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Chromosome 3q (22-ter) Encodes the Human Transferrin Receptor

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SUMMARY

The human transferrin receptor is an integral membrane glycoprotein of 180,000 molecular weight (mol. wt.) formed from two subunits of 90,000 mol. wt. A clone panel of Chinese hamster-human somatic cell hybrids was screened using a single cell plating cytotoxicity assay and rabbit antiserum raised to purified human transferrin receptor. Chromosome 3 displayed the highest rate of concordance with the presence of human transferrin receptor, as assayed by cytotoxicity. Antitransferrin receptor serum-resistant segregants of chromosome 3 positive, receptor-positive hybrids were selected, using antiserum and complement. The segregants consistently lost chromosome 3. ^{125}I human transferrin binding studies confirmed synteny between the functional human transferrin receptor and chromosome 3. Examination of hybrids with either translocated or deleted chromosome 3's allows regional mapping to 3q(22-ter).

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INTRODUCTION

Genetic studies of the human transferrin receptor have been undertaken [1]. Transferrin is the major extracellular iron transport protein. Iron delivery to cells is facilitated by the binding of transferrin to a specific cell surface receptor. Transferrin receptors have been identified on red cell precursors, on placental membranes, and on many nonhemoglobin synthesizing cells. The receptor is found in higher density on rapidly proliferating cells than on resting cells, and therefore is present in high concentration in many neoplastic cell lines [2–4]. The human transferrin receptor has been purified and characterized. It is an integral membrane glycoprotein of 180,000 mol. wt., formed from two equal subunits [5].

The transferrin receptor is not only of potential importance in understanding processes related to cellular proliferation but may also be related to the pathogenesis of iron-storage disorders, such as hereditary hemochromatosis. Therefore, utilizing antiserum raised against purified human transferrin receptor, we have undertaken genetic mapping of the human transferrin receptor.

While this work was ongoing, Goodfellow et al. reported that the antigen recognized by the monoclonal antibody OKT-9 is encoded by chromosome 3 [6, 7]. OKT-9 is a monoclonal antibody that precipitates the human transferrin receptor and therefore recognizes one of its antigenic determinants. They also demonstrated that OKT-9 precipitates a protein from mouse-human somatic cell hybrids containing human chromosome 3 with similar behavior on polyacrylamide gel SDS electrophoresis to the human transferrin receptor. In addition, Enns et al. demonstrated that chromosome 3 encodes the human transferrin receptor [8]. These investigators detected human transferrin receptor in human-mouse somatic cell hybrids using a radioimmunoassay with 10% cross-reactivity between human and mouse transferrin receptor. The identity of human transferrin receptor was confirmed by partial proteolytic digestion. Our results confirm and extend these observations. Using ^{125}I-labeled human transferrin binding studies carried out at 4°C, we show highly significant differences in specific human transferrin binding between Chinese hamster-human hybrids containing chromosome 3 and their chromosome 3 negative segregants, thereby demonstrating that chromosome 3 encodes a functional human transferrin receptor. As well as confirming that chromosome 3 encodes the human transferrin receptor, we additionally demonstrate that the transferrin receptor maps to the region q(22-ter) of chromosome 3.

MATERIALS AND METHODS

Antiserum to purified human transferrin receptor was obtained and characterized as described [5]. Human transferrin was purchased from Sigma, St. Louis, Mo. Chinese hamster transferrin was purified from Chinese hamster serum by Sephadex G-150 chromatography and DEAE cellulose ion-exchange chromatography according to the method of Sullivan et al. [9]. Purified human and Chinese hamster transferrins were radiolabeled with I^{125} using the chloramine T method [10]. In other experiments, purified Chinese hamster transferrin was radiolabeled using the water insoluble oxidant Iodogen, purchased from Pierce, Rockford, Ill. [11].

Chinese hamster-human somatic cell hybrids were produced by fusion of Chinese hamster auxotrophic mutants with either human lymphocytes or human fibroblasts, as described

[12]. The hybrids J1, 822-5a, 822-19b, 822-48a, 822-56a, and 822-59b had human fibroblasts used as the human donor cell line. The hybrids 750-7, 805-7, 55-C4, 153-E9A, and 706-B6 were formed from fusions utilizing human peripheral blood lymphocytes. The hybrids Q68-10A, Q68-11, Q68-12, Q68-14, Q68-16, and Q68-20 were formed from a fusion of the Chinese-hamster auxotrophic mutant Ade P^{CG} [13] and human tonsillar T lymphocytes, cultured in human T-cell growth factor as described [14]. These human lymphocyte cultures were defined as T cells as greater than 95% formed E-rosettes. Hybrid cells were grown in Ham's F12 or F12D media supplemented with 5% heat decomplemented fetal calf serum.

Human chromosomes were identified by karyotypic analysis that was performed by both trypsin banding and a modified Giemsa-11 procedure [15–17]. Human chromosomes were also identified by isozyme analysis, performed as described [12], with the exception that acylase-1 was used for chromosome 3 identification, as described by Voss et al. [18].

The human transferrin receptor was detected by a cytotoxicity assay. Five hundred cells were plated in a volume of 0.3 ml F12 medium supplemented with 5% heat decomplemented fetal calf serum containing 2% normal rabbit serum as a source of complement with varying concentrations of antihuman transferrin receptor serum in Flow 24 well plates. After 6 days of growth, plates were fixed with 10% ethanol and 3.5% acetic acid, and colonies stained with crystal violet. Surviving colonies were then counted, percentage survival compared with a control with no added antiserum calculated, and survival curves generated. An antibody and complement-mediated cytotoxic selection of human transferrin receptor negative segregants was employed. Cells (10^4) were plated in 60-mm plates containing F12 medium supplemented with 5% heat decomplemented fetal calf serum with 2% normal rabbit serum as a source of complement and 0.04% antihuman transferrin receptor serum. After approximately 1 week of growth, single colonies were isolated and confirmed resistant to antihuman transferrin receptor serum.

Cells used in ^{125}I-transferrin binding studies were grown in 100-mm plates and harvested at subconfluence while still growing actively. Care was taken to assure that all cells were at similar density when experiments were performed. Plates were washed once with PBS, then incubated for approximately 10 seconds with .025% trypsin and 1 mM EDTA. Trypsin was then inactivated with F12 media plus 5% fetal calf serum and the cells dislodged by pipetting. The cells were centrifuged at 1,500 rpm in an I.E.C. clinical centrifuge, the supernatant discarded, and the cells resuspended in F12 medium with 1 mg/ml ovalbumin and 10 mM, pH 7.4, HEPES buffer, and counted. They were again centrifuged at 1,500 rpm and resuspended in an appropriate volume of F12 with 1 mg/ml ovalbumin and 10 mM HEPES.

Binding assays were performed at 4°C in F12 media with 1 mg/ml ovalbumin and 10 mM, pH 7.4, HEPES buffer in a volume of 0.4 ml with 5×10^5 cells per assay tube. ^{125}I-labeled human or Chinese hamster transferrin was added and incubated for 4 hrs (the amount of time when saturation of binding was reached as determined by previous studies). The reaction was stopped by adding 2 ml cold media and the cells immediately centrifuged at 500 *g*. The supernatant was aspirated and the cell pellet counted in a Beckman gamma 8000 system. Specific binding was determined as the difference between binding in the absence and presence of a 100-fold excess of species nonradioactive transferrin.

RESULTS

Rabbit antihuman transferrin receptor serum was cytotoxic to the human cell line, HeLa-A, at a concentration of .005%, but did not display killing of Chinese hamster ovary cells below a 2% concentration (fig. 1*a*). These results indicated that the antihuman transferrin receptor serum was highly specific for human transferrin receptor and thus, at low concentrations, would not kill Chinese hamster human somatic cell hybrids that possess only Chinese hamster transferrin receptor.

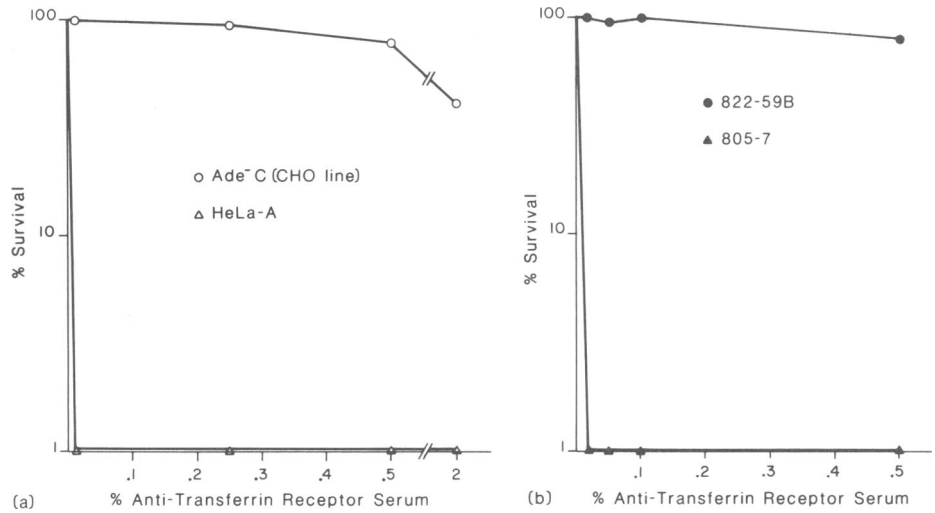


FIG. 1.—*a*, Complement-mediated cytotoxicity of rabbit antihuman transferrin receptor serum to the human cell line, HeLa-A, and Chinese hamster ovary cell line, Ade⁻C. *b*, Complement-mediated cytotoxicity of rabbit antihuman transferrin receptor serum to the human-Chinese hamster ovary somatic cell hybrids 805-7 and 822-59b.

A clone panel of 17 Chinese hamster-human somatic cell hybrids was analyzed for sensitivity to antihuman transferrin receptor serum. Rabbit antihuman transferrin receptor serum was cytotoxic to certain Chinese hamster-human somatic cell hybrids at a concentration of .005%–.010%, but not to others at concentrations up to 0.5% (fig. 1*b*). Cells killed by antihuman transferrin receptor serum at a .010% concentration were scored as positive.

Chromosome 3 demonstrated the highest rate of concordance with antihuman transferrin receptor serum sensitivity, giving a concordance rate of 94%. Concordance rates for other chromosomes ranged from 53% to 88%, with chromosome 10 having the next highest concordance rate (table 1).

The one discordant hybrid, 822-56a, which was killed in the cytotoxicity assay but was negative for acylase-1, the chromosome 3 isozyme, was subjected to karyotypic analysis, demonstrating that it did contain chromosome 3 but with a 3p deletion. This deletion is consistent with the regional mapping of acylase-1. Therefore, if this hybrid containing part of chromosome 3 is rated as concordant, the rate of concordance between chromosome 3 and sensitivity to antihuman transferrin receptor serum is 100%.

To confirm that the human transferrin receptor is encoded by chromosome 3, antihuman transferrin receptor serum-resistant segregants of chromosome 3-positive, acylase-1-positive, hybrids were selected by antibody and complement-mediated cytotoxicity, as described in MATERIALS AND METHODS. The three hybrids selected for segregants were the subclones 805-7-2, Q68-12-4, and Q68-20-1A. In each case, the three respective resistant segregants, designated by the suffix R (805-7-2-R, Q68-12-4-R, Q68-20-1A-R), were resistant to killing by antihuman transferrin receptor serum. In addition, each segregant had lost chromosome 3

TABLE 1

HUMAN CHROMOSOME CONSTITUTION OF 17 HYBRID CLONES AND RESULTS OF HUMAN TRANSFERRIN RECEPTOR ANALYSIS

HUMAN CHROMOSOME	750-7	805-7	822-5a	822-19b	822-48a	822-56a	822-59b	Q68-10A	Q68-11	Q68-12	Q68-14	Q68-16	Q68-20	J1	55-C4	153-E9A	706-B6	% CONCORDANT
1	−	+	−	+	−	−	−	−	+	−	−	−	+	−	−	−	−	82
2	−	−	−	+	+	+	−	−	+	+	+	+	−	−	−	−	−	65
3	−	+	−	+	−	−	−	−	−	+	−	−	+	−	−	−	−	94
4	+	+	−	−	−	+	+	+	−	+	+	−	+	−	−	−	−	71
5	−	+	−	+	+	+	−	−	+	+	+	+	+	−	−	−	−	76
6	−	−	−	+	+	−	−	−	+	+	+	+	−	−	−	−	−	59
7	−	−	−	−	−	−	+	−	−	+	+	+	−	−	−	−	−	59
8	−	+	−	−	−	−	−	+	+	−	+	+	+	−	−	−	+	53
9	+	−	+	−	−	−	−	−	+	+	+	−	−	−	−	−	−	53
10	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−	−	−	88
11	+	+	+	+	+	+	−	−	+	+	+	−	+	+	−	−	−	65
12	−	+	+	−	+	+	−	+	+	+	+	−	+	−	+	−	−	59
13	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	65
14	−	+	−	+	−	+	+	+	+	−	+	−	+	−	−	−	+	65
15	+	+	−	+	+	−	−	+	+	+	+	−	−	−	−	−	−	59
16	+	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	76
17	−	−	−	−	−	−	−	−	−	−	−	...	−	−	−	−	−	69
18	−	+	−	−	−	−	−	+	−	+	+	−	+	−	−	−	−	76
19	−	+	+	+	−	+	−	+	+	+	+	−	+	−	−	−	−	76
20	−	−	−	+	−	+	−	+	+	+	+	−	+	−	−	−	−	76
21	−	−	+	+	−	+	−	+	+	+	+	+	+	−	−	+	−	59
22	−	+	+	+	+	−	−	+	+	+	+	+	+	−	−	−	−	59
X	+	−	−	−	−	−	−	+	+	+	−	−	+	−	−	−	−	65
Transferrin Receptor	−	+	−	+	−	+	−	−	−	+	−	−	+	−	−	−	−	...

by isozyme and cytogenetic analysis. Chromosome 3 was the only consistent difference between the initial hybrids and their resistant segregants (table 2).

^{125}I human transferrin binding studies were carried out as described in MATERIALS AND METHODS. Three independent hybrids and their respective antihuman transferrin receptor serum-resistant segregants were tested. In each case, the hybrid containing chromosome 3 bound significantly more ^{125}I human transferrin than its antireceptor serum-resistant, chromosome 3-negative segregant (fig. 2). These results demonstrate a functional receptor for human transferrin that segregates concordantly with sensitivity to antihuman transferrin receptor serum and chromosome 3. The results suggested that, under the subsaturating and low temperature conditions of the assay, human transferrin has a higher affinity for the human transferrin receptor than for the Chinese hamster transferrin receptor. However, the above results had not ruled out a lower density or complete absence of Chinese hamster transferrin receptors on the chromosome 3-negative cells.

To demonstrate that Chinese hamster transferrin receptors were present on all the hybrids, ^{125}I Chinese hamster transferrin binding studies were carried out. One hybrid, Q68-20-1A, and its antitransferrin receptor resistant segregant, Q68-20-1A-R, were tested. Both specifically bound similar amounts of ^{125}I bound Chinese hamster transferrin, demonstrating that, as expected, the Chinese hamster cell lines possess specific binding sites for Chinese hamster transferrin (fig. 3).

Karotypic analysis of a series of hybrids containing deletions of chromosome 3 allowed regional mapping of the transferrin receptor gene. The acylase-1 negative, human transferrin receptor-positive hybrid 10B4C3 was subjected to cytogenetic analysis and found to have an intact 3q. Its antihuman transferrin receptor-resistant segregant, 10B4C3-R, has lost the 3q, thereby mapping the human transferrin receptor to 3q.

The hybrid UCH-2 contains 3q by cytogenetic analysis in addition to a small translocation of human genetic material to a Chinese hamster chromosome. An antihuman transferrin receptor-resistant segregant, UCH2-R, was selected. Cytogenetic analysis demonstrates that, in the segregant, 3q has translocated to a Chinese hamster chromosome with loss of 3q(22-ter) (fig. 4).

Therefore, the chromosome 3 or fragments thereof from receptor-positive and -negative cells are shown to differ in a pattern consistent with regional mapping

TABLE 2

HUMAN CHROMOSOMES CONTAINED IN HUMAN TRANSFERRIN RECEPTOR-POSITIVE HYBRIDS AND THEIR HUMAN TRANSFERRIN RECEPTOR-NEGATIVE SEGREGANTS

Hybrid	Human chromosomes	Human transferrin receptor
Q68-20-1A.....	3, 5, 10, 11, 14, 19, 21, X	+
Q68-20-1A-R.....	10, 11, 14, 19, 21, X	−
805-7-2.....	3, 4, 5, 6, 8, 10, 11, 12, 15, 16, 22, Y	+
805-7-2-R.....	4, 6, 10, 11, 22, Y	−
Q68-12-4.....	3, 4, 5, 6, 9, 10, 11, 15, 16, 18, 19, 20, 21	+
Q68-12-4-R.....	4, 5, 9, 10, 11, 15, 19, 21	−

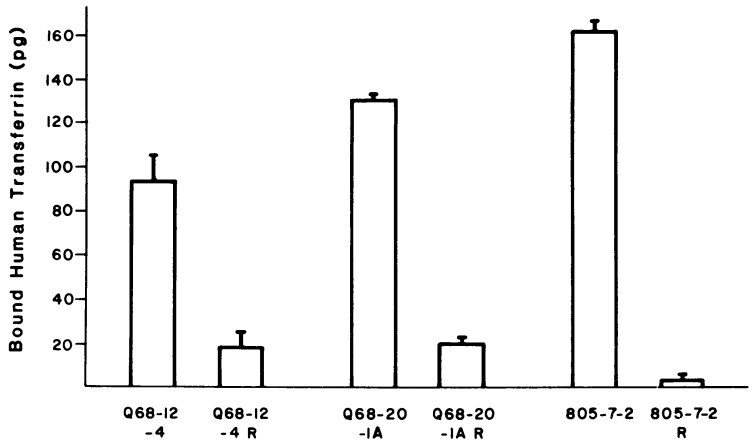


FIG. 2.—Specific binding of ^{125}I human transferrin to hybrid cells and their respective chromosome 3-negative segregants. Four ng of ^{125}I human transferrin was added per assay tube. *Bar* denotes standard deviation, representing variation both above and below the mean.

of the receptor to 3q(22-ter). This regional mapping is a conservative estimate, since the deletion identified in UCH2-R is the largest possible to have occurred.

DISCUSSION

We used two independent techniques to identify the human transferrin receptor. Rabbit antihuman transferrin receptor serum is highly species specific and distinguishes clearly between human and Chinese hamster cells. Using this assay and a clone panel of 17 human-Chinese hamster somatic cell hybrids, a concordance rate of 100% between sensitivity to the antiserum and chromosome 3, assayed by isozyme and cytogenetics, was obtained. To confirm this, antiserum-resistant segregants were selected from three independent hybrids. In each case, the resistant segregant had lost chromosome 3, and chromosome 3 was the only constant difference between the sensitive hybrids and their resistant segregants. Further analysis of hybrids containing partial deletions of chromosome 3 indicate that the human transferrin receptor maps to 3q(22-ter).

The antiserum used in this study was raised to human placental transferrin receptor. The human placental transferrin receptor has been shown to have immunologic cross-reactivity with the transferrin receptor on several different cell lines [2] and is identical with the human reticulocyte transferrin receptor as indicated by proteolytic digest analysis [19]. Thus, evidence to date suggests that one human transferrin receptor is present on all tissues containing transferrin receptor, and therefore it is likely that the receptor on the Chinese hamster-human somatic cell hybrids is the same protein.

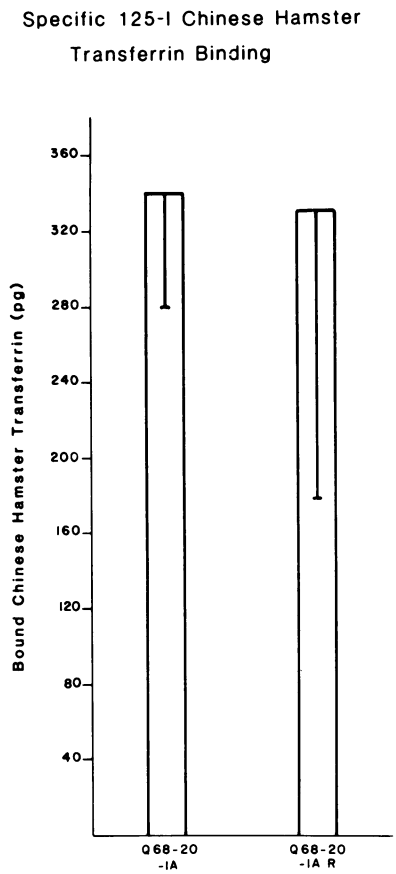
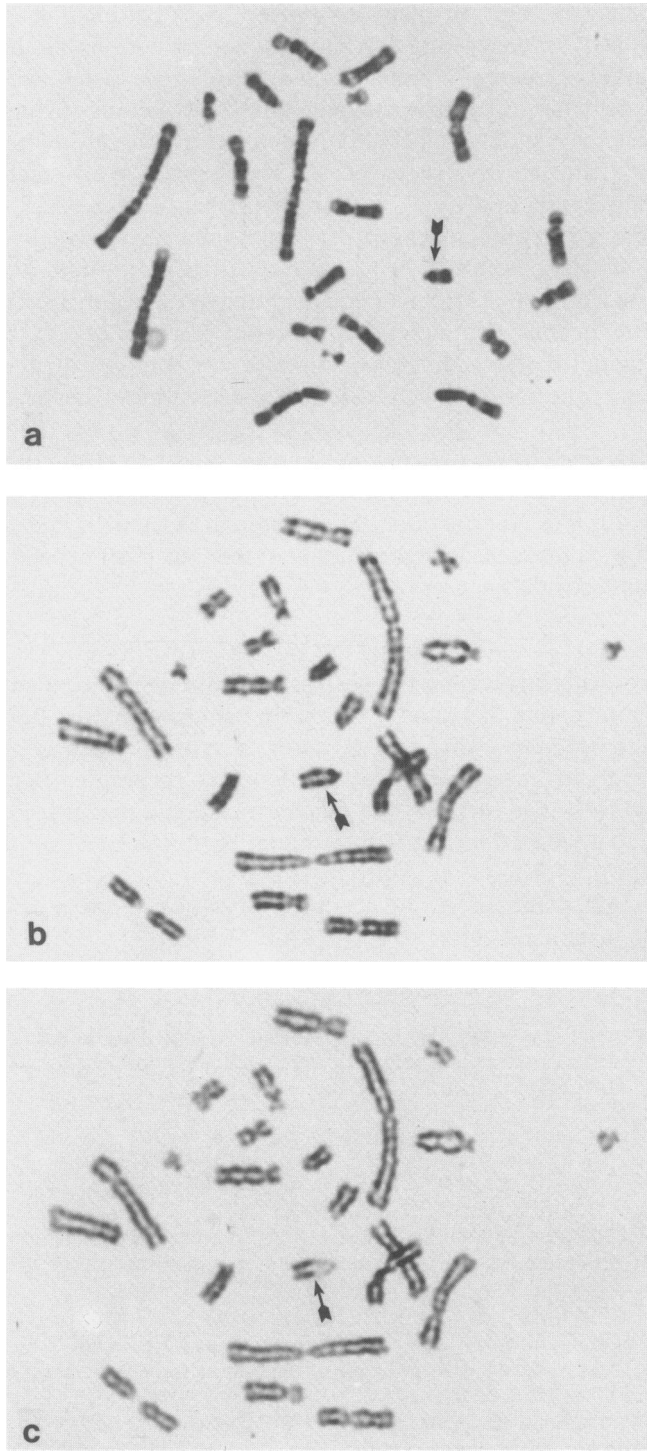


FIG. 3.—Specific binding of ^{125}I Chinese hamster transferrin to the hybrids Q68-20-1A (chromosome 3-positive) and Q68-20-1A-R (chromosome 3-negative). Ten ng of ^{125}I Chinese hamster transferrin was added per assay tube. *Bar* denotes standard deviation, representing variation both above and below the mean.

To demonstrate that sensitivity to antitransferrin receptor serum correlates with biologically functional human transferrin receptor, we took advantage of the species specificity of ^{125}I human transferrin binding at 4°C. The hybrids sensitive to antihuman transferrin receptor serum and their resistant segregants demonstrate markedly different specific ^{125}I human transferrin binding. Further elucidation of the biologic functions, other than binding, of the human transferrin receptor in these hybrid cells may lead to a greater understanding of the genetic control of such processes as receptor internalization and regulation.

FIG. 4.—*a*, Trypsin-banded metaphase spread of UCH-2. *Arrow* denotes 3q. *b*, Trypsin-banded metaphase spread of human transferrin receptor-negative segregant, UCH-2-R. *Arrow* denotes human 3q translocated to Chinese hamster chromosome with loss of 3q (22-ter). *c*, Giemsa-11-stained metaphase spread of UCH-2-R. *Arrow* denotes human 3q translocated to Chinese hamster chromosome. Differential staining shows breakpoint.


a
b
c

Human parent cells for the hybrids in these investigations were from three sources: fibroblasts, proliferating T lymphocytes, and peripheral blood lymphocytes. The actively growing T lymphocytes and fibroblasts would be expected to express high densities of transferrin receptor [2], whereas resting peripheral blood lymphocytes would not [20]. It is of interest, therefore, that a human chromosome 3 from any of these sources will express the human transferrin receptor when put into an actively growing Chinese hamster cell.

The transferrin receptor appears to be coordinately regulated with cellular proliferation. In fact, the transferrin receptor had been initially described as a tumor associated antigen [21]. Small cell carcinoma of the lung has recently been shown to contain a consistent deletion of chromosome 3 p(14-23) [22]. The fact that regional mapping shows the transferrin receptor to be encoded by 3q(22-ter) makes it unlikely to be involved in the deletion characteristic of small cell carcinoma of the lung.

Additionally, some forms of hemochromatosis, a genetic disorder of excessive iron storage, are linked to chromosome 6 as determined by family studies using HLA linkage [23, 24]. The fact that the transferrin receptor is encoded on chromosome 3 suggests that a structural mutation of the transferrin receptor is unlikely to be responsible for these cases.

CONCLUSION

The human transferrin receptor has been assayed by two independent techniques: antireceptor serum cytotoxicity and ^{125}I human transferrin binding. Both techniques indicate that the human transferrin receptor is syntenic with chromosome 3. Regional mapping to the segment 3q(22-ter) has been accomplished using a series of deletion hybrids. The fact that the human transferrin receptor is encoded by chromosome 3 suggests that a structural mutation in the transferrin receptor is not involved in the form of hemochromatosis linked to chromosome 6. Further somatic cell genetic studies will be useful in investigating the regulation of this important cell surface receptor.

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