Vaults. II. Ribonucleoprotein Structures Are Highly Conserved among Higher and Lower Eukaryotes

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Abstract. Vaults are cytoplasmic ribonucleoprotein structures that display a complex morphology reminiscent of the multiple arches which form cathedral vaults, hence their name. Previous studies on rat liver vaults (Kedersha, N. L., and L. H. Rome. 1986. *J. Cell Biol.* 103:699–709) have established that their composition is unlike that of any known class of RNA-containing particles in that they contain multiple copies of a unique small RNA and more than 50 copies of a single polypeptide of 104,000 M_r. We now report on the isolation of vaults from numerous species and show that vaults appear to be ubiquitous among eukaryotes, including mammals, amphibians (*Rana catesbeiana* and *Xenopus laevis*), avians (*Gallus Gallus*), and the lower eukaryote *Dictyostelium discoideum*. Electron microscopy reveals that vaults purified from these diverse species are similar both in their dimensions and morphology. The vaults from these various species are also similar in their polypeptide composition; each being composed of a major polypeptide with an approximate mass of 100 kD and several minor polypeptides with molecular masses similar to those seen in the rat. Antibodies raised against rat vaults recognize the major vault protein of all species including *Dictyostelium*. Vaults therefore appear to be strongly conserved and broadly distributed, suggesting that their function is essential to eukaryotic cells.

A LL known ribonucleoprotein (RNP)[1] particles play critical roles in cellular metabolism. One view of the biochemical evolution of life contends that the metabolism of primordial organisms was based on RNA rather than DNA, and that those RNA-containing structures that have survived into modern times represent molecular fossils which catalyze very ancient and fundamental life processes (Gilbert, 1986; Darnell and Doolittle, 1986; Weiner, 1988). Well-characterized examples of RNA-containing structures include the ribosome, signal recognition particle (SRP), and the small nuclear RNP particles or snRNPs (Lake, 1985; Walter and Lingappa, 1986; Dreyfuss et al., 1988; Birnstiel, 1988). All of these structures exhibit complex molecular composition and mediate important cellular functions. Eukaryotic ribosomes contain four different RNAs and over 70 polypeptides, almost all of which are present in single copy number. Similarly, SRP and the snRNPs contain single copies of different polypeptides in addition to a single species of RNA.

In contrast, vaults contain at least 55 copies of the major vault polypeptide of 104,000 M_r, as well as multiple copies (at least 8) of a single species of RNA (1). Such molecular redundancy is characteristic of viruses, cytoskeletal structures such as microtubules and stress fibers, and clathrin cages that surround coated vesicles. However, no previously characterized RNP displays this type of high-copy multisubunit composition, although some low-copy multiple subunit proteins exist in hnRNP (Lothstein et al., 1985) and in snRNP core proteins (Harris et al., 1988). Vaults are also unusual in size as they are relatively large, approximately threefold larger than eukaryotic ribosomes and 10-fold larger than SRP or the snRNPs. Vaults therefore appear to constitute a novel class of ribonucleoprotein structures that embody both cytoskeletal and ribonucleoprotein attributes. The present study demonstrates that vaults are present in mammals, amphibians, and the lower eukaryote *Dictyostelium discoideum*, and that both vault structure and protein composition are highly conserved.

Materials and Methods

Cell Lines

Normal human diploid fibroblast lines and rat fibroblasts were established in our laboratory from skin biopsies. Normal rat kidney epithelial cells (NRK-52E), *Portorous tridactylis* kidney cells (PT-KI), mouse hybridoma (SP 2/0-Ag14), and mouse myeloma cells (P3X63AgU.1) were obtained from the American Type Culture Collection (Rockville, MD 20852). Madin–Darby canine kidney (MDCK) cells, HeLa cells, and rat hepatoma cells were kind gifts from Dr. D. Haggerty (University of California, Los Angeles). Chinese hamster ovary cells were a gift from Dr. April Robbins

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1. *Abbreviations used in this paper*: RNP, ribonucleoprotein; SRP, signal recognition particle.

(National Institutes of Health, Bethesda, MD). *Dictyostelium discoideum* (axenic strain AX4) was kindly provided by Dr. W. Loomis (University of California, San Diego).

Cell Culture and Extraction for Western Blotting

Cells were cultured in Dulbecco's media supplemented with 5% FBS, 5% calf serum, and penicillin/streptomycin, harvested by brief trypsin treatment, washed with PBS, and stored frozen until use. Cell pellets were thawed into 50 mM MES (pH 6.5) containing protease inhibitors (1 mM PMSF, 5 μM leupeptin, 5 μM pepstatin, 5 μg/ml chymostatin, and 5 mM benzamidine) and 0.1% Triton X-100, and homogenized in a Teflon glass homogenizer. After an incubation of 1 h on ice, samples were microfuged for 5 min, and the entire supernatant was treated with 1% SDS. Total protein was determined (Fryer et al., 1986), and 50 μg of each cell extract was loaded per lane on a discontinuous polyacrylamide SDS gel (Laemmli, 1970) with modifications described previously (Kedersha and Rome, 1986a). The protein was electrophoretically transferred to nitrocellulose (Towbin et al., 1979) using a commercial blotting apparatus (Idea Scientific, Corvallis, OR). Gels were routinely stained with Coomassie brilliant blue after transfer to confirm that the transfer efficiency was >90% and that all samples transferred equally. The nitrocellulose was incubated for 1 h in 5% powdered milk in TBS, incubated for 1 h in the same buffer containing 0.5 μg/ml affinity-purified antivault antibodies, raised against intact vaults as described previously (Kedersha and Rome, 1986a) and purified on a column containing cyanogen-bromide-coupled intact vaults. Blots were washed, incubated for ∼1 h in a 1:1,000 dilution of goat anti–rabbit horseradish peroxidase conjugate (Bio-Rad Laboratories, Cambridge, MA), washed, and the bands were visualized with 4-chloro-1-naphthol substrate (Hawkes, 1982).

Purification of Vaults

Vaults were purified as described previously (Kedersha and Rome, 1986a; summarized in Fig. 1) using 10–80 g of liver from each vertebrate species and 10–20 g whole cells from *Dictyostelium*. The following modifications were used: in the purification of bullfrog, rabbit, and *Dictyostelium* vaults, a buffer containing 0.09 M MES and 0.01 M sodium phosphate (pH 6.5), 20 mM EDTA and 0.02% sodium azide was used in lieu of the previous MES buffer containing $MgCl_2$. Equilibrium sucrose gradients were fractionated, and the fractions were screened on Western blots using antivault antibodies to determine the fractions containing vaults (which were not always coincident with ferritin as previously determined for rat liver), which then were pooled, concentrated, and purified on preparative agarose gels as described previously (Kedersha and Rome, 1988b). Adjustment of the agarose gel buffer pH to 7.1 from 6.5 was necessary in both the *Rana* and the *Xenopus* preparations to obtain a clean separation of vaults from minor contaminants.

Dictyostelium vaults were purified from strain AX4, grown axenically in HL-5 medium as described (Sussman, 1987). Amoebae in logarithmic phase were harvested by centrifugation, washed twice in distilled water, and disrupted by homogenization in the presence of 0.05% NP-40 in MES buffer (0.09 M MES, 0.01 M NaPO₄, 1.5 mM $MgCl_2$, 1.0 mM EGTA; pH 6.5) containing 10% sucrose, protease inhibitors (50 μg/ml TPCK, 1 mM PMSF, 5 μg/ml chymostatin, and 5 mM benzamidine). Subsequent steps were performed essentially as described previously (Kedersha and Rome, 1986a). Our purest preparations of *Dictyostelium* vaults ran as a relatively broad band on the agarose gel, suggesting that the *Dictyostelium* vaults were not morphologically uniform despite an apparently homogeneous protein composition (see below).

Dictyostelium Developmental Study

A late log suspension culture of *Dictyostelium* (axenic strain AX4) grown in medium as described (Sussman, 1987) was harvested, rinsed in LPS buffer (Sussman, 1987), and plated on 43 mm black filters (Millipore Continental Water Systems, Bedford, MA) (5 × 10⁷ cells/filter). At 0, 4, 9, 22 ("maxifinger"), and 34 h (mature fruiting body), cells were harvested by flushing the filters with LPS buffer, centrifuged, and frozen at −80°F for several days. Frozen cell pellets were thawed into buffer containing 20 mM MES, 10 mM EDTA (pH 7.0), containing protease inhibitors (50 μg/ml TPCK, 1 mM PMSF, 5 μM leupeptin, 5 μM pepstatin, 5 μg/ml chymostatin, and 5 mM benzamidine), and 0.1% NP-40 detergent. Cells were homogenized with 10 strokes of a Teflon glass homogenizer, incubated 10 min on ice, and microfuged for 2 min. The pellets were washed once and the supernatants pooled. Samples of both the supernatant and the pellet were then adjusted to 2% SDS and boiled. Protein in each fraction was de-

termined by a modification of the Lowry assay (Fryer et al., 1986), and equal amounts of protein from each time point were loaded per lane of an SDS-PAGE slab gel.

Results and Discussion

To ascertain the distribution of vaults among vertebrate species, we purified vaults from the livers of chicken, cow, bullfrog (*Rana catesbeiana*), and the South African clawed frog *Xenopus laevis* in addition to rat. Minor modifications in the previously published purification scheme originally used for the purification of vaults from rat liver (Fig. 1) were used (see Materials and Methods). Particles with physical properties similar to rat vaults (sedimentation velocity and density on sucrose gradients, mobility in native agarose gels) were obtained from all vertebrates examined. When the purified structures were negatively stained and examined by electron microscopy, all displayed a remarkable morphological similarity to rat liver vaults (see Fig. 2 for rat, bullfrog and rabbit; cow and chicken vaults not shown). All vertebrate preparations yielded highly uniform, ellipsoid bodies measuring 35 × 65 nm, possessing the same elaborate substructure of multiple arches previously described for rat vaults (Kedersha and Rome, 1986a). Minor morphological differences appeared to be due to normal variation in the negative staining process, a procedure that gives highly variable results in sample intensity and detail even when applied repeatedly to the same particle preparation.

Vaults were also successfully obtained from the vegetative ameba of the lower eukaryote *D. discoideum*, using a similar purification strategy (see Materials and Methods). Initially, it appeared that the *Dictyostelium* vaults were more heterogeneous than vertebrate vaults when examined by EM; however, SDS-PAGE and immunological criteria (see below) indicated a high degree of apparent molecular homogeneity. Closer examination of the structures (Fig. 3) led us to conclude that most of the particles were in fact highly purified (>95%) and that our initial impression of heterogeneity was because of the increased number of views presented by these structures in negative stain. Fig. 3 *A* shows a field of *Dictyostelium* vaults where most of the particles appear to be largely intact; here the resemblance to vertebrate vaults is readily apparent. Intact *Dictyostelium* vaults (Fig. 3 *A*, ar-
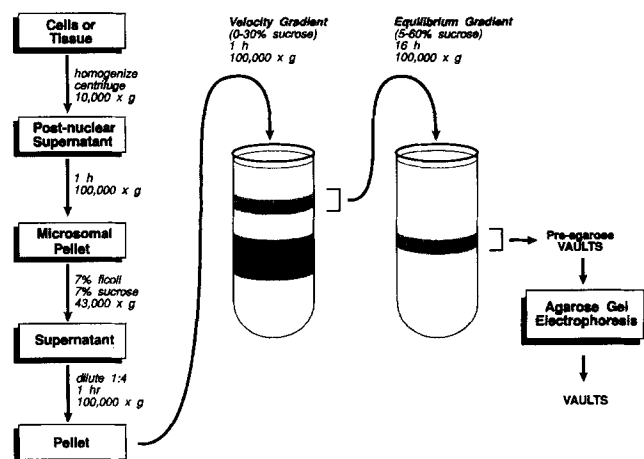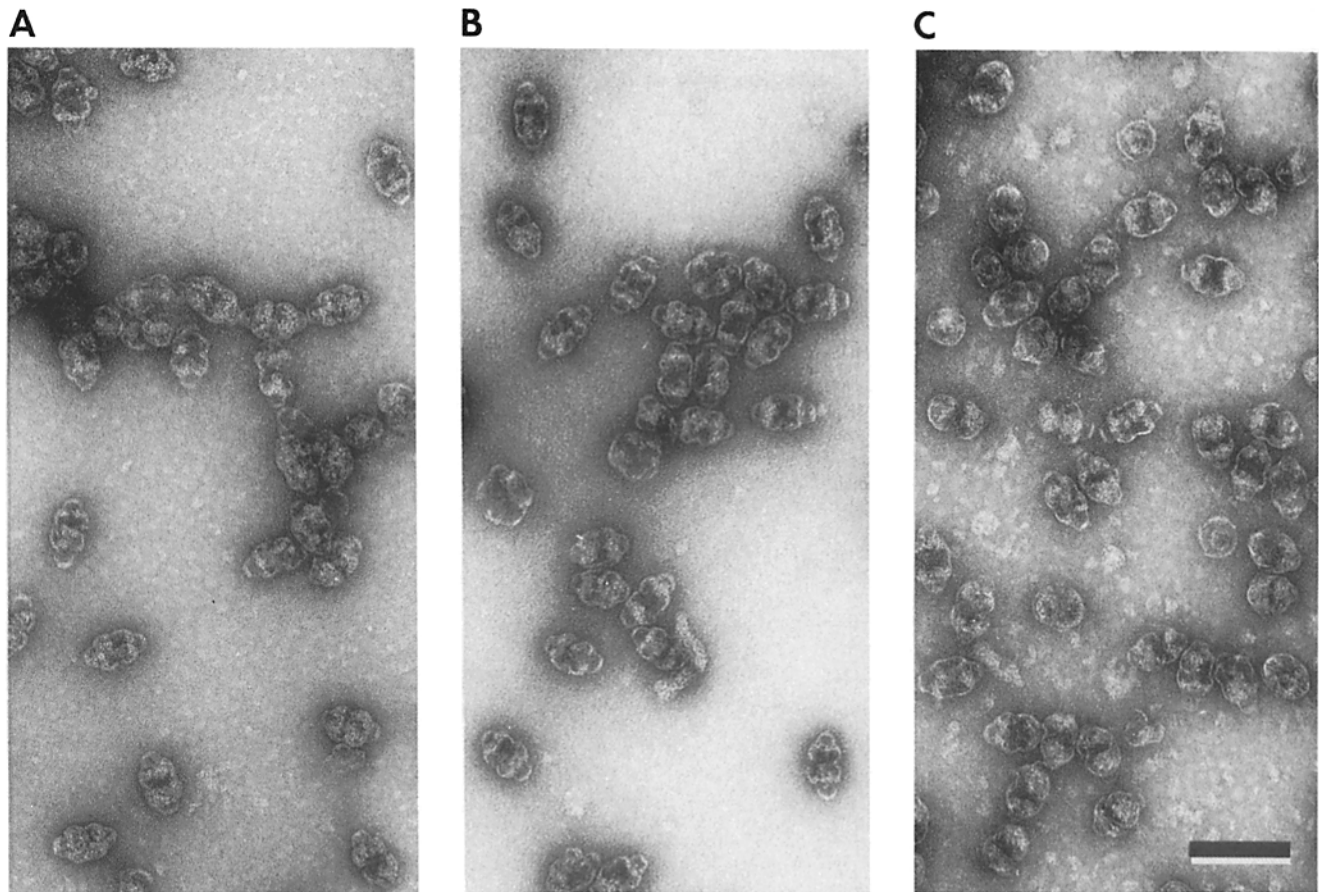

Figure 1. Purification of vaults.

Figure 2. EM of vaults purified from different higher eukaryotic species. Samples were absorbed onto carbon-coated grids, stained with 1% uranyl acetate, and air dried. Samples were viewed on an electron microscope operated at 80 kV. *Left*, vaults isolated from rat liver; *Center*, vaults isolated from bullfrog liver; *Right*, vaults isolated from rabbit liver. Bar, 100 nm.

rowheads) measured ~35 × 60 nm, slightly shorter than the ~35 × 65–nm vertebrate vaults. The strong conservation of structure suggests that vault morphology is important to vault function in all species. A different fraction of *Dictyostelium* vaults is shown in Fig. 3 *B*; here, most of the particles resemble vertebrate vaults broken in half perpendicular to the longitudinal axis. Such "broken vaults" are also seen in rat vault preparations stored for prolonged periods (1–2 yr) at 4°C (not shown). "Intact" vertebrate vaults resemble ellipsoids and as such possess two axes of symmetry, radial and bilateral. Cleavage or separation perpendicular to the longitudinal axis generates two elliptical cones that still retain radial symmetry but now have different ends, somewhat like half egg shells. Such morphology generates a greater variety of possible views and also increases the percentage of end views likely to occur. Numerous side views of such broken vaults (Fig. 3 *B, arrowheads*) and end views (Fig. 3 *B, arrows*) can be seen.

Several possibilities exist that could account for the much greater incidence of "half" vaults seen in the *Dictyostelium* preparations. Endogenous proteases or the use of detergent necessary to break the *Dictyostelium* could disrupt the vaults during purification; however, we obtained identical results both with and without protease inhibitors, and detergent treatment of rat liver vaults does not alter their morphology.

Another possibility is that subtle differences in *Dictyostelium* vault structure cause them to be more sensitive to the effects of the uranyl acetate used in the negative stain procedure. However, freeze-etch images show that the differences between vertebrate and *Dictyostelium* vaults are not because of the effects of uranyl acetate (John Heuser, unpublished observations). The possibility remains that the half vaults that predominate in the *Dictyostelium* preparations and that are frequently observed in vertebrate vault preparations are in fact physiologically relevant. The extremely high-copy number of both vault protein and RNA, as well as the apparent symmetry of the vault structures themselves, suggests that vaults are composed of multiple, identical subunits capable of assembly/disassembly under physiological conditions. Thus, the half vaults of *Dictyostelium* may arise as a consequence of the different physiologies of the ameboid *Dictyostelium* compared to the vertebrate liver, rather than representing species differences. *Dictyostelium* vaults could exist as half vaults in vivo, perhaps representing monomers which are in equilibrium with dimeric "whole vaults." The dimeric form may predominate in liver, while monomers are the predominant species in *Dictyostelium* ameba.

The protein subunit composition of the different vaults was examined by SDS-PAGE. Fig. 4 shows the pattern obtained from four different species of purified vaults. We were again
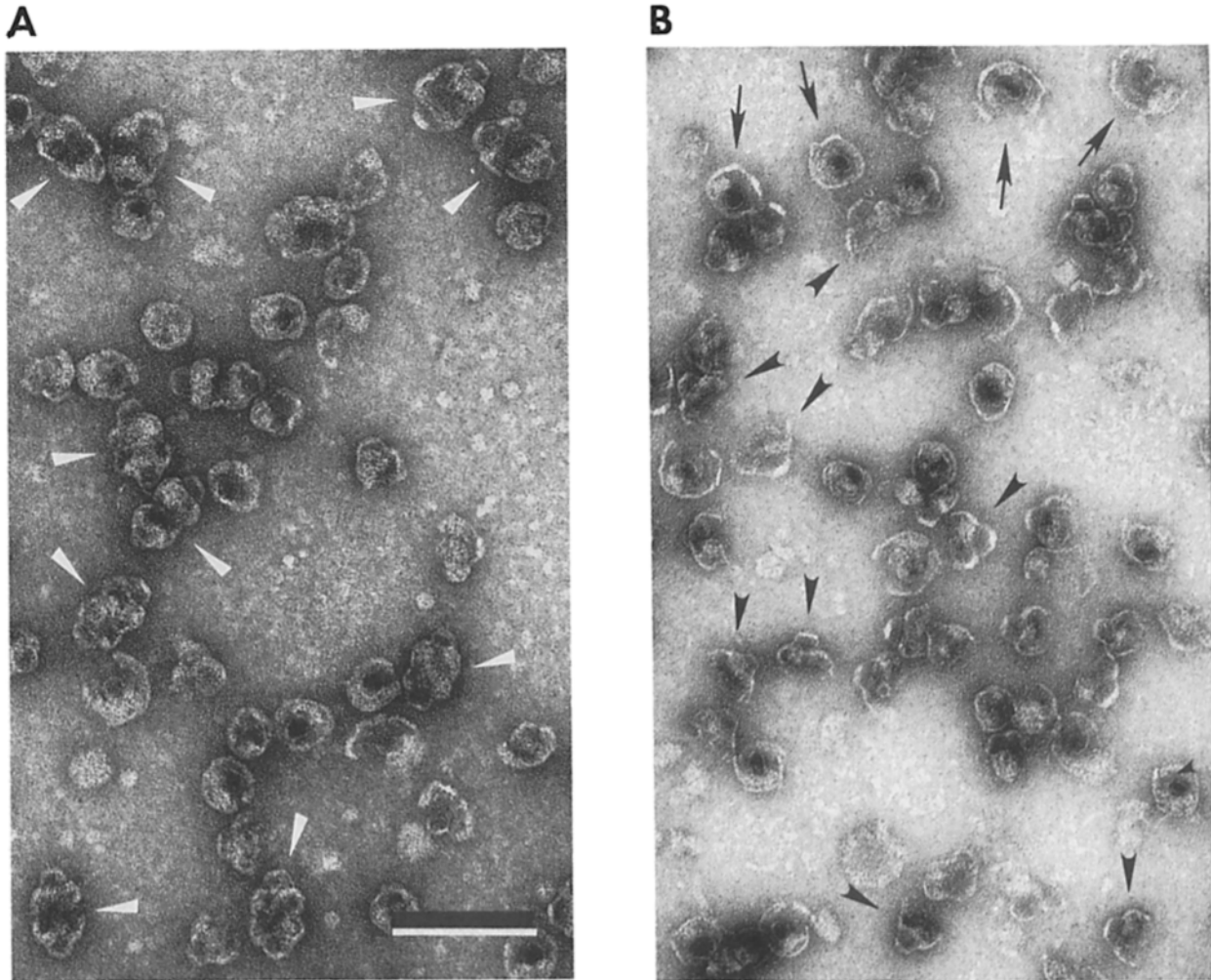
A

B

Figure 3. EM of vaults purified from *D. discoideum.* Samples were prepared as described in Materials and Methods. *A,* A field of *Dictyostelium* vaults in which more are "intact" as compared to vertebrate vaults. Best examples are indicated by the white arrowheads. *B,* A field of *Dictyostelium* vaults, apparently largely broken in half. Arrows indicate some prominent half vaults that appear to have been deposited on the grid on end, revealing the inside of each half. Arrowheads mark a number of examples of side views of these half vaults. Bar, 100 nm.

impressed by the similarities among species. Rat vaults contained four major protein species of $\sim$210,000, 192,000, 104,000, and 54,000 M_r (hereafter referred to as p210, p192, p104, and p54). Numerous minor bands of <50,000 M_r may be proteolytically derived from p104 during purification; such bands are not seen in immunoprecipitates (not shown). Vaults from all species examined (including bovine and *Xenopus* vaults not shown in Fig. 4) contained a predominant component of $\sim$100,000 M_r and two less prominent components of larger apparent molecular size, which in all vertebrate vaults appeared similar to the p210 and p192 of rat. The p54 species, previously seen in rat vaults (a possible breakdown product of p104), was also prominent in rabbit vaults. *Dictyostelium* vaults differed in polypeptide content in that (*a*) the p104 species could be resolved as a doublet, and (*b*) no apparent band was visible corresponding to p192, although a p210-like species was observed.

Vaults from the four different species were examined on Western blots (Fig. 4 *B*) using affinity purified polyclonal

antibodies raised against intact rat liver vaults (Kedersha, N. L., H. Roseboro, D. Barnes, and L. H. Rome, manuscript submitted for publication). All of the vertebrate p104 polypeptides were strongly recognized by the antibody, while only the larger of the *Dictyostelium* p104 doublet (p104α) was strongly positive. The smaller *Dictyostelium* species (p104β) was weakly recognized by the antibody (Fig. 4 *B*) and was only detectable when large amounts of protein (more than 1 μg/lane) were examined. We have purified *Dictyostelium* vaults under a variety of conditions (with and without protease inhibitors, EDTA, detergents) and consistently obtained p104α and p104β in a 1:1 ratio, suggesting that p104β is not proteolytically derived from p104α. Such a doublet is also obtained in immunoprecipitates (not shown). A similar p104 doublet has been observed in vaults purified from *Xenopus* oocytes (Kedersha, N. L., unpublished observations) in which both p104α and p104β are equally recognized by the antivault antibody. As vaults purified from adult *Xenopus* liver contain only one species of p104, it is likely that differ-
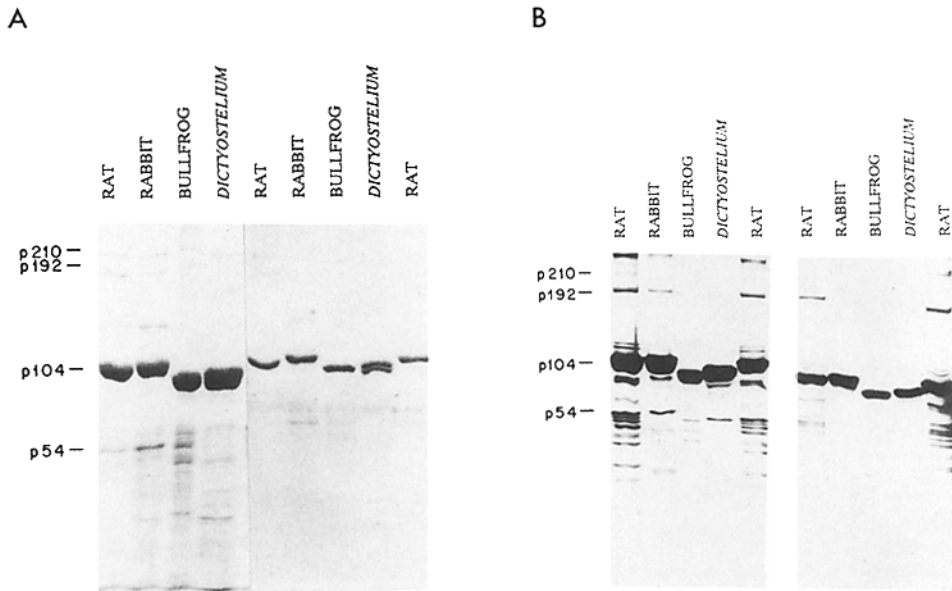
A

B

Figure 4. Protein composition and immunoreactivity of vaults from different species. Equal amounts of purified vaults from rat, rabbit, bullfrog, and *Dictyostelium* species were SDS-treated and subjected to SDS-PAGE. *A*, Samples stained with Coomassie brilliant blue, loaded at two concentrations (2 µg/lane, *left*, and 0.5 µg/lane, *right*). Note the apparent doublet in the p104 band seen in the *Dictyostelium* vaults at the lower concentration; *B*, duplicate gels were loaded with 4 µg of vaults/lane and visualized by either Coomassie brilliant blue (*left*) or transferred to nitrocellulose and subjected to Western blotting using affinity-purified anti–rat vault antibodies (*right*). The p104 band from all of the different vault samples is recognized by the antibody.

ent isoforms of p104 exist and may be developmentally regulated in vertebrates, while both are simultaneously present in *Dictyostelium*.

Bullfrog and rat vaults were subjected to RNase treatment and analyzed by SDS-PAGE and silver stain (Merril et al., 1983) (Fig. 5). Two bands (Fig. 5, lane *3*) were removed from bullfrog vaults by RNase treatment (Fig. 5, lane *4*). These bands were the only species stained by ethidium bromide, further suggesting that they were RNA. To confirm that these bands were indeed RNA, vaults from bullfrog and rabbit were phenol extracted, ethanol precipitated, labeled using T4 RNA ligase and ^{32}pCp, and resolved on an 8% urea-PAGE (not shown). Two major bands of <100 bases each were obtained from each species, confirming that both rabbit and bullfrog vaults contain similar small RNAs, both of which are somewhat smaller than that of the rat vault RNA. Bullfrog and rat vault RNAs have been sequenced and shown to be homologous despite their differences in sizes (Searles, R. P., V. K. Slagel, N. L. Kedersha, and L. H. Rome, manuscript in preparation). We can conclude that both rat and bullfrog vaults contain small RNAs. We were unable to detect similar RNase-labile species in the other classes of vaults examined (bovine, *Xenopus*); however, losses of vault RNA are likely to have occurred during the long (3-d) purification, since Southern blot analysis of genomic DNA suggests that vault RNA is present in a wide variety of other species (Searles, R. P., V. K. Slagel, N. L. Kedersha, and L. H. Rome, manuscript in preparation).

D. discoideum can be induced to undergo largely synchronous aggregation and fruiting under controlled conditions (Sussman, 1987). During this process, which is induced by starvation, mitosis ceases and both autophagy and locomotion are stimulated as the amebas aggregate into a multicellular structure that ultimately becomes a mature spore containing fruiting body (Loomis, 1982). This affords an excellent system in which mitosis and motility are segregated. When cultures of *Dictyostelium* were starved to induce synchro-

nous morphogenesis, major vault protein levels remained relatively constant throughout all stages of aggregation and migration, dropping only slightly in the terminal stage of fruiting (Fig. 6). Increased relative amounts of actin and myosin are seen during migratory periods (9 and 22 h), reflecting the changing needs of the organism. Upon reach-
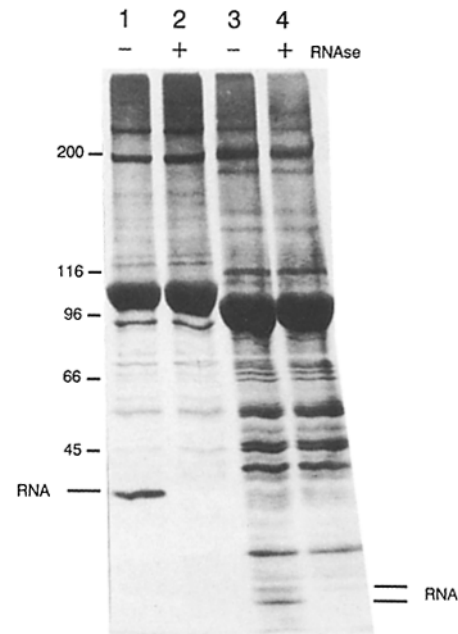

Figure 5. RNA content of rat and bullfrog vaults, shown as RNase labile bands on silver-stained SDS-PAGE. Lane *1*, Rat vaults; lane *2*, rat vaults with RNase pretreatment; lane *3*, bullfrog vaults; lane *4*, bullfrog vaults with RNase pretreatment. Arrows indicate the position of RNase labile species. In each case, ~2 µg of vaults were treated with 200 ng RNase A for 1 h at room temperature before electrophoresis.
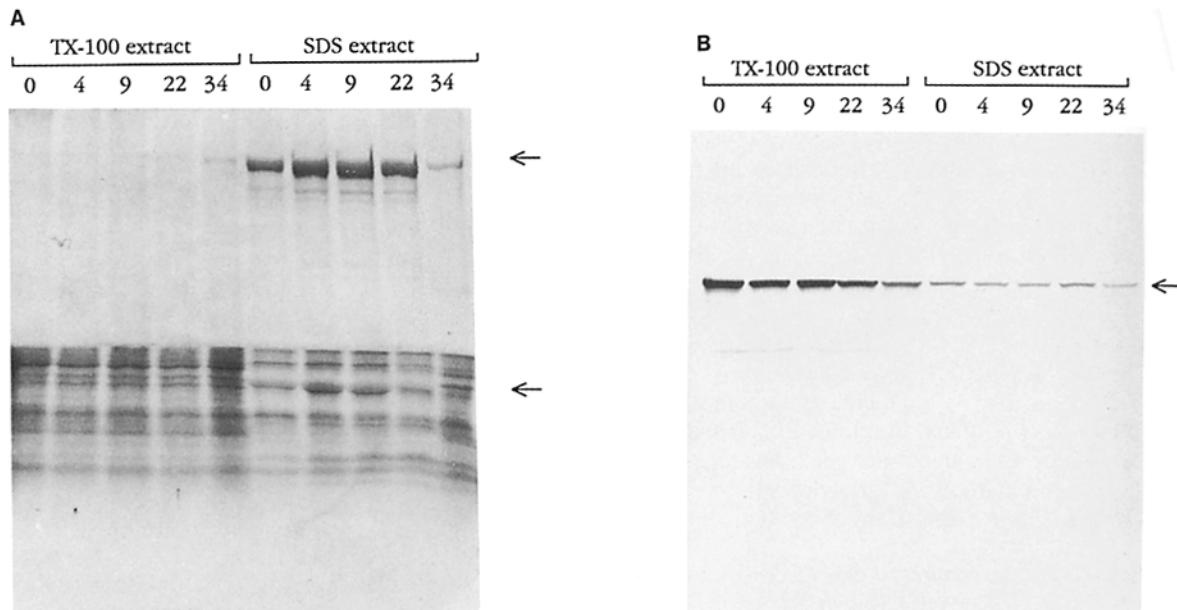
Figure 6. Vault expression during *Dictyostelium* development. *Dictyostelium* vegetative amebas were washed and transferred to filters (Millipore Continental Water Systems) as indicated in Materials and Methods. At various stages of differentiation, the cells were harvested and frozen. Both Triton-soluble and Triton-insoluble extracts were prepared and 2 μg of protein from each were resolved on SDS-PAGE and stained with Coomassie brilliant blue (*A*) or transferred to nitrocellulose and probed with affinity-purified antivault antibodies (*B*). Time points were taken at 0 (vegetative ameba), 4, 9 (aggregation), 22 (slug), and 34 h (mature fruiting bodies) as indicated. *A*, Upper arrow indicates migration position of myosin and lower arrow indicates actin. *B*, Arrow indicates major vault protein band.

ing the terminal fruiting stage (34 h), most of the actin and myosin are lost. The disappearance of the bulk of actin and myosin probably reflects the elevated levels of catabolism induced by starvation. The continued presence of the major vault protein (Fig. 6 *B*), albeit at slightly reduced levels, suggests that vaults remain biologically functional in the final stages of fruiting. Alternatively, they could constitute a component of the spores themselves. From this data, we conclude that vault function is important to nonmitotic, highly autophagocytic cells and that vaults may be required during
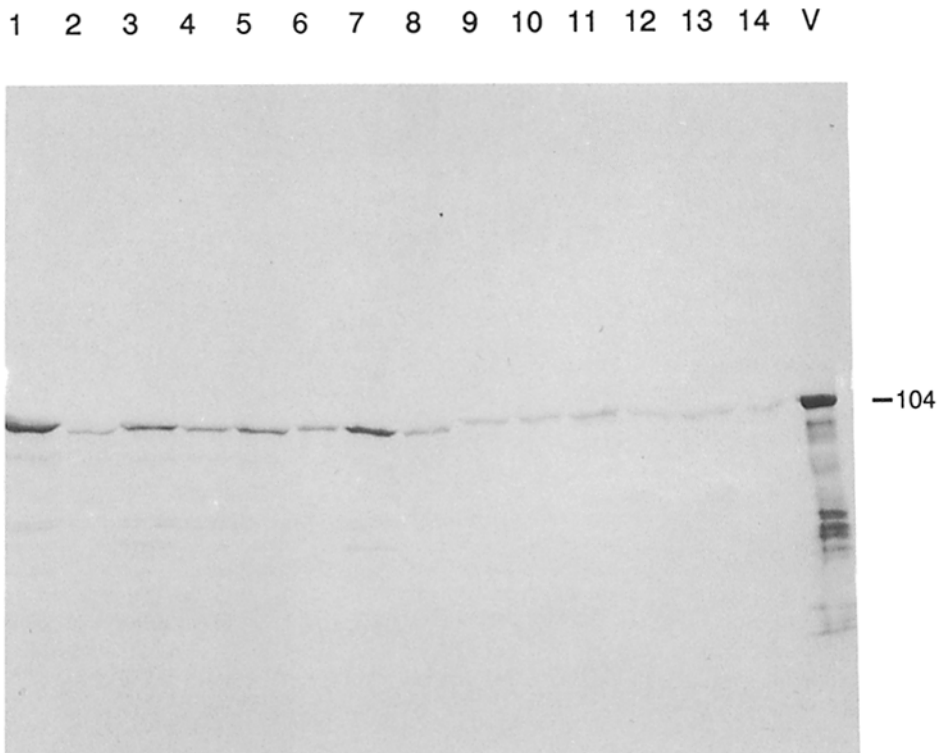

Figure 7. Occurrence of vault proteins in various cell lines. Western blot of total protein extracts of various cell lines using affinity-purified anti–rat vault antibodies. Each lane was loaded with 50 μg of protein. Lane *1*, normal rat kidney epithelial cells; lane *2*, rat hepatoma cells; lanes *3* and *4*, two different rat fibroblasts; lane *5*, *Portorous tridactylis* kidney cells (PT-K1 cells); lane *6*, MDCK cells; lane *7*, mouse hybridoma (SP 2/0- Ag14); lane *8*, Chinese hamster ovary cells; lane *9*, HeLa cells; lanes *10*, *11*, *12*, *13*, and *14*, different normal human diploid fibroblast lines established from skin biopsies; lane *15*, purified vaults from rat liver run as a standard.

germination. This observation is also supported by the analysis of the relative vault content in different vertebrate cell types, where macrophages are richest in vaults (Kedersha, N. L., unpublished observation).

Using Western blots of total protein extracts, we examined the phylogenetic distribution of vaults in a number of different established cell lines employing antivault antibodies previously described (Fig. 7). A variety of fibroblastic, epithelial, and myeloid cells from rat, human, dog, mouse, and hamster were examined; in all cases the major vault protein was detected. As was seen in the purified vault preparations (Fig. 4), slight differences were observed in the mobility of the nonrodent major vault protein bands. Vaults appear to be present in a wide range of cell types including adherent, nonadherent (myeloma SP2/0), clonal (myeloma SP 2/0 and hepatoma), normal diploid, and transformed cells. The presence of the major vault protein in a wide variety of cell types suggests that vault function is required by most types of metazoan cells.

Both the immunological and structural data suggest that vaults constitute a highly conserved, ubiquitous cellular structure. The data argue against the notion that vaults constitute a newly discovered class of viruses as their morphology and composition might suggest. The size of the rat vault RNA ($\sim$144 bases) is far too small to code for the major vault protein of 104,000 M_r or any of the other vault polypeptides, nor has any membrane been detected within the vault particles. Furthermore, the species-specific differences in electrophoretic mobility of the major vault protein suggest that it is not a widespread viral protein, but rather a genuine cellular constituent.

The function of a cellular organelle is realized through its composition and structure. The vault RNA component suggests that vault function may involve interaction with other nucleic acids, as other RNP structures, such as the ribosome, SRP, and the snRNPs interact with RNA and/or RNA-containing structures in the course of their normal functions. The cytoplasmic location of vaults and the complete lack of evidence for vaults in the nucleus (Kedersha, N. L., H. Roseboro, D. Barnes, and L. H. Rome, manuscript submitted for publication) precludes the involvement of vaults in nuclear events such as mRNA processing. The high-copy number of the major vault protein suggests that vaults may have cytoskeletal characteristics, and may require a rigid structure or undergo assembly/disassembly in the course of their function. The unusual marriage of cytoskeletal-like protein composition and specific ribonucleic acid content indicates that vault function may relate to both movement and metabolism. Although vault function remains an enigma, these structures

must be of major importance at the cellular level to account for their broad distribution among cell types and strong conservation of composition and morphology among species.

References

Birnstiel, M. L. 1988. Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles. Springer-Verlag New York Inc., New York. 216 pp.

Darnell, J. E., and W. F. Doolittle. 1986. Speculations on the early course of evolution. *Proc. Natl. Acad. Sci. USA.* 83:1271–1275.

Dreyfuss, G., L. Philipson, and I. W. Mattaj. 1988. Ribonucleoprotein particles in cellular processes. *J. Cell Biol.* 106:1419–1425.

Fryer, H. J. L., G. E. Davis, M. Manthorpe, and S. Varon. 1986. Lowry protein assay using an automatic microtiter plate spectrophotometer. *Anal. Biochem.* 153:262–266.

Gilbert, W. 1986. The RNA world. *Nature (Lond.).* 319:618.

Harris, S. G., S. O. Hoch, and H. C. Smith. 1988. Chemical cross-linking of Sm and RNP antigenic proteins. *Biochemistry.* 27:4595–4600.

Hawkes, R. 1982. Identification of concanavalin-A binding proteins after sodium dodecyl sulfate gel electrophoresis and protein blotting. *Anal. Biochem.* 123:143–146.

Kedersha, N. L., and L. H. Rome. 1986a. Isolation and characterization of a novel ribonucleoprotein particle: large structures contain a single species of small RNA. *J. Cell Biol.* 103:699–709.

Kedersha, N. L., and L. H. Rome. 1986b. Preparative agarose gel electrophoresis for the purification of small organelles and particles. *Anal. Biochem.* 156:161–170.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 166:553–557.

Lake, J. A. 1985. Evolving ribosome structure. *Annu. Rev. Biochem.* 54:507–530.

Loomis, W. F. 1982. *Dictyostelium discoideum.* A Developmental System. Academic Press Inc., New York. 551 pp.

Lothstein, L., H. P. Arenstorf, S.-Y. Chung, B. W. Walker, J. C. Wooley, and W. M. LeStourgeon. 1985. General Organization of Proteins in HeLa 40S Nuclear Ribonucleoprotein Particles. *J. Cell Biol.* 100:1570–1581.

Merril, C. D., D. Goldman, and M. L. Van Keuren. 1983. Silver staining methods for polyacrylamide gel electrophoresis. *Methods Enzymol.* 96:230–239.

Sussman, M. 1987. Cultivation and synchronous morphogenesis of *Dictyostelium* under controlled experimental conditions. *Methods Cell Biol.* 28:9–29.

Towbin, H., T. Staehdin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350–4354.

Walter, P., and V. R. Lingappa. 1986. Mechanism of translocation across the endoplasmic reticulum membrane. *Annu. Rev. Cell Biol.* 2:499–516.

Weiner, A. M. 1988. Eukaryotic nuclear telomeres: molecular fossils of the RNP world? *Cell.* 52:155–157.