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Evolution of Pandemic Influenza Virus Strains

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Evolution of pandemic influenza virus strains

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It was found by the technique of molecular hybridization that the pandemic influenza virus strains of 1957 (H2N2) and 1968 (H3N2) evolved by reassortment of RNA segments from the foregoing pandemic strains, replacing four genes including those coding for haemagglutinin (HA) and neuraminidase (NA) (1957) or only that coding for the haemagglutinin (1968), respectively. The earlier pandemic strains from 1918–9 (as deduced from the swine influenza strain (Hsw1N1), which is assumed to be a survivor of the Spanish influenza), from 1933–4 (H0N1), and from 1947 (H1N1) were derived from each other through a number of point mutations only. The Russian strain from 1977 (H1N1) is genetically almost identical with the FW strain from 1950 (H1N1).

In contrast to the conserved genes coding for the internal viral proteins, the genes coding for the viral surface glycoproteins consist of a relatively small highly conserved part which presumably is responsible for the functional integrity of the gene products, and the relatively large variable region which probably determines the antigenic property of their gene products. This structural feature of the surface glycoprotein genes explains the fast antigenic drift by mutations in the variable region and selection by the immune response of the host.

Introduction

Two different types of antigenic variation occur in influenza viruses: (1) the antigenic *drift* is thought to be caused by a number of point mutations of the genes coding for the two viral surface glycoproteins haemagglutinin (HA) and neuraminidase (NA), and selection by the immune response of the host; (2) the antigenic *shift* – this means the sudden appearance of a completely new human influenza virus strain – has been suggested to be due to replacement of the total HA and/or NA genes of a human strain for the corresponding genes of an animal influenza virus (for a review see Webster & Laver 1975).

Techniques of hybridization and assignment of RNA segments to gene functions

We have explored these two possible mechanisms by employing the technique of molecular hybridization. For this purpose we labelled the viral genome, which consists of eight single-stranded RNA segments. Each individual ^{32}P-labelled virion RNA (vRNA) segment was hybridized with a surplus of unlabelled complementary RNA (cRNA) isolated from infected cells. The RNase-resistant radioactivity of the double-stranded RNA was determined; this is 100% of the input radioactivity for a homologous hybridization. If a cRNA of a heterologous influenza A strain was used for hybridization, this value was in most cases less than 100% depending on the genetic relatedness, that is the base sequence homology between the allelic genes of the two strains under comparison.

If two allelic genes differ only by a few point mutations the test can be rendered so sensitive by determining the melting profiles in the presence of 1% formaldehyde that even such minor differences of a drift can be detected unequivocally (Scholtissek *et al.* 1976).

The separation of the eight ^{32}P-labelled vRNA segments of fowl plague virus (A/FPV/Rostock/34; Hav1N1) by polyacrylamide gel electrophoresis is shown in figure 1. The assignment of the various vRNA segments to gene functions was accomplished by using specific temperature-sensitive (*ts*) mutants of FPV for the production of recombinants in which the gene with the *ts* lesion was replaced (Scholtissek *et al.* 1976).

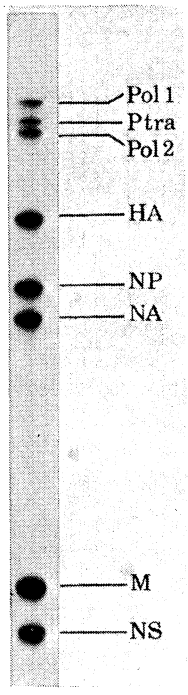

FIGURE 1. Separation of ^{32}P-labelled vRNA segments of fowl plague virus by polyacrylamide gel electrophoresis (left), and assignment of the segments to gene functions (right). Pol 1, polymerase 1 gene; Ptra, transport gene; Pol 2, polymerase 2 gene; HA, haemagglutinin gene; NP, nucleoprotein gene; NA, neuraminidase gene; M, matrix protein gene; NS, non-structural protein gene.

ANTIGENIC SHIFT BY REASSORTMENT OF VIRAL RNA SEGMENTS

A possible mechanism for the creation of a completely new influenza virus strain by an antigenic shift is outlined in figure 2. By double infection of the same host cell by two different influenza viruses the vRNA segments can be reassorted, and theoretically 254 ($2^8 - 2$) new combinations could emerge. For example, the recombinant shown in figure 2 could have gained the gene for the main immunogenic component (HA) of the animal strain for which no immunity would be found in the human population. This recombinant could therefore have the potential to cause a pandemic. If this theory is correct, one should find by comparison of two consecutive pandemic strains that most of the RNA segments exhibit a base sequence homology of nearly 100%, while at least the gene coding for HA should be significantly lower. The A/Singapore/1/57 (H2N2) strain has been compared by the hybridization technique with the preceding pandemic prototype strain of 1947 (A/FM/1/47; H1N1) and the succeeding pandemic strain of 1968 (A/Hong Kong/1/68; H3N2) (table 1). It is evident that

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the H1N1 and H2N2 strains have segments 1, 5, 7, and 8 in common. Two of the replaced genes are important in this respect, since they code for the surface glycoproteins HA (4) and NA (6). They are relatively variable compared with the rather conserved genes coding for the internal proteins. Concerning the H3N2 strain, only the HA gene is derived from another virus. Here we have exactly the situation as outlined in figure 2. Segment 4 of the Hong Kong strain has a base sequence homology of 92% to an avian influenza strain (A/duck/Ukraine/1/63; Hav7Neq2) indicating that these two strains had a close ancestor of their HA genes (Scholtissek *et al.* 1978 *a*). Thus, the data presented in table 1 are compatible with the idea that the H2N2 strain is derived from an H1N1 strain by reassortment and by keeping four segments of the foregoing strain. Similarly, the H3N2 strain is derived from the H2N2 strain by retention of seven segments of the latter strain.

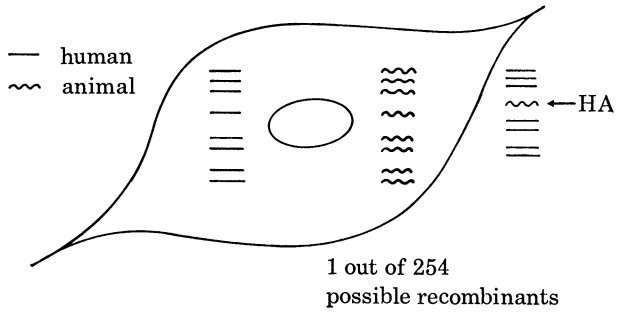

FIGURE 2. Scheme for the creation of a new pandemic human influenza strain by reassortment during double-infection of one host cell with human and animal influenza A virus.

TABLE 1. BASE SEQUENCE HOMOLOGIES (PERCENTAGES) BETWEEN ^{32}P-LABELLED vRNA SEGMENTS OF THE A/SINGAPORE/1/57 (H2N2) STRAIN (=100%) AND THE A/FM/1/47 (H1N1) AND A/HONG KONG/1/68 (H3N2) STRAINS

vRNA segments H2N2 ...	1	2	3	4	5	6	7	8
cRNA								
H1N1	98	70	76	24	94	29	97	98
H3N2	98	96	97	24	97	96	98	98

CREATION OF NEW PANDEMIC STRAINS BY STRONG ANTIGENIC DRIFTS

An analysis of earlier pandemic strains by the same technique has revealed that the PR8 strain from 1934 (A/PR/8/34; H0N1) is derived from the A/swine/1976/31 (Hsw1N1) strain (presumably a survivor of the Spanish influenza of 1918–9) *not* by reassortment. Furthermore, the FM1 strain of 1947 is derived from the PR8 strain *not* by reassortment, but by point mutations, since these strains exhibit nearly 100% base sequence homology in all eight RNA segments (Scholtissek *et al.* 1977 *a*). Although these strains do not cross-react in the neutralization or in the haemagglutination inhibition tests, other serological tests revealed corresponding cross-reactions. This is in accordance with the idea that these early pandemic strains are derived from each other by rather strong drifts and not by reassortment.

In this respect it is of special interest that the new Russian influenza strain of 1977 (A/USSR/90/77; H1N1) is virtually identical genetically with the Fort Warren isolate of 1950

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(A/FW/1/50; H1N1) when the melting profiles in the presence of formaldehyde are compared (figure 3). There are relatively great differences in the HA genes (figure 3*a*) between the FW strain and the drift viruses FM1 of 1947 and A/Loy/4/57 (H1N1) of 1957, while only minor differences were observed between the Russian strain and the FW strain at relatively high temperatures. Thus, the latter two strains still differ by a few point mutations in the HA gene. Concerning the nucleoprotein (NP) gene, which belongs to the highly conserved genes, such differences were not observed, although the FW virus differed slightly from the two drifted viruses FM1 and Loy (figure 3*b*). In this respect the variable NA gene resembles the HA gene, and the NP gene resembles the other conserved genes coding for the P-proteins,

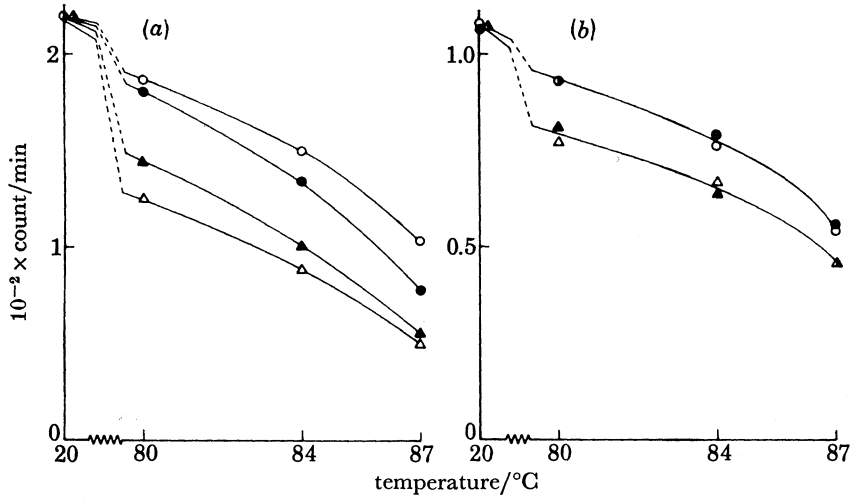

FIGURE 3. Melting profiles of RNA hybrid molecules of H1N1 influenza viruses. (*a*) The haemagglutinin gene (^{32}P-labelled segment 4) of the FW strain was hybridized with unlabelled cRNA of the FW strain (○), the USSR strain (●), the FM1 strain (▲), or Loy strain (△). (*b*) The nucleoprotein gene (^{32}P-labelled segment 5) of the FW strain was used for hybridization.

the matrix (M) protein and the non-structural (NS) protein (Scholtissek *et al.* 1978*b*). A mechanism has been proposed recently by which a human influenza strain might survive in an animal reservoir without a significant drift: the host range might be changed by recombination, and after many years by a second recombination the original host range might be regained, always keeping the surface glycoproteins of the original strain (Scholtissek *et al.* 1978*c*).

SPECIFIC PROPERTIES OF THE GENES CODING FOR THE SURFACE GLYCOPROTEINS

Since several of the pandemic strains emerged by rather strong drifts without obvious reassortment we have to consider why and how influenza A strains are able to drift to such an exceptional extent. Two facts are relevant in this context. (1) Only the genes coding for the surface glycoproteins HA and NA exhibit a relatively low base sequence homology of 30 or 20% respectively, as long as there is no serological cross-reaction between their gene products. (2) If the cRNA of two strains that exhibit the same low base sequence homology to the HA or NA genes of a third strain are mixed before hybridization, no increase in the RNA-resistant fraction is found as shown in table 2 for the pair Equi 2/Virus N with respect to FPV. This

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means that the homologous regions of the HA or NA genes between the various influenza strains are always identical and that they totally overlap (Scholtissek *et al.* 1977*b*). These data suggest that the genes coding for the surface glycoproteins consist of a relatively small conserved region(s) which might be responsible for the functional integrity of the gene products

TABLE 2. RNASE-RESISTANT RADIOACTIVITY (COUNT/MIN) OF ^{32}P-LABELLED RNA SEGMENTS 4 (HA) AND 6 (NA) OF FPV AFTER HYBRIDIZATION WITH cRNA OF FPV, EQUI 2 (A/EQUINE/MIAMI/1/63; HEQ2NEQ2), VIRUS N (A/CHICK/GERMANY/N/49; HAV2NEQ1) AND A MIXTURE OF THE LATTER

cRNA	HA gene	NA gene
FPV	3920	3180
Equi 2	1950	810
Virus N	1930	790
Equi 2 plus Virus N	2100	880

like receptor activity for HA or enzyme activity for NA, while the larger, highly variable regions might be involved in the antigenic specificity of the gene products. This means that most mutations within the conserved regions are lethal. On the other hand, within the variable region most mutations will not interfere with the function. Under the selection pressure of the immune system of the host, variants with altered antigenic properties would emerge easily.

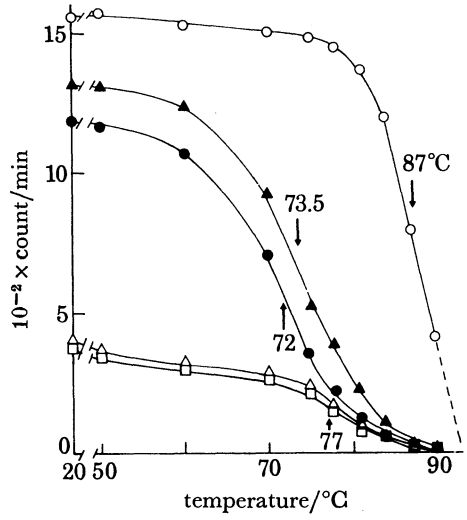

FIGURE 4. Melting profiles of homologous and heterologous hybrid RNA molecules of fowl plague virus segment 6 (neuraminidase gene). ○, Fowl plague virus (Hav1N1); ▲, A/chicken/Tajikistan/134/77 (Hav7N1); △, A/turkey/England/63 (Hav1Nav3); ●, A/PR/8/34 (H0N1); □, A/Singapore/1/57 (H2N2).

If this concept is correct, one should expect that the melting profile of hybrid RNA molecules between two antigenically unrelated strains – in spite of the fact that the base sequence homology is low – is relatively sharp and the melting point is relatively high. In contrast, for antigenically related strains, which exhibit a relatively high base sequence homology, the melting profiles should be flatter and the melting points significantly lower. As shown for the NA gene in figure 4, this is indeed so. The same was found for the HA gene, while with the conserved genes the melting profiles of the heterologous RNA hybrids are always sharp and the melting points are always high (Scholtissek 1979). In the latter case there is no selection

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pressure of the immune system, and presumably the total length of the gene is required for a functional gene product.

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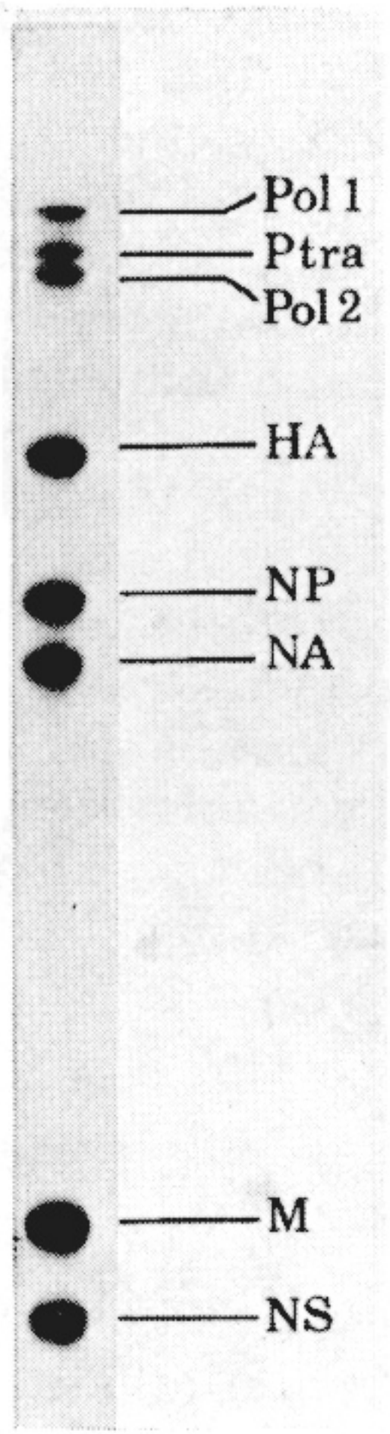

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