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Chromosome Changes Revealed by the Q-Band Staining Method during Cell Senescence of WI-38 (38381)

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Normal fibroblastic cell lines are finite in their life expectancy and can maintain positive growth for only approximately 50 generations (1–4). This phenomenon has been termed cell aging or senescence. The chromosome constitution of most normal human cells *in vitro* is diploid ($2n = 46$). Detailed studies have shown that departures from diploidy occasionally occur (3). For example, aneuploidy and the frequency of chromosome breaks and gaps are relatively invariable during the finite life (FL) *in vitro* and occur at a frequency of approximately 0.03 for both. However, the frequency of aneuploidy (both hypo- and hyper-diploidy) occasionally increases from 0.03 early in FL to 0.20 late in FL. The frequency of stem cell populations bearing rearranged chromosomal constitutions fluctuates during all phases of FL but is generally higher in older populations.

This report deals with two questions: (a) Do progressive modifications occur in the structure of chromosomes during the finite life of normal cell lines from human embryos?; and (b) What is the origin and fate of chromosome rearrangements which occasionally occur in these cell lines? These questions can be answered with greater refinement than before as a result of the introduction of new chromosome staining procedures (5).

Materials and Methods. Diploid fibroblasts, WI-38, were obtained on four separate occasions: three live stock ampules from Wister Institute, Philadelphia, and one from the Institute for Medical Research, Camden. The stocks were used to initiate separate aging experiments which were carried out serially over a three year period.

In order to detect subtle, progressive changes in banding associated with cell age, we examined the chromosome constitution of WI-38 during early (passage 27), middle (passages 27–40), and late (passage 40) phases of FL at weekly intervals. A total of 140 cells of high quality were randomly sampled during FL from experiments I and IV: 20 from early; 54 from middle; and 66 from late phase. The quinacrine mustard (QM) staining method (6, 7) was employed in this study. This technique permits the analysis of longitudinal differentiation along chromosome arms and allows the identification of each homologous chromosome pair. Moreover, this method reveals alterations in chromosome structure with a greater degree of discrimination than conventional chromosome staining procedures. Cultures were tested for pleuropneumonia-like organisms (PPLO) periodically by the Baltimore Biological Laboratories. No infections were detected.

Results. Our observations revealed no detectable alteration in the banding pattern of diploid chromosome sets during FL. This indicates that in the majority of cells no progressive change in chromosome structure occurs during the so-called aging process. Nevertheless, it is known from earlier studies that generally a small proportion of cells in normal cultures undergo chromosomal rearrangement, even during the early *in vitro* period (3). We have used the QM technique to obtain greater insight into the nature, origin and fate of such chromosomal alterations. In this report, we have selected three chromosome modifications which are particularly instructive.

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The first case involves an extra chromosome of the A group as determined by conventional chromosome staining with orcein. Staining with QM revealed, however, that the additional chromosome was actually a chromosome 1 with a deletion of the short arm (1p-, Fig. 1). Cells of this type also constituted a stem population comprising 3% of the population in experiment IV for 10 population doublings during the middle phase of senescence.

The second case involves a deletion of the short arm of chromosome 4 (4p-, Fig. 2). We have observed cells with this deletion in four separate experiments. In one experiment (experiment I) we could demonstrate that cells bearing the deletion constituted a replicating stem population since their frequency during several sequential passages rose slightly above 3%. Possibly cells of this type exist as a subpopulation in the very early generations of WI-38.

The third case which was observed in experiment IV involves an apparent instance of quasidiploidy (C+/E−) when studied by conventional staining methods. However, QM staining showed that an E-17 chromosome had been modified, most likely by the translocation of the distal two-third segment of 11q to its short arm (Fig. 3). Cells of this type, t(11q-,17) also constituted a stem line population.

Discussion. In early or later *in vitro* stages

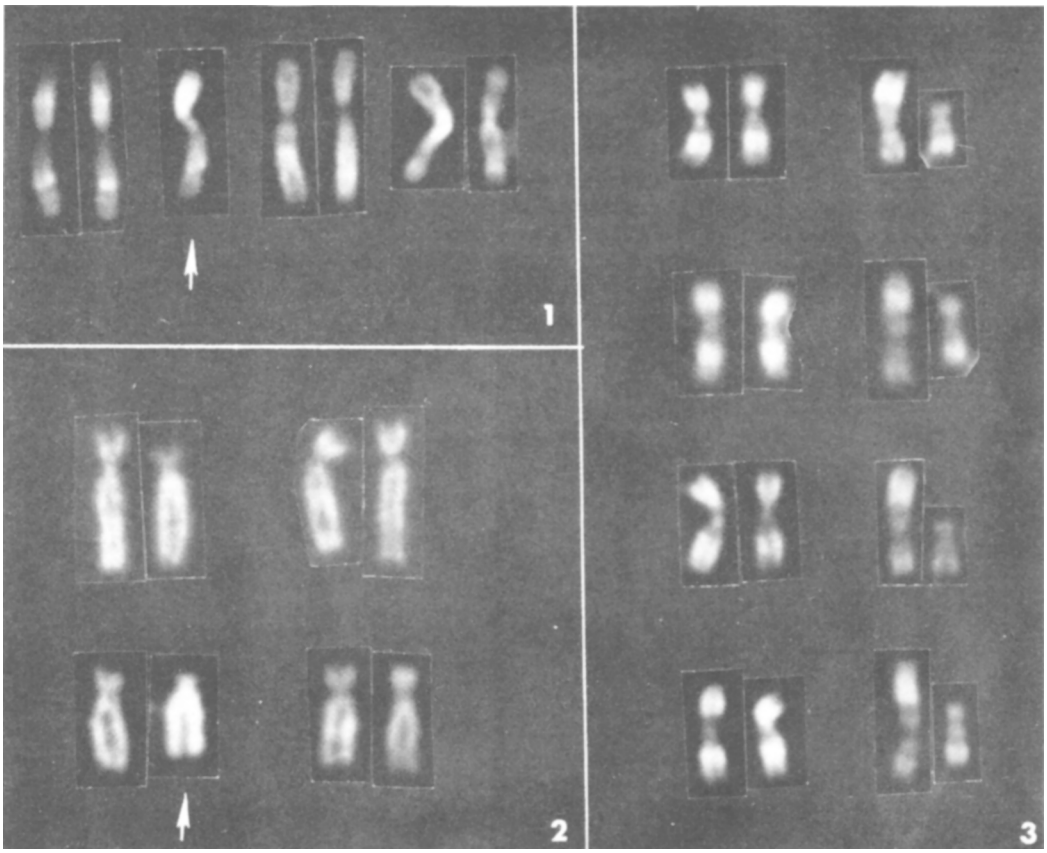

FIG. 1. The marker 1p-, the A-1 chromosome with a missing segment on the short arm (arrow), is shown together with other A group pairs. The marker appeared like an A-2 in aceto–orcein stained preparations.

FIG. 2. Number 4 chromosome (4p-, arrow) with an apparently missing segment in the short arm is shown together with normal B-4 (1st column) and B-5 pair (2nd column) for comparison.

FIG. 3. The marker t(11q-, 17) (second column at left) flanked by normal C-11 pair (first column) and an E-17 (second column at right) is selected from different *in vitro* stages (from top to bottom rows): 35th cell doubling (C.D.); 39th C.D.; 43rd C.D.; and 44th C. D. Note the stable appearance of the marker in sequential cell generations.

alike, diploid chromosome sets did not show any detectable changes in banding pattern. These observations are consistent with results from somatic cell hybridization studies which suggest that chromosomes from senescent WI-38 cell populations, which are rescued by cell fusion, function normally for indefinite periods of time in hybrid cells (8–10). These results, although by no means conclusive, provide additional evidence for the overall genetic stability of normal human, primary, fibroblast cultures propagated *in vitro*. The results also suggest that major genetic changes do not contribute to the "aging" of cultured fibroblasts.

Whereas the great majority of cells are normal diploids in primary human fibroblasts, the population at all stages of propagation also contains cells with chromosomal rearrangements. The population is probably composed of numerous minor stem cell populations which compete to dominate. In some instances a particular population may increase to 2–3% of the population, then decrease below the level of detection as in the case of 4p-. In other instances, the variant stem line may increase to 35% of the population as in the case of t(11q-, 17). It is necessary to keep the heterogeneity of primary cell populations in mind when using them for various experimental or industrial purposes, as for example human gene mapping by cell hybridization (11) or vaccine production.

Perhaps the frequency of chromosome abnormalities in primary cell populations *in vitro* is much greater than in comparable tissues *in vivo*. It is not possible now to distinguish between two alternative explanations for this difference: (a) higher rate of occurrence *in vitro*, or (b) selection against chromosomal mutants *in vivo*. However, it is of great interest that some of the chromosome variants observed in human *in vitro* cells resemble modifications associated with certain human congenital anomalies. For example, the 4p- (Fig. 1) observed in WI-38 cells resembles the chromosome associated with the 4p- syndrome (12). This suggests that either certain regions of chromosomes are more susceptible to breakage or exchange than are others (13, 14), or that certain chromosomal rearrangements affect cell viability less than do others.

Summary. Employing the quinacrine-mustard flurochrome staining procedure, Q-band karyotypes were analysed in selected metaphases from early, middle, and late stages *in vitro* of a human fibroblastic line WI-38. Systematic shifts in the banding patterns of the chromosomes were not detected, suggesting that the chromosomes are structurally stable during all stages of senescence. It can thus be concluded that progressive chromosomal change is not a correlate of the aging process. However, the chromosome constitution of cultured WI-38 fibroblast is not uniformly diploid as suggested by previous reports. Several recurring chromosomal rearrangements, namely 1p-, 4p-, and t(11q-, 17), were detected at sufficiently high frequencies to insure their generation by minor stem cell populations. It was also observed that certain chromosomal rearrangements in the stem cell populations, as for example 4p-, resembled those associated with certain congenital abnormalities. Possibly these chromosomal modifications arise preferentially and/or the cells which bear them are not seriously disadvantaged.

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1. Hayflick, L., and Moorhead, P. S., Exp. Cell Res. **25,** 585 (1961).

2. Hayflick, L., Exp. Cell Res. **37,** 614 (1965).

3. Ruddle, F. H., and Chen, T. R., "Nineteenth Annual Meeting of the Tissue Culture Association," abstract no. 141. p. 62. (1968).

4. Martin, G. M., Sprague, C. A., and Epstein, C. J., Lab. Invest. **23,** 86 (1970).

5. Paris Conference: Standardization in human cytogenetics. Cytogenetics **11,** 317 (1972).

6. Caspersson, T., Farber, S., Foley, G. E., Kudynowski, J., Modest, E. J., Simonsson, E., Wagh, U., and Zech, L., Exp. Cell Res. **49,** 128 (1969).

7. Caspersson, T., Zech, L., Johansson, C., and Modest, E. J., Chromosoma (Berlin) **30,** 215 (1970).

8. Davidson, R., and Ephrussi, B., Exp. Cell Res. **61,** 221 (1970).

9. Goldstein, S., and Lin, C. C., Exp. Cell Res. **70,** 436 (1972).

10. Ruddle, F. H., *in* "Advances in Human Genetics" (H. Harris, and K. Hirschhorn, eds.), vol. 3, p. 173. New York, Plenum (1972).

11. Ruddle, F. H., Nature **242,** 165 (1973).

12. Arias, D., Passarge, E., Engle, M. A., and German,

J., J. Pediat, **76,** 82 (1970).

13. Cohen, M. M., and Shaw, M. W., J. Cell Biol. **23,** 386 (1964).

14. Shaw, M. W., and Cohen, M. M., Genetics **51,** 181 (1965).

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