Multidirectional Action of Oligo in Plants

Subjects: Biochemistry & Molecular Biology | Biotechnology & Applied Microbiology
Contributor: Cezary Krasnodębski, Agnieszka Sawuła, Urszula Kaźmierczak, Magdalena Żuk

Oligo technology is a low-cost and easy-to-implement method for direct manipulation of gene activity. The major advantage of this method is that gene expression can be changed without requiring stable transformation. Oligo technology is mainly used for animal cells. However, the use of oligos in plants seems to be even easier. The oligo effect could be similar to that induced by endogenous miRNAs.

Keywords: oligo technology ; plant ASO ; DNA methylation ; gene modulation

1. Oligos Treatment—Alternative Method for Screening of Gene Functions

There is a constant search for new tools for genetic engineering and control of gene expression in organisms, both for basic research and for the development of genetically modified organisms in medicine, agriculture and industry. One of the methods increasingly used for this purpose is oligo treatment. Of particular note is the possibility of DNA methylation modulation by oligos, which leads to heritable changes in gene expression without creating GMO plants [1][2]. Oligo-induced changes are stable and show similar traits to the reference transgenic plants, but without altering the genome sequence [3]. Therefore, oligos provide new tools for plant improvement through noninvasive epigenetic modulation.

Due to the mode of action of oligos, similar to small RNAs, sequence-selective inhibition or enhancement of gene expression enables the elucidation of complex gene expression, especially gene functions and regulatory elements [4][5]. Most importantly, oligos enable the study of vital genes, which is virtually impossible using the classical method of gene knockdown. Gene silencing via RNAi and siRNA is also used for this purpose. Treatment with oligos does not require tedious construction preparation and plant transformation, whereas shRNA and artificial miRNA must be inserted into a plasmid before they can be introduced into the cell, which is difficult and time-consuming [6][7][8]. Furthermore, this means that the degree of inhibition of gene expression depends on the level of expression of the plasmid in the cell. A similar problem occurs when generating mutants with overexpression. Oligos, on the other hand, are dose-dependent and can be used to either increase or decrease expression, depending on the level of expression [9]. At the same time, pleiotropic effects were minimized, which is a common problem when generating mutants by genetic transformation [10][11]. The effects observed after transformation may not only be caused by gene silencing but may also depend on changes in genome sequences and structure caused by the insertion. Unfortunately, this may also lead to altered expression of other genes.

Apart from that, the external addition of oligos enables the study of genes at different stages of plant development and allows experiments to be conducted over time. Therefore, primary and compensatory effects can be distinguished [2]. Regulatory proteins can also be targets of oligos to alter the expression of specific genes or even entire signaling and metabolic pathways. This provides tremendous flexibility in studying gene function and its global impact on hormone balances.

Although small interfering RNAs (siRNAs) can also be delivered directly into the cell, their design and synthesis are more complicated and expensive. Oligos, unlike siRNAs, do not need to be fully complementary to exert an effect [12]. They allow for triggering a change at SNP sites, but because they are inaccurately designed with respect to genome sequences, oligos can lead to expression defects in nontarget genes.

In addition, homologous sequences can be targeted with a single oligo, meaning that a single oligo can inhibit more than one gene from the same gene family [13]. Important in the context of the applicability of oligo technology is the ability to target regulatory proteins, which allows the study and modulation of entire signaling pathways and the study of the influence of individual factors on cell function.

2. Methods of Introducing OLIGOs into the Plant Cell

Oligo technology is less commonly applied to plant cells than to animal cells, but it has already been shown to work in several species: flax, barley, tobacco, tea and recently cucumber and potato [1][2][14][15][16][17][18][19]. This technique was first successfully applied in plant cells to alter the expression of the gene encoding the transcription factor SUSIBA2 [10]. The researchers' results showed that antisense oligonucleotides were efficiently transported within the leaf and reached the nucleus and chloroplasts [10][15]. In general, plant cells are more susceptible to oligo treatment than animal cells. This is mainly due to the positively charged cell wall, which is not a barrier for the negatively charged oligonucleotide molecules. Moreover, oligonucleotides can enter plant cells through channels specific to sugar molecules [10]. The exact mechanism of import into a plant cell has not been fully described.

Oligonucleotides can be introduced into plant cells in several ways: infiltration under reduced pressure, infiltration through the stomata, spraying of cells, forced osmosis, and biolistic particle delivery system (gene gun) [20]. Depending on the plant species, tissue type and age (developmental stage), different delivery methods are most suitable. For details, please see **Table 1**.

Table 1. Method for delivering an oligonucleotide to a plant cell.

Method	Tissue Type/Developmental Stage	Advantages	Disadvantages
infiltration under reduced pressure [1][15]	**almost any type of tissue and any stage of development: whole plant, leaves, roots**	**quick and easy to perform method, many plants can be infiltrated at the same time, spiking is possible to a certain extent (easy to seedlings)**	**considerable stress, a large volume of oligonucleotide solution is needed at once and tissue residues contaminate the tissue, it is difficult to transfer the method to adult plants**
infiltration through the stomata [19]	**leaves only (the larger the better), best results with mature leaves, possible study in vivo**	**small volumes of the oligo solution required at once, no problem with impurities, no equipment required**	**more variable results, possibility of tissue damage, more difficult to do manually, especially troublesome with some small-leaved species such as flax**
spraying the cells/tissue [21]	**different developmental stages and organs, possible life examination for above-ground part of the plant only**	**simple scale-up, hardly any equipment required**	**more variable results, more difficult to control quantitative application of oligo**
uptake in a sugar solution [2][10][11][21]	**whole plant, leaves, roots, best results with seedlings, possible study in vivo**	**quick and easy to perform method, hardly any equipment re-quired**	**large volume of oligonucleotide solution is needed at once, it is difficult to transfer the method to adult plants**

3. Analysis Strategy for Oligo Actions

Oligos are usually designed for a specific target. Therefore, appropriate validation allows determining which one works "best," according to our expectations. Based on proper design of oligos, we can expect that they will act in a certain way on the target gene or sequences [1][22]. Nevertheless, not all aspects of oligos have been fully examined or explained yet. Therefore, it is necessary to determine at which stage of the gene expression oligo acts (e.g., transcription or translation) and the direction of those changes (down or up). For this purpose, it is recommended to check both the target transcript (e.g., by qPCR), and if it results in a protein product, also their value (e.g., by Western blot). In some cases, the enzymatic activity or the level of final metabolites are also determined [2][15][23]. Moreover, fluorescence labeling of oligos enables the determination of their subcellular localization. It is especially important when the target sequence is located outside the nucleus, e.g., in chloroplasts [15], which allows determining whether the oligos have reached their destination.

In plants, oligos have been shown affect DNA methylation, which can modulate the expression of the target gene. However, oligo usage may also lead to methylation modulation throughout the genome. The resulting epigenetic changes can be stable for up to 3 generations. Therefore, the total degree and profile of methylation would be worth checking with particular regard to the target gene [3].

References

1. Dzialo, M.; Szopa, J.; Czuj, T.; Zuk, M. Oligodeoxynucleotides Can Transiently Up- and Downregulate CHS Gene Expr ession in Flax by Changing DNA Methylation in a Sequence-Specific Manner. Front. Plant Sci. 2017, 8, 755.

2. Wojtasik, W.; Kulma, A.; Boba, A.; Szopa, J. Oligonucleotide treatment causes flax β-glucanase up-regulation via chan ges in gene-body methylation. BMC Plant Biol. 2014, 14, 261.
3. Dzialo, M.; Szopa, J.; Hnitecka, A.; Zuk, M. Transgenerational Perpetuation of CHS Gene Expression and DNA Methyla tion Status Induced by Short Oligodeoxynucleotides in Flax (Linum usitatissimum). Int. J. Mol. Sci. 2019, 20, 3983.
4. Gleave, M.E.; Monia, B.P. Antisense therapy for cancer. Nat. Rev. Cancer 2005, 5, 468–479.
5. Rayburn, E.R.; Zhang, R. Antisense, RNAi, and gene silencing strategies for therapy: Mission possible or impossible? Drug Discov. Today 2008, 13, 513–521.
6. Behlke, M.A. Chemical modification of siRNAs for in vivo use. Oligonucleotides 2008, 18, 305–319.
7. Sandy, P.; Ventura, A.; Jacks, T. Mammalian RNAi: A practical guide. Biotechniques 2005, 39, 215–224.
8. Scherer, L.J.; Rossi, J.J. Approaches for the sequence-specific knockdown of mRNA. Nat. Biotechnol. 2003, 21, 1457–1465.
9. Kurreck, J. Antisense technologies. Improvement through novel chemical modifications. Eur. J. Biochem. 2003, 270, 16 28–1644.
10. Sun, C.; Ghebramedhin, H.; Höglund, A.S.; Jansson, C. Antisense oligodeoxynucleotide inhibition as a potent diagnosti c tool for gene function in plant biology. Plant Signal. Behav. 2008, 3, 328–330.
11. Sun, C.; Höglund, A.S.; Olsson, H.; Mangelsen, E.; Jansson, C. Antisense oligodeoxynucleotide inhibition as a potent s trategy in plant biology: Identification of SUSIBA2 as a transcriptional activator in plant sugar signalling. Plant J. 2005, 44, 128–138.
12. Vickers, T.A.; Koo, S.; Bennett, C.F.; Crooke, S.T.; Dean, N.M.; Baker, B.F. Efficient reduction of target RNAs by small i nterfering RNA and RNase H-dependent antisense agents. A comparative analysis. J. Biol. Chem. 2003, 278, 7108–71 18.
13. Bennett, C.F.; Cowsert, L.M. Application of antisense oligonucleotides for gene functionalization and target validation. C urr. Opin. Mol. Ther. 1999, 1, 359–371.
14. Crooke, S.T.; Wang, S.; Vickers, T.A.; Shen, W.; Liang, X.H. Cellular uptake and trafficking of antisense oligonucleotide s. Nat. Biotechnol. 2017, 35, 230–237.
15. Dinç, E.; Tóth, S.Z.; Schansker, G.; Ayaydin, F.; Kovács, L.; Dudits, D.; Garab, G.; Bottka, S. Synthetic antisense oligod eoxynucleotides to transiently suppress different nucleus- and chloroplast-encoded proteins of higher plant chloroplast s. Plant Physiol. 2011, 157, 1628–1641.
16. Liang, X.H.; Shen, W.; Sun, H.; Migawa, M.T.; Vickers, T.A.; Crooke, S.T. Translation efficiency of mRNAs is increased by antisense oligonucleotides targeting upstream open reading frames. Nat. Biotechnol. 2016, 34, 875–880.
17. Liang, X.H.; Sun, H.; Nichols, J.G.; Crooke, S.T. RNase H1-Dependent Antisense Oligonucleotides Are Robustly Active in Directing RNA Cleavage in Both the Cytoplasm and the Nucleus. Mol. Ther. 2017, 25, 2075–2092.
18. Wdowikowska, A.; Janicka, M. Antisense oligonucleotide technology as a research tool in plant biology. Funct. Plant Bi ol. 2021, 49, 1–12.
19. Zhao, M.; Zhang, N.; Gao, T.; Jin, J.; Jing, T.; Wang, J.; Wu, Y.; Wan, X.; Schwab, W.; Song, C. Sesquiterpene glucosyl ation mediated by glucosyltransferase UGT91Q2 is involved in the modulation of cold stress tolerance in tea plants. N. Phytol. 2020, 226, 362–372.
20. Zhang, H.; Demirer, G.S.; Zhang, H.; Ye, T.; Goh, N.S.; Aditham, A.J.; Cunningham, F.J.; Fan, C.; Landry, M.P. DNA nan ostructures coordinate gene silencing in mature plants. Proc. Natl. Acad. Sci. USA 2019, 116, 7543–7548.
21. Xie, Z.; Sundström, J.F.; Jin, Y.; Liu, C.; Jansson, C.; Sun, C. A selection strategy in plant transformation based on antis ense oligodeoxynucleotide inhibition. Plant J. 2014, 77, 954–961.
22. Liang, X.H.; Sun, H.; Shen, W.; Wang, S.; Yao, J.; Migawa, M.T.; Bui, H.H.; Damle, S.S.; Riney, S.; Graham, M.J.; et al. Antisense oligonucleotides targeting translation inhibitory elements in 5’ UTRs can selectively increase protein levels. N ucleic Acids Res. 2017, 45, 9528–9546.
23. Holmes-Hampton, G.P.; Crooks, D.R.; Haller, R.G.; Guo, S.; Freier, S.M.; Monia, B.P.; Rouault, T.A. Use of antisense oli gonucleotides to correct the splicing error in ISCU myopathy patient cell lines. Hum. Mol. Genet. 2016, 25, 5178–5187.