THE PROPERTIES OF FREE AND MEMBRANE BOUND POLYSOMES STUDIED IN A CELL FREE SYSTEM OF *BOMBIX MORI* SILKGLANDS

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1. Introduction

Some attempts have been made to carry out a cell free system able to incorporate amino acids in fibroin by using silkgland extract, but the results reached are not conclusive [1–3]. The work of Shigematsu et al. [3] suggests that fibroin is produced by free ribosomes. However, according to a current hypothesis, membrane bound polysomes would be active for the synthesis of secretory proteins and free ones for the production of non exportable proteins [4]. Recent experiments with rat liver and ewe mammary gland are in good agreement with this hypothesis [5–8]. To solve this question concerning fibroin synthesis we tried to use an endogenous cell free system containing free or detached membrane bound polysomes.

The detailed studies of the properties of our cell free system will be published elsewhere. This paper concerns the functions of free and bound polysomes. Our results show that fibroin is made on bound ribosomes. This conclusion is based on two kinds of results, the pattern of incorporation of different amino acids and also its evolution during the fifth instar, during which the gland cells achieve their own growth and then produce almost exclusively fibroin.

2. Materials and technique

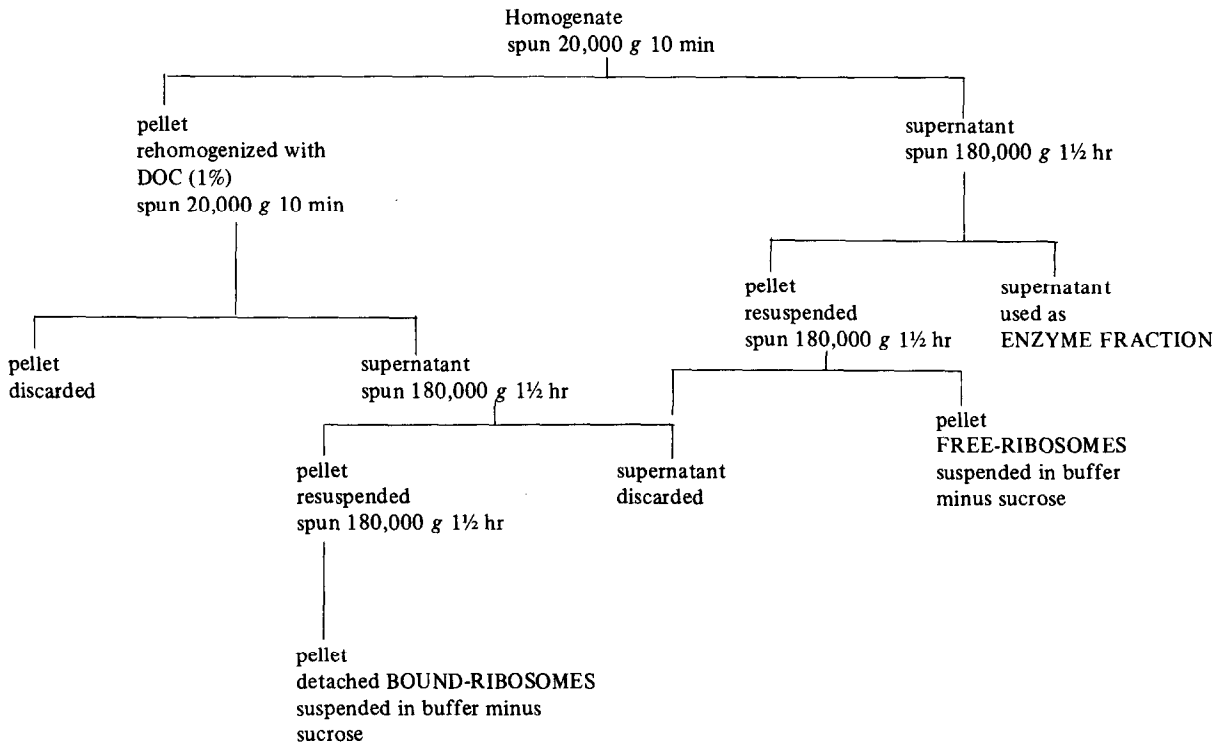
Silkworms are hybrids of *Bombyx mori* european strains 200 and 300. Fresh mulberry leaves are provided four times daily. In these conditions, silkglands stop their growth at the fifth day of the last instar which lasts ten days.

ATP, GTP, phosphoenolpyruvate and pyruvate kinase were purchased from Boehringer (Mannheim, Germany). Radioactive amino acids ^{14}C-glycine (34 mCi/mmole), ^{14}C-L-aspartic acid (60.5 mCi/mmole), ^{14}C-L-serine (78 mCi/mmole), ^{14}C-L-alanine (63 mCi/mmole) and ^{14}C-L-lysine (117 mCi/mmole) were supplied by CEA (France).

The procedure used for the preparation of particulate and soluble fractions is an adaptation of our previously described technique [9] and is summarized in table 1. When the homogenate is prepared without Na deoxycholate (DOC), most of the ribosomal material is sedimented at low speed and there is no microsomal peak if the 20,000 *g* DOC free supernatant was further analyzed on a sucrose gradient: this low speed sedimented ribosomal material consists of membrane bound ribosomes (monosomes and polysomes). A similar situation is also known to occur in some avian and mammalian cell types [10, 11]. The protein and RNA contents of the fractions are determined respectively by the Lowry [12] and the orcinol procedures [13]. The free amino acid content of the soluble fraction is measured by column chromatography as described by Chavancy [14].

The composition and the procedure of incubation of the cell free system (table 2) are adapted from the technique described by Schmitt et al. [15]. Counting is performed using a Packard Tricarb scintillation spectrometer, by the filter method of Mans and Novelli [16]. Counts vary from 500 to 10,000 cpm

Table 1
Fractionation of silkgland homogenate.



Homogenization is made in five volumes of tris-HCl buffer (50 mmoles, pH 7.5) containing KCl (50 mmoles) $MgCl_2$ (11.5 mmoles) and sucrose (25 mmoles).

depending of the amino acid tested, controls being always under 150 cpm. Results are expressed as pmoles incorporated per mg of ribosomal RNA, after having taken into account the enzyme preparation amino acids.

2. Results and discussion

The ability of the system to incorporate different amino acids varies with the age of animals and is specific for free and bound polysomes (table 2). It is known that during the first half of the last larval instar, the gland cells increase considerably in size, the production of silk being low; then, growth stops and the silk fibroin synthesis becomes predominant [17].

The incorporation pattern obtained with bound polysomes from old larvae is closely related to the very unusual glycine, alanine and serine content of silkfibroin [18]. The incorporation of aspartic acid and lysine is comparatively high, but that could be explained in two ways: first, other proteins are probably synthetized at the same time but in low amounts; second, as the system is not highly purified, we can assume that endogenously charged tRNAs are able to compete with the radioactive tracers.

Considering the high amount of glycyl, alanyl and seryl-tRNAs [19] this competition could be more important for minor amino acid incorporation than for major amino acid incorporation.

More significant is the evolution of the incorporation pattern during the fifth instar which is consistent with the increasing participation of fibroin production to the total protein synthesis.

Table 2
Cell-free amino acid incorporation into free and bound polysomes of silkgland.

Amino acid	Free ribosomes			Bound ribosomes			Silk composition* (%)
	3th day	5–7th day	8–9th day	3th day	5–7th day	8–9th day	
Glycine	501**	210	103	377	812	512	
	17.9%***	23.2%	25.4%	25.6%	46.3%	48.5%	52.3
Alanine	500	155	61	393	395	212	
	17.8%	17.1%	15.0%	26.7%	22.5%	20.1%	33.0
Serine	635	181	104	321	300	212	
	22.7%	20.0%	25.6%	21.8%	17.1%	20.1%	13.3
Aspartic	356	125	40	173	119	55	
acid	12.7%	13.8%	9.9%	11.8%	6.8%	5.2%	1.1
Lysine	809	235	98	209	126	64	
	28.9%	25.9%	24.1%	14.2%	7.2%	6.1%	0.3

Each assay tube contains in a final volume of 200 μl: a ribosome preparation containing 150–200 μg of RNA and 200–300 μg protein; an enzyme preparation containing 150–200 μg protein; 0.4 μmole ATP, 0.1 μmole GTP, 2 μmoles phosphoenolpyruvate, 6 μg phosphokinase, 7.5 μmoles KCl, 1.7 μmole $MgCl^{2+}$ and 0.5 μCi of radioactive amino acid. Incubation is performed at 37° for 45 min. Appropriate controls are kept at 0°.

* After Kirimura et Suzuki [18].
** pmoles of incorporated amino acid per mg of RNA.
*** Relative incorporation of each of the five tested amino acids.

The results obtained with free ribosomes differ from the precedents by two aspects:

a) The pattern of amino acid incorporation suggests the production of basic lysine-rich proteins.

b) This pattern is stable during the fifth instar.

It appears clearly that fibroin is made on membrane bound polysomes; this conclusion is in good agreement with electron microscopic observations [20, 21] which have shown that the granular reticulum grows when the fibroin production increases.

We can infer from our results that free polysomes are the principal site of the synthesis of cell proteins which takes place mostly in the first days of the instar. It is attractive to suppose that histone synthesis is the explanation for the highly active lysine incorporation.

Our results do not prove that no constitutive protein is made on bound ribosomal material (particularly in young animals) but at the end of growth a different equilibrium between free and bound ribosomes is realized when the protein metabolism of the cells becomes directed toward secretion.

References

[1] S.Tanaka and K.Shimura, J. Biochem. (Japan) 58 (1965) 145.
[2] T.Kobayashi, K.Otomo and K.Shimura, J. Biochem. (Japan) 60 (1966) 578.
[3] H.Shigematsu, H.Takeshita and S.Onoreda, J. Biochem. (Japan) 60 (1966) 140.
[4] P.Siekevitz and G.E.Palade, J. Biophys. Biochem. Cytol. 7 (1960) 619.
[5] C.M.Redman, J. Biol. Chem. 244 (1969) 4308.
[6] M.Takagi, T.Tanaka and K.Ogata, Biochim. Biophys. Acta 217 (1970) 148.
[7] M.C.Ganoza and C.A.Williams, Proc. Natl. Acad. Sci. U.S. 63 (1969) 1370.
[8] P.Gaye and R.Denamur, Biochem . Biophys. Res. Commun. 41 (1970) 266.
[9] J.C.Prudhomme, M.Guelin, L.Grasset and J.Daillie, Exptl. Cell. Res. 63 (1970) 373.
[10] P.Gerlinger, M.A.Le Meur, G.Beck and J.P.Ebel, Bull. Soc. Chim. Biol. 51 (1969) 1157.
[11] B.Goldberg and H.Green, J. Mol. Biol. 26 (1967) 1.
[12] O.M.Lowry, N.J.Rosebrough, A.L.Farr and R.J.Randall, J. Biol. Chem. 193 (1951) 265.
[13] W.C.S.Schneider, Methods Enzymol. 3 (1957) 680.
[14] G.Chavancy, Thèse de spécialité, Lyon (1970).

[15] R.J.Mans and G.D.Novelli, Arch. Biochem. Biophys. 94 (1961) 48.
[16] G.Schmitt, J.P.Beck, J.M.Guerne, F.Stutinsly and J.P.Ebel, Bull. Soc. Chim. Biol. 50 (1968) 21.
[17] J.Daillie, Comp. Rend. Acad. Sci. Paris 261 (1965) 4872.
[18] J.Kirimura and T.Suzuki, J. Agric. Chem. Soc. Japan 36 (1962) 336.
[19] J.P.Garel, P.Mandel, G.Chavancy and J.Daillie, FEBS Letters 7 (1970) 327.
[20] H.Akai, Sanshi Shikensho Hokoku 19 (1965) 375.
[21] Y.Tashiro, T.Masimoto, S.Matsuura and S.Nagata, J. Cell. Biol. 38 (1968) 574.