Chromosome Contraction in Somatic Prophase in Sugar Beet

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ABSTRACT


A preliminary study of chromosome contraction process, during the somatic prophase in sugar beet, Beta vulgaris L., was carried out based on measurements taken on somatic prophase chromosomes. An indirect method was proposed which allowed the analysis of the process without the need of determining the elapsed time from early prophase to any given substage. The results suggested that there was not a straight line relationship between time and total genome length. Comparisons among individual chromosomes revealed that the contraction process was not synchronous in all chromosomes.

INTRODUCCION

During early somatic prophase the two chromatids of each chromosome are twisted about each other in relational coils (SCHULZ-SCHAEFFER, 1980). The coils interlock in such a manner that chromatids cannot be separated from each other looking as a single unit. As prophase proceeds, the relational coiling disappears, the chromatids disengage themselves and lie side by side and a different type of coiling occurs within each chromatid. This causes a reduction in total length of the chromosomes and a apparent thickening that is often
referred to as chromosome contraction. As early as 1945, SVARDSON working with *Salmo alpinus*, reported that the contraction was not uniform in both arm, with a quicker contraction for the short than for the long arm. WICKBOM (1949) working with *Bufo bufo* reported that contraction is relatively stronger in the short than in the long chromosomes. On the opposite, BAJER (1959) did not find any differences between chromosome arms, nor among chromosomes in the contraction process of the plant species *Haemanthus*. In 1968 OHNUKI reported that the degree of coiling was specific for each chromosome, as consencuence of a differential contraction process. Recently numerous reports have studied the contraction process in somatic human chromosomes as a way to find more band which could permit a more detailed identification of chromosomic areas (YUNIS et al., 1980, 1981, SCHOLLMAYER et al., 1981; RØNNE; 1983; RICHER et al., 1983). However they did not studied the contraction process per se.

Sugar beet has long and identifiable chromosomes at somatic prophase (NAKAMURA, 1979, CISTUE, 1983) which may be used for the study of the contraction process. The objective of this study was to characterize biometrically the chromosome contraction process occurring during somatic prophase in sugar beet, based on measures taken on untreated somatic chromosomes at different substages between early prophase to metaphase.

**PROCEDURE**

To carry out this study it would be necessary to measure the length of the somatic chromosomes at different times, in order to fit simple mathematical expressions to try to reproduce the process. However, there is no easy and reliable method to measure the elapsed time from early prophase to a given mitotic substage. Although different techniques have been developed for cell division synchronization in sugar beet (CISTUE, 1979), small microvariations due to, for example, the relative position of the cells in the meristems may cause significant variations to the measuring process. To solve this problem, an alternative way to measure the elapsed time in the mitotic cycle was proposed (CUBERO, 1983) by using the total genome length as an indirect indicator of time. However, it was not know if there was a linear relationship between time and total genome length, what initially prevented from making inferences about the process from an absolute temporary point of view. However, by doing such an indirect
FIG. 1. —Contraction process measured as the regression line of chromosome length on total genome length.

FIG. 2. —Contraction of sugar beet chromosome 1 in somatic prophase.
determination of time, comparisons among the relative contraction process of individual chromosomes or chromosome arms could be still possible.

The null hypothesis to evaluate was that chromosome contraction was uniform per unit length, i.e. total contraction time was independent of chromosome length, and that there was synchronization among all chromosome arms.

Let $L_p$ total complement length at the beginning of the prophase, $L_m$ total complement length at the metaphase stage, $l_p$ length of a given chromosome or chromosome arm at the early prophase and $l_m$ length of a chromosome or chromosome arm at late metaphase. The slope of the straight line which represents the contraction rate of a chromosome or chromosome arm is (Fig. 1).

$$\beta_1 = \frac{l_p - l_m}{L_p - L_m}$$

But note that according to the null hypothesis, the chromosome contraction is uniform per unit length, and therefore the fraction of the complement due to a specific arm or chromosome remains constant at the different substages and therefore, $l_p/L_p = l_m/L_m$. The slope, under the null hypothesis, may be expressed as:

$$\beta_1 = \frac{l_p - l_m}{L_p - L_m} = \frac{l_p}{L_p} = \frac{l_m}{L_m}$$

The statistical procedure to evaluate the null hypothesis used, was to estimate by using the standard least-square method the slope of the regression line, $\beta_1$, between total genome length as independent value and the specific arm or chromosome length as dependent variable. A statistical test of significance of the difference between the estimated $\beta_1$ with any of the ratios $l_p/L_p$ or $l_m/L_m$, could provide an indirect way to accept or reject $H_0$.

Both $l_p/L_p$ and $l_m/L_m$ ratios were unknown. However, somatic metaphase chromosome lengths are more precisely determined, and better estimators may be found in the literature (BOSEMÄRK and BORMOTOV, 1971; CISTUE, 1983). Therefore, the $l_m/L_m$ ratios were used for the analyses.

MATERIALS AND METHODS

The plant material used in this study was diploid and one haploid plants of a homozygous sugar beet line.

Actively growing root tips were collected in the morning and kept in ice-cold water for pretreatment. The pretreatment period was less
FIG. 3. — Contraction of sugar beet chromosomes 1, 4, 5 and 8. Fig. 3a total chromosome length. Fig. 3b short and long chromosome arm contraction, positive values represent short arms, negative values long arms. All unit are in μm.
than an hour, and ice-cold water was used to avoid changes on the chromosome morphology due to artificial chemical or physical conditions. Root tips were then fixed in freshly prepared mixture of glacial acetic acid and ethanol (1:3) solution. Root tips were kept in the fixative for at least overnight. Very long fixing periods resulted in poor quality preparations. Root tips were stained in 2% lactopropionic orcein for 15-45 minutes. Slides were prepared by the squash method. To increase the staining of some preparations, a further staining with Giemsa was used. After removal of the cover slip, slides were placed in 96% ethanol for 2-4 hours. Air dried slides were then stained for 5-15 minutes in 1.5% Giemsa Merck no. 9204 in 1/15 M Sorensen’s phosphate buffer (pH=6.8). After optimal staining of the cells, the slides were rinsed in distilled water, air dried and mounted in Euparal. Selected cells were photographed on 9x12 cm plates (x5000) and measurements taken on prints (x20000).

Some difficulties were encountered during measurements, for example in ascertaining the total length of some chromosomes arms. This was specially difficult due to the cytological technique, squash, used, which could cause breakage or artificial elongation of chromosome arms. Moreover, the absence of characteristic telomeric structures on the chromosomes could in some cases difficult the determination of chromosome ends. To try to overcome these sources of systematic errors, only 14 selected cells were measured, 13 being diploid and 1 haploid, which made a total of 27 complements measured. All 14 cells showed characteristics somatic prophase, prometaphase or metaphase chromosome. Only 4 chromosomes were individually analyzed in each cell. They were the most characteristic chromosomes at prophase and easily identifiable (Cistue, 1983). chromosome 1 is the longest chromosome at prophase, it has a satellite in the long arm and the centromere in metaphase is located in median-submedian position. chromosome 4 is a long metacentric chromosome. chromosome 5 it has intermediate length and at metaphase has its centromere in a clear submedian position. Finally, chromosome 8 is a short chromosome with a centromere in median-submedian position.

RESULTS

Figure 2 shows the contraction process as observed cytologically in chromosome 1. It should be mentioned that not all individual chromosomes shown in this Figure were used for the analysis, since only
27 were finally measured. All chromosomes appear printed at the same magnification.

Table 1 summarizes the results of the analyses. It gives the estimated slopes of the straight lines, \( \hat{\beta} \), and their corresponden standard errors. Coefficients of determination of the straight lines, \( R^2 \), are also listed which were in all cases significant at the \( p = 0.001 \) level. These estimated, \( \hat{\beta} \), coefficients would represent the best estimators of the average contraction rate of each chromosome or chromosome arm. The results are shown graphically on Figures 3a and 3b. In Figure 3a all data values plus the straight lines are represented for total chromosome length as dependent variable, and total genome length as independent. In both cases the unit of measurement was \( \mu m \). Figure 3b shows the straight lines for short and long arms independently with total genome also as independent variable. The ordinate value of cero would represent the position of the centromere. This figure gives a visual representation of the actual chromosome contraction process in both arms of each chromosome. The higher the abscline value, that is total genome in \( \mu m \), the earlier in the prophase stage, that is the least contracted genome. Abscline values close to 50 \( \mu m \) represent metaphase stages.

Table 1 also gives the Student-t test used to evaluate the null hypothesis that \( lm/Lm \) values taken from CISTUE (1983) were not statistically different from the estimated regression coefficients as indirect method of testing that contraction is uniform per unit length. It is clear from this table that there was not a uniform behaviour of chromosomes. In only one chromosome, chromosome 4, the null hypothesis of uniform contration per unit length could not be rejected. There seemed to be uniform contraction per length in both arms, which indicated that the ratio length of short arm to length of long arm, r-ratio, remained constant. In this metacentric chromosome both arms contracted at the same rate. In two other chromosomes, 1 and 8, there were clear departures of the estimated regression coefficient, contraction rate, and the expected \( lm/Lm \) ratios, indicating that contraction was not uniform per unit length. The behaviour of chromosome 5 was intermediate to those mentioned.

**DISCUSSION**

As mentioned, it was not known if there were a linear relationship between time and total genome length. However, there is an indication
TABLE 1. — Regression coefficients of contraction process and test of uniform contraction per unit length.

| CHROMOSOME ARM | $\hat{b}_1$ | S.E.$\hat{b}_1$ | $R^2$ | $\frac{\text{lm/Lm}}{\text{lm/LM}}$ | $|t|$ |
|----------------|-------------|-----------------|-------|---------------------------------|------|
| 1 SHORT        | .100        | .0051           | .939  | .044                            | 10.98** |
| LONG           | .099        | .0048           | .943  | .075                            | 5.00** |
| TOTAL          | .199        | .0067           | .973  | .119                            | 11.94** |
| 4 SHORT        | .054        | .0037           | .897  | .053                            | 0.27 N.S. |
| LONG           | .057        | .0033           | .919  | .061                            | 1.21 N.S. |
| TOTAL          | .111        | .0067           | .916  | .114                            | 0.45 N.S. |
| 5 SHORT        | .030        | .0021           | .890  | .041                            | 5.23** |
| LONG           | .071        | .0031           | .954  | .069                            | 0.64 N.S. |
| TOTAL          | .101        | .0042           | .958  | .110                            | 2.38*  |
| 8 SHORT        | .027        | .0028           | .793  | .040                            | 4.64** |
| LONG           | .043        | .0054           | .718  | .060                            | 3.15** |
| TOTAL          | .070        | .0070           | .800  | .100                            | 4.28** |

*** Probability of Type I error at the 0.05 and 0.01 level.
that this is not the case. As seen on Figures 3a and 3b, total genome values ranging from 50 to 225 µm were measured. These values seemed to be clustered in three groups: the first between 50 and 100 µm could represent metaphase and prometaphase stages; the second between 140 and 165 µm could represent the midprophase substage; and the third from 200 to 225 µm or early prophase. Should a linear relationship between time and total genome length exist, and since no particular substages were sought, total genome values would have to be scattered uniformly between the minimum and maximum values. The absence of intermediate values could be a random consequence of the sampling process or perhaps to a non linearity between total genome length and elapsed time. Chi-square goodness of fit test of the observed values to a uniform distribution indicated the tendency of the observations to be not uniformly distributed ($X^2$ (4d.f.) = 8.74, $p = 0.067$) and, therefore, suggested indirectly that the contraction process in absolute terms did not proceed at a uniform rate. Contraction may have happened more rapidly from early to midprophase than from them on. This would be the reason why not a single cell with total genome length between 165 to 208 µm was observed. However, it is necessary to point out that data were not sufficient to allow a definitive conclusion in this respect.

The comparisons among individual chromosomes or chromosome arms (Table 1, Figures 3a, 3b) revealed that the contraction process in relative terms, that is from one chromosome to another, was not uniform per unit length in all chromosomes and therefore different from one chromosome to another. The regression coefficients, $\beta_4$, for chromosome 1 were larger than the expected Iμ/Iμ ratios, which suggested that contraction of this chromosome was relatively stronger than for the others. This was observed also cytologically because chromosome 1 was relatively longer at early prophase than at metaphase. The opposite was found for chromosome 8 with a smaller slope. Contraction was weaker in this chromosome since it was shorter in relative terms in prophase than in metaphase. Chromosome 4 maintained the same relative size from early prophase to late metaphase, since no significant differences between the slopes and the Iμ/Iμ ratios were detected.

The objective of this work was a first attempt to characterize the contraction process in prophase sugar beet chromosomes from a strictly cytological point of view using as only variable the length of the chromosome measurable at prophase. Based on these analyses it is clear that the behaviour of all chromosomes studied was not uniform. In one chromosome one arm behaved as expected according to the
null hypothesis of uniform contraction per unit length whereas the other did not (chromosome 5, Table 1, Figure 3). Undoubtedly the contraction process is controlled by the structure of the chromosomes. It is clear that any further study on the prophase contraction of chromosomes would have to consider different aspects of the chromosome structure such as presence of heterochromatic regions. However, sugar beet chromosomes are not easily banded, which would difficult the future work.

RESUMEN

Contracción cromosómica en profase somática de remolacha azucarera.

Se ha realizado un estudio del proceso de contracción cromosómica en remolacha, Beta vulgaris L., a partir de medidas cromosómicas tomadas en los estados de profase a metafase mitóticas. Se propone un método indirecto que permite el análisis del proceso sin necesidad de determinar el tiempo transcurrido desde el comienzo de la profase a cualquier subestado mitótico concreto. Los resultados sugieren que no existe una relación lineal entre tiempo y longitud total del complemento. El análisis del proceso entre cromosomas distintos reveló que la contracción no es uniforme para todos los cromosomas.

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