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Phil. Trans. R. Soc. Lond. B 1978 **283**, 407-409
doi: 10.1098/rstb.1978.0045

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Phil. Trans. R. Soc. Lond. B. **283**, 407–409 (1978) [407]
Printed in Great Britain

Molecular architecture of *Drosophila* chromatin

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[Plates 1–4]

The intact interphase genome of *Drosophila melanogaster* has been isolated by sucrose gradient centrifugation after gentle lysis of tissue culture cells in 0.9 M NaCl–0.4 % Nonidet P40. The non-viscous, folded DNA sediments as a single broad 5000*S* peak in a complex with RNA (a fraction of the nuclear nascent RNA) and protein (all of the four intranucleosome histones: H2A, H2B, H3 and H4).

The folded DNA is supercoiled and can be relaxed to more slowly sedimenting forms either by intercalating ethidium, or by nicking with DNase I. Incomplete DNase treatment gives partially relaxed complexes, indicating that each nick relaxes only a stretch of DNA (defined as a supercoiled DNA loop) without affecting the superhelical content of the rest of the genome. The concentration of superhelices in the *Drosophila* folded DNA is the same as in *Escherichia coli* and SV40 closed circular DNAs, namely about one negative turn every 200 base pairs in 0.15 M NaCl at 26 °C. The estimated average size of the supercoiled DNA loops, about 85000 base pairs, equals the size of the larger *Drosophila* chromomeres.

The results are interpreted in terms of a model for the folded *Drosophila* genome which has the DNA constrained (by both protein–DNA and RNA–DNA interactions) into independent supercoiled loops containing on the average 400 nucleosomes per loop. Each nucleosome is composed of a histone core with the DNA wound around it in a 360° left handed toroidal supercoil; each nucleosome toroidal supercoil plus its relaxed internucleosome DNA contains, on the average, 200 base pairs.

The intact interphase genome of *Drosophila melanogaster* has been isolated by sucrose gradient centrifugation after gentle lysis of tissue culture cells in 0.9 M NaCl–0.4 % Nonidet P40. The non-viscous, folded DNA sediments as a single broad 5000*S* peak in a complex with RNA (a fraction of the nuclear nascent RNA) and protein (all of the four intranucleosome histones: H2A, H2B, H3 and H4; histone H1 and the non-histone proteins are eluted from the DNA under our conditions of isolation).

The folded DNA is negatively supercoiled and can be relaxed to slower sedimenting forms either by intercalating ethidium or by single strand nicking with DNase I.

Ethidium intercalation in 0.9 M NaCl both removes the negative superhelical turns and dissociates the four intranucleosome histones from the DNA. The four histones are dissociated in equimolar concentrations, and the relative proportion of histones displaced from the DNA is a function of ethidium concentration. The histones are completely dissociated from the folded DNA at the ethidium concentration which removes all of the negative superhelices. Thus the data strongly suggest that the rotation of the Watson–Crick helix which accompanies ethidium intercalation causes the loss of nucleosomes from the DNA.

DNase I relaxes the complexes gradually. Incomplete DNase treatment gives partially relaxed complexes, indicating that each nick relaxes only a stretch of DNA (defined as a supercoiled DNA loop) without affecting the superhelical content of the rest of the genome. The

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estimated average size of the supercoiled DNA loops, about 85000 base pairs, equals the size of the larger *Drosophila* chromomeres.

Complete DNase I relaxation results in structures which have about one half of the original sedimentation velocity (2500 instead of 5000*S*) but which still retain all of the intranucleosome histones. This suggests that the DNase induced expansion of the folded genome is the result of a relaxation of the *inter*nucleosomal DNA coils and not the *intra*nucleosomal DNA (Benyajati & Worcel 1976).

We have now examined the folded *Drosophila* genomes by the electron microscope. The genomes can be readily isolated free from other cellular components by sucrose gradient centrifugation and can be prepared for electron microscopy by centrifugation onto an e.m. grid (Worcel & Benyajati 1977). The electron microscopy confirms our previous report, based on biochemical criteria, that the folded genome is free from nuclear membranes and other cellular structures. Each complex flattens out covering an area of 12–14 μm in diameter, suggesting that it may be derived from a single nucleus.

Each flattened genome appears to be made up of many overlapping layers of twisted fibres (figure 1). Fragments of the genome can be found lying next to the main complex, and in these cases the individual fibres can be easily resolved (figure 2). The fibres are remarkably uniform and have a diameter of about 10 nm. We have also examined by the electron microscope the DNase I relaxed genomes. When spun onto the e.m. grid, the flattened DNase relaxed complex covers a larger area than the native complex (about 16–20 μm in diameter instead of 12–14 μm), confirming our previous suggestion that DNase I nicking causes an expansion of the genome (Benyajati & Worcel 1976). More remarkably, the DNase relaxed genomes appear to be made up of entangled 'beaded strings' instead of 10 nm fibres. Figure 3 shows a negatively stained, DNase treated flattened complex: it appears to be more open than the native complex (compare with figure 1) and shows beads (nucleosomes) as well as strings (internucleosome DNA). Shadowing increases the contrast of nucleosomes and internucleosome DNA in the DNase relaxed genome (figure 4). It is clear from these studies that the original 10 nm fibre is no longer present in the DNase treated complexes.

Our results (Benyajati & Worcel 1976 and above) indicate that the 10 nm fibre (figures 1 and 2) may well be the native form of the H1 depleted chromatin (see Figs 3, 10 and 11, Benyajati & Worcel 1976). Nicking with DNase I causes an expansion of the genome (as detected both hydrodynamically, see Fig. 5, Benyajati & Worcel (1976), and by e.m. examination of the DNase treated complexes, figures 3 and 4), which is not accompanied by a loss of protein (see Fig. 11, Benyajati & Worcel 1976). It seems reasonable to conclude that, depending on whether the internucleosome DNA is relaxed or not, identical H1 depleted nucleosomes will adopt a beaded string or a 10 nm fibre conformation.

Based on these results and other available data, we have constructed a detailed space filling model for the higher order DNA coiling in chromatin, starting with the symmetrical nucleosome core previously described (Weintraub, Worcel & Alberts 1976). The model defines the path of the DNA helix and the nucleosome arrangement along the DNA coil for both the 10 nm and the 20–30 nm fibres.

Following Sobell *et al.* (1976), we suggest that the DNA is coiled in the 10 nm nucleofilament in a *uniform* left handed toroidal supercoil of about 90 base pairs per turn and 4.7 nm pitch; the 140 base pair symmetrical nucleosome cores align themselves along this uniform DNA superhelix so that the isologous (Monod, Wyman & Changeux 1965) outer surfaces of adjacent

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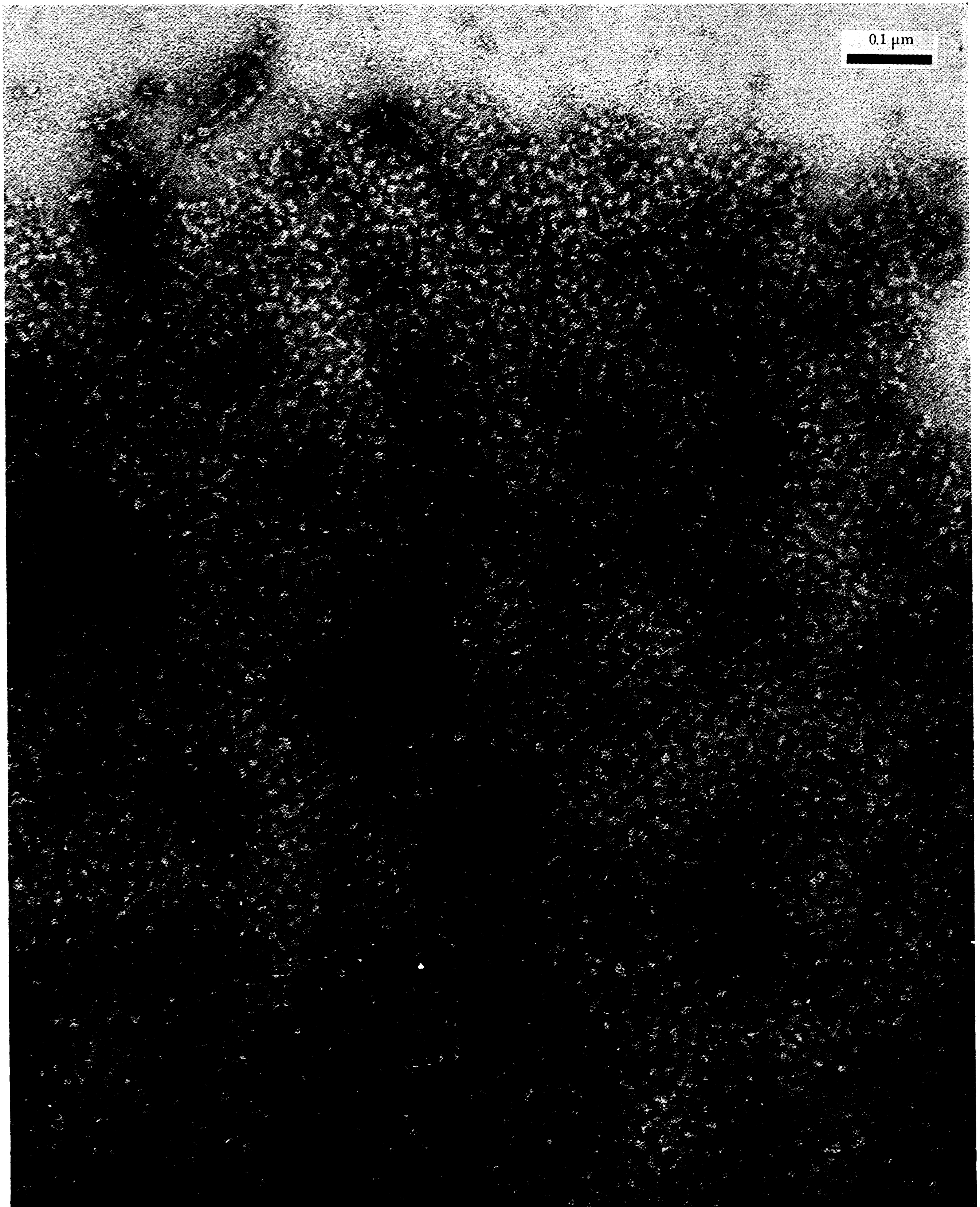

FIGURE 1. Electron micrograph of an area of a flattened *Drosophila* folded genome. The e.m. grid was negatively stained with 2% aqueous uranyl acetate. The edge of the flattened complex can be seen at the upper end of the photograph.

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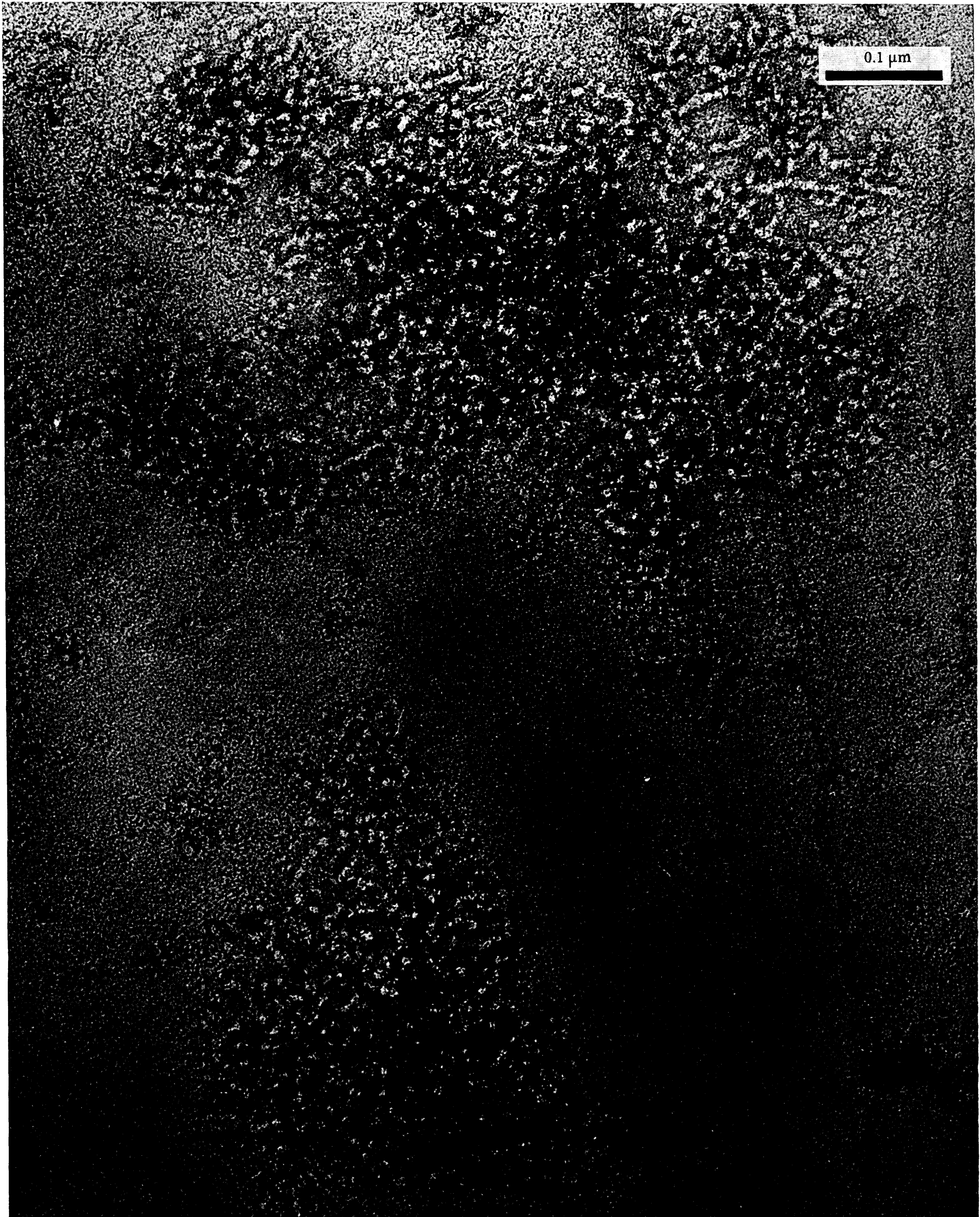

FIGURE 2. Electron micrograph of the 10 nm chromatin fibre in the H1 depleted folded *Drosophila* genome. The e.m. grid was negatively stained with 2% aqueous uranyl acetate. The two nucleofilament fragments shown are lying next to a flattened complex (not shown). Since we avoid handling the folded genomes, most breaks must occur when the complex adheres onto the charged e.m. grid; the attached DNA cannot unravel these conditions and the 10 nm fibre morphology is thus preserved in the fragment (Worcel & Benyajati 1977).

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FIGURE 3. Electron micrograph of an area of a flattened DNase I relaxed genome. The folded genome was relaxed with DNase I as previously described (Benyajati & Worcel 1976). The grid was negatively stained with 2% uranyl acetate. The edge of the flattened complex can be seen at the upper end of the photograph.

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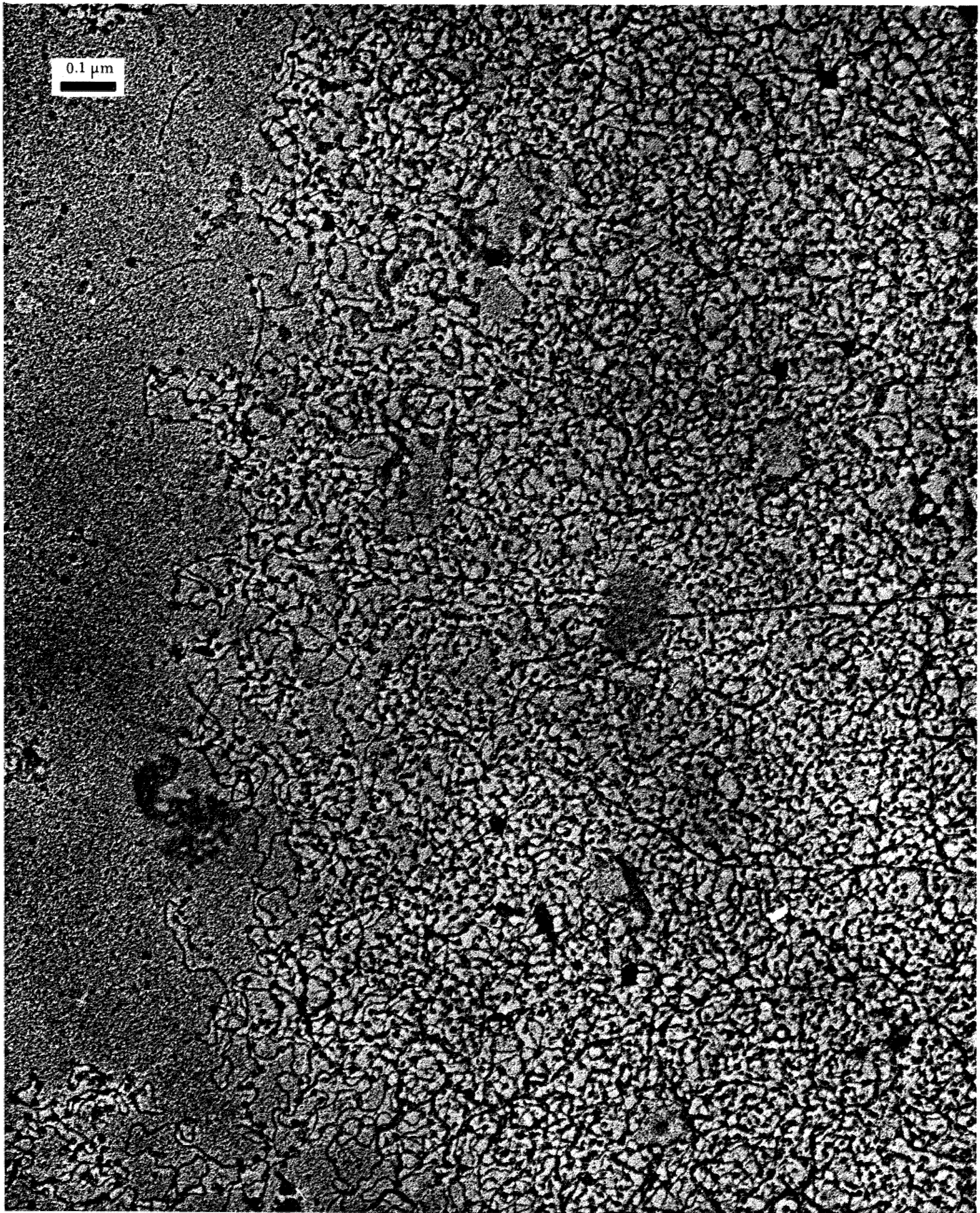

FIGURE 4. Electron micrograph of a flattened DNase I relaxed genome. The folded genome was prepared as described in the legend to figure 3, except that the grid was negatively stained and then shadowed with platinum. The edge of the flattened complex is on the left in this micrograph.

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nucleosomes touch and the internucleosome spacer DNA coils between them. A few single strand discontinuities (about one nick per 85000 base pairs; Benyajati & Worcel 1976) in the H1 depleted 10 nm fibre can thus relax the negatively supercoiled internucleosome DNA generating the 'beads on a string' appearance.

According to this general model of DNA coiling, in the *beaded string conformation* there will be a constant number of DNA superhelical turns (τ; Bauer & Vinograd 1968) per nucleosome (-1.5: 140 base pairs/-90 base pairs per turn) and a variable DNA superhelical concentration (which will be a function of the nucleosome repeat length). In the 10 nm *fibre conformation* the DNA superhelical concentration will be constant at $-1/90$ base pairs and the τ/nucleosome repeat will vary with the length of the repeat.

Using a space filling model (Worcel & Benyajati 1977) we can construct uniform 10 nm fibres containing different repeat lengths by (*a*) rotating each nucleosome about the axis of the DNA supercoil (changing the internucleosome contacts) and (*b*) sliding the DNA through the rotating nucleosome so as to regenerate the uniform -90 base pairs per turn supercoil. This coupled rotation and sliding will change both the τ/nucleosome repeat and the length of the repeat (Worcel & Benyajati 1977).

Nucleosome repeat lengths ranging from 150 to 210 base pairs have been reported in the literature (see, for example, Compton, Bellard & Chambon 1976). If the uniform DNA superhelix of -90 base pairs per turn is a common structural feature of all nucleofilaments as suggested, then it follows that the τ/nucleosome repeat will vary between -1.7 for a 150 base pair repeat (150 base pairs/-90 base pairs per turn) to -2.3 for a 210 base pairs repeat (210 base pairs/-90 base pairs per turn).

We propose that histone H1 binds to the uniform 10 nm diameter superhelix and coils it into tightly packed, 11 nm pitch super-super-helices ('solenoids'; Finch & Klug 1976) of *variable* diameter (between 20 and 30 nm). In our model, the 'thick' 20–30 nm fibre is stabilized by histone H1–H1 heterologous interactions (Monod *et al.* 1965) between adjacent helical turns of the nucleofilament, and the internucleosome spacer DNA is located on the outside. Symmetry considerations demand that changes in τ/nucleosome repeat in the nucleofilament (with accompanying changes in the length of the repeat) should lead to variations in the number of nucleosomes per helical turn and in the handedness of these turns in the 'thick' 20–30 nm fibre.

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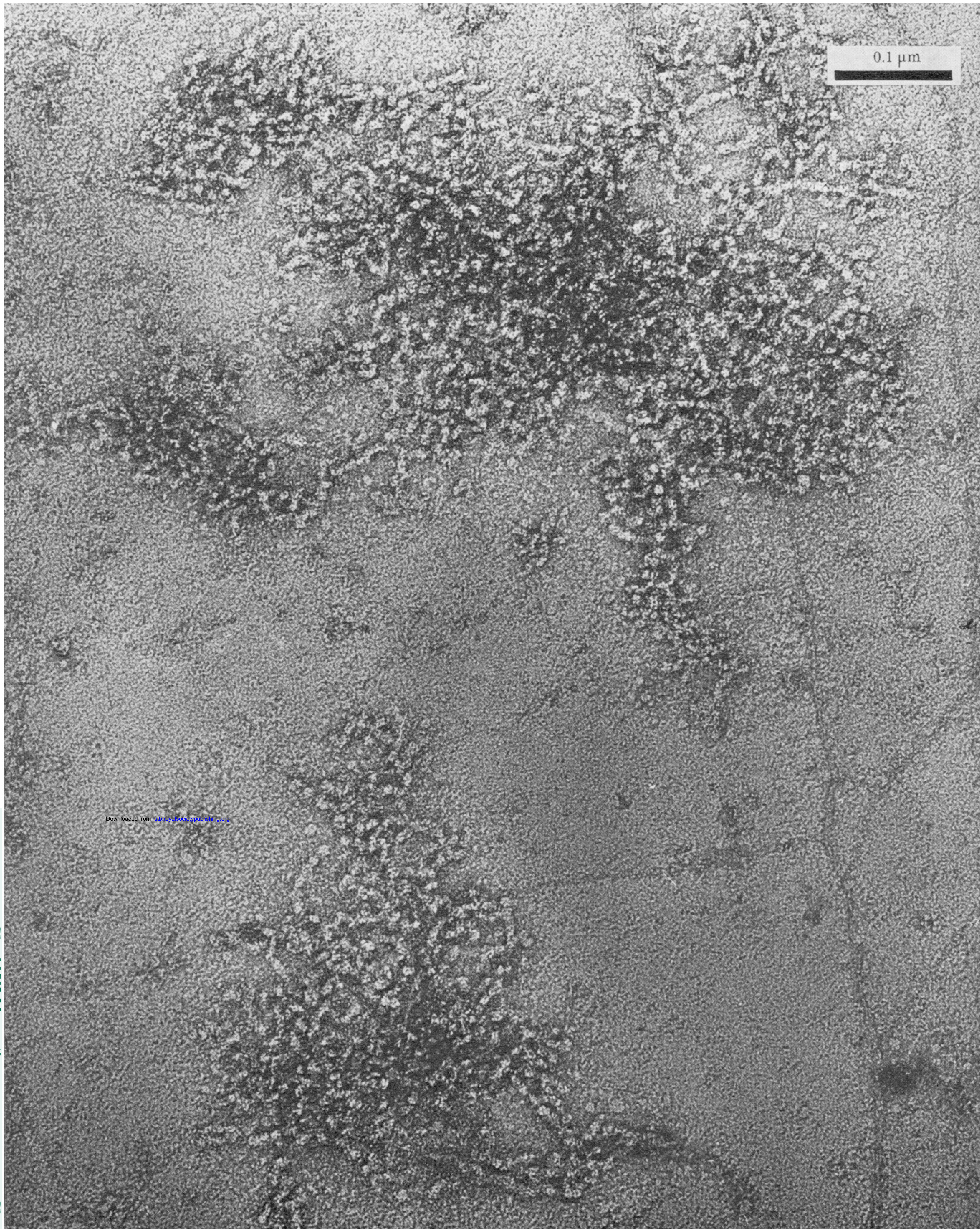

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FIGURE 2. Electron micrograph of the 10 nm chromatin fibre in the H1 depleted folded *Drosophila* genome. The e.m. grid was negatively stained with 2% aqueous uranyl acetate. The two nucleofilament fragments shown are lying next to a flattened complex (not shown). Since we avoid handling the folded genomes, most breaks must occur when the complex adheres onto the charged e.m. grid; the attached DNA cannot unravel these conditions and the 10 nm fibre morphology is thus preserved in the fragment (Worcel & Benyajati 1977).

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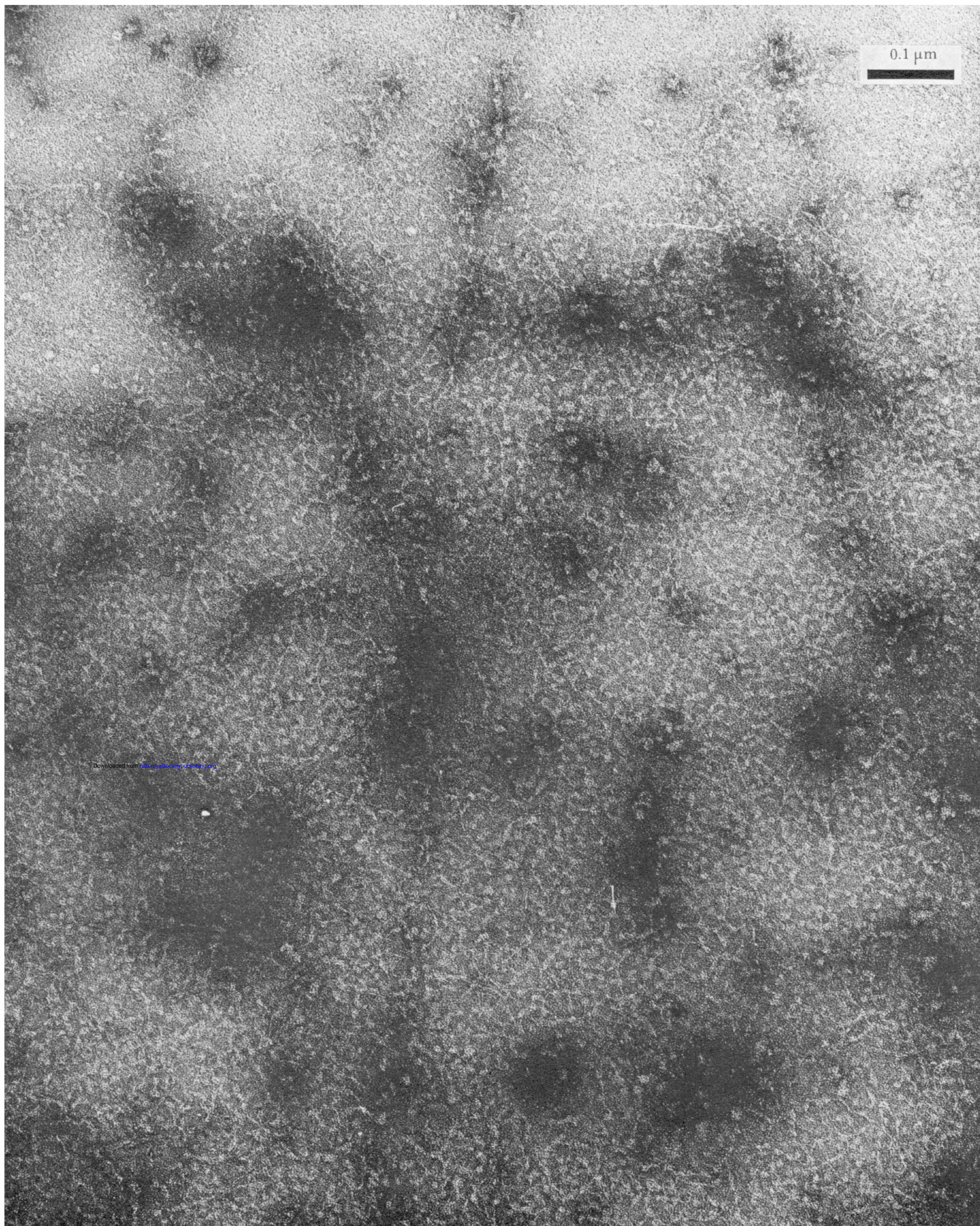

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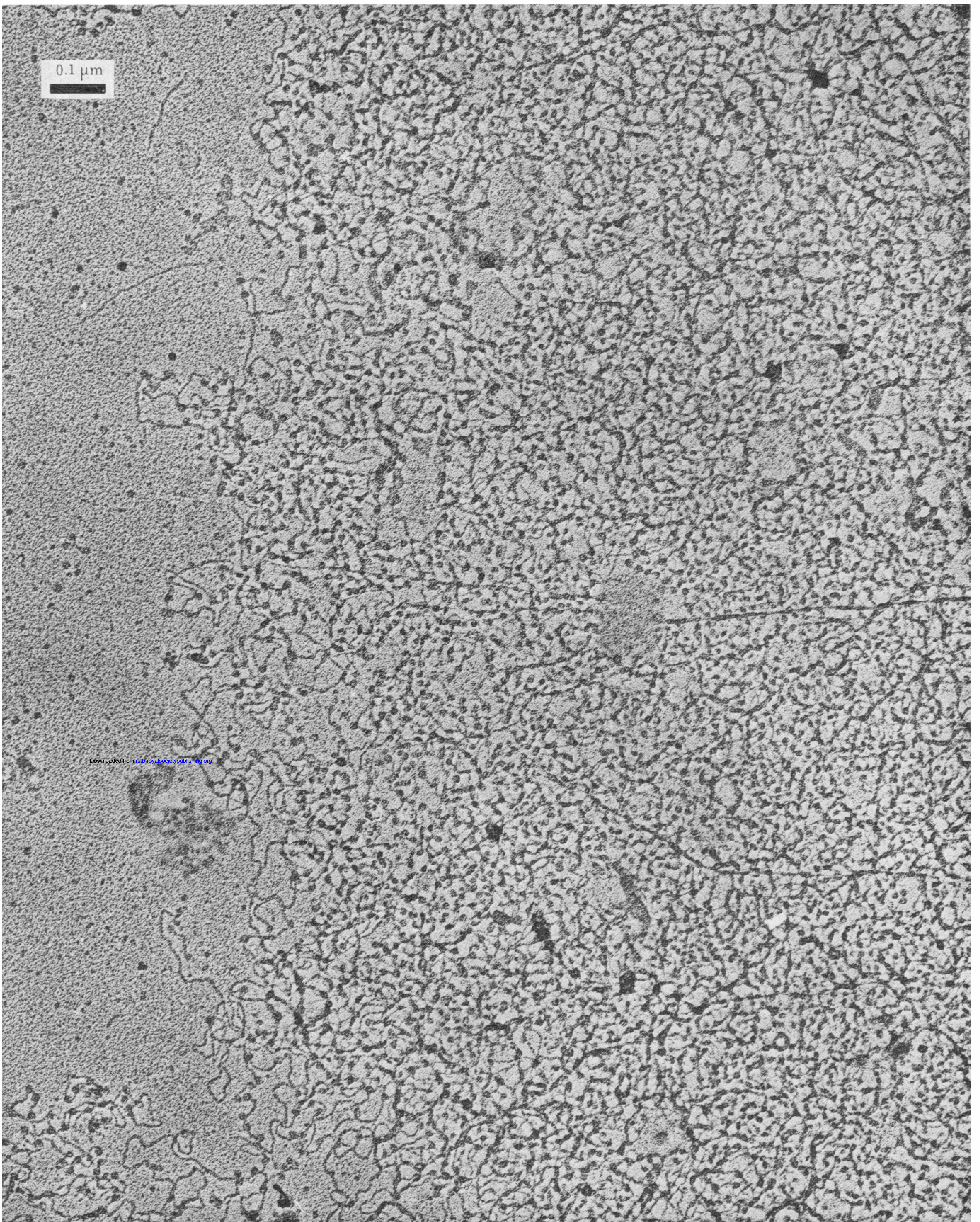

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