy, there persists a critical demand for more potent and precisely targeted treatments to address the evolving dynamics of this complex disease [2]. One area of investigation is peptide derived treatments. Over the last two decades anticancer peptide, a group of tumours fighting agents have proven to transform and revolutionize the pharmaceutical field [3].

Hematologic malignancies

Hematologic malignancies, commonly known as blood cancers or non-solid tumours also abbreviated as (HMs), comprise a heterogeneous and varied bracket of diseases marked by the unchecked proliferation of blood forming cells and lymphoid tissues. Hematologic malignancies broadly categorized into myeloid and lymphatic tumours [4]. Both brackets responsible for disrupting the hematopoietic processes, i.e. production and development of blood cells. Myeloid and lymphatic tumours can be distinguished on their origin in different immune-system cells. Myeloid tumour or leukaemia are characterized by the presence of an abnormally high number of myeloid cells throughout the bloodstream. Lymphocytic leukaemia or tumours also involve an overgrowth of lymphocytes, which can be found in lymphatic tissues, the bloodstream, bone marrow, and other body tissues. They are further categorized into prevalent subtypes, including leukaemia, multiple myeloma (MM), non-Hodgkin lymphoma (NHL), and Hodgkin lymphoma (HL) [4,5].

Among hematologic malignancies, Hodgkin lymphoma had the largest decline in the past few decades, with an age-standardized death rate (ASDR) of 0.34 per 100,000 population in 2019 [6]. Blood cancer comprises a substantial portion, approximately 6.5%, of global cancer cases. Currently, While the global incidence of leukemia is on the decline, certain developed regions like France, Spain, Slovenia, and Cyprus are witnessing a rise in cases. The prevalence of specific hematologic malignancies varies across countries and regions, influenced by distinct socioeconomic development stages [7]. Despite significant improvements in survival rates over recent decades, understanding the nuanced arrangements and temporal trends in illness and death rates related to hematologic malignancies remains imperative. This knowledge serves as a foundation for devising more targeted prevention strategies to further enhance the outcomes for individuals affected by these diverse malignancies [8].

Anticancer Peptides (ACPs): Mechanisms of Action

The anticancer peptides (ACPs) are sub-micron particles, usually consisting of fewer than 50 amino acids in terms of biological molecules. It displays a cationic nature, characterized by the existence of basic and nonpolar residues [9]. Since peptides have numerous advantages such as high specificity, minimal toxicity, effective tissue penetration, and versatility in modifications, it is opted-for treatment and therapy when contrasted to antibodies and molecules [10].

Notably, ACPs often share key characteristics with their predecessors, antimicrobial peptides (AMPs) as they are derived from (AMPs), which results in overlapping features between the two peptide classes [9, 11]. One fundamental feature of ACPs is their interaction with negatively charged cellular surfaces. In both bacterial and cancer cells, the cell membranes bear a negative charge, making them susceptible targets for these peptides [12]. This electrostatic interaction is believed to determine the selective toxicity of ACPs against cancer cells, distinguishing them from normal cells.

Besides inhibition by heparan sulfates (e.g., against LfcinB and KW5), resistance to anticancer peptides can arise from: Altered membrane lipid composition (less peptide binding) Protease-mediated degradation of peptides Efflux pump overexpression reducing intracellular peptide levels Tumour microenvironment barriers (acidic pH, dense ECM, proteases) Immune neutralization by antibodies [11, 13].

The cytotoxicity profiles of ACPs classify them into categories like, length: <20aa for short; >20aa for long, source (natural versus synthetic); structure (random coil, β-sheet, and α-helical); charge (amphipathic, cationic), the mode of action (non-lytic versus membrane-lytic) [14]. The preferential action of an anticancer peptides against cancer cells can be explained by various factors:

Increased Negative Charges

In normal cells, there's a structural arrangement in their membranes with asymmetric distribution. Anionic phosphatidylserine is mainly situated on the inner side of the cell membrane. While the outer side is typically composed of neutral lipids like phosphatidylcholine and sphingomyelin [15]. However, oncogenic cells disrupt the natural balance, as shown in Figure 1.

Factors like the acidic, low-oxygen environment, and elevated levels of reactive oxygen species in the tumour microenvironment (TME) cause phosphatidylserine and phosphatidylethanolamine to shift from the inner to the outer leaflet of the membrane. This alteration results in a high concentration of anionic phosphatidylserine being exposed on the outer membrane of the cancer cell [16, 17]. These biochemical vulnerabilities make them targets for these peptides.

Certain peptides, like NK-2, which originate from the central region of NK-lysin in pigs and T-cells, are effective against hematologic malignancies due to their positive charge. They work by selectively killing cancer cells through a process called necrosis. This ability is closely associated with the occurrence of phosphatidylserine (PS) on the surface of cancer cells. NK-2 can latch onto these molecules and disrupt the cancer cell's membrane, leading to its death [18]. The NK-2 peptide was found to be located alongside P-glycoprotein in cancer cells that are resistant to multiple drugs. This close association helped effectively target and eliminate these drug-resistant cells that had P-glycoprotein in the complex environment of tumours [19].

The environment around cancer cells is more acidic, with a pH shift from the normal 7.4 to 6.5 [20]. This acidic environment contributes to the development of the aggressive tumour characteristics seen in cancer [21]

Furthermore, Cancer cells often have higher levels of certain negative molecules like sialic acid and glycosaminoglycans. These molecules make the surface of the cancer cells more negatively charged. Additionally, hyaluronan, another anionic glycosaminoglycan, further enhances the overall negative charge within tumour tissue [22, 23]. While it's generally true that the increased electrostatic negativity on the surface

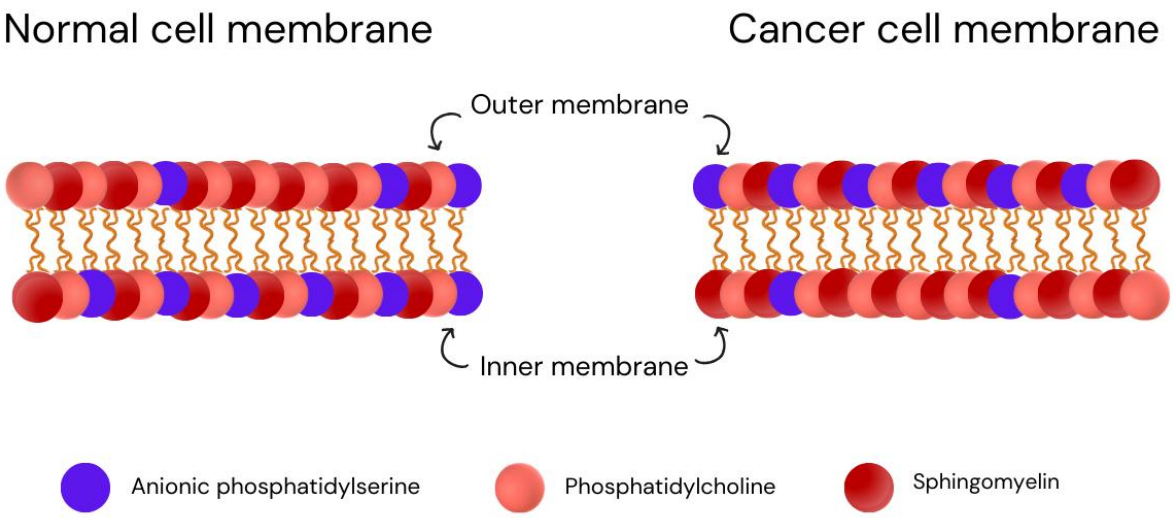


Figure 1 Difference between normal cell membrane and cancer cell membrane.

of oncogenic cells make them vulnerable to anticancer peptides, there's an interesting exception. Researchers discovered that when there is a lot of heparan sulfate on the outer leaflet of the cancer cell membrane, it can prevent anticancer peptides like LfcinB and KW5 from approaching the cell's inner membrane layer. As a result, this inhibits the peptides' ability to destroy the cancer cells. So, excessive heparan sulfate can act as a barrier and reduce their anticancer activity [24].

Pore formation

The ACP polybia-MPI, as well as bovine lactoferricin 6 (LfcinB6), have shown interesting properties in the context of cancer treatment [25, 26] Polybia-MPI, a short α-helical peptide, exhibits selectivity towards leukemia cells, and this selectivity may be attributed to variations in the level of exposed phosphatidylserine (PS) in the oncogenic cell membrane [26]

When tested for how well cells grow, survive, and respond to toxins, polybia-MPI was found to slow down the growth of both normal and drug-resistant cancer cells. At the same time, it increased the activity of lactate dehydrogenase (LDH), which indicates cell damage [26]. However, its effect on normal fibroblast cells was much less.

The biological membrane, the vital barrier defending the cell, is the first line of defense for a living cell. Pore forming proteins (PFP) play a key role in the host cell membrane alterations required to initiate the infection process. PFPs accomplish this process by changing from their soluble to membrane-bound forms. Because of this, these proteins frequently take on various structures and conformations, with one changing into the other during membrane interactions. The monomeric PFP subunit typically self-assembles into higher-order oligomeric species during this process, which are usually created in conjunction with a membrane scaffold. The development of effective drug molecules to treat a variety of infectious diseases has recently focused

attention on membrane interactions and biological system activities by membrane proteins, such as the PFPs-lipid bilayer interactions [27]. Peptides typically orient themselves more perpendicular to the membrane as the concentration increases after initially binding parallel to the membrane at low concentrations. Additionally, insertion into the bilayer and the eventual formation of trans-membrane pores take place at high peptide/lipid ratios. Numerous models have been developed to explain the interactions between ACPs and cancer cell membranes. Numerous models have been developed to explain how ACPs interact with cancer cell membranes like the barrel-stave model, carpet model, toroidal or two-state model, detergent-like effect model or inverted micelle model and in-plane-diffusion model [28]

The mechanism of action of polybia-MPI relies on its ability to disrupt and alter the cell membrane by creating pores, which was confirmed through imaging studies, as mentioned in figure 2 [26]. In pore formation, the positively charged polybia-MPI are attracted to the anionic components on the outer layer of cancer cell membranes. This electrostatic attraction helps the peptide to attach to the cancer cell. Once attached to the cancer cell membrane, these polybia-MPI can embed themselves into the lipid bilayer. This insertion is often facilitated by hydrophobic associations between the peptide and the nonpolar regions of the lipid molecules in the membrane. As the peptide inserts into the membrane, it can cause alterations in the shape and structure of the lipid bilayer by adopting a helical conformation that is capable of breaching the membrane. This disruption leads to the formation of pores or blebbing (bulging), and even bursting of the cell. Consequently, hematologic malignant cells die through a necrotic process, characterized by cell swelling and eventual bursting [26].

The anticancer peptide (ACP) Polybia-MPI and bovine lactoferricin 6 (LfcinB6) primarily follow the barrel-stave model among the models, which are later addressed [29]. In the barrel-stave model, peptides accumulate and embed perpendicularly into the cell membrane, forming a structure similar to a barrel with the nonpolar regions of the peptides associating with the hydrophobic lipid tails of the plasma membrane [30]. This model results in the formation of transmembrane pores, which can grow larger as more peptides aggregate and the cell's contents start leaking out, resulting in cell lysis [25,26,29]. In contrast, the Toroidal Pore Model describes the anticancer peptides stick to the anionic regions i.e. the head part of the cell's membrane as they embed themselves into the membrane. As they keep entering, the membrane starts bending, forming a shape like a toroidal pore with a hole through it. This pore is made up of the membrane's head parts and the peptides inside it [31,32]. Because both positive and negative charges are present in this pore, it becomes stable. This process causes the cancer cell's membrane to lose its integrity, its charge, and leads to the leaking of the cell's contents, eventually lysis of cell [32, 33].

In the Carpet-Like Model the anticancer peptides with a positive charge act like carpets on the outer leaflet of cancer cell membrane. They are shaped like spirals and stick to the negatively charged part of the cell's outer layer. When enough of these peptides gather, they disrupt the order of the cell's outer layer, causing instability and making it break down. This disruption leads to the cell's membrane falling apart, ultimately causing the cancer cell to break open [29, 34].

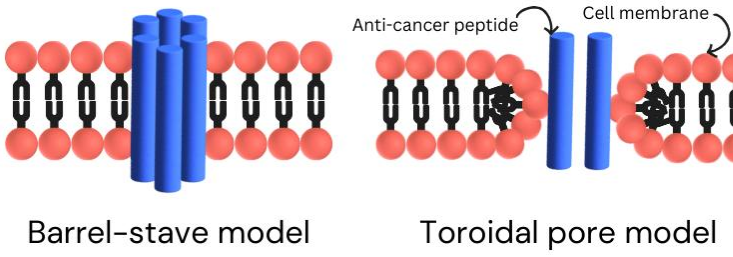


Figure 2 Diagram illustrating the models of action of anticancer peptides.

Peptide structure:

SK84 is a glycine-rich peptide derived from a species of fruit fly called Drosophila virilis, and it possesses the remarkable ability to disrupt the membranes of leukemia cells, as observed through scanning electron microscopy (SEM) [35]. This disruption doesn't happen because of electrostatic interactions seen with cationic peptides, as typically. SK84 seems to create membrane disruption through an alternative mechanism. This mechanism involves the creation of an elastic structure within the membrane, likely associated with the peptide's flexible N-terminal regions, which are rich in glycine [35]. In simpler terms SK84 gently pushes and pulls the membrane until it can't hold itself together anymore.

The peptide SK84 is quite selective. It's toxic to cancer cells but doesn't harm human red blood cells [35]. Leukaemia cells' distinct lipid makeup or membrane elasticity may be the cause of SK84's selectivity, whereas RBCs are structurally more resistant to mechanical disruption or do not have these weaknesses. This unique behavior makes SK84 a potential candidate for cancer treatment with a different mode of action compared to other anticancer peptides.

Immune responses

LTX-302 is a 9-amino acid peptide with a positive charge, derived from bovine lactoferricin. When tested, it was found to shrink tumours in models

where A20 cell lymphomas were implanted under the skin. Injecting LTX-302 directly into tumours caused damage to the cancer cell membranes, led to significant tumour death, and released tumour-associated antigens (TAAs). These tumour-associated antigens (TAAs) were then picked up by dendritic cells and presented to T cells, starting an immune response. The effectiveness of LTX-302 was shown in experiments with mice, where it not only had a local impact on the tumour but also triggered a strong and lasting immune response against the cancer [36, 37].

Emerging ACPs

Magainins, initially isolated from the skin of *Xenopus laevis*, are a group of peptides renowned for their potent antibiotic properties against diverse microorganisms. These peptides, typically made up of 21–27 amino acids and have a unique structure marked by positively charged and hydrophobic regions [38]. The synthetic magainin peptide derivatives exhibit the ability to selectively target tumour cells, inducing cytolytic activity. They show concentrations 5–10 times greater than what is required for antibacterial effects. This selectivity extends to maintaining relatively low toxicity levels in normal cells. The underlying mechanism of action involves the formation of α-helical channels on the membrane of tumour cells. This structural alteration impacts membrane permeability, leading to a quick and permanent cell damage [39].

PEP2 and PEP3 are short and synthetic peptides made from the end part of the ARTS protein, which promotes cell death. These peptides demonstrated efficient cell-killing capabilities specifically targeting human leukemia cells. By harnessing the proapoptotic properties of ARTS, PEP2 and PEP3 offer a potential avenue for inducing programmed cell death in leukemia cells, a crucial aspect in cancer treatment [40]. Another innovative approach involves peptides known as BIM SAHBA. This peptide combines parts of the BIM protein, which helps trigger cell death, with a stable section of the BCL-2 protein. It targets the BCL-2 pathway, disrupting proteins that help cancer cells survive and activating proteins that lead to cell death. This approach helps overcome the resistance to cell death seen in blood cancers like leukemia. Tests in mice showed that BIM SAHBA can reduce the growth of leukemia tumours that are resistant to drugs. "BIM SAHBA helps leukaemia cells overcome resistance to apoptosis, especially those

immune to BH3-mimetics and standard chemotherapies such as doxorubicin [41].

Anti-cancer peptide PNC-27 is a promising drug for clinical use. It interacts with a protein called hdm-2 on the cancer cell membrane, which causes pores to form and leads to cell death. It also disrupts the mitochondria inside the cancer cells. In PNC-27 treated cancer cells, the mitochondria lose the dye indicating healthy function, while the lysosomes retain their dye. Special imaging revealed that PNC-27 was located on the mitochondrial membranes.

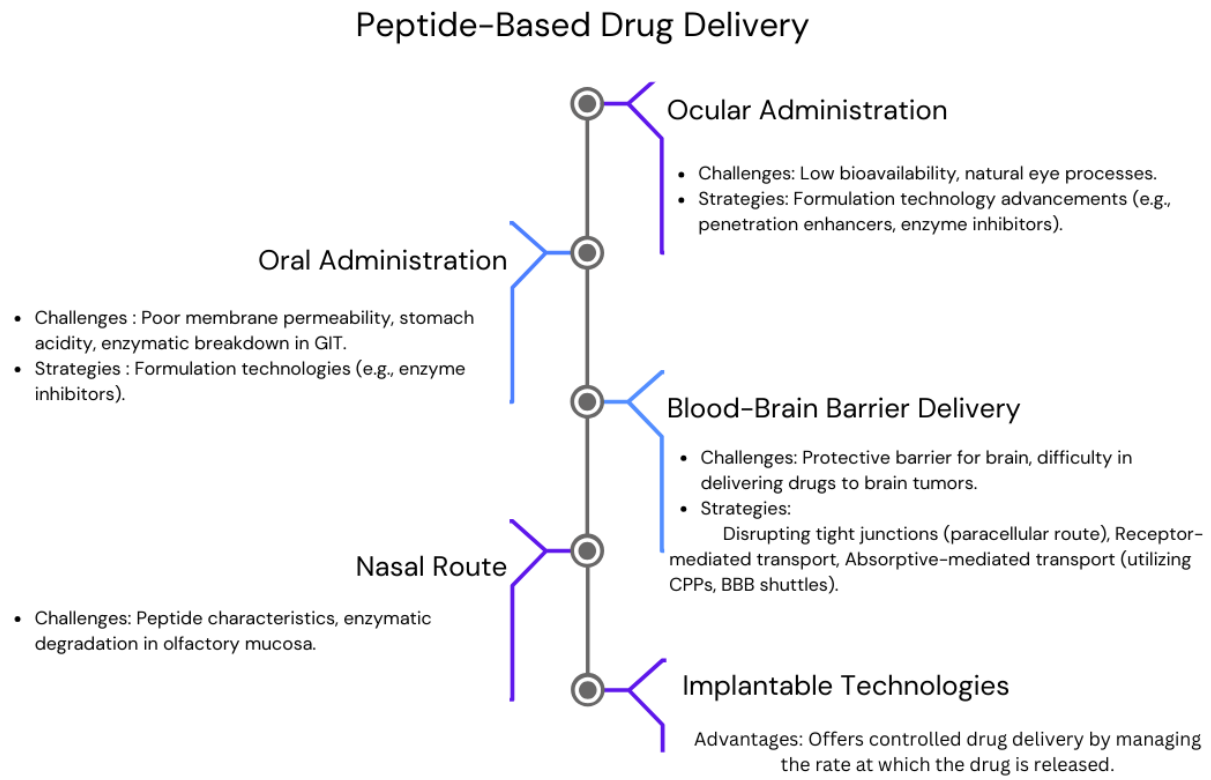
Pharmaceutical Applications of peptides

Recent strides in biopharmaceutical engineering have led to the creation of numerous peptide-based drugs [42, 43, 44]. The number of peptide drugs entering clinical trials has grown rapidly over the past 40 years. The market for peptide drugs, especially active pharmaceutical ingredients (APIs), has also expanded significantly. While peptide drugs used to be shorter, typically around 10 amino acids long, they are now often 30 to 40 amino acids long. Advances in technology have improved the ability to characterize and manufacture these larger peptides in large quantities. The method through which a drug is administered significantly influences its efficacy [45]. While the conventional needle-and-syringe approach is widely used, it presents issues with patient convenience, expense, and maintaining sterility. This section explores different administration routes proposed for peptides, aiming to overcome these limitations and enhance therapeutic outcomes, as shown in Figure 3. Peptides are typically delivered through invasive methods like injections, but several non-invasive options have been investigated, such as nasal, buccal, transdermal, and pulmonary routes, particularly for chronically administered drugs.

Oral drug administration is widely favoured for its convenience. But peptide drug molecules are generally not delivered orally. Due to poor membrane permeability, stomach acidity, and susceptibility to enzymatic breakdown in the gastrointestinal tract (GIT), but such challenges could be overcome by exploring various formulation technologies, including co-administering enzyme inhibitors with therapeutic peptides to enhance absorption and bioavailability [46].

Administration of drugs through the eyes proves beneficial for treating ocular malignant tumours, albeit facing challenges related to natural eye processes. The bioavailability of peptides could lead to potential cost issues. Ongoing advancements in formulation technology are made for optimizing the efficacy by incorporating penetration enhancers and enzyme inhibitors [47].

Figure 3: Peptide-based drug delivery



The nasal route for peptide delivery works by using different ways to get the peptides through the nasal membrane, such as passive diffusion mechanisms, carrier-mediated transport, and transcytosis. It appeals as a pain-free and non-invasive administration route for peptide delivery. While this method has benefits like increased permeation and rapid absorption, challenges persist too. These include constrained dosage and enzyme degradation in the olfactory mucosa [46].

Researchers are exploring peptides that can specifically target tumours or blood vessels, aiming to improve drug delivery to brain cancers. One approach involves disrupting the tight junctions between endothelial cells that make up the BBB (blood-brain barrier), allowing drugs to pass through the spaces between these cells (paracellular route). Another strategy maintains the integrity of the BBB (blood-brain barrier) but delivers drugs through receptor-mediated transport. In this method, drugs are attached to peptides that mimic specific ligands. Additionally, drugs can be transported through absorptive-mediated transport, where cell-penetrating peptides (CPPs) and BBB shuttles come into play [48, 49, 50]

Drug delivery across the blood-brain barrier primarily occurs through two routes: paracellular diffusion and the trans cellular route. Paracellular diffusion involves drugs moving between cells, but tight junctions normally prevent this process. To overcome this obstacle, researchers may disrupt or temporarily regulate the BBB. The transcellular route involves drugs passing through cells, traversing both the apical and basolateral membranes [50].

Light entering the eye, being focused by the cornea and lens onto the retina, and then being transformed into electrical signals by specialised cells are all examples of natural eye processes. The brain then decodes these signals as images after receiving them from the optic nerve. Recent advancements have also focused on implantable devices and technologies for delivering drugs via intracranial, intrathecal, or

intravaginal routes, with notable progress in intraocular and subcutaneous implants. It offers controlled drug delivery by managing the rate at which the drug is released. Some of these technologies have received FDA approval. Ongoing research aims to enhance both implantable devices and in situ-forming implants, which may use nanomaterial formulations in non-bioabsorbable and biodegradable polymers [43].

Known Side effects of ACPs

Hemolysis (Red Blood Cell Damage)

Negatively charged membranes interact with a variety of cationic anticancer peptides. High dosages can also damage red blood cell membranes, resulting in hemolysis, even though cancer cells have a higher negative charge than healthy mammalian cells [11]

Cytotoxicity to the normal cells

Off-target toxicity can result from certain ACPs' partial lack of selectivity and ability to harm non-cancerous mammalian cells [51]

Immunogenic complications

Peptides may trigger unintended immunological reactions, like hypersensitivity or the production of antibodies, which could lessen the effectiveness of treatment or have negative consequences [52].

Rapid degradation and half-life

Serum proteases frequently break down peptides rapidly, necessitating high or frequent dosages that might cause systemic toxicity [13].

Potential for organ toxicity

Peptide buildup or metabolism, particularly at higher doses, has been linked to hepatic or renal stress in certain in vivo studies [14]

ACPs used in combinations

Combination with chemotherapy:

Maganain II and Doxorubicin: By increasing doxorubicin uptake by membrane disruption, magainin II and doxorubicin demonstrated synergistic cytotoxicity in breast cancer cells [53].

Peptide & Cisplatin: Increases apoptosis in lung and ovarian cancer cells through cisplatin sensitization of tumour cells [54].

Combination with immunotherapy

LTS 315 (*oncolytic peptide*)*:* releases tumour antigens, triggers immunogenic cell death, and has been used in conjunction with immune checkpoint inhibitors (anti-PD-1, anti-CTLA-4) to increase T-cell-mediated tumour clearance [55].

Defensins (*e.g., hBD-2, hBD-3*): demonstrated to enhance checkpoint blockade and cancer vaccines by acting as chemoattractant for T cells and dendritic cells [56]

Combination with radiotherapy

LTS 315 + Radiation: improved local and systemic anticancer responses by increasing the immunogenicity of irradiation tumours, leading to improved tumour control in preclinical animals [55]

TP10 peptide (Transportan-10): encouraged glioblastoma cell apoptosis and DNA damage when paired with radiation [57].

Conclusion

In conclusion, cancer remains a formidable global public health issue, necessitating ongoing research and innovation in therapeutic approaches. The complex nature of cancer, with its diverse types and evolving challenges, demands potent and precisely targeted treatments. Peptide-based therapies, particularly Anticancer Peptides (ACPs) are promising for exploration over the last two decades, revolutionizing the pharmaceutical landscape. Hematologic malignancies, comprising a significant portion of global cancer cases, present a specific focus for ACP research. Notable ACPs, like NK-2 and Polybia-MPI, have demonstrated effectiveness against hematologic malignancies, showcasing the potential of these peptides in addressing blood cancers. Additionally, innovative ACPs like

SK84, LTX-302, Magainins, Pep2, Pep3, BIM SAHBA, and PNC-27 exhibit diverse mechanisms of action, further expanding the repertoire of peptide-based anticancer strategies. Beyond their therapeutic potential, ACPs offer advantages in terms of minimal toxicity, effective tissue penetration, and versatility in modifications. The emerging discoveries of ACPs, especially those inducing immune responses, hold promise for developing comprehensive cancer treatment strategies. Moreover, the pharmaceutical applications of peptides extend to alternative drug delivery routes, addressing challenges associated with conventional methods. Oral, nasal, ocular, and blood-brain barrier routes provide avenues for optimizing drug administration, enhancing bioavailability, and improving patient convenience.

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