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Suppression of the Temperature-Sensitive Phenotype of a Mutant of Reovirus Type 3

Abstract. *A revertant of a reovirus group A temperature-sensitive mutant was crossed with wild type. More than 50 percent of the progeny were temperature sensitive. In all of the temperature-sensitive progeny examined by recombination tests, the temperature-sensitive lesion was in group A. The results indicate that the revertant was phenotypically suppressed.*

Conditional lethal mutations are useful tools for the study of virus replication and genetics. Growth of conditional lethal mutants under nonpermissive conditions allows an absolute selection for reverse mutation or reversion. Revertants may not be true revertants to the wild type, but may represent the acquisition of second, suppressor mutations that allow the virus to bypass the original defect (*1*). In prokaryotic systems, these suppressor mutations are often in genes other than the one with the original lesion. Such extragenic suppressors are often in genes whose products interact physically with the original gene products (*2*). We have found that a revertant of a temperature-sensitive (ts) mutant of reovirus type 3 is phenotypically suppressed by a suppressor that can be separated from the ts mutation by reassortment of genome segments.

The group A mutant ts 201 of reovirus type 3 was isolated as a result of proflavin mutagenesis (*3*); it contains $\mu 1$ and $\mu 2$ polypeptides with altered electrophoretic mobility (*4*). Reversion of ts 201 to ts^+ phenotype is often accompanied by a change in the electrophoretic mobility of the $\mu 1$ and $\mu 2$ polypeptides (*5*). In several spontaneous ts 201 revertants, reversion to ts^+ phenotype was not accompanied by a change in mobility of $\mu 1$ and $\mu 2$ polypeptides. The possibility that these revertants were not true revertants but contained the ts 201 lesion in a suppressed form was examined in one revertant clone (clone 101).

To show that a revertant clone contains a suppressed ts lesion, it was necessary to show (i) that the reversion event occurred outside the gene with the ts lesion, and (ii) that the clone still contained the original ts lesion.

Since the genome of reovirus is segmented (*6*) and recombinants have been shown to arise by reassortment of genome segments in two-factor (*3, 7, 8*) and three-factor crosses (*5*), we reasoned that the suppressed ts lesion could be separated from its suppressor by reassortment if the ts lesion and the suppressor lay on different genome segments. Once separated from the suppressor mutation, the ts phenotype of the ts lesion would once against be expressed. Accordingly, clone 101 was backcrossed to wild type, and progeny plaques were picked from plates grown at permissive temperature. After two passages at permissive temperature, we determined the titer of the progeny clones. As controls, single infections were performed with clone 101, wild type, and ts 201. Progeny plaques were picked and passaged, and their titers were determined (Fig. 1). The distribution of temperature phenotypes shows two populations among the progeny of the cross; one population has a wild-type efficiency of plating (EOP) and the other has an EOP that is temperature sensitive. [The ts 201 control clones (data not shown) had an EOP ranging from 5×10^{-3} to 5×10^{-5}.] This result represents unequivocal evidence that clone 101, although phenotypically ts^+, contains a phenotypically suppressed ts lesion.

Since clone 101 was selected as a revertant of the group A mutant ts 201, one would expect the ts lesion appearing among the progeny to be group A. However, it has been shown that extragenic suppressors often have intrinsic temperature phenotypes (*2*). Thus ts lesions among the progeny would be expected to be either the ts lesion from which the revertant was selected or a new ts phenotype associated with the suppressor mutation. To test whether the ts progeny clones contained group A ts lesions or lesions in other groups, selected clones were crossed (*3*) against the group A mutant ts 201 and the group B mutant ts 352 at permissive temperature. All progeny clones tested failed to recombine with the group A test virus, whereas they did recombine with the group B test virus, indicating that they contained the group A lesion (data not shown).

Thus clone 101 does contain a phenotypically suppressed ts lesion, and this le-

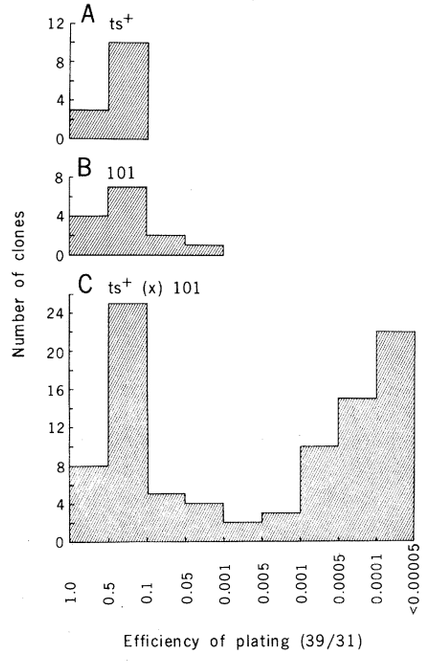

Fig. 1. Distribution of EOP of progeny and control clones. A suspension culture of 10^7 mouse L cells were mixedly infected with a multiplicity of infection of 10, each with freshly cloned 101 and wild type. Two hours after infection, unadsorbed virus was removed by centrifuging the infected cells and resuspending them in fresh medium. Forty-eight hours after infection, the cells were sonicated to release cell-associated virus and to disrupt viral aggregates. Appropriate dilutions were plated on L cell monolayers and incubated for 13 days at 31°C. The culture plates were overlaid with neutral red agar and, after overnight incubation, plaques were picked. The plaques were passaged twice on L cell monolayers at 31°C. The titer and efficiency of plating of second passage virus was determined by plating on L cell monolayers at 39°C and 31°C. Plates at 39°C were overlaid and counted on day 5; plates at 31°C were overlaid and counted on day 13 after infection. Wild-type and clone 101 controls were the same, except that for single infection a multiplicity of infection of 20 was used. The EOP is the ratio of the titer at 39°C to that at 31°C. (A) EOP of wild-type control clones. (B) EOP of clone 101 control clones. (C) EOP of clone 101 (×) wild-type progeny clones.

sion, when separated from the suppressor, is the expected group A lesion. The ability to segregate ts and ts^+ progeny from the cross of clone 101 with wild type indicates that the site of the suppressor mutation can be separated from the suppressed ts lesion, presumably by reassortment of genome segments, and that the suppressor must lie in a second gene.

We have not yet defined the site of the suppressor mutation, or the specific mechanism of this phenotypic suppression. However, the ability to separate the suppressor mutation from the ts lesion by reassortment indicates that the suppression is extragenic rather than intragenic.

In prokaryotic systems extragenic suppressors often are in genes whose products interact physically with the original gene product. These suppressor mutations frequently have temperature phenotypes of their own (*2*) which make them useful in the study of protein interactions, as well as making reversion a useful method for the selection of new mutations. We have been unable to identify a new temperature-sensitive phenotype among the progeny of this backcross. This indicates that the suppressor mutation described does not have an intrinsic temperature-sensitive phenotype.

Our results suggest a previously undetected perhaps general mechanism by which the ts phenotype of eukaryotic virus mutants may be altered. The presence of pseudorevertants in this virus system suggests that reversion in animal viruses can occur by mechanisms similar to those elucidated in other biological systems and that when revertants are examined, standard genetic considerations apply.

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Control of Gamete Formation (Exflagellation) in Malaria Parasites

Abstract. *The only stages of malaria parasites capable of establishing an infection in a mosquito are the gametocytes that circulate in the blood of the vertebrate host. Within minutes of ingestion by a mosquito the gametocytes transform into mature gametes in the process of "exflagellation." This process is controlled in vitro solely by the change in* pH *in the blood as it moves from the environment of the circulation to that of the atmosphere, the* pH *rise being mediated by the fall in carbon dioxide tension as the blood equilibrates with the atmosphere.*

All parasitic organisms cyclically transmitted by arthropod vectors experience a profound change in environment at least twice during their life cycle—once as the parasite is transferred from the vector to the vertebrate host and again as the parasite moves back from the vertebrate to the vector. Accompanying these environmental changes most parasites undergo extensive and often rapid biological transformations. Such transformations are well recognized among arthropod-transmitted parasitic protozoa and are especially dramatic in Haemosporidia, including malaria parasites. Although in several instances considerable effort has been devoted to describing the morphological and biochemical developments associated with these changes (*1*), there is little understanding concerning the factors initiating or controlling the transformation of malaria parasites from the forms present in the vertebrate host to those that establish the infection in the mosquito vector—a problem that has vexed malariologists for almost a century.

Malaria parasites multiply in the red blood cells of their vertebrate host by repeated cycles of an asexual process known as schizogony. Certain parasites, themselves the products of schizogony, do not undergo further division in the red cell but become gametocytes, the precursors of the gametes. While in the blood stream the mature gametocytes remain quiescent. Within the stomach of the mosquito following a blood meal, however, the gametocytes undergo rapid transformation. In malarious blood removed from the stomach of a freshly engorged mosquito the membrane of the red cell enclosing each gametocyte disintegrates releasing a macrogamete (female gamete) or a microgametocyte (male gametocyte). This process is specific to red cells containing mature gametocytes; no similar disintegration of the host cell normally occurs in either red cells infected with asexual parasites or immature gametocytes or in uninfected red cells. The microgametocyte completes its development in the dramatic process of exflagellation, releasing eight highly motile, flagellated microgametes. The process is completed 10 to 20 minutes after ingestion of blood; fertilization, in which an entire microgamete enters or fuses with a macrogamete, follows rapidly (*2*).

The intervention of the mosquito is not essential for the initiation of exflagellation. The sequence of events described above may be readily observed in gametocyte-carrying blood directly exposed to air, for example, in a drop of blood on a microscope slide. It is clear, therefore, that changes taking place in blood as it is transferred from the circulation to the atmosphere are capable of initiating the transformation of gametocytes to gametes.

Although the factors controlling its initiation have not been extensively studied, it has been well established that exflagellation is suppressed by maintaining infected blood at CO_2 tensions corresponding to those of circulating blood (*3–5*). Hitherto the role of CO_2 in controlling this process has been obscure, although Bishop and McConnachie (*4*) believed that it was independent of its effect on the *p*H of the blood. Our results, obtained with the chicken malaria parasite, *Plasmodium gallinaceum*, demonstrate that the effect on exflagellation of different CO_2 tensions correlates solely with their effect on the *p*H of the surrounding medium.

Immediately upon withdrawal from the heart or wing vein of an infected chicken (parasitemia, 40 to 60 percent; gametocytemia, 0.5 to 2 percent), blood was diluted in not less than 50 volumes of a minimal saline solution (10 m*M* tris, 166 m*M* NaCl, 10 m*M* glucose; *p*H 7.4), which we refer to as suspended animation (SA) solution. In it both emer-