Reply to Jack Heinemann on the issue silencing due to homologous promoters

In my initial comment I have questioned the statement “Having the same or similar promoters may lead to gene silencing…” in the draft outline and asked for an example.

Jack Heinemann has responded as follows:
“It is a well-documented phenomenon that, for example, when two genes run by homologous promoters come to be together in the same plant cell, both genes may be silenced (De Schrijver, A. et al., 2007). This can result in inadvertent silencing of agronomic traits. “The cauliflower mosaic virus 35S (35S) promoter has been extensively used for the constitutive expression of transgenes in dicotyledonous plants. The repetitive use of the same promoter is known to induce transgene inactivation due to promoter homology” (p. 988 Bhullar, S. et al., 2003). In an elegant study, Al-Kaff et al. showed directly that infection of susceptible plants with the cauliflower mosaic virus (the source of the 35S promoter) can cause silencing of a herbicide tolerance transgene with a 35S promoter (Al-Kaff, N. S. et al., 2000).

Al-Kaff, N. S., Kreike, M. M., Covey, S. N., Pitcher, R., Page, A. M. & Dale, P. J. (2000). Plants rendered herbicide-susceptible by cauliflower mosaic virus-elicited suppression of a 35S promoter-regulated transgene. Nat. Biotechnol. 18, 995-999.

De Schrijver, A., Devos, Y., Van den Bulcke, M., Cadot, P., De Loose, M., Reheul, D. & Sneyers, M. (2007). Risk assessment of GM stacked events obtained from crosses between GM events. Trends Food Sci Technol 18, 101-109.“

I have checked the three literature references and came to the conclusion that my initial comment is still valid.

A. De Schrijver at al. (2007) is a Viewpoint Article not a Research Article with the view expressed:
In addition, one should take into consideration that in the case of GM stacked events, the combined presence of transgenes might influence expression. For example, gene silencing that involves transgene/transgene interactions might occur in case homologous DNA sequences, e.g. expression controlling elements, are brought together (Fagard & Vaucheret, 2000).

The article of **M. Fagard and H. Vaucheret (2000)** cited is a Review Article referring to a Research Article of **Mette, M.E., von den Winden, J., Matzke, M.A., Matzke, A.J. (1999)**.

Production of aberrant promoter transcripts contributes to methylation and silencing of unlinked homologous promoters in trans. EMBO J. 18: 241-248

Citations from that article:

To test the hypothesis that transcriptional silencing and methylation of target gene promoters could result from a trans-acting promoter RNA, we constructed a chimeric gene consisting of a nopaline synthase promoter (NOSpro) under the control of a cauliflower mosaic virus (CaMV) 35S promoter (35Spro) and used it to transform plants expressing an unmethylated NOSpro-nptII target gene. Transformed plants were analyzed for NOSpro RNA synthesis, and for activity and methylation of the NOSpronptII gene. Although production of a full-length, polyadenylated NOSpro RNA did not lead to inactivation or methylation of the target locus, the synthesis of nonpolyadenylated NOSpro transcripts from a 35Spro-NOSpro inverted repeat (IR) was associated with silencing and methylation of the NOSpro-nptII target gene. This suggests that aberrant promoter RNAs can mediate the methylation of unlinked homologous promoters in trans.

Here, we have tested whether a transcriptional trans-silencing locus can act by producing transcripts of promoter sequences. Full-size, polyadenylated NOSpro RNA, even when abundant, did not induce trans-silencing or methylation of the NOSpro-nptII target gene. Silencing and promoter methylation of NOSpro-driven target genes was observed, however, when non-polyadenylated NOSpro RNAs that deviated from the expected size were synthesized from an IR comprising 35Spro-NOSpro sequences. Decreasing NOSpro transcription by repressing the 35Spro partially alleviated silencing and reduced the methylation of the NOSpro-nptII target gene, indicating a role for aberrant NOSpro RNA in this trans-silencing phenomenon.

Conclusion:

Full-length transcript of the *nos* promoter (transcribed via the 35S promoter) did not lead to silencing of an other ***nos*** in *trans*. In one out of 9 transformants a rearrangement of the transforming DNA hat occurred during the process of transformation, resulting in inverted repeat structure of two elements both carrying the 35S promoter and the *nos* promoter, whereas the terminator sequenced was deleted. This transformant produced an aberrant *nos* promoter transcript capable of silencing a *nos* gene in *trans*. Silencing was mediated by an aberrant RNA and not by the homolog as such of the two copies of the *nos* promoter present.

S. Bhullar et al. (2003) mention in there introduction that *homology-based silencing has been reported to occur extensively in transgenic plants* and refer mainly to a second review

(**Vaucheret H. and Fagard M, (2001)**, transcriptional gene silencing in plant: targets, inducers and regulators, Trends Genet 17: 29-35).
This review refers to **Mette M.F. et al. (2001)**, Transcriptional silencing and promoter methylation triggerd double stranded RNA. EMBO J. 19: 5194-5201.
With regard to promoter homology this article refers back to **Mette et al. (1999)** as mentioned above.

Al-Kaff et al. (2000) report a host defense response to CaMV infection that leads also to silencing of a recombinant gene under the control of the CaMV 35S promoter.
Citation:
Our experiments have shown that virus infection can lead to loss of herbicide tolerance in transgenic plants expressing the BAR gene. This effect is a consequence of a host defense response to CaMV infection causing inactivation of virus replication by gene silencing leading to silencing of a transgene homologous to the invading virus. From analysis of different transgenic lines, we have found no evidence that transgene position effects or copy number play a role in the virus-elicited suppression of BAR, NPT II, or GUS transgenes reported here or previously. Gene silencing in cruciferous hosts of CaMV can affect viral functions at the transcriptional and posttranscriptional levels, with a concomitant effect on transgenes sharing promoter or RNA homology with the virus.

Overall conclusion:
All these articles do not indicate that silencing may occur only on the basis of homology of promoters. However, I am aware that in the early days of gene silencing research sequence homology mediating direct DNA-DNA contact was discussed as a possible step that may lead to gene silencing