THE APPEARANCE OF ALTERRED PROTEIN PRODUCTS IN MUTAGENIZED VESICULAR STOMATITIS VIRUS

by

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Submitted in Partial Fulfillment of the Requirements of the
University Undergraduate Fellows Program

1981-1982

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April 21, 1982

ABSTRACT- Mutants of vesicular stomatitis virus (VSV) were generated by treatment during the virus' infective phase with the known frameshift mutagen ICR-191. Both mutant and wild-type viral proteins were labelled with ^{35}S-methionine, extracted, and examined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), looking for mutant proteins that exhibited differing mobilities from their corresponding wild-type protein. Two alterred protein products were observed. One involved the G protein while the other involved either the N or NS proteins.

ACKNOWLEDGMENTS- I would like to express my thanks and appreciation to Dr. Raymond Vonder Haar and the Medical Biochemistry Department of the Texas A&M College of Medicine for their assistance and support of my involvement in the University Undergraduate Fellows Program.

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INTRODUCTION

The title of our original research proposal was, "Frameshift Mutagenisis in Vesicular Stomatitis Virus," and our goal was to identify virus mutants that were the result of a frameshift mutation. The title of our final paper is, "The Appearance of Alterred Protein Products in Mutagenized Vesicular Stomatitis Virus." This indicates the fact that we have identified mutant proteins by virtue of their differing migration rates in an SDS-polyacrylamide gel as compared to the migration of the wild-type protein; however, at this point we can not conclusively say that these mutants are actually frameshifts even though they were generated following treatment with a known frameshift mutagen. Further research is required to more conclusively determine the nature of the alterred protein bands we have observed; we are presently continuing to conduct such experiments.

Research is being done in this area for several reasons. The first is a result of the fact that many known carcinogens have been shown to be frameshift mutagens in the Ames test. Some of these mutagens occur routinely in hair dyes, pesticides, and as by-products in oil refinery processes. Another reason for interest in this area is due to the fact that while there have been several mechanisms proposed to explain the occurence of frameshift mutations, no one mechanism has been agreed upon and it is hoped that work in this area will further our understanding of frameshift mutagenisis in general. The particular viral system that we are using is also interesting in that VSV has a ssRNA genome and frameshift mutations have not yet been demonstrated in RNA genomes; it is also proposed that the genome must be double stranded in nature for frameshifts to occur. Therefore, VSV should prove to be an interesting test system to work with.

REVIEW OF THE LITERATURE

Vesicular stomatitis virus (VSV) represents a very useful system for researchers. For this reason there is a wealth of information on VSV in the literature. There has been much work done to characterize the nature of the five (5) viral proteins coded for by the ssRNA genome. The five proteins are referred to as the N, NS, L, M, and G proteins. The NS and L proteins stand for the nonstructural and large proteins respectively and both are probably involved in the replication of the viral genome since they are only found in significant quantities when the virus is in its infective phase. The N protein stands for the nucleocapsid protein which is found complexed with the ssRNA genome of the virus. The M and G proteins stand for the membrane and glycoprotein proteins respectively and both are involved in the viral envelope. Mutants of these proteins have been investigated fairly extensively. The entire genome of the virus, to my knowledge, has not been sequenced. However, portions of the genome and of the virus' defective interfering particles have been determined. Defective interfering particles of VSV have been investigated also. These particles are formed following serial undiluted passages of the virus and are incapable of replicating themselves. They must co-infect with the standard VSV particles in order to replicate. Evans, Pringle and Szilagyi reported alterred NS proteins in mutant VSV following treatment with the base analog 5-fluorouracil in a paper published in the Journal of Virology, August, 1979, p. 325-333, entitled, "Temperature Sensitive Mutants of Complementation Group E of Vesicular Stomatitis Virus New Jersey Serotype Possess Altered NS Polypeptides." Chatis and Morrison reported G mutants in a paper entitled,"Mutational Changes in the Vesicular Stomatitis Virus Glycoprotein Affect the Requirement of Carbohydrate in Morphogenesis," also published in the Journal of Virology, Jan. 1981, p.307-316. While VSV mutants have been investigated previously in the

literature our research with VSV is original in investigating viral mutants isolated following treatment with the known frameshift mutagen ICR-191.

THE APPEARANCE OF AN ALTERRED PROTEIN PRODUCT IN MUTAGENIZED VESICULAR STOMATITIS VIRUS*

Abstract- Mutants of vesicular stomatitis virus (VSV), were generated by treatment during the virus' infective phase with the known frameshift mutagen ICR-191. The mutant and wild-type viral proteins were labelled with ^{35}S-methionine, extracted, and examined by SDS-polyacrylamide gel electrophoresis, (SDS-PAGE).

The occurence of a frameshift mutation within a gene can exhibit drastic effects, often resulting in a loss of the functional protein product coded for.[1] There have been several mechanisms proposed to explain frameshift mutations; however, no one mechanism has been agreed upon and there is a disagreement in the literature over the actual cause(s) of frameshift mutations. One mechanism proposes that intercalating agents like ICR-191, (see Figure 1, page 2), that can insert themselves between adjacent base pairs in the DNA cause the addition of an extra base in the progeny DNA. Upon transcription this will result in the formation of an mRNA molecule that will also have an extra base, (complememtary to the one found in the parental DNA), and will finally be expressed in the formation of a mutant protein with incorrect amino acid incorporation following the point of mutation due to the shift in the reading frame, (see Figure 2, page 2). The generation of an early termination codon is also possible in the shifted reading frame and the identification of the shortened mutant protein that would result is an indication of a possible frameshift mutation. Many known carcinogens have been shown to be frameshift mutagens in the Ames test[2-4]therefore, it is hoped that research in this area will also further our understanding of events that can lead to neoplastic transformation in animal systems. We have treated VSV with ICR-191 during the virus' infective phase and have investigated the mutant proteins by SDS-PAGE. The proteins of both wild-type and mutant VSV were radioactively labelled with ^{35}S-methionine, extracted, and their mobilities observed on gels.

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* The style and format of the New England Journal of Medicine is used.

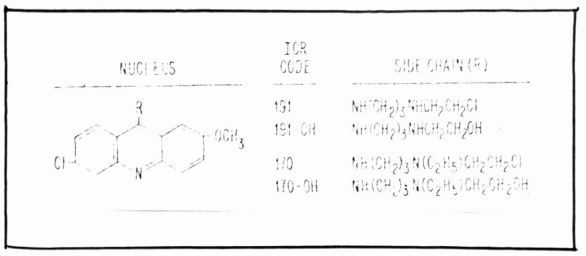

Figure 1- The structure of ICR-191 and other ICR compounds.[5]

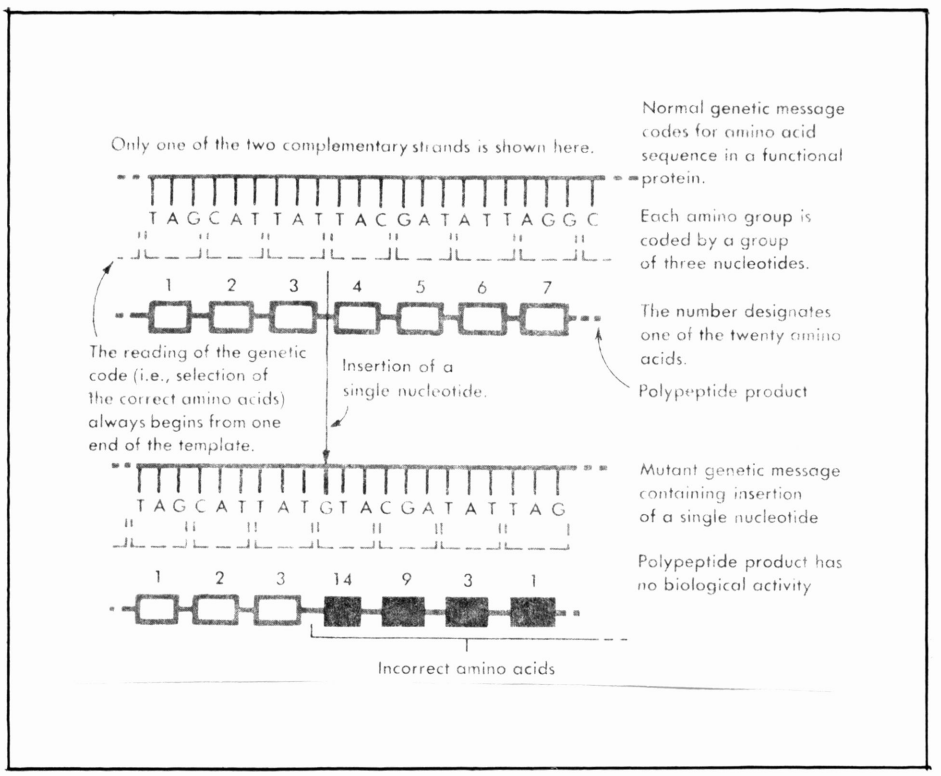

Figure 2- The effect of a frameshift mutation in the mRNA molecule.[6]

METHODS

Tissue Culture

Cultured mouse L-cells were used as the host cells for the viral infection. The cells were allowed to grow to a confluent monolayer in modified MEM plus Earle's Salts, (Irvine Scientific).

Infection

The viral stock solutions were diluted to obtain an m.o.i. of 100. The growth media was removed from the cultured host cells which were then infected with 0.1 ml of the appropriate diluted viral solution. The virus was allowed to absorb for an hour in an incubator maintained at $30^{o}C$ in a 5% CO_2 , 100% humidity atmosphere. After one hour 0.9 ml of growth media was added to the cells, bringing the total volume to 1.0 ml. The virus was allowed to infect the host L-cells for a 3 hr period.

Labelling

Following the infection procedure, the growth media was removed and replaced with a labelling media containing ^{35}S-methionine, glutamine, and a vitamin solution. The cells were placed back into the incubator and allowed to produce labelled protein for another 3 hr period. During this incubation it is necessary to isolate the cells from the 5% CO_2 atmosphere in the incubators because the labelling media is sensitive to CO_2 and will become acidic if exposed to it resulting in cell death and a decrease in labelled protein. This can be achieved by simply wrapping the tissue culture plates in Saran wrap. When labelling is complete, the media is discarded into radioactive waste and the cells are extracted.

Extraction

The cells are extracted with an extraction buffer which contains: 2% Triton X-100, 25 mM Tris, 3.5 mM $MgCl_2$, 300 mM KCl at a pH of 7.4. A 100 µl volume of extraction buffer is used to extract each well of the tissue culture plate.

Protein Concentration

The extract is centrifuged in an Eppendorf microcentrifuge and the supernatant is concentrated on a Speed Vac Concentrator, (Savant Instruments), to produce a dried, concentrated protein residue.

SDS-PAGE

The concentrated protein is resuspended in sample buffer solution, (0.5 M Tris-Cl @ pH 6.8, 20% SDS, 2.5 ml β-mercaptoethanol, 0.05% bromophenol Blue, 6.25 ml glycerol, and 2.5 ml D-H_2O in a total volume of 25 ml), in preparation for loading onto the gel apparatus. The protein mixture is electrophoresed at 200 volts in a 10% polyacrylamide gel until the bromophenol blue marker in the sample buffer reaches the bottom of the gel. The gel is dried and exposed against a photogaphic film to produce an autoradiogram.

RESULTS

Two alterred protein products are observed in the resulting autoradiogram, (see Figure 3, page 5). The alterred band in the upper portion of the gel occurs in the G protein and exhibits varying mobilities from the wild-type G protein. An alterred band also appears in the region just following the N and NS protein bands which have not been resolved on this particular representative gel. This second band appears to be a shortened protein that may have resulted from the generation of a premature termination codon in either the N or NS genes.

DISCUSSION

The particular mutants we investigated were generated by treatment with ICR-191, a known frameshift mutagen. Therefore, it is inviting to assume that any mutant protein observed would be the result of a frameshift mutation. However, the fact that we observe this same or a very similar band in approximately half of the mutants we investigated weakens the argument

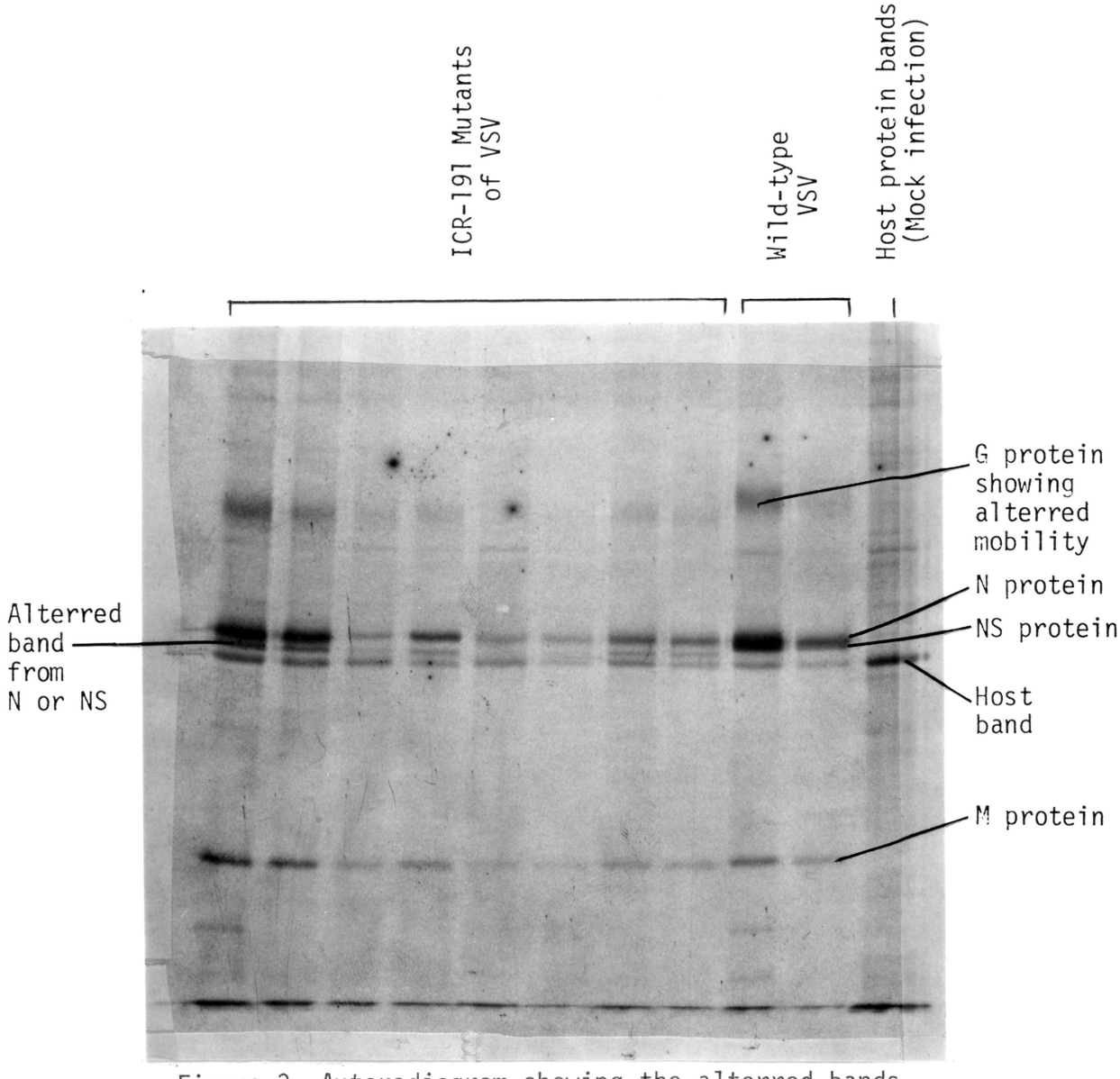

Figure 3- Autoradiogram showing the alterred bands.

that the appearance of the band slightly smaller than the N and NS proteins is actually the result of a frameshift mutation. It is unlikely that intercalation would occur in the same position in so many of the mutants generated unless it involves a mutational "hot spot" in the viral genome. Further work needs to be done in this area to more conclusively determine the nature of the alterred protein band we have observed migrating slightly faster then the N and NS proteins. Complementation tests should be performed to determine whether the N or NS gene is involved. The gene implicated by the complementation tests could then be sequenced and compared to the analysis of the wild-type gene to determine whether or not a frameshift event has actually been observed.

The varying rates of migration for the G protein has been observed previously in the literature and is attributed to the post-translational effect of glycosylation.[7,8] After the G protein is synthesized it must be glycosylated before it can be incorporated into the viral envelope. The varying mobilities observed for the G band are therefore assumed to be due to differing degrees of glycosylation.

An alterred mobility for the NS band has also been noted in the literature.[9] However, our band may be due to either the N or NS bands and so might be quite different in origin. The mutants investigated in that study were generated following treatment with the base analog 5-fluorouracil as opposed to ICR-191 in our study.

In conclusion, we have demonstrated that it may be possible to observe frameshift mutations in a ssRNA genome, a fact that had not yet been determined. We have also observed a possible, albeit questionable frameshift mutation and have postulated additional steps that need to be taken in order to more conclusively determine the nature of the possible frameshift band observed. Unfortunately, these steps involving the sequence analysis of the genes in question exceed the time restrictions of the University Undergraduate Fellows Program.

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