Electron microscopic visualization of a discrete class of giant translation units in salivary gland cells of *Chironomus tentans*

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Lysates were made of whole salivary glands from *Chironomus tentans* and electron microscopic spreads prepared according to Miller and Beatty (1969). The sedimented material consisted mainly of ribosomes, most of which were present in giant polysomes which showed gradients of material protruding laterally from the ribosomes, interpreted as nascent polypeptide chains. Each giant polysome contained one such gradient. On the basis of the presence of a small group of terminal ribosomes without nascent chains at the 3' end, 19 apparently complete polysomes were selected, representing a discrete class of polysomes containing between 66 and 92 ribosomes (79 ± 7 , $m \pm s.d.$). Arguments are presented that they constitute the translation units for giant secretory proteins coded by RNA from the large Balbiani rings, BR1 and BR2.

Key words: polysomes/secretory proteins/Balbani rings/messenger RNA/nascent polypeptides

Introduction

Our understanding of various aspects of transcription has benefited considerably from the visualisation of active transcription units in electron microscopic spreads (Miller and Beatty, 1969). This technique has had less impact on our understanding of translation, however, largely because nascent protein is not easily visualized, either because the protein is too short and/or assumes a globular conformation and cannot be distinguished from the ribosomes, or because nascent chains may be proteolyzed. With the exception of polysomes from silk glands with putative nascent silk fibroin chains (McKnight *et al.*, 1976), no translating polysomes of known function seem to have been visualized. Even in the case of silk fibroin, the units were believed to be incomplete. Another suitable material for visualization of nascent polypeptides is the salivary gland of *Chironomus tentans*. Here synthesis is predominantly of a large secretory protein initially reported to have molecular weight (mol. wt.) of 569 000 (Grossbach, 1973), but later found to consist of two components termed Ia and Ib. The mol. wt. of component Ib was determined to be $\sim 850\,000$ (Rydlander and Edström, 1980). Components Ia and Ib, identified by Rydlander and Edström (1980), probably correspond to proteins SPI (mol. wt. 1 400 000) and SPII (mol. wt. 1 000 000) reported by Hertner *et al.* (1980). These two proteins (Ia and Ib) are coded for by RNA from the large puffs, Balbiani rings 1 and 2 (BR1 and

BR2) (Rydlander *et al.*, 1980). The product of BR2 is probably component Ib since in normal cells the amount of component Ib is much greater than that of Ia, and the BR2 puff is much larger than the BR1 puff (Edström *et al.*, 1980).

Component I (Ia and Ib collectively) is much larger than most primary translation products and the possibility that it is formed by post-translational crosslinking of smaller units has been discussed (Grossbach, 1973). This is unlikely, but has not been entirely excluded. Daneholt *et al.* (1977) isolated large polysomes containing 75S BR-RNA. Such polysomes contained most of their nascent polypeptide chains as a population of molecules increasing in size up to that of the finished component I (Rydlander and Edström, 1980). Even this observation does not, however, exclude the possibility of crosslinking of smaller nascent polypeptides during translation. Since the 75S mRNA cannot yet be translated to completion *in vitro*, definitive evidence for giant translation units could only be provided by electron microscopic spreads. Synthesis of component I accounts for $\sim 50\%$ of the total protein synthesis in the salivary gland (Grossbach, 1973). If component I is indeed synthesized as a simple polypeptide chain, we would expect most ribosomes to accumulate in long polysomes because of the length of time required to translate very large mRNAs. For these reasons, we anticipated that it might be possible to visualize giant translation units in unfractionated cytoplasm.

Results

Polysomes containing nascent polypeptide chains

The predominant component in the spreads was ribosomes organized into polysomes. Occasional monosomes were seen. Masses of secretory material and chromatin with well-preserved nucleosomal organization were also observed.

Fig. 1. Survey electron micrograph of spread contents from lysed salivary glands from *Chironomus* showing ribosomes arranged in long, partially aggregated polysomes. The bar represents 1 μ m.

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Fig. 2. Individual giant polysomes (a – b) and four aggregated polysomes (e) showing gradients of poly peptide chains. The polysome in a contains 74 ribosomes of which four are 3' terminal with no attached poly peptides (74–4), in b (which also contains a small polysome to the lower right) 77–3, in c 66–2, in d (which shows part of an adjoining large polysome to the upper left) 71–2. Terminal 3' groups of ribosomes without attached poly peptides are also seen in e, indicated with arrows. Material similar to the nascent protein is seen to the right in e in aggregations. The bar in each case represents 0.5 μ m.

At low magnification the majority of ribosomes were present in very large polysomes (Figure 1). Higher magnifications (Figure 2) revealed polysomes with lateral protrusions from the ribosomes interpreted as nascent polypeptide chains. The length gradient of these protruding structures confirmed this interpretation and allowed identification of the 5' and 3' ends of the polysomes. In occasional stretched polysomes, individual ribosomes with attached nascent polypeptides could be clearly seen (Figure 3). The smaller and less dense ribosomal subunit contacts the mRNA on one side and the larger and denser subunit on the other. The nascent polypeptide, which in this case has relatively little secondary structure, appears to emerge between the two subunits or from the large subunit.

The compaction of the nascent polypeptide varied between different preparations but was uniform within a given spread. Usually the nascent chain was ~ 100 Å in diameter and reached a length of 500–1000 Å at the 3' end of the polysomes. Similar material could also be seen in aggregated masses (e.g., Figure 2e). At the 5' end, nascent chains were apparent after 5–8 ribosomes. The ribosomes were not evenly distributed along the mRNA but often lay in groups of 2–4 sometimes in repeats (e.g., Figure 1).

Fragments of polysomes with long polypeptide chains were not very common. Occasional small polysomes without visible nascent chains were observed (Figure 2b, lower right).

A discrete class of giant polysomes

Inspection of several long polysomes revealed that practically all of them terminated at the 3' end with a group of ribosomes without nascent chains (Figure 2). The number of such "terminal ribosomes" varied between one and seven with an average of three. Nineteen large polysomes with 3' terminal groups of ribosomes were found lying sufficiently free from other material to allow the individual ribosomes to be counted. Only polysomes which had been selected for photography before the terminal ribosome group had been identified as a reproducible structure were used for the following statistical analysis. Polysomes with terminal ribosomes were classified according to the number of ribosomes, and were found to be distributed between 66 and 92 with a mean value of 79 ± 7 (s.d.) (Figure 4). Within this range we found only one polysome without terminal ribosomes.

Free mRNA protruding from the end of the polysomes, as described for giant polysomes from sea urchin embryos (Whiteley and Mizuno, 1981), was never observed. The distance to the 3'-terminal group was, however, sometimes longer than any other inter-ribosomal distance (Figure 2b).

Discussion

A discrete class of giant polysomes with nascent polypeptide chains carrying on average 79 ribosomes and with nontranslating terminal ribosomes was observed in lysates from *C. tentans* salivary gland cells. The large majority of the ribosomes that sedimented appeared to be present in giant polysomes. Our belief that the polysomes were intact was reinforced by the observation that polysome fragments exhibiting distinct nascent chains were rarely seen. Thus, we did not observe fragments with nascent chains distinctly larger than those present near the 3' end of the intact giant polysomes in a given preparation. The presence of a group of 3'-terminal ribosomes without nascent chains provided further evidence that the polysomes were intact at least at this end since a corresponding structure was not observed in some

Fig. 3. High magnification power view of an exceptional preparation in which the large polyribosomes were stretched during specimen preparation. The two ribosomal subunits and their relation to mRNA and the nascent protein chain can be seen. The bar represents 0.25 μm .

Fig. 4. Histogram showing the distribution of 19 polysomes having terminal ribosomes without nascent chains classified according to number of ribosomes (right) and a histogram of the number of terminal ribosomes per polysome (left).

smaller, apparently fragmented, polysomes at the point of breakage. It is likely that the 5' end, as seen in the spreads, is also representative of the native condition since nascent polypeptide chains start to appear not far from this end, i.e., after 5–8 ribosomes.

It is uncertain whether in eukaryotes monomeric ribosomes are released from polysomes after translation. If they are released as such, they constitute only a minor part of the monomer population (Henshaw *et al.*, 1973). The related question of whether complete ribosomes remain on the polysome after termination of translation is not easily solved using a biochemical approach. The present electron micrographs provide direct evidence for such a phase in the ribosome cycle and suggest that $\sim 4\%$ of the ribosomes in the giant polysomes are terminal or post-translational.

The number of ribosomes on the giant polysomes we have visualized, corresponds rather well with the size of *Chironomus* salivary gland polysomes isolated by sedimentation in sucrose gradients (Daneholt *et al.*, 1977), the heaviest class of which contained on average 60 ribosomes. Furthermore, the polysomes from the sucrose gradients were shown to contain 75S BR-RNA, only slightly degraded, and this RNA is now known to code for the giant secretory protein, component I (Rydlander *et al.*, 1980). Such polysomes also contain nascent polypeptide chains up to the size of component I (Rydlander and Edström, 1980). It is thus likely that the polysomes we have visualized and described here are identical to those studied biochemically.

We had originally expected polysomes for proteins with mol. wts. of the order of 10^6 to contain > 200 ribosomes. The number of ribosomes per polysome determined for globin chains (Warner *et al.*, 1962), ovalbumin (Palacios *et al.*, 1972) and serum albumin (Wieringa *et al.*, 1978) leads to the estimate of one ribosome for ~ 30 amino acids of the transla-

tion product. Component Ib probably has 7000–9000 amino acids and thus would, by extrapolation, require polysomes with >200 ribosomes. Available data should, however, be related to the protein size. The polysomes of vitellogenin (mol. wt. 220 000) have 40–60 amino acids/ribosome (Wieringa et al., 1978; Roskam et al., 1976). Silk fibroin with ~5000 amino acids, is formed by polysomes containing 45–112 ribosomes (Lizardi, 1980) which gives 45–111 amino acids/ribosome. It is therefore possible that the spacing between ribosomes increases with polysome size. An exception to this rule is myosin for which 30–35 amino acids/ribosome can be estimated (Heywood et al., 1967). If the giant polysomes we have seen synthesize component I, a value of ~100 amino acids/ribosome can be derived. This is not necessarily in disagreement with the expectations for a translation product of this size.

The polysomes we describe were prepared in the presence of high concentrations of cycloheximide after prior treatment of the animals with the same drug. Cycloheximide might increase the loading of polysomes if initiation is the rate-limiting step in translation, as has been reported for globin polysomes (Lodish, 1971). Of the investigations cited above, the silk fibroin polysomes were obtained after cycloheximide treatment similar to ours. The addition of cycloheximide, however, had no evident effect on ribosome loading, either with silk fibroin polysomes (McKnight et al., 1976) or with giant polysomes from sea urchin embryos (Whiteley and Mizuno, 1981). It does not appear likely, therefore, that the increase in ribosome spacing with increased polysome size is due to differences in treatment of the polysomes.

A mol. wt. for the secretory protein of ~10⁶ does not quite account for the total length of BR2 mRNA which has a mol. wt. of 12.3 × 10⁶ (Case and Daneholt, 1978), corresponding to 12 000–13 000 triplets. Since the polysomes we report do not show much untranslated mRNA, which is clearly visible in spread sea urchin polysomes (Whiteley and Mizuno, 1981), it is possible that the mol. wt. of component I has been underestimated. This appears to be more likely than an overestimation of the size of 75S mRNA. It is unlikely that the polysomes we see produce proteins other than component I. The proteins next in size in *Chironomus* salivary glands are component II (mol. wt. 235 000) and III (mol. wt. 160 000) (Rydlander and Edström, 1980) or protein 2 and 3 (Grossbach, 1977) estimated to have mol. wts. of 140 000–160 000, or SPIII (mol. wt. 160 000) (Hertner et al., 1980). If the 79-unit polysomes translated a polypeptide of mol. wt. 200 000–250 000, there would be ~25 amino acids/ribosome, which is less than reported for even the smallest polysomes.

Most of the rapidly labelled polypeptide chains isolated from salivary gland polysomes are members of a nascent population the size of which extends up to that of the finished component I (Edström and Rydlander, 1980). The predominant class of polysomes we have visualized have 79 ± 7 ribosomes. The simplest and most reasonable interpretation is that we are seeing the polysomes which synthesize component I proteins.

Materials and methods

Animals

Late fourth instar *C. tentans* larvae were cultivated as previously described (Rydlander and Edström, 1980). A dozen animals were placed in 200 ml culturing medium with 20 µg/ml cycloheximide (Sigma) (McKnight et al., 1976) one day before sacrifice.

Preparation of polysomes for electron microscopy

Polysomes were prepared by spreading as described by Miller and Bakken (1972) with minor modifications. One gland was placed at room temperature in 50 µl incubation medium containing 0.5% Joy (Procter and Gamble, Cincinnati, OH), 0.05% sodium deoxycholate (Merck), 0.1% Sarkosyl NL 97 (Ciba-Geigy), 20 µg/ml cycloheximide adjusted to pH 8.5 with a pH 10 borate-KOH buffer. During incubation the gland was punctured and cut open with insect needles. After 5–8 min, when most of the cytoplasm had leaked out, the residue was removed and 200 µl of distilled water buffered to pH 9 with the borate buffer was added, after which the mixture was left for 5 min at room temperature. Specimen preparation was carried out in a microcentrifugation chamber (Miller and Bakken, 1972) in the bottom of which was placed a glow discharged, carbon-coated 400 mesh grid overlaid with 10% formaldehyde in 0.1 M sucrose adjusted to pH 9.0 with the borate buffer. 2–3 drops of the cell lysate were carefully layered over this solution and centrifuged for 15–20 min at 1500 g (3000 r.p.m.) at 8°C in a Beckman TJ-6 centrifuge, after which the grid was removed and immersed for 10 s in 0.4% Kodak Photo-Flo 200 adjusted to pH 9 with the pH 10 borate buffer. This solution was drained off and the grid left to dry, after which it was rinsed with absolute ethanol and 0.4% Photo-Flo and dried again. The grids were rotary shadowed at 8°C with 0.2 mm platinum wire wound around tungsten wire in an Edwards 306 vacuum coater with Baltzers quartz thin film monitor attachment.

Samples were examined in a Zeiss EM10A electron microscope calibrated with cross grating replica and a Mono stable facility which guarantees ± 1.5% magnification reproducibility. Quantitative measurements were made on the photomicrographs using a Peak scale lupe x10 with No. 8 graticule.

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