Possible functions of a RNA-dependent RNA polymerase in eukaryotic cells

R. Gopalakrishna

RNA-dependent RNA polymerase (RdRP) activity, although implicated in viral RNA replication, has also been observed in healthy eukaryotic cells. Recently, a gene encoding such an enzyme has been cloned from tomato leaves. Molecular analysis confirms its presence in many eukaryotic cells. Although a role for RdRP has been predicted in post-transcriptional gene silencing, some of the other possible functions that this enzyme can serve and the advantages that can accrue to eukaryotic cells that possess an active RdRP are discussed here.

REPLICATION of genetic material is one of the most important mechanisms which allows life to pervade the biological milieu. The enzyme systems required for replication of the two basic genetic molecules – DNA and RNA – are the DNA-dependent DNA polymerase (DdDP) and the RNA-dependent RNA polymerase (RdRP), respectively. Of these, the latter has primarily been implicated in viral RNA replication.

RdRP activity has been detected in many eukaryotic tissues. Initially, RNA-dependent RNA polymerase activity had been observed in healthy tissues of chinese cabbage[1], cauliflower[2], tobacco[3–5], tomato[6] and cucumber[7]. Moreover, RdRP activity[1,3,5,8] as well as the amount of the enzyme[9] were found to increase when tissues were infected with RNA viruses or viroids. Such an observation led to the conclusion that RdRP activity, observed in eukaryotic cells, may be used for replication of viral or viroid RNAs[10–12]. Later, however, it has been shown that there are actually two immunologically distinct RdRP complexes[9], one host-encoded and the other viral in origin[13]. These two enzymes also appear to be functionally different since the host-encoded activity does not appear to be involved in viral replication. Nevertheless, levels of this enzyme too were observed to increase significantly upon viral infection[9]. Other studies have shown that viral-encoded RdRP, but not the host-encoded one, is involved in viral RNA replication of cowpea mosaic virus[14] and turnip yellow mosaic virus[15]. Moreover, viroid replications have been found to occur through DNA-dependent RNA polymerase II[16–18].

There are also reports of RdRP activity in rat brain cells[19] and in rabbit reticulocyte cells[20]. A partially-purified RdRP from rabbit reticulocytes showed maximal activity when haemoglobin mRNAs were used as templates[20]. This enzyme is insensitive to actinomycin D, α-amanitin and rifampicin, showing that it is not contaminated with any DNA-dependent polymerase. It is, however, sensitive to rifamycin AF/013 which appears to be a general inhibitor for both DNA and RNA polymerases[20]. Moreover, there is evidence for the presence of negative strands corresponding to the mRNA of cytochrome oxidase III (unedited, partially edited and fully edited), cytochrome b, MURF 2, and cytochrome oxidase I in *Trypanosoma brucei*[21,22]. These molecules are synthesized even in the presence of actinomycin D; this could conceivably occur in a DNA-independent, but RNA-dependent, manner with the possible involvement of RdRP in this process[22].

Although a number of laboratories have attempted to understand the reasons for the existence of RdRP in eukaryotic cells, these attempts almost invariably proved futile until recently. The kind of discouragement that the researchers received in this endeavour is, in fact, reflected in an article by Fraenkel Conrat[11], a pioneering researcher on RNA viruses. 'Because the two groups finding such enzymes in healthy plants were composed of virologists, the consensus of the scientific community, including other virologists, was that these observations were probably due to RNA virus contamination or cryptic viruses of these plants. Actually, no consensus was needed – disregarding such findings by each individual reader of these papers suffered to make them almost nonexistent, and the authors soon abandoned this line of research or at least refrained from publishing any more on this topic.'[11]

Recently, a RdRP enzyme has been purified from tomato leaves[23], and its properties characterized *in vitro*[24]; this seems to be the only completely purified RdRP activity from an eukaryotic source. Although this enzyme is active in healthy leaves, its activity increases three-fold when tomato leaves are infected with the potato spindle tuber viroid[23]. Like the earlier-identified enzyme from rabbit reticulocytes, this enzyme is resistant to both actinomycin D and α-amanitin. It, however, appears to be unique in its ability to use both single-stranded RNA and DNA templates as substrates *in vitro* and generate complementary RNA strands from them[24].

The presence of a RdRP gene in eukaryotes has finally been validated by the successful cloning of such a gene from tomato[25]. The cloning strategy included the synthesis of degenerate primers, based on the amino acid sequence of the enzyme, followed by PCR and screening of cDNA and genomic libraries. Homologues of this gene have also been identified in other plants including petunia, *Arabidopsis*, tobacco and wheat. Moreover, DNA sequence comparison indicated the presence of sequence homologues in the yeast *Schizosaccharomyces pombe* and in the nematode *Caenorhabditis elegans*. The authors postulate a role for RdRP in a complex, not yet completely understood, process of post-transcriptional gene silencing[25,26].

Although RdRP activity had been observed in plants as well as in animal tissues for quite some time, its function has never been investigated properly. However, the purification of the enzyme and the subsequent cloning of its gene has intensified investigations in this direction. A role for RdRP has now been predicted in post-transcriptional gene silencing[26,27] and in viral resistance[28]. Strong evidence in this direction also comes from a quelling-defective (qde) mutant of *Neurospora crassa*, impaired in gene-silencing[29]. This mutant bears a mutation in a particular DNA sequence which is highly homologous to the identified tomato RdRP gene. Since RdRP is an enzyme which is basically involved in the replication of RNA, it may have many more important functions in the cells. Integrating the various lines of evidence obtained so far, I predict below some of the other possible functions that this enzyme may serve within eukaryotic cells.

Putative functions of RdRP: Some predictions

If RdRP does occur regularly in most eukaryotic cells, it is conceivable that it might have had an adaptive role during the evolutionary history of such cells. RdRP, as its name indicates, synthesizes (+)RNA from (–)RNA or vice versa and (+) or (–)RNA from (±) double stranded RNAs. Hence, the primary importance of this enzyme might lie in its ability to regulate the levels of RNA in the cell. RdRP may thus possibly be involved in a number of regulatory processes.

mRNA amplification

In many circumstances, the cell or an organism may require very high amounts of a specific protein. Usually this is achieved by several strategies like an increase in the rate of synthesis, enhanced stability, or increased translation efficiency of specific mRNAs, or even combinations of these processes. In addition, it is possible that RdRP-mediated RNA synthesis may also lead to an increase in the number of transcribable mRNA molecules, which perhaps can be designated as (I)mRNAs (Intermediary mRNAs) (Figure 1). These (I)mRNAs can now act as templates for synthesis of more translatable (+) mRNAs by RdRP and thus, more protein. Such mRNA amplification might be the function of the putative RdRP reported in rabbit reticulocytes exhibiting increased synthesis of haemoglobin[20]. Support for this hypothesis comes from the fact that this activity preferentially uses haemoglobin mRNA as template over bacteriophage RNA and poly(AG)[20]. RNA synthesis in these cells has also been shown to occur in a RNA-dependent manner than in a DNA-dependent one[20].

It might, however, be experimentally difficult to detect RdRP-mediated RNA amplification in a cell since it would be indistinguishable from other processes such as increased transcription rates or enhanced stability of the message. Pulse-chase experiments could conceivably rule out increased messenger stability, while experiments with cell-free extracts *in vitro*, in the presence of specific inhibitors for DNA-dependent RNA polymerases, might be required to distinguish between the processes of mRNA synthesis (from DNA) and its amplification (from RNA).

(I)mRNA as a surrogate gene

Under conditions when a gene i
scriptionally inactive, as occurs
is heterochromatinized or im
during specific stages of develo
or when subjected to environ
stress, (I)mRNA molecules may
surrogate genes. RdRP activity
thus ensure the continued synth
functional messenger molecules
these conditions. It is also possil
the 3′- and 5′-untranslated regior
ent in different mRNA may
regulatory sequences in con
this function. Moreover, such
could exist in the cell without c
ing with their DNA counterpa
space in the nuclear genome. Al
there is yet no direct evidence
presence of surrogate genes, on
predict that (I)mRNA molecule
be present during the quiescent
such cells or in tissues such as
seeds.

(I)mRNA as a buffering agent

In their capacity as surrogate
(I)mRNA molecules can also

Fig. 1

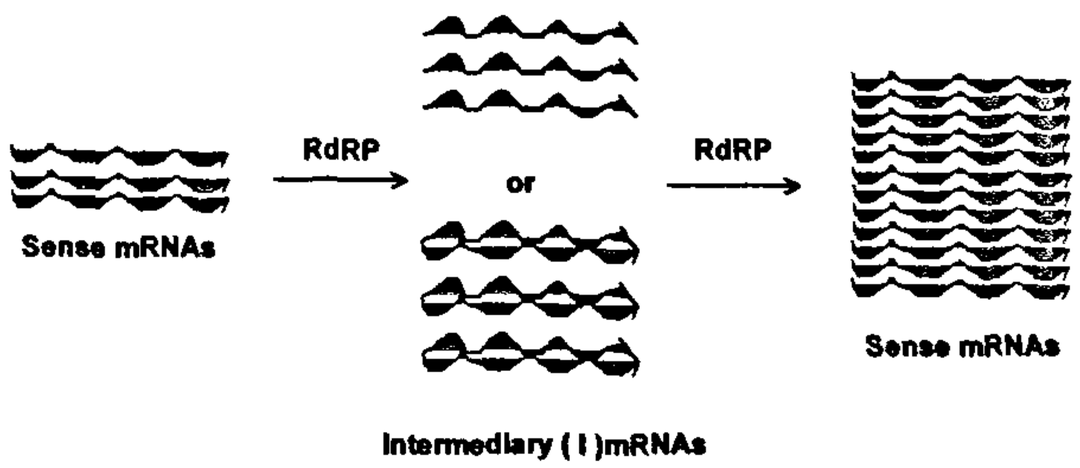

Figure 1. Amplification of sense mRNA through intermediary (I)mRNA.

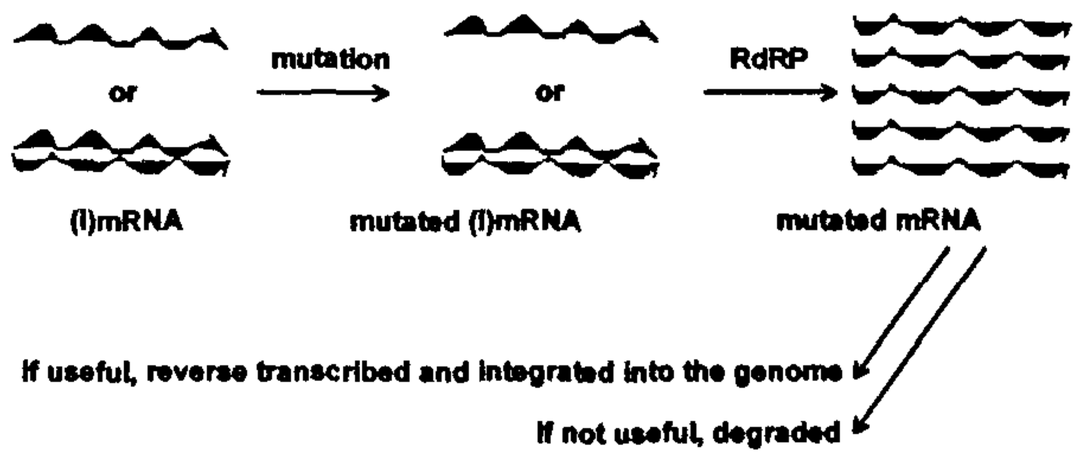

Figure 2. Buffering capacity of (I)mRNA.

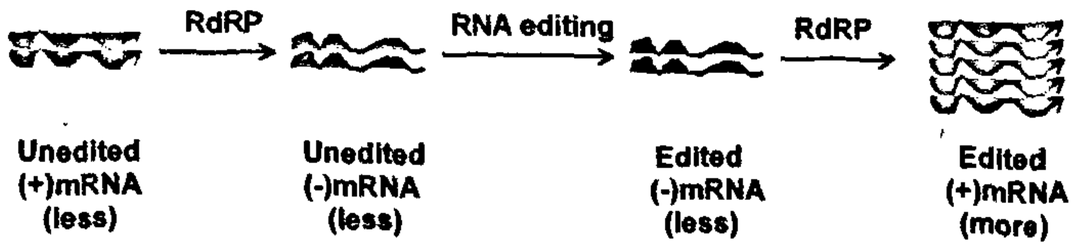

Figure 3. Involvement of RdRP in increasing efficiency of RNA editing.

buffers on which mutations (addition, deletion or changes in base sequence) can occur, resulting in corresponding changes in RdRP-synthesized translatable mRNAs and in the synthesized protein (Figure 2). If advantageous to the cell, such altered (+)mRNA molecules can even be reverse transcribed and permanently incorporated into the nuclear genome. Deleterious changes can easily be lost by the degradation of unwanted (+)mRNA molecules as well as the mutated (I)mRNAs.

One can question whether even mRNAs transcribed directly from DNA can be used for such mutations. But, in that case, a specific mutation needs to be incorporated into a large number of particular mRNA molecules to see the effect in terms of mutated protein in the cell. In contrast, a mutation on a small number of (I)mRNA molecules can lead to the synthesis of readily-translatable mutated mRNAs by RdRP, thus producing more mutated protein within the cell.

A role for RdRP in RNA editing

RdRP activity can also participate in an amplification process following the editing of RNA (Figure 3). A large number of edited (+)mRNA molecules can be produced directly by the subsequent replication of a few edited (–)mRNA products. This process would make a cell more bioenergetically efficient by allowing it to save the energy which it would have otherwise squandered by the editing of every (+)mRNA molecule produced by transcription. This is indeed supported by the documented presence of unedited, partially edited and fully edited negative mRNA strands, corresponding to cytochrome oxidase III and some other enzymes, which are synthesized in a DNA-independent manner in *Trypanosoma brucei*[21,22].

(I)mRNA as a template for synthesis of antisense RNA

It is conceivable that an increase in mRNA may have to be regulated in a cell under certain conditions. In such a situation, this excess mRNA can act as templates for synthesis of antisense mRNA by RdRP. Alternatively, RdRP can produce intermediary (I)mRNA which can then act as template for synthesis of more antisense mRNA. Antisense mRNA, thus produced, could then regulate the expression and subsequent translation of (+)mRNA according to the particular needs of the cell. In fact, it has been suggested that in transgenic tobacco plants infected with the tobacco etch virus, late-developing resistance to the virus may be due to post-transcriptional silencing of viral RNA mediated by RdRP[27,28]. It has also been suggested that RdRP may have a role in the production of small cRNA (complementary RNA) which can act as antisense molecules to remove the excess and/or aberrant RNA molecules from the cell by a specific degradation process[26].

What are these (I)mRNAs? What clues do we have?

(I)mRNAs are thus intermediary molecules hypothesized to be capable of acting as template for RdRP. Does RdRP use single-stranded or double-stranded RNA as template? In other words, what are the advantages and disadvantages of (I)mRNAs being either single-stranded or double-stranded molecules?

The only RdRP activity purified so far – from tomato – preferentially uses single-stranded RNA or DNA as template over their double-stranded counterparts to yield small RNA molecules[24]. However, one should not forget that the template for RdRP activity has only been examined in isolation, *in vitro*[24]. It is possible that the presence of different ancillary proteins *in vivo* might aid RdRP to identify its original template either as single-stranded or double-stranded molecules on the basis of their sequence or the structure arising due to such sequences. Therefore, it may be premature to rule out the possibility that RdRP might use dsRNA as templates *in vivo*. Identification of such preferential target sequences will not only help us to discover the actual templates being used *in vivo*, but also unravel the genes regulated by these intermediary RNA molecules through RdRP.

Interestingly, recent experiments with double-stranded RNA-mediated inactivation of specific genes have led to a lot of discussion[30–32]. RNAi (RNA-mediated genetic interference) has been observed in *C. elegans*[33,34], *Drosophila*[35] and *Trypanosoma*[36], where introduction of double-stranded RNA into cells leads to the inactivation of corresponding genes. The interference effect is long-lasting, sometimes even extending to the F1 generation. This has led to the conclusion that either RNAi is catalytically amplified or that it induces a long-lasting signal[33]. But since molecular analysis has indicated that it might actually occur post-transcriptionally[37], it has been predicted that some polymerases might amplify the RNAi[31]. It is possible that such dsRNA (RNAi) can act as templates for synthesis of antisense mRNA by RdRP; these can then titrate the high amounts of sense mRNA in the cell. It therefore becomes important to examine whether all dsRNA can bring about specific genetic interference, and whether dsRNAs involved in RNAi can trigger RdRP synthesis. Whether RdRP indeed has any such role in RNA-mediated genetic interference can perhaps be examined in the quelling-defective mutant (RdRP mutant) of *Neurospora crassa*[38].

These are some of the predicted functions of RdRP which would account for its presence in eukaryotic cells. As mentioned earlier, however, it is of primary importance that the presence of RdRP is first demonstrated in the different eukaryotic cells where the above-described processes need to occur. Attempts should be made to purify RdRP activities from these cells using tech-

niques that have already proved successful[23,24]. For this purpose, biochemical assay systems, which are absolutely specific for the enzyme, would also have to be developed and standardized. Finally, strategies should be designed to clone and subsequently characterize putative RdRP genes from other eukaryotic sources; one such route could employ Southern and Northern analysis with heterologous probes from RdRP genes of tomato and *Neurospora crassa*. Such investigations into the existence and functions of RdRP in eukaryotic cells, if successful, could then conceivably lead to more elaborate mechanisms for nucleic acid formation processing. Indeed, such a hypothesis, has already been proposed earlier[39].

1. Astier-Manificier, S. and Cornuet, P., *Biochim. Biophys. Acta*, 1971, **232**, 484–493.
2. Astier-Manificier, S. and Cornuet, P., *C. R. Acad. Sci.*, 1978, **D287**, 1043–1046.
3. Duda, C. T., Zaitlin, M. and Siegel, A., *Biochim. Biophys. Acta*, 1973, **319**, 62–71.
4. Duda, C. T., *Virology*, 1979, **92**, 180–189.
5. Takanami, Y. and Fraenkel-Conrat, H., *Biochemistry*, 1982, **21**, 3161–3167.
6. Boege, F. and Sänger, H. L., *FEBS Lett.*, 1981, **121**, 91–96.
7. Khan, Z. A., Hiriyanna, K. T., Chavez, F. and Fraenkel-Conrat, H., *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 2383–2386.
8. Romaine, C. P. and Zaitlin, M., *Virology*, 1978, **86**, 241–253.
9. van der Meer, J., Dorssers, L., van Kammen, A. and Zabel, P., *Virology*, 1984, **132**, 413–425.
10. Fraenkel-Conrat, H., *Trends Biochem. Sci.*, 1979, **4**, 184–186.
11. Fraenkel-Conrat, H., *Proc. Natl. Acad. Sci. USA*, 1983, **80**, 422–424.
12. Fraenkel-Conrat, H., *Crit. Rev. Plant. Sci.*, 1986, **4**, 213–216.
13. Dorssers, L., van der Meer, J., van Kammen, A. and Zabel, P., *Virology*, 1983, **125**, 155–174.
14. Dorssers, L., van der Krol, S., van der Meer, J., van Kammen, A. and Zabel, P., *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 1951–1955.
15. Candresse, T., Mouches, C. and Bove, J. M., *Virology*, 1986, **152**, 322–330.
16. Mühlbach, H.-P. and Sänger, H. L., *Nature*, 1979, **278**, 185–188.
17. Spiesnaeger, E., Mühlbach, H.-P., Tabler, M. and Sänger, H. L., *Biosci. Rep.*, 1985, **5**, 251–265.
18. Schindler, I.-M. and Mühlbach, H.-P., *Plant Sci. (Shannon)*, 1992, **84**, 221–229.
19. Mikoshiba, K., Tsukada, Y., Haruna, I. and Watanabe, I., *Nature*, 1974, **249**, 445–447.
20. Downey, K. M., Byrnes, J. J., Jurmark, J. S. and So, A. G., *Proc. Natl. Acad. Sci. USA*, 1973, **70**, 3400–3404.
21. Volloch, V., Schweitzer, B. and Rits, S., *Nature*, 1990, **343**, 482–484.
22. Volloch, V., Schweitzer, B., Zhang, X. and Rits, S., *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 10671–10675.
23. Schiebel, W., Haas, B., Marinkovic, S., Klanner, A. and Sänger, H. L., *J. Biol. Chem.*, 1993, **268**, 11851–11857.
24. Schiebel, W., Haas, B., Marinkovic, S., Klanner, A. and Sänger, H. L., *J. Biol. Chem.*, 1993, **268**, 11858–11867.
25. Schiebel, W., Pélissier, T., Riedel, L., Thalmeir, S., Schiebel, R., Kempe, D., Lottspeich, F., Sänger, H. L. and Wassenegger, M., *Plant Cell*, 1998, **10**, 2087–2101.
26. Wasenegger, M. and Pélissier, T., *Plant Mol. Biol.*, 1998, **37**, 349–362.
27. Flavell, R. B., *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 3490–3496.
28. Lindbo, J. A., Silva-Rosales, L., Proebesting, W. M. and Dougherty, W. G., *Plant Cell*, 1993, **5**, 1749–1759.
29. Cogoni, C. and Macino, G., *Nature*, 1999, **399**, 166–169.
30. Montgomery, M. K. and Fire, A., *Trends Genet.*, 1998, **14**, 255–258.
31. Sharp, P. A., *Genes Dev.*, 1999, **13**, 139–141.
32. Hunter, P. C., *Curr. Biol.*, 1999, **7**, R440–R442.
33. Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E. and Mello, C. C., *Nature*, 1998, **391**, 806–811.
34. Timmons, L. and Fire, A., *Nature*, 1998, **395**, 854.
35. Kennerdell, J. R. and Carthew, R. W., *Cell*, 1998, **95**, 1017–1026.
36. Ngo, H., Tschudi, C., Gull, K. and Ullu, E., *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 14687–14692.
37. Montgomery, M. K., Xu, S. and Fire, A., *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 15502–15507.
38. Cogoni, C. and Macino, G., *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 10233–10238.
39. Ikegami, M. and Fraenkel-Conrat, H., *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 2122–2124.

ACKNOWLEDGEMENT. I thank Anindya Sinha and Kakoli Mukhopadhyay for encouraging and helping me to write this paper, and Amitabha Chaudhuri, Niranjan V. Joshi, R. Uma Shaanker and Mitradas M. Panicker for critical comments on the original version of this paper. I thank CSIR for partial funding.

R. Gopalakrishna is in the Department of Crop Physiology, University of Agricultural Sciences, GKVK Campus, Bangalore 560 065, India.