CHROMOSOME STRUCTURE IN VICIA FABA AND NARCISSUS JONQUILLA
BY $^3$H-THYMIDINE AUTORADIOGRAPHY AND TRYPsin DIGESTION

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Chromosomes treated with trypsin have been studied by several investigators in order to observe the structure of the chromosome as to its strandedness and replication. The fact that chromatids consist of at least two strands when trypsin is applied, is agreed by Trosko and Wolff (1965) and Ghosh and Ghosh (1970), the former authors observing still smaller units. Furthermore, Ghosh and Ghosh (1970) have incorporated $^3$H-thymidine before applying trypsin to study the replication of chromosomes in Vicia faba. They have concluded that the mode of chromatid replication is semi-conservative. However, opinions vary concerning this subject.

The purpose of the present investigation is to study the distribution of $^3$H-thymidine and the changes which may occur in mitotic chromosomes and in the chromatin of interphase nuclei in V. faba and Narcissus jonquilla when digested with trypsin.

MATERIALS AND METHODS

Vicia faba L.

When the lateral roots of V. faba were about 0.5 cm the beans were skewered with a stainless wire and grown in distilled water for 2-3 days till the roots were about 1 cm long. They were then immersed in a $^3$H-thymidine (TRA 61 Thymidine 6-T(n) Batch 135 obtained from Japan Radiochemical Assoc.; S.A. 5 Ci/mM) solution, which was diluted with distilled water to make up a concentration of $^3$H-thymidine 2 $\mu$Ci/ml, at 20°C for 8 hr. The 8 hr treatment was calculated to be about half of the cell cycle of root tip cells in V. faba from the report of Webster and Davidson (1968). After treating with $^3$H-thymidine the roots were washed in several changes of distilled water and transferred to 0.01% colchicine solution for 8 hr.

The meristematic regions of the roots were cut off and fixed in cold 2% neutral formalin for 1-2 hr. To observe the effect of trypsin digestion the method by Trosko and Wolff (1965) was followed. However, in order to isolate the nuclei and chromosomes two layers of Tetoron T-2000 (screen gauze) supplied by Teijin Co. were used as filters. When the slides were air-dried, they were hydrolyzed in 1 N HCl at 60°C for 20 min and stained with Feulgen's nuclear reaction.

For autoradiographic observation stripping film (Fuji stripping film ET-2F) was applied on the dry, stained material adhered to the gelatin coated slide glass. This was stored in a tightly sealed container at 5°C for 2-3 weeks. These slides were then
developed for 4 min at 20°C with Rendol.

To observe the cells without the silver grains and to make comparisons with those of the labelled photographs, silver grains were removed by applying a solution made up of 13% Na$_2$S$_2$O$_3$·5H$_2$O and 10% K$_3$Fe(CN)$_6$ (1:1) for 10 min followed by washing in tap water for 15 min.

*Narcissus jonquilla* L.

Bulbs of *N. jonquilla* were grown at 20°C in a liter glass container filled with distilled water and when the lateral roots were about 1-1.5 cm long the bulbs were skewered and immersed in a $^3$H-thymidine (TRA 61 Thymidine 6-T(n) Batch 137 obtained from Japan Radiochemical Assoc.; S.A. 5 Ci/mM) solution, which was diluted with distilled water to make up a concentration of $^3$H-thymidine 2 $\mu$Ci/ml, at 20°C for 12 hr,

![Fig. 1. Interphase nuclei of the root-tip cells of Vicia faba. A: Control, B: $^3$H-thymidine incorporated, C: Trypsin digested, D: $^3$H-thymidine incorporated, followed by trypsin digestion. $\times$1,300.](image-url)
this treatment being selected due to the results of a previous report (Hirahara 1971). The rest of the procedure was the same as that described for \textit{V. faba} except for the exposure time being 2.5 to 3 months.

**RESULTS**

\textit{Interphase}: Results of the \(^3\)H-thymidine incorporated interphase nuclei in \textit{Vicia faba} after digesting with trypsin are shown in Fig. 1.

The nuclei digested with trypsin (Fig. 1C) appear quite expanded approximately

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\textbf{Fig. 2.} Interphase nuclei of the root-tip cells of \textit{Narcissus jonquilla}. A: Control, B: \(^3\)H-thymidine incorporated. C: Trypsin digested, D: \(^3\)H-thymidine incorporated followed by trypsin digestion. Nucleolini can still be observed after trypsin digestion. \(\times 1,300\).
three to four times that of the control (Fig. 1A, B). The nucleolus seems to have lost its shape but the chromatin could still be identified. A network structure which is Feulgen-positive can be observed stretching toward the outer surface and loosening at the edges. This observation has also been reported by Trosko and Wolff (1965).

Further studies have been made by the authors by incorporating $^3$H-thymidine prior to the use of trypsin, and the result is illustrated in Fig. 1D. Silver grains are usually found condensed in $^3$H-thymidine incorporated nuclei (Fig. 1B), however, when digested with trypsin the grains are somewhat condensed near the center but gradually spreading outward still keeping its network pattern. This observation coincides with the structure obtained from cells without the incorporation of $^3$H-thymidine (Fig. 1C).

In the case of Narcissus jonquilla two nucleolini can be observed within the nucleolus as already reported previously (Hirahara and Tatuno 1967), except quite

Fig. 3. Metaphase chromosomes of Vicia faba. A: Trypsin digested, B: $^3$H-thymidine incorporated, followed by trypsin digestion. Numbers 1 to 9 indicate the sections where grain count was made. ×1,300.
expanded like the rest of the chromatin (Fig. 2C, D). In some nuclei the nucleoli could not be differentiated due to the rest of the chromatin being extremely digested. In general, however, similar observations as seen in V. faba were noted. Some were found to be digested even more so than that of V. faba although the network structure could still be seen but quite extended (Fig. 1D).

Metaphase: As illustrated by Trosko and Wolff (1965), after trypsin digestion the chromosomes from the meristematic root tips were observed to separate into two strands of chromatids. Furthermore, each chromatid is found to split longitudinally into two halves with still finer splittings at some locations. At the primary constriction staining is light but fine fibers can be seen joining the two arms of the chromosomes. These observations were clearly seen in both V. faba (Fig. 3A) and N. jonquilla (Fig. 4A). By trypsin digestion the coiling of the chromatid can be clearly seen, tightly more so in N. jonquilla.

Fig. 4. Metaphase chromosomes of Narcissus jonquilla. A: Trypsin digested, B: 3H-thymidine incorporated, followed by trypsin digestion. ×1,300.
Incorporation of $^3$H-thymidine prior to trypsin digestion revealed silver grains over the whole expanded chromosome (Figs. 3B, 4B). The appearance of the grains were distinct, which enabled one to differentiate the grains located over the longitudinal splits of chromatid and those elsewhere.

A summary of grain counts observed over nine sections of the chromosomes in *V. faba* is given in Table 1. As indicated in the Table, the total number of grains among the four longitudinal splits show only a slight difference of one or two grains. From this data it may be concluded that there is no difference between the splits, however, to confirm this finding statistical analyses were made by first rearranging the number of grains in order of the highest number within a section in one group and the lowest number in another group. By so doing, in the case of *V. faba* the mean value for the longitudinal split having the greatest number of grains was 5.4 ± 0.3 and the least 4.7 ± 0.1. When t-test was made using these two extreme lots, the t-value was calculated to be 1.6. At the 0.01 probability level the t-value must be more than 2.9 to be considered significantly different. Thus, with a value of 1.6 it can be said that there is no significant difference among the four longitudinal splits formed within the same metaphase chromosome. Therefore, in relation to DNA synthesis it can be concluded that the four splits are homologous. Similar results were also obtained from *N. jonquilla*. Analyses for the finer splittings could not be made as it was difficult to make specific determinations.

**DISCUSSION**

It was confirmed that by trypsin treatment the nucleus expands and the network structure can be obtained in both *Vicia faba* and *Narcissus jonquilla*. Such structure was found to be Feulgen-positive. It was interesting to find that when $^3$H-thymidine was incorporated prior to trypsin digestion the silver grains could be seen arranged
over the structure, further proving that DNA is present.

In spite of the artefacts (due to fixation, heat treatment, trypsin digestion, etc.) that can be expected to occur, in the case with the metaphase chromosomes it was found that $^3$H-thymidine also appeared over the obtained longitudinal splits of chromatids similar to Trosko and Wolff (1965). According to the grain counts on each split it can be said that the number of grains showed no significant difference, this indicating that each split was labelled similarly. Thus, it can be presumed that the individual longitudinal split formed by trypsin digestion is compatible with both the artefact-split of unineme chromatid discussed by Taylor (1966) and the half-chromatid of bineme scheme reported by Peacock (1963) and Darlington and Haque (1969).

It has been a matter of question as to what happens to the nucleoli. Trosko and Wolff (1965) have reported that it disappears leaving a trypsin resistant core, which may be the nucleolini. In the case of *N. jonquilla* it was observed that the nucleolini expanded like the rest of the chromatin, however, in the case of *V. faba*, such clear findings could not be seen.

**SUMMARY**

By incorporating $^3$H-thymidine to the meristematic root tips of *Vicia faba* and *Narcissus jonquilla* followed by trypsin digestion, it was revealed that the silver grains were arranged in a network pattern in the isolated interphase nuclei. In the metaphase chromosomes the grains were found to be localized along the digested chromatids and their number showed no significant difference from one longitudinal split to the other found within the same chromosome.

**LITERATURE CITED**


