Fluorescent chromosome banding in inbred chicken: quinacrine bands, sequential chromomycin and Dapi bands

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Summary. Highly inbred White Leghorn chickens were used for an investigation of the banding pattern of the macrochromosomes. A standard was set for the Q-bands. GC-specific fluorochrome chromomycin and the AT-specific Dapi were used in a sequential stain. The comparison of these two stains disclosed quantitative differences in the base distribution of the DNA. Factors responsible for the binding mechanism and the appearance of the bands are discussed.

Key words: Inbred chicken – Chromosomes – Banding pattern

Introduction

The domestic fowl (Gallus domesticus) is cytogenetically the most investigated bird. Its diploid chromosome number is 78 (Pollock and Fechheimer 1981), and its chromosomes vary in size from 8 µm to particles almost invisible under the light microscope. The largest eight pairs and the sex chromosomes are designated as macrochromosomes; the other 30 pairs as microchromosomes. The sex determining chromosomes are reversed in comparison to mammals with the cock being homozygous (ZZ) and the hen hemizygous (ZW).

The aim of this article is to characterize the banding pattern of the chicken macrochromosomes by using different fluorescent dyes, namely quinacrine stain, chromomycin and Dapi sequential staining, respectively. The use of highly inbred strains of chicken cell material offers a precise description of bands since the polymorphic appearance of chromosome structures should be limited. Up to now, only a few studies have been published describing bands of chicken chromosomes; namely Q-band (Stahl and Vagner-Capodano 1972), G-band (Stock et al. 1974; Wang and Shoffner 1974; Pollock and Fechheimer 1981; Carlenius et al. 1981) and R-band (Carlenius et al. 1981). The banding pattern can be subdivided into two categories: AT-rich regions staining with quinacrine mustard (Comings 1978), and 4,6-Diamidino-2-phenylindol·2 HCl (Dapi) (Lin et al. 1977) and GC-rich DNA regions staining with Chromomycin A3 (Schweizer 1976).

Chromosomes stained with Dapi show a qualitatively similar, though not identical, banding pattern as Q-bands. The dye chromomycin produces a type of R-band pattern. Sequential staining of Dapi and chromomycin of the same metaphase allows the distinction of differences between the distribution of base pairs (Schnedl 1981; Schweizer et al. 1978). The two fluorescence dyes can clearly be separated with the use of different excitation wavelengths of the filters.

Materials and methods

Metaphase preparations were made from embryonic liver cells taken from nine highly inbred (inbreeding coefficient = 94%) White Leghorn strains (Abplanalp et al. 1981) bred at the experimental farm, Chamau, of the ETH Zurich. For producing embryonic liver cells, the hens were inseminated twice a week with undiluted ejaculates and eggs collected daily for ten days. Fertilized eggs were incubated 11–13 days in a standard incubator at 37.6 °C. Four hours before the preparation of the chromosomes, 0.4 ml Colcemid (10 µg/ml) was injected into the aircell of the fertile eggs and the eggs returned to the incubator. Thereafter, embryos were taken out of the egg and liver cells were directly prepared without the preceding incubation. The method was modified after Fechheimer (Fechheimer et al. 1970). As hypotonic fluid, 1% prewarmed (39 °C) sodium citrate with added Colcemid (0.3 ml...
Colcemid – 10 μg/ml – per 10 ml sodium citrate) was chosen. A hypotonic treatment of 30 min at 39°C was applied. For the first two fixations ice-cold methanol/acetic acid mixture in the proportion 3:1 was used. In the last fixation the proportion was 4:1. The samples were put into the freezer before the fixations and also before preparing the slides. Two to three drops of the cell suspension were put onto a slide and were air-dried overnight.

**Staining procedures**

**Quinacrine mustard (Sigma, St. Louis, USA).** Quinacrine mustard was dissolved in Sorensen phosphate buffer, pH 6.8, (i.e. 0.1 mg/ml) with the addition of some thymol to prevent bacterial development. The dry chromosome preparations were put into the dye solution and stained for 30 min. After staining, the slides were rinsed with tap water and put into the buffer solution (pH 6.8) for three min. Afterwards the slides were covered with a 3:1 glycerolphosphate buffer mixture (glycerol for fluorescence microscopy by Merck, Darmstadt, BRD) and the coverslip sealed with nail polish (modification after Caspersson et al. 1970).

**Dapi and chromomycin.** The combination of Dapi (4,6-Diamidino-2-phenylindol·2 HCl; Serva Heidelberg, BRD) and chromomycin A3 (Serva Heidelberg, BRD) staining provides an opportunity to observe banding differences at the same metaphase. Air-dried slides were incubated in McIlvaine buffer (pH 7) for 15 min and subsequently stained with Dapi (0.3 μg Dapi/ml buffer) for 15 min. The slides were then rinsed in McIlvaine buffer (pH 7) to which was added 10 mM MgCl2. The chromomycin solution (0.1 mg chromomycin/ml buffer with MgCl2) was dropped onto the slides and those kept in the dark for 30 min. The slides were then rinsed in the buffer and sealed with glycerol buffer mixture (modification after Schweizer 1980).

**Microscopic observation and photography**

A Zeiss Microscope No. III with fluorescent equipment and a 63x oil immersion objective was used. To obtain clear banding patterns from the quinacrine stain, the preparations were immediately photographed with a Zeiss Filter No. 48.77.06 (wavelength 436 nm) in the blue field. The best banding effect from the Dapi staining resulted when the preparations were stored up to 14 days in the refrigerator, so that the stain could stabilize (Zeiss Filter No. 48.77.02, wavelength 365/366 nm). Chromomycin fades very quickly, and it was only possible to take one photograph per metaphase. The filters for chromomycin A3 were in the blue field (wavelength 436 nm), Zeiss Filter No. 48.77.06. On the other hand, the fluorescence of Dapi is quite stable and several pictures could be taken. All the fluorescence photographs were taken on Kodak 2415 film. The analysis of the karyotypes was done by means of paper prints.

**Results and discussion**

**Q-banding**

The quinacrine bands of the nine identifiable chromosome pairs are distinct, as shown in Fig. 1. The microchromosomes fluoresce weakly and the intensity of the fluorescence is quite variable.

The Q-banding of these chicken chromosomes is schematically shown in Fig. 2. Based on our preparations, a standard banded karyogram is proposed. The diagram takes into consideration the distribution and the relative width of the bands. Until now only one description has been published (Stahl and Vagner-Capodano 1972) and it seems to be preliminary and incomplete. In Fig. 2 we tried to subdivide the chromosome arms into regions and landmarks (Paris Conference 1972). The Q-bands are characteristic for the individual chromosome and consist of reproducible patterns (Caspersson et al. 1970; Schweizer 1976). With the aid of the banding, the chromosomes may be clearly identified.

![Fig. 1. Karyotype of a female animal with the sex chromosomes ZW, stained with the Q-band technique](image-url)