been taken as an indication that Leu-enkephalin may be a "purer" agonist than Met-enkephalin (*12*). Intraventricular Met-enkephalin lowered but Leu-enkephalin enhanced pain threshold in a test involving sustained low-intensity pain (*13*). Furthermore, intraventricular administration of a high dose (320 μg) of Met-enkephalin elicited in rats a stuporous immobility that could be prevented by naloxone. The same dose of Leu-enkephalin, on the other hand, induced naloxone-insensitive rotational behavior (*14*). Pharmacologic differences between both peptides are consistent with the suggestion that Met- and Leu-enkephalin may derive from different precursors (*15*).

The anti-amnesic effect of the pentapeptides was observed after systemic administration of low doses. This is in sharp distinction to the analgesic effects of Leu- and Met-enkephalin, which are only seen after intracranial administration of huge quantities of these substances (100 μg or more) (*13, 14, 16*). Behavioral activity of low doses of systemically administered Met-enkephalin has also been shown by Plotnikoff *et al.* (*17*). The anti-amnesic effect of enkephalins is presumably not mediated through opiate receptors. This is suggested by the failure of naloxone to prevent this behavioral effect of the pentapeptides (*18*).

Several explanations are possible to account for the anti-amnesic effect of the enkephalins. Thus, the prevention of CO_2-induced amnesia by Met-enkephalin (the preacquisition effect) may be due either to a facilitation of memory consolidation or to a protection of the animals against the adverse effects of the amnesic agent. The preretrieval effect of the enkephalins may either result from facilitated retrieval of a weak, spared memory trace or from a specific reversal of a CO_2-induced disturbance of memory retrieval. Further studies are necessary to clarify the modes of action of the enkephalins. The present data suggest that the enkephalins may directly or indirectly modulate memory processes but it is premature to postulate a crucial role of these peptides in memory.

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8. No avoidance: entrance latencies of 0 to 10 seconds. This category is based on my experience that virtually all day 4 entrance latencies of nonshocked rats fall in this range (*2*). Incomplete avoidance: entrance latencies of 11 to 179 seconds. Complete avoidance: failure of an animal to enter within 180 seconds. The validity of the present categorization is apparent from the fact that an analysis of the categorized data yields essentially the same pattern of statistical significance as the analysis of the raw data (*7*). The categorized data can be analyzed with the Yates test [F. Yates, *Biometrika* **35**, 178 (1948)].
9. Met-enkephalin and Leu-enkephalin were synthesized by H. M. Gfeven, Organon.
10. Mean entrance latencies at the acquisition trial were as follows. Experiment 1: saline-treated animals (N = 50), 1.1 seconds; Met-enkephalin, 0.3 μg (N = 10), 1.3 seconds; 3 μg (N = 10), 1.0 second; 30 μg (N = 20), 1.1 seconds. Experiment 2: saline (N = 60), 1.2 seconds; Met-enkephalin, 0.0003 μg (N = 10), 1.0 second; 0.003 μg (N = 10), 1.0 second; 0.03 μg (N = 20), 1.3 seconds. Experiment 3: saline (N = 60), 1.6 seconds; Leu-enkephalin, 0.03 μg (N = 10), 1.5 seconds; 3 μg (N = 10), 1.8 seconds; 30 μg (N = 40), 1.3 seconds.
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Polyribosomes Associated with Forming Acrosome Membranes in Guinea Pig Spermatids

Abstract. *Ribosomes, some of which are arranged in polyribosomal configurations, are attached to specialized regions of the acrosomal membrane in guinea pig spermatids. This finding indicates a new functional dimension for the acrosomal membrane, that of protein synthesis, and suggests that during acrosome formation, proteins of the acrosomal membrane or acrosomal contents need not be synthesized before or during passage through the Golgi apparatus.*

Protein synthesis is generally thought to be restricted to polyribosomes or polyribosome-rich regions of the cell. For the most part, two classes of polyribosomes are recognized: those bound to rough endoplasmic reticulum and those that occur free in the cytoplasm (*1*). Possible exceptions to this classification are the Golgi apparatus polyribosomes first reported by Franke *et al.* (*2–4*). These polyribosomes are not membrane-bound in the strict sense; rather they represent a special class of free polyribosomes found within the Golgi apparatus zone of exclusion (*5*). Golgi apparatus polyribosomes have been isolated from rat liver and are functional in protein synthesis (*4*).

We report here particles resembling ribosomes that are associated with parts of the acrosomal membrane of developing spermatids (Fig. 1, A and B). The particles are confined to the part of the acrosomal membrane covering the acrosomal vesicle (headcap); no particles are found on any part of the acrosomal membrane in contact with the acrosomal granule. These ribosome-like particles (RLP's) react to fixative and stain like ribosomes. For example, RLP's are preserved after glutaraldehyde–osmium tetroxide fixation but are lost after potassium permanganate fixation. Also, when the method of Bernhard (*6*) is used to distinguish between RNA and DNA, the glutaraldehyde-fixed RLP's have characteristics of RNA; that is, they stain positive with uranyl acetate and lead citrate and do not destain after treatment with EDTA.

Most RLP's are dispersed over the surface of the acrosomal membrane (Fig. 1, A to D). Aggregates of three or more RLP's are common (Fig. 1D). These aggregates of RLP's often extend outward from the membrane surface (Fig. 1D). The RLP's attached to the membrane appear loosely bound and do not seem to penetrate the membrane surface (Fig. 1, C and D). In many instances, the RLP's appear attached to the acrosomal membrane by short segments of filamentous material (Fig. 1C).

Ribosome-like particles are present on the acrosomal membrane beginning with the clear-cut differentiation of the headcap (stage IV to stage V) up to the beginning of nuclear condensation. At this stage of development, the endoplasmic reticulum becomes closely aligned along the surface of the acrosome and the RLP's are no longer evident. The number of RLP's associated with the acrosomal membrane seems to increase during

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spermatid maturation. Ribosome-like particles were present in all seven of the guinea pigs used for this study. They do not seem to be present (at least in easily recognizable numbers) in mouse and rat spermatids.

While the tentative identification of the particles reported here as being associated with the forming acrosome membrane of guinea pig spermatids is purely morphological, there are almost no additional criteria that we can apply at the structural level to their identification. They have an appearance in the electron microscope and a size distribution similar to those of ribosomes found free in the cytoplasm and ribosomes associated with rough endoplasmic reticulum. They exist in a polyribosomal configuration. They react as ribosomes to various fixatives and stains.

We cannot determine whether the acrosome-associated polyribosomes observed are functional in protein synthesis, nor can we determine whether the products of synthesis by these polyribosomes are likely to be membrane constituents or constituents of the acrosomal content. The RLP's differ from endoplasmic reticulum polyribosomes in that they are not as closely bound to the membrane surface. In this respect, the RLP's appear to be intermediate in their degree of membrane association between the ribosomes associated with endoplasmic reticulum and the ribosomes associated with Golgi apparatus.

The observations are significant in two respects. First, to our knowledge, this is the first report of membrane-associated polyribosomes with any structure other than nuclear envelope or rough endoplasmic reticulum. The polyribosomes previously reported to be associated with Golgi apparatus (*2–4*) and functional in protein synthesis (*4*) are probably a class of free polyribosomes associated with, but not actually attached to, the Golgi apparatus membranes. Second, the presence of polyribosomes associated with the membrane of the forming acrosome would mean that the protein biosynthetic capacity could be a function of the acrosome itself. Therefore, the formation and maturation of the acrosome may be less dependent on contributions of the endoplasmic reticulum and the Golgi apparatus than was previously suspected.

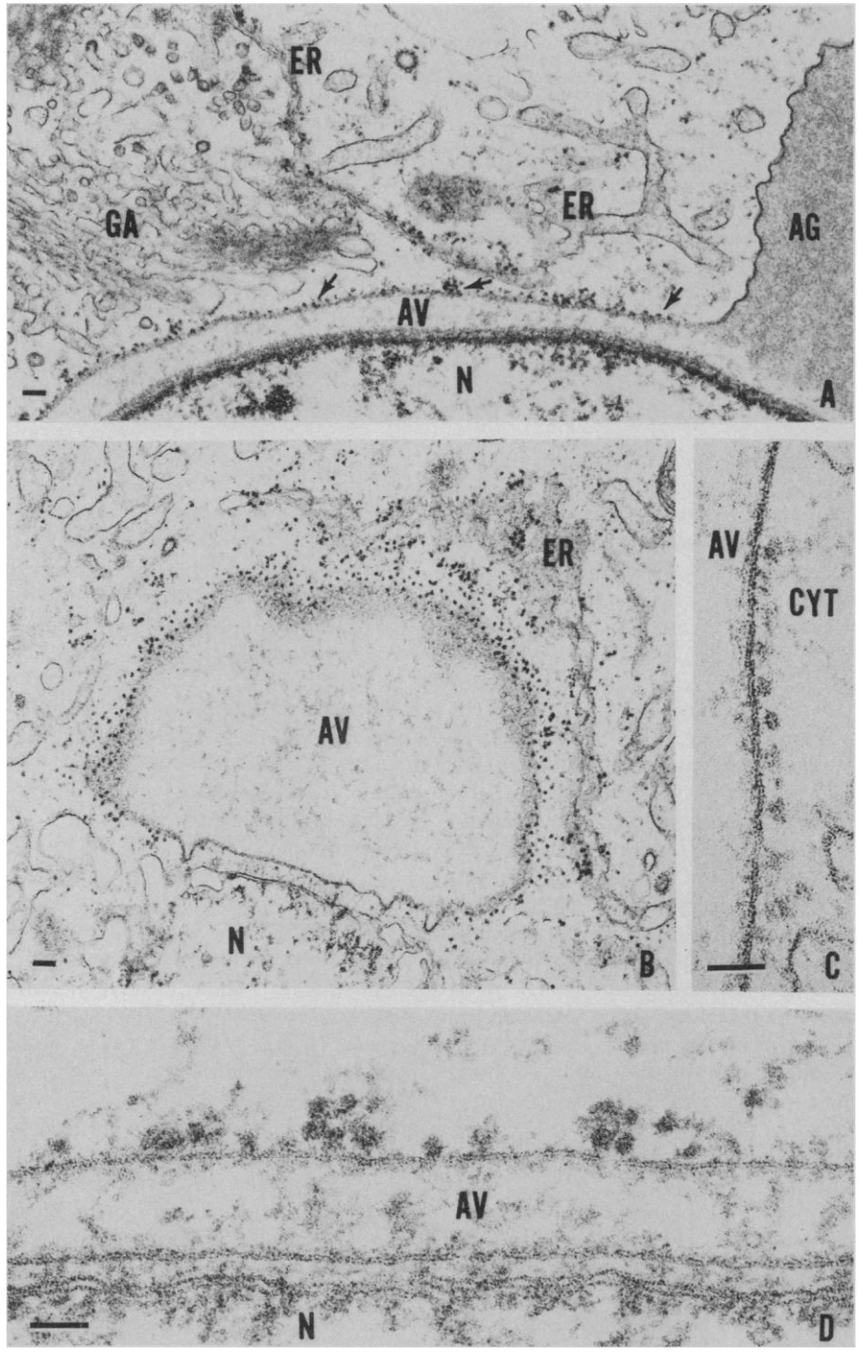

Fig. 1. Sections through guinea pig spermatids showing ribosome-like particles (RLP's) attached to, or associated with, the forming acrosome. (A) The RLP's (arrows) were restricted to the membrane of the acrosomal vesicle (*AV*); no RLP's were associated with any part of the acrosomal membrane that was in contact with the acrosomal granule (*AG*). (B) The propensity of RLP's for the acrosomal vesicle was most evident in tangentially oriented sections. (C) The RLP's were usually slightly separated from the surface of the acrosomal membrane. Sometimes they appeared to be connected to the acrosomal membrane by filamentous material. (D) Aggregates of RLP's resembling polyribosomes were common along the surfaces of the acrosomal membrane. Abbreviations: endoplasmic reticulum (*ER*), cytoplasm (*CYT*), nucleus (*N*), and Golgi apparatus (*GA*). Scale bars: (A and B) 0.1 μm and (C and D) 0.05 μm.

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