Androgen Regulated Expression of the α_{2u}-Globulin Gene in Pancreatic Hepatocytes of Rat

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Abstract. Under a copper-deficient regimen, pancreatic cells in the adult rat can be found to undergo differentiation into hepatocytes. Pancreatic hepatocytes induced in male and female rats were examined for the expression of the androgen-inducible hepatic protein, α_{2u}-globulin. α_{2u}-Globulin protein was demonstrable by immunoperoxidase method in all the pancreatic hepatocytes of male rats. Northern blot analysis confirmed the presence of 1.3 kb α_{2u}-globulin mRNA transcript in the pancreas of male rats with hepatocytes. Orchiectomy resulted in marked decrease of α_{2u}-globulin protein and its mRNA. Administration of dihydrotestosterone to castrated rats resulted in increased levels of α_{2u}-globulin mRNA and the amount of α_{2u}-globulin protein in the pancreatic hepatocytes. Unlike normal males, in intact and ovariectomized females α_{2u}-globulin was not detectable in pancreatic hepatocytes. These results indicate that similar to hepatic parenchymal cells pancreatic hepatocytes synthesize α_{2u}-globulin under androgenic regulation. Furthermore, unlike in liver where it is expressed predominantly in perivenular and midlobular hepatocytes, there is no localized difference in the expression of this gene in the transdifferentiated pancreatic hepatocytes.

ALTHOUGH all the parenchymal cells of the mammalian liver are derived from a single diverticulum of the foregut entoderm, they exhibit morphological and functional heterogeneity (6, 32). Some proteins are synthesized by all the hepatocytes present in different portions of the lobule, while other proteins are predominantly or exclusively synthesized by periportal or perivenular cells (9, 10, 19, 21). Although the exact reason for such heterogeneity is presently unclear, roles of oxygen tension, substrate concentration, endocrine and paracrine factors have been implicated (2, 7, 31).

Hepatic α_{2u}-globulin is a low molecular weight lipid carrying protein which belongs to a superfamily of ligand binding proteins (17). It is the principal urinary protein of the mature male rat; the urinary source of α_{2u}-globulin is the liver. The hepatic synthesis of α_{2u}-globulin is regulated by several hormones including androgen, growth hormone, and insulin (1, 11, 29, 30). α_{2u}-Globulin synthesis in the liver of male rats appears at ~35 d, reaching adult level by 65 d and maintained at maximum levels until old age (1, 14, 30). α_{2u}-Globulin appears to be synthesized preferentially in the perivenular cells under normal physiological conditions (1, 31). Administration of androgens leads to increased numbers of hepatocytes synthesizing α_{2u}-globulin and increased content of this protein in perivenular and midlobular cells (1).

Development of hepatocytes in the pancreas of adult rats and hamsters has been described recently by several investigators. The experimental conditions that lead to the development of pancreatic hepatocytes include administration of carcinogens and feeding diets deficient in copper and methionine (8, 15, 22, 27, 28, 33). In addition, spontaneous development of hepatocytes in pancreas of old rats was also described (4).

In the present study we have examined the expression of α_{2u}-globulin in pancreatic hepatocytes differentiating in male and female rats following copper deficiency induced pancreatic atrophy (20, 26, 35). Multiple islands of liver cells that develop in the adult pancreas within several weeks after switching the copper-deficient rats to normal diet exhibit many liver specific functions. It is of great interest to determine if these liver specific functions in the transdifferentiated cells are regulated in the same fashion as those in the normal hepatocytes. Results presented in this article show that the transdifferentiated hepatocytes not only express the α_{2u}-globulin gene, the synthesis of α_{2u}-globulin mRNA is also regulated by the androgen.

Materials and Methods

Induction of Pancreatic Hepatocytes

F344 Rats weighing 80–90 g were obtained from Charles River Breeding Laboratories (Wilmington, MA). 12 normal and 6 castrated male and 3 normal and 3 ovariectomized female rats were used to induce pancreatic hepato-

cytes as described elsewhere (26). Orchiectomy and ovariectomy were performed 1 wk before the start of the experiment. Briefly, rats were fed a copper deficient diet (United States Biochemical Corporation, Cleveland, OH) supplemented with 0.6% trien (Aldrich Chemical Co., Milwaukee, WI) (designated CuDT diet) for 8–9 wk. After 8 or 9 wk on CuDT diet rats were fed normal rat chow (Purina, St. Louis, MO). Rats were killed under light ether anesthesia between 15 and 20 wk after transfer to normal diet. Three castrated males, containing hepatocytes in their pancreas, were given α-dihydrotestosterone (Sigma Chemical Co., St. Louis, MO) subcutaneously as an emulsion daily for 8 d at a dose of 30 mg/kg body weight and killed 1 d after the last injection (31).

Tissue Preparation and Immunoperoxidase Staining

Portions of pancreas were fixed in 10% neutral buffered formalin for 24 h and processed for light microscopy. Paraffin sections (4-μm-thick) were stained with rabbit anti-rat α_{2u}-globulin (IgG 10 μg/ml) using avidin-biotin peroxidase complex as described (17). Peroxidase activity was developed using diaminobenzidine as substrate and counterstained with hematoxylin. Specificity of the staining was confirmed by using appropriate controls. In addition, adjacent sections were routinely stained with hematoxylin and eosin for routine histological evaluation.

Northern and Dot Blot Hybridization

Total RNA from pancreas and liver was extracted as described before (28) according to the procedure outlined by Chirgwin et al. (3). The RNA was analyzed by Northern and dot blot hybridization (18) using nick translated ^{32}P-labeled α_{2u}-globulin cDNA (13) or albumin cDNA (34). The relative concentration of specific mRNAs was measured by densitometric scanning of the autoradiographs.

Results

Histological examination of pancreas of both male and female rats maintained first on CuDT for 8–9 wk, and then on normal diet for 15–20 wk, showed fatty infiltration and randomly distributed multiple foci of hepatocytes (Fig. 1 *A*). Orchiectomy and ovariectomy did not significantly affect the development of hepatocytes in the pancreas. The hepatocyte foci were of variable sizes containing a few to as many as 50–100 cells. The pancreatic changes in both males and females were comparable.

α_{2u}-Globulin Immunoperoxidase Stain

The cytoplasm of pancreatic hepatocytes in normal males showed uniform staining for α_{2u}-globulin in all the hepatocyte foci (Fig. 1, *B* and *C*). No appreciable variation in staining intensity was observed between the cells situated at the periphery and center of these hepatic foci. The intensity of staining in pancreatic hepatocytes was equal to or slightly greater than that observed in the centrilobular cells of liver (Fig. 1, *C* and *D*). In castrated males the pancreatic hepatocytes were generally negative for α_{2u}-globulin (Fig. 2 *A*). An occasional cell showed weak positive staining. Administration of dihydrotestosterone to orchiectomized rats resulted in restoration of positive staining in pancreatic hepatocytes (Fig. 2 *B*). The intensity of staining in the hepatocytes of these animals is comparable to that observed in intact animals. However, some difference in the staining between the individual hepatocytes is noted. Pancreatic hepatocytes in both the ovariectomized and intact females were uniformly negative for this protein (not illustrated). All the other constituent cells of pancreas (i.e., acinar, ductal, and islet cells) were uniformly negative for α_{2u}-globulin. The staining pattern in the livers of rats in different groups was similar to that reported in the literature (1, 31).

Northern and Dot Blot Analysis

Specific mRNA levels were measured in the pancreatic hepatocytes of normal males, orchiectomized males, and rats given dihydrotestosterone after orchiectomy by hybridizing total cellular RNA with α_{2u}-globulin and albumin cDNA probes. Albumin mRNA signals were comparable in the pancreas of all the rats containing hepatocytes (Fig. 3 *A*). α_{2u}-Globulin mRNA levels in the pancreas of intact males was high, which decreased markedly in orchiectomized rats and increased after administration of dihydrotestosterone to orchiectomized rat (Fig. 3 *B*). Dot blot analysis showed that the levels of α_{2u}-globulin mRNA in the intact and testosterone administered males were comparable, whereas in orchiectomized rat it was ~4.4-fold lower. Since the amount of hepatocyte specific total RNA may vary depending upon the relative abundance of pancreatic hepatocytes in the pancreas, we calculated the albumin–α_{2u}-globulin ratios as a relative indicator of change. The relative ratios of albumin and α_{2u}-globulin mRNA in the liver and the pancreas containing hepatocytes was 1:0.87 (range 0.84–0.93) and 1:0.71 (range 0.67–0.76) respectively. In orchiectomized rats the albumin and α_{2u}-globulin mRNA ratio decreased to 1:0.2 (range 0.1–0.25) and returned to 1:0.76 (range 0.71–0.83) after testosterone administration (mRNA levels were obtained from three separate experiments). Such an effect of orchiectomy is comparable to that seen in the normal liver.

Discussion

Transdifferentiation of pancreatic cells to hepatocytes in the pancreas of rats and hamsters has been observed under various experimental conditions (4, 8, 15, 22, 23, 24, 26, 27, 33). The copper depletion and repletion model of pancreatic hepatocytes (26, 28) uses copper-deficient diet (20) supplemented with trien, a mild nontoxic copper chelator (35). Morphological and functional studies unequivocally indicate that the pancreatic hepatocytes are fully differentiated cells. They synthesize albumin, respond to xenobiotics like normal liver cells and contain liver specific mitochondrial enzyme carbamyol phosphate synthetase (22, 23, 36). Unlike the normal liver cells that are arranged as 1-cell-thick plates separated by sinusoids, the pancreatic hepatocytes are arranged as clusters and sheets. No sinusoids are observed between the hepatocytes. Recent studies indicate that ductular and periductal cells serve as progenitor or stem cells (16, 28).

In the present study pancreatic hepatocytes are induced in the male and female rats maintained on CuDT for 8 wk followed by normal diet. The incidence and distribution of pancreatic hepatocytes is similar to that described before (25, 26). The immunohistochemical studies of pancreas and blot analysis of pancreatic RNA from intact male rats showed the presence of α_{2u}-globulin protein and mRNA coding for that protein. However, the distribution of α_{2u}-globulin is different from that observed in the normal liver. In the pancreas, all hepatocytes showed even staining pattern, whereas in the liver only perivenular cells actively synthesize this protein (1, 5, 31). This difference in the synthesis of α_{2u}-globulin by liver cells and pancreatic hepatocytes may be dependent on the microenvironment and or local factors. In the liver a vascular gradient is produced because of unidirectional blood flow in the hepatic sinusoids (7). In pancreatic hepatocytes

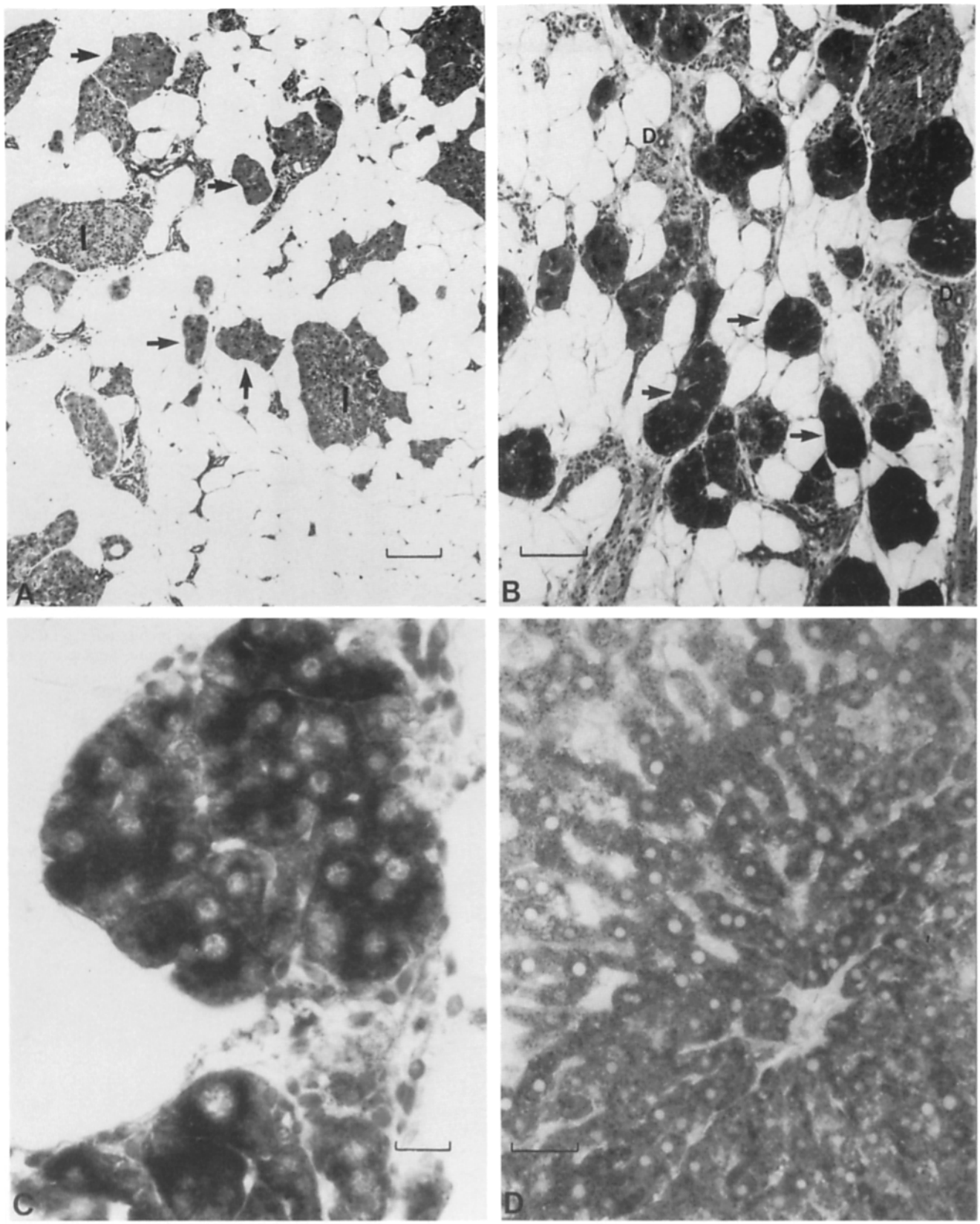

Figure 1. Sections of pancreas of a male rat maintained on normal diet for 16 wk after 8 wk of copper-deficient diet. (*A*) Hematoxylin- and eosin-stained section showing several foci of hepatocytes (*arrows*) around the islets of Langerhans (*I*) and in the fatty stroma; localization of α_{2u}-globulin by immunoperoxidase in pancreatic hepatocytes (*B* and *C*) and normal liver (*D*). Pancreatic hepatocytes show intense staining reaction (*arrows*). Islets of Langerhans (*I*) and ducts (*D*) are negative for this protein. Bars, (*A* and *B*) 100 μm; (*C*) 25 μm; (*D*) 50 μm.

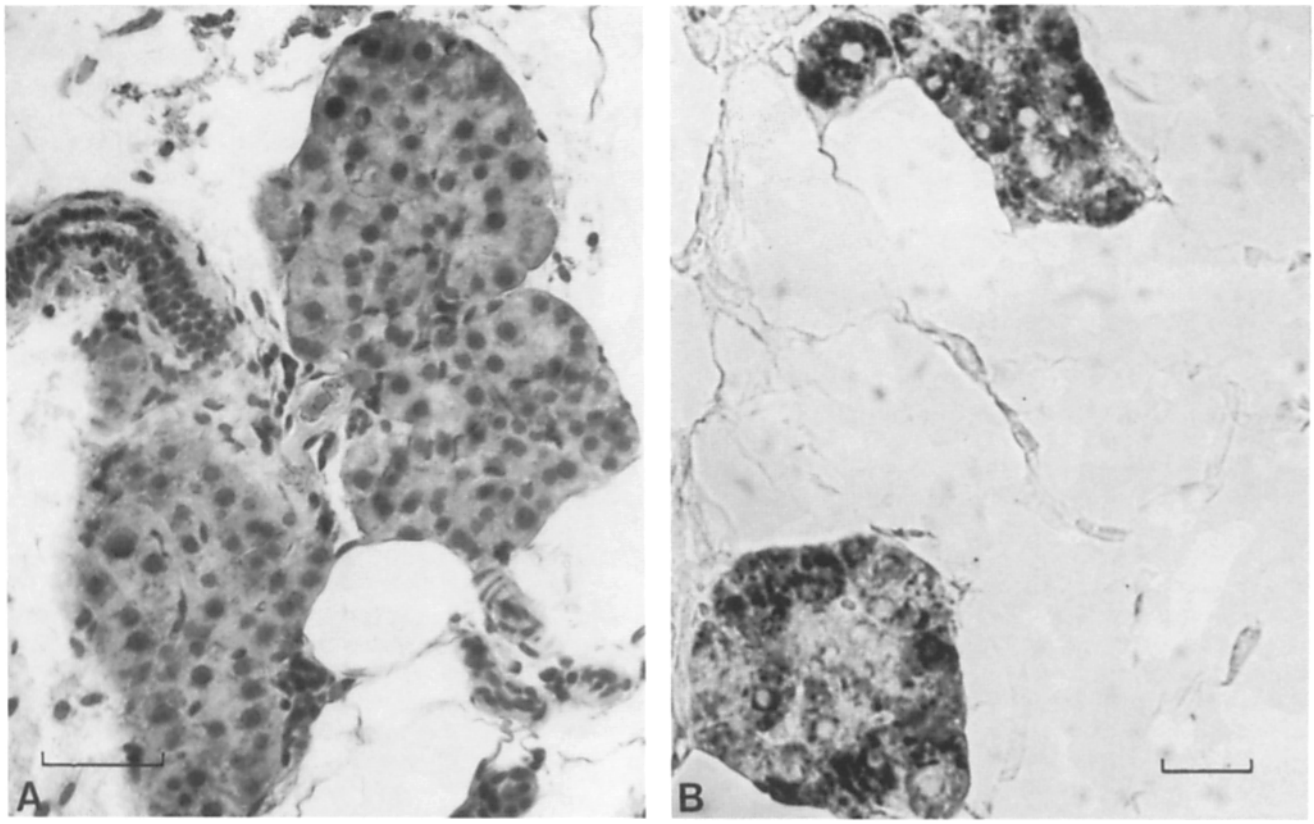

Figure 2. Pancreatic hepatocytes stained for α_{2u}-globulin by immunoperoxidase method. (*A*) Hepatocytes from an orchiectomized rat show no staining reaction; (*B*) hepatocytes from a rat treated with dihydrotestosterone following orchiectomy show intense staining reaction. Bar, 50 μm.

because of lack of regulated sinusoidal vascular flow differences in the milieu may not exist between the cells located at different areas of the hepatic foci. In this context, it is pertinent to point out that the distribution of α_{2u}-globulin is different in different types of tissues. In the lacrimal and preputial gland α_{2u}-globulin is synthesized by all the acinar cells, whereas in submaxillary, meibomian and sebaceous glands only selective cells contain this protein (5, 17).

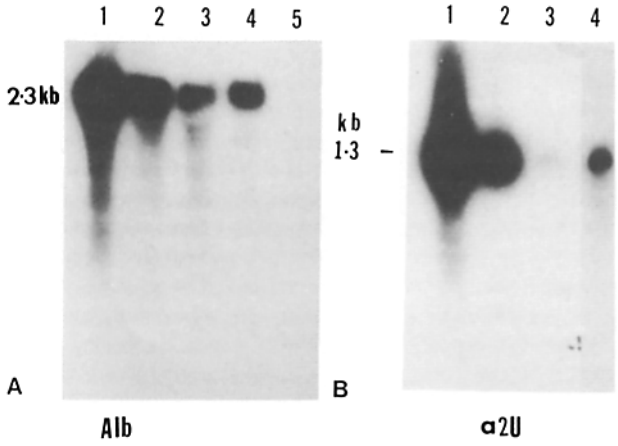

Figure 3. Northern blot analysis of albumin cDNA (*A*) and α_{2u}-globulin cDNA (*B*) in the liver and pancreas containing hepatocytes. Lanes *1*, liver of male rat; lanes *2*, pancreas of male rat with hepatocytes; lanes *3*, pancreas of orchiectomized male; lanes *4*, pancreas of orchiectomized rat given dihydrotestosterone for 8 d; lane *5* in *A*, control rat pancreas. Total RNA (20 μg/lane) was analyzed by Northern blotting with ^{32}P-labeled albumin cDNA (*A*) and α_{2u}-globulin cDNA (*B*). The sizes of albumin mRNA and α_{2u}-globulin mRNA are 2.3 and 1.3 kb, respectively.

Although there is difference in the localized distribution of α_{2u}-globulin in α_{2u}-globulin producing cells in the liver and pancreas, its synthesis in both organs appears to be under the control of sex hormones. By immunoperoxidase stain no α_{2u}-globulin was observed in normal and ovariectomized females. In males, orchiectomy resulted in a marked decrease but not total absence of both α_{2u}-globulin and its mRNA in pancreatic hepatocytes. This finding is consistent with that observed in the livers of orchiectomized rats in which α_{2u}-globulin mRNA has decreased to 15–20% of control values (12, 30). Administration of testosterone to castrated rats resulted in the appearance of immunohistochemically detectable amounts of α_{2u}-globulin and ~4.4-fold increase in the mRNA.

Hormonal regulation of α_{2u}-globulin synthesis is varied in different tissues. In the liver and lacrimal gland α_{2u}-globulin synthesis is dependent on sex hormones, whereas in submaxillary and preputial glands its synthesis is independent of sex hormonal regulation (14, 17). Even in the liver although α_{2u}-globulin synthesis appears to be under androgen regulation, concerted action of several hormones may be necessary (1, 5). It will be of interest to examine whether pancreatic hepatocytes are also under complex hormonal regulation.

Thus, we have clearly demonstrated that differentiated hepatocytes generated from pancreatic cells of an adult rat express the liver-specific α_{2u}-globulin gene under androgenic regulation. This finding not only substantiates our earlier observations concerning the bonafide hepatocytic phenotype of these cells, it also clearly shows that newly expressed genes are maintained under strict liver-specific control. In light of the fact that α_{2u}-globulin is not essential for the maintenance of the liver phenotype and hepatocytes cultured in vitro rapidly loses its synthesis, the regulated expression of the α_{2u}-globulin in the pancreatic hepatocytes is highly intriguing.

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