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STRUCTURAL VARIATIONS IN THE *H-2* GENES OF AKR LYMPHOMAS

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SUMMARY

K36.16 is an AKR *H-2*^{k} thymoma which expresses an aberrant H-2D^d-like allospecificity, does not have a detectable amount of the H-2K^k syngeneic antigen and grows very easily in syngeneic mice. By DNA-mediated gene transfer experiments, we were able to obtain transformed clones which do express the H-2K^k molecules and are rejected by AKR mice. Southern hybridization was performed to assess whether any gross changes had occurred in the K36.16 *H-2K* locus or elsewhere in the MHC, which might explain the lack of H-2K expression and/or the presence of the aberrant H-2D^d-like allospecificity. Specific H-2 class I DNA probes were used to compare the K36.16 genomic DNA with normal AKR thymus DNA after digestion with a variety of restriction enzymes. After hybridization with the pH-2IIa probe a 2·8 kb 'Hind III' fragment was identified in the K36.16 genomic DNA which is absent from AKR DNA. The pH-2IIa probe detects the third, transmembrane and cytoplasmic domains of class I genes. Although these changes are indicative of MHC genome modifications it is not yet possible to link these specific Southern blot pattern variations with the phenotypic changes mentioned above.

INTRODUCTION

The mouse major histocompatibility complex (MHC) is a closely-linked group of genes on chromosome 17 that encodes a set of structurally related cell surface glycoproteins. Three of these proteins, H-2K, H-2D and H-2L, termed class I MHC antigens, are cell surface glycopolypeptides of 40–45 Kd that are non-covalently linked to β2-microglobulin, a 12 Kd protein encoded by a gene on chromosome 2 (Klein, 1975; Festenstein & Démant, 1978; Hood *et al.*, 1985). Just as the cytotoxic T lymphocytes (CTL) which recognize viral antigens do so in the context of self-MHC class I antigens (Zinkernagel & Doherty, 1979), so the CTL which recognize oncogenic, virally-induced tumour target cells are also restricted to MHC antigens encoded by the *H-2K* and/or *H-2D* genes. However, the involvement of H-2K and/or H-2D specificities is not the same in all cases: thus, particular tumour

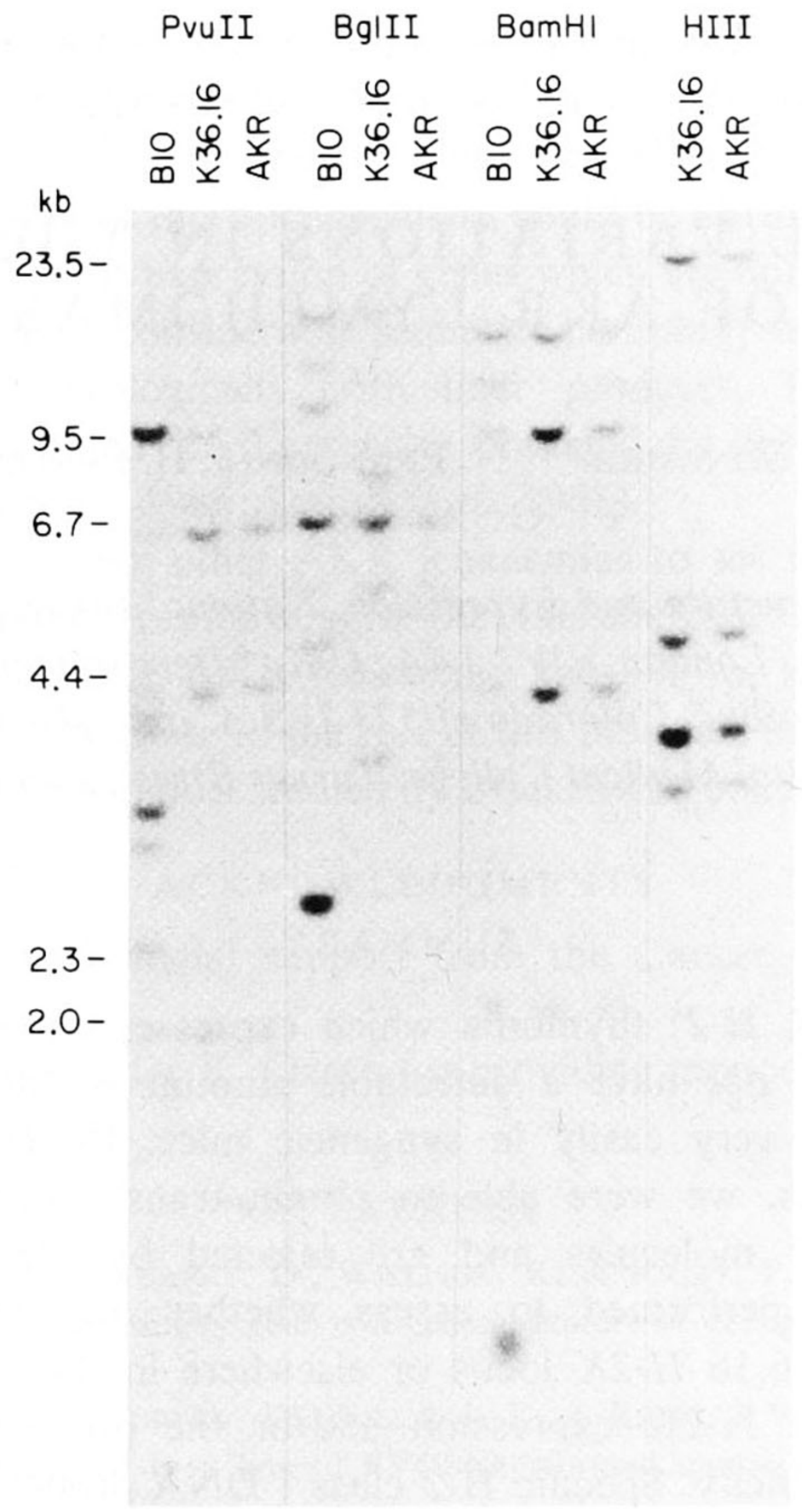

FIG. 1. Autoradiogram obtained after hybridization of enzyme digests of normal AKR thymus or K36.16 DNA to a 5′ flanking *K* region probe (Weiss *et al.*, 1984).

antigens may be recognized preferentially by CTL in association with either the H-2K and/or H-2D antigens depending on the origin of the tumour and the *H-2* haplotype of the CTL (Blank & Lilly, 1977; Gooding, 1980; Weiss *et al.*, 1980; Schmidt & Festenstein, 1982; Flyer *et al.*, 1985). The immune regulation of tumour growth, development and host resistance is therefore not only dependent on the nature of the tumour cell antigens, but also on the quantity and quality of their MHC class I antigen expression. It is not surprising, then, to find that many virally- and chemically-induced tumours frequently exhibit altered profiles of MHC class I products on their surfaces (Festenstein & Schmidt, 1981; Bernards *et al.*, 1983). Most importantly, we and others have shown that the oncogenicity and metastatic properties of several mouse tumours can be abrogated by the experimental manipulation of their genome causing the re-expression of missing class I MHC molecules (Hui *et al.*, 1984; Tanaka *et al.*, 1985; Wallich *et al.*, 1985).

MATERIALS AND METHODS

Gene probes

The following probes were used: pH-2IIa, pH-2III (Steinmetz *et al.*, 1981) and a probe from the 5′ flanking region of the H-2K^b gene (Weiss *et al.*, 1984). The pH-2IIa probe is a

3′ cDNA gene probe which detects the alpha-3, transmembrane and cytoplasmic domains of MHC class I gene. pH-2III is a 5′ cDNA gene probe which detects the first three exons of MHC class I genes.

Southern blots (*Southern, 1975*)

After digestion with restriction endonucleases, DNA samples (5–10 μg/track) were separated in 0·7% (w/v) agarose gels. The DNA was then denatured and transferred on to nitrocellulose filters. The filters were hybridized to ^{32}P-labelled probes. The filters were then washed, dried and autoradiographed.

RESULTS AND DISCUSSION

DNA from normal AKR thymus, K36.16 thymoma and normal C57BL/10 thymus was digested with endonucleases and hybridized with the probes mentioned above. No differences were seen between the K36.16 tumour DNA and the DNA of the strain of origin (AKR) using either the 5′ flanking probe (Fig. 1), or the pH-2III probe (Fig. 2). But, as expected, differences were seen with the C57BL/10 (*H-2^b*-thymus DNA) (Fig. 1). However, hybridization of the Hind III digested DNA with the pH-2IIa probe revealed a fragment of about 2·8 kb which is present in the K36.16 genomic DNA but absent from that of the AKR thymus DNA (Fig. 3). Although these results rule out gross

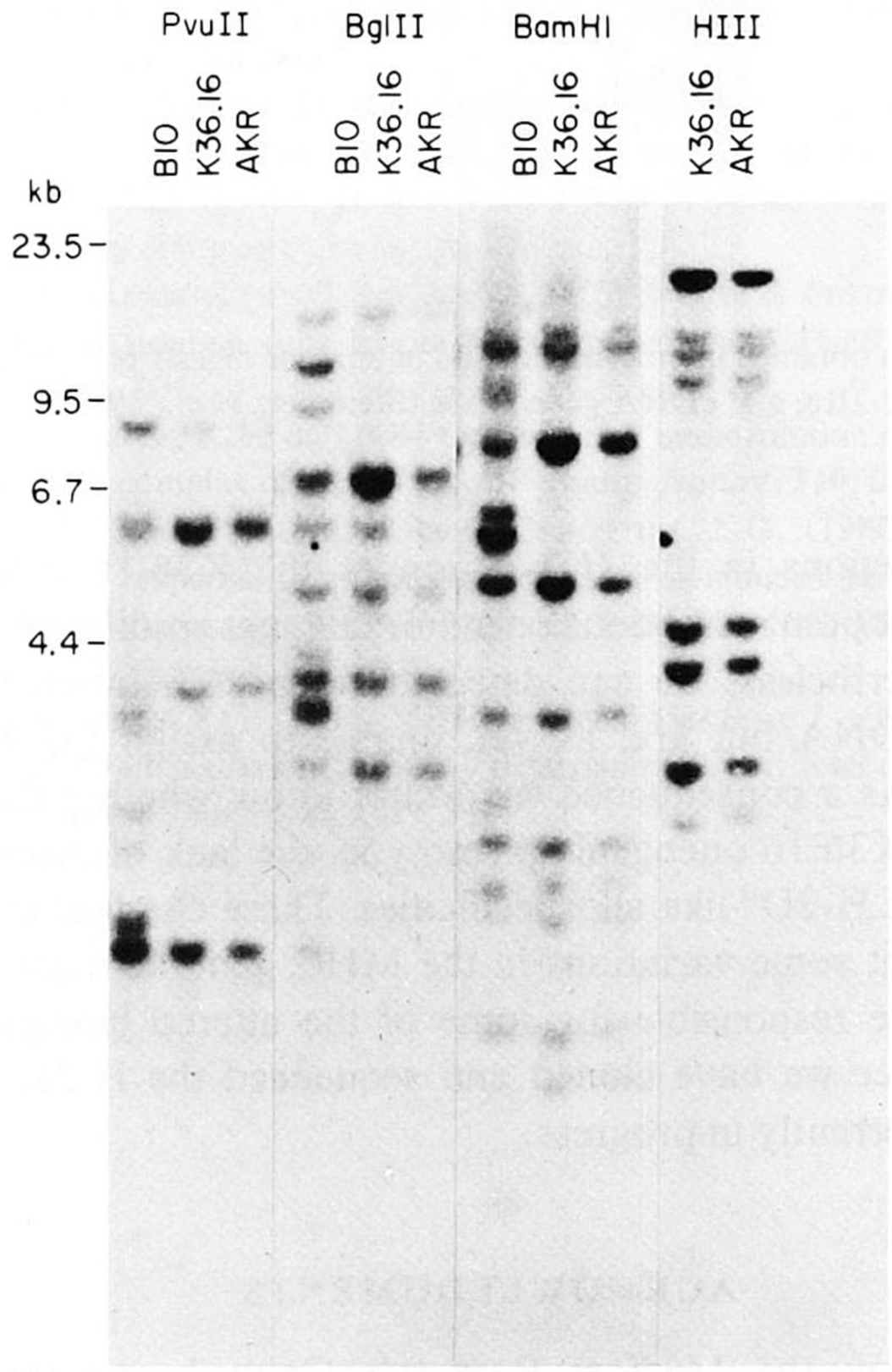

FIG. 2. Autoradiogram obtained after hybridization of enzyme digests of normal AKR thymus or K36.16 DNA to pH-2III, a 5′ cDNA gene probe (Steinmetz *et al.*,1981).

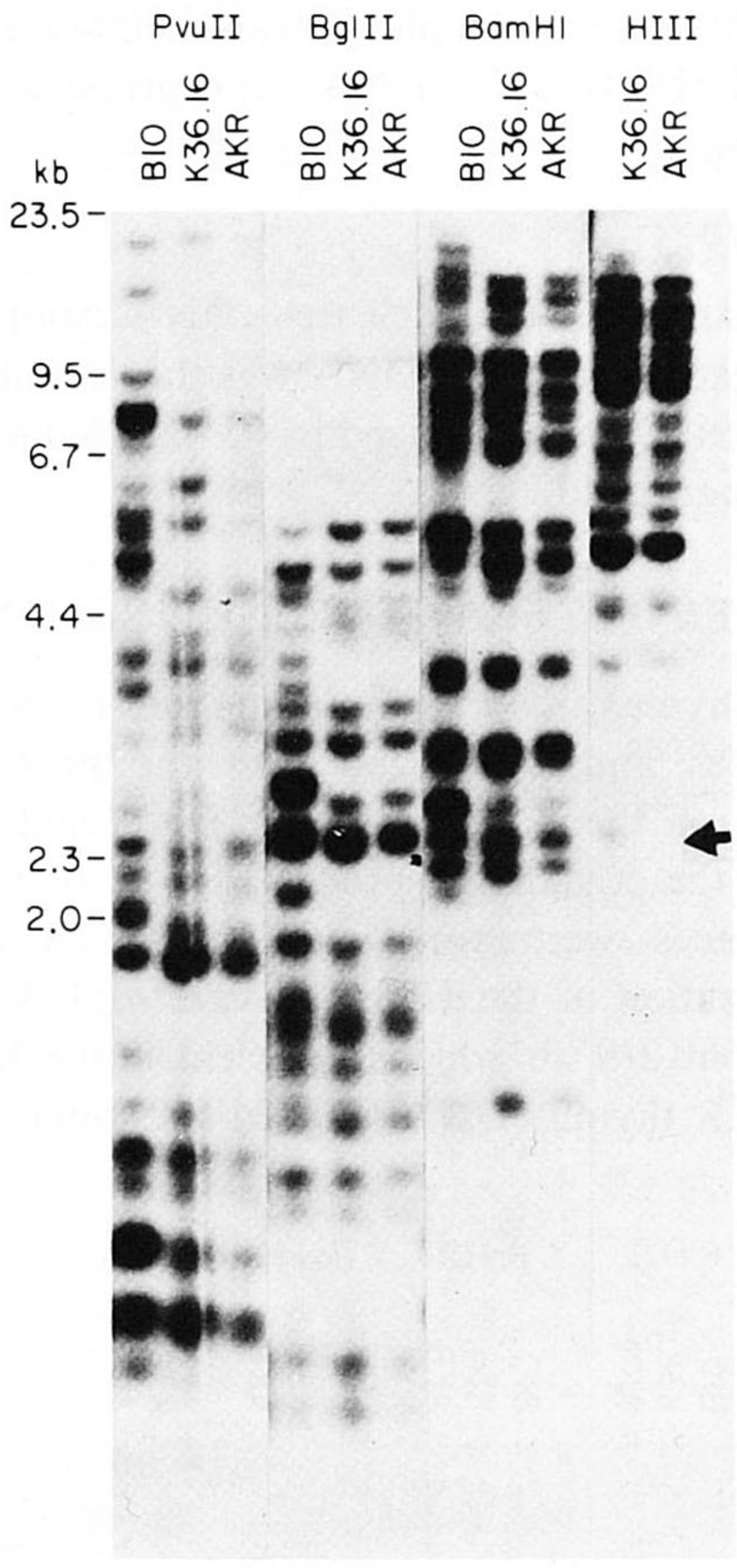

FIG. 3. Autoradiogram obtained after hybridization of enzyme digests of normal AKR thymus or K36.16 DNA to pH-2IIa, a 3′ cDNA gene probe (Steinmetz *et al.*, 1981).

rearrangements and deletions in the *H-2* region of the K36.16 cells, the assay is not sensitive enough to detect point mutations or minor changes resulting from gene conversion or small deletions. Nevertheless, we can detect differences between the AKR thymoma and the normal AKR DNA but are, as yet, unable to assign the 2·8 kb band to any particular MHC gene. As a consequence we do not know whether this particular change has any relation to the K36.16 oncogenic phenotype, the lack of expression of the H-2K^k molecule or the aberrant H-2D^d-like allospecificities. These changes in the Southern blots, nevertheless, indicate that some variations in the MHC genome must have taken place in the tumours and may be responsible for some of the altered biological properties. This should become clear once we have cloned and sequenced the H-2K gene from K36.16. These experiments are currently in progress.

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