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Transgenic plants and vaccines

Eradication of infectious diseases by vaccination has been a major thrust area in this century and will continue to be a major challenge in the next century. Despite technological advances and revolutionary discoveries in vaccine development ever since Edward Jenner's first documentation of small pox vaccination two hundred years ago, many vaccine-preventable diseases are still rampant in several parts of the world, especially in developing countries. Prevalence of these diseases is often due to non-affordability rather than non-availability of effective vaccines. Thus, while we are being threatened by dreadful diseases such as AIDS and malaria for which a vaccine still remains a distant dream, we have to find ways to produce and market vaccines at an affordable cost for diseases such as rabies, hepatitis, etc. The review article by Sharma *et al.* (**page 524**) describes the progress made on the production of biopharmaceuticals such as vaccines and antibodies in plants and suggests that 'biofarming' is a viable choice. While plants have been used as a source of natural medicines for several centuries, they are now being exploited as low-cost production systems and effective delivery systems for vaccines and biopharmaceuticals. Antigens of several pathogenic bacteria and viruses have been expressed in diverse plants such as banana, tomato, potato, etc. and consumption of these transgenic plant products or injection of plant extracts results in the induction of specific immune response leading to protective immunity. While some edible vaccines are currently undergoing human clinical trials, it is heartening to note that Charles Artnzen, who pioneered edible vaccine research, mentioned recently that transgenic banana containing enterotoxigenic *E. coli* enterotoxin is already available for vaccination of children in the form of infant feed formulation in Mexico. Let us hope that plants, in addition to their role as producers in the ecosystem will also serve as protectors of humans and animals against dreadful diseases by providing therapeutic drugs and preventive vaccines.

P. N. Rangarajan

Non-coding RNAs: Versatile roles in cell regulation

For the past four decades or so, the 'central dogma of molecular biology' has been a very powerful driving force that has led Biology to its current exciting state. The basic tenet of this dogma is that the genetic information is carried in the form of the linear base sequence of a DNA molecule which can replicate to produce identical daughter DNA molecules having the same information content and which can transcribe to produce the messenger RNA (mRNA); the mRNA in turn is translated to produce a polypeptide using the dictionary of genetic code and finally, the linear sequence of amino acids of a polypeptide determine its functions and ultimately the phenotype. Parallel, and often independent, studies established that a significant proportion of the genome of any species was present as a genetically 'inert' component in the form of heterochromatin, usually enriched in repetitive and simple DNA sequences of little transcriptional and translational potential. The riddle of heterochromatin and related issues led to formulation of the C-value paradox that most species have far excess DNA content in their genomes than required for the protein-coding functions and that closely-related species show unexplainable variations in their DNA (and thereby genetic information) content. To explain the C-value paradox and the presence of heterochromatin and/or simple repetitive DNA sequences in the genomes, notions of 'selfish', 'parasitic', 'junk' DNA, etc. were propounded. A common belief in these notions, although often not explicitly stated, was that an RNA molecule, to be relevant from the genetic information point of view, must be translated into a polypeptide and if it did not fulfil this requirement, it belonged to the 'selfish', 'parasitic' or 'junk' or any other such non-essential class. Of course the other classes of 'non-coding' RNA molecules (like the ribosomal RNAs (rRNA), the transfer RNAs (tRNA), small nuclear RNAs (snRNA), etc.) that were instrumental in processing of the precursors of the 'informational' messenger RNAs (mRNA) and their subsequent translation into polypeptides were accepted to be essential components of the genome in any organism. Such a discrimination was strengthened by the fact that in multi-cellular organisms, at least three types of RNA polymerases exist and generally the protein-coding or 'informationally relevant' mRNAs were produced by the RNA polymerase II (pol II) while the pol I and pol III transcribed those regions of DNA that were responsible for rRNAs, tRNAs, snRNAs, etc.

Discovery of reverse transcriptase that can produce a DNA copy from an RNA template necessitated the first modification in the central dogma that the information flow between DNA and RNA could be in either direction. The unanticipated process of RNA-editing that could change the sequence of an RNA molecule in a defined manner revealed novel modes of generating genetic information that did not exist in the genomic sequence. RNA molecules were also shown to have specific enzymatic activities as ribozymes. Finally, the realization that the first biological molecule may have been an RNA, established the importance of 'RNA world'.

It is interesting that in spite of the increasingly known instances of the varied roles of RNA in living systems, a general prejudice has survived among biologists against the pol II transcribed RNA molecules that do not carry open-reading frames and are thus not translatable. Although the pol II transcribed 'non-coding' small nucleolar RNA or

snRNA have gained universal acceptance due to their essential function in processing of precursor of rRNAs, it is often suspected that a pol II transcribed non-coding RNA molecule may ultimately become translatable due to alternative splicing, RNA-editing or some as yet unknown process. And if it is not, it must belong to the 'selfish' or 'parasitic' class of DNA and be of little relevance to the organism. Notwithstanding such prejudices, recent years have witnessed increasingly convincing instances of pol II transcribed RNAs that perform diverse but vital functions without having to suffer 'editing' or other modifications to conform to the conventional role of 'coding' mRNAs. (Lakhotia, S. C. *Indian J. Biochem. Biophys.*, 1996, **33**, 93–102.)

The small collection of articles in the special section on 'Non-coding RNA' in this issue highlights the varied roles of RNA molecules in processes ranging from regulation of trancriptional competence of an entire chromosome (Xist transcripts, Spusta and Goldman, **page 530**) or a cluster of adjacent imprinted genes (H19 RNA, Kanduri *et al.*, **page 539**) in mammalian cells to regulation of the intra-nuclear RNA-processing machinery (the *hsrω* gene transcripts, Lakhotia *et al.* **page 553**) and male fertility (Y-chromosome transcripts, Hennig, **page 550**) in *Drosophila*. Xu *et al.* (**page 545**) describe the *His-1* gene which is highly conserved in vertebrates and whose over-expression results in neoplastic transformation. Bhattacharya *et al.* (**page 564**) summarize the information on a number of non-coding RNAs in a parasitic amoeba while Das Gupta (**page 568**) focuses on a novel activity of the 23S rRNA in the initial folding of the nascent polypeptides as they are synthesized. *These few examples serve to bring in focus the fact that some pol II transcribed RNA molecules perform important roles in cell regulation without the necessity of their coding for a protein product.*

As is clear from the discussions in this collection of papers, there is no 'prototype mechanism' through which the different non-coding RNAs discharge their functions. Some of these RNAs have nuclear functions while others are distributed in cytoplasm also. Some bind to particular class/es of proteins while for others the targets are yet to be identified. Some non-coding RNAs function as anti-sense molecules that interfere with transcription and/or translation. RNA may function as molecular chaperone as well.

Unlike the prokaryotes, eukaryotes use only a small fraction of their genome for protein coding but extensively process the transcripts through alternative splicing, editing, etc. to generate many more messages than directly coded by the genome and, therefore, the prokaryotic and eukaryotic genomes have been designated as 'hard-wired' and 'soft-wired', respectively. (Herbert, A. and Rich, A., *Nature Genet.*, 1999, **21**, 265–269.) As a result, the same genotype in an eukaryote can generate cells with different 'ribotypes' due to the various RNA processing events. The C-value paradox and notions of 'selfish' or 'parasitic' or 'junk' DNA, etc. are primarily based on the belief that the DNA genotype controls phenotype only through the protein-coding function of the mRNA. This, however, is only partly true. It is often forgotten that besides the language of base sequence of DNA, the genetic material in eukaryotes has a higher order language of chromatin organization as well. Among a variety of factors that are already known to affect the higher order chromatin organization and consequently gene expression and 'ribotype' of a cell, RNA is one as exemplified by the inactive X-chromosome in female mammals. Since the fine-tuned 'ribotype' of a cell results in individual cell phenotype, the 'ribotype' actually is subjected to natural selection. Additionally, since the 'ribotype' can also generate new components of the genotype through reverse transcription, RNA molecules in a cell remain the prime players.

In the primeval 'RNA world', a variety of functions were carried out by RNA till, for reasons of stability, DNA was selected as the repository of genetic information. However, RNA continues, even in the present 'DNA world' of evolved life forms, to function in diverse ways, including generation of new genetic information through reverse transcription. If rRNAs, tRNAs, snRNAs, snoRNAs, ribozymes, etc. can carry out specific and complex reactions in a cell, there is, *a priori*, no reason why the pol II transcribed RNA molecules cannot do anything else than being templates for polypeptides. Since RNA, unlike DNA, is essentially a single stranded molecule, different sequences can assume different higher order structures due to intra-molecular base-pairings and, much like the polypeptides, the higher order structures would determine their specific reactivity and functions. Mutations in polypeptide chains have often generated new functions and metabolic pathways. Likewise, it is possible that mutations in the so-called 'non-coding' RNA species can also generate newer regulatory circuits and thus facilitate evolution of more complex life forms.

The volume of data on genomic sequences in different organisms is increasing very rapidly and powerful programmes are being employed to ascertain the 'coding potential' of genomes in different organisms. In view of a better appreciation of functions of RNA as RNA molecules, rather than only as templates for polypeptide production, it will be very rewarding to search the genomic sequence data bases for unusual transcription units whose products may not have coding potential. Positive action in this direction will certainly provide the much needed comprehensive insights into the complexities of eukaryotic cell. Dogmas are helpful in providing directions for searches in a defined framework but they need continued revisions and modifications so that newer directions are found. Followers of the central dogma of molecular biology need to become less dogmatic since the living world is full of diversity and surprises.

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