

Synthesis and processing of the α heavy chains of secreted and membrane-bound IgA

(murine B lymphoma/hybridoma/two-dimensional gel electrophoresis/ α chain mRNA/*in vitro* translation)

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ABSTRACT We have compared the synthesis and processing of immunoglobulin α chains in two murine cell lines, a B cell lymphoma that expresses membrane-bound IgA and a hybridoma that secretes IgA. Results of biosynthetic labeling experiments demonstrated that membrane-bound and secreted α chains have two distinct intracellular precursors, of different molecular weights and isoelectric points. RNAs from both of these cell lines direct the synthesis *in vitro* of two α polypeptides of M_r 59,000 and 62,000, the larger one being the precursor for membrane-bound α chain and the smaller one being the precursor for secreted α chain. These cell lines each contain three RNAs, 1.7, 2.1, and 3.1 kilobases in length, which hybridize with cDNA for the α constant region and which are present in different concentrations. Our results suggest that the smallest RNA encodes the secreted α chain and one or both of the larger RNAs encode(s) the membrane-bound α chain.

Immunoglobulin (Ig) molecules have two major functions: to serve as antigen receptors on the surface of B lymphocytes and to serve as effector antibodies after secretion from plasma cells. Recently it has been shown that the μ polypeptide chains of membrane-bound IgM have a hydrophobic domain at their COOH terminus that is missing from the μ chains of secreted IgM and that may play a role in the insertion of the μ chains into the lipid bilayer of the cellular membrane (1-5). B lymphocytes expressing IgM contain two different sized μ mRNAs, one of which codes for membrane-bound μ chain and the other of which codes for secreted μ chains (4-6).

To determine whether there are differences between the α polypeptide chains of membrane-bound and secreted IgA, we examined the synthesis and processing of α chains in two murine cell lines: a B lymphoma, BFO.3, expressing membrane-bound IgA, and a hybridoma, ID150, secreting large amounts of IgA. This report describes biosynthetic and *in vitro* translation studies which demonstrate that membrane-bound and secreted IgA molecules contain different α polypeptide chains that are encoded by different α mRNAs.

MATERIALS AND METHODS

Cell Lines. BFO.3, a cloned cell line expressing surface-associated IgA (α , λ), was obtained by culturing I.29 leukemic tumor cells *in vitro* (7). The IgA-secreting hybridoma, ID150, was obtained by hybridization of I.29 cells with P3-NS.1-Ag-8 myeloma cells (8, 9). Both cell lines synthesize IgA and no other class of Ig. These cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, penicillin (100 unit/ml), and streptomycin (100 μ g/ml).

Cell Surface Labeling, Biosynthetic Labeling, and Immunoprecipitation. The cell surface was labeled either by lacto-

peroxidase (10) or with NaB^3H_4 and NaIO_4 (11), using 5 mCi of ^{125}I or 0.5 mCi of NaB^3H_4 , respectively, for 50×10^6 cells (1 Ci = 3.7×10^{10} becquerels).

Cells were biosynthetically labeled by incubation at 37°C in a CO_2 incubator in methionine-free RPMI 1640 medium containing 10% dialyzed fetal calf serum and [^{35}S]methionine (New England Nuclear), or by incubation in glucose-free RPMI 1640 medium containing 10% dialyzed fetal calf serum and ^3H -labeled sugar (New England Nuclear).

Lysis and immunoprecipitation by anti- α antiserum and *Staphylococcus aureus* Cowan I strain were performed according to Siden *et al.* (12), using either rabbit anti-mouse α chain antiserum (Bionetics, Kensington MD) or affinity-purified goat anti-mouse α chain antibody made against IgA from MOPC 460 (gift from P. Kincade). The material immunoprecipitated by both antibodies was identical as analyzed by NaDodSO_4 /gel electrophoresis.

NaDodSO_4 /Polyacrylamide Gel Electrophoresis and Two-Dimensional (2D) Gel Electrophoresis. Immunoprecipitates were analyzed by electrophoresis in 10% polyacrylamide slab gels containing NaDodSO_4 (13) or by a combination of isoelectric focusing and electrophoresis on 2D gels (14). ^{14}C -Labeled proteins (Bethesda Research Laboratories, Rockville MD) were used as molecular weight markers. After electrophoresis, gels were treated with 2,5-diphenyloxazole (15), dried, and exposed to Kodak XR film at -80°C.

Isolation of RNA, RNA Blotting, and *In Vitro* Translation. RNA was isolated from cytoplasmic fractions that were prepared from ID150 and from BFO.3 cells by lysing them in a Dounce homogenizer in 0.5% Nonidet P-40/10 mM Tris-HCl (pH 7.4)/140 mM NaCl/1.5 mM MgCl_2 , followed by centrifugation at $650 \times g$ for 10 min to pellet the nuclei. The cellular homogenates appeared to contain intact nuclei and a small fraction of whole cells by examination in the light microscope. The cytoplasm was adjusted to 10 mM ribonucleoside-vanadyl complexes (16)/10 mM Na_2EDTA (pH 7.0)/0.5% NaDodSO_4 . RNA was extracted with phenol/chloroform. Poly(A) $^+$ RNA was isolated by two successive adsorptions to oligo(dT)-cellulose. To determine the size of the α chain RNAs, RNA was denatured with dimethyl sulfoxide and glyoxal, fractionated by electrophoresis on 1% agarose gels in 10 mM phosphate at pH 7.0 (17), and blotted (18) to diazophenyl-thioether paper, which was prepared according to B. Seed (see footnote 51 in ref. 18). The RNA blots were hybridized as described (19) and then washed in $0.1 \times \text{SSPE}$ ($1 \times \text{SSPE} = 0.15 \text{ M NaCl}/1 \text{ M Na}_2\text{EDTA}/10$

Abbreviations: 2D, two-dimensional; kb, kilobase(s); α_m , membrane-bound α chain; α_s , secreted α chain.

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mM sodium phosphate, pH 6.8) at 52°C. The probe was a nick-translated DNA fragment containing the α constant region sequence, which was isolated from the cDNA plasmid p α (J558)¹³ (20) (given by K. B. Marcu, State University of New York at Stony Brook) by digestion with *Msp* I restriction endonuclease. *In vitro* translation of cytoplasmic RNA was performed in the rabbit reticulocyte system (Bethesda Research Laboratories) (21).

RESULTS

Biosynthetic Labeling of BFO.3 and ID150 Cells. The B lymphoma cell line, BFO.3, expresses membrane-bound IgA but secretes very little IgA (ref. 7; unpublished data). The hybridoma line, ID150, secretes large amounts of IgA (8) but expresses little or no membrane-bound IgA (unpublished data). The α heavy chains synthesized by both cell lines have the same idiotype (I.29 idiotype) (refs. 7 and 8; unpublished data).

To compare the IgA molecules synthesized by BFO.3 and ID150, the cells were incubated for 6 hr in medium containing [³⁵S]methionine and lysed with detergent buffer, and the IgA was immunoprecipitated with anti- α chain antiserum. The biosynthetically labeled IgA molecules were analyzed by 2D gel electrophoresis. As shown in Fig. 1A, lysates of BFO.3 cells contained two spots, a and a', a cluster of small spots, b, and

a more acidic spot, c, of higher molecular weight, all in the position of serum α chain (M_r 61,000–67,000). BFO.3 cells also had one λ chain (ref. 7; unpublished data), migrating with an apparent M_r of 23,000. By contrast, intracellular IgA from the lysate of ID150 cells showed two α spots, which correspond to a and a' of BFO.3, and an additional light chain spot, which migrated with an apparent M_r of 25,000 (Fig. 1B). This light chain is presumably a κ chain contributed by the NS.1 myeloma (8, 22).

IgA was immunoprecipitated from the medium in which the ID150 cells had been labeled and was analyzed on a 2D gel. In addition to the spots observed in the lysate of ID150 cells, a smear of spots extending from a' in the acidic direction and an acidic spot, d, with an apparent M_r of 23,000 were observed (Fig. 1C). Spot d probably corresponds to the J chain, which is associated with secreted IgA and which migrates similarly to the immunoglobulin light chain on NaDodSO₄/polyacrylamide gels (23, 24). It was also detected in the lysates of ID150 cells upon longer exposure of the film (data not shown). Spot d was not detected in BFO.3 cells.

Identification of Membrane-Bound α Chains (α_m) by Cell Surface Labeling. To determine which spots represent α_m , the surface of BFO.3 cells was labeled by radioiodination to label the tyrosine residues (10), and with NaB³H₄ and NaIO₄ to label the sialic acid residues (11) (Fig. 1D and E, respectively). BFO.3 cells labeled by both methods showed only one α chain spot, which corresponded to spot c of internally labeled BFO.3 cells, indicating that the most acidic α chain (spot c) was α_m .

Lysates of biosynthetically labeled ID150 cells did not show any spot that corresponded to α_m (Fig. 1B), and little or no α_m could be detected by radioiodination or by immunofluorescence (unpublished results). Therefore, it appears that spots a and a' may be intracellular precursors for secreted α chain (α_s) and that these α chains become more acidic shortly before or during secretion.

Pulse-Chase Labeling of BFO.3 Cells. BFO.3 cells contain α_m (spot c) and also intracellular precursor(s) for α_s (spots a, a', or both). There are two possibilities for the cluster of small spots, b, found in the lysates of BFO.3 but not of ID150 cells (Fig. 1A and B). (i) Cluster b may represent intracellular precursors for α_m . (ii) Cluster b may represent intermediates in the processing of α_s —i.e., more acidic forms of the same polypeptide chain(s) as in spots a and a'. If the conversion of these putative intermediates to mature α_s were more rapid in ID150 than in BFO.3 cells, it is possible that they might not be detected in lysates of ID150 cells labeled for 6 hr (due to the large size of spots a and a').

To distinguish these possibilities and to analyze the turnover of α chains in BFO.3 cells, pulse-chase experiments were performed. BFO.3 cells were labeled for 10 min with [³⁵S]methionine and chased for various times. As shown in Fig. 2A, BFO.3 cells labeled for 10 min had spots a, a', and a very intense spot(s) b, but did not have spot c. After a 45-min chase, spot c appeared (Fig. 2B). As the duration of the chase increased, spot(s) b became progressively fainter (Fig. 2C–F), almost disappearing by 6 hr (Fig. 2E). Spot(s) b could not be detected after a 24-hr chase (Fig. 2F). Spot c was still quite dark at 6 hr, but very faint after a 24-hr chase. These results suggest that spot(s) b represents an intracellular precursor for α_m (spot c) and that these proteins are rapidly turned over and shed from the membrane. Spots a and a' persisted 24 hr after the chase (although they became less intense), indicating that spots a and a' are not precursors for spots b and c, and they have a very slow turnover in BFO.3 cells.

Sugar Labeling of BFO.3 and ID150 Cells. Our results suggest that there are different intracellular precursors for α_s and α_m and that these α chains increase in size and become more

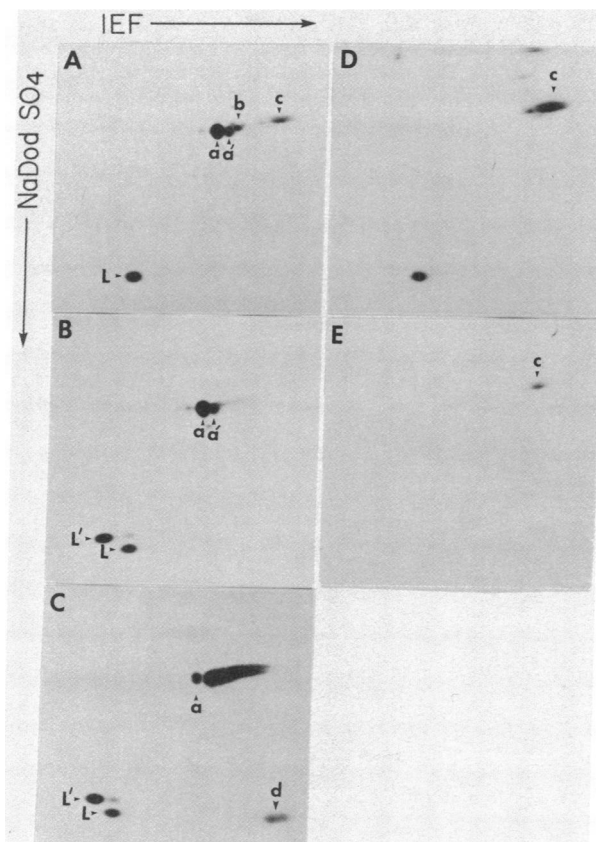


FIG. 1. 2D gel analysis of IgA molecules of BFO.3 and ID150 cells. Lysates or culture supernatants of radiolabeled cells were immunoprecipitated with anti- α chain antiserum. (A) Lysate of BFO.3 cells labeled with [³⁵S]methionine for 6 hr; (B) lysate of ID150 cells labeled with [³⁵S]methionine for 6 hr; (C) culture supernatant of ID150 cells labeled with [³⁵S]methionine for 6 hr; (D) lysate of radioiodinated BFO.3 cells; (E) lysate of BFO.3 cells labeled with NaB³H₄. All gels are shown with the acidic side on the right and decreasing M_r from top to bottom. An apparent M_r of each spot was calculated from the migration of labeled marker proteins that were electrophoresed on the second dimension of the same or a parallel gel. L, light chain; IEF, isoelectric focusing.

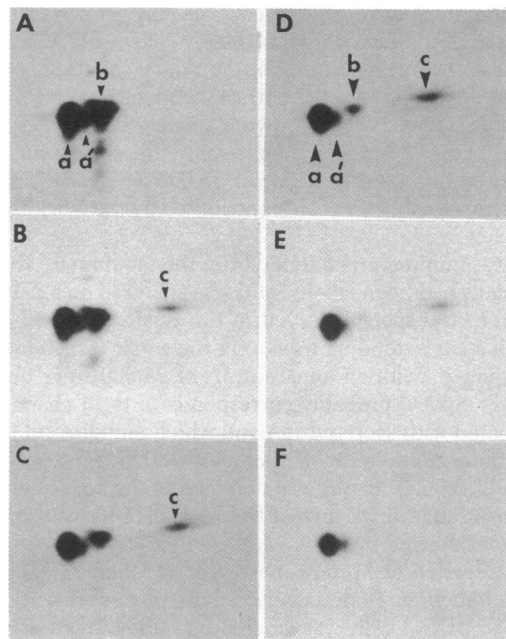


FIG. 2. 2D gel analysis of pulse-chase-labeled α chains. Cells (20×10^6) were labeled for 10 min with [35 S]methionine, washed, and divided into six portions. Each portion was chased for 0 min (A), 45 min (B), 90 min (C), 3 hr (D), 6 hr (E), or 24 hr (F) in nonradioactive medium. After the chase, cells were lysed, and IgA was immunoprecipitated with anti- α chain antiserum.

acidic during their maturation. To determine whether glycosylation is involved in the processing of the α chains, BFO.3 and ID150 cells were labeled for 6 hr with three different sugars, either with [3 H]mannose to label the core sugars or with [3 H]fucose or [3 H]galactose to label the terminal sugars (25). As shown in Fig. 3, it appears that in both ID150 and BFO.3 cells mannose was mostly incorporated into the same spot (a) that was pulse-labeled by methionine. By contrast, fucose and particularly galactose were incorporated to a greater extent into the most acidic spots. Because the patterns obtained by labeling with fucose differed from those obtained by labeling with galactose (galactose being incorporated into the more acidic molecules), the heterogeneity of the pI and molecular weight of the α chains may reflect the kinds and amounts of added sugars.

mRNA for α Chains of BFO.3 and ID150 Cells. Biosynthetic labeling experiments suggest that there are two distinct precursors for α_m and α_s . To provide further evidence for the existence of two different α polypeptides, we attempted to determine whether these cells contained more than one α chain mRNA. Cytoplasmic RNA from BFO.3 and ID150 cells was analyzed by gel electrophoresis, blotting to diazotized paper, and hybridization with a cloned cDNA, p α (J558)¹³, coding for the α constant region (20). Three species of poly(A)⁺ RNAs, 1.7, 2.1, and 3.1 kilobases (kb) in length, were detected in BFO.3 cells (Fig. 4). Poly(A)⁺ RNA from ID150 cells also contained these same species, although the amount of the 1.7-kb RNA was much greater in ID150 than in BFO.3 cells. (In Fig. 4 it appears that the 1.7- and 2.1-kb α RNAs are larger in ID150 than in BFO.3 cells, but this may be due to a gel artifact caused by the large amount of the 1.7-kb RNA in the ID150 lane.)

The 1.7-kb α RNA probably encodes α_s because the relative amounts of the 1.7-kb RNA in ID150 and BFO.3 cells correlate well with the relative abundance of secreted IgA in these two cell lines. Furthermore, the size of α mRNA in myeloma cells is also 1.7 kb (unpublished data). Either or both of the two larger RNAs that hybridize with p α (J558)¹³ probably encode α_m .

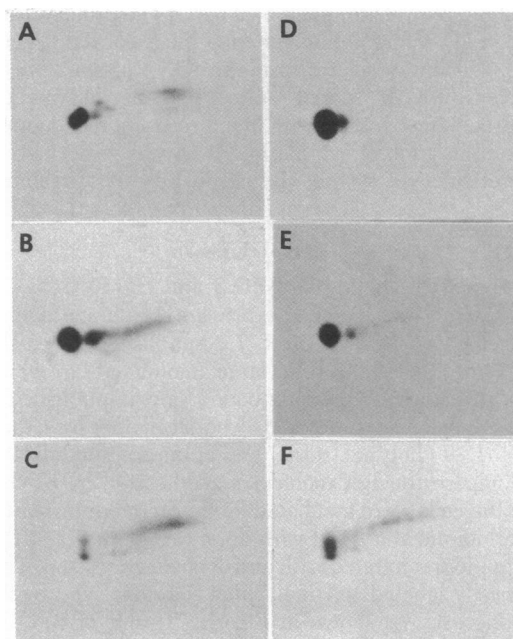


FIG. 3. 2D gel analysis of intracellular α chains of BFO.3 and ID150 cells incubated with 3 H-labeled sugars. Cells were incubated for 6 hr with 3 H-labeled sugars and lysed, and α chains were immunoprecipitated. (A–C) BFO.3 cells labeled with [3 H]mannose (A), [3 H]fucose (B), [3 H]galactose (C). (D–F) ID150 labeled with [3 H]mannose (D), [3 H]fucose (E), or [3 H]galactose (F).

In Vitro Translation of RNA from BFO.3 and ID150 Cells. To determine whether the α RNAs detected by hybridization with p α (J558)¹³ can actually function as mRNAs for α chain,

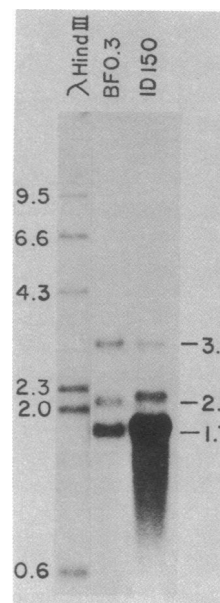


FIG. 4. Ig α chain sequences in cytoplasmic RNA from BFO.3 and ID150 cells. Poly(A)⁺ RNAs from BFO.3 (3 μ g) and from ID150 (2 μ g), and *Hind*III fragments of λ bacteriophage DNA (labeled with 32 P by polynucleotide kinase) were denatured with dimethyl sulfoxide/glyoxal, fractionated by electrophoresis in an agarose gel, and blotted to diazotized paper. The blot was hybridized with a 32 P-labeled α cDNA probe and exposed for 4 hr at -80°C to X-ray film, using an enhancing screen. The sizes in kb of the α RNAs present in BFO.3 cells (indicated on the right) were determined relative to the sizes of *Hind*III fragments of λ DNA, which are indicated on the left.

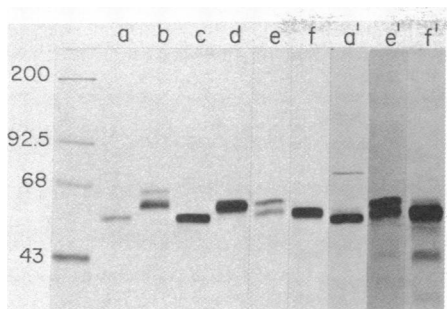


FIG. 5. *In vitro* translation of RNA from BFO.3 and ID150 cells. α chains synthesized *in vitro* or labeled *in vivo* with [35 S]methionine for 6 hr either in the presence of tunicamycin (2.5 μ g/ml) (after a 2-hr preincubation with tunicamycin) or in the absence of tunicamycin were immunoprecipitated and analyzed on a NaDodSO₄/polyacrylamide gel. Tunicamycin inhibited mannose incorporation into IgA by 92%. *In vitro* translation of total cytoplasmic RNA from BFO.3 and from ID150 cells was performed in a rabbit reticulocyte system. Lanes: a, lysate from BFO.3 cells labeled in the presence of tunicamycin; b, lysate from BFO.3 labeled in the absence of tunicamycin; c, lysate from ID150 (with tunicamycin); d, lysate from ID150 (without tunicamycin); e, cell-free translation product of total cytoplasmic RNA from BFO.3 (40 μ g); f, cell-free translation product of total cytoplasmic RNA from ID150 (8 μ g); a', 3-fold longer exposure of a; e', 5-fold longer exposure of e; f', 5-fold longer exposure of f. The leftmost lane shows marker proteins, with $M_r \times 10^{-3}$.

cytoplasmic RNAs from BFO.3 and ID150 cells were translated *in vitro*. Two α polypeptides (M_r 59,000 and 62,000), in approximately equal amounts, were obtained by the *in vitro* translation of RNA from BFO.3 cells (Fig. 5, lane e). By contrast, one α polypeptide chain (M_r 59,000) was the predominant translation product of RNA from ID150 cells (Fig. 5, lane f), although a very faint larger α chain (M_r 62,000) was detected when the gel was exposed to film five times longer (lane f'). Because ID150 cells synthesize predominantly secreted IgA, it appears likely that the M_r 59,000 chain is the polypeptide precursor for α_s , and the M_r 62,000 polypeptide is the precursor for α_m .

The sizes of the α chains synthesized *in vitro* were compared with the sizes of α chains synthesized *in vivo* in the presence and absence of tunicamycin (Fig. 5, lanes a–d, a'). Tunicamycin inhibits N-linked glycosylation (26, 27). The α chains synthesized *in vitro* were smaller than the α chains synthesized *in vivo* in the absence of tunicamycin (BFO.3, lane b; ID150, lane d) but larger than the α chains synthesized in tunicamycin-treated cells (BFO.3, lanes a, a'; ID150, lane c), probably because the α chains synthesized *in vitro* are not glycosylated but do retain the NH₂-terminal signal peptides (28, 29). The largest band in lane b probably corresponds to spot c in Fig. 1A and the smaller one may correspond to spots a, a', and b. The band in lane d probably corresponds to spots a and a' in Fig. 1B.

The amount of α_m synthesized in BFO.3 cells in the presence of tunicamycin appeared to be much less than that obtained by *in vitro* translation. It is possible that this result was observed because α_m turns over more rapidly than does α_s in BFO.3 cells, and during the 6-hr labeling period the pool of [35 S]methionine was exhausted.

DISCUSSION

Two Distinct Precursors for α Chain. Our data demonstrate that two distinct polypeptides serve as precursors for α_m and α_s . Evidence supporting this conclusion was provided by comparing the α chains synthesized in the B lymphoma, BFO.3, which expresses membrane-bound IgA and secretes very little IgA, and the hybridoma, ID150, which secretes large amounts of IgA but bears little or no membrane-bound IgA. These cell

lines were derived from the same leukemic tumor and synthesize IgA of the identical idiotype.

The polypeptides obtained by *in vitro* translation of cytoplasmic RNAs from these two cell lines differed in size. RNA from ID150 cells directed predominantly the synthesis of a M_r 59,000 α polypeptide, whereas translation of RNA from BFO.3 cells yielded a M_r 59,000 α polypeptide but, in addition, approximately equal amounts of a M_r 62,000 α polypeptide. From the relative amounts of the M_r 59,000 and 62,000 α polypeptides obtained, it appears that the M_r 59,000 polypeptide is the precursor for α_s , and the M_r 62,000 polypeptide is the precursor for α_m .

The kinetics of biosynthetic labeling of the α chains in BFO.3 cells also supported the presence of two distinct precursors for α_m and α_s , because the rates of turnover of these precursors were inconsistent with the existence of a single precursor for both α_m and α_s . These results are in agreement with reports by others that μ and γ 2a chains of membrane-bound and secreted IgM and IgG2a, respectively, contain different polypeptide chains (2–6, 30, 31).

Differential Turnover of α Chains *in Vivo*. BFO.3 cells synthesize approximately equal amounts of the two polypeptide precursors for α_m and α_s (Fig. 2A and Fig. 5, lane e), although BFO.3 cells secrete very little IgA into the medium (ref. 7; unpublished data). The results of the pulse-chase experiments showed that the polypeptide precursor for α_s is turned over very slowly in BFO.3 cells (Fig. 2). Furthermore, BFO.3 cells contain cytoplasmic granules or vesicles, which intensely fluoresce when fixed cells are stained with anti- α conjugated to rhodamine (7). These granules or vesicles may sequester the precursor(s) for secreted IgA. A lack of glycosylation is probably not the explanation for why these α chains are not secreted, because tunicamycin does not inhibit the secretion of IgA from ID150 cells (data not shown). The half-life of the α chains in ID150 cells is less than 2 hr and therefore the turnover of α_s is much more rapid in ID150 than in BFO.3 cells (data not shown).

ID150 cells also contain mRNAs that can be translated *in vitro* to yield the polypeptide precursors for α_s and α_m (although they do have much more of the mRNA coding for α_s). However, biosynthetic labeling of ID150 for 6 hr failed to show any spot corresponding to α_m (Fig. 1B). To attempt to detect the synthesis of the precursor for α_m in ID150 cells, the α chains of ID150 cells were labeled by a 5-min pulse with [35 S]methionine and then analyzed on a 2D gel. A cluster of spots resembling the b spot(s) of BFO.3 cells was detected (data not shown), but the fate of these spots could not be followed during the chase period, because α_s were so much more predominant and thus obscured spot(s) b. It seems likely that a small amount of the precursor(s) for α_m is synthesized in ID150 cells, and either it is rapidly turned over or the amount of membrane-bound IgA present on ID150 cells is so small relative to the amount of secreted IgA that it could not be detected.

Processing of the α Polypeptide Chains. Sugar labeling experiments indicated that the heterogeneity of the α chain was correlated with the presence of different kinds and amounts of sugars. Furthermore, when [35 S]methionine-labeled lysates of BFO.3 and of ID150 cells and the culture supernatant of ID150 were treated with sialidase, the acidic spots (c and the smear corresponding to α_s) shifted toward the basic direction (data not shown); when tunicamycin was present during biosynthetic labeling with [35 S]methionine, the acidic spots corresponding to the mature forms of α_s and α_m disappeared (data not shown). These results indicate that glycosylation is involved in the processing of precursor molecules for α_m and α_s . It is unknown why fucose, which is believed to be a terminal sugar, is incorporated into the precursor molecule (spot a) as well as into the more

acidic α chains, although Sidman (32) reported that fucose labeled the intracellular precursor of μ chain similarly to mannose.

Three α RNAs. Membrane-bound and secreted μ chains are encoded by two different mRNAs (2.7 and 2.4 kb), which differ in sequence at their 3' ends (4–6). BFO.3 cells contain approximately equal amounts of three RNAs that hybridize with the α probe, 1.7, 2.1, and 3.1 kb in length, whereas ID150 cells contain relatively much more of the 1.7-kb α RNA but do also contain the two larger RNAs. Only two α polypeptides appeared to be obtained by *in vitro* translation of RNA from BFO.3 cells. It is possible that one of the larger α RNAs is not a functional mRNA or that the two larger α RNAs encode the same size polypeptide chain, or, perhaps, the identical polypeptide chain. It does not appear likely that one of the two larger α RNAs is an incompletely processed nuclear RNA that has leaked into the cytoplasmic fraction, because the amounts of these two larger RNAs are comparable to the amount of 1.7-kb RNA in BFO.3 cells. Furthermore, when α sequences present in total cell or nuclear RNAs from BFO.3 and ID150 cells were examined on RNA blots, the nuclear species larger than 3.1 kb were present in much lower amounts than were any of the three cytoplasmic α RNAs, and the amounts of the three cytoplasmic α RNAs relative to each other were the same as in the cytoplasm (data not shown).

Transcription of the α RNAs. Early *et al.* (33) reported that the two different mRNAs for μ chains were transcribed from the same μ gene and were produced either by differential termination of transcription, or by alternative splicing of a single RNA transcript. It appears likely that the three α RNAs are also transcribed from one α gene, because Southern blots of genomic DNAs from BFO.3 and ID150 cells indicate that these cells contain one rearranged α gene with the identical restriction map in both cell lines (unpublished data). Because the relative amounts of the three α RNAs differ between the lymphoma and hybridoma cells, mechanisms to regulate the termination of transcription or the preferential use of certain splice sites in the α RNA transcript(s) must exist.

Note Added in Proof. The three RNAs detected in BFO.3 and ID150 cells are transcribed from the expressed α gene. This statement is supported by the facts that (i) BFO.3 cells contain two heavy chain chromosomes, one of which contains a rearranged α gene and one of which retains the α gene in the germline configuration, and (ii) each of the three RNAs detected with the α cDNA probe also hybridizes with a genomic DNA probe for the J_{H3} and J_{H4} gene segments. By translation of RNA fractionated on a sucrose gradient, we have determined that the largest α RNA (3.1-kb) codes for α_m (unpublished).

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