The Molecular Biology of Picornaviruses

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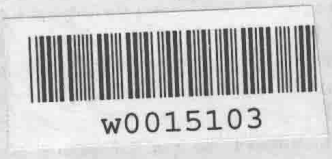

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WHY THIS N.A.T.O. MEETING?

WHY THIS BOOK?

In the year 1961, a paleontologist discovered the foot prints of a dinosaurian, an iguanodon, which had lived in the United Kingdom a few hundred millions years ago. There were 13 pairs of foot prints, a heretofore unknown large number of reptilian fossil foot prints. Moreover, the steps, clearly preserved in limestone, were lying in a perfectly straight line. From these remarkable facts the paleontologist drew two remarkable conclusions: (a) the iguanodon was going somewhere, and (b) it was going there with a definite purpose.

Our work on the effect of temperature on viral development was started a long time ago. A paleontologist searching in a library would discover 21 fossil papers preserved in various fossil periodicals. He would see that they are lying in an extremely tortuous line. From this remarkable fact, the paleontologist would draw two remarkable conclusions: (a) we were going nowhere, and (b) we had no definite purpose. Yet our aim was to disclose the mechanism by which supra- and infra-optimal temperatures influence viral development. Despite the fact that our way was twisted, we have nevertheless landed somewhere and I would like to tell you where we are on Friday, June the 8th, 1962.

Andre Lwoff
Cold Spring Harbor Symp. Quant. Biol. (1962), *27*, 159

This book is a sort of natural "fall-out" of the homonymous N.A.T.O. Advanced Study Institute held in Maratea (Gulf of Policastro), Italy, in September 1978, and it faithfully reflects the format and purposes of this conference.

In organizing this meeting, I knew all too well that "the way had been twisted, (but) we had nevertheless landed somewhere..."

The intent, therefore, was to provide for a fresh and original review of all relevant topics and issues in the field, following a comprehensive and coherent programme.

Such an ambitious goal could only be reached thanks to the unlimited collaboration of the lecturers: They were requested to produce nothing less than "freer, broader, speculative and personal "considerations of the subjects" they had to cover... And so they did: their presentations unfolded a fantastic picture, a most fascinating and meaningful identification of the field, its present problems and trends.

But participants at this conference contributed many valuable observations while discussing specific points. Unfortunately, more often than not, it proved impossible to identify them in the records. Accordingly, we incorporated here and there some of these "anonymous" contributions as part of the edited version of the texts.

To all of the participants, I would like to express my gratitude for their actively taking part in all the scientific (and social...) activities (lectures, round tables, posters, encounters) of this N.A.T.O. ASI. The result was a stimulating atmosphere, conducive to authentic scientific exchanges. Hence, the success of this meeting should be credited only to them.

Finally, on behalf of all participants, I would like to thank Professor Sissini, the Mayor of Maratea, and Mr. B. Vitolo, the Chairman of its Tourist Office: their enthusiastic support greatly contributed to make of our time in their wonderful seaside resort an unforgettable one.

R. Perez-Bercoff
Zurich, October 1978

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THE STRUCTURE OF THE PICORNAVIRION

THE PICORNAVIRION: STRUCTURE AND ASSEMBLY

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INTRODUCTION

The mammalian picornaviruses (from pico-small, and rna-containing a ribonucleate genome) comprise a large group of agents, which can presently be classified according to a scheme such as that shown in Table I.

Although there are some differences among subgroups which are manifest in the properties of pH stability and buoyant density in cesium salts, the evidence at this time points to a fundamental similarity of structure and assembly for all of these viruses. Consequently, in this brief review attention will be concentrated on the cardioviruses and poliovirus with the implication that the characteristics of these agents will be applicable - with only minor variations - to all mammalian picornaviruses.

I. STRUCTURE OF THE VIRION

A. Physical-Chemical Properties

The picornavirus particle is composed of a molecule of single-stranded RNA (30% by weight) enclosed in a capsid of protein (70%). There is no good evidence for the presence of carbohydrate or lipid in the virion (1). As viewed in the electron microscope by negative staining, the particle is isometric with a dry diameter of 27-28 nm. In solution it behaves as a spheroid (frictional ratio of 1.05-1.10) with a diameter of about 30 nm, and containing some 0.25 g water per gram of dry virus (2).

Table I

VERTEBRATE PICORNAVIRUSES

Genus Enterovirus	: Polio (3 serotypes) Coxsackie A (23) Coxsackie B (6) Echo (31) Enteroviruses of mice,swine,cattle Enterovirus 70 (conjunctivitis virus)		Sedimentation coefficient ~155 S Buoyant density (CsCl) ~1.34 g/ml Virions stable at pH 3-10 Empty capsids produced in vivo
Genus cardiovirus	: EMC ME Mengo Columbia-SK MM	Serologically very closely related	Sedimentation coefficient ~155 S Buoyant density ~1.34 g/ml Virions labile 5<pH<7 in the presence of 0.1 M Cl^- or Br^- No empty capsids in vivo
Genus Rhinovirus (Human)	: More than 120 serotypes		Sedimentation coefficient ~155 S Buoyant density ~1.40 g/ml Virions labile pH<5 Empty capsids produced in vivo
Genus Aphtovirus	: Foot-and-Mouth Disease Virus 7 serotypes		Sedimentation coefficient ~145 S Buoyant density ~1.43 g/ml Virions labile pH <6.5 Empty capsids produced in vivo

Summarizing the best available values, the picornavirion has a sedimentation coefficient ($S^0_{20,w}$) of 150-160S, a diffusion coefficient ($D^0_{20,w}$) of 1.44-1.47 x 10^{-7} cm^2/sec, and a partial specific volume ($\bar{v}$) of 0.68-0.70 ml/g. Substituting these values in the Svedberg equation, one arrives at a "molecular weight" for the particle of about 8.3-8.5 x 10^6 (2, 3).

B. The RNA Component

The picornavirus genome is a single polyribonucleotide chain of molecular mass (Na^+ form) of 2.4-2.7 x 10^6 daltons (4, 5). No unusual nucleotides have been detected therein and, with the exception of some human rhinovirus genomes, approximately equimolar amounts of adenylate, cytidylate, guanylate and uridylate are present (6). The nucleotide residues are not, however, uniformly distributed along the genome. At the 3'-end is a tract of 20-50 adenylate residues which are required for the infectivity of isolated virion RNA (7, 8), and in the case of the cardioviruses and FMDV there is a cluster of 100-200 cytidylate residues located near the 5'-end (9, 10). Recently, a small protein (MW ~ 4000), called VPg, has been found to be covalently linked to the 5'end of the virion RNA of polio, EMC and FMD viruses (11, 12, 13).

This protein may play important roles in the replication of the genome and/or assembly of the virion (14).

C. The Protein Component

When total protein is extracted from the virus particle and subjected to analytical ultracentrifugation or density gradient sedimentation, it behaves as a relatively homogeneous entity with a molecular mass of approximately 30,000 daltons. However, as early as 1963 Maizel reported that the protein component of the poliovirion could be separated electrophoretically into several non-identical polypeptide species (15). With the discovery that the electrophoretic mobility of a polypeptide in polyacrylamide gels containing sodium dodecyl sulfate (SDS) is inversely proportional to the logarithm of its molecular weight (16), it became possible to determine simultaneously both the number of different polypeptides which comprise the capsid protein and their molecular weights. Also, if the virions have been produced in cells maintained in medium containing radioactive amino acids, measurement of the amount of radioactivity associated with each polypeptide enables one to calculate the number of copies of each in the capsid. The results of such analyses for selected picornaviruses are summarized in Table II. More complete data and references are given by Rueckert (17).

Table II

POLYPEPTIDE COMPOSITIONS OF PICORNAVIRAL CAPSIDS

Polypeptide Designation[b]		Molecular mass[a]/Estimated number of copies per virion						
		Poliovirus (Type 1)	Coxsackie Virus (Type B3)	Bovine Enterovirus	ME	Mengo	Rhinovirus (Type 1A)	FMDV (Type O)
VP0	ε	41/-	38/1-2	-	41/2	39/1-2	37/2	40/4
VP1	α	35/-	29/60	34/60	33/60	33/59	34/60	34/60
VP2	β	28/-	25/60	28/60	31/58	30/58	30/58	30/60
VP3	γ	24/-	21/60	26/60	25/60	24/59	26/60	26/60
VP4	δ	5.5/-	5/60	9/30	7.3/58	7.4/58	7/58	13/30

[a] Values are given in 10^3 daltons

[b] The VP0-4 system of nomenclature has been used for the enteroviruses and FMDV; the ε-δ system has been used for the cardioviruses and rhinovirus 1A

A reasonable interpretation of these data is that the picornavirus capsid is composed of four major polypeptide species: VP1 or α (MW ∿ 34,000), VP2 or β (MW ∿ 30,000), VP3 or γ (MW ∿ 25,000) and VP4 or δ (MW 5000-8000); and that there are approximately 60 copies of each in a complete capsid (17). The molecular weights of VP4 shown above for BEV and FMDV may be overestimates because of the unreliability of molecular weight determination for polypeptides of this size by SDS gel electrophoresis (e.g. the molecular weight for the δ polypeptide of Mengo virus is 7350 based upon amino acid composition analyses, whereas its apparent molecular weight by SDS gel electrophoresis is 10,600 (18). Thus it is probable that both BEV and FMDV contain a full complement of VP4 polypeptides. The polypeptide VP0 (ε) is a precursor of VP2 and VP4 (β and δ), and is not genetically distinct (19). It is likely that all picornavirions contain one or more of these uncleaved precursors.

Individual capsid polypeptides have been isolated from cardioviruses and FMDV by electrophoretic or chromatographic procedures and their amino acid compositions determined (18, 20, 21). The composition characteristics common to all of these polypeptides are as follows. There is an excess of potentially acidic (asx, glx) over basic (lys, arg) amino acid residues, a low sulfur content (cys + met = 2-4 mole %), and a substantial number of prolines (5-8 mole %) and non-α-helix forming residues (val + ile + ser + cys + thr + gly = 35-40 mole %). Approximately 50 mole % of the amino acid residues are apolar. Considering the Mengo virion, the excess of acidic amino acid residues is reflected in the low isoelectric points of the individual capsid polypeptides (isoelectric pH's for α, β, γ and δ are 5.1, 5.3, 5.8 and 4.6 respectively (22). Optical rotatory dispersion and circular dichroism measurements indicate an α-helical content of only 5-10% for the Mengo capsid polypeptides *in situ* (2, 23), in keeping with the large proportion of helix-disrupting and non-helix-forming amino residues. The apolar character of the capsid polypeptides is manifest in their insolubility in aqueous solvents at neutral pH.

Amino- and carboxyl-terminal amino acid sequences have been determined for the Mengo capsid polypeptides (24), and these are given in Table III. Dansylation of intact poliovirions has revealed three amino terminals, these being asp, ser and gly (25). The three large polypeptides from FMD virions (types A, O and C) have been found to have the amino terminal residues asp, thr and gly (21, 26) and their carboxyl-terminal residues (type A) are gln, glu and leu (27). Although the correlation of the N- and C-terminals for individual FMD polypeptides has not been done, it is tempting to speculate that all picornavirus capsid polypeptides have the following structure:

$$X\text{—}\underset{\delta}{\text{VP4}}\text{—ala} \qquad \text{asp—}\underset{\beta}{\text{VP2}}\text{—gln} \qquad \begin{matrix}\text{ser}\\ \text{thr}\end{matrix}\text{—}\underset{\gamma}{\text{VP3}}\text{—}\begin{matrix}\text{gln}\\ \text{glu}\end{matrix} \qquad \text{gly—}\underset{\alpha}{\text{VP1}}\text{—leu}$$