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# Ankyrin-like proteins of variola and vaccinia viruses

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Computer analysis of full coding sequences of variola major virus strain India-1967 genome and vaccinia virus strain Copenhagen genome have been carried out. A wide set of proteins containing ankyrin-like repeats have been identified for both viruses. Only three proteins of this family of the studied viruses are highly homologous. The rest of the proteins are different. The possible role of such proteins in determination of virus tissue tropism is discussed.

Variola virus; Vaccinia virus; Ankyrin-like protein

## 1. INTRODUCTION

Metabolism of animal viruses, depending on their peculiarities, suggests the use of structures of cytoplasmic or nuclear protein cell skeleton, as well as cell membranes. In particular virus-specific cytopathic effect can be caused by a specific reconstruction of the elements of cell skeleton and certain membranes. The aim of this reconstruction is to create the conditions for viral propagation [1]. The cytoskeleton proteins are coded by a large set of genes, which are characterized by tissue-specific expression. This accounts for the difference in the protein contents of the 'framework' of various cell types, which affects the function of these cells [2]. Various cell types may also differ in their membrane contents. The above differences can also affect such parameters of virus propagation inside the organism as tissue tropism.

The detection of genes which determine the tissue tropism of viruses is important not only for fundamental understanding of virus–cell interactions, but for the creation of more safe live virus vaccines. Poxviruses and primarily vaccinia virus (VAC) in recent years have been the most widely used as vectors for the production of live polyvalent vaccines by gene engineering methods. The given virus is capable of propagation in a broad spectrum of continuous animal cell lines.

VAC does not propagate on the CHO cell line, although this line is permissive for the other orthopoxvirus, cowpox virus (CPV). It was shown [3] that the CPV gene coding for the 77 kDa protein is responsible for this property of the virus and when integrated into the VAC genome allows the latter virus to propagate in CHO cells. Another gene which is necessary for VAC virus propagation in a number of human cell cultures was revealed in the genome of this virus [4]. The obtained results proved that the presence of the gene family which regulates the ability of these viruses to multiply in different cell types is characteristic of large cytoplasmic viruses such as poxviruses.

Computer analysis revealed that the above genes of orthopoxviruses code for proteins, containing the ankyrin-like repeats. The given observation is of considerable interest since ankyrin and ankyrin-like proteins control the interactions between the integral membrane proteins and elements of the cell cytoskeleton [2,5].

Taking into account the importance of this type of protein in determining the properties of viruses we carried out the computer analysis of nucleotide sequences of variola (VAR) major virus genome, strain India-1967 and VAC strain Copenhagen. The presence of families of ankyrin-like proteins, specific for each virus, was revealed.

## 2. MATERIALS AND METHODS

Earlier we cloned the fragments of DNA of VAR strain India-1967 in molecular vectors of *E. coli* [6]. The Maxam and Gilbert technique [7] was used to determine the full coding sequence of this virus genome; it contains 193 open reading frames (ORF). The nucleotide sequence of VAR DNA was compared with the sequence of VAC strain Copenhagen published previously [8].

Structure function analysis of viral DNA and proteins was carried out using the packages of applied programs 'Alignment service', 'Image', 'Nucleowriter', 'Gene Tools', which we had created earlier. The specialized package 'Q-Search' was used to search the consensuses and regions of viral proteins, which are the most homologous to the cell ones. Data bases SWISS-PROT (Release 21) and EMBL (Release 28) were used in this work.

## 3. RESULTS AND DISCUSSION

The performed analysis of VAR and VAC proteins showed that both viruses code for a large set of ankyrin-

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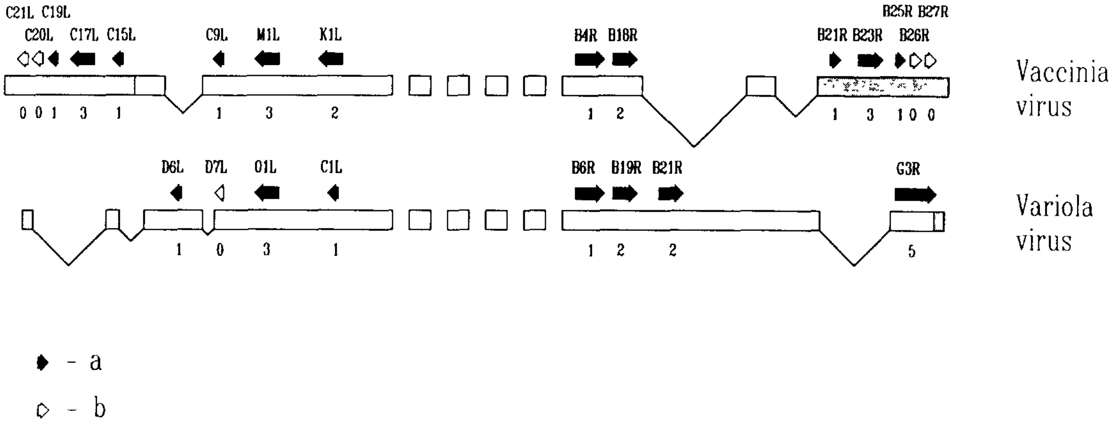

Fig. 1. Scheme of the location of the genes coding for the ankyrin-like proteins (a) and the proteins which in the course of divergent evolution have lost the ankyrin repeats (b) in the genome of the vaccinia virus strain Copenhagen, and variola virus strain India-1967. Genes are indicated by arrows. Digits opposite genes show the number of ankyrin repeats in the corresponding protein. Terminal inverted repeats of viral DNAs are crosshatched.

like proteins (Fig. 1). M1L and K1L genes of VAC were found to determine the virus host range in cell cultures [8]. Three genes of the studied viruses are highly homologous (M1L, B4R and B128R of VAC and O1L, B6R and B19R of VAR, respectively). The rest of the genes are different. The VAR analogues of C9L and K1L genes of VAC code for significantly shorter proteins. Three ankyrin-like proteins (C19L, C17L and C15L) are located in the long terminal repeat of VAC genome, i.e. they are present in two copies (at the right genome end, designated B25R, B23R and B21R, respectively). VAC sequence corresponding to G3R ORF of VAR consist of three short ORFs. Ankyrin-like structure is preserved only in B25R (Fig. 1). It should be noted that G3R of VAR is the most typical ankyrin-like protein among those detected in poxviruses. It contains five ankyrin repeats (Fig. 2). We revealed only three such repeats in the 77 kDa protein of CPV.

The individual repeats or their groups in ankyrin-like proteins is supposed to form binding sites for various integral membrane proteins and of the cell cytoskeleton. The differences in amino acid sequences of each repeat could account for their different specificity [5]. Ankyrin of human erythrocytes contains 23 tandem repeats of 33 amino acid residues [9].

Three proteins regulating tissue differentiation were detected in invertebrates. Each of these proteins contains 6 ankyrin repeats. Yeast was reported to have ankyrin-like proteins which contain two ankyrin repeats each and control the cell cycle [5].

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 67   GMSPLHCYVMN-TRFKPSVLKILLHNGVNNFDNKDN
103   GHIPLHHYLI-YSLSIDNKVFDILTDPIDDFSKSSD
225   GNTPLHTYLQQYTKHSPRVVYALLSRGADTRIRNNF
261   DCTPIMEYIKN-DCVTGHILIMLLNWHEQKYGKLQK
337   TMTPLHTAFQN---CNNNVASYLVYIGYDINLPTKD

      G-TPLH-A-----------V--LL--GA------N-
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Fig. 2. Amino acid sequence of the ankyrin-like repeats tandemly organized within G3R variola virus protein. The numbers of the first amino acid residues in each repeat is marked on the left. The bottom line presents the consensus sequence of the ankyrin repeat [5].

For VAC it was shown [10] that the infection of non-permissive CHO cells resulted in a rapid inhibition of the protein synthesis both of the host cell and the virus. The mutant of VAC with deleted K1L gene (Fig. 1) is not capable of multiplication in cultures of human MRC-5, KB or HEp-2 cells. This process is accompanied by a rapid inhibition of protein synthesis both of the virus and the infected cell [11]. The experimental data and the results of our analysis suggest that ankyrin-like proteins coded by poxviruses are important for the formation of viroplasma from the proteins of a certain type of the cell and the virus. Such macrostructures are necessary for normal synthesis of proteins and assembly of orthopoxvirus virions [12]. We suppose that different sets of the ankyrin-like proteins, as well as the level of their synthesis, allow the fine regulation and determination of tissue tropism of virus propagation in the infected animal.

The sequence data from this article have been deposited with the EMBL Data Library under accession number X69198.

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