A maize root tip system to study DNA replication programmes in somatic and endocycling nuclei during plant development

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Abstract

The progress of nuclear DNA replication is complex in both time and space, and may reflect several levels of chromatin structure and 3-dimensional organization within the nucleus. To understand the relationship between DNA replication and developmental programmes, it is important to examine replication and nuclear substructure in different developmental contexts including natural cell-cycle progressions in situ. Plant meristems offer an ideal opportunity to analyse such processes in the context of normal growth of an organism. Our current understanding of large-scale chromosomal DNA replication has been limited by the lack of appropriate tools to visualize DNA replication with high resolution at defined points within S phase. In this perspective, we discuss a promising new system that can be used to visualize DNA replication in isolated maize (Zea mays L.) root tip nuclei after in planta pulse labelling with the thymidine analogue, 5-ethyl-2'-deoxyuridine (EdU). Mixed populations of EdU-labelled nuclei are then separated by flow cytometry into sequential stages of S phase and examined directly using 3-dimensional deconvolution microscopy to characterize spatial patterns of plant DNA replication. Combining spatiotemporal analyses with studies of replication and epigenetic inheritance at the molecular level enables an integrated experimental approach to problems of mitotic inheritance and cellular differentiation.

Key words: DNA replication, EdU, endocycle, flow cytometry, high-resolution microscopy, maize, mitotic cell cycle, root development, S phase.

Introduction

Eukaryotic DNA replication is distributed in both time and space, with different regions of the genome replicating at different times in S phase and at different locations within the nucleus (reviewed by Bryant and Aves, 2011; Gilbert et al., 2010). In multicellular organisms, gene-dense euchromatic regions replicate early, and repetitive heterochromatic regions replicate later within the S phase of the cell cycle (Rhind and Gilbert, 2013). It has been reported in mammals and Xenopus that early replication occurs in the interior of the nucleus while late replication mostly occurs at the periphery (Pope and Gilbert, 2013). There is also evidence in mammals that clusters of replicons, known as replication domains, are regulated as units that can be visualized in cells pulse-labelled with nucleotide analogues (Gilbert et al., 2010).

Chromatin structure and replication programmes change during development and cell differentiation. Several examples of developmental regulation of replication timing have been described in mammals (Gilbert et al., 2010; Pope and Gilbert,
Less is known in plants, but there is evidence that replication time and origin use are subject to hormonal control (Ishida et al., 2010; Bryant and Aves, 2011). A significant amount of information has been gathered about the proteins and epigenetic modifications associated with plant DNA replication (Shultz et al., 2007; Costas et al., 2011a,b), and a replication timing profile has been produced for Arabidopsis chromosome 4 for cultured cells (Lee et al., 2010). However, our understanding of the larger-scale dynamics of chromosomal DNA replication is still very limited for plants.

Although some aspects of the DNA replication process are broadly conserved across eukaryotes (Bryant and Aves, 2011; DePamphilis and Bell, 2011), studying replication in plants presents unique opportunities. Unlike animals, plant meristems are typically indeterminate and continue to give rise to new organs after the initial embryonic stage, allowing studies of genome duplication and maintenance in organized tissues rather than in cells artificially maintained in culture (Costas et al., 2011b). In addition, many plant tissues undergo developmentally programmed endocycles, in which S phase is uncoupled from mitosis resulting in multiple rounds of chromosomal DNA replication and increased ploidy. We have only begun to understand potential differences in chromatin structure, replication programmes, and epigenetic inheritance between mitotic and endocycle S phases in plants (Hayashi et al., 2013).

Maize (Zea mays) has several features, including well-characterized genetics and a fully sequenced genome, that make it an excellent plant system for studying DNA replication. The relatively large and distinct chromosomes of maize are well suited for studies of cytogenetics and subnuclear structure (Birchler and Bass 2009). In addition, given its prominence as a crop species worldwide, understanding how maize replicates its genome may have important agricultural implications. This is especially true for important seed tissues such as the endosperm of cereal grain species, which undergo programmed endoreduplication and produce large amounts of valuable products such as seed protein, starch, and oils (Sabelli and Larkins, 2009)

The maize B73 genome is similar in size (2.3 Gb) to the human (3.2 Gb) and mouse (2.5 Gb) genomes and is comprised of about 85% transposable elements (Lander et al., 2001; Waterston et al., 2002; Schnable et al., 2009). Interestingly, the maize genome is c.17-times larger than the genome of Arabidopsis thaliana (Poethig, 2001), which has been used as a model for plant DNA replication studies (Van’t Hof et al., 1978; Jacob et al., 2010; Lee et al., 2010; Costas et al., 2011a,b). In spite of the large difference in genome size, it is estimated that the maize and Arabidopsis genomes contain 32 500 and 25 000 genes, respectively—a difference of only about 30%.

Comparison of DNA replication in maize and Arabidopsis will lead to a better understanding of chromatin dynamics in higher plants. As a first step towards such an understanding, we discuss in this perspective paper some of the advantages, challenges and opportunities associated with investigating the spatiotemporal aspects of DNA replication in maize. In particular, we highlight the utility of our recently developed maize root tip system using a combination of fluorescent labelling, flow cytometry, and high-resolution 3D microscopy to characterize spatiotemporal replication patterns in nuclei from naturally cycling cells.

### Isolating and staging S-phase nuclei for analysis

**EdU as a tool for plant research**

In recent years, 5-ethyl-2′-deoxyuridine (EdU), a nucleoside analogue of thymidine, has been used increasingly to detect cells actively replicating their DNA. This technology takes advantage of a copper(I)-catalysed azide-alkyne cyclodaddition (often referred to as ‘click chemistry’) first described by Rostovtsev et al. (2002) and Tornøe et al. (2002). Click chemistry can be used to detect EdU incorporated into newly replicated DNA by reaction of the terminal alkynyl on EdU with an azide attached to a fluorescent dye such as Alexafluor 488 (Salic and Mitchison, 2008). Darzynkiewicz et al. (2011) reviewed the benefits of using EdU for detecting DNA synthesis compared to earlier techniques. In particular, the elimination of the acid or heat denaturation treatment needed for antibody detection of 5-bromo-2′-deoxyuridine, a commonly used thymidine analogue, greatly enhances maintenance of DNA and protein structural integrity (Darzynkiewicz et al., 2011).

Kotogány et al. (2010) were the first to look in depth at the feasibility of using EdU in plant suspension cultures and roots for flow cytometry and microscopy. Since then, a number of research groups have used EdU for plant cell-cycle studies, establishing its utility in a variety of plant tissues (Table 1). Most of these studies have addressed three broad areas: (1) the proliferation patterns and fraction of cells entering S phase in various plant tissues (Ayaydin et al., 2011; Kelliher and Walbot, 2011; Ilina et al., 2012; Kuznetsova and Sheval, 2013); (2) changes in DNA replication in response to various mutations, transgenes or chemical treatments (Vanzraelen et al., 2009; Zhou et al., 2010; Schubert et al., 2011; Tresch et al., 2011; Zhu et al., 2011; Xu et al., 2012, 2013; Bazin et al., 2013; Xiong et al., 2013); and (3) the characterization of replication patterns at a chromosome or a chromatin level (Heckmann et al., 2011; Greer et al., 2012; Xu et al., 2012; Hayashi et al., 2013; She et al., 2013). More recently, EdU labelling over discrete time intervals has been used in combination with confocal microscopy (Hayashi et al., 2013) or flow cytometry (E. Wear, L. Hanley-Bowdoin, and W. Thompson, unpublished data) to determine the average length of S phase in plant tissues.

EdU-labelling techniques have been combined with immunocytology and fluorescent in situ hybridization (FISH) to explore patterns of DNA replication, replication-associated epigenetic marks, and chromatin packaging in plant nuclei. Multiple groups have studied the progression of premitotic replication in germ line cells using EdU in conjunction with DNA probes or antibodies that target centromeric or...
telomeric DNA, histone proteins, or developmentally related proteins (Greer et al., 2012; Higgins, 2013; She et al., 2013). In particular, Higgins presented detailed methods for EdU labelling and isolating barley inflorescences and subsequent immunolocalization and FISH preparations. Others have investigated the colocalization of EdU with DNA probes or histone marks and the overall chromosomal organization following mitotic replication on holocentric chromosomes (Heckmann et al., 2011) or supernumerary B chromosomes (Klemme et al., 2013). There has also been interest in identifying ‘early’ and ‘late’ replication patterns and associating them with euchromatic and heterochromatic regions of DNA (Heckmann et al., 2011; Greer et al., 2012; Klemme et al., 2013).

Most of the studies in Table 1 used microscopy to visually stage plant nuclei in S phase, which only distinguishes large differences in EdU labelling and does not provide measures of DNA content. As a consequence, visual staging of EdU-labelled nuclei cannot resolve them into different parts of S phase. The few studies in Table 1 that combined EdU labelling with flow cytometry were analytical in nature and did not use flow sorting to enrich for discrete S-phase populations. Hence, more precise methods for staging plant nuclei within S phase are needed for fine-scale observations and measurements of DNA replication patterns both spatially and in relation to chromatin features. To address this need, we developed a method whereby EdU-labelled nuclei are isolated using a flow cytometer to separate S-phase nuclei into sequential stages for 3D microscopic analysis. We combined this strategy with analyses of nuclei from cells at different positions in the developmental gradient of the growing maize root tip to establish a new experimental system for studying plant DNA replication. Approaches to visualize and analyse DNA replication patterns in time and space are emphasized here. We are currently using these approaches to compare DNA replication programmes in mitotic and endocycling plant cells.

Flow sorting for temporal resolution within S phase
Flow cytometers were originally developed for use with mammalian blood cells but evolved to include a wide range of applications in the plant sciences (reviewed by Galbraith, 2010). The introduction of the chopping procedure to release intact nuclei from plant tissue homogenates (Galbraith et al., 1983) made it possible to run a single particle suspension through a flow cytometer and sort nuclei with various fluorescent tags. This isolation method has been adapted to fixed tissues through the use of gentle blending (Lee et al., 2010), after which nuclei can be subjected to the click reaction to couple a fluorescent Alexa-488 azide to EdU incorporated into nascent DNA.

Many DNA replication studies have used a flow cytometer to isolate different fractions of S phase based on DNA content as measured by 4′,6-diamidino-2-phenylindole (DAPI)
or propidium iodide staining (Chen et al., 1995; Woodfine et al., 2004; Lee et al., 2010). While this is a useful technique, it is not suitable for some applications because it does not separate replicating from non-replicating cells or nuclei (Fig. 1B and D). By adding a second parameter to the flow sorting (e.g. EdU incorporation measured after coupling to an Alexa-488 azide), we can readily distinguish nuclei in S phase from other cell-cycle phases. EdU-labelled nuclei, even those in very early or very late S phase, are resolved by flow cytometry as an arc distinct from unlabelled G1 and G2 nuclei in a bivariate plot of DAPI versus Alexa-488 (Fig. 1A and C). Gating of the arc can be used to further separate S phase into early, middle, and late stages (Fig. 1A, rectangles labelled E, M, and L). This approach for staging nuclei allows us to start with an asynchronous population of nuclei and avoid artefacts sometimes associated with synchronization procedures such as sucrose starvation or incubation with nucleotide synthesis inhibitors (Cooper, 2003).

Some technical considerations must be addressed when combining flow sorting and high-resolution microscopy. First, the fixation strategy and buffers must be carefully selected to balance the need to ensure a single particle suspension for the flow cytometer with the need to preserve chromatin structure and nuclear architecture for downstream analysis. Second, depending on the experimental goals, the length of the EdU pulse can be important. For example, a short pulse will label DNA proximal to replication forks, while a long pulse will label more of the genome and include sequences

![Fig. 1. Labelling actively replicating DNA in maize root tips. (A–D) Root tips from 3-d-old seedlings were pulse-labelled with EdU for 20 min, followed by fixation and nuclei isolation and the nuclei were analysed by flow cytometry using a BD Influx instrument equipped with 355 nm (UV) and 488 nm (blue) lasers. Bivariate plots of DNA content (based on DAPI fluorescence in the 460 ± 50 nm detection range) and EdU incorporation (based on Alexa fluor (AF) 488 fluorescence in the 530 ± 40 nm detection range) are shown in A and C. The corresponding univariate histograms of DNA content are shown in B and D, respectively, from 0–1 mm root segments (A, B) and 1–3 mm segments (C, D); the gates corresponding to early (E), middle (M), and late (L) subpopulations of S phase are indicated as rectangles for the mitotic cell cycle (A) and the endocycle (C). (E) Bivariate flow cytometry profiles of mitotic and endocycling nuclei from consecutive 1-mm root segments up to 5 mm labelled with EdU for 1 h: the identical black boxes in each profile indicate the gates used for analysis of the percentage of nuclei with 2C, 4C, and 8C DNA content shown in the root schematic below. (F) Merged confocal images of root tip cross-sections taken at the points indicated by the black arrowheads, showing DAPI-stained DNA (red), and EdU label in newly replicated DNA (green). Bar, 100 μm.](https://academic.oup.com/jxb/article-abstract/65/10/2747/574174)
that are distal as well as proximal to active forks. In many cases, knowing the duration of S phase in the material under study will allow a more informed decision on pulse length.

Programmed endocycling in plants and the developmental biology of roots

Endocycling: a widespread phenomenon in plants

A shift from a mitotic cell cycle to endoreduplication or an endocyte, in which cells replicate their DNA without subsequent cell divisions, has been documented during development for roots, shoots, leaves, and endosperm of diverse plant species (Joubes and Chevalier, 2000; Lee et al., 2009; Hayashi et al., 2013). The formation of many specialized plant tissues and organs is known to require endocycling. Examples include trichomes, leaf epidermal cells, maize endosperm, and tomato fruit among many others (Joubes and Chevalier, 2000). Recently, Breuer et al. (2010) reviewed some of the developmental controls of the endocyte in Arabidopsis leaves and roots, emphasizing the importance of the endocyte for normal organ growth. They and others have also noted that the transition from the mitotic cell cycle to the endocyte is often coupled with a shift from cell proliferation to cell expansion and differentiation (Breuer et al., 2010; Hayashi et al., 2013).

Opportunity afforded by root tip organization

Developmentally distinct regions along the maize root have been identified morphologically (Baluska et al., 1990; Baluska and Mancuso, 2013). The tip contains dividing cells, followed by a region termed the ‘transition zone’ or ‘post-mitotic isodiametric zone’, located approximately 1–2.5 mm from the root apex. In this region, cell division mostly halts and cells begin to increase in length and width before reaching a zone of rapid elongation. Endopolyploidy has been observed for maize roots (Biradar et al., 1993; Ogawa et al., 2010). However, there are only a few reports that integrate ploidy information with plant root developmental programmes (Hayashi et al., 2013). Using flow cytometry, we found that the first 10 mm of the maize root tip contain 2C, 4C, and 8C cells, but very few cells of higher ploidy (data not shown). To increase our resolution, we dissected a series of 1-mm segments from the apex up to 5 mm of EdU-labelled maize roots and examined the distribution of isolated nuclei in the mitotic cell cycle and the endocyte by 2-colour flow cytometry, as summarized in Fig. 1. The flow cytometry data were first filtered to remove cellular debris based on plots of side scatter (90° angle light scatter) and DAPI fluorescence. Identical gates were then set to analyse the percentage of 2C, 4C, and 8C nuclei in each root segment (Fig. 1E). This analysis revealed that the first 1 mm of the root consists primarily of cells in the mitotic cycle with 37% 2C, 52% 4C, and 4% 8C nuclei (n = 39 351). The endocyte is increasingly prominent beyond 1 mm, with the 2–3 mm segment containing 9% 2C, 44% 4C, and 38% 8C nuclei (n = 27 238). Both types of replication have essentially ceased by 4–5 mm from the tip. Strikingly, most maize root cells exit the cell cycle with either 4C (43%) or 8C (36%) DNA content, with only a small fraction (10%) having 2C content. We also observed the distribution of EdU-labelled cells across different cell types by imaging cross-sections at intervals along the root (Fig. 1F).

Our experiments established the feasibility of combining careful dissection and flow sorting to target either the mitotic cycle or the endocyte for a molecular analysis of DNA replication in an organized plant tissue. There are several advantages of studying DNA replication patterns using root tips instead of cell cultures. By focusing on zones with active division, we can obtain preparations highly enriched in nuclei undergoing replication. Fixed root tips also provide relatively clean nuclei preparations, ideal for flow sorting. In addition, the spatial separation of mitotic and endocycling cells, in combination with flow sorting, affords an opportunity to compare DNA replication programmes before and after a normal developmental transition between the two cell-cycle types.

Collection and analysis of 3D fluorescent images of nuclei

Flow sorting considerations for 3D imaging using multiple wavelengths

Formaldehyde is an excellent fixative for chromatin and has been widely used for decades to preserve nuclear architecture and chromatin structure for microscopy (Belmont et al., 1989; Sweldow et al., 1993). We have used deconvolution microscopy to obtain high-resolution fluorescent 3D images of fixed nuclei using optical sectioning. In the past, we often employed a 3D acrylamide FISH technique to preserve nuclei within cellular or tissue contexts (Bass et al., 1997, 2000a,b, 2003). However, we found that nuclei fixed in situ and then purified from the maize root tip system retained their generally spherical shape without acrylamide embedding, which considerably simplifies the mounting and imaging procedures. We also found that nuclei fixation with formaldehyde can be carried out in the presence of various buffers, including phosphate-buffered saline or Buffer A. Buffer A was specifically developed to preserve the native chromatin state for cytological analysis of nuclear architecture and chromatin structure (Sedat and Manuelidis, 1978; Belmont et al., 1989). The formaldehyde plus Buffer A chromatin fixation has been adapted for use in plants and is also compatible with additional staining techniques such FISH or immunocytochemistry (Howe et al. 2013). Phosphate-buffered saline has the advantage of being more compatible with flow cytometry and, as such, is our buffer of choice for experiments that combine sorting and microscopy. In addition, nuclei separated by 2-colour flow cytometry are already fixed, labelled with Alexa-488, stained with DAPI and, thus, ready for immediate 2-colour imaging. Fixed and sorted nuclei are stable in various buffers, and can be stored for later use at 4°C for a few months in air-tight, light-free tubes (data not shown).
Other useful features of wide-field epifluorescence deconvolution microscopy with a sensitive CCD camera include the ability to collect two additional wavelengths using conventional red (Cy3, Rhodamine, Texas Red, ROX, Alexa-546) or far-red (Cy5, Alexa-694) fluorophores and obtain intensity data over a wide linear range (1–4096 counts/pixel before deconvolution) for every wavelength. These capacities enhance comparison of EdU-labelling patterns with those of other chromatin or nuclear feature staining methods such as in situ hybridization or immunocytochemistry.

Display of 3D image data on 2D media

Collection of multicolour data adds extra dimensions of information. For each wavelength, a series of optical sections are photographed using a CCD camera at defined Z-axis step sizes, providing 3D digital data. The different wavelengths represent a fourth dimension. For 3D imaging of fixed samples, separate images are collected for each wavelength at each focal plane, before the stage is moved to the next optical section. The interleaved image data are sorted by wavelength and subjected to computational deconvolution to correct for chromatic aberrations (Chen et al., 1995). In this way, photon counts are conserved and redistributed within the 3D space of a cube of images, accomplishing a level of deblurring and brightening that distributes pixel signal intensities over a wide dynamic range. Linear scaling up to 32 000 counts can be achieved without the use of photomultiplier tubes or artificial gain. As a result, the deconvolved images contain considerable substructure (for example, within the heterochromatic knobs, as shown in Fig. 2). This substructure is visible even for regions of the 3D dataset that may have narrow portions (10%) of the intensity range in any given experiment.

In Fig. 2, a representative 2-colour (DAPI and Alexa-488) 3D image of a single nucleus from a somatic cell in late S phase illustrates several ways to visualize the information contained in a 3D dataset using 2D media, such as computer screens or the printed page. The 3D dataset is comprised of optical sections of pixels with X–Y dimensions of 0.07 μm Z-step sizes. These dimensions provide over 0.25, or 0.3 μm Z-step sizes. These dimensions provide over

Spatial and fluorescence signal measurements from image datasets

Another useful feature of 3D digital data collection is that the datasets can be used as raw material for experiments
New approaches to studying plant DNA replication allow for measurements and quantitative hypothesis testing that take advantage of the spatial resolution and dynamic range of signal intensity data. In the case of DNA replication time during S phase, it is often of interest to compare the replicated DNA captured with EdU pulse labelling to the total genomic DNA. Here, two types of

Fig. 2. Collection and display of 3D image data from a representative somatic nucleus pulse-labelled during late S phase. Root tips were pulse-labelled with EdU and fixed with formaldehyde. Nuclei isolated from the labelled root tips were stained for EdU incorporation using a Click-It EdU Alexa-488 imaging kit, separated by flow cytometry, and either imaged directly (A–C, E) or subjected to 3D acrylamide FISH (D) to visualize the location of knob and centromere sequences; images in A–C are all from a single 3D dataset comprised of two-colour optical sections that imaged a single nucleus from late S phase. (A) Digital data are recorded in voxels (3D pixels with real-space X, Y, and Z dimensions). (B) Whole-nucleus through-focus projections generated by two common methods are compared: projections using the average method are shown in the left column, while those in the right column were made with the maximum intensity method. (C) Sequential projections illustrate the entire data set as a series of partial projections with each projection representing one-fifth of the Z-sections after 3D cropping to the edges of the nucleus: this approach optimizes the information–space relationship for showing 3D data in 2D formats; double arrows indicate loci (also depicted in B and C) that may appear close together in projection, but spatially separate along the Z-axis. (D) Suitability of DAPI-stained and EdU-Alexa-488-labelled nuclei for 3D FISH using the method described by Howe et al. (2013). The knob and centromere probes are fluorescent oligonucleotide probes (knob probe ‘NUBI-R’ is 5′-Rhodamine-AACATATGTGGGGTGAGGTGTATG-3′ and centromere probe ‘MCCY’ is 5′-Cy5-GAAAAACGAAGAAATGGTTCTGGTG-3′, from Koumbaris and Bass, 2003). k indicates the locations of two late-replicating knobs. Bars, 3 μm.
3D measurements prove useful. One type measures the correlation of signals (DAPI versus Alexa-488) across the population of volumetric pixels, while the other extracts real-space measurements—such as volumes and centres of space and ‘mass’—as summarized in Fig. 3. Here we illustrate the use of such techniques to quantify the differences between early and middle S phase. For instance, the early S-phase nucleus (Fig. 3A left) appears to have more separation between the DAPI and Alexa-488 signals than does the nucleus from middle S phase (Fig. 3A right). This difference was quantified using Pearson’s correlation coefficient (Fig. 3 legend) and the visual impression was verified numerically with a statistical R-value.

We also measured nuclear volumes and the non-uniform distribution of signals using image segmentation (Fig. 2B legend). The nuclear volume was segmented using computer-assisted or manual edge tracings and the resulting polygons—one per optical section—were connected to produce a solid surface object with spatial coordinates linked to that dataset. The solid object serves to delineate the inside and outside of the nucleus, providing information on nuclear volume, and the degree to which the DAPI and Alexa-488 signals are codistributed across the entire 3D dataset.

Together, the two approaches illustrated here—image projections for visual data inspection and collection of signal intensity and real-space measurements for quantitative analysis—are useful starting points for 3D cytology and particularly valuable for DNA replication studies. As large digital datasets become more common, sharing and reanalysing archived cytological data is expected to become more commonplace (Allan et al., 2012), and approaches similar to those illustrated here are expected to come into widespread use for 3D datasets.

**New insights and future prospects**

Using EdU and click chemistry to fluorescently label nascent DNA is an important technical advance in the general field of DNA replication. Although the EdU-labelling technology has been increasingly applied to plant systems (Table 1), its full potential is only now being realized with its adaptation to other technologies like flow cytometry. In this report, we discuss the experimental advantages for combined application of EdU and flow cytometry to label and fractionate replicating nuclei from maize root tips for DNA replication studies. Unlike cell-culture-based

![Fig. 2B](image74x341to314x770)
systems typically used to study eukaryotic DNA replication, maize root tips provide a source of actively dividing nuclei undergoing a natural developmental programme in the context of an organized tissue. In particular, the maize root tip system can be used to examine DNA replication during the developmental transition from a mitotic cell cycle to an endocycle—a common transition during plant cell differentiation.

As described here, the maize root tip system can be combined readily with high-resolution microscopy to compare the spatial patterns of DNA replication during S phase in mitotic and endocycling cells. The maize root tip system can also be adapted to genomic protocols like DNA-Ip, ChIP, and Hi-C to study replication programmes, chromatin landscapes, and long-distance interactions in EdU-labelled nuclei. These types of studies will help define the regions of the maize genome that replicate during early, middle, or late S phase and any associated chromatin features. Comparison of the temporal, spatial, and epigenetic properties of replicating DNA in maize root tips will provide insight into general characteristics of a plant nucleus in S phase and those that change when a cell transitions from a proliferating state to an endocycling state during differentiation.

Knowledge of the mechanisms that mediate and control DNA replication is essential for understanding plant growth and development. We now have the tools to explore these important processes in plants and to use this information to address important agronomic questions such as reducing phenotypic variation associated with passage through S phase or increasing grain yield by modulating DNA replication during endosperm development.

Supplementary material

Supplementary data are available at JXB online.

Supplementary Table S1. Movies from a 4-wavelength 3D deconvolution image dataset of a nucleus in late S phase showing EdU-labelled DNA synthesis along with knob and centromere repeat FISH signals.

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