EXPERIENTIA

Vol. 38 - Fasc. 3 Pag. 285-410 15.3.1982

GENERALIA

Organization and expression of the poxvirus genome

by Riccardo Wittek

Institut für Virologie der Universität Zürich, Winterthurerstrasse 266a, CH-8057 Zürich (Switzerland)

Summary. Poxviruses comprise a large group of very complex animal DNA viruses which replicate in the cytoplasm of infected cells. Vaccinia virus, the most studied poxvirus, has a linear, double stranded DNA genome with an approximate molecular weight of 120×10^6 (180 kilobase pairs). The two strands of the DNA molecule are naturally cross-linked at both termini. In addition, the vaccinia virus genome contains very long inverted terminal repetitions of approximately 10 kilobase pairs which are further characterized by the presence of direct tandem repeats of a 70-base-pair sequence arranged in two blocks of 13 and 17 copies, respectively. A central region of the genome is highly conserved between different orthopoxviruses. In contrast, the ends are hypervariable and may contain extensive deletions and complex, symmetrical sequences rearrangements.
Vaccinia virus gene expression is divided into two stages. Early in infection, RNA complementary to one half of one strand-equivalent of the genome is transcribed within subviral particles by the virion-associated RNA polymerase. Later in infection, after DNA replication, RNA complementary to one entire strand-equivalent is transcribed. RNA made late in infection is very heterogeneous in length and a large fraction of it contains self-complementary sequences. Late genes are clustered near the central region of the genome. Vaccinia virus mRNAs do not appear to be synthesized by a splicing mechanism.

1. Introduction

Poxviruses have been isolated from a wide variety of animals including mammals, birds and insects. They are characterized by their complex particle morphology (fig. 1), DNA genome structure and cytoplasmic site of replication. Systematically, they are grouped in the familiy poxviridae (Fenner, 1976) containing 6 genera with more than 40 species (table). The most studied members of the poxvirus family belong to the genus orthopoxvirus (synonym: vaccinia-variola subgroup) and variola virus, the agent of smallpox disease in man, is clinically by far the most important. Smallpox has been a threat to the human population since ancient times and in 1967 the disease was still endemic in 33 countries. In that year, it caused an estimated 10–15 million cases with some 2 million deaths (World Health Organization, 1980).
In 1958, the World Health Assembly called for a worldwide campaign for the eradication of smallpox, which was finally achieved in 1980 (World Health Organization, 1980). The eradication programme was based on the hypothesis that variola virus has no animal reservoir and that once the chain of transmission from human to human was interrupted, the virus would become extinct. Judged by the success of the WHO programme, this assumption has proved to be correct. However, a number of poxviruses, phenotypically indistinguishable from or closely resembling variola viruses, has been isolated apparently from animal tissues. This raises again the question of an animal reservoir of variola viruses, which could threaten the permanence of eradication. Only by detailed knowledge of the genome structure of these isolates and comparison with the orthopoxviruses in general will it be possible to determine whether such a reservoir exists. Similarly, the question of whether variola virus can be derived from another, possibly avirulent, orthopoxvirus by simple genetic variation, can be evaluated only if the DNA structure of the viruses in question is well characterized.
Besides the continuing interest in poxviruses as agents of disease in man and animals, these viruses also offer an ideal model system for studying the organization and expression of viral genomes. Vaccinia virus, which, as a potent and reliable vaccine, has played an

Family Poxviridae Genus	Species[1]
Orthopoxvirus	Variola virus, vaccinia virus, rabbitpox virus, cowpox virus, monkeypox virus, ectromelia virus
Avipoxvirus	Fowlpox virus, canary pox virus
Capripoxvirus	Sheep pox virus, goat pox virus
Leporipoxvirus	Myxoma virus, rabbit fibroma virus
Parapoxvirus	Orf virus, stomatitis papulosa virus
Entomopoxvirus	Melolontha melolontha entomopoxvirus

[1] Only a small number of selected species are listed.

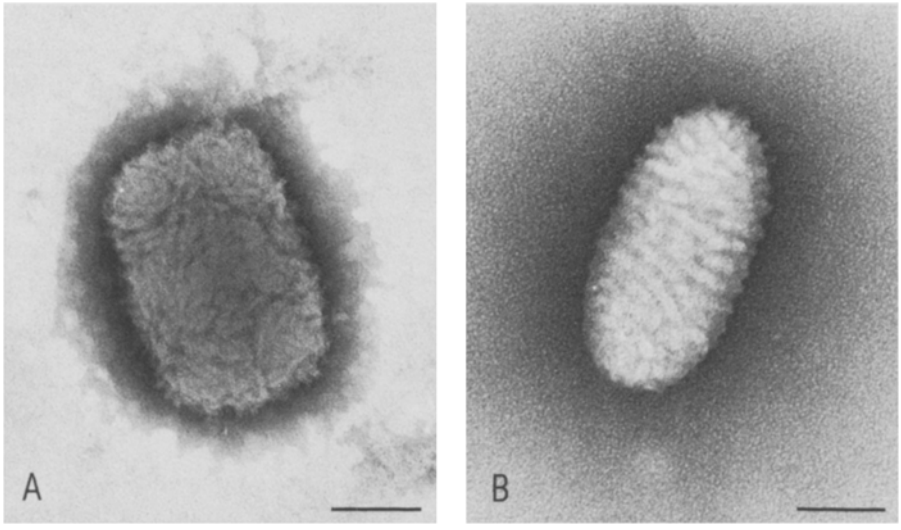

Figure 1. Electron micrographs of a negatively stained orthopoxvirus (A) and parapoxvirus (B). The length of the bar represents 100 nm.

essential role in the eradication of smallpox, is the most studied poxvirus. This virus multiplies to high titers in both primary and established cell lines, effectively inhibits host cell macromolecular syntheses and is easy to purify. Additionally, poxviruses are among the few viruses that replicate DNA in the cytoplasm of the infected cell. This physical separation of viral and host cell DNA greatly facilitates biochemical analysis.

Application of restriction endonucleases and of recombinant DNA technology has provided recent insights into the molecular organization and expression of the poxvirus genome. Much information on these aspects has accumulated in the past 5 years and has begun a new era in poxvirus research.

In the 1st part of this article, I will summarize recent findings related to genome structure and discuss the significance of some of this work for the smallpox eradication programme. The 2nd part will deal with gene expression and focus on transcriptional and translational mapping of the vaccinia virus genome. Readers interested in other aspects of poxviruses are referred to comprehensive reviews by B. Moss (1974, 1978).

2. *Organization of the poxvirus genome*

2.1. *Size and base composition of DNA*

The genome of poxviruses consists of a single linear, double stranded DNA molecule (review: Moss, 1974, 1978). The molecular weight of the vaccinia virus genome is approximately 122×10^6, corresponding to 180 kilobase pairs (Geshelin and Berns, 1974). This size, determined by measurements of DNA molecules in electron micrographs, has been confirmed in several laboratories by summation of the molecular weights of DNA restriction fragments (Gangemi and Sharp, 1976; Müller et al., 1978; Mackett and Archard, 1979). The genomes of other orthopoxviruses vary in size from about 118×10^6 for rabbitpox virus (Wittek et al., 1977) to about 145×10^6 for cowpox virus (Mackett and Archard, 1979).

Fowlpox, an avipoxvirus, appears to have the largest genome of all vertebrate poxviruses examined with a mol. wt of approximately 200×10^6 (Gafford and Randall, 1967, 1970). Müller et al. (1978) obtained a smaller size of 160×10^6 daltons and more work may be needed to determine accurately the size of fowlpox DNA.

Parapoxviruses have the smallest genome of all poxviruses so far examined with a mol. wt of about 85×10^6 (Menna et al., 1979).

The genome of orthopoxviruses is characterized by a low G+C content of 36–37 mole% (Joklik, 1962a,b). In contrast, parapoxviruses have a higher G+C content of approximately 65% (Wittek et al., 1979).

2.2. *Physical conformation of DNA*

Alkali denatured poxvirus DNA rapidly reforms the double stranded structure upon neutralization (Szybalski et al., 1963; Jungwirth and Dawid, 1967; Gafford and Randall, 1970; Berns and Silvermann, 1970). This unusual property has been attributed to the presence of cross-links in the DNA since all agents expected to remove protein, RNA or lipids did not affect this 'snap-back' property. Geshelin and Berns (1974) have further characterized and localized the cross-links in the vaccinia virus DNA. Electron micrographs of partially denatured DNA showing single stranded loops at each end were consistent with the concept that vaccinia virus DNA contains a covalent cross-link at each end of the molecule. Digestion of vaccinia DNA with single strand-specific endonuclease eliminates the cross-links and permits strand separation indicating that the native genome corresponds structurally to a single-stranded circular poly-

nucleotide chain continuously base-paired except for hairpin loops of single stranded DNA at each terminus.

Terminal cross-links are not unique to vaccinia virus DNA but seem to be a general characteristic of poxvirus genomes. Several investigators have made use of the cross-links to identify terminal DNA restriction fragments by their ability to reform duplex structures rapidly after release from denaturing conditions (DeFilippes, 1976; Jaureguiberry, 1977; Wittek et al., 1977; Wittek et al., 1978a; Mackett and Archard, 1979; Menna et al., 1979). The genomes of all poxviruses so far examined, including vaccinia, rabbitpox, cowpox, monkeypox, ectromelia, variola and even parapoxviruses appear by this criterion to be cross-linked at both termini.

The biological significance of the cross-links is unknown but for semiconservative replication to take place, they must be removed from the infecting DNA and this occurs soon after penetration into the cell (Pogo, 1977). The formation of the cross-links is a late step in the maturation of progeny DNA (Esteban and Holowczak, 1977). Recently, Pogo (1980) described an in vitro system capable of cross-linking DNA.

2.3. Presence of inverted, terminal repetitions in poxvirus DNA

Cross-hybridization between restriction fragments derived from the opposite ends of the rabbitpox virus DNA strongly suggested the presence of identical sequences at the termini of the genome (Wittek et al., 1977). A detailed restriction endonuclease analysis of the termini from DNA of rabbitpox virus or vaccinia virus (Lister strain) showed that these genomes contain large inverted terminal repetitions of 3.4–3.6 and 7.4–8.0 $\times 10^6$ daltons, respectively, (Wittek et al., 1978b). Garon et al. (1978), observed an inverted terminal repetition in the WR strain of vaccinia virus DNA by electron microscopy: self-annealing of single-stranded DNA, obtained by denaturation of virion DNA after treatment with single strand-specific endonuclease to remove the cross-links, generates single-stranded circles containing a duplex projection. The length of this 'panhandle' structure (3.54 + 0.12 μm) corresponds to a mol. wt of approximately 6.9 $\times 10^6$, equivalent to about 10,500 nucleotide base pairs, for the inverted terminal repetition.

Terminal repetition is not unique to the DNA of vaccinia or rabbitpox virus. Mackett and Archard (1979) have demonstrated such terminal repetitions in DNA from Monkeypox, cowpox or ectromelia virus. In contrast, terminal fragments of DNA from variola and some related viruses fail to cross-hybridize strongly with each other and thus may lack such an obvious repeat structure (Mackett and Archard, 1979; Dumbell and Archard, 1980). Similarly, the opposing termini of DNA from parapoxviruses do not cross-hybridize strongly and thus probably contain only very short, terminal repetitions (Menna et al., 1979).

2.4. Presence of tandem repeats within the terminal repetitions

Repetitive elements within the vaccinia virus genome were postulated on the basis of DNA reassociation kinetics (Grady and Paoletti, 1977; Pedrali-Noy and Weissbach, 1977). A DNA fragment containing almost the entire terminal repetition of vaccinia virus DNA has recently been cloned in a coliphage lambda vector (Wittek et al., 1980a). Using this cloned fragment, Wittek and Moss (1980b) have recently located and characterized repetitive elements present close to the end of the vaccinia virus genome. These consists of a 70 base pair sequence (bp) tandemly repeated in 2 blocks of 13 and 17 copies, respectively. The first repeating unit starts at approximately 150 base pairs from the cross-linked terminus of the DNA and the 2 sets are separated from each other by a sequence of 435 base pairs. Since these tandem repeats are part of the inverted terminal repetition, vaccinia virus DNA contains a total of 60 copies of the repeating 70 bp unit.

Preliminary evidence suggests that such tandem repeats are also present in the genome of the Lister strain of vaccinia virus and the closely-related rabbitpox virus (B. Moss, unpublished). A sequence related to the vaccinia 70 bp tandem repeat unit is present within the inverted terminal repetitions of the genomes of cowpox, ectromelia and monkeypox viruses although, in the case of cowpox virus at least, the size and organization of the tandem repeats differs from that in vaccinia virus DNA (L. Archard, M. Mackett and K.R. Dumbell; unpublished).

The function of such tandem repeats is not known. Wittek and Moss (1980b) proposed a model in which these play a role in DNA replication. Assuming a strand displacement mechanism of replication, initiation at the 3′ end of a displaced strand can proceed via a circular intermediate formed by annealing of the inverted terminal repetitions of that strand as proposed by Sambrook (cited by Daniell (1976)) and Lechner and Kelly (1977) for adenovirus DNA replication (fig. 2). Clearly, the presence of tandem repeats within the inverted terminal repetition would greatly increase the rate of nucleation and thus the formation of a putative circular intermediate. The principal structural elements of the vaccinia virus genome are summarized in figure 3.

2.5. Conservation and variation in wild-type poxvirus genome structure

Viruses belonging to the genus orthopoxvirus are very closely related serologically and differentiation is based mainly on phenotypic criteria such as host

range, pock morphology and ceiling growth temperature. These criteria, however, have frequently not allowed unequivocal classification of poxvirus isolates. Restriction endonuclease analysis of poxvirus genomes has proved to be the most precise and reliable method of differentiation. Two closely related strains of vaccinia virus could readily be distinguished by a small difference in the restriction patterns of their DNAs (Gangemi and Sharp, 1976). Müller et al. (1978) compared the cleavage patterns of rabbitpox, vaccinia, cowpox, ectromelia and fowlpox virus DNAs and from the similarities and dissimilarities concluded that vaccinia and rabbitpox virus are genetically the most closely related viruses investigated, although their DNA fragment patterns are clearly distinguishable. By the same criteria, cowpox and ectromelia virus both show about the same degree of relatedness to each other as to rabbitpox and vaccinia virus. In contrast, the cleavage pattern of the DNA of fowlpox, an avipox virus, shows no similarity with those of orthopoxvirus DNAs thus indicating a very low degree of genetic relatedness. Hybridization experiments revealed no sequence relationship of restriction fragments of DNA from representative orthopoxviruses to those of DNA from representative avipox or parapox viruses (N. McBride, M. Mackett and L. C. Archard, unpublished).

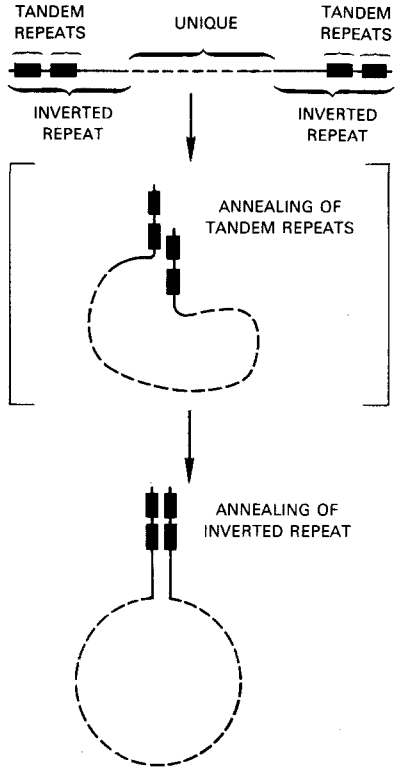

Figure 2. Model showing the cyclization of single strands of vaccinia virus DNA that are displaced during replication. The nucleation event consists of the annealing of 70 bp repeats. This is followed by hybridization of the remaining 7000 nucleotide portion of the inverted terminal repetition and final realignment to maximize base pairing. From Wittek and Moss, 1980b. Copyright: MIT Press; with permission.

Physical maps showing the order of restriction fragments clearly allow a more detailed comparison of the genomes of closely related viruses. Examination of the restriction maps of rabbitpox virus and vaccinia virus DNA reveals that the large number of fragments comigrating in the 2 DNAs, are all derived from an internal part of the genomes (Wittek et al., 1977). The differences between the restriction patterns and the length of the genomes are due mainly to sequence divergence near the ends of the molecules. Thus, a large internal region appears to be highly conserved between the 2 genomes. By mapping a large number of restriction sites on the same 2 DNAs Schümperli et al. (1980) have estimated more precisely a mol. wt of over 100×10^6 for the conserved region of these 2 closely related genomes. In addition, the inverted terminal repetition of both genomes contains a subset of common sequences (Wittek et al., 1978b). The physical maps of a large number of DNAs prepared by Mackett and Archard (1979) have allowed the most detailed comparison of orthopoxvirus genome structures. These authors have included rabbitpox, 4 vaccinia virus strains, 3 monkeypox virus strains, 2 variola virus strains, 4 cowpox virus strains and 2 ectromelia virus strains in their analysis (fig. 4). The physical maps are characteristic of species but similar for strains of the same orthopoxvirus species. Species-specific differences in genome structure consists mainly of extensive near terminal variations in sequence and in length and some such sequences are species unique (Mackett and Archard, 1979). In contrast, all

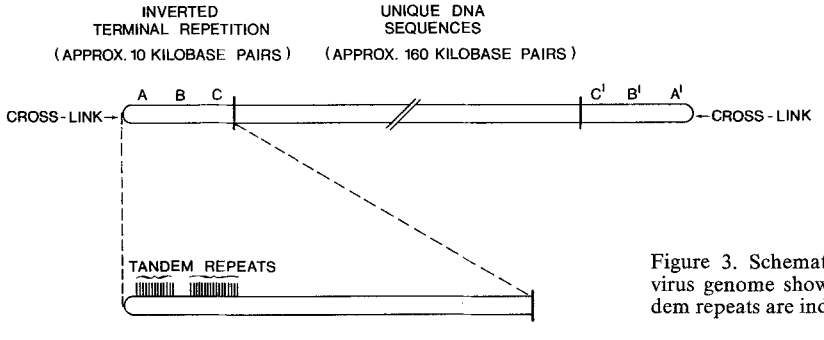

Figure 3. Schematic representation of the vaccinia (WR strain) virus genome showing the principal structural elements. The tandem repeats are indicated by *Hinf* I restriction sites.

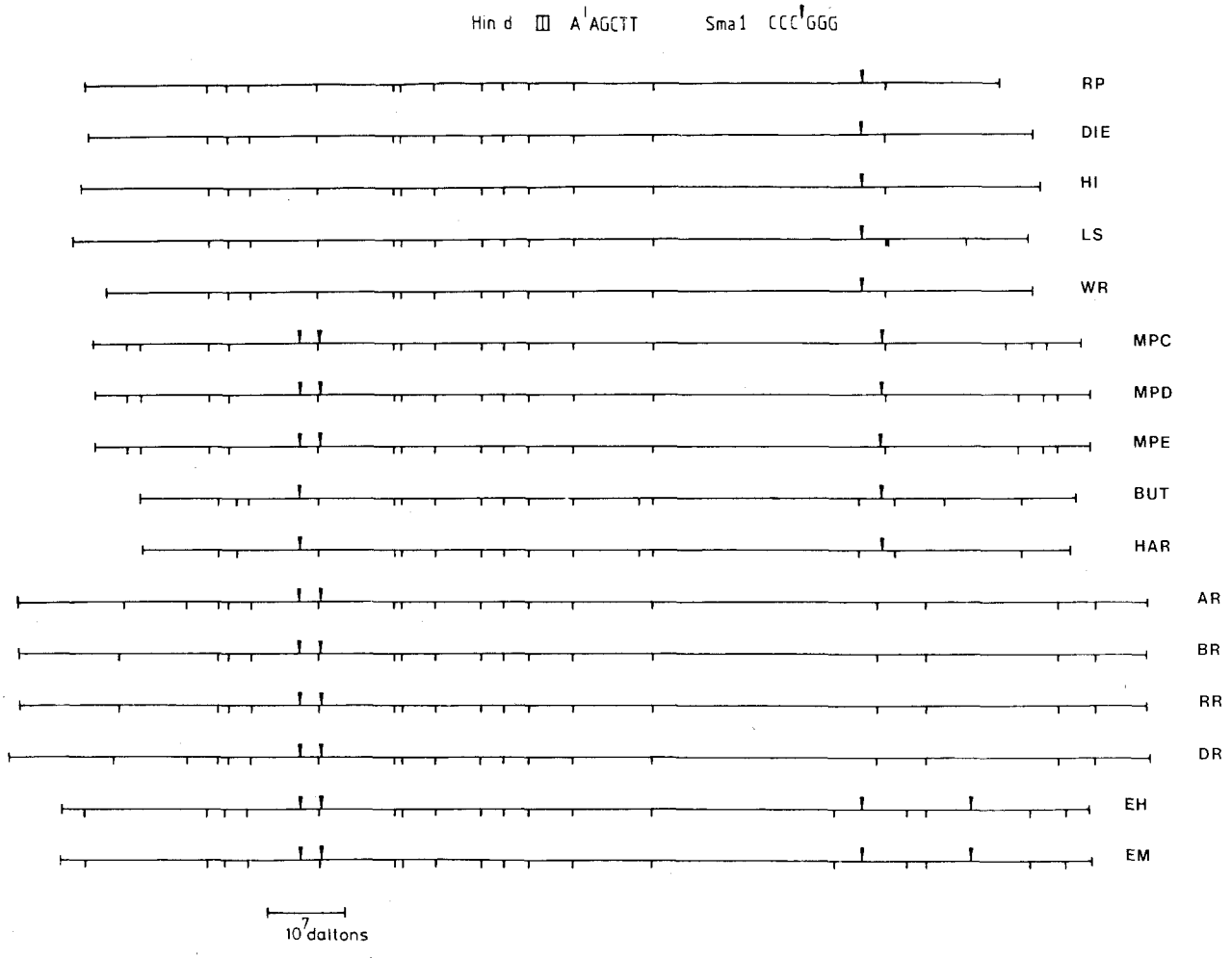

Figure 4. Physical map locations of *Hin*dIII or *Sma*I restriction fragments of DNA from rabbitpox strain Utrecht (RP); vaccinia strain DIE, Hall Institute (HI), Lister (LS) or Western Reserve (WR); monkeypox strain Congo (MPC), Denmark (MPD) or Espana (MPE); variola strain Butler (BUT) or Harvey (HAR); Cowpox red strain Austria (AR), Brighton (BR), Ruthin (RR) or Daisy (DR) and ectromelia strain Hampstead (EH) or Moscow (EM). Modified from Mackett and Archard, 1979. Copyright: Society for General Microbiology; with permission.

orthopoxvirus DNAs examined show a high degree of sequence conservation judged by location of restriction sites, in a central region consisting of approximately 30×10^6 daltons of the genome. However, species-specific differences in the distribution of restriction sites even within this region are not uncommon. Opposing terminal restriction fragments from the genomes of particular orthopoxviruses cross hybridize strongly and hybridize also with terminal fragments from the genomes of other species indicating that the terminal repetitions contain subsets of sequences common to most orthopoxvirus DNAs.

Little is known about genetic variation among parapoxviruses. Differentiation by serology is difficult, owing to their close antigenetic relationships. However, restriction enzyme analysis of various isolates of stomatitis papulosa and of orf viruses has indicated that parapoxviruses may represent a complex group of viruses containing several species (Wittek et al., 1980c).

2.6. Aberrations in poxvirus genome structures

The analysis of plaque (or pock) isolates of serially propagated stock virus and of orthopoxvirus variants has yielded a series of interesting mutants in genome structure. These mutants (described in the following section) provide further evidence that the termini of poxvirus genomes are hypervariable and may undergo extensive deletions and complex sequence rearrangements. In all known cases however, symmetrical termini are preserved.

Non-selectable variants

When DNA of vaccinia virus propagated from a commercially available smallpox vaccine was analyzed by restriction endonucleases, the terminal restriction fragments migrated as diffuse bands in gel electrophoresis suggesting length heterogeneity in the DNA molecule population (Wittek et al., 1978a). Pock purified virus from the same stock, yielded end

fragments of discrete size. Evidence for length heterogeneity in vaccinia virus DNA has also been obtained by McCarron et al., (1978). Although the molecular mechanism of the generation of terminal heterogeneity is not known, it is possible that recombination events within the terminal repetitions lead to different numbers of the small tandem repeats described previously. Another example of variability at the ends of orthopoxvirus DNA has been reported by McFadden and Dales (1979) who analyzed the genomes of a number of temperature-sensitive mutants of vaccinia virus (IHD-W strain). Approximately 20% of all clones examined showed near-terminal deletions of up to 250 base pairs.

These deletions never occurred asymmetrically but were present always as mirror-image deletions of both termini of the DNA molecule suggesting that conjunction between termini occurs at some point during viral DNA replication. The deletions are not related to the temperature sensitive phenotype since spontaneous ts^+ revertants retain the mirror image deletions.

A complex and bizarre variant in vaccinia virus DNA structure has been reported by Moss et al., (1981). From a serially propagated stock of vaccinia virus, 20 plaque isolates were picked randomly and their DNA analyzed by cleavage with restriction endonucleases. Four of these isolates did not contain the usual double molar end-fragment resulting from symmetrical cleavage within the inverted terminal repetition, but instead had a series of at least 8 distinct bands differing from each other by approximately 1.6–1.7 kilobase pair increments. A detailed examination of one such variant revealed that 1 set of tandem repeats and the unique sequence separating it from the 2nd set was reiterated many times. Since this feature was not eliminated by repeated plaque purification, the authors concluded that a population of DNA molecules with various numbers of reiterations can evolve rapidly from the DNA of a single virus particle. However, this genome structure appears to be unstable and, at each successive round of plaque purification, about 20% of the isolates containing such reiterations revert to the prototype containing only 2 blocks of tandem repeats. The authors propose the generation of unstable variants and reversion to more stable forms by unequal crossover (fig. 5).

Besides these relatively subtle variations at, or very close to the ends of the genome, a number of variants containing very large deletions have been described. Panicali et al. (1981) have isolated from serially propagated stocks of the WR strain of vaccinia virus a deletion mutant which is missing a region of 6.3×10^6 daltons of DNA mapping close to the left hand end of the genome. The deletion does not extend into the inverted terminal repetition but maps close to the junction of terminally repeated and unique DNA sequences. Mutants containing such large deletions appear to arise with high frequency since 2 such mutants were detected in a limited number of plaque isolates analyzed. This may be explained by the fact that, in the particular culture system, these variants apparently have no disadvantage and show similar kinetics of replication and yields of progeny when compared to wildtype virus.

Selectable variants

Whereas some mutants were detected by screening a number of individual, randomly picked, plaque isolates from a serially propagated stock virus, mutants exist which exhibit variant phenotypes.

A variant of vaccinia virus was originally recognized as a plaque morphology mutant after nitrous acid mutagenesis of a virus stock (Drillien et al, 1981). Restriction endonuclease analysis of its DNA revealed that this mutant had deleted a region of approximately 12.6×10^6 daltons from the left hand end of the genome including a portion of the inverted terminal repetition. The new fragment, presumably containing the non-deleted portion of the inverted terminal repetition fused to the DNA sequences located beyond the deletion, retained the terminal cross-link. This variant proved to be a host range mutant unable to multiply in most human cell lines. Although this mutant displays altered biological behavior, it is viable in some cell lines. Therefore, as much as 10% of left hand sequences of vaccinia virus DNA appear to be dispensable under certain conditions.

Orthopoxviruses such as rabbitpox, cowpox and monkeypox which normally produce ulcerated, haemorrhagic lesions (pocks) on the chorioallantoic mem-

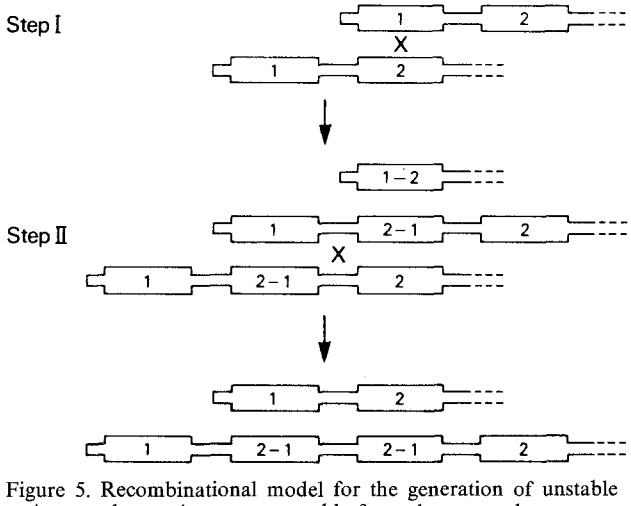

Figure 5. Recombinational model for the generation of unstable variants and reversion to more stable forms by unequal crossover. Ends of DNA molecules are represented. Each numbered segment encloses a set of 13–17 direct tandem repeats. In step I, recombination occurs between 2 sets of repeats to generate the unstable variant. In step II, recombination occurs within intervening regions (as shown) or between sets of tandem repeats. Further recombinational events lead to additional reiterations as well as to the original genome structure. From Moss et al., 1981, with permission.

brane of fertile hen's eggs generate non-haemorrhagic (white-pock) variants with a frequency of about 1%. Such variants behave as deletion mutants in that spontaneous reversion does not occur although, in the case of different classes of rabbitpox mutants, recombination may generate the wild-type phenotype (Lake and Cooper, 1980). A number of laboratories have investigated the genome structure of these white pock variants and the results are summarized briefly.

The white pock variants of rabbitpox virus can be subdivided into 2 classes based on their host-range properties.

The 1st class are mutants which fail to multiply in a pig kidney cell line. The same cell line is however, permissive for a 2nd class of white-pock mutants. The genome structure of host-range and non host-range white pock variants of rabbitpox virus has been analyzed in 2 laboratories. Lake and Cooper (1980) and Moyer and Rothe (1980a) have detected deletions of 3×10^6 to 20×10^6 daltons at the left hand terminus of the genome of all host-range mutants examined. In some mutants, the deletion extends well into the terminal repetition. In contrast, non host-range white pock mutants examined by Lake and Cooper (1980) are all characterized by extensive deletions (approximately 10×10^6 daltons mapping at the right-hand terminus of the genome). It is therefore not surprising that in mixed infections between host-range and non host-range mutants, recombinants are readily obtained which have the wild-type pock morphology and no host-range restriction and that no genetic interaction is detected in crosses involving only host-range or non host-range white pock mutants.

The most obvious feature of the mutants described above is the loss of DNA sequences from either terminus of the genome. In addition, white-pock mutants of rabbitpox virus (Moyer et al., 1980b) and of monkeypox virus (Esposito et al., 1981) have been described which are characterized by net additions of DNA. Detailed restriction endonuclease analysis, however, has shown, that these variants had also deleted sequences from either terminus of the genome but that this deletion was compensated by sequences copied from the opposing terminus. In the case of one of the rabbitpox virus mutants, a deletion of 6×10^6 daltons of the extreme left-hand sequences had been compensated by 28×10^6 daltons of DNA copied from the extreme right-hand end to yield a net increase in the molecular weight of the genome of 22×10^6 daltons (Moyer et al., 1980b). Similarly, sequences from the lefthand terminus were copied to a deleted right-hand terminus in the case of 2 monkeypox virus mutants described by Esposito et al., (1981). This transposition event resulted in the net increase in the total genome size of 8×10^6 daltons.

White pock variants of cowpox virus which are characterized by extensive deletions of 16×10^6 to 18×10^6 daltons from the right hand terminus of the genome (Archard and Mackett, 1979) proved to have a similarly complex genome structure. Further investigation showed that these deletions were compensated variably by sequences copied from the opposing terminus with the effect of restoring both an inverted terminal repetition and terminal covalent cross-link. The sequence copied to the deleted terminus was in one case larger than and, in another case, smaller than the original deletion (L.C. Archard, M. Mackett and K.R. Dumbell, in preparation).

Copying of sequences from 1 terminus to compensate the deleted opposing terminus may be a rather frequent event in poxvirus DNA replication. This again implies conjunction of termini at some stage of DNA replication and may explain the generation of some of the monkeypox mutants described by Dumbell and Archard (1980) and the vaccinia mutants described by Panicali et al. (1981) or Drillien et al. (1981). The overall effect is to restore symmetry of genome structure i.e. an inverted terminal repetition of some size, (plus terminal cross-link) which is probably a prerequisite for DNA replication if this depends on the annealing of opposing termini of displaced single strands.

2.7. Origin and nature of variola-like ('whitepox') viruses

The WHO smallpox eradication programme was based on the premise that smallpox is transmitted exclusively between humans with no animal reservoirs of the virus. Smallpox-like disease in man is caused occasionally by monkeypox virus although this agent is readily distinguishable from variola both phenotypically and by comparison of DNA restriction patterns (Esposito et al., 1978). However, as mentioned in the introduction, 6 poxviruses isolated apparently from animal tissues have proved to be indistinguishable from variola viruses by phenotypic criteria. Since these 6 isolates produce typically variola-like, white pocks on the chick chorioallantoic membrane, they have collectively been called 'whitepox' viruses. A possible explanation for the origin of 'whitepox' viruses has been presented in 2 reports (Marennikova and Shelukhina, 1978; Marennikova et al., 1979). Either by inoculating hamsters with monkeypox virus or by cloning monkeypox virus on the chick chorioallantoic membrane, white-pock variants were isolated which had all ascertainable properties of 'whitepox' viruses. The implication, that monkeypox virus which, on rare occasions, infects humans and produces a smallpox-like disease, can segregate a genetic variant apparently identical to variola virus, is alarming and could threaten the permanence of eradication. Dumbell and Archard (1980), therefore compared the genome structures of several white-pock

variants of monkeypox virus with parental monkeypox virus, with variola virus and with 2 'whitepox' viruses. The physical maps of parental monkeypox virus DNA were clearly distinguishable from those of variola virus. The white pock variants of monkeypox showed variation in genome structure ranging from apparently simple deletions close to the right-hand terminus to complex rearrangements involving both termini symmetrically. However, all monkeypox-white pock variants examined nevertheless retained the typical monkeypox genome structure in a large internal region of their DNA although some resembled variola virus in 3 of 4 phenotypic criteria examined. In contrast, both 'whitepox' viruses examined were essentially indistinguishable from variola virus and the minor differences in genome structure observed were due mainly to near-terminal variation which is common even in strains of the same orthopox virus species. Thus, from the physical maps, it appears unlikely that viruses with a variola-like genome structure can be derived from monkeypox virus by simple genetic variation.

The origin of 'whitepox' viruses is still in some doubt but, if animal reservoirs of variola-like viruses do exist, they have not been a source of reinfection of man in areas where smallpox has been eradicated.

3. Expression of the poxvirus genome

3.1. Temporal regulation of gene expression

Theoretically, the vaccinia virus genome is capable of coding for approximately 180 average-sized polypeptides and more than 100 polypeptides have been detected in purified virions (Essani and Dales, 1979). Evidence that expression of this large amount of genetic information is well regulated temporally has been obtained mainly by pulse-labelling infected or control cells with radioactive amino acids (review: Moss, 1974, 1978). Identification of labelled polypeptides as virus-specific is greatly facilitated by the rapid shut-off of host cell protein synthesis in infected cells (Salzman and Sebring, 1967; Moss and Salzman, 1968).

In the first few hours after infection, a class of early virus-specific polypeptides is detected. This pattern changes drastically after the onset of viral DNA replication and a 2nd class of late, or post-replicative, virus-specific polypeptides appears. Early polypeptides include many enzymes, some involved in DNA replication, whereas proteins synthesized late in infection are predominantly structural (review: Moss, 1974, 1978).

3.2. Core associated RNA polymerase

A characteristic feature of poxviruses in the DNA-dependent RNA polymerase associated with purified virus particles (Kates and McAuslan, 1967; Munyon et al., 1967). In addition, enzymes involved in the modification of the 5′ and 3′ ends of nascent RNA molecules to yield typical eucaryotic mRNA are located in vaccinia virus particles (review: Moss, 1978). Treatment of purified virions with a reducing agent and non-ionic detergent results in the disruption of the outer viral protein coat and in the release of viral cores. These cores contain the viral DNA and the enzymes involved in synthesis and modification of RNA. In appropriate incubation mixtures, such cores synthesize viral RNA for several hours and in large amounts. This RNA is referred to as in vitro RNA.

Vaccinia virus cores occur naturally during the earliest stages of infection of animal cells (Dales, 1963) and these cores also synthesize messenger RNA in vivo prior to the complete uncoating of the viral DNA (Kates and McAuslan, 1966). Due to the presence of an RNA polymerase in the cores, initial RNA synthesis does not require de novo protein synthesis. However, when RNA made in the presence of inhibitors of protein synthesis is translated in vitro, only early polypeptides are detected (Cooper and Moss, 1979a). The switch from early to late gene expression requires not only protein synthesis, which is a prerequisite for complete uncoating of the viral DNA, but also DNA replication (review: Moss, 1974, 1978).

3.3. Sequence relationships and relative abundance of early and late RNA

The fraction of the genomes transcribed early or late in infection was estimated by annealing of labelled DNA with an excess of RNA (Oda and Joklik, 1967; Kaverin et al., 1975; Paoletti and Grady, 1977; Boone and Moss, 1978). Although the values obtained vary, early RNA, made prior to DNA synthesis, was found to hybridize with approximately 25% of the total DNA. This corresponds to one half of the double-stranded genome if only 1 strand-equivalent is transcribed. RNA isolated late in infection hybridized to 1 entire strand-equivalent and hybridizations of DNA with a mixture of early and late RNAs suggested that the sequences transcribed early are a subset of the sequences present late (Oda and Joklik, 1967; Kaverin et al., 1975; Boone and Moss, 1978). Although synthesis of many early polypeptides cannot be detected at late times in infected cells, early RNA still appears to be present, in low amounts as judged by in vitro translation experiments (Cooper and Moss, 1979a).

3.4. Characteristics of in vitro RNA

The bulk of the RNA synthesized in vitro by vaccinia virus cores sediments in sucrose gradients as 10–12S species (Kates and Beeson, 1970a) and is identical in this respect to early in vivo RNA (Oda and Joklik, 1967). Further, in vitro RNA also is capped and

polyadenylated (review: Moss, 1978). In experiments using virus DNA immobilized on nitrocellulose filters, in vitro RNA was found to hybridize to between 14% (Kates and Beeson, 1970b) and 50% (Nevins and Joklik, 1975) of 1 strand-equivalent of the viral genome. The latter value is in good agreement with that obtained with in vivo early RNA (Oda and Joklik, 1967).

However, significantly higher values, similar to those obtained with in vivo late RNA, were found by RNA excess hybridizations in solution (Boone and Moss, 1978; Paoletti and Grady, 1977).

When RNA transcribed in vitro is isolated and translated in a cell-free system, the products resemble early proteins (Beaud et al., 1972; Fournier et al., 1973; Jaureguiberry et al., 1975; Nevins and Joklik, 1975).

Recently, a coupled transcription-translation system has been developped for vaccinia virus (Pelham, 1977; Cooper and Moss, 1978; Bossart et al., 1978; Pelham et al., 1978) and this system has been used to characterize further the polypeptides made from in vitro RNA. Representative polypeptides synthesized in vitro were subjected to partial proteolysis and shown to be identical to corresponding early polypeptides isolated from infected cells (Cooper and Moss, 1978).

A transcriptional and translational map of early RNAs transcribed in vivo from the inverted terminal repetition (Wittek et al., 1980d; Cooper et al., 1981a) was used to compare individual in vitro RNAs with respect to length, map positions and coding capacities (Venkatesan and Moss, 1981). By these criteria, 3 RNAs synthesized in vitro were found to be identical to the corresponding early RNAs made in vivo.

3.5. High molecular weight RNA

The bulk of the RNA synthesized in vitro by vaccinia virus cores sediments in sucrose gradients as 10–12S species (Kates and Beeson, 1970a). In addition, a small amount of larger RNA, sedimenting heterogeneously between 20 and 30S, has been found (Paoletti, 1977a). This high molecular weight RNA remains core-associated but pulse-chase experiments indicated that a fraction of it can be chased into RNA sedimenting at 8–12S which is subsequently extruded from the virus. Further, nucleic acid hybridization - competition studies indicated that virion-extruded 8–12S RNA contains sequences found in the high molecular weight, virion-associated RNA (Paoletti, 1977b). A soluble, endoribonuclease activity capable of cleaving high molecular weight RNA specifically into limit-sized fragments sedimenting at 8–12S was found associated with purified vaccinia virus particles (Paoletti and Lipinskas, 1978) and a possible precursor role of the high molecular weight RNA was therefore suggested (Paoletti, 1977b; Paoletti and Grady, 1977). However, using a coupled transcription-translation system, a linear relationship between the molecular weight of the proteins made in vitro and the rate of UV-inactivation was found (Pelham. 1977; Bossart et al., 1978). This argues strongly against tandem, polycistronic transcription of their mRNAs. Further, in 3 mRNAs studied in detail by Venkatesan and Moss (1981), capping occurs at the sites of initiation indicating that each RNA is synthesized from an individual promotor. The alternative explanation therefore, that high molecular weight RNA results from a defect in the termination of transcription, i.e. a read-through mechanism, may be favored.

High molecular weight RNAs are not unique to in vitro RNA but have recently also been detected in RNA preparations isolated from infected cells (Wittek et al., 1980d; Wittek et al., 1981; Cooper et al., 1981b). Again, the biological significance, if any, remains unclear.

3.6. Unusual properties of the late RNA

Virus specific double-stranded RNA has been demonstrated at both early and late times after vaccinia virus infection (Colby and Duesberg, 1969; Duesberg and Colby, 1969; Colby et al., 1972; Boone et al., 1979). Relatively little early, polyadenylic acid-containing RNA formed double-stranded structures upon self-annealing. However, late in infection, approximately 15% of the RNA was ribonuclease resistant after self-annealing (Boone et al., 1979). The same percentage of double-stranded RNA was also detected by annealing of labeled in vitro RNA to an excess of unlabeled late RNA (Paoletti and Grady, 1977). The biological significance of symmetrical transcription late in infection is not clear but explains the observation that self-annealed late RNA is not translatable unless denatured (Cooper and Moss, 1979a).

Another unusual property of late RNA was revealed by size fractionation under denaturing conditions (Cooper et al., 1981b) followed by in vitro translation. Most individual polypeptides were made by a continuous spectrum of mRNAs ranging from more than 6000 bases down to a size just large enough to contain the required coding information. It is possible that late in infection the termination mechanism becomes less effective and that the large mRNAs represent read-through transcription products.

3.7. Transcriptional mapping of the vaccinia virus genome

Late genes are clustered near the center of the genome

The extent of transcription of different regions of the vaccinia virus genome early and late in infection has been estimated by hybridization of labeled RNA to DNA restriction fragments (Cabrera et al., 1978). Except for a relatively small region near the center of the genome which is not transcribed in vitro by the

virion associated RNA polymerase and only partially transcribed in vivo in the absence of protein synthesis, RNA complementary to all other restriction fragments was detected both early and late in infection. However, hybridization-competition experiments indicated that a central region of the genome is transcribed to a greater extent late in infection.

A map of early and late genes was obtained by in vitro translation of RNA selected on restriction fragments of the viral DNA. Using the 3 largest *Hin*dIII fragments which together comprise over 50% of the genome, a total of 34 polypeptides were mapped (Cooper and Moss, 1979b).

Several early, but very few late polypeptides were coded by RNA selected on the opposing terminal fragments B or C. In contrast, both early and late genes were found in the *Hin*dIII fragment which maps adjacent to the right-hand *Hin*dIII terminal fragment B. Translational mapping of the remaining portion of the vaccinia virus genome was greatly facilitated by the availability of cloned DNA restriction fragments (Belle Isle et al., 1981). An additional 75 early and more than 40 specific late polypeptides were identified by polyacrylamide gel electrophoresis, among the translation products of RNA purified by hybridization selection to these fragments. Significantly, most late mRNAs appear to be clustered near the center of the genome. Interestingly, this region of the genome is highly conserved between different orthopoxviruses (Wittek et al., 1977; Mackett and Archard, 1979).

This clustering of late genes was not observed previously (Chipchase et al., 1980), possibly due to difficulties in synchronization of infection in the particular cell system used.

Analysis of individual mRNA encoded within the left 21 kilobase pairs of the vaccinia virus genome

The most detailed transcriptional and translational maps currently available have been established for the leftmost 21 kilobase pairs of the vaccinia virus genome (Wittek et al., 1980a; Wittek et al., 1980d; Cooper et al., 1981a; Wittek et al., 1981; Cooper et al., 1981b). Three continuous DNA fragments representing a stretch of DNA which includes the inverted terminal repetition have been cloned in coliphage lambda. Together these fragments comprise more than 10% of the genome. A total of 11 specific early and 2 specific late polypeptides were identified by in vitro translation of RNA selected on these fragments and the corresponding mRNAs were analyzed with respect to map position, length, direction of transcription and colinearity with the genome (fig. 6). Besides a putative role in DNA replication (Wittek and Moss, 1980b), the inverted terminal repetition of the vaccinia virus genome was found to encode 4 early mRNAs which direct the synthesis of 7500 (7.5K), 19K, 42K, or 21K polypeptides in vitro (Wittek et al., 1980a; Wittek et al., 1980d; Cooper et al., 1981a). Two mRNAs are transcribed towards the end of the genome and two in the opposite direction.

Interestingly, the direction of transcription of all mRNAs encoded by the unique DNA sequences is also towards the end of the genome. Three mRNAs coding for 14K, 32K or 38K polypeptides were found to be clustered between approximately 16.5 and 18.5 kilobase pairs from the end of the genome (fig. 6). At least 2, and possibly all 3 RNAs overlap each other (Cooper et al., 1981b). Since vaccinia virus replicates in the cytoplasm of infected cells, the question of whether the mRNAs are spliced is of particular interest. No evidence for splicing was obtained by detailed nuclease S1 analysis of the RNAs transcribed from the left 21 kilobase pairs of the vaccinia virus genome (Wittek et al., 1980d; Wittek et al., 1981; Cooper et al., 1981b).

4. The reproduction cycle of poxviruses

As discussed in the previous sections, reproduction of poxviruses involves a series of complex, temporally

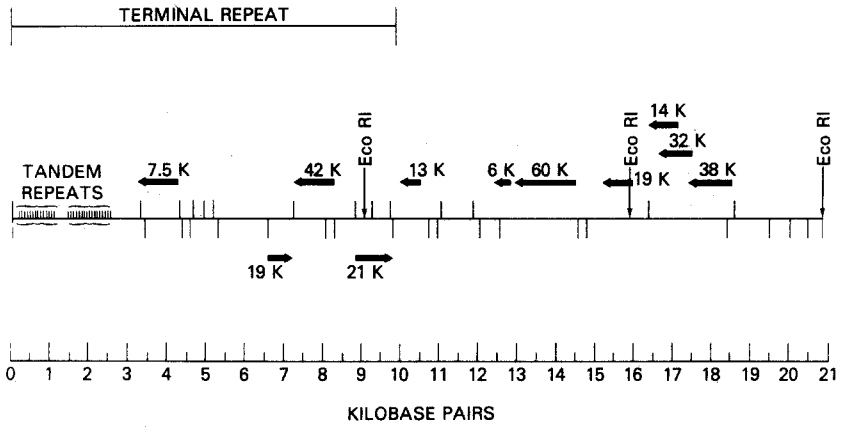

Figure 6. Transcriptional and translational map of the left 21 kilobase pairs of the vaccinia virus genome. Early mRNAs are shown with the sizes of the polypeptides that they encode. The 2 late mRNAs have been omitted. Except for the tandem repeats at the far left, the vertical lines above and below the horizontal are *Hpa*II and *Hin*cII sites, respectively. From Cooper, Wittek and Moss, J. Virol., in press. Copyright: American Society for Microbiology, with permission.

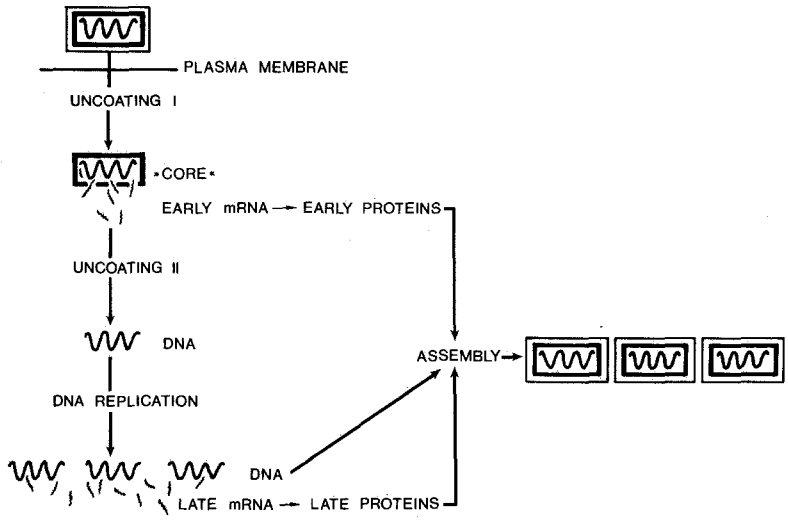

Figure 7. Reproduction of poxviruses.

well regulated events. These steps are depicted in figure 7 and outlined below.

a) The virus particles penetrate into the host cell.
b) The outer membranes of the virions disintegrate by a process referred to as uncoating I. This process leads to the generation of subviral particles (= cores).
c) RNA polymerase and enzymes involved in the modification of RNA are activated and a first burst of early RNA synthesis occurs within the cores.
d) The RNA is extruded and translated on host cell ribosomes into early proteins.
e) The viral DNA is released from the cores (= uncoating II) and replicated.
f) Late RNA is transcribed presumably from progeny DNA and translated into late polypeptides.
g) Early and late polypeptides, as well as DNA are assembled in a complex morphogenesis process.

Acknowledgments.
I am very greatly indebted to Len Archard for his critical reading of the manuscript and for communicating unpublished information. I thank Mathias Ackermann, Ernst Peterhans and Robert Wyler for helpful suggestions, Sonja Pletscher and Anita Hug for preparing the illustrations and Erika Boeniger and Christina Gerber for assistance with the preparation of the manuscript.

References

Archard, L.C., and Mackett, M., 1979. Restriction endonuclease analysis of red cowpox virus and its white pock variant. J. gen. Virol. *45*, 51-63.
Beaud, G., Kirn, A., and Gros, F., 1972. In vitro protein synthesis directed by RNA transcribed from vaccinia DNA. Biochem. biophys. Res. Commun. *49*, 1459-1466.
Belle Isle, H., Venkatesan, S., and Moss, B., 1981. Cell-free translation of early and late mRNA's selected by hybridization to cloned DNA fragments derived from the left 14 million to 72 million daltons of the vaccinia virus genome. Virology *112*, 306-317.
Berns, K.I., and Silverman, C., 1970. Natural occurrence of cross-linked vaccinia virus deoxyribonucleic acid. J. Virol. *5*, 299-304.
Boone, R.F., and Moss, B., 1978. Sequence complexity and relative abundance of vaccinia virus mRNA's synthesized in vivo and in vitro. J. Virol. *26*, 554-569.
Boone, R.F., Parr, R.P., and Moss, B., 1979. Intermolecular duplexes formed from polyadenylated vaccinia virus RNA. J. Virol. *30*, 365-374.
Bossart, W., Nuss, D.L., and Paoletti, E., 1978. Effect of UV-irradiation on the expression of vaccinia virus gene products synthesized in a cell-free system coupling transcription and translation. J. Virol. *26*, 673-680.
Cabrera, C.V., Esteban, M., McCarron, R., McAllister, W.T., and Holowczak, J.A., 1978. Vaccinia virus transcription: hybridization of mRNA to restriction fragments of vaccinia DNA. Virology *86*, 102-114.
Chipchase, M., Schwendimann, F., and Wyler, R., 1980. A map of the late proteins of vaccinia virus. Virology *105*, 261-264.
Colby, C., Jurale, C., and Kates, J.R., 1971. Mechanism of synthesis of vaccinia virus double-stranded ribonucleic acid in vivo and in vitro. J. Virol. *7*, 71-76.
Colby, C., and Duesberg, P.H. 1969. Double-stranded RNA in vaccinia virus infected cells. Nature *222*, 940-944.
Cooper, J.A., and Moss, B., 1978. Transcription of vaccinia virus mRNA coupled to translation in vitro. Virology *88*, 149-165.
Cooper, J.A., and Moss, B., 1979a. In vitro translation of immediate early, early, and late classes of RNA from vaccinia virus-infected cells. Virology *96*, 368-380.
Cooper, J.A., and Moss, B., 1979b. Translation of specific vaccinia virus RNAs purified as RNA-DNA hybrids on potassium iodide gradients. Nucl. Acids Res. *6*, 3599-3612.
Cooper, J.A., Wittek, R., and Moss, B., 1981a. Hybridization selection and cell-free translation of mRNA's encoded within the inverted terminal repetition of the vaccinia virus genome. J. Virol. *37*, 284-291.
Cooper, J.A., Wittek, R., and Moss, B., 1981b. Extension of the transcriptional and translational map of the left end of the vaccinia virus genome to 21 kilobase pairs. J. Virol. *39*, 733-745.
Dales, S., 1963. The uptake and development of vaccinia virus in strain L cells followed with labelled deoxyribonucleic acid. J. Cell Biol. *18*, 51-72.
Daniell, E., 1976. Genome structure of incomplete particles of adenovirus. J. Virol. *19*, 685-708.
DeFilippes, F., 1976. Restriction enzyme digests of rapidly renaturing fragments of vaccinia virus DNA. J. Virol. *17*, 227-238.
Drillien, R., Koehren, F., and Kirn, A., 1981. Host range deletion mutant of vaccinia virus defective in human cells. Virology *111*, 488-499.
Duesberg, P.H., and Colby, C., 1969. On the biosynthesis and structure of double-stranded RNA in vaccinia virus-infected cells. Proc. natl Acad. Sci. USA *64*, 393-403.
Dumbell, K.R., and Archard, L.C., 1980. Comparison of white-pock (h) mutants of monkeypox virus with parental monkeypox and with variola-like viruses isolated from animals. Nature *286*, 29-32.

Esposito, J.J., Obijeski, J.F., and Nakano, J.H., 1978. Orthopoxvirus DNA: strain differentiation by electrophoresis of restriction endonuclease fragmented virion DNA. Virology *89,* 53–66.

Esposito, J.J., Cabradilla, C.D., Nakano, J.H., and Obijeski, J.F., 1981. Intragenomic sequence transposition in monkeypox virus. Virology *109,* 231–243.

Essani, K., and Dales, S., 1979. Biogenesis of vaccinia: Evidence for more than 100 polypeptides in the virion. Virology *95,* 385–394.

Esteban, M., and Holowczak, J.A., 1977. Replication of vaccinia DNA in mouse L cells. I. In vivo DNA synthesis. Virology *78,* 57–75.

Fenner, F., 1976. The classification and nomenclature of viruses. Summary of results of meetings of the International Committee on Taxonomy of viruses in Madrid, September 1975. J. gen. Virol. *31,* 463–470.

Fournier, F., Tovell, D.R., Esteban, M., Metz, D.H., Ball, L.A., and Kerr, I.M., 1973. The translation of vaccinia virus messenger RNA in animal cell-free systems. FEBS Lett. *30,* 268–272.

Gafford, L.G., and Randall, C.C., 1967. The high molecular weight of the fowl pox virus genome. J. molec. Biol. *26,* 303–310.

Gafford, L.G., and Randall, C.C., 1970. Further studies on the high molecular weight fowlpox virus DNA and its hydrodynamic properties. Virology *40,* 298–306.

Gangemi, J.D., and Sharp, D.G., 1976. Use of a restriction endonuclease in analyzing the genomes from two different strains of vaccinia virus. J. Virol. *20,* 319–323.

Garon, C.F., Barbosa, E., and Moss, B., 1978. Visualization of an inverted terminal repetition in vaccinia virus DNA. Proc. natl Acad. Sci. USA *75,* 4863–4867.

Geshelin, P., and Berns, K.I., 1974. Characterization and localization of the naturally occurring cross-links in vaccinia virus DNA. J. molec. Biol. *88,* 785–796.

Grady, L.J., and Paoletti, E., 1977. Molecular complexity of vaccinia DNA and the presence of reiterated sequences in the genome. Virology *79,* 337–341.

Jaureguiberry, G., Ben-Hamida, F., Chapeville, F., and Beaud, G., 1975. Messenger activity of RNA transcribed in vitro by DNA-RNA polymerase associated to vaccinia virus cores. J. Virol. *15,* 1467–1474.

Jaureguiberry, G., 1977. Cleavage of vaccinia virus DNA by restriction endonuclease *Bal*I, *Eco*RI, *Bam*HI. Isolation of the natural cross-links. FEBS Lett. *83,* 111–117.

Joklik, W.K., 1962a. The purification of four strains of poxvirus. Virology *18,* 9–18.

Joklik, W.K., 1962b. Some properties of poxvirus deoxyribonucleic acid. J. molec. Biol. *5,* 265–274.

Jungwirth, C., and Dawid, I.B., 1967. Vaccinia DNA: Separation of viral from host cell DNA. Arch. Virol. *20,* 464–468.

Kates, J.R., and McAuslan, B.R., 1966. Messenger RNA synthesis by a coated viral genome. Proc. natl Acad. Sci. USA *57,* 314–320.

Kates, J.R., and McAuslan, B.R., 1967. Poxvirus DNA-dependent RNA polymerase. Proc. natl Acad. Sci. USA *58,* 134–141.

Kates, J.R., and Beeson, J., 1970a. Ribonucleic acid synthesis in vaccinia virus. II. Synthesis of polyriboadenylic acid. J. molec. Biol. *50,* 19–33.

Kates, J.R., and Beeson, J., 1970b. Ribonucleic acid synthesis in vaccinia virus. I. The mechanism of synthesis and release of RNA in vaccinia cores. J. molec. Biol. *50,* 1–18.

Kaverin, N.V., Varich, N.L., Surgay, V.V., and Chernos, V.I., 1975. A quantitative estimation of poxvirus genome fraction transcribed as 'early' and 'late' mRNA. Virology *65,* 112–119.

Lake, J.R., and Cooper, P.D., 1980. Deletions of the terminal sequences in the genomes of the white-pock (u) and host-restricted (p) mutants of rabbitpox virus. J. gen. Virol. *48,* 135–147.

Lechner, R.L., and Kelly, T.J., Jr, 1977. The structure of replicating adenovirus 2 DNA molecules. Cell *12,* 1007–1020.

Mackett, M., and Archard, L.C., 1979. Conservation and variation in orthopoxvirus genome structure. J. gen. Virol. *45,* 683–701.

McCarron, R.J., Cabrera, C.V., Esteban, M., McAllister, W.T., and Holowczak, J.A., 1978. Structure of vaccinia DNA: Analysis of the viral genome by restriction endonucleases. Virology *86,* 88–101.

McFadden, G., and Dales, S., 1979. Biogenesis of poxviruses: mirror-image deletions in vaccinia virus DNA. Cell *18,* 101–108.

Marennikova, S.S., and Shelukhina, E.M., 1978. Whitepox virus isolated from hamsters inoculated with monkeypox virus. Nature *276,* 291–292.

Marennikova, S.S., Shelukhina, E.M., Maltseva, N.N., and Matsevich, G.R., 1979. Monkeypox virus as a source of whitepox viruses. Intervirology *11,* 333–340.

Menna, A., Wittek, R., Bachmann, P.A., Mayr, A., and Wyler, R., 1979. Physical characterization of a stomatitis papulosa virus genome: a cleavage map for the restriction endonucleases *Hin*dIII and *Eco*RI. Arch. Virol. *59,* 145–156.

Moss, B., and Salzman, N.P., 1968. Sequential protein synthesis following vaccinia virus infection. J. Virol. *2,* 1016–1027.

Moss, B., 1974. Reproduction of poxviruses; in: Comprehensive virology vol. 3, pp. 405–473. Ed. H. Fraenkel-Conrat, and R.R. Wagner. Plenum Press, New York.

Moss, B., 1978. Poxviruses; in: The molecular biology of animal viruses vol. 2, pp. 849–890. Ed. D.P. Nayak. Marcel Dekker, New York.

Moss, B., Winters, E., and Cooper, N., 1981. Instability and reiteration of DNA sequences within the vaccinia virus genome. Proc. natl Acad. Sci. USA *78,* 1614–1618.

Moyer, R.W., and Rothe, C.T., 1980a. The white pock mutants of rabbit poxvirus I. Spontaneous host range mutants contain deletions. Virology *102,* 119–132.

Moyer, R.W., Graves, R.L., and Rothe, C.T., 1980b. The white-pock (u) mutants of rabbit poxvirus. III Terminal DNA sequence duplication and transposition in rabbit poxvirus. Cell *22,* 545–553.

Müller, H.K., Wittek, R., Schaffner, W., Schümperli, D., Menna, A., and Wyler, R., 1978. Comparison of five poxvirus genomes by analysis with restriction endonucleases *Hin*dIII, *Bam*I and *Eco*RI. J. gen. Virol. *38,* 135–147.

Munyon, W., Paoletti, E., and Grace, J.T., Jr, 1967. RNA polymerase activity in purified infectious vaccinia virus. Proc. natl Acad. Sci. USA *58,* 2280–2287.

Nevins, J.R., and Joklik, W.K., 1975. Poly (A) sequences of vaccinia virus messenger RNA: Nature, mode of addition and function during translation in vitro and in vivo. Virology *63,* 1–14.

Oda, K., and Joklik, W.K., 1967. Hybridization and sedimentation studies on 'early' and 'late' vaccinia messenger RNA. J. molec. Biol. *27,* 395–419.

Panicali, D., Davis, S.W., Mercer S.R., and Paoletti, E., 1981. Two major DNA variants present in serially propagated stocks of the WR strain of vaccinia virus. J. Virol. *37,* 1000–1010.

Paoletti, E., and Grady, L.J., 1977. Transcriptional complexity of vaccinia virus in vivo and in vitro. J. Virol. *23,* 608–615.

Paoletti, E., 1977a. In vitro synthesis of a high molecular weight virion associated RNA by vaccinia. J. biol. Chem. *252,* 866–871.

Paoletti, E., 1977b. High molecular weight virion-associated RNA of vaccinia. A possible precursor to 8–12 S mRNA. J. biol. Chem. *252,* 872–877.

Paoletti, E., and Lipinskas, B.R., 1978. Soluble endoribonuclease activity from vaccinia virus: specific cleavage of virion-associated high-molecular-weight RNA. J. Virol. *26,* 822–824.

Pedrali-Noy, G., and Weissbach, A., 1977. Evidence of a repetitive sequence in vaccinia virus DNA. J. Virol. *24,* 406–407.

Pelham, H.R.B., 1977. Use of coupled transcription and translation to study mRNA production by vaccinia cores. Nature *269,* 532–534.

Pelham, H.R.B., Sykes, J.M.M., and Hunt, T., 1978. Characteristics of a coupled cell-free transcription and translation system directed by vaccinia cores. Eur. J. Biochem. *82,* 199–209.

Pogo, B.G.T., 1977. Elimination of naturally occurring crosslinks in vaccinia virus DNA after penetration into cells. Proc. natl Acad. Sci. USA *74,* 1739–1742.

Pogo, B.G.T., 1980. Terminal crosslinking of vaccinia DNA strands by an in vitro system. Virology *100,* 339–347.

Salzman, N.P., and Sebring, E.D., 1967. Sequential formation of vaccinia virus proteins and viral deoxyribonucleic acid replication. J. Virol. *1,* 16–23.

Schümperli, D., Menna, A., Schwendimann, F., Wittek, R., and Wyler, R., 1980. Symmetrical arrangement of the heterologous regions of rabbit poxvirus and vaccinia virus DNA. J. gen. Virol. *47,* 385–398.

Szybalski, W., Erikson, R.L., Gentry, G.A., Gafford, L.G. and Randall, C.C., 1963. Unusual properties of fowlpox virus DNA. Virology *19,* 586–589.

Venkatesan, S., and Moss, B., 1981. In vitro transcription of the inverted terminal repetition of the vaccinia virus genome: correspondence of initiation and cap sites. J. Virol. *37,* 738–747.

Wittek, R., Menna, A., Schümperli, D., Stoffel, S., Müller, H.K., and Wyler, R., 1977. *Hin*dIII and *Sst*I restriction sites mapped on rabbitpox virus and vaccinia virus DNA. J. Virol. *23*, 669–678.
Wittek, R., Müller, H.K., Menna, A., and Wyler, R., 1978a. Length heterogeneity in the DNA of vaccinia virus is eliminated on cloning the virus. FEBS Lett. *90*, 41–46.
Wittek, R., Menna, A., Müller, H.K., Schümperli, D., Bosely, P.G., and Wyler, R., 1978b. Inverted terminal repeats in rabbit poxvirus and vaccinia virus DNA. J. Virol. *28*, 171–181.
Wittek, R., Kuenzle, C.C., and Wyler, R., 1979. High C+G content in parapoxvirus DNA. J. gen. Virol. *43*, 231–234.
Wittek, R., Barbosa, E., Cooper, J.A., Garon, C.F., Chan, H., and Moss, B., 1980a. Inverted terminal repetition in vaccinia virus DNA encodes early mRNAs. Nature *285*, 21–25.
Wittek, R., and Moss, B., 1980b. Tandem repeats within the inverted terminal repetition of vaccinia virus DNA. Cell *21*, 277–284.
Wittek, R., Herlyn, M., Schümperli, D., Bachmann, P.A., Mayr, A., and Wyler, R., 1980c. Genetic and antigenetic heterogeneity of different parapoxvirus strains. Intervirology *13*, 33–41.
Wittek, R., Cooper, J., Barbosa, E., and Moss, B., 1980d. Expression of the vaccinia virus genome: analysis and mapping of mRNA's encoded within the inverted terminal repetition. Cell *21*, 487–493.
Wittek, R., Cooper, J., and Moss, B., 1981. Transcriptional and translational mapping of a 6.6 kilobase-pair DNA fragment containing the junction of the terminal repetition and unique sequence at the left end of the vaccinia virus genome. J. Virol. *39*, 722–723.
World Health Organization, 1980. The global eradication of smallpox. World Health Organization, Geneva.

SPECIALIA

The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – Ответственность за короткие сообщения несёт исключительно автор. – Solo los autores son responsables de las opiniones expresadas en estas comunicationes breves.

Identification of an acyclic diterpene alcohol in the defense secretion of soldiers of *Reticulitermes lucifugus*[1]

R. Baker, A.H. Parton and P.E. Howse

Department of Chemistry, The University, Southampton, SO9 5NH (England), 8 July 1981

Summary. The defense secretion of soldiers of *Reticulitermes lucifugus* has been shown to contain, predominantly, (R)-(−)-(E,E)-geranyllinalool together with germacrene A and β-farnesene.

Soldiers of the termite *Reticulitermes lucifugus*, which is found in many areas of Southern Europe, produce a cephalic (frontal) gland secretion in response to provocation.

We wish to report results of chemical analysis of the secretion, including the identification of an acyclic diterpene alcohol, (R)-(-)-(E,E)-geranyllinalool (**1**) not previously found in termite defense secretions. Preliminary trials of biological activity using synthetic racemic (**1**) show that it is toxic to *Atta cephalotes*, *Camponotus vagus* and *Crematogaster scutellaris* on topical application. In feeding tests, quantities equivalent to those found in 10 soldiers were repellent to the 2 latter species[2]. Further tests are underway to determine the mechanisms of repellency and toxicity. Other components of the secretion are the sesquiterpene hydrocarbons germacrene A (**2**) and β-farnesene (**3**).

Soldiers of *Reticulitermes lucifugus* collected from South-West Spain (near Cadiz) were immersed in dichloromethane and the solution decanted and concentrated. Gas chromatography – mass spectrometry of the extract indicated the presence of sesquiterpene hydrocarbons (M^+ 204, approximately 10 µg/insect) and 1 diterpene alcohol (M^+290, approximately 40 µg/insect). Hydrocarbons of the type commonly found in insect cuticle were also recognized in the extract. A small sample of secretion collected from live soldiers, however, contained the terpene components only.

The diterpene was isolated by HPLC (reverse phase); 7 mg, prepared from approximately 200 soldiers, was used to obtain a 100 MHz ^{1}H-NMR-spectrum which confirmed the structure to be (E,E)-geranyllinalool by comparison with the previously published spectrum[3]. The chemical shifts of methyl groups on the double bonds (6H, 1.58δ; 3H, 1.60δ and 3H, 1.66δ, in CCl_4) enable the double bond geometry to be assigned E,E. Furthermore the optical rotation ($[\alpha]_D^{22} = -20° \pm 5°$) indicated that (**1**) was the enantiomer with R-(-)-configuration[3].

The major sesquiterpene component underwent rearrangement during gas chromatography and HPLC. When the