Chromosomes of SV40 Transformed Human Amnion Cells After Mycoplasma Infection[1] (35020)

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The incidence of SV40 transformation was reduced and the appearance of transformed foci delayed by concurrent VS40-mycoplasma infection of primary human amnion cell cultures (1, 2) and several characteristics of the strains of transformed cells were affected. For example, the number of culture passages and population doublings during serial cultivation prior to "crisis" were reduced. The virus production pattern was changed, but differently for individual strains of transformed cells. After mycoplasma elimination during serial culture, the number of passages and cell divisions, and the period of time before "crisis" increased. Several established lines were recovered from "crisis" (3).

Since characteristic chromosome changes were observed in mycoplasma-infected FL human amnion cells (4–6), including a gradual reduction in chromosome numbers, increase in chromosome aberrations, and the appearance of several new chromosome varieties, it was pertinent to investigate whether concurrent mycoplasma-SV40 infection of primary amnion cells resulted in further alterations of the chromosome picture. The present report describes modifications in the chromosome numbers and frequencies of abnormalities which were related to the presence of mycoplasma in the transformed cultures.

Materials and Methods. Virus. The SV40 strain VA 45-54 GMK 4 has been previously described (7).

Mycoplasma. The origin of mycoplasma strain HT (8) and its identification have also been reported (9), as have methods for detection, elimination, and quantitative determinations of colony-forming units for this strain (2).

Cells. Methods for establishing primary cultures of human amnion cells have been previously described (2), and the techniques and media used for subculture of strains of transformed cells have been reported (10). The cultural characteristics of the strains analyzed in the present paper have been described (2).

SV40 transformed amnion cell cultures were prepared for chromosome analysis by a modified version of the suspension technique with ignition of the fixative as developed by Moorhead *et al.* (11). The same technique was used for the cultures resulting from concurrent SV40-mycoplasma infection. The chromosomes of cells of many culture passages were studied for chromosome numbers, abnormalities, and new chromosome varieties.

Results. Effects on chromosome numbers. The number of chromosomes in SV40 transformed human amnion cells in the absence of mycoplasma has been reported (10). During a period of time following transformation, the distribution of numbers in repeated experiments, was in the diploid range. However, after some culture passages, cells with hypertriploid or hypotetraploid numbers were observed. Thus, based on all our collected data, including cells from many different amniotic membranes, 15 to 25% metaphase plates were in the high range between 3 and 5.5 months after SV40 infection, as illustrated by 7 strains; 26 to 50% at 3 to 6 months as illustrated by 10 strains; and more than 50% at 3.5 to 6 months as illustrated by 5 strains. For 6 strains, close to 100% cells

[1] This investigation was supported in part by NCI Research Grant No. CA-08748.

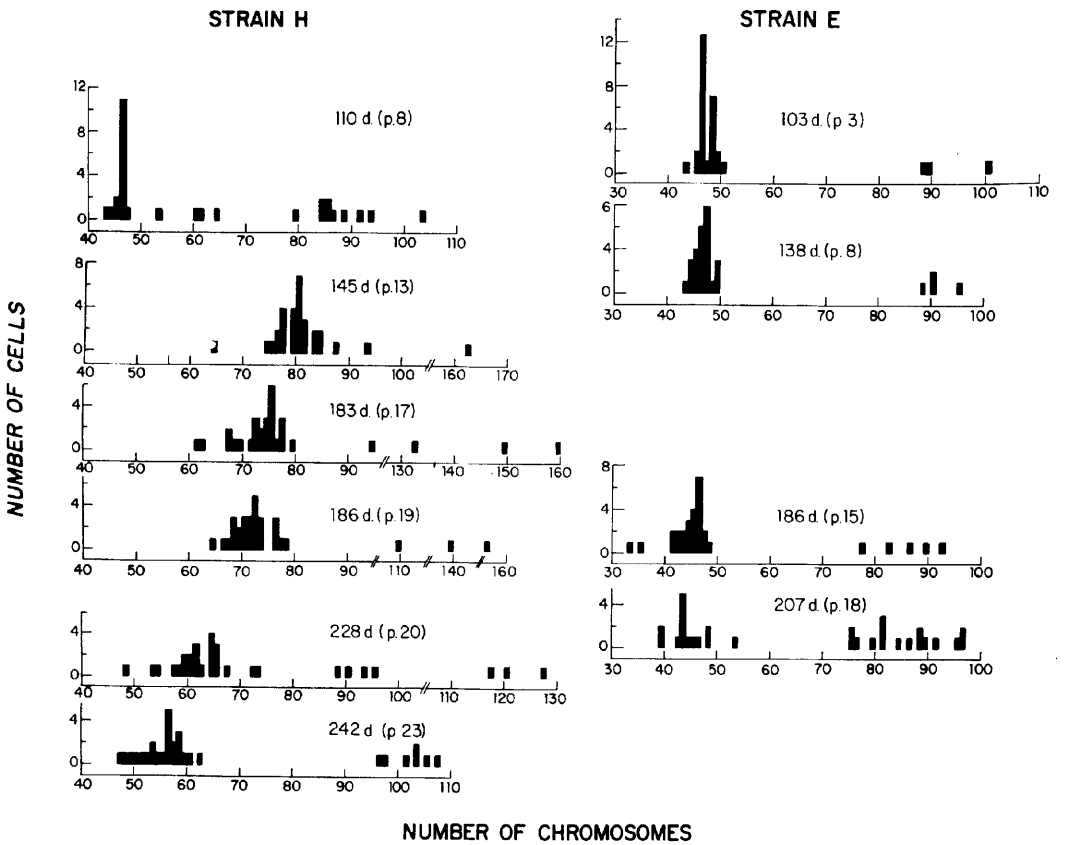

FIG. 1. (left side) Chromosome numbers of SV40 transformed amnion cells (strain H (resulting from infection with SV40 without mycoplasma); Expt. A245) from culture passage 8 to 23, 110 to 242 days after infection. (right side) Chromosome numbers of strain E (resulting from infection with SV40 and mycoplasma); Expt. A245. Culture passages 3 to 18, 103 to 207 days after infection.

were hypertriploid or hypotetraploid between 3 and 6 months after infection.

The effects of concurrent mycoplasma-SV40 infection were studied in Expt. A245 (2). The chromosome numbers for two strains of transformed cells of this experiment, strain H (infected only with SV40) and strain E (infected with SV40 and mycoplasma) are depicted in Fig. 1. The numbers for strain H had changed to the hypertriploid–hypotetraploid range when examined in culture passage 13, 145 days after infection. In the following culture passages examined there was a gradual reduction of the numbers of chromosomes. In the latest passage, day 242 after infection, most metaphase plates contained between 50 and 60 chromosomes. "Crisis" was observed at 259 days after infection. In contrast, the major proportion of cells of strain E maintained chromosome numbers in the diploid range up to the 18th culture passage, 207 days after infection. At this time approximately half the cells were in the hypertriploid to hypotetraploid range. "Crisis" was observed for strain E at 237 days after infection. Chromosome numbers were determined for another strain (strain F) of transformed cells, originating from the same amniotic membrane, and also infected with SV40 and mycoplasma. Changes towards higher chromosome numbers were observed 2 to 3 weeks earlier. In the latest passages prior to "crisis," which occurred at day 259 after infection, the major proportion of metaphase plates were hypertriploid.

Mycoplasma elimination from cells of

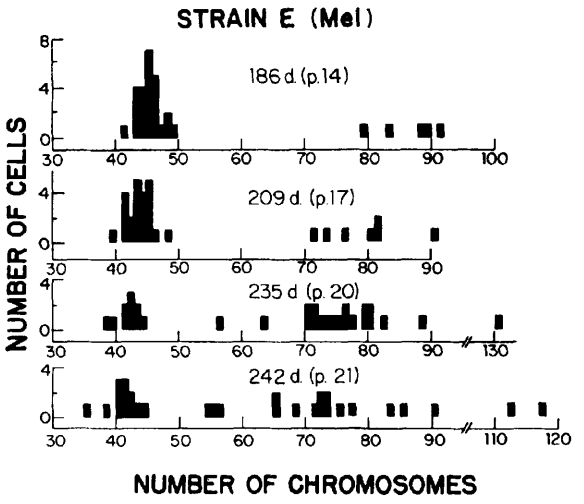

FIG. 2. Chromosome numbers of strain E (Mel), derived from strain E by elimination of mycoplasma from day 170 after infection. Culture passages 14 to 21, 186 to 242 days after infection.

strain E, by a short treatment with Aureomycin from day 170 after SV40 infection, resulted in a strain [strain E (Mel)] which differed from strain E in respect to growth potential and virus production, as reported (2). However, chromosome numbers for cells of strain E (Mel) did not differ notably, at similar times, from those of strain E which still carried the mycoplasma infection, as shown in Fig. 2. During the latest passages of strain E (Mel), approximately half of the examined cells were diploid, whereas, most other cells were hypertriploid or hypotetraploid.

Chromosome abnormalities. Previously reported data, representing several independent experiments, have shown that many abnormalities, including chromosome breaks and translocational exchanges, could be observed in the chromosomes of SV40 transformed amnion cells (10). Dicentric chromosomes were common, and their frequency increased with the time of cultivation. Acentric fragments and minute chromosomes were also more frequent in the later culture passages, as was the occurrence of ring chromosomes. Secondary constrictions were unusually clearly pronounced in many metaphase plates. Uncoiled, disintegrated, or puffy chromosomes were observed. A large subtelocentric and a large telocentric chromosome were seen at different frequencies during the various culture passages prior to "crisis."

As shown in Table I, all these chromosome abnormalities were seen after concurrent SV40 and mycoplasma infection. It is apparent that many of the abnormalities occurred at lower frequencies in the transformed cells also infected with mycoplasma. This difference became highly pronounced with increasing time after infection. Thus, in the earlier months after infection and transformation there was a higher frequency of di- and polycentric chromosomes, minute chromosomes, ring chromosomes, secondary constrictions, and presence of the large telocentric chromosome in the strains of transformed cells not infected with mycoplasma. Chromosome breaks and gaps were slightly increased in the presence of mycoplasma. Other abnormalities were approximately equal. However, in the later culture passages, all the listed abnormalities were reduced in frequency in the mycoplasma infected and transformed cell strains, except for translocational exchanges and secondary constrictions which occurred at a similar frequency.

Cells of strain E (Mel) contained abnormalities to the same extent as strain E, with one exception (Table II). There was a definite increase in the frequency of the new large

TABLE I. Percentage of Metaphase Plates with Chromosome Abnormalities and with New Chromosome Varieties in SV40 Transformed Amnion Cells in the Absence (SV40) or Presence (SV40 + M) of Mycoplasma.

Data are pooled for various times, early and late after infection.

Infected with:	SV40	SV40 + M	SV40	SV40 + M
After infection (days):	110, 145	103, 110, 138	183, 186, 228, 242	186, 207, 242
No. of metaphase plates:	60	90	120	150
Metaphase plates with abnormalities:		Percent		
Di- and polycentric chromosomes	65	26	96	77
Acentric fragments	13	15	33	13
Minute chromosomes	16	2	47	31
Ring chromosomes	6	0	15	7
Chromosome breaks	2	9	21	15
Gaps	0	4	14	7
Translocational exchanges	0	0	4	3
Secondary constrictions	12	5	7	7
Large telocentric	17	2	8	5
Large subtelocentric	0	2	24	9

telocentric chromosome. In comparable culture passages, this chromosome was present in 13 to 37% of the metaphases in strain E (Mel), and only in 0 to 3% of the cells in strain E.

Discussion. In addition to the differences shown in Table I, other changes in chromosome abnormalities were observed after mycoplasma infection; for example, the proportion of poly- to dicentric chromosomes was reduced and the number of abnormalities per metaphase plate, or abnormalities per chromosome, was lower.

Since the chromosome numbers for cells from which mycoplasma was eliminated [strain E (Mel)] were similar to those still carrying the infection (strain E), and abnormalities occurred at the same frequencies in the two strains, it appears that the mycoplasma-induced changes were irreversible, as reported for mycoplasma-modified FL cells (5). One exception was the large telocentric chromosome, which was observed only at high frequencies after mycoplasma elimination. It resembled a chromosome observed in mycoplasma-modified FL cells (4, 5). Reduction of

TABLE II. Percentage of Metaphase Plates with Chromosome Abnormalities and with New Chromosome Varieties in Strains E and E (Mel) at Various Times After Infection.

	Strain E		Strain E (Mel)			
After infection (days):	186	207	186	209	235	242
Metaphase plates with abnormalities:			Percent			
Di- and polycentric chromosomes	50	70	63	63	87	87
Acentric fragments	0	7	0	23	3	10
Minute chromosomes	30	17	23	17	10	10
Ring chromosomes	0	7	0	10	10	10
Chromosome breaks	7	13	7	13	7	13
Gaps	Ob[a]	20	7	10	23	20
Translocational exchanges	3	0	3	3	3	0
Secondary constrictions	3	3	20	0	3	10
Large telocentric	0	3	13	23	33	37
Large subtelocentric	10	17	0	13	17	0

[a] Observed but not counted.

chromosome numbers was also a notable effect of mycoplasma after infection of the heteroploid FL cells. Chromosome aberrations, however, increased in mycoplasma infected FL cells, but after mycoplasma elimination they decreased in frequency, to the level observed in uninfected FL cells. Oscillation between the diploid level and hypotetraploid elements has been characteristic for both epithelial and fibroblastic (12) SV40 transformed human cell populations. Thus, the relative absence of hypertriploid–hypotetraploid cells after mycoplasma infection may be the result of an adaptation or a reaction to a "selection pressure" in the population towards cells with lower numbers, and may be interpreted either by a delay of the appearance of or a specific elimination of cells with higher numbers conceivably by cell destruction. There is evidence from studies of other human cells systems that cells with higher chromosome numbers are more susceptible to destruction by mycoplasma than the diploid cell from which they are derived (for example, FL cells and primary amnion cells). Recent experiments (to be published) have indicated a similar difference for SV40 transformed amnion cells. However, there are reasons to assume that the observed changes in chromosome number and abnormalities may include mycoplasma effects on the cell genome; for example, the close mycoplasma-cell association (13), the selection of the cell site, rather than the fluid culture phase, for the location of mycoplasma propagation (14), the intracellular presence (15), the altered nucleic acid metabolism which can be observed in mycoplasma-infected cell cultures (16, 17), and the irreversibility of chromosome changes observed in the present system. Chromosome effects after mycoplasma infection of diploid human fibroblasts, have been interpreted by a competitive effect for nucleic acid precursors which may interfere with the host cell DNA synthesis (18).

Chromosome analysis of three established, post-"crisis" lines derived from replicate "crisis" cultures of strain E (Mel), has previously been published (3). Many aberrations were observed in the lines and the range of chromosome numbers differed among the lines. There was no evidence, therefore, that the pre-"crisis" pattern of SV40 plus mycoplasma was characteristic of these post-"crisis" lines. As was found for other established SV40 transformed amnion lines, increasing chromosome number was generally correlated with an increase in di- or polycentrics, and a decrease in acentric fragments and minute chromosomes. As reported (3), recovery from "crisis" has been an exceptional occurrence for SV40 transformed amnion cells, and the recovery of the 3 lines from mycoplasma-modified strains may have been related to the different chromosome picture observed prior to "crisis." "Crisis" is preceded by a cellular change towards a decreased resistance of SV40 infection (19). This change, in the absence of mycoplasma, coincides with the increase in chromosome number and frequency of many abnormalities. Thus, when cells with high chromosome numbers and many abnormalities are mostly eliminated after mycoplasma infection, the higher number of cells with lower chromosome numbers and fewer abnormalities may be advantageous for survival and subsequent recovery as established cell lines.

Summary. Concurrent mycoplasma-SV40 infection of cultures of primary amnion cells changed the chromosome picture of the resulting SV40 transformed cells. Chromosome numbers were maintained in the diploid range for a longer period, and most abnormalities were present at lower frequencies, especially during the later period of cultivation prior to "crisis." Chromosome numbers and frequencies of abnormalities in the mycoplasma-modified cells were not reversed after mycoplasma elimination. However, a large telocentric chromosome was more frequent after mycoplasma elimination. The recovery of several post-"crisis" cell lines from a mycoplasma-modified SV40 transformed strain of cells may be correlated with the lower chromosome numbers and the fewer abnormalities.

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Received Feb. 24, 1970. P.S.E.B.M., 1970, Vol. 135.