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References

McCarthy, D. A. (1990). Paper models illustrating virus symmetry. *Journal Of Biological Education (Society Of Biology)*, *24*(1), 32.

PAPER MODELS ILLUSTRATING VIRUS SYMMETRY

Abstract

Building paper models helps students to understand the concept of icosahedral symmetry in small virus capsids. Instructions are given for constructing two models, one to illustrate the general principles of symmetry in T = 1, T = 3, and T = 4 viruses, and the other to illustrate the disposition of protein subunits in the T = 3 plant viruses and the picornaviruses.

Introduction

Virions (or virus particles) are the extracellular forms of a virus and are the means by which the viral genome is usually transmitted from one host, or host cell, to another. The viral genome, which may comprise one or several molecules of RNA or DNA, is usually but not necessarily covered and protected by a capsid (or coat). Capsids are constructed with helical, icosahedral, or complex symmetry and comprise multiple copies of one or more protein species. The structural unit visible by electron microscopy from which capsids are built is termed a capsomere. Together, the viral nucleic acid and capsid form a nucleocapsid; this may be naked (or non-enveloped) as in tobacco mosaic virus (TMV), polio virus, and adenovirus, or, as in many instances, such as influenza virus and herpes virus, may be enveloped (i.e. surrounded by a membrane). Nucleocapsids which are rod-like, such as TMV, are constructed with helical symmetry and the capsomeres which are visible by electron microscopy are the protein monomers. Whilst nucleocapsids which are spherical or isometric, such as the bacteriophage phiX 174, polio virus, or polyoma virus, are usually constructed with icosahedral symmetry and the capsomeres may be protein monomers but

more usually are aggregates of three, five, or six protein molecules. It is the construction of virus capsids with icosahedral symmetry and in particular those of the small RNA-containing viruses with which the exercise described in this paper is concerned.

The regular icosahedron is a platonic solid which has 20 equal-sized triangular faces, the edges of which can be projected on to the surface of a concentric sphere in order to create a lattice which divides the spherical surface into 20 equal triangles (figures 1a and 1c). Because protein molecules are asymmetric in three-dimensions, there is a limit to the number that can occur in strictly equivalent positions over the surface of a spherical shell. That limit is 60 and for simplicity can be envisaged as three protein subunits per triangular face. Only viruses with the smallest genomes, such as the satellite of tobacco necrosis virus (STNV), have shells comprising 60 copies of a single polypeptide species. Other viruses with larger genomes require larger shells to accommodate their genomic nucleic acids (see table 1). As the M_r of most virion proteins lies within the range 20×10^3 -- 60×10^3 irrespective of virion diameter, more than 60 polypeptide chains must be used to form larger capsids if the shell thickness is to be maintained. This raises the question of how the extra polypeptides are distributed. For instance, from chemical studies it is known that picornaviruses such as poliovirus and human rhinovirus (HRV, a common cold virus) have capsids comprising 60 copies of each of four different poly-peptide species, whereas the tomato bushy stunt virus (TBSV) capsid comprises 180 copies of a single poly-peptide species (Rayment, 1984). Where capsids are formed from a multiple of 60 chemically identical subunits, i.e. 60N subunits, the subunits must be located in N different environments. The question of how a single polypeptide species might bond in different environments was first addressed by Caspar and Klug (1962), who suggested that the faces of an icosahedron could be subtriangulated in a regular manner to form a series of larger polyhedra called icosadeltahedra. If subunits are now distributed so that there are three per facet (or subtriangle) inter-subunit bonding would then be similar (quasi-equivalent) but not identical (vide infra and figures 1b and 1c). Geometrical considerations limit the possible surface lattices for icosadeltahedra to 20T according to the formula

$$T = h^2 + hk + k^2$$

where T is the extent to which one face of the original icosahedron is subtriangulated (T = 'Triangulation number') and h and k are any pair of integers with no common factor. The possible surface lattices therefore conform to the series T = 1, 3, 4, 7, etc. Within this series three classes of icosadeltahedra can be recognized depending on the orientation of the sub-triangles (facets) relative to the face of the original icosahedron. In the first two classes, the P = 1 series (i.e. when h or k = 0; T = 1, 4, 9, etc.) and the P = 3 series (when h = k and h, k not equal to 0; T = 3, 12, 27, etc.) the facets lie respectively within the original face or are shared equally between two adjacent faces, but in the third class, the P = 7 series (when h not equal to k and h, k not equal to 0; T = 7, 13, 19, 21, etc.) the subtriangulation is skewed relative to the original face and is defined as laevo (or left-handed; $h > k > 0$) and dextro (or right-handed; $k > h > 0$). This can also be illustrated

graphically on an equilateral triangular net (e.g. Casjens, 1985). Because three subunits can be accommodated on each facet and an icosadeltahedron has 20T facets the total number of subunits should be 60T; thus theoretically, icosahedral shells should comprise only 60, 180, 240, 420, etc., asymmetric protein subunits. Where just one polypeptide species is used, it will occur in T different but quasi-equivalent positions, thus values of T are numerically equal to N. Moreover, in the T = 1 capsids all subunits would be pentavalent (i.e. have five nearest neighbours), whereas as in the larger shells, that is T = 3, 4, 7, etc., only those subunits located at the vertices of the original icosahedron would be pentavalent, whilst the remainder would be hexavalent (figures 1b and 1c). A T = 1 capsid would comprise 12 pentamers (12 x 5=60 subunits); a T = 3 capsid, 12 pentamers plus 20 hexamers (12x5 + 20x6=180 subunits); a T=4 capsid, 12 pentamers plus 30 hexamers (12 x 5+30 x 6=240 subunits), etc. In capsids where T>3 the bonding between subunits on any given facet (i.e. around the local or quasi three-fold symmetry axis of the facet) would be similar to that around the true three-fold axes of the original faces. However, where facets meet to produce local or quasi six-fold axes, inter subunit bonding will be hexameric and will differ from that around the five-fold axes (i.e. the vertices of the original icosahedron) which will be pentameric (illustrated in models A and B).

Within the last few years the structures of several T = 1 viruses, for instance STNV, and the picornaviruses, poliovirus, human rhinovirus 14 (HRV 14), Mengovirus, and foot-and-mouth disease virus (FMDV), and some T=3 viruses, such as tomato bushy stunt virus (TBSV), southern bean mosaic virus (SBMV), and turnip crinkle virus (TCV), have been determined at atomic resolution (e.g. see Rossmann and Erickson, 1985; Hogle et al., 1987; Luo et al., 1987; Acharya et al., 1989). These studies have revealed many similarities in the construction of small spherical virus shells, especially the way most of the chain is folded into a beta-'jelly-roll' structure in the shell or 'S' domain to create the thickness of the shell and the inter-subunit contact faces. In SBMV and TBSV the capsid proteins occur in three distinct environments, denoted A, B, and C, to which they adapt by 'switching' between alternative conformations without altering the structure of the S domain (Harrison, 1984a). The capsid geometries of the picornaviruses are similar to those of the plant viruses SBMV, TBSV, and TCV, with the three larger proteins VP1, VP2, and VP3 (VP stands for virion protein) occupying analogous positions to the 'A', 'C', and 'B' subunits (illustrated in figure 3 and model B). The 'C' subunit of the plant viruses has an ordered N-terminal arm which interacts with another across the two-fold axis and with two others around the three-fold axis to form an internal T = 1 framework. The N-terminal ends (approximately 50 to 60 amino acids) of the plant virus subunits and VP1, VP2, and VP3 in the picornaviruses are in the interior of the virion and interact with the RNA to stabilize the virion. VP4 in the picornaviruses is a relatively small protein (69-85 residues) and its location is often not well defined in electron density maps. Because the major proteins in picornavirus shells (VP1-VP3) have a similar three-dimensional structure, the picornaviruses can be considered to have a pseudo T = 3 construction.

The prediction (Caspar and Klug, 1962) that sub-units would be distributed according to the principles of subtriangulation has clearly been fulfilled but the suggestion that there would be slight

deformations of subunit structure and of inter-subunit bonds in the quasi-equivalent positions, for example in the T = 3 plant viruses, has not. Instead, it has been found that the structure of the subunit, at least in the S domain, is maintained constant, whilst different types of inter-subunit contacts occur at the different quasi-equivalent positions. Unexpectedly, in polyoma and simian virus 40 (SV 40), the only more complex viruses which have yet been analysed by low resolution x-ray crystallography and electron microscope image analysis, it has been found that 360 copies of a single protein species (VP1, M_r 40-42 x 10^3 occur in a T=7 design and not 420 copies (i.e. 60 x 7) as might be anticipated. All of the capsomeres are pentamers, even those around the local or quasi six-fold axes which are involved in hexavalent bonding. Larger icosahedral virus shells such as those of reovirus, herpes virus, and adenovirus, which appear at first to be T=9, T = 16, and T=25 structures, are more complex and like bacteriophage phiX 174 have chemically different protein subunits occurring in different locations. For example, in adenovirus the subunits at the vertices (pentons) differ from those on the faces (hexons). Altogether six protein species (proteins II, III, IIIa, VI, VIII, and IX), excluding the penton fibre (protein IV) form the shell. Hexons are in fact trimers of protein II and penton bases are pentamers (or possibly trimers) of protein III. Thus, in the larger viruses it appears that subtriangulation of an icosahedron is used as an effective way to obtain close packing of the subunits irrespective of the symmetry of the local interactions (Harrison, 1984b).

A model-building exercise will serve to demonstrate the basic principles of icosahedral symmetry (model A) and to illustrate the capsid geometry of the picornaviruses and the T= 3 plant viruses (model B). The exercise has been done with advantage for several years by undergraduate and postgraduate students of both science and medicine.

Procedure Model A

1. Examine the sheet from which the model will be made (figure 2). Note that the solid lines will become the edges of the triangles which comprise the 20 faces of the regular icosahedron.

2. Parts of the lattice have been subtriangulated to produce a T= 3 and a T= 4 arrangement, and 'commas' (used to illustrate asymmetric protein subunits) have been placed on these lattices. Note that the asymmetric units (commas) always have local symmetry with respect to each other. However, when the model is assembled, it is seen that they occur in different packing environments. For example, in the T = 3 structure, the tails of the 'A' subunits pack around the five-fold axes, whereas those of the 'B' and 'C' subunits alternate around the three-fold axes.
3. The stippled kidney-shaped units represent the positions in which the subunits of STNV would be found relative to the icosahedral lattice. This serves to emphasize the point that structural units are only positioned with icosahedral symmetry. Unlike the commas used in the diagrammatic T=3 and T=4 structures, they do not necessarily lie neatly within the lattices defined by the particle axes.
4. To assemble the model, cut carefully along the dotted lines. Score, then crease the paper along all the solid lines. Fold up the model to make a closed shell, glueing the tabs together with a quick-

setting adhesive.
5. Using figure 1, identify the axes of rotational symmetry on the model. Verify that only 60 structural units can be placed in identical environments. Distinguish the different packing environments in the T = 3 and T = 4 lattices.

Model B

1. Examine the hexagonal outline (figure 3) from which the model will be built. Altogether, 12 of these will be needed and when the model is finished they will form the vertices at the five-fold axes of symmetry.

2. The relative locations of the VP1, VP2, and VP3 protein subunits of the picornaviruses and of the A, C, and B types of subunit found in TBSV, TCV, and SBMV are illustrated. The picornavirus structural protein VP4 is an internal protein located below VP1 and VP2; it is not shown on the model.
3. The fundamental geometrically arranged asymmetric building block for these viruses is therefore the triangle comprising VP1, VP2, and VP3 (VP4 is hidden), or for the plant viruses it is A, C, and B. There will be a total of 5 x 12 such units, i.e. 60, in the capsid. In the picornaviruses, one of these units corresponds to the 6S (VP1, VP3, VP0) complex seen during virus assembly in vivo. The mark (*) and the curved line on the C subunit illustrates the ordered arm and the contacts which it makes internally.
4. To assemble the model, cut carefully along the dotted lines. Score, then crease the paper along the thicker solid lines. Fold one section to make a cup-shaped pentagon and glue the two tabs marked 'X' together using a quick-setting adhesive. This structure would correspond to the 14S pentamer observed during picornavirus assembly in vivo.
5. Repeat the procedure with the 11 remaining hexagonal outlines. Then glue all twelve cup-shaped pieces together by means of their unlabelled tabs, so that a closed shell is formed.
6. Identify the true axes of symmetry and the local quasi, or pseudo, axes of symmetry which exist. The true axes of symmetry are those of the original icosahedron; find the five-fold axes first and the others should become apparent more easily. The local, quasi, or pseudo axes of symmetry are the new two-fold, three-fold, and six-fold axes formed by subtriangulation. This symmetry arises solely from the arrangement of contiguous subunits and does not extend to the whole particle. There are no five-fold axes of purely local, quasi, or pseudo symmetry. Note that although the A, B, and C subunits of the plant viruses pack with the same local three-fold symmetry, they occur in environments which are similar but not identical.
7. The wedge-shaped subunits on the model represent reasonably well the surface area occupied by each polypeptide chain; the long axis of the beta-'jelly roll' or beta-'barrel' structure lies more or less parallel to the longer axis of the subunits.

Additional information

1. Completed models can be strengthened by spraying with clear cellulose lacquer.

2. Remember that of necessity these paper models are angular in outline whereas in reality the viruses are spherical.

3. When assembled, model B (picornavirus or T = 3 plant virus) has a diameter of approximately 140 mm whereas the true diameter of virions is about 28 nm. The magnification factor is therefore 140/28 x 10^6 or 5 x 10^6. A 10 mum diameter cell at the same magnification would be 50 m across.

Supplies

Master sheets for the two models can be obtained on request from the author at the address below. Multiple copies for class use can then be made, preferably on thin card using a plain-paper photocopier. Alternatively, master sheets can be made by enlarging photocopies made directly from the Journal.

Table 1 Properties of some icosahedral viruses, their capsids and genomes

Legend for Chart:

A - Virus
B - Diameter[a]
C - Capsid proteins[b], Protein M^r
D - M_t
E - Copies/Virion
F - Triangulation number[c]
G - Genome[d] Type
H - M_r

A	B	C	D
	E		
	F	G	H
STNV	17.6	--	21.6
	60		
	1	R(1)	0.4
phiX 174	congruent 30	faces	50
	60		
	[1]	D(1)	1.7
		vertices	20

	60		
	spike	37	12
Picornavirus	congruent 27	VP1	congruent 34
	60		
	1 (pseudo 3)	R(1)	2.4
		VP2	congruent 29.5
	60		
	VP3 congruent	25	60
	VP4 congruent	8	60
SBMV	28.4		28.2
	180		
	3	R(1)	1.2
TBSV	30.8		40.0
	180		
	3	R(1)	1.5
Caliciviruses	38		congruent 67
	180		
	3	R(1)	2.8
Nudaurelia beta virus	35-38		congruent 60
	240		

		4	R(1)	1.8
Polyoma/SV_{40}	49	VP1	42.4	
		360		
		7d	D(2)	3.3
Reovirus		76	mu1C	72
		congruent 400		
		[9]	R(2)	15
			sigma 1	42
		congruent 24		
			sigma 3	34
		congruent 1000		
Herpesvirus		100-110		142-155
		congruent 810		
		[16]	D(2)	96
				35-37
		congruent 1470		
				32-36
		congruent 1720		
				plus 3-4
		minor protein species		

Adenovirus	75	II	110
	720		
	[25]	D(2)	20-24
		III	85
	(36-60?)		
		IIIa	65
	60		
		IV	62
	36		
		IX	12
	(720?)		

1 In nm. Herpesvirus nucleocapsid is enveloped giving a virion diameter of 200-300 nm.

2 M_r x 10^{-3}. Polyoma, SV_{40}; reovirus, herpesvirus and adenovirus have additional internal proteins not mentioned in the table. Adenovirus capsid proteins are: II (hexon), III (penton base), IIIA (peripentonal hexon associated), IV (fibre), IX (hexon associated). The distribution of the reovirus and herpes virus proteins is not yet certain.
3 d = dextro. Values in square brackets indicate an apparent triangulation number only, for the subunits on the faces of these viruses differ chemically from those at the vertices.
4 4D = DNA, R = RNA, l = single stranded, 2 = double stranded. The reovirus genome is divided into 10 segments which are encapsidated together; the M, given is the sum of the M[su r]'s of the segments.
DIAGRAM: Figure 1a A regular icosahedron, viewed along a three-fold axis of rotational symmetry. The two-fold axes go through opposite edges, the three-fold axes go through the centres of opposite faces and the five-fold axes go through opposite vertices.

DIAGRAM: Figure 1b Subtriangulating one face of an icosahedron (outline marked by the continuous line) creates new six-fold axes of symmetry. The first two subtriangulated lattices (T = 3 and T = 4) are shown; note that each of the new subtriangles in the T = 3 icosadeltahedron is created across two of the original faces of the icosahedron, whereas those of the T = 4 lie within

the original face.

DIAGRAM: Figure 1c Orthogonal projections of the spherical triangulated lattices corresponding to T = 1, 3, and 4.

DIAGRAM: Figure 2 A plane hexagonal net to illustrate the distribution of subunits in T = 1, T = 3, and T = 4 virus structures. This diagram should first be enlarged by photocopying to produce an A4 size 'master' copy, which can then be used to produce multiple copies by photocopying or printing on to thin card. Copies of this card can be used to construct model A.

DIAGRAM: Figure 3 A plane hexagonal net illustrating the distribution of subunits in for example, poliovirus and TBSV (see text). This diagram should be enlarged by photocopying so that the length of any edge is 50 mm. Four copies at this enlargement can be mounted on an A4 sheet to produce a 'master' from which multiple copies may then be made by photocopying or printing on to thin card. Three copies of this master sheet on thin card will be needed to construct model B.

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