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Department of Cell Biology, Institute of Biology,
UNICAMP, Campinas (SP), Brazil

Absorption spectrum of FEULGEN-stained polytene chromosomes of *Drosophila melanogaster*

By MARIA LUIZA S. MELLO and SHIRLEI M. RECCO

With one figure

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Summary

Hetero- and euchromatic polytene chromosome regions of early and fully grown larvae of *Drosophila melanogaster* had their FEULGEN absorption curves determined cytophotometrically. Hydrolysis conditions adequate to induce maximal DNA depurination were used. No differences in curve shapes specially concerning the absorption shoulder at the $520 \leq \lambda \leq 550$ nm spectral range were detected with advancing polytenization or when comparing the absorption patterns for the hetero- and euchromatic regions to each other. It is considered that prominence of the FEULGEN absorption shoulder could be related to the amount of SCHIFF molecules di-substituted with apurinic acid aldehydes and to the relatively larger resistance of these apurinic acid fragments towards solubilization with acid hydrolysis due to their binding to nuclear proteins. It is therefore assumed that no significant differences owing to the above-cited factors exist for the chromosome regions analyzed at 2 phases of the polytenization process. Consequently, the replication of reiterated sequences from the main band DNA which occurs in polytene chromosome regions of *Drosophila melanogaster* appears not to affect the Feulgen absorption spectrum of this material.

Introduction

It has been suggested that the absorption shoulder which appears in the FEULGEN spectrum under certain fixing and hydrolysis conditions (MOSES 1951; SWIFT 1953; KASTEN 1956; MELLO and ZANARDI 1976; MELLO 1978) could be related to factors favoring the appearance of SCHIFF reagent molecules di-substituted with vicinal apurinic acid aldehydes (DEITCH 1966; MELLO 1978). Among the factors inducing prominence of the FEULGEN absorption shoulder, chromatin compactness and richness in repetitive DNA (probably associated with *lag* in the extraction rate of apurinic acid fragments due to their binding to nuclear proteins) have been enlisted (MELLO 1980).

It has been reported that with the advancement of the polytenization process in the sciarid fly, *Bradysia spatitergum*, a shoulder or secondary peak develops in the FEULGEN absorption spectrum, provided that maximal depurination of the DNA had occurred (MELLO and SILVA 1980). This finding would be in agreement with the supposition that the number of repetitive DNA sequences could increase during

polytenization, favoring enhancement of more di-substituted SCHIFF molecules adjoined per measuring area (MELLO and SILVA 1980). For the case of *Bradysia spatitergum* it has been assumed that reiterated sequences from both satellite and main band DNA types would belong to the replicating category. In the chromosomes of salivary glands of *Drosophila melanogaster*, however, only the repetitive sequences from main band DNA have proven to replicate during polytenization; these sequences are distributed along the chromosomal loci of euchromatin and β-heterochromatin (GALL et al. 1971).

In the present work, the FEULGEN absorption spectra of the chromocenter and of euchromatin of *Drosophila melanogaster* at early and late stages of the polytenization process are compared with one another. The aim was to find whether increase in number of repetitive sequences from main band DNA during polytenization could affect the FEULGEN curve shape, inducing prominence of the absorption shoulder.

Materials and methods

Polytene chromosomes of the salivary glands of *Drosophila melanogaster* were obtained from 1st and 3rd instar larvae. The glands were squashed in a drop of 45 % acetic acid and the coverslips were immediately removed by freezing on dry ice. The preparations were immersed in 70 % ethanol for 1 to 2 min, air dried, and then subjected to FEULGEN reaction in such a way that maximal depurination was produced [(hydrolysis conditions: 4 *M* HCl at 25 °C for 1 *h* 30 min); (KJELLSTRAND 1977, 1979)]. Canada balsam ($n_D = 1.54$) was used as mounting medium.

Absorbances were determined for the chromocenter and an euchromatic zone (region 2 of the X-chromosome). Identification of the chromosome regions was based on maps by BRIDGES (1935). As the measuring spot area had to be constant for the various chromosomal area chosen for analysis, and the width of the polytene chromosome arms at the 1st larval instar is small compared to the diameter of commercially available measuring diaphragms, the region 2 of the X chromosome could not be measured isolatedly at this developmental stage. In this case, cytophotometry was carried out only when the X-2 region appeared positioned contiguously to an euchromatic zone of another chromosome. Consequently, the absorption curves of euchromatin at the 1st larval instar were plotted with absorbance values due mostly to X-2 region but also contributed by other enchromatic regions. The measuring spot area, however, could be entirely contained in the chromocenter area of the early grown larvae.

The microspectrophotometric data were obtained with a *Zeiss* photomicroscope equipped with a Ol photometer and an EMI 6256 photomultiplier. Operating conditions were: Plan 100/1.25 objective, optovar 1.25, measuring diaphragm diam.: 0.16 mm, field diaphragm diam.: 0.2 mm, Kpl W MPM 12.5 eye-piece, and *Schott* continuous monochromator ruler. The area of the specimen measured per plug point was equal to 1.29 μm^2. Considering that the width of the effective light beam was equal to 0.2 mm, undesirable spectral range was blocked almost completely (ZEISS 1977).

Spectral absorption curves were plotted with the absorbance values. Five curves were determined for each experimental condition.

Results

The FEULGEN absorption curves for the hetero- and euchromatic regions of the polytene chromosomes of young and fully grown larvae of *Drosophila melanogaster* displayed one maximum positioned at $\lambda = 565$ nm and a shoulder at the $520 \leq \lambda \leq 550$ nm spectral range (Fig. 1). The prominence of this shoulder appeared not

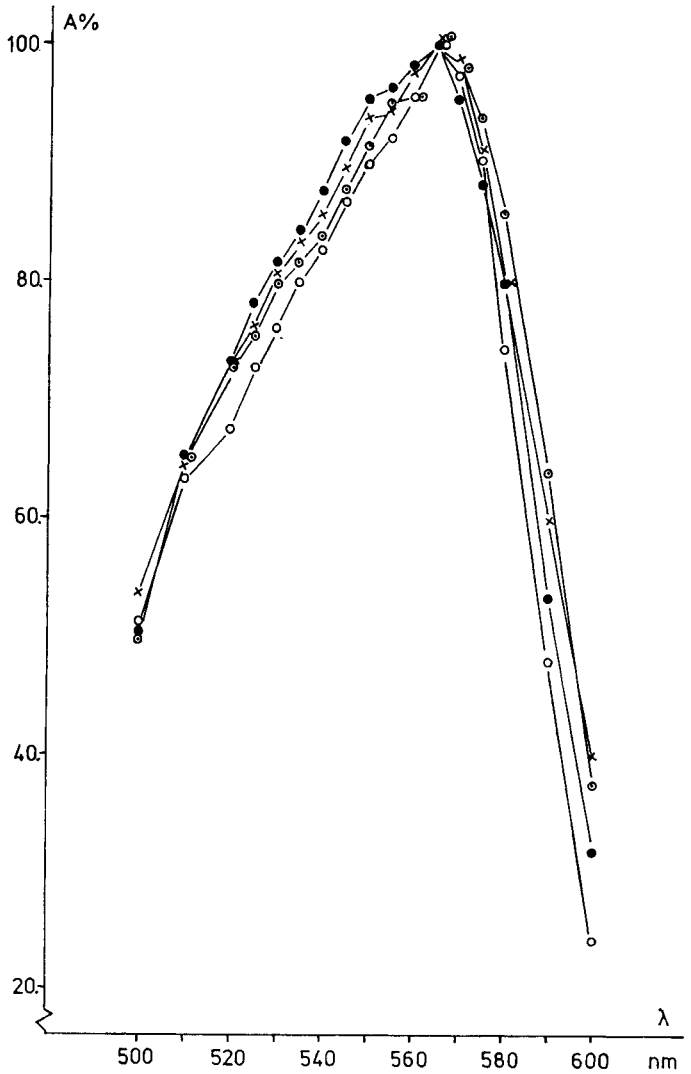

Fig. 1. FEULGEN spectral absorption curves for polytene chromosomes of *D. melanogaster*. *A* = absorbances; ● and × = chromocenter of 1st and 3rd instar larvae, respectively; ○ and ⊙ = euchromatin of 1st and 3rd instar larvae, respectively. Each point in the curves is the arithmetic mean of 5 measurements. The confidence intervals for relative absorbances at $500 \leq \lambda \leq 560$ nm are enlisted in Table 1.

remarkable specially when compared with that depicted by the FEULGEN-stained chromosomes at highly polytenized states of another fly, *Bradysia spatitergum* (MELLO and SILVA 1980). While mean values close to 89.8 % (S_1 nuclei) and 94.2 % (S_2 nuclei) have been reported as relative absorbances at $\lambda = 420$ nm (secondary peak) for *Bradysia*, *Drosophila* values were equal to 67.4 to 73.2 % at this wavelength.

The profiles of the various curves of *D. melanogaster* are practically identical in the $500 \leq \lambda \leq 565$ nm spectral range, when comparing data from euchromatin and chromocenter of 1st and 3rd instar larvae with one another (Table 2).

Table 1. Confidence intervals ($P_{0.05}$) for the relative absorbances of FEULGEN-stained polytene chromosomes of *D. melanogaster* at $500 \leq \lambda \leq 560$ nm

λ	Larval instars			
	1st		3rd	
[nm]	chromocenter	euchromatin	chromocenter	euchromatin
500	0.75	4.50	3.73	3.96
510	3.35	3.48	3.47	3.09
520	4.48	2.74	3.70	3.48
525	1.24	4.10	2.49	2.85
530	1.24	4.70	3.48	4.10
535	0.99	2.85	3.70	2.98
540	2.61	3.55	2.61	2.60
545	1.00	2.71	2.50	3.61
550	1.74	4.45	2.98	3.11
555	1.25	3.09	0.87	2.70
560	0.75	2.36	0.50	0.98

Table 2. *t*-test for the relative absorbances of Feulgen-stained polytene chromosomes of *D. melanogaster* at wavelengths of the shoulder region. d.f. = 8; $t_{0.01} = 3.36$; $t_{0.05} = 2.31$.

Comparisons	λ [nm]	*t*	Comparisons	λ [nm]	*t*
chromocenter (1st vs. 3rd instars)	520	0.05	1st instar (chromocenter vs. euchromatin)	520	3.18
	530	0.53		530	3.24
	540	1.58		540	3.06
	550	1.29		550	3.07
euchromatin (1st vs. 3rd instars)	520	3.14	3rd instar (chromocenter vs. euchromatin)	520	0.49
	530	1.69		530	0.62
	540	0.69		540	1.28
	550	0.66		550	1.55

Table 3. FEULGEN relative absorbances ($\bar{X} \pm S$) at some wavelengths of the high spectral region n = 5.

λ [nm]	Larval instars			
	1st		3rd	
	chromocenter	euchromatin	chromocenter	euchromatin
590	53.1 ± 7.9	48.0 ± 6.5	60.0 ± 6.1	63.6 ± 7.7
600	31.9 ± 10.9	24.5 ± 9.5	40.2 ± 7.4	37.6 ± 8.0

The relative broadness and apparent differences in the shape of the curves after $\lambda = 570$ nm (Table 3; Fig. 1) have been also observed in other materials (MELLO 1978, 1980; MELLO and SILVA 1980). They are possibly related to decrease in sensitivity of the *Zeiss EMI 6256* photocathode at the high wavelength range (MELLO 1980).

The largest absorbances estimated at $\lambda = 565$ nm were equal to 0.320 (transmittance, 47.8%, comprising an error of 3% [Ewing 1969]; *chromocenter*) and 0.226 (transmittance, 59.4%, comprising an error of 3.2% [Ewing 1969]; *euchromatin*) both for 1st instar larvae. On the other hand, the smallest absorbances at the same wavelength were 0.208 (transmittance, 61.9%, comprising an error of 3.7% [Ewing 1969]; *chromocenter*) and 0.094 (transmittance, 80.5%, comprising an error of 6.1% [Ewing 1969]; *euchromatin*) both for 3rd instar larvae. Consequently, it is expected that no departure from Beer law due to systematic errors involving the consideration of extremely high or low absorbances (Ewing 1969) has apparently occurred for the spectral range covering the absorption peak and shoulder regions.

Discussion

Based on Feulgen spectral absorption patterns, it is suggested that the relative amount of Schiff reagent molecules di-substituted with vicinal apurinic acid aldehydes (Deitch 1966, Mello 1978) for hetero- and euchromatic regions of maximally depurinated polytene chromosomes of young and fully grown larvae of *D. melanogaster* does not much differ when compared with one another. This amount, however, appears to differ from that shown by similarly stained nuclei of the sciarid, *Bradysia spatitergum*, with advancing polytenization (Mello and Silva 1980).

Satellite repetitive DNA has been pointed out to occur in the chromocenter of the polytene chromosome-containing nuclei of *D. melanogaster* (Hennig et al. 1970, Jones and Robertson 1970, Rae 1970, Botchan et al. 1971, Gall et al. 1971). Repetitive sequences from the main band DNA, on the other hand, are distributed along the chromosomal loci of euchromatin and β-heterochromatin of this species (Gall et al. 1971, Bonner and Wu 1973). It has been demonstrated that contrary to whar happens with the reiterated sequences from the satellites, those from the main band DNA do replicate during polytenization (Gall et al. 1971). Consequently, the replication of the reiterated sequences from the main band DNA of the regions chosen for analysis appears not to affect significantly their Feulgen absorption spectrum. The fact of the X-2 region having to be measured together with a small part of another euchromatic zone at nuclei of early grown larvae is assumed not to invalidate the conclusions. In fact, the absorbance contribution of the euchromatic region positioned contiguous to the X-2 zone was apparently small. Even if, the availability of vicinal aldehydes for binding of Schiff molecules had been doubled due to chromosome contiguity, it would not surpass the result due to the various DNA replication cycles which are expected to occur in X-2 from the 1st up to the 3rd larval instars. At the 3rd larval instar the measuring spot was well contained in the X-2 zone.

The relative small prominence of the absorption shoulder of *Drosophila* curves when compared with those for sciarids (Mello 1980, Mello and Silva 1980) may be concerned with a probably significant part played by the repetitive sequences from satellite DNA fractions (and rDNA?) in the spectral absorption patterns of the latter. It may also be speculated that in the sciarids, apurinic acid fragments containing certain reiterated sequences would have their solubilization rate slowed down due to nuclear proteins associated to them (Kjellstrand and Lamm 1976).

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Author's address: Prof. Dr. MARIA LUIZA S. MELLO, Departmento de Biologia Celular, I.B., UNICAMP, Brasil - 13100 Campinas (SP).