Volume 268, number 2, 427-430 FEBS 08632 August 1990

Minireview

Regulation of plant gene expression by antisense RNA

J.N.M. Mol, A.R. van der Krol, A.J. van Tunen, R. van Blokland, P. de Lange and A.R. Stuitje

Department of Genetics, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

Received 14 May 1990

Regulation of gene expression by antisense RNA was first discovered as a naturally-occurring phenomenon in bacteria. Recently natural antisense RNAs have been found in a variety of eukaryotic organisms; their in vivo function is, however, obscure. Deliberate expression of antisense RNA in animal and plant systems has lead to successful down-regulation of specific genes. We will review the current status of antisense gene action in plant systems. The recent discovery that 'sense' genes are able to mimic the action of antisense genes indicates that (anti)sense genes must operate by mechanisms other than RNA-RNA interaction.

Antisense RNA; Sense RNA; Co-suppression; Gene interaction; Gene silencing

1. INTRODUCTION

The antisense technology is based on blocking the information flow from DNA via RNA to protein by the introduction of an RNA strand complementary to (part of) the sequence of the target mRNA. This so-called antisense RNA is thought to basepair to its target mRNA thereby forming double-stranded RNA. Duplex formation may impair mRNA maturation and/or translation or alternatively may lead to rapid mRNA degradation. In any event the result mimicks a mutation.

Izant and Weintraub [1] were the first to demonstrate the effectiveness of antisense gene constructs in eukaryotic cells. Numerous reports have appeared since then on the effective down-regulation of genes either transiently, using cloned DNA, RNA or oligonucleotides or by stable transformation using antisense DNA (for reviews, consult [2-4]). The antisense approach in eukaryotes has evolved now from a model system to an approach well integrated in the field of molecular and applied genetics.

Plants were the first multicellular organisms in which endogenous genes were successfully down-regulated by antisense counterparts. This was made possible by their unique capacity to regenerate from a single cell. We will review our current knowledge about plant gene regulation by antisense DNA/RNA and speculate on possible mechanisms of action in view of the recent discovery [5,6] that 'sense' versions of antisense genes sometimes down-regulate homologous gene expression.

Correspondence address: J.N.M. Mol, Vrije Universiteit, Dept of Genetics, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

2. NATURAL ANTISENSE RNA

Antisense regulation was first discovered in naturally occurring bacterial systems (for review, see [7]). Eukaryotic cells contain RNAs which are complementary to portions of known mRNAs (reviewed in [2]). Recently more examples have appeared in the literature. Kapler and Beverly [8] report the presence of RNAs complementary to the dihydrofolate reductase-thymidylate synthase region of the protozoan parasite Leishmania. RNAs complementary to the myelin basic protein gene in mouse have been reported by Tosic et al. [9]. Murine erythroleukemia cells accumulate an antisense RNA involved in the maturation of the transformation-associated protein p.53 [10]. For plant systems only circumstantial evidence is available for the presence of naturally-occurring antisense RNAs.

3. BACTERIAL ANTISENSE AND SENSE GENES IN PLANTS

The first report on artificial antisense regulation of gene expression in plants came from Ecker and Davis [11]. They reported effective transient inhibition of chloramphenicol acetyl transferase (CAT) activity in carrot cells by co-introduction of sense and antisense cat genes in protoplasts. By double transformation of tobacco plants with nos and antisense nos genes, NOS activity can be modulated [12]. More recently other bacterial genes such as bar [13] and gus [14] have been successfully down-regulated by antisense techniques. Table I gives a summary of the state of the art.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

Table 1

Successful inhibition of plant gene expression by antisense RNA

Gene encoding	Reference	Comment
Chloramphenicol acetyl transferase	[11] Ecker and Davis (1986) [26] Delaunay et al. (1988)	Transient expression Double transformation
Nopaline synthase	[12] Rothstein et al. (1987) [27] Sandler et al. (1988)	Double transformation Double transformation
Phosphinotricin acetyl transferase	[13] Cornelissen and Vandewiele (1989)	Confers resistance to bialaphos
β-Glucuronidase	[14] Robert et al. (1989)	
Chalcone synthase	[15] Van der Krol et al. (1988)	Role in flower pigmentation
Polygalacturonase	[18,19] Smith et al. (1988, 1990) [20] Sheehy et al. (1988)	Role in fruit softening
Ribulose bisphosphate carboxylase (SSU)	[21] Rodermel et al. (1988)	Role in CO_2 fixation and photorespiration
Peroxidase	Rothstein, unpublished	Role in lignin formation and wound healing
Cinnamyl alcohol dehydrogenase	[23] Schuch et al. (1990)	Role in lignin formation
Granule-bound starch synthase	[22] Visser et al. (1990)	
Potato virus coat protein	[25] Hemenway et al. (1988)	
Cucumber mosaic virus coat protein	[24] Cuozzo et al. (1988)	

4. PLANT ANTISENSE AND SENSE GENES IN PLANTS

The first authentic plant gene successfully down-regulated by antisense technology was the gene for chalcone synthase (chs) encoding the key enzyme of flavonoid biosynthesis [15]. Since the substrates of CHS are colorless, an evenly-reduced pigmentation of the corolla was obtained in independent transformants, as expected. The amount of pigmentation correlates with the level of residual chs mRNA [16]. Moreover, the effect is specific of the chs mRNA; chi and dfr mRNA levels are unaltered. Unexpectedly, pigmentation patterns were obtained in rings and sectors that showed variation with light and hormone (GA_3) regime [15,16]. Some of the antisense pigmentation phenotypes are shown in Fig. 1. Due to the conserved nature of chs genes, the antisense gene from petunia also works in other solanaceae such as tobacco and potato.

The different phenotypes observed in independent transformants are attributed to position effects of the antisense chs gene on its own expression. The effects of different promoters, antisense gene fragments and chromosomal location have been described [17]. An important conclusion is that subgenomic fragments of antisense genes can be ineffective in establishing a phenotype. This may be due to decreased stability of corresponding mRNAs.

Attempts to enhance coloration by introduction of sense chs or sense dfr genes in petunia had an inverse effect [5,6]. Antisense-like effects were observed ranging in phenotype from patterns to fully white (cf. Fig. 1). Transcript analysis showed that transcript levels of both the endogenous and that of the transgene were specifically reduced (*co-suppression*). The two halves of chs cDNA are equally effective in establishing a sense effect (R. van Blokland, unpublished data). This indicates that the protein product encoded by the transgene is not a prerequisite. Whether transcription of the transgenes is necessary is under investigation.

Inactivation of chi mRNA should lead to accumulation of yellow chalcones. However, attempts to down-regulate chi gene expression by antisense or sense have failed so far (A. van Tunen, unpublished data). Possibly specific sequences or structures are required to get sense or antisense effects.

Polygalacturonase (PG) plays an important role in fruit ripening. Smith et al. [18,19] and Sheehy et al. [20] have observed a dramatic reduction of pg mRNA and PG protein levels after introduction of an antisense pg gene in tomato. This could lead to increased shelf life.

Even the most abundant protein present in plants can be reduced effectively using antisense techniques (Rubisco ss; [21]). Amylose-free potatoes were obtained by Visser et al. [22] by introduction of an antisense granule-bound starch synthase (gbss) gene. Cinnamyl alcohol dehydrogenase (cad) is involved in lignin formation. Schuch et al. [23] have shown that antisense cad genes completely abolish lignin synthesis.

Attempts to reduce plant RNA virus-specific mRNA

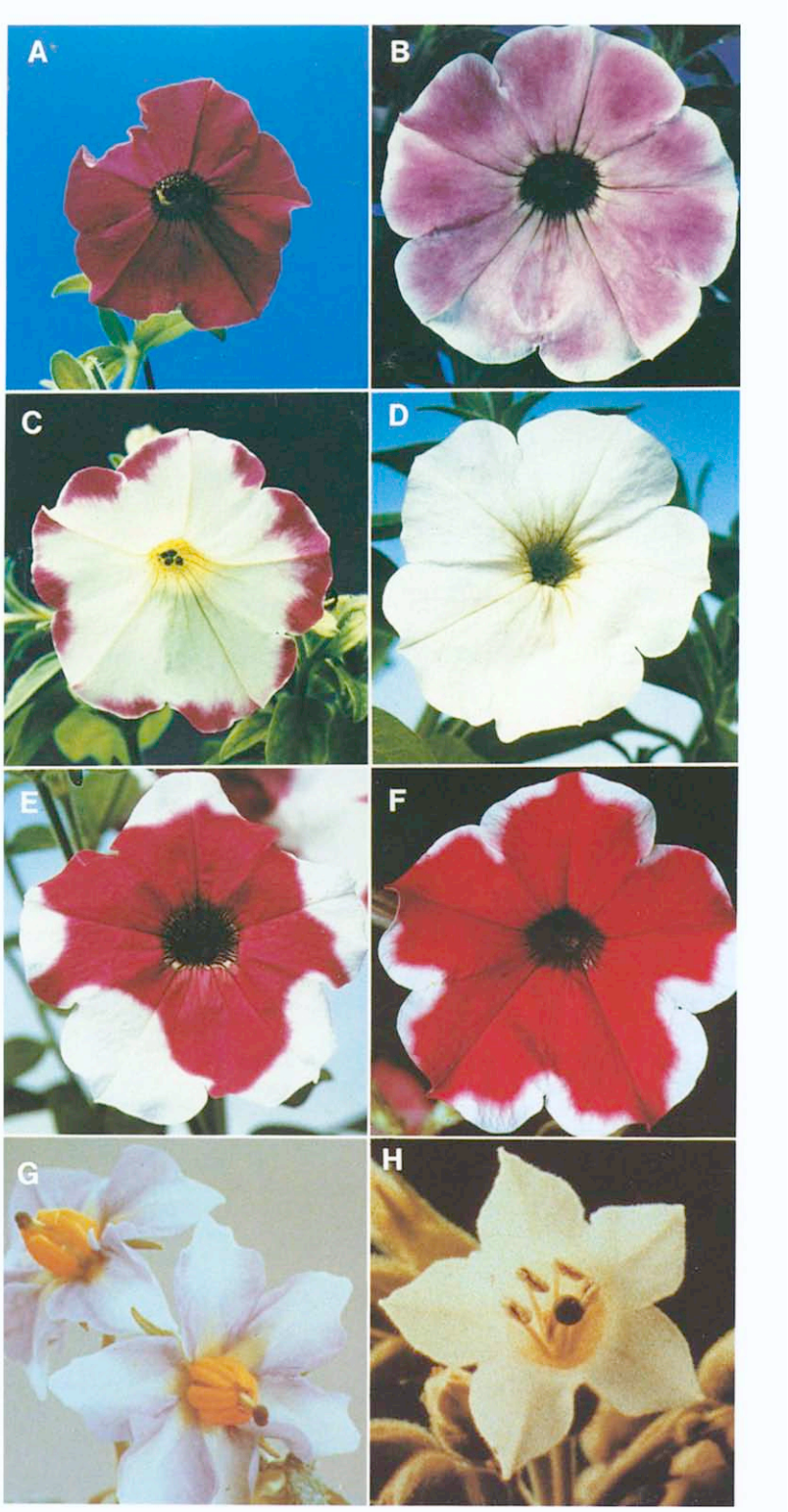

Fig. 1. Different flower phenotypes of petunia, potato and tobacco transgenic plants. (A) Control VR flower. (B–D) Flowers from petunia plants transformed with 'antisense' chs gene. (E) Flower from petunia plant transformed with 'sense' chs gene. (F) Flower from petunia plant transformed with 'sense' dfr gene. (G) Flower from potato plant transformed with 'antisense' petunia chs gene (courtesy Dr R. Visser, Dr E. Jacobsen). (H) Flower from tobacco plant carrying 'antisense' petunia chs gene.

levels (e.g. spec. coat protein) by introduction of antisense genes were rather unsuccessful [24,25]. Only at relatively low inoculum concentration was some degree of protection observed. The ineffectiveness of this system is probably associated with the cytoplasmic life cycle of these RNA viruses (PVX, CMV). The DNA

containing Gemini viruses on the other hand can be efficiently inhibited by an antisense viral gene (A1-1; C. Lichtenstein, unpublished data). Table I gives a complete summary of the data presented.

5. ON THE MECHANISM OF ANTISENSE ACTION

Evidence has accumulated over the past years that in prokaryotes antisense RNA forms double-stranded complexes with mRNA, thereby preventing translation. Model experiments in eukaryotes have indicated that duplex RNA can be formed in vivo and that such structures are poorly processed and translated (see [3]). In *Xenopus* oocytes a double-stranded RNA unwinding activity has been detected, the significance of which is still unclear.

Several lines of evidence suggest that the formation of double-stranded RNA cannot fully account for the phenotypic effects observed in the flower pigmentation system of petunia. First, neither duplex chs RNA nor free antisense RNA could be detected in floral tissue of independent transformants [16]. Second, antisense chs cDNA driven by weak promoters can be very effective. Combined with the observation that antisense chs RNA is less stable than chs mRNA [17] we conclude that substoichiometric amounts of antisense RNA are very effective. Third, addition of chs or dfr sense genes leads to antisense-like effects [5,6]. In this case RNA-RNA interaction is unlikely to occur. At present we cannot discriminate in the latter case between RNA-DNA and DNA-DNA interaction. It can be envisaged that such an interaction may trigger a mechanism to silence the interacting gene(s), e.g. by base methylation. (For an extensive discussion of gene silencing mechanisms, see [5,6]).

Irrespective of the mechanism of action of sense and antisense genes in eukaryotes our experiments with the chi gene and chs gene fragments indicate that specific sequence elements must exist that mediate the silencing. It is encouraging that sense effects are not only seen in the flavonoid pathway, but also in the starch biosynthetic pathway [22].

6. CONCLUSIONS AND PROSPECTS

Sense and antisense nucleic acids are useful tools to modulate the expression of specific genes. The technique enables one to shut off the expression of entire multigene families and could be very useful to 'probe' kryptic genes, e.g. genes that are differentially expressed in a temporal and/or spatial way. The mechanism of action of sense and antisense genes is of interest. Our data suggest that there may be similarities in the way they exert their effect. Future work will concentrate on the possible involvement of RNA-DNA and/or DNA-DNA interactions in the gene silencing.

Acknowledgement: The authors are indebted to Mrs Hansje Bartelson for typing the manuscript.

REFERENCES

[1] Izant, J.G. and Weintraub, H. (1984) Cell 36, 1007-1015.
[2] Van der Krol, A.R., Mol, J.N.M. and Stuitje, A.R. (1988) Gene, 72, 45-50.
[3] Van der Krol, A.R., Mol, J.N.M. and Stuitje, A.R. (1988) Biotechniques 6, 958-976.
[4] Weintraub, H.M. (1990) Antisense RNA and DNA. Sci-Am. 1, 34-40.
[5] Van der Krol, A.R., Mur, L.A., Beld, M., Mol, J.N.M. and Stuitje, A.R. (1990) Plant Cell 2, 291-299.
[6] Napoli, C., Lemieux, C. and Jorgensen, R. (1990) Plant Cell 2, 279-289.
[7] Simons, R.W. (1988) Gene 72, 35-44.
[8] Kapler, G.M. and Beverly, S.M. (1989) Mol. Cell. Biol. 9, 3959-3972.
[9] Tosic, M., Roach, A., De Rivaz, J.C., Dolivo, M. and Matthieu, J.M. (1990) EMBO J. 9, 401-406.
[10] Khochbin, S. and Lawrence, J.-J. (1989) EMBO J. 8, 4107-4114.
[11] Ecker, J.R. and Davis, R.W. (1986) Proc. Natl. Acad. Sci. USA 83, 5372-5376.
[12] Rothstein, S.J., DiMaio, J., Strand, M. and Rice, D. (1987) Proc. Natl. Acad. Sci. USA 84, 8439-8443.
[13] Cornelissen, M. and Vanderwiele, M. (1989) Nucleic Acids Res. 17, 833-843.
[14] Robert, L.S., Donaldson, P.A., Ladaigue, C., Altosaar, I., Arnison, P.G. and Fabijanski, S.F. (1989) Plant Mol. Biol. 13, 399-409.
[15] Van der Krol, A.R., Lenting, P.E., Veenstra, J., Van der Meer, I.M., Koes, R.E., Gerats, A.G.M., Mol, J.N.M. and Stuitje, A.R. (1988) Nature 333, 866-869.
[16] Van der Krol, A.R., Mur, L.A., De Lange, P., Gerats, A.G.M., Mol, J.N.M. and Stuitje, A.R. (1990) Mol. Gen. Genet. 220, 204-212.
[17] Van der Krol, A.R., Mur, L.A., De Lange, P., Mol, J.N.M. and Stuitje, A.R. (1990) Plant Mol. Biol. 14, 457-466.
[18] Smith, C.J.S., Watson, C.F., Ray, J., Bird, C.R., Morris, P.C., Schuch, W. and Grierson, D. (1988) Nature 334, 724-726.
[19] Smith, C.J.S., Watson, C.F., Morris, P.C., Bird, C.R., Seymour, G.B., Gray, J.E., Arnold, C., Tucker, G.A., Schuch, W., Harding, S. and Grierson, D. (1990) Plant Mol. Biol. 14, 369-379.
[20] Sheehy, R., Kramer, M. and Hiatt, W.R. (1988) Proc. Natl. Acad. Sci. USA 85, 8805-8809.
[21] Rodermel, S.R., Abbott, M.S. and Bogorad, L. (1988) Cell 55, 673-681.
[22] Visser, R.G.F., Feenstra, W.J. and Jacobsen, E. (1990) in: Applications of Antisense Nucleic Acids and Proteins (Mol, J.N.M. and Van der Krol, A.R. eds) Marcel Dekker, New York (in press).
[23] Schuch, W., Knight, M., Bird, A., Frima-Pettenati, J. and Boudet, A. (1990) (in press).
[24] Cuozzo, M., O'Connel, K.M., Kaniewski, W., Fang, R.-X., Chua, N.-H. and Turner, N.E. (1988) Bio/Technology 6, 549-557.
[25] Hemenway, C., Fang, R.-X., Kaniewski, W.K., Chua, N.-H. and Tumer, N.E. (1988) EMBO J. 7, 1273-1280.
[26] Delauney, A.J., Tabaeizadeh, A. and Verma, D.P.S. (1988) Proc. Natl. Acad. Sci. USA 85, 4300-4304.
[27] Sandler, S.J., Stayton, M., Townsend, J.A., Ralston, M.L., Bedbrook, J.R. and Dunsmuir, P. (1988) Plant Mol. Biol. 11, 301-310.