PRELIMINARY NOTE

BBA 41133

Membranifibrils in cristae and grana

Linear organization has been observed on the surface of mitochondrial cristae and chloroplast grana membranes. Treatment of the membranes with detergents releases a fibrous structure which we interpret as the organizational basis for the linear arrangement in the membrane. We propose to call these fibrous structures membranifibrils.

Linear arrangements of particles on chloroplast membranes have been recognized since PARK AND PON[1] described quantosomes. Negatively stained chloroplasts also show linear structure[2]. Similar linear organization has not previously been recognized on mitochondrial cristae, probably because the twisting arrangement as shown in Fig. 1 is more common than straight lines often found in chloroplasts. In a few cristae we have observed straight linear arrangement. We also find linear elements on cristae examined by the freeze etch technique[3].

Detergent treatments will release membranifibrils from both cristae and grana membranes. The fibrils are 70–90 Å in diameter with 90-Å headpieces or F_1 knobs attached at 120-Å intervals along the fiber. Short sections of membranifibrils have been previously extracted from cristae (electron transport particles) by deoxycholate treatment[4]. The fibers were interpreted as basepieces of the elementary particles[4,5]. We have extracted the fibers from cristae by treatment with 3 % Triton X-114 (Rohm and Haas Co.) in 0.4 M KCl. After centrifugation at 78000 × *g*, the fibers remain in the supernatant. They can be purified by precipitation with saturated ammonium sulfate. The purified fiber fraction is shown in Fig. 1. The preparation contains almost no cytochrome and 30 % lipid. Fibrous structures with headpieces attached are also present in the rutamycin-sensitive ATPase prepared by cholate and ammonium sulfate treatment of cristae reported by KOPACZYK *et al.*[8]. Their preparation contains only 10 % lipid.

Fibers were extracted from chloroplasts by treatment with 4 % Triton X-100. The supernatant obtained after centrifugation at 78000 × *g* was centrifuged at 144000 × *g* for 1 h to remove a membraneous pellet. The fibers are present in the clear, light green supernatant. This fraction has enhanced photoreaction one activity, as measured with methyl viologen, and thus resembles the particles described by VERNON, SHAW AND KE[6], except that their particles did not show linear structure. The chloroplast membranifibrils are shown in Fig. 2. The light fraction (144000 × *g*), obtained by digitonin fractionation of chloroplasts[7], also contains the membranifibrils. These fiber structures can be observed after both negative stain and freeze etch procedures.

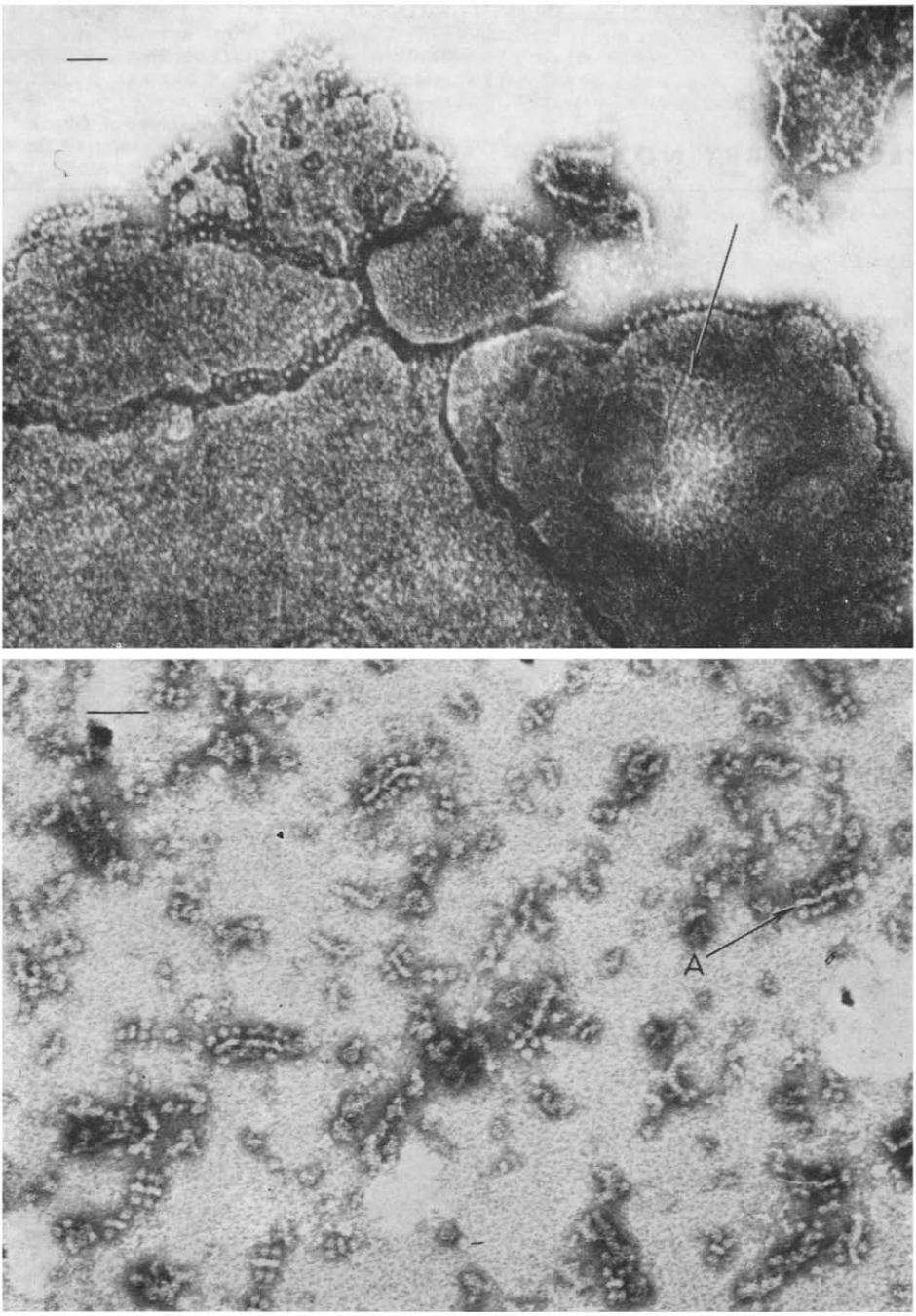

Fig. 1. Top: Fragments of beef heart mitochondrial cristae showing linear arrangement of headpieces on the surface of the membranes. Negative stain with phosphotungstate[2]. Marker 500 Å. Bottom: Membranifibrils isolated from heart mitochondria by Triton treatment. Note the thinner fiber elements at A. Negative stain uranyl acetate. Marker 500 Å.

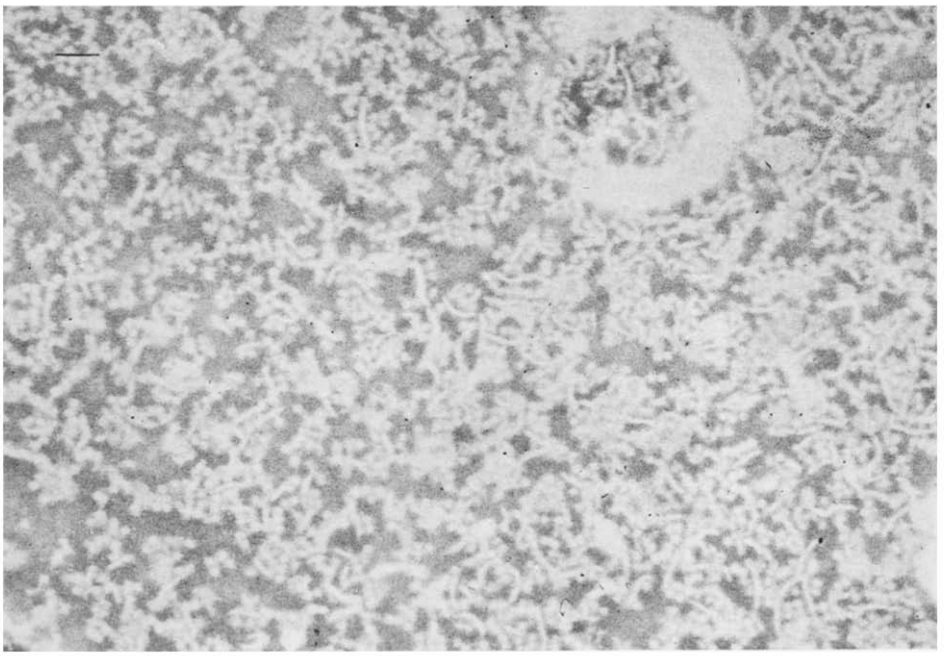

Fig. 2. Membranifibrils isolated from spinach chloroplast grana by Triton treatment. Negative stain with phosphotungstate. Marker 500 Å.

Extraction of membranifibrils with 4% aqueous acetone or chloroform–methanol (2:1, v/v) leaves 30-Å fibers as the basis of the membranifibril organization. Thin fibers similar to the extracted form can be seen at A in Fig. 1.

The fibrils to which the knobs are attached correspond to the basepieces along the edge of the cristae membrane. Knobs on the surface of the membrane edge would give the occasional thickening seen in the basepiece region. Since the membranifibrils contain no cytochrome *a* or *b* and very little *c* it is unlikely that cytochrome-dependent electron transport function is associated with the site of attachment of the knobs. The cytochromes could be located in the membrane between and under the fiber or basepiece region.

Evidence has previously been presented for fibers in plasma membrane[9]. The membranifibrils in mitochondria and chloroplasts suggest that fibrous protein may be more common in membranes than generally expected.

Supported under a grant AM 04663 from the National Institute of Arthritis and Metabolic Diseases. J.W.S. was supported by training grant GM 01195 and F.L.C. by career grant K6-21839 from the National Institute of General Medical Science.

Department of Biological Sciences,
Purdue University,
Lafayette, Ind. (U.S.A.)

J. W. STILES
J. T. WILSON
F. L. CRANE

1 R. B. PARK AND N. G. PON, *J. Mol. Biol.*, 6 (1963) 150.
2 W. P. CUNNINGHAM AND F. L. CRANE, *Exptl. Cell Res.*, 44 (1966) 31.
3 H. MOOR AND K. MUHLETHALER, *J. Cell Biol.*, 17 (1963) 609.
4 T. ODA AND S. SEKI, *6th Intern. Congr. Electron Microscopy, 1966*, Maruzen, Tokyo, 1966, p. 369.
5 H. FERNANDEZ-MORAN, T. ODA, P. V. BLAIR AND D. E. GREEN, *J. Cell Biol.*, 22 (1964) 63.
6 L. P. VERNON, E. R. SHAW AND B. KE, *J. Biol. Chem.*, 241 (1966) 4101.
7 N. K. BOARDMAN AND J. M. ANDERSON, *Nature*, 203 (1964) 166.
8 K. KOPACZYK, J. ASAI, D. W. ALLMANN, T. ODA AND D. E. GREEN, *Arch. Biochem. Biophys.*, 123 (1968) 602.
9 R. M. GLAESER, T. HAYES, H. MEL AND C. TOBIAS, *Exptl. Cell Res.*, 42 (1966) 467.

Received September 2nd, 1968

Biochim. Biophys. Acta, 162 (1968) 631–634

TITLES OF RELATED PAPERS IN OTHER SECTIONS

The following papers that have recently appeared in other sections of BIOCHIMICA ET BIOPHYSICA ACTA may be of interest to the readers of this specialized section:

BBA–BIOMEMBRANES

Transport and transport-associated phosphorylation of 2-deoxy-D-glucose in yeast (BBA 75194)
by J. VAN STEVENINCK (Leiden). 163 (1968) 386

BBA–ENZYMOLOGY

Malate dehydrogenase (decarboxylating) (NADP) isozymes of Opuntia stem tissue: Mitochondrial, chloroplast, and soluble forms (BBA 65778)
by S. K. MUKERJI AND I. P. TING (Riverside, Calif.). 167 (1968) 239
The reduction of ferricytochrome *c* during the enzymic oxidation of DOPA (BBA 65805)
by D. KERTESZ (Marseille) 167 (1968) 250
Electron–nuclear double resonance from flavin free radicals in NADPH dehydrogenase ("old yellow enzyme") (BBA 61174)
by A. EHRENBERG, L. E. G. ERIKSSON AND J. S. HYDE (Stockholm and Palo Alto, Calif.) 167 (1968) 482
Étude de la fraction oxydante de la glycéraldéhyde-3-phosphate déshydrogénase obtenue par hydrolyse chymotrypsique (BBA 65813)
par B. CANTAU, C. BRUNEL ET J. PUDLES (Orsay) 167 (1968) 511
Der Einfluss von Askorbinsäure auf die zytoplasmatische AMP-Aminohydrolase des Rattenhirns (BBA 63352)
by H. KLUGE UND V. WIECZOREK (Jena). 167 (1968) 621
Influence du β-mercaptoéthanol sur l'état d'agrégation et l'activité ATPasique de la H-méromyosine de carotide de bovidé (BBA 63359)
par A. GASPAR-GODFROID (Liège) 167 (1968) 622

BBA–NUCLEIC ACIDS & PROTEIN SYNTHESIS

Ultraviolet irradiation studies on the cytoplasmic determinant of the yeast mitochondrion (BBA 95985)
by N. G. MAROUDAS AND D. WILKIE (London) 166 (1968) 681