Selective Staining of X Chromosome Segments in Schistocerca gregaria after Denaturation and Reassociation Procedures

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Abstract. The DNA of fixed mitotic and meiotic chromosomes and of spermatides of Schistocerca gregaria males was heat denaturated and then differentially reassociated in a Giemsa buffer or in acridine orange buffer solution. After this procedure, two to three large, selectively stained regions are seen in the X chromosome of spermatocytes and spermatides. Denaturation and reassociation experiments have shown that after differential reassociation such a selective stainability of chromosome regions is characteristic for the presence of fast-reassociating, i.e., repetitive DNA (Stockert and Lisanti, 1972). The possible presence of repetitive DNA in the X chromosome regions concerned can not be the only reason for the occurrence of the heavily stained segments after reassociation because (1) these segments are obtained in positively heteropycnotic X chromosomes, but not in negatively heteropycnotic Xs and (2) they do not occur in positively heteropycnotic X chromosomes when the histones have been extracted before the denaturation and reassociation processes. Contrary to the latter statement, the heavily stained X chromosomal regions are preserved when the histones are removed after the denaturation and reassociation steps. – It is assumed that the heavily stained X chromosome segments represent DNA reassociation complexes which are only formed if histones are present. It is discussed whether the formation of the X chromosome complexes depends on a specific chromatin configuration within positively heteropycnotic X chromosomes.

Introduction

After application of denaturation and differential reassociation techniques it is possible to localize regions of repetitious DNA in fixed chromosomes (Stockert and Lisanti, 1972; de la Chapelle et al., 1973a, 1973b; Comings et al., 1973). Repetitious DNA has a faster rate of reassociation than non-repetitious DNA (Britten and Kohne, 1968). Therefore, under differential reassociation conditions, repetitious DNA is reassOCIated, whereas non-repetitious DNA is single-
stranded. Differential reassociation can be visualized because reassociated (double-stranded) DNA fluoresces green and single-stranded DNA fluoresces red after acridine orange staining (Kasten, 1967; Stockert and Lisanti, 1972). Denaturation and reassociation methods, which lead to a differential acridine orange staining, also give rise to a characteristic staining pattern with Giemsa (Stockert and Lisanti, 1972).

There is no clear-cut explanation as to what extent the chromosomal proteins affect denaturation and reassociation. As will be shown in *Schistocerca gregaria* males within the positively heteropycnotic X chromosome, large, dark-stained regions are obtained after denaturation and Giemsa reassociation, which do not occur when histones are removed prior to the denaturation and reassociation processes and which are generally absent in the negatively heteropycnotic X chromosomes.

**Material and Methods**

Testes from adult *Schistocerca gregaria* males were freed from investing fat bodies in Ringer solution and fixed in alcohol acetic acid. Squash preparations from testis tubules were stored in 100% isopropanol for 6 days. After air drying the slides, denaturation was performed in 0.1 x SSC, 97°C for 2.5 min. Then the slides were immediately placed in ice cold 0.1 x SSC. Differential reassociation followed by treating in 10% Giemsa or in 0.03% acridine orange in 0.025 m phosphate buffer, pH 7.0, for 2.5 to 10 min. The preparations were washed in buffer and distilled water, then air dried. Acridine orange-stained slides were mounted in buffer under a coverslip for fluorescence microscopical observation; the Giemsa-stained preparations were mounted in euparal.

Histone extraction was done in 0.1 N HCl, 60°C for 30 min. Histones were removed either before or after denaturation and reassociation. In the latter case reassociation was carried out in phosphate buffer only. Then the histones were extracted and, after washing, the slides were dried and stained as described above.

**Results**

The chromosome complement of *Schistocerca gregaria* males is 2n=22+X. The single X chromosome is allocyclic with respect to the autosomes (John and Naylor, 1961). In its positive heteropycnotic state (in pre-meiotic interphase, meiotic prophase, interkinesis, post-meiotic interphase I, spermatids) it is a compact body. In its negatively heteropycnotic state (in mitotic pro- and metaphase) it is undercondensed in comparison to the autosomes and is not compact.

After the denaturation and Giemsa reassociation procedures, two to three large heavily stained segments occur within the positively heteropycnotic X chromosome (Figs. 1a, 2a, 3a). Additionally, a heavily stained small region is found in most X chromosomes, presumably representing the centromere. The autosomes (Fig. 2a) appear pale with the exception of small, deeply stained knobs which correspond to the centromeric and N-band regions (Hägele, 1979).

The large heavily stained X chromosome segments can not be obtained after denaturation and reassociation when the X is in the negatively heteropycnotic state (Fig. 3b). In this case only the centromeric region is selectively stained.