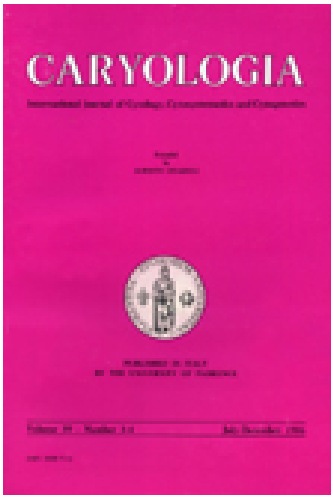

Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tcar20

Autoradiographic Study of the Turnover of Chromatin-Associated Phospholipids in Vicia Faba L.

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Published online: 31 Jan 2014.

To cite this article: P. B. Gahan, M. P. Viola-Magni & C. F. Cave (1986) Autoradiographic Study of the Turnover of Chromatin-Associated Phospholipids in Vicia Faba L., Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics, 39:3-4, 281-285, DOI: 10.1080/00087114.1986.10797790

To link to this article: http://dx.doi.org/10.1080/00087114.1986.10797790

CARYOLOGIA Vol. 39, n. 3-4: 281-285, 1986

AUTORADIOGRAPHIC STUDY OF THE TURNOVER OF CHROMATIN-ASSOCIATED PHOSPHOLIPIDS IN *VICIA FABA* L.

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SUMMARY — Autoradiographic data from labelling studies on the incorporation of [^{14}C] ethanolamine into nuclear phospholipids of meristem cells from roots of *Vicia faba* indicated the presence of a continuous labelling pattern for the nuclear membrane throughout the cell cycle. In contrast, a periodic labelling pattern was observed in chromatin of interphase nuclei which closely paralleled the synthesis pattern for DNA. This corroborated biochemical data from previously published studies of hepatocyte nuclear membranes and chromatin.

INTRODUCTION

Studies on both rat liver and higher plant nuclei and mitotic chromosomes (CHAYEN *et al.* 1957; GAHAN 1965; VIOLA-MAGNI *et al.* 1985*a*; GAHAN *et al.* 1985) have indicated the presence of chromatin-associated phospholipids. Recent analyses on chromatin isolated from hepatocytes from rats injected with [^{32}P]O^{2-}_4 have supported the hypothesis that phospholipids are synthesized on the microsomes and are then transported to the chromatin. Whilst the nuclear membrane phospholipids seem to be synthesized continuously throughout the cell cycle, the synthesis of chromatin-associated phospholipid appears to be cyclic (VIOLA-MAGNI *et al.* 1985*b*).

In the present communication, information is presented showing that autoradiographic data from labelling studies with [^{14}C] ethanolamine on plant tissues indicate a continuous labelling pattern of nuclear membranes throughout the cell cycle. In contrast, a periodic labelling pattern is observed in interphase chromatin which closely parallels the synthesis pattern for DNA, so corroborating the biochemical data obtained for hepatocyte nuclear membranes and chromatin by VIOLA-MAGNI *et al.* (1986).

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MATERIAL AND METHODS

1. *Plants.* — Seeds of *Vicia faba* var. «green windsor» were soaked in water overnight, the testas removed and the seeds planted in moist paper towelling to germinate in the dark at 19°C. Five days later the seedlings were transplanted to grow in aerated tap-water at 19°C with 16 hours light and 8 hours dark. Plants were transferred to grow under similar conditions with ethan-1-ol-2-amine-2-[C^{14}] (Specific activity 4.86 or 9.3mCi/mM) added to the tap water at the concentration of 2 μCi/ml continuously for up to 48 hours. Some plants were fed with [^{3}H] thymidine (2 μCi/ml; specific activity 5Ci/mM) for 3 hours and chased for periods up to 36 hours.

2. *Tissue preparation.* — The terminal 1 cm of lateral roots of *V. faba* were removed at time intervals from 0 to 48 hours after feeding with [^{14}C] ethanolamine and fixed in i) formal-calcium (BAKER 1946) for 48 hours at 4°C or ii) LEWITSKY's (1931) fluid for 6 hours at 20°C, or iii) formal-calcium saturated with Reinecke's salt (LA COUR *et al.* 1958) for 6 hours. The material was washed well with running tap water for 18 hours, dehydrated, embedded in paraffin wax and sectioned at 5 μm. Unfixed, frozen sections (GAHAN 1884) were fixed for 1 hour in either formal-calcium or Lewitsky's fluid or formal-calcium saturated with Reinecke's salt. After fixation they were washed well with tap water. Root tips fed with [^{3}H] thymidine were sampled every 3 hours up to 36 hours after labelling and prepared as Feulgen squashes (GAHAN 1984).

3. *Biochemical analysis.* — Roots of *V. faba* were assayed for their percentage of labelling by [^{14}C] ethanolamine present in the phospholipids as previously described (CAVE and GAHAN 1970).

4. Autoradiographs were prepared with Kodak AR10 stripping film (GAHAN 1984) and exposed for 3,4, 10 or 21 days prior to photographic processing. [^{3}H] thymidine autoradiographs were observed directly, whilst those of [^{14}C] ethanolamine were stained with methyl green-pyronin prior to mounting in Euparal.

RESULTS AND DISCUSSION

Autoradiographs prepared from roots fed with [^{3}H] thymidine and scored for the percentage of labelled mitosis versus time after pulsing from 1-36 hours (CLEAVER 1967; GAHAN 1984), provided mean values of the phases of the cell cycle as G_1 = 4.0 hours; S = 9.0 hours, G_2 = 3.5 hours and M = 2.0 hours, yielding a total cell cycle time of 18.5 hours. These values are similar to those obtained for *V. faba* by other workers (in SCHULTZE 1969).

Control autoradiographs made with unlabelled sections showed no artefactual autoradiographs arising from the effects of fixation (PELC 1958), pressure, heat or chemography (ROGERS 1979; GAHAN 1984). Background counts from all experimental preparations yielded c.a. 0.5 grain per nucleus.

Autoradiographs of root sections fixed in formal-calcium and exposed for 3-4 days at -25°C showed all nuclei to be labelled by 12h of continuous feeding with [^{14}C] ethanolamine and all mitotic figures by 6 hours. If the exposure time

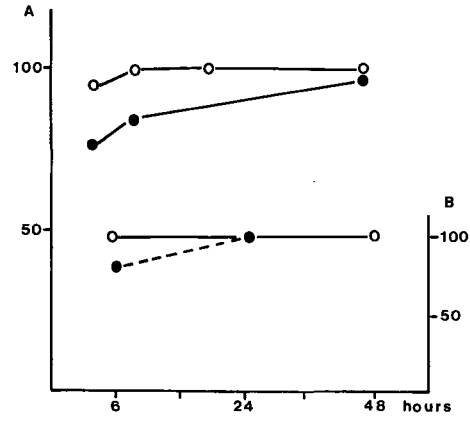

Fig. 1. — Analysis of autoradiographs of root apices of *Vicia faba* fed continuously with [^{14}C] ethanolamine followed by fixation in formal-calcium for 48h at 4°C to remove nuclear membrane phospholipids. Autoradiographs exposed for 3-4 days at −25°C. A. Percentage of interphase nuclei labelled at 6, 12, 24 and 48 hours. B. Percentage of mitotic figures labelled at 6, 24 and 48 hours. The autoradiographic exposure times were 3-4 days (closed circles) and 21 days (open circles).

of the autoradiographs was extended to 21 days then 10% labelling of interphase nuclei occurred after only 6 hours of feeding. An analysis of the level of labelling of these nuclei by grain counts, showed an increased intensity of labelling with increased feeding time after fixation in formal-calcium-Reinecke fixative (Fig. 1). The level of labelling per nucleus was also found to increase (Tab. 1). Biochemical assays showed more than 90% ot the [^{14}C] to be in the lipid fraction.

The cells fixed with formal-calcium will have had the phospholipid fraction totally removed from the mitotic chromosomes and partially from the nuclear membranes (CHAYEN *et al.* 1957; GAHAN 1965; VIOLA-MAGNI *et al.* 1985*a*). Thus, autoradiographs will show both chromatin-associated and some

TABLE 1 — *Percentage of nuclei for each range of grain numbers derived from autoradiographs of root apical cell nuclei from «Vicia faba» after continuous feeding with* [^{14}C] *ethanolamine, fixation in formal-calcium for 48 hours at 4°C and autoradiographic exposure for 3 days at −25°C.*

Feeding time	Grain numbers corrected for background					
	0-9	10-19	20-29	30-39	40-49	50-59
6h	60	35	5	0	0	0
12h	0	45	40	5	0	0
24h	0	25	45	25	5	0
48h	0	0	25	50	20	5

residual nuclear membrane [^{14}C] ethanolamine. The observed pattern of labelling may be interpreted to indicate continuous phospholipid synthesis of the nuclear membrane fraction throughout the cell cycle except for the period of late prophase to mid-telophase (Tab. 1; Fig. 1), since once labelled, the interphase nuclei remain labelled.

Although the nuclear membrane labelling inhibits a direct observation upon the labelling of the chromatin phospholipids in interphase nuclei, the presence of a cyclic labelling pattern may be deduced from the autoradiographs derived from a continuous feeding experiment on application of the methodology described by CLEAVER (1967). An assessment of the time taken (a) for all interphase nuclei to become labelled should yield $t_1 + t_2$ i.e. $G_1 + G_2 + M$; (b) to reach a maximum grain count on interphase nuclei should yield t_s; and (c) to reach the 50% level on the graph describing percentage of labelled mitotic figures versus time of incubation should yield t_2 i.e. $G_2 + 0.5$ M.

In the current experiments, the length of the cell cycle and mitosis as derived from labelling with [^{3}H] thymidine, are 18.5 hours and 2 hours, respectively. The data calculated from autoradiographs with [^{14}C] ethanolamine after fixation in formal-calcium i.e. a «chromatin fraction» (Fig. 2) show $t_1 + t_2 \simeq 12$ hours; $ts \simeq 6.5$ hours and $t_2 \simeq 3$ hours. This means that if the cell cycle and mitosis take 18.5 hours and 2 hours, respectively, then the periods of lipid labelling will be $G_1 \nearrow 8$ hours; $S \nwarrow 6.5$ hours and $G_2 \simeq 2$ hours. These figures are approximations since nuclear membrane labelling (Tab. 1) will cause the estimate for t_s to be low. Nevertheless, the evidence indicates the presence of a cyclic labelling pattern which has a periodicity similar to that for the DNA cycle. This interpretation reinforces data from biochemical experiments on hepatocytes from regenerating rat liver showing a cyclic synthesis of chromatin-associated phospholipids (VIOLA-MAGNI *et al.* 1985*b*).

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Received 3 December 1985; revision accepted 27 August 1986