Transcripts of Crystallin Genes in Neural Retina of Chick Embryos

ニワトリ胚神経性網膜におけるクリスタリン遺伝子の転写産物のRot解析法及びノーザンブロット法による検出

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Further detection of transcripts of crystallin genes in neural retina of chick embryos

（ニワトリ胚神経性網膜におけるクリスタリン遺伝子の転写産物のRnt解析法及びノーザンブロット法による検出）

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（論文内容の要旨）

1個の受精卵が分裂をくり返し，特有の形態と機能をもった組織を形成し，複雑な器官より構成された動物の個体を形づくる。動物のある種の組織の一部が損傷を受けたときに，再生と呼ばれている修復する機構が生物体に備わっている。特に，イモリの眼の水晶体は，その全組織が失なわれたときに，別の組織から再生されることが知られている。この現象は，種々の生物体の組織細胞を生体外の細胞培養系に移したときにも観察され，「分化の転換」と呼ばれ，多くの組織細胞にみられる。特に，ニワトリ胚の神経性網膜細胞から水晶体への転換は，その典型的な例として広く知られている。

申請者は，水晶体に特異的なクリスタリン mRNAを指標にして，この「分化の転換」の機構を，転写調節のレベルで調べた。水晶体に特異的なクリスタリン mRNA に対する1本鎖 DNA プローブを得るために，poly A RNAを抽出し，ショ糖密度勾配遠心で純度約95%のδ－クリスタリン mRNA を得て，これを鋳型として，^{3}Hラベルされた cDNA を作製した。このプローブと，3.5日と8.5日目胚の神経性網膜細胞より得られたpoly A RNA を再会合させて，Rot 解析を行なった。両者のpolyA RNA の約0.0028%がδ－クリスタリンcDNA と会合しうることが判明した。このことは，水晶体に特異的に発現しているクリスタリン遺伝子が，他の初期胚の組織でも低頻度（1細胞当たり10～20分子程度）に発現していることを示唆している。

これまでに得られている知見では，組織特異的に発現している遺伝子の転写産物が，別の組織に存在することは知られていない。上記の事柄を，より正確にするために，遺伝子工学の手法でクローン化されたδ－クリスタリンとα－クリスタリンcDNA を用いて以下のような，ノーザンブロット法で検討した。3.5日目胚の神経性網膜細胞より分離した poly A

RNA をアガロースゲル電気泳動で，RNA のサイズに応じて分離したのち，フィルターに固定し，^{32}P ラベルされたδ－クリスタリンｃDNA をプローブとしてハイブリダイズさせた。成熟 mRNA に相当する17Ｓのバンド以外に，より分子量の大きい RNA の位置にもいくつかのバンドが検出された。同様の実験を，α－クリスタリンｃDNA を用いて行なった結果，成熟 mRNA のバンドは検出できなかったが，より分子量の大きい precursor RNA と思われるバンドが検出された。これらの DNA プローブを用いた，ニワトリ全 DNA のサザーンブロット解析の結果は，δ－とα－クリスタリン遺伝子だけがハイブリダイズすることが知られているので，初期胚の神経性網膜細胞には，分化転換したのち形成される水晶体に特異的に発現している転写産物が，非常に少量ではあるが確かに含まれていると結論づけられる。

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（論文審査の結果の要旨）

動物の発生の初期の胚の各部分は，将来，正常に発生を続ける場合の予定運命よりは広い予定能力をもっている。このことは，生物の発生現象における最も特徴的で，しかも基本的な性質である。この事実は，古典的な発生研究において，実験形態学的な手法を用いて繰返し確められてきたところであるが，どうしてそのようなことが起こるのか，その分子的な背景は全く明らかにされていない。しかし，1950年代の研究において，胚の各部分は成体においてそれに匹敵する部分よりは，じつは多様な蛋白質を少量ながら合成していて，これが広い予定能力の基礎になるのではないか，という考え方があった。この考え方を，当時とは全く別の，より進んだ技術を導入しつゝ検討したのが本申請者の論文である。

若いニワトリの胚では脳，網膜などの組織にはレンズに分化する能力のあることがすでに明らかにされている。レンズという組織細胞は形態的に際立った特徴があるだけでなく，その分子組成においても著しく他の組織とは異なっていて，特にニワトリにおいては，成体になるとレンズ以外には存在しないδ－クリスタリンと呼ぶ蛋白質が大部分を占めている。この論文の主眼は，このδ－クリスタリンというレンズに特異的な蛋白質をコードするδ遺伝子の活性が，胚においてもやはりレンズに限られるのか，それともレンズ以外の脳や網膜にもあるのか，という問題を分子間雑種の技術を駆使して検討することである。

得られた結果は，δ－クリスタリンの遺伝子の転写活性は若い胚ではレンズに止まらず，ごく低くはあるが脳，網膜その他いくらかの組織にもあることを示している。しかし，発生が進むにつれてこの転写活性はレンズの部分に限られてくることになる。特に興味深いのは，レンズ以外の部分における転写産物は未成熟で，分子量の大きなメッセンジャーＲＮＡであることを明らかにした点である。実際にδ－クリスタリンという蛋白への

はん訳の可能な転写産物は，若い胚においてでも，レンズでだけ作られることが示されたのである。

これらの結果は，発生生物学の方法と分子生物学の方法とを合一することによって初めて得られるものである。特にニワトリの若い胚から充分な量のサンプルを得ることは頗る困難であるが，申請者は技術的に熟達していて信頼のおける結果を得ることに成功している。分子間雑種による実験結果の解釈を妥当なものと判定された。

従って，この研究は発生生物学上の最も古典的で基本的な，広い発生能力という概念の分子的背景がどのようなものであるかをうかがう上で，重要な貢献をなしたものと考えられ，理学博士の学位を受ける価値のあるものと結論されるのである。

なお，主論文及び参考論文に報告されている研究業績を中心とし，これに関連した研究分野について試問した結果，合格と認めた。

Further Detection of Transcripts of Crystallin Genes in Neural Retina of Chick Embryos

(Transdifferentiation/ Rot analysis/ Northern blot)

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ABSTRACT

Crystallins are proteins specific to lens tissue. It was previously shown that the gene coding for δ-crystallin are transcribed in several non-lens tissues (neural retinas, brains, limb buds) of early chick embryos (1). In the present work the amount of transcripts of the δ-crystallin gene in embryonic neural retina was determined by liquid hybridizations. For this, poly(A)$^+$RNAs were isolated from neural retinas collected from 1,700 of 3.5-day-old embryos and 140 of 8.5-day-old embryos. The experiments of reassociation reaction between these poly(A)$^+$RNAs and δ-crystallin cDNA showed that about 0.0028% of poly(A)$^+$RNAs of neural retina in these embryos are δ-crystallin transcripts.

Northern blot analyses using α- and δ-crystallin cDNAs as probes showed that (i) the mature size of transcripts and larger RNA species were detected in neural retina of 3.5-day-old chick embryos by the δ-crystallin probe (ii) though the mature size of transcripts was not detected by the α-crystallin probe, larger RNA species which are probably precursors of α-crystallin transcripts were detected in embryonic neural retina. This indicates that the α-crystallin gene as well as the δ-crystallin gene are transcribed in embryonic neural retina.

INTRODUCTION

δ-crystallin is a protein highly specifically located in lens. It has been disclosed, however, that the gene coding for this protein is transcribed in several non-lens tissues of chicken embryos, by means of Northern blot analyses using cloned δ-crystallin cDNA (1). By means of liquid hybridization, Clayton et al. (2) reported that the embryonic neural retina (NR) contains RNA sequences which hybridize with cDNA to the most abundant mRNAs in lens. These findings are of special interest to interpret the wide distribution of the potentiality of lens differentiation in several non-lens tissues of embryos in terms of gene expression (3,4,5).

In the previous paper (1) the ratio of the mature δ-crystallin mRNA to total poly(A)$^+$RNAs in non-lens tissues was estimated to be 0.001% as judged from the intensity of the band in Northern blot. In addition, we have observed larger RNA species hybridizing with δ-crystallin cDNA in non-lens tissues besides the mature mRNA. These larger RNA species are probably precursors of δ-crystallin transcripts, because no other DNA sequence hybridizing with the same probe is detected besides the δ-crystallin gene in all the chicken genome(6). The present work was conducted to confirm the presence of δ-crystallin transcripts in embryonic NR and to estimate the amount of total

δ-crystallin transcripts in NR more precisely by means of Rot analyses.

It was also examined whether the α-crystallin gene is also transcribed in NR or not, by Northern blot using a cloned α-crystallin cDNA as a probe.

MATERIALS AND METHODS

Preparation of poly(A)$^+$RNAs

NRs were isolated from 1,700 of 3.5-day-old and 140 of 8.5-day-old chicken embryos. Lenses and livers were collected from 100 of one-day-old chickens. These tissues were homogenized in the solution containing 0.1 M Tris-HCl (pH 9.0), 0.5% Nonidet P-40 and 10 mM vanadyl-ribonucleosidecomplexes (7). The homogenate was centrifuged at 4°C for 5 min at 10.000 x g. The supernatant fraction was extracted with an equal volume of phenol saturated with 0.1 M Tris-HCl buffer (pH9.0) containing 10 mM EDTA. RNA was precipitated with ethanol at -20°C and suspended in water as cytoplasmic RNA. Cytoplasmic RNA was put on an oligo(dT)cellulose column (Collaborative Research). The bound RNA was collected as poly(A)$^+$RNA (8). From 3,400 of 3.5-day-old NR and 280 of 8.5-day-old NR, 127 μg and 220 μg of poly(A)$^+$RNA were purified respectively.

Preparation of ^{3}H-labeled δ-crystallin cDNA (δ-cDNA)

δ-crystallin mRNA (δ-mRNA) was purified by essentially the same procedure as used by Yasuda et al (6). Lens poly(A)$^+$RNA was fractionated by sucrose density gradient (5 % - 20%) centrifugation. Each RNA fraction was assayed by SDS gel electrophoresis (9) of the in vitro translation products in the reticulocyte lysate (Amersham, Fig. 1A). The fractions with high δ-mRNA and very low non-δ-mRNA were combined and further purified by another density gradient

centrifugation (Fig. 1B). δ-cDNA labeled with ^{3}H-dCTP (50 Ci/mmol, Amersham) was synthesized by reverse transcriptase using the purified δ-mRNA as a template (10). δ-cDNA was denatured in a mixture consisting of 1 M glyoxal, 50% dimethylsulfoxide (DMSO) and 10 mM sodium phosphate (pH 7.0) for 60 min at 50°C (11), electrophoresed on a 1% agarose gel, and fluorographed. Fig. 2 shows the molecular size of the δ-cDNA, indicating that most of the synthesized cDNA migrated similarly to δ-mRNA. The specific activity of synthesized δ-cDNA was 1.2×10^{6} cpm/μg and this was used as a probe in liquid hybridization reactions.

Liquid hybridization reactions

The hybridization reactions were performed in 20-50 μl of the reaction mixture containing 0.6 M NaCl, 0.2 mM EDTA, 20 mM Tris-HCl (pH 7.4), 0.1 ng of ^{3}H-cDNA and various amounts of RNA (12). The mixture in an 1.5 ml polyethylene tube was overlaid with the buffer-saturated paraffin liquid and the reaction was carried out at 75°C for various periods, to give the appropriate Rot values. Hybridization was stopped by diluting the mixture in 1 ml of cold 50 mM sodium acetate (pH 4.5), 2mM $ZnSO_4$, 0.1 M NaCl and 10 μg/ml of heat denatured-sonicated salmon sperm DNA. Each sample was divided into two aliquots. One of them was incubated for 1 hr at 37°C in the presence of 20 U of S_1 nuclease (Sigma, Type III), and the ^{3}H-cDNA precipitable with 10% TCA was collected on Whatman GF/C glass-fiber filters. The filters thus obtained were counted in 10 ml of Toluen Scintillation

solution. The percentage of ^{3}H-cDNA hybridized was calculated from the ratio of ^{3}H-cDNA precipitated in a tube containing S_1 nuclease to that in a tube without the enzyme.

Probes for Northern blots

A cloned δ-cDNA, designated pBδ 5, was obtained from Dr. K. Yasuda (6). By Pst I digestion, 0.8 kb of δ-cDNA fragment inserted in this plasmid was prepared and then was radiolabeled with ^{32}P by nick translation (Amersham nick translation kit, PB. 5100). A cloned α-crystallin cDNA (α-cDNA) was obtained from K. Okazaki (paper in preparation).

Northern blot analyses

RNAs were denatured in a mixture of 1 M deionized glyoxal, 50% DMSO and 10 mM phosphate buffer (pH 7.0) at 50 °C for 1 hr (11). The samples were electrophoresed on horizontal 1.0% agarose gels and transferred to nitrocellulose filter by a technique developed by Thomas (13). The blot was baked in a vacuum oven for 4 hr at 80°C and prehybridized in a mixture of 50% (v/v) formamide, 5 x SSC (1 x SSC contains 0.15 M NaCl and 0.015 M trisodium citrate), 5 x Denhardt's solution (1 x Denhardt's solution contains 0.02% each bovine serum albumin, Ficoll, and polyvinylpyrrolidone), 50 mM sodium phosphate (pH 6.5), 1% glycine, and denatured salmon sperm DNA at 0.25 mg/ml for 15 hr at 42°C. The nick-translated probes were denatured at 100°C for 3 min, cooled and added to a hybridization buffer containing 50% (v/v) formamide, 5 x SSC, 1 x Denhaldt's

solution, 50 mM sodium phosphate (pH 6.5), and 10% (w/v) dextran sulfate. The RNA blot was hybridized for 20 hr at 42°C, washed with four changes of 2 x SSC containing 0.1% SDS for 5 min each at room temperature, and then washed with two changes of 0.1 x SSC containing 0.1% SDS for 15 min each at 50°C. The blot was exposed to X-ray film at -70°C.

RESULTS

Kinetics of hybridization of poly(A)$^+$RNAs of embryonic NR to ^{3}H-labeled-cDNA

Results of the liquid hybridizations between δ-cDNA and poly(A)$^+$RNAs extracted from embryonic NR are given in Fig. 3. As controls, assays were also made of purified δ-mRNA, and cytoplasmic RNAs of lens and liver. The percentage of cDNA hybridized with δ-mRNA, whole cytoplasmic RNA of lens and poly(A)$^+$RNA of 8.5-day-old embryonic NR, increased up to 81%, 84% and 91%, respectively. Poly(A)$^+$RNA prepared from 8.5-day-old embrionic NR reacted 36,000 times slowly ($Rot_{1/2}$ of 9.0×10^1) than purified mRNA. As for cytoplasmic RNA of liver only 15% was obtained, even at a Rot value of 1.0×10^3. The percentage of cDNA hybridized with poly(A)$^+$RNA of 3.5-day-old embryonic NR increased up to 45% from a Rot value of 1.0×10^1 to 1.0×10^2. Thus, the Rot curve of 3.5-day-old embryonic NR is very close to that obtained with poly(A)$^+$RNA from 8.5-day-old embryonic NR. The results demonstrate that the mRNA sequence specific to δ-crystallin cDNA is present in embryonic NR, though at a very low concentration. As calculated from $Rot_{1/2}$ values, the ratio of the δ-specific mRNA sequence in embryonic NR to total poly(A)$^+$RNA is about 0.0028% (see Table 1).

Northern blot analyses using α- and δ-crystallin cDNAs as probes

The δ-specific mRNA sequence in poly(A)$^+$RNA extracted from 3.5-day-old embryonic NR was visualized as bands by Northern blot analysis using cloned δ-cDNA as a probe. In the blot shown in Fig. 4A, 10 μg of poly(A)$^+$RNA of 3.5-day-old embryonic NR was run. For comparison, 100ng of lens cytoplasmic RNA extracted from 1-day-old chickens was run in parallel. A band containing RNA of the same molecular size as δ-mRNA is clearly seen in poly(A)$^+$RNA of 3.5-day-old embryonic NR. This is a confirmation of the previous demonstration of the presence of this lens specific messenger in embryonic NR (1). In addition, larger RNA species hybridized with δ-cDNA were also observed as reported in the previous paper.

In the present study, Northern blot analysis was extended to the detection of α-crystallin specific mRNA sequence in the same poly(A)$^+$RNA extracted from embryonic NR. Fig. 4B shows that the results of the hybridization with cloned α-crystallin cDNA (α-cDNA). The RNA blot hybridized with ^{32}P-labeled δ-cDNA was washed in the buffer containing 5 mM Tris-HCl at pH 8.0, 0.2 mM EDTA, 0.05% sodiumpyrophosphate and 0.002% each of bovine serum albumin, Ficoll, and polyvinylpyrrolidone for 2 hr at 65°C. The RNA blot was then hybridized with nick-translated α-cDNA. The band corresponding to α-crystallin mRNA is not seen in poly(A)$^+$RNA of 3.5-day-old embryonic NR, although such band

is clearly seen in lens cytoplasmic RNA. The other, larger RNA species hybridizing with α-cDNA are detected in NR. This indicates that α-crystallin transcripts is present in 3.5-day-old embryonic NR, though not being processed to mature mRNA in this non-lens tissues.

Above results were also confirmed by the densitometric analyses as given in Fig. 5.

DISCUSSION

In this paper, the previous finding of the transcription of lens-specific δ-crystallin mRNA in NR of early chick embryos (1) was well confirmed by means of the liquid hybridizations. However, the ratio of δ-crystallin transcripts to total poly(A)$^+$RNAs in NR is much lower than in lens cells. Only 0.0028% of poly(A)$^+$RNA is δ-crystallin transcripts in embryonic NR, as calculated from the $Rot_{1/2}$ values. It is possible to estimate the copy numbers of δ-crystallin transcripts per cell of 3.5-day-old NR from the $Rot_{1/2}$ values. If δ-crystallin gene is transcribed equally in NR cells of 3.5-day-old embryos, δ-crystallin transcripts will accumulate to approximately 10 copies per cell.

In the present study, the $Rot_{1/2}$ value of 8.5-day-old embryonic NR is almost the same as that of 3.5-day-old embryonic NR. However, it has been shown in the previous paper (1) that the amount of mature δ-mRNA decreases with development and disappears in 1-day-old chicken NR. This apparent discrepancy is accounted for the presence of larger RNA species hybridizing with δ-cDNA. These larger RNA species, which are detected in Northern blots by using the double-stranded δ-cDNA as a probe, reassociated with the single-stranded DNA complementary to δ-mRNA in liquid hybridizations. Thus, larger RNA species derived from the coding strand of the δ-crystallin gene are probably precursors of the mature δ-mRNA. It has been shown that the

amount of larger RNA species reached a peak in 5.5 - 8.5-day-old embryos in the Northern blot in the previous paper.

The ratio of the mature δ-mRNA in 3.5-day-old embryonic NR to total poly(A)$^+$RNAs was estimated to be not more than 0.001%, as judged from the intensity of the band in the previous paper. The data of liquid hybridization show that the ratio of δ-crystallin transcripts to poly(A)$^+$RNAs of NR is 0.0028%. This suggests that the larger RNA species observed as smeary bands in Northern blots are major components of δ-crystallin transcripts in embryonic NR. It remains to be examined whether the mature size δ-mRNA in NR can be translated in vivo with the same efficiency as in the homologous lens cells.

There are three major classes of crystallins (α-, β- and δ-crystallin) in chicken lenses (Fig. 1A). These crystallins are expressed coordinately with differentiation of lens in vivo (14) and with transdifferentiation of NR into lens in vitro (15,16). The Northern blot using α-cDNA shows that the α-crystallin gene is also transcribed but putative precursor RNAs are not processed in NR.

These results seems to support the irreversible gene repression model for control of development (17), in which at early stages of development all genes are transcriptionally accessible and the diminution of developmental potential is accounted for as resulting from progressive, irreversible repression of previously active genes. It is supposed from the present author's results

that the expression of the genes transcribed in heterologous embryonic tissues might be regulated at the step of the RNA processing.

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REFERENCES

1. AGATA, K., K. YASUDA and T. S. OKADA, 1983. Develop. Biol., 100, 222-226.
2. CLAYTON, M. R., I. THOMSON and D. I. DE POMERI, 1979. Nature, 282, 628-629.
3. OKADA, T. S., 1980. In "Current Topics in Developmental Biology" (A. A. MOSCONA, A. MONROY and H. K. HUNT, eds.), vol.16, pp.349-390.
4. -----, 1983. Cell Differ., 13, 177-183.
5. CRAYTON, M. R., 1982. In "Differentiation in vitro" (M. M. YOEMAN and D. E. S. TRUMAN, eds.), pp.83-120.
6. YASUDA, K., H. KONDOH, T. S. OKADA, N. NAKAJIMA and Y. SHIMURA, 1982. Nucleic Acids Res., 10, 2879-2891.
7. BERGER, S. L. and C. S. BIRKENMEIER, 1979. Biochemistry, 18, 5143-5149.
8. AVIN, H. and P. LEDER, 1972. Proc. Natl. Acad. Sci. USA., 69, 1408-1412.
9. LAEMMLI, U. K., 1970. Nature, 227, 680-685.
10. CHANG, A. C. Y., J. H. NUNBERG, R. J. KAUFMAN, H. A. ERLICH, R. T. SCHIMKE and S. N. COHEN, 1978. Nature, 275, 617-624.
11. McMASTER, G. K. and G. G. CARMICHAEL, 1977. Proc. Natl. Acad. Sci. USA., 77, 4835-4838.
12. YASUDA, K., K. OKUYAMA and T. S. OKADA, 1983. Cell Differ., 12, 177-183.

13. THOMAS, P. S., 1980. Proc. Natl. Acad. Sci. USA., 77, 5201-5205.

14. ZWAAN, J. and A. IKEDA, 1968. Exp. Eye Res., 7, 301-311

15. THOMAS, I., C. E. WILKINSON, J. F. JACKSON, D. I. DE POMERAI, R. M. CLAYTON, D. E. S. TRUMAN and R. WILLIAMSON, 1978. Develop. Biol., 65, 372-382.

16. ARAKI, M., M. YANAGIDA and T. S. OKADA, 1979. Develop. Biol., 69, 170-181.

17. CAPLAN, A. I. and C. P. ORDAHL, 1978. Science, 201, 120-130.

Table 1. Dosage of δ-crystallin specific sequence

Sourse of material	RNA·sample	$Rot_{1/2}$	Dosage*
1-day-old chicken lens	purified δ-mRNA	2.5×10^{-3}	1.0
1-day-old chicken lens	total cytoplasmic RNA	3.0×10^{-1}	0.0083
3.5-day-old embryo NR	poly(A)$^+$RNA	9.0×10^{1} **	0.000028**
8.5-day-old embryo NR	poly(A)$^+$RNA	9.0×10^{1}	0.000028

* Dosage of δ-crystallin specific sequence is calculated relative to the level of purified δ-mRNA, which has been given the value 1.

**These values are estimated, as the hybridization with poly(A)$^+$RNA of 3.5-day-old embryonic NR did not reach the saturation level at the highest Rot values available.

FIGURE LEGENDS

Fig. 1. SDS-polyacrylamide gel electrophoresis of the *in vitro* translation products of the fractionated lens poly(A)$^+$RNAs. Panel A shows the products of lens poly(A)$^+$RNAs fractionated by first step centrifugation. Each crystallin class (α-, β-, δ-), as indicated on the right side of the panel, is synthesized. Panel B shows that the product of the fraction of second step centrifugation is only δ-crystallin. This purified fraction was used as a template for synthesis of ^{3}H-δ-cDNA.

Fig. 2. Molecular size analysis of synthesized ^{3}H-δ-cDNA. Denatured ^{3}H-δ-cDNA was electrophoresed on a vertical agarose gel and fluorographed. Ribosomal RNAs of chicken and *E. coli* were used as molecular marker.

Fig. 3. Liquid hybridization reactions of ^{3}H-δ-cDNA to poly(A)$^+$RNAs of embryonic NR. (★-★), 3.5-day-old embryonic NR; (●-●), 8.5-day-old embryonic NR. Kinetics of hybridization with purified δ-mRNA (○-○), cytoplasmic RNA of lens (□-□) and cytoplasmic RNA of liver (△-△) of 1-day-old chickens are also given.

Fig. 4. Northern blot analysis of poly(A)$^+$RNA of 3.5-day-old embryonic NR. Lens cytoplasmic RNA of 1-day-old chicken (100 ng) and poly(A)$^+$RNA of 3.5-day-old embryonic NR (10 μg) were electrophoresed. Panel A shows blots hybridized with δ-cDNA. Panel B shows blots of the same sample hybridized with α-cDNA.

Fig. 5. Scans of the autoradiographs of Northern blots given in Fig. 4. Migration of RNA was from left to right. A, Scans of blots hybridized with δ-cDNA. Arrow indicates the peak of the mature size δ-mRNA. B, Scans of blots hybridized with α-cDNA. Arrow indicates the peak of the mature size α-mRNA.

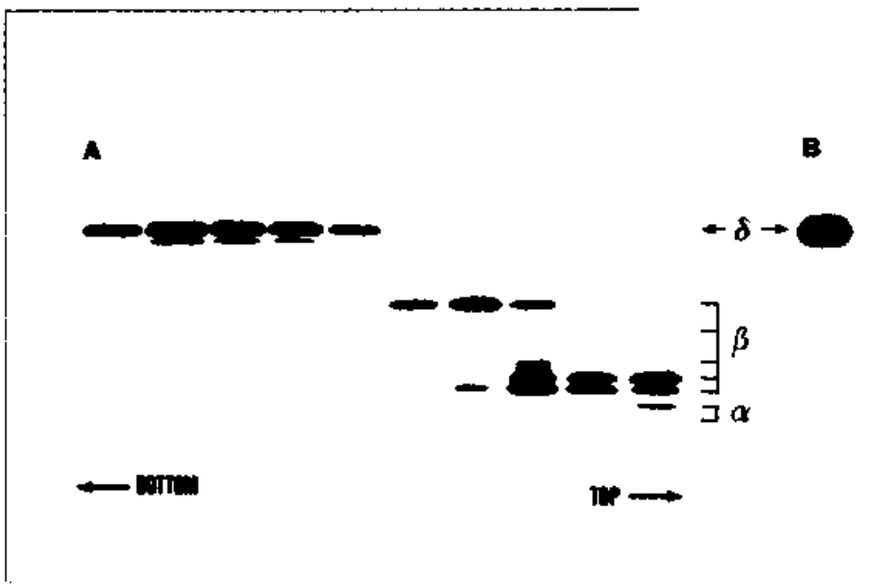

Fig. 1 K. AGATA

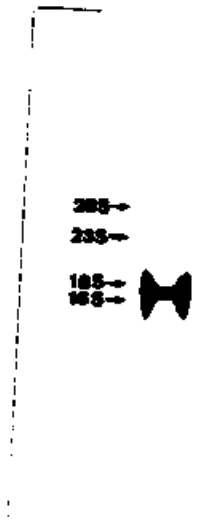

Fig.2 KAGATA

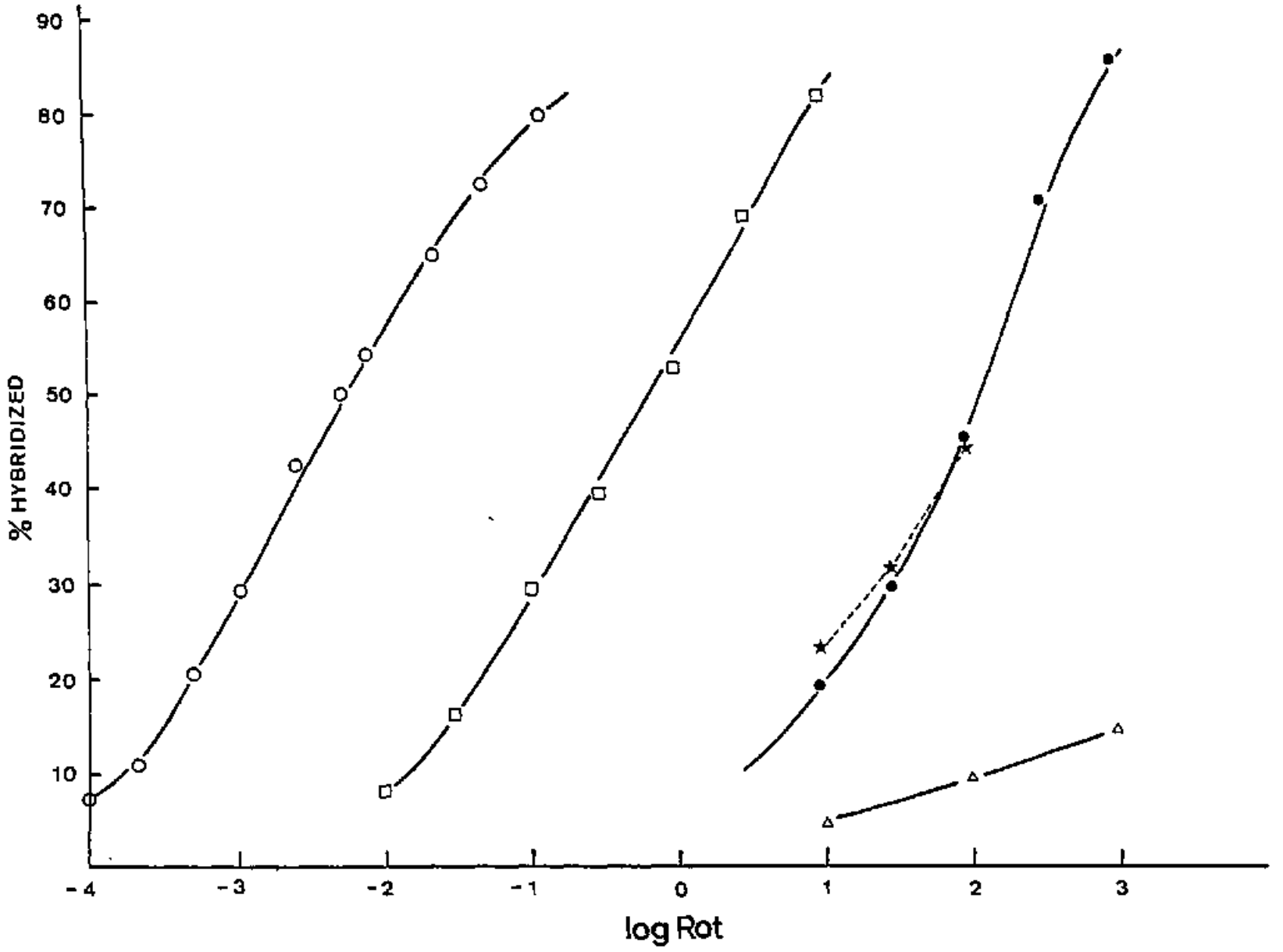

Fig. 3. K. AGATA

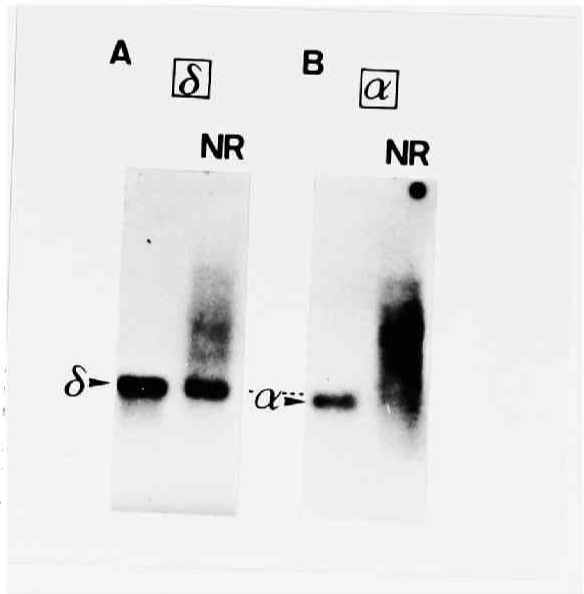

Fig 4. k AGATA

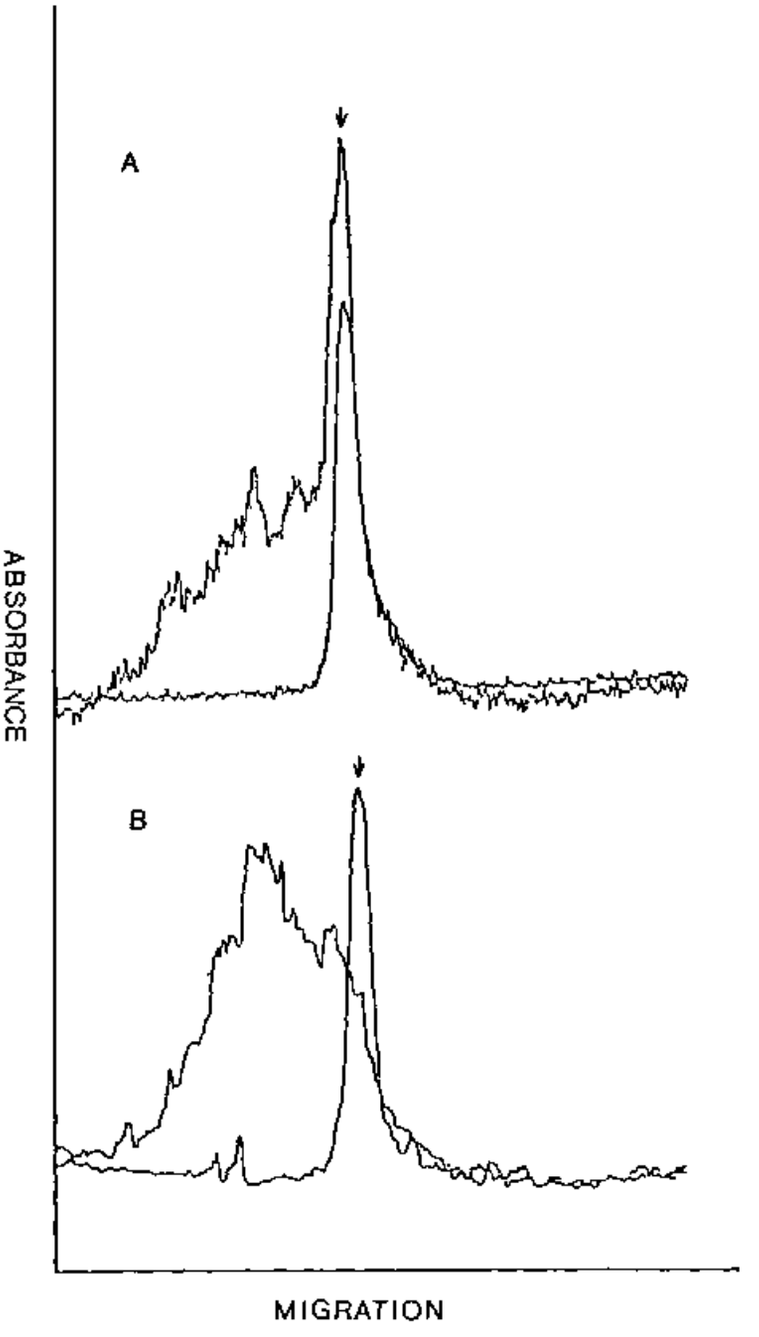

Fig. 5. K. AGATA