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# INHIBITORY OR STIMULATORY EFFECT OF HUMAN GENES ON THE EXPRESSION OF ADRENAL FUNCTION IN HUMAN LEYDIG $\times Y_1$ CELL HYBRIDS

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**Summary**—In order to investigate the expression and the regulation of steroidogenesis, human Leydig cells were fused with a functional mouse adrenal cell line ($Y_1$). Six independent hybrid clones were analysed for hormone receptors and for cAMP and steroid response to ACTH, hCG, 8Br-cAMP or forskolin.

All hybrids had lost hCG receptors and their ability to produce testosterone. With respect to the response of adenylate cyclase to ACTH and/or forskolin, hybrids could be classed into two groups. In the first group, the pattern of response was qualitatively similar to $Y_1$ parental cells; The second group was far less responsive to ACTH than are $Y_1$ cells, and when added together, forskolin and ACTH only had an additive effect. All hybrids responded to ACTH and 8Br-cAMP with an increased production of pregnenolone ($P_5$). The amounts of $P_5$ produced both under basal conditions and following 8Br-cAMP stimulation were significantly higher in three hybrids when compared to $Y_1$ cells. However, the ability of two of these three hybrids to produce 20α-dihydroprogesterone (20α $OHP_4$) was very low. The metabolism of [$^{14}$C]$P_5$ revealed that in one of these hybrids, there was a loss of 3β-hydroxysteroid dehydrogenase/isomerase whereas in the other case, there was a low 20α-hydroxylase activity. The inhibition of cell growth by ACTH was related to the ability of the hormone to stimulate cAMP. Conversely, the inhibitory growth effects of 8Br-cAMP were not always inversely correlated with the ability of this nucleotide to stimulate $P_5$ production.

Since hybrids contained two mouse genomes and retained variable human chromosomes, these results suggest that extinction or enhancement of murine genes coding for some of the enzymes involved in steroidogenic response to ACTH was due to the regulation by human genes.

## INTRODUCTION

Somatic cell hybrids have provided a useful tool to examine the regulation of various differentiated functions such as liver function, hemoglobin synthesis and pigment formation [1–3]. In most cases interspecific crosses between expressing and non-expressing cells led to the extinction of the specialized function [1, 2, 4]. However, hybrids between hepatoma cells and cells of non hepatic origin have been shown to synthetize hepatic products of both parental species [5, 6] while the conservation of some of the steps of differentiated phenotypes have been observed [7]. In such hybrids the two parental cells generally originated from different lineages.

We investigated the behaviour of differentiated functions in hybrids constituted of two cells sharing the same embryonic origin by fusing human Leydig cells with the mouse adrenal cell line $Y_1$. Leydig cells possess LH-hCG receptors and respond to this hormone by increasing their cAMP and testosterone production [7]. On the other hand, $Y_1$ cells possess ACTH receptors: ACTH stimulates their cAMP and steroid synthesis and inhibits their cell multiplication [8–11]. None of the six independent hybrids analysed could express some of the specific Leydig cell functions. Differences in the pattern of expression of adrenal function between hybrids were found and these differences may be related to human gene regulation. This hybridization thus gave rise to the establishment of permanent cell lines defective in some steroidogenic steps while ACTH increases their cAMP and steroid production and inhibits their cell growth.

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## EXPERIMENTAL

### *Materials*

ACTH-(1-24) was a gift from Dr Rittel (Ciba, Basel). hCG (CR 121) was a gift from Dr E. Canfield. Iodination of hCG was performed by the lactoperoxidase method as described [12]. [$^{14}$C]Pregnenolone (S.A. 49 mCi/mmol) and $^3$H-labelled pregnenolone, progesterone, 20α-dihydroprogesterone were obtained from Amersham, England. Polyethylene glycol 1000 was obtained from Beckin Chemicals Co. (Philipsburg, NJ). WIN 24540 (4α-5-epoxy-17β-hydroxy-5α-androstane-2α-carbonitrile) was purchased from Winthrop laboratory.

*Cells*

Functional mouse adrenocortical cells used in this study were derived from the $Y_1$ mouse tumour cell line [8] and were provided by Dr B. Schimmer (Toronto). They were thymidine kinase deficient ($TK^-$) and resistant to $2.10^{-6}$ M ouabain.

Cells were routinely cultured in RPMI 1640 medium (Gibco Bio Cult., Glasgow, Scotland) supplemented with 10% fetal calf serum, 100 IU/l penicillin and 6 $\mu$g/ml streptomycin in a humidified 5% $CO_2$–95% air atmosphere at 37°C.

Human Leydig cells were prepared from testes of newborn infants (who died 2 days after birth) by the method previously described [7]. These cells possessed LH-hCG receptors and responded to hCG by increasing their cAMP and testosterone production [7]. They were cultured in RPMI 1640 medium containing 0.1% fetal calf serum, insulin (5 $\mu$g/ml), transferrin (5 $\mu$g/ml) and vitamin E (0.2 $\mu$g/ml) for 3 days. They could not grow when $2.10^{-6}$ M ouabain was added to the medium.

*Cell hybridization*

Hybridization was performed on parental monolayer cell mixture (ratio 1:1) using polyethylene glycol (PeG 1000), [13]. Forty-eight hours after fusion, hybrid populations were grown in HAT medium (Hypoxanthine 0.1 mM, aminopterine 0.01 mM, thymidine 0.04 mM), containing 10% fetal calf serum (FCS) so that parental mouse cells, thymidine kinase deficient, could not grow. Human cells were selected against by ouabain $2.10^{-6}$ M [13]. Hybrid clones became visible within 20–30 days and a single hybrid was selected from each flask. After four to six passages, ouabain was removed from the culture medium but HAT selection was maintained during the whole experiment.

*Chromosome studies*

Chromosome studies and steroid production analysis were performed on cells originating from the same subculture: chromosomes were analysed after R and G banding [14, 15] and approx 15 metaphases were counted for each preparation.

*cAMP and steroid production*

Cells were incubated at 37°C in the presence of 1-methyl-3-isobutylxanthine (0.5 mM) with or without the effector at the concentrations indicated in figures. An aliquot of the medium was taken at 1 h for cAMP determination, and the incubation continued for 3 additional hours. At the end of the incubation, the remaining medium was saved for steroid determination. cAMP, $P_5$(*), 20$\alpha$-$OHP_4$ and testosterone were determined in the culture medium by specific radioimmunoassays [18–21]. When $P_5$ production was measured, cells were incubated with $5.10^{-6}$ M WIN 24540 and $10^{-5}$ M spironolactone in order to inhibit the 3$\beta$-hydroxysteroid dehydrogenase and 17$\alpha$-hydroxylase respectively [16, 17].

*[$^{14}C$]-Pregnenolone metabolism*

Cells were incubated with $10^{-7}$ M ACTH for 24 h, then the medium was removed and replaced by fresh medium containing $5.10^{-5}$ M [$^{14}$C]$P_5$ (S.A. = 50 mCi/mmol). After 2 h at 37°C, the medium was removed and stored at −20°C.

Before extraction, $^3$H-labelled and unlabelled (50 $\mu$g) $P_5$, $P_4$, 20$\alpha$-$OHP_4$, 17-$OHP_5$, 11$\beta$-20$\alpha$-$OHP_4$ and testosterone were added. The medium was subsequently extracted 3 times with 3 vol of ethyl acetate.

The dry extracts dissolved in 50$\mu$l acetonitrile were chromatographed in high pressure liquid chromatography using a Waters chromatograph equipped with a C-18$\mu$ Bondapak column. The column was eluted with a gradient of water–acetonitrile beginning with 70% water in acetonitrile to pure acetonitrile. The gradient was an exponential concave curve generated by a programmer (model 660 Waters). The running time was 90 min and the flow rate 0.8 ml/min. Fractions of 0.4 ml were collected. $\Delta^4$-3-Ketosteroids were detected at 240 nm by a spectrophotometer (Fig. 1). The fractions corresponding to each peak were pooled and purified as follows: Peak i, which contained $P_4$ and $P_5$ was submitted to thin layer chromatography using the chloroform–acetone (96:4) solvent system. After two migrations, the areas corresponding to $P_4$ and $P_5$ were eluted and counted. 17$OHP_5$ and 17$OHP_4$, 11$\beta$-20$\alpha$$OHP_5$ and 11 keto-20$\alpha$$OHP_4$, 20$\alpha$$OHP_5$ and 20$\alpha$$OHP_4$ were purified as described [22].

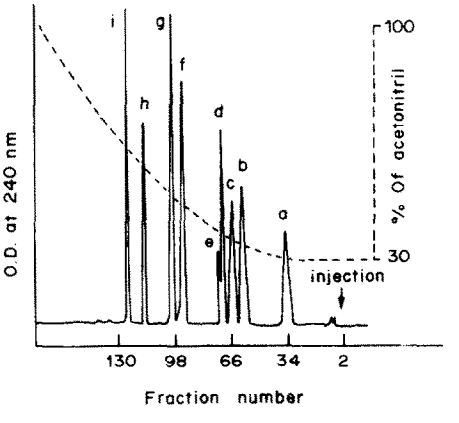


Fig. 1. Chromatographic separation of steroids by HPLC using a C-18 $\mu$ Bondapack column. The column was eluted with a gradient of water-acetonitrile (---). a = cortisol, b = corticosterone, c = 11-deoxycortisol, d = 11$\beta$-20$\alpha$-$OHP_4$, e = 11 ceto-20$\alpha$-$OHP_4$, f = deoxycorticosterone, g = 17$OHP_5$ and 17$OHP_4$, h = 20 $OHP_5$ and 20$\alpha$-$OHP_4$, i = $P_5$ and $P_4$.

**Abbreviations*: $P_5$ = pregnenolone; $P_4$ = progesterone, 20$\alpha$$OHP_5$ = 20$\alpha$-dihydropregnenlone; 20$\alpha$$OHP_4$ = 20$\alpha$-dihydroprogesterone; 17$OHP_5$ = 17$\alpha$-hydroxypregnenolone; 17$OHP_4$ = 17$\alpha$-hydroxyprogesterone; 11$\beta$-20$\alpha$-$OHP_4$ = 11$\beta$-hydroxy-20$\alpha$-dihydroprogesterone; 11 keto 20$\alpha$ $OHP_4$ = 11 keto-20$\alpha$-dihydroprogesterone.

*Tumorigenicity*

To determine whether hybrid cells retained the adrenal parental capacity to produce tumors, $5.10^6$ parental or hybrid cells were injected subcutaneously into, at least, 5 adult isogenic LAF mice. Hybrid cell lines were considered as non-tumorigenic when no tumor was observed 3 months after injection.

*Cell growth*

Cells were seeded at low density (10,000–30,000 cells/cm$^2$) in RPMI medium containing 5% fetal calf serum. The next day the medium was removed and replaced by a fresh one with or without ACTH ($10^{-8}$ M) or 8-bromo cyclic AMP (8br-cAMP) ($10^{-3}$ M). The cells in triplicate dishes for each culture condition were counted daily for 6 days using a Coulter counter.

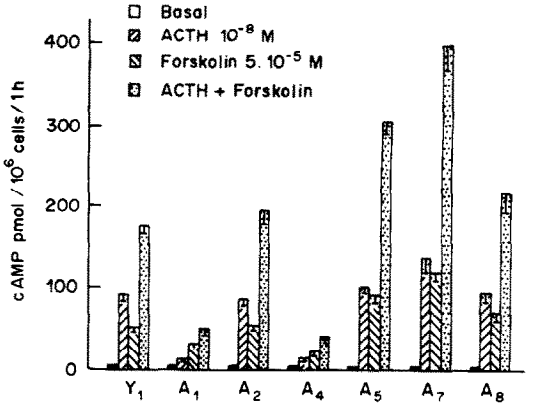


Fig. 2. cAMP production by $Y_1$ and hybrids. $10^6$ cells were incubated for 1 h in the absence (▣) or presence of $10^{-8}$ M ACTH (▨), $5 \times 10^{-5}$ M forskolin (▧) or both (■). The results are the mean ± SD of triplicate determinations of three different dishes.

## RESULTS

Six independent aminopterin and ouabain resistant clones were selected and developed. They contained a quasi tetraploid set of mouse chromosomes and only one incomplete complement of human chromosomes. They therefore derived from fusion which involved two murine cells and a single human cell. With the exception of clone $A_4$, they segregated human chromosomes at random excepting chromosome 17 which was selected for by HAT medium. Six to ten human chromosomes per cell were retained by clones $A_1$, $A_5$ and $A_7$, only one for $A_2$ and $A_8$ and none for $A_4$ cells. Tumorigenicity assays revealed that only $Y_1$ parental cell line and clone $A_4$ were able to induce tumors when injected in isogenic mice. However, clone $A_4$ was able to proliferate in HAT medium.

*cAMP production under several conditions*

None of the hybrids increased their basal cAMP production following hCG stimulation (data not shown). This refractoriness was probably due to a lack of hCG receptors since non-specific binding of $^{125}$I-hCG could be demonstrated (data not shown). On the contrary, ACTH was able to stimulate cAMP production by each hybrid but the magnitude of the response varied from hybrid to hybrid (Fig. 2). Indeed, the response of $A_1$ and $A_4$ was lower than that of parental cell line $Y_1$, which in turn was lower than that of hybrids $A_5$ and $A_7$. The absolute value of cAMP production following ACTH stimulation varied from one experiment to another, but a similar pattern of response was observed in four different experiments.

The response to forskolin alone or added together with ACTH was investigated. ACTH stimulated cAMP production of $Y_1$ and $A_2$ cells was significantly higher than that induced by the diterpen ($P < 0.05$) whereas in clones $A_1$ and $A_4$, the opposite was observed. In other hybrids these two effector activities were similar.

With respect to the response to ACTH plus forskolin, hybrids could be classed in two groups. In the first one, which included $A_2$, $A_5$, $A_7$ and $A_8$, a synergistic effect was observed and the pattern of response was qualitatively similar to that of $Y_1$ cells. However, this synergistic effect of forskolin and ACTH was more pronounced in clones $A_5$ and $A_7$ than in $Y_1$ cells. In hybrids of the second group ($A_1$ and $A_4$), ACTH plus forskolin induced only an additive effect.

*Production of 20α-dihydroprogesterone and testosterone*

The main steroid produced by $Y_1$ cells is 20αOHP$_4$ [21] while the main steroid produced by human Leydig cells is testosterone [7]. Therefore, the secretion of both steroids was measured following 4 h incubation in the absence or presence of ACTH-[1–24], hCG or 8-Br-cAMP. By using a specific and sensitive assay [18], testosterone could not be detected under any experimental conditions. On the other hand, 20αOHP$_4$ was detected. The results of a representative experiment, out of five, are shown in Fig. 3. Basal production was low, except in hybrid $A_1$, which was 10–15 times higher than that of parental cells $Y_1$. hCG was unable to stimulate 20αOHP$_4$ in any clone. Both ACTH-(1–24) and 8Br-cAMP induced a 8- to 10-fold increase in 20α-OHP$_4$ production by $Y_1$ cells, and by $A_2$ and $A_5$ hybrids. Steroid production by $A_1$ and $A_8$ hybrids following 8Br-cAMP stimulation was significantly higher than that of $Y_1$ cells. However, the response of these two hybrids to ACTH was significantly lower than that observed after 8Br-cAMP. Finally, $A_4$ and $A_7$ hybrids did not respond to either ACTH or 8Br-cAMP.

Two hypotheses may explain the differences observed in 20αOHP$_4$ production by hybrids: either a

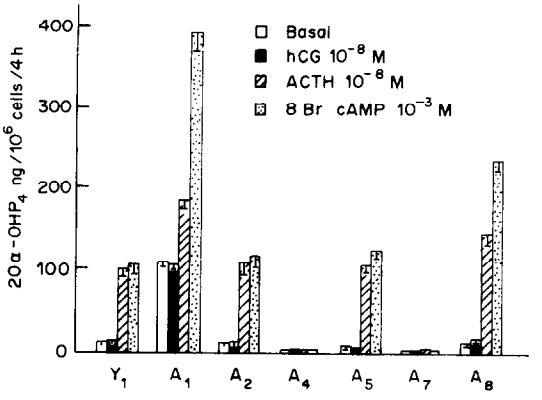


Fig. 3. 20α-OHP$_4$ production by $Y_1$ and hybrids. $10^6$ cells were incubated for 4 h at 37°C in the absence (□) or presence of $10^{-8}$ M hCG (■), $10^{-8}$ M ACTH (▨) or $10^{-3}$ M 8Br-cAMP (▩). The results are the mean ±SD of triplicate determinations of three different dishes.

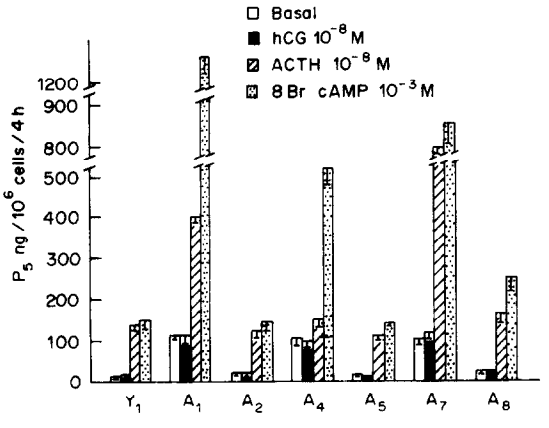


Fig. 4. $P_5$ production by $Y_1$ and hybrids. $10^6$ cells were preincubated with WIN 24540 ($10^{-6}$ M) and spironolactone ($10^{-5}$ M) for 15 min at 37°C. Then, the cells were further incubated for 4 h in the absence (□) or presence of $10^{-8}$ M hCG (■) or $10^{-8}$ ACTH (▨) or $10^{-3}$ M 8Br-cAMP (▩). THe results are the mean ±SD of triplicate determinations of three different dishes.

modification of their capacity to produce pregnenolone or an inability to transform this steroid in 20αOHP$_4$. We therefore investigated these two possibilities.

*Production of pregnenolone*

The production of this steroid was studied in the presence of inhibitors of pregnenolone metabolism. Under these conditions, less than 10% of $P_5$ was metabolized. The results of a representative experiment out of seven are shown in Fig. 4. Basal $P_5$ production by $Y_1$ cells was similar to that of hybrids $A_2$, $A_5$ and $A_8$ but lower than that of $A_1$, $A_4$ and $A_7$. Again hCG had no effect on $P_5$ production. Both ACTH and 8Br-cAMP stimulated 6- to 10-fold $P_5$ production by $Y_1$. The maximal response of $A_2$, $A_5$ and $A_8$ was close to that of $Y_1$ parent, whereas that of three other hybrids ($A_1$, $A_4$ and $A_7$) was significantly higher. However, the response of $A_1$ and $A_4$ to ACTH was lower than that to 8Br-cAMP.

*Metabolism of [$^{14}$C]pregnenolone*

After a 24 h ACTH incubation, cells were incubated with [$^{14}$C]$P_5$ for 2 h without the hormone and metabolites analyzed as described in Experimental. The results (Table 1) show that about 60% of $P_5$ was metabolized by $Y_1$ and $A_1$ cells. However, this metabolism was higher in $A_2$ and $A_8$ cells, and lower in $A_5$ and $A_7$ and particularly in $A_4$, which metabolized only 2% of $P_5$. The main metabolite formed by $Y_1$ was 20α-OHP$_4$, confirming previous results [9, 19]. This was also the case with $A_2$ and $A_5$ hybrids, whereas in $A_1$ and $A_8$, $P_4$ was the main product. Finally, all hybrids had almost completely lost the 11β-hydroxylase activity. It must be pointed out that, except in clones $A_4$ and $A_5$, the addition of radioactivity found in the metabolites indicated in Table 1, did not account for the [$^{14}$C]$P_5$ added. This was more evident in $A_2$ and $A_7$ clones. These results indicate that a part of [$^{14}$C]$P_5$ was metabolized into other compounds. Indeed, the profile of the HPLC showed two other main radioactive peaks which represented between 0.5–7% of [$^{14}$C]$P_5$ added. The identification of these compounds is in progress.

*Effect of ACTH and 8Br-cAMP on cell growth*

Generation times of $Y_1$ cells was about 22 h (Fig. 5). A 6 day treatment with ACTH or 8Br-cAMP reduced $Y_1$ cell multiplication by 70 and 87% respectively, confirming previous results [10, 11, 23]. The proliferation of $A_2$ and $A_5$ (data not shown) and $A_8$ hybrids (Fig. 5), both under basal conditions and in the presence of ACTH or 8Br-cAMP was similar to that of parental cell $Y_1$. On the other hand, $A_1$, $A_4$ and $A_7$ hybrids were resistant to the growth inhibitory effect of ACTH. Moreover $A_7$ was partially resistant to the growth inhibitory effect of 8Br-cAMP, while the nucleotide killed $A_1$ cells within 2 days.

Table 1. Metabolism of [$^{14}$C]pregnenolone by Y-1 and hybrid cells

| | Y-1 | $A_1$ | $A_2$ | $A_4$ | $A_5$ | $A_7$ | $A_8$ |
|---|---|---|---|---|---|---|---|
| Pregnenolone | 41 | 40 | 25 | 98 | 70 | 58 | 17 |
| Progesterone | 8 | 39 | 3 | 1.2 | 2.1 | 9 | 47 |
| 20α-Dihydroprogesterone | 20 | 8 | 27 | 0.7 | 24 | 0.5 | 24 |
| 11β-Hydroxy-20α-dihydroprogesterone | 14 | 0.3 | <0.01 | <0.01 | 0.2 | <0.01 | 0.2 |

Cells were incubated with [$^{14}$C]pregnenolone for 2 h at 37°C. The steroids in the medium were analyzed as indicated in Experimental. The results are expressed as % of the radioactivity found in the medium.

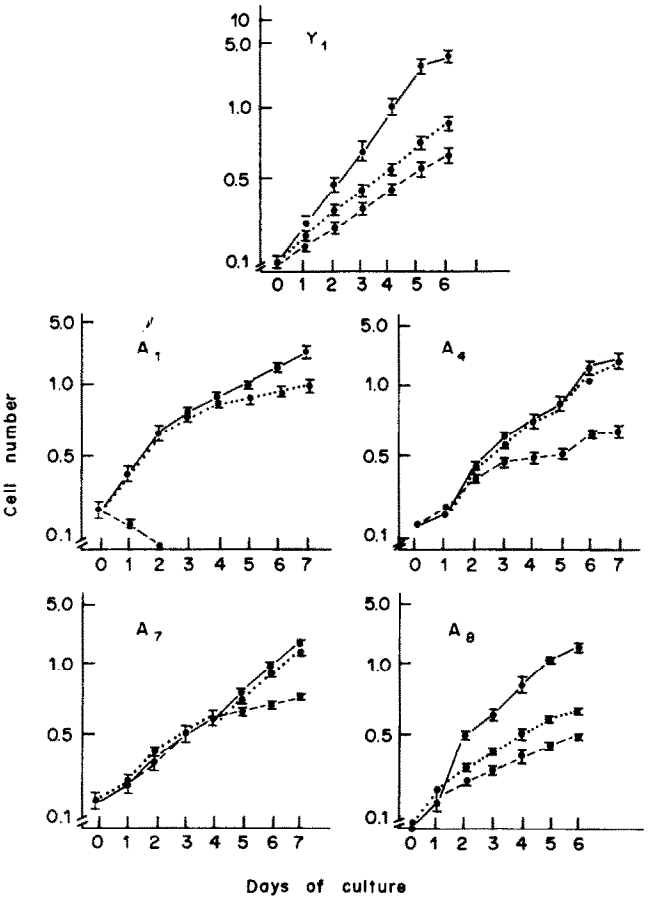


Fig. 5. Effects of ACTH ($10^{-8}$ M) or 8Br-cAMP ($10^{-3}$ M) on cell growth. Cells were plated at low density (10,000–30,000 cells/cm$^2$) in medium containing 5% fetal calf serum. The following day, the medium was removed and replaced by fresh medium without (——) or with $10^{-8}$ M ACTH (·····) or 8Br-cAMP (– – –). The medium was changed daily. On the days indicated, the cells in three dishes for each condition were counted.

## DISCUSSION

This study was an attempt to understand the mechanisms of cell differentiation in adrenal and Leydig cells by the means of cell hybridization. Fusion of human Leydig cells with the mouse adrenal cell line $Y_1$ led to the isolation of six independent hybrid clones.

The hybrid nature of five out of the six clones isolated in the present study was inferred by their ability to grow in selective medium, by their inability to produce tumor when injected in isogenic mice and by the presence of human chromosomes. On the other hand, clone $A_4$ fulfilled only one of these requirements, that is, the ability to grow in selective medium. This discrepancy could be due either to a complete loss of human chromosomes with a reverse mutation of thymidine kinase gene or to the retention of some human genes, including that of thymidine kinase and their subsequent recombination with mouse chromosomes. Nevertheless, the hybrid nature of this clone is likely when one considers the many biochemical differences which exist between $A_4$ and parental $Y_1$ cell.

None of the six hybrids retained functional LH-hCG receptors or the capacity to produce testosterone, which involves three enzymes (17α-hydroxylase, 17-20 lyase and 17-ketoreductase). Indeed, all the clones had lost the 17α-hydroxylase activity since neither 17α-$OHP_5$ nor 17α-$OHP_4$ was detected following their incubation with [$^{14}$C]$P_5$. It is difficult to determine whether the loss of human chromosomes alone was responsible for the failure to express some of the specific Leydig cell functions or whether the murine genome exerted a negative regulation on the human function since the ploidy of the mouse partner was much higher than that of the human parent.

The study of expression of adrenal function was more instructive. Analysis of cAMP production after ACTH or forskolin or ACTH plus forskolin stimulation showed different patterns of response among the hybrids.

In normal adrenal cells, as well as in $Y_1$ cells, ACTH is more potent than forskolin, and ACTH plus forskolin had a synergistic effect on cAMP production [24]. The response pattern of hybrids $A_2$, $A_5$, $A_7$ and $A_8$ was qualitatively similar to that of $Y_1$

parent while clones $A_1$ and $A_4$ were far less sensitive to both ACTH and forskolin, and, when added together, there was only an additive effect. It is unlikely that these differences could be due to an increased phosphodiesterase activity since the stimulations were always performed in the presence of a phosphodiesterase inhibitor. The most likely explanation for these alterations is a lesion of either ACTH receptors and/or of the coupling between hormone–receptor complex and adenylate cyclase. Indeed $A_1$ and $A_4$ clones might be compared to $Y_1$ mutants in which ACTH has little or no effect on cAMP production and whose adenylate cyclase remained sensitive to other effectors [25].

The cholesterol side chain cleavage activity was maintained in each clone, since all of them were able to produce pregnenolone. The capacity to produce this steroid by clones $A_1$, $A_4$ and $A_7$ was significantly higher ($P < 0.001$) than that of $Y_1$ cells. It is interesting to note that $P_5$ production by $A_1$ and $A_4$ cells after ACTH stimulation was lower than that after 8Br-cAMP, indicating that in these two clones, the rate limiting step in $P_5$ formation, after ACTH, was cAMP production.

The inability of $A_4$ and $A_7$ cells to produce $20\alpha OHP_4$ seems to be due to two different alterations. In $A_4$, the explanation is the loss of $3\beta$-hydroxysteroid dehydrogenase/isomerase activity. A decrease or loss of this enzymatic activity has been often observed in human adrenocortical tumors [Review in 26], in rat adrenocortical cells transformed by Kirsten murine sarcoma virus [27] and in $Y_1$ cells transformed by simian adenovirus SA-7 [20]. Moreover, since in normal and virus-transformed $Y_1$ cells $20\alpha$-reductase can utilize $P_5$ as substrate [22], it is likely that $A_4$ cells had also lost this activity. $A_7$ cells were able to transform $P_5$ into $P_4$, but very small amounts of this steroid were transformed into $20\alpha OHP_4$, indicating an almost complete loss of $20\alpha$-hydroxylase activity. A decreased activity of this enzyme was probably present in $A_1$ and $A_8$, as indicated by the accumulation of $P_4$.

*In vitro*, ACTH inhibits adrenal cell DNA synthesis [10, 11, 23, 28–31]. This effect seems to be mediated by cAMP and cAMP-dependent protein kinase, since the nucleotide and its derivatives produced the same effects [10, 23, 29, 31] and since mutants of $Y_1$ cells with defects in cAMP-dependent protein kinase were resistant to growth inhibition by cyclic nucleotides [11, 32]. Moreover, in some of these mutants the steroidogenic effects of ACTH and cAMP derivatives correlated closely with their growth inhibitory effects [32]. The mediator role of cAMP on ACTH-induced growth cell inhibition is supported by our results in clones $A_2$, $A_5$ and $A_8$. In addition, this hypothesis explains why $A_1$ and $A_4$ clones were resistant to ACTH (low cAMP production) and sensitive to 8Br-cAMP. However, this hypothesis does not explain the results observed for clone $A_7$, which was resistant to the growth inhibitory effect of both ACTH and 8Br-cAMP, but sensitive to their steroidogenic action. This discrepancy suggests a defect of some step beyond protein kinase activation involved in growth inhibition but not in the steroidogenic action of ACTH and 8Br-cAMP.

A mutual extinction has generally been observed in hybrids originating from two parental cells expressing different specific functions according to the law of exclusivity [4]. We report here not a complete extinction but rather a differential expression of murine genome associated with the retention of human chromosomes. Mouse genes coding for enzymes involved in steroidogenesis are probably subjected to a control by the remaining human genes; such a negative regulation reflects normal mechanisms responsible for cell differentiation and acts probably via diffusible substances [33].

Interspecific hybridization of human Leydig cells with a non-differentiated murine cell line has recently allowed the isolation of hybrids retaining at least two steps of Leydig cell steroidogenesis and to study the regulation of one of these enzymes [7]. Conversely, by crossing two parental cells sharing the same embryonic origin, we obtained permanent cell lines lacking some steps of $Y_1$ cell specific functions. Our results thereby show the independence of extinction of these steps.

In summary, our results show that the expression and regulation of steroidogenic enzymes are controlled by different mechanisms since they are independently expressed among hybrids. We believe that somatic hybridization can provide valid experimental models for exploring regulation of steroidogenic cells.

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