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MICROCOCCAL NUCLEASE AND DNase I DIGESTION OF DNA FROM AGING HUMAN DIPLOID CELLS

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Nuclei prepared from confluent and mitotically arrested populations of human diploid fibroblast-like cells of different in vitro ages were subjected to digestion by micrococcal nuclease and DNase I. There was no age or culture state variation in the susceptibility of DNA to micrococcal nuclease digestion. There was, however, an age related inhibition of DNA digestion by DNase I in nuclei from older confluent but not older arrested cells. It is suggested that this is the result of an age related masking by nucleosome core histones which limits the accessibility of DNA to enzymatic activities in older confluent cells.

INTRODUCTION

Human diploid fibroblast-like cells in culture provide an established in vitro model system for the study of cellular senescence (1). Among the theories proposed to explain the limited in vitro lifespan exhibited by HDF have been those that attribute functional failure and cell death to age related alterations in genetic information (2-4). These theories have been supported by evidence of an increased incidence of altered DNA in older HDF populations (5-7) which agrees with earlier findings from other model systems (8-10). Additionally, age related alterations in chromosomal proteins have indicated that certain proteins may be more tightly bound to DNA in both older populations of HDF (11, 12) and older animals (13, 14). Alterations in DNA-protein binding characteristics can result in restricted accessibility of DNA to enzymatic activities. Such restrictions could explain the age related decline of DNA repair synthesis in HDF (15) and provide a mechanism for the increase in DNA abnormalities observed in older populations (5-10).

ABBREVIATIONS HDF - human diploid fibroblast-like cell;
PDL - population doubling level.

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The influence of in vitro age on DNA accessibility to enzymatic activities was determined by measuring DNA susceptibility to micrococcal nuclease and DNase I. These enzymes were reacted with nuclei prepared from different age HDF that had recently attained confluence or had been maintained in an essentially non-mitotic, arrested state (16). Micrococcal nuclease was employed to detect changes in accessibility associated with the nucleosome linker region (17), and DNase I was used to determine those associated with the nucleosome core particle (18). Arrested populations were included because previous results showed that older cells maintained in this culture state removed DNA damage more rapidly than confluent populations (7), indicating an alteration in enzyme access and thus providing a possible system to further investigate chromatin structure and aging.

MATERIALS AND METHODS

The cells, designated CF-3, used in these experiments were derived from newborn foreskin and have a characteristic in vitro lifespan of 65 $\pm$ 10 population doublings. Cells were grown in T-75 plastic flasks (Falcon), and the culture conditions for both growth to confluence and maintenance in an arrested state have been described previously (16).

Log phase cultures were radiolabeled for 48 hr with either [^{3}H] or [^{14}C] thymidine (New England Nuclear) at 0.1 and 0.05 μC/ml, respectively. The radioactive medium was replaced with unlabeled medium for an additional 72 hr when confluence was attained. Nuclei were prepared from radioactively labeled cells at confluence or after they had been maintained in an arrested state for 9 days.

Cells were removed from the growth surface by scraping into phosphate buffered saline at 4°. Nuclei were prepared by placing the cells in distilled water for 2 min on ice and expelling them four times through a 22 gauge hypodermic needle. Nuclei were pelleted by low speed centrifugation and washed twice in the appropriate assay buffer before resuspension in assay buffer for enzymatic digestion. The distilled water and the assay buffer washes contained 1.0 mM phenylmethanesulfonyl fluoride.

Digestion of DNA by micrococcal nuclease and DNase I (both purchased from Worthington) was followed by the release of acid soluble radioactivity under conditions described by Smerdon et al. (19) for micrococcal nuclease and Nelson et al. (20) for DNase I.

RESULTS

The data from the digestion assays were grouped according to the in vitro age of the cells. Low PDL cells were those that had completed less than 50% of their characteristic in vitro lifespan and high PDL cells were those that had

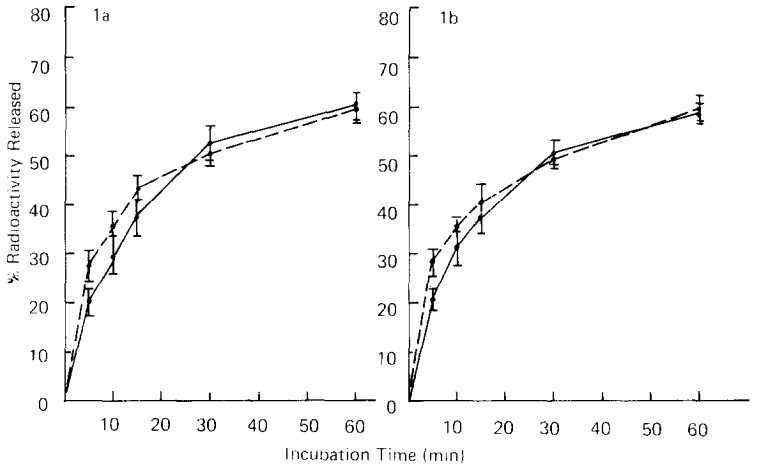

FIG. 1. Release of radioactivity from nuclei obtained from confluent (●——●) and arrested (●---●) populations of low (1a) and high (1b) PDL cells incubated with micrococcal nuclease. Each point represents the mean ± SE from 6 to 9 experiments. The range of cellular doubling levels used in these experiments was 13 to 36 for low PDL cells and 39 to 65 for high PDL cells.

completed more than 50% of their lifespan. The results presented in Figure 1 were obtained when micrococcal nuclease was used to digest the DNA from confluent and arrested low (Fig. 1a) and high (Fig. 1b) PDL cells. These results show that there are no major effects of *in vitro* age on the ability of micrococcal nuclease to access and digest HDF DNA under these experimental conditions. The only variation between the confluent and arrested cells occurred at the 5 min time point. While this may indicate a slightly greater accessibility in arrested cells during the initial steps of the digestion process, any statistically significant alteration in sensitivity was lost by the 10 min time point.

When DNA was digested with DNase I, the results presented in Figure 2 were obtained. There was no difference in the release of radioactivity from confluent or arrested low PDL cells (Fig. 2a). There was, however, a significant inhibition of release from confluent high PDL cells (Fig. 2b). This inhibition was not evident in arrested populations of high PDL cells that yielded release curves similar to those obtained from low PDL cells. These data indicated that DNA from confluent high PDL populations was less susceptible to DNase I digestion but that this inhibition could be overcome in arrested cultures.

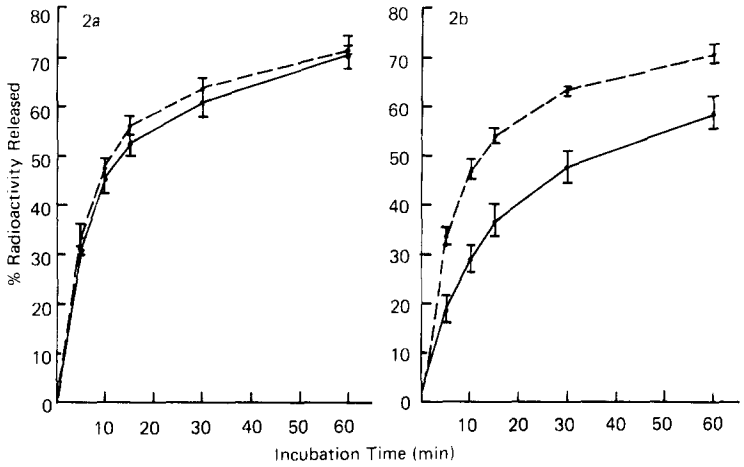

FIG. 2 Release of radioactivity from nuclei obtained from confluent (●——●) and arrested (●---●) populations of low (2a) and high 2b) PDL cells incubated with DNase I. Each point represents the mean ± SE from 7 to 12 experiments. The range of cellular doubling levels used in these experiments was 14 to 30 for low PDL cells and 39 to 65 for high PDL cells.

DISCUSSION

Previous attempts to detect age related differences in the ability of micrococcal nuclease and DNase I to access and digest DNA have employed material from in vivo systems and have indicated little or no variation with age. Gaubatz et al. (21) reported minor differences in the ability of both enzymes to digest DNA from certain mouse tissues, and Hill and Whelan (22) found no age related variation in the accessibility of DNase I to DNA from mouse chromatin. Other information concerning the possible masking of DNA by chromosomal proteins in older cells and animals, however, have suggested that susceptibility of DNA to enzymatic attack may be inhibited with age (11-14). Further it has been demonstrated that alterations in the association of nuclear proteins, primarily histones, with DNA can effect the rate and extent of micrococcal nuclease and DNase I digestions (20, 23, 24).

The results presented here show for the first time in the HDF model system an age related inhibition of enzyme access to DNA. This inhibition does not appear to be associated with the nucleosome linker region, since no significant variation in DNA susceptibility to micrococcal nuclease was detected (Fig. 1). However, the data do suggest that DNA associated with the nucleosome core

particles from older cells is less susceptible to enzymatic attack (Fig. 2). Since this restricted accessibility occurs only in confluent high PDL cells, the data indicate that its cause(s) is reversible and amenable to study in the confluent/arrested cell system.

One candidate for study is the reversible process involved in the post-synthetic acetylation of core particle histones. The rate of histone acetylation has been reported to decline in older HDF (11). Also, the extent of histone acetylation has been shown to effect both the binding of core histones to DNA (20) and the digestion of DNA by DNase I (20, 23, 25). The decline of histone acetylation with age would result in the tighter association of core histones with DNA. This closer association would reduce enzyme accessibility and could result not only in the reduction in proliferative activity (1), but also in DNA repair synthesis (15). Altering the culture conditions to place the cells in an arrested state may allow for a greater degree of histone acetylation and enzyme access in older populations. This would offer an explanation for the results reported here as well as those previously noted concerning the removal of DNA damage in arrested high PDL cells (7). Additional experiments to determine the extent of histone acetylation in confluent and arrested HDF may offer a better insight into the relationship between age related alterations in chromatin and the increase in DNA abnormalities.

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