Synthesis, processing, and secretion of rat immunoglobulin E made in *Xenopus* oocytes

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Rat immunoglobulin E (IgE) synthesized in *Xenopus laevis* oocytes, injected with rat plasmacytoma mRNA, was analysed by specific immunoprecipitation and SDS-polyacrylamide gel electrophoresis under reducing as well as non-reducing conditions. The results indicate that the oocytes will translate and correctly process the rat IgE heavy and light chains, resulting in secretion of a correctly assembled, normal immunoglobulin molecule. The normal, extensive glycosylation of the IgE heavy chain (ε-chain) is faithfully carried out by the oocytes; therefore, this posttranslational modification is apparently of an unspecific nature, and does not depend upon a mechanism specific for plasma cells.

Immunoglobulin E *Glycoprotein* *Posttranslational protein processing*
(Rat plasmacytoma, Xenopus laevis *oocyte)*

1. INTRODUCTION

Xenopus laevis oocytes, injected with eukaryotic mRNAs coding for secretory proteins, will carry out many of the appropriate posttranslational processes and modifications of the polypeptides translated from these foreign mRNAs (see [1] for references). Therefore, *Xenopus* oocytes offer a very useful system for the study of cellular manufacture and export of proteins. However, the oocytes may not perform certain posttranslational modifications such as prosequence removal from proinsulin [2] and promelittin [3]. The oocytes may glycosylate foreign proteins; but in some instances, glycosylation was found to be incomplete [4] or to result in a heterogeneous product, different from the authentic mature protein [1].

We have analysed the fate of rat immunoglobulin E (IgE) translated from mRNA injected into *Xenopus* oocytes. IgE is a glycoprotein with an M_r of 190000 consisting of two light chains each with an M_r of 24000 and two heavy (ϵ) chains each having an M_r of 70000. The ϵ-chain is glycosylated at six sites that contain a variety of carbohydrates [5]. Here we present evidence that the rat IgE can be translated and correctly processed by the oocytes injected with IgE mRNA, and that the IgE molecules are secreted from the cell as a tetramer with a size identical to that of authentic IgE. Correct glycosylation of the heavy chain is observed to take place in two discrete steps, the latter of which is tightly associated with secretion.

2. MATERIALS AND METHODS

Total poly(A)-containing RNA was isolated from a rat plasmacytoma IR162 [6] by procedures

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described earlier [7]. The mRNA was translated in a rabbit reticulocyte lysate system made mRNA dependent by digestion with micrococcal nuclease [8], using conditions detailed in [7].

Xenopus oocytes were injected with about 50 nl each of poly(A) containing RNA at a concentration of 2 mg/ml. Batches of 12 oocytes were incubated at 20°C for 20 h in 30 μl Barth's saline solution containing 1 mCi/ml L-[^{35}S]methionine (Amersham, 500–1000 Ci/mmol). At the end of the incubation period, the oocytes were separated from the incubation medium. The oocytes were homogenized in 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 5 mM magnesium acetate (20 μl/oocyte), centrifuged at 130000 × *g* for 20 min in a Beckman airfuge, and the supernatant fraction saved for analysis.

Rabbit antibodies specific to rat IgE, prepared as described by Karlsson et al. [9], were a generous gift from H. Bennich. Immunoprecipitation was performed essentially as in [7], and the immunoprecipitates were analysed by SDS-polyacrylamide gel electrophoresis [10], using reducing as well as non-reducing conditions.

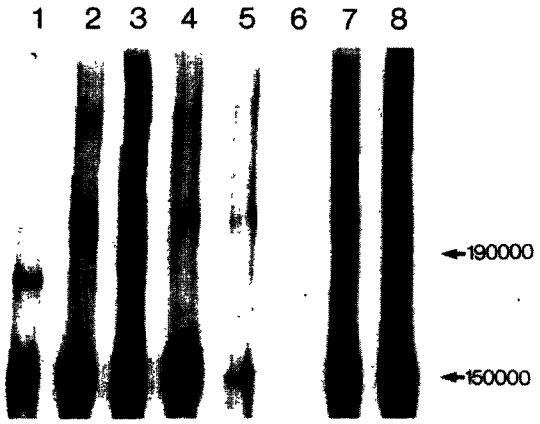

Fig.1. Autoradiogram of a non-reducing, 6.5% SDS-acrylamide gel, loaded with immunoprecipitates of the following oocyte extracts or incubation media: (lanes 1,2) cell extract from oocytes injected with IR162 mRNA (exposed for 3 days); (lanes 3,4) incubation medium from oocytes injected with IR162 mRNA (exposed for 7 days); (lanes 5,6) cell extract from uninjected control oocytes (exposed for 3 days); (lanes 7,8) incubation medium from uninjected control oocytes (exposed for 7 days). Immunoprecipitation was with antibodies against rat IgE (lanes 1,3,5,7) or with non-immune IgG (lanes 2,4,6,8). The 190000 Da arrow shows the migration of mature rat IgE. The 150000 Da arrow shows the migration of rabbit IgG.

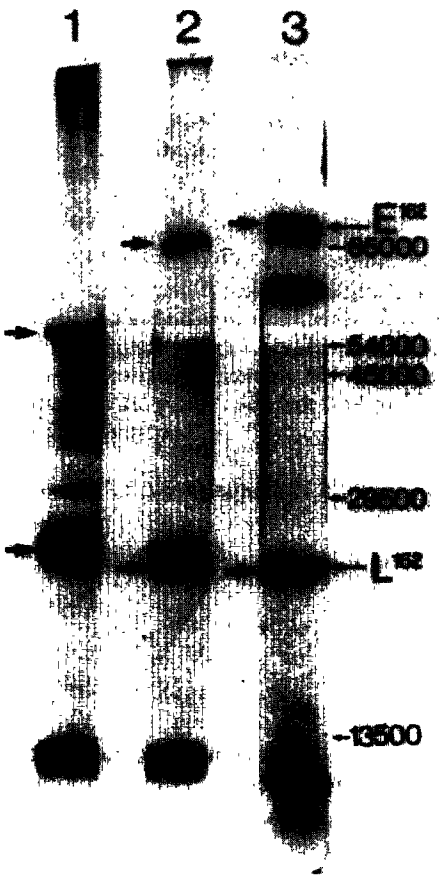

Fig.2. Autoradiogram of a reducing 12.5% SDS-acrylamide gel, loaded with immunoprecipitated IR162 mRNA products from injected *Xenopus* oocytes or from a rabbit reticulocyte lysate cell-free assay. (Lane 1) Cell-free translation products; (lane 2) cell extract from injected oocytes; (lane 3) incubation medium from injected oocytes; immunoprecipitation was with antibodies against rat IgE. The autoradiogram was exposed for 12 h. The arrows indicate the immunoglobulin subunit chains or their precursors. L162 and E162 indicate the positions of migration of mature rat IgE light and heavy chains, respectively. The other bands seen in the lanes were also present in precipitates obtained with non-immune IgG (not shown). M_r markers were BSA (65000), rabbit γ-chain (54000), ovalbumin (45000), carbonic anhydrase (29500) and cytochrome *c* (13500).

3. RESULTS

Fig.1 shows an autoradiogram of a non-reducing SDS gel of the immunoprecipitates of cell extract and incubation medium, from oocytes injected with rat plasmacytoma poly(A) RNA as well as from control oocytes. One protein with an apparent M_r of 180000 is specifically precipitated with anti-IgE from the lysate of injected oocytes (fig.1, lane 1). This protein is absent from the immunoprecipitate of the control oocyte cell extract (fig.1, lane 5), indicating that it has been synthesized under direction of the injected mRNAs. The protein migrates faster than mature IgE. Also, the medium from injected oocytes contains a protein which binds specifically to anti-rat IgE (fig.1, lane 3); this latter protein, however, has an apparent M_r of 190000 and co-migrates with mature IgE.

The proteins precipitated with antibodies against IgE were separated on a reducing SDS-polyacrylamide gel, shown in fig.2. Upon reduction, the 180 kDa intracellular protein separates into a 24 and a 65 kDa polypeptide (fig.2, lane 2); the 190 kDa protein from the medium is resolved into a 24 and a 70 kDa subunit co-migrating with authentic rat IgE light and heavy chains, respectively (fig.2, lane 3). For comparison, the primary translation products of the IgE heavy and light chain mRNAs have M_r values of 54000 and 26000, respectively (fig.2, lane 1). The identities of these subunit chains have been confirmed by tryptic peptide mapping of the eluted gel bands. As noted by Hellmann et al. [11], a truncated light chain, with an apparent M_r of about 10500, is also present in the translation products of IR162 mRNA.

4. DISCUSSION

We conclude from the above data that rat IgE heavy and light chains are translated and correctly processed by *Xenopus* oocytes injected with rat plasmacytoma mRNA. The initial light chain mRNA translation product is converted to a polypeptide of slightly smaller M_r, corresponding to removal of a signal peptide from the primary translation product. The ϵ-chain mRNA translation product, having an apparent M_r of 54000 in a cell-free translation assay, is converted to the correct size, M_r 70000, of mature ϵ-chain. Furthermore, the translation products are secreted from the oocytes as a 190 kDa moiety, corresponding to a correctly assembled, normal immunoglobulin complex of two heavy and two light chains.

The posttranslational modification of the ϵ-chain results in a molecule of considerably larger size than the initial translation product. This must be due to glycosylation of the ϵ-chain since no other modification would give rise to such a large increase in M_r. The resulting molecule has the correct size when compared to authentic, mature ϵ-chains. We therefore conclude that the chains are correctly glycosylated by the oocytes, since in view of the large size difference between the glycosylated and unglycosylated forms, any major deviations from the normal glycosylation pattern would have been detected.

The glycosylation of ϵ-chains in the *Xenopus* oocytes occurs in two discrete steps. Intracellularly, the initial translation product of 54 kDa is converted to a 65 kDa form, presumably due to attachment of *N*-linked precursor oligosaccharide chains (core glycosylation) during passage through the membrane of the rough endoplasmic reticulum, followed by trimming of the oligosaccharide core [12]. Extracellularly, the secreted ϵ-chain is clearly even larger, reflecting processing of the oligosaccharide core, including addition of terminal sugars, e.g. galactose and sialic acid. In this study, terminal glycosylation appears to be tightly coupled to secretion, although glycosylation is probably not required for transport out of the cell [1,4].

Within the limits of detection, all IgE is secreted from the oocytes as a complex of the size expected for a normal IgE molecule of two heavy chains and two light chains as also found for immunoglobulin G [13]. Intracellularly, we detect apparently correctly assembled IgE molecules of a slightly smaller size than the secreted form, indicating that assembly of the heavy and light chains takes place before terminal glycosylation. This finding is in agreement with the assumption by Vasalli et al. [14] that chain assembly takes place while the heavy chains are growing on the ribosomes.

Xenopus oocytes injected with foreign mRNA are known to carry out many correct posttranslational modifications of heterologous proteins, e.g. signal sequence removal and assembly of multimeric proteins such as immunoglobulins [13]. The oocytes do not, however, perform a number

of presumably tissue-specific functions such as proinsulin cleavage [2]. *Xenopus* oocytes may glycosylate foreign proteins; in some cases, however, glycosylation was incomplete [4] or the resulting products were heterogeneous with respect to size and composition [1]. The present results, however, demonstrate that *Xenopus* oocytes have an enzyme system that will glycosylate the rat ϵ-chain to yield a homogeneous, correctly sized molecule. Therefore, although glycosylation may in some instances be influenced by host-dependent factors [15], the extensive glycosylation of the IgE ϵ-chain is apparently determined by information residing in the ϵ-chain itself and is not dependent on a plasma cell-specific mechanism.

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