LENGTH POLYMORPHISM OF RESTRICTION FRAGMENTS OF ONCOGENE c-fos AND c-src LOCI IN SPONTANEOUSLY HYPERTENSIVE (SHR) AND CONTROL (WKY) RATS

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The hypertension which develops in spontaneously hypertensive rats (SHR, Okamoto-Aoki strain) is a multifactorial inherited disease which is regarded as an adequate model of primary (essential) hypertension in man [10]. During the last two decades the SHR line has been used worldwide as experimental objects for intensive physiological and biochemical research aimed at studying the primary disturbances leading to development of the disease. An important step in this research was the formulation of the membrane concept of the pathogenesis of primary hypertension, based on the finding of disturbances in a number of ion-transporting membrane systems [2]. It has recently been found that many similar changes can be reproduced by activation of intracellular protein kinases [8]. This has led to the hypothesis of the possible participation of oncogene products in the genesis both of membrane disturbances and of hypertension itself [1]. Since it is difficult in a purely physiological approach to prove whether changes arising under polygenic control are primary relative to the elevation of blood pressure (BP), which also is a quantitative polygenic trait, we attempted to find qualitative, monogenic traits. Analysis of the linking of these traits with the BP level or with other physiological-biochemical markers in segregating generations of hybrids (F_2, back cross) has led to the identification of the loci of the genome whose inheritance is an essential condition for the development of hypertension. Restriction fragment length polymorphism (RFLP) is the most convenient source of such traits.

Under these circumstances we decided to investigate polymorphism between SHR (hypertensive) and WKY (normotensive control) lines with respect to loci of a number of oncogenes.

EXPERIMENTAL METHOD

Restriction endonucleases EcoRI, SalI, BamHI, PstI, HindIII, and PvuII were obtained from the Vilnius Ferment Research-Production Combine. Plasmids containing v-src, v-fos, v-myc, and v-ras genes were obtained from the bank of the Molecular Biology of Viruses Laboratory, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Total DNA was isolated from the rat liver by extraction with phenol and chloroform [3]. Restriction of 10 μg DNA was carried out with 50 U of restriction endonucleases in appropriate buffers at 37°C for 5 h. The restriction fragments were separated in 0.8% agarose gel with a current of 20 mA for 14 h. Denaturation in 0.5 M NaOH, subsequent neutralization with 1 M Tris-HCl, and blotting on nitrocellulose were carried out as described previously [5]. After baking of the blots at 80°C in vacuo hybridization was carried out with plasmids labeled with ^{32}P by nick-translation [6]. Autoradiography of the blots was then undertaken at −70°C for 5-10 days.

EXPERIMENTAL RESULTS

Examination of Figs. 1 and 2 showed the presence of RFLP for the BamHI site in the region of the c-fos gene and for the EcoRI and HindIII sites in the c-src region.

The SHR genome is characterized by a band with molecular weight of 4 kbp, containing a sequence complementary to v-fos. The weak intensity of the signal compared with the main band can be taken to indicate limited homology or the presence of a short segment of a homologous sequence within the restriction fragment.

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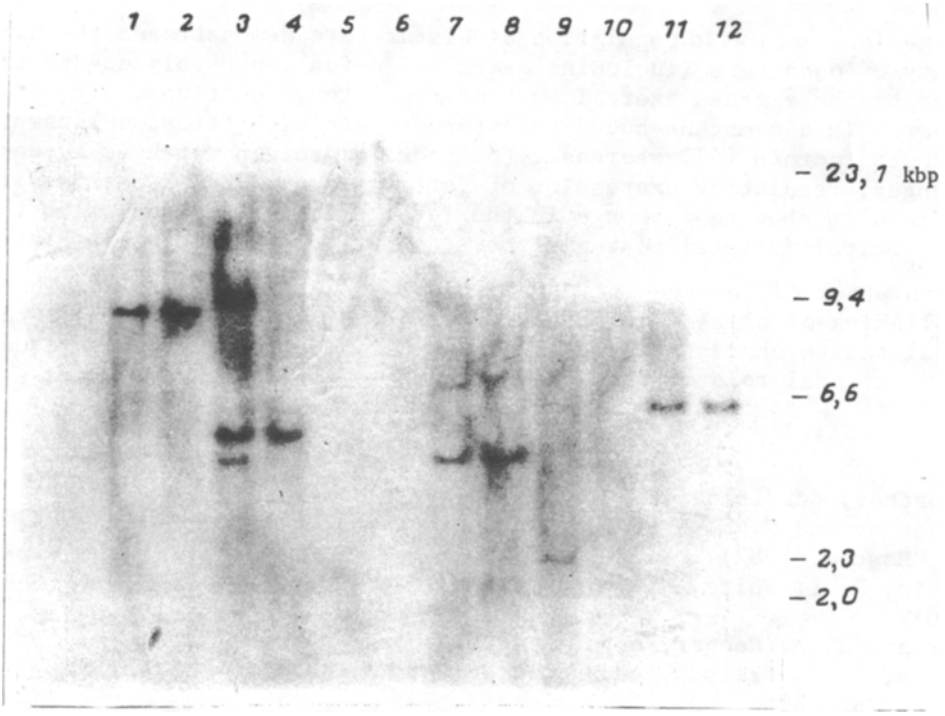

Fig. 1. Intralinear polymorphism of BamHI restriction site in region of locus of homologous v-fos, detected by Southern blotting. Odd lanes — DNA of SHR rats, even lanes — DNA of WKY rats, restricted by HindIII (lanes 1, 2), BamHI (3, 4), SalI (5, 6), PstI (7, 8), PvuII (9, 10), EcoRI (11, 12).

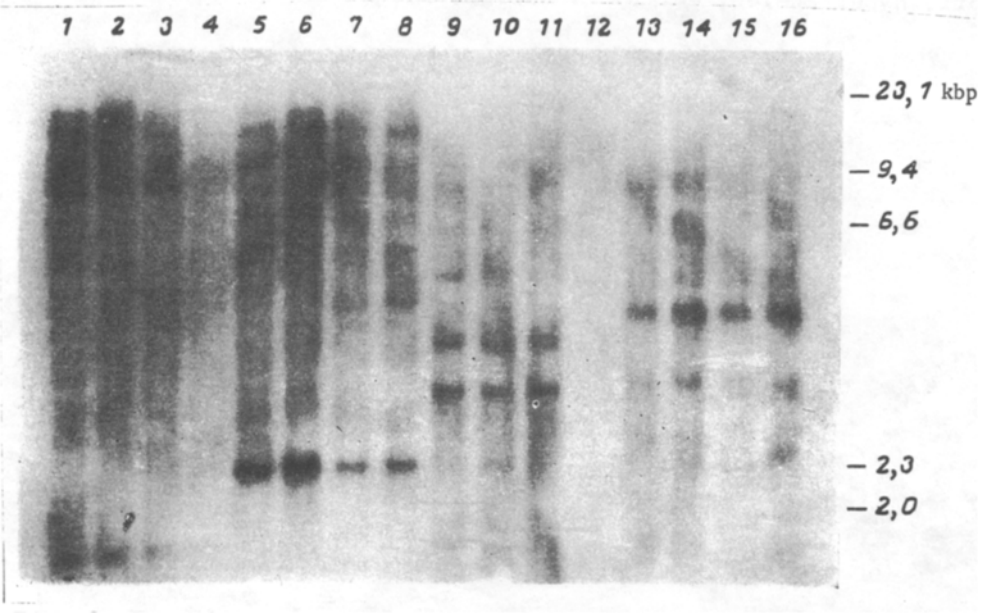

Fig. 2. Interlinear polymorphism of EcoRI (lanes 1-8) and HindIII (9-16) restriction sites in region of locus of homologous v-src. Lanes 1-4 and 9-12) DNA of SHR rats from four different litters; lines 5-8 and 13-16) DNA of WKY rats from four different litters.

Polymorphism for two restriction sites at once was found in the region of the c-src gene. In this case the main fragments containing sequences complementary to v-src were found to be polymorphic. These were the 1.6 kbp band for SHR and the 2.4 kbp band for WKY in the case of EcoRI, and also the 3.4 kbp band for SHR and the 4.1 kbp band for WKY in the case of HindIII. The phenotypic consequences of this polymorphism are not yet known. Pre-

vious investigations on a wild population of Wistar rats demonstrated the high conservatism of the majority of oncogenes (including c-arc and c-fos), possibly due to the fact that the proteins coded by these genes exert important regulatory functions. The protein coded by c-src (P^{60} c-src) is a membrane-bound receptor protein with tyrosine kinase activity, controlled by growth factors [4], whereas c-fos codes a protein which is evidently an intranuclear messenger, regulating expression of certain genes. This hypothesis is confirmed by experimental results showing a change in the level of c-fos transcription through the action of the principal intracellular mediators: cAMP, Ca^{++}, and protein kinase C [9].

The polymorphism discovered for c-src and c-fos genes makes it possible to move on to the study of linking of alleles of these genes in (SHR × WKY)F_2 hybrids with physiological and biochemical traits characteristic of hypertension. It may also prove useful for the study of the functional role of these genes in the cell.

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