Expression of insulin receptors and insulin action in cell hybrids and cybrids

C. Ronald KAHN, Vilma LAURIS, Max FEHLMANN and Marco CRETТАZ

Research Division, Joslin Diabetes Center, and Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02215, U.S.A.

(Received 10 January 1983/Accepted 15 April 1983)

The expression of insulin receptors and insulin action was studied in cell hybrids and cybrids produced by fusion of the BWIJ mouse hepatoma cell line with nucleated and enucleated mouse L-cells (LEA-2A) respectively. The BWIJ parent and the cybrids expressed high numbers of insulin receptors, whereas the hybrids resembled the L-cell parent with low numbers of receptors. Likewise, the hybrids resembled the LEA-2A cells with high levels of glycogen synthase, whereas the BWIJ cells and cybrids had much lower levels. Both parents, the cybrids, and the hybrids, expressed insulin stimulation of α -aminoisobutyric acid influx, but the dose-response curves indicated an increased insulin sensitivity in the cells with the higher receptor concentration. Insulin also stimulated $^{86}\text{Rb}^+$ uptake in the hepatoma parent, hybrids and cybrids, but not in the L-cell parent. These data suggest that insulin receptors, like other hepatoma-specific properties, behave as a 'luxury function' of the hepatoma cell line and are extinguished when the hepatoma cell is fused with a less differentiated cell type. The biological activities associated with insulin action, on the other hand, are much more complex in their expression and probably the result of the interaction of multiple factors that vary in their expression in cell hybrids and cybrids.

Somatic-cell genetics has provided an important approach to our understanding of the control of gene expression in eukaryotic cells (Ephrussi, 1972; Weiss, 1976; Ringertz & Savage, 1976). When two cells are fused in the process of hybridization, there is an opportunity for interactions between the various factors derived from the two genomes in a common cytoplasm. Hybrids between two cell types, one of which expresses a specific differentiated function and one of which does not, may result in a variety of responses. Most often, the specific functions of the more differentiated cell are lost (extinguished), but occasionally there may be activation of the non-expressing genome or, in the case of cells that present two different functions, co-expression of both genomes (Weiss, 1976). The activation or extinction process usually affects all or most of the proteins characteristic of the particular parental cell, suggesting the presence of diffusible substances in one cell that can influence the expression of genes in the other. Cytoplasmic organelles and cytoplasmic factors may also modify or regulate gene expression (Grun, 1976; Gillham, 1978). The influence of these factors can be

demonstrated by nuclear transportation or cell hybridization. In the latter approach, one cell is enucleated, and the remaining cytoplasm is fused with a whole cell to form a cybrid.

We have previously prepared and characterized several hepatic functions in hybrids and cybrids between a line of cultured mouse hepatoma cells (BWIJ) and a chloramphenicol-resistant fibroblastic mouse L-cell (LEA-2A) (Kahn *et al.*, 1981). Since the liver is a major target tissue for insulin action and rich in insulin receptors, the hybrids and cybrids also permit an opportunity to investigate the expression of these membrane and hormonal functions.

Materials and methods

Materials

The cell lines used in the present study are described in detail by Kahn *et al.* (1981) and in the Results section. There were two parental cell lines, a mouse hepatoma (BWIJ) and a fibroblastoid L-cell (LEA-2A) and the hybrids and cybrids produced by their fusion. The hybrids are referred to as 'HyLEB's' and the cybrids as 'CyLEB's' as previously described. Culture medium for all cell lines was Coon's modified Ham's F-12 (Coon & Weiss,

Abbreviations used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; SDS, sodium dodecyl sulphate; AFP, α -foetoprotein.

1969) supplemented with 5% (v/v) foetal-bovine serum from Flow Laboratories (Rockville, MD, U.S.A.). The medium for all the cell lines, except BWIJ, contained also 50 µg of chloramphenicol/ml. All cells were maintained in 37°C humidified incubator with CO₂/air (1:19) with changes of medium three times weekly. For transfer, the cells were detached with 0.05% trypsin/0.02% EDTA as described by Cassio & Weiss (1979).

Tissue-culture dishes and flasks were either from Falcon Plastics or COSTAR (Cambridge, MA, U.S.A.) Culture medium was from Gibco. Bovine serum albumin was purchased from Sigma. UDP-[¹⁴C]glucose, Na¹²⁵I, α-amino[1-¹⁴C]isobutyric acid (sp. radioactivity 60 mCi/mmol) and ⁸⁶Rb⁺ (sp. radioactivity 1.55 mCi/mg) were obtained from New England Nuclear Corp. Pig insulin was from Elanco Products Co., a division of Eli Lilly Co. (Indianapolis, IN, U.S.A.). All other chemicals were of analytical grade supplied by the usual chemical companies. ¹²⁵I-insulin (100–150 µCi/µg) was prepared as described by Freychet *et al.* (1972).

Methods

Insulin binding. Insulin binding was carried out in F-12 medium without glucose and supplemented with 20 mM-Hepes and 1% bovine serum albumin, pH 7.8. About 48 h before the experiment, cells were detached by trypsin/EDTA and seeded in six-well plates at $(1.5\text{--}2) \times 10^6$ cells per 35 mm-diameter well. On the day of the experiment, confluent cultures were incubated at 15°C for 3 h with binding medium containing 30 pM-¹²⁵I-insulin and different concentrations of unlabelled insulin ranging from 1 nM to 2 µM. At the end of incubation, the monolayers were washed four times with 2 ml of cold phosphate-buffered saline (137 mM-NaCl/2.6 mM-KCl/8.1 mM-Na₂HPO₄/1.5 mM-KH₂PO₄/0.5 mM-MgCl₂/0.7 mM-CaCl₂), the cells were solubilized in 1 ml of 0.01% SDS, and counted for radioactivity in a Tracor model 1290 γ-radiation counter. Cell protein was determined by the method of Bradford (1976), with Bio-Rad reagent. Insulin-binding results were expressed per mg of protein.

Amino acid transport (Fehlmann *et al.*, 1981). For amino acid-transport measurements, cells were grown to confluency. Medium was aspirated and replaced by fresh serum-free F-12 medium containing 1% bovine serum albumin with or without insulin. Cells were then incubated for 6 h at 37°C. At the end of this 6 h incubation, the medium was aspirated and the transport assays were initiated by adding 1 ml of amino acid-free Krebs–Ringer bicarbonate buffer containing α-amino[¹⁴C]isobutyric acid (0.02 µCi/dish), unlabelled aminoisobutyrate (final concn. 0.01 mM) and [³H]inulin (1 µCi/dish); the latter was used for determination of the extracellular space. After 15 min at 37°C, the

reaction was terminated by aspirating the buffer and washing the plates with 2 × 4 ml of chilled phosphate-buffered saline. Cells were in 0.01% SDS and counted for ¹⁴C and ³H radioactivity. Transport data were corrected for extracellular trapping and normalized per mg of cell protein.

Rb⁺ uptake (Fehlmann & Freychet, 1981). Cells were grown to confluency. Medium was aspirated and replaced by 1 ml of serum-free F-12 medium containing 1% bovine serum albumin and ⁸⁶Rb⁺ (1 µCi/dish), with or without insulin at 100 nM and with or without 1 mM-ouabain. After 1 h of incubation at 37°C, medium was aspirated and the plates were washed twice with 4 ml of chilled saline (see above). Cells were ‘digested’ in 0.01% SDS and radioactivity was measured on the ³²P channel of a liquid-scintillation spectrometer.

Glycogen synthase activity. At 48–72 h before the experiment, cells were detached and 7×10^6 cells seeded in 100 mm-diameter dishes. On the day of the experiment the confluent monolayers were pre-incubated with 3 ml of binding medium (pH 7.4) for 3 h at 37°C in a humidified incubator. Incubation was continued for an additional 30 min in the presence or absence of 1 µM-insulin. The reaction was stopped by aspirating the media, and the cells were washed once with 3 ml of cold 100 mM-NaF/10 mM-EDTA and harvested in test tubes by scraping with a rubber policeman, followed by two rinses with 3 ml of cold NaF/EDTA solution. The cell suspension was centrifuged for 2 min at 4°C at 500 g and resuspended in 100 µl of cold NaF/EDTA in 1.5 ml Microfuge tubes. The cells were disrupted by sonication for 5 s at position 3 on a Branson Sonifier 200, centrifuged at 100 000 g for 30 s in a Beckman model B Microfuge, and the supernatant was assayed for glycogen synthase activity by a slight modification (M. Crettaz & C. R. Kahn, unpublished work) of the method of Thomas *et al.* (1968). Glycogen synthase activity was expressed in nmol of UDP-glucose incorporated into glycogen/min per mg of protein. Total glycogen synthase activity was measured in the presence of glucose 6-phosphate, the independent or I-form in the absence of glucose 6-phosphate; the glucose 6-phosphate-dependent or D-form was the difference between the total activity and the activity of I-form.

Results

Characteristics of the cell lines (Table 1)

BWIJ is a subclone of the BW1 mouse hepatoma line (Szpirer & Szpirer, 1975; Cassio & Weiss, 1979). These cells have an epitheloid morphology, are hyperdiploid with 64 chromosomes, and secrete both albumin and α-foetoprotein (AFP) in substantial amounts (Table 1). These cells also produce other liver-specific proteins, such as aldolase B,

transferrin, and third component of complement, but lack some of the properties of the differentiated adult liver in that they have very low levels of tyrosine aminotransferase and gluconeogenic enzymes (Kahn *et al.*, 1981; Szpirer & Szpirer, 1975; Cassio & Weiss, 1979).

LEA-2A is a mouse L-cell derived by fusion of a cytoplasm of a chloramphenicol-resistant mouse cell line, 501-1, with a thymidine kinase-deficient mouse L-cell, LM (TK⁻) (Bunn *et al.*, 1974). These cells have a fibroblast morphology, possess an average of

45 chromosomes, and exhibit no albumin or AFP production (Kahn *et al.*, 1981).

As previously described (Kahn *et al.*, 1981), hybrid cell lines were established by fusion of intact BWIJ and LEA-2A cells. The four hybrids studied here were derived from independent fusions and are referred to as HyLEB 1A, 2B, 7 and 8. All are chloramphenicol-resistant, have a morphology intermediate between that of BWIJ and LEA-2A and approximately the sum of the chromosome numbers of the parental cell lines. Only one of the hybrid clones, HyLEB 7, produced albumin and AFB, at about 5–10% of the rate of the BWIJ parent, but as expected, in most hybrids, albumin and AFP production were decreased to below detectable levels, i.e., they were extinguished (Table 1) (Kahn *et al.*, 1981).

Cybrids, produced by fusion of enucleated LEA-2A cells and intact BWIJ cells, were also chloramphenicol-resistant, but had a morphology and karyotype similar to that of the BWIJ parent (Table 1). The three cybrids (CyLEB's) studied were derived from independent fusions. All produced albumin at about half the rate of the BWIJ parent. All also produced AFP, and in CyLEB 2 and 5A at a somewhat higher rate than the parent, consistent with about 20% of the cells being 2s in chromosome number.

Table 1. Characteristics of parental cell lines, hybrids, and cybrids

Data were derived from those of Kahn *et al.* (1981). Abbreviations used (morphology): E, epithelial; I, intermediate; F, fibroblastic.

	Morphology	Mean chromosome no.	Production (mg/24 h per 10 ⁶ cells)	
			Albumin	AFP
Parental lines				
BWIJ	E	64	8.2 ± 1.5	13.1 ± 3.6
LEA-2A	F	45	<0.02	<0.02
Hybrids				
HyLEB 1A	I	101	<0.02	<0.02
2A	I	91	<0.02	<0.02
7	I	101	0.33	1.1
8	I	108	<0.02	<0.02
Cybrids				
CyLEB 1A	E	61	5.3	10.2
2	E	61*	4.6	19.7
5A	E	62*	3.3	26.3

* About 20% of the cells were 2s (tetraploid).

¹²⁵I-insulin binding studies

Both the BWIJ and the LEA-2A cell lines had demonstrable insulin receptors by specific binding of ¹²⁵I-insulin, but the levels varied dramatically (Fig. 1).

Fig. 1. ¹²⁵I-insulin binding to parental cells, hybrids and cybrids

Confluent cell cultures in 35 mm-diameter wells were incubated in 1 ml of buffered medium with 20 pM-¹²⁵I-insulin and unlabelled insulin to produce the indicated concentrations for 3 h at 15°C. The percentage of ¹²⁵I-insulin bound per mg of total cell protein is plotted as a function of hormone concentration.

When expressed per mg of cell protein, the BWIJ bound over 50% of the tracer ^{125}I -insulin, the highest level that we have ever observed in a cultured cell line. This was over 95% specific as determined by the ability of a high concentration ($1\mu\text{M}$) of unlabelled insulin to inhibit the tracer binding. By contrast, although specific ^{125}I -insulin binding could be demonstrated to the LEA-2A parent, it was just above the minimum level detectable under the conditions of this assay (i.e., in a 35 mm-diameter tissue-culture well).

All four hybrid cell lines exhibited a marked decrease in ^{125}I -insulin binding when compared with BWIJ parent, at a tracer concentration ranging from 5 to 10% of that present in the hepatoma. The level of binding was about twice that of the low-binding parent, LEA-2A, when expressed per mg of cell protein. No significant difference could be detected in insulin binding to HyLEB-7 when compared with the others, although this hybrid did express some albumin and AFP production.

In contrast with the hybrids, the three cybrids all exhibited high levels of ^{125}I -insulin binding. CyLEB 2 and 5A actually exhibited a somewhat higher level of binding than the BWIJ parent. This correlated with their ability to produce somewhat higher levels of AFP, and with the fact that these cybrid lines contained significant percentages of cells with a 2s chromosome number.

The concentration of unlabelled insulin required to produce 50% inhibition of specific ^{125}I -insulin binding was similar for BWIJ and all of the cybrids and hybrids, ranging from 0.25 to 1.35 nM. The concentration of insulin required for half-maximal inhibition of specific insulin binding to LEA-2A cells was about 40 nM, suggesting that at least part of the difference in binding exhibited by this cell line was due to a lower affinity of the receptor for insulin.

Scatchard analysis of insulin binding

Scatchard (1949) analysis of insulin binding to both parental cell lines and one exemplary hybrid and cybrid cell line is shown in Fig. 2. All four cell types exhibited curvilinear Scatchard plots consistent with multiple classes of sites, negative cooperativity, or both (Ginsberg, 1977). Although there may be considerable error in the estimate of total receptor concentration (R_0) from such curvilinear plots, by comparing the bound/free ratio for tracer insulin and the estimate R_0 from the Scatchard plot, it is possible to make some assessment of the relative roles of changes in affinity and binding capacity. Most of the differences in binding between BWIJ, HyLEB-7 and CyLEB-2 can be explained by differences in receptor concentration (per mg of protein). Most of the difference between HyLEB-7 and LEA-2A, on the other hand, appears

Fig. 2. Scatchard plots of ^{125}I -insulin binding to the parental cells, a hybrid and a cybrid clone. The data were derived from the binding data in Fig. 1 and plotted by the method of Scatchard (1949).

Fig. 3. Effect of insulin (INS) on amino acid uptake by cells

Confluent cell cultures were incubated with or without $0.1 \mu\text{M}$ -insulin for 6 h at 37°C , then pulsed with α -amino ^{14}C isobutyrate, as described under 'Methods', for 15 min. Transport data were corrected for extracellular trapping (measured by using ^3H -insulin) and normalized per mg of cell protein. All results are the means from two separate experiments, each done in triplicate. *P* values are as indicated.

to result from a change in affinity, since no significant difference in R_0 could be detected.

Insulin stimulation of amino acid influx

α -Aminoisobutyric acid is a non-metabolisable analogue of alanine and has been used to measure transport of amino acids in many cells in culture (Fehlmann *et al.*, 1981). Both parental cell lines and the one exemplary hybrid and cybrid studied exhibited an Na^{2+} -dependent α -aminoisobutyrate-transport capacity (Fig. 3). When expressed per mg of protein, the basal level of influx was greatest for the fibroblastic LEA-2A cell line and similar for BWIJ, HyLEB-7 and CyLEB-2. Insulin ($1 \mu\text{M}$) stimulated α -aminoisobutyrate influx in all four lines by about the same absolute increment, but owing to the differences in basal transport, this amounted to a 35% stimulation in HyLEB-7 ($P < 0.0005$) and only a 15% stimulation in LEA-2A ($P < 0.005$).

Dose-response curves for insulin were constructed for all four cell lines and are shown in Fig. 4. To compare the curves, the data for each cell line have been normalized as the percentage of maximal insulin effect for that particular line. Insulin stimulation was most sensitive in the BWIJ hepatoma and CyLEB-2; half-maximal stimulation occurred at about 0.3 nM -insulin. The dose-response curve for insulin stimulation of α -aminoisobutyrate influx in LEA-2A was parallel with that in BWIJ and CyLEB-2, but was about one order of magnitude to

Fig. 4. Dose-response curves for insulin stimulation of influx

The experiments were conducted as described in the legend to Fig. 3, but different insulin concentrations (indicated in the Figure) were used. The data plotted are the means of two separate experiments, each done in duplicate.

the right. This corresponds, at least qualitatively, to the differences in insulin binding in these cell lines.

The dose-response curve for the hybrid cell line HyLEB-7 was most noteworthy. The curve had a much more gradual slope than any of the others and thus appeared to start with the curve for one parent (BWIJ) and end with the curve for the other (LEA-2A) (Fig. 4). Although it is possible to analyse this curve as consisting of separate components similar to those of the two parental cell lines, the data are not of sufficient precision to warrant this interpretation.

Rb^+ uptake and glycogen synthase

Two other potential effects of insulin were examined in these cell lines. $^{86}\text{Rb}^+$ is a measure of $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase transport activity and is therefore present in virtually all cells. All four of the cell lines under study here also exhibited $^{86}\text{Rb}^+$ uptake in the basal state, and as with α -aminoisobutyrate influx, the basal level was slightly higher in the LEA-2A cells than in the other cell lines (Fig. 5). $^{86}\text{Rb}^+$ uptake was inhibited by about 70–80% by ouabain in all cell lines. In this system an insulin effect was apparent for only one of the two parental cell lines, BWIJ ($P < 0.005$). Insulin effects of a similar order of magnitude were demonstrable for both HyLEB-7 ($P < 0.001$) and CyLEB-2 ($P < 0.01$). Unfortunately, owing to the small amount of insulin stimulation, it was impossible to construct dose-response curves for these effects.

Glycogen synthase activity could be detected in both parental cell lines, and all hybrids and cybrids tested (Fig. 6). In the BWIJ hepatoma, however, the levels were very low, similar to what has been observed in foetal liver (Eisen *et al.*, 1973). CyLEB-

Fig. 5. Effect of insulin (INS) on $^{86}\text{Rb}^+$ uptake

Confluent cells were incubated with or without $0.1 \mu\text{M}$ -insulin for 1 h at 37°C in 1 ml of medium containing $1 \mu\text{Ci}$ of $^{86}\text{Rb}^+$ in the presence or absence of 1 mM -ouabain. After washing, cells were solubilized with SDS and the radioactivity measured. The total bar represents uptake in the absence of ouabain; the shaded area is uptake in the presence of ouabain. The data are the means of duplicate experiments. P values are as indicated; NS, not significantly different.

Fig. 6. Glycogen synthase activity in cell lines

Total glycogen synthase activity in each cell line was measured as described under 'Methods'. The proportion of the I-form was 10–20% of the total in all cases and did not change upon insulin treatment.

2 and -5A showed similar levels. LEA-2A and all three hybrid lines, on the other hand, had substantial levels of glycogen synthase. In many cells, insulin action can also be measured by its effect in converting the glucose 6-phosphate-dependent form (D-form) of the enzyme into the glucose 6-phosphate-independent form (I-form) (Thomas *et al.*, 1968). In both parental lines and in the cybrids and hybrids, the amount of I-form in the basal state was 10–20% of the total. Insulin had no effect on either the I-form percentage or total glycogen synthase activity in any of the cell lines tested.

Discussion

Insulin receptors are present on many tissues of mammals. In general, target tissues, such as liver, adipose cells and muscle, have relatively high numbers of receptors, whereas non-target tissues such as erythrocytes or circulating lymphocytes have few or no receptors. In cells that undergo differentiation in culture, insulin receptors and insulin-stimulated biological effects are expressed to various extents as would usually be predicted by the nature of the tissue. Thus in 3T3-L1 cells, which undergo an adipocyte-like differentiation, there is an increase in both insulin receptors and bioeffect with differentiation (Rubin *et al.*, 1978; Karlsson *et al.*, 1979), whereas, in Friend erythroleukaemia cells, insulin receptors decrease in number as the cells undergo erythroid differentiation (Ginsberg *et al.*, 1979). Thus insulin receptors and action behave as specific differentiated functions of target cells. The factors controlling the expression of these functions remain unknown.

One approach to understanding the control of expression of differentiated functions is somatic-cell genetics. Hybridization of somatic cells permits confrontation of two genomes in different functional states. Genetic analysis of corticotropin (Schimmer *et al.*, 1981), glucocorticoids (Pfahl & Bourgeois, 1980), and β -adrenergic (Coffino & Yamamoto, 1976) action has been conducted with lymphoid cell lines. At present little information on insulin binding or action in cell hybrids exists.

Straus & Williamson (1978) have studied insulin action in hybrids of mouse melanoma cells fused with mouse-embryo fibroblasts. Both parental cells and the hybrids exhibited only modest levels of insulin receptors. Insulin stimulated DNA synthesis in only one of the two parental cells, the fibroblast. However, insulin stimulation of DNA synthesis was also observed in the hybrids, leading to the conclusion that insulin action was dominant (Straus & Williamson, 1978).

Another interesting genetic study of insulin has been conducted by Pawelek and associates (Murray *et al.*, 1982), who used a series of melanoma cells selected for differential growth response to insulin: the wild-type being inhibited in its growth by insulin, whereas other variants exhibit stimulation of growth or no effect by insulin. Hybrids between insulin-dependent and insulin-resistant clones are generally insulin-dependent, suggesting again that insulin-dependence is dominant. Insulin inhibition of growth (wild-type) was, however, dominant over both insulin-dependence and -resistance. Most of these differences appear to be due to post-receptor events in insulin action, since differences in insulin binding to these cells were small. Also, since insulin stimulation of DNA synthesis may be mediated through receptors for the insulin-like growth-factor

receptors or the insulin receptor (King *et al.*, 1980), the interpretation of both of these experiments is limited.

In the present study we have prepared hybrids between hepatoma cells and fibroblastic L-cells and studied the expression of insulin receptors and the metabolic actions of insulin. Although the mouse hepatoma BWIJ exhibits the highest levels of insulin receptors of any cell in culture that we have studied, hybrids between BWIJ and LEA-2A cells exhibit almost no insulin binding, i.e., there is a marked extinction of this differentiated function. This is similar to the loss of other hepatic functions, such as albumin and AFP production, that occur in these hybrids (Kahn *et al.*, 1981). Thus, in a descriptive sense, the insulin receptor and other hepatoma-specific functions usually appear to be 'recessive', rather than 'dominant', in these hybrids. Extinction of a differentiated function is often observed in hybrids, and this has suggested that the cells which are not expressing the differentiated function produce diffusible substances whose final effects are negative, i.e., they prevent expression of the function (Weiss, 1976). This may be rather misleading, however, since the interpretation is entirely dependent on knowing whether the controlling factors act primarily as repressors or promoters for the expression of the differentiated function. Obviously more study will be needed to determine the exact mechanism in each case.

Insulin binding to cells is a function of both receptor concentration and affinity, and, as might be expected, these may be regulated independently (Bar *et al.*, 1979). Most of the loss of binding in hybrids as compared with the parent hepatoma is due to a decrease in receptor number. Hybrids, however, do bind slightly more insulin than the 'low-binding' parent (LEA-2A), and this appears to be due to a change in receptor affinity. This may be due to differences in the membrane environment or post-translational modifications in the receptor, such as the state of glycosylation (Hedo *et al.*, 1981; Ronnett & Lane, 1981). Evidence has also been presented for another membrane protein that may regulate insulin receptor affinity (Maturo & Hollenberg, 1978; Harmon *et al.*, 1980) and, if such a protein exists, it may well be under separate genetic control.

Metabolic effects of insulin in the hepatoma cell can be studied as stimulation of α -aminoisobutyrate and $^{86}\text{Rb}^+$ uptake. Both of these are effects expressed in normal freshly isolated hepatocytes and other differentiated hepatoma cells and were present in the BWIJ parent. LEA-2A cells, on the other hand, responded to insulin with an increase in α -aminoisobutyrate uptake (although with a less-sensitive dose-response curve), but failed to respond in terms of Rb^+ influx. Hybrids responded to insulin

both in terms of ion flux and amino acid uptake. In the case of α -aminoisobutyrate influx, the dose-response curve was noteworthy in that it appeared to be more spread out, beginning with that for the BWIJ cells and ending with that for the LEA. The fact that hybrids showed insulin-stimulated Rb^+ influx and a more-sensitive stimulation of α -aminoisobutyrate influx than the LEA-2A parent suggests that, despite the low numbers of receptors in both the hybrids and L-cell, there was improved coupling between the receptor and response in the hybrids. In the case of Rb^+ uptake this probably reflects changes at the membrane level, whereas stimulation of α -aminoisobutyrate influx requires intracellular metabolism and protein synthesis (Fehlmann *et al.*, 1981).

Neither parent nor the hybrid exhibited insulin-stimulated glycogen synthase. Interestingly, the total enzyme levels were lowest in the hepatoma parent and high in LEA-2A. Hybrids, like LEA, had high levels of enzyme, suggesting dominance of the 'less differentiated' state in the hybridization.

In contrast with the hybrids, cybrids prepared between BWIJ cells and enucleated LEA-2A behave, in all respects, like the nucleated BWIJ parent. These cybrids produce albumin and AFP, have large numbers of insulin receptors, are sensitive to insulin stimulation of α -aminoisobutyrate influx and Rb^+ uptake, and have low levels of glycogen synthase. Thus, in this fusion, which takes advantage of the chloramphenicol-resistance mutation in the mitochondrial DNA of the LEA-2A cell, there is no detectable alteration of insulin binding or action.

In summary, we find that in hybrids between two cells that express a very large difference in the insulin-receptor content, insulin receptors behave as a 'luxury' function, and expression is extinguished. This is not simply due to an alteration in the membrane upon fusion, since this extinction is not observed in cell cybrids. Insulin action in the hybrids and cybrids, on the other hand, appears to be the interaction of multiple factors. Many insulin effects are retained in the hybrids despite the loss in receptors. In general, however, the dose-response curves for insulin indicate that the hybrids that have lost receptors are less sensitive to hormonal stimulation than the parent hepatoma line. Further studies of the hybrids should help elucidate the mechanism of coupling between the insulin receptor and its intracellular response systems.

This work was supported by National Institutes of Health (NIH) grant AM 31036. M. F. was supported by a Fellowship (Fl. F05.TWO 3079-0) from the Fogarty International Center of the NIH.

References

- Bar, R. S., Harrison, L. C., Muggeo, M., Gorden, P., Kahn, C. R. & Roth, J. (1979) *Adv. Intern. Med.* **24**, 23–46
- Bradford, M. (1976) *Anal. Biochem.* **72**, 248–254
- Bunn, C. L., Wallace, D. C. & Eisenstadt, J. M. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1681–1685
- Cassio, D. & Weiss, M. C. (1979) *Somatic Cell Genet.* **5**, 719–738
- Coffino, P. & Yamamoto, K. R. (1976) in *Control Mechanisms in Cancer* (Criss, W. E., ed.), pp. 5766, Raven Press, New York
- Coon, H. G. & Weiss, M. C. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **62**, 852–859
- Eisen, H. J., Goldfine, I. D. & Ginsmann, W. H. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3454–3457
- Ephrussi, B. (1972) *Hybridization of Somatic Cells*, Princeton University Press, Princeton
- Fehlmann, M. & Freychet, P. (1981) *J. Biol. Chem.* **256**, 7449–7453
- Fehlmann, M., Morin, O., Kitabgi, P. & Freychet, P. (1981) *Endocrinology* **109**, 253–261
- Freychet, P., Kahn, C. R., Roth, J. & Neville, D. M., Jr. (1972) *J. Biol. Chem.* **247**, 3953–3961
- Gillham, N. W. (1978) *Organelle Heredity*, Raven Press, New York
- Ginsberg, B. H. (1977) in *Biochemical Actions of Hormones*, vol. 4 (Litwack, C. T., ed), pp. 313–349, Academic Press, New York
- Ginsberg, B. H., Brown, T. & Raizada, M. (1979) *Diabetes* **28**, 823–829
- Grun, P. (1976) *Cytoplasmic Genetics and Evolution*, Columbia University Press, New York
- Harmon, J., Kahn, C. R., Kempner, E. & Schlagel, W. (1980) *J. Biol. Chem.* **255**, 3412–3419
- Hedo, J., Kasuga, M., Van Obberghen, E., Roth, J. & Kahn, C. R. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4791–4795
- Kahn, C. R., Gopalakrishnan, T. V. & Weiss, M. C. (1981) *Somatic Cell Genet.* **7**, 547–565
- Karlsson, F. A., Grunfeld, C., Kahn, C. R. & Roth, J. (1979) *Endocrinology* **104**, 1383–1392
- King, G. L., Kahn, C. R., Rechler, M. M. & Nissley, S. P. (1980) *J. Clin. Invest.* **66**, 132–140
- Maturo, J. M., III & Hollenberg, M. D. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3070–3074
- Murray, M., Fleischmann, R. & Pawalek, J. (1982) *Cold Spring Harbor Conf.* **9**, 911–919
- Pfahl, M. & Bourgeois, S. (1980) *Somatic Cell Genet.* **6**, 63–74
- Ringertz, N. R. & Savage, R. E. (1976) *Cell Hybrids*, Academic Press, New York
- Ronnett, G. & Lane, M. D. (1981) *J. Biol. Chem.* **256**, 4704–4707
- Rubin, C. S., Hirsch, A., Fung, C. & Rosen, O. M. (1978) *J. Biol. Chem.* **253**, 7570–7578
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672
- Schimmer, B. P., Rae, P. A., Gutmann, N. J., Watt, V. M. & Tsao, J. (1981) *Cold Spring Harbor Conf. Cell Prolif.* **6**, 261–297
- Straus, D. S. & Williamson, R. A. (1978) *J. Cell Physiol.* **97**, 189–198
- Szipir, J. & Szipir, C. (1975) *Cell* **6**, 53–60
- Thomas, J., Schlender, K., & Larner, J. (1968) *Anal. Biochem.* **25**, 486–493
- Weiss, M. C. (1976) *Proc. Int. Congr. Hum. Genet. 5th, Mexico City* 284–292