Diffusive and Nondiffusive Proteins In Vivo

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Proteins within the living cell potentially possess different spatial localizations and a wide range of diffusivities. At one extreme, proteins may be essentially nondiffusive—that is, highly localized structural components of the cytoplasm and/or nucleus, with very long or infinite residence-times. An extensive, proteinaceous cytomatrix network consisting of the polymerized proteins of microtubules, microfilaments, and intermediate filaments, as well as a host of tubule- and filament-associated proteins that apparently serve as structural interconnectors and functional modulators of the cytomatrix, does pervade the cell (1). Among the recognized cytomatrix proteins, intermediate filament proteins appear to be archetypical nondiffusive proteins.

At the other extreme, some proteins may diffuse freely, with essentially zero residence-times, within the aqueous phase which percolates through the interstices of the cytomatrix. The existence of such an aqueous intracellular phase is demonstrated by the free diffusion of tracer macromolecules microinjected into cells (2). Movements of freely diffusing proteins within the cell would be hindered only by random collisions with organelles and the structured proteins and water of the cytomatrix.

Between these nondiffusive and diffusive extremes, proteins may be partially cytomatrix associated and partially diffusive, with intermediate residence-times. Actin and tubulin, for example, seem to exist in both diffusive and nondiffusive forms. Largely on the basis of the extensive in vitro biochemistry of these two proteins, it is thought that the dynamic exchange of proteins between diffusive (monomer) and nondiffusive (polymer) states can be fundamental to their roles in cellular function (3–5).

It is not known how proteins are distributed among these classes in vivo. Notions about the intracellular states of proteins are usually derived from experiments that disrupt (homogenize, fix, or permeabilize) cellular structure in aqueous media. Some proteins, normally diffusive in vivo, may precipitate onto structured elements during such treatments. Conversely, to assume that proteins that are soluble after such treatments (so-called "cytosolic" proteins) are actually diffusive in the living cell is patently unwise (6). It has not been possible to distinguish diffusive proteins populating the aqueous phase in vivo from nondiffusive intracellular proteins or to measure the diffusive/nondiffusive partitioning of individual proteins within intact cells.

Our intent has been to eliminate the uncertainties inherent in aqueous cell disruption methods and to measure (*a*) the precise intracellular locations of individual proteins and (*b*) the amounts of their diffusive and nondiffusive forms in vivo. These parameters cannot be measured by aqueous methodologies because both are sensitive to the composition and ordering of the in vivo aqueous milieu: uncontrolled changes in these parameters occur during cell disruption (7, 8) and also must be suspected of accompanying chemical fixation and cell permeabilization. By application of techniques designed to obviate these difficulties, we have sought to determine protein spatial and biochemical relationships lost by other methods and to better understand protein functions within the living cell.

To this end we have developed an experimental system that allows us to measure the regional concentrations and the amounts of diffusive and nondiffusive forms of individual proteins within the full-grown oocyte of *Xenopus laevis*. A gelatin reference phase (RP)[1] is microinjected into the oocyte and constitutes a defined aqueous compartment that equilibrates with diffusive intracellular proteins but excludes organelles and nondiffusive proteins. The RP and the nucleus and cytoplasmic samples are subsequently isolated from the cell by cryomicrodissection—a cryogenic method that eliminates artifactual relocation of proteins. The protein contents of cryomicrodissected samples are then separated, characterized, and quantitated on two-dimensional polyacrylamide gels. We describe here our initial measurements for many individual proteins, including actin, one of the most abundant cytomatrix elements in the oocyte.

Experimental System—Sampling Proteins in the Living Cell

CELLS: Large oocytes (late stage V and early stage VI) (9) of *Xenopus laevis* were manually isolated from surgically resected ovaries into a Ringer's solution containing (mmol/L): 82.5 NaCl, 2.5 KCl, 1.0 $CaCl_2$, 1.0 $MgCl_2$, 1.0 Na_2HPO_4, 3.3 NaOH, 5.0 HEPES buffer, and 1.0 sodium pyruvate, pH 7.6 (10, 11). Cells were maintained in this medium continuously until frozen for cryomicrodissection (see below). The

[1] *Abbreviations used in this paper:* HEPES, 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid; SDS, sodium dodecyl sulfate; RP, reference phase; N, nucleus; C, cytoplasm; IEF, isoelectric focusing.

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great size of the *Xenopus* oocyte (1,200–1,400 μm in diameter) and its nucleus (350–400 μm in diameter) makes it ideal for microinjection and cryomicrodissection. Furthermore, *Xenopus* oocytes contain all the major cytoskeletal proteins—actin (12), tubulin (13), and intermediate-filament proteins (14), as well as a demonstrable aqueous phase for macromolecular diffusion (2).

INTRACELLULAR GELATIN REFERENCE PHASE: An aqueous RP was introduced into living oocytes as described (15, 16). In brief, a 10–15% solution of purified gelatin was microinjected into the cell with hydraulic pressure through a glass micropipette with a tip diameter of 2–5 μm (Fig. 1 *a*). Injections were directed into the vegetal portion of the cell without touching the nucleus, which is accentrically located toward the animal pole. The gelatin was quickly gelled in place in the cytoplasm (within 15 s of microinjection) by cooling the cell for 1–2 min in iced Ringer's solution. RP-containing oocytes were then incubated at 13°C, a temperature that keeps the RP gelled but does not affect cell viability (as measured by maintenance of intracellular cation levels and continuation of protein synthesis). The gelled gelatin, typically occupying 4–12% of the cell volume (one to three times the nuclear volume), is a loose fibrous protein network (85–90% water) that excludes organelles and nondiffusive proteins but reaches equilibrium with proteins and other solutes that are diffusive within the cell. Several controls validate the efficacy of the intracellular RP as a sampler of diffusive proteins:

(*a*) Theoretical calculations (not shown) based upon measurements of macromolecular diffusion in cross-linked gels (17) indicate that the gelatin RP will equilibrate with globular diffusive proteins of up to ~100,000 daltons within 20 h.

(*b*) In vitro gelatin water has the solvent characteristics of ordinary water for small solutes and proteins (^{125}I-myoglobin). Equilibrium dialysis experiments show that neither exclusion from gelatin water nor binding to the gelatin matrix is detectable; and no effects of tracer electrical charge on kinetics or equilibria have been observed (16, 18).

(*c*) After ^{125}I-myoglobin has been introduced into the oocyte dissolved in RP gelatin, it reaches diffusive equilibrium between the RP, cytoplasm, and nucleus within 4 h. For myoglobin, as well as for the dextran tracers discussed immediately below, the nucleus/cytoplasm equilibrium distribution is the same in RP-containing and control (non-RP-injected) oocytes, demonstrating that the intracellular equilibrium distributions of diffusive macromolecules are not affected by the presence of the RP.

(*d*) We have injected [^{3}H]dextrans 45 Å in diameter (roughly equivalent in size to globular proteins of 40,000 daltons) dissolved in the RP gelatin into the oocyte. The dextrans were positively charged (aminated), negatively charged (sulfated), or neutral. The kinetics of tracer dextran intracellular distributions were subsequently determined by scintillation counting of RP, nucleus, and cytoplasm samples isolated by cryomicrodissection (see below). Diffusional equilibration between the RP and cytoplasm was reached between 10 and 20 h (Fig. 2 *a*). At equilibrium, RP/cytoplasm concentration ratios were 2.0 (the equilibrium ratio that characterizes [^{3}H]-

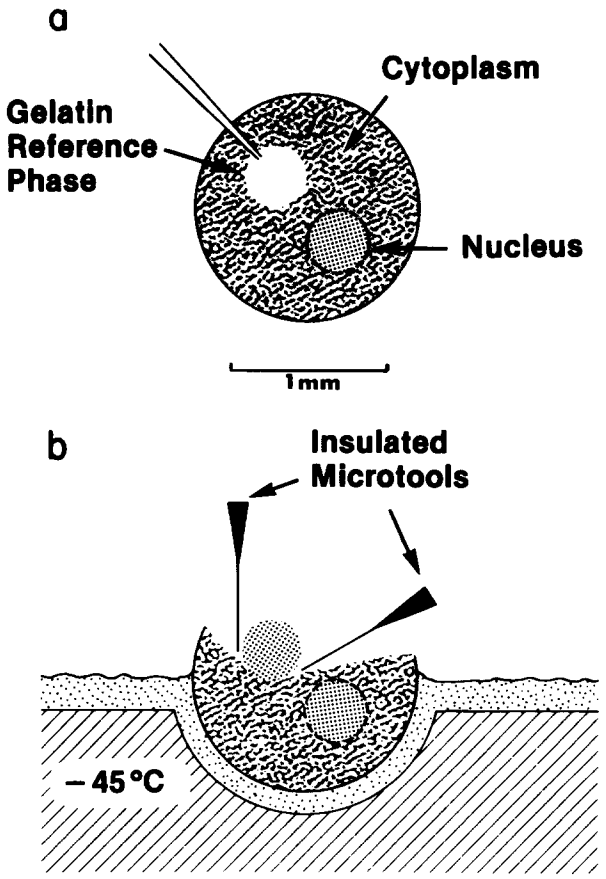

FIGURE 1 (a) Microinjection of an intracellular RP into the living *Xenopus laevis* oocyte. (*b*) Retrieval of the RP, cytoplasm, and nucleus by cryomicrodissection at −45°C.

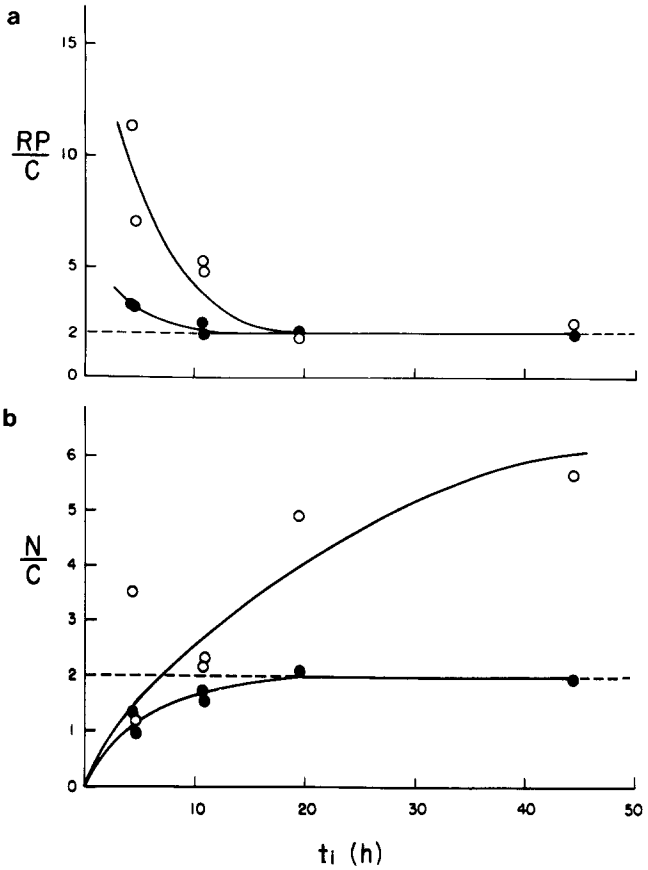

FIGURE 2 The intracellular distributions of negatively charged (O) and neutral or positively charged (●) dextrans as functions of time (t_i) after microinjection. (*a*) Ratio of RP to cytoplasm concentrations. (*b*) Ratio of nucleus to cytoplasm concentrations.

sucrose and ^{125}I-myoglobin tracers in *Xenopus* oocytes), because about one-half of the oocyte's cytoplasmic water is unavailable as a solvent for diffusive molecules. The restricted ability of intracellular water to serve as a solvent has been termed hydrate crystal solute exclusion (19), and is largely due, in the oocyte, to yolk platelets. As with in vitro dialysis studies, no charge effects were observed for the RP/cytoplasm distribution of dextrans, indicating that no electrical potential exists to complicate the diffusive equilibration of macromolecules between the RP and surrounding cytoplasm (Fig. 2*a*). Similar kinetics and equilibria were measured for the nucleus/cytoplasm movement of neutral and positively charged dextrans (Fig. 2*b*), although negatively charged dextrans displayed nuclear accumulation, presumably due to binding to nondiffusive nuclear elements (20).

(*e*) When RP-containing oocytes were frozen within 1 min after injection, the cryomicrodissected RP contained no detectable polypeptides. This demonstrates that neither the RP gelatin nor cellular proteins trapped in the RP at the time of microinjection contribute significantly to the polypeptide contents of the RP at equilibrium.

(*f*) Precise computer quantitation was carried out for two cells: one incubated for 21 h between RP injection and cell freezing; the other incubated for 30 h. No systematic differences in the RP/cytoplasm distributions were seen for the six polypeptides measured in both cells. The largest of these was ~70,000 daltons (see Table II, discussed below).

On the basis of these results, we believe that the intracellular RP equilibrates with the diffusive proteins of the oocyte within 20 h (the minimum diffusion time used in our experiments), at least for proteins 70,000 daltons or smaller, and possibly for larger ones.

OOCYTE CRYOMICRODISSECTION: After diffusional equilibrium was reached, subsequent protein movements were prevented by quenching RP-containing cells to liquid nitrogen temperature. Cryomicrodissection (18, 21) was used to isolate frozen RP, nucleus, and cytoplasmic samples. The method eliminates artifactual relocations of all diffusive solutes, including macromolecules (18, 21, 22). The frozen oocyte was removed directly from liquid nitrogen to the −45°C dissection stage of the cryomicrodissection apparatus, and the nucleus, cytoplasm, and RP were dissected freehand with insulated, stainless steel microtools (Fig. 1*b*). The nucleus and RP, due to their high water contents, are hard, icelike chunks embedded within the softer cytoplasm. This tactile difference, in addition to the distinctive yellow hue of the cytoplasmic yolk, facilitates the retrieval of clean, intact RP, nucleus, and cytoplasm samples. Samples were transferred to small tared aluminum foil envelopes, which were pressure sealed, and the envelopes were weighed in a low-temperature chamber for sample wet weight determinations. Some samples, including at least one piece of cytoplasm from each cell, were dried, reweighed to determine water content, and analyzed for Na^+ and K^+ content by atomic-absorption spectroscopy. The water and cation contents of healthy RP-containing oocytes are similar to those of uninjected control cells (16). Only cryomicrodissected samples from cells judged healthy on the basis of cytoplasmic or RP cations were analyzed for protein content (Table I).

ANALYSIS OF PROTEIN CONTENT BY TWO-DIMENSIONAL ELECTROPHORESIS: Individual cryomicrodissected samples of known water content were dissolved directly (not previously thawed) in lysis buffer containing, per liter, 9.5 mol of urea, 20 g of NP-40 detergent, 23.5 g of mixed ampholytes (0.05%, pH 9–11; 0.2%, pH 3.5–5; 0.2%, pH 4–6; 0.3%, pH 8–9.5%, 0.4%, pH 2–11; 0.6%, pH 5–7; 0.6%, pH 6–8), and 5 g of dithiothreitol, and then loaded with microsyringes onto first-dimension isoelectric focusing (IEF) tube gels. The IEF gels were 40-g/L acrylamide, with diameters of 0.95 mm and lengths of 95.0 mm. Focusing between 10 mmol/L H_3PO_4 anode buffer and 20 mmol/L NaOH (sample end) cathode buffer was carried out overnight for 5,000 volt-hours, constant voltage. (Some tube gels were frozen, and 5-mm sections were eluted in water for pH gradient determination.) Focusing gels were extruded from their tubes, applied directly to the tops of 110 × 160 × 0.38-mm SDS slab gels (after O'Farrell [23], with 4% stacking and 10% running regions), and sealed to the slabs with a 10-g/L agarose solution containing, per liter, 50 mmol of Tris HCl (pH 6.8), 10 g of SDS, 1 mmol of dithiothreitol, and 50 mg of bromophenol blue. Agarose strips containing molecular-mass marker proteins were applied at one or both ends of the tube gel. Electrophoresis in the second dimension was for 3 h at 15 mA per gel (constant current). The slab gels were fixed in 38% formaldehyde/ethanol/water (1:1.8:4.2 by vol) for 30 min, washed three times (5 min/wash) in ethanol/water (1:9 by vol) and fixed for 30 min in glutaraldehyde/water (1:9 by vol). The glutaraldehyde was rinsed out with six 10-min water washes.

TABLE I

Water and Cation Contents of Cryomicrodissected Samples from Healthy Xenopus laevis *Oocytes*

Sample	Wet weight	H_2O content	Na^+	K^+
	μg	%	meq/L	
Nucleus	34.1 ± 0.6	87.2 ± 0.3	10 ± 4	103 ± 4
Reference phase	30–100	85–90	<25	>100
Cytoplasm	50–200	53.0	37 ± 3	110 ± 5

RP wet weight and percent H_2O were determined by the amount and the water content of the microinjected gelatin. In healthy cells RP cation contents closely parallel nuclear cations (37). (Cells damaged during microinjection or handling showed intracellular cation concentrations tending toward those of the Ringer's solution, 88 mM Na^+ and 2.5 mM K^+, and were discarded.)

Proteins were silver-stained by a modification of the method of Oakley et al. (24), which allows visualization of the total protein population (as opposed to only newly synthesized proteins by isotopic labeling and autoradiography). Gels were soaked for 10 min in an ammoniacal silver solution made by adding (slowly, with stirring) 20 mL of 194-g/L $AgNO_3$ to a mixture of 7 ml of NH_4OH, 105 ml of 3.6-g/L NaOH, and 368 ml of water. The silver solution was followed by three 2-min water washes and development in citric acid (0.26 mmol/L)-formaldehyde (6.3 mmol/L) solution. After development, the gels were washed extensively in water. All steps were performed with continuous agitation and with solution/gel volume ratios >50:1. Gels to be comparatively analyzed were electrophoresed together (in both dimensions), fixed, processed, and stained simultaneously with the same batch of each solution, and processing intervals at each step were equalized. The dimensional ranges of these gels (pI ~3.5–7.5 and mass ~10,000–150,000 daltons) display the majority of cellular proteins, with yolk proteins and some basic proteins (histones, ribosomal and high-mobility group proteins) being excluded.

With a few gels we quantitated the amounts of individual

polypeptide spots. Over the appropriately controlled ranges, integrated densities of silver-stained spots were linearly related to the amount of protein present (25, 26). Quantitation of two-dimensional gels requires expensive computer and image analysis hardware and sophisticated software (27). Our present access to this technology is limited, but initial measurements on the proteins of two cells demonstrate the power of the methodology. Stained gels were placed between two sheets of porous, transparent cellophane dialysis membrane and heat-vacuum dried. The dried transparencies were taken to the Laboratory of General and Comparative Biochemistry at the National Institute of Mental Health, Bethesda, MD, where, with the generous assistance of Drs. Carl R. Merril and David Goldman, quantitative computer analysis was performed. Gels were scanned with a 1,000-HS densitometer (Optronics International, Inc., Chelmsford, MA), and a two-dimensional optical density scan of each gel was recorded onto computer disk storage. Gel images were recalled, displayed, and manipulated on a cathode-ray tube, and integrated densities of individual polypeptide spots, with background correction, were determined with an IP 5000 image processor (DeAnza Systems, Inc., San Jose, CA) and a PDP 11/60 computer (Digital Equipment Corp., Marlborough, MA).

Some Proteins Diffuse In Vivo—Others Apparently Do Not

With the combination of the RP, cryomicrodissection, and two-dimensional gel analysis, we can now begin to glean information on the localizations and diffusivities of individual proteins within the living cell. Diffusive proteins equilibrate with the RP, whose water possesses the solvent properties of ordinary water. Proteins that are present in the cytoplasm and/or nucleus but that do not enter the RP are operationally defined as nondiffusive and include cytomatrix proteins as well as some proteins incorporated into cellular membranes and impermeable vesicles.

Individual cryomicrodissected samples from single cells were run simultaneously on two-dimensional gels, separating the polypeptides of nonyolk[2] proteins by isoelectric point in the first (horizontal) dimension and mass in the second (vertical) dimension. RP gels (Fig. 3*a*) show that hundreds of proteins exist in diffusive form within the intact, living cell. Polypeptides that diffuse into the RP are not distinguished by size or electrical charge but are distributed widely over the ranges mapped by the gels, pI ~3.5–7.5 and ~10,000–150,000 daltons. (Many of these polypeptides are probably coassembled in vivo in multichain proteins, whose aggregate, native characteristics are not known.) One of the most prominent spots seen in the RP is actin (spot 31). We discuss actin in detail below, illustrating how quantitative gel analysis can measure the in vivo localizations and diffusivities of individual proteins.

[2] Isolated yolk platelets were solubilized and run on our gel system; only one peripheral region contained detectable proteins. Proteins in this region were present on all gels loaded with cytoplasmic samples but only occasionally (and much more faintly) on gels of nucleus or RP samples, presumably representing trace cytoplasmic contamination (see outlined region, Fig. 3*a*). Oocyte yolk is known to contain polypeptides (phosphitin and lipovitellin) in this size range, 30,000–35,000 daltons (28), but they apparently migrate only a short distance into our first-dimension gels and do not confuse the display of nonyolk proteins.

Gels show qualitative and quantitative differences in the polypeptide contents of RP, nucleus, and cytoplasm. To accurately compare gels and to measure the amounts of individual polypeptides in these compartments, we quantitatively analyzed gels loaded with RP, nucleus, and cytoplasm samples from the same cell by computer. Specific polypeptides were identified by their positions, and their integrated spot densities were measured on RP, nucleus, and cytoplasm gels and converted to in vivo concentrations by dividing by the known amounts of sample water actually loaded. The numbered positions of 90 polypeptides so analyzed are superimposed on a nuclear gel in Fig. 3*b*. Measured RP/cytoplasm concentration ratios are shown in Table II. Values have been calculated for those spots whose integrated densities on both the RP and cytoplasm gels were within the linear response range of the silver stain. For some polypeptides, RP spot densities were above the stoichiometric response range, and we have assigned lower limits to their ratios. (For those polypeptides with both RP and cytoplasm spot densities below the linear range, no values were calculated.) The close agreement of the data for two cells, and for two independent experiments with materials from the same cell (Table II), illustrates the accuracy and reproducibility of the analysis.

Twenty-three of the measured polypeptides did not enter the RP in detectable amounts. These proteins were also undetected in the cytoplasm but were readily detectable in the nucleus and hence possess very high nucleus/cytoplasm concentration ratios (Table III). We believe that these proteins have high nuclear binding capacities or that they are components of the nuclear matrix itself. (We do not know whether the prevalence of these nuclear concentrating proteins, 23 of the 90 polypeptides we have measured, reflects generally higher concentrations of nondiffusive proteins in the oocyte nucleus vis-à-vis cytoplasm or whether it may simply result from a nonrandom selection of polypeptides for quantitation.) The most prominent such protein is nucleoplasmin (29), spots 60 and 61 on our map (Fig. 3*b*), a phosphorylated nuclear protein with widespread occurrence among eukaryotic cells (30).

In evaluating quantitative RP/cytoplasm data, recall that a protein that exists only in diffusive form is expected to display a RP/cytoplasm concentration ratio of 2.0, due to its exclusion from ~50% of the oocyte's cytoplasmic water (discussed above). Of the 45 proteins listed in Table II, as many as 12 (numbers 2–4, 9–12, 37–39, 58, and 59) potentially behave in this fashion. However, the majority (at least 33) have RP/cytoplasm concentration ratios of between 0.1 and 1.6, corresponding, after correction for the nonsolvent cytoplasmic water, to 5–80% diffusive form. Thus, 73% or more of the proteins listed in Table II have a measurable portion of their molecules in nondiffusive form. When the 23 proteins for which we have found no diffusive forms (Table III) are also considered, 82% (56/68) of the measured oocyte polypeptides exhibit some nondiffusive forms.

Actin: Diffusive/Nondiffusive Ratios—Cytoplasm and Nucleus

Among the polypeptides that we have quantitated, one known cytomatrix protein, actin, has been identified. The β and γ isoforms of actin are synthesized by *Xenopus* oocytes throughout oogenesis and have been previously identified by two-dimensional gel electrophoresis (12, 14). The full-grown

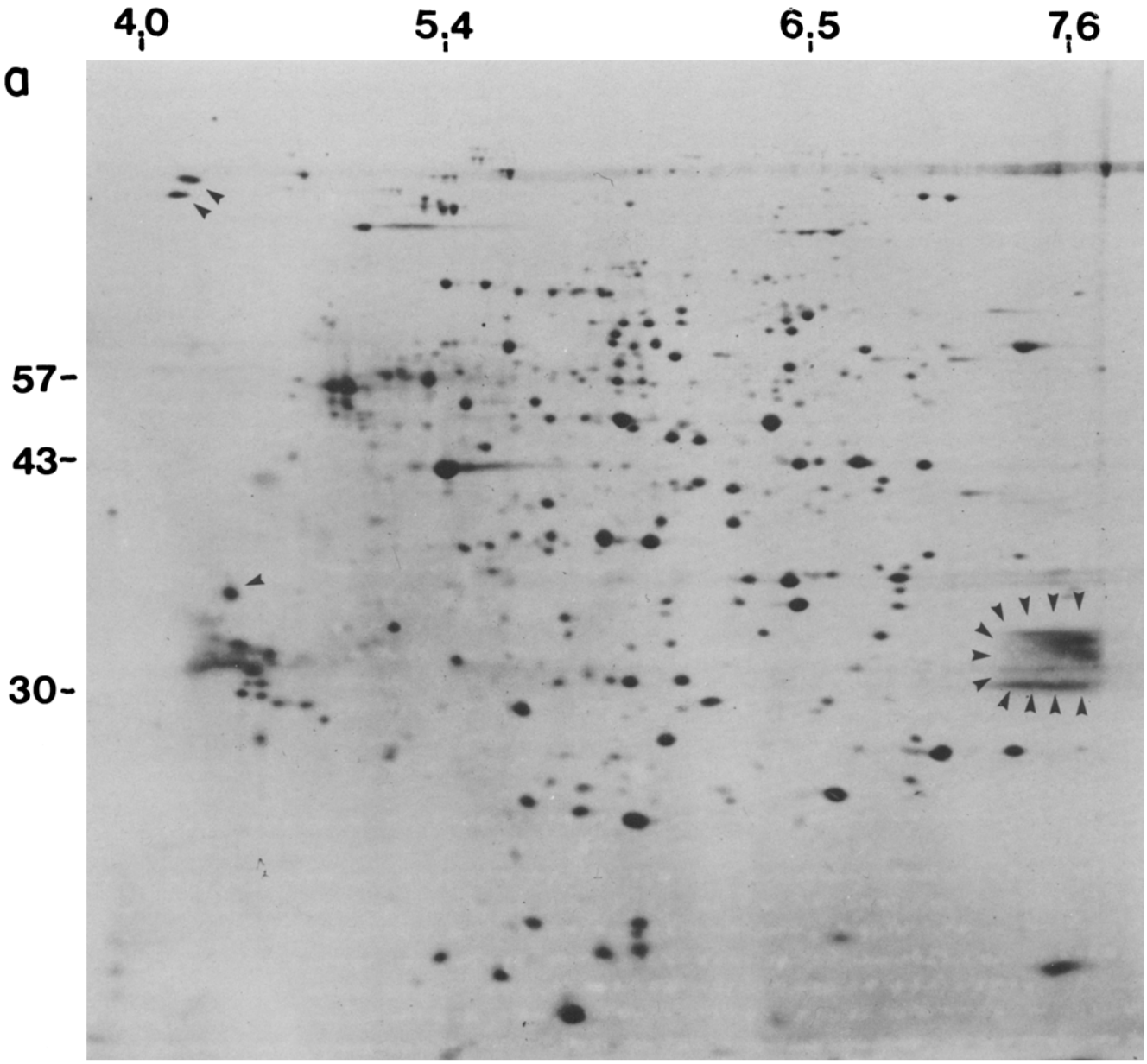

FIGURE 3*a* Two-dimensional gel display of the polypeptides in the RP. This particular RP, from an oocyte stimulated to mature with progesterone, is identical to those of RPs from unstimulated control oocytes, except for the presence of three polypeptides (62, 74, and 75, arrows)—see text for discussion. Outlined with arrows is a small amount of yolk polypeptides, which are sometimes present but always confined to this peripheral region. Molecular mass (daltons) calibrations are given on the vertical axis; isoelectric points on the horizontal axis.

Xenopus oocyte contains 1.4–1.7 μg of actin per cell (31). The actin content of the nucleus was found to be 0.13–0.15 μg (32, 33), but these measurements were made on aqueously isolated nuclei and may be low due to diffusional losses (see below). Oocyte actin can polymerize; actin microfilaments have been identified in oocyte cytoplasm by electron microscopy and myosin labeling (34), and both oocyte cytoplasm and nucleus form actin-based gel networks in vitro under the appropriate conditions (33, 35).

Polypeptide 31 (Fig. 2 and Table II), a prominent spot on all cytoplasmic, RP, and nuclear gels, is identified as β- and γ-actin by its molecular weight, isoelectric point, and electrophoretic mobility relative to purified muscle (α-) actin. We measured the nucleus/cytoplasm concentration ratio for actin in two RP-injected cells (911 and 26, Table II), and obtained values of 3.0 and 2.6. Measurements of actin in two control oocytes (not RP injected) gave 2.7 and 3.0. (As with the diffusive myoglobin and dextran tracers discussed above, the intracellular distribution of actin is not altered by microinjection or the presence of the RP.) Using the mean of these determinations, 2.8, we calculated the amounts and concentrations of actin in the oocyte nucleus and cytoplasm (Table IV). Our value for nuclear actin, 0.20 μg, is somewhat larger than the values obtained in previous determinations performed on aqueously isolated oocyte nuclei (32, 33). However, the difference, 0.05–0.07 μg, is similar to the amount of actin that we calculate to be diffusive within the in vivo nucleus (see below), which is consistent with diffusive protein loss from the aqueously isolated oocyte nucleus (8).

We can also estimate the in vivo distribution of oocyte

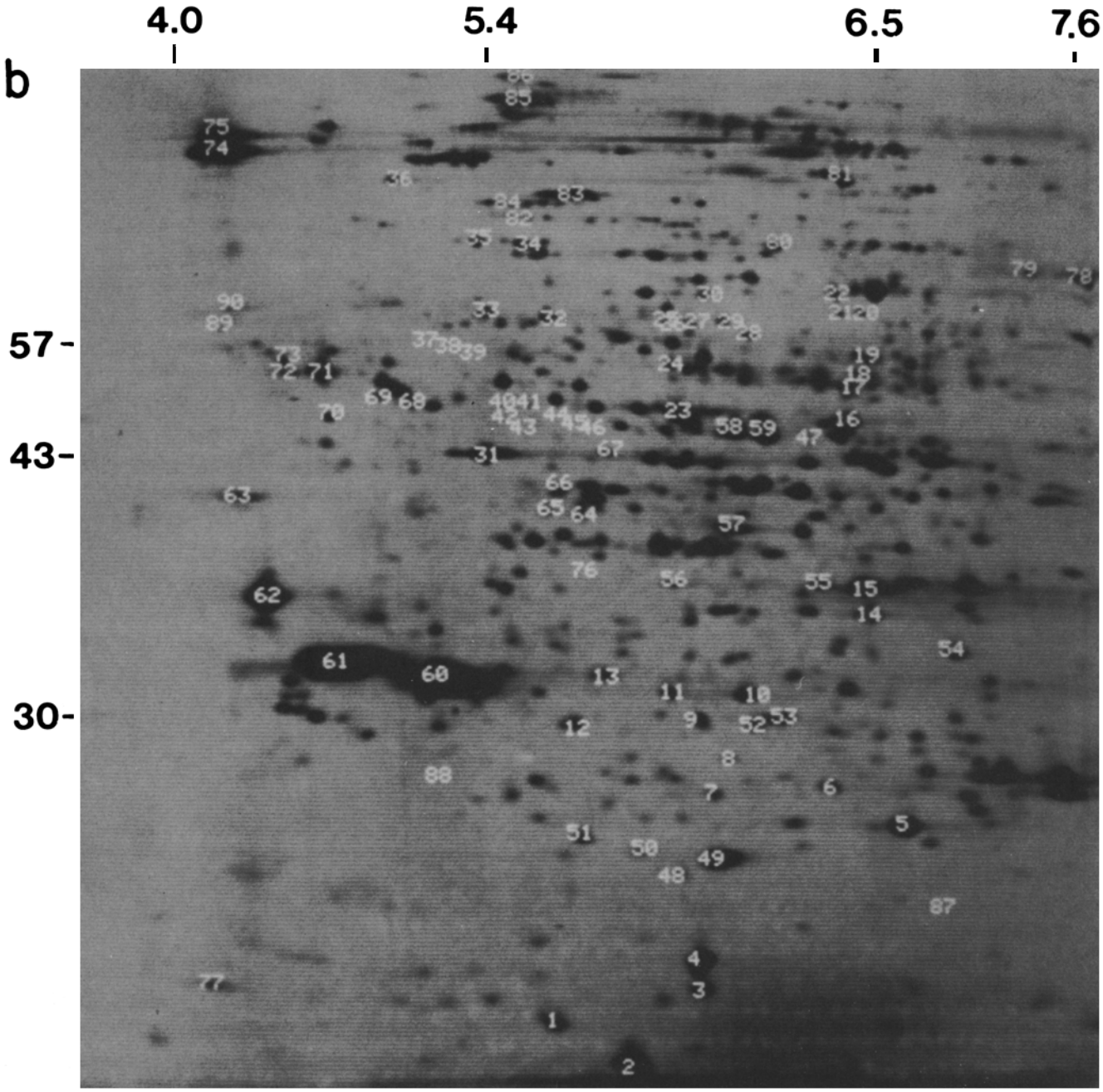

FIGURE 3*b* "Map" gel showing the locations of 90 polypeptides selected for quantitative computer analysis. The polypeptide positions are shown superimposed on a gel loaded with a single oocyte nucleus. Molecular mass (daltons) calibrations are given on the vertical axis; isoelectric points on the horizontal axis.

actin between diffusive and nondiffusive forms. Merriam and Clark (31), using centrifugation of disrupted oocytes, concluded that 75–95% of the actin in *Xenopus* oocytes is in a soluble, nonfilamentous state. Table II shows that in the oocyte the RP actin (polypeptide 31) concentration is 85% of that in the cytoplasm, or 1.95 mg/ml. Because the RP is in diffusional equilibrium with only 50% of the cytoplasmic water (discussed above), we conclude that 43% (1.95/[2.29 × 2]) of cytoplasmic actin can enter the RP and thus is in diffusive form.

A parallel calculation can be made for nuclear actin. Clark and Merriam (32) found that ~75% of the oocyte nuclear actin was diffusible after aqueous nuclear isolation, but—as they pointed out—this result does not necessarily reflect the state of in vivo nuclear actin. Clark and Rosenbaum (33), using a DNAase I inhibition assay (36), determined that oocyte nuclear actin was 63% G-(globular) actin and 37% F-(filamentous) actin. The study using the latter method also was performed on aqueously isolated nuclei. Our RP data indicate that only 1.95/6.47, or 30%, of oocyte nuclear actin is diffusive.

The present determinations of the diffusive/nondiffusive distributions of oocyte cytoplasmic and nuclear actin are based on data from only one cell. However, they are the first such data available from a healthy, intact cell. The difference between our results and those previously obtained support

TABLE II

Reference Phase/Cytoplasm Concentration Ratios

Polypeptide	Cell*			Mean‡	Polypeptide	Cell			Mean‡
	911	26H	26L			911	26H	26L	
1	—	0.3	—	0.3	28	0.4	0.4	—	0.4
2	>1.1	>1.2	—	—	29	0.5	—	—	0.5
3	>2.4	—	—	—	30	0.5	—	—	0.5
4	>2.5	—	—	—	31	>0.4	0.7	0.9	0.8
5	>0.5	0.6	—	0.6	32	0.2	0.2	—	0.2
6	1.1	—	—	1.1	33	0.2	—	—	0.2
8	0.9	—	—	0.9	34	0.2	—	0.3	0.2
9	>1.9	—	—	—	35	>0.4	0.6	—	0.6
10	>1.5	—	—	—	36	0.4	—	—	0.4
11	>2.1	—	—	—	37	>1.6	—	—	—
12	>2.2	—	—	—	38	>1.3	—	—	—
14	>0.6	0.7	—	0.7	39	>0.6	—	—	—
15	>1.0	1.1	—	1.1	41	0.5	—	—	0.5
16	>0.5	1.0	1.0	1.0	42	0.5	—	—	0.5
17	0.6	—	—	0.6	43	0.6	—	—	0.6
18	0.3	0.3	—	0.3	44	0.5	—	—	0.5
19	0.5	0.6	—	0.5	45	0.3	—	—	0.3
20	0.4	0.5	—	0.5	46	0.6	—	—	0.6
21	0.7	—	—	0.7	47	0.4	—	—	0.4
22	1.6	—	—	1.6	58	>1.1	—	—	—
23	>0.8	1.1	1.2	1.1	59	>1.9	—	—	—
24	—	0.4	—	0.4	69	<0.1	0.1	—	0.1
25	—	0.5	—	0.5					

* Data are from two computer-analyzed cells. For cell 911, solubilized RP and cytoplasm samples were loaded onto single gels; for cell 26, solubilized RP and cytoplasm samples were split into light (L) and heavy (H) loads, and each was run on a different gel, yielding two independent pairs of gels for analysis (26L and 26H).

‡ Not including indefinite values.

TABLE III

Polypeptides Not Detected in the Reference Phase: Minimum Nucleus/Cytoplasm Concentration Ratios

Polypeptide	N/C	Polypeptide	N/C
13	20	74	250
60	600	75	190
61	590	77	20
62	210	78	75
63	50	79	20
64	40	80	40
67	25	81	50
68	20	82	15
70	20	83	100
71	100	84	50
73	20	85	135
		86	30

For each polypeptide, nuclear concentrations were measured, but cytoplasmic concentrations were undetectably low. Lower limits to the ratios of nucleus to cytoplasm concentrations (N/C) were calculated, assuming a cytoplasmic concentration equal to the limit of measurement sensitivity.

TABLE IV

Actin in the Oocyte In Vivo

Compartment	Amount	Concentration	Percent diffusive
	μg	mg/ml	
Cytoplasm	1.35	2.29	43
Nucleus	0.20	6.47	30

Data calculated from our determination of the nucleus/cytoplasm concentration ratio, 2.8, the measurement of Merriam and Clark (31) for total oocyte actin, 1.4–1.7 μg/oocyte (median 1.55 used here), and our measurements for whole cell wet weight (1.15 mg), the nuclear wet weight, and the nuclear and cytoplasmic percent H_2O contents (Table I). Calculation of percent diffusive is described in text.

the view that aqueous cell disruption methods alter the in vivo diffusive/nondiffusive partitioning of proteins.

Diffusivities of Specific Proteins Change during Meiosis

The *Xenopus* oocyte also offers an opportunity to measure changes in the relative proportions of diffusive/nondiffusive forms of specific proteins during a cell division process. The cells used in all the experiments described above were full-grown ovarian oocytes, arrested in first meiotic prophase (equivalent to the G_2 phase of the mitotic cycle). It is possible, however, to stimulate the oocyte to resume in vitro meiosis (mature) with progesterone. During maturation the nucleus migrates to the cell surface and, as in mitosis, undergoes nuclear envelope dissolution.

Dissolution of the nucleus before mitotic cell division is characterized by release to the cytoplasm of proteins normally concentrated in the interphase nucleus. These proteins later reaccumulate in the reforming daughter nuclei. Central to an understanding of nuclear reaccumulation mechanisms is knowledge of the nature of the physical states of nuclear proteins during their period in cytoplasm. Are they freely diffusive, and do they concentrate in the new nucleus by polymerizing from solution? Or are they maintained in association with nondiffusive cytoplasmic structures that play some role in determining their subsequent nuclear location? We thought that meiotic and mitotic divisions may be enough alike that data gathered with the RP in the oocyte would provide clues to help us in answering these questions.

We examined two maturing oocytes (without RPs) that had not yet progressed as far as nuclear breakdown. Computer analysis of the gels of nucleus and cytoplasm from these cells showed that several of the nuclear concentrating proteins listed in Table III exhibited reduced nucleus/cytoplasmic concentration ratios compared to those of first meiotic prophase (control) oocytes; others showed no change in their intracellular distributions (data not shown). Thus, some oocyte nuclear proteins are released to the cytoplasm during meiotic nuclear breakdown. We determined whether any of these assume diffusive forms and enter the intracellular RP. Fig. 3*a* shows the polypeptide contents of an RP from a matured oocyte, after nuclear breakdown. Polypeptides 62, 74, and 75 (refer to map gel, Fig. 3*b*), not detectable in the RP of unmatured oocytes, are present in this RP. (Except for these newly diffusive polypeptides, the gel shown in Fig. 3*a* is identical to gels of RPs from control oocytes.) Other oocyte nuclear proteins, especially nucleoplasmin (polypeptides 60 and 61), apparently are not released in a diffusive form, because they do not appear in the RP shown in Fig. 3*a*. Thus, meiotic nuclear breakdown entails the release of some nuclear proteins into the cytoplasm in diffusive forms, whereas others remain associated with nuclear remnants in nondiffusive or particulate forms or perhaps form stable associations with nondiffusive cytoplasmic elements.

Summary and Prospectus

These initial RP studies in the amphibian oocyte indicate that neither of two extreme models is correct: all cellular proteins are not fixed and nondiffusive; likewise, all are not free to diffuse within the cell. This finding, though not entirely unexpected, is the first to be presented for proteins in intact, living cells. Moreover, measurements on the most prominent oocyte polypeptides indicate that over 80% exist, at least in part, in nondiffusive form. Two interesting opportunities are immediately suggested: (*a*) the identification of these measured polypeptides as proteins of known function (e.g., cytoskeletal components, enzymes, receptors, gene regulators) and (*b*) detection of changes in the relative amounts of their diffusive and nondiffusive forms and correlation of such changes with changes in cell physiology and biochemistry.

For actin, the cytomatrix protein that we have measured, we find two parameters to be larger than previously determined using oocytes disrupted in aqueous media: the amount of actin in the nucleus and the relative proportion of actin in nondiffusive form. However, our data do support an important conclusion drawn previously for several cell types: the intracellular concentration of diffusive actin is higher than the critical concentration for polymerization, 0.1–0.5 mg/ml, determined in vitro (38–41). In the oocyte, as in other cells, it appears that in vivo factors restrain the polymerization of actin relative to its behavior in purified in vitro preparations.

In attempting to elucidate the nature and mechanisms of in vivo factors that regulate the diffusive/nondiffusive ratio of actin and other intracellular proteins, it will be important to work with intact cells. The intracellular RP, when coupled with cryomicrodissection and quantitative two-dimensional gel electrophoresis, provides a unique window to the state of proteins in the living cell.

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