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Immune Recognition of Influenza Virus Haemagglutinin [and Discussion]

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Immune recognition of influenza virus haemagglutinin

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[Plate 1]

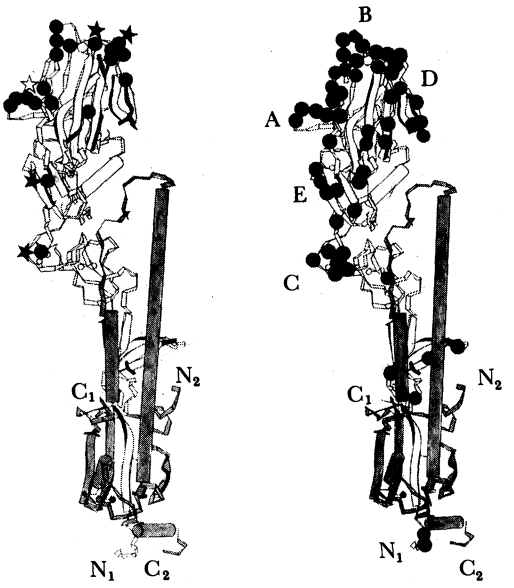
Haemagglutinin glycoproteins are the components of influenza virus membranes against which infectivity-neutralizing antibodies are directed. Sequence analysis of natural and laboratory-selected variant haemagglutinins indicates the regions of the molecule recognized by antibodies and by helper T cells; the identity of these regions and the relations between them are discussed.

Introduction

Haemagglutinin (HA) is the component of the influenza virus membrane against which neutralizing antibodies are directed, and as a consequence variants generated during the antigenic drift for which influenza viruses are notorious contain antigenically modified HAs. To understand the molecular basis of this variation, and of the recognition of HA by antibodies, many investigations have been reported involving HA amino acid sequences and three-dimensional structure analyses (reviewed in Wiley & Skehel 1987) and in the first part of this paper the main conclusions drawn from them will be reviewed. In the second part, experiments will be described that extend these analyses of the immune recognition of HA to major histocompatibility complex (MHC) class-II-restricted helper T cells.

The HA of the 1968 Hong Kong influenza virus, A/Aichi/2/68 (X-31: Kilbourne 1969) is a trimer of identical subunits, each consisting of two disulphide-linked polypeptide chains, HA_1 and HA_2. Both polypeptides are glycosylated at six sites in HA_1, residues 8, 22, 38, 81, 165 and 285, and at one site in HA_2, 154. The molecule is associated with the lipid membrane of the virus particle by a hydrophobic 'anchor' near the carboxy terminus of HA_2 and the projecting portion of the molecule is released as a soluble glycoprotein by digesting viruses with bromelain. Analyses of crystals formed by these soluble fragments indicate that the amino terminus of HA_1 is also near the lipid membrane of the virus. The HA_1 chain extends from the base of the molecule through a fibrous stem into a peripheral β-structure-rich region, and then returns to the fibrous region and terminates about 30 Å† from the virus membrane. The most prominent features of the part of the subunit composed of HA_2 residues are two antiparallel α-helices, one 29 Å long that proceeds distally from the membrane end of the molecule to connect through an extended chain with the other helix, which stretches 76 Å back towards the membrane. Schematic diagrams of a subunit are shown in figure 1.

† 1 Å = 10^{-10} m = 10^{-1} nm.



matic diagrams (Lesk & Hardman 1982) of HA subunits showing the amino and carboxy termini of the glycopolypeptide components, HA_1 and HA_2 (for review see Wiley & Skehel 1987). On the left subunit the symbols indicate the locations of single amino acid substitutions in antigenic mutants selected by growing Hong Kong influenza virus in the presence of different monoclonal antibodies (Laver *et al.* 1979; Daniels *et al.* 1983). The amino acid substitutions are: 54, Asn→Lys; 63, Asp→Asn, Asp→Tyr; 128, Thr→Asn; 134, Gly→Trp; 135, Gly→Arg; 143, Pro→Thr, Pro→Ser; 144, Gly→Asp; 145, Ser→Asn; 146, Gly→Asp; 156, Lys→Glu; 157, Ser→Leu; 158, Gly→Glu; 188, Asn→Asp; 189, Gln→Lys, Gln→His; 193, Ser→Arg. On the right subunit the amino acid substitutions detected in the HAs of Hong Kong influenza viruses isolated between 1968 and 1986 are shown (Both *et al.* 1983; Skehel *et al.* 1983, and unpublished results).

Antibody recognition

The surface of the membrane distal globular domain formed by the HA_1 chain contains the sites that are recognized by infectivity-neutralizing antibodies. This has been most convincingly concluded from analyses for the HAs of monoclonal-antibody-selected antigenic variants that contain one or occasionally two amino acid substitutions compared with the wild-type molecule. The molecular locations of the substitutions, which are exclusively in the HA_1 polypeptide (figure 1), define five antigenically important regions: site C, a surface bulge about 60 Å from the top of the molecule; site E, a region of β-structure which extends distally from it; site A, a prominent loop nearer the tip of the molecule; site B, a loop and a short α-helix at the top, and site D, a region of β-structure near the interface between subunits in the trimer.

The nature of the amino acid substitutions is also given in figure 1. They include residues with different sidechain length or sidechain charge, and in one case a substitution that creates a new site for glycosylation.

Two sorts of evidence indicate that the locations of these amino acid substitutions indicate the sites where the selecting antibodies bind to the wild-type HA. The first comes from X-ray crystallography of mutant HAs and concerns two mutants, one with an amino acid substitution at position HA_1 146 (Gly→Asp) (Knossow *et al.* 1984) and one at HA_1 188 (Asn→Asp) (Weis 1987). No structural changes other than those in the areas of the new amino acid sidechains were detected in either mutant; this indicates directly that the structural change that prevents binding of the selecting antibody is exclusively at the site of amino acid substitution and

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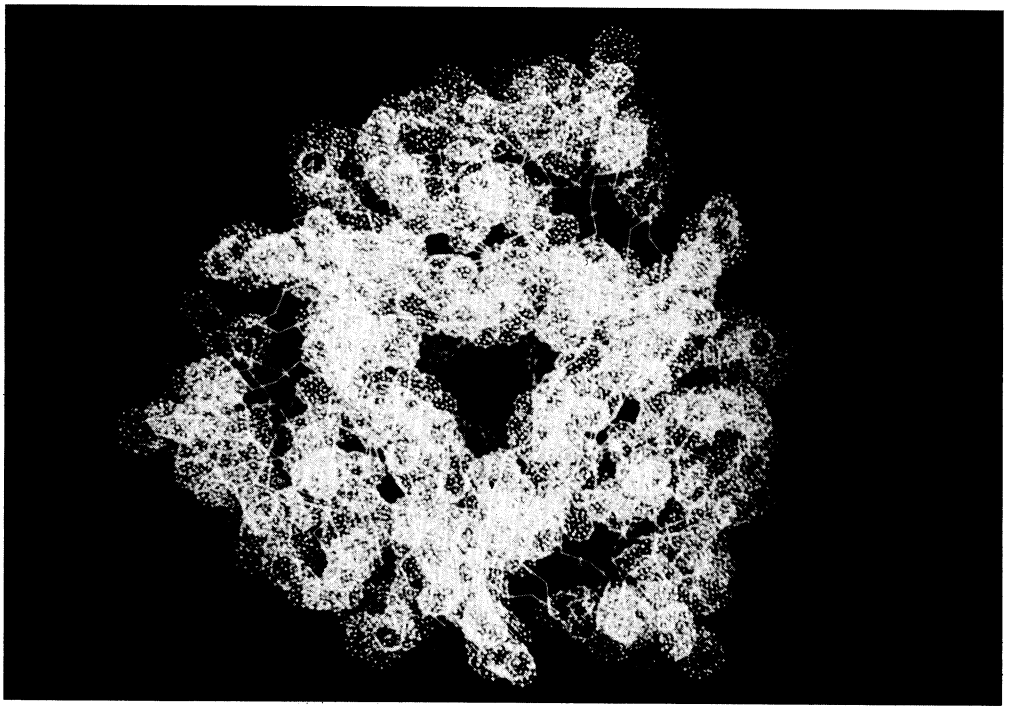

FIGURE 2. Dotted spheres mark the locations of amino acid residues that have changes in the HAs of viruses isolated between 1968 and 1986 on a diagram of the HA trimer viewed from the membrane distal top of the molecule. The three regions devoid of spheres are the sialic acid receptor-binding sites of the three subunits (Weis *et al.* 1988).

(*Facing p.* 480)

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therefore that this site defines the position of antibody binding. The second comes from electron microscopy of monoclonal antibody HA complexes formed with antibodies that select substitutions in sites A, B and E (Wrigley *et al.* 1983). In each case the antibody is seen to associate with the HA in the region containing the amino acid substitution.

On the basis of this evidence, infectivity-neutralizing antibodies bind to a number of regions on the membrane distal surface of the HA in interactions that are destabilized by single amino acid substitutions in antigenically variant HAs. These conclusions are in accord with the locations of amino acid substitutions that have been detected in the HAs of antigenic variants isolated in the period of antigenic drift in Hong Kong influenza viruses between 1968 and 1986. The substitutions cluster in the five antigenically important regions of the HA shown in figure 1; they involve changes in amino acid sequence similar to those listed and, as the period of antigenic drift has extended, they have involved most of the residues on the surface of the membrane distal HA_1 domain. This is particularly clear when the HA is viewed from the top of the molecule, down the threefold axis of symmetry (figure 2, plate 1) when the locations of the amino acid substitutions are seen to encircle the conserved receptor binding site (Weis *et al.* 1988).

Helper T-cell recognition

Studies of the specificity of helper T cells have also involved analyses of the differential recognition of monoclonal-antibody-selected antigenic mutants in these cases by T-cell clones. In a series of studies Gerhard and colleagues defined two regions of the PR8 (H_1 subtype) HA by the inability of mutants with amino acid substitutions at HA_1 122 and HA_1 140 to stimulate the production of IL2 by distinct T-cell hybridomas (Hurwitz *et al.* 1984; Hackett *et al.* 1983). They also characterized the former site, using peptide analogues to stimulate IL2 production, and further defined in this way the importance for recognition of residue HA_1 122 and an additional recognition region near the carboxy terminus of HA_1. This region, HA_1 302–315, was also shown to be recognized by human T-cell clones by Lamb & Green (1983). Regions of the HA recognized by T-cell clones have also been investigated by Thomas and colleagues in similar experiments, by using synthetic peptides and monoclonal antibody selected variants to stimulate the proliferation of clones isolated from Hong Kong virus-infected mice (Thomas *et al.* 1986, 1987). T-cell clones from infected CBA mice were shown to be stimulated by peptides equivalent to HA_1 residues 48–68; 118–138, 226–245, 246–265 and 269–288. In Hong Kong HAs the sequences of two of these regions, 226–245 and 246–265, are relatively conserved during antigenic drift. However, HA_1 48–68 contains residues that form part of antibody-binding site C, and peptide analogues of this region with amino acid sequences different from the wild-type HA sequence were used to implicate residue HA_1 54 in recognition by specific clones. Amino acid substitutions at residue HA_1 54 in monoclonal-antibody-selected variants were used previously to define antigenic site C (Laver *et al.* 1979; Wiley *et al.* 1981). Also in these studies, the importance of residue 135 in antigenic site A for recognition by clones stimulated by peptide HA_1 118–138 was shown by the inability of a monoclonal-antibody-selected variant containing the substitution HA_1 135 (Gly→Arg) to stimulate proliferation.

This apparent overlap of the recognition specificities of T-cell clones and antibodies is more marked in a study of T-cell clones isolated from infected Balb/C mice in which the majority of clones, over 70 to date, recognize residues in either antigenic sites B or E. Antigenic site E is defined (figure 1) by amino acid substitutions at HA_1 63, and a group of clones responding

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to peptide HA_1 53–78 failed to be stimulated by mutants with Asp→Tyr or Asp→Asn substitutions at this position. The short α-helix, residues HA_1 189–199, which is a component of antigenic site B, is recognized by another group of clones. In these cases substitutions in monoclonal-antibody-selected variants at HA_1 193 and HA_1 198 are seen to influence T-cell proliferation, and differences in the recognition specificities of different clones are reflected in their responses to variants containing Ser→Asn, Ser→Arg or Ser→Ile substitutions at residue HA_1 193.

The recognition specificities of these clones were also analysed by using synthetic peptides. A peptide equivalent to site E, HA_1 56–76, stimulated those clones that were influenced by amino acid substitutions at residue HA_1 63, and similar observations have been reported by Brown *et al.* (1988) for mice of the $H2^d$ haplotype. Similarly, analogues of peptide HA_1 186–200 containing Ser→Asn or Ala→Glu substitutions at residues HA_1 193 or HA_1 198 either stimulated or failed to stimulate clones shown to recognize equivalent substitutions in mutant HAs.

These observations on the recognition specificities of HA-specific Balb/C helper T-cell clones suggest that common features of the HA are recognized by T cells and antibodies but as the mechanisms of recognition involved are clearly different – antibody binding is sensitive to HA conformation whereas T cells recognize protein fragments in association with MHC class II molecules – the molecular basis for the commonality is not clear. It is possible, however, that after infection HA-specific immune B cells functioning as antigen-presenting cells may preferentially re-present in association with class II molecules those regions of the processed HA with which their surface antibodies combine in the native molecule. In this way they may influence the range of specificities demonstrated by T cells isolated from convalescent mice.

Finally, a number of observations have been made in the course of these studies using synthetic peptides and mutant HAs to define regions recognized by helper T cells that are not as readily interpretable as the results described above. For example, a mutation at HA_1 17 (His→Arg) that destabilizes the HA, rendering it susceptible to proteolysis, prevents recognition by a number of HA-specific T-cell clones which do not, however, respond to peptide analogues of this region of the wild-type molecule. It is possible that the mutant HA yields different protein fragments during its proteolytic processing by the antigen-presenting cells. In a second case a deletion mutant of HA lacking residues 224–230 is not recognized by a number of clones specific for HA_1 but is rendered 'visible' by reduction and alkylation. The region recognized by these clones has been mapped distant to the deletion, between residues HA_1 236 and 245, and the deletion may remove a site normally involved in HA processing. Thirdly, one of the monoclonal-antibody-selected mutants that defines antigenic site E introduces a site for glycosylation at residue 63. This substitution is recognized by a T-cell clone, which fails to proliferate in response to the mutant HA. However, inhibition of glycosylation by tunicamycin leads to the production of a non-glycosylated protein to which the clone responds, albeit in a cytotoxicity assay, which suggests that glycosylation influences recognition by this clone.

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Discussion

Sir Aaron Klug, F.R.S. (*MRC Laboratory of Molecular Biology, Cambridge, U.K.*). Do the antigenic sites correlate with mobile regions of the protein, and is the correlation better than with regions on the surface, which are merely accessible?

J. J. Skehel. Figure D1 shows the relation between antigenic regions, temperature factors, and accessibility on the haemagglutinin.

The amino acid sequence of the X31 HA_1 polypeptide chain is listed on the horizontal axis. The lower set of curves are temperature factors (maximum $B = 50$) from the refined HA structure of Knossow *et al.* (1986). The upper set of curves are accessible surface areas (arbitrary scale) to probes of different radii.

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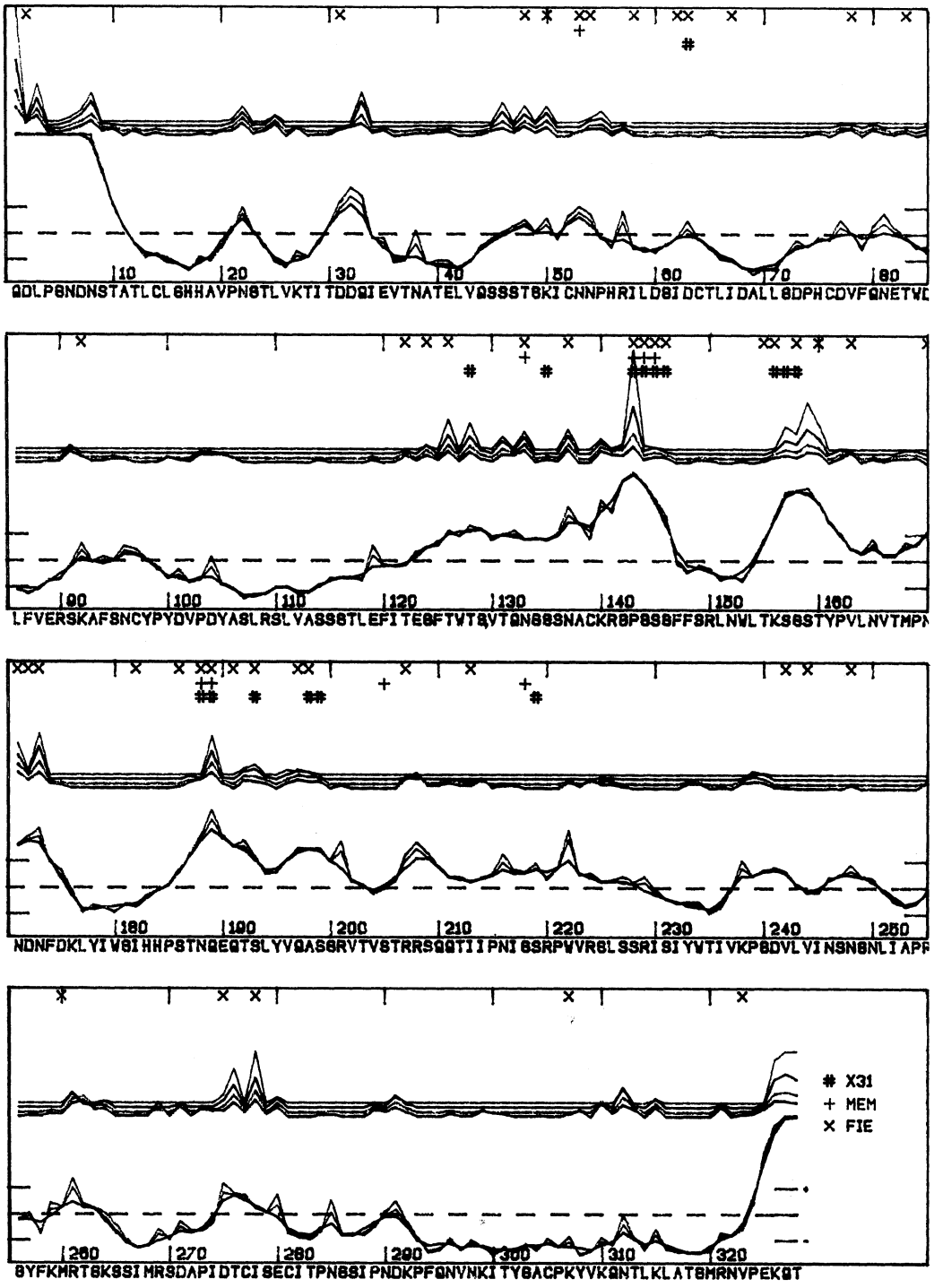

FIGURE D1. See text for description.

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Antigenic regions are specificed by three symbols: ×, amino acid substitutions in the HAs from viruses of the H_3 subtype isolated between 1968 and 1979 (Both & Sleigh 1981); +, single amino acid substitutions in monoclonal-antibody-selected mutants of A/Memphis/102/72 (Laver *et al.* 1980); #, single amino acid substitutions in monoclonal-antibody-selected mutants of X-31 (Daniels *et al.* 1983). (Probe surfaces calculated by Handschumacker-Richards program ACCESS: plot generated by BPLOT program by W. Bennett.)

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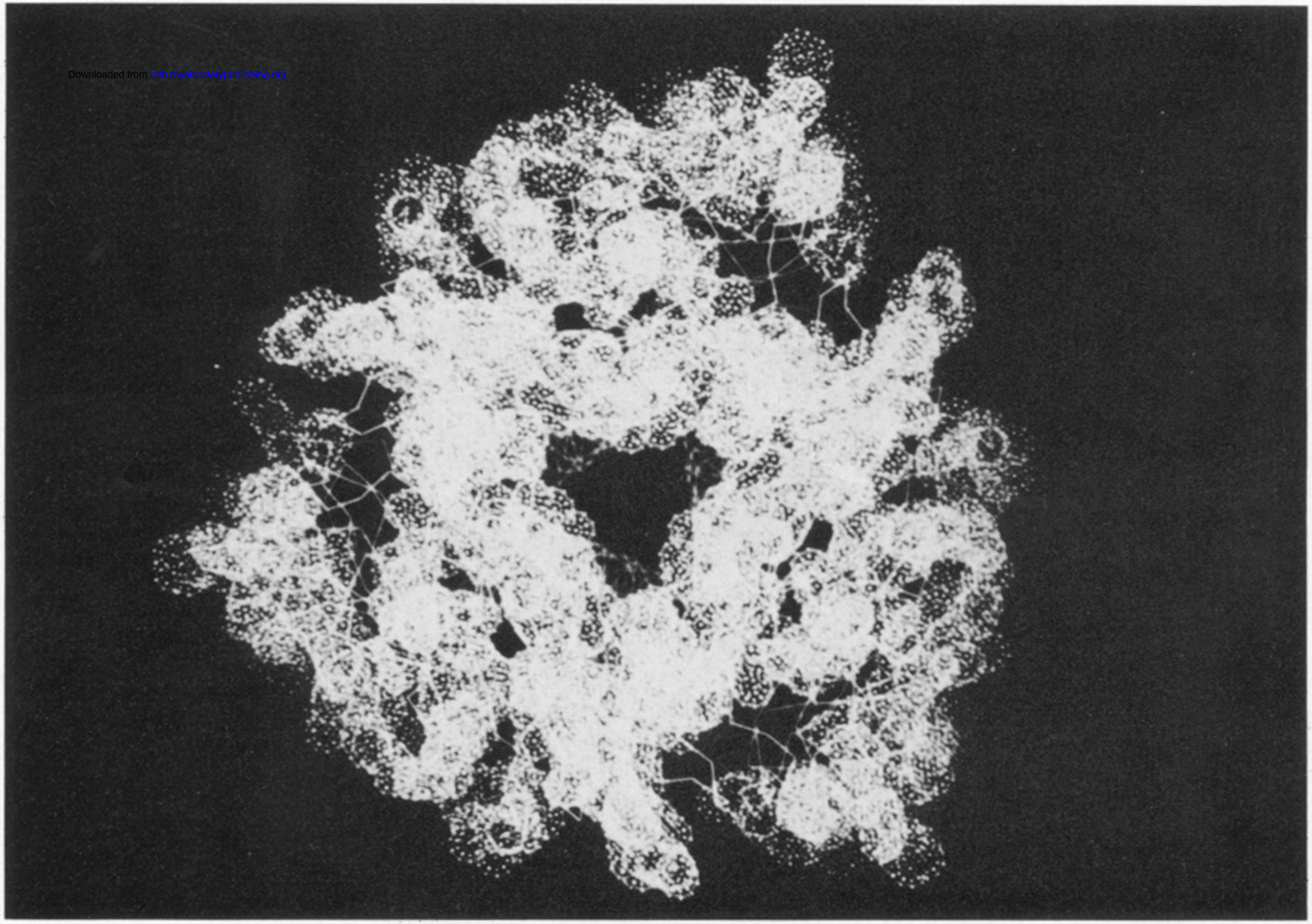

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