Gene silencing: A problem in transgenic research

Tapan Kumar Mondal, Pradip Kumar Kundu and P. S. Ahuja

Transferring a gene from one plant to another is an area of immense importance in transgenic research today. Successful gene transfer depends on the integration and expression of the foreign DNA within the plant cell. Some recent reports suggest that if the foreign DNA, which is homologous to the endogenous gene gets integrated into a plant chromosome, then both the transgene and the endogenous gene are likely to become inactivated[1]. Even if multiple copies of the transgene are integrated, all or some of the copies become inactive. Such a phenomenon of gene inactivation in response to transgene transfer is known as gene silencing or transgene inactivation. Genes can be inactivated/silenced due to sequence homology either between the transgene and the endogenous gene or among transgenes themselves. The sequence homology may be present either in the promoter region or in the protein coding region. This fact is broadly defined as the 'homology dependent gene silencing'. Depending upon the mode of gene silencing, the mechanism can be classified under three types namely: (i) *cis*-inactivation, where silencing occurs among multiple copies of the transgene integrated either as inverted or as direct repeat on the same chromosome; (ii) *trans*-inactivation, where silencing occurs either in the transgene or in the endogenous gene when the integration occurs on a different chromosome. It can be either at allelic or non-allelic chromosomal locations with respect to the transgene insert; (iii) co-suppression/sense suppression, when expression of both the endogenous and the transgene coordinately gets suppressed. A well-documented example was reported with chalcone synthase (CHS) gene which is responsible for flower colour in petunia plant. When this gene was transferred in petunia to get a darker flower, interestingly, some white flowers were produced. This was attributed to the fact that the endogenous and the transferred CHS gene were silenced in white flowers[2].

Inactivation of a gene might occur at the transcriptional or at post-transcriptional level and different models/hypotheses were put forth for its explanation. Transcriptional gene inactivation can be explained by ectopic pairing[3], where the pairing of chromosomes occurs between an allelic and non-allelic (ectopic) homologous sequences due to some 'homology searching mechanism' present in the plant system. The ectopic pairing, in contrast to the normal crossing-over, is transient and does not involve any exchange of genetic material but occurs between very small homologous segments of non-homologous chromosomes. Rather, there is an exchange of some chromatin material causing heterochromatization which is known as a non-functional segment of chromosome for gene expression. It is also believed that some methylation does occur during ectopic pairing.

Transcriptional gene inactivation can also be explained by paramutation, in which the gene expression of one allele can be changed by exposure to another allele in a way that persists even after segregation of the two alleles. The allele that induces mutation to another allele (paramutable allele) is termed as paramutagenic allele. Paramutation was first observed in R locus of maize and was found only in plants[6]. Thus, the transgene or the endogenous gene or multiple copies of the transgene become paramutagenic inducing mutation in the corresponding homologous allele present in the plant genome. Paramutation also involves hypermethylation at the promoter region of the homologous DNA segment and causes gene suppression.

Post-transcriptional level was explained by (i) biochemical switch model[4], where accumulation of high level of gene product, i.e. RNA, reaching the critical threshold level, due to presence of more copies of the same gene leads to RNA degradation by RNAase. The degradation induces a type of feedback mechanism causing methylation of the homologous DNA sequences which causes suppression of the respective DNA. (ii) Antisense RNA hypothesis[5]. According to this hypothesis, sometimes antisense RNAs may be produced inside the plant cell, when the endogenous plant promoter is located at 3′ end to the transgene insert or they may be generated from the unintended RNA (abberant/faulty RNAs) produced by a plant-based RNA dependent RNA polymerase. These abberant RNAs are produced as a result of (a) formation of secondary structures of DNA during transcription, (b) methylation of the corresponding DNA (sense strand) and (c) delay in RNA processing when heterogeneous nuclear RNA of transgene and its endogenous gene gets accumulated due to the same processing track. Once the antisense RNA is produced, it pairs with the corresponding sense RNA and blocks the translation. Further, this double stranded RNA structure is degraded by a double strand specific RNAase, present inside the plant cell.

It is noteworthy to mention that at a time, more than one mechanism may operate simultaneously to silence a gene. Methylation plays a vital role in gene silencing both at transcriptional and post-transcriptional levels. The methylation occurs at 5′ position of cytosine residue by an enzyme called DNA methylase. In eukaryotes over 90% methylation occurs at CG dinucleotide sequences. But how does the *de novo* methylation occur? Two different nucleic acid interactions are believed to cause *de novo* methylation: DNA–DNA or DNA–RNA pairing (Figure 1).

The DNA–DNA pairing forms a hairpin structure which triggers the DNA methylase to cause methylation at both the strands. The DNA–DNA pairing and methylation was first observed in a filamentous fungus *Ascobolus immersus*, where the duplicated DNA sequences pair among themselves and form a hairpin structure leading to transcriptional inactivation of the gene and the process is called 'methyl induced premeotically' (MIP). DNA–RNA pairing also serves as a signal for *de novo* methylation. It has been observed in the transgenic plants which contain viral cDNA that after the production of RNA, from viral cDNA, the DNA becomes

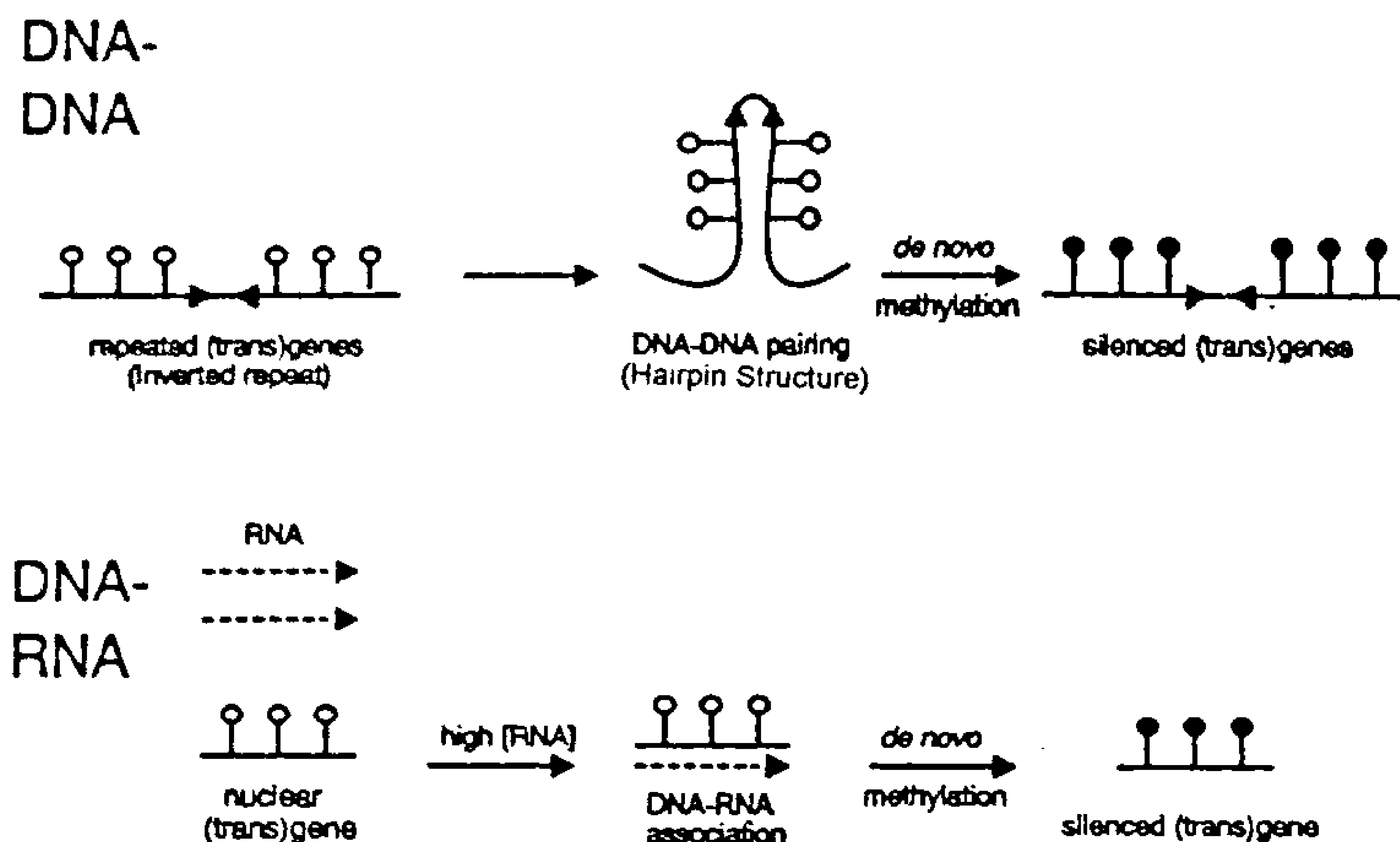

Figure 1. Hypothetical models of nucleic acid interaction involved in transgene silencing (Reproduced from ref. 7 with permission).

methylated probably through DNA–RNA pairing. The above observations were extended further to plant gene suppression involving DNA–RNA pairing which possibly triggers *de novo* methylation.

How do plants recognize the transgene as a foreign element and why do they have a tendency to suppress the homologous gene? It is well known that plants recognize an invasive DNA as 'foreign element' because of different CG contents[7] and like other living organisms, the plant cell also tries to eliminate the foreign body by activating its defense mechanism, which involves DNA methylation. DNA methylation serves as a useful tool in plants against the viral multiplication and also to prevent expression and spread of some transposable elements.

How can one overcome the transgene silencing? Gene silencing can be checked successfully firstly by using a drug named 5-azacytidine which is a modified cytosine base, incorporated during DNA replication. This drug is added in tissue culture medium during regeneration of the transgenic plant. Secondly, single copy gene insert can be selected among the transgenic progenies. However, further research is required to combat with this undesirable problem encountered during transgenic plant research.

1. Gupta, P. K., *Curr. Sci.*, 1996, **70**, 654–660.
2. Napoli, C., Lemieux, C. and Jorgensen, R., *Plant Cell*, 1990, **2**, 279–289.
3. Jorgensen, R., *Ag. Biotech. News Inf.*, 1991, **9**, 265–273.
4. Meins, F. Jr., *Annu. Rev. Genet.*, 1989, **23**, 395–408.
5. Meyer, P., *TIBTECH*, 1995, **13**, 332–337.
6. Matzke, A. M., Matzke, J. M. A. and Eggleston, B. W., *Trends Plant Sci.*, 1996, **1**, 382–388.
7. Matzke, A. M. and Matzke, J. M. A., *Plant Physiol.*, 1995, **107**, 679–685.

The authors are in the Biotechnology Division, Institute of Himalayan Bioresource Technology, Palampur 176 061, India.

The India–Asia collision warps and thaws Tibet's bowels

A. V. Sankaran

The Himalayas, along with the Tibetan Plateau, formed as a result of a classic continent–continent collision that took place about 50 million years (Ma) ago between India and Asia[1], is an uniquely elevated region of the Earth. The colliding boundaries of the two continents, forms today, the well-known Zangbo Suture Zone which coincides with the Yarlung–Zangbo river valley. The complex geological structure of the region has given rise to considerable debate among the earth scientists trying to interpret its orogenic evolution. According to some of them, the Indian plate, along with the crust and mantle below, is sliding underneath the Asian Plate, while a few others believe that the two plates are colliding head-on; and these tectonic processes are still active. Whatever be the mode of plate movements here, they have warped the colliding faces and pushed the crust deep down and created features, quite unique for the lithosphere of this plateau. For example, the region has an anomalously thick crust (almost twice the normal continental thickness), and an unusually high amount of heat flow. Earlier geophysical surveys have indicated that the unusual crustal thickness here is due to convergence during the collision of the two continents[2], while the abnormal heat flow has been attributed to the existence of molten granite at a depth of 10 to 20 km[3].

During the last couple of decades, this warped and contorted region had attracted a few international teams of geoscientists who undertook surveys to evaluate its geology and tectonic evolution. The latest to carry out such joint studies, is a team of scientists from USA, Canada, Germany and China. They undertook detailed geological and geophysical investigations to bring out an indepth profiling of Tibet and the Himalayas and advance existing