Construction of a representative cDNA library from mRNA isolated from mouse oocytes

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A representative cDNA library has been constructed from the small quantities of poly(A)$^+$ RNA present in unfertilised mouse oocytes. The construction of this library has been achieved by use of cow pea mosaic virus RNA as a carrier during isolation of polyadenylated message and during subsequent cloning procedures. This approach may be applicable to any system in which amounts of mRNA are limiting.

cDNA cloning *(Mouse oocyte)* *Cow pea mosaic virus* *RNA* *maternal RNA*

1. INTRODUCTION

Analysis of the molecular biology of the preimplantation mammalian embryo is difficult, principally because of its small size and limited available numbers. A single mouse oocyte contains only 0.35 ng of total RNA of which about 2% is estimated to be poly(A)$^+$ RNA [1], and only 15–30 oocytes are recoverable from a single female by superovulation. Nonetheless, a considerable body of information has accumulated about the biochemistry of the earliest stages of mouse development (review [2,3]). In particular, it has been established recently that the maternally inherited RNA within the fertilised oocyte is functional during the first 2 cell cycles only and is then destroyed and replaced by newly synthesised embryonic RNA [4–7]. Moreover, during the period of expression of maternal RNA, some species of mRNA appear to be expressed selectively [8,9]. We are interested to know how the selective expression of mRNA is achieved and how the destruction of maternal RNA is regulated. To pursue these studies, we have constructed a cDNA library. The limiting quantities of material necessitated development of a highly efficient cloning procedure, and the use of a carrier RNA to reduce losses of oocyte mRNA. The representative nature of the library was assayed by searching it for sequences which corresponded to a species of mRNA the abundance of which had previously been estimated [7].

2. MATERIALS AND METHODS

2.1. *Collection of oocytes*

Mice were superovulated and unfertilised oocytes were isolated exactly as described in [6]. The oocytes were collected in 2 batches and snap-frozen in liquid nitrogen for storage at −70°C prior to RNA extraction.

2.2. *Isolation of RNA*

Total RNA was extracted from oocytes as in [7], with the important difference that no tRNA was used as carrier. Commercially available tRNA was found to contain small amounts of double-stranded DNA which tends to carry through the oligo(dT) chromatography step and clone preferentially, causing a marked reduction in the efficiency of cDNA cloning. RNA from cow pea mosaic virus (CPMV) was a gift from Drs J. Davies and G. Lomonossoff (John Innes Institute, Colney Lane, Norwich) and was prepared as in

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[13]. Poly(A)$^+$ mRNA was isolated as described [8].

2.3. *Synthesis and cloning of double-stranded cDNA*

Double-stranded cDNA was synthesised as described by Watson and Jackson [12] and inserted into the *Eco*RI site of λ gt10 [13].

2.4. *Analysis of recombinants*

Replica filters of plaques were made as described by Benton and Davis [14]. After baking for 2 h, all filters were prehybridised for 2 h at 65°C in 5 × SSC containing 5 μg/ml herring sperm DNA (1 × SSC: 0.5 M NaCl, 0.5 M Na citrate), 0.1% SDS and 2 × Denhardts (1 × Denhardts: 0.2 g Ficoll, 0.2 g polyvinylpyrrolidone, 0.2 g BSA fraction 5 in 1 l). Hybridisations were performed overnight in the same solution at 65°C. All filters were washed in 1 × SSC + 0.1% SDS at 55°C for 2 h with several changes of buffer. Molecular probes were prepared from CPMV RNA and poly(A)$^+$ mRNA from mouse liver by 5′-end labelling after partial base cleavage, using [γ-^{32}P]ATP and polynucleotide kinase according to Smith et al. [15]. The actin clone, which encoded a 300 bp fragment from the 3′-coding region of skeletal actin, was isolated from a cDNA library made from *Xenopus laevis* RNA, a gift from Dr T. Mohun (Department of Zoology, Cambridge). This clone was constructed in mp8M13 by insertion into the *Sma*I site and radiolabelled as described by Hu and Messing [16].

3. RESULTS

3.1. *Evaluation of CPMV RNA as a suitable carrier*

Total RNA was extracted from 16000 oocytes. It was estimated from A_{260} measurements that 5.0 μg total RNA was recovered. As only approx. 2% of this material, or 0.1 μg RNA, would be polyadenylated, the losses during oligo(dT) purification and subsequent generation of double-stranded cDNA would have been unacceptable in the absence of a carrier. A suitable carrier would need to satisfy certain criteria, namely (i) it should be polyadenylated, (ii) it should not share sequences in common with the carried material, (iii) it must not clone preferentially, and (iv) it should contain only a limited number of species of RNA (preferably 1) of known sequence. CPMV RNA is know to satisfy some of these requirements, being easily purified in large amounts, one of the few plant viruses that is polyadenylated [10], and containing only 2 components which have been sequenced recently [17].

To determine whether CPMV also fulfilled criteria (ii) and (iii), a pilot cloning experiment was performed in which CPMV RNA and poly(A)$^+$ mRNA from mouse liver were mixed in a ratio of 9:1 for use in preparation of double-stranded cDNA and cloning into λ gt10. The recombinants arising from this cloning experiment were then plated at medium density, duplicate filters were prepared and probed with CPMV RNA and mouse liver poly(A)$^+$ RNA which had been kinase labelled to the same specific activity. A representative result is shown in fig.1. No plaques contained sequences which hybridised to both CPMV RNA and mouse liver poly(A)$^+$ RNA, and it can also be seen that the relative frequencies of recombinants containing CPMV and mouse liver sequences reflect the proportion in which these RNA species were mixed at the beginning of the cloning procedure. It was therefore concluded that CPMV RNA fulfilled the required criteria for use as a suitable carrier.

3.2. *Construction of a mouse oocyte cDNA library*

1.9 μg CPMV RNA was added to the total RNA extracted from the oocytes, the mixture submitted to oligo(dT) chromatography and the poly(A)$^+$ mRNA reverse transcribed, converted into double-stranded cDNA and cloned. We obtained 5 × 10^8 plaques per μg of double-stranded cDNA packaged. To determine the number of recombinants arising from the oocyte material, and hence to determine the complexity of our library, a number of controls were performed during the packaging reaction. The number of plaques arising from the packaging of cut and religated λ gt10 vector alone gives an estimate of the plaques arising from non-recombinants in this system, and these were found to occur at a frequency of 25%. Thus of 100 clones 25 should be false positives, 71 should contain CPMV sequences and 4 should contain oocyte sequences. We therefore screened 65 plaques with

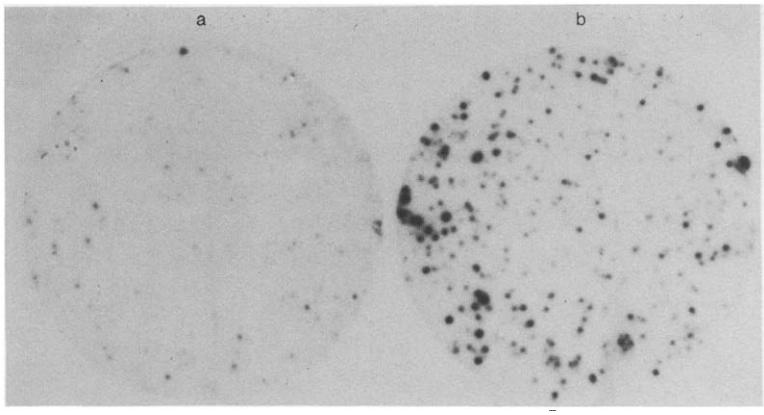

Fig.1. Autoradiograms of duplicate filters that were hybridised with kinase-labelled poly(A)$^+$ mRNA from (a) mouse liver and (b) CPMV.

kinase-labelled CPMV RNA, and found that as predicted only 41 plaques reacted (fig.2).

3.3. *Test for representative nature of the library*

Having shown that the frequency of CPMV sequences represented in the library is as expected, it was next necessary to examine whether the mRNA species extracted from the oocytes were represented faithfully in the library. To avoid using valuable oocyte material, it was decided to investigate the frequency of representation of a species of cDNA corresponding to an mRNA the abundance of which in the oocyte had been estimated previously. Actin was selected since it has been estimated to be present at a level of approx. 1% in the mRNA in the oocyte [7]. We used as our probe an M13 clone isolated from an *X. laevis* cDNA library, which contained a sequence corresponding to the 300 bp 3′-coding region of skeletal actin. This sequence has been shown from computer analysis to be highly conserved across species, being 85% homologous to the mouse skeletal actin sequence studied by Minty et al. [18]. A total of 4000 plaques were screened, of which we would expect 25% to be false positives, and, of the remaining 3000, 5% should contain oocyte sequences. Of these about 1% should contain actin sequences, i.e. 1–2 plaques should hybridise with the labelled actin probe. The results of such a hybridisation are shown in fig.3, in which 3 plaques are reactive, a result in accord with expectation.

4. DISCUSSION

Our data demonstrate that it is possible to construct cDNA libraries for systems in which only very small amounts of material are available. Analysis of further clones from this library has shown that inserts of up to 2 kb are present, which should permit a detailed study of the mRNAs represented. Additionally clones have also been isolated which contain sequences corresponding to RNA species the presence of which in oocytes has been detected more directly, e.g. laminin B subunit, SET 1 repeat [19,20]. This approach to making a cDNA library should be of general value not only to developmental biologists but also should have wider application, for example, to pathologists who have only small samples of biopsy material for analysis. A similar library has now been made from blastocyst poly(A)$^+$ mRNA using the same approach.

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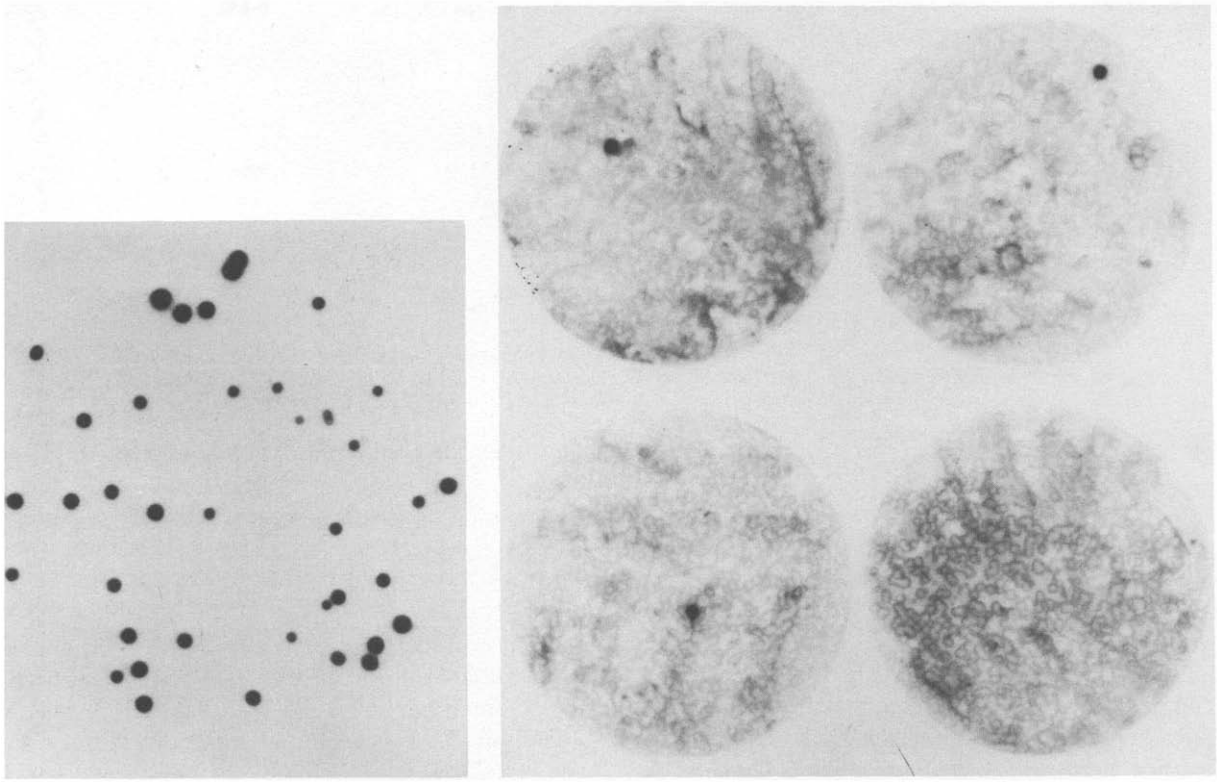

Fig.2. Autoradiogram of a filter hybridised with kinase-labelled CPMV RNA.

Fig.3. Autoradiograms resulting from the hybridisation of radiolabelled actin probe to 4000 plaques.

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