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Photomap of the polythenic chromosomes of Drosophila and in situ mapping

Pierre Teodosio[1]

[1]LaBECom

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LaBECom

Pierre Teodosio
LaBECom, UNIVISA

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1 Making the photomap of polythenic chromosomes

2 1 The slides of politenic chromosomes of D. malerkotliana of the population of Parque Dois Irmãos (Recife) were prepared by crushing the dissected salivary glands of zero-hour pre pupae, fixed with a solution of acetic acid, water and lactic acid, in a 3:2:1 ratio. The material was stained with aceto-lactic orcein (1g of orcein-MERCK, 45ml of acetic acid, 25ml of lactic acid 85%, and 30ml of distilled water), according to ASHBURNER (1967). The best slides were photographed under Leica Microscope in increase of 100 X 1.25 with Kodak TMAX Iso 100 film.

3 In situ hibridization

4 For in situ hybridization, the ENGELS et al. (1986) technique was used. After dissection of the larvae, the salivary glands were fixed in 45% acetic acid and crushed with cover slip in a lactic-acetic solution (lactic acid, water and acetic acid, 1:2:3). The slides were stored at 4°C for at least 18 hours. The slides were removed and the slides were submitted to several washes in 2X SSC and ethanol. The slides were air dried and stored at 4°C. After these steps, the best slides were selected and only those with intact chromosomes, spread properly, were stored for further hybridization.

5 For the marking and mapping of the Hsp83 locus in D. malerkoltiana, the clone lambda 6 of the Hsp83 gene of D. melanogaster (HOLMGREN et al., 1981), inserted in the plasmid pBR322, was used as probe. The plasmid was amplified by the transformation of the Xm1 strain of the bacterium Escherichia coli, cultivated in LB medium with ampicillin (100°g/ml) (SAMBROOK et al., 1989) and was extracted by non-phenolic method (PHILIPPSEN et al., 1991).

6 To prepare the probe, 1μg of the plasmid containing the gene fragment, marked with biotin by nick-translation with gibco's Bionick kit, was used. The probe marking was tested in "dot blot" with revelation with Estreptavidin – Alkaline Phosphatase (SA-AP), Nitroblue tetrazolium chloride (NBT), 5-bromine-4-chloro-3-indolylphosphate p-Toluidine salt (BCIP). 500ng of probe per blade was used under high astringency conditions (temperature of 36º C and 50% of formamide) for 48 hours. The revelation was performed with SA-AP, NBT and BCIP, followed by the lacto-acetic orcein counter-dye at 0.2%. After drying the blades, the material was permanently assembled with Entellan.

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