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Transfer of the marker for morphologically transformed phenotype by isolated metaphase chromosomes in hamster cells

IN spite of extensive investigations of the process of carcinogenesis *in vitro*, the basic mechanisms of the primary events are poorly understood[1]. In general, it is found that the first manifestation of the transformed phenotype involves morphological alteration of the cells. This is usually followed by an ill-defined process resulting in several further changes in phenotype including the ability of the cells to grow in agar (aga$^+$), and to produce tumours in animals. In part because of the variety of phenotypes observed, but also because of the lack of suitable methods of analysis, it has been difficult to examine the process of transformation at the genetic level. We have recently initiated experiments designed to use chromosome transfer techniques to attempt such a genetic analysis[2,3] and work in several laboratories has shown that single gene markers can be transferred from cell to cell at frequencies of 10^{-6}–10^{-7} by means of the metaphase chromosome[4–9]. Most of the initial work was done with the genes for hypoxanthine phosphoribosyl transferase and thymidine kinase, but dominant markers such as resistance to methotrexate and ouabain can also be so transferred[9], as can the wild-type alleles for three auxotrophic markers[10]. In seeking to delineate the genetic elements of carcinogenesis, we have examined the ability of purified metaphase chromosomes from Chinese hamster ovary (CHO) cells to transfer the first recognisable phenotype observed during cell carcinogenesis, that is, morphological transformation. Because of the low frequency of chromosome transfer, an appropriate method for selection of cells (transferents) which have incorporated the relevant markers from the background of recipient cells is necessary. Our experiments were based on the assumption that if transformation gene(s) could be transferred to senescent cells, then they should be able to produce colonies, whereas the majority of the cells which do not receive the appropriate marker, will not form colonies. The results presented here show that senescent hamster cells can be rescued and become transformed by transfer of purified metaphase chromosomes from CHO cells; the frequency of this event is similar to that of single gene markers.

Primary Chinese hamster lung cells were subcultured through five generations, and cloned. When the cloned cultures had reached confluence (~ 15 further generations), they were split into two subcultures, in 100-mm plates, and grown again. At the end of this time the cells were growing slowly, and were obviously in senescence. As might be expected, each clone gave rise to different numbers of cells at this stage. Nevertheless, in this way, a number of sets of two plates, each pair containing similar numbers of cells at the same level of senescence, were available as recipients. Purified metaphase chromosomes were isolated from CHO cells and aliquots added to one plate from each clone, as previously described[2,9]. The plates were incubated at 37 °C in α-medium[11] containing 15% FCS for 2 d. Cells were then trypsinised, counted, replated in the same medium and incubated at 37 °C for 3 weeks. Colonies were then counted and isolated using a stainless steel cylinder and trypsinisation. The results of the first such experiment are presented in Table 1 which shows that colonies were obtained in five of the six cultures treated with metaphase chromosomes (line 1), whereas no colonies were obtained when the senescent cells were plated without previous addition of metaphase chromosomes (line 2). No colonies were obtained when metaphase chromosomes were added to the plates in the absence of recipient cells (line 3). The results of a similar experiment, with two additional controls, are shown in Table 2. Again, colonies were obtained when CHO chromosomes were added to the senescent cells (line 1) and no colonies were found in the absence of chromosomes (line 4) and in the absence of recipient cells (line 5). We also prepared metaphase chromosomes from two primary strains developed from Chinese hamster lung and Chinese hamster ovary, both in their sixth transfer passage (1:4 splits), CHLP6 and CHOP6, respectively. As may be seen in Table 2 (lines 2 and 3), these chromosomes were also unable to rescue senescent cells.

Table 1 Rescue of Chinese hamster lung (CHL) cells from senescence after treatment with purified metaphase chromosomes of CHO cells

Donor for chromosomes*	No. of cells plated × 10^5 CHL clone no.†						No. of colonies obtained CHL clone no.‡						No. of colonies per no. of cells or cell equivalent chromosomes × 10^6
	1	2	3	4	5	6	1	2	3	4	5	6	
1 CHO	3	5	10	1	2	4	2	6	13	1	0	5	10.4
2	4	4	10	2	3	5	0	0	0	0	0	0	<0.4
3 CHO							0	0	0	0	0	0	<0.1

*A total of 2 × 10^6 cell equivalent chromosomes was added per plate.
†At day 2 after treatment cells treated with chromosomes (line 1) or untreated cells (line 2) were trypsinised and counted. The cells were plated in 20 ml of α-medium containing 15% FCS, at a concentration of up to 5 × 10^5 cells per 100-mm plate. Incubation of chromosomes alone (line 3) was continued in the above medium.
‡Colonies obtained after incubation at 37 °C for 3 weeks.

Table 2 Rescue of Chinese hamster lung (CHL) cells from senescence after treatment with purified metaphase chromosomes of CHO cells

Donor for chromosomes*	No. of cells plated × 10^5 CHL clone no.†					No. of colonies obtained CHL clone no.‡					No. of colonies per no. of cells or cell equivalent chromosomes × 10^6
	11	12	13	14	15	11	12	13	14	15	
1 CHO	6	4	10	10	1	3	2	10	7	0	7.1
2 CHLP6	6	8	8	9	2	0	0	0	0	0	<0.4
3 CHOP6	7	5	8	8	2	0	0	0	0	0	<0.4
4	5	4	9	10	2	0	0		0	0	<0.4
5 CHO						0	0	0	0	0	<0.1

*, †, And ‡ as in legend to Table 1.

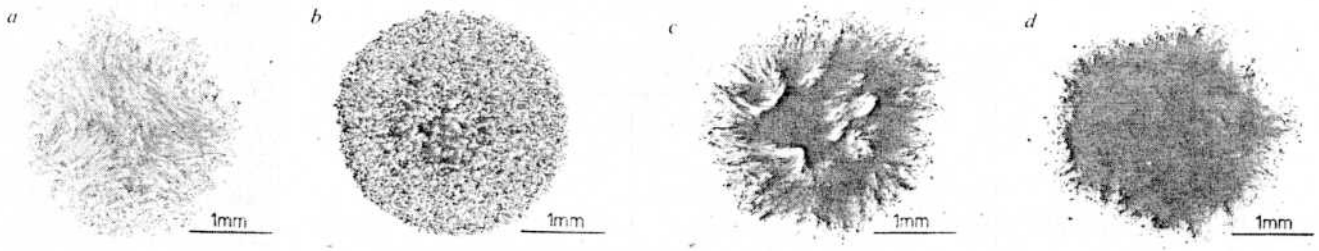

Fig. 1 Normal and transformed colonies. Normal recipient Chinese hamster lung cells (*a*), donor CHO cells (*b*) and two phenotypically different morphologically transformed Chinese hamster lung cells with metaphase chromosomes of CHO cells (*c*, *d*).

The experiments described in Tables 1 and 2 provide strong evidence that CHO metaphase chromosomes can rescue senescent cells. Figure 1 shows the morphology of clones from two of these transferent clones, as well as that of the donor and primary recipient cells. It is clear that the rescued cells have been altered morphologically and have the appearance of transformed cells. This transformed phenotype is maintained on continued culture of these clones. Four of the transferents were karyotyped in detail with trypsin–Giemsa banding. Of 26 cells examined, 60% had a normal diploid karyotype, 15% showed loss of one or more chromosomes, and 25% showed structural rearrangements.

of recipient cells by metaphase chromosomes is caused by the transfer of a single gene.

Although the transferent cells obtained in these experiments show a transformed morphology, we have found that they do not form colonies in agar (aga$^-$) or tumours when injected into recipient animals[2], but they can be converted into cells which do form colonies in agar (aga$^+$) and which can produce tumours in animals, by a second addition of CHO metaphase chromosomes, and appropriate selection[2]. It has not been possible to produce aga$^+$ cells directly by addition of CHO chromosomes to primary cells. In terms of their ability to be converted to aga$^+$, the primary transferents behave

Table 3 Rescue of Chinese hamster lung (CHL) cells from senescence after treatment with fractionated metaphase chromosomes of CHO cells

Chromosomal fraction*	No. of cells plated × 10^5 CHL clone no.†				No. of colonies obtained CHL clone no.‡				No. of colonies per no. of cells or cell equivalent chromosomes × 10^6
	21	22	23	24	21	22	23	24	
1 Large(*A*)	7	5	10	6	8	7	12	8	12.5
2 Medium(*B*)	4	6	9	7	0	0	2	0	0.8
3 Small(*C*)	5	4	8	7	1	0	0	0	0.4
4	5	4	9	8	0	0	0	0	<0.4
5 *A*+*B*+*C*					0	0	0	0	<0.2

*, † and ‡ as in legend of Table 1.

From these results, we conclude that the addition of CHO metaphase chromosomes to senescent cells provides genetic information resulting in the transformation phenotype, and in rescue of senescence. Further support for this view has come from an experiment with fractionated chromosomes. Previously, we showed that metaphase chromosomes could be separated into three size classes in sucrose gradients (large *A*, medium *B*, small *C*), and that individual markers could be localised to specific size classes[9,10]. Metaphase chromosomes from CHO cells were fractionated in this way, and each fraction was tested for the ability to rescue senescent recipient hamster cells. As may be seen in Table 3, lines 1–3, activity was found in the large size (*A*) class of chromosomes but few or no colonies were obtained with the other fractions. As before, no colonies were obtained in the absence of chromosomes (line 4) or recipient cells (line 5). Transferent colonies obtained in this experiment were also transformed in phenotype.

Thus, the ability to transform cells behaves similarly to other genetic markers and can be located on a specific size class of chromosomes.

The negative findings with the *B* and *C* chromosome size classes provides further evidence that the transformed colonies obtained result from addition of donor chromosomal material, rather than spontaneous transformation in the recipients, and also tend to rule out any mechanism of 'induction' of transformation in recipients due to addition of chromosomal material.

The frequency of appearance of transferents in all of the experiments (Tables 1–3) is similar to that observed for a variety of single gene markers[2,9,10]. This fact, in addition to the localisation of the activity to one size class argues for the view that the transformation identically to cells which have been transformed by benzo(*a*)pyrene, or cells which have transformed spontaneously in culture[2]. These results provide further confirmation that the rescued senescent cells are transformed, and that a gene involved in a first step in transformation has been identified. The successful isolation of such transferents also provides evidence that this gene acts dominantly. Our results do not, however, show that this is the only gene involved in the primary transformation step. Further experiments involving a variety of donors, and recipients, will be required to examine questions of this kind.

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