SYNTHESIS AND GLYCOSYLATION OF THE MOPC-46B IMMUNOGLOBULIN KAPPA CHAIN IN XENOPUS LAEVIS OOCYTES

Robert L. Jilka, Ralph L. Cavalieri, Lyn Yaffe and Sidney Pestka

Roche Institute of Molecular Biology
Nutley, New Jersey 07110

Received October 19,1977

SUMMARY: Polyadenylated mRNA isolated from MOPC-46B plasmacytoma, which secretes a glycosylated kappa chain, was injected into Xenopus laevis oocytes. Analysis of the resulting product showed that [1-^{14}C]mannose was incorporated into the MOPC-46B kappa chain. Light chains synthesized in oocytes injected with mRNA from MOPC-321 plasmacytoma, which secretes a nonglycosylated kappa chain, failed to incorporate label from [1-^{14}C]-mannose. Thus, protein glycosylation in the oocyte is apparently specific in that carbohydrate is incorporated only into the kappa chain synthesized as a glycoprotein by myeloma cells. It is thus evident that the general signals for glycosylation have remained stable during independent evolution of the amphibia and mammalia.

INTRODUCTION

Newly synthesized proteins are not necessarily functional. Many require secondary cleavages of the primary polypeptide chain or other modifications of a covalent nature such as phosphorylation, acetylation, or glycosylation. Such changes not only may be essential for gene product function, but represent the last steps at which gene expression can be controlled. To study the synthesis and post-translational modification of proteins, Xenopus laevis oocytes have been injected with a variety of mRNAs. These investigations have shown that oocytes contain the enzyme systems for proline hydroxylation (1), serine phosphorylation (2), NH_2-terminal acetylation (3), and viral polypeptide cleavage (4). The ability of Xenopus oocytes to glycosylate a specific protein programmed by exogenous mRNA has not been demonstrated; however, while our studies were underway, it was reported that Xenopus oocytes can incorporate fucose into nonspecific immunoglobulin chains synthesized in response to rat splenic mRNA (5). Accordingly, we

ISSN 0006-291X

examined the ability of Xenopus oocytes to synthesize and glycosylate an immunoglobulin kappa chain following injection of mRNA from MOPC-46B murine plasmacytoma. The MOPC-46B plasmacytoma secretes a light chain (κ^{46B}) which is glycosylated (6). Our results are reported in this communication.

MATERIALS AND METHODS

Isolation of mRNA. Polyadenylated mRNA was isolated by phenol extraction and oligo(dT)-cellulose chromatography from MOPC-46B and MOPC-321 tumors using procedures described earlier (7). The mRNA from MOPC-321 murine plasmacytoma, which secretes a nonglycosylated kappa chain (8), was used as a control.

Translation of mRNA in Xenopus laevis Oocytes. Messenger RNA was injected into Xenopus oocytes using techniques described by Gurdon and associates (9,10). No human chorionic gonadotropin was administered to the frogs prior to removal of oocytes. Fifty nanoliters of an 88 mM NaCl, 15 mM Tris·HCl (pH 7.6) solution containing 1 µg of mRNA/µl were injected into each of 100 oocytes. Groups of 10 oocytes were incubated for 20 hours at 23°C in 0.1 ml of incubation medium consisting of 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.11 mM $CaCl_2$, 7.5 mM Tris·HCl (pH 7.6), penicillin (18 U/ml) and streptomycin (18 µg/ml). For radiolabeling either 100 µCi/ml of a mixture of 15 tritiated amino acids (specific activity range for each amino acid: 1-60 Ci/mmol) or 10 µCi/ml of [1-^{14}C]mannose (53 Ci/mol) were used (New England Nuclear, Boston, Mass.). Glycoprotein biosynthesis in the oocyte was followed with [1-^{14}C]mannose because κ^{46B} contains this sugar in the core portion of its oligosaccharide moiety (11,12). After incubation, the oocytes were homogenized, centrifuged at 3000 x g at 5°C for 10 minutes in an SS-34 rotor of a Sorvall RC2-B centrifuge. The supernatant was removed and stored at -20°C.

Immune Precipitation of Kappa Chain. Antisera to mouse kappa chain was prepared by immunization of rabbits with κ^{46B} urinal protein (13). This antisera showed extensive cross reaction with mouse myeloma kappa chains, including κ^{321}, and was used to precipitate κ^{321} and κ^{46B} made in oocytes. Glutaraldehyde cross-linked IgG fraction of goat antiserum to rabbit IgG was prepared according to Palacios et. al. (14). Labeled oocyte homogenate (20-50 µl) was added to 2 ml of a solution containing 15 mM sodium phosphate (pH 7.0), 0.15 M NaCl, and 2% (v/v) Triton X-100. After adding 1.5 µl of rabbit antiserum to mouse kappa chain, the mixture was incubated at 37°C for 15 minutes. Then, 150 µg of cross-linked goat anti-rabbit IgG was added and incubated with shaking at 23°C for 2 hours. Non-immune rabbit serum was used in control samples. The suspension was then centrifuged at 10,000 rpm in an SS-34 rotor of a Sorvall RC2-B centrifuge. The supernatant was discarded. The pellet was washed by resuspension in 2 ml of H_2O, then resedimented as above. The pellets were resuspended in 60 µl of gel sample buffer consisting of 3% sodium dodecyl sulfate ($NaDodSO_4$), 0.5 M β-mercaptoethanol, 0.01 M Tris·HCl (pH 6.8), and 10% glycerol, heated at 90°C for two minutes, and then centrifuged as above. The supernatant was used for $NaDodSO_4$-polyacrylamide gel electrophoresis.

$NaDodSO_4$-Polyacrylamide Gel Electrophoresis. Slab gel electrophoresis was performed by discontinuous $NaDodSO_4$-polyacrylamide gel electrophoresis

at an acrylamide concentration of 12.5% (7). Gels were prepared for autofluorography according to procedures described by Bonner and Laskey (15).

RESULTS AND DISCUSSION

MOPC-46B and MOPC-321 mRNA injected into Xenopus oocytes programmed the synthesis of κ^{46B} and κ^{321}, respectively. The light chains were identified by NaDodSO$_4$-polyacrylamide slab gel electrophoresis of immunoprecipitates of labeled oocyte homogenates (Fig. 1, slots 4-6 and 11-13). Oocytes injected with κ^{46B} mRNA incorporated [1-^{14}C]mannose into immunoprecipitated κ^{46B} (Fig. 1, slots 8-10) which migrated with amino acid labeled κ^{46B} (Fig. 1, slots 4-6). Light chain synthesized in response to mRNA from MOPC-321 cells failed to incorporate [1-^{14}C]mannose (Fig. 1, slots 14-16). We conclude that the κ^{46B} apoprotein synthesized was specifically glycosylated by oocyte enzymes. Mannose was incorporated into κ^{46B}, but not into κ^{321}. An insignificant amount of label from [1-^{14}C]-mannose was incorporated into endogenous oocyte proteins (Fig. 1, slot 7). This indicates that drift of the label from [1-^{14}C]mannose into amino acids as a result of oocyte metabolism was minimal and does not affect the conclusion that κ^{46B} was specifically glycosylated.

The primary structures of the secreted κ^{46B} (5) and κ^{321} (8) chains, though similar, differ in the region where carbohydrate is linked to κ^{46B}. This linkage occurs at asparagine-28 in the amino acid sequence Asn-Ile-Ser (6). According to the "sequenon" model of glycoprotein biosynthesis (17), the sequence Asn-X-$\frac{\text{Ser}}{\text{Thr}}$ is required for recognition by the enzymes responsible for glycosylation. This is presumably the reason κ^{321}, which does not contain such a sequence, is not glycosylated in MOPC-321 tumor cells. Glycoprotein biosynthesis in oocytes seems to occur in a similar manner, for only κ^{46B} and not κ^{321} incorporated [1-^{14}C]mannose. Thus, lack of κ^{321} glycosylation in oocytes is presumably due to the absence of a proper amino acid sequence. These conclusions extend the observation of Deacon

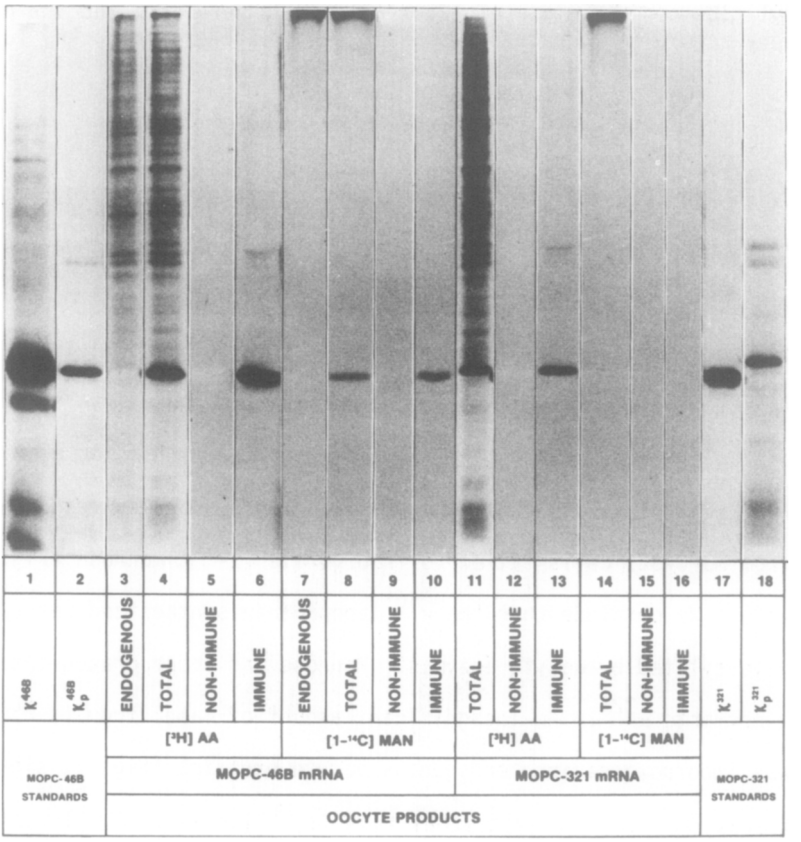

Fig. 1. Autofluorogram of $NaDodSO_4$-polyacrylamide gel electrophoresis of MOPC-46B and MOPC-321 kappa chains synthesized in Xenopus laevis oocytes. Polyadenylated mRNA from MOPC-46B or MOPC-321 tumors was injected into oocytes incubated with [^{3}H]labeled amino acids or [1-^{14}C]mannose as described under "Materials and Methods." Electrophoretic analysis of labeled oocyte homogenates, immunoprecipitated kappa chains, and appropriate standards are shown as follows: 1, MOPC-46B tritiated secreted kappa chain (13); 2, MOPC-46B tritiated precursor kappa chain synthesized in a reticulocyte lysate (16); 3, tritiated endogenous oocyte proteins; 4, tritiated proteins synthesized in oocytes injected with MOPC-46B mRNA; 5, non-immune rabbit serum immunoprecipitate of products in 4; 6, rabbit anti-mouse kappa chain immunoprecipitate of products in 4; 7, [1-^{14}C]mannose labeled oocyte endogenous proteins; 8, [1-^{14}C]mannose labeled proteins synthesized in oocytes injected with MOPC-46B mRNA; 9, non-immune rabbit serum immunoprecipitate of products in 8; 10, rabbit anti-mouse kappa chain immunoprecipitate of products in 8; 11, tritiated proteins synthesized in oocytes injected with MOPC-321 mRNA; 12, non-immune rabbit serum immunoprecipitate of products in 11; 13, rabbit anti-mouse kappa chain immunoprecipitate of products in 11; 14, [1-^{14}C]mannose labeled proteins synthesized in oocytes injected with MOPC-321 mRNA; 15, non-immune rabbit serum immunoprecipitate of products in 14; 16, rabbit anti-mouse kappa chain immunoprecipitate of products in 14; 17, MOPC-321 tritiated secreted kappa chain; 18, MOPC-321 tritiated precursor kappa chain synthesized in a reticulocyte lysate system (16).

and Ebringer (5) that fucose is incorporated into immunoglobulins synthesized in oocytes injected with rat splenic mRNA. Our data support not only carbohydrate addition, but also indicate that oocyte glycosylating enzymes apparently recognize specific amino acid sequences.

Although lack of carbohydrate addition to κ^{321} in the oocyte is best ascribed to the absence of the sequence Asn-X-$\begin{matrix}\text{Ser}\\\text{Thr}\end{matrix}$ in κ^{321}, one might suggest that κ^{321} was not synthesized on membrane-bound polysomes where incorporation of carbohydrate into protein is probably initiated (11,18). However, a preliminary analysis of the NH_2-terminal sequence of κ^{321} synthesized in oocytes yielded a sequence identical to that of the secreted chain (unpublished observations). This suggests that the κ^{321} NH_2-terminal precursor region, observed in cell-free translation systems (Fig. 1, slot 18) (19), was properly cleaved in the oocyte, implying synthesis on the rough endoplasmic reticulum. Others have observed that mRNA programming the synthesis of secreted proteins are similarly translated on membrane-bound polysomes when injected into Xenopus oocytes (20).

Apparently, Xenopus oocytes are capable of many appropriate modifications of proteins synthesized in response to exogenous mRNA. This report demonstrates that only the kappa chain which normally contains carbohydrates is glycosylated in the oocyte. Possibly any protein made in oocytes programmed by exogenous mRNA will be glycosylated provided it is normally made as a glycoprotein. Specific immunoglobulins synthesized and assembled in oocytes in response to injected splenic mRNA from immunized rats (21) are also probably glycosylated considering that heavy chain glycosylation occurs in oocytes injected with nonspecific rat splenic mRNA (5). Similarly, interferon is probably glycosylated during biosynthesis in Xenopus oocytes following injection of mRNA from induced human fibroblasts or leukocytes (22). Further studies will be required to determine whether the structure of the oligosaccharide attached to proteins made in the oocyte subsequent to mRNA injection is identical to the carbohydrate normally

found on these proteins. Nevertheless, it is apparent that Xenopus oocytes perform modifications similar or identical to those occurring in the distant cells from which the mRNAs were derived. It is thus evident that the general signals for these modifications have remained stable throughout most of vertebrate evolution during the last 400,000,000 years when amphibia and mammalia evolved.

ACKNOWLEDGEMENTS: We thank Dr. Philip Tucker and Mr. David Weiss for preparation of the mRNAs, Dr. Hans Hager for goat antiserum to rabbit IgG, Mr. Phillip Familletti for technical assistance, and Miss Susan Emmer for assistance in the preparation of the manuscript.

REFERENCES

1. Lane, C. D., and Knowland, J. S. (1975) in Biochemistry of Animal Development (Weber, R., ed.) Vol. 3, pp. 145-181, Academic Press, New York.
2. Gurdon, J. B. (1974) in The Control of Gene Expression in Animal Development, p. 41, Harvard University Press, Cambridge, Mass.
3. Berns, A. J. M., van Kraaikamp, M., Bloemendal, H., and Lane, C. D. (1972) Proc. Natl. Acad. Sci. U.S.A. 69, 1606-1609.
4. Laskey, R. A., Gurdon, J. B., and Crawford, L. V. (1972) Proc. Natl. Acad. Sci. U.S.A. 69, 3665-3669.
5. Deacon, N. J., and Ebringer, A. (1977) FEBS Letters 79, 191-194.
6. Melchers, F. (1969) Biochem. 8, 938-947.
7. Green, M., Zehavi-Willner, T., Graves, P. N., McInnes, J., and Pestka, S. (1976) Arch. Biochem. Biophys. 172, 74-89.
8. McKean, D., Potter, M., and Hood, L. (1973) Biochem. 12, 749-759.
9. Moar, V. A., Gurdon, J. B., Lane, C. D., and Marbaix, G. (1971) J. Mol. Biol. 61, 93-103.
10. Gurdon, J. B., Lane, C. D., Woodland, H. R., and Marbaix, G. (1971) Nature 233, 177-182.
11. Hsu, A.-F., Baynes, J. W., and Heath, E. C. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 2391-2395.
12. Parkhouse, R. M. E. and Melchers, F. (1971) Biochem. J. 125, 235-240.
13. Tucker, P., and Pestka, S. (1977) J. Biol. Chem. 252, 4474-4486.
14. Palacios, R., Palmiter, R. D., and Schimke, R. T. (1972) J. Biol. Chem. 247, 2316-2321.
15. Bonner, W. M., and Laskey, R. A. (1974) Eur. J. Biochem. 46, 83-88.
16. Pelham, H. R. B., and Jackson, R. J. (1976) Eur. J. Biochem. 67, 247-256.
17. Marshall, R. D. (1974) Biochem. Soc. Symp. 40, 17-26.
18. Toneguzzo, F., and Ghosh, H. P. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 1516-1520.
19. Schechter, I., and Burstein, Y. (1976) Biochem. J. 153, 543-550.
20. Zehavi-Willner, T., and Lane, C. (1977) Cell, 11, 683-693.
21. Deacon, N. J. and Ebringer, A. (1976) Biochem. Soc. Trans. 4, 818-820.
22. Cavalieri, R. L., Havell, E. A. Vilcek, J., and Pestka, S. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 3287-3291.