

(FR3T3-A2N2) and by the ts mutant (=FR3T3-tsaN1) are not significantly inhibited by somatostatin compared with the controls (fig. 2, c, d and e).

**Discussion.** Neither our *in vivo* nor our *in vitro* examinations were able to establish an inhibitory effect of somatostatin on tumor cell growth. These results are in accordance with our own experience from an unpublished pilot study in which we could not find an antitrophic effect of somatostatin, injected once daily, on various tumor types in mammals (mouse mammary tumor, Viennese leukemia, MCA-induced transplantable sarcoma, hamster melanoma, rat hepatoma, Yoshida, Lewis lung tumor, mouse myeloma, Ehrlich ascites tumor, osteosarcoma)<sup>8</sup>.

We deliberately used very high doses of somatostatin in both the *in vivo* and the *in vitro* studies. Even when taking into account the short half-life time and the rapid metabolism of somatostatin *in vivo*, this high dose should give a sufficient concentration of somatostatin. So we do not believe that the failure of tumor growth inhibition is due to low dose administration. We suggest that the antitrophic effect of somatostatin reported in the literature cannot be attributed to a direct somatostatin action on growing cells but is due to an inhibition of hormones or metabolic

mediators responsible for the trophic action. E.g., the inhibition of gastrointestinal cell proliferation by somatostatin is explained by inhibition of gastrin<sup>3</sup>, the main trophic hormone of the stomach<sup>9</sup>; the inhibition of ribosomal protein synthesis is due to cyclic AMP inhibition by somatostatin<sup>4,5</sup>. We conclude from our results that there is no indication for applying somatostatin as an antitumor agent in cancer treatment.

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## Argyrophilic intranuclear bodies of plant cells

P.W. Barlow

*Agricultural Research Council Letcombe Laboratory, Wantage, Oxon, OX12 9JT (England), 5 February 1981*

**Summary.** Within interphase nuclei of meristematic cells of plants there are small spherical bodies that stain intensely with silver. Their number is related to the DNA content of the species.

Silver nitrate has proved to be a useful reagent with which to stain the nuclei of plant and animal cells<sup>1-8</sup>. It reveals certain details of nuclear structure that are not shown up by the more commonly employed nuclear stains. In addition to the nucleolus and the nucleolar organizer region of mitotic chromosomes, both of which have a strong affinity for silver, another class of argyrophilic body is often seen within interphase nuclei of plants<sup>5,8,9</sup>. A nucleus may contain many of these bodies. They are small and spherical and lie in the nucleoplasm. At the ultrastructural level they probably correspond to the so-called 'dense bodies' that lie within areas of diffuse chromatin<sup>8,10</sup>. Since no suitable name has yet been given to them, they will be called argyrophilic intranuclear bodies (AIBs). Results are presented here on the number of AIBs within nuclei of root meristem cells of several plant species.

**Materials and methods.** Young root tips were removed from seedlings and were fixed and impregnated with AgNO<sub>3</sub> according to Risueño et al.<sup>5</sup>. The silver-impregnated apices were embedded in wax and sectioned longitudinally at a thickness ranging from 5 to 16 µm according to the species. This ensured that a high proportion of nuclei in any section were uncut. After dewaxing, the sections were mounted in Canada Balsam under a coverslip. Nuclei in the middle of the stelar portion of the meristem were selected for measurement of nuclear and nucleolar volumes and for counting the number of AIBs per nucleus.

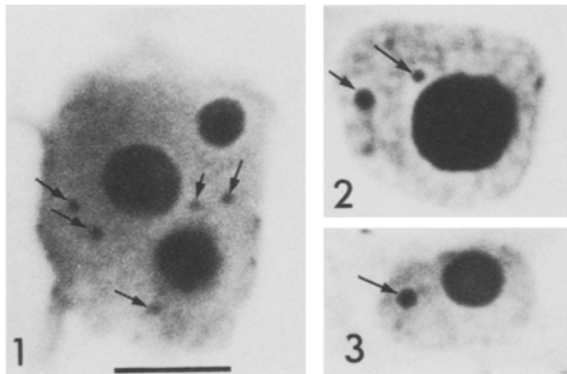
**Results.** Figures 1-3 show the appearance of AIBs within nuclei of 3 species after silver staining. The diameter of the AIBs is rather variable, but the maximum diameter is about 1 µm. The largest AIBs seem to be present in species which have the fewest mean number of AIBs per nucleus (compare figs 1 and 3).

The species investigated have a wide range of nuclear DNA contents. The relationship between the mean number of AIBs per nucleus and the 2C DNA content (fig. 4) shows that species with a 2C DNA content of 5 pg or less lack, or have very few, AIBs, while in species with 5 pg of DNA or more the mean number of AIBs per nucleus increases with increasing DNA content.

The relationship between the number of AIBs and nucleolar volume bears on the question of whether the nucleolus and AIBs have a common structural basis<sup>9-12</sup>. Although the mean nucleolar volume of early interphase nuclei and log<sub>10</sub> 2C DNA content show a positive correlation ( $p < 0.01$ ) in the species examined, the correlation between nucleolar volume and number of AIBs is rather weak ( $p > 0.05$ ). Moreover, a detailed examination shows that within the root apex of any one species the mean number of AIBs per early interphase nucleus is independent of the mean nucleolar volume of the same sample of nuclei (table).

**Discussion.** At least 2 interpretations are possible for the origin of the AIBs in plant cell nuclei. One of these interpretations, for which there is circumstantial evidence suggested by the appearance of silver-stained early interphase nuclei<sup>4,13</sup>, is that the AIBs are clumps of prenucleolar material that have persisted into interphase<sup>11,12</sup>. The prenucleolar material originates from a pellicle of ribonucleoprotein (RNP) that surrounds the mitotic chromosomes<sup>13</sup>; the AIBs of interphase nuclei may correspond to those portions of this pellicle which did not coalesce to re-form the nucleolus at the end of telophase. The numerical interrelationships between AIBs and DNA content, and also nucleolar volume, seem consistent with this proposed origin and composition of the AIBs, since the more abundant the nucleolar and prenucleolar material, the more numerous

the AIBs that might be formed at early interphase. However, this can be only a partial explanation since it does not account for why species with low nuclear DNA contents have virtually no AIBs. In addition, prenucleolar material not used to construct the new nucleolus might be expected eventually to disperse during interphase, yet the AIBs seem to persist throughout interphase, especially in species with high nuclear DNA contents. Nuclear structure may play a role in their apparent persistence. The organization of the chromatin within the interphase nucleus is determined by its DNA content<sup>14</sup>. Species with 2C nuclear DNA contents below about 5 pg have an areticate nuclear organization (where dense chromatin is scarce and is confined to points against the inside of the nuclear envelope), while species with 2C DNA contents above 5 pg have a reticulate structure (where dense chromatin is more abundant and forms a meshwork within the nucleus). Prenucleolar bodies that do not become incorporated into the nucleolus possibly disperse (perhaps at the nuclear envelope<sup>9</sup>) more easily in areticate nuclei than in reticulate nuclei where they become trapped within, and persist amongst, the meshwork of dense chromatin.



Figures 1-3. Interphase nuclei from root meristems of 3 species impregnated with AgNO<sub>3</sub> to show the argyrophilic nucleolus and intranuclear bodies (AIBs) (arrowed). Figure 1. *Avena sativa*. Figure 2. *Pisum sativum*. Figure 3. *Daucus carota*. Scale bar represents 5  $\mu$ m.

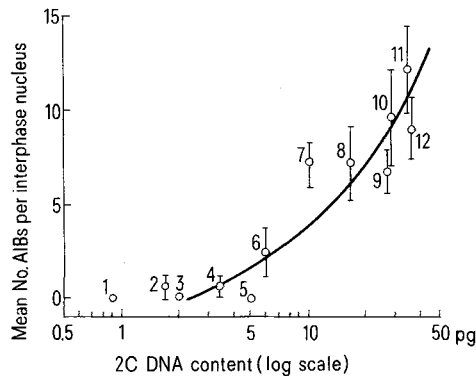


Figure 4. Relationship between the mean number of AIBs in interphase nuclei in root meristems of various plant species and the 2C DNA content of the species. Each point represents 1 species. The error bar on each point denotes half the standard deviation. The number beside each point identifies the species: 1, *Raphanus sativus*; 2, *Lupinus angustifolius*; 3, *Daucus carota*; 4, *Brassica napus*; 5, *Helianthus annuus*; 6, *Zea mays*; 7, *Pisum sativum*; 8, *Secale cereale*; 9, *Avena sativa*; 10, *Vicia faba*; 11, *Allium cepa*; 12, *Triticum aestivum*.

Nucleolar volume and number of AIBs in early interphase nuclei<sup>a</sup> in different locations within the root meristem of 2 species

Species	Location in meristem	Mean nucleolar volume ( $\mu$ m <sup>3</sup> )	Mean number of AIBs
<i>Pisum sativum</i>	Quiescent centre (QC)	6.9	2.8
	Stele 350 $\mu$ m above QC	13.7	6.6
	Stele 1200 $\mu$ m above QC	19.9	4.4
<i>Zea mays</i>	QC	8.2	4.9
	Cap initials	12.7	4.3
	Stele 250 $\mu$ m above QC	19.1	5.1

<sup>a</sup>Early interphase nuclei were those that constituted the fraction at the smaller end of the complete range of nuclear volumes measured: this fraction was 15% of the total sample of 120-200 nuclei.

A 2nd interpretation of the AIBs, for which there is some preliminary experimental evidence<sup>15</sup>, is that they are aggregates of RNP which are synthesized in the nucleus (perhaps originating from the nucleolus) and are in transit to the cytoplasm<sup>9</sup>. In this case the number of AIBs would be the resultant of their rate of production and their rate of export from the nucleus; this could account for the various mean number of AIBs recorded for nuclei in different regions of the root apex (table) since the nuclei in these regions have different rates of RNA synthesis and transport<sup>16</sup>. The rate of export of AIBs from the nucleus might also be influenced by the organization of the chromatin; reticulate chromatin may be more of an impediment to their export than areticate chromatin. It should be possible to devise experiments which would establish the origin and function of the AIBs.

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