

The processing and secretion of rat serum albumin by oocytes from *Xenopus laevis*

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Microinjection of rat liver mRNA into *Xenopus* oocytes led to the synthesis of intracellular proalbumin and the secretion of mature albumin into the incubation medium. The ionophore monensin abolished the secretion of albumin but not the processing of the precursor. A variety of protease inhibitors were added to the incubation medium but there was no detectable inhibition of proalbumin cleavage.

Proalbumin processing; Oocyte; Albumin secretion; mRNA; (*Xenopus laevis*, Rat liver)

1. INTRODUCTION

A variety of eukaryote secreted proteins undergo post-translational proteolysis before release from the cell [1,2]. In many cases proteolytic cleavage of the proform is obligatory for physiological activity [2,3]. The appearance of one or two basic residues at processing sites is common among proproteins [4,5] however, this feature alone does not constitute a signal for proteolysis and other structural and tissue specific markers must exist.

Xenopus oocytes injected with exogenous mRNAs have been used as a surrogate system to investigate protein modifications and their effect on intracellular protein transport and secretion [6,7]. However, the limited ability of the oocyte to proteolytically cleave propeptide precursors may indicate not only the tissue specific nature of the signals but also the restricted distribution of the proteases involved [8,9]. To examine this phenomenon we have chosen to study the process-

ing of rat proalbumin in the oocyte. Serum albumin from several species exists as an intracellular proform, bearing an amino-terminal peptide which is proteolytically cleaved shortly before secretion [10]. Rat serum albumin (RSA) has a hexapeptide propiece with two arginine residues at the carboxy terminus [11,12]. We examined the ability of the oocyte to proteolytically cleave the rat albumin precursor and the effect of monensin on the coupling of processing to secretion. We also report the effect of various protease inhibitors on the intracellular cleavage of rat proalbumin.

2. MATERIALS AND METHODS

Female Wistar rats (100-150 g) were used for mRNA preparations. Oligo(dT) cellulose was from BRL Laboratories (Science Park, Cambridge); L-[³⁵S]methionine (>800 Ci·mmol⁻¹) and Amplify were from Amersham International (Amersham, Bucks). The ionophore, monensin, and protease inhibitors, *N*-*p*-tosyl-L-lysyl-chloromethyl ketone (TLCK), leupeptin and diisopropyl fluorophosphate (DFP), were supplied by Sigma (Poole, Dorset) as were all other analytical grade reagents.

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Anti-rat serum albumin was raised in sheep (Wellcome Research). RSA and proRSA were prepared by the method of Quinn et al. [12]. Poly(A⁺) RNA was prepared as described [7] and stored under liquid nitrogen. Oocytes from *Xenopus laevis* were maintained in Barth's saline [6] and microinjected with RNA. Injected oocytes were incubated overnight at 21°C in Barth's saline with or without the appropriate inhibitor. Details of inhibitor concentrations are in the figure legends. Surviving oocytes were cultured in Barth's saline containing 0.7 mCi·ml⁻¹ [³⁵S]methionine with or without inhibitor for 6 h then transferred to unlabelled medium for 24 h. The time course experiment shown in fig.1 involved the removal of batches of 5 labelled oocytes and their surrounding medium at the times described. Homogenisation and immunoprecipitation of oocyte extracts and incubation media with anti-rat serum albumin were done as described elsewhere [7]. Polyacrylamide isoelectric focussing gels were prepared by a modification of the method of Ames and Nikaido [13]. 6% slab gels were prepared (total volume 30 ml) and polymerised by the addition of 1.4 ml of 0.14 mg·ml⁻¹ riboflavin/2% TEMED. Poured gels were illuminated for 30 min using a photographic light box at a distance of 5 cm. Polymerisation by this method was more reliable than the use of ammonium persulphate. RSA and proRSA were added as reference markers to each sample before isoelectric focussing. Gels were washed in 10% acetic acid to remove ampholines then stained with Coomassie blue. Destained gels were prepared for fluorography using Amplify and following the manufacturers instructions.

3. RESULTS AND DISCUSSION

Fig.1 shows the synthesis of rat albumin by oocytes injected with rat liver poly(A⁺) RNA. After 3 h labelling an intracellular species appears which cofocusses with rat proalbumin (track 1) but no labelled protein has been secreted (track 5). At later times in the incubation both albumin and proalbumin appear in oocyte extracts (tracks 2 and 3) but only processed albumin is secreted (tracks 6 and 7). If labelled oocytes are incubated in unlabelled medium for a further 16 h then most of the intracellular proalbumin disappears (track 4)

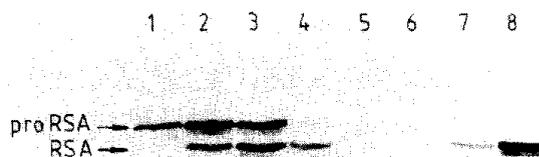


Fig.1. The synthesis and secretion of rat serum albumin in oocytes. Samples are derived from oocyte extracts (tracks 1–4) or incubation media (tracks 5–8) at the following times: 3 h (tracks 1 and 5), 5 h (tracks 2 and 6), 8 h (tracks 3 and 7) and after a 16 h 'chase' in unlabelled medium (tracks 4 and 8). The migration of marker proalbumin and serum albumin are as indicated on the figure.

and the secreted albumin band intensifies (track 8). It is clear from this experiment that the oocyte cleaves the basic hexapeptide from rat proalbumin and that this processing step is tightly coupled to secretion of the mature protein.

Little is known about the mechanism which links proteolytic cleavage to export and the associated intracellular proteases. We decided to investigate the phenomenon in the oocyte using inhibitors of intracellular transport and proteolysis. The carboxylic ionophore monensin inhibits the intracellular transport of secretory [14–16] and membrane [16,17] proteins. The presumed target of the ionophore is some element in the Golgi complex [18]. Fig.2 shows that in oocytes monensin inhibits the secretion of rat albumin. In the control experiment half of the synthesised albumin was secreted from the oocytes (tracks 1 and 2). In other experiments >80% of the intracellular albumin was exported. These quantitative differences are probably caused by batch variation among oocytes so non-inhibitor controls are included in all experiments. 15 μM monensin completely inhibits albumin export (tracks 3 and 4) and small amounts of proalbumin accumulate in the oocyte. Increasing the monensin concentration (tracks 5 and 6) depresses synthesis and causes the ratio of proalbumin to albumin in the cell to increase. Monen-

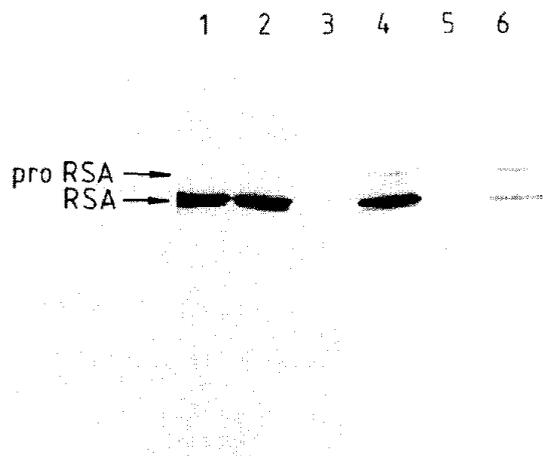


Fig.2. The effect of monensin on proalbumin processing and secretion. Tracks 1, 3 and 5 are incubation media, tracks 2, 4 and 6 oocyte extracts. No inhibitor (tracks 1 and 2), 15 μ M monensin (tracks 3 and 4) and 30 μ M monensin (tracks 5 and 6). Injected oocytes were incubated in Barth's saline containing the stated concentration of monensin for 2 h before incubation in [35 S]methionine containing medium. The position of albumin and proalbumin is as indicated.

sin did not produce the complete abolition of proalbumin processing seen in hepatocytes treated with the inhibitor [19,20]. The observed intracellular accumulation of albumin in the oocyte implies that the ability of monensin to block albumin secretion is not closely linked to inhibition of proteolytic processing. This may mean that proalbumin is cleaved before it reaches the Golgi complex in oocytes, assuming that monensin acts on some Golgi element to block secretion.

The specific protease(s) responsible for proprotein cleavage have not been fully identified. It has been suggested that a membrane bound form of the enzyme cathepsin B is the convertase in rat liver [21]. We studied the effect of three protease inhibitors on oocyte mediated processing of proalbumin. Leupeptin and TLCK had no discernable effect on proalbumin conversion. Both inhibitors decreased activity of the rat liver processing enzyme at concentrations below those used here [21]. Diisopropylfluorophosphate (DFP), a general inhibitor of serine proteases, also failed to inhibit processing at 200 μ M. We also examined the effect of DFP at 100 μ M on isolated hepatocytes, but failed to find any inhibition of proalbumin pro-

cessing (not shown). This concentration of DFP is enough to inhibit completely the cholinesterase activity of rat liver [22]. These results strongly suggest that in the oocyte, as well as in the rat hepatocyte, the converting enzyme is not a serine protease, a possibility that has been put forward for the propeptide convertase of human liver [23].

Our results show that *Xenopus* oocytes process rat proalbumin to a form minus the three arginine residues found in the propeptide, cleavage probably occurs after the carboxy-terminal double arginine residues. However, it is clear that double basic residues are not always recognised as a signal for proteolysis in the oocyte. The frog cell also cleaves at a single arginine of pro-vasopressin but ignores the double basic residues between vasopressin and oxytocin found in the same molecule [24]. It may be that the intramolecular position of the cleavage signal is important since the oocyte also fails to convert proinsulin and secretes the intact precursor [25].

There may be two distinct levels of intracellular proteolysis. A general processing step catalysed by a protease common to a variety of different cell types, and a more tissue specific step involving enzymes with a more restricted distribution. It is well known that the presence of certain proteases can determine the choice between alternative physiological signals. The proprotein neuropeptide precursors are cleaved at different sites depending on the cell type in which they are synthesised, thus the nature of the processing enzyme(s) in situ governs the type of neuropeptide secreted [26].

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