Reciprocal modulation of growth and differentiated functions of mature rat hepatocytes in primary culture by cell-cell contact and cell membranes

(cell density dependency/plasma membranes/cell growth/liver functions)

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ABSTRACT In primary monolayer cultures of rat mature hepatocytes, many metabolic functions as well as cell growth are regulated by cell density. There are two types of regulatory response of these functions to change of cell density. Growth-related functions, such as DNA synthesis, induction of glucose-6-phosphate dehydrogenase, 2-aminobutyric acid transport, synthesis of cellular protein, and cholestero genesis, are stimulated by low cell density. In contrast, functions related to hepatocyte-specific characters, such as the inductions of tyrosine aminotransferase, serine dehydratase, and malic enzyme and synthesis of triglycerides, are stimulated by high cell density. The reciprocal responses of these cellular activities to cell density were mimicked by addition of plasma membranes purified from adult rat liver to hepatocytes cultured at low cell density. The modulator activity was heat labile and trypsin sensitive. The activity was also found in plasma membranes from kidney, brain, and erythrocytes, although the specific activities of these preparations seemed to be different. These results suggest that the reciprocal regulations of cell growth and hepatocyte-specific functions are mediated by some surface components via cell-cell contact.

Adult rat hepatocytes in primary culture retain in vitro levels of various liver functions and respond to various hormones (1–4). However, adult rat hepatocytes do not proliferate, even when they are cultured in 10% fetal calf serum. Several investigators have found that insulin and epidermal growth factor (EGF) stimulated DNA synthesis in mature rat hepatocytes in primary culture, although the cells did not divide (5–8). Recently, we found that mature hepatocytes could proliferate when cultured at lower cell density in the presence of insulin and EGF, and we suggested that a density-dependent mechanism regulates entry of cells into the cell cycle (9). Michalopoulos et al. (8) also observed the importance of cell density for DNA synthesis and cell proliferation in primary cultured hepatocytes. Subsequently, we noticed density-dependent control of many other cellular activities of mature hepatocytes besides proliferation and we classified the activities of the cells into two types, growth-related functions and hepatocyte-specific characters.

This paper reports that the two types of function are controlled reciprocally by cell density and that their regulation at high cell density can be mimicked by addition of hepatic plasma membranes to cultures at low density. We suggest that cell growth and various liver functions are regulated by cell surface components via cell-cell contact in vitro.

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MATERIALS AND METHODS

Materials. The materials used for cell isolation and culture were as reported (10). Insulin, glucagon, tryptophan (type III), trypsin inhibitor (type I-S), acetyl-L-carnitine A, and malonyl-coenzyme A were obtained from Sigma; dexamethasone (Dex) was from Schering (Berlin); 3,3',5'-triiodo-DL-thyronine (T₃) was from Nakarai Chemical (Kyoto, Japan); EGF was from Collaborative Research (Waltham, MA); NADP and glucose 6-phosphate were from Oriental Yeast Co. (Osaka, Japan); Percoll was from Pharmacia; [1-14C]acetate (57.6 mCi/mmol; 1 Ci = 3.7 × 10¹² Bq), [5,5'-3H]uridine (55 Ci/mmol), and [methyl-3H]thymidine (54.2 Ci/mmol) were from the Radiochemical Centre.

Cell Isolation and Monolayer Culture. Parenchymal hepatocytes were isolated from male Wistar rats weighing 150–200 g by in situ perfusion on the liver with collagenase, essentially as described by Seglen (11). The isolated cells were suspended at 5 × 10⁶ cells per ml in Williams medium E containing 2% calf serum and 2 mM insulin. The cell density at inoculation was varied from 5 × 10⁵ to 2.5 × 10⁶ cells per 5-cm (diameter) Corning plastic culture dish. Over 80% of the cells became attached in 1 hr at 37°C under 5% CO₂/3% O₂ in air. After culture for 2 hr, the medium was changed to hormone-free Williams medium E containing 5% calf serum. In experiments on the effect of addition of plasma membranes, rat hepatocytes were inoculated at a low density (7 × 10⁵ cells per 5-cm dish). After 2 hr, the medium was changed to hormone-free medium containing 5% calf serum and then various amounts of plasma membranes were added to the cultures. Hormones were added to the medium after 22 hr of culture. Incubation was continued for 24 or 48 hr further and then various activities were assayed.

Assay of DNA Synthesis. DNA synthesis was assayed by measuring incorporation of [³H]thymidine into DNA with or without hydroxyurea in 2 hr. Radioactivity in the hot trichloroacetic acid-soluble fraction was measured as described (6).

Assays of Enzyme Activities. The cells were harvested with a rubber policeman in 0.2–1.0 ml of 20 mM potassium phosphate buffer (pH 7.5) containing 0.1 mM dithiothreitol and 5 mM EDTA for the glucose-6-phosphate dehydrogenase (Glc-6-P-DHase) assay, in 0.1–0.5 ml of 5 mM Tris-HCl buffer (pH 7.5).

Abbreviations: EGF, epidermal growth factor; T₃, 3,3',5'-triiodo-DL-thyronine; Glc-6-P-DHase, glucose-6-phosphate dehydrogenase; T-NH₂Tase, tyrosine aminotransferase; Ser-dehydratase, serine dehydratase; AIB, 2-aminobutyric acid; Dex, dexamethasone; mU, milliunit(s).

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7.4) containing 1 mM dithiothreitol, 0.1 mM EDTA, and 0.25 M sucrose for assay of malic enzyme, and in 0.1–0.5 ml of 0.1 M potassium phosphate buffer (pH 8.0) containing 1 mM EDTA and 50 μM pyridoxal phosphate for assays of serine dehydratase (Ser-dehydratase) and tyrosine aminotransferase (T-\(\text{NH}_2\text{Tase}\)). The volume of homogenizing buffer was adjusted to give a constant protein concentration. The cells were disrupted by two cycles of freeze-thawing, and tyrosine the enzyme stant protein concentration.

Malic enzyme (13), with bovine Units per washed lipids N2, Amol washed silica gel was expressed as mM 5 were with n-hexane/ethyl ether/acetic ether/acetic 70:20:1 (vol/vol). Lipids were extracted from the extract of Bligh and Dyer (17). The extract was evaporated under N\(_2\), and the concentrated extract in CHCl\(_3\) was spotted on silica gel 60 plates (Merck, Darmstadt, FRG) and developed with n-hexane/ethyl ether/acetic anhydride, 70:20:1 (vol/vol). Spots were located with iodine vapor, scraped off into vials, and assayed for radioactivity in toluene scintillator.

Assays of Lipogenesis. Monolayers of hepatocytes in 5-cm dishes were incubated with 2 ml of culture medium containing 5 mM Na-[\(^{14}\)C]acetate (1 μCi/ml) for 3 hr. The cells were washed twice with phosphate-buffered saline (P\(_2\)/NaCl), harvested in 1 ml of P\(_2\)/NaCl, and homogenized in a Polytrom homogenizer. Lipids in the homogenates were extracted by the method of Bligh and Dyer (17). The extract was evaporated under N\(_2\), and the concentrated extract in CHCl\(_3\) was spotted on silica gel 60 plates (Merck, Darmstadt, FRG) and developed with n-hexane/ethyl ether/acetic anhydride, 70:20:1 (vol/vol). Spots were located with iodine vapor, scraped off into vials, and assayed for radioactivity in toluene scintillator.

Assays of AIB Transport and Protein Synthesis. Transport of AIB was determined by the method of Kletzien et al. (18). Cells were preincubated with insulin (0.1 μM) for 2 hr and then washed and incubated in Hanks/Hepes solution containing 0.2 mM [\(^{14}\)C]AIB (0.1 μCi/ml) for 30 min. Then the medium was discarded and the cells were washed three times with ice-cold P\(_2\)/NaCl and solubilized in 0.2 M NaOH and their radioactivity was measured. Syntheses of intra- and extracellular proteins were measured by incubating the cells in leucine-free Williams medium E containing 1-[\(^{1-5}\)H]leucine (0.1 μCi/nmol) and then measuring radioactivity incorporated into intra- and extracellular proteins as reported (10).

Preparations of Plasma Membranes from Various Tissues. Hepatic plasma membranes were purified from adult rat liver on a Percoll gradient as described (19); the membranes were purified about 20-fold from the homogenate, judging by their 5’ nucleotidase activity. Renal basolateral membranes were purified by the method of Sacktor et al. (20); about 6.5-fold purification from a homogenate of renal cortex was achieved, judging from the activity of Na\(^{+}\),K\(^{+}\)-ATPase. Synaptosomal membranes were purified by the method of Jones and Matus (21) from a cerebral homogenate; these membranes were purified 9-fold, judging by their Na\(^+\),K\(^+\)-ATPase activity. Erythrocyle ghosts were prepared by the method of Dodge et al. (22). The purified membranes were sterilized by irradiation from an UV lamp at a distance of about 5 cm for 10 min.

Treatments of Purified Plasma Membranes. A suspension of purified plasma membranes (2 mg of protein per ml) in P\(_2\)/NaCl was heated for 10 min in boiling water, cooled, and used for assay of modulation of activity. A suspension of the membranes (2.5 mg of protein per ml) in 10 mM Hepes buffer (pH 7.5) containing 0.15 M NaCl and 10 mM CaCl\(_2\) was mixed with 1/9 vol of 4 mg of trypsin preparation per ml, which contained 50% of the activity, and incubated for 3 hr at 37°C. The reaction was stopped by the addition of the same volume of 6 mg of soybean trypsin inhibitor per ml. A control suspension of plasma membranes was incubated without trypsin for 3 hr at 37°C and then was mixed with trypsin previously inactivated with soybean trypsin inhibitor.

RESULTS

Effects of Cell Density on Various Metabolic Activities of Hepatocytes. Previously we showed that mature rat hepatocytes do not proliferate when cultured at high cell density, even in the presence of insulin and EGF, but that they can proliferate when cultured at low cell density with these hormones (9). These findings prompted us to examine whether other metabolic activities of hepatocytes depend on cell density. Isolated hepatocytes were plated at various inocula of \(5 \times 10^4\) to \(2.5 \times 10^5\) cells per 5-cm dish. Microscopic examination showed that at the lowest cell density (\(2.5 \times 10^5\) cells per cm\(^2\)) the cells were present in cultures singly or as small aggregates, whereas at the highest cell density (\(12.5 \times 10^6\) cells per cm\(^2\)) they were present as a sheet with tight cell–cell contact almost completely covering the surface of the plate. Fig. 1 shows that at cell densities of \(>5 \times 10^4\) cells per cm\(^2\), the inductions of T-\(\text{NH}_2\text{Tase}\) and Ser-dehydratase by Dex and Dex with glucagon, respectively, were markedly increased, whereas the inductions of DNA synthesis and Glc-6-P-DHase by insulin with EGF were markedly decreased. At confluency (\(12.5 \times 10^6\) cells per cm\(^2\)), the inductions of T-\(\text{NH}_2\text{Tase}\) by Dex and Ser-dehydratase by Dex with glucagon reached maximal levels of 10–13 times the control values, whereas in sparse cultures \(2.5 \times 10^6\) cells per cm\(^2\) their inductions were less than twice the control levels. The stimulation of cAMP formation by glucagon did not show cell density-dependent regulation (data not shown). This fact suggests that the density-dependent change in induction of Ser-dehydratase by glucagon with Dex is not due to modulation of the glucagon receptor-adenylate cyclase system in the plasma membranes.

An interesting finding was that Glc-6-P-DHase, which is

![Fig. 1. Cell density-dependent controls of DNA synthesis and inductions of Glc-6-P-DHase, T-\(\text{NH}_2\text{Tase}\), and Ser-dehydratase in primary cultured hepatocytes. The numbers of cells indicated were inoculated and cultured (see text). Activities were measured 24 hr after hormone addition, except Glc-6-P-DHase activity, which was assayed 45 hr after hormone addition. c, DNA synthesis induced by insulin (0.1 μM) with EGF (20 ng/ml); ∆, Glc-6-P-DHase activity induced by insulin with EGF; o, T-\(\text{NH}_2\text{Tase}\) activity induced by Dex (10 μM); A, Ser-dehydratase activity induced by Dex with glucagon (0.5 μM). The basal activities (no hormone) at different cell densities were within ranges of 1,468–1,697 dpm/hr per mg of protein for DNA synthesis, and 4–6 μU/mg of protein for Ser-dehydratase, respectively. Values are means for duplicate experiments.](image-url)
thought to be a key enzyme for the supply of NADPH in lipogenesis, was induced much more at low cell density, whereas malic enzyme, which is another key enzyme for the supply of NADPH in lipogenesis, was induced by insulin and T3 much more at high cell density (Table 1). Therefore, Glc-6-P-DHase may be more important in supplying ribose 5-phosphate for cell growth than for lipogenesis, as discussed earlier (23), whereas malic enzyme may be more important for lipogenesis. Consistent with this idea, the density-dependent regulation of malic enzyme was found to correlate with that of synthesis of triglycerides.

Another interesting reciprocal relation in lipogenesis was found: cholesterol synthesis was greater at low cell density, whereas triglyceride synthesis was much higher at high cell density (Table 1). Possibly, cholesterol is required for synthesis of cell membranes during growth, whereas triglycerides are deposited in resting cells. Similarly, synthesis of cellular protein was stimulated at low cell density, whereas synthesis of serum proteins was not affected by cell density. The rate of AIB transport, which is generally thought to depend on cell growth, was also stimulated at low cell density. Thus, there seem to be two types of responses of various metabolic activities to change of cell density: one is that of activities related to cell growth and anabolism, and the other is that of activities related to the differentiated characters of hepatocytes. As summarized in Table 1, the properties related to cell growth tended to respond more to low cell density, whereas those related to specific characters of hepatocytes were stimulated more at high cell density than at low cell density. The basal activities of these functions without added hormones also changed in the same way as with hormones, although these changes were <10% of those with hormones (data not shown). Moreover, some functions, such as the syntheses of protein and cholesterol, changed markedly by cell density without added hormones. These results suggest that cell density affects both the basal expression of cellular functions and their responses to hormones.

Effects of Plasma Membranes on Growth and Specific Functions of Hepatocytes. Next we examined the mechanism responsible for the cell-density dependent controls for these many cellular activities of adult rat hepatocytes. Two mechanisms are possible: one is that the cell-density-dependent regulations of cell growth and various cellular activities may result from accumulation of some soluble factors released from hepatocytes into the medium. However, this possibility is excluded by the fact that addition of conditioned medium from cultures at high cell density to cultures at low density did not mimic the effect of high cell density (data not shown). The other possibility is that direct cell–cell contact, perhaps involving interaction of complementary cell surface components on adjacent cells, provides a signal for regulation of cell growth and various differentiated characters of hepatocytes. If this possibility is correct, contact between intact cells would not necessarily be required for density-dependent regulation. This consideration prompted us to examine whether cell surface components of isolated plasma membranes could mimic the effect of increase in cell density on the growth and various cellular activities of cells at low density.

Isolated plasma membranes were prepared from adult rat liver. The inductions of T-NH₂Tase by Dex (Fig. 2) and malic enzyme by insulin with T3 (Fig. 3) were markedly enhanced in hepatocytes at low cell density by addition of these plasma membranes. The effect of the plasma membranes was dose-dependent and reached a maximum at 300 μg of membrane protein per 5-cm dish. This amount of membranes corresponds to that of about 2.5 × 10⁶ cells, calculated on the basis of the purity and yield of membranes during purification. In contrast,

![Graph](image-url)

**Fig. 2.** Stimulation of T-NH₂Tase induction and inhibition of DNA synthesis in sparse cultures of hepatocytes by addition of hepatic plasma membranes. Hepatocytes were seeded at an initial cell density of 7 × 10⁶ cells per 5-cm dish (3.5 × 10⁶ cells per cm²) and cultured. The membranes were added to the culture medium 2 hr after plating. Dex (10 μM) for T-NH₂Tase induction and insulin (0.1 μM) with EGF (20 ng/ml) for DNA synthesis were added to the culture medium 22 hr after addition of the membranes. T-NH₂Tase activity and DNA synthesis were assayed 24 hr after hormone addition. ○, T-NH₂Tase activity without Dex; ●, T-NH₂Tase activity with Dex; Ψ, DNA synthesis with insulin and EGF. The basal activity (mean ± SD) of DNA synthesis was 1,605 ± 450 dpm/hr per mg of protein (three experiments). Values are means for duplicate experiments.

<table>
<thead>
<tr>
<th>Function</th>
<th>Stimulators</th>
<th>25,000 per cm²</th>
<th>75,000 per cm²</th>
<th>125,000 per cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA synthesis</td>
<td>Insulin</td>
<td>100</td>
<td>36</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>+ EGF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIB transport</td>
<td>Insulin</td>
<td>100</td>
<td>67</td>
<td>42</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracellular</td>
<td>Insulin</td>
<td>100</td>
<td>84</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Secreted</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Lipogenesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Insulin</td>
<td>100</td>
<td>75</td>
<td>37</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Insulin</td>
<td>100</td>
<td>33</td>
<td>88</td>
</tr>
<tr>
<td>Enzyme induction</td>
<td>Glc-6-P-DHase</td>
<td>100</td>
<td>38</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>+ EGF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malic enzyme</td>
<td>Insulin</td>
<td>100</td>
<td>38</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>+ T3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-NH₂Tase</td>
<td>Dex</td>
<td>100</td>
<td>47</td>
<td>100</td>
</tr>
<tr>
<td>Ser-dehydratase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ glucagon</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The membrane preparation strongly inhibited DNA synthesis stimulated by insulin with EGF in cells at low cell density (Fig. 2). This inhibition was also dose dependent and was maximal with about 150 μg of membrane protein per 5-cm dish, which is less than half the amount for maximal stimulations of T-NH₂Tase and malic enzyme. Addition of plasma membranes also affected the basal activities of the functions shown in Figs. 2 and 3 and Table 2 of cells at low density without added hormones, although the changes were very small. These results suggest that the reciprocal controls of DNA synthesis and differentiated characters of hepatocytes are mediated by some cell surface components via direct cell–cell contact.

The ability of hepatic plasma membranes to mimic the reciprocal effect increased cell density on cellular activities was almost lost by digestion of the membranes with trypsin (Table 2). Moreover, the modulator activity of hepatic plasma membranes was significantly decreased by heat treatment (in boiling water for 10 min) of the membranes. Thus, the active component(s) present in the cell surface may be a protein(s).

Next we examined whether the cell surface modulator(s) is specific to hepatocyte membranes. Table 3 shows that plasma membranes purified from rat kidney, brain, and erythrocytes have similar modulator activities, as shown by their enhancement of T-NH₂Tase induction, although the activities of preparations from different tissues varied. Renal membranes seemed to contain at least the same amount of cell surface modulator(s) as hepatic membranes, although the specific activities of the membranes could not be compared exactly because the purities of these membranes were different.

It should be mentioned that the effects of membranes on DNA synthesis and T-NH₂Tase induction were similar whether the membranes were isolated from intact adult liver or from hepatocytes cultured at high density.

**DISCUSSION**

Cell–cell contact is known to be an important mechanism in tissue formation, cytodifferentiation, and cell growth. Studies on this phenomenon have mainly been carried out on the slime mold (24–26) and chicken neural retina (27–29). Recently, it was found that in slime molds, some surface components differing from cell adhesive factors regulate induction of the post-aggregative enzyme UDP-glucose pyrophosphorylase (26).

The phenomenon of “contact inhibition” of cell proliferation has also been studied extensively in 3T3 cells and is usually explained as due to either depletion of growth factors (30, 31) or secretion of growth inhibitors (32, 33). As an alternative, Glaser

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**Table 3. Tissue distribution of cell surface modulator in plasma membranes**

<table>
<thead>
<tr>
<th>Plasma membranes</th>
<th>T-NH₂Tase activity, mU/mg of protein</th>
<th>DNA synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without Dex</td>
<td>With Dex</td>
</tr>
<tr>
<td>None</td>
<td>6.0 ± 0.3</td>
<td>9.2 ± 0.7</td>
</tr>
<tr>
<td>Liver</td>
<td>62.5 ± 0.3</td>
<td>7.6 ± 0.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>125 ± 0.3</td>
<td>7.0 ± 0.7</td>
</tr>
<tr>
<td>Brain</td>
<td>250 ± 0.3</td>
<td>7.5 ± 0.7</td>
</tr>
</tbody>
</table>

**Experimental conditions were as described for Fig. 2. Values are means for duplicate dishes. Dex was added at 10 μM.**

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**Table 2. Effects of various treatments of plasma membranes on their cell surface modulation activity**

<table>
<thead>
<tr>
<th>Hepatic membranes</th>
<th>Dose, μg of protein per 5-cm dish</th>
<th>T-NH₂Tase activity, mU/mg of protein</th>
<th>DNA synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without Dex</td>
<td>With Dex</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>10.1 ± 0.3</td>
<td>18.8 ± 0.3</td>
</tr>
<tr>
<td>Untreated 25</td>
<td></td>
<td>9.3 ± 0.2</td>
<td>41.7 ± 0.9</td>
</tr>
<tr>
<td>Untreated 50</td>
<td></td>
<td>9.2 ± 0.7</td>
<td>86.5 ± 0.7</td>
</tr>
<tr>
<td>Untreated 100</td>
<td></td>
<td>17.6 ± 0.2</td>
<td>141.9 ± 0.6</td>
</tr>
<tr>
<td>Untreated 200</td>
<td></td>
<td>33.9 ± 0.4</td>
<td>147.1 ± 0.9</td>
</tr>
<tr>
<td>Trypsinized 100</td>
<td></td>
<td>8.4 ± 0.2</td>
<td>28.9 ± 0.2</td>
</tr>
<tr>
<td>Trypsinized 200</td>
<td></td>
<td>7.4 ± 0.4</td>
<td>45.8 ± 0.2</td>
</tr>
<tr>
<td>Heated 200</td>
<td></td>
<td>9.9 ± 3.2</td>
<td>55.8 ± 3.5</td>
</tr>
</tbody>
</table>

Membranes were treated as described in the text. Hepatocytes were plated and treated as for Fig. 2. The membranes used in these experiments were purified 38-fold from liver homogenates, judging by their 5' nucleotidase activity. Values are means ± SD of three experiments. Basal activity for DNA synthesis without hormone with different amounts of membranes was as shown in Fig. 2.
and his colleagues (34, 35) proposed that it might be due to inhibitors on the cell membrane, because they found that 3T3 cell growth was inhibited by addition of plasma membranes of 3T3 cells. However, details of the regulatory mechanism of cell–cell contact are still unknown.

In this work we showed that growth-related functions and hepatic characters were regulated reciprocally by cell density and that these regulations were mediated by a cell surface component of the plasma membrane via direct cell–cell contact. The surface component seems to be a protein(s), because its activity was heat labile and sensitive to trypsin (Table 2). Recently, we succeeded in solubilizing the cell surface modulator(s) from hepatic membranes with a nonionic detergent, octylglucoside, with 4 M guanidine-HCl and partially purified the modulator(s) by Sephacryl S-400 filtration. This membrane-free modulator(s) showed activities for both modulation of growth-related functions and hepatocyte-specific characters. The details of properties of the partially purified modulator will be reported elsewhere. These results in vitro suggest that various functions of hepatocytes in vitro at the mature state and during development and regeneration are all regulated by cell–cell contact and that stable functions are not maintained unless the cells form tissue and receive complex regulation from their cytosocial environment. In liver regeneration there is swelling of liver lobules and disappearance of gap junctions morphologically and decreased transfer of substances of low molecular weight and of electrical coupling functionally (36, 37). Liver tissue consists of not only parenchymal hepatocytes but also other cells, such as cells of the bile ducts, endothelium, and connective tissues. The participation of these other types of cells in regulation of liver functions cannot be excluded, but the population of parenchymal cells is the largest and these cells are in contact with each other. Therefore, the effect of cell–cell contact of hepatocytes on their activities must be the most important.

The molecular mechanism of intracellular transduction of membrane signals to regulate growth and liver functions is unknown. It should involve control of gene transcription of liver functions, because the changes in activity of Glc-6-P-DHase and Ser-dehydratase in cells at different densities are parallel with their mRNA levels and rates of enzyme syntheses (unpublished data). Moreover, the cell density-dependent regulation does not depend on the type of hormone or its action but depends on the type of cellular activity. These considerations support the idea that the mechanism of density-dependent regulation is a nuclear event involving control of gene transcription.

Cell surface modulators involved in the regulations of cell functions by cell density were not present only on hepatocyte membranes but were also found in the plasma membranes of other tissues (Table 3). Their wide distribution seems reasonable, because cell surface modulators do not induce new expression of dormant genes but regulate phenotypic genes that have already been switched on during terminal differentiation. Therefore, cell surface modulators of various tissues could have a common mechanism in regulating the phenotypes of various tissues, although these phenotypes are tissue specific.

It would be interesting to know whether the same factor in cell membranes is responsible for inhibition of growth-related functions and stimulation of hepatocyte-specific characters. This problem must be examined by further purification and characterization of the factor(s) responsible for these activities. Our recent findings show that solubilized and partially purified factor regulates reciprocally several activities reported in this paper. This suggests that density-dependent changes in activities may all be mediated by one or a few kinds of factors. This information would be helpful in understanding the process of assembly of liver cells to organize liver tissue in vitro and the mechanisms of change of gene expression in liver during development, regeneration, and carcinogenesis.

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