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Cellular Responses Regulated by Rho-Related Small GTP-Binding Proteins

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Cellular responses regulated by rho-related small GTP-binding proteins

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SUMMARY

Rho-related proteins are members of the ras superfamily of small GTP-binding proteins. Their function in fibroblasts has been analysed using microinjection of living cells. Rho appears to link plasma membrane receptors to the assembly of focal adhesions and actin stress fibres. The closely related protein rac, on the other hand, links receptors to the polymerization of actin at the plasma membrane to form membrane ruffles and pinocytotic vesicles. In phagocytic cells, rac has been shown to be required for activation of a membrane-bound NADPH oxidase in response to receptor activation. These systems provide the basis for a working model for the mechanism of action of the rho family of small GTPases.

1. THE RAS SUPERFAMILY

Around 40 members of the ras superfamily of small GTP-binding proteins have now been described. These can be divided into four subfamilies based on sequence and functional similarities: (i) ras-like; (ii) rho-like; (iii) rab-like; and (iv) ARF-like. Ras controls signal transduction pathways linking receptors to cell growth and differentiation, whereas rab and ARF proteins are involved in the assembly and trafficking of intercellular vesicles (Hall 1990).

2. THE RHO SUBFAMILY OF SMALL GTPASES

Nine members of the rho superfamily have been identified so far. Rho (A,B,C), rac (1,2), CDC42/G25K, rhoG, and TC10 (Madaule *et al.* 1987; Yeramian *et al.* 1987; Chardin *et al.* 1988; Didsbury *et al.* 1989; Munemitsu *et al.* 1990; Shinjo *et al.* 1990; Drivas *et al.* 1990; Vincent *et al.* 1992). Most are expressed in all cell types, although rac2 appears to be found predominantly in haematopoetic cells (Olofssan *et al.* 1988; Didsbury *et al.* 1989; Shirsat *et al.* 1990; Moll *et al.* 1991). RhoB and RhoG are serum inducible gene products (Jahner & Hunter 1991; Vincent *et al.* 1992).

(*a*) *Exchange factors*

A number of potential regulators of the rho subfamily are known. SmgGDS was originally identified as an exchange factor for rap and Ki-ras but later shown to be active on rho and rac (Hiraoka *et al.* 1992). The product of the *dbl* oncogene is an exchange factor for CDC42, although it is not yet clear that malignant transformation by *dbl* is mediated through CDC42 (Hart *et al.* 1991). Interestingly, a family of proteins has been described containing a dbl-related domain, including bcr (the breakpoint cluster region gene product), the *vav* oncogene and the N-terminal domain of rasGRF, a ras nucleotide exchange factor (Adams *et al.* 1992; Shou *et al.* 1992). It is not yet clear whether these dbl-like proteins have any exchange activity for members of the rho subfamily. A factor, rhoGDI, originally identified through its ability to inhibit the release of guanine nucleotides from rho, was later shown to solubilize rho-like proteins from membranes (Hiraoka *et al.* 1992; Isomura *et al.* 1991). The role of this protein *in vivo*, however, is unclear.

(*b*) *GTPase activating proteins*

In 1990 we reported the purification of a GTPase activating protein (GAP) specific for rho, rhoGAP (Garrett *et al.* 1991). Partial amino acid sequence revealed that two other known proteins were highly related, bcr and n-chimaerin, the product of a brain specific cDNA (Diekmann *et al.* 1991). Since then two other highly related proteins have been reported, p190, a protein that binds to rasGAP after growth factor stimulation of cells, and 3BP-1, a protein capable of interacting with the SH3 domain of c-abl (Settleman *et al.* 1992*a*,*b*; Ciccheti *et al.* 1992). The GAP activities of these proteins on the different members of the rho subfamily are summarized in table 1. p85, the regulatory subunit of PI-3 kinase, is more distantly related to the rhoGAP family and we have been unable to detect any GAP activity towards rho, rac or CDC42 (Otsu *et al.* 1991). The *in vivo* roles of these GAPs are unknown, they could act as downregulators or as target proteins for the rho-like GTPases (Hall 1992*a*).

(*c*) *Cellular localization*

The cellular location of rho-like proteins has been

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Table 1. *Proteins of the rhoGAP family*

	GAP activity		
	rho	rac	CDC42
rhoGAP	+	+	+
bcr	−	+	+
chimerin	−	+	+
p190	+	+	+
3BP-1	n.d.	n.d.	n.d.
p85	−	−	−

somewhat confusing, as they have been purified from both cytosolic and membrane compartments. We have recently addressed this problem by microinjecting cells with an expression vector containing rho cDNA attached to an N-terminal epitope tag (Adamson *et al.* 1992). The cellular localization of expressed protein can then be visualized by immunofluorescence using a tag-specific antibody. The results obtained with the three different rho proteins are shown in figure 1. Rho A and C (figure 1, right panel) are predominantly cytosolic with a little at the plasma membrane, whereas rho B (figure 1, left panel) is in a vesicular compartment which we have identified as early and late endosomes (but not mature lysosomes). We have argued from this and other work that rho proteins cycle on and off the plasma membrane and that this is an integral feature of their mechanism of action (Adamson *et al.* 1992; Hall 1992*b*).

3. A FUNCTION FOR RHO IN FIBROBLASTS

Confluent Swiss 3T3 cells, starved of growth factors overnight, show a punctate and disorganized distribution of polymerized actin (figure 2, left panel). Microinjection of activated recombinant rho protein into the cells induces the assembly of well-defined actin stress fibres (figure 2, right panel) and the concomitant assembly of focal adhesions (Ridley & Hall 1992). Re-addition of serum produces the same effect. We speculated therefore that the effects of serum on cells might be mediated through endogenous rho proteins. To test this we have made use of a bacterial ADP-ribosyltransferase, C3 transferase, from *Clostridium botulinum*. C3 transferase has been shown to inactivate rho by ADP-ribosylating an asparagine residue at codon 41 of rho A, B and C. (Sekine *et al.* 1989; Chardin *et al.* 1989; Rubin *et al.* 1988; Paterson *et al.* 1990). When quiescent cells are preinjected with C3 transferase and then challenged with serum, the assembly of focal adhesions and of actin stress fibres is blocked (Ridley & Hall 1992). We conclude from this and other experiments that rho regulates a signal transduction pathway linking membrane receptors to the assembly of focal adhesions and actin stress fibres.

4. FUNCTION FOR RAC IN FIBROBLASTS

Rac has also been shown to have dramatic effects on actin polymerization. Microinjection of recombinant rac protein into quiescent fibroblasts induces actin

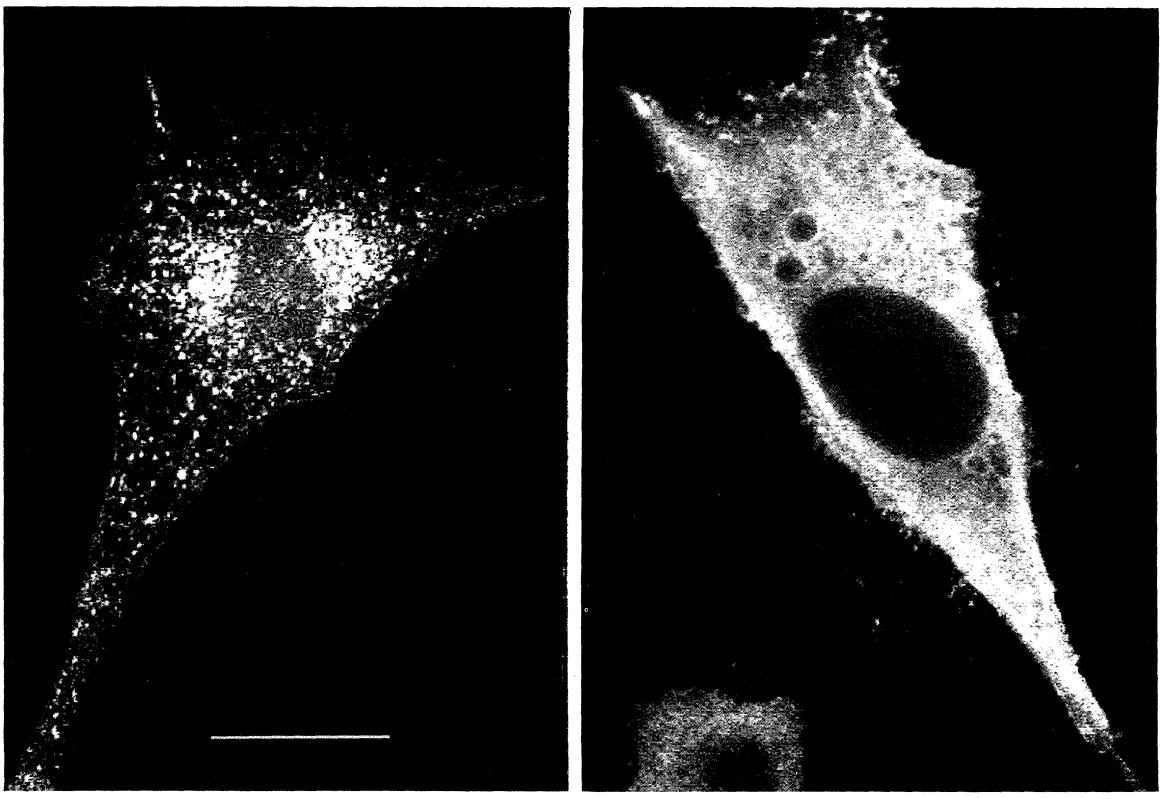

Figure 1. Cellular localization of rho proteins. A eukaryotic expression vector containing epitope-tagged rhoB (left panel) or rhoC (right panel) cDNA was microinjected into the nucleus of Rat2 cells. Sixteen hours later cells were fixed and rho proteins visualized with an anti-epitope antibody. Bar 15 μm.

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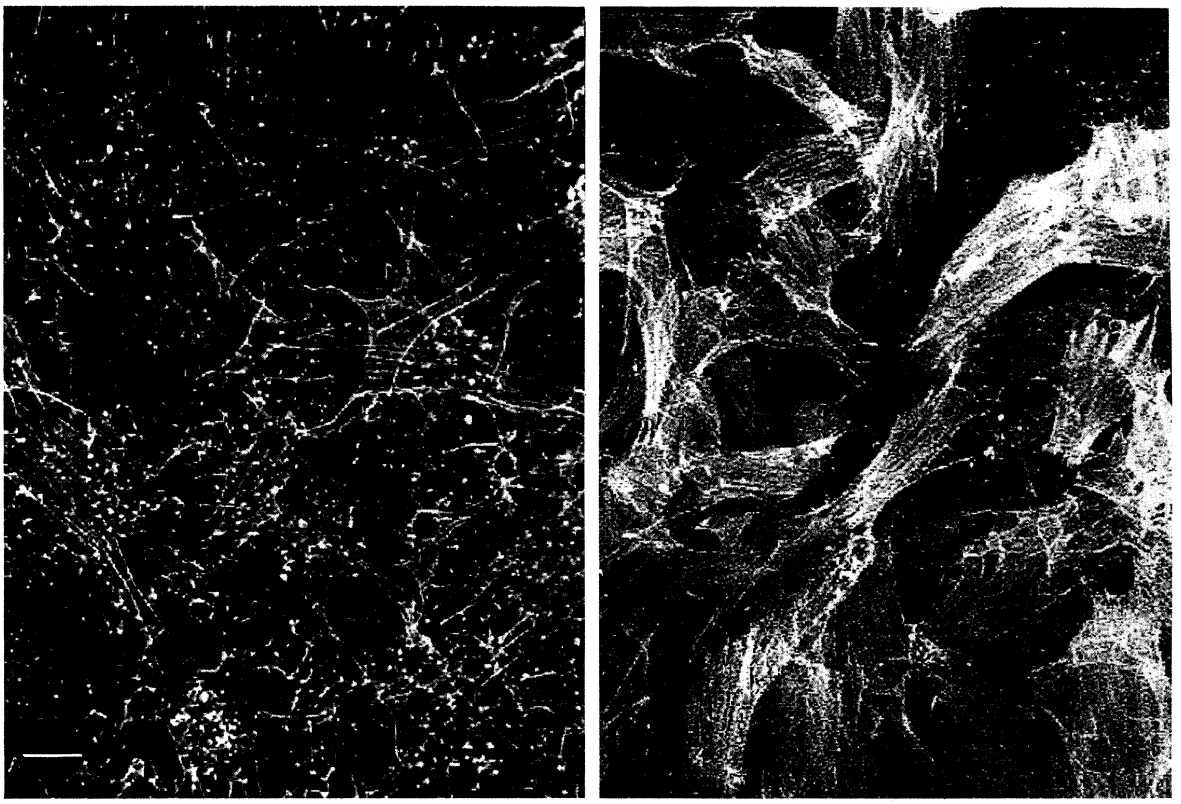

Figure 2. Microinjection of activated rho into quiescent SWISS 3T3 cells. Confluent cells were starved of serum overnight and injected with buffer (left panel) or recombinant Val14 rhoA (right panel) and fixed 30 min later. Polymerized actin was visualized with TRITC labelled phalloidin. Bar 15 μm.

polymerization at the plasma membrane leading to membrane ruffling (seen in figure 3) and pinocytosis (Ridley *et al.* 1992). No effects are observed on stress fibres.

To examine this in more detail, we looked at the effects on actin organization, of adding individual growth factors to serum-starved cells. PDGF, EGF and insulin each induce rapid (5 min) accumulation of polymerized actin at the plasma membrane to produce ruffles followed at later times (30 min) with some stress fibre formation. Serum, however, produces a different effect; a rapid (5 min) induction of the assembly of focal adhesions and stress fibres with very little ruffling. Analysis of serum showed that lysophosphatidic acid (LPA) or a closely related molecule is responsible for activating rho proteins leading to stress fibre formation (Ridley & Hall 1992).

Because the effects of PDGF and microinjected rac were similar we tested whether the observed effects of this growth factor on actin polymerization were mediated by endogenous rac proteins. Microinjection of rac containing a dominant negative mutation (N17rac) into quiescent fibroblasts completely blocked PDGF induced ruffling, but had no effect on serum induced stress fibre formation (Ridley *et al.* 1992). It appears, therefore, that rac regulates the polymerization of actin at the plasma membrane in response to certain growth factors.

5. A FUNCTION FOR RAC IN PHAGOCYTES

Professional phagocytes are responsible for combating microbial infections. One of their major lines of attack is to phagocytose the invading bacteria and to activate a membrane-bound NADPH oxidase to produce toxic superoxide radicals. Activation of the oxidase is complex, and requires the assembly of two cytosolic proteins, p47 and p67 with the two oxidase subunits at the plasma membrane. A defect in any one of these four components has serious clinical consequences as seen in patients with chronic granulomatosis disease (CGD) (Morel *et al.* 1991).

The activation of the oxidase has been partly reconstituted in an *in vitro* assay using membranes and cytosol from neutrophils or macrophages. Detailed biochemical analysis of cytosolic components has revealed that rac, is also essential for activation of the oxidase (Abo *et al.* 1991; Knaus *et al.* 1991). It has now been shown that an active oxidase complex can be assembled *in vitro* using purified NADPH oxidase (two subunits), recombinant p47 and p67, and recombinant rac in the GTP-bound form. It has been suggested, therefore, that the role of rac in this system is to promote the assembly of the multimolecular complex.

6. A GENERALIZED FUNCTION FOR RHO AND RAC

In fibroblasts rho controls the assembly of focal adhesions; clusters of integrin receptors associated with cytosolic proteins such as vinculin and talin which serve as anchors for actin stress fibres (Burridge *et al.* 1988). In phagocytes rac promotes the assembly

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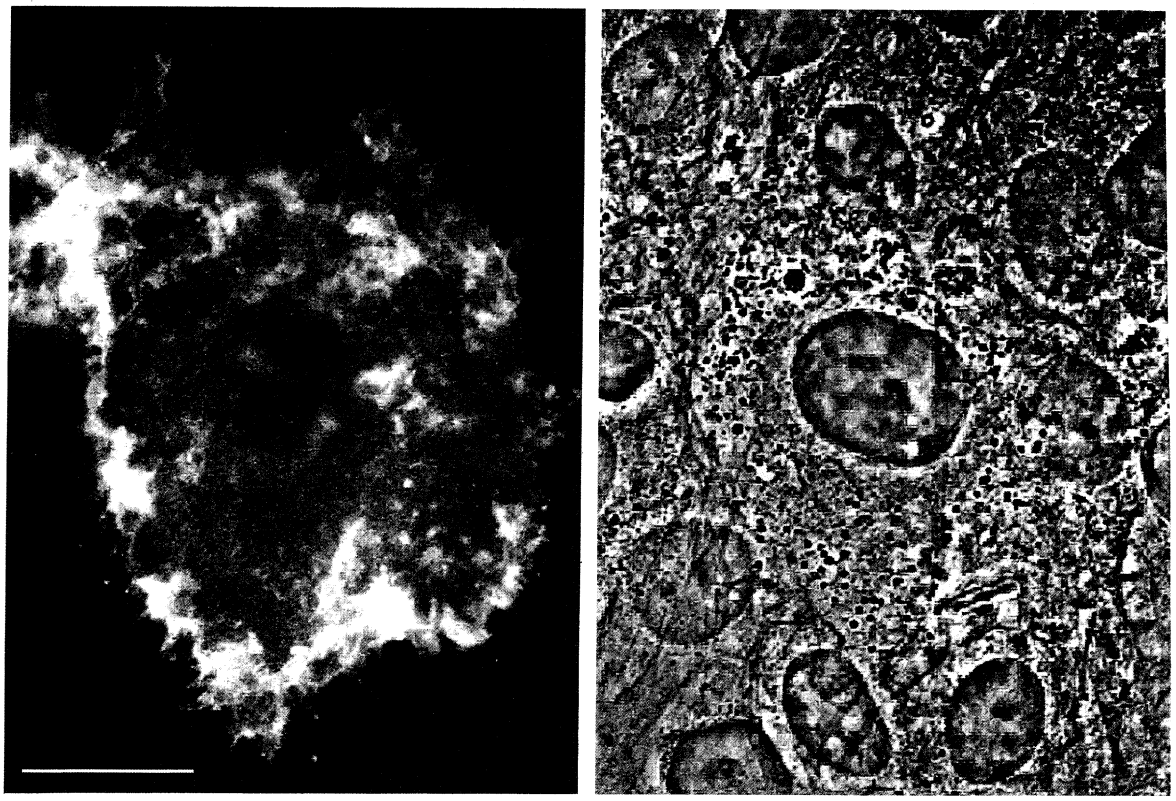

Figure 3. Microinjection of activated rac into Rat2 cells. A cDNA expression vector containing epitope tagged rac1 cDNA was injected into the nucleus of a Rat2 cell in a confluent monolayer. Sixteen hours later, cells were fixed and the distribution of rac observed using an anti-epitope antibody (left panel). The right panel shows under phase contrast the effect of rac expression on cell morphology and the stimulation of membrane ruffles by rac can be seen in the left panel using immunofluorescence. Bar 15 μm.

of cytosolic proteins at a membrane-bound NADPH oxidase. *In vivo* this is closely coordinated with phagocytosis, a process which involves changes in polymerized actin at the plasma membrane not unlike the changes we have observed in fibroblasts after injection of rac. We have argued previously that rac may serve to coordinate the activation of NADPH oxidase with phagocytosis (Ridley *et al.* 1992; Hall 1992*b*). To complete the story (so far) the CDC42 gene has been studied in some detail in *S. cerevisiae*. Budding of a daughter cell from its mother occurs at a particular site on the plasma membrane, the bud site. CDC42 is required for the correct assembly of components of the bud at the bud site (Adams *et al.* 1990).

There appears to be a striking underlying similarity between these diverse biological processes. Rho-like proteins, in the GTP form, promote the assembly of cytosolic proteins onto a plasma membrane target in response to an extracellular signal. Furthermore the formation of these multimolecular complexes is accompanied by discrete changes in the organization of polymerized actin. Many questions remain to be answered not least the mechanism by which the GTPase promotes protein assembly and the nature of the link with actin polymerization. In addition, the mechanism by which ras GTPases are activated by membrane receptors is unclear and the role of the growing number of rhoGAP-related proteins remains to be investigated.

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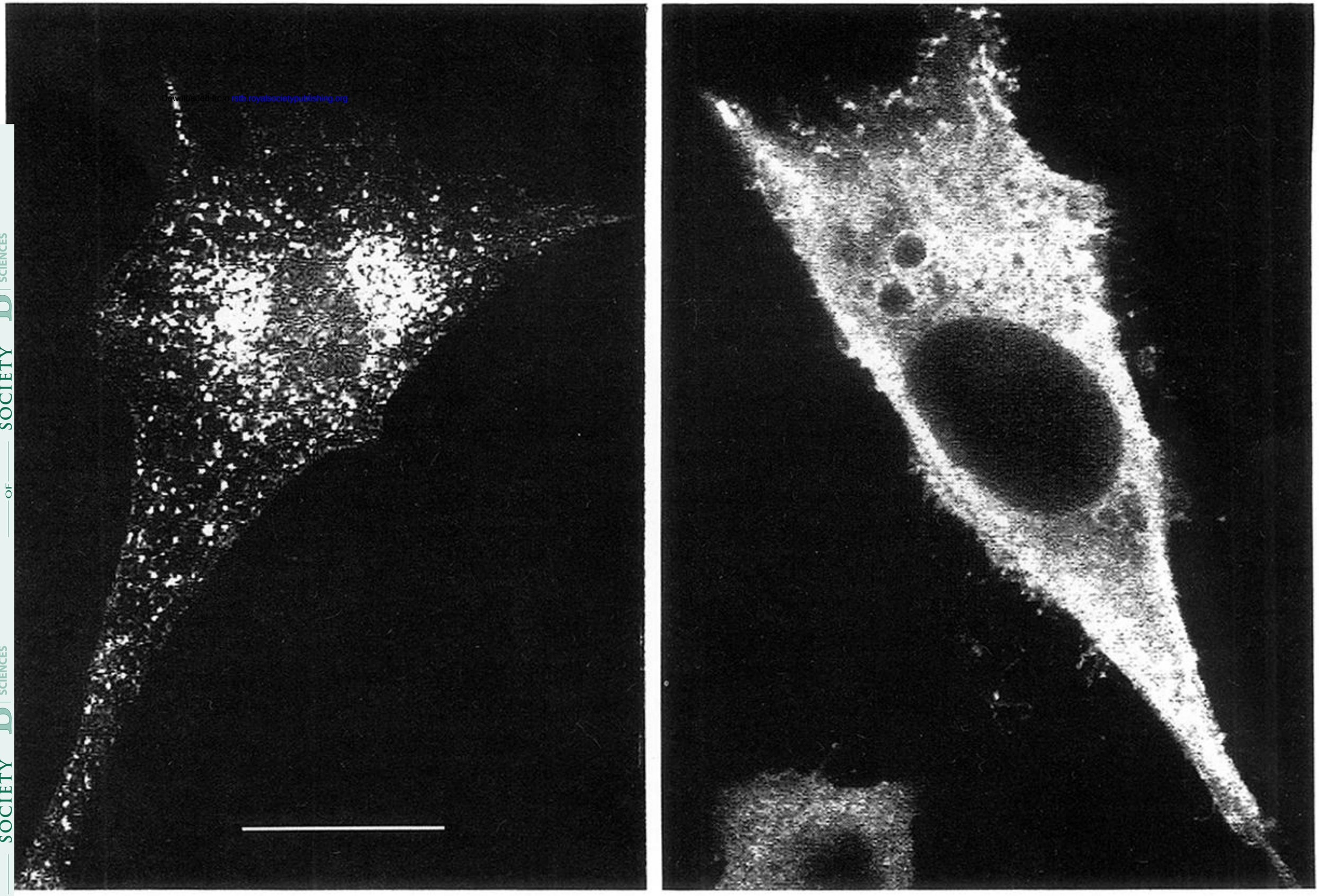

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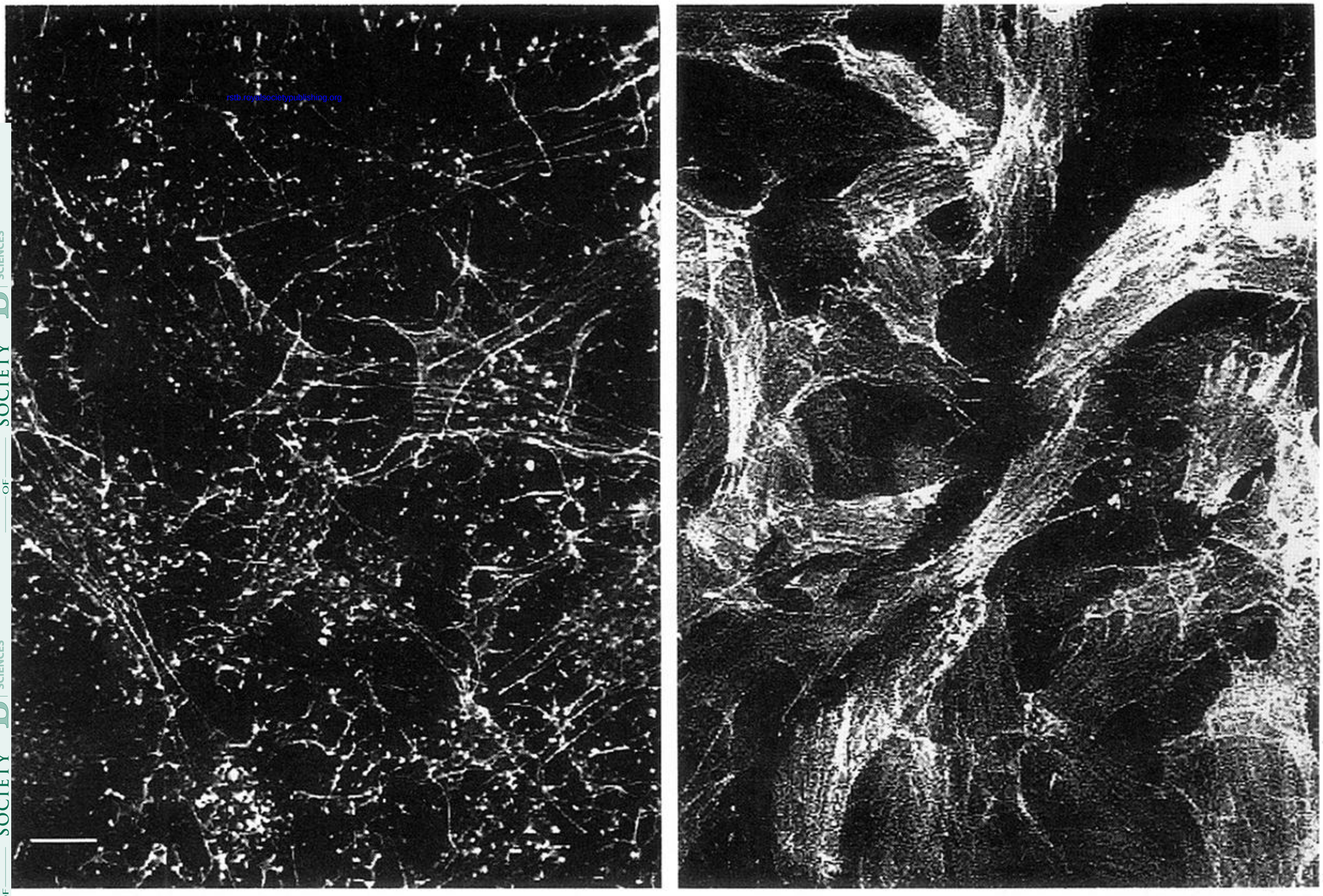

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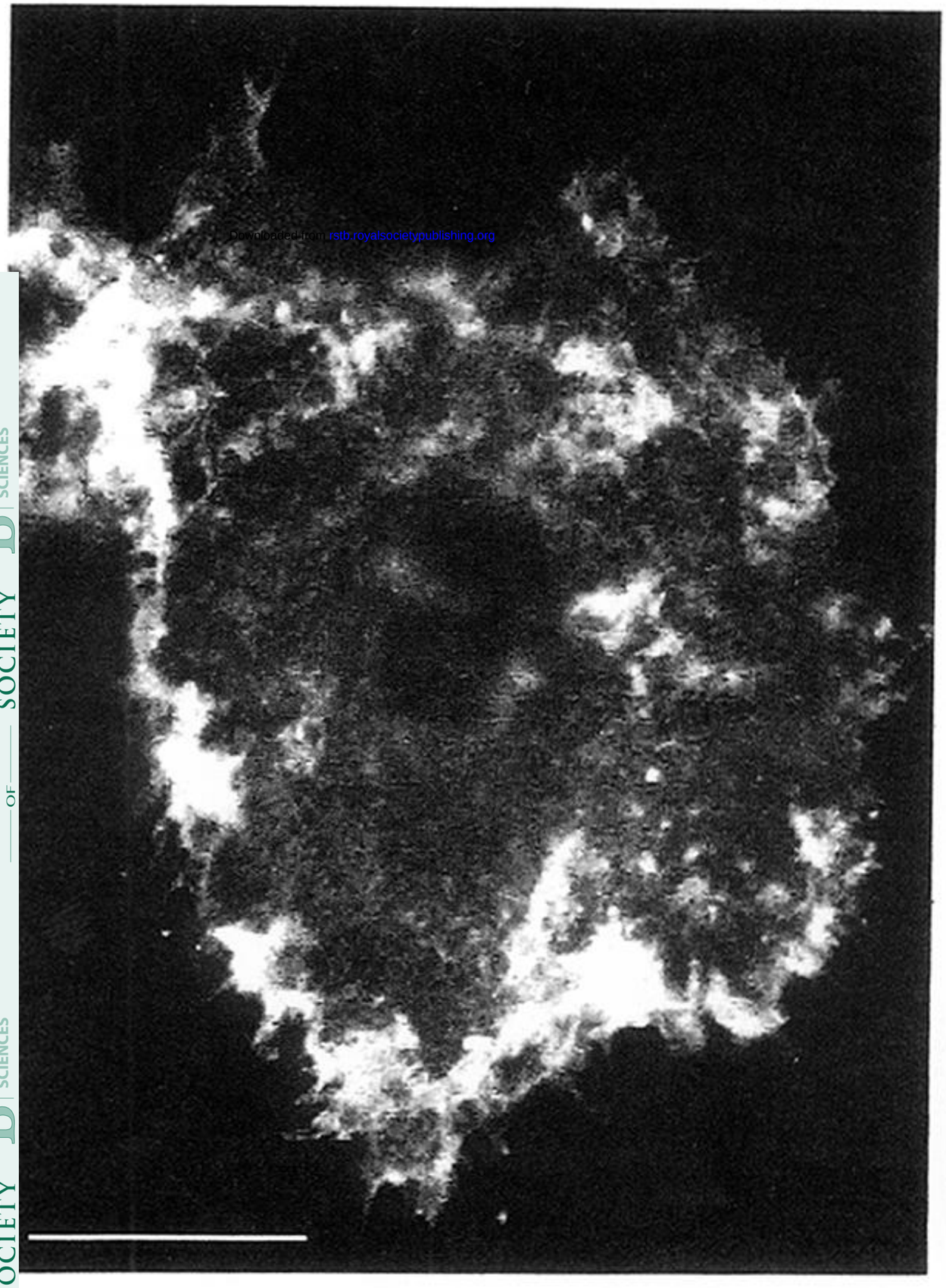

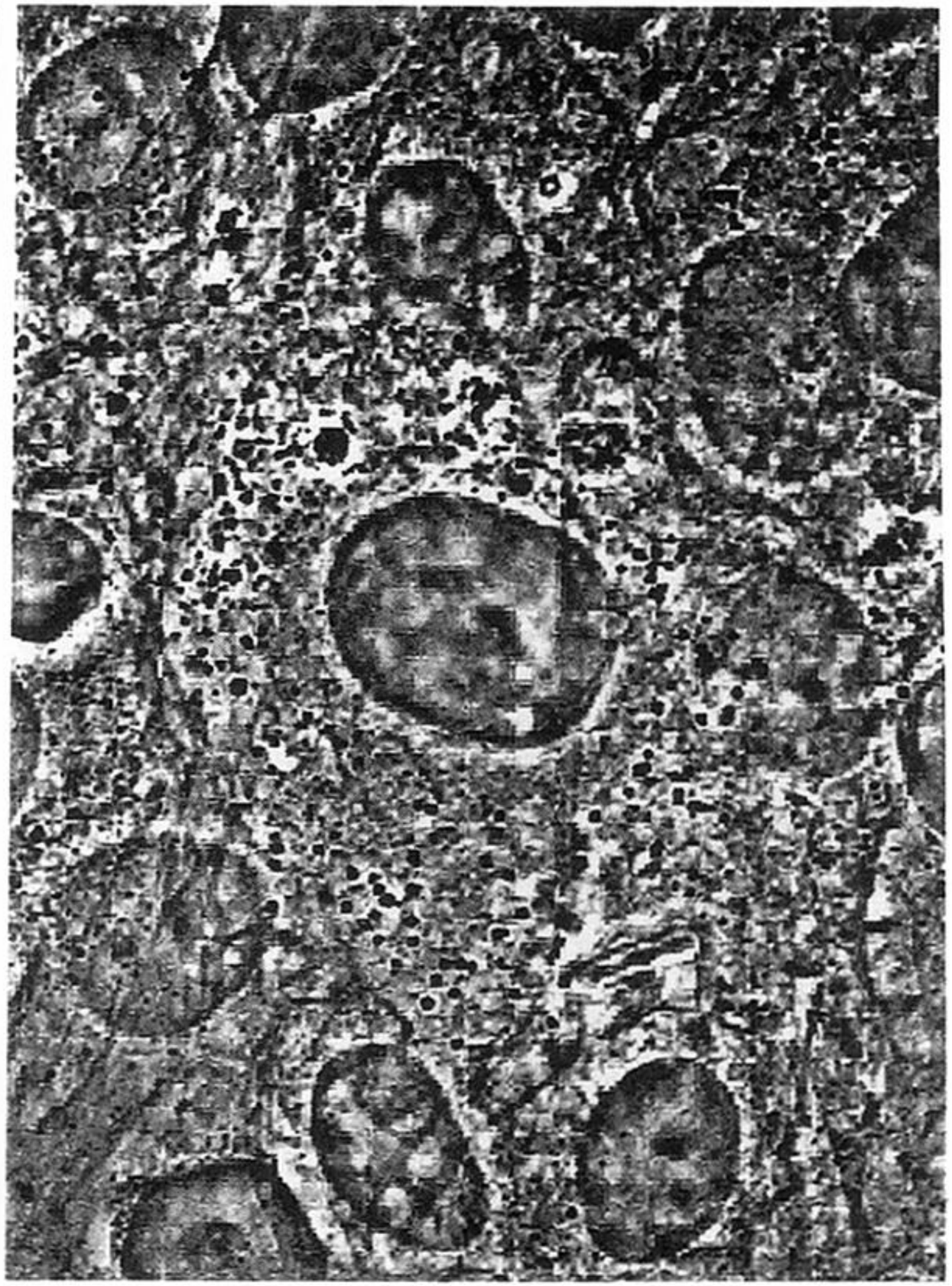

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