Protein Coding Assignment of the S Genes of the Avian Reovirus S1133

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The protein coding assignments for each of the S genes of the avian reovirus S1133 have been determined. In vitro translation of RNA derived from individual S dsRNA genome segments demonstrated that the largest S gene, S1, codes for the smallest protein, σc; the S2 for σA; the S3 gene for σC; and the S4 gene for σS. No other gene products could be identified. By examination of appropriate reassortant viruses, these assignments were confirmed.

Although the avian reoviruses demonstrate differences from the mammalian reoviruses in biological and serological properties, they share many morphological and physico-chemical characteristics which have resulted in their classification into the same group of viruses (1-5). Recent biochemical studies (1, 3, 6-8) have confirmed the basic genetic and structural similarities between these two groups of viruses. Both contain a genome consisting of 10 segments of double-stranded (ds) RNA separable into three size classes. Each gives rise to 10 distinct viral-specified products, eight of which are found in the complete, infectious virion. Furthermore, the arrangement and relative quantities of these structural proteins also appear to be quite similar.

In these investigations the S1133 and Lasswade strains of avian reovirus (7) were utilized. For the in vitro translation studies, the S1133 virus was grown in chick embryo fibroblasts (CEF) and purified by repetitive cesium chloride equilibrium gradient centrifugation as previously described (8). Viral dsRNA was then extracted and run on a 5% Tris-acrylamide gel. dsRNA segments were visualized after staining with ethidium bromide and the gel was cut to permit separation of individual S gene segments. The dsRNA was removed from the gel by electroelution and then extracted twice with phenol:chloroform:isoamyl alcohol and once with chloroform before being ethanol precipitated overnight. Prior to use, each of the RNA preparations was quantitated and appropriate aliquots melted in 90% DMSO for 30 min at 50°. The separated RNA strands were precipitated and resuspended in the translation mixture at the time of use. The translation protocol consisted of the standard BRL rabbit reticulocyte in vitro translation system modified by adjusting the final potassium (K+) ion concentration to 40 mM with KAc, this concentration giving optimal [35S]methionine incorporation. For most experiments, 5 μg of dsRNA were utilized per reaction mixture. Incorporation of radioisotope was found to be linear in this range, declining at higher RNA concentrations. The reaction was allowed...
to proceed at 30° for 60 min and then terminated by placing on ice. Aliquots were precipitated with TCA to determine total incorporation and the remainder subjected to immune precipitation utilizing rabbit antisera to S1133 as has been previously described (9). This material was then analyzed by electrophoresis on standard 9% polyacrylamide gels followed by fluorography.

Figure 1 demonstrates the products obtained upon in vitro translation of each of the S genes of the avian reovirus S1133. To permit identification of each band, an immunoprecipitate from a lysate of [35S]methionine-labeled cells previously infected with S1133 was run in parallel in all experiments. As can be seen, the S1 gene, the largest of the S genome segments, gave rise to the smallest reovirus protein, σc. The possibility of an additional protein product, either a primary translational product or a cleavage product, was considered but no evidence of any smaller molecular weight polypeptides was found. The S2 gene product was shown to comigrate with the σA protein band from S1133. In vitro translation of the S3 gene occasionally produced two bands when analyzed as described, the major incorporation occurring in a band with mobility of the σB reovirus protein. In addition, a less dense band migrating in the position of σNS was often found in these preparations. As the σNS protein could be shown to be the product of the S4 gene, the possibility that the S3 dsRNA was contaminated by a minor amount of S4 dsRNA was examined by re-running the S3 dsRNA on a second polyacrylamide gel after it had been electroeluted from the first gel. Invariably a small amount of RNA, detectable by ethidium bromide staining, could be seen to migrate in the position of the S4 dsRNA band. Prior treatment of extracted total viral RNA with proteases, or the use of higher SDS concentrations, failed to prevent this apparent "sticking" of S4 dsRNA to the S3 dsRNA.

In order to confirm these protein coding assignments, a second independent approach was undertaken. Reassortant viruses were produced by dual infection of CEF with both the S1133 and Lasswade strains of avian reovirus. Previous studies (6, 7) had demonstrated that two of the four S dsRNA genome segments of these two viruses could easily be differentiated by significant differences in their mobility upon PAGE. A third S genome segment of each virus (S3 of Lasswade and S4 of S1133) comigrated, but assignment of this band could be made to either parental virus by assuming that any viable reassortant would contain one of each of the S genes rather than two copies of the same gene. The S1 genes of each of these viruses also comigrated but could not be differentiated. Thus, the parental origin of three of the four S genes in the reassortant viruses could be unequivocally determined.

Figure 2A depicts the pattern of migration of the dsRNA genome segments for a group of reassortant viruses produced using S1133 and Lasswade as the parental strains. In those instances in which there is comigration of the dsRNA genome segments (indicated by the dashed line), the parental origin, determined as described above, has been indicated. No assignment of S1 genes has been made.
Figure 2B demonstrates the pattern of migration upon PAGE of the immunoprecipitated [\(^{35}\)S]methionine-labeled viral proteins obtained from lysates of CEF infected with each of the reassortant viruses shown in Fig. 2A. Examination of this figure demonstrates that two bands, those corresponding to the viral proteins \(\sigma_B\) and \(\sigma_{NS}\), can clearly be distinguished among the reassortant viruses. Reassortant virus A and F alone contain a \(\sigma_B\) band that is significantly retarded compared to those seen for reassortant viruses B, C, D, and E. Reassortants A and F both contain the S3 gene from Lasswade; additionally, the mobility of the \(\sigma_B\) protein band of these viruses is found to be identical to that of the \(\sigma_B\) band of Lasswade when examined under similar conditions. In the case of the \(\sigma_{NS}\) band, reassortants D and F both demonstrate a band with mobility retarded relative to the same band seen in the other reassortants examined, and they alone have the S4 gene from S1133. Again, the comigration of this band with that of the parental S1133 has been confirmed by simultaneous PAGE of radiolabeled protein from each virus. Because the relative molecular weights of the S genome segments and the \(\sigma\) proteins have been assumed to vary in direct relation to their mobility upon PAGE, it was unexpected to find the supposedly largest gene, S1, coding for the lowest molecular weight \(\sigma\) protein, \(\sigma_C\). The relative molecular weights of the S2, S3, and S4 genome segments and their respective protein products were as might be expected.

Several explanations for the findings in regard to the S1 genome segment can be considered. First, it is possible that there is a second protein product produced from the S1 gene. Despite examination for such a lower molecular weight species, none has been found. Furthermore, in the mammalian reovirus system, in which all of the genes have been cloned (11), no evidence exists for such a situation. Second, and more likely, the mobility of either the dsRNA or the protein may not truly reflect its molecular weight but may be altered by secondary structures or other factors. Finally, there may be a silent or nontranslated portion of the S1 gene. This can only be determined after the gene has been fully sequenced.

One additional observation is the fact that there appears to be no detectable post-translational modification of the avian reovirus \(\sigma\) proteins. The mobility upon PAGE of the products of in vitro translation comigrate with the true viral proteins obtained from infected cell ly-
sates. This suggests, but obviously does not prove, that modifications, such as glycosylation or phosphorylation, do not occur with these avian reovirus proteins upon infection.

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