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The Organization of Transcription on Lampbrush Chromosomes

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Phil. Trans. R. Soc. Lond. B 1978 **283**, 359-366
doi: 10.1098/rstb.1978.0037

Phil. Trans. R. Soc. Lond. B. **283**, 359–366 (1978) [359]
Printed in Great Britain

The organization of transcription on lampbrush chromosomes

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[Plates 1–4]

The meiotic lampbrush chromosomes of amphibian oocytes display readily distinguishable regions of transcription (lateral loops) which extend from axial condensates of chromatin (chromomeres). The chromomeres contain most of the chromosomal DNA which, along with histone, is tightly compacted as regular arrays of DNP. Many RNA transcripts are generated on the lateral loops, and heterogeneous non-histone proteins associate with these transcripts, forming periodic condensates of 20–30 nm ribonucleoprotein (RNP) particles. These unit particles aggregate in various ways and to varying degrees and thereby confer distinctive gross morphologies to particular loops. There are about 10^4 lateral loops per haploid complement of newt chromosomes and this figure is similar to the experimentally derived number of different messenger RNA sequences found in oocytes. From cytological and biochemical studies it is now possible to consider individual lateral loops from various aspects: as morphologically distinct units; as units of inheritance; as units of functional activity; as units of transcription; as units of transcribed repetitive sequences; and as units containing one coding sequence. The difficulties in arriving at a simple explanation of the organization of transcription in lampbrush chromosomes are discussed.

1. The distribution of transcriptionally active chromatin

In studying the lampbrush chromosomes of amphibian oocytes we are particularly fortunate in being presented with a clear visual distinction between regions of transcriptional activity and regions of condensed chromatin. The lateral loops of these chromosomes are the manifestation of synthetic activity, and consist for the most part of large numbers of nascent RNA transcripts with which specific, non-histone proteins associate to form ribonucleoprotein (RNP) complexes. The loops extend as symmetrical pairs from regions along the chromosome axis where the two sister chromatids are tightly packed in the form of synthetically inactive chromomeres. These features can be seen in figure 1. Taking into account the length of lampbrush chromosomes relative to their DNA content, it would seem that in the chromomeres the DNA fibre is foreshortened by a factor of 10^3, whereas cytological observations support the view that the stretches of DNA fibre which form the axis of the loops are subjected to little compaction. Therefore, from an examination of the different chromosomal components it can readily be estimated that, at least at any one time during oogenesis, only a small fraction of the genome is being transcribed. On this basis, and in agreement with molecular hybridization data (Sommerville & Malcolm 1976), the level of transcription in oocytes of the newt *Triturus* is about 5 % of the genome. Nevertheless, this value is higher than that of most somatic cells and, owing to the high density of RNA polymerase binding (Miller, Beatty, Hamkalo & Thomas 1970) and the enormous size of many amphibian genomes, summates to a very high level of transcriptional activity. This activity, it is interesting to note, is distributed rather evenly throughout the genome. That is, the lateral loops are located at fairly regular intervals throughout the

entire lengths of all the chromosomes. Likewise, there are no extensive regions of condensed chromatin.

The chromomeres of lampbrush chromosomes, unlike those of polytene chromosomes, are not always discrete and unitary structures, for they are frequently associated with more than one loop pair (figure 1) and they undergo various extents of fusion as oogenesis proceeds. Rather than the chromomeres, it is the loops that impart an individual and characteristic structure to lampbrush chromosomes. Therefore we can consider the phenotype of the lampbrush chromosome to be determined by its transcriptional activity.

2. Structural and functional features of transcriptionally active chromatin

Because transcriptional activity is restricted to the lateral loops of lampbrush chromosomes, it is important to establish the nature of loop organization in terms of the arrangement of nucleotide sequences, the structure of the RNA transcripts, and the types of protein which associate with the different primary transcripts. Such information is essential for an understanding of gene activity in oocytes. From cytological and biochemical studies we can now consider loop organization from the following viewpoints.

Description of plate 1

Figure 1. High voltage electron micrograph showing the distribution of chromatin in lampbrush chromosomes. The chromomeric regions (ch) consist of tightly packed deoxynucleohistone which is synthetically inactive; the lateral loops (l.l.) consist for the most part of ribonucleoprotein and are the manifestation of RNA transcription. It is obvious that several pairs of lateral loops may derive from a single chromomeric mass.

Figure 2. Isolated primary transcript RNP, after treatment with 85% formamide, showing the linear continuity of unit RNP beads.

Figure 3. High voltage electron micrograph showing a conspicuously granular loop matrix which is formed by the aggregation of 30 nm unit RNP beads.

Figure 4. The longer arms of bivalent XII of *Triturus cristatus cristatus*. The giant granular loops characteristic of this subspecies are marked g.g.l.

Figure 5. The ends of the longer arms of bivalent XII of an F_1 hybrid between *Triturus cristatus carnifex* ♀ and *T.c. cristatus* ♂. The giant granular loop pair (g.g.l.) is heterozygous, being present on the *cristatus* chromosome but absent from its *carnifex* homologue.

Description of plate 2

Figure 6. Electrophoresis of nucleoproteins in SDS–15% acrylamide gel: (*a*) *Triturus* liver histones; (*b*) *Triturus* oocyte primary transcript RNP proteins; (*c*) the proteins found in association with *Triturus* oocyte 5*S* RNA and tRNA as RNP particles which sediment at about 40*S*. The gel was calibrated using marker proteins of known molecular weight.

Figure 7. Immunofluorescence of lampbrush loops which react specifically with antiserum prepared against primary transcript RNP proteins in the molecular mass range 40000–50000 (see figure 6*b*). In this reaction virtually all loops fluoresce.

Figure 8. Immunofluorescence of a pair of dense-matrix loops on the long arm of chromosome X which react specifically with antiserum prepared against the 49000 molecular mass protein of oocyte 40*S* RNP (see figure 6*c*). This is tentatively assigned as the site of transcription of 5*S* RNA.

Figure 9. Immunofluorescence of one of several loop pairs which react specifically with antiserum prepared against the 38500 molecular mass protein (see figure 6*c*). This is tentatively assigned as a site of tRNA transcription.

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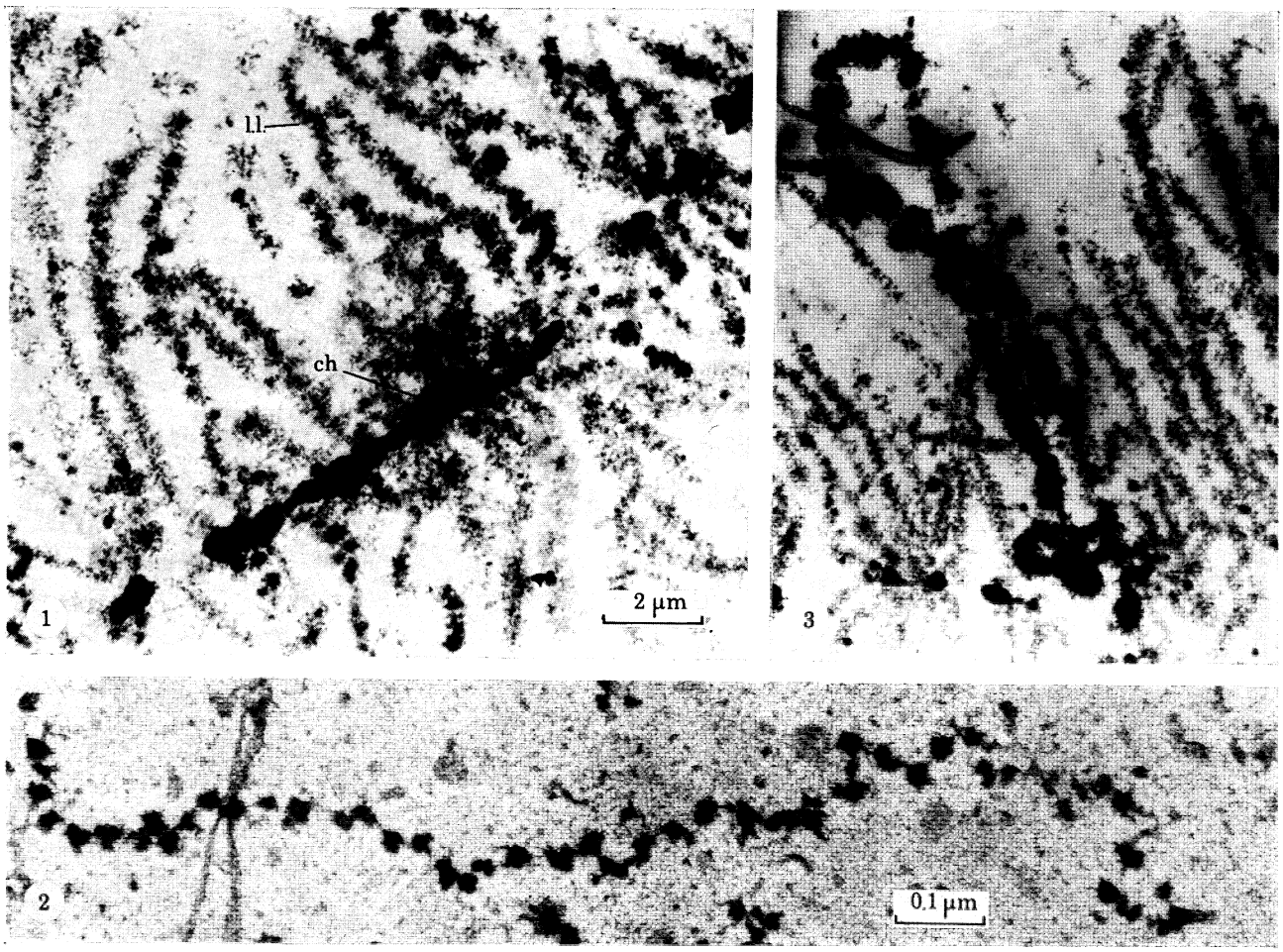

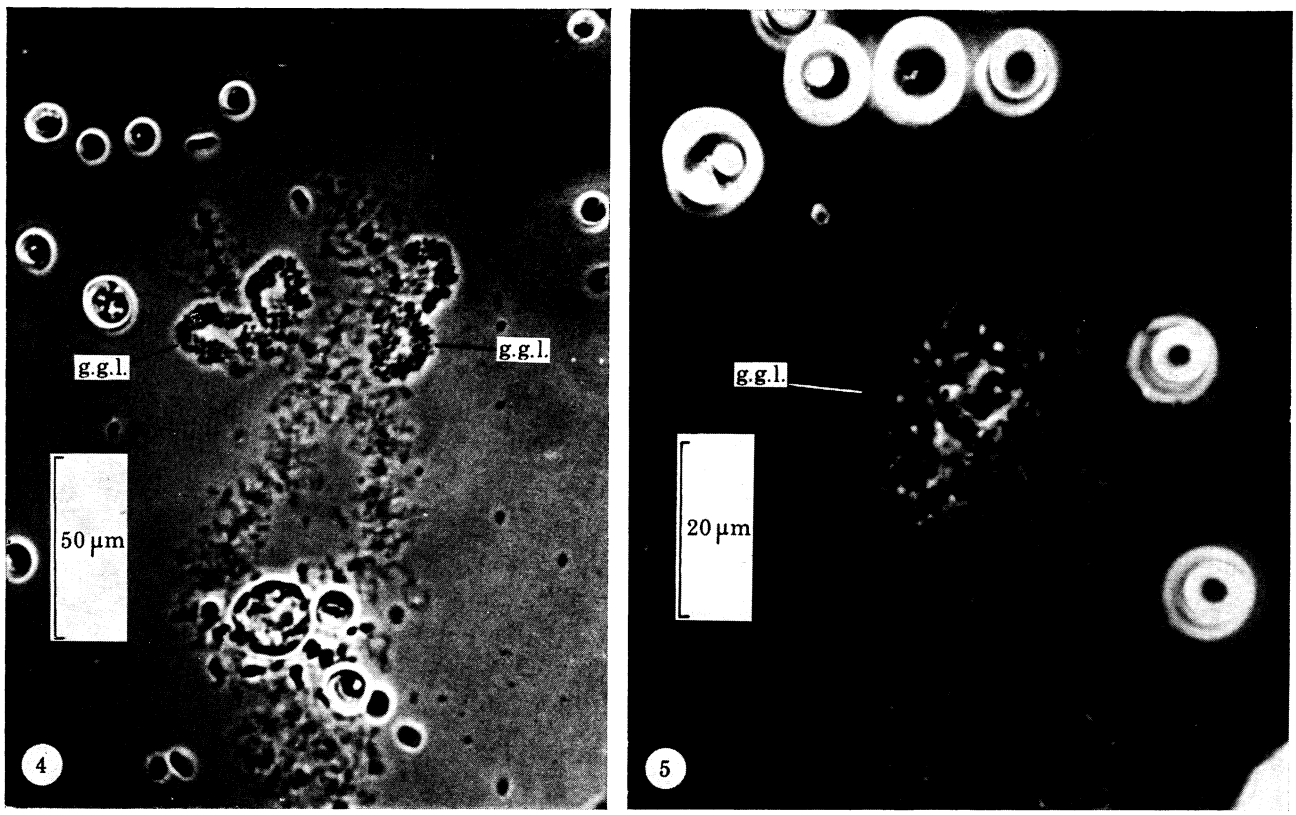

FIGURES 1–5. For description see opposite.

(*Facing p.* 360)

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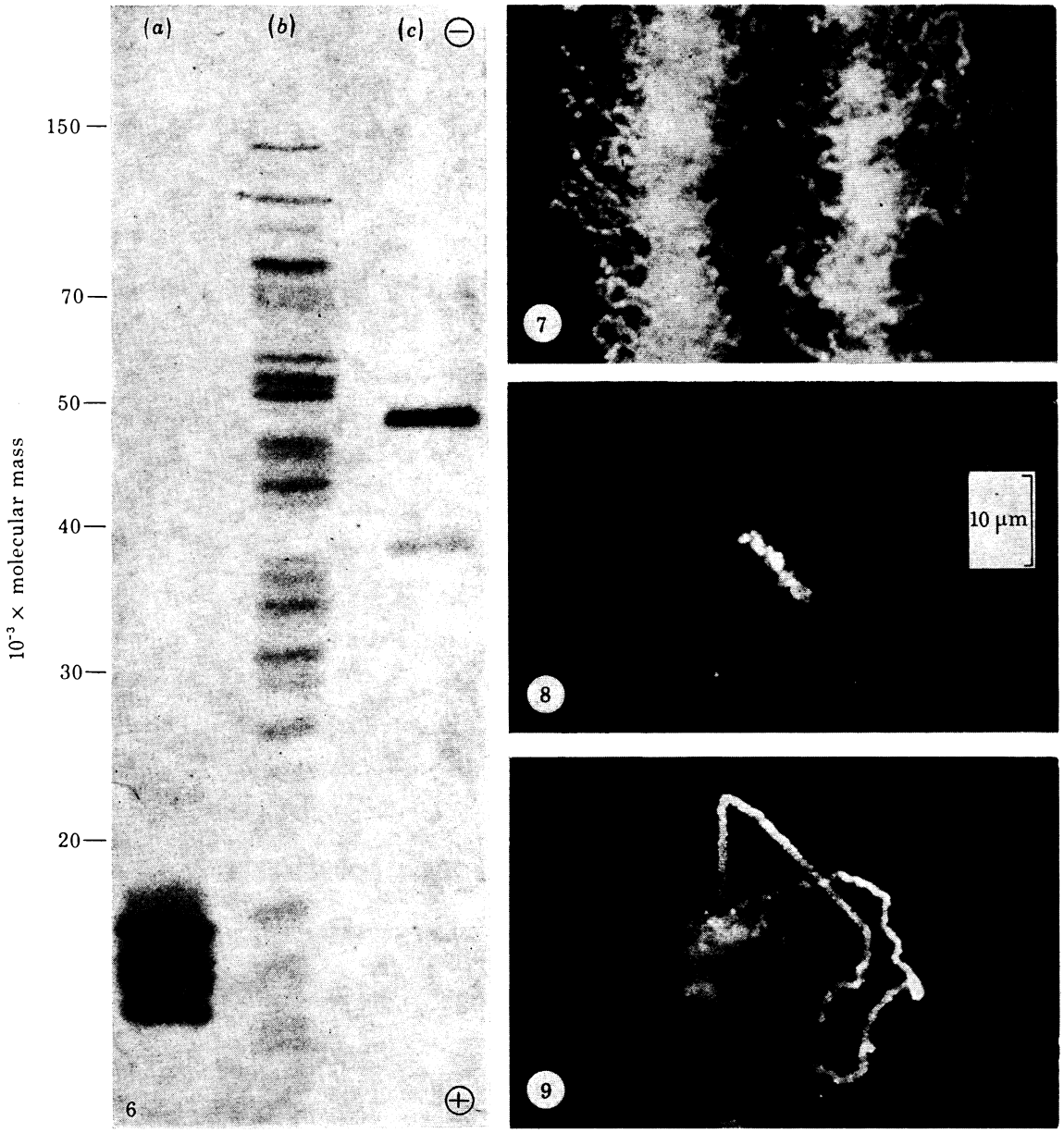

FIGURES 6–9. For description see page 360.

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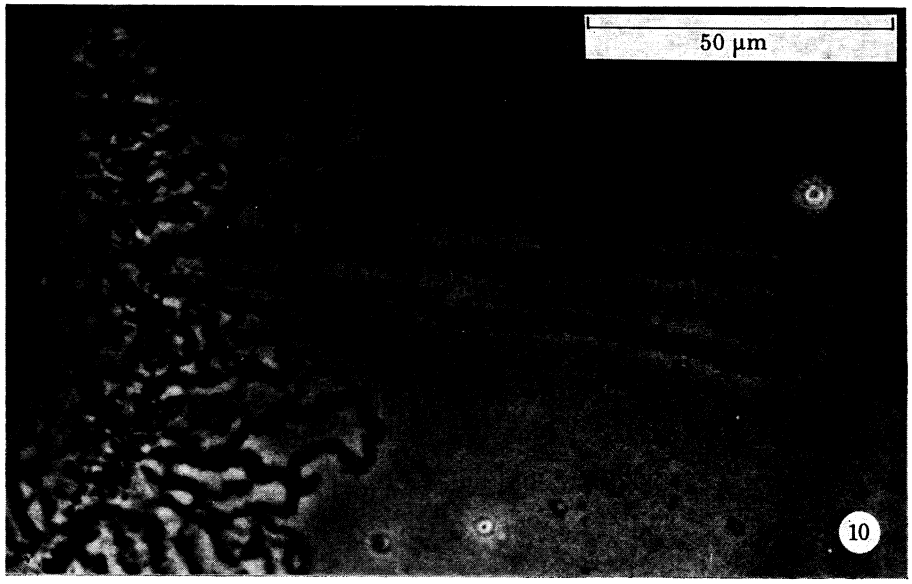

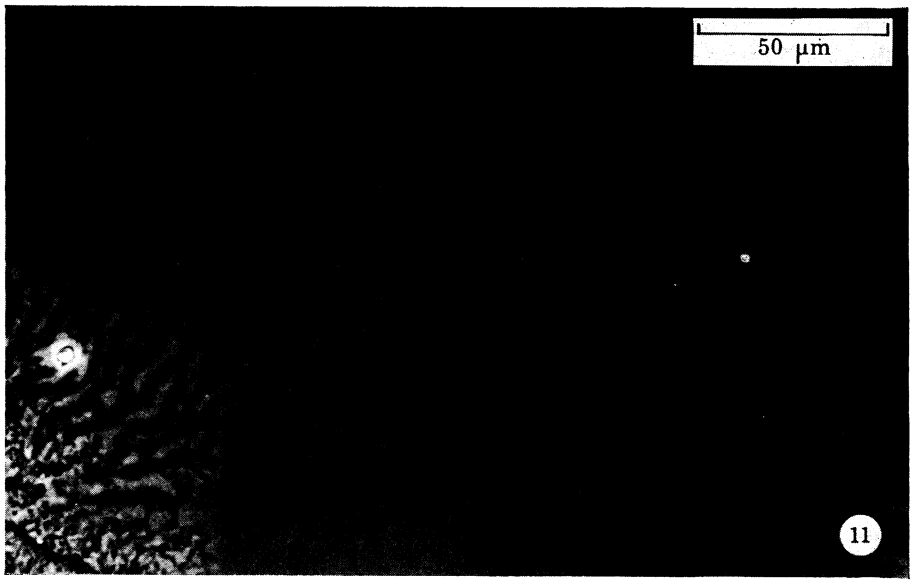

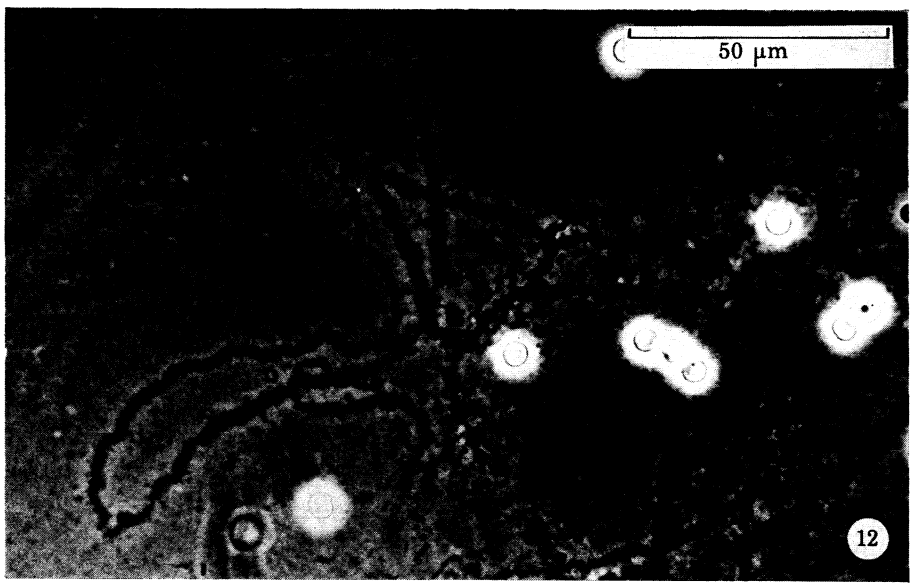

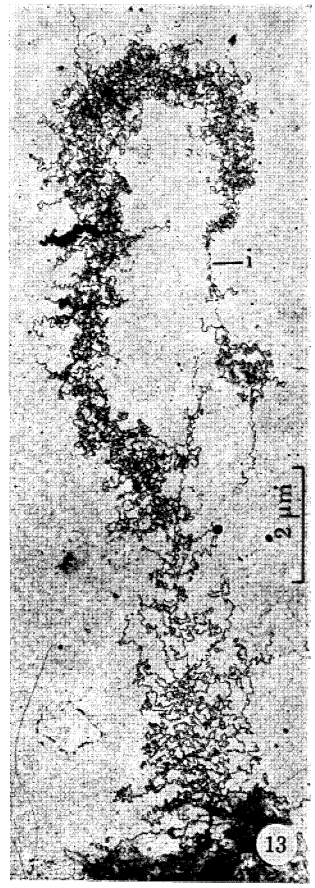

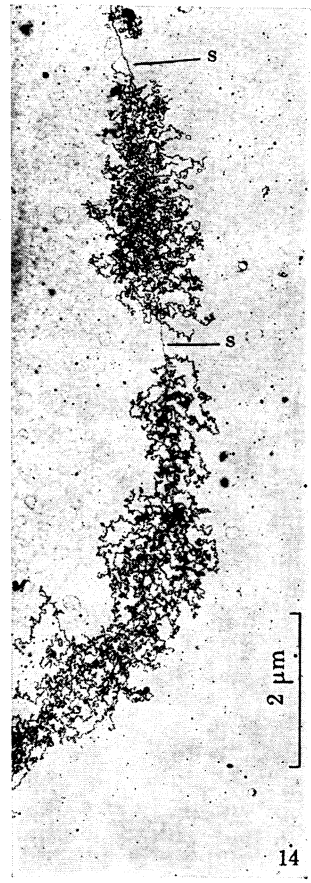

FIGURES 10–14. For description see page 361.

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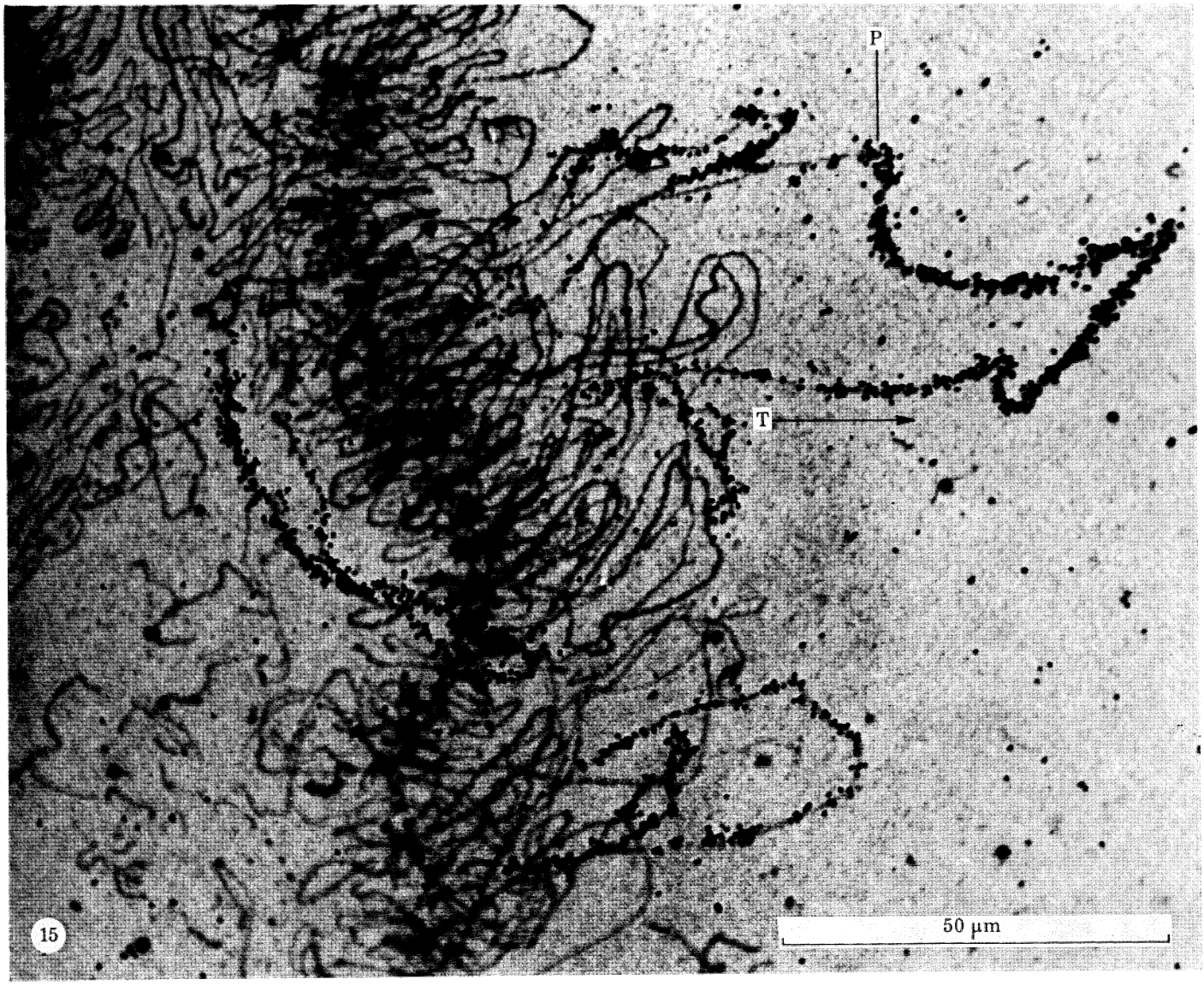

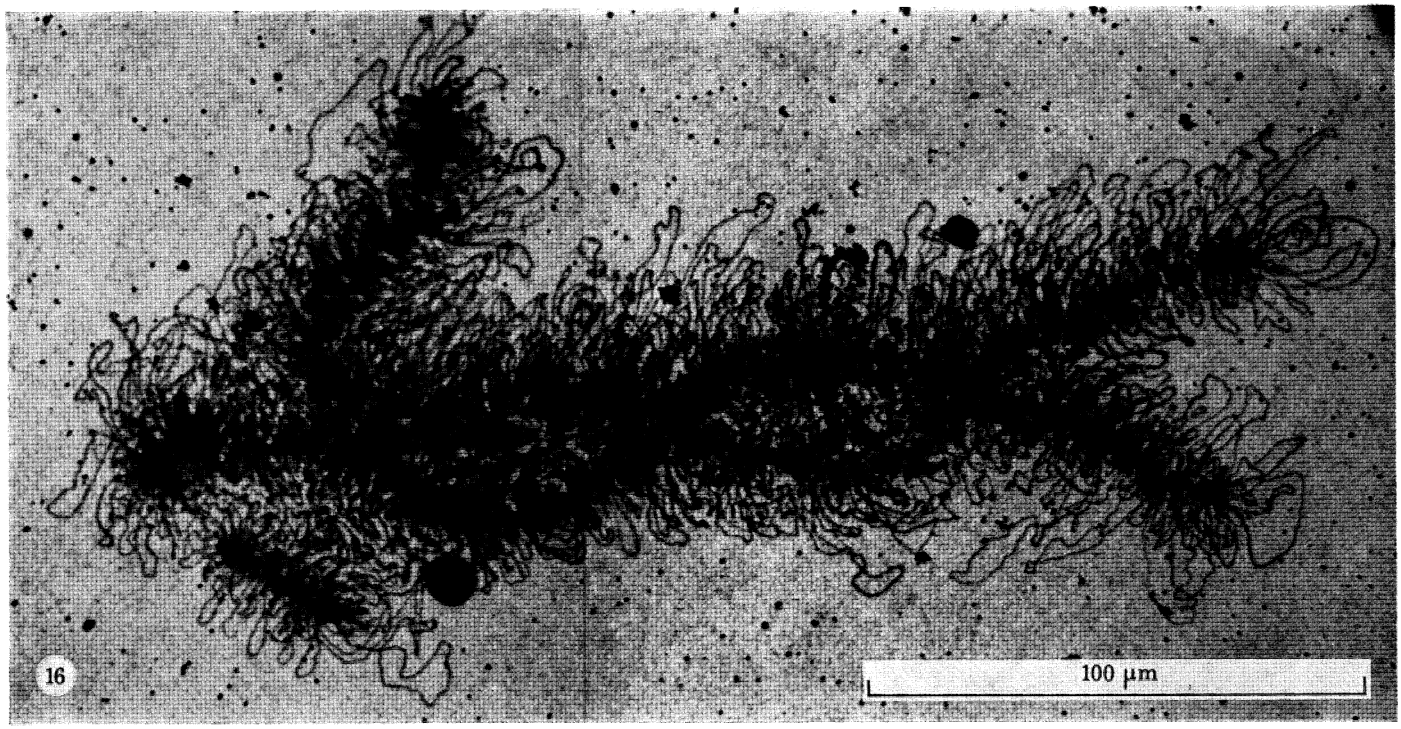

FIGURES 15 AND 16. For description see opposite.

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(*a*) *The loop as a morphologically distinct unit*

Although the condensed chromatin which constitutes the chromomeric regions of lampbrush chromosomes is apparently invariant in its structural organization, it has long been recognized that many of the lateral loop pairs have distinctive morphologies (reviewed by Callan 1963). Variations in size, shape and density are derived from the particular deployment of the RNP products which constitute loop matrix and therefore reflect features of the level and pattern of transcriptional activity, the extent of enzymatic modification of the transcripts (processing) and the degree of molecular interaction between ribonucleoproteins. Electron micrographs show that the RNP matrix of most loops is made up of strings of particles which are 20–30 nm in diameter (Malcolm & Sommerville 1974; Mott & Callan 1975). The linear structure of these beaded strings is seen to advantage in preparations of isolated nuclear RNP which have been treated with 85 % formamide (figure 2), the beads consisting mainly of non-basic protein, the thin connecting fibre of RNA (Malcolm & Sommerville 1977).

As far as we can ascertain, all morphologically distinct loops owe their individuality to varying degrees of aggregation of the unit RNP particles (Malcolm & Sommerville 1974; Mott & Callan 1975). Figure 3 shows the aggregation of 30 nm particles to form a conspicuously granular loop matrix. The mechanism whereby the RNP transcripts of certain loops are aggregated in a particular fashion is not understood.

Description of plate 3

Figure 10. Long lateral loop on the heteromorphic arm of chromosome I of *Triturus cristatus cristatus* which is evidently a single transcriptional unit.

Figure 11. One of the giant lateral loops on chromosome II of *Notophthalmus viridescens* within which there are three transcriptional units. Photograph provided by Miss S. E. Hartley.

Figure 12. Part of a preparation of the lampbrush chromosomes of *Notophthalmus viridescens* in the process of digestion by the sequence-specific endonuclease Hae III. Whereas all other loops (and main chromosome axes) are extensively fragmented, the giant loops of chromosome II remain unbroken.

Figure 13. A matrix of RNP fibrils, presumably derived from a single loop, showing a gradation of transcript lengths from one end (i) towards the other. Chromosomes from *Triturus cristatus carnifex* were dispersed and spread in 0.1 mM borate buffer, pH 9, and prepared for electron microscopy by a method similar to that described by Scheer *et al.* (1976).

Figure 14. A region of transcription showing a pattern of multiple matrices of non-identical length. The space(s) between each unit is presumably a site of termination and initiation of transcription. From the same chromosome preparation as in figure 13.

Description of plate 4

Figure 15. Part of lampbrush chromosome I of *Triturus cristatus carnifex* ♀ 7 hybridized *in situ* with tritiated, nick-translated, *Echinus* histone DNA fragment λ27. The labelled loops lie at 27, 32 and 45 units from the telomere of the longer arm of this chromosome, in the heteromorphic region (for the definition of 'unit', refer to Callan & Lloyd 1960). T with nearby arrow indicates the presumed direction of transcription. P marks the presumed site of processing of histone RNA transcripts.

Figure 16. Lampbrush bivalent X of *Triturus cristatus carnifex* ♀ 8 hybridized *in situ* with tritiated, nick-translated, *Psammechinus* histone DNA fragment λPm 22. The labelled pair of loops, for which this newt is heterozygous, lie at 43 units from the telomere of the longer arm of this chromosome, just to the 'right' of the centromere.

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(*b*) *The loop as a unit of inheritance*

The structural individuality of certain lampbrush loops is so distinct and reproducible from preparation to preparation that maps can be drawn which record their chromosomal location (Callan & Lloyd 1960, 1975). These chromosome maps are characteristic of a species or subspecies; therefore there appears to be a continuity in inheritance of loop morphology. This point is illustrated by considering the giant granular loops which are situated near the end of the longer arm of chromosome XII of *T. cristatus cristatus* (figure 4). These loops are present in the homozygous state in *T. c. cristatus* but are absent in the other three subspecies *T. c. carnifex*, *T. c. danubialis* and *T. c. karelinii*. In an F_1 ♀ hybrid *carnifex* ♀ × *cristatus* ♂ the giant granular loops are seen on only one chromosome, that inherited from the *cristatus* parent (figure 5). Backcrosses of this F_1 hybrid to *carnifex* ♂♂ give a 1:1 ratio of females with and without one pair of giant granular loops. Wherever they have been studied, all examples of heterozygosity such as this, both within and between subspecies of *T. cristatus*, show a straightforward inheritance of loop morphology in the following generations (see, for example, Callan & Lloyd 1960). Thus the loops represent an inherited pattern of transcriptional activity which may be distinguished by a specific deployment of RNP products.

(*c*) *The loop as a unit of functional activity*

More than 95% of the RNP matrix of lampbrush loops consists of protein. This protein can be extracted from an isolated primary transcript RNP fraction (Sommerville 1973) and has been shown to contain a large number of polypeptides which are heterogeneous both in molecular mass (10000–150000; figure 6 and Sommerville & Hill 1973) and in net charge (pI 4–9). Because of this heterogeneity, one aspect of loop function in which we have been particularly interested relates to questions about the specificity of protein–RNA binding, and how this might influence the processing and fate of certain transcripts and the nucleotide sequences they contain. For instance, do all the RNA transcripts of one loop bind the same proteins? Do different loops bind different proteins? In an attempt to answer these questions we have prepared antibodies against isolated size fractions of nuclear RNP protein, and also against the proteins associated with other classes of RNP, and have used them as immunofluorescent probes to detect their chromosomal location. The findings are that most nuclear proteins are located on nearly all loops (figure 7) and so far we have detected only one protein present in nuclear RNP which has a specific localization; in this instance this is a protein of about 35000 molecular mass which is found on several loops pairs on different chromosomes (Scott & Sommerville 1974). On the other hand, the two proteins that are complexed with 4*S* and 5*S* RNA in oocytes (see figure 6) appear to have specific chromosomal sites; the one of molecular mass 49000 on one dense-matrix loop pair on the long arm of chromosome X (figure 8), the other of molecular mass 38500 on several long loops on various different chromosomes (e.g. figure 9). Whether these sites are the loci for transcription of 5*S* RNA and tRNA respectively, has yet to be conclusively demonstrated by nucleic acid–chromosome hybridization.

Therefore the protein components of RNP transcripts are present at their site of formation. In all instances of a specific protein localization these proteins are present on transcripts at all points round individual loop pairs. In the sense of protein binding being an important stage in the processing of RNA transcripts, whole loops appear to function as single units.

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(*d*) *The loop as a unit of transcription*

In many instances the RNP matrix of the loop increases in amount from one chromomeric insertion to the other (figure 10). These loops presumably represent a continuous stretch of RNA transcription. Indeed, when lampbrush chromosomes are dispersed and spread in 'pH 9 water', many of the matrices of linear RNP fibrils that result from this procedure are of about the same average length as the loops from which they were derived (figure 13). However, in many lateral loops there is no obvious polarity in the distribution of RNP, and in certain instances there are multiple thin to thick distributions of RNP. For example, the giant loops on chromosome II of *Notophthalmus viridescens* consist of up to three or more long (150 μm) transcriptional units (figure 11). These different transcriptional patterns do not involve any localized variations in synthesis, because the rate of [^{3}H]uridine incorporation into RNA is the same at all points round the loop; rather the transcriptional patterns are due to termination/initiation at intermittent points on the loop axis, foreshortening of the RNP transcripts by packing (see Malcolm & Sommerville 1977) and possibly processing of the transcripts by scission near their 5′ ends.

The variations in transcription pattern are seen even more clearly in spread chromosome preparations. Here are seen multiple transcriptional matrices, often of different dimension and occasionally with non-transcribed regions between them, presumably derived from the one loop (figure 14). A number of distinct forms of transcriptional complex have been classified by Scheer, Franke, Trendelenburg & Spring (1976).

Nevertheless complex matrix patterns do not by themselves constitute proof that the adjacent units are transcribing different sequences, even if they vary in overall form and have different polarities of transcription. Although they may well represent coordinately active regions, nucleotide sequence identification within loops is required before any definite conclusion can be drawn concerning genetic function. Later, in § 2*g*, we shall see that there can indeed be a complex genetic organization within a loop.

(*e*) *The loop as a unit of transcribed repetitive sequences*

From biochemical investigations, we are familiar with the concept of certain genetic sequences existing as blocks of tandem repeats. Particularly well characterized are the 18*S* and 28*S* ribosomal genes (reviewed by Tobler 1975), the 5*S* ribosomal genes (Brownlee, Cartwright & Brown 1974), the tRNA genes (Clarkson, Birnstiel & Serra 1973) and the histone genes (reviewed by Kedes 1976).

How are these sequences arranged on lampbrush chromosomes? Ribosomal genes are mostly located on extrachromosomal nucleoli, therefore their transcriptional activity is not relevant to the present discussion. The genes for 5*S* RNA have a chromosomal location, and Pukkila (1975) has shown, by hybridizing labelled 5*S* DNA to lampbrush chromosomes of *N. viridescens*, that nascent 5*S* RNA sequences, and probably also their spacer sequences, are transcribed round the lengths of specific loops. These loops presumably contain many tandem repeats of the 5*S* sequence. We may also refer back to the immunofluorescent labelling of presumptive 5*S* and tRNA transcripts where again whole loops are reactive.

One set of transcribed sequences which are likely to be tandemly repeated, and are certainly unique in other respects, are to be found in the giant loops on chromosome II of *N. viridescens*. As already mentioned, these loops may consist of a series of three transcriptional units, each

about 150 μm in length. Like almost all other loops, the giant loops are fragmented by the action of various restriction endonucleases (Gould, Callen & Thomas 1976). However, the giant loops are refractile to a nuclease extracted from *Haemophilus aegyptius* (Hae III) which produces breaks at the sequence 5′ GGCC (figure 12). Therefore this sequence, which on a random basis is expected to occur about 10 times per micrometre of DNA, is completely absent from the DNA axis of these loops. The simplest explanation of this finding is that the axial DNA of the giant loops consists of a large number of tandem repeats which happen to lack the sequence 5′ GGCC. Therefore there are probably many sequence repeats within each repeated transcriptional unit.

The giant loops of *N. viridescens* are also bizarre in their incorporation of RNA precursors, showing an exceptionally low level of labelling with [^{3}H]guanosine. The estimated base composition of the RNA transcripts is adenine, 25; cytidine, 38.5; uridine, 27.5 and guanosine, 9 (S. E. Hartley & H. G. Callan, unpublished). In order to obtain this pattern of incorporation there must be a marked asymmetry in the distribution of bases in the DNA duplex. It is also interesting to note that the overall rate of RNA synthesis in the giant loops, as determined by grain counts, is about half that of other loops. A possible function for the extensive transcription of RNA sequences containing very little guanosine remains unexplained.

(*f*) *The loop as a unit containing one coding sequence*

The extent of tandem gene reiteration in the generality of loops appears to be small. Kinetic studies have shown that cDNA transcribed from the total polyadenylated mRNA fraction of oocytes preferentially hybridizes with non-repetitive DNA sequences (Rosbash, Ford & Bishop 1974; Sommerville & Malcolm 1976). Also, this same cDNA hybridizes to its mRNA templates with rate kinetics which suggest that there are about 10^4 different types of mRNA sequence present in oocytes (Rosbash *et al.* 1974; Sommerville & Malcolm 1976). Because this number approximates to the number of cytologically observable loops, it is tempting to speculate that there is, on average, one coding sequence per loop. The possibilities of some coding sequences sharing single loops and other loops transcribing RNA which does not become adenylated may balance out. In any event, individual loops and their complete transcripts are one or two orders of magnitude longer than the length of sequence required to code for single proteins. What are the other sequences transcribed on loops? We have previously suggested (Sommerville & Malcolm 1976), from a consideration of the kinetics of hybridization and the thermal stabilities of hybrid molecules, that they are in part ancestral coding sequences that have deviated and become informationally redundant.

(*g*) *The distribution of loops with related function*

It is often assumed that there is a single discrete location for each coding sequence. Control sequences may be dispersed throughout the genome but sequences coding for any one protein should be restricted to one locus, in this discussion a single loop. In the absence of a thorough genetic analysis the simplest approach to this problem is to study the chromosomal localization of identified genetic sequences using specific labelled probes. Using this technique the diversity in location of 5*S* gene families has been demonstrated in the genome of *Xenopus* (Pardue, Brown & Birnstiel 1973). The example that we should like to discuss is the apparent distribution of histone genes on the lampbrush loops of *Triturus cristatus carnifex*.

A histone DNA fragment 6000 bases long, derived from the sea-urchin *Psammechinus miliaris*

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and amplified in a λ vector bacteriophage (Gross, Schnaffner, Telford & Birnstiel 1976) was kindly provided by Dr K. Gross. Labelled sequences were obtained by nick-translating this fragment and were hybridized with lampbrush chromosomes of *Tritirus* (Old, Callan & Gross 1977). Lambda-cloned *Echinus esculentus* histone DNA was also used and gave identical results. The transcription of histone genes was revealed as a pattern of activity which was complex in several respects.

First, the loops on the heteromorphic arms of chromosome I which hybridize histone DNA show, when they are conveniently disposed, and beginning from the thin matrix insertion, a short unlabelled portion with progressively increasing matrix, a labelled portion where grain density rises rapidly, a plateau region of uniform grain density, a region of diminishing grain density where the grains are distributed in irregular clusters, followed by an unlabelled region of variable length leading back to the thick matrix insertion in the chromosome axis (figure 15). The matrix distribution on such a loop is that characteristic of a single transcriptional unit. Its pattern of labelling suggests that the portion of the loop axis where transcription begins contains no histone sequences; there follows a short stretch of histone sequences (where grain density rises) and then a long stretch, devoid of histone sequences, running to the transcriptional terminus where the loop returns to the chromosome axis; histone mRNA sequences are conveyed with the continuing transcripts beyond the stretch of histone sequences in loop axis DNA along the length of the plateau region, and are processed and shed (though not their accompanying sequences lying proximal to the loop axis) in the region of diminishing and non-uniform grain density.

Secondly, histone sequences are found at several loci on different chromosomes, unlike the situation obtaining in *Drosophila* (Pardue 1975). The major loci are 3 or 4 on chromosome I; these loci are invariant in oocytes of a single newt, but show some variation between individual newts. In addition to these major loci, others may be present on chromosomes VI, X and XI, and these again show variation from one newt to another.

Thirdly, the loci involved in transcribing histone coding sequences may exhibit heterozygosity. Thus one newt is heterozygous for the labelling *in situ* of a loop pair on chromosome X (figure 16) whereas another shows no labelling on X but is heterozygous for the labelling of a loop pair on V.

Whether this complex pattern of transcriptional activity is peculiar to histone genes and other repetitive sequences, or whether it represents a more general type of sequence organizations remains to be seen.

3. Genetic implications of transcriptional activity

The process of transcription in lampbrush chromosomes is not as straightforward as might at one time have appeared. The frequently observed complex organization of transcriptional units may imply that some loops contain more than one type of genetic sequence and that their activity is regulated in a coordinate fashion.

The distribution of the same or similar sequences at multiple loci, such as is the case with ribosomal genes in many amphibia, with 5*S* RNA genes in *Xenopus* and *Notophthalmus* and probably also with histone genes in *Triturus*, means that there may be considerable mixing of elements within the genome. Variation in the extent of transcriptional activity between individual animals may be a fairly common phenomenon provided there exists a large number of

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sequence repeats which are likely to be distributed between different chromosomal loci. In the examples of complete absence, or heterozygosity, of the transcription of certain histone loci in *Triturus*, it is not known, as yet, to what extent the numbers of histone genes vary, or whether we are seeing the differential expression of possibly non-equivalent sequences. However, it has been demonstrated that there may be considerable heterozygosity in the number of ribosomal genes at certain nucleolar organizer loci in *Notophthalmus* (Hutchison & Pardue 1975).

The formation of substantial amounts of RNP matrix on those loops engaged in the transcription of 5*S* RNA sequences, and also on putative tRNA loops, suggests that transcript lengths include much more than one unit sequence; rather they include several tandem repeats plus their interspersed spacer sequences. Also, the transcription pattern of histone sequences, as seen by the continuity of loop matrix and the distribution of hybridized histone DNA, is interpreted to mean that transcription is continuous through histone gene cluster repeats, as well as through non-histone sequences on both sides of the histone gene region. The organization of transcription of repeated genetic sequences presumably facilitates the processing and rapid accumulation of cytoplasmic RNA. The situation pertaining to non-repetitive sequences has still to be elucidated: the reason for transcribing whole loops which may contain only one or a few coding sequences is not obvious. Why so much RNA is transcribed on loops and so little apparently used in translation is a problem not confined to oocytes, yet exaggerated in those genomes of high *C* value. However, it is important to remember that oocytes are rather special cells and that some of the phenomena described may relate to processes peculiar to oogenesis rather than to eukaryotic cell activity in general.

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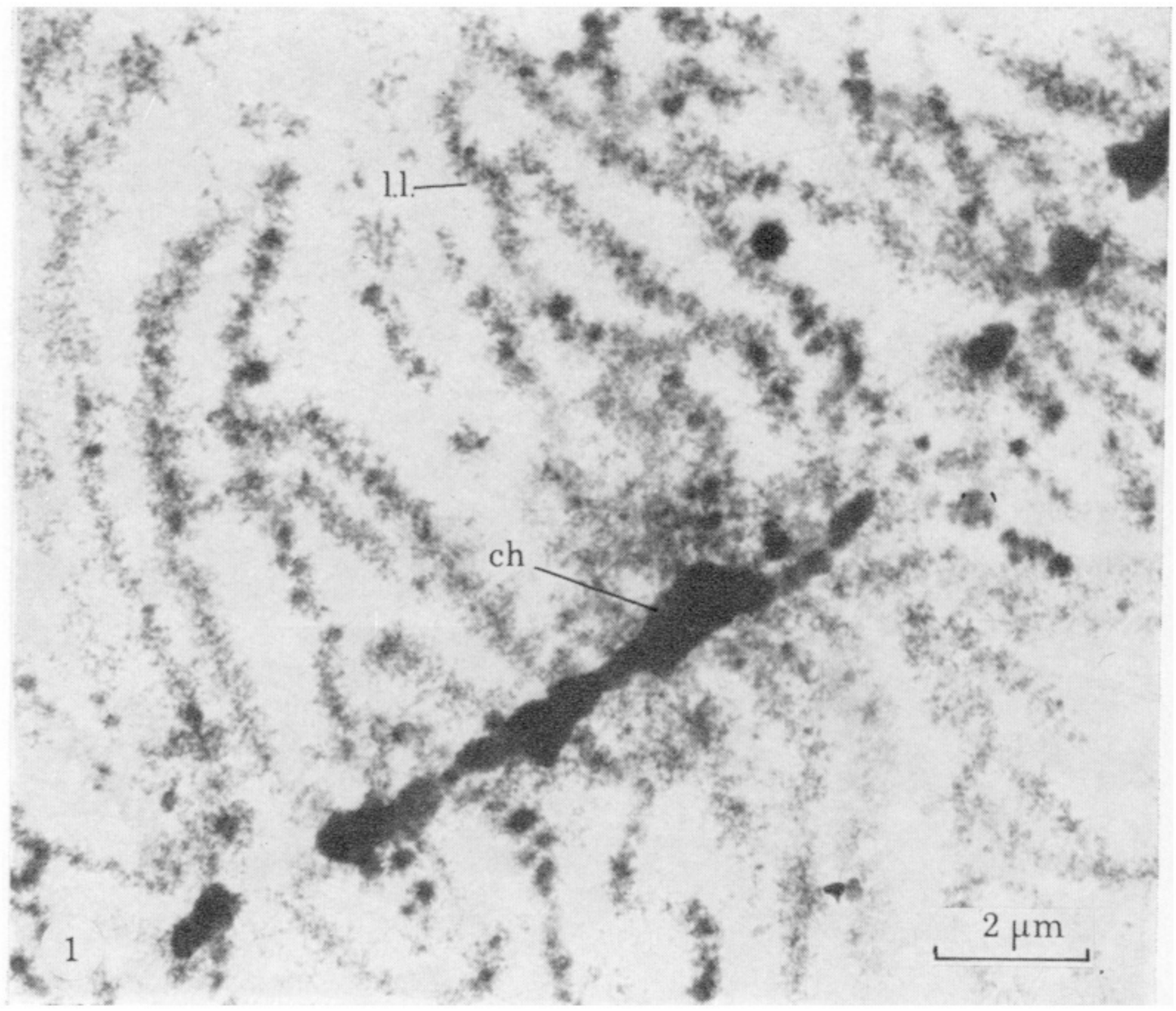
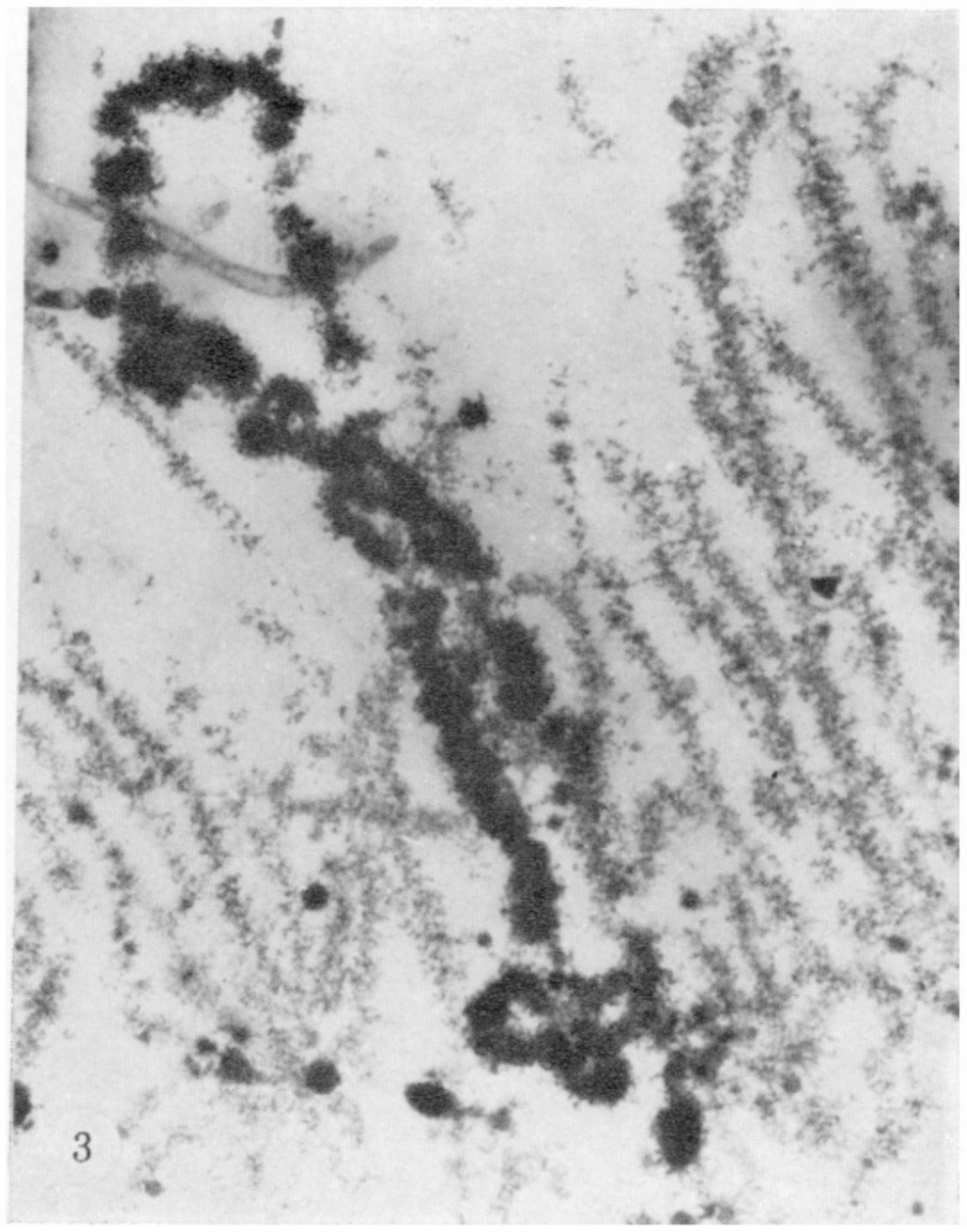
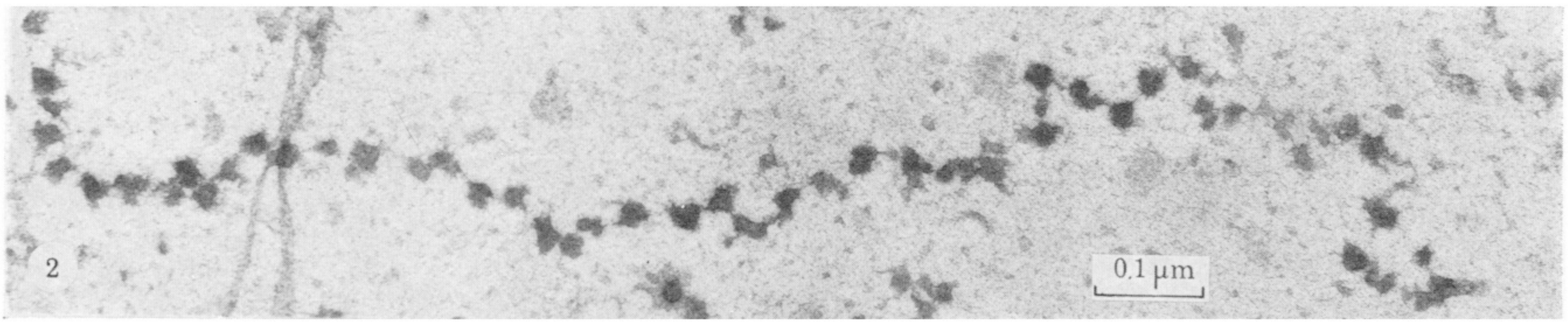
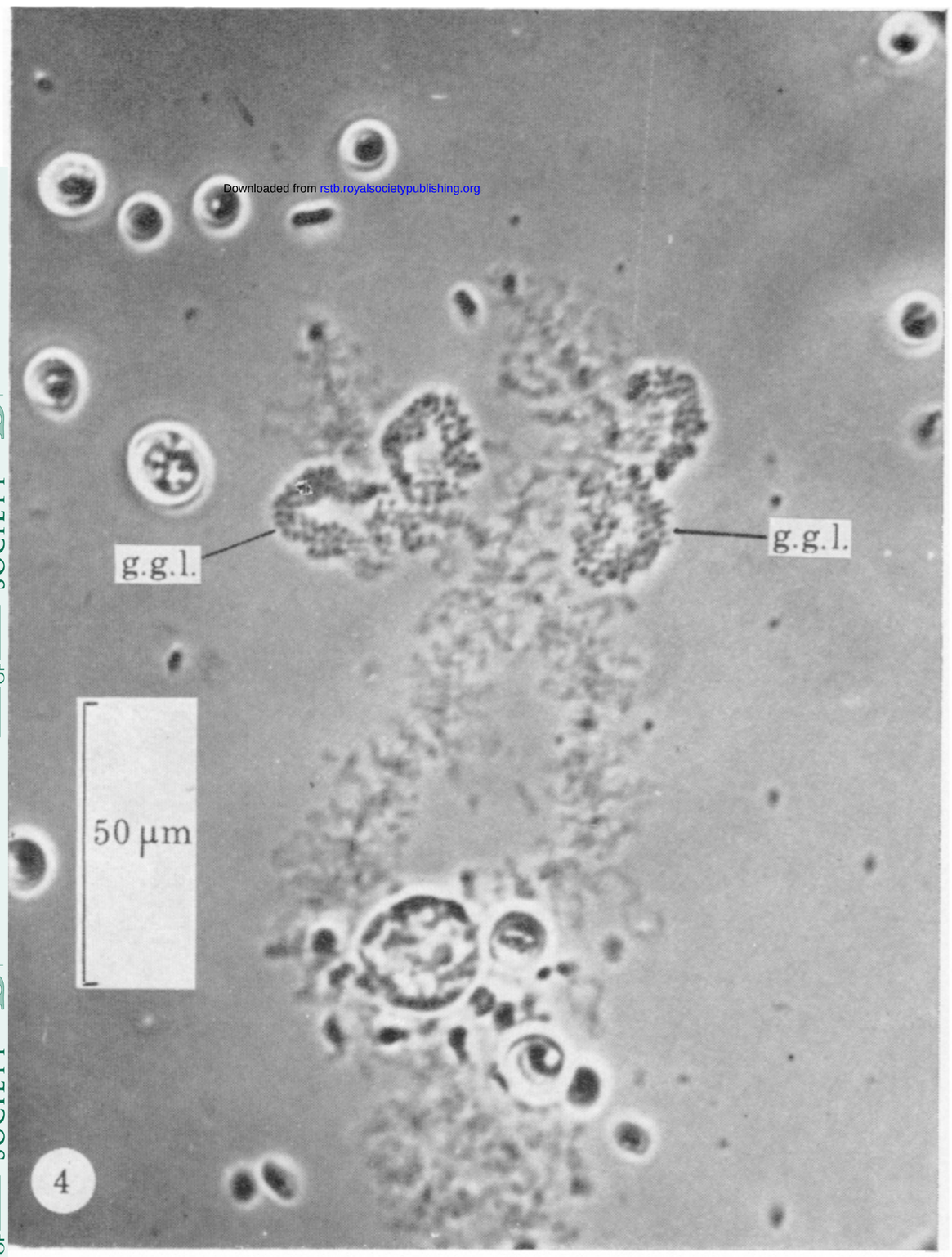
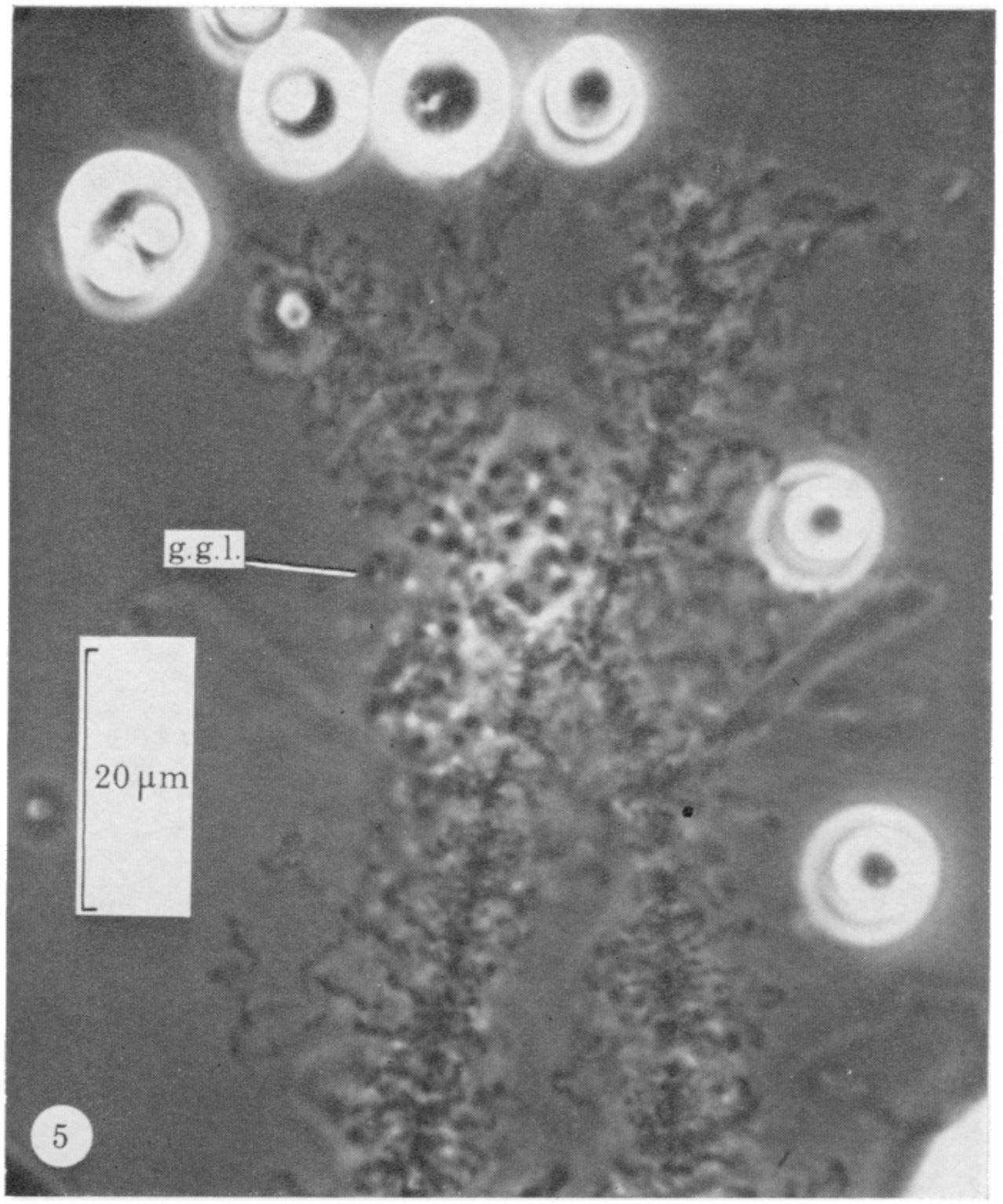


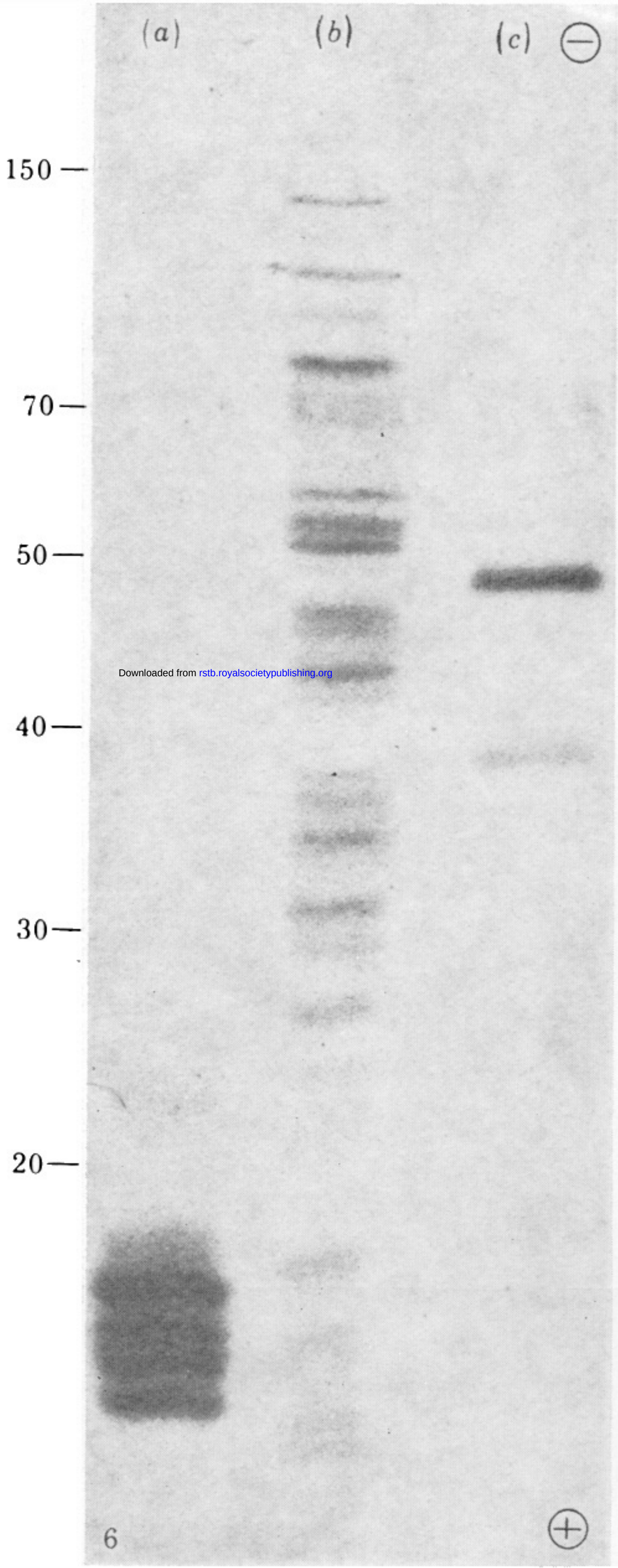


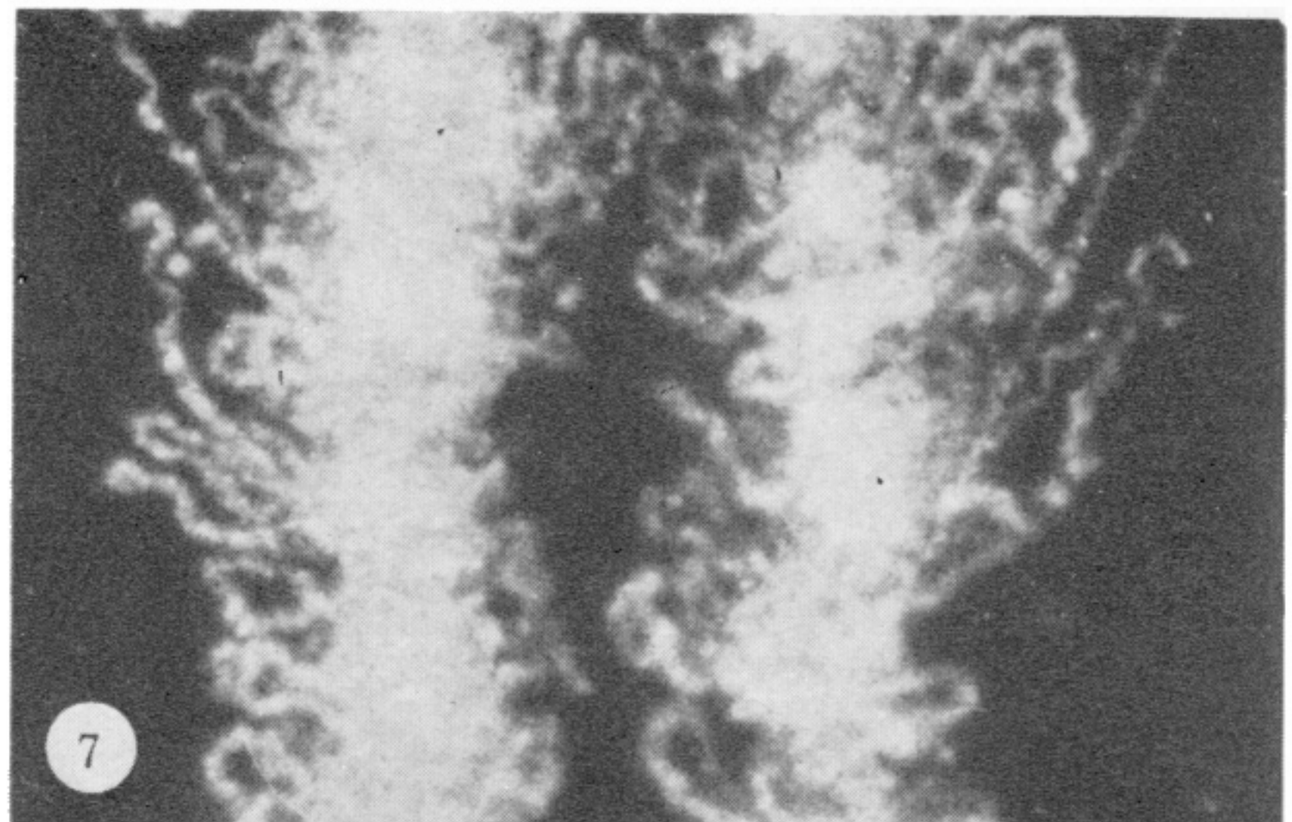


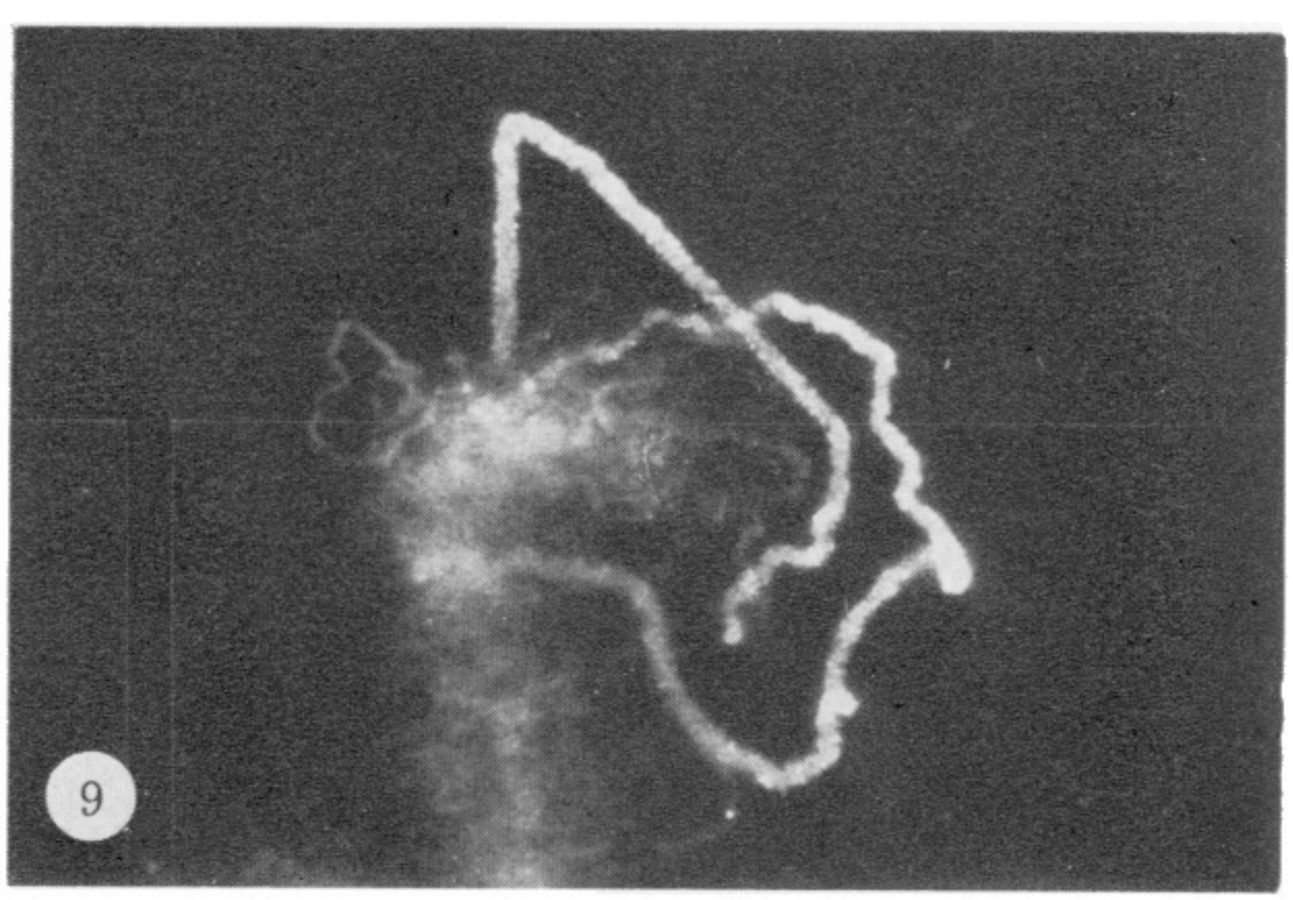




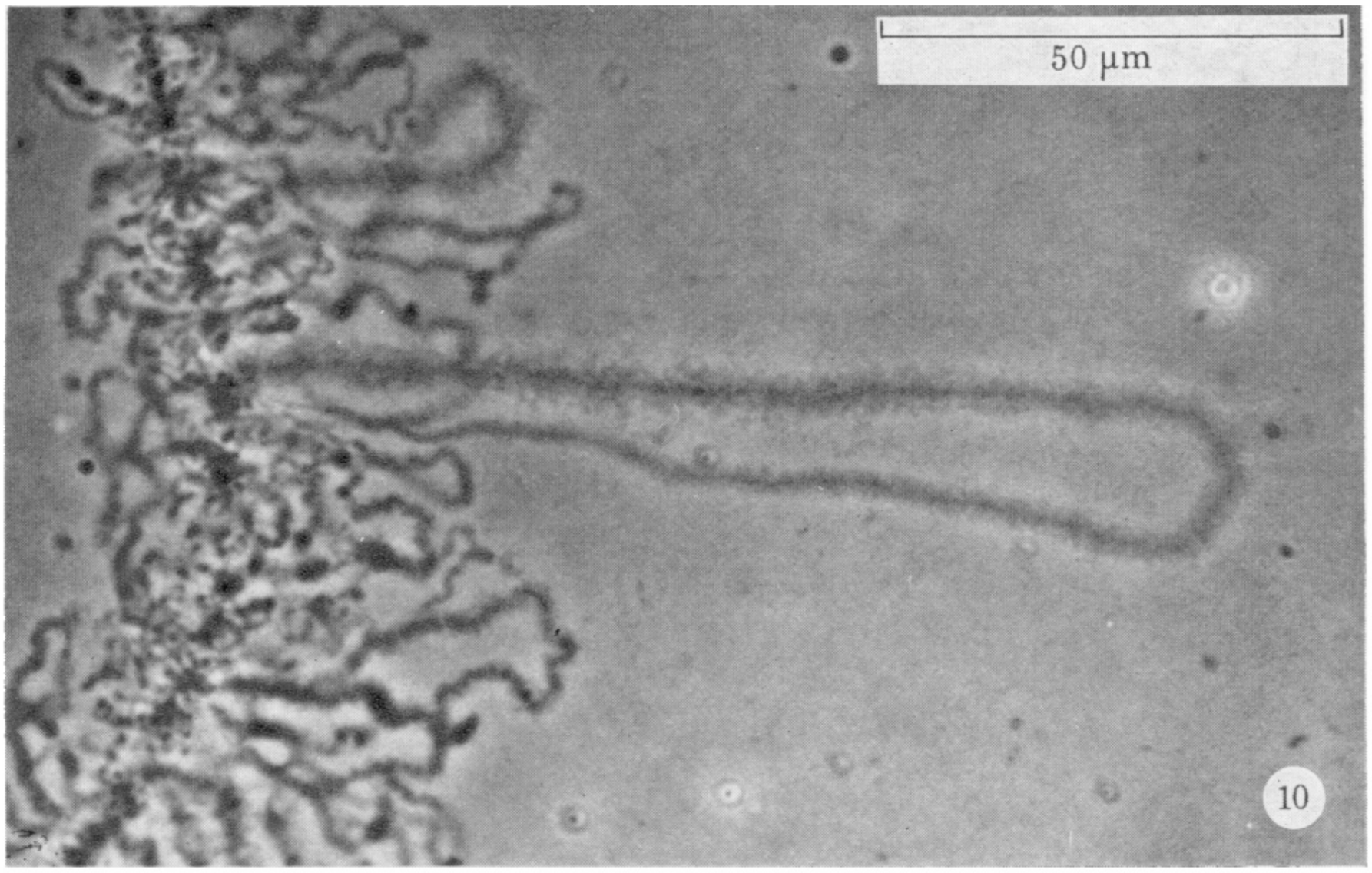
FIGURES 1–5. For description see opposite.

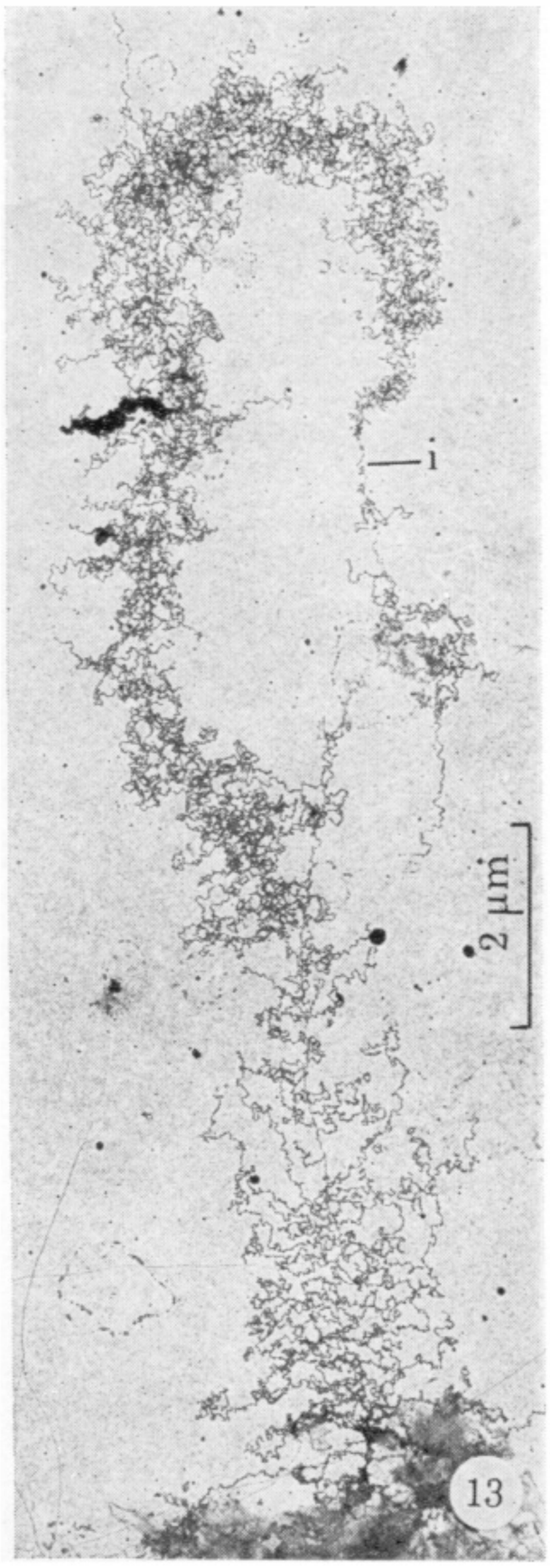


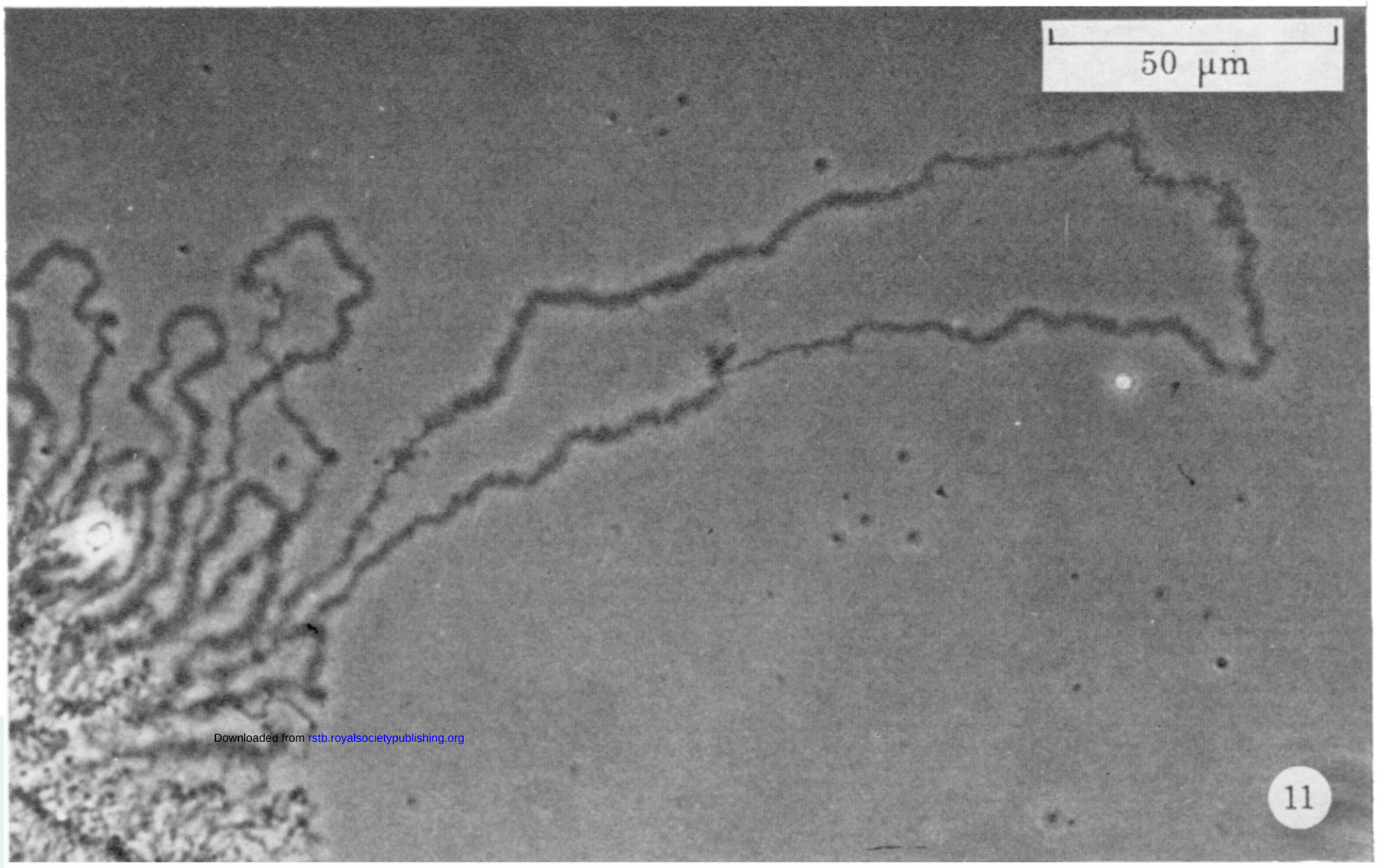


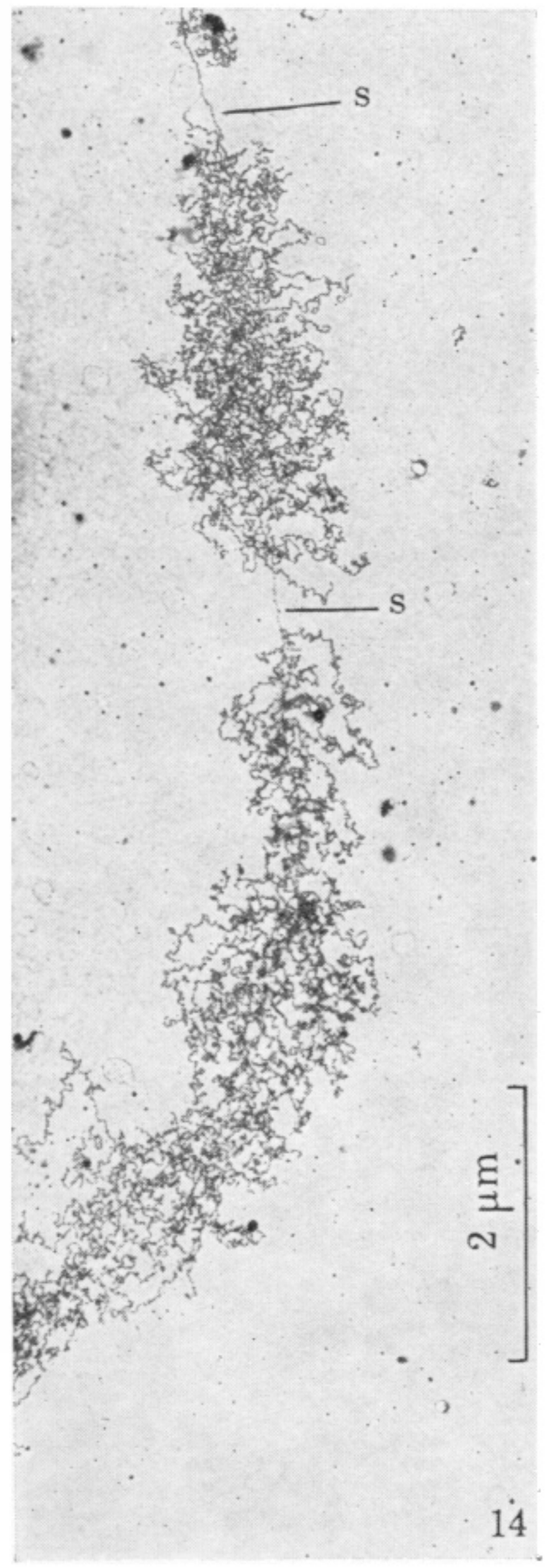


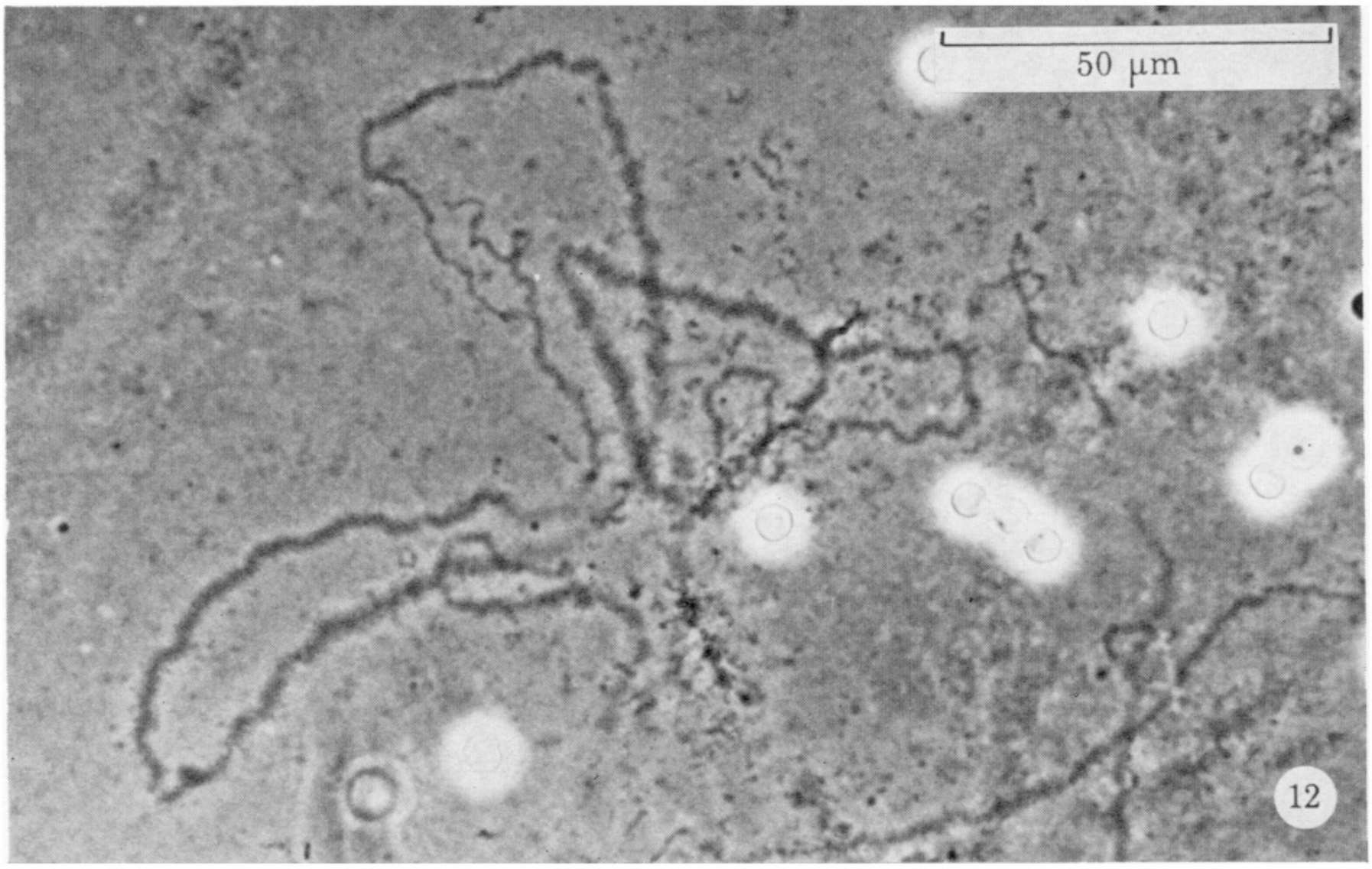
FIGURES 6–9. For description see page 360.











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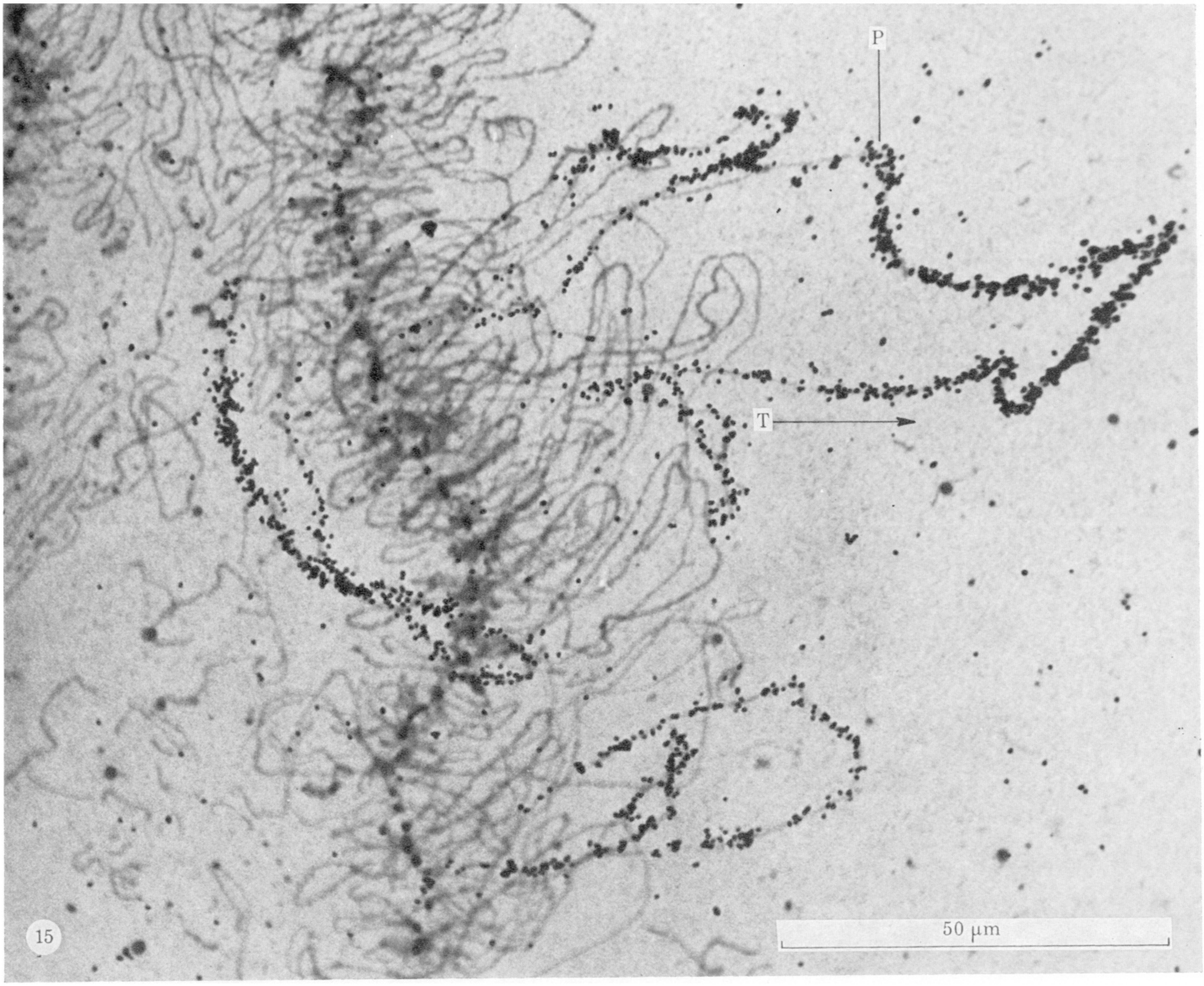
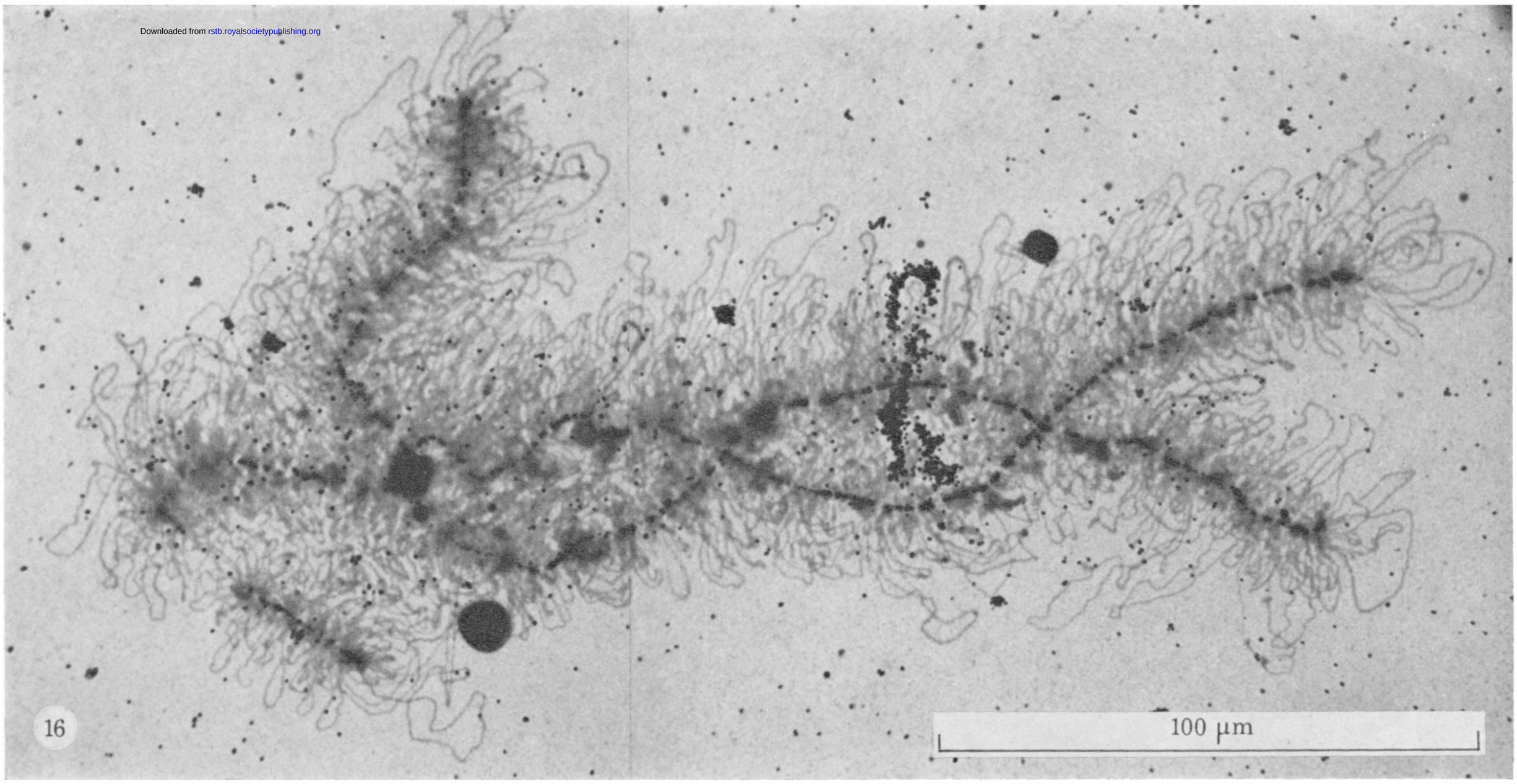
FIGURES 10–14. For description see page 361.





FIGURES 15 AND 16. For description see opposite.