ond theory is that, in the presence of BrdU, some DMs may not replicate their DNA at all or replicate only once during the two cell cycles. For harlequin chromatin staining to appear, BrdU must be incorporated in the DNA of DMs for two consecutive cycles. Consequently, this research indicates for the first time that the DM-DNA may have two modes of replication. Usually, it may replicate once during S phase of the cell cycle concurrently with chromosomal DNA. Sometimes, however, it might escape replication during S phase, while chromosomal DNA does replicate. The differential DNA replication patterns of DMs might produce uneven numbers of DMs in subsequent cell generations and might cause numerical heterogeneity of DMs in a cell population undergoing anomalous mitotic segregation.

1 This work was supported by a grant from the Whitehall Foundation, Inc. awarded to A.B.M.
2 Spriggs, A.I., Boddington, M.M., and Clarke, C.M., Br. Med. J. *2* (1962) 1431.
3 Cowell, J.K., A. Rev. Genet. *16* (1982) 21.
4 Mukherjee, A.B., and Krawczun, M.S., Cancer Genet. Cytogenet. *10* (1983) 11.
5 Barker, P.E., and Hsu, T.C., J. natl Cancer Inst. *62* (1979) 257.
6 Cox, D., Yuncken, C., and Spriggs, A.I., Lancet *2* (1965) 55.
7 Levan, A., Levan, G., and Mitelman, F., Hereditas *86* (1977) 15.
8 Barker, P.E., Drwinga, H.L., Hittelman, W.N., and Maddox, A., Exp. Cell Res. *130* (1980) 353.
9 Igarashi, A., J. gen. Virol. *40* (1978) 531.
10 Herrera, R.J., Ph. D. Thesis, Fordham University, New York, N.Y., 1982.

0014-4754/85/010085-02$1.50 + 0.20/0

Glucagon and pancreatic polypeptide immunoreactivities co-exist in a population of rat islet cells

Hue-lee Cheng Kaung

Department of Anatomy, 4–135 Jackson Hall, University of Minnesota, School of Medicine, Minneapolis (Minnesota 55455, USA),
18 January 1984

Summary. In mammalian pancreas, glucagon and pancreatic polypeptide have been shown to be present in distinct cell types. The present communication reports that, in rat pancreas, in addition to glucagon and pancreatic polypeptide cell populations, there is a small population of cells which contain both glucagon and pancreatic polypeptide immunoreactivities.
Key words. Co-existence of glucagon; pancreatic polypeptide.

Immunocytochemical studies have shown that, in mammals and many other vertebrates, the four major pancreatic hormones, insulin, glucagon, somatostatin and pancreatic polypeptide, are located in four distinct cell types[1–3]. We reported earlier that, in frog pancreas, glucagon and pancreatic polypeptide overlap in distribution, i.e. a majority of glucagon containing cells also contain pancreatic polypeptide[4,5]. The present communication reports that in one mammalian species, rat, there is also a small degree of overlap in cellular distribution of glucagon and pancreatic polypeptide. In the rat pancreas, in addition to glucagon containing cells and pancreatic polypeptide containing cells, there is another population of cells which contain both glucagon and pancreatic polypeptide immunoreactivities. This observation suggests that glucagon and pancreatic polypeptide may be phylogenetically and biochemically closely related.

Materials and methods. Pancreases from five normal Sprague-Dawley adult rats were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer and embedded in Epon 812. Plastic sections of 1–1.5 μm were collected on glass slides. Before immunohistochemical staining, the plastic sections were deplasticized with alcoholic sodium hydroxide solution according to Lane and Europa[6]. The nature of the hormonal content of the pancreatic islet cells was demonstrated by staining the deplasticized section of pancreas with rabbit antiserum to porcine glucagon[7] or rabbit anti-serum to bovine pancreatic polypeptide[8] by the preroxidase anti-peroxidase (PAP) technique of Sternberger[9]. The anti-glucagon serum was used at 1:2000 dilution and the anti-pancreatic polypeptide serum was used at 1:5000 dilution. Specificity of each antiserum was demonstrated by staining adjacent sections with the antiserum absorbed with homologous or heterologous antigen (100 μg/ml diluted antiserum) prior to staining.

The distribution of glucagon and pancreatic polypeptide immunoreactivities in pancreas were studied by the following two experiments: (1) Two adjacent plastic sections were deplasticized and stained for glucagon and pancreatic polypeptide, respectively. The staining patterns of islets of these two adjacent sections were photographed and compared. (2) A plastic section was first stained with glucagon antiserum. The positively stained cells were visualized using 4-chloro-1-naphthol as the reducing agent in the PAP method. The staining pattern of islets was photographed. The same section was then treated with alcohol and $KMnO_4/H_2SO_4$ solution according to the method of Tramu et al.[10] to remove the reaction product of 4-chloro-1-naphthol and tissue-bound antibodies. The section was then restained with a second antibody, anti-bovine pancreatic polypeptide by the PAP method. The stained islets were again photographed. The staining patterns of the same islet in the two staining sequences were compared. To test the effectiveness of the antibody removal procedure in this experiment, a control section was run simultaneously with the experimental section. The control section was stained for glucagon identically as for the experimental section during the first staining sequence. After being subjected to procedure for removal of the bound antibodies, this section was stained with normal rabbit serum instead of the anti-pancreatic polypeptide serum in the second staining sequence. The control section was negatively stained after the second staining sequence of PAP procedure, indicating complete removal of bound antibodies of first staining sequence.

Results and discussion. Comparison of adjacent sections, respectively stained with anti-glucagon and anti-pancreatic polypeptide sera, and sequential staining of a same section with the two antisera showed that a small number of islet cells are immunoreactive to both antisera in addition to sole glucagon immunoreactive cells and pancreatic polypeptide immunoreactive cells.

Figure 1 shows part of an islet in two adjacent sections stained respectively with antiglucagon (fig. 1A) and antipancreatic

polypeptide (fig. 1B) sera. In addition to distinct glucagon immunoreactive cells, a cell which contains both glucagon and pancreatic polypeptide immunoreactivities is seen in this islet (arrows). The thickness of the sections (approximately 1 μ) indicates the unlikeliness of such an observation of overlap being due to the presence of two separate cells in these two adjacent sections.

Figure 2 shows another islet, first stained with antiserum to glucagon (fig. 2A) and then stained again with antiserum to bovine pancreatic polypeptide after removal of reaction product and bound antibodies (fig. 2B). Again, it is demonstrated that, in addition to cell populations that react to glucagon antiserum and bovine pancreatic polypeptide antiserum, respectively, there is a population of cells which react to both antisera (arrows in fig. 2). The complete removal of the bound first antibody was demonstrated by the absence of staining in all previously stained islets when normal rabbit serum was used in place of second primary antibody in the second staining sequence. Thus, the positive stain observed in figure 2B is due to the presence of antigen which bound to anti-bovine pancreatic polypeptide serum.

That the co-localization of glucagon and pancreatic polypeptide in certain islet cells is not due to the cross-reactivity of the antisera and antigens involved is demonstrated in the fact that glucagon and pancreatic polypeptide immunoreactivities are not co-localized in most islet cells. Further, specificity control experiments showed that each of the two antisera abolished its stainability when absorbed with its respective antigen, but did not diminish its staining reactivity when absorbed with heterologous antigen.

In the double staining experiment, the glucagon immunoreactivity appeared more uniformly distributed in the cells (2A), whereas subsequent staining of the same cells with anti-pancreatic polypeptide serum resulted in discrete patches of positive stain (2B). This does not reflect true difference in the distribution of these two immunoreactivities. Rather, it represents artifact in the antipancreatic polypeptide serum stained cells caused by antibody removal procedure. Cells in a section will show patchy staining pattern with either glucagon or pancreatic polypeptide antiserum if subjected to antibody removal treatment prior to staining. It is not clear how the antibody removal procedure affected the staining pattern.

Examination on sections taken from head, body, and tail of pancreas showed that the glucagon-pancreatic polypeptide cells were present in islets of all portions of the pancreas. However, not all islets contain these dual hormone containing cells. Preliminary quantitation in 40 randomly selected islets from sections of five animals showed that of all the cells that contain

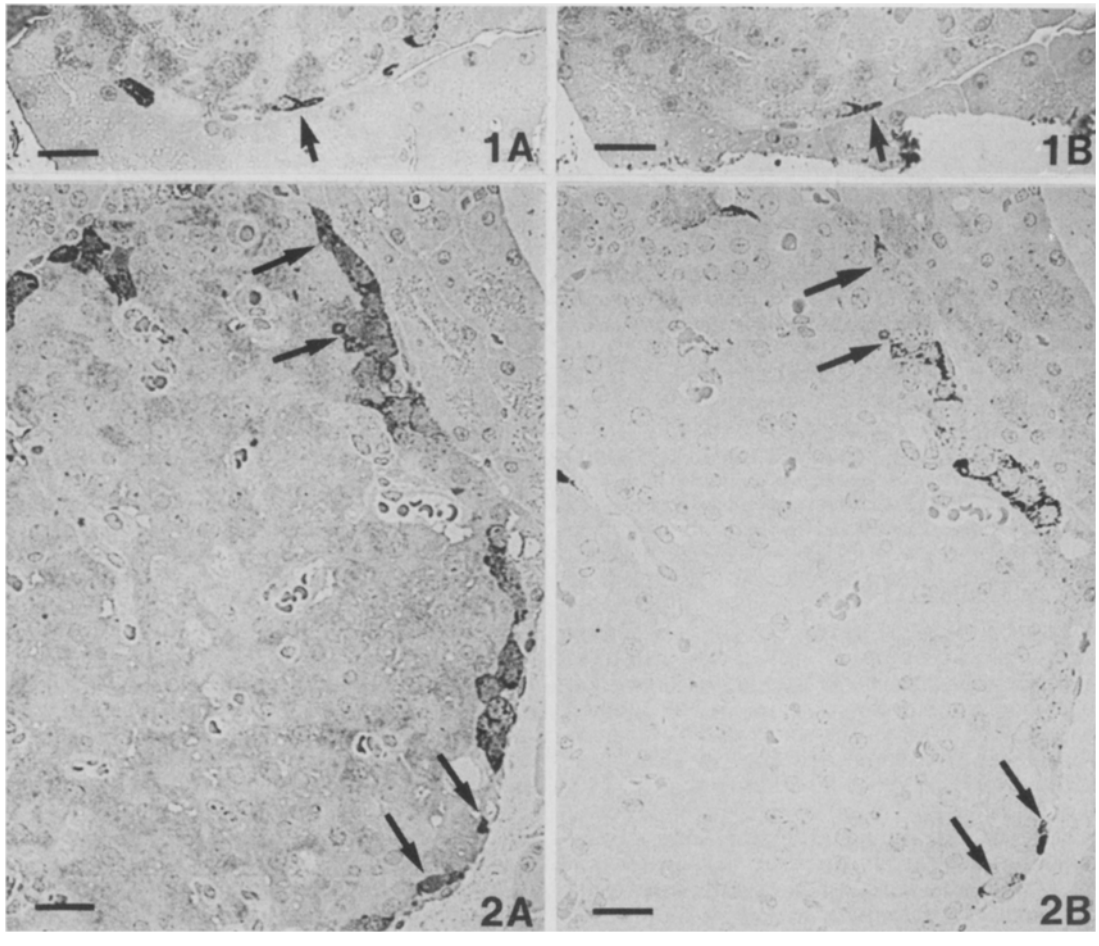

Figure 1. Two adjacent plastic sections of the same islet stained with antiglucagon serum (1A) and anti-bovine pancreatic polypeptide serum (1B). Arrows point to the same cell which is stained in both sections. Both sections were stained by PAP method using DAB as chromogen and counter-stained with hematoxylin. Bar = 20 μm.

Figure 2. A plastic section was first stained with antiglucagon serum (2A) and then decolorized, bound antibodies eluted and then stained with anti-bovine pancreatic polypeptide serum (2B). Arrows point to the cells that are immunostained by both glucagon and bovine pancreatic polypeptide antisera. Figures 2A and 2B were both stained by PAP method using 4-chloro-1-naphthol as chromogen. Bar = 20 μm.

glucagon and/or pancreatic polypeptide immunoreactivities, approximately 75% of the cells contain only glucagon immunoreactivity, 15% contain only pancreatic polypeptide immunoreactivity, whereas 10% of the cells contain both immunoreactivities. The detailed distribution of these three cell types in islets of different regions of the pancreas remains to be studied. Presence of multiple peptides in one cell type is not uncommon in nervous and endocrine systems[11], including the A cells in endocrine pancreas[12–14]. In some instances, such presence of multiple peptides can be attributed to the presence of a large precursor hormone[15,16]. However, presence of separate synthetic machineries in the same cells is also possible[17]. The possibility that the present observation represents the identification of a new peptide which contains the immunodeterminants of both glucagon and pancreatic polypeptide also exists. Relevant to the present observation are the reports of presence of bovine pancreatic polypeptide, glucagon and glicentin immunoreactivities in human colorectal mucosa[18] and in cat intestine[19]. Glicentin is known to contain the immunodeterminants of glucagon[20,21]. But, the relationship of glicentin to bovine pancreatic polypeptide is unknown.

Nevertheless, the presence of glucagon and pancreatic polypeptide immunoreactivities in the same cells in frog[4,5] and, to a lesser extent, in rat suggests a close relationship of the two peptides. It is tempting to speculate that, perhaps at some point in vertebrate evolution, these two peptides were handled simultaneously in their production, and that these glucagon-pancreatic polypeptide cells in rat represent a primitive cell type which handles glucagon and pancreatic polypeptide production in a similar manner as frog islet cells do.

In addition to immunohistochemical studies in other vertebrates, future biochemical and physiological studies are necessary to understand the exact nature of the relationship between glucagon and pancreatic polypeptide and the significance of the glucagon and pancreatic polypeptide containing cells in rat islets.

1 Erlandsen, S.L., in: International Academy of Pathology Monograph, The Pancreas, p. 140. Eds P.J. Fitzgerald and A.B. Morrison. Williams and Wilkins, Baltimore 1980.
2 Larson, L.-I., Sundler, F., and Hakanson, R., Diabetologia *12* (1976) 211.
3 Orci, L., Diabetes *31* (1982) 538.
4 Kaung, H.C., Anat. Rec. *193* (1979) 584.
5 Kaung, H.C., and Elde, R., Anat. Rec. *196* (1980) 173.
6 Lane, B.P., and Europa, D.L., J. Histochem. Cytochem. *13* (1965) 579.
7 Gift from Dr R. McEvoy, Mound Sinai School of Medicine, NY.
8 Gift from Dr R. Chance, Eli Lilly Corp., Indianapolis, IN.
9 Sternberger, L.A., Immunocytochemistry, 2nd edn., p. 126. John Wiley and Sons, New York 1979.
10 Tramu, G., Pillez, A., and Leonardelli, J., J. Histochem. Cytochem. *26* (1978) 322.
11 Larsson, L.-I., Invest. Cell Path. *3* (1980) 73.
12 Smith, P.H., Merchant, F.W., Johnson, D.G., Fujimoto, W.Y., and Williams, R.H., Am.J. Anat. *149* (1977) 585.
13 Grube, D., Maier, V., Raptis, S., and Schlegel, W., Histochemistry *56* (1976) 13.
14 Grube, D., Voigt, K.H., and Weber, E., Histochemistry *59* (1978) 75.
15 Eipper, B.A., and Mains, R.E., J. Supramolec. Struct. *8* (1978) 247.
16 Crine, P., Gianoulakis, C., Seidah, N.G., Gossard, F., Pezalla, P.D., Lis, M., and Chretien, M., Proc. natl. Acad. Sci. USA *75* (1978) 719.
17 Watson, S.J., Akil, H., Fischli, W., Goldstein, A., Zimmerman, E., Nilaver, G., and van Wimersma Greidanus, T.B., Science *216* (1982) 85.
18 Fiocca, R., Capella, C., Buffa, R., Fontana, P., Solcia, E., Hage, E., Chance, R.E., and Moody, A.J., Am. J. Pathol. *100* (1980) 81.
19 Ravazzola, M., and Orci, L., Histochemistry *67* (1980) 221.
20 Moody, A.J., Jacobson, H., and Sundby, F., in: Gut Hormones, p. 369. Ed. S.R. Bloom. Churchill Livingstone, Edinburgh 1978.
21 Holst, J.J., Biochem. J. *187* (1980) 337.

0014-4754/85/010086-03$1.50 + 0.20/0

Loss of antibody production accompanied by chromosome loss in a cloned hybrid line secreting antibodies to sheep red blood cells

J.S. Nowak

Institute of Human Genetics Polish Academy of Sciences, PL-60-781 Poznań (Poland), 3 January 1984

Summary. Somatic cell hybrids between Sp2/O-Ag14 mouse myeloma cells and lymphocytes derived from BALB/c mice hyperimmunized with sheep red blood cells (SRBC) were produced. One hybrid producing IgG_1 antibody to SRBC was selected, cloned twice and subsequently transferred to BALB/c mice. After a number of transfers it was found that the antibody titer in ascites fluid gradually decreased. Cytogenetic analysis revealed gradual chromosome loss in the hybrid clone, which produced progressively less antibody.

Key words. Myeloma cells, mouse; lymphocytes, mouse; cloned hybrid cell line; somatic cell hybrids; antibody production; chromosome loss.

The somatic cell hybridization technique developed by Kohler and Milstein[1,2] has made it possible to generate hybrid cell lines synthesizing monospecific antibodies. However, the production of antibody is often lost when the hybrids are grown in mass culture. This could probably be due to a number of factors. In the work described in this report it was found that the loss of specific antibody production is accompanied by, and is most likely due to chromosome loss.

Materials and methods. BALB/c mice were injected twice with 2×10^7 SRBC i.p. at two-week-intervals and boosted after three weeks with the same dose. Spleens were taken three days later. 4×10^6 pooled spleen cells from three mice were fused with 2×10^6 Sp2/O-Ag14 myeloma cells using polyethylene glycol 1500 (Merck), essentially as described previously by Galfre et al.[3]. Specific antibodies to SRBC were detected using a standard hemagglutination assay. Cloning and recloning of hybrid cultures was performed under limiting dilution conditions[4]. The karyotype of ascites fluid cells was analyzed according to the method of Ford[5].

Results and discussion. Spleen cells from BALB/c mice hyperimmunized with SRBC were fused with Sp2/O-Ag14 myeloma cells and plated in two 96-well tissue culture clusters at 0.2 ml