Myosin-like and other proteins associated with clathrin-coated vesicles in growing hyphae of Neurospora crassa

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MYOSIN-LIKE AND OTHER PROTEINS ASSOCIATED WITH CLATHRIN-COATED VESICLES IN GROWING HYPHAE OF *NEUROSPORA CRASSA* [2]

BY

Khan HOANG-VAN and Gilbert TURIAN [1]

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ABSTRACT

The clathrin-coated vesicular fraction isolated from hyphae of *Neurospora crassa* resolves on SDS-PAGE gels into a 205 Kd protein corresponding to rabbit muscle myosin plus clathrin, tubulin and probable kinesin proteins.

RÉSUMÉ

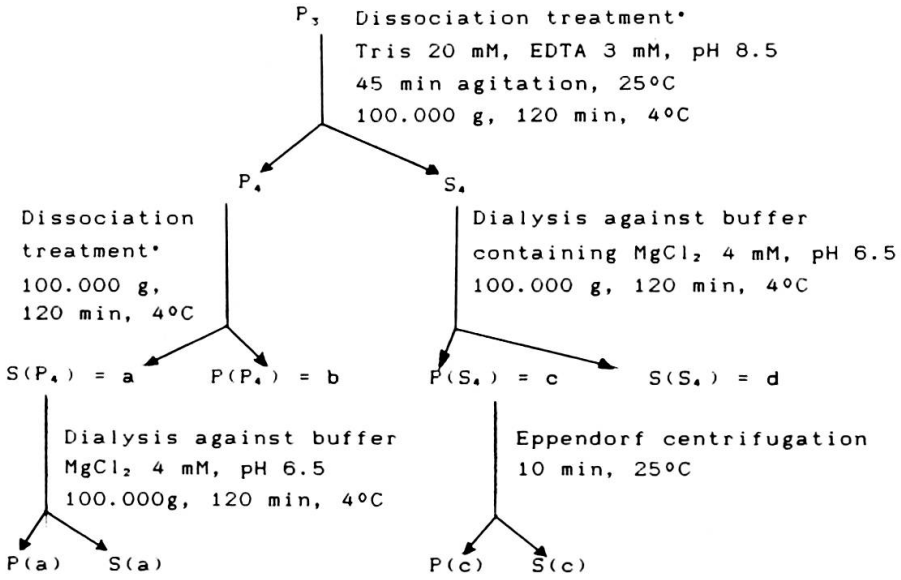
La fraction de vésicules enveloppées à clathrine isolée des hyphes de *Neurospora crassa* se résout sur gels SDS-PAGE en une protéine de 205 Kd correspondant à la myosine musculaire en plus d'autres protéines, clathrine, tubuline et, probablement, kinésine.

In our search for the types of vesicles implicated in the apical traffic of elongating hyphae of *Neurospora crassa,* we have isolated coated vesicles and identified their clathrin (Caesar-Ton That *et al.,* 1986, 1987) as recently confirmed by Rosa & Maccioni (1987). Parallely, we had measured myosin-like Ca^{2+}-ATPase activity in young hyphae of the same mold (van Tuinen *et al.,* 1986). Now, we have obtained evidence for the constant presence in extracts of hyphal coated vesicles of a protein electrophoretically separating at the same Rf than animal myosin of 205 Kd molecular weight. Other vesicular associated major proteins were liberated by our sequential dissociation treatments, according to the following procedure:

[1] Microbiologie générale, 3, place de l'Université, CH-1211, Genève 4, Switzerland.

[2] Bien que cet article n'ait pas fait l'objet d'une communication, il est publié ici, en fin de fascicule, pour des raisons d'actualité scientifique (note de la rédaction).

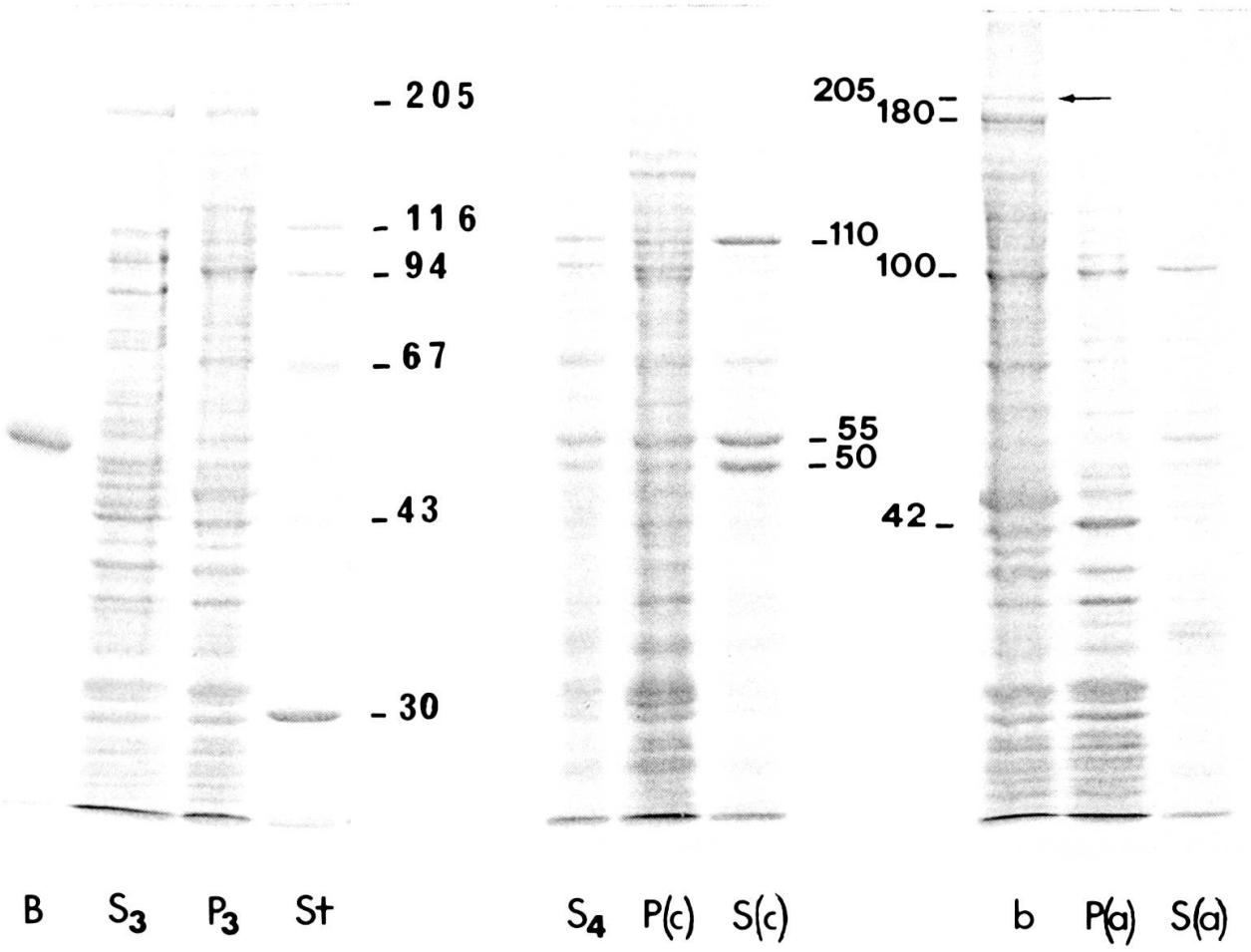
60 g of wet-weighed mycelium of *N. crassa,* grown for 16 h at 25°C in liquid Vogel (1956) synthetic medium, was frozen and ground to a fine powder in a mortar with liquid nitrogen, then homogenized in a Waring Blendor (Bender & Hobein, Zürich) with buffer-Tris 20 mM, pH 7.5, containing EGTA 3 mM, $MgCl_2$ 3 mM, and proteolytic inhibitor (β-mercapto-ethanol 1 mM, pepstatine 0.25 μg/ml, benzamidine 10 mM). The homogenate after disruption of cells was centrifuged at 48,000 g for 45 min to remove extraneous debris and large organelles. The supernatant was carefully retained and centrifuged at 100,000 g for 120 min. The resulting high speed pellet was resuspended in 12 ml of isolating buffer (MES 100 mM, pH 6.5, $MgCl_2$ 1 mM, EGTA 1 mM, 0.02% NaN_3 and proteolytic inhibitors) containing Triton 1%, and sucrose 6%, Percoll (Pharmacia) 6%, agitated for 30 min and homogenized in a glass Dounce homogeneizer. The resuspended high speed pellet was centrifuged for 120 min at 100,000 g to remove the non-coated vesicles in supernatant (S_3) and to obtain a coated vesicle pellet (P_3). The next treatments from P_3 to the final fractions were made according to the following flow-sheet:



From the $P(S_4)$ or (c) fraction, after its homogeneization in the isolating buffer and after Eppendorf centrifugation, the supernatant produced contained, after SDS-PAGE, 3 major bands corresponding to microtubule proteins (α and β, plus 110 Kd = kinesin?).

In fraction $S(P_4)$ or (a), one of the major protein bands on SDS-PAGE corresponds to actin (42 Kd). The thin band at 205 Kd on top of fraction $P(P_4)$ or (b) passed on SDS-PAGE corresponds to the rabbit muscle myosin. This myosin-like band is additionally associated with clathrin-coated vesicles (180 Kd, 100 Kd, 38 Kd, etc.).

Definitive proof for the myosin nature of our 205 Kd protein should rest on its positive reaction with antimyosin antibodies. For now, a faint staining of our 205 Kd band has been observed with monoclonal antimyosin antibodies revealed by the peroxidase procedure; this encourages us to further work along this line as now in progress.



SDS-PAGE (gradient 8-10%) of homogenate from *N. crassa:*
the myosin-like band of b (arrow) corresponds to the 205 Kd rabbit muscle myosin standard (Sigma).
Other standards (St) are: 116 Kd = β-galactosidase, 94 Kd = phosphorylase b, 67 Kd = BSA,
43 Kd = ovalbumin, 36 Kd = carbonic anhydrase; B = bovine brain α-tubulin,
180 Kd = clathrin heavy chain, 110 Kd = kinesin, 50-55 Kd = α,β- tubulins.
Gels were stained with Coomassie Brillant Blue R 250.

REFERENCES

CAESAR-TON THAT, T. C., HOANG-VAN, K., TURIAN, G. and HOCH, H. C. (1986). Isolation and characterization of coated vesicles from filamentous fungi. *Acta Biol. Hung.* 37 Suppl.: 105.

—— (1987). Isolation and characterization of coated vesicles from filamentous fungi. *Eur. J. Cell Biol.* 43: 189-194.

ROSA, A. L. and MACCIONI, H. J. F. (1987). Clathrin coated vesicles in *Neurospora crassa*. *Molecul. Cell. Biochem.* 77: 63-70.

VOGEL, H. J. (1956). A convenient growth medium for *Neurospora*. *Microbial Genet. Bull.* 13: 42-43.

van TUINEN, D., R. ORTEGA PEREZ and G. TURIAN (1986). A search for myosin in elongating hyphae of *Neurospora crassa*. *Bot. Helv.* 96: 299-302.