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Review

Nuclear extrachromosomal DNA of higher plants

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Recent reports indicate that minichromosomes and other small genetic entities may occur in the nuclei of uninfected higher plants. They become especially abundant under some special growth conditions and, sometimes, resemble extrachromosomal genes of ciliated protozoa. An example of such a gene-sized DNA species was isolated from resting wheat embryos. The presence of telomeric sequences at its termini and the ability to replicate autonomously in wheat nuclei made it possible to distinguish this nuclear minichromosome from chromosomal DNA fragmentation products. The biological significance of plant minichromosomes remains to be elucidated.

Cell nuclei are known to contain various small genetic entities in addition to standard chromosomes. The largest of these belong to a class of B chromosomes which may be present in many plant and animal species [1, 2]. Another class of small chromosomes, called microchromosomes, occurs commonly in birds and reptiles [3]. All these small structures are large enough to be discernible under the light microscope as metaphase chromosomes but are probably indistinguishable from chromosomal DNA in interphase nuclei.

In this minireview, I would like to concentrate on gene-sized DNA species that may be isolated in the form of extrachromosomal DNA. Although the occurrence of nuclear exDNA in higher plants still remains a matter of controversy, progress in preparative and analytical methods brings new information on this topic rapidly.

AUTHENTIC AND ARTEFACTUAL exDNA SPECIES

As reviewed by Rush & Misra [4], a number of reports on the isolation of various exDNA species appeared before 1985. Unfortunately, the extraction procedures usually led to an extensive fragmentation of chromosomal DNA. Standard isolation and fractionation methods therefore yielded putative exDNA preparations that were composed mainly, if not exclusively, of chromosomal DNA degradation products. Various structural features, e.g., circularity [5, 6] or single strandedness [7, 8], as well as differences in

Abbreviations: ABA, abscisic acid; ARS, autonomously replicating sequence; exDNA, extrachromosomal DNA; ORF, open reading frame; PCR, polymerase chain reaction; PFGE, pulsed field gel electrophoresis; RAP1, repressor/activator protein 1.

buoyant densities [9, 10] and hybridization patterns [6, 11] were, therefore, utilized to distinguish between true exDNA and preparative artefacts. None of these approaches has, however, been commonly accepted. Instead, attempts to prepare exDNA with the omission of the stage of total DNA have become predominant.

As early as in 1976, Hirt [12] developed a simple method for selective extraction of viral DNA from virus-infected mammalian cells. A non-enzymic lysis followed by high speed centrifugation allowed him to separate viral (extrachromosomal) DNA from all the cellular DNA species. No simultaneous fragmentation of chromosomal DNA was caused and no traces of cellular DNA could be detected in the viral DNA preparation. Thus, the method could be successfully used for the isolation of cellular exDNA if it occurred in the analysed cells. Indeed, the method was soon applied for the isolation of bacterial plasmids as described in a commonly used manual [13].

The first attempt to apply the method of Hirt to plant material was described by Van't Hof & Bjerknes [9]. To adjust experimental conditions to the specificity of plant material, they used isolated nuclei, instead of intact tissue or cell suspension, as the immediate source of crude exDNA. Special attention was then paid to extensive purification of the nuclei which, however, resulted in exDNA preparations that, similarly as chromosomal DNA, were rich in repetitive sequences and rRNA genes [14]. In our laboratory, the crude nuclear pellet was used as the exDNA source. The preparations obtained differed from randomly degraded chromosomal DNA in respect to buoyant density [10] and contained telomeric sequences [15, 16].

Another way to omit the total DNA stage from preparative procedures was found by Schwartz & Cantor [17]. Yeast cells were embedded in an agarose gel and after lysis with proteinase K, subjected to the PFGE. The solidified agarose prevented DNA from shearing during all the treatments and PFGE conditions allowed separation of both chromosome-sized and extrachromosomal DNA molecules simultaneously. The method, however, needed some modification to be applied to plant material. The first attempts included preparation of protoplasts as a preliminary step [6, 18]. More recently, tissue slices have been used [19]. Some recent data on nuclear exDNA of higher plants are summarized in Table 1. The table does not include plasmids as, to my knowledge, no report on their occurrence in plant nuclei has appeared so far.

Table 1. Examples of small genetic entities occurring in plant nuclei

Entity	Plant species	DNA size (bp)	Reference
B chromosome	*Crepis capillaris*	10^7–10^8	[2]
Minute chromosome	*Scilla siberica*	$<10^7$	[24]
	Triticum aestivum	10^5–10^7	[25]
Circular exDNA	*Oryza sativa*	4.5×10^4	[6]
Minichromosome	*Triticum aestivum*	637	[15]
Transposon exDNA	*Zea mays*	1.4×10^3	[45]
	Arabidopsis thaliana	431	[11]
γ-Induced exDNA	*Zea mays*	variable	[47]

MINUTE CHROMOSOMES

Minute and double minute chromosomes have been defined as extrachromosomal, acentric, autonomously replicating elements [20]. They have been extensively studied in animal cell cultures where their appearance

may be related to gene amplification [21], chromosomal deletions [20] and chromatin diminution [22].

In higher plants, minute-like structures were first observed in the orchid by Nagl [23] in 1983. More recently, Deumling and Clermont [24] presented cytogenetical evidence for a massive appearance of very small (< 1 μm) Feulgen-positive chromatin particles in cultured cells of another monocot, *Scilla siberica*. These minute chromosomes often arranged in metaphase-like arrays during mitosis. Quite similar elements were then found in cell and tissue cultures of wheat [25]. This time, minutes and double minutes were observed as granular chromatin particles containing 0.17 to 5.54 pg of DNA. The DNA species of these bodies ranged in size from 2.56×10^5 to 1.05×10^7 base pairs (bp). A close relationship between formation of the minute chromosomes and DNA amplification and cell differentiation in embryogenic calli was pointed out [25]. It remains to be established whether minute chromosomes occur in plants growing under natural environmental conditions.

SMALL POLYDISPERSE CIRCULAR DNA

A review of Gaubatz [26] summarizes recent data on small polydisperse circular DNA of animal cells. Similar extrachromosomal circular DNA has been reported to occur in the cell nuclei of higher plants since many years. In particular, a series of circular exDNA species was observed by electron microscopy in calli induced from immature embryos of wheat [5]. No individual specimen of this DNA class was, however, described in detail.

More recently, Cuzzoni *et al.* [6] used PFGE to isolate exDNA of rice. They demonstrated that a circular extrachromosomal molecule of about 45 kb was produced in cultured rice cells as a result of amplification of a repeated sequence. A counterpart of the exDNA sequence was present in the chromosomal DNA of rice plants.

MINICHROMOSOME-LIKE STRUCTURES

In this context, a minichromosome means a gene-size, double-stranded, linear or circular DNA molecule containing a native coding sequence and its own origin of replication; a linear minichromosome should, in addition, have telomeric repeats at its termini. A typical example of such exDNA species was first identified by Blackburn & Gall [27] in the macronuclei of *Tetrahymena thermophila,* and has since been referred to as minichromosome [28]. Plasmids, on the other hand, can hardly be considered as minichromosomes since they carry genetic information that is alien to the host cells.

Although nuclear minichromosomes occur in some protozoa [29–31] and, less frequently, in fungi [32–34], there is a shortage of data on their occurrence in vertebrates and higher plants. My interest to plant minichromosomes emerged from studies on seed germination. Some time ago, we found that, while RNA and protein synthesis starts at the onset of germination, DNA replication is activated in germinating wheat embryos with a considerable delay [35]. Some incorporation of labelled DNA precursors could, however, also be observed at the early germination time. Unexpectedly, the early synthesized DNA product corresponded neither to nuclear chromosomal nor to organellar DNA [36]. After a series of inconclusive experiments, the possibility of autonomous replication of nuclear exDNA in germinating wheat embryos was pointed out [37]. Circumstantial evidence further suggested that nuclear exDNA does occur in wheat embryos and is rich in telomeric type sequences [10]. Hirt extractions followed by PCR amplification of sequences flanked by telomeric repeats then allowed us to isolate and clone a 637 bp exDNA sequence that was structurally similar to *Tetrahymena* minichromosomes [15]. Blot-hybridization experiments further indicated that much larger (2–20 kb) telomere-rich exDNA species also occur in the nuclei or resting wheat embryos [16].

As it is schematically shown in Fig. 1, the cloned exDNA sequence [15] contains 28 bp

inverted terminal repeats consisting of the same heptamer units that were earlier identified by Richards & Ausubel [38] in chromosomal telomeres of *Arabidopsis thaliana*. The presence of a yeast type ARS core element and RAP1 binding-like site, in addition to two in-phase ORFs and an intriguing 22 bp direct repeat, further makes the cloned sequence similar to a functional, albeit unusually small, genetic entity. The direct repeat contains a motif (CATG) that is common to prokaryotic iterons [39] and ABA-responsive elements of the wheat genome [40]. Current experiments (Szurmak & Buchowicz, this issue, p. 79) indicate that the putative plant minichromosome can replicate autonomously in isolated wheat nuclei.

EXTRACHROMOSOMAL COPIES OF TRANSPOSABLE ELEMENTS

The use of the Hirt method allowed Flavell & Ish-Horowicz [43] to isolate and characterize a defined specimen of nuclear exDNA from cultured *Drosophila* cells. This was represented by small (about 5 kb) circular DNA molecules hybridizing to the transposable element *copia*. A restriction map of the circles was virtually identical with that of a cloned *copia* element. Similar extrachromosomal copies of transposable elements, both circular and linear, have since been found in a wide variety of eukaryotic organisms, including vertebrates [44]. They are, however, rather rare in higher plants. There seem to

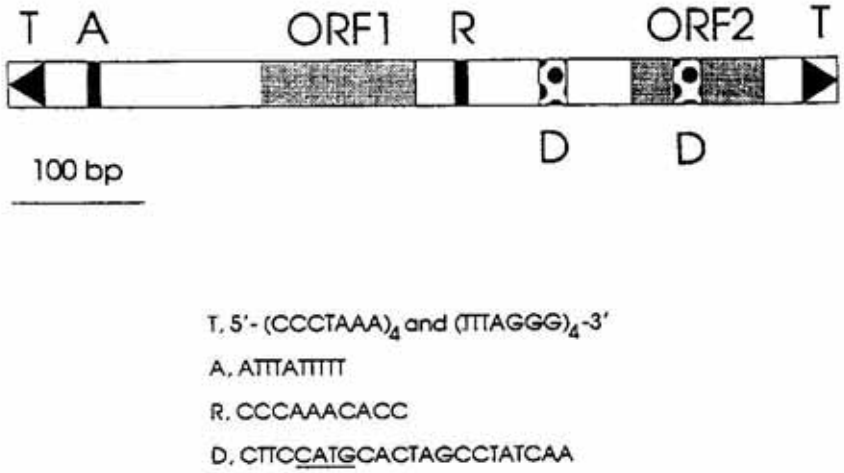

Figure 1. Structural organization of a wheat minichromosome.

Some sequence elements are given below the scheme: T, telomere; A, ARS core; R, RAP1-binding site; D, direct repeat with a motif (underlined) common to ABA-responsive elements and iterons. The complete sequence can be found under EMBL/GenBank accession number X73235 and in a recent report [15].

The origin of the wheat minichromosome is unknown. It should, however, be emphasized that essentially the same sequence (97% identity in a 510 bp overlap) was identified by Cheung *et al.* [41] in chromosomal DNA of wheat. It is known that in plants growing under natural environmental conditions, chromosomal DNA is always exposed to lesions that may result in its fragmentation [42]. Those of the fragmentation products which, fortuitously, will contain telomeric sequences, a replication origin and regulatory elements may be expected to replicate autonomously and, hence, to exist in plant nuclei as minichromosomes.

be only two well characterized examples of extrachromosomal copies of plant transposon sequences.

The first of these was found by Sundaresan & Freeling [45] in maize. The copy was circular, corresponded to an earlier reported sequence of the maize *Mu* transposon and occurred in all maize lines carrying a chromosomal form of the transposon. The second one also originated from a nonretroviral-like transposon, namely *Tat 1* of *A. thaliana* [11]. This time, however, the copy was linear and occurred in a few of the examined populations.

The biological significance of extrachromosomal forms of mobile elements is unknown.

According to the prevailing view [4, 44], they may represent either intermediates or by-products of DNA transposition. This interpretation does not, however, include all the possibilities. Some extrachromosomal copies of retrotransposons are capable of autonomous replication and may be used as cloning vectors [46]. Thus, they conceivably might exist independently of transposition events.

RADIATION-INDUCED CHROMOSOMAL BREAKAGE

Recently, Brock & Pryor [47] described the appearance of a nuclear minichromosome in maize as the consequence of gamma irradiation of pollen. The minichromosome comprised part of the short arm of chromosome 10 of maize and was cytogenetically unstable. Similarly derived minichromosomes were also observed in other plants, including wheat [48].

In vertebrates, such minichromosomes may be generated by both radiation-induced and spontaneous chromosomal breakages. Spontaneously formed linear fragments usually have telomeric sequences at their ends and represent a suitable substrate for chromosomal healing [49]. It seems justified to expect that similar events may also be quite frequent in plants. So far, healed broken chromosomes have been detected in a telotrisomic line of barley [50].

PERSPECTIVES

It is difficult to understand the reason for which plant cell nuclei may be endowed with small genetic entities. According to the present state of knowledge, standard chromosomes are sufficient to ensure all the functions that may be governed by genetic material in resting, growing, and differentiating cells. Nevertheless, minute chromosomes, small circular DNA species, linear minichromosome-like structures, and extrachromosomal copies of transposon sequences do appear in many plant species. Usually, they are hardly detectable but, under some stress conditions, become abundant enough to be investigated with standard cytogenetical methods. It may be expected that extensive use of PCR techniques will soon make it possible to define new examples of minichromosome type structures in the nuclei of higher plants.

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