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# Transfection with plasmid pSV2gptEJ induces chromosome rearrangements in CHEF cells

(c-Ha-ras/cytogenetics/p21/tumor formation)

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ABSTRACT **In previous cytogenetic studies, trisomy for 3q was found to be the most frequent chromosome change associated with induced tumorigenicity by a variety of agents in Chinese hamster cells. Here we describe similar chromosome changes in 11 lines of CHEF/18 cells transfected with the mutant c-Ha-*ras* containing plasmid pSV2gptEJ. All 11 lines contained the transfected *EJ* gene and expressed increased levels of p21, the *EJ* gene product. Ten of the 11 lines were tumorigenic and all but 2 of these were trisomic for all or part of 3q. One line remained diploid and was nontumorigenic despite expressing elevated p21. Two tumorigenic lines from "hit-and-run" transfection with pSV2gpt were shown to express only control levels of p21, but they were trisomic for 3q. These results show that increased p21 expression is neither necessary nor sufficient for inducing tumorigenicity of CHEF cells. We propose that tumorigenicity in the transfected CHEF/18 cells of this study was induced by chromosome rearrangements, especially trisomy for 3q, that occurred at increased frequencies following transfection with pSV2gptEJ.**

Some 10–20% of human solid tumors and tumor-derived cell lines contain DNA with transforming activity when assayed for focus formation with NIH 3T3 cells (1). Much of this transforming activity has been associated with genetic changes in one of the *ras* genes: *HRAS*, *KRAS*, or *NRAS* (2). Primarily on the basis of these data, the *RAS* genes have been considered among the most potent transforming genes yet identified in human cancer.

On the other hand, direct tests of the transforming activity of the same human-derived mutant *HRAS* gene transfected into normal diploid human fibroblasts have given contrary results. The presence of the transfected gene and increased levels of its p21 gene product have induced neither the transformed morphology nor tumor-forming ability (3). Furthermore, the transfected cells remained diploid throughout these tests and later senesced (4).

It was of interest, therefore, to determine whether the tumorigenic consequences of c-Ha-*ras* gene expression in rodent cells were a primary effect of the transfected gene, or whether chromosome changes had been induced as well, leading to additional genetic changes. Since 3T3 cells are already aneuploid, and most primary rodent cells become aneuploid and immortal during experimental procedures involved in transfection and subsequent testing, it was decided to use CHEF cells for these studies. CHEF/18 cells are a doubly recloned fibroblastic line derived from a Chinese hamster embryo. The cells are stably diploid and nontumorigenic (5, 6).

In previous studies, nontumorigenic anchorage-independent and low serum mutants of CHEF/18 cells were either diploid or contained a variety of chromosome rearrangements including two regions of chromosome 1 associated with anchorage independence (7, 8). The tumorigenic and tumor-derived lines from other experiments contained aberrations affecting chromosome 3, principally trisomy for 3q. In subsequent studies, tumorigenic lines recovered from foci after treatment of cells with 5-azacytidine and corresponding tumor-derived lines showed a consistent trisomy for 3q (9). Similarly, trisomy for 3q was uniformly present in tumorigenic lines recovered as foci after transfection with pSV2gpt plasmids not containing a transforming gene. In these lines, the plasmid DNA was not present, indicating a "hit-and-run" origin of tumorigenicity (10). A similar association of changes in chromosome 3 with tumorigenesis was found in tumors derived from the related cell line CHEF/16 (11). Also, Ray *et al.* (12) described trisomy for 3q as a frequent aberration in spontaneously transformed primary Chinese hamster fibroblasts.

We show here that trisomy for 3q is the commonest rearrangement found in a series of CHEF/18 cells transfected with the mutant c-Ha-ras containing plasmid pSV2gptEJ. All 11 lines derived from foci contained the transfected *EJ* gene and expressed increased levels of p21, the *EJ* gene product. Ten of the 11 lines were tumorigenic and 8 of them were trisomic for all or part of 3q. One line remained diploid and was nontumorigenic despite the presence of elevated levels of p21. These results do not support a direct transforming role for the *EJ* gene, but rather suggest that p21 acts as a growth stimulator and that plasmid integration induces chromosome rearrangements leading to tumorigenicity.

## MATERIALS AND METHODS

**Origin of Cell Lines.** The origin and properties of the CHEF/18, CHEF/16, and 205-30 cell lines have been described (5, 6). CHEF/18-1D-3 is a diploid recloned subline from CHEF/18. Cell line 205-30 is a thioguanine-resistant derivative of CHEF/18 (6); gpt-3 and gpt-4 are plasmid-induced hit-and-run tumorigenic lines from CHEF/18-1D-3 (10). The L1–L8 and C1, C3, and C4 lines were derived from foci of independent origin after transfection of CHEF/18-1D-3 were either the linear (L) or circular (C) plasmid pSV2gptEJ (3). The term EJ refers to the mutant c-Ha-*ras* gene cloned from a human bladder carcinoma cell line (13). Each focus-derived line was purified by two passages in mycophenolic acid to remove any residual nontransfected cells. CHEF/16 cells are diploid but have lost their anchorage and high serum requirements for growth and are tumorigenic in the nude mouse assay (6).

**Cell Culture.** Cells were grown and maintained in α minimal essential medium (αMEM) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT)/2 mM glutamine/

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Abbreviation: kb, kilobase(s).

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penicillin (100 units/ml)/streptomycin (100 μg/ml). All cells were grown in humidified 6.5% $CO_2$/93.5% air at 37°C and pH 7.2.

**Cloning Properties.** Cloning efficiencies on plastic were determined by inoculating 200 or 500 cells onto 100-mm dishes in medium containing 10% or 3% fetal bovine serum, respectively. After 2 weeks, the plates were fixed in 10% formalin, stained with crystal violet, and scored. Anchorage independence was estimated by culturing $10^2$ or $10^3$ cells in medium containing 1.3% methylcellulose, using 60-mm dishes coated with 0.6% agar as described (5). Cells were fed weekly, and macroscopic colonies were scored at 4 weeks.

**Detection of EJ Sequences in Cell DNA.** Genomic DNA from each cell line was extracted as described (3). Approximately 10 μg of DNA digested with *Bam*HI (New England Biolabs) or *Bam*HI and *Sst* I (Bethesda Research Laboratories) was loaded per lane for electrophoresis. Agarose gel electrophoresis (1%), transfer to nitrocellulose filters, hybridization to $^{32}$P-labeled EJ insert [6.6 kilobases (kb)], and autoradiography were carried out as described (3).

**Assay of p21 Encoded by *EJ*.** Labeling of cells with [$^{35}$S]methionine and immunoprecipitation of p21 protein followed published procedures with minor modifications (4). Immunoprecipitation was carried out with $1 \times 10^7$ cpm of trichloroacetic acid-precipitable material using the rat monoclonal antibody preparation 238 (14), provided by Mark Furth. Normal rat serum (Cappel Laboratories, Cochranville, PA) served as control. Immunoprecipitates and controls were electrophoresed in a 12.5% polyacrylamide slab gel, treated with Enlight (New England Nuclear), dried, and exposed to x-ray film (Kodak XR-5) at −70°C. Underexposed p21 fluorograms were scanned to quantitate the p21 content of each cell line and normalized to a reference peptide on the same gel.

**Tumorigenicity Assay.** Suspensions of $10^7$ cells in 200 μl of medium were injected subcutaneously into the flanks of athymic BALB/c mice bred in this laboratory (6). Tumors reaching >1 cm diameter were scored as positive.

**Chromosome Analysis.** For chromosome analysis, $5 \times 10^5$ cells were plated in 75-cm$^2$ flasks and incubated at 37°C for 24–72 hr. Cells were harvested after treatment for 1–2 hr with Colcemid (0.05 μg/ml), exposed to a hypotonic 0.075 M KCl solution for 5–7 min, centrifuged, and fixed in freshly prepared Carnoy's fixative. Chromosomes were G-banded using 3- to 10-day-old slides according to published procedures (15). Fifteen to 20 cells were karyotyped from each cell line and 50 cells were counted. Only clonal abnormalities were considered. Chromosome classification and nomenclature follow international recommendations (16–18).

## RESULTS

**Growth Characteristics of Cell Lines Tested.** The cell lines comprised three readily distinguished morphological categories. The normal fibroblastic morphology of CHEF/18-1D-3 (group 1) was also seen in the thioguanine-resistant mutant 205-30 of CHEF/18 origin and in the hit-and-run line *gpt-3*. Transformed cells of group 2, including L4, L6, L7, L8, gpt-4, C1, C4, and CHEF/16, were characterized by a densely packed fusiform and refractile morphology in monolayer culture. In contrast, cells of group 3—namely, L1, L2, L3, L5, and C3—were small and round or cuboidal and showed disorganized typically transformed growth. Morphology correlated well with anchorage independence. The group 3 cell lines all grew very well in methylcellulose, whereas those of groups 1 and 2 grew poorly or not at all.

CHEF/18-1D-3 and 205-30 were not tumorigenic in nude mice (Table 1). All of the pSV2gptEJ-transfected lines except C4 were tumorigenic, although some tumors regressed after reaching >1 cm in diameter. Tumors appeared 2–4 weeks after injecting $10^7$ cells per site. The *gpt-3* line was less tumorigenic than the others. L1, C1, and L8 formed particularly rapid and progressively growing tumors, some of which were highly vascularized.

Table 1. Characteristics of the cell lines

| Cell line | Colony formation* | Tumors/ sites | pEJ† Gene copies | pEJ† p21 | Complete or partial trisomy 3q |
|---|---|---|---|---|---|
| | | Group 1 | | | |
| 18-1D-3 | $<10^{-3}$ | 0/4 | — | — | — |
| 205-30 | $<10^{-3}$ | 0/4 | — | — | — |
| gpt-3 | $<10^{-3}$ | 1/8 | — | — | +‡ |
| | | Group 2 | | | |
| CHEF/16 | 100 | 15/15 | — | — | — |
| L4 | 10 | 2/8 | + | + | + |
| L6 | 18 | 2/8 | ++ | ++ | +‡ |
| L7 | 7 | 4/8 | ++ | ++ | + |
| L8 | 3 | 8/8 | ++ | ++ | + |
| C1 | ND§ | 4/4 | ++ | + | +‡ |
| C4 | 26 | 0/4 | ND§ | ++ | — |
| gpt-4 | ND§ | 4/4 | — | — | + |
| | | Group 3 | | | |
| L1 | 64 | 8/8 | +++ | +++ | —‡ |
| L2 | 100 | 4/6 | ++ | ++ | + |
| L3 | 100 | 5/8 | + | + | +‡ |
| L5 | 100 | 4/8 | +++ | ++ | +‡ |
| C3 | 100 | 4/8 | ++ | ND§ | —‡ |

*Colony formation (percentage in methylcellulose/percentage on plastic) × 100.
†Increase in gene copy number and p21 expression over control level (—).
‡Other chromosome rearrangements also present.
§Not determined.

**Presence of the EJ DNA in Transformed Cell Lines.** The DNAs extracted from the transformed lines and controls were cleaved with the restriction enzymes *Bam*HI and *Sst* I to distinguish between endogenous and plasmid-derived Ha-*ras* gene sequences (Fig. 1). Double digests of the plasmid DNA produced three fragments that hybridized with the 6.6-kb Ha-*ras* probe in Southern blots (1.0, 2.5, and 2.9 kb), whereas the untransfected CHEF cells showed two weakly hybridizing bands of 1.6 and 0.8 kb (Fig. 1). All of the

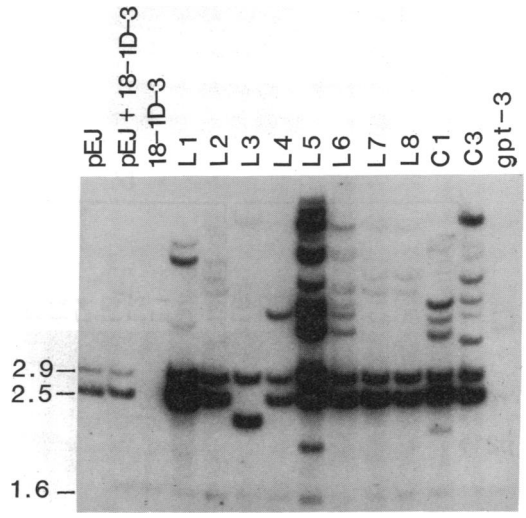


FIG. 1. Blot hybridization of genomic DNAs. Genomic DNA from each cell line was digested with the restriction enzymes *Bam*HI and *Sst* I, electrophoresed in 1% agarose, transferred to nitrocellulose, and hybridized with the $^{32}$P-labeled 6.6-kb *Bam*HI fragment of the *EJ* gene.

EJ-transformed lines contained hybridizing DNA of both plasmid and endogenous origin. (The 1-kb *Sst* I/*Sst* I fragment and the 0.8-kb endogenous fragment are not shown.) The 2.9- and 2.5-kb bands were found in all the EJ-transformed lines except for L3, in which the 2.5-kb fragment representing the 3′ region of the gene was reduced to 2.2 kb. As shown, most lines contained about the same number of gene copies. L1 and L5 each had additional copies, whereas L3 and L4 each contained on average one copy of the *EJ* gene as determined by quantitative comparison with the EJ cell line (data not shown). No *ras* DNA of plasmid origin was found in the hit-and-run lines *gpt-3* and *gpt-4*.

**Expression of Ha-*ras* Encoded p21 Protein.** All of the EJ-transformed cell lines expressed increased levels of the p21 gene product (Fig. 2). Lines 18-1D-3 and the hit-and-run transformants *gpt-3* and *gpt-4* served as negative controls, expressing normal levels of p21. Relative quantitation of p21 was determined by densitometry using underexposed films. With these data, the lines could easily be rank-ordered. L1 expressed ≈30-fold higher p21 levels than the controls, and L2, L5, L6, L7, L8, and C4 were similar in levels that were 15- to 20-fold higher than controls. L3, L4, and C1 expressed levels ≈10-fold above the controls, as is evident from Fig. 2.

**Chromosome Banding Analysis.** The stem-line karyotypes and the derivation of all clonal marker types in the transfected lines are summarized in Table 2. The parental line 18-1D-3 showed a diploid male karyotype, except that a small unidentifiable segment was present at the terminal end (q8) of one chromosome 5 and was also present in all the L and C lines.

L1 had a pseudodiploid stem-line characterized by a 4q+ marker and a 6q+ marker (marI). In ≈25% of the analyzed cells, the segment attached to 6q was longer than in the other cells (marII). From these cells, it was evident that the segment translocated onto 6q represents almost the whole short arm of one chromosome 2 and that marI is a deleted marII. All cells showing marII had also a 2p− marker resulting from a 2;4− translocation.

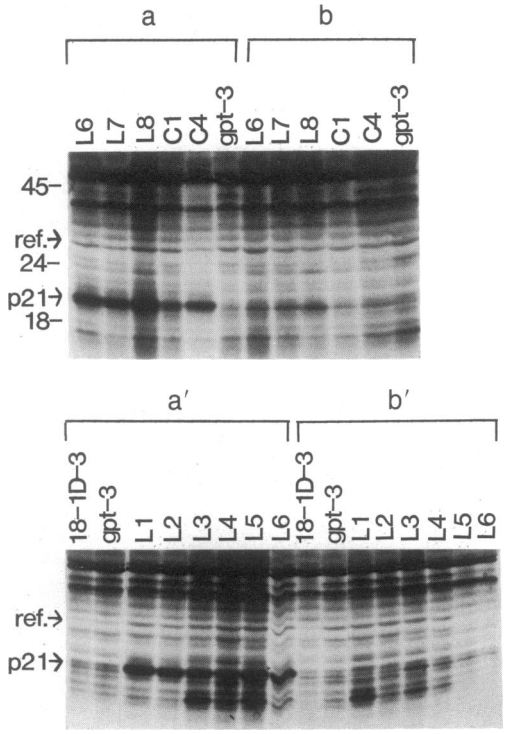


FIG. 2. Electrophoresis of p21. $^{35}$S-labeled proteins from each cell line were immunoprecipitated with rat monoclonal antibody 238 (*a* and *a′*) or normal rat serum (*b* and *b′*) and electrophoresed in 12.5% polyacrylamide slab gels. Band sizes were determined by using molecular weight standards.

Table 2. Cytogenetic findings in the 11 EJ transformants and the two plasmid-induced hit-and-run transformants

| Cell line | Stem-line karyotype | Markers | Mode of origin |
|---|---|---|---|
| L1 | 22,XY,4q+,6q+ | 4q+ | t(4;7)(q3;q2) |
| | | 6q+(I) | t(2;6)(p2;q8), del der(6)(2p6) |
| | | 2p− | t(2;4?)(p6;q3) |
| | | 6q+(II) | t(2;6)(p2;q8) |
| | | Yp− | del(Y)(p0) |
| L2 | 23,XY,+3q | 3q | del(3)(cen) |
| | | Yp+ | t(Y;?)(p2;?) |
| L3 | 22,XY,+3q−,−8 | 3q− | del(3)(q2) |
| L4 | 23,XY,+3q | 3q | del(3)(cen) |
| L5 | 23,XY,inv(3p), +3p+q− | inv(3p) | inv(3)(p2p3) |
| | | 3q− | inv(3)(p0q2), del der(3)(q4-5) |
| L6 | 23,XY,3p+,+3q | 3p+ | t(3;?)(p4;?) |
| | | 3q | del(3)(cen) |
| L7 | 23,XY,+3q | 3q | del(3)(cen) |
| L8 | 23,XY,+3q | 3q | del(3)(cen) |
| C1 | 23,XY,+3q/ | 3q | del(3)(cen) |
| | 23,XY,+5,7q− | 7q− | del(7)(q4) |
| | | Yp− | del(Y)(p0) |
| C3 | 22,XY,3p+q− | 3p+q− | t(3;7)(p2;q2), del der(3)(q5) |
| C4 | 22,XY | — | — |
| gpt-3 | No consensus stem-line* | 7q− | del(7)(q4) |
| | | 3q | del(3)(cen) |
| gpt-4 | 23,XY,+3q | 3q | del(3)(cen) |

*The most common changes observed were a 7q− marker, −8, and +3q.

L2 was hyperdiploid and trisomic for 3q (Fig. 3*a*). In addition, one-third of the stem-line cells showed a Yp+ marker. L4, L7, L8, and gpt-4 were also trisomic for 3q. In L8, a few of the cells were also trisomic for chromosome 5.

L3 was pseudodiploid and was characterized by an extra chromosome 3 showing a terminal deletion in the long arm as well as loss of one chromosome 8 (Fig. 3*b*). Since the banding pattern of the remaining 3q segment (cen → q2) is very similar to that of 8q we cannot exclude the possibility that the 3q− marker results from a whole-arm translocation between 3p and 8q. However, the presence of two normal copies of chromosome 8 in ≈20% of the analyzed cells in an earlier passage suggests that the 3q− marker represents a deletion rather than a 3;8− translocation.

L5 had a hyperdiploid stem-line partially trisomic for chromosome 3 (Fig. 3*c*). Two of the three chromosomes 3 present were consistently rearranged; one copy had a paracentric inversion involving the short arm, and the other had a pericentric inversion and a terminal deletion of the long arm. L6 was also hyperdiploid and showed two different chromosome 3 rearrangements (Fig. 3*d*). All analyzed cells had a 3p+ marker with an extra band of unknown origin at the terminal end. In addition, 75% of the analyzed cells had an extra 3q.

C1 had two different hyperdiploid stem-lines, each constituting ≈50% of the analyzed cells. One showed an extra 3q as the sole abnormality, while the other showed trisomy 5 and a terminal long-arm deletion of one chromosome 7. A few cells in both populations showed an identical Yp− marker. C3 was pseudodiploid and was characterized by a 3p+q− marker resulting from a terminal long-arm deletion and a translocation involving 3p and 7q (Fig. 3*e*). C4 had a normal diploid karyotype without gross chromosomal deviations.

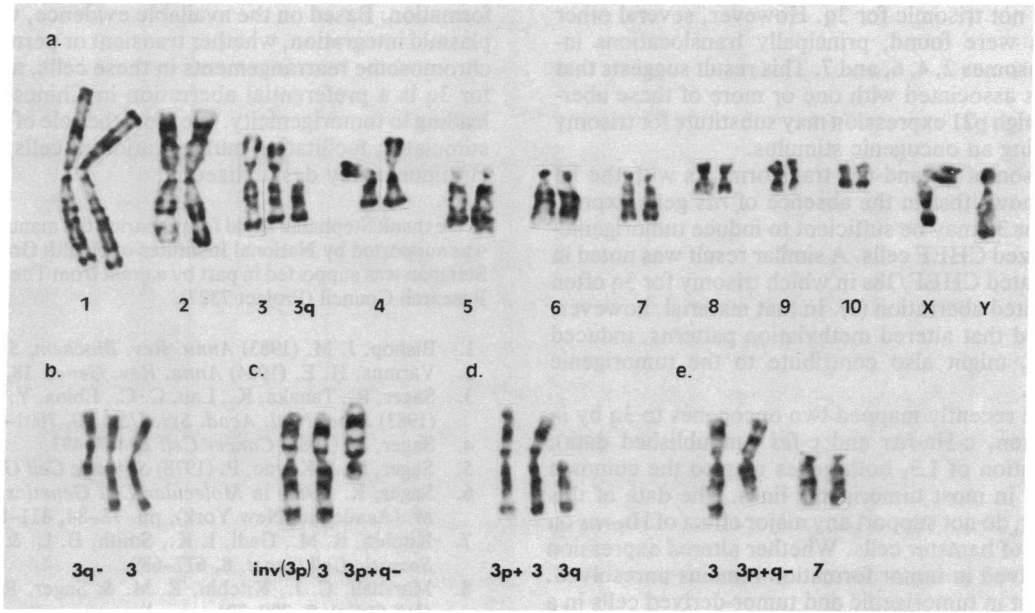


FIG. 3. Complete and partial G-banded stem-line karyotypes of the c-Ha-*ras*-transformed lines with chromosome 3 abnormalities. (*a*) L2; (*b*) L3; (*c*) L5; (*d*) L6; (*e*) C3. (×1500 to ×2100.)

The hit-and-run line gpt-3 showed a complex pattern of rearrangements and no definite stem-line could be discerned. Each of the analyzed cells was karyotypically different. However, some common markers were observed—i.e., a long-arm deleted chromosome 7 found in ≈60% of the analyzed cells, monosomy 8 and +3q found in 40% and 33% of the cells, respectively. In addition, the long arm of chromosome 4 was involved in different types of structural rearrangements in 25% of the analyzed cells.

Most lines contained a small fraction (<5%) of tetraploid cells, constituting exact doubling products of stem-line cells. No cells with normal karyotypes were found in L1–L8, C1, C3, or in gpt-3 and gpt-4.

## DISCUSSION

This study was designed to examine the effects of transfection with the plasmid pSV2gptEJ on tumor-forming ability and chromosomal aberrations in the nontumorigenic cell line CHEF/18. Since CHEF/18 cells are diploid, they provide excellent material for detecting karyotypic changes associated with transfection, with mutant c-Ha-ras expression, and with tumorigenesis. Eleven transfected lines recovered as foci were compared with two lines from foci induced by transient transfection with pSV2gpt—i.e., hit-and-run transfection (10). Several findings resulted from this study.

(*i*) In comparison with the control cells (group 1), the transfected lines fell clearly into two groups, with those of group 3 being much more transformed morphologically than group 2. Only the lines of group 3 were anchorage independent, but both groups were tumorigenic in the nude mouse assay. These results show clearly that tumor-forming ability and anchorage independence are genetically independent in CHEF/18 cells, confirming and extending our previous studies (7, 8).

(*ii*) The role of the c-Ha-*ras* gene was assessed by determining the number of transfected gene copies and by measuring the levels of p21 expression. Most lines were similar in transfected gene copies except L3 and L4, which had fewer, and L1 and L5, which had more copies than the rest. Also, most of the lines were similar in p21 expression, with a few exceptions. The hit-and-run lines expressed only control levels of p21. Line L3 of group 3 as well as L4 and C1 of group 2 expressed only ≈10-fold increased p21 over controls, whereas L1 of group 3 expressed the highest p21, ≈30-fold over controls. Thus, the extent of p21 expression correlated with the number of transfected gene copies but not with the morphological and anchorage differences between groups 2 and 3.

(*iii*) All but one of the transfected lines were tumorigenic. The exception, C4, was also the only line that remained diploid. All of the others, both EJ and hit-and-run transfectants, underwent chromosome rearrangements. This result confirms and extends previous studies, which also showed that diploid CHEF/18 cells are not tumorigenic (7).

(*iv*) Detailed karyotypic analysis revealed a common feature present in all but three of the EJ-transfected lines, and in both hit-and-run transformants—namely, trisomy for all or part of 3q. One further line, C3, showed rearrangements involving both arms of one chromosome 3. The common region of 3q trisomic in all lines was localized to 3cen → q2 (Fig. 4). Complete or partial trisomy for 3q seems to be the most consistent primary change in transformed Chinese hamster cells, whether transformation is induced by cloned oncogenes (present report), chemical carcinogens (7), 5-azacytidine (9), or is of spontaneous origin (12). The second most commonly affected chromosome in this series of *ras* and hit-and-run transformants was chromosome 7. Lines L1, C1, C3, and gpt-3 each showed structural rearrangements involving the segment 7q2-4.

(*v*) Line L1, which expressed the highest levels of p21 in

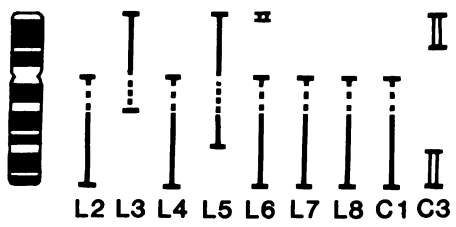


FIG. 4. Schematic representation of complete or partial trisomy 3 and partial monosomy 3 in all lines with chromosome 3 rearrangements. Solid lines indicate trisomic regions, and open bars indicate monosomic regions. Dotted lines correspond to the shortest overlapping region that is trisomic in all lines—i.e., 3cen → q2.

this study, was not trisomic for 3q. However, several other rearrangements were found, principally translocations involving chromosomes 2, 4, 6, and 7. This result suggests that genetic changes associated with one or more of these aberrations and/or high p21 expression may substitute for trisomy of 3q in providing an oncogenic stimulus.

(*vi*) Comparison of hit-and-run transformants with the EJ transfectants shows that in the absence of *ras* gene expression, trisomy for 3q may be sufficient to induce tumorigenicity in immortalized CHEF cells. A similar result was noted in azacytidine-treated CHEF/18s in which trisomy for 3q often was the only noted aberration (9). In that material, however, it was suggested that altered methylation patterns, induced by azacytidine, might also contribute to the tumorigenic phenotype (19).

(*vii*) We have recently mapped two oncogenes to 3q by *in situ* hybridization, c-Ha-*ras* and c-*fes* (unpublished data). With the exception of L3, both genes map to the common region trisomic in most tumorigenic lines. The data of this paper, however, do not support any major effect of Ha-*ras* on transformation of hamster cells. Whether altered expression of c-*fes* is involved in tumor formation remains unresolved.

(*viii*) 3p is lost in tumorigenic and tumor-derived cells in a very consistent manner. In all our previous studies of CHEF cells, only one tumorigenic line was found with three normal copies of chromosome 3 (9). We infer from these results that 3p has a selective disadvantage in tumorigenicity, perhaps owing to the presence of a tumor-suppressor gene. These results are in line with studies showing that Syrian hamster tumors induced by cotransfection with plasmids carrying v-Ha-*ras* and v-*myc* have a nonrandom chromosome abnormality—namely, loss of chromosome 15 (20). Similarly, the reexpression of tumor-forming ability in human cell hybrids of HeLa × normal cells has been associated with the loss of human chromosome 11 (21, 22). Interestingly, human chromosome 11p shows homology to Chinese hamster chromosome 3 in the presence of Ha-*ras* (see above). Whether this homology also includes the region carrying the suppressor gene remains to be determined.

In summary, these results implicate three factors in the induction of tumorigenicity in CHEF/18 cells: trisomy for 3q, increased p21, and perhaps other aberrations present in L1. Of these, only the 3q aberration appears to be a sufficient factor. However, it should be noted that focus formation was ≈10-fold more frequent after pSV2gptEJ than after pSV2gpt transfection (10), thus implicating the *EJ* gene in focus formation. Based on the available evidence, we propose that plasmid integration, whether transient or permanent, induces chromosome rearrangements in these cells, and that trisomy for 3q is a preferential aberration in Chinese hamster cells leading to tumorigenicity. We view the role of p21 as a growth stimulator, facilitating multiplication of cells that have been chromosomally destabilized.

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1. Bishop, J. M. (1983) *Annu. Rev. Biochem.* **52,** 301–354.
2. Varmus, H. E. (1984) *Annu. Rev. Genet.* **18,** 553–612.
3. Sager, R., Tanaka, K., Lau, C. C., Ebina, Y. & Anisowicz, A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7601–7605.
4. Sager, R. (1984) *Cancer Cell* **2,** 487–493.
5. Sager, R. & Kovac, P. (1978) *Somatic Cell Genet.* **4,** 375–392.
6. Sager, R. (1984) in *Molecular Cell Genetics*, ed. Gottesman, M. (Academic, New York), pp. 75–84, 811–828.
7. Kitchin, R. M., Gadi, I. K., Smith, B. L. & Sager, R. (1982) *Somatic Cell Genet.* **8,** 677–689.
8. Marshall, C. J., Kitchin, R. M. & Sager, R. (1982) *Somatic Cell Genet.* **8,** 709–721.
9. Gadi, I. K., Harrison, J. J. & Sager, R. (1984) *Somatic Cell Genet.* **10,** 521–529.
10. Lau, C. C., Gadi, I. K., Kalvonjian, S. & Sager, R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2839–2843.
11. Kitchin, R. M. & Sager, R. (1980) *Somatic Cell Genet.* **6,** 615–629.
12. Ray, F. A., Bartholdi, M. F., Kraemer, P. M. & Cram, L. C. (1986) *Cancer Genet. Cytogenet.* **21,** 35–51.
13. Shih, C. & Weinberg, R. A. (1982) *Cell* **29,** 161–169.
14. Furth, M. E., Davis, L. J., Fleurdylis, B. & Scolnick, E. M. (1982) *J. Virol.* **43,** 294–304.
15. Stenman, G. & Mark, J. (1983) *J. Oral Pathol.* **12,** 458–464.
16. Kakati, S. & Sinha, A. K. (1972) *Genetics* **72,** 357–362.
17. Stubblefield, E. (1974) in *The Cell Nucleus*, ed. Busch, E. (Academic, New York), Vol. 2, pp. 149–162.
18. Standing Committee on Human Cytogenetic Nomenclature (1978) *Cytogenet. Cell Genet.* **21,** 309–404.
19. Harrison, J. J., Anisowicz, A., Gadi, I. K., Raffeld, M. & Sager, R. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6606–6610.
20. Oshimura, M., Gilmer, T. M. & Barrett, J. C. (1985) *Nature (London)* **316,** 636–639.
21. Stanbridge, E. J., Flandermeyer, R. R., Daniels, D. W. & Nelson-Rees, W. A. (1981) *Somatic Cell Genet.* **7,** 699–712.
22. Klinger, H. P. & Shows, T. B. (1983) *J. Natl. Cancer Inst.* **71,** 559–569.