Analysis of cell ploidy in histological sections of mouse tissues by DNA–DNA in situ hybridization with digoxigenin-labelled probes

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Summary

DNA–DNA in situ hybridization, with two digoxigenin-labelled, chromosome-specific DNA probes, was used to determine the number of copies of a given chromosome in interphase nuclei and so identify putatively polyploid nuclei in histological sections of several mouse tissues. One hybridization site per diploid genome was expected for tissues with hemizygous markers: male mice hybridized with a Y chromosome probe (pY353/B) or hemizygous transgenic mice hybridized with a β-globin probe (pMPβ2). Nuclei with more than one hybridization site were considered putative polyploids. Three groups of experiments were undertaken: (1) evaluation of the method, using mouse liver sections; (2) studies of tissues already known to contain polyploid nuclei, and (3) studies that resulted in the discovery that the mouse ovary contains polyploid nuclei. First, control studies showed that the ability to detect the target DNA sequences was affected by section thickness. Studies of nuclear ploidy in the developing mouse liver revealed a pattern similar to that established by previous studies using DNA content as a criterion for ploidy. At birth, only about 5% of the liver nuclei were polyploid; this increased to 10–15% by 10–20 days and was followed by a sharp increase in the frequency of tetraploid nuclei between 20 and 40 days (to about 35%) and a more gradual increase in higher order polyploid nuclei. Secondly, this technique was used to confirm that polyploid (mostly tetraploid) nuclei were present in the bladder epithelium, heart, uterine decidua and placental trophoblast. Higher order polyploidy was seen in large bone marrow cells (megakaryocytes) but not in the even larger trophoblast giant cells of the placenta, thus confirming previous claims that these cells are polytene rather than polyploid. Thirdly, putatively tetraploid nuclei were found in the ovarian follicle and corpus luteum. As far as we are aware, this is the first time polyploid nuclei have been reported for the mouse ovary.

Introduction

Polyploid cells have been identified in interphase nuclei of animal tissues by a variety of techniques which rely on the proportional relationship between the number of sets of chromosomes (diploid (2n), tetraploid (4n), etc.) and the DNA content of the nucleus (2C, 4C etc.). Early work relied on visual assessment of nuclear size and staining intensity with Feulgen–PAS (e.g. Alfert & Geshwind, 1958). This was later superseded by quantitative estimates of nuclear DNA involving either microdensitometry of Feulgen-stained nuclei (e.g. Epstein, 1967; Barlow & Sherman, 1972; Gladwin et al., 1988) or quantitative fluorescence of nuclei stained with a DNA-binding fluorochrome (e.g. Chatelain & Burstein, 1984).DNA–DNA in situ hybridization with a chromosome-specific DNA probe offers an alternative approach for detecting polyploid nuclei.

The technique of hybridizing chromosome-specific DNA probes, labelled with a radioactive or immunogenic marker, to interphase cells in order to detect the presence of chromosome-specific DNA sequences has been used by cytogeneticists in order to infer the number of copies of a particular chromosome in interphase cells (e.g. Burns et al., 1985; Julien et al., 1985; Lichter et al., 1988a, b; Pinkel et al., 1988). This technique has been dubbed ‘interphase cytogenetics’ and is useful for prenatal diagnosis of chromosome anomalies such as aneuploidy (Lichter et al., 1988b) and chromosome translocations (Pinkel et al., 1988; Ellis et al., 1990).

The same general approach has been used to identify apparently polyploid cells in cell spreads of human blastocysts (West et al., 1987, 1988), human amniotic fluid cells (West et al., 1989) and mouse hepatocytes (Varmuza...
Polyplody detected by in situ hybridization

et al., 1988). One advantage of this approach is that it can distinguish between polyploid and polytene cells, both of which may be large and have a high DNA content (Bower, 1987; Varmuza et al., 1988). When applied to histological sections rather than cell spreads, DNA–DNA in situ hybridization can also provide spatial information about the distribution of cells within a tissue, and for this reason it has been employed as a cell marker for studies with mouse chimaeras (Thomson & Solter, 1988a, b; Nagy et al., 1990; Palmer & Burgoyne, 1991b; Patek et al., 1991) and mosaics (Palmer & Burgoyne, 1991a). The studies by Thomson and Solter (1988a, b), Varmuza et al. (1988), Nagy et al. (1990) and Palmer and Burgoyne (1991b) made use of a transgenic strain of mice that carries about 1000 copies of the mouse β-globin gene inserted at one site in chromosome 3 (Lo, 1986), which we have also used in the present study.

We have used DNA–DNA in situ hybridization in histological sections to study the ploidy of cells in various mouse tissues. These studies are divided into three groups of experiments. First, we used sections of mouse liver to evaluate the method because the extent of polyploidy in this tissue is well understood. Secondly, we tested whether the method could detect polyploid nuclei in a range of tissues, that were already known to contain cells with DNA contents in the polyploid range (Brodsky & Uryvaeva, 1985). Thirdly, we investigated whether the mouse ovary contained any putatively polyploid nuclei.

Materials and methods

Mice

Most studies were carried out with mice hemizygous for a transgene (here abbreviated to Tg) comprising approximately 1000 tandemly repeated copies of a mouse β-globin plasmid inserted near the telomere of chromosome 3 (Lo, 1986). Homozygous mice, derived from Dr Cecelia Lo's original transgenic strain 83, were obtained from Dr Roger Gosden (Department of Physiology, University of Edinburgh). These mice had a mixed genetic background (contributions from inbred strains CBA, C57BL/6 and SJL) and were maintained in the Centre for Reproductive Biology. Males, homozygous for the β-globin transgene (Tg/Tg), were crossed to wild-type (C57BL/WS x CBA/Ca)F1 females, with no transgene (+/+), to produce hemizygous (Tg+/+) offspring. Pregnancies were obtained by natural matings. The stage of pregnancy was timed relative to the day of the vaginal plug, which was designated 0.5 day p.c. (post coitum) because mating was assumed to have occurred during the preceding night. Conceptuses analysed with the Y chromosome specific probe (see below) were produced by mating two mice without the transgene. CBA/Ca mice were obtained from the Institute of Cell, Animal and Population Biology, University of Edinburgh. All other mice were bred in the Centre for Reproductive Biology.

Labelling DNA probes with digoxigenin

Two DNA probes were used: the Y chromosome-specific probe, pY353/B (kindly provided by Dr Colin Bishop, Institut Pasteur, Paris), and the β-globin probe, pMFB62 (kindly provided by Drs John Ansell and Bruce Lyons, Institute of Cell, Animal and Population Biology, University of Edinburgh). Plasmid pMFB62 is derived from the plasmid (pMB1) which was inserted into the strain 83 transgenic mice and is described fully by Lo (1986).

The appropriate plasmid was linearized using the restriction endonuclease EcoRI (Boehringer, Mannheim), and then 0.5–1 µg of denatured DNA was labelled with digoxigenin–dUTP by random primed labelling, using the DNA labelling and non-radioactive detection kit (Boehringer, 1093657). For a typical labelling reaction 1 µg of linearized probe was denatured in a total volume of 10 µl. To this was added 2 µl 10× hexanucleotide mixture and 2 µl of 10× dNTP labelling mixture from the Boehringer kit. The volume was made up to 19 µl with sterile distilled water and 1 µl Klenow enzyme (2 U µl−1) added. The reaction mixture was incubated at 37°C for 6 h and the reaction was stopped by the addition of 2 µl of 0.2 M EDTA, pH 8.0. The labelled DNA was precipitated by the addition of 2 µl 3 M sodium acetate and 60 µl of cold ethanol and left overnight at −20°C. The labelled DNA was then spun down and dried under vacuum, and the DNA pellet was dissolved in 50 µl TE buffer (10 mM Tris, pH 7.5, 1 mM EDTA) to provide a stock concentration of 20 µg ml−1 of labelled DNA.

Dot blot hybridizations were carried out to check that the DNA was labelled with digoxigenin. Labelled DNA was serially diluted in TE buffer, and three samples (0.5 pg, 1.0 pg and 1.5 pg of DNA) were spotted onto nitrocellulose filter paper (Hybond-N, Amersham), about 1 cm apart, and baked in an oven at 80°C for 2 h. The labelled DNA was detected by alkaline phosphatase immunocchemistry (see below), following the manufacturer's instructions for the DNA labelling and non-radioactive detection kit (Boehringer, Mannheim). A positive signal (blue-coloured spot) was usually apparent for each spot if the DNA labelling was successful, and the probe was used in a hybridization experiment only if the 1 pg DNA sample produced a clear signal.

Tissue preparation and in situ hybridization

Mice were killed by cervical dislocation and the tissue immediately dissected out and fixed. Livers, hearts and some 11.5-day conceptuses (for the analysis of the placentas with the Y chromosome probe) were fixed in 4% paraformaldehyde, and all other tissues were fixed in 3:1 (v/v) ethanol:acetic acid. The method of tissue preparation and in situ hybridization depended on which fixative was used, so these are described separately.