Volume 243, number 2, 323–327 FEB 06743 January 1989

Changes in DNA methylation are associated with loss of insecticide resistance in the peach-potato aphid *Myzus persicae* (Sulz.)

Linda M. Field, Alan L. Devonshire, Richard H. Ffrench-Constant and Brian G. Forde

AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden AL5 2JQ, England

Received 11 November 1988

Insecticide resistance in peach-potato aphids arises from the overproduction of one of two very closely related esterases (E4 or FE4) that detoxify insecticidal esters, and this is associated with amplification of E4-related DNA sequences. Some highly resistant aphid clones can spontaneously lose their elevated esterase and hence their resistant phenotype. We report here that such aphids also lose their elevated E4 mRNA whilst retaining their amplified genomic sequences. We have also shown that the amplified E4-related sequences are highly methylated at *Msp*I sites in all resistant aphid clones examined, but not in those that have lost resistance.

Insecticide resistance; Gene amplification; DNA methylation; Esterase; (*Myzus persicae*)

1. INTRODUCTION

Insecticide resistance in the peach-potato aphid, *Myzus persicae* (Sulz.), is caused by the increased synthesis of an esterase (E4 or FE4) that both sequesters and hydrolyses insecticidal esters [1], and this in turn results from increased levels of translatable esterase mRNA [2]. Furthermore, an E4 cDNA clone has been used to show that resistance level is correlated with the degree of amplification of E4-related sequences in the aphid genome [3]. Increased esterase (and consequently resistance) can be lost in the absence of insecticide selection [4,5], but this reversion has only been observed in some of the aphid clones with a specific A1,3 chromosomal translocation [6]. Translocated aphids overproduce the esterase E4 [5], whereas resistant aphids of normal karyotype contain the closely related esterase, FE4 [7] and have stable resistance [5]. Unlike susceptible aphids, revertants retain their translocation and can be re-selected for resistance by exposure to insecticide [5].

Here, we report the use of the E4 cDNA [3] to investigate changes in esterase mRNA and DNA associated with reversion and re-selection of insecticide-resistant aphids.

Correspondence address: L.M. Field, AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts, AL5 2JQ, England

2. MATERIALS AND METHODS

2.1. *Aphid clones*

The resistant aphid clones examined were R_1 and FerR, which have a normal karyotye and 4- [8] and 16-fold [7] increases in esterase, and R_3, which has the A1,3 chromosomal translocation and a 64-fold increase in esterase [7]. The revertant (RevA) was established as a subclone of an extremely resistant aphid clone; it retained its chromosomal translocation but had esterase levels similar to the susceptible clone US1L [5]. RevAd, a subclone of RevA that had been re-selected for moderate resistance by exposure to insecticide, had E4 at the same level as FE4 in R_1 [5].

2.2. *Esterase cDNA*

The isolation and identification of the E4 cDNA clone, pMp24, have been described [3].

2.3. *RNA and DNA dot blots*

Total aphid RNA was prepared by extraction with guanidinium thiocyanate and ultracentrifugation through CsCl [9], and the polyadenylated fraction was isolated by oligo(dT)-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

cellulose chromatography [2]. This poly(A^+) RNA was serially diluted in 2 × SSC (SSC: 150 mM NaCl, 15 mM trisodium citrate), boiled for 1 min, snap frozen, thawed and loaded onto a nylon membrane (Biodyne A) using a BRL Hybridot^R apparatus.

Aphid DNA was prepared as in [3] and sheared by 3 passes through an 18-gauge needle. It was then serially diluted, and each adjusted to 32 μg DNA/ml with sheared non-homologous DNA (herring sperm). Samples (250 μl) were boiled for 1 min, cooled on ice and adjusted to 2 × SSC before loading onto a membrane as above. The membrane was treated with 0.5 M NaOH, 1.5 M NaCl (2 × 5 min) to denature the DNA and then with sodium acetate, pH 5.5 (2 × 5 min).

Membranes were baked at 80°C for 2 h and then pre-hybridized at 42°C for 2 h in 50% formamide, 5 × SSC, 50 mM Na phosphate (pH 6.5), 0.1% SDS, 250 μg/ml herring sperm DNA, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin. Hybridization was at 42°C for 16 h in the same solution containing 50 ng/ml pMp24 which had been ^{32}P-labelled by nick translation. Membranes were washed in 2 × SSC, 0.1% SDS at 65°C and autoradiographed at −80° using intensifying screens, and Kodak X-Omat S film, pre-flashed to improve its linearity of response [10].

2.4. *Southern blots*

Total aphid DNA (10 μg) [3] was digested (2 h at 37°C) with *Msp*I or *Hpa*II (50 U) in the buffers supplied, electrophoresed in 0.8% agarose gels (in TAE buffer) denatured, neutralized and capillary-blotted [9] onto a nylon membrane (Biodyne A). The membrane was processed for hybridization to pMp24, washed and autoradiographed as for the dot blots.

3. RESULTS AND DISCUSSION

Dot blots of poly(A^+) RNA from susceptible (S) and R_3 aphids probed with E4 cDNA (fig.1A) showed a > 32-fold difference in E4 RNA level that is in agreement with the overproduction (approx. 64-fold) of E4 protein by R_3 aphids [8]. The revertant aphid clone, RevA, which has an E4 protein content similar to S [5], also has very low E4 RNA, approx. 32-fold less than R_3 (fig.1B). The slightly higher E4 mRNA content in RevA compared to S probably reflects the intraclonal variation in E4 content that is typical of revertant clones and on which insecticidal selection acts [5]. Also shown in fig.1B is hybridization of pMp24 to RNA from the subclone of RevA (RevAd) that had been reselected for partial recovery of resistance by exposure to insecticide; the 4-fold increase in abundance of E4 RNA, compared with RevA, is in accord with their relative esterase levels [5].

To establish whether the lower E4 RNA abundance in RevA is correlated with loss of E4-related amplified sequences, we probed dot blots of total

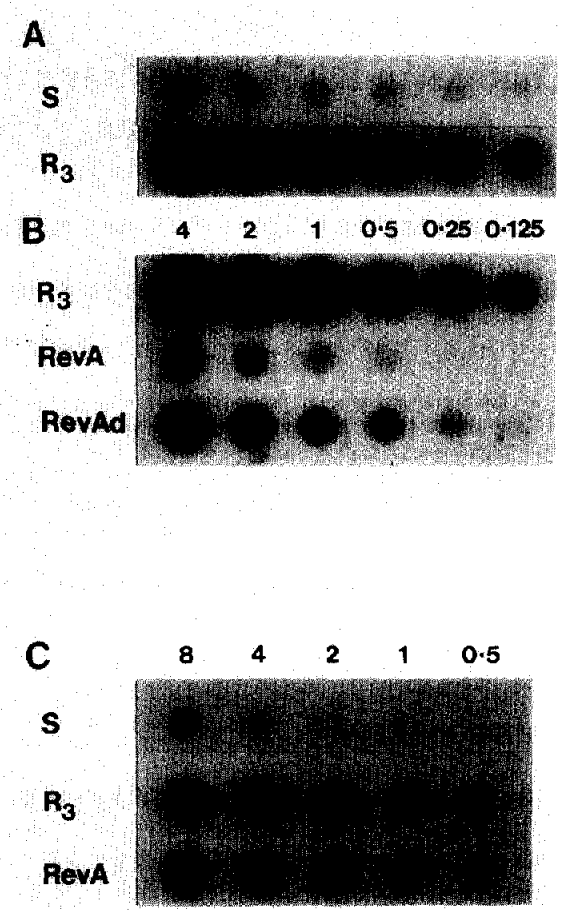
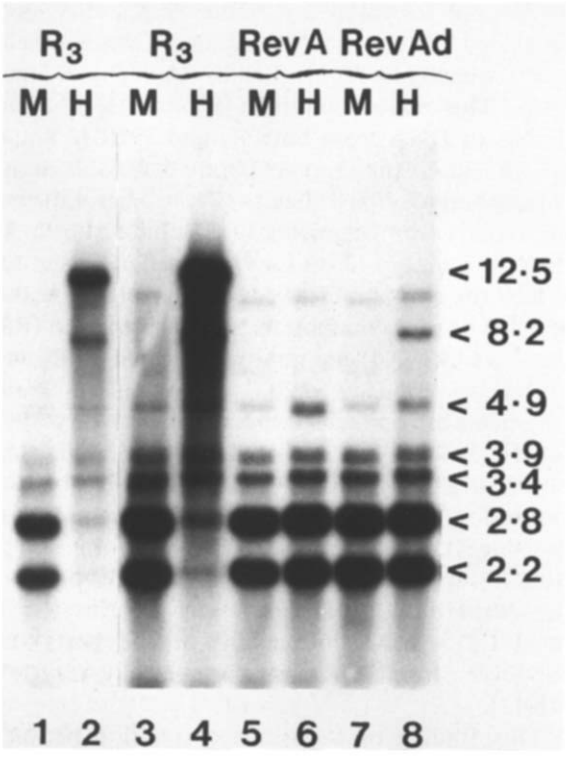


Fig.1. Hybridization of E4 cDNA (pMp24) to (A,B) poly(A^+) RNA and (C) DNA from susceptible (S), extremely resistant (R_3), revertant (RevA) and reselected revertant (RevAd) aphids.

aphid DNA from S, R_3 and RevA with pMp24 (fig.1C). The intensity of hybridization to RevA was the same as to R_3, demonstrating that there is no change in copy number associated with reversion. This retention of amplified esterase sequences in revertant aphids was confirmed with four other independently isolated revertant clones (not shown).

In mammalian cell cultures it is well established that gene amplification is responsible for resistance to cytotoxic drugs. In general, such resistance is stable when the amplified genes are integrated into the chromosome and unstable when they occur on extrachromosomal elements which do not segregate equally and can therefore be lost during


R3
R3
RevA
RevAd
M H M H M H M H
< 12·5
< 8·2
< 4·9
< 3·9
< 3·4
< 2·8
< 2·2
1 2 3 4 5 6 7 8

of normal karyotype, R_1 and FerR, also have amplified E4-related DNA sequences which are cut less frequently by *Hpa*II than by *Msp*I (fig.3, lanes 3–6). The major amplified fragments in *Msp*I digests of DNA from both R_1 and FerR (2.8 and 1.8 kb; fig.3, lanes 3,5) are barely detectable in the corresponding *Hpa*II digests (fig.3, lanes 4,6), being replaced by fragments of 17 and 12 kb in R_1 (lane 4) and 17, 12 and 4.7 kb in FerR (lane 6). Whilst the non-amplified *Msp*I fragments (3.4, 3.9 and 4.9 kb) are common to both translocated (R3, RevA and RevAd) and non-translocated (S, R_1 and FerR) aphids (figs 2,3), the amplified *Msp*I fragments are quite distinct according to karyotype (cf. fig.3, lanes 3,5, with fig.2, lane 1). This supports earlier evidence (with *Eco*RI digests) that the restriction pattern of amplified E4-related DNA sequences correlated with karyotype [3]. In susceptible (S) aphids there were no differences between the *Msp*I and *Hpa*II restriction patterns (fig.3, lanes 1,2) which is in line with other reports that the level of 5mC in insects is normally very low [16,17].

This finding of a positive correlation between methylation of amplified E4-related sequences and esterase gene expression is in direct contrast to most other studies, which link increased methylation with suppression of gene activity [13–15]. However, there is a further exception to this generalization; melanoma cell lines express a major histocompatibility gene only when a particular *Msp*I site is methylated [18]. There are also examples of hypermethylated genes being transcribed [19,20], and of other hypomethylated genes being inactive [21,22].

Most of the present knowledge of gene amplification and the role of DNA methylation in gene expression is based on studies with cell cultures; the present work extends this to an intact higher organism, which nevertheless has considerable analogy with cell cultures in view of its parthenogenetic mode of reproduction. Because of the variegated way in which aphids lose resistance we have not studied changes in methylation during reversion; nor have we shown that the methylated CpGs are located within the esterase genes. It is therefore premature to speculate on the role of methylation and demethylation in the acquisition and loss of insecticide resistance. However, *M. persicae* clearly is able to methylate CpG sequences

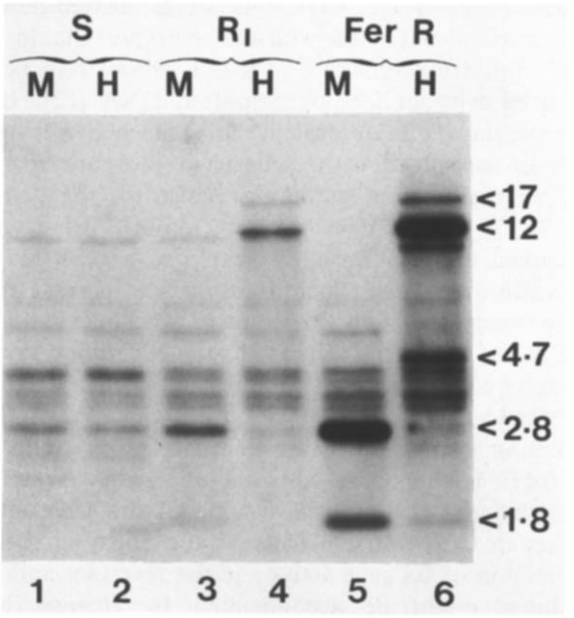


Fig.3. Hybridization of E4 cDNA (pMp24) to Southern blots of DNA from susceptible (S) and two non-translocated resistant aphid clones (R_1 and FerR) digested with either *Msp*I (M) or *Hpa*II (H).

selectively and generally to maintain that methylation pattern from one generation to the next.

Lack of evidence for DNA methylation in other insects [16,17] has been used to argue against its importance as a universal primary regulator of gene expression in complex organisms [13–15]. However, a sensitive HPLC assay for 5mC showed that, whilst *Drosophila* DNA did not contain detectable amounts of 5mC, the larval silk glands of *Bombyx mori* showed both tissue- and stage-specific cytosine methylation [23]. Work in progress on *M. persicae* should establish whether methylation of the amplified esterase genes is essential for their expression, and whether demethylation plays a critical part in the events leading to loss of insecticide resistance.

Acknowledgements: We thank M.F. Stribley for maintaining the aphid clones, R.L. Dunckley for technical assistance and R.M. Sawicki for useful comments on the manuscript.

REFERENCES

[1] Devonshire, A.L. and Moores, G.D. (1982) Pestic. Biochem. Physiol. 18, 235–246.

[2] Devonshire, A.L., Searle, L.M. and Moores, G.D. (1986) Insect Biochem. 16, 659–665.
[3] Field, L.M., Devonshire, A.L. and Forde, B.G. (1988) Biochem. J. 251, 309–312.
[4] Sawicki, R.M., Devonshire, A.L., Payne, R.W. and Petzing, S.M. (1980) Pestic. Sci. 11, 33–42.
[5] Ffrench-Constant, R.H., Devonshire, A.L. and White, R.P. (1988) Pestic. Biochem. Physiol. 30, 1–10.
[6] Blackman, R.L., Takeda, H. and Kawakami, K. (1978) Nature 271, 450–452.
[7] Devonshire, A.L., Moores, G.D. and Chiang, C. (1983) in: IUPAC Pesticide Chemistry Human Welfare and the Environment (Miyamoto, J. and Kearney, P.C. eds) pp.191–196, Pergamon, Oxford.
[8] Devonshire, A.L. and Sawicki, R.M. (1979) Nature 280, 140–141.
[9] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) in: Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[10] Lasky, R.A. and Mills, D. (1973) Eur. J. Biochem. 56, 335–341.
[11] Schimke, R.T. (1982) in: Gene Amplification in Mammalian Somatic Cells (Chater, K.F. et al. eds) Proceedings of the Fifth John Innes Symposium, pp.235–251.
[12] Sugimoto, Y., Roninson, I.B. and Tsuruo, T. (1987) Mol. Cell Biol. 7, 4549–4552.
[13] Doerfler, W. (1983) Annu. Rev. Biochem. 52, 93–124.
[14] Riggs, A.D. and Jones, P.A. (1983) Adv. Cancer Res. 40, 1–30.
[15] Kolata, G. (1985) Science 228, 1183–1184.
[16] Adams, R.L.P., McKay, E.L., Craig, L.M. and Burdon, R.H. (1979) Biochim. Biophys. Acta 563, 72–81.
[17] Rae, P.M.M. and Steele, R.E. (1979) Nucleic Acids Res. 6, 2987–2995.
[18] Lee, J.S. and O'Neill, L. (1987) Immunogenetics 26, 92–98.
[19] Gerber-Huber, S., May, F.E.B., Westley, B.R., Felber, B.K., Hosbach, H.A., Andres, A.-C. and Ryffel, G.U. (1983) Cell 33, 43–51.
[20] Graessmann, M., Graessmann, A., Werner, E. and Simon, D. (1983) Proc. Natl. Acad. Sci. USA 88, 6470–6474.
[21] Van der Ploeg, L.H.T. and Flavell, R.A. (1980) Cell 19, 947–958.
[22] Ott, M.-O., Sperling, L., Cassio, D., Levilliers, J., Sala-Trepat, J. and Weiss, M.C. (1982) Cell 30, 825–833.
[23] Patel, C.V. and Gopinathan, K.P. (1987) Anal. Biochem. 164, 164–169.