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Comparison of the genetic maps of variola and vaccinia viruses

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The complete genetic map of the variola major virus strain India-1967 is built basing on the sequence data. The suggested map is compared with the maps of the sequenced genomic regions of Copenhagen and Western Reserve strains of vaccinia virus and Harvey strain of variola major virus. The principle differences revealed in the genomic organization of these viruses are discussed.

Variola virus; Vaccinia virus; Genetic maps

1. INTRODUCTION

Several laboratories have been carrying out the mapping and functional studies of numerous genes of vaccinia virus (VAC) using a variety of methods. However, the major part of such studies involve the laboratory strain Western Reserve (WR) [1–3]. Along with the gene mapping the major part of WR genome has been sequenced [1,2]. Recently the complete DNA sequence of VAC strain Copenhagen (COP) was determined [4]. Though most of the genes of these two VAC strains coincide, yet some differences are revealed [4]. Systematic investigations of genomic organization of not only VAC but the other closely related orthopoxviruses, in particular variola (or smallpox) virus, monkeypox and cowpox viruses, are required in order to reveal those genes determining the most important biological features of human pathogenic orthopoxviruses.

Within the frames of International Program supervised by World Health Organization we have performed the sequencing of variola major virus strain India-1967 DNA. In the present work we carry out the comparison of genetic map of variola virus strain under study with that of VAC strains COP and WR.

2. MATERIALS AND METHODS

DNA of the variola major virus strain India-1967 (VAR-IND) [5] was hydrolyzed by *Hin*dIII and *Xho*I restriction enzymes and cloned in bacterial plasmids [6]. Nucleotide sequencing and computer analysis were carried out as described elsewhere [5]. VAR-IND DNA sequence data from this article have been deposited with the EMBL Data Library under the accession number X69198.

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Abbreviations: VAR-IND, VAR-HAR, variola virus strains India or Harvey, respectively; VAC-COP, VAC-WR, vaccinia virus strains Copenhagen or Western Reserve, respectively; aa, amino acid residues; kbp, kilobase pairs; ORF, open reading frames.

3. RESULTS AND DISCUSSION

Variola and vaccinia viruses differ considerably concerning a number of biological features such as the range of the hosts, pathogenicity, persistence ability, and others [7]. Therefore the comparison of genetic maps of these viruses which are related to the same genus Orthopoxvirus of Poxviridae family are of obvious interest. Results of such comparative analysis of these viral genomes are graphically displayed in Fig. 1. We revealed 195 potential open reading frames (ORFs) in the complete coding sequence of VAR-IND genome, these ORFs as a rule being more than 65 aa long.

One can see that the interspecific variation as well as the differences between the strains of a species is concentrated in the terminal genomic regions. Moreover, the intraspecific variable regions are considerably smaller comparing with the interspecific ones for VAR/VAC.

The long terminal inverted repeat (TIR) of VAC-COP is of 12 kbp whereas that of VAC-WR is 10 kbp long [9], the VAC-WR TIR beginning closer to the center of the viral genome and several genes being deleted comparing with strain VAC-COP (Fig. 1). TIR of variola virus is about 0.7 kbp long and is presented by the truncated terminal region comparing with vaccinia viruses which contains the short tandem repeats and the unique segment of 0.3 kbp (Fig. 1); the constituents of the latter TIR are likely to represent the minimal genetic element necessary for the proper DNA replication of poxviruses [10]. Organization of this VAR terminal DNA region is similar to that of telomeric region of

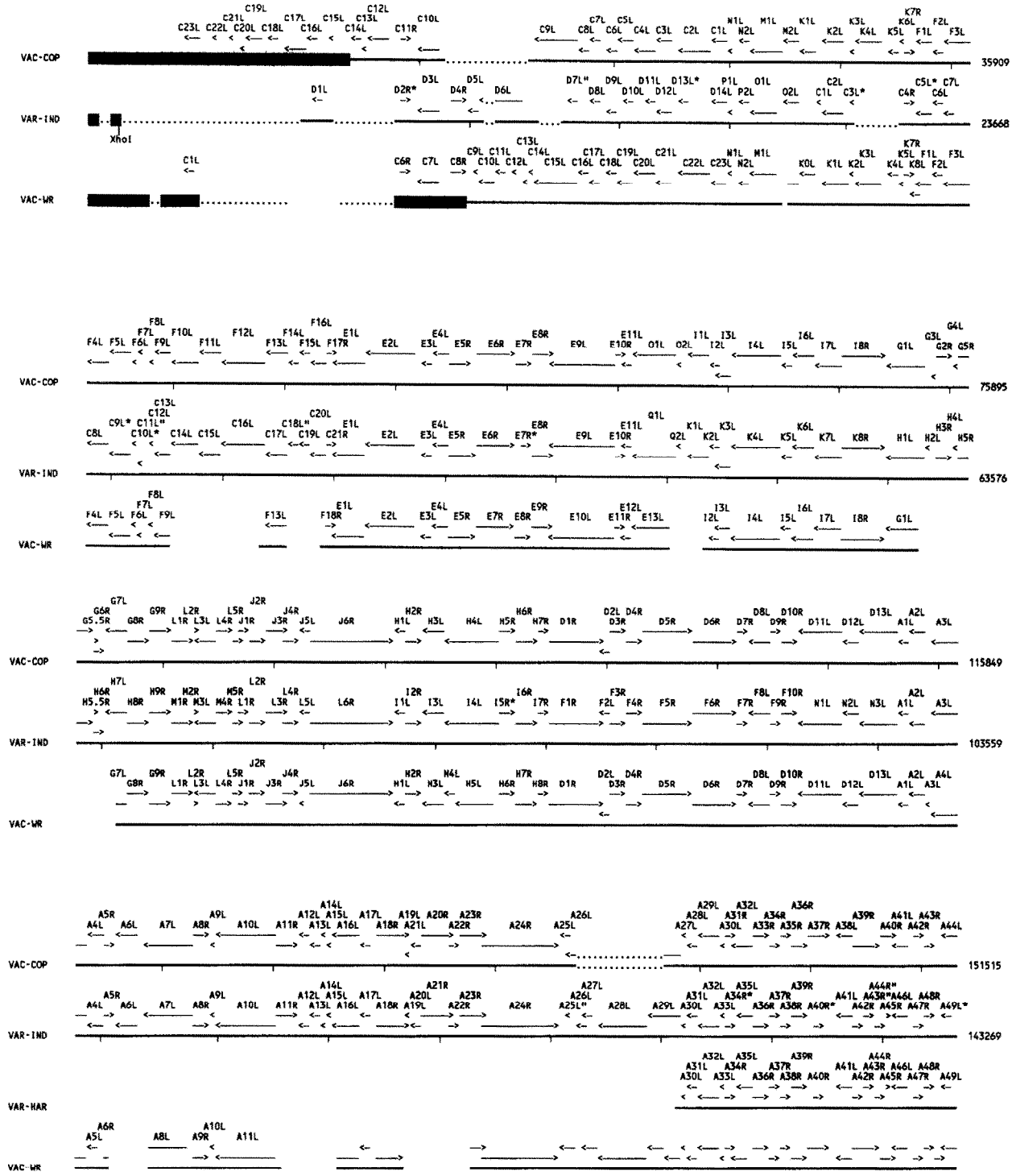

Fig. 1. Graphical alignment of orthopoxviruses genomes. VAC-COP, vaccinia virus strain Copenhagen [4]; VAR-IND, variola major virus strain India-1967; VAC-WR, vaccinia virus strain WR [1,2], VAR-HAR, variola major virus strain Harvey [8]. Long terminal inverted repeats (TIR) of viral DNA are bordered by triple asterisks. Unique regions within TIR which separate the blocks of short tandem repeats (54 bp; 125 bp and 70 bp) are marked by parti-colored rectangles. Arrows show the size and direction of open reading frames. Dots indicate the deleted regions in both DNA and proteins of a virus with respect to the other one which exceed 50 bp and 17 aa, respectively. Gaps in VAC-WR sequence correspond to the unsequenced genomic regions. The extreme left and right *Xho*I restriction sites are marked on VAR-IND DNA. Size of DNA sequence (in base pairs) between these extreme *Xho*I sites are shown near the right end of VAR-IND genome. For VAR-COP DNA the sizes of both the entire genome and the region corresponding to the *Xho*I–*Xho*I segment of VAR-IND (in brackets) are shown.

SFV DNA [11]. This similarity is likely to point not only to the common evolutionary origin of the viruses which belong to different poxvirus genera but also to suggest the similar evolutionary development of their DNA rep-

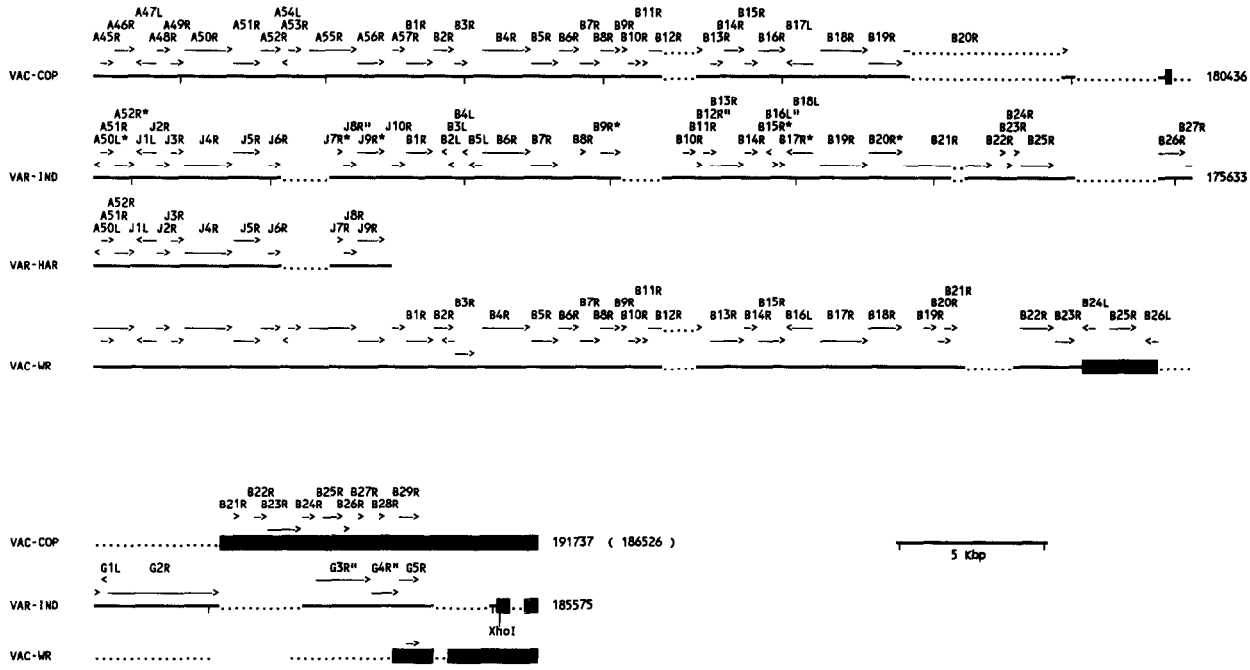

Fig. 1 (*continued*).

lication apparatus. Regions of short terminal tandem repeats are considerably longer in the VAC genome (Fig. 1). It is not clear yet how these differences can affect the features of orthopoxviruses.

The other characteristic feature of VAR genome genetic organization with respect to that of VAC is the occurrence of considerable number of truncated ORFs in the terminal regions of the genome (for example, VAR ORFs D7L, D10L, D13L, C1L, C7L, A38R, A40R, A45R, J6R, and others). In rare instances there may be VAR ORFs of considerably greater size (G3R, G4R) [12,13] comparing with the genes from the analogous VAC genomic regions. Noteworthy, that genetic map of the sequenced DNA segment of variola major virus strain Harvey [8] almost completely coincides with VAR-IND genetic map of this region (Fig. 1). Thus we suppose it highly reliable that the observed differences between VAR and VAC in genetic organization of their terminal regions are responsible for in vivo species-specific characteristics of these viruses. It should be noted that VAR-HAR was isolated in 1944 from a soldier who had returned with a convoy from Gibraltar [12], while VAR-IND strain was isolated in 1967 from a patient in India [14]. Thus, the strains compared differ considerably in both the time of isolation and their geographical origin.

Comparison of the genetic maps of vaccinia and variola viruses suggests that VAR and VAC are likely to have the common ancestor; subsequently in the course of evolution numerous mutational and recombinational events had taken place which resulted, in particular, in the 'truncation' of a number of VAR ORFs which in VAC genome are of larger size. Several ORFs have been conserved in the genome of VAR or had undergone slight changes comparing to the ancestor virus whereas in VAC genome the corresponding ORFs had been deleted (as the region of ORFs B26R, B27R, G2R of VAR) or truncated (region of VAR ORFs G3R, G4R). The supposed natural evolutionary changes had brought into being such highly virulent anthroponosic pathogen as variola virus. On the other hand, vaccinia virus was formed in the course of mostly artificial evolution which had been directed to select attenuated highly immunogenic orthopoxvirus. Sequencing of the genomes of cowpox and monkeypox viruses is necessary to make more justified and valid conclusions concerning evolutionary origin of human pathogenic orthopoxviruses.

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