Acta Biochimica Polonica

Vol. 44 No. 1/1997

79–82

QUARTERLY

Short communication

Autonomous replication of a wheat DNA sequence in isolated wheat nuclei*

Blanka Szurmak and Jerzy Buchowicz

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, A. Pawińskiego 5a, 02-106 Warsaw, Poland

Received: 11 December, 1996

Key words: autonomous DNA replication, nuclear extrachromosomal DNA, *Triticum aestivum*

A fragment of wheat nuclear DNA was shown to be able to replicate autonomously in wheat nuclei. The fragment was 637-bp long and contained telomeric repeats at both termini. Its replication was manifested by the appearance of a radioactive reaction product of the same approximate size. Further analyses showed that the linear, double-stranded reaction product was labelled along its entire length and contained the same number of similarly located *Hae*III sites as did the fragment tested. Prokaryotic DNA remained unlabelled under the same assay conditions.

It has been know for many years that, due to the enormous size and peculiar topography of eukaryotic DNA, its replication should start at multiple sites simultaneously, to be completed within the S-phase of the cell cycle [1, 2]. In budding yeasts, such sites are scattered through the entire genome to endow it with a large number of sequence-specific origins of replication [3, 4]. A strong coherence in the mechanisms that control eukaryotic cell division has emerged from studies on yeasts, to the extent that we have come to expect that the initiation of replication charted in these microorganisms will be found to be very similar in higher eukaryotes. Tran *et al.* [5] demonstrated, however, that autonomously replicating sequences of yeast do not function as replication origins in cultured human cells. Smith & Calos [6] concluded further that animals may lack specific origin sequences. Some other observations, on the contrary, have indicated a rather high degree of origin specificity in cultured human [7] and mammalian [8–11] cells.

These controversial data pointed to the urgent need to know whether higher plants, the largest group of multicellular organisms, utilize specific DNA sequences for the initiation of replication. Recent findings of Van't Hof & Lamm [12] indicate that plants may use more than one mechanism to replicate their genome. Here, we demonstrate that a fragment of nuclear extrachromosomal DNA from resting wheat embryos becomes highly radioactive when it is incubated with isolated wheat nuclei and a labelled DNA precursor. The precursor incorporation seems to result from a replicative DNA synthesis and to depend on a specific sequence signal.

*This work was supported by a grant No. 6 P203 048 04 from the State Committee for Scientific Research.

MATERIALS AND METHODS

Cloned DNA fragment. As described in a previous report [13], the plasmid pTa637 was constructed by ligation of *Sma*I-digested pUC119 to nuclear extrachromosomal DNA of resting wheat embryos. The *Triticum aestivum* insert was 637-bp long (EMBL/GenBank accession number X73235) and contained short telomeric repeats. To release the wheat sequence, the intact (circular) plasmid pTa637 was digested with *Kpn*I and *Pst*I under conditions recommended by the manufacturer (Amersham). The digestion products were separated by electrophoresis (0.8% agarose gel, 40 mM Tris/acetate, 1 mM EDTA, pH 7.5) on two identical gels. One of the gels was stained with ethidium bromide. The pTa637 insert was eluted from the second unstained gel [14].

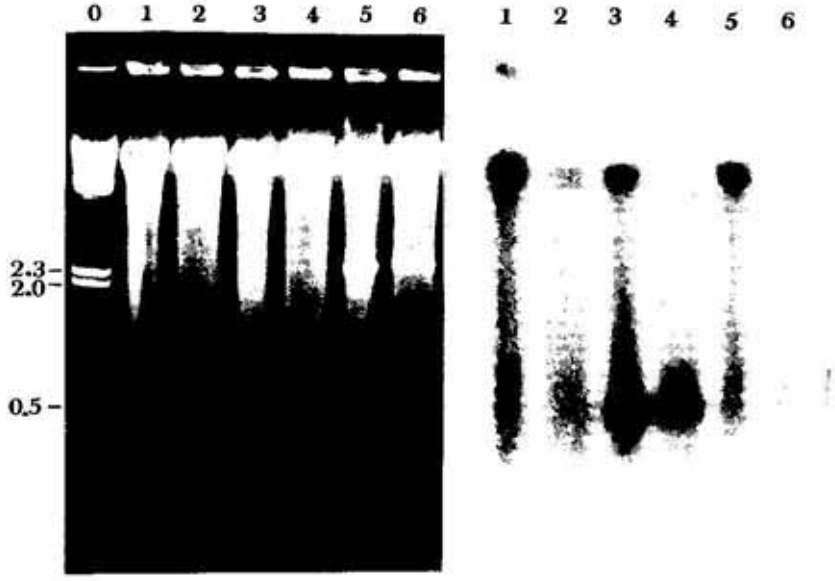

Figure 1. Electrophoretic separation of products formed under conditions of the assay for autonomous DNA replication in wheat.

Pictured are the results of ethidium bromide staining (left) and autoradiography (right). Lane 0, size markers (kb); lanes 1 and 2, no exogenous DNA; lanes 3 and 4, pTa637 insert; lanes 5 and 6, pUC119 (linearized with *Pst*I). For lanes 2, 4 and 6, DNA was digested with S1 nuclease prior to the electrophoresis.

Isolated nuclei. Mature embryos of the common wheat *T. aestivum* (cv. Begra) were germinated at 21°C for 10 h and then used as the source of cell nuclei. The nuclear preparation (900 ***g*** pellet) was obtained by a typical procedure with experimental details given earlier [13].

Assay for autonomous DNA replication in wheat. Approximately 10^6 of freshly prepared nuclei were suspended in 0.1 ml of a mixture [15] containing 200 mM sucrose, 100 mM sorbitol, 37 mM Tris/HCl (pH 8.0), 30 mM KCl, 12 mM $MgCl_2$, 7 mM EGTA, 5 mM dithiothreitol, 1.5 mM $CaCl_2$, 1 mM ATP, 0.15 mM each of dATP, dGTP and dTTP, 1 μCi of [α-^{32}P]dCTP (400 Ci/mmol) and 1 μg of exogenous DNA. After incubation at 21°C for 20 min, the nuclei were spun down (900 × ***g***, 6 min, 4°C), suspended in 1 ml of incubation mixture in which [^{32}P]dCTP had been replaced by unlabelled dCTP (0.15 mM) and pelleted again. Total DNA was then isolated by the phenol method [16] and analysed by gel electrophoresis and autoradiography (2-day exposure). In some experiments (as specified in figure legends), DNA was digested with S1 nuclease (1 unit/20 μg, 20 min, 37°C).

RESULTS

In a series of preliminary experiments (Szurmak, unpublished), the pTa637 insert was found to confer the ability to a yeast plasmid (pFL34) to be maintained in the autonomous state in yeast. To test its ability to replicate in its native cellular environment, the insert was incubated with wheat nuclei in the presence of [α-^{32}P]dCTP. As Fig. 1 shows, the tested fragment becomes highly radioactive when incubated under the assay conditions. The labelled product is insensitive to S1 nuclease. Some endogenous components also become radioactive but no labelling of added prokaryotic DNA (pUC119 linearized with either *Kpn*I or *Pst*I) can be observed. Probably, the labelling depends on

a sequence signal that is present in wheat DNA but absent from the prokaryotic DNA sample.

The apparently double-stranded nature of the radioactive product seemed to indicate that it could not result from an unspecific, terminal addition-type reaction. However, to exclude such an alternative, the reaction product was subjected to digestion with *Hae*III. This enzyme was chosen for the restriction analysis as it cuts the linear 637 bp sequence into three unequal parts (187, 408 and 42 bp, respectively), whose radioactivities could be detected by autoradiography. All the three *Hae*III fragments showed a significant radioactivity (Fig. 2). Moreover, the largest fragment, corresponding to the central part of the 637 bp sequence, appeared on the autoradiogram as the strongest band. Clearly, the reaction product was labelled along the entire sequence and no preferential labelling of its termini occurred. Such a labelling pattern may, however, be common to both replicative synthesis and extensive repair. To distinguish between these two possibilities, labelling of the newly synthesized strand with BrdUTP followed by isopycnic centrifugation should be carried out. Alternatively, the absence of gaps and nicks in the fragment tested should be demonstrated. We chose the latter approach.

Figure 2. Restriction analysis of a product formed under conditions of the assay for autonomous DNA replication in wheat.

The product corresponding to the main band of radioactivity in lane 4 of Fig. 1 was eluted from the gel and digested with *Hae*III for 3 h under conditions recommended by the manufacturer (Amersham). The completely digested product was mixed with an unlabelled 637 bp fragment (1 μg) which had been digested with *Hae*III for 1 h. The mixture was subjected to gel electrophoresis followed by ethidium bromide staining (left, where the mobility of size markers is also shown) and autoradiography (right; 5-day exposure).

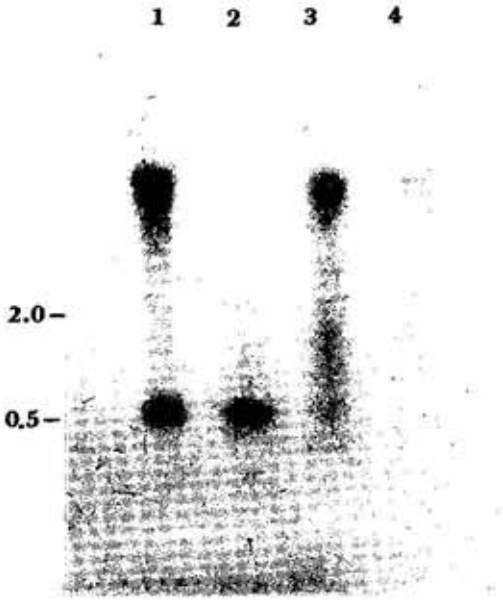

Figure 3. A comparison of the labelling of the free pTa637 insert and intact pTa637 under conditions of the assay for autonomous DNA replication in wheat.

Both insert (637 bp) and intact plasmid (3799 bp) were purified by gel electrophoresis before incubation with the nuclei. Lanes 1 and 2, pTa637 insert; lanes 3 and 4, intact pTa637. Positions of size markers (kb) are indicated on the left. For lanes 2 and 4, DNA was digested with S1 nuclease prior to the electrophoresis.

No radioactive reaction product appeared when intact (circular) pTa637 was incubated under conditions that ensured extensive labelling of the free insert (Fig. 3). Apparently, the cloned sequence was perfectly double-stranded and could not serve as a substrate for repair enzymes. Thus, its labelling resulted, most probably, from a replicative DNA synthesis. The reason for which the intact plasmid pTa637 (containing the entire 637 bp sequence) could not serve as a template for this replication is unknown.

DISCUSSION

We have examined the ability of a wheat DNA fragment to replicate autonomously in isolated wheat nuclei. The fragment chosen for this investigation corresponded to a 637

bp sequence of nuclear extrachromosomal DNA [13] whose properties were discussed in a minireview [17].

Essentially the same sequence was identified by Cheung *et al.* [18] in chromosomal DNA of wheat. The extrachromosomal 637 bp fragment differs, however, from its chromosomal counterpart with the presence of a very AT-rich, highly asymmetric element of the sequence, 5′-ATTTATTTTTCTTTTT-3′. This structural difference arises the question whether such a 16 bp element can contribute to the maintenance of the whole 637 bp sequence in the extrachromosomal state in wheat embryo nuclei.

Independently of the answer to this particular question, our observations, at present, allow us to assume that in higher plants, similarly as in yeast, specific sequences (present in the insert but absent from vector) may be involved in the initiation of replication. A similar conclusion was reached earlier by Van't Hof & Lamm [12] on the basis of their experiments on rDNA replication in synchronized root cells of pea.

REFERENCES

1. Huberman, J.A. & Riggs, A.D. (1968) *J. Mol. Biol.* **32**, 327–341.
2. Chargaff, E. (1976) *Progr. Nucleic Acid Res. Mol. Biol.* **16**, 1–24.
3. DePhamphilis, M.L. (1993) *Annu. Rev. Biochem.* **62**, 29–63.
4. Toyn, J.H., Toone, W.M., Morgan, B.A. & Johnson, L.H. (1995) *Trends Biol. Sci.* **20**, 70–73.
5. Tran, C.T., Caddle, M.S. & Calos, M.P. (1993) *Chromosoma* **102**, 129–136.
6. Smith, J.G. & Calos, M.P. (1995) *Chromosoma* **103**, 597–605.
7. Giacca, M., Zentilin, L., Norio, P., Diviacco, S., Dimitrova, D., Contreas, G., Biamonti, G., Perini, G., Weighardt, F., Riva, S. & Falaschi, A. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7119–7123.
8. Zannis-Hadjopoulos, M., Nielsen, T.O., Todd, A. & Price, G.B. (1994) *Gene* **151**, 273–277.
9. Brun, C., Dijkwel, P.A., Little, R.D., Hamlin, J.L., Schildkraut, C.L. & Huberman, J.A. (1995) *Chromosoma* **104**, 92–102.
10. Brinton, B.T. & Heintz, N.H. (1995) *Chromosoma* **104**, 143–151.
11. Kelly, R.E., DeRose, M.L., Draper, B.W. & Wahl, G.M. (1995) *Mol. Cell. Biol.* **15**, 4136–4148.
12. Van't Hof, J. & Lamm, S.S. (1992) *Plant Mol. Biol.* **20**, 377–382.
13. Bucholc, M. & Buchowicz, J. (1994) *Plant Mol. Biol. Rep.* **12**, 204–208.
14. Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
15. Roman, R., Caboche, M. & Lark, K.G. (1980) *Plant Physiol.* **66**, 726–730.
16. Hattori, J., Gottlob-McHugh, S.G. & Johnson, D.A. (1987) *Anal. Biochem.* **165**, 70–74.
17. Buchowicz, J. (1997) *Acta Biochim. Polon.* **44**, 13–20.
18. Cheung, W.Y., Money, T.A., Abbo, S., Devos, K.M., Gale, M.D. & Moore, G. (1994) *Mol. Gen. Genet.* **245**, 349–354.