Protein Loss during Nuclear Isolation

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ABSTRACT Cryomicrodissection makes possible the measurement of the entire in vivo protein content of the amphibian oocyte nucleus and provides a heretofore missing baseline for estimating protein loss during nuclear isolation by other methods. When oocyte nuclei are isolated into an aqueous medium, they lose 95% of their protein with a half-time of 250 s. This result implies an even more rapid loss of protein from aqueously isolated nuclei of ordinary-size cells.

Cell nuclei are isolated in aqueous media in many laboratories, and their analyses are used to characterize structures and functions of the in vivo nucleus. Because the nuclear envelope contains pores permeable to macromolecules (1–4), it is understood that some proteins must be lost (5). However, the magnitude of the loss is unknown, because the in vivo (pre-isolation) protein content of nuclei has not been determined and compared to the protein remaining in isolated nuclei. We present here a two-step approach to this problem. First, we determined the in vivo protein content of the large nucleus (400–500-μm diameter) of the amphibian oocyte isolated by cryomicrodissection. Second, with this in vivo content as a baseline, we measured the kinetics of protein loss from oocyte nuclei isolated directly into an aqueous medium.

MATERIALS AND METHODS

Cryomicrodissection (6, 7) is a method in which individual oocytes are frozen in liquid nitrogen and subsequently maintained at less than −45°C while the nucleus is microsurgically isolated with fine-tipped stainless steel microtools (Fig. 1). The low temperature prevents diffusive relocations of nuclear and cytoplasmic solutes from their in vivo locations. Clean, intact nuclei cyomicrodissected from *Xenopus* oocytes (stages V and VI) (8) varied somewhat in wet weight from animal to animal, but their size distribution was narrow for cells from the same animal (standard error of the mean <5%). Nuclear water contents, determined from wet and dry weights of cryomicrodissected nuclei, were relatively constant (even between animals) at 87.2 ± 0.3%, with the dry mass consisting almost entirely of protein. The nuclei of the oocytes from the two animals used in the present study had protein contents of 3.8 ± 0.4 and 5.5 ± 0.7 μg (Fig. 2, upper and lower curves, respectively.)

To isolate nuclei into aqueous solution, we punctured and compressed individual oocytes with forceps (9, 10) until the nucleus was extruded. The medium was Ca^{2+}-free and formulated (legend, Fig. 2) to mimic the oocyte's intracellular free monovalent cation concentrations (7). After extrusion, each nucleus was gently pipetted through the medium to remove traces of adherent cytoplasm, incubated without agitation in fresh medium for time t_i, and assayed for protein content. Nonspherical (damaged) nuclei were discarded.

RESULTS AND DISCUSSION

The aqueous isolation procedure we used is gentle compared to the mass cell shearing or homogenization employed in most studies. Nevertheless, even under these conditions, loss of nuclear protein was 90% by 1 h, and asymptoted to ~95% within a few minutes thereafter (Fig. 2, lower curve). These results agree remarkably with those of an earlier study by Macgregor (11), who monitored optical interference in isolated newt oocyte nuclei and estimated 1 h loss of mass (protein) to be >80%. Fig. 2 shows the half-time for loss from the *Xenopus* oocyte nucleus, of both total and recently synthesized ([^{3}H]leucine-labeled) protein populations, was ~250 s.

What do these findings tell us about protein loss from smaller, more conventional-size nuclei? Loss is a complex process involving a protein population that includes a range of molecular sizes, charges, and diffusivities. Furthermore, at least two rate processes are involved: (*a*) diffusion within the bulk of the nucleus and (*b*) permeation through the nuclear surface. Both of these are influenced by nuclear size. Loss over time (t) from a spherical nucleus, if determined entirely by surface permeability, would be inversely related to the nuclear radius; if determined entirely by bulk diffusion, it would be inversely related to the radius squared (12). Typical somatic cell nuclei have radii about 1/100 that of the oocyte nucleus. Protein loss from these nuclei, if limited by permeation, would be about 100 times faster, and, if limited by diffusion, about 10,000 times faster than loss from the oocyte nucleus. The resulting half-times of loss would be 2–3 s or less. Most nuclear proteins in vivo exist at least partially as diffusive molecules (13), and conventional aqueous isolation procedures take minutes or hours. Hence, any protein which remains in the nucleus following aqueous isolation is likely to be part of the nuclear matrix or other structural elements, or tightly associated with chromosomes.

Can the situation be improved? Nuclei imbibe water and swell when isolated in salt and sucrose solutions (14–17). Macromolecules such as serum albumin or polyvinylpyrrolidone added to the isolation medium decrease protein loss (11, 18). Because these agents simultaneously reduce nuclear swelling (see also references 9, 14, and 16), they presumably slow protein loss by limiting swelling-induced nuclear envelope or

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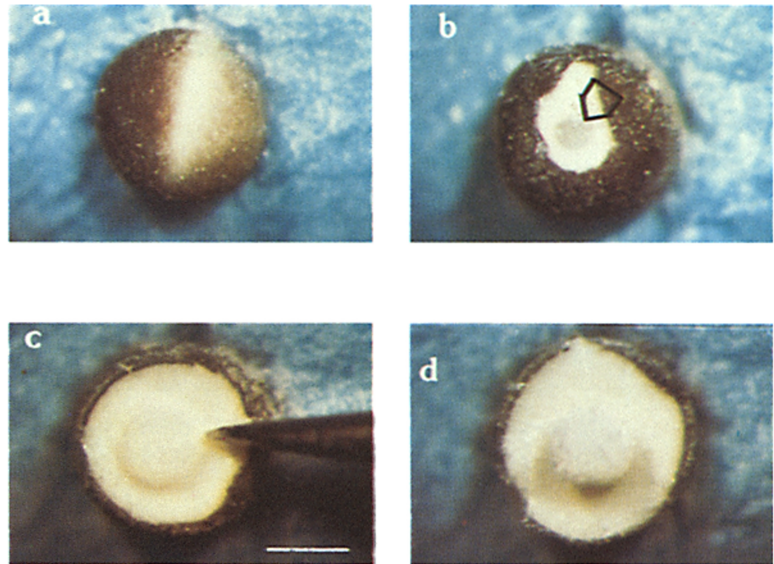
FIGURE 1 Cryomicrodissection of a full-grown (Stage VI) (8) *Xenopus laevis* oocyte nucleus. (*a*) The cell, frozen in embedding medium (blue), is shown on the −45°C dissection stage, as viewed by the operator prior to dissection. The animal (dark pigment) and vegetal hemispheres are left and right, respectively. This animal-vegetal polarity assists isolation of the nucleus, which is located in the animal hemisphere. (*b*) With the animal-pole upward, cytoplasm is carefully scraped away with stainless-steel dissecting tools. The nucleus (arrow), more crystalline and translucent due to its high water content, contrasts with the more opaque, yolk-filled cytoplasm. (*c*) Removal of cytoplasm from around the nucleus continues until, as shown here, the nucleus (diameter 500 μm, white bar) is about half-free. (*d*) Excavation of cytoplasm is continued. Subsequent steps (not shown) include severing the last cytoplasmic stalk connecting the nucleus to the cell, cleaning the remaining cytoplasm from the nuclear surface, transfer of the nucleus to a pre-tared aluminum foil packet, and analysis.



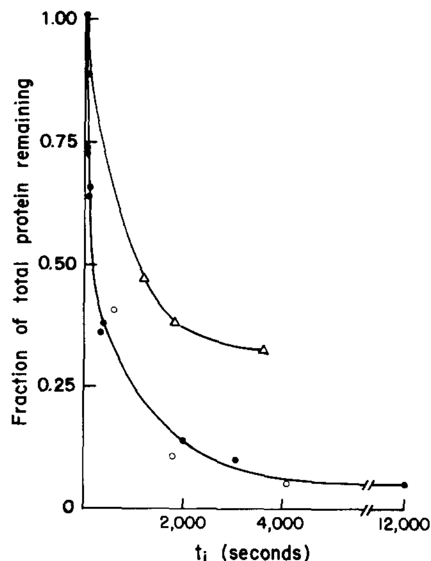


FIGURE 2 Protein loss from *Xenopus laevis* oocyte nuclei in aqueous medium. Nuclear protein content remaining at time (t_i) after aqueous nuclear isolation expressed as the fraction of the in vivo protein content. The in vivo ("zero-time") protein content was determined on cryomicrodissected nuclei. To measure the rate of aqueous loss, we individually extruded nuclei of [^{3}H]leucine-labeled *Xenopus* oocytes into an intracellular medium (102.0 mM KCl, 11.1 mM NaCl, 7.2 mM K_2HPO_4, 4.8 mM KH_2PO_4, pH 7.0). At times t_i after isolation, we measured the total TCA-precipitable radioactivity by liquid scintillation counting (●, individual nuclei), and total protein (○, pooled nuclei, $n \geq 3$) by the Bio-Rad Protein Assay (Bio-Rad Laboratories, Richmond, CA) using BSA as a standard. A second experiment (△) shows the kinetics of protein loss from nuclei isolated into the intracellular medium containing 2% (55 μM) polyvinylpyrrolidone, molecular weight 360,000.

nucleoplasmic changes. Polyvinylpyrrolidone added to the medium in our experiments decreased the fraction of protein lost in 1 h from 90% to 65% (Fig. 2, upper curve). Although polymer slows loss, this is probably of limited practical value, because even the unswollen nucleus is quite permeable. The half-time of diffusive exchange of an average protein (46 Å in diameter) from the in vivo oocyte nucleus is 2–3 h. Exchange half-time for a 4-μm diameter nucleus with comparable properties is 2 min or less. (Calculations based on a combined diffusion-permeation model, Eq. 6.43 in reference 12, and the intracellular diffusion coefficient [2.5×10^{-7} cm^2/s] and nuclear envelope permeability [5.4×10^{-7} cm/s] in reference 2.)

The oocyte nucleus is unusual in its large size and low DNA/volume ratio. This could imply that the present findings lack generality. However, in other respects, the oocyte nucleus closely resembles other eucaryotic nuclei. For example, it possesses a proteinaceous skeletal matrix (19) and apparently similar water and solute content (20–24). Furthermore, the oocyte nuclear envelope and pore complex lamina resemble those of other cells in permeability (2, 22), ultrastructure (25), and polypeptide composition (26). In view of this, we suggest that it would be prudent to view, as seriously incomplete, models of the eucaryotic nucleus based primarily on data obtained using aqueous isolation. Even gentle aqueous methods remove the nucleus from its controlled in vivo environment, sever its structural connections with cytoplasm, and perfuse it with a fluid whose composition cannot be matched to that of the in vivo milieu. Under these conditions, loss of proteins, including those which are normally diffusive in the cell and those that are reversibly associated with intranuclear structures, must be expected to be considerable. In the oocyte nucleus—the only experimental system for which full quantitation has been achieved—these losses are enormous.

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