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Flavanols in the nuclei of the tea bush (*Camellia sinensis*) – broadening the perspectives to human health

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(Received January 16, 2013)

Summary

The nuclei of the tea shrub *(Camellia sinensis* L.) contain flavanols. Based on the dynamic, fine-tuned subnuclear changes of the flavanol pattern during the cell cycle, it can be postulated that these blue staining phenols play a role in organizing basic mechanisms of chromatin remodelling. Silenced interphase nuclei of mature parenchyma cells indicate a pronounced diffuse distribution of blue stained flavanols using the selective reagent *p*-dimethylaminocinnamaldehyde (DMACA). By contrast, in activated nuclei the flavanols reflect a variable, mosaic-like blue patterning enclosed by white interchromatin spaces. Subnuclear expression of euchromatin displays relative tiny blue dots of flavanols as compared with the larger-sized blobs of heterochromatin. From metaphase to telophase, the chromosomes stain a fairly dark blue on flavanols with a more or less diffuse appearance. Those nuclei running through mitotic interphases from G 1 to G 2 have well-defined flavanol-free nucleoli. The flavanol pattern of meristematic chromosomes found in the tea plant is basically also valid for herbaceous plants, such as *Hyacinthus romanus* L., *Tulipa gesneriana* L. and *Allium cepa* L., which genuinely do not contain nuclear flavanols. This was verified by incubation of their rootlets in solutions of green tea and epigallocatechin gallate (EGCG). Also human nuclei demonstrate an easy import of added flavanols and the resulting blue stained chromatin reflects in much the same way the structural modifications as already described for the plant nuclei. Flavanols have the potential to associate to the histones of chromatin which inhibits a possible oxidation. Even small fragments of histones can aggregate to catechin as shown on the basis of kinetic measurements for the H4-core peptide HAKRKT and its acetylated product HAK(ac)RK(ac)T.

Introduction

In many tree species the flavanols, generally known as catechins, are deposited preferentially in vacuoles of enlarging cells. Histological studies, UV-VIS spectroscopic titrations and reaction kinetic investigations as well laser microsurgery indicated that also nuclei of certain tree species are targets for flavanols (FEUCHT and POLSTER, 2001; POLSTER et al., 2003; POLSTER et al., 2006). Recently, these results were analytically confirmed by high resolution two photon excitation techniques applied to nuclei of *Taxus baccata* (MUELLER-HARVEY et al., 2012). Many coniferous species show a significant nuclear affinity for flavanols in contrast to woody angiosperms as dealt in a recent review (FEUCHT et al., 2012 b). A prominent example of the angiosperms displaying a high affinity of nuclei for flavanols is the tea plant (FEUCHT et al., 2004). HPLC investigations showed the presence of catechin (C), epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCG), procyanidin B2 and epicatechin gallate (ECG) in leaves and anthers of the tea plant (FEUCHT et al., 2005). Most of these flavanols were examined on their association behaviour towards histone proteins (FEUCHT et al., 2004, 2005, 2007).

Newest results indicate that the aggregation of flavanols to histones seems to be dependent on the epigenetic histone code (FEUCHT et al., 2012 a). These findings appear to be of particular importance because histones are known to be linked with epigenetic and transcriptional activities (JENUWEIN and ALLIS, 2001). Acetylation and methylation imposing relaxation or compaction of nucleosomes play also a major role in histone modifications (GASSER, 2002).

Biological activities of phenolics located in nuclei have been exhaustively discussed by BIDEL et al. (2010). It is interesting that the subnuclear flavanol distribution of the tea bush is basically similar to that found in the distantly related hemlock as a coniferous species (FEUCHT et al., 2011). The present publication pays particular attention to the fine-mapping of the nuclear flavanols during cell cycling of *Camellia sinensis*. To carry on a further step, green tea flavanols were added to three herbaceous species and human nuclei lacking flavanols in the native state.

Material and methods

Biological Material

Five tea bushes (*Camellia sinensis* L.), 8 years old, were grown in the greenhouse. During the early phase of growth young anthers of sprouting flowers were sampled. The anthers between 100-150 μm in length were most active in cell cycling. Over a period of three years a total of about 3.500 to 4.000 nuclei of the tea bush were studied.

Young rootlets of *Allium cepa* L., *Hyacinthus romanus* L. and *Tulipa gesneriana* L. were used to study the affinity of their nuclei for flavanols. The plants were cultivated in a greenhouse and the rootlets were sampled at a length of about 3 to 7 mm. Then, they were incubated for 3 h in green tea. A total of about 500 nuclei per plant species were examined by microscopy.

In the case of human cells a total of **about 200 nuclei from nasal mu**cosa, saliva secretion and skin (forehead) epidermis were sampled. Then, they were incubated as outlined above in green tea and EGCG solutions. In this context, DNA patches (calf thymus, Sigma) were likewise incubated for 3-4 h in EGCG (concentrations as described above).

Histology

To study the dynamics of flavanol patterning nuclei from very young and older anthers are directly stained and studied by microscopy (Zeiss Axiom apparatus). Direct staining of fresh cells with the DMACA reagent is necessary because any embedding procedures would result in some loss of the soluble flavanols. The staining reagent (0.5% *p*-dimethylaminocinnamaldehyde dissolved in 1.5 M sulphuric acid and methanol 1:4, v/v) is very specific for flavanols. The cells are stained on a microscope slide for 15-25 min. Then, most of the reagent is withdrawn with a strip of filter paper, and after adding few drops of water the flavanols switched rapidly to bright blue. The stained tissues are gently squashed with a cover-glass, so that the cells of the tissues separated easily from one another. For

DNA detection DAPI staining (4`, 6-diamidino-2-phenylindole di-hydrochloride, Serva) the Zeiss fluorescence apparatus (III RS) was used (Fig. 2b).

Histological absorption measurements in the visible range

Light microscopic images of the anther nuclei were obtained with a Zeiss Axioscope, then scanned with a Nicon Coolscan IV ED. Thereafter, a DigiEye 2.3 apparatus from VeriVide Ltd. Leicester, United Kingdom, as described by (LAU et al 2011), is used to determine the relative absorption in % of the scanned blue coloured nuclei at 640 nm. This measurement method is based on a non-contact imaging system. The image is captured by a high resolution digitalcamara in a unique controlled lighting environment, the DigiEye Cube. The measurements device was calibrated according to the DigiEye calibration routine. This experimental set up leads to most reliable absorption measurements. The captured image is then displayed on a calibrated monitor. DigiEye is well suited to measure very small and irregular shaped samples by selecting and retrieving colour data from any pixel in the high-resolution image. Thus far, the method permits a more refined analysis of the nuclei from the tea plant. As stated by LAU et al. (2011) the values measured by the DigiEye have very strong positive correlations with those measured by classical spectrophotometry.

Chemicals for kinetic measurements

Using the UV-VIS spectroscopy the influence of histone peptides on the oxidation degradation behaviour of catechin was studied in dependence on time (Fig. 4). The peptides HAKRKT and HAK(ac)RK(ac)T were prepared from Peptides & Elephants GmbH (Nuthetal, Germany; article no: EP-01641 and EP-01716); (+)-Catechin (puriss.; article no: 6200) and Tris = Tris-(hydroxymethyl)amino methane (PUFFERAN®, ≥ 99.9%, p.a.) were obtained from Carl Roth (Karlsruhe, Germany), ethanol (for spectroscopy; "Uvasol") from Merck (Darmstadt, Germany), oxygen 4.5 (content 12 L; „Minican"; 12 bar) from Linde (Unterschleißheim, Germany), hydrochloric acid (min. 25%; puriss. p.a.) from Riedel-de Haën (Sigma Aldrich, Seelze, Germany) and magnesiumchloride hexahydrate (AnalaR Normapur, 99.0-102.0%) from VWR (Leuven, Belgium). The preparation of the buffers and catechin-peptide-solutions as well the procedure of the UV-VIS spectroscopic kinetic measurements are described in FEUCHT et al. (2012 a).

Results

Differential flavanol patterning of activated and silenced nuclei of tea cells

The cytological approach using directly the fresh nuclei provides a global view on the entire nuclei. The most striking feature of the silenced anther nuclei is pronounced diffuseness of the slightly undulated blue stained nucleoplasm (Fig. 1 a, b). Other silenced, inactive tissues from the tea bush, such as petioles and sepals from fully developed flowers indicate a similar diffuse appearance (not shown). On the contrary, each interphase nucleus from the meristematic cluster (Fig. 1 c) shows its own chromatin landscape. By now, transcriptional mRNA synthesis is high because prominent nucleoli are evident. These nuclei, measuring between 7 to 9 μm in diameter, are more or less loosened and display a fine-structured network of blue chromatin mottling on a mosaic-like white background (Fig. 1 c). Stronger magnification of those activated interphase nuclei (Fig. 1 d-g) reveals fairly blue heterochromatin blobs as well as smaller pale blue dots of euchromatin. The mosaic structures of the four nuclei show a wide variability since the white flavanol-free areas are considerably different in both, size and subnuclear distribution.

The most opened and active configuration is reflected by Fig. 1 g, the chromatin-free nucleolus of which is, as usual, without flavanols. Sometimes larger blobs of heterochromatin or nuclear organizer regions (NORs) come to lie close to the nucleolar periphery.

Fig. 1 g shows clearly half the flavanol absorption of Fig. 1 f . During S phase a doubling of DNA takes place, and then the histones and also the flavanols should attain a two-fold increase which is really exemplified by an increase of absorption from 24 to 48 units (Fig. 1 g and f). By anaphase, the separating sister chromatids are pushed to the opposite poles and then contracted to form two synchronously structured telophase nuclei (Fig. 1 h). The two daughter nuclei exhibit rather small colourless interchromatin spaces being interspersed throughout the closely located, dark blue mottled chromatin blobs.

The absorption data from the activated nuclei (Fig. 1 c) range from 44 to 66 and indicate a higher variation than those of the silenced ones. A dense and diffuse blue cluster of silenced cells (Fig. 1, a) ranges from 64 to 70 absorption units and shows a low variation.

Application of flavanols to nuclei of herbs and humans

It is clearly of evident importance to know whether in herbs or humans which are genuinely without nuclear flavanols take them up in vitro. There is no doubt that a general affinity of chromosomes for flavanols is of immense significance for the plant kingdom and in particular for human health.

Three plant species which genuinely do not contain nuclear flavanols were investigated (Fig. 1 i-t). EGCG (10 μM) in watery solution was externally supplied. It is striking that supplied EGCG (Fig. 1 i, k, m) and flavanols of the green tea (Fig. 1 j, l, n) are capable to bind to nuclei. The rootlets of the three herbaceous species were investigated in the following order (Fig. 1): *Hyacinthus* (i, j), *Tulipa* (k, l) and *Allium* (1 m, n). After an incubation period of 3 hours each nucleus reflects a certain individuality of the flavanol landscape.

The characteristic symptoms found in the nuclei of *Hyacinthus* have more or less sharply mottled mosaic structures and show a change from fairly blue (i) to a rather faint blue (j). Two large nucleoli are seen in each nucleus.

In *Tulipa*, treatment with EGCG (Fig.1 k) resulted in a rather diffuse, only lightly mottled flavanol pattern throughout the nucleus. In such a diffuse blue environment even the two nucleoli display a tinge of blue. A very contrasting crisp structure is shown when using the treatment with green tea extract (Fig. 1 l).

In *Allium,* the nucleoplasm is strongly granulated by both euchromatin and heterochromatin (Fig. 1 m, incubated in EGCG) whereas the following nucleus displays a fine mottled lighter blue and a dispersal of dense heterochromatin blocks (Fig. 1 n, green tea). Additionally, the very large nucleolus is nearly completely encircled by a visibly dense blue border line.

Finally, human nuclei were sampled from epithelial cells of nasal mucosa (o, p), saliva secretion (q, r), and from cells of the forehead surface (s, t). It is to assume that some nuclei are affected by certain degrees of ageing which might affect structural aspects and also the flavanol pattern.

Each of the three groups was treated for 2 to 4 h with EGCG (Fig. 1 o, q, s) or green tea extract (Fig. 1 p, r, t). Nasal mucosa imbibed in EGCG shows a rather deep flavanol staining which is characterized by a conspicuous dot-like mottling (Fig. 1 o), especially near the nuclear periphery. The second nucleus from nasal mucosa treated with green tea extract is rather low in amount of flavanols, except the darker blue envelope area (Fig. 1 p). The low affinity for flavanols, except the envelope region, might be due to an advanced stage of degeneration.

Both nuclei from saliva secretions (Fig. 1 q, r) indicate a marked flavanol reaction. The first one (Fig. 1 q), treated with EGCG, reveals an apparent dark blue, diffuse flavanol reaction with condensed dark
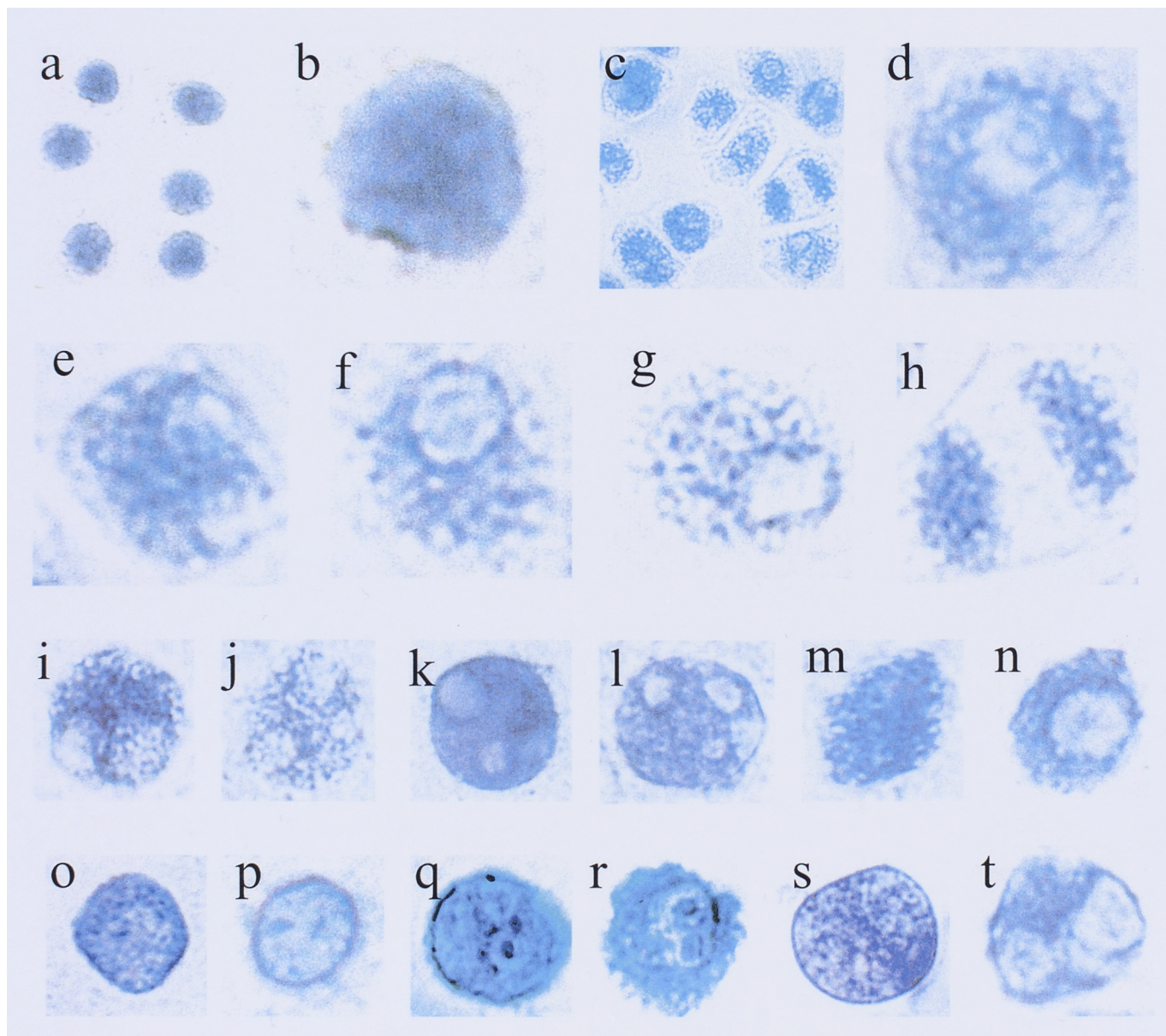
Fig. 1: Endogenous nuclear flavanols from *Camellia sinensis* and externally added flavanols (EGCG and green tea) to nuclei of herbaceous plants and humans.

Absorbance units (AU) measured at 640 nm are given in [brackets] as single values or as the mean ± standard deviation for the nuclei visible in the figures.

Diameters of the nuclei from tea bush range from 7 to 9 μm including those of Fig. 1a and 1c.

a. Silenced nuclei from the mature anther epidermis (7 μm in diameter) [AU 66.6 ± 2.1].

b. Magnification of one diffuse nucleus from Fig. a [AU 68.4].

c. Activated nuclei of a young anther during maximal cell cycling representing a meristematic cluster [60.1 ± 7.8].

d-g. Magnification of activated nuclei showing the typical mosaic pattern of flavanols. [AU: d, 57.2; e, 59.0; f, 48.1; g, 24.4]

h. Developing daughter cells of telophase with euchromatin interspersed with sharply mottled, darkly stained blobs of heterochromatin [AU 57.4; 63.5].

Herbaceous nuclei from *Hyacynthus* (i, j, *Tulipa* (k, l) and *Allium*

(m, n). EGCG (i, k, m) [AU: i, 66.0; k, 71.9; m, 74.1] and green tea (j, l, n). [AU: j, 44.0; l, 63.9; n, 60.0], Diameters of the nuclei range from 8-9 μm.

Human nuclei from nasal mucosa (o, p), saliva secretion (q, r) skin (forehead) epidermis (s, t). EGCG (p, r, t) [AU: p, 51.1; r, 78.0; t, 49.2] and green tea (o, q, s) [AU: o, 73.4; q, 79.; s, 65.3] Diameters of the nuclei range from 5-6 μm.

blotches, and a large part of the envelope line is exceptionally dark blue. The second nucleus (Fig. 1 r), imbibed in green tea, shows curious morphological details. The envelope is without flavanol reaction, probably a sign of degeneration. Also the organization of the nucleolar region appears to be anomal, as evidenced by two heavily stained dots and a partially darkly blue border line around the nucleolus.

An epidermal skin cell (forehead) treated with EGCG developed throughout the nuclear area a number of tiny but deep blue spots which are clearly interrupted by fairly pale blue areas (Fig. 1 s). In

addition, this nucleus is characterized by a sharply delimited, dark blue envelope. The second nucleus from skin, treated with green tea extract (Fig. 1 t), shows a moderate blue chromatin without pronounced blobs of heterochromatin. The two rather enlarged nucleoli are characteristic of a stronger transcriptional activity.

Summing up, the nuclei of the herbaceous plants and human cells range in flavanol absorption values from 44 to 79 which are roughly comparable with those of the tea nuclei.

Flavanols during mitosis of tea cells

The morphological change from G2 to the mitotic structures is dramatic because the nuclear envelope is removed and elongated chromosome structures become visible (Fig. 2 and 3). It is commonly accepted that metaphase chromosomes are very compacted compared with the interphase (arrow, Fig. 2 a). Pronounced DNA density during metaphase is affirmed by the bright UV-fluorescence when stained with DAPI (Fig. 2 b) whereas the interphase (arrow) appears lighter blue. Fig. 2 c shows the transit stage from metaphase to anaphase. At the spindle axis there is a fairly blue staining deposition of chromatin and some pale chromosomes begin to move toward the poles. A certain degree of diffusion is recognizable. Fig. 2 d shows an example in which a more concrete, long armed and zigzagging chromosome is visible.

Hook shaped chromosome segments showing blue streaks can be seen in Fig. 2 e. Other chromosome segments (Fig. 2 f) stain a darker blue, appear denser crowded and curved to semi-circles. The next Fig. 2 g shows one of the two daughter nuclei during early telophase configuration, with rather uniform organization of chromosomes which are more or less bended. Finally, both daughter nuclei of a progressed telophase (Fig. 2 h) appear in a more diffuse state, in contrast to the pronounced fine-grained blue mosaic as seen in Fig. 1 h.

The frequently dark blue areas of nuclei support the idea that flavanols, aside from their affinity to histones, probably also bind to DNA. Fig. 2 i shows a sample of DNA (calf thymus) incubated for 3 h in a watery solution of EGCG (50 μM) with dark blue staining.

Stability of catechin in presence of modified histone peptides

In a recent paper, it was shown that not only histone proteins but also fragments of them synthetically produced have the potential to reduce more or less strongly the oxidative degradation of catechin (FEUCHT et al., 2009 and 2012 a). Thus, the fragment 71-85 of the H4-core protein (TYTEHAKRKTVTAMD) leads to a significant decrease of the rate of the oxidative degradation. Even distinctly smaller peptides of the H4-core fragment can exert an influence on the protection of catechin degradation.

The time dependent degradation of catechin in the presence of oxygen is shown for the wavelength 434 nm in Fig. 4 (two curves). In the presence of the peptide HAKRKT being a segment of TYTEHAKRKTVTAMD the oxidative degradation is significantly retarded (see Fig. 4). When the two lysine amino acids are acetylated according to the epigenetic histone code, the peptide HAK(ac)RK(ac)T reduces also the degradation of catechin, however to a less extend than the unmodified peptide. It is well documented that both lysine positions of the peptide can be acetylated in the H4-core protein (ZHANG et al., 2003; HYLAND et al., 2005).

Discussion

Flavanols involved in fine-mapping of the chromatin landscape

In the literature, a large body of data is offered indicating that nuclear chromatin arrangement is precisely regulated by both genetic and epigenetic information (EXNER and HENNING, 2008). The find-
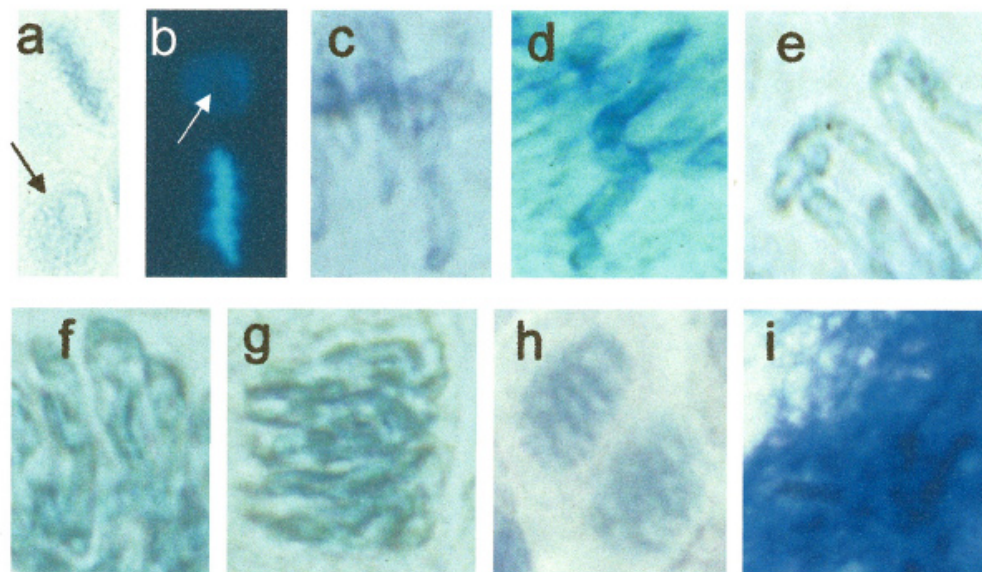

Fig. 2: Curved and compacted chromosomes during mitosis.
    a. Compacted metaphase compared with a loosened interphase nucleus (arrow).
    b. UV micrograph to visualize DNA of both metaphase and interphase (arrow).
    c. Transit from metaphase to anaphase with longer and shorter arms of diffuse chromosomes.
    d. Stretched chromosome with a zigzag configuration and condensed flavanols confined to distinct sectors.
    e. Curved chromosomes during anaphase with pale blue and moderate blue sectors.
    f. Semicircles of chromosomes during anaphase.
    g. One daughter cell at early telophase shows bending and intermingling chromosomes with variable density of flavanols.
    h. Diffuse early telophase configuration.
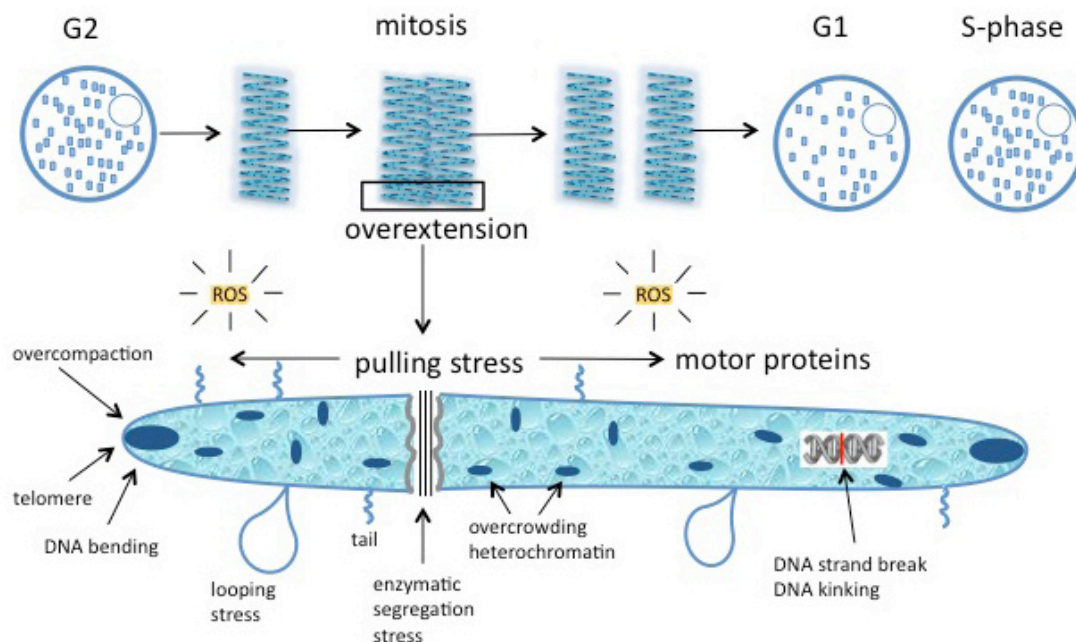   i. DNA (calf thymus) incubated in watery EGCG solution (20 μM).

Fig. 3: Four proposed properties of flavanols to counteract harmful effects during mitotic destabilization of the chromosomes.
1. Affinity of flavanols to DNA and histones might provide more stability against too strong bending, compaction, stretching and breakage of chromosomes, especially with regards to exposed tails and loops.
2. Flavanols are involved in optimal balancing of enzymatic activities, for example of separases, RNAses and polymerases.
3. Flavanols act in oxygen radical scavenging to allow a reductive nuclear milieu being favourable for mitosis and growth promotion.
4. Flavanols bind to specific histone proteins in dependence on the epigenetic histone code, and thus influence directly the gene activity.
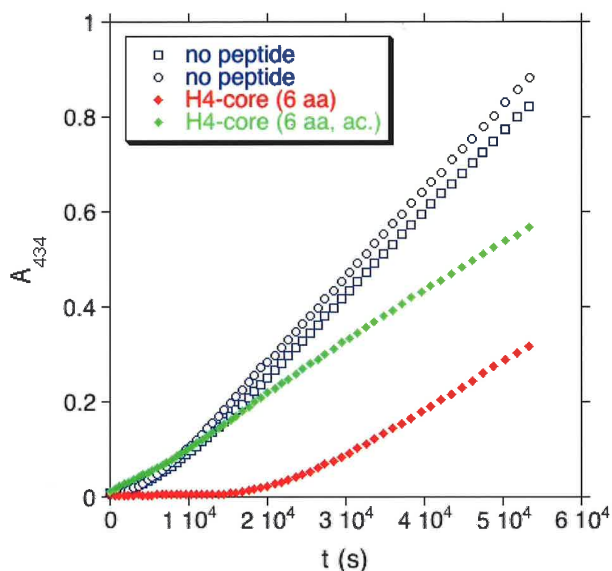

Fig. 4: Absorbance-time (A_{434} vs. t) curves of catechin degradation (0.2 mg catechin/mL, 0.69 mM) in the presence of peptides HAKRKT and HAK(ac)RK(ac) each about 1 mg/mL. Further conditions: 20 °C, 0.1 M Tris buffer pH 8 with 3.3% ethanol, 10 mM $MgCl_2$ (see FEUCHT et al., 2012 a), and gassed with O_2; aa means amino acid, 6 refers to hexapeptide, ac represents acetylated.

ing that the blue staining flavanols associate with histones (POLSTER et al., 2006) prompted us to study more thoroughly possible links between the chromatin activity and patterning of flavanols. Recent studies with hemlock (*Tsuga canadensis)* indicated that the blue coloured flavanol landscape from activated chromatin is organized as a mosaic-like structure being quite different from the evenly diffuse

blue appearance of silent chromatin (FEUCHT et al., 2011). Evidently, the flavanol characteristics of the tea nuclei (Fig. 1 d-g) share a strong similarity with those of the very distantly related hemlock fir.

Other plant species which genuinely are without nuclear flavanols, as shown in the rootlets of *Allium cepa* L., *Hyacinthus romanus* L. and *Tulipa gesneriana* L. obviously respond with blue colouration when flavanols are applied externally. Moreover, also human nuclei follow this pattern. Notably, all these flavanol-treated nuclei (Fig. 1 i-t) indicate a similar spatial mosaic arrangement as shown by those of the tea plant (Fig. 1 d-g).

In humans, it would be of particular medical concern to apply new methods which allow an import of externally supplied flavanols into the nuclei to yield protection against oxidative damage. New analytic methods being much more sensitive in detecting lowest amounts of nuclear flavanols would be very promising (MUELLER-HARVEY et al., 2012). Destruction of hamster nuclei by aflatoxins could be significantly reduced by adding catechin in vitro (BAUER et al., 2009). Various tissues of the cow (brain, kidney, liver and spleen) were shown to be a strong sink for added flavanols (POLSTER et al., 2002). When young piglets were fed with apple- or red-grape pomace various flavanols could be detected in ileum, liver and kidney and white blood cells responded with changes in mRNA expression (SEHM et al., 2011).

Intranuclear flavanol distribution and flavanol-free nuclear spacing

Regarding the blue-white mosaic pattern of active nuclei (Fig. 1 c-g) there is evidently a large-scale change in both size and staining intensity of the chromatin blobs. Consequently, the variation of the nuclear flavanols (Fig. 1, a1-a6 and c1-c11) is significantly higher in meristematic clusters. The distinct values for blue colour absorption are 7.4 % as compared with 1.4 % of the silenced ones. Obviously, flavanols respond to changes in activity of local intranuclear micro-

domains as already shown for hemlock (FEUCHT et al., 2011). In Fig. 1 f twice the absorption value of flavanols is found compared with Fig. 1 g. It is to note here that during S phase a doubling of DNA from 2 C to 4 C takes place. This is accompanied by a doubling of histones which, in turn, would imply that also the flavanols increase on parallel lines. In either case, the S phase period might span over a time space of about 5 to 8 hours. So, by this time there might be single nuclei which just have reached only amounts of 30% or 60% and so on regarding the final values of DNA, histones plus flavanols. In essence, variability in flavanol aggregation within a cluster of nuclei should be expected.

Two cell clusters of the same anther can be similar or different in density of flavanols (Tab. 1). Such a feature is due to meristematic cell lineages starting from one initial cell and finally consisting of 4 or seldom 8 daughter cells (FEUCHT et al., 2011). Lateron, the lineage is not prolonged, however, by longitudinal divisions of 2 or 4 lineage cells, about 8 to 12 daughter cells are established which are visibly synchronized in size, shape and density of nuclear flavanols. A cluster rather low in flavanols may relate to different aspects. For example, the import of histones into the nuclei can be restricted by a limited availability of sufficient cellular energy and ATP (BREEWER and GOLDFARB, 1990). A most extreme flavanol loading of nuclei from yellow flecked upper epidermal cells might be due to special stress conditions imposed by fungal infection (Tab. 1). Irradiation stress is less likely since the tea shrubs are grown in the greenhouse.

Are flavanols involved in modulating the histone-DNA contacts and gene transcription?

Transcription activities are particularly focussed to distinct regions of chromosomes as for instance to lysine residues of the core histones H3 or H4 (MERSFELDER and PARTHUN, 2006). So far, if flavanols interact with acetylated or methylated histone domains, then they inevitably should modulate distinct patterns of gene expression. Roughly, light or dark blue flavanol patterning should result in expression and repression of the tea nuclei.

The flavanol-free interchromatin 'channels' (Fig. 1 d-g) can be considered as transport highways to achieve high transcription activity. They facilitate movement of many compounds, for example non-histone proteins and small RNAs which are necessary for any transcriptional activity of a nucleus (GASSER, 2002; MISTELI, 2007). Non-histone proteins coordinate the multiple activities of transcription (GORSKI et al., 2006) or special protein kinases together with RNAs spread out to distinct target sites within nuclei and chromosomes (LI and REINBERG, 2011).

Despite the relative low amount of *DNA* in heterochromatin according to RICHARDS and ELGIN (2002), nevertheless a deep blue staining was found for flavanols (compare Fig. 1 g). These roughly spherical bodies of highly folded heterochromatins are considered to be engaged primarily in gene silencing. However, FRANSZ et al. (2003) argue that methylation combined with silencing, for example of histone lysines, is not generally sufficient to form heterochromatin. Perhaps, in chromatin blobs the dense flavanols may additionally be involved in impeding faulty intermingling of genes. As discussed by DORIER and STASIAK (2010) such an intermingling is a high-risk incident.

Cross-linking of proteins with polymeric phenolic compounds up to tannins is, roughly speaking, widely accepted. However, monomeric catechins, as located in nuclei (FEUCHT et al., 2008) by far have not gained a proper scientific interest regarding the affinity of phenols to proteins.

The variable mode and strength of flavanol-binding to specific histone sites can be manipulated by a number of facilities, such as van der Waals interactions, hydrogen bonds, hydrophobic effects,

electrostatic forces and molecular size of the interacting protein and phenol structures (HAGERMAN, 2012). There is surely a potential link between different binding modes and biological activity during gene transcription. Yet, reversible binding should be of crucial importance for chromatin remodelling.

Do nuclear flavanols operate as antioxidants?

Principally, DNA wrapped round the histones is continuously exposed to oxidative stress (APEL and HIRT, 2004) and mitosis, depleted from the nuclear membrane, surely suffers from more oxidative damage.

Fragmentation of DNA can be markedly reduced by monomeric catechin at 0.5 mM (HUANG et al., 2006). Flavanols (catechins), donating electrons or hydrogens, are well-known to operate in an antioxidative fashion (STANGL et al., 2006). Transcriptionally important basic amino acids of histones, such as lysine, arginine and histidine, are protected against oxidative stress by proanthocyanidins, mostly dimeric catechins, of pomegranate juice (GUO et al., 2008). Collectively, transcription is prone to increased rates of oxidative DNA damage. This point needs more attention. Histone buffer solutions, even if gassed with oxygen, nevertheless are capable to inhibit oxidation of catechin (FEUCHT et al., 2009).

There is another crucial point. During mitosis, the nuclear membrane becomes disassembled so that the chromatides are additionally exposed to a novel source of cytoplasmic oxygen radicals. Even a low-level oxidative stress can be responsible for cell cycle arrest (CLOPTON and SALTMAN, 1995). Under such conditions also the auxin molecules (IAA) involved in mitosis might suffer from rapid oxidative degradation. Auxin nestled in a reductive cell environment enforces the transcriptional processes during cell cycling (EXNER and HENNING, 2008; SPOEL et al., 2010). In this connection, it should be emphasized that catechin together with auxin is capable to double the fresh weight of callus tissues as compared with auxin applied alone (FEUCHT and NACHIT, 1977). Using callus cultures (FEUCHT and TREUTTER, 1995), after a 4 weeks growth period the content of residual IAA was roughly 8-fold in the culture media containing IAA plus catechin compared with IAA alone. Most exciting, however, in these experiments was a highly significant increase of proteins in the catechin-treated fast dividing callus tissues compared with the controls.

Yet, it is not correct to delimit the role of ROS (reactive oxygen species) molecules solely as being hazardous by-products of metabolic activities (NOCTOR et al., 2007). At subtoxic levels, they also operate as dynamic signals which activate transcription factors (VAN BREUSEGEM et al., 2001). Both, ROS-mediated signaling of histidine kinase and gene expression are tightly integrated into the cell cycling processes (APEL and HIRT, 2004).

Support flavanols the physical stability of overstretched, sharply bent or over-compacted chromatin?

From prophase to telophase the nucleosome territories are exposed to extreme physical stress (Fig. 2 c-h, Fig. 3). At transit from prophase to metaphase kinesin-like motor proteins manipulate a tight congression of chromosomes along the metaphase plate. Then, at transit from metaphase to anaphase the chromatides suffer from strong physical tension by too much pulling forces from microtubule motors. Intrachromosomal cohesion is known to be achieved by adherence-type proteins. Cohesins keep sister chromosomes together and readily also small chromatin granules (PERIC-HUPKES and VAN STEENSEL, 2008). Is there a functional similarity or even cooperation of cohesines and flavanols in stabilizing supercoiled DNA as well as in the case of extended DNA stretches looping out from chromosomes?

During the cell cycle, special conditions account for a more fluid appearance of blue-coloured chromatin, at least temporally (Fig. 2 c, h). Indeed, diffusion-driven mechanisms are in play during microtubule flux of the poleward sliding nucleosomes (BERG et al., 1981). Hereby, proteins or histones might be covered by a fluid hydration shell allowing a lower friction during anaphase movement.

In this context, of utmost importance is the finding that flavanols also bind to DNA as shown for EGCG by chemo-physical methods (KUZUHARA et al., 2006). A similar result, based on histochemical blue staining reaction, is obtained if DNA (from calf thymus) is incubated in EGCG or green tea flavanols (Fig. 2 i). DNA and RNA are faced by nucleases and these enzymes are repressed by EGCG (TICHOPAD et al., 2005). Active euchromatin was found to be very sensitive to nucleases (PAUL and FERL, 1998). Complexation of various polyphenols with DNA results in a reduction of strand breaks (BIDEL et al., 2010).

Apart from the pulling stress as discussed above, a basic problem of the nucleus is that the thousand-fold compaction of the genome, as produced by curled and folded structures up to 700 nm, provide an enormous intranuclear pressure. Here again, the pressure stress might be damped down by flavanols attached to histones and supercoiled DNA. Phenolic hydroxyl groups build hydrogen clamps, for instance with proline-rich protein complexes which, as explicitly emphasized by HASLAM (1998), are exposed to conditions of overstretching, bending and distortion. If a single hydrogen bond (O-H---O) is broken, then the whole group of a hydrophilic, hydrogen-bonded network will dissipate (HASLAM, 1998).

All the features discussed here advocate for an intricate involvement of the nuclear flavanols in regulating nucleosome conformation. However, no individual factor such as the flavanol molecule is capable of playing an isolated physiological role. As most of these complex interactions as yet are barely understood, the flavanols located in nuclei need much more detailed basic research.

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