Well-defined genome architecture in the human sperm nucleus

A. O. Zalensky1, M. J. Allen1,2, A. Kobayashi1, I. A. Zalenskaya1, R. Balhorn2, E. M. Bradbury1,3

1 Department of Biological Chemistry, University of California at Davis, Davis, CA 95616, USA
2 Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA
3 Los Alamos National Laboratory, Los Alamos, NM 87545, USA

Received: 19 August 1994; in revised form: 12 December 1994 / Accepted: 22 December 1994

Abstract. Using fluorescence in situ hybridization, conventional epifluorescence microscopy, and laser scanning confocal microscopy followed by three-dimensional reconstruction we describe a well-defined higher order packaging of the human genome in the sperm cell nucleus. This was determined by the spatial localization of centromere and telomere regions of all chromosomes and supported by localization of subtelomere sequences of chromosome 3 and the entire chromosome 2. The nuclear architecture in the human sperm is characterized by the clustering of the 23 centromeres into a compact chromocenter positioned well inside the nucleus. The ends of the chromosomes are exposed to the nuclear periphery where both the subtelomere and the telomere sequences of the chromosome arms are joined into dimers. Thus chromosomes in the human sperm nucleus are looped into a hairpin-like configuration. The biological implications of this nuclear architecture in spermatogenesis and male pronuclear formation following fertilization are discussed.

Introduction

The spatial arrangements of chromosomes and/or their well-defined domains such as centromeres and telomeres (further referred to as nuclear architecture) have been discussed for more than a century (Rabl 1885; Boveri 1888; Comings 1980; Hadlaczky et al. 1986; Hilliker and Appels 1989; Manuelidis 1990). More recently the development of in situ hybridization techniques has resulted in systematic studies on the localization of chromosomes in interphase nuclei (Emmerich et al. 1989; Manuelidis and Borden 1988; Weier et al. 1991; Lichter et al. 1991; McNeil et al. 1991). Information on the three-dimensional (3D) organization of eukaryotic chromosomes began to emerge as a result of application of optical sectioning (Agard and Sedat 1983; Hiraoka et al. 1990) and laser scanning confocal microscopy (Vourc’h et al. 1993; Kaplan et al. 1993). Individual chromosomes have been shown to occupy distinct territories within mammalian nuclei (Lichter et al. 1988). The non-random positioning of telomeres and centromeres appears to depend on cell type (Manuelidis 1984; Billia and Boni 1991) and the stage of the cell cycle (Vourc’h et al. 1993). Nuclear architecture of the eukaryotic genome might also be mediated by chromatin attachments to the nuclear matrix and nuclear membrane (reviewed by Nelson et al. 1986; Laemmlı et al. 1992) and higher order chromatin structures (reviewed by Garrard 1990).

The mammalian spermatozoon is a very distinctive cell type with most of the genomic DNA tightly packed into a nucleoprotamine complex (Balhorn 1989). The strong DNA-protamine interactions result in the DNA supercompaction needed to shut down all gene activity and to protect the vital genetic information during transfer between generations. Upon fertilization remodeling of the sperm chromatin structure to a nucleosomal state occurs, a process that requires a number of enzymatic activities and histone pool accumulation, which are provided by the egg cytoplasm (Poccia 1989; Zirkin et al. 1989). It is not known whether the sperm nucleus itself participates actively in chromosome release and decondensation during fertilization. If it does, this would require a specific nuclear architecture for the sperm cell (Powell et al. 1990). There are some indications that a nuclear architecture does exist. It has been shown that hamster sperm DNA is organized into loop domains anchored to a structure called the nuclear annulus (Ward 1993). We previously described the preservation of the centromere as a chromosome domain in human sperm and demonstrated specific interactions between centromeres of individual nonhomologous chromosomes (Zalensky et al. 1993). Recently we have also described a prominent telomere-telomere dimer interaction that is characteristic of human sperm as well as sperm of other mammals (Zalensky et al. submitted)
In the current work we have determined the spatial localization of telomere and centromere DNA sequences in human sperm cells using fluorescence in situ hybridization (FISH) and laser scanning confocal microscopy followed by 3D image reconstruction. The relative spatial positioning of telomeres, centromeres and total nuclear DNA has been established. The data show that two major non-random nuclear positions can be assigned within the DNA volume: (1) the centromeric regions of chromosomes cluster to form a chromocenter in the interior of the nucleus; and (2) the telomeres appear to be scattered about on the periphery of the nuclear volume. Additionally FISH labeling revealed that the subtelomeric sequences of the $\mathbf{p}$ and $\mathbf{q}$ arms of chromosome 3 colocalize to nearby regions in the nuclear periphery suggesting that the previously observed telomere-telomere dimers (Zalensky et al. submitted) are intrachromosomal. These results strongly suggest that a well-defined nuclear architecture exists in human sperm, which is described in a model supported by the spatial localization of chromosome 2.

Materials and methods

Preparation of sperm cells for hybridization. Human sperm cells were prepared from semen obtained from healthy donors. Semen was filtered, cells were pelleted by centrifugation (10 min at 4,000 g), and washed twice with PBS. To make cells permeable for hybridization probes and antibodies, outer membranes were partially destroyed using nonionic detergent (Gusse et al. 1986). Cell suspensions were treated with 20 mM 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate (CHAPS) prepared in PBS for 20 min at room temperature, then washed free of detergents with PBS. In some experiments (indicated in text) the CHAPS concentration and time of treatment were decreased to 2 mM and 5 min respectively. Partially demembranated cells were swollen by treatment with 10–1,000 µg/ml heparin, 1 mM dithiothreitol (DTT) in PBS for 30 min at room temperature. For the experiments including confocal microscopy a standard concentration of heparin (40 µg/ml) was always used. The pretreatment procedure was described previously for human sperm nuclei (Zalensky et al. 1993). Swollen cells were adsorbed onto microscope slides and dehydrated in a series of ethanol (70%, 80%, 95%) washes, for 2 min each.

DNA probes for in situ hybridization. All DNA probes (biotinylated and digoxigenin labeled) were obtained from Oncor, Gaithersburg, Md. The following probes were used: a selection of $\alpha$-satellite sequences that label centromeres of all human chromosomes (catalog no. P5095); the consensus telomeric repeat (TTAGGG)$_n$, complementary to all human telomeres (no. P5097); unique subtelomeric sequences of the $\mathbf{p}$ and $\mathbf{q}$ arms of chromosome 3 subcloned from a 450 kb yeast artificial chromosome (YAC) clone (nos. P5405; P5406); a chromosome 2 painting probe (no. P5206).

Separate hybridization with telomere and centromere probes. Hybridization, detection and amplification procedures were as recommended by the Oncor manual (catalog no. 1370-CF). Microscopic slides with adsorbed sperm cells (pretreated as described above) were denatured in 70% formamide, 2xSSC at 70°C for 2 min, dehydrated in cold ethanol (70%, 80%, 90%, 100%) and air dried (1xSSC is 0.15 M NaCl, 0.015 M sodium citrate). The biotinylated DNA probes, which were provided in a hybridization mixture containing 50% formamide, 2xSSC plus standard block-

Hybridization with chromosome 3 $\mathbf{p}$ and $\mathbf{q}$ subtelomeric specific DNA probes. Slides were prepared and denatured for hybridization as described. A mixture of equal volumes of digoxigenin labeled DNA probes corresponding to $\mathbf{p}$ and $\mathbf{q}$ arm specific subtelomeric sequences of chromosome 3 was applied to slides without probe denaturation, as recommended by the manufacturer. Hybridization was allowed to proceed for 16–24 h at 37°C. Slides were washed twice in 1xSSC at 72°C for 3 min. Hybridization signals were detected with FITC labeled sheep anti-digoxigenin antibodies. Signals were amplified with rabbit anti-sheep antibodies followed by FITC labeled goat anti-rabbit antibodies. Finally, total DNA was counterstained with PI and slides were mounted using Fluorosave (Calbiochem) and coverslips.

Simultaneous hybridization with telomere and centromere probes. For dual hybridization we used a biotinylated telomere probe and a digoxigenin labeled centromere probe. Equal amounts of probe DNAs were mixed then denatured and applied to slides. Hybridization and posthybridization washings were performed as described above in section three of Materials and methods. Hybridization was detected using Texas Red labeled sheep anti-digoxigenin antibodies (centromere) and FITC labeled avidin (telomere). The signals were subsequently amplified for (1) centromere hybridization using rabbit anti-sheep antibodies followed by Texas Red conjugated goat anti-rabbit antibodies and (2) telomere hybridization using anti-avidin antibodies followed by FITC labeled avidin. Each step was followed by washes using PBD buffer (three times for 2 min). For details of procedure, see Manual for Rapid Chromosome In Situ Hybridization (Oncor 1993). Cells were counterstained for total DNA with DAPI.

Simultaneous hybridization with centromere and chromosome 2 painting probes. The conditions for hybridization and post-hybridization washes for the chromosome-specific painting probe and “all human centromeres” probe were different. Sperm adsorbed on slides were first hybridized with the biotin conjugated chromosome 2 painting probe and washed. This was followed by hybridization with the digoxigenin labeled centromere probe and posthybridization washing. In more detail, the chromosome painting probe was denatured at 70°C for 10 min and reannealed at 37°C for 2.5 h, hybridization was performed for 12–24 h at 37°C. Posthybridization washings were: 15 min in 50% formamide, 2xSSC at 43°C; 15 min in 0.1xSSC at 60°C. Slides were then soaked for 2 min in 50% formamide 2xSSC and the digoxigenin-labeled centromere probe was applied. The second hybridization was performed for 4–12 h and followed by washings as described for the centromere probe alone. Hybridization of the digoxigenin-labeled centromere probe was detected with Texas Red conjugated sheep anti-digoxigenin antibodies followed by one round of amplification. Afterward chromosome 2 hybridization signals were detected using avidin-FITC followed by two rounds of amplification. Finally, total nuclear DNA was stained with DAPI.