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Secretory protein synthesis in *Chironomus* salivary gland cells is not coupled with protein translocation across endoplasmic reticulum membranes

Electron microscopic evidence

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Fragments of rough endoplasmic reticulum containing polysomes bound to the membranes only at the 5′ end were visualized in electron microscopic spreads from *Chironomus thummi* salivary gland cells. The length of the nascent protein molecules in the polysomes increased from the 5′ to the 3′ (free) polysome end. The data obtained disagree with the generally accepted model according to which synthesis of secretory proteins is concomitant with the protein transport across the endoplasmic membrane

Polysome; Protein, secretory; Salivary gland; mRNA; Endoplasmic reticulum; *(Chironomus thummi)*

1. INTRODUCTION

On the basis of biochemical [1] and morphological [2] data, it has been demonstrated that the ribosomes bound to the rough endoplasmic reticulum (ER) are the sites of synthesis of secretory proteins. Concomitant emergence of nascent proteins into the ER lumen and their synthesis have been suggested as a common mechanism providing protein translocation across the ER membrane [3,4].

Miller's technique has made it possible to visualize native translation units synthesizing giant secretory proteins (molecular mass up to 16^6 Da) [5]. These proteins are coded for by 75 S RNA from the large puffs; Balbiani rings of *Chironomus* polytene chromosomes [6]; they constitute about 50% of all the proteins synthesized in *Chironomus* salivary gland cells [7]. Also it has been found that the translation units were frequently distributed in the form of polyribosome (PS) aggregates. The mechanism of formation of these aggregations is unknown.

In the present work it is shown that the PS aggregation on spread preparations results from association of the PS with the ER membranes only at the 5′ end. Thus, mRNA binding to the ER membrane may take place before the initiation of translation, and it does not seem to be coupled with protein translocation.

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2. MATERIALS AND METHODS

Third and fourth instar *Chironomus thummi* larvae were used. The salivary glands of two animals isolated in 0.1 M NaCl, 0.001 M $MgCl_2$ solution were placed in 30 ml of a solution containing 0.1% detergent Joy, 0.05% sodium sarcosylate, 0.1% PMSF, 0.1 mM sodium tetraborate, pH 8.5. In 5–8 min, the glands were transferred to 0.1 mM sodium tetraborate, the cells were carefully cut open with needles and incubated for 15 min at room temperature. The obtained samples were centrifuged according to Miller [8] (10000 rpm) onto carbon-coated grids. The grids were rotary-shadowed with platinum-palladium and examined in electron microscope JEM-100C.

3. RESULTS AND DISCUSSION

The released content of the cell was incubated for 15 min in 0.1 mM borate buffer and spread. Along with nuclear structures and polytene chromosome fragments, the spread preparations contained numerous free PSs of different sizes. Most of these free PSs were giant and included (fig.1a) 5 nm thick mRNA molecules with 79–146 ribosomes. The length of the protein molecules in the PS increased from the 5′ to the 3′ (free) PS end. The protein molecules had globular enlargements (fig.1b).

It is noteworthy that almost two-thirds of the PSs were associated with numerous oval or elongated vesicular bodies (fig.1c,d). These structures were most likely the fragments of rough ER. This was derived from the earlier observations that 90% of 75 S RNA involved in high molecular protein synthesis was associated with the ER membranes in *Chironomus* salivary gland cells [9,10]. Because centrifugation rate

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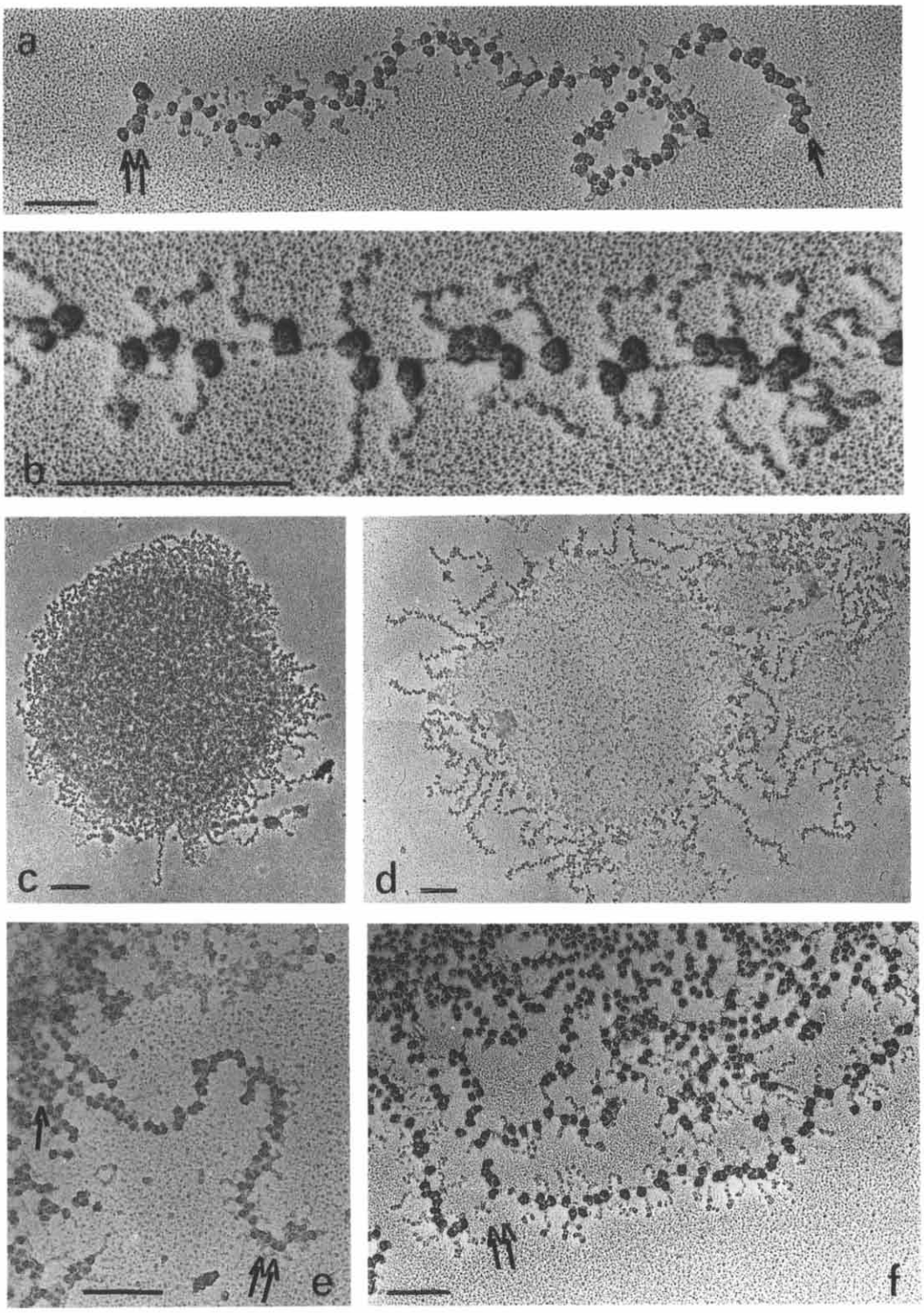

Fig.1. Electron micrographs of spread contents from the salivary glands of *Chironomus thummi*. Individual giant polyribosomes (a,b) and endoplasmic membrane-bound polyribosome complexes (c–f) isolated from the secretory cells. Single and double arrows indicate the 5′- and 3′-ends of mRNA, respectively. Bar = 0.2 μm.

was relatively low, the preparations contained mainly large 2–10 μm vesicles. As a rule, the PS were observed to be associated with the membrane surface at the 5′ end of mRNA only.

The radially arranged mRNA with ribosomes formed a halo around the microsomal vesicles. When the integrity of the ER vesicles was well-preserved (fig.1c), the PS usually covered the whole membrane surface,

while when integrity was lost (fig.1d), the PS were mostly located along the border of the partly destroyed structure; no PS were observed in the central regions which appeared to be the inner surface of the membrane vesicles.

Nascent protein molecule protrusions from the ribosomes (similar to those in the giant free PS) could be registered in many bound PSs (fig.1e,f). A length gradient of protein molecules increasing from the PS-membrane attachment site to the 3′ (free) PS end was noteworthy. This length gradient was not always visible because of the occasionally insufficient unfolding of RNA molecules. The attached PS were of different sizes, and the number of ribosomes per PS varied from 15 to 100. Some mRNA did not contain ribosomes at the 3′ end. These molecules were presumably at the early stages of protein synthesis.

If the above-described structures are assumed to be real translation complexes, it will appear that protein synthesis in the bound PS is not coupled with the translocation of protein across the ER membrane. In our case the mechanism of PS binding to the membrane seems to differ from that according to which the PS-synthesizing proteins, cotranslationally translocated across the membrane, are bound. It has been observed that 50% of the 75 S RNA remains bound to the ER membranes in *Chironomus tentans* salivary gland cells after removal of the ribosomes [9]. The 50% binding may be provided by either nascent proteins that have emerged into the ER membrane lumen or nonfunctional mRNA-ER membrane binding independent of the PS nascent proteins and ribosomes. The present electron microscopic data support the latter possibility. The gradient length of the nascent proteins suggests that mRNA should be bound to the ER membrane in a site preceding the initiating codon.

Judging by the size of the synthesized proteins in the giant PS, they are high-molecular and coded for by mRNA from the Balbiani rings (BRb and BRc) [11] of *Chironomus thummi* polytene chromosomes.

According to our data, these giant PS constitute about 50% of the PS isolated from these salivary glands. High-molecular proteins of the salivary gland secretion are glycosylated and phosphorylated like most of the secretory proteins [12,13], i.e. they must be translocated across the ER membrane prior to secretion. A signal sequence for these proteins has not been determined so far, although it appears plausible for at least one of the *Chironomus thummi* proteins [14].

However, the present electron microscopic data indicate that the transport of high-molecular and other secretory proteins in *Chironomus* salivary gland cells can occur posttranslationally like in yeast [15]. Thus, the present results suggest that the processes of protein synthesis and translocation should not be necessarily simultaneous and coupled in secretory cells.

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