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NOTES

Classification of Rhabdovirus Proteins: a Proposal

R. R. WAGNER, L. PREVEC, F. BROWN, D. F. SUMMERS, F. SOKOL, AND R. MACLEOD

Department of Microbiology, University of Virginia, Charlottesville, Virginia 22901; Department of Biology, McMaster University, Hamilton, Ontario, Canada; The Animal Virus Research Institute, Pirbright, Surrey, England; Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461; The Wistar Institute, Philadelphia, Pennsylvania 19104; and Department of Botany, University of Illinois, Urbana, Illinois 61801

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A simple alphabetical classification is offered as a means to codify the proteins of rhabdoviruses, using vesicular stomatitis virus as the prototype.

This proposal emanated from discussions at the "Colloque International sur les Rhabdovirus" (A. Berkaloff, convenor), held in Roscoff, France, June 16–18, 1972. There was general agreement among the participants there assembled that communication in this field and related areas would be significantly improved by agreement to use standard nomenclature. Considerable confusion had arisen in the past due to different numerical designations of the proteins of vesicular stomatitis (VS) virus by individual investigators. The original descriptions and numbering of VS viral proteins (11, 20) have been rendered obsolete by further investigations. Moreover, the designation VP4 in one laboratory is the equivalent of protein V in another.

Most, if not all, investigators now agree that VS virions contain at least five separate polypeptides identifiable by polyacrylamide gel electrophoresis (13, 17, 22). Y. Kang and L. Prevec (*unpublished report*) have estimated that the aggregate molecular weight of these five polypeptides is equivalent to 383,000, which accounts for almost all the coding potential of the VS viral genome of 3.6×10^6 to 4.0×10^6 daltons (4, 17). They have also proposed estimates of the molecular weights of each of the polypeptides of the Indiana serotype which are essentially in agreement with other data (17, 23); the Kang-Prevec values will be used in this report. Conceivably, other small polypeptides encoded by the VS viral genome could be discovered. Therefore, any system of nomenclature for these proteins must take this consideration into account. Such an eventuality has obviated the usual numerical systems based on decreasing molecular weight that have been devised, rather haphazardly, for proteins of other viruses.

The system we propose is based on the use of letters to designate structural and chemical properties of the viral polypeptides. This nomenclature applies primarily to VS virus, but there is ample evidence to indicate that it can apply equally well to the proteins of rabies virus (10, 18) and to the proteins of a representative plant rhabdovirus, potato yellow dwarf virus (15). The three major virion proteins of VS virus are relatively easy to characterize and are strikingly similar in molecular weight to the corresponding three virion proteins of rabies and potato yellow dwarf viruses. The two minor proteins of VS virus are more difficult to characterize with assurance and are more likely to undergo reclassification in the future. In addition, current evidence is insufficient to identify with confidence which of the five proteins, if any, is the transcriptase of VS virus (2, 3, 9) or any other rhabdovirus (1); no similar enzymes have yet been demonstrated in rabies virus but a polymerase has been found in a plant rhabdovirus (9a). Nevertheless, enough is known about all five identifiable VS viral proteins, as well as those of other representative rhabdoviruses, to propose a nomenclature with some assurance that it will not require drastic revision in the near future. The letter designations proposed for the VS viral proteins are listed below in decreasing order of molecular weight (M.W.) as estimated by polyacrylamide gel electrophoresis of Indiana serotype polypeptides. Slightly, but significantly, different values have been noted for the molecular weights of other VS viral serotypes and other rhabdoviruses. The rationale behind the designation of each protein is also summarized.

L protein (M.W. $\cong$ 190,000). This large (L) protein was originally thought to be an aggregate or uncleaved precursor of the other viral proteins

(11, 20). It is almost invariably present near the top of acrylamide gel electropherograms of VS viral proteins dissociated in sodium dodecyl sulfate, mercaptoethanol, 8 M urea, and by boiling (20). A large protein is also frequently present in disc electrophoretic profiles of proteins extracted from rabies virus (18) and stained gels of potato yellow dwarf virus (15). Analysis by electrophoresis on polyacrylamide gels of the L-protein peptides cleaved by cyanogen bromide revealed a fingerprint pattern distinct from that of three of the other VS virion proteins (S. U. Emerson, *personal communication*). M. Stampfer and D. Baltimore (*personal communication*) have obtained similar evidence by tryptic peptide fingerprints of the distinctiveness of the L protein. There is also some evidence that the L protein is a product of the 28*S* messenger ribonucleic acid (RNA) of VS virus (Stampfer and Baltimore, *personal communication*). The L protein is associated with VS virion nucleocapsids (3, 9, 21) and intracellular nucleocapsids (19).

G protein (M.W. ≅ 69,000). This protein is so designated because of ample evidence that it is the only identifiable VS virion protein which is glycosylated (4, 8, 17, 22). A similar glycoprotein is present in rabies virus (10, 18) and can be demonstrated in protein electropherograms of potato yellow dwarf virus by staining with periodic acid-Schiff's reagent (15). It is conceivable that more than one glycoprotein of these viruses migrates identically on polyacrylamide gels and, therefore, cannot be resolved by this method. If other techniques reveal additional glycoproteins, they can be designated G_1, G_2, etc. In fact, the evidence that migration on gels, possibly related to degree of glycosylation, can differ for glycoproteins extracted from intracellular components and released VS virions (13) may necessitate a modification of this nomenclature, possibly by the use of numerical subunits. Rhabdovirus glycoproteins have been identified as the only components of the spikes protruding from the viral envelope and can be removed relatively cleanly with proteolytic enzymes or nonionic detergents (7, 9, 10, 16). The G protein is primarily associated with the plasma membrane of the VS virus-infected cell (8, 19).

N protein (M.W. ≅ 50,000). The evidence seems clear that this major virus component is the structural protein of the nucleocapsid of all rhabdoviruses studied to date (7, 11, 15, 18, 21). It is tightly bound to the virion RNA and renders it resistant to ribonuclease (5). The N protein complexed with viral RNA can be effectively separated from virion envelope proteins by treatment with deoxycholate (5, 18, 21) and can be freed of other nucleocapsid proteins by exposure to a high-salt environment (14).

NS protein (M.W. ≅ 40,000–45,000). This designation is the least satisfactory of all those proposed for the VS viral proteins and will undoubtedly be superseded when more information is available concerning the function of this protein. The original term NS_1 stood for nonstructural protein number 1 because large amounts of this protein were found to be synthesized in VS virus-infected cells despite failure originally to detect it in released purified virions (13, 22). The possibility of the existence of another smaller nonstructural protein, termed NS_2 (22), now seems unlikely and is not considered in this report. Subsequently, Mudd and Summers (17) identified on high resolution gels a minor VS virion protein IV which migrated to about the same position as protein NS_1. This observation was confirmed by other investigators who provided additional evidence that this protein was a minor component of the VS viral nucleocapsid core in association with proteins N and L as well as the viral RNA (3, 14). We are also following the suggestion made by several investigators to drop the subscript 1 and simply refer to this protein tentatively as NS.

M protein (M.W. ≅ 29,000). This designation is proposed because of general agreement that this major nonglycosylated protein is an integral component of the VS viral membrane (8, 19, 22) but is less accessible than the G protein to proteolytic enzymes and nonionic detergents (7, 10). The earlier designation of S (for surface) protein (22) is incorrect and should be abandoned. The hypothesis has been advanced that the M protein is the matrix protein that serves to bind the ribonucleocapsid to the VS viral envelope (6). In this respect, as well as others, the rhabdovirus M protein is quite similar to, but not interchangeable with, the single matrix protein of paramyxoviruses (16). Rabies virus, in contrast, contains two M proteins that migrate rapidly on polyacrylamide gels (10, 18). We propose that these rabies proteins be designated M_1 for the larger and M_2 for the smaller component. The M proteins of the New Jersey serotype and Cocal virus are slightly smaller than the M protein of the Indiana serotype (20, 23; P. Talbot and F. Brown, *unpublished data*). Wunner and Pringle (23) have also reported other differences among polypeptides of the Indiana, New Jersey, and Cocal serotypes, but these data have not yet been confirmed by other investigators.

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