# POLIOVIRUS SEQUENCES CLONING INTO VACCINIA VIRUS

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Vaccinia virus is the prototypic member of the poxvirus family. Due to its biological properties and its successful history as an immunizing agent in smallpox vaccination this virus became a strong candidate for generating live recombinant vaccines (Panicali & Paoletti, 1982; Mackett et al., 1982). The progress in molecular biology study of this virus permited the use of genetic engineering technics to express foreign antigens (Brown et al., 1986).

Some biological properties of the virus contributed to its use as an expression vector. The large genome of the virus (187 kb) allows stable integration of foreign DNA sequences as large as 25 kb. The virus DNA replicates within the cytoplasm of infected cells and the infectious cycle shows distinct stages from uncoating to assembly. The virions have all enzymatic machinery necessary for transcription, modification and viral genome replication (Brown et al., 1986). The DNA promoter sequences are different from those sequences found in both pro and eukariotic cell (Hanggi et al., 1986). Those sequences are recognized only by the viral RNA polymerase. As a consequence, the key to obtain foreign gene expression is to put the sequence to be expressed under the control of a viral promoter.

The purified vaccinia DNA is not self infectious and therefore the "in vitro" manipulation of vaccinia genome is not possible. The approach used to obtain the recombinant virus includes the construction of a bacterial plasmid that contains the vaccinia promoter sequence followed by the foreign gene and flanked by non essential vaccinia virus DNA sequences. A cell culture is then co-infected with intact vaccinia virus and the recombinant plasmid. "In vivo" recombination event occurs, at a low frequency, between the homologous sequences present in the hybrid bacterial plasmid and the viral genome. This results in the formation of stable recombinant infectious particles that can be easily selected if the foreign sequences interrupts a marker gene in the vaccinia genome. Many recombinant vaccinia virus have already been obtained expressing antigens of hepatitis B, rabies, influenza, malaria and HIV-1 (Tartaglia & Paoletti, 1988).

Our purpose is the study of poliovirus antigens expression by using vaccinia virus as a vector. The causative agent of poliomyelitis is one of the best known animal viruses as respect to its molecular biology and immunochemical properties (Koch & Koch, 1985). Therefore it is interesting to verify how vaccinia viruses express poliovirus sequences.

Poliovirus is a virus of the Picornaviridae family whose RNA genome has already been cloned by the cDNA strategy (Koch & Koch, 1985). The viral particle contains four structural proteins (VP1, VP2, VP3 and VP4) arranged into an icosahedral symmetry. The fine structure of purified virions was obtained by X-ray crystallography (Hogle et al., 1985). Those results were related to the immunochemical studies and the main epitopes of the virus surface could be visualized. Van der Werf et al. (1983) showed by genetic engineering manipulation that aminoacids comprised between positions 92 and 105 of VP1 polypeptide were important for the immune response.

CLONING OF VACCINIA SEQUENCES INTO BACTERIAL PLASMID: A bacterial plasmid was constructed by cloning the *Hind* III-J fragment of vaccinia genome. The thymidine kinase (TK) gene is localized into this fragment and is a very useful marker because viruses with foreign insertions can be selected for TK-phenotype (Weir et al., 1982).

Vaccinia virus particles, strain Lister 180, were purified from vaccinal scars on sheep skin. The virus DNA was digested with *Hind* III-J and the J fragment was isolated from 0.7% agarose gel electrophoresis by electroelution and then ligated to *Hind* III cleaved pBR322 derivative in which the *Eco* RI and *Cla* I sites had been eliminated. The restriction map of this new plasmid named pIB 038 showed that the unique sites for *Eco* RI and *Cla* I are inside the TK gene. This plasmid was used to insert polio sequences in the correct reading frame.

CLONING OF SABIN-PV1 UP1 IMMUNODOMINANT EPITOPE SEQUENCE: By analyzing the hydrophobicity profile of VP1 polypeptide from poliovirus type 1 (Sabin strain) we were able to detect a hydrophylic region which was significantly different from the Mahoney strain. This region was comprised between aminoacids 93 to 102 and must be responsible for the immunodominant epitope of this particular strain. The DNA sequence that codes for this region was chemically synthesized. The final sequence contains, besides the nucleotides that code for the epitope aminoacids, a *Cla* I site at the 5' end and a *Eco* RI site at the 3' end. At the 3' end there is also an additional sequence coding for a *Bam* HI site. Therefore, the complete sequence can be inserted into pIB 038 in only one orientation and in the correct reading frame. The extra *Bam* HI site permited the rapid screening of a clone that contained the new plasmid designated pEP 072.

CLONING OF MAHONEY VP1 SEQUENCE: We used the VP1 sequence of Mahoney poliovirus that was cloned as a cDNA in a bacterial plasmid. This plasmid designated pCW 18Δ0 contains the DNA sequence coding for the COOH terminus of VP3, the whole VP1 and the NH2 terminus of 2A, a non structural protein (Wychowski el al., 1986). This fragment was rescued with a double digestion *Bal* I - *Bgl* II and *Eco* RI linkers were put at both ends. The fragment was then inserted into the *Eco* RI site of pIB 038. The resulting molecules were used to

transform competent **E. coli** cells. One of the positive clones (pBP 012) has the insert in the correct orientation. The DNA of this plasmid was partially sequenced by the dideoxy method and it was shown that the insertion was in the correct reading frame.

DISCUSSION: We have constructed two bacterial plasmids containing one the main epitope of VP1 from Sabin type 1 poliovirus (pEP 072), and other with the complete VP1 Mahoney sequence (pBP 012). In these plasmids, the polio sequences were inserted into the vaccinia TK coding sequence and flanked with vaccinia sequences. The homologous recombination of this plasmid with wild vaccinia virus will provide the opportunity to analyze the expression of hybrids Tk-polio polypeptides under the TK promoter control in a recombinant vaccinia virus.

The recombinant viruses so obtained could be probed with monoclonal or polyclonal antibodies. The results obtained could be related to the immunochemical studies made in the whole poliovirus.

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