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Abundance and distribution of RNA polymerase II in Arabidopsis interphase nuclei

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Abstract

RNA polymerase II (RNAPII) is responsible for the transcription of most eukaryotic protein-coding genes. Analysing the topological distribution and quantification of RNAPII can contribute to understanding its function in interphase nuclei. Previously it was shown that RNAPII molecules in plant nuclei form reticulate structures within euchromatin of differentiated Arabidopsis thaliana nuclei rather than being organized in distinct ‘transcription factories’ as observed in mammalian nuclei. Immunosignal intensity measurements based on specific antibody labelling in maximum intensity projections of image stacks acquired by structured illumination microscopy (SIM) suggested a relative proportional increase of RNAPII in endopolyploid plant nuclei. Here, photoactivated localization microscopy (PALM) was applied to determine the absolute number and distribution of active and inactive RNAPII molecules in differentiated A. thaliana nuclei. The proportional increase of RNAPII during endopolyploidization is confirmed, but it is also shown that PALM measurements are more reliable than those based on SIM in terms of quantification. The single molecule localization results show that, although RNAPII molecules are globally dispersed within plant euchromatin, they also aggregate within smaller distances as described for mammalian transcription factories.

Key words: Arabidopsis thaliana, endopolyploidy, photoactivated localization microscopy, RNA polymerase II, structured illumination microscopy, transcription factory.

Introduction

Most eukaryotic genes are transcribed by RNA polymerase II (RNAPII) (Kornberg, 1999; Sims et al., 2004). Post-translational modifications of the C-terminal domain (CTD) of the largest subunit of RNAPII are important for controlling transcription, mRNA processing, chromatin remodelling, and RNA export (Hajheidari et al., 2013). The quantity of active RNAPII in nuclei reflects the degree of transcription. The co-localization of transcripts and RNAPII has been proven by electron (Iborra et al., 1996) and light microscopy (Grande et al., 1997; Osborne et al., 2004). Depending on its position on a gene and the stage of transcription, RNAPII is differentially phosphorylated. Inactive RNAPII is mainly unphosphorylated. Its activation requires the phosphorylation at Ser2 and Ser5 of the heptapeptide YSPTSPS present as tandem repeats in the RNAPII CTD (Hirose and Okkuma, 2007; Hajheidari et al., 2013). Antibodies specific for the phosphorylation state of the peptide allow the discrimination between active and inactive RNAPII (Bourdon et al., 2012). To initiate transcription and for the binding onto promoters of genes, phosphorylation at Ser5 is necessary (Hengartner et al., 1998; Komarnitsky et al., 2000). For the elongation step of transcription, phosphorylation at Ser2 is required (Nie et al., 2004). At the end of transcription, the Ser5 phosphorylation is removed, whereas RNAPIISer2ph accumulates at the 3’ end of the genes (Egloff and Murphy, 2008).
In mammals, RNAPII is thought to be organized in distinct so-called ‘transcription factories’ (Jackson et al., 1993, 1998; Chakalova and Fraser, 2010; Ferrai et al., 2010; Rieder et al., 2012; Papantonis and Cook, 2013). In human and murine cells, the size of transcription factories ranges between ~40 nm and 200 nm in diameter and increases with higher transcriptional activity (Iborra et al., 1996; Eskiw et al., 2008; Eskiw and Fraser, 2011). The number of transcription factories in a nucleus seems to be cell type and species specific.

In mouse nuclei of different cell types, ~100–1500 transcription factories were found, whereas those in mouse and human erythroblasts amounted to ~100–550 and ~1500, respectively (Osborne et al., 2004; Brown et al., 2008; Eskiw and Fraser, 2011).

These numbers may also vary depending on the imaging technique applied. In HeLa cells (a human tumour cell line) ~300–500 factories were identified by widefield fluorescence microscopy (Jackson et al., 1993), ~2100 by combining electron and confocal microscopy (Iborra et al., 1996), ~2400 by electron microscopy alone (Jackson et al., 1998), and ~850–3900 by deconvolution microscopy (Fay et al., 1997). Pombo et al. (1999) found ~10 000 RNAPIII foci by cryo-sectioning. In addition, the methodologies detecting different molecules related to transcription (e.g. mRNA, RNA polymerase, and splicing factors) could also induce the variability in the number of foci detected.

In a single transcription factory, the number of RNAPII molecules ranges from four to 30 (Iborra et al., 1996; Jackson et al., 1998; Martin and Pombo, 2003). These numbers convert to a minimum of ~400 (100 × 4) and a maximum of ~117 000 (3900 × 30) RNAPII molecules per nucleus, based on the estimation of 100–3900 factories per nucleus. Obviously, this high variability was not only caused by the different cell types analysed, but rather by the different imaging methods applied.

The recently developed super-resolution microscopy techniques such as photoactivated localization microscopy (PALM) and spectral position determination microscopy (SPDM) allow localization of single molecules at high precision beyond the diffraction limit of light allow a more reliable counting of molecules in cells (Lando et al., 2012; Deschout et al., 2014). SPDM allowed the determination of the number of P-glycoprotein efflux transporter molecules present per cell at the blood–brain barrier and showed that they cluster (Huber et al., 2012). Moreover, by two-colour PALM, quantitative information on the tumour necrosis factor receptor 1 cluster sizes and copy numbers were obtained in HeLa cells (Fricke et al., 2014).

Super-resolution microscopy also enables assessment of the third dimension in biological specimens (Hajj et al., 2014). 3D-PALM was used to measure the spatial distribution of H2B nucleosomes to determine the degree of chromatin condensation in human osteosarcoma cells (Recamier et al., 2014), and reflected light-sheet super-resolution microscopy was applied to quantify in 3D the absolute number of RNAPII molecules in the same cells (Zhao et al., 2014).

The combination of multicolour structured illumination microscopy (SIM) and localization microscopy was applied to map in three dimensions Bld12p/CrSAS-6 molecules in basal bodies of Chlamydomonas (Rossberger et al., 2013; Hamel et al., 2014).

In Arabidopsis, SIM has already been used successfully to identify fluorescent structures in various tissues (Bell et al., 2013; Komis et al., 2014) and sorted nuclei (Schubert et al., 2013; Schubert, 2014).

Here for the first time the numbers of RNAPII molecules in differentiated endopolyploid plant nuclei were determined by applying 3D-PALM. It is believed that this approach is well suited to approximate absolute numbers better than other technologies. In single- and two-colour experiments, it is shown that RNAPII molecules are dispersed evenly within euchromatin, but that they may also form clusters. In addition, it is confirmed that transcription increases with a higher degree of endopolyploidy.

Materials and methods

Preparation of nuclei and immunolocalization

Differentiated rosette leaves from 4-week-old Arabidopsis thaliana (L.) Heynh. (Columbia) plants grown under short-day conditions (8 h light/16 h darkness) were fixed for 20 min under vacuum in 4% formaldehyde in TRIS buffer (pH 7.5) and homogenized in TRIS buffer. Suspended nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) (1 μg ml⁻¹) and flow-sorted according to their ploidy level using a FACS Aria flow cytometer (BD Bioscience) onto 22 × 22 mm high precision coverslips (Marienfeld, Germany) in a drop of buffer containing 100 mM TRIS, 50 mM KCl, 2 mM MgCl₂, 0.05% Tween, 5% sucrose, then air-dried and used for immunolabelling.

For co-localization and quantification of active and inactive modifications of RNAPII, immunostaining was performed according to Jasencakova et al. (2000).

The non-phosphorylated (inactive) enzyme was detected with mouse monoclonal antibody (1:300; Abcam, ab817) and goat anti-mouse Alexa 488 (1:200; Invitrogen) or goat anti-mouse-Cy5 (1:300; Jackson ImmunoResearch).

RNAPIISer5ph (active; phosphorylated at Ser5) was detected with rabbit polyclonal antibody (1:200; Active Motif, 39233) and goat anti-rabbit Alexa488 (1:200; Jackson ImmunoResearch), and RNAPIISer2ph (active; phosphorylated at Ser2) with rat monoclonal antibody (1:200; Millipore, 04-1571) and goat anti-rat Alexa488 (1:200; Jackson ImmunoResearch).

Structured illumination microscopy (SIM)

To analyse the substructural organization of RNAPII molecules beyond the classical Abbe-Rayleigh limit of ~250 nm, SIM was applied that yields a 2-fold improvement in all spatial directions. Coverslips bearing the labelled nuclei were placed into Chamilide™ magnetic chambers (Live Cell Instrument, South Korea) and submerged in phosphate-buffered saline (PBS; pH 7.5) supplemented with 1% β-mercaptoethanol prior to SIM imaging on a Zeiss ELYRA PS.1 microscope (Carl Zeiss Microscopy, Germany) equipped with a Plan-Apochromat 63×1.4 objective. Optimal grid sizes for each wavelength were chosen according to the recommendations of the manufacturer. For 3D-SIM, stacks with a step size of 110 nm were acquired sequentially for each fluorophore starting with the highest wavelength dye. The centre of the stack was chosen to coincide with the main plane along the axis of the ellipsoidal nuclei to allow the alignment of SIM and PALM images. The correction of chromatic aberrations was performed with the ZEN Channel alignment tool using a template obtained from imaging TetraSpeck fluorescent
microspheres (200 nm in diameter; InVitroGen) and affine correction. Thus, the corrections achieved a precision of <100 nm.

**Photoactivated localization microscopy (Palm)**

The same set-up was subsequently used to perform 3D-PALM with the PRLIM (phase ramp localization imaging microscopy) implementation (Baddley et al., 2011). In general, the EM-CCD gain was set to 200 V. To avoid z-drift, the Definite Focus of the instrument was used, which kept the z-position within 30 nm. The focal position was chosen to coincide with the central slice of the SIM stack which corresponded to the main plane along the long axis of the nuclei. The z capture range was ~2 μm, which allowed the whole z distance of the nuclei to be covered for counting. All dye molecules were transferred into their dark state by using high laser power (~10 kW cm⁻²) of the imaging laser followed by 3D-PALM to record the number and localization of single blinking molecules at a lateral resolution of ~20 nm and an axial resolution of ~80 nm. PALM two-colour experiments were performed first for the long wavelength dye (Cy5) followed by the short wavelength dye (Alexa488). The PALM experiments continued for one dye until the blinking molecules observed were negligible, which needed ~30 000 frames at an integration time of 20 ms for PALM acquisition. This allowed the whole nuclei to be covered for counting. For peak finding, the peak localization precision was determined according to the Thompson function using a theoretical point spread function (PSF), and the localization precision of 2D-PALM data, the identified peaks were fitted to a 2D Gaussian function to account for overlapping signals. For peak finding, the peak detection method of the ZEN software was applied. A multi-emitter model was used that proved to be the most efficient in the experiments conducted here as it can detect multiple emitters.

**Processing and analysis of PALM data**

To generate PALM images (vector maps), the PALM processing function of the ZEN software was applied. A multi-emitter model was used to account for overlapping signals. For peak finding, the peak mask size was set to 9 pixels, and the noise filter to 6. For localization of 2D-PALM data, the identified peaks were fitted to a 2D Gaussian function using a theoretical point spread function (PSF), and the localization precision was determined according to the Thompson formula for 2D-PALM (Thompson et al., 2002). For 3D data, the identified peaks were fitted to an experimentally acquired PSF and the precision determined by simulations. The created vector map of localizations was then drifted corrected using the model-based approach with a precision of <20 nm in the x, y, and z direction. For 3D data, the identified peaks were fitted to an experimentally acquired PSF and the precision determined by simulations. The created vector map of localizations was then drifted corrected using the model-based approach with a precision of <20 nm in the x, y, and z direction. The identified peaks were fitted to a 2D Gaussian function using a theoretical point spread function (PSF), and the localization precision was determined according to the Thompson formula for 2D-PALM. The same set-up was subsequently used to perform 3D-PALM with the PRLIM (phase ramp localization imaging microscopy) implementation (Baddley et al., 2011). In general, the EM-CCD gain was set to 200 V. To avoid z-drift, the Definite Focus of the instrument was used, which kept the z-position within 30 nm. The focal position was chosen to coincide with the central slice of the SIM stack which corresponded to the main plane along the long axis of the nuclei. The z capture range was ~2 μm, which allowed the whole z distance of the nuclei to be covered for counting. All dye molecules were transferred into their dark state by using high laser power (~10 kW cm⁻²) of the imaging laser followed by 3D-PALM to record the number and localization of single blinking molecules at a lateral resolution of ~20 nm and an axial resolution of ~80 nm. PALM two-colour experiments were performed first for the long wavelength dye (Cy5) followed by the short wavelength dye (Alexa488). The PALM experiments continued for one dye until the blinking molecules observed were negligible, which needed ~30 000 frames at an integration time of 20 ms for PALM acquisition. This allowed the whole nuclei to be covered for counting. For peak finding, the peak detection method of the ZEN software was applied. A multi-emitter model was used that proved to be the most efficient in the experiments conducted here as it can detect multiple emitters.

**Correlation between SIM and PALM images**

Channels of the SIM and PALM images were subsequently aligned with the ZEN software (Carl Zeiss Microscopy, Germany) inbuilt Channel Alignment tool using structural features common in both channels. SIM and PALM images were acquired on the same microscope with the same objective, hence magnifications are identical. Only the pixels size (lateral 80 nm for SIM, 20 nm for PALM) and sectioning (110 nm for SIM, 40 nm for PALM) were different. Prior to alignment, the PALM images were rendered to a precision corresponding to the resolution of SIM; that is, 120 nm laterally and 360 nm axially. In addition, the pixel size and slice thickness of the PALM image were set to the pixel size and slice thickness of the SIM image. After channel alignment, manual correction with the ZEN channel shift tool was employed. In particular, the nuclear envelope and the nucleolus were excellent landmarks for matching the images as they could be easily identified since the nucleolus and the region beyond the nucleus were mainly devoid of staining. Such an alignment approach was within the precision of 1 voxel (corresponding to 80 nm laterally and 360 nm axial). After alignment, the localization precision of PALM was changed back to Gaussian and the pixel size and slices were reduced to the initial values.

**Results**

By applying SIM, it has been proven that the relative amounts of RNAPII enzymes in differentiated 2C–32C leaf nuclei of *A. thaliana* proportionally increase with increasing endopolyploidy (Schubert, 2014). To determine the number of RNAPII molecules per nucleus to a better extent and to check whether transcription factories may exist in plants, nuclei were labelled with specific antibodies against active and inactive RNAPII modifications and 3D-PALM was applied in combination with SIM.

After acquiring SIM images to determine the distribution of the RNAPII enzymes at lateral and axial resolutions of 120 nm and 360 nm, respectively, reticulate structures for the active and inactive RNAPII variants, which were not detectable by classical wide-field microscopy, became visible (Fig. 1A). Then, most of the molecules were transferred into their dark state by high laser power (~10 kW cm⁻²) followed by 3D-PALM to record the number and localization of single blinking molecules at a lateral resolution of ~20 nm and an axial resolution of ~80 nm (Fig. 1B, C). When molecules were rendered to the obtained localization precision, both active and inactive RNAPII variants distributed uniformly throughout the nucleus, with the exception of the nucleolus that was devoid of RNAPII (Fig. 1B; Supplementary Fig. S1 available at *JXB* online). The combination of SIM with
PALM and the simultaneous labelling of different RNAPII variants with the fluorophores Alexa488 and Cy5 in the same nucleus allowed single molecules to be localized within the reticulate structures (Fig. 1C). No co-localization of active and inactive RNAPII appeared, suggesting that both variants occupy distinct areas within euchromatin. This finding...
is quite interesting because theoretically it could be expected that partially phosphorylated RNAPII molecules would also be identified by both antibody variants.

Due to the presence of more molecules, the active RNAPII showed a higher global density than the inactive variant (Fig. 2A). However, both variants exhibited similar cluster behaviour with regularly occurring sites of higher accumulation (Figs 2B, C, 3). The distance between single RNAPII molecules within small clusters ranged between 20 nm and 40 nm for both active and inactive variants (Fig. 4A). In addition, there are regions in which the small clusters further accumulate at distances between 50 nm and 150 nm, and 100 nm and 200 nm (large clusters) for active and inactive RNAPII, respectively (Fig. 4B). The distances between the large clusters amounted to 200-300 nm for active and 200-400 nm for inactive RNAPII (Fig. 4C). Thus, in addition to a global dispersion of both RNAPII variants within euchromatin, RNAPII molecules may also aggregate at two different levels. Despite a similar mean single molecule distance of 27 nm, the active RNAPII molecules aggregate more densely in small and large distance clusters than the inactive molecules. Small and large clusters correspond to the size known for animal transcription factories. The diameters of small clusters ranged between 60 nm and 90 nm, that of round large clusters between 250 nm and 400 nm, and that of large ellipsoid clusters between 120-250 nm and 250-700 nm, respectively. On average, in the same euchromatic area (measured in a 110 nm slice of 10 different 8C nuclei), 50–75 large active RNAPII clusters (containing 2-8 small clusters) correspond to 25–40 large inactive RNAPII clusters (containing 2–5 small clusters). The mean small cluster distance was 130 nm and 170 nm and for large clusters it was 265 nm and 335 nm for active and inactive RNAPII, respectively.

3D-PALM also allowed the estimation of the copy numbers of active and inactive RNAPII within the whole nucleus. In differentiated 2C and endopolyploid 4C–16C leaf nuclei, the mean number of RNAPIISer2ph increased and ranged between ~13 000 in 2C and ~58 000 in 16C nuclei (Fig. 5A; Supplementary Table S1A at JXB online), thus confirming the data obtained by Schubert (2014) based on signal intensity measurements in maximum intensity projections of SIM image stacks. However, the molecule numbers did not increase proportionally as expected theoretically in the case of exact enzyme duplication during endoreduplication and as has been found by Schubert (2014).

In a second experiment in 8C nuclei, the mean number of both active (RNAPIISer2ph and RNAPIISer5ph) variants amounted to ~53 000 and thus was clearly higher than the number of the inactive molecules (~22 000; Fig. 5B; Supplementary Table 1B at JXB online). In different experiments it became obvious that active RNAPII is ~2.5 times as abundant as the inactive variant (Fig. 5B, C). In contrast, the signal intensity measurements by Schubert (2014) indicated similar amounts of active and inactive RNAPII enzymes.

To test whether an increased transcriptional activity (indicated by a higher number of active RNAPII molecules) induces the decrease of already available inactive molecules, 8C nuclei were labelled simultaneously with antibodies against inactive and active (phosphorylated at Ser2) RNAPII, and 3D-PALM was applied (Figs 1, 5C). Considering the amount of both RNAPII variants in single nuclei, it seems that such a correlation does not exist because in most nuclei a low or high number of inactive RNAPII molecules is accompanied by a corresponding active molecule number (Fig. 5C; Supplementary Table S1C at JXB online).

In summary, it is concluded that (i) the reticulate structures formed by the different RNAPII modifications and identified by SIM contain, as proven by PALM, several globally mainly dispersed single molecules which may cluster within a size known from animal transcription factories; (ii) RNAPII quantification by 3D-PALM is more reliable than signal intensity measurements in maximum intensity projections generated from SIM image stacks; and (iii) the 3D-PALM measurements confirm that the number of RNAPII molecules increases with the degree of endopolyploidy.

Discussion

3D-PALM reliably counts and co-localizes single molecules in isolated nuclei

In this report, a version of 3D-PALM called phase ramp imaging localization microscopy (PRILM), a method that among other 3D technologies provides the largest capture range in the axial direction per measurement position (Baddeley et al., 2010), was applied. Due to the outstanding signal-to-noise ratio achieved by the staining procedure on isolated nuclei and their flattening on the coverslips, a z dimension of ~2 μm was obtained, namely flat enough to be fully covered in their z-extension by PRILM during only one measurement step. Thus, it was possible to avoid multiple measurements at different z-planes that potentially can affect the counting efficiency.

Regardless of the approach, the true number of molecules depends on different factors such as labelling efficiency, molecule density, and dye properties (Dempsey et al., 2011; Durisic et al., 2014). To ensure that as many molecules as possible were labelled antibodies were chosen which provided the best labelling efficiencies at concentrations above saturation, and dyes with high quantum yields were used. As internal controls, the secondary antibodies were swapped between the different colours to show that they had no influence on the overall counting efficiencies. To reduce the possibility of counting a molecule twice, the events were grouped in such a way that those lying within the localization precision were regarded as originating from the same molecule. Recordings were made until no significant blinking appeared. In all three experiments using different nuclei from different preparations (Fig. 5; Supplementary Table S1 at JXB online), similar mean numbers of RNAPII molecules per nucleus were obtained (compare RNAPIIinactive and RNAPIISer2ph), indicating the reliability of the 3D-PALM measurements.

Although a constant increase in gene copy numbers is accompanied by an increasing endopolyploidy level, the theoretically expected doubling of RNAPII molecules was not observed (Fig. 5A). The increases varied between 1.38- and 2.2-fold,
with an average increase of 1.68-fold. This might reflect counting inaccuracies. For example, when comparing the counts of three different preparations of 8C nuclei, variations by a factor of 1.3–1.8 were observed (compare RNAPIIser2ph in Fig. 5A, B, and C). On the other hand, with the doubling of the gene copy numbers, other cellular factors could also possibly limit a parallel doubling of RNAPII molecules.

Despite these considerations, the 3D-PALM counting allowed a more accurate estimate of the RNAPII amount to be obtained than in a previous intensity-based approach (Schubert, 2014). Intensity-based methods lack absolute quantitative information as higher intensities could be caused by more molecules less densely arranged or fewer molecules with a denser arrangement. PALM allows unambiguous discrimination between density and amount of molecules by accurately localizing and counting them. Active RNAPII seems to be more tightly arranged probably due to its ~3-fold higher abundance than the inactive variant (Fig. 2A).

**Fig. 2.** Distribution of inactive and active RNAPII molecules in an 8C A. thaliana nucleus (Nucleus 1 in Supplementary Table S1C at JXB online) recorded by 3D-PALM. (A) Density map of inactive (not phosphorylated) and active (phosphorylated at Ser2) RNAPII in a 110 nm slice. The density colour code represents molecules per $\mu m^3$. (B) Gauss-rendered (displayed in a heat map; $1=100\%$ intensity) 3D-PALM images to emphasize intensity differences. (C) Enlarged image of the area boxed in (B) showing the intensity distribution (see the scale in B) with localized molecules (red dots) within the heat map. Short distance clusters are encircled in red, long distance clusters in grey.
RNA polymerase II molecules are more densely packed, the probability of fluorophore quenching might be higher.

**RNA polymerase II appears globally dispersed, but also aggregates within euchromatin**

In mammals, genes with similar functions may be co-localized and co-expressed in postulated distinct transcription factories via out-looping from chromatin (Iborra et al., 1996; Edelman and Fraser, 2012; Rieder et al., 2012; Papantonis and Cook, 2013), as has been proven, for example, for active immunoglobulin genes from three different mouse chromosomes (Park et al., 2014). In contrast, in a recent report, it has been found in human osteosarcoma cells by applying reflected light-sheet super-resolution microscopy that >70% of the transcription foci originate from single RNA polymerase II molecules with a mean distance of ~230 nm (Zhao et al., 2014). In the same report, no clustering between RNA polymerase II molecules was detected within the range of ~40–200 nm (mean=130 nm), which was predicted for transcription factories (Iborra et al., 1996; Eskiw and Fraser, 2011). Thus, the majority of the RNA polymerase II molecules may exist in a solitary fashion inside the mammalian nucleus, and Zhao et al. (2014) conclude that the idea of a coordinated transcription of mammalian genes in prevalently existing transcription factories needs to be revisited. These findings deviate from the previous results because, in addition to a global RNA polymerase II dispersion, an aggregation of RNA polymerase II molecules within a size predicted for animal and human transcription factories was also found. Thus it is argued that in plant nuclei transcription factories may exist and that they are homogeneously dispersed within euchromatin. The slightly higher distances of inactive RNA polymerase II may be due to its lower abundance compared with active RNA polymerase II.

Based on a global transcription factor localization analysis within a single human cell type, Yan et al. (2013) described the majority of these factors to be arranged in clusters also containing cohesins. They may be important for the re-establishment of transcription factor clusters after DNA replication and for chromatin condensation and thus for maintaining the transcriptional memory of dividing cells.

Markaki et al. (2010) describe a network of channels and lacunas, called the interchromatin compartment, throughout mammalian nuclei which contain decondensed chromatin. In this region, nascent DNA, nascent RNA, RNA polymerase II, and histone modifications for transcriptionally active chromatin, are highly enriched.

Similarly, the distribution of RNA polymerase II as resolved by SIM was identified in a network-like manner within euchromatin of rye and Arabidopsis. RNA polymerase II was absent from nucleoli and heterochromatin (Schubert, 2014). The inactive form...
Schubert and Weisshart showed more distinct signals than the active RNAPII forms (phosphorylated at Ser2 and Ser5, respectively). In accordance with this, it is shown here by 3D-PALM that within these reticulate structures single molecules are dispersed and additionally that both the inactive and the active RNAPII molecules may aggregate, but not active and inactive RNAPII together.

Interestingly, the structural maintenance of chromosome (SMC) cohesin and condensin complex subunits SMC3 and CAP-D3, respectively, show a reticulate distribution within euchromatin of differentiated endopolyploid Arabidopsis nuclei (Schubert et al., 2013) similar to that of RNAPII. In these nuclei, single chromatid segments are mainly not cohesive within euchromatic chromosome territories, possibly making multiple gene copies accessible for transcription (Schubert et al., 2012). Despite a stable global interphase chromatin organization, intra- and interchromosomal associations indicative for co-ordinated transcription may appear, but more seldom at interstitial euchromatin segments than at subtelomeres.

Fig. 4. Distances between single molecules (A), small (B), and large clusters (C).

Fig. 5. Mean (A, B, C) and individual (C2) numbers of RNAPII molecules in differentiated A. thaliana leaf nuclei (95% confidence intervals are indicated; for statistics, see Supplementary Table S1 at JXB online). (A) Mean number of RNAPIISer2ph in 2C–16C nuclei. The theoretical RNAPII distribution (red dots) indicates the values expected in the case of exact enzyme duplication during endoreduplication. (B) Mean number of inactive and active (RNAPIISer2ph, RNAPIISer5ph) RNAPII molecules in 8C nuclei. (C1) Mean number of inactive RNAPII and RNAPIISer2ph in simultaneously labelled 8C nuclei. (C2) Numbers of inactive and active (RNAPIISer2ph) RNAPII molecules in nine different nuclei simultaneously labelled with specific antibodies.
and pericentromeres (Grob et al., 2013; Schubert et al., 2014; Feng et al., 2014). According to data found in Hi-C experiments, epigenetic marks of active chromatin did not show a co-localization with highly associated chromatid segments in Arabidopsis, suggesting a lack of clustering of the most actively transcribed genes (Feng et al., 2014). This observation is supported by the finding that interstitial euchromatin segments containing highly co-expressed genes do not associate more often than those containing genes co-expressed at a low level (Schubert et al., 2014). Thus, it seems that in Arabidopsis most of the transcriptional activity is not only localized in potential transcription factories but is also homogeneously distributed within euchromatin, indicating that most of the Arabidopsis genes are not induced by co-expression as described for yeast, Drosophila, and mammals (Osborne et al., 2004, 2007; Brown et al., 2006, 2008; Tanizawa et al., 2010; Gibcus and Dekker, 2012, 2013; Hou and Corces, 2012). Similarly, Abranches et al. (1998) found a uniform distribution of transcription sites throughout the nucleoplasm of wheat.

Based on previous findings and those presented here regarding the Arabidopsis interphase chromatin arrangement (Schubert et al., 2012), and the distribution of cohesin and condensin proteins (Schubert et al., 2013) and RNAPII molecules (Schubert, 2014), a model of the organization of endopolyploid plant interphase nuclei is proposed in which SMC proteins are involved in maintaining a euchromatin structure allowing flexible dispersed transcription (Fig. 6).

**How many genes are transcribed in a nucleus?**

In contrast to temporally induced genes, from prokaryotes to mammals, due to stochastic fluctuations in transcription, the expression of constitutive genes encoding essential subunits of protein complexes can vary by at least ~50% among genetically identical cells. Nevertheless, the resulting proteins show a similar abundance and less variability (Golding et al., 2005; Sigal et al., 2006; Raj et al., 2006, 2010; Zenklusen et al., 2008; Cohen et al., 2009; Pare et al., 2009; Taniguchi et al., 2010; Reiter et al., 2011; Gandhi et al., 2011). In addition, the gene activation causes burst-like expression of all genes within a larger chromatin segment (Raj et al., 2006). This supports the idea that transcription is intrinsically stochastic, providing a flexibility important for cells to respond to changing environments and sudden stress, and to provide a cell population heterogeneity needed during cellular differentiation and development (Kaern et al., 2005; Li and Xie, 2011; Velez-Bermudez and Schmidt, 2014). Little et al. (2013) found that, despite fluctuating transcription, the accumulation of mRNAs is similar across essential Drosophila embryo patterning genes generating precise protein distributions. Furthermore, these authors suggest that fluctuations in mRNA production are context independent and are a fundamental characteristic of transcription, thus resolving the apparent paradox between stochastic transcription and developmental precision.

Stochastic fluctuations in transcription may explain the high variability in the numbers of RNAPII molecules found between individual nuclei of the same ploidy level (Supplementary Table S1 at JXB online). Compared with the observed difference of ~50% regarding active RNAPIIs, it even reached as much as 70% for inactive RNAPII. However, the heterogeneity of the number of RNAPII molecules may also be caused by the different leaf cell types from where the sorted nuclei originate.

It has been suggested that ~75% of the human genome is associated with transcriptional activity (Djebali et al., 2012). A lower amount was determined for A. thaliana where the...
global level of expression depends on growth conditions (laboratory controlled or field conditions) and the accession analysed (Schmid et al., 2005; Kliebenstein et al., 2006; Richards et al., 2012). In the shoot, 45–61% of genes were expressed at each time point under field conditions (Richards et al., 2012).

*Arabidopsis thaliana* has 27 379 genes (Arabidopsis Genome Initiative, 2000) and for rye ~26 000 genes are expected, as found for the closely related barley (International Barley Genome Sequencing Consortium, 2012). This may explain the similar amounts of active as well as inactive RNAPII found by SIM signal intensity measurements in meristematic nuclei of both species (Schubert, 2014).

Here it is confirmed by single molecule localization that the amount of RNAPII increases with endopolyploidy in plants (Bourdon et al., 2012; Schubert, 2014). Thus, increasing the transcriptional activity of cells and tissues seems to be an important function of endopolyploidy.

Assuming that active RNAPII enzymes are phosphorylated only either at Ser2 or at Ser5 and not in parallel, the mean sum of RNAPII molecules can be calculated (22340 inactive + 54480 Ser2ph + 52900 Ser5ph; Fig. 5B; Supplementary Table S1B at JXB online) and thus amounts to ~107 000 active and in total to ~130 000 molecules per 8C nucleus.

By reflected light-sheet super-resolution microscopy, Zhao et al. (2014) determined that there were ~80 200 ± 8800 active RNAPII molecules within the transcription foci of human osteosarcoma cell nuclei, a number in agreement with that previously reported in human nuclei based on other methods (Sugden and Keller, 1973; Jackson et al., 1998, 2000; Pombo et al., 1999).

Assuming that these human nuclei are diploid and that half of them are in G1 phase (containing two unreplicated chromatids, i.e. two copies per gene) and the others are in G2 (containing four replicated chromatids, i.e. four copies per gene) and regarding that the human genome contains 20 687 protein-coding genes (Pennisi 2012), the mean gene copy number per nucleus should be 62 061 (20 687 × 2 + 20 687 × 4 = 124 122 ± 2). Thus ~1.3 (80 200 ÷ 62 061) active RNAPII enzymes are present per gene copy.

In differentiated 4C *A. thaliana* nuclei containing four chromatids, 109 516 (27 379 × 4) gene copies are available. In addition, ~36 000 (~18 000 RNAPIISer2ph + ~18 000 expected RNAPIISer5ph because the amount is similar in 8C nuclei) (Fig. 5B; Table 1) active RNAPII molecules should be present per nucleus. These are fewer (36 000 ÷ 109 516 = 0.3) active RNAPII molecules per gene copy than found in cultured and cycling human cells, probably caused by the differentiated status of these plant cells. Fewer RNAPII molecules per gene copy is in accordance with the observation that more human genes are associated with transcriptional activity than those of *A. thaliana* (see above). Interestingly, with 0.3–0.5 RNAPII molecules per gene copy, the ratio is similar at all four ploidy levels in *A. thaliana*.

By two-colour labelling and 3D-PALM, it was observed that in addition to active RNAPIISer2ph molecules responsible for transcriptional elongation, inactive RNAPII molecules are present in these nuclei with a frequency of about half of that of active enzymes. Because the density of active RNAPII on genes depends on the initiation frequency, elongation frequency, and processivity (RNAPII remaining on the template after each catalytic event) (Ehrensberger et al., 2013), it is difficult to calculate the number of transcribed genes per nucleus. However, assuming that only one RNAPII Ser2ph molecule responsible for transcriptional elongation would be associated with only one gene, as mostly found in *Drosophila*, mouse, and human nuclei (Laird and Chooi, 1976; McKnight and Miller, 1976; Fakan et al., 1986; Jackson et al., 1998), then every second to third gene would be active in 2C–16C *A. thaliana* leaf nuclei (Table 1). Due to the presence of mainly non-cohesive chromatids in highly endopolyploid *A. thaliana* nuclei (Schubert et al., 2012), the resulting ‘open chromatin’ structure (Zhang et al., 2014) may allow the parallel transcription of multiple gene copies by RNAPII as demonstrated at the four well-separated chromatids in *Drosophila* embryo nuclei (Little et al., 2013). Thus, assuming that ~50% of the different genes are active in *A. thaliana* (see above), on average approximately one copy of the same gene would be transcriptionally active in 2C–16C nuclei. However, it could also be possible that less than the half of the genome of endopolyploid nuclei is transcribed, but instead in parallel more gene copies per gene (e.g. up to 16 in 16C nuclei) are transcribed in differentiated endopolyploid nuclei.

**Supplementary data**

Supplementary data are available at JXB online. Figure S1. Gauss rendered PALM image of RNAPIISer2ph localized within reticulate structures acquired by SIM.

**Table 1.** Number of active RNAPIISer2ph and RNAPIISer5p molecules in differentiated 2C–16C leaf nuclei determined by 3D-PALM (see Supplementary Table S1A at JXB online) in comparison with the available gene copy number calculated on the basis of the 27 379 genes evident in *A. thaliana* (TAIR9; Arabidopsis Genome Initiative, 2000).

| Ploidy | No. of gene copies | No. of RNAPIISer2ph | No. of RNAPIISer5p | Total active RNAPII molecules | Active RNAPII molecules per gene |
|--------|--------------------|---------------------|-------------------|-----------------------------|---------------------------------|
| 2C     | 54 758             | 12 630              | 12 630            | 25 260                      | 0.5                             |
| 4C     | 109 516            | 17 960              | 17 960            | 35 920                      | 0.3                             |
| 8C     | 219 032            | 40 130              | 40 130            | 80 260                      | 0.4                             |
| 16C    | 438 064            | 57 590              | 57 590            | 115 180                     | 0.3                             |

* A similar amount expected as found for RNAPIIphSer2 based on the data shown in Supplementary Table S1.
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References

Abranches R, Beven AF, Aragon-Alcaide L, Shaw PJ. 1998. Transcription sites are not correlated with chromosome territories in wheat nuclei. Journal of Cell Biology 143, 5–12.

Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408, 796–815.

Baddeley D, Chapin VO, Schermelleh L, et al. 2010. Measurement of replication structures at the nanometer scale using super-resolution light microscopy. Nucleic Acids Research 38, 68.

Baddeley D, Crossman D, Rossberger S, Cheyne JE, Montgomery JM, Jayasinghe ID, Cremer C, Cannell MB, Soeller C. 2011. 4D super-resolution microscopy with conventional fluorophores and single wavelength excitation in optically thick cells and tissues. PLoS One 6, e20645.

Bell K, Mitchell S, Paultre D, Posch M, Oparka K. 2013. Correlative imaging of fluorescent proteins in resin-embedded plant material. Plant Physiology 161, 1565–1603.

Bourdon M, Pirrello J, Cheniclet C, et al. 2012. Evidence for karyoplasmic homeostasis during endoreduplication and a ploidy-dependent increase in gene transcription during tomato fruit growth. Development 139, 3817–3826.

Brown JM, Green J, das Neves RP, et al. 2008. Association between active genes occurs at nuclear speckles and is modulated by chromatin environment. Journal of Cell Biology 182, 1083–1097.

Brown JM, Leach J, Reittie JE, Atzberger A, Lee-Prudhoe J, Wood WG, Higgs DR, Iborra FJ, Buckley VJ. 2006. Coregulated human globin genes are frequently in spatial proximity when active. Journal of Cell Biology 172, 177–187.

Chakalova L, Fraser P. 2010. Organization of transcription. Cold Spring Harbor Perspectives in Biology 2, a00729.

Cohen AA, Kalisky T, Mayo A, et al. 2009. Protein dynamics in individual human cells: experiment and theory. PLoS One 4, e4901.

Dempsey GT, Vaughan JC, Chen KH, Bates M, Zhuang X. 2011. Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging. Nature Methods 8, 1027–1036.

Deschout H, Cella Zanacchi F, Mlodzianoski M, Diaspro A, Bewersdorf J, Hesse ST, Braeckmans K. 2014. Precisely and accurately localizing single emitters in fluorescence microscopy. Nature Methods 11, 253–266.

Djebali S, Davis CA, Merkel A, et al. 2012. Landscape of transcription in human cells. Nature 489, 101–108.

Durisc N, Cuervo LL, Lakadamyali M. 2014. Quantitative super-resolution microscopy: pitfalls and strategies for image analysis. Current Opinion in Chemical Biology 20, 22–28.

Edelman LB, Fraser P. 2012. Transcription factories: genetic programming in three dimensions. Current Opinion in Genetics and Development 22, 110–114.

Egloff S, Murphy S. 2008. Cracking the RNA polymerase II CTD code. Trends in Genetics 24, 280–288.

Ehrensberger AH, Kelly GP, Svejstrup JQ. 2013. Mechanistic interpretation of promoter-proximal peaks and RNAPII density maps. Cell 154, 713–715.

Eskiw CH, Fraser P. 2011. Ultrastructural study of transcription factories in mouse erythroblasts. Journal of Cell Science 124, 3676–3683.

Eskiw CH, Rapp A, Carter DR, Cook PR. 2008. RNA polymerase II activity is located on the surface of protein-rich transcription factories. Journal of Cell Science 121, 1999–2007.

Fakan S, Leser G, Martin TE. 1986. Immunoelectron microscope visualization of nuclear ribonucleoprotein antigens within spread transcription complexes. Journal of Cell Biology 103, 1153–1157.

Fay FS, Taneya KL, Shenoy S, Lifshitz L, Singer RH. 1997. Quantitative digital analysis of diffuse and concentrated nuclear distributions of nascent transcripts, SC35 and poly(A). Experimental Cell Research 231, 27–37.

Feng S, Cokus SJ, Schubert V, Zhai J, Pellegrini M, Jacobsen SE. 2014. Genome-wide Hi-C analyses in wild-type and mutants reveal high-resolution chromatin interactions in Arabidopsis. Molecular Cell 55, 694–707.

Ferrai C, Xie SQ, Luraghi P, Munari D, Ramirez F, Branco MR, Pombo A, Crippa MP. 2010. Poised transcription factories prime silent uPA gene prior to activation. PLoS Biology 8, e1000270.

Fricke F, Mcalkus S, Wangorsch G, Greiner JF, Kaltischmidt B, Kaltischmidt C, Widera D, Dandekar T, Heilemann M. 2014. Quantitative single-molecule localization microscopy combined with rule-based modeling reveals ligand-induced TNF-R1 reorganization toward higher-order oligomers. Histochemistry and Cell Biology 142, 91–101.

Gandi SJ, Zenklenus D, Lionnet T, Singer RH. 2011. Transcription of functionally related constitutive genes is not coordinated. Nature Structural and Molecular Biology 18, 27–34.

Gibcus JH, Dekker J. 2012. The context of gene expression regulation. F1000 Biology Reports 4, 8.

Gibcus JH, Dekker J. 2013. The hierarchy of the 3D genome. Molecular Cell 49, 773–782.

Golding I, Paulsson J, Zawiliwski SM, Cox EC. 2005. Real-time kinetics of gene activity in individual bacteria. Cell 123, 1025–1036.

Grande MA, van der Kraan I, de Jong L, van Driel R. 1997. Nuclear distribution of transcription factors in relation to sites of transcription and RNA polymerase II. Journal of Cell Science 110, 1781–1791.

Grob S, Schmid MW, Luedtke NW, Wicker T, Grossniklaus U. 2013. Characterization of chromosomal architecture in Arabidopsis by chromosome conformation capture. Genome Biology 14, R129.

Hajheidari M, Koncz C, Eck D. 2013. Emerging roles for RNA polymerase II in Arabidopsis. Trends in Plant Science 18, 633–643.

Haj B, El Beheimy M, Izeddin I, Darzaqz X, Dahan M. 2014. Accessing the third dimension in localization-based super-resolution microscopy. Physical Chemistry Chemical Physics 16, 16340–16348.

Hamel V, Guichard P, Fournier M, Guiet R, Flücker I, Seitz A, Gönczy P. 2014. Correlative multicolor 3D SIM and STORM microscopy. Biomedical Optics Express 5, 3326–3336.

Hengartner CJ, Myer VE, Liao SM, Wilson CJ, Koh SS, Young RA. 1998. Temporal regulation of RNA polymerase II by Srb10 and Kin28 cyclin-dependent kinases. Molecular Cell 2, 43–53.

Hirose Y, Okhuma Y. 2007. Phosphorylation of the C-terminal domain of RNA polymerase II plays central roles in the integrated events of eucaryotic gene expression. Journal of Biochemistry 141, 601–608.

Hou C, Corces VG. 2012. Throwing transcription for a loop: expression of the genome in the 3D nucleus. Chromosoma 121, 107–116.

Huber O, Brunner A, Maier P, Kaufmann R, Couraud PO, Cremer C, Fricker G. 2012. Localization microscopy (SPDM) reveals clustered formations of P-glycoprotein in a human blood–brain barrier model. PLoS One 7, e44776.

Iborra FJ, Pombo A, Jackson DA, Cook PR. 1996. Active RNA polymerases are localized within discrete transcription ‘factories’ in human nuclei. Journal of Cell Science 109, 1427–1436.

International Barley Genome Sequencing Consortium. 2012. A physical, genetic and functional sequence assembly of the barley genome. Nature 491, 711–716.

Jackson DA, Hassan AB, Errington RJ, Cook PR. 1993. Visualization of foci sites of transcription within human nuclei. EMBO Journal 12, 1059–1065.

Jackson DA, Iborra FJ, Manders EM, Cook PR. 1998. Numbers and organization of RNA polymerases, nascent transcripts, and transcription units in HeLa nuclei. Molecular Biology of the Cell 9, 1523–1536.

Jackson DA, Pombo A, Iborra F. 2000. The balance sheet for transcription: an analysis of nuclear RNA metabolism in mammalian cells. FASEB Journal 14, 242–254.

Jasencakova Z, Meister A, Walter J, Turner BM, Schubert I. 2000. Histone H4 acetylation of euchromatin and heterochromatin is cell cycle dependent and correlated with replication rather than with transcription. The Plant Cell 12, 2087–2100.

Kaern M, Elston TC, Blake WJ, Collins JJ. 2005. Stochasticity in gene expression: from theories to phenotypes. Nature Reviews Genetics 6, 451–464.
Kliebenstein DJ, West MA, van Leeuwen H, Kim K, Doerge RW, Michelmore RW, St Clair DA. 2006. Genomic survey of gene expression diversity in Arabidopsis thaliana. Genetics 172, 1179–1189.

Komarnitsky P, Chen EJ, Buratowski S. 2000. Different phosphorylated forms of RNA polymerase II and associated mRNAs processing factors during transcription. Genes and Development 14, 2452–2460.

Kornberg RD. 1999. Eukaryotic transcriptional control. Trends in Cell Biology 9, M46–M49.

Laird CD, Chooi WY. 2007. Quantitative single-molecule microscopy reveals that CENP-A(Cnp1) deposition occurs during G2 in fission yeast. Open Biology 2, 120078.

Li GW, Xie XS. 2011. Central dogma at the single-molecule level in living cells. Nature 475, 308–315.

Little SC, Tikhonov M, Gregory T. 2013. Precise developmental gene expression arises from globally stochastic transcriptional activity. Cell 154, 789–800.

Löschberger A, Franke C, Krohne G, van de Linde S, Sauer M. 2014. Correlative super-resolution fluorescence and electron microscopy of the nuclear pore complex with molecular resolution. Journal of Cell Science 127, 4351–4355.

Markaki Y, Gunkel M, Schermelleh L, Beichmanis S, Neumann J, Heidemann M, Leonhardt H, Eick D, Cremer C, Cremer T. 2010. Functional nuclear organization of transcription and DNA replication: a topographical marriage between chromatin domains and the interchromatin compartment. Cold Spring Harb Symposia on Quantitative Biology 75, 475–492.

Martin S, Pombo A. 2003. Transcription factories: quantitative studies of nanostructures in the mammalian nucleus. Chromosome Research 11, 461–470.

McKnight SL, Miller OL Jr. 1976. Ultrastructural patterns of RNA synthesis during early embryogenesis of Drosophila melanogaster. Cell 8, 305–319.

Ni Z, Schwartz BE, Werner J, Suarez JR, Lis JT. 2004. Coordination of transcription, RNA processing, and surveillance by P-TEFb kinase on heat shock genes. Molecular Cell 13, 55–65.

Osborne CS, Chakalova L, Brown KE, et al. 2004. Active genes dynamically colocalize to shared sites of ongoing transcription. Nature Genetics 36, 1065–1071.

Osborne CS, Chakalova L, Mitchell JA, Horton A, Wood AL, Bolland DJ, Corcoran AE, Fraser P. 2007. Myc dynamically and preferentially relocates to a transcription factory occupied by Igh. PLoS Biology 5, e192.

Papantonis A, Cook PR. 2013. Transcription factories: genome organization and gene regulation, Chemical Reviews 113, 8683–8705.

Pare A, Lemons D, Kosman D, Beaver W, Freund Y, McGinnis W. 2009. Visualization of individual Scr mRNAs during Drosophila embryogenesis yields evidence for transcriptional bursting. Current Biology 19, 2037–2042.

Park SK, Xiang Y, Feng X, Garrard WT. 2014. Pronounced cohabitation of active immunoglobulin genes from three different chromosomes in transcription factories during maximal antibody synthesis. Genes and Development 28, 1159–1164.

Pennisi E. 2012. Genomics. ENCODE project writes eulogy for junk DNA. Science 337, 1159–1161.

Pombo A, Jackson DA, Hollinshead M, Wang Z, Roeder RG, Cook PR. 1999. Regional specialization in human nucleolus: visualization of discrete sites of transcription by RNA polymerase III. EMBO Journal 18, 2241–2253.

Raj A, Peskin CS, Tranchina D, Vargas DY, Tyagi S. 2006. Stochastic mRNA synthesis in mammalian cells. PLoS Biology 4, e309.

Raj A, Rifkin SA, Andersen E, van Oudenaarden A. 2010. Variability in gene expression underlies incomplete penetrance. Nature 463, 913–918.

Recamier V, Izeddin I, Bosanac L, Dahan M, Proux F, Darzacq X. 2014. Single cell correlation fractal dimension of chromatin: a framework to interpret 3D single molecule super-resolution. Nucleus 5, 75–84.

Reiter M, Kirchner B, Muller H, Holzhauer C, Mann W, Pfaffl MW. 2011. Quantification noise in single cell experiments. Nucleic Acids Research 39, e124.

Richards CL, Rosas U, Banta J, Bhambrha N, Purugganan MD. 2012. Genome-wide patterns of Arabidopsis gene expression in nature. PLoGS Genetics 8, e1002662.

Rieder D, Trajanoski Z, McNally JG. 2012. Transcription factories. Frontiers in Genetics 3, 221.

Rossberger S, Best G, Baddeley D, Heintzmann R, Birk U, Dithmar S, Cremer C. 2013. Combination of structured illumination and single molecule localization microscopy in one setup. Journal of Optics 15, 094003.

Schmid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, Scholkopf B, Weigel D, Lohmann JU. 2005. A gene expression map of Arabidopsis thaliana development. Nature Genetics 37, 501–506.

Schübel V. 2014. RNA polymerase II forms transcription networks in rye and Arabidopsis nuclei and its amount increases with endopolyplody. Cytogenetic and Genome Research 143, 60–77.

Schübel V, Berr A, Meister A. 2012. Interphase chromatin organisation in Arabidopsis nuclei: constraints versus randomness. Chromosoma 121, 369–387.

Schübel V, Lermontova I, Schübel I. 2013. The Arabidopsis CAP-D proteins are required for correct chromatin organisation, growth and fertility. Chromosoma 122, 517–533.

Schübel V, Rudnik R, Schübel I. 2014. Chromatin associations in Arabidopsis interphase nuclei. Frontiers in Genetics 5, 389.

Sigal A, Milo R, Cohen A, Geva-Zatorsky N, Klein Y, Liron Y, Rosenfeld N, Danon T, Perzov N, Alon U. 2006. Variability and memory of protein levels in human cells. Nature 444, 643–646.

Sims RJ 3rd, Mandall SS, Reinberg D. 2004. Recent highlights of RNA-polymerase-II-mediated transcription. Current Opinion in Cell Biology 16, 263–271.

Sugden B, Keller W. 1973. Mammalian deoxyribonucleic acid-dependent ribonucleic acid polymerases. 1. Purification and properties of an -amanitin-sensitive ribonucleic acid polymerase and stimulatory factors from HeLa and KB cells. Journal of Biological Chemistry 248, 3777–3788.

Taniguchi Y, Choi PJ, Li GW, Chen H, Babu M, Hearn J, Emili A, Xie XS. 2010. Quantifying E. coli proteome and transcriptome with single-molecule sensitivity in single cells. Science 329, 533–538.

Tanizawa H, Iwasaki O, Tanaka A,纹理分析的 RNA polymerase II forms transcription networks in Arabidopsis nuclei. Cytogenetic and Genome Research 143, 60–77.

Thompson RE, Larson DR, Webb WW. 2002. Precise nanometer localization analysis for individual fluorescent probes. Biophysical Journal 82, 2775–2783.

Velez-Bermudez IC, Schmidt W. 2014. The conundrum of discordant protein and mRNA expression. Are plants special? Frontiers in Plant Science 5, 619.

Yan J, Enge M, Whittington T, et al. 2013. Transcription factor binding in human cells occurs in dense clusters formed around cohesin anchor sites. Cell 154, 801–813.

Zenklausen D, Larson DR, Singer RH. 2008. Single-RNA counting reveals alternative modes of gene expression in yeast. Nature Structural and Molecular Biology 15, 1263–1271.

Zhang W, Zhang T, Wu Y, Jiang J. 2014. Open chromatin in plant genomes. Cytogenetics and Genome Research 143, 18–27.

Zhu Z, Ruy R, Gebhardt JC, Suter DM, Chapman AR, Xie XS. 2014. Spatial organization of RNA polymerase II inside a mammalian cell nucleus revealed by reflected light-sheet superresolution microscopy. Proceedings of the National Academy of Sciences, USA 111, 681–686.