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Reflections on the Early Development of Poxvirus Vectors

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Abstract

Poxvirus expression vectors were described in 1982 and quickly became widely used for vaccine development as well as research in numerous fields. Advantages of the vectors include simple construction, ability to accommodate large amounts of foreign DNA and high expression levels. Numerous poxvirus-based veterinary vaccines are currently in use and many others are in human clinical trials. The early reports of poxvirus vectors paved the way for and stimulated the development of other viral vectors and recombinant DNA vaccines.

Keywords

recombinant vaccines; vaccinia virus; vaccine vectors; recombinant DNA; DNA cloning

1. Recombinant Vaccinia Virus DNA

The prospect of genetically engineering viruses was raised by the construction of recombinant SV40 DNA in 1972 [1]. However, safety fears led to the famous 1975 Asilomar Conference, which was followed by a voluntary moratorium on cloning DNA. In 1977, the National Institutes of Health issued the first Guidelines for Recombinant DNA Research. At the time, Riccardo Wittek was making plans to come from Zurich to my laboratory at the National Institute of Allergy and Infectious Diseases where we hoped to clone and determine the structure of the long inverted terminal repetitions of the vaccinia virus genome. However, under the 1977 rules, cloning of viral DNA was only allowed under BSL-4 laboratory conditions, effectively precluding such research. Fortunately, revised Guidelines drafted in 1978 and issued on January 2, 1979 permitted cloning of viral DNA in a BSL-2 laboratory, albeit only attenuated phage lambda vectors could be used at first. We were very excited at the prospects as we anticipated that molecular cloning would be a paradigm shift that would totally change the nature of virus research. The last few weeks of 1978 were busy ones as Riccardo and other members of my laboratory prepared vaccinia virus and lambda DNA so that the ligations could be legally performed on the day the rules changed. In November 1979, we submitted the first of a series of papers to Nature and Cell describing the cloning of vaccinia virus DNA and mapping viral transcripts [2–4]. Eventually the ability to molecularly clone DNA, as well as other major advances in technology, led to the determination of the complete, nearly 200,000 base pair, genome sequence of vaccinia virus by Paoletti and co-workers [5] and more recently to the complete

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transcriptome by Yang and others in my group [6, 7]. Today, complete genome sequences are available for representative members of all chordopoxvirus genera.

2. Poxvirus Expression Vectors

Early genetic studies by Woodroofe and Fenner [8] indicated that homologous recombination could occur between the genomes of two replicating poxviruses. Subsequent marker rescue studies demonstrated that fragments of genomic [9] and cloned [10] DNA could recombine with the genome of vaccinia virus in infected cells. These results suggested that it might be possible to insert foreign DNA into the vaccinia virus genome, an experiment that had become permissible with revised National Institutes of Health Guidelines. At the same time that we were cloning and characterizing the vaccinia virus genome, we were mapping mRNAs and gaining an understanding of how mRNA synthesis is regulated [3, 10, 11]. At this opportune time, Michael Mackett and Geoffrey Smith came to my laboratory from the UK for postdoctoral training. Their joint project was to develop vaccinia virus into an expression vector. Dennis Panicali and Enzo Paoletti had the same goal, although our approaches were somewhat different. In 1982 both of our laboratories reported the use of vaccinia virus as a eukaryotic cloning and expression vector [12, 13]. Over the following two years my colleagues and I showed that hepatitis B antigen and influenza hemagglutinin could be expressed from infectious vaccinia viruses and that chimpanzees and hamsters, respectively, could be protected against disease [14–16]. During this same period Paoletti and co-workers [17, 18] also described the potential use of recombinant vaccinia virus as a vaccine vector. (Parenthetically, some officials at the World Health Organization, who were advocating the cessation of vaccination following the eradication of smallpox, viewed the possibility of recombinant vaccinia virus vaccines with anguish.) With the description of a general method for production and selection of recombinant vaccinia viruses (Fig. 1) and the distribution of plasmid insertion vectors [19], the use of vaccinia virus expression vectors for immunology and infectious disease research became widespread. As all chordopoxviruses have a similar arrangement of genes, interchangeable promoters, and conserved RNA polymerase and transcription factors, the principles developed for vaccinia virus expression vectors could be used for other poxviruses as exemplified with other orthopoxviruses, avipoxviruses, leporipoxviruses, parapoxviruses and yatapoxviruses [20–25].

While infectious recombinant vaccinia viruses quickly achieved success as veterinary vaccines e.g. rabies [26, 27], the history of side effects of the smallpox vaccine made it important to attenuate recombinant vaccinia virus for further human use. This led to the development of naturally host-restricted avipoxviruses [22, 28, 29] and vaccinia virus deletion mutants such as NYVAC [30], MVA [31–33] and DNA-replication-deficient strains [34] as vaccine vectors.

3. Enhancements of Poxvirus Vectors

As studies of poxvirus replication and host interactions advanced [35], many incremental improvements of the expression vectors were made. These included stronger promoters [36–38], removal of poxvirus transcription termination signals from inserted genes [39], removal of immunomodulatory genes [40, 41], silent codon mutations to enhance stability [42], alternative selection systems [43–46], and recombineering with bacterial artificial chromosomes [47, 48]. More innovative was the development of an inducible vaccinia virus expression system, based on the bacteriophage T7 RNA polymerase and *Escherichae coli* operator and repressor for bioproduction of proteins [49, 50]. Furthermore, the T7 expressing vaccinia virus was used to generate infectious negative-stranded RNA viruses from cDNA clones [51–53].

Additional modifications of recombinant vaccinia virus, aimed at reducing virulence and enhancing immunogenicity by expressing cytokine genes such as interferon-gamma [54], IL-2 [55, 56], IL-12 [57], IL-15 [58] and others, are currently receiving attention. Another important development was heterologous priming with an unrelated recombinant virus [59–61] or more commonly recombinant DNA [62, 63], which greatly enhances the cytotoxic T cell response elicited upon boosting with recombinant vaccinia virus.

4. Conclusions

The engineering of recombinant poxvirus vectors for research and as candidate vaccines preceded and contributed to the subsequent development of other viruses and DNA for related purposes. Since their initial description in 1982, there have been numerous improvements in efficacy and safety. Currently, recombinant poxviruses are being used as veterinary and wildlife vaccines and clinical trials are in progress to prevent infectious diseases such AIDS, influenza, malaria and tuberculosis and to treat cancers.

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Highlights

- Poxvirus vectors can accommodate and express large amounts of heterologous DNA.
- An important use of poxvirus vectors has been to characterize targets of immunity.
- Poxvirus-based veterinary vaccines are licensed and human ones are in clinical trials.

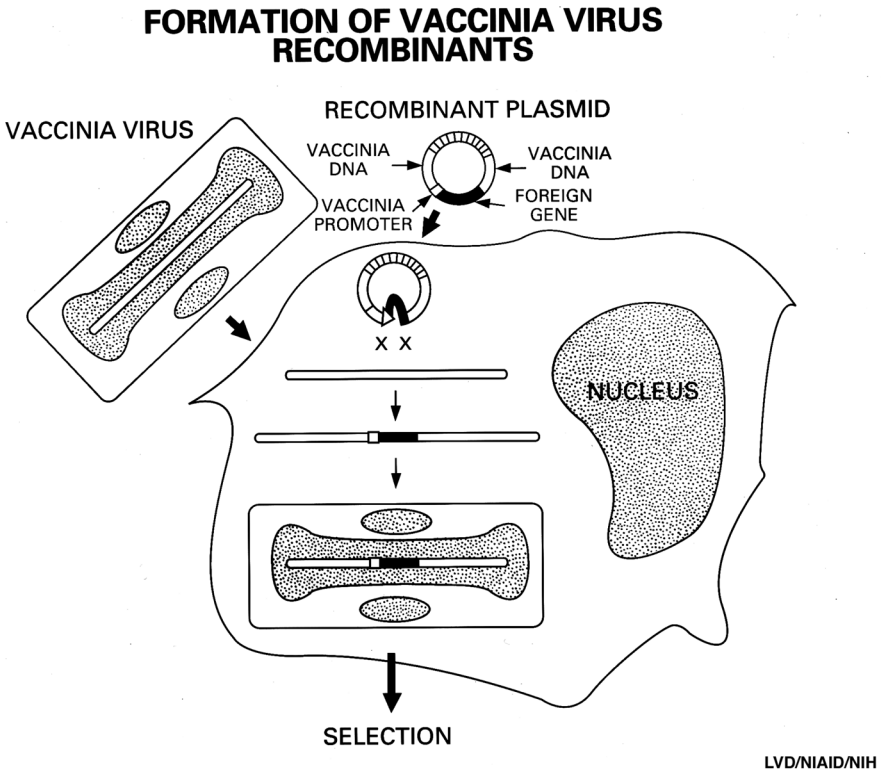

Fig. 1. Formation of vaccinia virus recombinants

The recombinant plasmid insertion vector contains a foreign gene preceded by a vaccinia virus promoter and flanked by vaccinia virus DNA that targets homologous recombination in cells infected with vaccinia virus. The recombinant virus can be identified or selected by a variety of methods.