Close Examination of Cell Nuclei Reveals a ‘Ring’ Surrounding the Nuclear Membrane

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Summary  Human cells from a variety of diseases, as well as controls, were fixed in Carnoy’s solution, air-dried onto slides, incubated in phosphate buffer, and stained with Giemsa. Close microscopic examination revealed that in most cells, the nucleus contained a ring-like structure at the nuclear periphery. The ‘ring’ resembled a cusp or seam in structure, and was even seen in cells that had not been incubated. It is not known whether this ‘ring’ is a real structure or an artifact of the staining procedure. While the function of the ‘ring’ is unknown, it may act as a seam to maintain the integrity of the nuclear membrane or may serve as a storage magazine for the synthesis/repair of the nuclear membrane.

We have recently reported the finding that lymphoblasts from the premature aging disorder Werner syndrome (WS) display rings of unstable heterochromatin (hc) upon high temperature phosphate buffer incubation and Giemsa staining (Edelman and Lin 2000, 2001). A similar finding resulted when we studied Fanconi Anemia (FA) cells (Lin and Edelman 2001). The rings usually encircled the nuclear membrane and were thought to be composed of hc which was sloughed-off from chromosomes during the incubation procedure. Such rings were not seen in control cells. These findings were used to formulate and advance the hypothesis that unstable hc is a characteristic of WS/premature aging disorders.

Continuation of our work indicates that nearly all, if not all cells contain a ring-like structure which appears to encircle or straddle the nuclear membrane. We have not determined if this ‘ring’ is real or an artifact of the cell preparation and staining procedure. If this structure is indeed real, we offer some speculation as to its possible functions.

Materials and methods

Cells of various genetic diseases as well as controls were obtained from various sources. Lymphocytes from individuals with xeroderma pigmentosum and progeria were obtained as cell cultures from the Coriell Cell Repositories, Camden, New Jersey, U.S.A. Cells were harvested by standard methods using colcemid, hypotonic solution (0.075 M KCl), Carnoy’s fixative (methanol-acetic acid, 3:1) and air-dried onto ice water-chilled glass slides. Lymphocytes of Fanconi anemia cell lines were obtained from the laboratory of Dr. Grover C. Bagby, Jr., M.D. and Ms. Winifred Keeble of Oregon Health Sciences University, as well as from Dr. Alan D’Andrea, M.D., and Dr. Akiko Shimamura, M.D., Ph.D. of the Dana Farber Cancer Institute of Harvard University. WS lymphoblasts and control cells were provided by Dr. George M. Martin, M.D., School of Medicine, University of Washington. Slides were allowed to age for at least one day after air-drying.

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Some slides were merely stained in 3% Giemsa (Gurr’s Improved R66 dissolved in Sorensen’s phosphate buffer, 0.066 M) for 15 min. Other slides were incubated in the same buffer in Coplin jars in a water bath for 2 h before Giemsa staining. Additional slides were treated with high-intensity incandescent light for 75 min using our sister chromatid differential staining (SCD) method reported previously (Edelman and Lin 1991) before Giemsa staining. Cells were then examined and photographed using a Zeiss photomicroscope.

Results and discussion

In nearly all cells examined, close examination revealed a ‘ring’-like or ‘cusp’-like structure at the periphery of the nucleus, and this structure appeared to be part of the nuclear membrane. Often it helped to manipulate the fine adjustment knob slowly in one or both directions in order to see the

Fig. 1. ‘Rings’ surrounding the nuclear membrane in various cells after Giemsa staining, with or without prior high-temperature phosphate buffer incubation: (a) xeroderma pigmentosum lymphocytes (no incubation); (b, c) progeria lymphocytes (incubation); (d) progeria (no incubation); (e) Fanconi anemia (no incubation); (f) Fanconi anemia (incubation); (g) Werner syndrome (incubation with exposure to high-intensity incandescent light). Arrows point to location of ‘rings’. Bar=10 μm.
‘ring’ in better detail. Cells that were subjected to high temperature incubation (63°C) in phosphate buffer usually exhibited the most distinct ‘rings’, but cells subjected to high intensity incandescent light exposure in phosphate buffer also exhibited rings. Even cells that were untreated before being stained with Giemsa usually exhibited ‘rings’, but they were more difficult to visualize.

Figure 1 shows a variety of cells in which ‘rings’ can be seen. Among the cells shown with nuclear ‘rings’ of varying clarity are those from patients with xeroderma pigmentosum, the premature aging disorders progeria and WS, and Fanconi anemia.

It should also be noted that nuclear ‘rings’ were also seen in control cells. In addition, preliminary work in our laboratory on cells from various animals (mice, rats, gerbils, hamsters, etc.) and even plants indicate the presence of nuclear ‘rings’. These ‘rings’, then, may be a component of cells in general.

WS cells incubated in phosphate buffer for 2 h before Giemsa staining are shown in Fig. 2. Arrows were not drawn to depict the ‘rings’ since they are very distinct. The rings are darkly colored because they most likely contain unstable hc which was sloughed-off the chromosomes and chromocenters as we reported previously (Edelman and Lin 2000, 2001). Originally, then, nuclear ‘rings’ were first thought to be a feature only of WS cells, and later we found them to be in FA cells (Lin and Edelman 2001). In this current study we are reporting that nuclear ‘rings’ may actually be a feature, in general, of all, or nearly all cells; they are just more distinct in WS and FA cells, especially after high temperature phosphate buffer treatment.

Although we cannot determine whether these nuclear ‘rings’ are real structures or merely artifacts of the cell preparation and staining procedure, we offer the following speculation as to their functions: (a) they may function as a seam or cusp to maintain the integrity of the nuclear membrane and/or (b) they may serve as a storage magazine for the synthesis/repair of the nuclear membrane, in much the same way as a Schwann cell functions for the cell membrane in neurons.

We believe additional studies, including fine structure/electron microscopic studies, are needed to determine the ‘realness’ of these nuclear ‘rings’ and their probable structure and function.

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References