land development, irrigation, transportation of planting material, organic manure, barbed wire fencing, etc. Since each state and Union Territory has its own State Medicinal Plants Board (SMPB) working for NMPB, the funding provided by NMPB will be routed through respective SMPBs.

For developing herbal gardens in schools, the concerned SMPBs will arrange to provide technical support with the help of state forest/horticulture/agriculture departments of Agricultural Universities/ Research Institutions, whatsoever is near the school. Besides, the SMPB will provide quality planting material. Only use of organic manure/bio-fertilizer is suggested for raising herbal gardens. A school may grow about 5–10 medicinal plant species out of the total 32 prioritized species of the Board. However, the selection of medicinal plant species for developing herbal gardens is not restricted to prioritized species of the Board. Marketing of cultivated medicinal plant species will be made through networking of SMPB, drug manufacturers and traders.

In order to meet the objectives, the Herbal Garden Scheme of the NMPB has been circulated to all SMPBs for wider dissemination of the scheme. Within six months (April to September 2006) a total of 359 project proposals on School Herbal Gardens have been received from 13 States/Union Territories. After screening and internal reviews of all the proposals received, 238 proposals were found suitable for financial assistance, which costs Rs 33,32,000. The state-wise break up of the proposals received and the amount of funds to be allocated to respective SMPBs are given in Table 1. Within a short period of six months, submission of project proposals by 359 schools located at different corners of the country, reflects the interest and awareness of schools in medicinal plants. This interest will be a milestone in developing the medicinal plants sector and the conservation of biological diversity in the days to come.

Chandra Prakash Kala* and **Bikram Singh Sajwan,** National Medicinal Plants Board, Chandralok Building, 36 Janpath, New Delhi 110 001, India
*e-mail: cpkala@yahoo.co.uk

RNA interference – gene silencing by double-stranded RNA: The 2006 Nobel Prize for Physiology or Medicine

The classical view of the flow of genetic information at the molecular level envisages that within a living cell, the information encoded in the master molecule, the deoxyribonucleic acid (DNA), is first transcribed into RNA with the help of an RNA polymrase enzyme, and is then translated into proteins using the protein synthesis machinery available within the cell (Figure 1). This flow of genetic information from DNA via mRNA to protein was termed the central dogma of molecular biology by the British Nobel Laureate Francis Crick. However, except for some housekeeping genes, synthesis of proteins due to individual genes is not constitutive, and is now known to be regulated at different levels. For instance, initially in early 1960s, it was shown that the coding sequences in bacteria and other prokaryotes are organized in operons, which are under the control of regulator, promoter and operator genes. This work was recognized by the award of 1965 Nobel Prize for Physiology or Medicine to François Jacob, Jacques Monod and André Lwoff. Several modifications of central dogma and the classical operon concept were discovered later during 1970s, 1980s and 1990s. For instance, in 1970, it was shown that in some RNA viruses, RNA can be used for the synthesis of DNA (as an intermediate molecule) using an enzyme now popularly described as reverse transcriptase; this discovery was recognized by the award of 1975 Nobel Prize for Physiology or Medicine to David Baltimore, Renato Dulbecco and Howard Temin. During early 1980s, it was also shown that the genetic information within a eukaryotic cell occurs as split genes with intron and exon sequences, and that intron sequences are spliced out after transcription during RNA processing. This discovery was recognized by the award of 1993 Nobel Prize for Physiology or Medicine to Richard J. Roberts and Phillip A. Sharp. More recently during mid-1990s, it was shown that a large part of DNA in eukaryotes is actually used for synthesis of non-coding RNA (ncRNA), which plays an important role in regulating the expression of genes at the post-transcriptional level. It was shown that the ncRNA gives rise to double-stranded RNA (dsRNA), which is responsible for gene silencing; the phenomenon was described as RNA interference (RNAi). This 'discovery of RNAi involving gene silencing by dsRNA' has been recognized by the award of the 2006 Nobel Prize for Physiology or Medicine to two American scientists, Andrew Fire and Craig C. Mello. They reported for the first time in 1998 that in a very specific manner, gene silencing can be achieved through dsRNA-mediated degradation of mRNA[1]. This mechanism of RNAi is activated when specific RNA molecules occur in the cell as dsRNA, which activates biochemical machinery degrading mRNA molecules having nucleotide sequence identical to that of the dsRNA. When such mRNA molecules disappear due to dsRNA-mediated degradation, obviously the corresponding protein cannot be synthesized, so that the corresponding gene is apparently silenced.

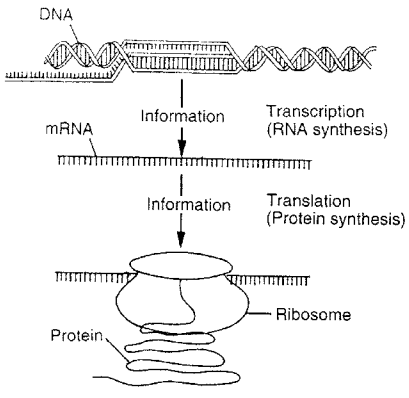

Figure 1. Central dogma showing the flow of information from DNA to protein via RNA.

Initial discovery of gene silencing in *Petunia*

Around 1990, molecular biologists obtained a number of unexpected results that were difficult to explain. The most striking effects were observed by plant biologists who were trying to increase the colour intensity of the petals in petunias by introducing a gene inducing the formation of red pigment in the flowers. But instead of intensifying the colour, this treatment led to a complete loss of colour and the petals turned white[2–4]. The mechanism causing these effects remained enigmatic until Fire and Mello discovered that dsRNA is involved in RNA interference[1] (see next para). This discovery won them the 2006 Nobel Prize for Physiology or Medicine within a short period of eight years after the discovery.

Discovery of the role of dsRNA in RNAi

Although the role of dsRNA in RNAi was suggested even by some earlier studies conducted in plant systems, the first direct evidence of the role of dsRNA in RNAi was made when Fire and Mello were investigating how gene expression is regulated in the nematode worm *Caenorhabditis elegans* (Figure 2). In their seminal study, they observed that when single-stranded RNA molecules including 'sense' strand encoding a muscle protein or the corresponding 'antisense' strand were separately injected in the body of the worm, there was no change in the behaviour of the worm. But when the sense and the antisense RNA were injected together, they observed that the worms displayed peculiar, twitching movements that were characteristic of the worms that completely lacked a functioning gene for the muscle protein, thus suggesting gene silencing due to dsRNA (Figure 2). Fire and Mello published[1] their findings in *Nature* on February 19, 1998, thus clarifying many confusing and contradictory experimental observations. This paper heralded the start of a new research field in molecular biology, as evident from the fact that this paper has been cited in as many as over 2500 research papers according to *Web of Science* (Thompson Scientific).

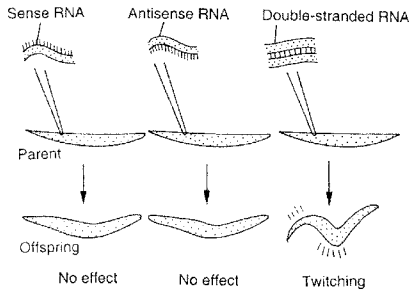

Figure 2. A simplified outline of the experiment of Fire and Mello, demonstrating that injection of single stranded sense or antisense RNA for muscle protein into the worm *C. elegans* had no effect, but injection of double-stranded RNA led to twitching that is characteristic of the worm carrying a defective gene for the muscle protein.

Fire and Mello had speculated that when sense and antisense RNA molecules meet, they base-pair and form dsRNA, which silences the gene carrying the matching nucleotide sequence. They tested this hypothesis by injecting dsRNA molecules encoding several worm proteins other than the muscle protein used initially. In every experiment, injection of dsRNA led to silencing of the gene containing the matching nucleotide sequence[5]. Thus, after a series of simple but elegant experiments, Fire and Mello deduced that dsRNA can silence genes through the phenomenon of RNAi, and that this RNAi is specific for the gene whose nucleotide sequence matches with the injected RNA molecule. It was also found that RNAi can spread across cells and can also be inherited. It was enough to inject tiny amounts of dsRNA to achieve this effect. Fire and Mello therefore proposed that RNAi is a catalytic process, which was later shown to involve amplification of siRNA (small interfering RNA).

Discovery of RNAi machinery

After the discovery of the role of dsRNA in RNAi in 1998, the components of the RNAi machinery were identified during the following years using biochemical and genetic approaches. It was found that dsRNA is cleaved into small fragments (called small interfering RNA or siRNA) by an RNase-III-like endonuclease called Dicer or Dicer-like (DCL). Another protein complex, RISC (RNA-induced silencing complex) binds these dsRNA fragments; one of the RNA strands is eliminated but the other (antisense strand) remains bound to the RISC complex and serves as a probe to detect the target mRNA molecules. When an mRNA molecule is available to pair with the RNA fragment within the RISC, it is bound to RISC, cleaved and degraded (Figure 3). The corresponding gene for this particular mRNA is thus apparently silenced (for a review, see ref. 6). It was also shown that although dsRNA is needed to trigger RNAi, the chemical composition of antisense RNA (relative to that of the sense strand) was more important.

A remarkable feature of RNA silencing is also its ability to spread across

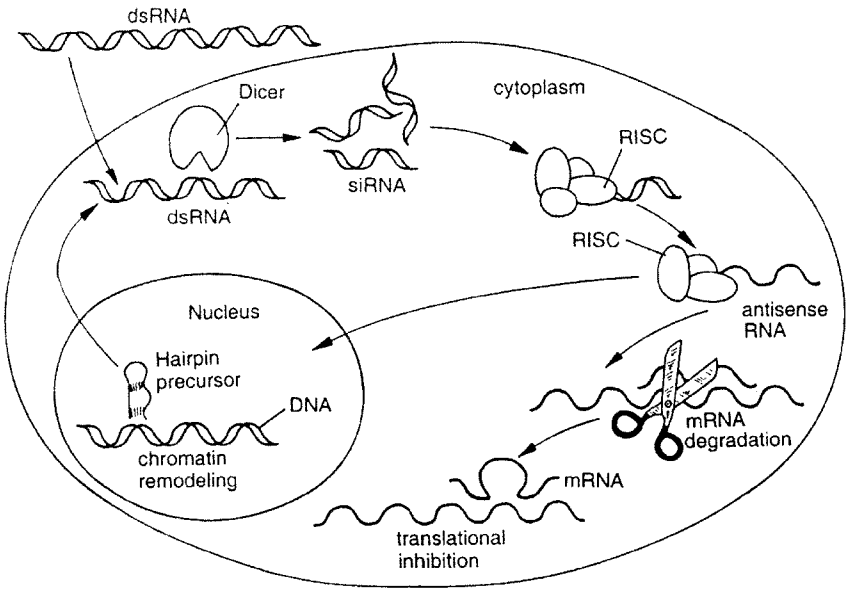

Figure 3. Mechanism of RNA interference, showing the roles of Dicer and RISC in post-transcriptional gene silencing (PTGS).

cells within a tissue and then to the entire organism. Silencing can thus be initiated locally but manifested throughout the organism. RNA-dependent RNA polymerases (RdRPs) are implicated in the amplification of siRNAs, so that the production and spread of the signal persists for a long time.

RNAi in defense against viruses and transposons

Although RNAi was initially discovered as a phenomenon for gene silencing, later it was found that it is also involved in protection of cells against molecular parasites like RNA viruses and transposons. Many viruses either contain dsRNA or they form dsRNA as an intermediate product during their multiplication. When such a virus infects a cell, its dsRNA molecule immediately binds to Dicer (Figure 4 *a*), the RISC is activated, the viral RNA is degraded, and the cell survives the infection.

It is also known that transposons that are present in all organisms can cause cell damage, if they end up in the wrong place. Many transposons operate by copying their DNA to RNA, which is then reverse-transcribed back to DNA and inserted at another site in the genome. Part of this RNA is often dsRNA that yields siRNA, which can be targeted by RNAi machinery. In this way, RNAi also protects the genome against transposons. However, this RNAi mechanism of protection against transposons differs from another recently discovered mechanism, which involves silencing of transposons due to another small RNA species, the repeat-associated interfering RNA (rasiRNA), which is longer (24–29 nt) than siRNA (~20–25 nt) and originates mainly from the antisense strand[7].

Other small ncRNAs (miRNA and piRNA) in RNAi

RNAi is now known to regulate gene expression in the cells of all organisms including plants, humans and worms. Hundreds of genes in our genome encode small RNA molecules, which mainly include another category called microRNAs or miRNA (Figure 4 *b*). They contain pieces of the code of other genes. Such siRNA and miRNA molecules can form dsRNA and activate the RNAi machinery to block protein synthesis. We now understand that genetic regulation by miRNAs plays an important role in the development of the organism and the control of cellular functions. In July 2006, occurrence of another small ncRNA called Piwi interacting RNAs (piRNA) was reported[8], thus suggesting that a variety of small ncRNAs may be involved in regulation of gene expression through gene silencing.

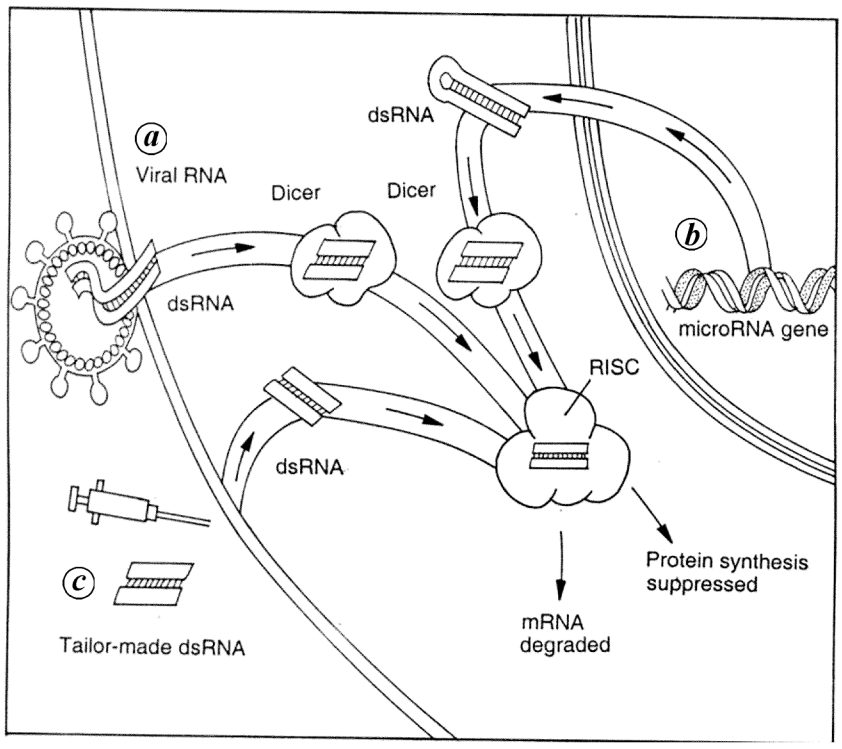

Figure 4. Diagrammatic representation of three processes involving RNAi within a cell: ***a***, Defense against RNA viruses; ***b***, RNAi and dsRNA involving gene-encoded microRNA; ***c***, Gene silencing due to tailor-made dsRNA.

Application of RNAi in medicine and agriculture

Utility of RNAi in biomedical research and health care

RNAi is currently being used for a variety of purposes including biomedical research and health care. In order to meet this objective, dsRNA molecules have been designed for silencing of specific genes in humans and animals (Figure 4 *c*). Such silencing RNA molecules are introduced into the cell to facilitate activation of the RNAi machinery. This method has already become an important research tool in biomedicine. Several recent publications show successful gene silencing in human cells and experimental animals. For instance, a gene causing high blood cholesterol levels was recently shown to be silenced by treating animals with silencing RNA. Plans are also underway to develop silencing RNA as a treatment for virus infections, cardiovascular diseases, cancer, endocrine disorders and several other conditions.

Utility of RNAi in crop improvement

RNAi is also being used for downregulating genes in crop plants in a very specific manner without affecting the expression of other genes, thus increasing their productivity. For instance, the transient satellite-virus-based SVISS technology developed by Bayer CropScience allows the production of high levels of dsRNA in plants, which triggers efficiently transient RNAi. The SVISS technology has been implemented as a research tool to discover and validate gene functions of candidate herbicide target genes and genes involved in abiotic stress response. For the abiotic stress-related PARP pathway in canola and corn and the enzymatic pathway underlying seed shattering in oilseed rape, it has been demonstrated

that stable transformation of crops with RNAi constructs results in stable modification of biochemical pathways which can result in improved productivity and quality of crops in the field.

Is the work on plant systems ignored in the award of Nobel Prize?

After the award of the 2006 Nobel Prize to Fire and Mello for the dicovery of the mechnism involved in RNAi, questions have also been raised arguing that recognition of the earlier work done on RNAi in plant systems was ignored. For instance, in a letter published in *Nature* on October 26, 2006, it has been argued that several aspects of the discovery that were cited in favour of the award including sequence specificity, RNA degradation and post-transcriptional nature of gene silencing were earlier demonstrated in plant systems, and were ignored during the award of Nobel Prize to Fire and Mello[9], since none of the scientists, who devoted themselves to RNAi work on plants shared the Nobel Prize.

1. Fire, A., Xu, S. Q., Montgomery, M. K., Kostas, S. A., Driver, S. E. and Mello, C. C., *Nature*, 1998, **391**, 806–811.
2. Napoli, C., Lemieux, C. and Jorgensen, R., *Plant Cell*, 1990, **2**, 279–289.
3. Van der Krol, A. R., Mur, L. A., Beld, M., Mol, J. N. M. and Stuitje, A. R., *Plant Cell*, 1990, **2**, 291–299.
4. Van Blokland, R., Van der Geest, N., Mol, J. N. M. and Kooter, J. M., *Plant J.*, 1994, **6**, 861–877.
5. Caplen, N., Parrish, S., Imani, F., Fire, A., and Morgan, R., *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 9742–9747.
6. Kooter, J. M., *Plant Epigenetics* (ed. Meyer, P.), Blackwell Press, Oxford, 2005.
7. Vagin, V. V., Sigova, A., Li, C., Seitz, H., Gvozdev, V. and Zamore, P. D., *Science*, 2006, **313**, 320.
8. Lau, N. C., Seto, A. G., Kim, J., Kuramochi-Miyagawa, S., Nakano, T., Bartel, D. P. and Kingston, R. E., *Science*, 2006, **313**, 363–367.
9. Bots, M., Maughan, S. and Nieuwland, J., *Nature*, 2006, **443**, 906.

ACKNOWLEDGEMENTS. I thank Indian National Science Academy (INSA) for the award of a position of INSA Senior Scientist, during the tenure of which this article was written. Thanks are also due to CCS University, Meerut for offering me the position of Honorary Emeritus Professor, and to Head, Department of Genetics and Plant Breeding, CCS University for providing facilities.

P. K. Gupta, Molecular Biology Laboratory, Department of Genetics and Plant Breeding, Ch. Charan Singh University, Meerut 250 006, India
e-mail: pkgupta36@hotmail.com

MEETING REPORT

Free-electron lasers and their applications*

The Indo-French workshop brought together accelerator physicists and users from the CLIO (Collaboration for an Infrared FEL at Orsay) Free-Electron Laser (FEL) in Orsay, France, accelerator physicists from the Compact Ultrafast Terahertz FEL (CUTE-FEL), and the latter's potential users in India. CLIO is an infrared FEL facility that has been operating for the last fifteen years at wavelengths from 3 to 100 μm. The CUTE-FEL is a terahertz FEL that is being built at the Raja Ramanna Centre for Advanced Technology. Ten scientists from France and twenty scientists and two students from India attended the workshop.

The workshop commenced with introductory remarks by Shiva Prasad, Director of IFCPAR, who welcomed the participants and briefed them on the objectives of the Centre. J.-M. Ortega then spoke on the CLIO FEL, emphasizing the unique features of the FEL – broadly tunable spectral range, appropriate pulse structure of picosecond pulses at MHz repetition rate, and high peak and average power (around 10 MW and 10 W respectively). He also pointed out the importance of value-addition to the facility by providing conventional lasers that are synchronized to the FEL pulses. S. Krishnagopal described the parameters of the CUTE-FEL being developed in India, and briefed the participants on the present status of activities. He emphasized the fact that a major part of the effort has been in the development of the technology of the linear accelerator and undulator that are part of the FEL. A standing-wave Plane Wave Transformer (PWT) linac has been developed, only the second in the world, and a 10 mA electron beam has been accelerated in this 21 cm long structure to 3.5 MeV, corresponding to an accelerating gradient of around 20 MV/m. A 5 cm period, 2.5 m long, planar undulator, using NdFeB magnets, has also been built and characterized. The remaining talks on the first day were devoted to further details of the two FELs. There were also talks from V. B. Asgekar on undulator development and Cherenkov FEL activities at Pune University, and from Ravi Kumar on FEL-related activities at the Institute for Plasma Research.

From the second day onwards discussions turned to applications of FELs. The French participants presented interesting work done on the CLIO FEL. They emphasized that for all of this work the CLIO FEL was a unique tool either because of the high power (enabling nonlinear studies), or the wide frequency tuning, or the convenient pulse structure.

C. Desfrançois discussed the structure of protonated peptides and drugs in the gas phase using the technique of infrared multi-photon dissociation (IRMPD) spectroscopy. He compared the IR spectrum of the biological peptide sequence RGD (argininеglycine–aspartic acid) with the one of a cyclic peptide containing the same RGD sequence (Arg–Gly–Asp–Phe–Val), and showed that the recognized RGD loop structure encountered in RGD-containing proteins is conserved in the gas-phase. He also showed the first gas-phase IR spectra of the very powerful

*A report of the Indo-French workshop on free-electron lasers and their applications, held in Goa during 20–24 March 2006, under the auspices of the Indo-French Centre for the Promotion of Advanced Research (IFCPAR).