EXPRESSION OF BENZ[a]ANTHRACENE-INDUCIBLE ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN MOUSE-HAMSTER AND MOUSE-HUMAN SOMATIC-CELL HYBRIDS

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SUMMARY. Aryl hydrocarbon hydroxylase (AHH) activity is inducible in mouse 3T3 fibroblasts by benz[a]anthracene, whereas no detectable basal or inducible levels occur in hamster BHK cells and barely detectable inducible levels of this enzyme are found in human D98 cultures. AHH induction is present--at about the same degree as that in the mouse parent line--in 6 of 9 mouse-hamster hybrids and in 3 of 3 mouse-human hybrids. AHH induction is gene-dose dependent in the only mouse-hamster clone having an excess of mouse chromosomes.

INTRODUCTION. Aryl hydrocarbon hydroxylase (AHH) is an example of the membrane-bound multicomponent mono-oxygenases, or so-called "drug-metabolizing enzymes," which require NADPH, NADH, and molecular oxygen for the oxidative metabolism of drugs, insecticides, polycyclic hydrocarbons, and many lipophilic endogenous substrates (1-4). Recent studies from this laboratory have shown (5-9) that the expression of AHH induction by aromatic hydrocarbons segregates *in vivo* as a simple autosomal dominant trait in the mouse. In fact, this genetically determined difference in AHH induction can be detected *in utero* and in cell culture (6, 9).

The fusion of mammalian cells in culture (10-12) may also provide valuable information for studying the mechanism of enzyme induction in eukaryotic cells. More specifically, how would AHH induction by expressed in hybrids formed by the combination of a parent line in which there is little or no inducible enzyme activity? We have found that AHH induction by aromatic hydrocarbons such as

benz[a]anthracene (BA) occurs in mouse 3T3-4(e) cells, whereas there are no detectable basal or inducible levels of the enzyme activity in hamster BHK(T6a) cells and very low inducible levels of AHH activity in human D98/AH2 cells. In this paper we report on the expression of BA-inducible AHH activity in various hybrids formed by fusion of the mouse parent with either the hamster or the human parent line.

MATERIALS AND METHODS

The chromosomal complement has been thoroughly characterized for several somatic-cell hybrids formed by fusion of the 3T3 mouse line and the BHK hamster line (13) and for hybrid HLE-cl-C, formed by combination of the 3T3 mouse line and the human D98 line (14). These clones were very kindly provided for us by Dr. Howard Green, Massachusetts Institute of Technology, Cambridge, Mass. Clone 3T3-4(E) is deficient in thymidine kinase, and both clone BHK(T6a) and clone D98/AH2 lack hypoxanthine phosphoribosyltransferase. Thus, any mouse-hamster or mouse-human cell hybrid inheriting the simultaneous expression of both enzymes (12) is able to grow in the presence of 100 μM hypoxanthine, 0.40 μM methotrexate, and 16 μM thymidine in Eagle's medium (HMT medium). After the clones had been received from Dr. Green and grown in our laboratory for 2 to 3 weeks, chromosomal analyses (15) were repeated on the parent lines and on randomly chosen hybrids at the same time as AHH induction was determined. Because essentially no differences were found between the chromosomal complements previously reported (13) and our chromosomal preparations, we have used in this report the former data for estimating the chromosomal contribution in the mouse-hamster hybrids. Two additional clones of a 3T3-4(E) x D98/AH2 fusion were isolated by techniques similar to those (14) previously described, and these hybrids are designated DHE-cl-1 and DHE-cl-2. The chromosomal content of these clones was examined as described previously (15). All of the hybrid clones were maintained in HMT medium so as to prevent revertant cells from appearing and to minimize further chromosomal changes (12). Other chemical reagents, tissue culture materials, and procedures for growing the cells and treating the cultures

TABLE I. RELATIONSHIP BETWEEN CHROMOSOMAL COMPLEMENT AND MAXIMALLY INDUCIBLE AHH ACTIVITY IN THE MOUSE 3T3 AND THE HAMSTER BHK PARENT LINES AND IN THEIR HYBRID CLONES

Designated name of cell line		Estimated number of chromosomes[a]		Specific AHH activity after BA
		Mouse origin	Hamster origin	
Parents				
3T3-4(E)		68	0	2.9[b]
BHK(T6a)		0	64	< 0.02
Hybrids				
1. balanced	1B	59	36	1.5
	7A	65	37	2.8
2. mouse excess	7B	115	48	6.2
3. slight hamster excess	2A	33	73	1.8
	8A	39	70	2.1
4. marked hamster excess	4C	36	131	2.1
	4B	39	95	< 0.02
5. hamster excess and mouse deficiency	3B	16	114	< 0.02
	6B	9	92	< 0.02

[a]Estimates as previously reported (13).

[b]The basal specific AHH activity ranged between 0 and 0.10 for the 3T3 parent and the clones having the inducible AHH activity and was not necessarily proportional to the maximally inducible level. The fully induced AHH specific activity in 3T3 cells ranged between 1.5 and 2.9 in 9 separate experiments.

with BA have been described in detail (3).

Both AHH activity and protein concentration were determined in duplicate from the homogenate of 1×10^6 to 6×10^6 cells grown in one 100-mm cell culture dish (3). One unit of AHH activity is defined (3) as that amount of enzyme catalyzing per min at 37° the formation of hydroxylated product causing fluorescence equivalent to that of 1 picomole of 3-hydroxybenzo[a]pyrene. Specific AHH activity is therefore expressed as units per mg of cellular protein. No difference in AHH induction by BA was found between any of the cultures grown in regular Eagle's medium and any of those grown in HMT medium.

RESULTS AND DISCUSSION

Maximally inducible levels of AHH activity in the mouse 3T3 parent line and

TABLE II. RELATIONSHIP BETWEEN CHROMOSOMAL COMPLEMENT AND MAXIMALLY INDUCIBLE AHH ACTIVITY IN THE MOUSE 3T3 AND THE HUMAN D98 PARENT LINES AND IN 3 HYBRIDS

Cell Line	Chromosomal mode (and range)[a]	Bi-armed chromosomes	Estimated number of human chromosomes	Estimated number of mouse chromosomes	Specific AHH activity after BA
Parents					
3T3-4(E)	68 (68-69)	0	0	68	2.9[b]
D98/AH2	63 (61-64)	51	63	0	0.40
Hybrids					
HLE-c1-C	161 (142-176)	17	21[c]	140[d]	3.0
DHE-c1-1	73 (72-76)	7	9	64	2.6
DHE-c1-1	75 (74-79)	8	10	65	1.5

[a]A minimum of 20 metaphases was evaluated for each parent line and 10 metaphases for each hybrid. The range is shown in parentheses.

[b]The basal specific AHH activity ranged between 0 and 0.10 for both parents and the 3 hybrid clones and was not necessarily proportional to the maximally inducible level.

[c]Approximated by counting the number of bi-armed chromosomes per metaphase, and then solving the equation $x/b = 63/51$, where b = the number of bi-armed chromosomes, and x = the total number of chromosomes of human origin.

[d]Estimated by the difference between the total number of chromosomes and the number of chromosomes estimated to be of human origin.

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in all hybrids were found after 4 to 5 days of continuous treatment of the cultures with 13 μM BA; the medium was always replaced daily with fresh inducer-containing medium. Table I shows the maximally induced AHH activities in the mouse and hamster parent lines and in 9 mouse-hamster hybrids. Clones 1B and 7A which contained a balanced chromosomal complement, clones 2A and 8A which had a slight hamster excess, and clone 4C which possessed a marked hamster excess, demonstrated the same approximate extent of BA-inducible AHH activity as that of the mouse 3T3 parent. Hybrid 7B, the only clone with approximately a 2S complement of mouse chromosomes, possessed twice the level of inducible AHH activity found in the 3T3 parent line. AHH induction therefore appeared to be 3T3 gene-dose dependent in this particular hybrid. Clone 4B which had a marked hamster chromosomal excess, and clones 3B and 6B which had a marked hamster excess coupled with a deficiency of mouse chromosomes, had no inducible AHH activity.

Table II shows the magnitude of AHH induction in the mouse and human parent lines and in 3 mouse-human hybrids. The HeLa-derived D98/AH2 parent displayed some inducible AHH activity. The hydroxylase activity in 2 of the 3 hybrid clones was induced to levels similar to those found in the mouse parent, and the induced enzyme in the third clone was at least half as high as that of the 3T3 parent line. If one assumes random chromosomal loss, then approximately two-thirds of the total human chromosomes found in the parent D98/AH2 line (i.e., 63) are most likely present at least once in one of these 3 hybrids. Thus, most human gene products found in the human parent line in which AHH induction was very low may be expected to exist in one or another of these hybrids.

These data demonstrate the dominant expression of BA-inducible AHH activity. In contrast to other enzyme induction studies involving fused-cell hybrids: the suppression of glycerol-3-phosphate dehydrogenase induction (16) in glial cell-fibroblast combinants by hydrocortisone and the suppression of tyrosine aminotransferase induction (17-20) by steroids in hybrids between hepatoma cells having the inducible enzyme and other cell lines having no inducible tyrosine aminotransferase activity. This apparent dominant expression and gene-dose dependence of AHH induction has also been found (20) in hybrids formed by the fusion of mouse 3T3 fibroblasts and rat hepatoma tissue culture (HTC) cells, the latter having no detectable basal or inducible AHH activity. AHH induction *in vivo* by aromatic hydrocarbons (5-9) is presumably the first example in mammalian genetics wherein the induction of enzyme activity is regulated by one chromosome and perhaps by genes at a single locus. We may be observing this same effect among these hybrids in cell culture. BA-inducible AHH activity either is present--in 6 of the 9 mouse-hamster hybrids and in all 3 of the mouse-human hybrids--to about the same extent as that in the mouse parent line or is totally absent--as in 3 of the mouse-hamster hybrids. And, 2 of the 3 hybrids lacking the inducible AHH activity were markedly deficient in mouse chromosomes. It will be of interest to see if any other traits as they are demonstrated to

be dominant *in vivo* will also be expressed dominantly in the appropriate somatic-cell hybrids.

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