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Understanding TnpB's Role in Crop Improvement

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The study focuses on the role of TnpB to improve the crop traits that can enhance the growth and the functionalities of the TnpB for precise gene editing application through opening new research opportunities in the OMEGA-R system. Further, it includes the evolutionary journey and comparison with CRISPR-Cas to portray the effectiveness. Along with this, future approaches, advantages and genome editing application helps to understand the implementation strategies of TnpB for crop improvement to mitigate the food scarcity within India and encourage sustainability further.

Abbreviations

TALENs	'Transcription activator-like effector nucleases'
CRISPR	'Clustered regularly interspaced short palindromic repeats'
TAMs	'Target-adjacent motifs'
ISDra2	'Insertion Sequence Deinococcus radiodurans 2'
OMEGA	'Obligate Mobile Element Guided Activity'
single-AAV methods	'Recombinant adeno-associated virus vectors'
PAM	Protospacer Adjacent Motif
ISAam1	Integrated Site-specific Transposase from A. malum

Introduction

Food security is one of the major factors which can be challenged due to the inflation level fluctuation in agriculture and cereals. Though, different survey reports stated that price fluctuation in maize, wheat, and rice with 2 percent, 20 percent and 31 percent respectively. Further, it is observed that with 11 percent higher inflation in 76.5 percent low-income countries (World Bank, 2025).Development of conventional breeding with different molecular techniques is now essential to implement for encouraging sustainable agriculture, thus European countries have taken different initiatives by aligning with the U.S SDGs with the European Green Deal. Such as zinc-finger nucleases, transcription activator-like effector nucleases (TALENs), and the meganuclease and clustered regularly interspaced short palindromic repeats (CRISPR) systems helps to develop faster development of new plant types and enable the mitigation wide range of food security within the country further (Tyczewska *et al*., 2023, Das *et al.*, 2023, Munaweera *et al*., 2022).

Understanding TnpB

TnpB, a transposon-encoded IS200/IS605-type RNA-guided nuclease, has been highly publicized as the game-changing genome-editing tool, but its evolutionary history as selfish genetic elements raises questions about its accuracy and reliability compared to CRISPR systems (Karvelis *et al.*, 2021).

Evolutionary Background: Integrated in IS200/IS605 transposons, TnpB is an evolutionary precursor of CRISPR-Cas12 enzymes but its parasitic nature signals self-replication rather

than stable editing, which limits its application to intricate genomic function (Karvelis *et al*., 2021, Altae-Tran *et al*., 2021). Structure and Function ωRNAs direct TnpB to cut double-stranded DNA at specific positions determined by restrictive target-adjacent motifs (TAMs) like 5'-TTGAT, at a frequency of roughly once every 512 base pairs, restricting its target range considerably; its suboptimal cleavage to begin with also renders its engineered variants like TnpBmax extremely useful for functional purposes (Karvelis *et al*., 2021).

Significance: Small TnpB (~400 amino acids) size is within the handling capability of AAV delivery, and its programmability provides editing capability, but its restrictive TAM requirements, differing efficiency, and untested off-target profile detract from claims of superiority when not optimized substantially (Karvelis *et al*., 2021, Altae-Tran *et al*., 2021).

TnpB in Genome Editing

Comparison with CRISPR-Cas systems: TnpB and CRISPR-Cas systems both employ RNA-guided DNA targeting. However, they differ in structure, evolution, and application. TnpB, derived from the IS200/605 transposons, is assumed to be the evolutionary ancestor of Cas12 nucleases while both have a RuvC-like cleavage domain (Altae-Tran *et al*., 2023; Nety, 2023).

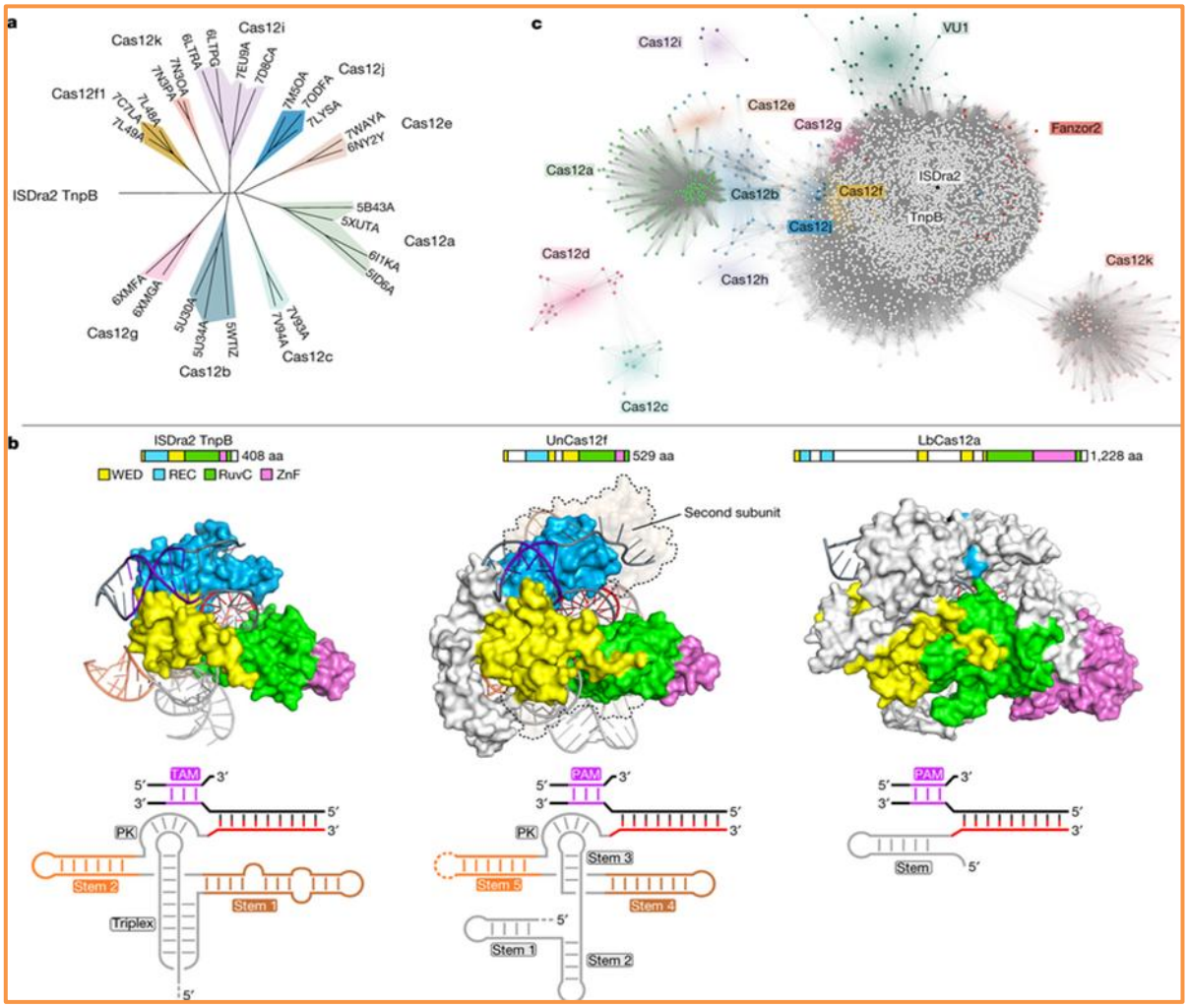

Figure: Visual comparison between TnpB and Cas12 protien (Source: Rai and Dutta, 2024)

TnpB's is smaller in size (about 400 amino acids) than Cas12 (1,000-1,300 amino acids). This difference facilitates in vivo administration and reduces immunogenicity concerns (Wang *et al*., 2024). TnpB uses a single ωRNA (or reRNA) guide, similar to Cas12's crRNA, but requires a TAM such as TTTAA or TTGAT for identification. This requirement is more stringent than Cas12's TTTV PAM and provides better specificity in studied models (Xu *et al*., 2023; Li *et al*., 2024). The results of editing can also vary. TnpB, unlike several Cas effectors, frequently produces deletions with little collateral activity,

making it suitable for precise applications (Altae-Tran *et al.*, 2023). TnpB variants such as ISDra2 showed 90-100% efficiency in rice without off-target effects, outperforming previous Cas12f attempts (Li *et al*., 2024).

In animals, TnpB corrected tyrosinemia in mice with AAV, which is challenging for bigger Cas9 due to packaging constraints (Li *et al*., 2024).Truncated versions of TnpB build on this advantage, demonstrating significant in vitro and in vivo activity comparable to optimized Cas12 but with less complexity (Wang *et al.*, 2024). Cas12 evolved from TnpB several times, demonstrating TnpB's adaptability and greater functional variety beyond immunity, including potential regulatory roles (Altae-Tran *et al*., 2023). Thus, whereas CRISPR-Cas technologies are useful for multiplex editing, TnpB shines in small, focused settings.

Potential Applications in Crop Improvement

TnpB is a progenitor of CRISPR–Cas nucleases and can be reprogrammed for genome editing in human cells (Karvelis *et al.*, 2021). Different researchers have stated in their study that Transposons reshape genomes; 'IS200/IS605' elements encode TnpA which is essential transposase and TnpB plays an accessory role which is still unclear. However, study shows TnpB is an RNA-guided nuclease, using a transposon-derived RNA to cut DNA at the 5′-TTGAT motif.

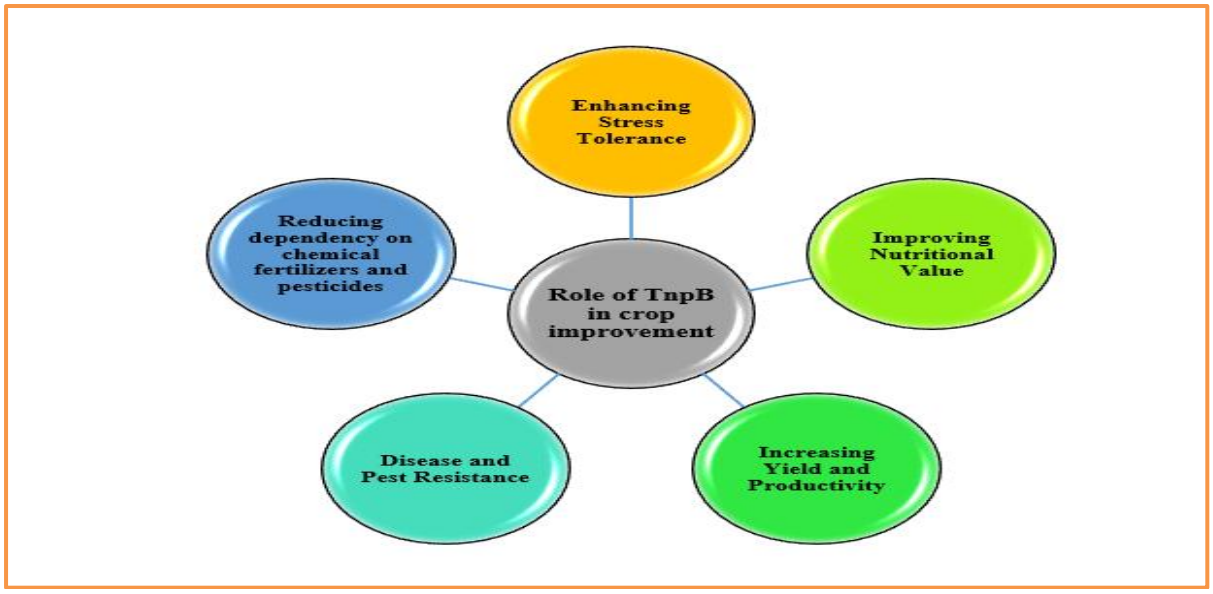

Figure: Different role of crop improvement by TnpB (Source: Self-Designed)

In crop improvement, TnpB's programmable activity can be applied to stress-responsive genes, enabling precise modifications that enhance tolerance to drought, salinity, heat, and pathogens. Though, OMEGA-R system helps to harness different RNA guided DNA cleavage through different high-throughput genome editing approaches and effective mutagenesis applications (Liu *et al*., 2025, Teng *et al*., 2024). The flexibility of the OMEHA-R system helps to reduce the dependency of TAM dependency through genetic engineering approaches and by direct evolution of stress-related alleles modification of different complex traits such as multi stress resistance (Liu *et al*., 2025). Similar to the CRISPR-Cas system, TnpB has developed a new era of genome editing techniques within agriculture by improving different areas of development such as stress resistance, nutritional value addition, increasing yield potential through regulatory genes which are responsible for flowering time, photosynthesis efficiency and productivity. Along with this, it created new research on the OMEGA-R system (Obligate Mobile Element Guided Activity) to improve the disease and pest resistance like bacterial blight in rice through engineering bacterial pathogens, and reduce the chemical fertilizer dependency to promote sustainable agriculture further (Tripathi *et al*., 2024).

Advantages of TnpB Over Existing Tools

A transposon-encoded RNA-guided nuclease, TnpB offers numerous advantages for gene editing. Its modest size, roughly 400 amino acids, makes it much smaller than classic CRISPR effectors like Cas9 (1,300 amino acids) and Cas12 (1,000 amino acids). This lower size makes it easier to deliver in vivo medicines using adeno-associated virus (AAV) vectors disenabling the problem of packaging via viral methods of delivery. In a study crried on with mice which had a liver disease called tyrosinemia, the TnpB-ωRNA system was able to reinstate liver function. This showed that the use of a single AAV (a common type of viral vector) to deliver a cure for diseases with just one dose is possible (Li *et al.*, 2024).

The TnpB are RNA guided nuclear which eliminates DNA at the ensuring sudden modification. This mechanism is observed both in animals and plants. In case of a study of rice genome greatest accuracy is observed supported with non significant off targets mechanism in two variations: ISDra2 and ISYmu1 (Li *et al.*, 2024). Due to the diversity of TnpB occurs for (IS200/605 transposons) it helps in the selection for the scientists. However, some versions are minute and show significant effect on anumal cells (Altae-Tran *et al.*, 2023; Nett, 2023).

Challenges and Limitations

In practical filed it was very difficult for the refreshers to develop more concise application which can be RNA guided DNA nuclease and Beneficial for more accurate and less Time-consuming approach. Some systems have low initial editing efficiency, necessitating adjustments to the ωRNA guide or protein variations to improve activity. This was observed in archaeal TnpB, which needed reprogramming for proper DNA cleavage (Xu *et al*., 2023).

Off-target activity is often modest, but it remains a worry in complex genomes, necessitating more safety enhancements (Altae-Tran *et al*., 2023).In case of vertebrate, fish, reptile, or amphibian system TnpB has no flexibility on TAMs like TTGAT regions which can be extensively improved by PAM (Nety, 2023). Furthermore, In crops like ISAaml has no action can be demonstrated which indicates towards the discrepancies among orthologs so far (Li *et al*., 2024).In the era of genomic advancements for modifying DNA sequence without altering the sequence helps to develop effective desirable traits within the crops for mitigating food scarcity within the India.

Future Prospects

Using a transposon like TnpB can develop precise genome editing approach to implement advanced technological opportunities towards the medical filed Corley diagnosis the Cardio Vascular diseases to identify and help to find strategic process to overcome further (Li *et al*., 2023). Further, it can accelerate future prospects by developing various newly applied changes in the field of epigenetic like modification in DNA bases through cutting or alternation of histone groups. Moreover, studying different gene expressions which can be bounded in case of CRIPR-Cas thus may be extensively successful through TnpB.

Conclusion

The study concludes by illustrating the importance of the TnpB through portraying the role for improving crops regulatory genes related to stress resistance, nutritional value enhancement, disease or pest resistance through different advanced genomic approaches. Although, it encourages researchers' attention for encouraging technological advancement within the life science subject areas for future research. As well as helps to increase the more precise usage of gene engineering applications for sustainable agriculture.

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