

# Two *Drosophila melanogaster* Proteins Related to Intermediate Filament Proteins of Vertebrate Cells

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**ABSTRACT** Monoclonal antibodies were prepared against a 46,000 mol wt major cytoplasmic protein from *Drosophila melanogaster* Kc cells. These antibodies reacted with the 46,000 and a 40,000 mol wt protein from Kc cells. Some antibodies showed cross-reaction with 55,000 (vimentin) and 52,000 mol wt (desmin) proteins from baby hamster kidney (BHK) cells that form intermediate sized filaments in vertebrate cells. In indirect immunofluorescence, the group of cross reacting antibodies stained a filamentous meshwork in the cytoplasm of vertebrate cells. In Kc cells the fluorescence seemed to be localized in a filamentous meshwork that became more obvious after the cells had flattened out on a surface. These cytoskeletal structures are heat-labile; the proteins in Kc or BHK cells rearrange after a brief heat shock, forming juxtannuclear cap structures.

Beside the effects on various levels of gene expression (reviewed in reference 1), an increase in temperature may also cause quite dramatic rearrangements in the cytoplasm. In our recent communication (6) we described two proteins from *Drosophila melanogaster* Kc cells (mol wt 46,000 and 40,000) that appear to change their cellular distribution after a brief heat shock. Here we report on further characterization of these proteins. To this end, monoclonal antibodies have been prepared against the 46,000 mol wt protein and used to determine its location in the cytoplasm of Kc cells and to analyse the relationship between the two proteins from *Drosophila* and to cross-reacting proteins from hamster cells. Indirect immunofluorescence studies led us to the conclusion that these two proteins are contained in a heat-labile cytoskeleton in *Drosophila*.

Three different cytoskeletal structures have been described and characterized by immunofluorescence microscopy: microfilaments, microtubules, and intermediate-sized (10-nm) filaments (for review see reference 11 and 38). Our 46,000 mol wt *Drosophila* protein cannot be actin, because *Drosophila* actins have molecular weights of 44,000 (36) and do not comigrate with the two proteins described here. *Drosophila* tubulins do not differ in size from tubulins in other organisms and have molecular weights of ~54,000 and 52,000 (14). The third class of cytoplasmic filamentous structures is characterized by a diameter of ~10 nm. A variety of mesenchymal cells and cells growing in culture contain a type of intermediate-sized filaments (26) whose major protein components are vimentin (7)

or desmin (25). Here we present evidence that *Drosophila* cells also contain a cytoplasmic meshwork that consists of proteins related to the intermediate filament proteins of vertebrate cells but have different molecular weights.

## MATERIALS AND METHODS

### *Protein Extractions from Kc Cells and Organisms*

*D. melanogaster* Kc cells were grown under constant stirring at a temperature of 25°C in D20 medium (5) and harvested at a density of  $5-9 \times 10^6$  cells/ml. Cells were heat-shocked by immersing the cultures in a 70°C water bath under continuous stirring until the temperature of the culture had reached 37°C. Cultures were shifted to a 37°C water bath and kept for an additional 5 min at elevated temperature. Cells from 500-ml cultures were collected by centrifugation, washed once in 150 mM NaCl, 10 mM Tris/HCl, pH 7.5, 5 mM MgCl<sub>2</sub>, 0.2 mM phenylmethylsulfonylfluoride (PMSF), and taken up in 20 ml of the same buffer. Cells were homogenized without detergent with a motor-driven teflon homogenizer until ~50% of the cells were broken. Cell debris was removed by centrifugation at 10,000 g for 15 min, and this postmitochondrial supernate subjected to a 100,000 g centrifugation in a SW56 rotor (Beckman Instruments, Inc., Fullerton, Calif.) for 5 h. The pellet is referred to as microsomal fraction whereas the supernate represents the soluble fraction. Nuclei were prepared and extracted with buffered 0.4 M NaCl as described recently (6).

Total proteins were extracted from salivary glands, Oregon R embryos (16-20 h old), third instar larvae, pupae, or adults by homogenization in SDS-sample buffer (62.5 mM Tris/HCl, 2.3% SDS, 5% 2-mercaptoethanol, 10% glycerol, pH 6.8) and brief heating to 100°C. Undissolved material was sedimented by centrifugation for 30 min at 10,000 g, and some 60 µg of protein were applied to acrylamide gels.

## Polyacrylamide Gel Electrophoresis and Detection of Antigens by Protein Blotting

Analysis of protein populations was done on 0.8 mm thick 10–16% polyacrylamide-SDS slab gels with a 4.5% stacking gel. Gels were run for 15 h at 6 mA, then at 12 mA until the dye had reached the bottom, and stained with Coomassie Blue in methanol/acetic acid. Buffers and stocks were prepared essentially as described by O'Farrell (29).

Proteins were transferred from the gel to nitrocellulose with 0.075 M Tris, 0.58 M Glycine, pH 9.0 for 24 h. For the reaction with antibodies, the filters were first incubated for 30 min in a 1% bovine serum albumin (BSA) solution in phosphate buffered saline (PBS) and then with ~0.7 ml per protein lane of antibody-containing supernatant fluid from the hybridoma lines. The incubation with antibodies was performed in sealed plastic bags for 5 h at room temperature or overnight at 4°C under constant shaking. Afterwards the filters were washed twice for 30 min in PBS and incubated for 5 hr with 300,000 cpm/protein lane of rabbit antimouse (RAM) Fab labeled with <sup>125</sup>Iodine according to Greenwood et al. (17) and dissolved in PBS, 0.1% BSA at a concentration of some 10<sup>5</sup> cpm/ml. Unreacted radioactivity was removed by two washes with PBS containing 0.05% NP40 for 1 h each. The filters were dried and exposed with amplifying screen Quanta 2 or 3 (DuPont Co., München, W. Germany) on Kodak X-Omat films at -70°C. This blotting procedure will be described in detail elsewhere (31).

### Preparation of Monoclonal Antibodies

Immunization of mice with proteins recovered from polyacrylamide gels was done as described by Lane and Robbins (24). A 0.4 M NaCl nuclear extract from heat-shocked cells was applied to a 1.5 mm thick preparative 10–16% polyacrylamide-SDS gel. After electrophoresis the 46,000 mol. wt. protein band was cut out of the gel with a razor blade, using stained vertical strips of the gel as references. The remaining gel was stained to ensure that the right protein had been accurately removed. The gel fragments were suspended in PBS and homogenized by sonification. To this suspension, 0.2 vol of complete Freund's adjuvant (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) was added and 150 µl were injected into 6-wk-old female mice (32). We estimate that these 150 µl contain ~20 µg of the 46,000 mol wt protein. 2 wk later the mice were boosted with 50 µl of an identically prepared protein/gel suspension without adjuvant. The mice were boosted again 3 d before the cell fusion was done (3.5 to 4 mo after the primary immunization) using 500 µl of the same suspension and antibody-secreting cell lines were prepared by fusion of mouse spleen cells with myeloma cell lines P3-NS1/1-Ag4-1 (23) or P3-X63-Ag8-65/3 (21) as first described by Köhler and Milstein (22) using the modifications introduced by Haemmerling (15) and described in detail elsewhere (32). Antibodies secreted from cell lines after cloning twice in soft agar were referred to as monoclonal antibodies. The arbitrary system we use for naming lines, clones, and antibodies is described in Saumweber et al. (32).

### Radioimmunoassay (Solid Phase Test)

Hybridoma cell lines producing antibodies were tested using a modified solid-phase radioimmunoassay (32). Briefly, 100 µl of antigen in PBS (either postmitochondrial supernates from normal Kc cells or 0.4 M NaCl nuclear extracts from heat-shocked cells) were adsorbed onto the surface of each well of a PVC microtiter plate (V-plate disposable 220–25, Flow Laboratories, Bethesda, Md.) for 15 min at room temperature. The wells were rinsed with PBS containing 0.1% BSA for 1 h on ice. Wells were washed twice with PBS and incubated for at least 5 h with 100 µl of hybridoma cell culture supernatant fluid. After two washes with PBS, 100 µl of PBS containing 0.1% Triton X-100 and 10<sup>5</sup> cpm of <sup>125</sup>I-RAM Fab-fragments were added and incubated for 5 h at room temperature. Wells were again washed twice with PBS and the plate was exposed on Kodak X-Omat films with amplifying screen at -70°C. For negative controls, either the adsorption of antigens or incubation with antibody-containing culture supernate was omitted.

### Indirect Immunofluorescence Assays

To localize the 46,000 mol. wt. protein in the Kc cells, we modified the procedure described by Jockusch et al. (20). 20 ml of Kc cells (at ~5×10<sup>6</sup> cells/ml) were collected and washed once in PBS. For fixation they were taken up in 2 ml of PBS containing 3.7% formaldehyde and incubated for 20 min at room temperature. Cells were sedimented again and washed with PBS. The cell pellet was suspended in 50% fetal calf serum in PBS, applied to slides, and air-dried. Before incubation with antibodies the dried cells were soaked for 5 min in PBS, then briefly immersed (5 s) in PBS/0.1% NP40 and washed again for 5 min in PBS.

To flatten out Kc cells on glass slides, they were grown for several days in medium containing 4 µg/ml cholesterol and 0.2% ethanol (16). Cells were fixed as described below for baby hamster kidney (BHK) cells.

BHK cells were grown in Eagle's medium with 10% fetal calf serum and allowed to attach to microscope slides for 24 h. They were then washed, fixed with 3.7% formaldehyde, and treated with methanol and acetone at -20°C (30).

Salivary glands were dissected from *D. melanogaster* Oregon R larvae in PBS containing 1% Triton and fixed for 30 s in the same solution containing 3.7% formaldehyde. After fixation the glands were transferred to 3.7% formaldehyde in 45% acetic acid and slightly squashed with a cover slip to avoid breaking of nuclei. Slides were plunged into liquid nitrogen, the cover slip was removed, and samples were washed for 10 min in 95% ethanol and twice in PBS. Heat shock of Kc cells was performed for 5–10 min at 37°C. Salivary glands were heat shocked for 10 min at 37°C in PBS; BHK cells were incubated for 45 min at 42°C. 20 µl of hybridoma culture supernate were applied and the slides were incubated in a moist chamber for 30 min. After washing twice for 5 min each with PBS, incubation with fluorescent second antibody was performed for 30 min in the dark using 10 µl of fluorescein isothiocyanate-coupled rabbit antimouse immunoglobulins (Miles Laboratories, Inc., Elkhart, Ind.) diluted 40-fold with PBS. Specimens were washed again twice with PBS and, after mounting a cover slip, photographed with phase contrast or incident UV illumination in a Zeiss universal microscope.

## RESULTS

We recently described two proteins (mol wt 46,000 and 40,000) that appear to change their cellular distribution after a 5-min heat shock of *D. melanogaster* Kc cells (6). To determine the localization of these proteins within Kc cells, we fractionated cells into nuclear, mitochondrial, microsomal, and soluble cytoplasmic components using differential centrifugation. No detergent was used in this procedure and physiological pH and ionic strength were used. Proteins from each fraction were dissolved in SDS sample buffer and analysed on SDS-polyacrylamide gels. Fig. 1 shows that the two proteins (arrows) are associated with the microsomal fraction of non-heat-shocked Kc cells and that they appear in the nuclear fraction after a 5-min heat shock. It should also be noted that the 46,000 mol wt protein is a major component of the protein complement of the fraction operationally designated "microsomes". In total cell extracts this protein is also easily detectable as a major component. The protein could be solubilized from the microsomal fraction by treatment with 1% SDS or 2 M NaCl, whereas RNase (50 µg/ml), 10 mM EDTA, or 1% NP40 are

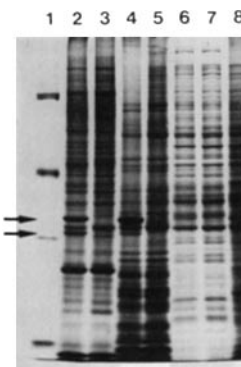


FIGURE 1 SDS-acrylamide gel electrophoresis of proteins extracted from various fractions of Kc cells before and after a 5-min heat shock. Size markers in lane 1 with molecular weights of 165,000, 155,000, 68,000, 39,000, and 21,500. Proteins of the nuclear fraction extracted with 0.4 M NaCl after heat shock (lane 2) and from control cells (lane 3). Proteins of the microsomal fraction from control cells (lane 4) and from heat-shocked cells (lane 5). Soluble (100,000 × g supernatant) proteins from control (lane 6) and from heat-shocked cells (lane 7). Total SDS-extractable Kc cell proteins (lane 8). Arrows indicate the 46,000 and 40,000 mol wt proteins.

without effect (data not shown). We conclude that both proteins are tightly bound to microsomal or other structures contained in this fraction or form 100,000 *g*-sedimentable structures themselves.

### Immunological Characterization of the Antigen

We have used antibodies prepared against the 46,000 mol wt protein to localize this protein in Kc cells and salivary glands of *D. melanogaster*. Kc cell nuclei were extracted after a 5-min heat shock with 0.4 M NaCl in 20 mM Tris-HCl (pH 7.5), 0.5 mM EDTA, 0.2 mM PMSF. This nuclear extract was separated on a preparative SDS-acrylamide gel, the 46,000 mol wt protein band was cut out and used for immunization of white mice (see reference 32). Permanent cell lines secreting monoclonal antibodies were established as described in Materials and Methods. Hybridoma lines from several cell fusions that produced sufficient titers of antibody were selected by solid-phase radioimmunoassay. Supernates from ten hybridoma lines that gave a positive response in the radioimmunoassay also gave a bright fluorescence with Kc cells (see below). To demonstrate that the antibodies were directed against the 46,000 mol wt protein that was used for immunization, proteins from the microsomal and nuclear fractions of control or heat-shocked Kc cells were separated on polyacrylamide gel (compare Fig. 1) and tested after blotting to nitrocellulose with antibody-containing cell culture supernates. Bound antibodies were detected with iodinated RAM-Fab $\gamma$ -fragments. The autoradiograph in Fig. 2a clearly shows binding of the antibodies to the 46,000 and 40,000 mol wt proteins and reveals the quali-

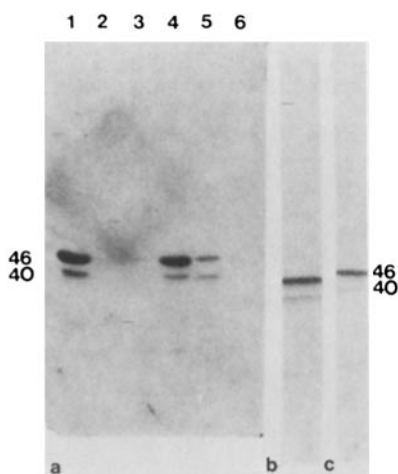


FIGURE 2 Autoradiograph of proteins blotted onto a nitrocellulose filter and reacted first with supernatants from hybridoma cell lines a, b: Ag25; c: Ah3/7/7) and then with iodinated rabbit antimouse Fab $\gamma$ -fragments. Different protein samples were loaded on the acrylamide gel that was used for blotting. Compare with Coomassie blue-stained protein samples in Fig. 1. Fig. 2a: SDS-extractable proteins from nuclear fraction of heat-shocked cells (lane 1), SDS-extractable proteins from nuclear fraction of control (25°C) cells (lane 2), microsomal fraction of heat-shocked cells (lane 3, see Fig. 1, lane 5), microsomal fraction of control cells (lane 4, see Fig. 1, lane 4), 0.4 M NaCl nuclear extract from heat-shocked cells (lane 5, see Fig. 1, lane 2) and from control cells (lane 6, see Fig. 1, lane 3). Fig. 2 b and c: Total SDS-extractable Kc cell proteins (see Fig. 1, lane 8) reacted with antibody containing supernatant from hybridoma cell line Ag25 (Fig. 2 b) or Ah3/7/7 (Fig. 2 c). The numbers 46 and 40 indicate the position of the respective 46,000 and 40,000 mol wt proteins on the stained gels.

tative redistribution of these two proteins after heat shock as visualized earlier by Coomassie Blue staining of the gels. However, a quantitative analysis of redistribution cannot be achieved with the blotting procedure. All antibody-containing supernates from cell lines positive in the radioimmunoassay reacted with the same two proteins. We conclude that these two proteins share common determinants. In some blots an additional weak protein band at 27,000 mol wt was detected. This lower molecular weight protein could be a degradation product arising during the preparation. When a total cellular protein extract obtained by boiling in SDS-electrophoresis sample buffer was tested using the protein blotting method, only two bands of 46,000 and 40,000 mol wt could be detected (Fig. 2 b, c).

It is obvious from the stained protein gels (see Fig. 1) that the 46,000 mol wt protein is a major component of the "microsomal" fraction and the whole cell. To examine the question whether this protein is also present in the living organism, total proteins were extracted in SDS buffer from salivary glands of third instar larvae and analyzed by the protein blotting procedure. After reaction with Ah6 antibody the autoradiograph also showed the presence of the 46,000 mol wt protein in salivary glands (data not shown).

### Localization of Antigen in Kc Cells and Salivary Glands by Indirect Immunofluorescence

The antibodies produced by ten hybridoma cell lines that originated from different fusion experiments all gave the same reaction pattern with Kc cell proteins shown in Fig. 2 b, c. However, when their reaction with Kc cells was tested by indirect immunofluorescence, two groups could be distinguished which gave different fluorescence patterns. Seven hybridomas (Ag6, Ag9, Ag25, Ag29, Ag32, Ah6, Bf2) produced antibodies that stained the cytoplasm of Kc cells very brightly (Fig. 3 a, b). In some cases, especially when lower antibody concentrations were used, the fluorescence seemed to be localized in a filamentous network and brighter, defined spots became apparent in the cytoplasm. These filaments became more obvious after the cells had been flattened out on a glass surface by treatment with cholesterol (Fig. 4 a, b). Nuclei always remained dark. Because the subcloned Ah6/5/9 hybridoma produced IgM, we used IgM from the W6/1 cell line (2) directed against group A erythrocytes as control (Fig. 3 e). The second group of antibodies (Ah3, Ah9, Bf16) also stained the cytoplasm of Kc cells but showed a somewhat different pattern: very strong fluorescence was observed in defined areas of the cells (Fig. 3 c, d). Almost every Kc cell contained one or more of these patches that were also detectable by phase contrast. The number of these patches per cell increased significantly after treatment with colcemid (not shown).

To study the presence of the antigens in a tissue, we chose salivary glands of *D. melanogaster*. Because the 46,000 mol wt protein was detectable in glands by the protein blotting procedure, immunofluorescence microscopy also showed cytoplasmic staining (Fig. 5 a, b). Type I and type II antibodies did not exhibit obvious differences. The significance of this weak fibrillar staining in the cytoplasm is presently difficult to assess. However, it should be noted that no such cytoplasmic staining in salivary gland cells was observed when monoclonal antibodies directed against nuclear proteins were used as control (see also 32).

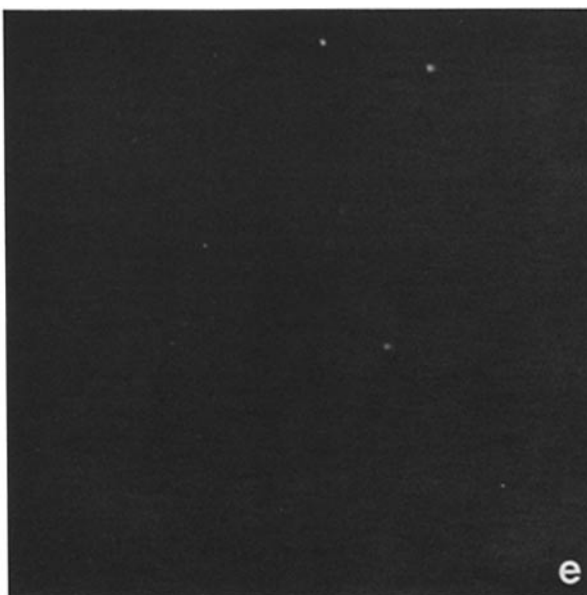
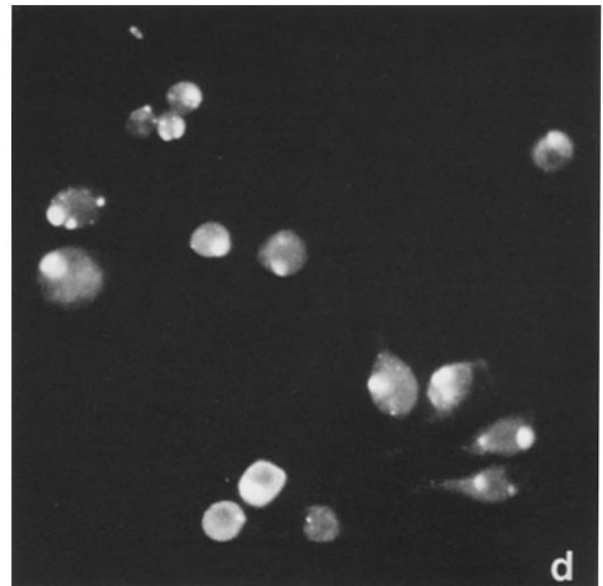
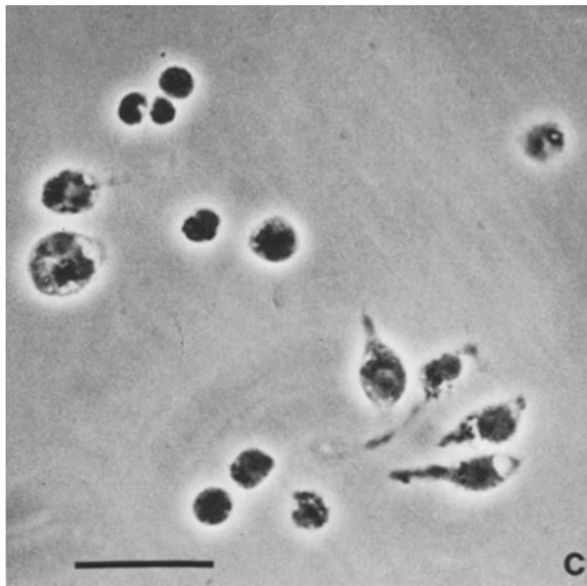
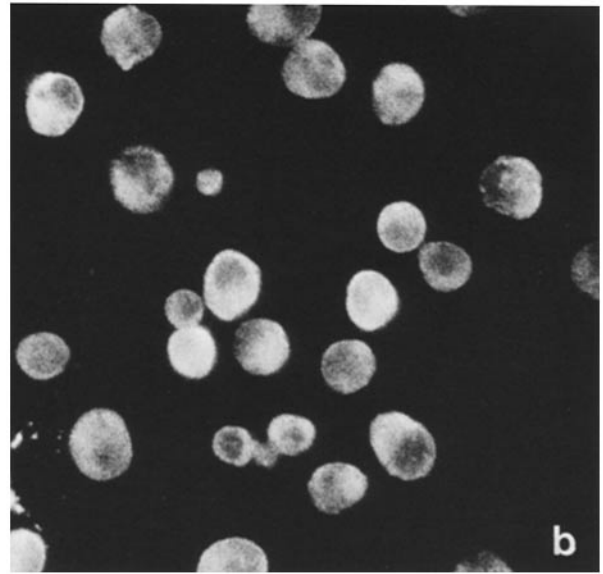
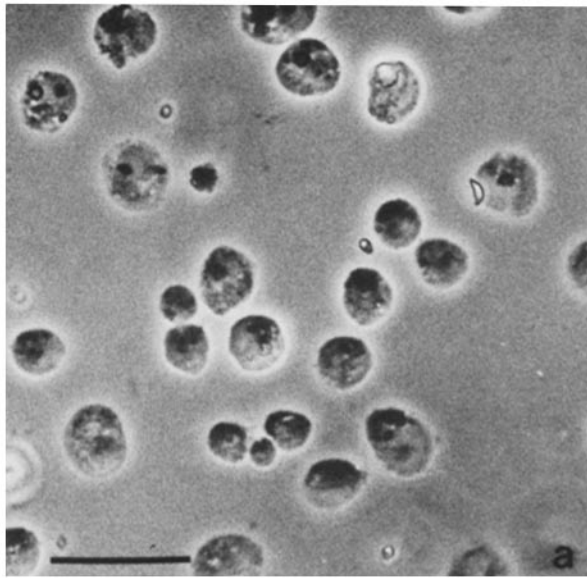


FIGURE 3 Indirect immunofluorescence staining of Kc cells grown at 25°C. Phase-contrast micrograph (a, c) and indirect immunofluorescence obtained with monoclonal antibodies from the Ah6/5/9 hybridoma cell line (type I) (b), and with monoclonal antibodies from the Ah3/7/7 hybridoma cell line (type II) (d). Control: indirect immunofluorescence with the same concentration of IgM from the hybridoma cell line W6/1 (Sera-Labs, Crawley) producing antibodies against group A erythrocytes (e). Bars, 25  $\mu$ m.

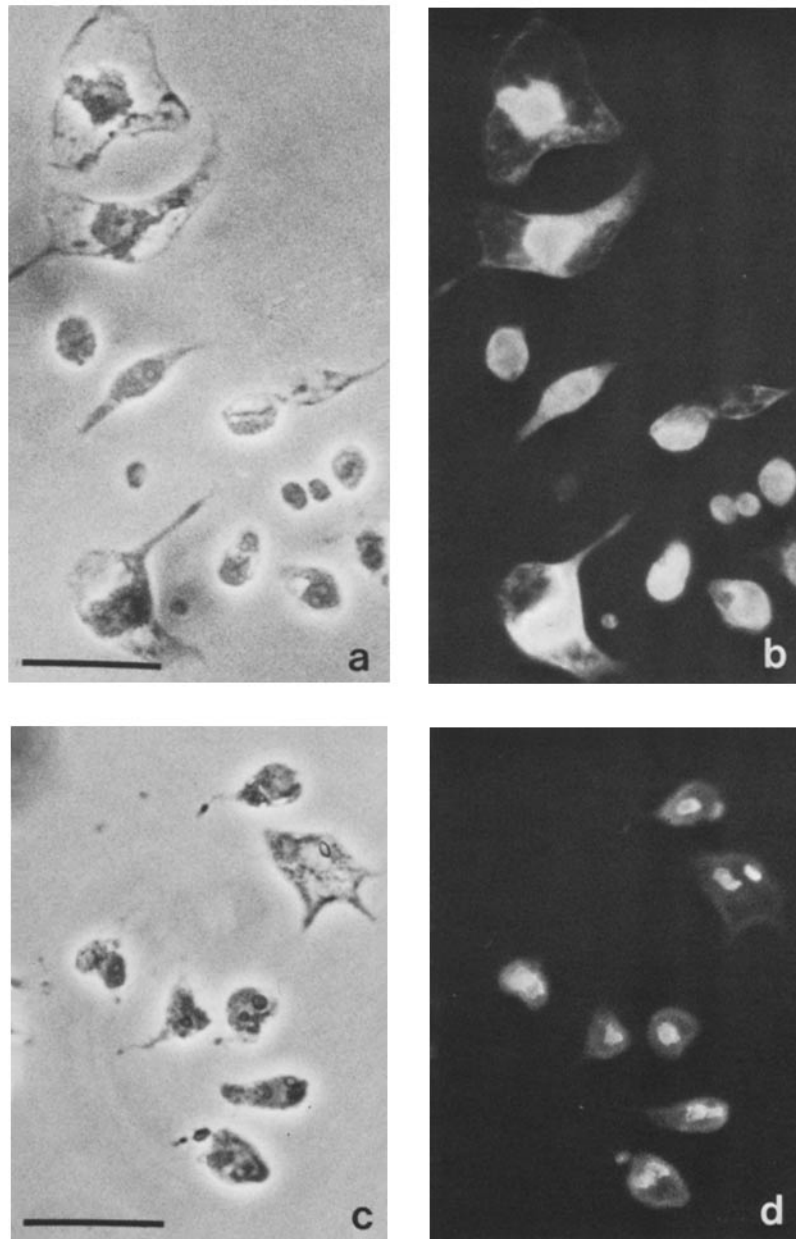


FIGURE 4 Effect of elevated temperature on cytoplasmic filaments in *Drosophila* Kc cells. Indirect immunofluorescence with Ah6/5/9 antibody reveals a filamentous network in the cytoplasm of flattened-out cells (a, b). After a 5-min heat shock (c, d) the antigen is aggregated as shown by indirect immunofluorescence with Ah6/5/9 antibody (d). Bars, 25  $\mu$ m.

### Crossreaction of Antibodies with Intermediate Filament Proteins of Vertebrates

Because proteins forming cytoskeletons are believed to be relatively well conserved in evolution, we investigated the cross-reaction of our monoclonal antibodies with components of other cell lines.

Intermediate filaments have the characteristic property of aggregating and forming perinuclear rings or juxtannuclear "cap" structures in cells treated with colcemid (11, 19). We isolated the juxtannuclear aggregates of 10-nm filaments from BHK cells after colcemid treatment following the procedure described by Starger and Goldman (33). Protein samples from various stages of purification were run on SDS polyacrylamide gels (Fig. 6a) and transferred to nitrocellulose as usual. As shown in Fig. 6b, Ah6/5/9 and Ag25 antibodies bound two

proteins in the total protein extract from BHK cells. These two proteins were highly enriched by colcemid treatment. We conclude that these two BHK proteins (mol wt ~55,000 and 52,000) are identical to the two proteins of similar molecular weight that are believed to form intermediate filament structures in these cells (34).

Using indirect immunofluorescence, we found positive response with bovine kidney cells, chicken fibroblasts, and BHK cells when antibodies of the first group of hybridomas (e.g. Ag25, Ah6/5/9) were used. Type I antibodies stained a filamentous network in the cytoplasm of all cell types as shown in Fig. 7a, b for BHK cells. Occasionally, more condensed areas in the vicinity of the nuclei were stained more intensely. The second group of antibodies (e.g. Ah3/7/7), which reacted with the same protein bands on the protein blots (see Fig. 2), did not show any significant cross-reaction with these vertebrate cell lines.

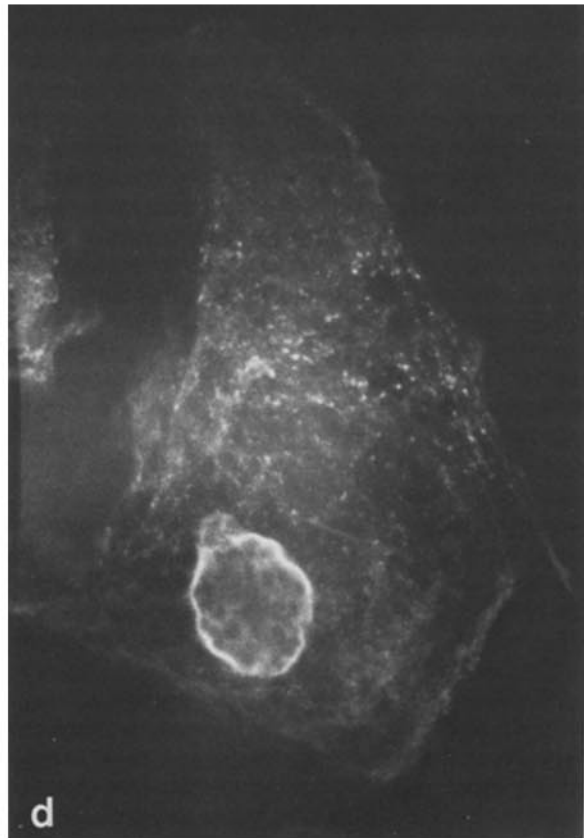
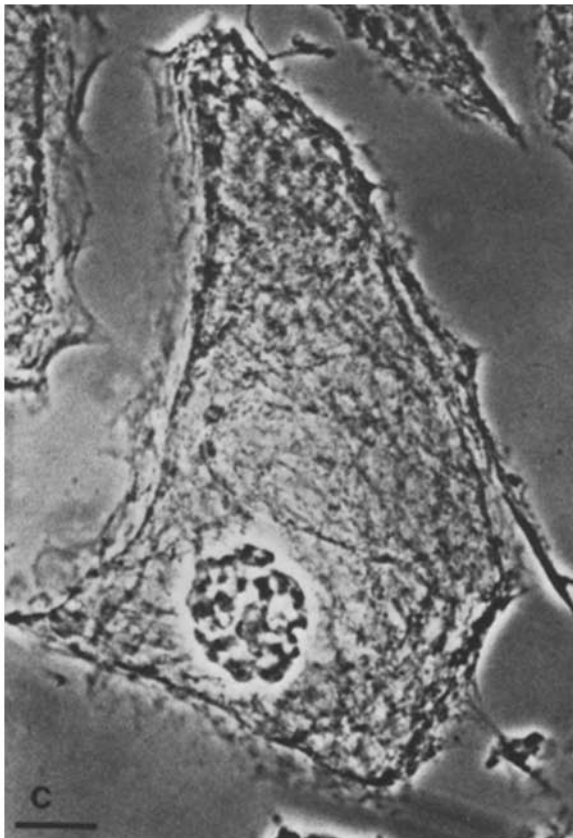
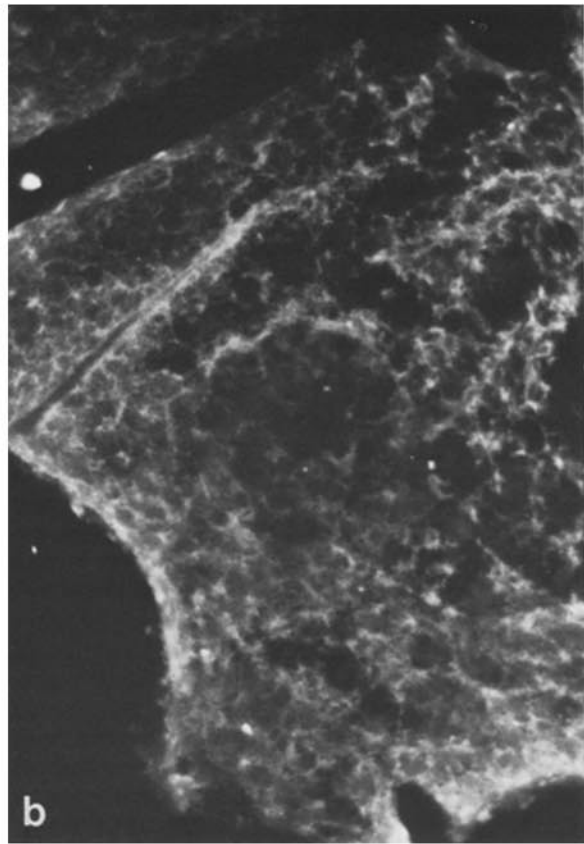
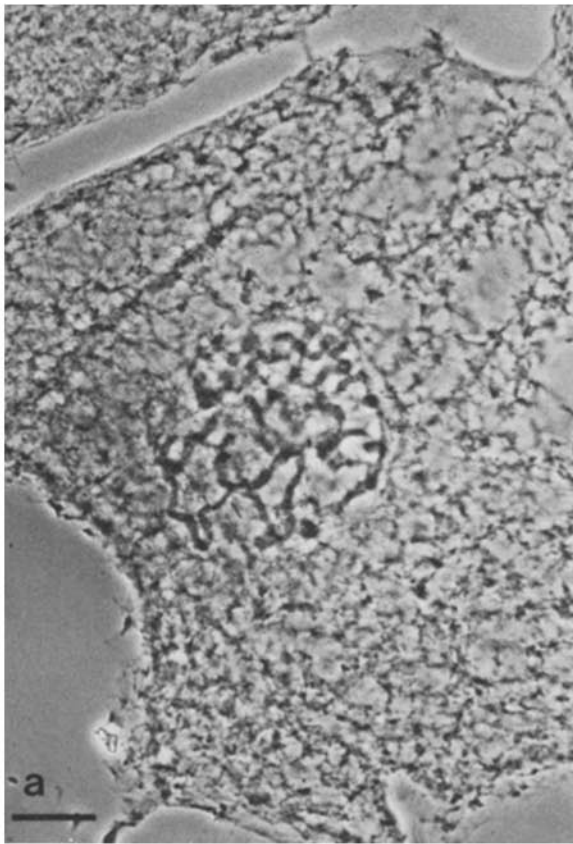


FIGURE 5 Phase-contrast photograph (a, c) and indirect immunofluorescence staining (b, d) of *Drosophila* salivary gland cells with Ah6/5/9 antibody. Salivary gland cells from larvae grown at 25°C (a, b), and after heat shock (c, d). Bars, 25  $\mu$ m.

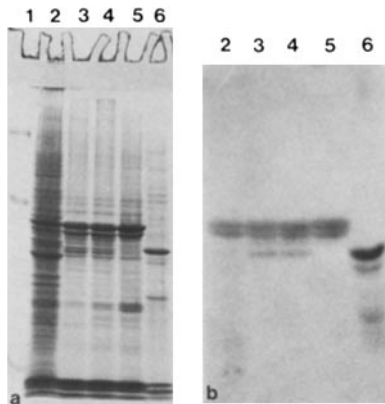


FIGURE 6 (a) SDS-polyacrylamide gel electrophoresis of total BHK proteins (lane 2), and protein fractions of various stages of vimentin purification from these cells (33) (lanes 3, 4, 5). Proteins after final PBS-EDTA washes (lane 3), protein after DNase I treatment (lane 4), proteins extracted from the perinuclear "caps" after colcemid treatment of BHK cells (lane 5). Total SDS-soluble nuclear proteins of *Drosophila* Kc cells after heat shock (lane 6, see Fig. 3, lane 1). (b) The gel shown in (a) was blotted and the proteins were immobilized on nitrocellulose filter incubated with antibodies directed against the 46,000 mol wt *Drosophila* protein in the supernatant fluid of the Ag25 or Ah6/5/9 hybridoma cell line. After incubation with  $^{125}\text{I}$ -rabbit antimouse Fab $\gamma$ , the filter was exposed on x-ray film. Numbers indicate the corresponding lanes of the acrylamide gel.

### Effect of Heat Shock on Intermediate Filaments

The cellular redistribution of the *Drosophila* 46,000 and 40,000 mol wt proteins after heat shock was followed by analyzing protein patterns from various cellular fractions (see Figs. 1 and 2). We have also studied the effect of heat shock on the distribution of these proteins by indirect immunofluorescence in intact *Drosophila* or BHK cells.

Kc cells were kept for 10 min at 37°C and fixed with formaldehyde. With antibodies from the first group (e.g. Ah6/5/9) no obvious shift in the localization of fluorescence could be detected after heat shock when the cells were round (data not shown). However, flattened-out Kc cells showed higher concentrations of antigen in the perinuclear region after heat shock (Fig. 4c, d). With antibodies from the second group of hybridomas (e.g. Bf16), ~70% of the bright dots stained in normal cells were no longer stained after a 5-min heat shock. The dark structures in the cell remained visible by phase-contrast microscopy but showed no fluorescence. In this case we did not observe a more intense fluorescence of the nuclei after heat shock (data not shown).

Redistribution of the antigens was also observed in salivary glands by indirect immunofluorescence after heat shock. After a 5 to 10-min treatment of the glands at 37°C in PBS, the cytoplasmic filaments decorated with Ah6/5/9 antibodies appeared less intense than in control glands, and most of the fluorescence was observed in the peripheral region of the nuclei (Fig. 5c, d). As in control glands, the polytene nuclei remained dark after heat shock and, as far as we can judge, chromosomes did not fluoresce.

The effect of elevated temperature on the intermediate filaments of BHK cells was also tested (4). As shown in Fig. 7c, d, the cytoskeleton stained with Ah6/5/9 in control cells (Fig. 7a, b) was no longer stained after 45 min at 42°C and juxtannuclear "caps" of the filament material appear in higher frequency in

the cells, quite analogous to the effect observed after colcemid treatment (11).

### DISCUSSION

In this paper we describe two proteins of *D. melanogaster* (mol wt 46,000 and 40,000) that are located in the cytoplasm of tissue culture cells and salivary glands. Because the concentration of these proteins in cultured Kc cells is high, they could be easily detected by protein blotting and even by Coomassie Blue staining.

The two proteins have several characteristic properties: (a) In fractionated Kc cells they pellet at 100,000 g with the so-called microsomal fraction from which they are not extractable with 1% NP40. (b) Both proteins bind monoclonal antibodies prepared against only the 46,000 mol wt protein and thus must have common antigenic determinants. (c) These proteins are associated with a cytoskeleton as demonstrated by indirect immunofluorescence using our monoclonal antibodies. (d) The cross-reaction of the type I antibodies (e.g. Ag25, Ah6/5/9) with vertebrate cell lines provide further evidence for the cytoskeletal nature of this protein. In BHK cells these antibodies bind to two proteins of molecular weights of ~55,000 and 52,000, respectively, that could be enriched from perinuclear cap structures induced by colcemid (34). The larger of these two proteins is identical to "vimentin" (7, 18, 37), whereas the smaller protein has been reported to be identical to a different intermediate filament termed "desmin" (10, 25, 37).

Vimentin has been found in a variety of vertebrate cells and the cross-reaction of antibodies against purified vimentin from one source with other cell types indicates its evolutionary conservation (3, 7, 8). However, the occurrence of vimentin or related proteins in invertebrates has not been reported. Our data indicate that *D. melanogaster* Kc cells, and also salivary gland cells, contain proteins with antigenic determinants also found on vimentin and desmin of BHK cells. From our findings with immunofluorescence microscopy, we conclude that these proteins can form cytoplasmic meshwork structures resembling arrays described for the intermediate filament meshworks observed in vertebrate cells. A striking difference, of course, is the different molecular weight of these proteins in *Drosophila*. The monoclonal antibodies against the isolated 46,000 mol wt *Drosophila* protein bind to the protein used for immunization as well as to a 40,000 mol wt protein. We therefore believe that these two proteins share common determinants and may be structurally related. Although both proteins are detected after rapid disruption of Kc cells in SDS buffer and in the presence of protease inhibitor, we cannot completely rule out the possibility that the 40,000 mol wt protein is a degradation product of the 46,000 mol wt protein. Further comparative protein analysis will be necessary.

All ten antibodies react with these two proteins immobilized on nitrocellulose filters. However, two classes of antibodies could be distinguished according to their staining properties of *Drosophila* or vertebrate cells. Type I antibodies, produced by seven hybridoma lines, showed a bright staining of cytoplasmic structures in Kc cells with some brighter patches. These antibodies showed cross-reaction with all vertebrate cell lines tested. Type II antibodies, derived from three hybridoma lines, stained only distinct patches in the cytoplasm of Kc cells but did not cross-react with vertebrate cells. Therefore, there are at least two different antigenic determinants common to both proteins. We suggest that the type II determinant may not be available to antibodies when the proteins form a cytoskeleton.

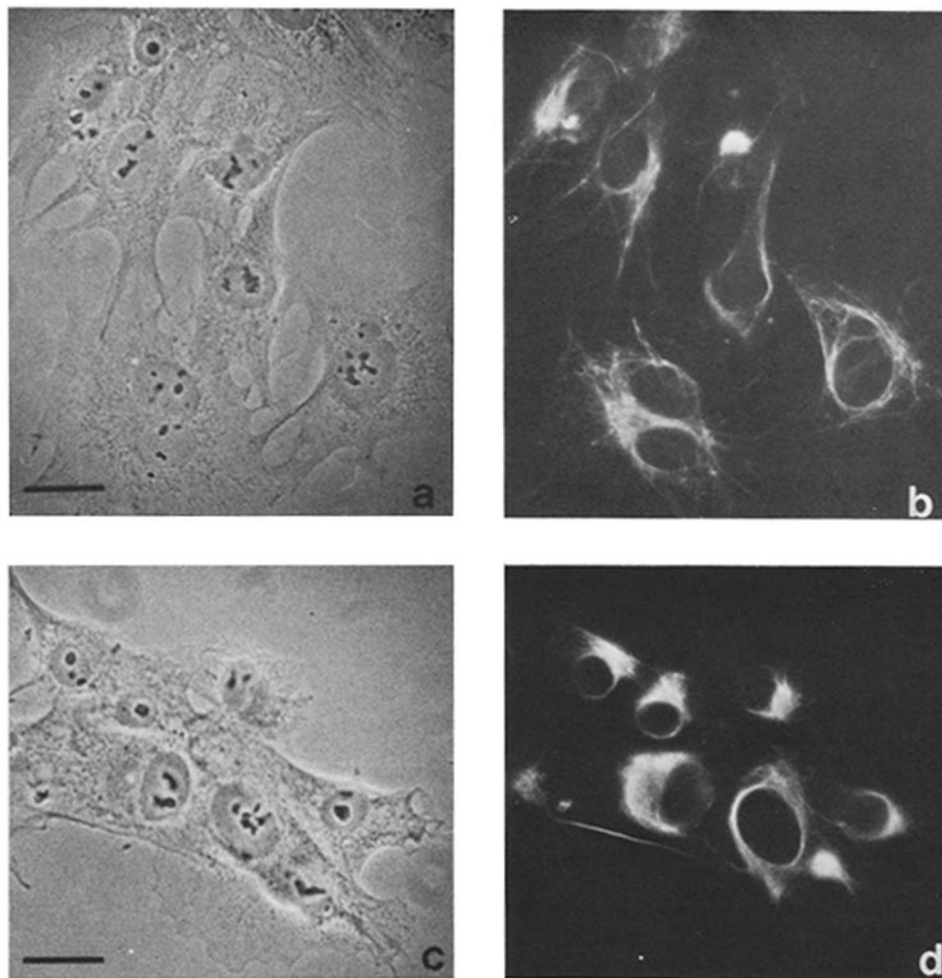


FIGURE 7 Crossreaction of antibodies directed against the 46,000 mol. wt. *Drosophila* protein with cytoskeletal proteins of BHK cells. Phase-contrast pictures (a, c) and indirect immunofluorescence staining with antibodies from the supernatant fluid of the Ag25 or Ah6/5/9 hybridoma cell line (type I) (b, d). Cells grown at 37°C (a, b) and after heat shock (c, d). Elevated temperature causes the cytoskeletal filaments (b) to aggregate at the nuclei (d). Bars, 25  $\mu\text{m}$ .

The "patches" in the cytoplasm of the Kc cells might be areas of aggregates of the proteins in a nonfilamentous fashion, thus exposing both antigenic determinants. Whether this material can be organized in filaments in Kc cells that are attached to a surface remains to be shown by electron microscopy. It seems that large amounts of protein are present in these patches, which explains why these two proteins are major components of total protein extracts from Kc culture cells. This is consistent with the finding of Franke et al. (8) that almost all cultured vertebrate cells contain massive amounts of vimentin.

The cross-reaction of the type I antibodies with intermediate-filament proteins from BHK cells strongly suggests that the 46,000 and 40,000 mol wt *Drosophila* proteins are related to the 55,000 and 52,000 mol wt proteins of BHK cells. Type II monoclonal antibodies may be directed against a determinant either not accessible to the antibody, or not conserved in evolution. Similar conclusions were drawn by Franke and co-workers (8) who described guinea pig antibodies to murine vimentin with different ranges of cross-reactivity, suggesting that vimentin possesses conserved and variable regions. When we tested the guinea pig antivimentin serum with the broadest species specificity (GP<sub>4M</sub>, 8) in the protein blotting assay, it reacted very strongly with the BHK vimentin but not with the desmin band. Only a very weak, hardly detectable cross-reaction with the 46,000 mol wt *Drosophila* protein could be

observed (data not shown). The determinant that binds our monoclonal type I antibodies is found, not only on BHK vimentin, but also on the lower molecular weight protein which is probably desmin. From peptide analysis it has been suggested (10, 35) that vimentin and desmin share common determinants, although no cross-reaction between these proteins has so far been detected with polyvalent sera (3, 7, 13 and this report).

The correlation between the cytoplasmic rearrangement of intermediate filaments in BHK cells, possibly analogous structures in salivary glands and Kc cells of *Drosophila* and the other observed heat-shock effects (1) is unknown. The function of intermediate filaments in nonmuscle cells is unclear, but they may play a role in maintaining the spatial organization of the cytoplasm (26, 27, 33). In addition, attachment of certain polyribosomes to intermediate filaments has been described (9, 28, 30). *Drosophila* and BHK cells, both of which show changes in protein synthesis after heat shock (1, 4), also show aggregation of intermediate-filament material around cell nuclei at elevated temperature. It is conceivable that the breakdown of the intermediate-filament cytoskeleton after heat shock leads to the observed changes in protein synthesis.

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