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# LOCATION OF TS DEFECTS IN THE GENOME OF COLD-ADAPTED RECOMBINANT INFLUENZA A VIRUS VACCINE STRAINS

T. E. MEDVEDEVA, *K. V. LISOVSKAYA, F. I. POLEZHAEV, L. M. GARMASHOVA, G. I. ALEXANDROVA, *Y. Z. GHENDON

Research Institute for Experimental Medicine
Academy of Medical Sciences of the U.S.S.R., 197022 Leningrad,
and *Moscow Research Institute for Viral Preparations,
Ministry of Public Health of the U.S.S.R.

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*Summary.* — The ts phenotype and location of ts mutations were studied in the genome of parent viruses and those obtained by recombination of cold-adapted strains A/Leningrad/134/17/57 or A/Leningrad/134/47/57 with epidemic H1N1 and H3N2 influenza A virus strains. The epidemic H1N1 and H3N2 strains under study possessed a ts phenotype and contained ts mutations in one or two genes. The ts phenotype was lost following three clonings at 40 °C, suggesting that influenza virus strains isolated from humans may be heterogenous and contain virions either carrying or not carrying the ts mutations in their genomes. Two cold-adapted strains possessing a distinct ts phenotype contained ts mutations in three (A/Leningrad/134/17/57 virus after 17 passages at 25 °C) or in five (A/Leningrad/134/47/57 variant after 30 additional passages at 25 °C) genes coding for non-glycosylated proteins. When compared with cold-adapted donor strains, the recombinants had either the same set or additional ts mutations. However, no ts mutation was detected in a gene which had been inherited from the donor strain. It is suggested that, in addition to the analysis of the genome composition, in cold-adapted recombinant influenza virus strains recommended as vaccine candidates it is necessary to control the number of genes containing ts mutations.

*Key words: influenza virus; recombination; ts mutants; cold-adapted variants*

## *Introduction*

To the most prospective donors of attenuation for developing a safe human influenza virus vaccine belong the cold-adapted strains obtained upon passaging at a lowered temperature, such as strain A/Leningrad/134/17/57 (Alexandrova, Smorodintsev, 1965) and strain A/Ann Arbor/6/60 (Maassab, 1969). In the earlier investigation we used ts mutants of fowl plague virus belonging to different recombination groups, for analyses the ts mutations in two cold-adapted variants of influenza virus A/Leningrad/134/57.

Such mutations were obtained after 17 passages in chick embryos (CE) at 25 °C (A/Leningrad/134/17/57) in three genes coding for the P3, NP and M proteins, and after 30 additional passages under the above mentioned conditions (A/Leningrad/134/47/57) in five genes coding for P3, P2, NP, M, and NS proteins (Ghendon *et al.*, 1981; Lisovskaya *et al.*, 1981).

The aim of our study was to evaluate the number and location of ts mutations in the genomes of recombinants of two above-mentioned cold-adapted variants of A/Leningrad/134/57 virus and epidemic H1N1 and H3N2 influenza A virus strains, which should be safe for vaccination of both adults and children.

## *Materials and Methods*

*Viruses.* The used ts mutants of fowl plague virus (FPV) were ts 29, ts 131, ts 166, ts US1, ts 303/1 and ts mN3 which were shown (Ghendon *et al.*, 1981) to have ts mutations in genes 1, 2, 3, 5, 7 and 8 coding for P3, P1, P2, NP, M and NS proteins, respectively. As donors of attenuation served the cold-adapted variants of influenza virus A/Leningrad/134/57 (H2N2), namely A/Leningrad/134/17/57 strain which had had 21 passages in CE at 32 °C and 17 passages at 25 °C, and A/Leningrad/134/47/57 strain which is a variant of A/Leningrad/134/17/57 virus obtained after additional 30 passages at 25 °C. Epidemic influenza A virus strains were the following: A/Khabarovsk/7/77 (H1N1), a variant of strain A/USSR/090/77 (H1N1); strain A/Leningrad/322/79 (H1N1), a variant of strain A/Brazil/11/78 (H1N1); strain A/Bangkok/1/79 (H3N2). All underwent 2—3 passages in CE at 32 °C. The recombinant vaccine strains H/32/5 (H1N1), 17/32/2 (H1N1) and 17/42/3 (H3N2) for the use in adults were obtained on the basis of the above epidemic viruses and attenuated donor strain A/Leningrad/134/17/57 (Ghendon *et al.*, 1981; Polezhaev, Alexandrova, 1979). Recombinant vaccine strains 47/25/1 (H1N1) and 47/7/2 (H3N2) for children were obtained by recombination of epidemic strains A/Leningrad/322/79 (H1N1) and A/Bangkok/1/79 (H3N2) with the cold-adapted A/Leningrad/134/47/57 strain (Ghendon *et al.*, 1984).

*Recombination test* was carried out according to the method of Ghenkina and Ghendon (1979) described elsewhere (Ghendon *et al.*, 1981). The method is based on recombination of human influenza virus strains with FPV ts mutants in which the gene has been identified carrying a ts mutation. Recombination is performed in CE fibroblasts in which human influenza viruses fail to form plaques. Cultures were incubated at a high non-permissive temperature. If the influenza virus strain genes carried no ts mutation, they would recombine with a ts mutant. The resulting recombinants formed plaques under the conditions used. Briefly, CE fibroblasts were infected with the influenza virus strains under study (1—2 $EID_{50}$/cell) using various dilutions of FPV ts mutans. The infected cell monolayer was overlaid with agar and incubated at 36 and 40 or 42 °C for 72 hr when the plaques were counted.

*Evalution of temperature-sensitivity of viruses.* Groups of 5 CE were co-infected with $10^3$ $EID_{50}$ of the viruses under study and incubated at 32 °C for 48 hr. Temperature-sensitivity of virus reproduction (a ts phenotype) was evaluated by comparative titration of allantoic fluids from 5 CE at optimal (32 °C) and non-permissive (40 °C) temperatures. When the difference in titres was $\leq$ 2.0 log $EID_{50}$, the viruses were considered to posses a $ts^+$ phenotype; when this difference was more than 5.0 log $EID_{50}$, they were considered to possess a $ts^-$ phenotype; when the difference was within the range of 2.0 — 5.0 log $EID_{50}$, the viruses possessed a $ts\pm$ phenotype.

## *Results*

### *Analysis of ts phenotype and the location of ts mutans in the the parent influenza virus strains*

Table 1 shows that epidemic parent viruses inoculated into CE incubated at 40 °C possessed a distinct ts phenotype, the difference in titres at 32 and 40 °C being 4.25—6.0 log $EID_{50}$/0.2 ml. At the same time, the variant of A/Krasnodar/101/59 (H2N2) strain used as control possessed a distinct

**Table 1. Ts phenotype and location of ts mutations in the epidemic influenza A virus strains**

| Strain | Ts phenotype | | | Analysis of ts mutations at various temperatures[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Reproduction in CE at | | Difference at 32 °C and 40 °C | ts 29(1) | | ts 131(2) | | ts 166(3) | | ts US1(5) | | ts 303/1(7) | | ts mN3(8) | |
| | 32 °C | 40 °C | | 36 °C | 42 °C | 36 °C | 42 °C | 36 °C | 40 °C | 36 °C | 42 °C | 36 °C | 42 °C | 36 °C | 40 °C |
| A/Khabarovsk/7/77 (H1N1) | 7.5* | 1.5 | 6.0 | **6.0**** | **<2.0** | 6.4 | 5.9 | **6.6** | **<2.0** | 6.3 | 4.3 | 6.3 | 3.6 | 6.2 | 5.5 |
| A/Leningrad/322/79 (H1N1) | 6.5 | 0 | 6.5 | **6.3** | **<2.0** | 6.1 | 5.8 | **6.8** | **<2.0** | 6.4 | 4.3 | 6.1 | 3.8 | 6.5 | 5.8 |
| A/Bangkok/1/79 (H3N2) | 6.5 | 1.75 | 4.75 | 6.5 | 4.7 | 6.2 | 5.4 | 6.8 | 5.8 | 6.3 | 5.2 | **6.2** | **<2.0** | 6.2 | 4.4 |
| A/Krasnodar/101/59 (H2N2) | 9.5 | 8.75 | 0.75 | 6.5 | 5.8 | 6.8 | 5.4 | 6.5 | 6.5 | 6.2 | 5.3 | 6.8 | 3.8 | 6.3 | 5.9 |
| A/Leningrad/134/17/57 (H2N2) | 8.5 | 1.5 | 7.0 | **6.8** | **<2.0** | 7.0 | 6.3 | 6.4 | 5.6 | **6.2** | **<2.0** | **7.2** | **<2.0** | 6.8 | 5.4 |
| A/Leningrad/134/47/57 (H2N2) | 9.5 | 2.0 | 7.5 | **6.1** | **<2.0** | **6.5** | **< 2.0** | 6.7 | 5.8 | **6.9** | **<2.0** | **6.3** | **<2.0** | **6.0** | **< 2.0** |

* log $EID_{50}$/0.2 ml; the virus yields at optimal temperature (32 °C) were simultaneously determined in CE at 32 °C and 40 °C.
** log PFU/ml; [1]mutant gene in brackets.

**Table 2. Ts phenotype and location of ts mutations in the cloned epidemic influenza A virus strains**

| Strain | Ts phenotype | | | Analysis of ts mutations | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Reproduction in CE at | | Difference in titres at 32 °C and 40 ° | FPV mutants (mutant gene in brackets) at various temperatures | | | | | | | | | | | |
| | | | | ts 29(1) | | ts 131(2) | | ts 166(3) | | ts US1(5) | | ts 303/1(7) | | ts mN3(8) | |
| | 32 °C | 40 °C | | 36 °C | 42 °C | 36 °C | 42 °C | 36 °C | 40 °C | 36 °C | 42 °C | 36 °C | 42 °C | 36 °C | 40 °C |
| A/Khabarovsk/7/77 (H1N1) | 6.25* | 5.25* | 1.0* | 6.4** | 3.5** | 6.6** | 6.0** | 6.6** | 5.4** | 6.0** | 5.3** | 6.2** | 3.5** | 6.7** | 5.6** |
| A/Leningrad/322/79 (H1N1) | 6.5 | 5.0 | 1.5 | 6.2 | 3.6 | 6.5 | 5.2 | 6.5 | 5.2 | 6.9 | 5.5 | 6.1 | 3.3 | 6.7 | 5.3 |
| A/Bangkok/1/79 (H2N2) | 8.25 | 5.25 | 3.0 | 6.5 | 4.7 | 6.2 | 5.4 | 6.8 | 5.8 | 6.3 | 5.2 | 6.2 | 3.9 | 6.2 | 4.4 |

* log $EID_{50}$/0.2 ml ** log PFU/ml

$ts^+$ phenotype and its titres decreased at 42 °C only by 0.75 log $EID_{50}$/0.2 ml. In the experiments with cold-adapted strains A/Leningrad/134/17/57 and A/Leningrad/134/47/57 virus the titres decreased at 40 °C by 7.0—7.5 log $EID_{50}$/0.2 ml.

Taking into consideration the temperature-sensitivity of virulent parent strains which were intended for preparing recombinant vaccine strains, we studied them in a recombination test to identify genes carrying the ts mutations (Table 1). Ts 29 and ts 166 mutants failed to recombine with 2 H1N1 strains — A/Khabarovsk/7/77 and A/Leningrad/322/79 — suggesting that they had ts defects in genes 1 (P3) and 3(P2). When A/Bangkok/ 1/79 (H3N2) strain was crossed with mutant ts 29, ts 131, ts 166, ts US1 and ts mN3, it formed $ts^+$ recombinants, but failed to recombine with ts 303/1 mutant, i.e. it had a ts mutation in gene 7 coding for the M protein. Therefore, with respect to a ts phenotype, virulent strains A/Khabarovsk/322/79 (H1N1) and A/Bangkok/1/79 (H3N2) may be referred to the category of natural ts mutans containing one or two mutations in the genes coding for non-glycosylated proteins.

Table 1 shows that the cold-adapted attenuated donor strain A/Leningrad/ /134/17/57 (H2N2) used to prepare recombinant vaccine strains safe for adults had ts defects located in genes 1 (P3), 5(NP) and 7(M) as confirmed by the fact that it failed to recombine with ts mutants ts 29, ts US1, and ts 303/1, respectively, containing the above mutations. Thirty additional passages of the strain A/Leningrad/134/17/57 at a low temperature resulted in the appearance of additional ts mutations in genes 2(P1) and 8(NS). Thus, the cold-adapted attenuated donor strain A/Leningrad/134/47/57 used to prepare recombinant vaccine strains for children had 5 ts mutations in genes 1, 2, 5, 7 and 8. The variant of A/Krasnodar/101/59 (H2N2) strain which possessed a $ts^+$ phenotype and was used as control, recombined with all PFV ts mutants studied.

Recombination of the cold-adapted strain with epidemic viruses A/Khabarovsk/7/77, A/Leningrad/322/79 and A/Bangkok/1/79 possessing a ts phenotype was carried out after three clonings of these viruses using limited dilutions technique in fertile CE at 40 °C to obtain lines of these viruses capable of reproducing in CE at a high temperature. As follows from Table 2, the epidemic strains had lost the ts phenotype entirely or partially after three clonings at 40 °C, the difference in infectious titres at 32 and 40 °C of the cloned variants of A/Khabarovsk/7/77, A/Leningrad/322/79 and A/Bangkok/ /1/79 viruses being 1.0, 1.5, and 3.0 log $EID_{50}$/0.2 ml, respectively.

Analysis of the ts mutations in the genome of these viruses by recombination with six ts mutants belonging to different complementation groups showed that A/Khabarovsk/7/77 (H1N1), A/Leningrad/322/79 (H1N1) and A/Bangkok/1/79 (H3N2) strains which had been cloned at 40 °C contained no ts mutations in the genes coding for non-glycosylated proteins (Table 2), which were detected in a heterogenous population of original strains. Distinct differences in a ts phenotype of the epidemic strains cloned at 40 °C and of cold-adapted variants of influenza virus facilitated subsequent selection of

**Table 3. Ts phenotype and location of ts mutations in the recombinant influenza A virus strains**

| Parent viruses | Recombinant vaccine strains | Ts phenotype | | | Analysis of ts mutations | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Reproduction in CE at | | Difference in titres at 32 °C and 40 °C | FPV ts mutants (mutant gene in brackets) at various °C | | | | | | | | | | | |
| | | | | | ts 29(1) | | ts 131(2) | | ts 166(3) | | ts US1(5) | | ts 303/1(7) | | ts mN3(8) | |
| | | 32 °C | 40 °C | | 36 | 42 | 36 | 40 | 36 | 42 | 36 | 42 | 36 | 42 | 36 | 40 |
| A/Khabarovsk/ 7/77 K*(H1N1) | H/32/5** (H1N1) | 7.5[a] | 1.5[a] | 6.0[a]. | 6.1[b] | <2.0[b] | 5.7[b] | 5.9[b] | 6.0[b] | <2.0[b] | 6.2[b] | 5.3[b] | 6.6[b] | <2.0[b] | 6.3[b] | 5.0[b] |
| A/Leningrad/ 134/17/57 (H2N2) | | | | | | | | | | | | | | | | |
| A/Leningrad/ 322/79 K(H1N1) | 17/32/2** (H1N1) | 8.5 | 1.25 | 7.25 | 6.8 | <2.0 | 6.8 | 5.9 | 6.2 | <2.0 | 6.8 | <2.0 | 6.5 | <2.0 | 6.5 | 5.8 |
| A/Leningrad/ 134/17/57 (H2N2) | | | | | | | | | | | | | | | | |
| A/Leningrad/ 322/79 K(H1N1) | 47/25/1*** (H1N1) | 8.5 | 1.25 | 7.25 | 7.5 | 4.7 | 6.2 | <2.0 | 6.0 | <2.0 | 6.7 | <2.0 | 6.3 | <2.0 | 6.9 | < 2.0 |
| A/Leningrad/ 134/47/57 (H2N2) | | | | | | | | | | | | | | | | |
| A/Bangkok/ 1/79 (H3N2) | 17/42/3** (H3N2) | 7.75 | 0 | 7.75 | 7.3 | <2.0 | 6.3 | 6.2 | 6.3 | 5.1 | 6.5 | <2.0 | 6.4 | <2.0 | 6.7 | 5.2 |
| A/Leningrad/ 134/17/57 (H3N2) | | | | | | | | | | | | | | | | |
| A/Bangkok/ 1/79 (H3N2) | 47/7/2*** (H3N2) | 8.25 | 0 | 8.25 | 7.2 | 2.0 | 6.0 | 2.0 | 6.0 | 6.3 | 6.1 | 2.0 | 6.5 | 2.0 | 6.4 | 2.0 |
| A/Leningrad/ 134/47/57 (H2N2) | | | | | | | | | | | | | | | | |

* K — a virus variant cloned at 40 °C
** recombinants H/32/5, 17/32/2 and 17/42/3 — vaccine strains for adults
***recombinants 47/25/1 and 47/7/2 — vaccine strains for children
[a] log $EID_{50}$/0.2 ml; [b]log PFU/ml

temperature-sensitive recombinants possessing a ts phenotype at a high temperature, like the attenuated donor strain.

*Analysis of ts phenotype and location of ts mutations in the recombinant vaccine influenza virus strains*

As shown in Table 3, the recombinants H/32/5 (H1N1), 17/32/2 (H1N1), 17/42/3 (H3N2), 47/25/1 (H1N1) and 47/7/2 (H3N2) possessed a distinct ts phenotype and the difference in the titres at 32 °C and 40 °C was 6.0—8.25 log $EID_{50}$/0.2 ml. Thus, their ts phenotype was similar to that of the cold--adapted but differed from that of the epidemic viruses (Table 1). The location of ts mutations in the genomes of two groups of recombinant vaccine influenza A virus strains intended for the use in adults or children was also identified using a test of recombination with FPV ts mutants. Crossing of the recombinant vaccine strain 17/42/3 (H3N2) intended for the use in adults with mutants ts 131 and ts mN3 yielded $ts^+$ recombinants. At the same time $ts^+$ recombinants were not formed in the experiments with mutants ts 29, ts US1 and ts 303/1 which contained ts mutations in genes 1, 5 and 7 coding for the P3, NP and M proteins, respectively. These data indicated the presence of ts mutations in three genes (1, 5 and 7) in the genome of the recombinant 17/42/3 which corresponded to ts mutations in the genome of the attenuated donor strain A/Leningrad/134/17/57 (Table 1). The recombinant 17/32/2 (H1N1) also had ts mutations in genes 1, 5 and 7, but, in addition, a ts mutation was detected also in gene 3 although the parent cold-adapted strain contained no ts mutation in this gene. The recombinant H/32/5 (H1N1) had ts mutations in genes 1 and 7 as similar as the attenuated donor strain; an additional ts mutation was detected in gene 3, but no ts mutation present in gene 5 of the cold-adapted donor was detected in this gene of the recombinant.

The recombinant vaccine strain for children 47/7/2 (H3N2) had ts mutations in five genes (1, 2, 5, 7, 8) which were located alike to those of the cold-adapted donor strain. Another recombinant vaccine strain for children 47/25/1 (H1N1) did not interact with mutants ts 131, ts 166, ts US1, ts 303/1 and ts mN3 carrying ts mutations in genes 2, 3, 5, 7, 8, respectively. Therefore, this recombinant contained a ts mutation in gene 3 derived from the cold-adapted donor strain, although the latter contained no ts mutation in this gene (Ghendon *et al.*, 1984).

## *Discussion*

The primary aim of our work was to study ts mutations in the genomes of parent viruses and of recombinant influenza A (H3N2 and H1N1) vaccine virus strains obtained by recombination of cold-adapted attenuated donor strains with epidemic influenza viruses and intended to be used in adults and children. For this purpose we used a test of recombination with FPV ts mutants with a known gene carrying a ts mutation. Earlier this technique was successfully applied to identify location of ts mutations in the genome of cold-adapted influenza virus donor strains (Ghendon *et al.*, 1981; Lisovskaya

*et al.*, 1981). Preliminary studies of epidemic parent influenza A virus strains showed that all three strains under study (two H1N1 and one H3N2) possessed the ts phenotype and contained ts mutations at least in 1—2 genes (genes 1 and 3 in H1N1 strains and gene 7 in H3N2 strain). Three subsequent clonings of the epidemic strains at 40 °C using limited dilutions technique in chick embryos resulted in the variants possessing a $ts^+$ or $ts^\pm$ phenotype, in the genomes of which no ts mutations were detected, at least not in the genes coding for non-glycosylated proteins.

As shown by others (Polezhaev, Alexandrova, 1979; Oxford *et al.*, 1980; Chu *et al.*, 1982), among recent H1N1 and H3N2 influenza virus strains there were many of those possessing a ts phenotype. Chu *et al.* (1982) showed that ts mutation was present in the NP gene in a number of H3N2 strains, while in the H1N1 strains it was found in the M gene. The authors also showed that populations of a number of strains were heterogenous and contained virions possessing both ts and $ts^+$ phenotypes. Apparently, in our studies the population of epidemic influenza virus strains was heterogenous as well and possessed a ts phenotype. Subsequent cloning at 40 °C yielded clones possessing either a $ts^+$ or a $ts^\pm$ phenotype.

The cold-adapted influenza virus strains used in our experiments which had had 17 and 47 passages at 25 °C contained ts mutations in three (1, 5, 7) and five (1, 2, 5, 7, 8) genes, respectively, and possessed a distinct ts phenotype. The recombinants obtained on the basis of these donor strains contained in their genomes the same set of ts mutations like their cold-adapted parents (17/42/3, 47/7/2), and also additional ts mutations which were not detected in the cold-adapted donor (17/32/2, H/32/5, 47/25/1). In the recombinant H/32/5 a ts mutation was not present in one of the genes, although the analysis of the genome composition of this recombinant using molecular cRNA/vRNA hybridization (Ghendon *et al.*, 1981) showed that this gene had been inherited by the recombinant from its cold-adapted parent. The data obtained show that recombination between the epidemic influenza virus strains and the cold-adapted attenuated donor strains, as well as the subsequent cloning of recombinants may involve the appearance of additional ts mutations and the loss of ts mutations in individual genes inherited from the cold-adapted parent. The appearance of new ts mutations is supposed to be due to the fact that obtaining recombinants and their subsequent cloning should be sometimes performed in 2—3 passages at a lowered temperature, as was the case with the recombinant 47/25/1 (Ghendon *et al.*, 1984). It is natural that additional passages at lowered temperature may cause the appearance of new ts mutations. At the same time, disappearance of ts mutations is not necessarily due to reversion, but rather due to extragenic suppression of this property, which was discovered during recombination of ts mutants of orthomyxoviruses (Ghendon *et al.*, 1982; Scholtissek, Spring, 1982). Since recombinants between cold-adapted attenuated donor strains and new epidemic influenza virus strains are intended to be used as live influenza vaccines, it seems expedient to analyse not only the genome composition of each recombinant vaccine strain candidate in order to make sure that it

inherited the genes coding for non-glycosylated proteins from a cold-adapted donor, but also to identify the location of ts mutations in each of these genes. The latter is necessary to be sure that all ts mutations of the attenuated donor strain are actually present in the genome of the recombinant obtained.

## References

Alexandrova, G. I., and Smorodintsev, A. A. (1965): Obtaining of an additionally attenuated vaccinating cryophil influenza strain. *Rev. roum. d'Inframicrobiol.* **2**, 179—186.

Chu, C. M., Tian, S. F., Ren, G. F., Zhang, L. X., and Liu, C. Q. (1982): Occurence of temperature-sensitive influenza A viruses in nature. *J. Virol.* **41**, 353—359.

Ghendon, Yu. Z., Klimov, A. I., Alexandrova, G. I., and Polezhaev, F. I. (1981): Analysis of genome composition and reactogenicity of recombinants of cold-adapted and virulent virus strains. *J. gen. Virol.* **53**, 215—224.

Ghendon, Yu. Z., Markushin, S., Lisovskaya, K., Penn, G. R., and Mahy, B. W. J. (1982): Extragenic suppression of a ts phenotype during recombination between ts mutants of two fowl plague virus strains with a ts mutation in gene 1. *J. gen. Virol.* **62**, 239—248.

Ghendon, Yu. Z., Polezhaev, F. I. Klimov, A. I., Lisovskaya, K. V., Medvedeva, T. E., and Alexandrova, G. I. (1984): Recombinant of cold-adapted attenuated influenza A vaccines for use in children. 1. Obtaining and molecular-genetic analysis of the cold-adapted donor and recombinants. *Infect. Immun.*, in press.

Ghenkina, D. B., and Ghendon, Yu. Z. (1979): Recombination and complementation between orthomyxoviruses under conditions of abortive infection. *Acta virol.* **23**, 97—106.

Lisovskaya, K. V., Garmashova, L. M., and Medvedeva. T. E. (1981): Analysis of ts mutations of cold-adapted influenza A/Leningrad/134/57 virus variants. *Acta virol.* **25**, 415—417.

Maassab, H. F. (1969): Biologic and immunologic characteristics of cold-adapted influenza virus. *J. Immunol.* **102**, 728—732.

Oxford, J. S., Corcoran, T. S., and Schild, G. C. (1980): Naturally occcuring temperature-sensitive influenza A viruses of H1N1 and H3N2 subtypes. *J. gen. Virol.* **48**, 383—389.

Polezhaev, F. I., and Alexandrova, G. I. (1979): Isolation of thermosensitive influenza virus strains during an epidemic of A/Victoria in 1975—76 (in Russian). *Vop. Virus.* **24**, 430.

Scholtissek, C., and Spring, S. B. (1982): Extragenic suppression of temperature-sensitive mutations in RNA segment 8 by replacement of different RNA segments with those of other influenza A virus prototype strains. *Virology* **118**, 28—34.