The homologies of gas vesicle proteins

Ann E. Griffiths, A. E. Walsby* and P. K. Hayes

Department of Botany, University of Bristol, Woodland Road, Bristol BS8 1UG, UK

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In addition to GvpA, the main structural protein, an SDS-soluble protein has been found in gas vesicles isolated from six different genera of cyanobacteria. N-terminal sequence analysis of the first 30 to 60 residues of the gel-purified proteins showed that they were homologous to GvpC, a protein that strengthens the gas vesicle in Anabaena flos-aquae. The proteins from some of the organisms showed rather low homology, however, and this may explain why the genes that encode them have not been found by Southern hybridization studies. The gas vesicles of another cyanobacterium, Dactylococcopsis salina, contained two SDS-soluble proteins (M, 17000 and 35000) that were identical in sequence for the first 24 residues but not thereafter; these two proteins showed no clear homology to GvpC. The sequence of GvpA, the main structural gas vesicle protein, was very similar in each of the organisms investigated. GvpA from the purple bacterium Amoebobacter pendants was different for the first 8 residues but 51 of the next 56 residues were identical to those of the cyanobacterial GvpA. Analysis of the GvpA and GvpC sequences provides support for the idea that the low diversity of GvpA reflects a high degree of conservation rather than a recent origin followed by lateral gene transfer between different bacteria.

Introduction

Gas vesicles provide many micro-organisms with buoyancy. They occur in at least five of the eleven phyla in the Bacteria (the cyanobacteria, green sulphur bacteria, green non-sulphur bacteria, purple bacteria and Gram-positive bacteria), and two of the phyla in the Archaea (sensu Woese et al., 1990) (the extreme halophiles and methanogens). In cyanobacteria the gas vesicle has the form of a hollow cylindrical tube closed by hollow conical end caps (Bowen & Jensen, 1965; Jost, 1965). Both the end caps and the central cylinder are made up of ribs 4-6 nm wide, which, in gas vesicles of Anabaena flos-aquae, have a crystallographic structure with subunits repeating at intervals of 1.15 nm (Blaurock & Walsby, 1976). Direct amino acid sequencing has shown that these gas vesicles contain a small, mainly hydrophobic protein of 70 amino acid residues, which corresponds in size to that of the crystallographic unit cell (Walker et al., 1984; Hayes et al., 1986). Similar proteins have been demonstrated in gas vesicles of five other genera of cyanobacteria (Walker et al., 1984; Hayes et al., 1986), and two species of halobacteria (Walker et al., 1984; Surek et al., 1988).

Tandeau de Marsac et al. (1985) isolated the gene (gvpA) that encodes this protein, which is now termed GvpA, from Calothrix sp. PCC 7601. Downstream from gvpA was a second gene that encoded an identical protein and an open reading frame, gvpC, encoding a putative protein of 162 amino acids that had four contiguous repeats of 33 amino acid residues (Damerval et al., 1987).

Damerval et al. (1989) showed by Southern hybridization that of 24 gas-vacuolate strains of cyanobacteria all contained a gene homologous to gvpA from Calothrix sp. but only 12 contained sequences homologous to gvpC from the same organism. They concluded that either the product of gvpC was not essential for gas vesicle formation, or the gene had diverged to the extent that it could no longer be detected by DNA:DNA hybridization.

Hayes et al. (1988) isolated a gene homologous with the Calothrix gvpC from Anabaena flos-aquae and showed that its product, the protein GvpC, was a component of the gas vesicle. GvpC accounted for only 2-9% of the protein molecules present in the structure, with GvpA making up the remainder (Walsby & Hayes, 1988; Hayes et al., 1988). Walsby & Hayes (1988) showed that the GvpC could be removed from the gas vesicle by rinsing with detergent; the structure remained intact but was weakened. It was concluded that GvpC was located on the outside of the gas vesicle and it was suggested that its function might be to strengthen the ribbed structure formed by GvpA. This was confirmed by Hayes et al. (1992), who demonstrated that GvpC could be

* Author for correspondence. Tel. (0272) 303761.

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reattached to gas vesicles that had been stripped of their native GvpC, and that this restored their strength.

The fact that the basic gas-filled structure of the gas vesicle survives when GvpC is removed may indicate that this protein is not absolutely essential for its formation. We have analysed the proteins in gas vesicles isolated from a number of cyanobacteria to determine whether the GvpC-like proteins were universal constituents of these structures. We investigated representitives of three of the five taxonomic sections (I, III and IV) described by Rippka et al. (1979). Some Section V organisms (branched filaments) may produce gas-vacuolate hormogonia but are not available in culture; no gas-vacuolate organisms are known from Section II.

Methods

Organisms and culture conditions. Gas vesicles were isolated from the bacteria listed in Table 1, which gives information on the taxonomic groupings and strain numbers from the following collections: Culture Collection of Algae and Protozoa (CCAP) at the Institute of Freshwater Ecology, Ambleside, Cumbria, UK; Pasteur Culture Collection (PCC), Institut Pasteur, Paris, France; Culture Collection of Algae (CYA) at the Norwegian Institute for Water Research, Oslo, Norway. Anabaena flos-aquae was grown in the medium of Walsby & Booker (1980). Aphanizomenon flos-aquae and Calothrix sp. were grown in medium B + N of Armstrong et al. (1983). Dactylococcopsis salina was grown at 32 °C in the medium described by Walsby et al. (1983), but with lower concentrations of CaCl₂, 2H₂O (0.08 g 1⁻¹), K₂HPO₄ (8 mg 1⁻¹) and Na₂CO₃ (2 mg 1⁻¹), and a higher concentration of Na₂EDTA (3 mg 1⁻¹). Microcystis sp. was grown in medium BG 11 of Rippka et al. (1979). The green strain of Oscillatoria agardhii was grown in the medium of Van Liere & Mur (1978). Spirulina sp. was grown in medium containing the following ingredients in 1 litre: NaCl (1 g), NaNO₃ (2.5 g), CaCl₂ (0.04 g), FeSO₄ 7H₂O (0.01 g), Na₂EDTA (0.08 g), K₂SO₄ (1 g), MgSO₄. 7H₂O (0.2 g), K₃HPO₄ (0.5 g), NaHCO₃ (16.8 g), and 1 ml of the trace element mixture of Stanier et al. (1971). All cultures were grown under warm white fluorescent light and bubbled continuously with air. Cells of Amoebobacter pendens were supplied by Professor Norbert Pfennig at the University of Konstanz, Germany.

Gas vesicle isolation. Cultures were left to stand at low irradiance (<4 μmol m⁻² s⁻¹) for up to 72 h. Floating cells or filaments were drawn off from the liquid surface using a cannula attached to an evacuating Buchner flask. Cells of Aphanizomenon flos-aquae and Anabaena flos-aquae were lysed by the addition of 0.7 M-sucrose, and incubated at 37 °C for 2 h. Cells of Amoebobacter pendens, Microcystis sp., Oscillatoria agardhii CYA 29 and Spirulina sp. were lysed by the addition of lysozyme (at concentrations of 0.75, 4, 4 and 5 mg ml⁻¹, respectively), 0.05 M-Tris pH 8.0, and 0.1 M-EDTA, and incubated at 37 °C overnight. Cells of Dactylococcopsis salina were lysed by the addition of 4 mg lysozyme ml⁻¹ in 3% (w/v) NaCl, and 2% (v/v) Triton X-100. Lysis was confirmed under the light microscope. Gas vesicles were collected by accelerated flotation (Walsby, 1974). After three successive centrifugations, gas vesicles were filtered through 1.0 μm pore size Nuclepore filters, concentrated as described above, and then stored at 4 °C in 0.05% (w/v) ammonium bicarbonate solution (or 3% w/v, NaCl for Dactylococcopsis salina) containing 5 mM-NaCN to act as a preservative (Powell et al., 1991).

Electrophoretic purification of gas vesicle proteins. Collapsed gas vesicles were resuspended in 2.5% (w/v) SDS, 0.05% (w/v) bromophenol blue and 0.05% (w/v) xylene Cyanol ff, and loaded into wells of 10% (w/v) polyacrylamide gels (Laemmli, 1970). Protein bands were stained with Coomassie blue. The size of the protein in each band was estimated by comparison with proteins of known M₆, (Rainbow, Amersham).

For sequencing studies, gels were lightly stained (1 min) and the required bands were cut out and eluted in 30 mM-Tris (pH 8.5), 0.5 mM-EDTA, 1 mM-sodium thioglycollate and 0.1% (w/v) SDS. A current of 8-10 mA per tube was applied over 15 h (BioRad Miniprotean I). Lysis was confirmed under the light microscope. Gas vesicles were filtered through 1.0 μm pore size Nuclepore filters, concentrated as described above, and then stored at 4 °C in 0.05% (w/v) ammonium bicarbonate solution (or 3% w/v, NaCl for Dactylococcopsis salina) containing 5 mM-NaCN to act as a preservative (Powell et al., 1991).

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**Table 1. Soluble proteins in gas vesicles isolated from cyanobacteria and a purple bacterium**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain</th>
<th>Section*</th>
<th>Estimated M₆†</th>
<th>Reference to strain</th>
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<tr>
<td>Anabaena flos-aquae</td>
<td>CCAP 1403/13F</td>
<td>IV</td>
<td>28400‡</td>
<td>Walsby &amp; Hayes (1988)</td>
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<td>Aphanizomenon flos-aquae</td>
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<td>20000§</td>
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<td>PCC 8305</td>
<td>I</td>
<td>35200</td>
<td>Walker et al. (1984)</td>
</tr>
<tr>
<td>Microcystis sp.</td>
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<td>17300</td>
<td></td>
</tr>
<tr>
<td>Oscillatoria agardhii (green)</td>
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<td>I</td>
<td>25200‡</td>
<td>Walsby &amp; Hayes (1988)</td>
</tr>
<tr>
<td>Spirulina sp.</td>
<td>CYA 29</td>
<td>III</td>
<td>21700</td>
<td>Walsby &amp; Bleything (1988)</td>
</tr>
<tr>
<td>Amoebobacter pendens</td>
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<td>III</td>
<td>22100</td>
<td>P. Newton (unpublished)</td>
</tr>
<tr>
<td></td>
<td>Konstanz 5813</td>
<td>–</td>
<td>ND</td>
<td>Eichler &amp; Pfennig (1986)</td>
</tr>
</tbody>
</table>

* The systematic sections of cyanobacteria defined by Rippka et al. (1979).
† The M₆ values estimated from the mobilities of bands in the SDS-PAGE system used. The discrepancies with the known values (‡, §) indicate that the value given for other species may also be overestimated. ND, Not determined.
‡ The M₆, indicated by the deduced amino acid sequence (Hayes et al., 1988) is 21985.
§ The M₆, indicated by the deduced amino acid sequence (Damerval et al., 1987), including N-terminal Met, is 19179.
Results

Electrophoretic separation of gas vesicle proteins

When entire gas vesicles from each of the cyanobacteria were analysed by SDS-PAGE, Coomassie blue stained three components: (1) the residual protein that failed to penetrate the gel, (2) a minor component that lodged at the junction of the stacking and separating gels, and (3) usually a single band in the separating gel with a mobility indicating an \( M_r \) in the range of 19000 to 30000 (Fig. 1). Previous studies on gas vesicles from *Anabaena flos-aquae* showed that the protein in the well was GvpA and the protein in the mobile band was GvpC (Hayes et al., 1988; Walsby & Hayes, 1989). Western blotting studies previously suggested that the protein at the top of the separating gel was GvpA (Walsby & Hayes, 1989). To obtain confirmation of this, entire gas vesicles isolated from *Anabaena flos-aquae* were run on SDS-polyacrylamide gels, and the protein lodged at the top of the separating gel was eluted and sequenced. The sequence obtained was Ala-Val-Glu-Lys-Thr-Asn-Ser-Ser-Ser-Ser-Leu-Ala, which is the N-terminal sequence of GvpA from *Anabaena flos-aquae* (Walker et al., 1984, and see Fig. 2). In similar gels that had been stained for a longer time it was possible to see, by examination with a hand lens, that this band comprised a family of very narrow

![Fig. 1. SDS-polyacrylamide gel of proteins from isolated gas vesicles: lane 1 'Rainbow' size markers with \( M_r \) values; lane 2 *Microcystis* sp. showing, from the top, GvpA in the well, GvpA at the top of the running gel, GvpC, and a trace of lysozyme; lane 3, *Dactylococcopsis salina* showing two mobile bands.](image)

![Fig. 2. Amino acid sequences for GvpA aligned to show the high degree of sequence homology within this family of proteins. Boxed and shaded areas show where the amino acid residues are identical for 50% or more of the proteins. Sequences for *Anabaena flos-aquae*, *Calothrix* sp. (PCC 7601) and *Pseudanabaena* sp. (PCC 6901) have been derived from their gene sequences (P. K. Hayes, unpublished; Tandeau de Marsac et al., 1985; Damerval et al., 1991). Partial amino acid sequences for *Aphanizomenon flos-aquae*, the red *Oscillatoria* agardhii CYA 18 (PCC 7821; CCAP 1459/37) and *Microcystis* sp. are from Walker et al. (1984) and Hayes et al. (1986). Residues of uncertain identity at positions 37 and 42 in the Aphanizomenon and red Oscillatoria sequences have been previously listed as P or P/E (Walker et al., 1984). (See Table 1 for other strain numbers).](image)
Table 2. Distance matrix analysis of GvpA sequence

The number in each box is the percentage of amino acid residues in the shorter sequence of each comparison that are identical to residues in the other sequence. For strain numbers see Table 1 and Fig. 1.

<table>
<thead>
<tr>
<th>Organism</th>
<th>No.</th>
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<td>60</td>
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bands whose intensity decreased with distance from junction of the gels.

N-terminal amino acid analysis of three new GvpAs

The N-terminal amino acid sequences of GvpA, obtained by analysing entire gas vesicles, of the green-coloured strain of Oscillatoria agardhii CYA 29, Spirulina sp. and Amoebobacter pendens are shown in Fig. 2. Comparisons are made to sequences of GvpA in Anabaena flos-aquae, and other cyanobacteria previously investigated (Walker et al., 1984; Tandeau de Marsac et al., 1985; Hayes et al., 1986; Damerval et al., 1991) using distance matrix analysis (Table 2).

The SDS-soluble gas vesicle proteins

Gas vesicle proteins of Aphanizomenon flos-aquae, Calothrix sp., the green Oscillatoria agardhii, Spirulina sp. and Microcystis sp. were separated by SDS-PAGE. In each organism, a protein of M, between 19 000 and 30 000 was observed (see Table 1).

Previous studies on Dactylococcopsis salina gas vesicles indicated only a single mobile band when gas vesicles were separated by SDS-PAGE (Hayes et al., 1986). In this study, however, two mobile bands were consistently found, one of M, 35 000 as previously seen, and a second of M, 17 000. The two bands were obtained even after rinsing the isolated gas vesicles with 2% (v/v) Triton X-100, 0.001% (w/v) SDS, and vigorous vortex-mixing in water. They were present in approximately equimolar quantities.

Contamination by lysozyme

In some of the samples (Microcystis, Spirulina, Oscillatoria, Amoebobacter) there was an additional minor band with a mobility indicating a M, of about 14 000, which ran coincidentally with lysozyme. This band was gel-purified from a sample of Amoebobacter gas vesicles; N-terminal sequence analysis gave the amino acid sequence Lys-Val-Phe-Gly-Arg-(unknown)-Glu-Leu-Ala-Ala-Ala-Met-Lys-Arg-His-Gly-Leu-Asp-Asn-Tyr-Arg-Gly-Tyr-Ser-Leu-Gly-Asn, which identified this protein as lysozyme (Jung et al., 1980). It appeared that the lysozyme originally added to lyse the cells of these organisms had become adsorbed to the gas vesicles during the isolation procedure.

N-terminal amino acid analysis of the GupC-like proteins

N-terminal amino acid sequences obtained from the proteins eluted from the single mobile bands from Aphanizomenon flos-aquae, Oscillatoria agardhii CYA 29, Spirulina sp., and Microcystis sp., and the two mobile bands from Dactylococcopsis salina, are shown in Fig. 3.
**Aphanizomenon flos-aquae**

**Anabaena flos-aquae**

**Calothrix sp.**

**Microcystis sp.**

**Green Oscillatoria agardhii**

**Spirulina sp.**

**Dactylococcopsis salina**

**Table 3. Distance matrix analysis of GvpC-like proteins**

The first number in each box is the percentage of amino acid residues in the shorter sequence of each comparison that are identical to residues in the other sequence, and the second number (in italics) is the percentage of residues that are similar. Similar residues are as defined by Devereux et al. (1984): neutral, weakly hydrophobic (P, A, G, S, T); hydrophilic, acid amine (Q, N, E, D); hydrophilic, basic (H, K, R); hydrophobic (L, I, V, M); hydrophobic, aromatic (F, Y, W). For strain numbers see Table 1 and Fig. 1.
Also shown are the N-terminal portions of the deduced amino acid sequences of GvpC from Calothrix sp. and Anabaena flos-aquae which have been obtained from the gene sequences (Damerval et al., 1987; Hayes et al., 1988). The presence of GvpC in Anabaena flos-aquae gas vesicles was previously confirmed by N-terminal sequencing of the gel-purified protein (Hayes et al., 1988), and in Calothrix by SDS-PAGE, which revealed a 20000 M₄ band (Damerval et al., 1991), which is close to the M₄ of the gvpC gene product, 19179 (Damerval et al., 1987). The N-terminal sequences of these seven proteins are compared to one another by distance matrix analysis in Table 3.

**Discussion**

**GvpA**

Our results confirm the presence of two types of protein in the gas vesicles of Anabaena flos-aquae. The identification of the series of bands at the top of the stacking gel as GvpA indicates that this protein forms aggregates that are not easily disrupted. Walker & Walsby (1983) found that the Anabaena gas vesicle membranes could be completely dissolved to form a clear solution in 80% (v/v) formic acid, but only the M, 22000 band, now known to be GvpC, was subsequently obtained by SDS-PAGE. Damerval et al. (1991) showed that a single mobile band of M, 7000 could be obtained from gas vesicles of Pseudanabaena sp. PCC 6901 after dissolving them in formic acid; this is probably GvpA, but its identity should be confirmed by N-terminal sequence analysis.

Our three new GvpA sequences provide further evidence of the high degree of conservation of this protein (Table 2). It is of interest that the sequences of the four Section III cyanobacteria – Pseudanabaena, Spirulina and the two Oscillatoria strains – are identical up to residue 40, and are distinct from all of the others in having valine at residue 5 and serine at residue 40.

The first eight residues of the *Amoebobacter pendens* GvpA differ from the cyanobacterial sequence but of the next 56 residues 51 are identical to the consensus sequence; the five changes are all conservative (four of them Ile→Val, and the fifth Arg→Lys) and two of them occur in other cyanobacteria. In fact this section shows more similarity to the consensus cyanobacterial sequence than the corresponding portion of *Dactylococcopsis* GvpA. The *Amoebobacter* GvpA shows greater homology to the cyanobacterial consensus sequence (80%) than to the sequence of Halobacterium halobium GvpA (58%).

Although gas vesicles of different cyanobacteria (and other organisms) are remarkably similar in overall shape and fine structure, they do differ in cylindrical diameter (Hayes & Walsby, 1986; Walsby & Bleything, 1988). These differences in gas vesicle diameter could be determined by the amino acid sequence of GvpA or GvpC, or by other gene products that might be involved in assembly of the structure. Diameter is important because it determines the critical collapse pressure of the gas vesicle and its efficiency in providing buoyancy (Walsby & Bleything, 1988).

**GvpC**

Preparations of gas vesicles isolated from seven genera of cyanobacteria have now been shown to contain SDS-soluble proteins. In four of these genera, *Anabaena, Aphanizomenon, Calothrix and Microcystis*, comparison of the sequences of these proteins indicates significant homology (Fig. 3, Table 3); in the other three genera the homology is less clear-cut.

Only 20% of the residues in the Oscillatoria protein are identical to those in the Anabaena GvpC, but 36% are identical to those in Microcystis GvpC. A number of the different residues indicate conservative changes; including these conservative changes yields 52% residues that are similar to those in Microcystis GvpC. For the Spirulina protein there are only 23% of residues identical to Anabaena GvpC and 31% to Microcystis GvpC, but the percentages of similar residues are 40% and 57%, respectively. These comparisons suggest that the proteins of these two species are also part of the GvpC family. Further evidence that they are true components of the gas vesicle might be provided by showing that they can restore the strength of the gas vesicles on being readsorbed by them, or by showing that the encoding genes form part of a gvp operon.

The two SDS-soluble proteins obtained from Dactylococcopsis salina gas vesicles show little homology with the other GvpCs and we have no direct evidence that these two proteins are the homologues or even the functional analogues of GvpC. The remarkable feature of the two sequences is that the first 24 residues are identical; a gene duplication must have been responsible for this.

The results of this survey provide evidence that SDS-soluble outer-proteins are widespread, if not universally present, on the gas vesicles of cyanobacteria. Recent studies on the mechanical properties of gas vesicles (Walsby, 1991) indicate that GvpC increases the critical pressure of the structure by postponing buckling. This property would be especially important in wide gas vesicles, such as those of the halobacteria (Walsby, 1971). There is no direct information at present on whether halobacterium gas vesicles contain a functional analogue of GvpC, but in the gvp operon of *Halobacterium halobium* there is a gene downstream from *gvpA* that encodes a protein which, like the cyanobacterial GvpCs,
has a high content of acidic amino acids and a repeating structure, in this case seven repeats of length between 32 and 40 residues (Jones et al., 1991; Horne et al., 1991). The N-terminal sequence of this protein shows no homology to the cyanobacterial GvpCs (see Table 3) although the repeating sections show some weak homology with those in Calothrix GvpC (Jones et al., 1991). It is not known if it is this protein or GvpA that constitutes the mobile band when gas vesicle proteins of halobacteria are separated by electrophoresis (see Simon, 1981; Surek et al., 1988).

It remains now to question whether there are cases where gas vesicles lack GvpC. The observation (Damerval et al., 1989) that Southern hybridization fails to find homologues of the Calothrix gvpC gene in certain cyanobacteria could be accounted for by the low degree of conservation of the encoded proteins. This would explain, for example, why homologous genes were found in species of Anabaena and Microcystis, where there is distinct homology between the proteins, but not in species of Oscillatoria, where the homology is less. Damerval et al. (1991) found no evidence of GvpC in gas vesicles isolated from Pseudanabaena, however, and they found no gvpC-like gene immediately downstream of the single gvpA gene in this organism. It is possible, of course, that this outer protein could have been lost during the isolation procedure, and that the encoding gene could be located further away from gvpA. Nevertheless, there is no direct evidence of GvpC in the Pseudanabaena gas vesicles and as Damerval et al. (1991) have pointed out, they may derive their stability from their small dimensions. A direct investigation of the relationship between the diameter and critical pressure of the Pseudanabaena gas vesicle is now required, using the methods developed by Walsby & Bleything (1988).

Evolution of GvpA and GvpC

Gas vesicles are found in two groups of micro-organisms, the Bacteria and Archaea, that diverged at an early stage of evolution (Woese, 1987; Woese et al., 1990). If gas vesicles existed before the divergence of these two groups, the high degree of homology between extant GvpAs would indicate that there had been a very low rate of mutation in the gvpA genes (Damerval et al., 1987). Alternatively, the gene may have developed more recently, mutated at a higher rate, and then become transferred laterally between the different organisms (Walker et al., 1984). The much smaller degree of homology of GvpC is consistent with a long evolution of its encoding gene. It is unlikely that GvpC predated GvpA, and we therefore favour the idea that both genes are ancient and that the slower mutation rate of GvpA must be due, at least in part, to this small protein having to fulfil a number of requirements to produce a gas-filled structure.

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References


