MOLECULAR AND CELLULAR BIOLOGY, Aug. 1982, p. 914–920
0270-7306/82/080914-07$02.00/0

Vol. 2, No. 8

Small Nuclear Ribonucleoproteins of *Drosophila*: Identification of U1 RNA-Associated Proteins and Their Behavior During Heat Shock†

ERIC D. WIEBEN AND THORU PEDERSON*

Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

Received 12 February 1982/Accepted 4 May 1982

In *Drosophila*, two nuclear proteins of approximately 26,000 and 14,000 molecular weight are recognized by a human autoimmune antibody for mammalian ribonucleoprotein (RNP) particles that contain U1 small nuclear RNA. The antibody-selected *Drosophila* RNP contains, in addition to these two proteins, a single RNA species that has been identified as U1 by hybridization with a cloned *Drosophila* U1 DNA probe. Small nuclear RNP isolated from human cells under the same conditions as used for *Drosophila* and selected by the anti-U1 RNP-specific antibody contains eight proteins, two of which are similar in molecular weight to the two *Drosophila* U1 RNP proteins. Thus, even though the nucleotide sequences of *Drosophila* and human U1 RNA are about 72% homologous, and the corresponding RNPs are both recognized by the same human autoantibody, *Drosophila* U1 RNP appears to have a simpler protein complement than its mammalian counterpart. The two *Drosophila* U1 RNA-associated proteins are synthesized at normal or slightly increased rates during the heat shock response and are incorporated into antibody-recognizable RNP complexes. This raises the possibility that U1 RNP is an indispensable nuclear element for cell survival during heat shock.

U1 RNA is one of several small, abundant nuclear RNA species found in eucaryotic cells (13). U1 RNA exhibits a high degree of evolutionary conservation: the sequences of rat, chicken, and *Drosophila* U1 RNAs show 99, 94, and 72% homology, respectively, with human U1 RNA (2, 11). The 5′ end of U1 RNA contains a conserved region which is potentially complementary to consensus sequences at splice junctions of mRNA precursors (8, 11, 12, 14), suggesting that U1 RNA may be involved in mRNA splicing. This possibility is supported by the observations that adenovirus mRNA splicing in isolated nuclei is inhibited by a polyclonal antibody specific for U1 RNA-protein complexes (18), and that U1 RNA is base paired with heterogeneous nuclear RNA in vivo (3).

U1 RNA is associated with small ribonucleoprotein (RNP) particles in the nucleus, termed snRNP. In mouse ascites cells, U1 snRNP contains seven polypeptides having molecular weights between 12,000 and 30,000 (9). These proteins may be important structurally, but it is also possible that the U1 RNA-associated proteins actively participate in the splicing reaction. A paradigm for this is *Escherichia coli* RNase P, which is complexed with an RNA cofactor essential for the enzyme's activity in tRNA processing (15). It is therefore of interest to know whether U1 RNPs display the same degree of evolutionary conservation as the RNA itself. In this paper we report that *Drosophila* U1 RNA is complexed with only two proteins. In addition, we find that the synthesis of these proteins and their assembly into U1 snRNP is sustained during the heat shock response.

† This is paper no. 20 in a series entitled "Ribonucleoprotein Organization of Eucaryotic RNA." The preceding paper in the series is J. P. Calvet, L. M. Meyer, and T. Pederson, Science, in press.

MATERIALS AND METHODS

Sera. Anti-RNP serum was obtained from an adult, female patient with mixed connective tissue disease. Anti-Sm serum was from an adult, female patient with systemic lupus erythematosus. Control serum was obtained from healthy laboratory personnel. Immunoglobulin G (IgG) was purified as described previously (4) and stored at −20°C.

Cells and radioisotopic labeling. The serum-independent *Drosophila* cell line Kc0 was used for these experiments. Cells were cultured at 25°C in Echalier and Ohanessian's D-20 medium (5). For short-term labeling with [^{35}S]methionine (90 min or less), cells were collected by centrifugation, suspended at approximately 2×10^7 cells per ml in minimal medium (D-20 lacking lactalbumin hydrolysate and yeastolate), and incubated for the desired period with [^{35}S]methionine at 160 μCi/ml. In other cases, proteins were

labeled by incubating cells for 22 h in standard D-20 medium containing [^{35}S]methionine at 10 μCi/ml. Incorporation of [^{35}S]methionine into total trichloroacetic acid-insoluble material was monitored as described previously (10). For experiments with labeled RNA, cells were incubated for 22 h in standard D-20 medium containing [^{3}H]uridine at 100 μCi/ml.

Preparation of nuclear extracts. Cells were harvested by centrifugation at 600 × *g* for 5 min and then suspended in cell lysis buffer (10 mM NaCl, 3 mM $MgCl_2$, 10 mM Tris-hydrochloride [pH 7.5], 0.25 M sucrose, 0.25% Nonidet P-40). The nuclei were collected by centrifugation in an Eppendorf microfuge (5 s), washed once with cell lysis buffer, and suspended in 0.1 M NaCl–1 mM $MgCl_2$–10 mM Tris-hydrochloride (pH 8.5). After brief sonication to disrupt nuclei (10 s at setting 7; Heat Systems Ultrasonics model W-375), unbroken nuclei, nucleoli, and chromatin were pelleted by centrifugation for 3 min in an Eppendorf microfuge. The cleared supernatant was used for all further analyses.

Isolation of antibody-reactive RNA and protein. IgG (100 μg) was added to nuclear extract samples from up to 2 × 10^7 cells. After 20 min at 2°C, approximately 200 μl of protein A-Sepharose (Pharmacia Fine Chemicals) was added, and the incubation was continued for another 20 min. The protein A-Sepharose was pelleted by centrifugation and washed extensively with NET2 (0.15 M NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 50 mM Tris-hydrochloride, pH 7.5). IgG and antibody-selected material was then eluted with 0.1 M glycine-hydrochloride, pH 3.0. Proteins were concentrated by precipitation with 9 volumes of acetone containing 50 mM HCl. RNA was recovered by phenol-chloroform extraction in the presence of 0.5% sodium dodecyl sulfate.

Electrophoresis. Proteins collected by acetone precipitation were dissolved in sample buffer and electrophoresed in 11% polyacrylamide gels containing 0.1% sodium dodecyl sulfate as previously described (7). RNA was electrophoresed in 10% polyacrylamide gels (27:1 acrylamide-bisacrylamide) containing either 7 M urea and 50 mM Tris-borate (pH 8.3) or 98% formamide and 40 mM sodium phosphate (pH 7.0) (3). Gels were fluorographed using En^{3}Hance (New England Nuclear Corp.). The preparation of diazobenzloxymethyl paper, electrophoretic transfer of RNA, and hybridization to cloned DNA were all as described previously (3).

RESULTS

Patients with rheumatic diseases such as systemic lupus erythematosus or mixed connective tissue disease produce circulating antibodies against nuclear antigens, often including RNPs (4, 9). Some individuals, such as the mixed connective tissue disease patient whose serum was used in this study, produce high-titer antibodies which are essentially monospecific for U1 snRNP. As shown in Fig. 1, lanes 2 and 3, U1 RNA was the sole species of HeLa cell small nuclear RNA (snRNA) recognized by this patient's IgG. Control IgG from a healthy individual did not precipitate any snRNAs (lane 1). The anti-U1 RNP antibody did not precipitate protein-free U1 RNA prepared by phenol extraction (data not shown). Therefore, as with previously described anti-U1 RNP sera (4, 9), the antigen recognized by this antibody is a site(s) on the U1 RNA-protein complex. Figure 2 demonstrates that this antigenic site was preserved in the U1 snRNP of *Drosophila*. Three *Drosophila* snRNAs were selected by the human anti-U1 RNP antibody (Fig. 2, lane 2). The larger species was coelectrophoretic with HeLa U1 RNA and was conclusively identified as *Drosophila* U1 by hybridization with a cloned *Drosophila* U1 DNA probe after transfer to diazobenzyloxymethyl paper (Fig. 2, lane 3). The two bands smaller than U1 (Fig. 2, lane 2) were observed in variable amounts in antibody-selected *Drosophila* snRNP. They also hybridized to *Drosophila* U1 DNA (Fig. 2, lane 3) and probably correspond to the *Drosophila* U1 RNA fragments reported by Mount and Steitz (11). No *Drosophila* snRNAs were selected by control, nonimmune human IgG (see Fig. 5, lane 6). That a human antibody specific for U1 snRNP cross-reacts with the insect nuclear antigen indicates not only that the U1 RNA sequences are similar (11), but also that the evolutionary conservation extends to the

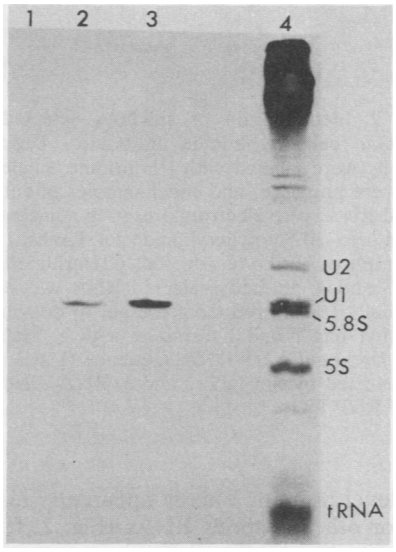

FIG. 1. [^{3}H]uridine-labeled RNA selected from HeLa cell nuclear extracts by anti-U1 RNP serum. HeLa cells were labeled with [^{3}H]uridine, and nuclear extracts were reacted with anti-U1 RNP IgG. The antibody-selected material was deproteinized by phenol extraction and electrophoresed in a 10% polyacrylamide gel containing 98% formamide. Lanes: 1, control (nonimmune) human IgG reacted with nuclear extract from 5 × 10^6 HeLa cells; 2, anti-U1 RNP IgG reacted with nuclear extract from 1.5 × 10^6 cells; 3, same as lane 2, but nuclear extract from 5 × 10^6 cells; 4, total snRNAs from HeLa cells.

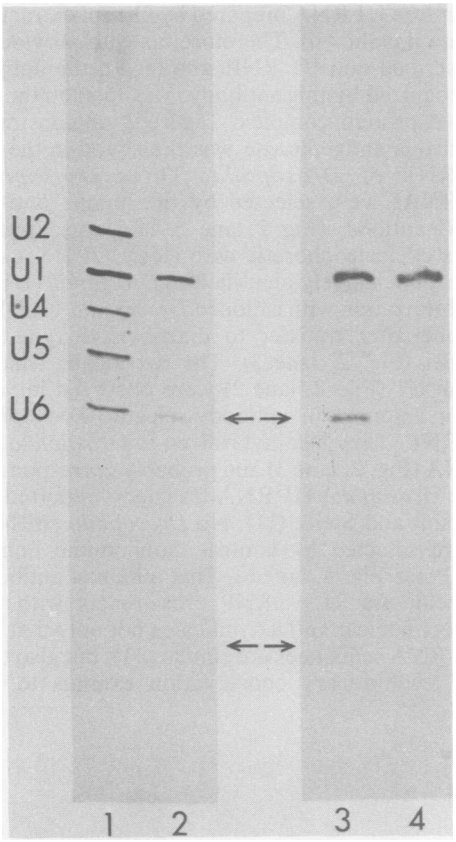

FIG. 2. Identification of snRNAs selected from *Drosophila* cells by human antibodies. *Drosophila* Kc0 cells were labeled with [^{3}H]uridine, nuclear extracts were prepared, and equal samples of antibody-selected RNA were electrophoresed in adjacent lanes of a 7 M urea–10% polyacrylamide gel. Lanes: 1 and 2, fluorograph of antibody-selected, [^{3}H]uridine-labeled RNA; 3 and 4, antibody-selected RNA was electrophoretically transferred from the gel to diazobenzyloxymethyl paper and hybridized with a ^{32}P-labeled cloned *Drosophila* U1 DNA sequence (11); 1 and 4, RNA selected by Sm IgG; 2 and 3, RNA selected by anti-U1 RNP IgG.

RNP level as well. This is apparently also the case for other U-series RNAs (Fig. 2, lane 1). Another antibody, Sm, that is known to precipitate mammalian snRNPs containing U1, U2, U4, U5 and U6 RNAs, also specifically reacted with *Drosophila* RNP complexes containing RNAs of comparable size. Note also that, of the several small RNAs precipitated by Sm antibody (Fig. 2, lane 1), only the band corresponding to U1 RNA hybridized to the *Drosophila* U1 DNA probe (Fig. 2, lane 4).

To investigate the proteins complexed with human and *Drosophila* U1 snRNP, [^{35}S]methionine-labeled nuclear extracts were reacted with antibody, and the selected proteins were displayed by gel electrophoresis. Figure 3 (lane HeLa) shows that the HeLa cell nuclear proteins selected by Sm antibody consisted of eight proteins (B and C are doublets in lighter exposures) ranging in molecular weight from 32,000 ("A") to approximately 10,000 ("F"). In contrast, *Drosophila* RNP selected by the U1 RNA-specific antibody contained only two proteins (Fig.

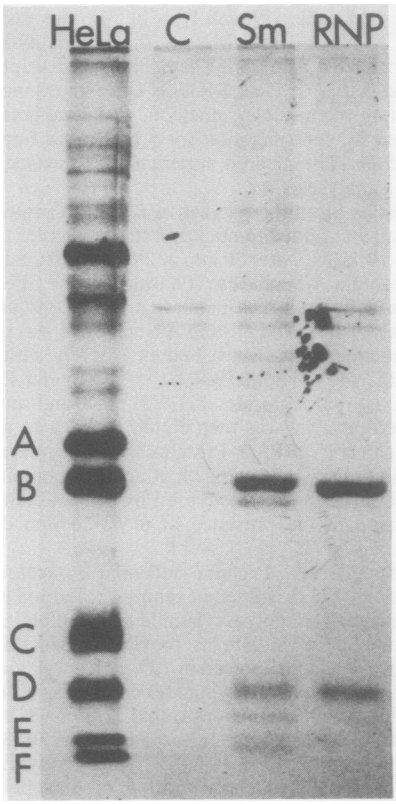

FIG. 3. ^{35}S-labeled proteins selected from *Drosophila* cells by human autoimmune sera. Kc0 cells were labeled for 45 min with 160 Ci of [^{35}S]methionine per ml. Nuclear extracts were prepared, and antibody-selected material was isolated. After elution from protein A-Sepharose, proteins were concentrated by precipitation with acetone containing 50 mM HCl. Samples were dissolved in sample buffer, electrophoresed in 11% polyacrylamide gels, and fluorographed. Lanes: HeLa, proteins selected from HeLa cell nuclear extracts by Sm IgG; C, control IgG; Sm, Sm IgG; RNP, Anti-U1 RNP IgG. The HeLa snRNP proteins A through D appear to correspond to the four largest snRNP proteins identified by Lerner and Steitz (9) in mouse cells. Although proteins E and F have molecular weights similar to those of the three smallest proteins identified in mouse cells, they are labeled according to alphabetical sequence and could be the human analog of any two of the murine E, F, and G proteins.

3, lane RNP). These migrated with the human B and D proteins and had estimated molecular weights of 26,000 and 14,000, respectively. These *Drosophila* proteins were not selected by control IgG (Fig. 3, lane C). Lane Sm of Fig. 3 shows the *Drosophila* nuclear proteins selected by the Sm antibody. In addition to the 26,000- and 14,000-molecular-weight species, four other proteins were present: one just below the 26,000 molecular weight band, one between the human D and E proteins, and two others comigrating with E and F. Thus, *Drosophila* U1 snRNP (Fig. 3, lane RNP) apparently contains a subset of the proteins bound to U1, U2, U4, U5, and U6 RNAs (Fig. 3, lane Sm).

The foregoing results indicate that, in *Drosophila*, U1 RNA exists as an RNP complex with two proteins of 26,000 and 14,000 molecular weight. To examine this further, [^{35}S]methionine-labeled nuclear extracts were fractionated in sucrose gradients, followed by anti-U1 RNP antibody selection of proteins across the gradient. Both the 26,000- and 14,000-molecular-weight proteins selected by the anti-U1 RNP antibody resided in structures sedimenting at 9 to 18S (Fig. 4). This is the sedimentation behavior expected if these proteins were associated with U1 RNA in a multiprotein complex and suggests sizes similar to those previously reported for mouse ascites U1 snRNP (8).

Exposure of *Drosophila* embryos or tissue culture cells to elevated temperatures (36°C) elicits a profound inhibition of total protein synthesis and the concomitant activation of a set of genes which encode a small group of polypeptides known as the heat shock proteins (1). Because some of these proteins are nuclear (16, 17), and because the heat shock response includes a change in gene transcription patterns (1, 6), it was of interest to probe the structure of U1

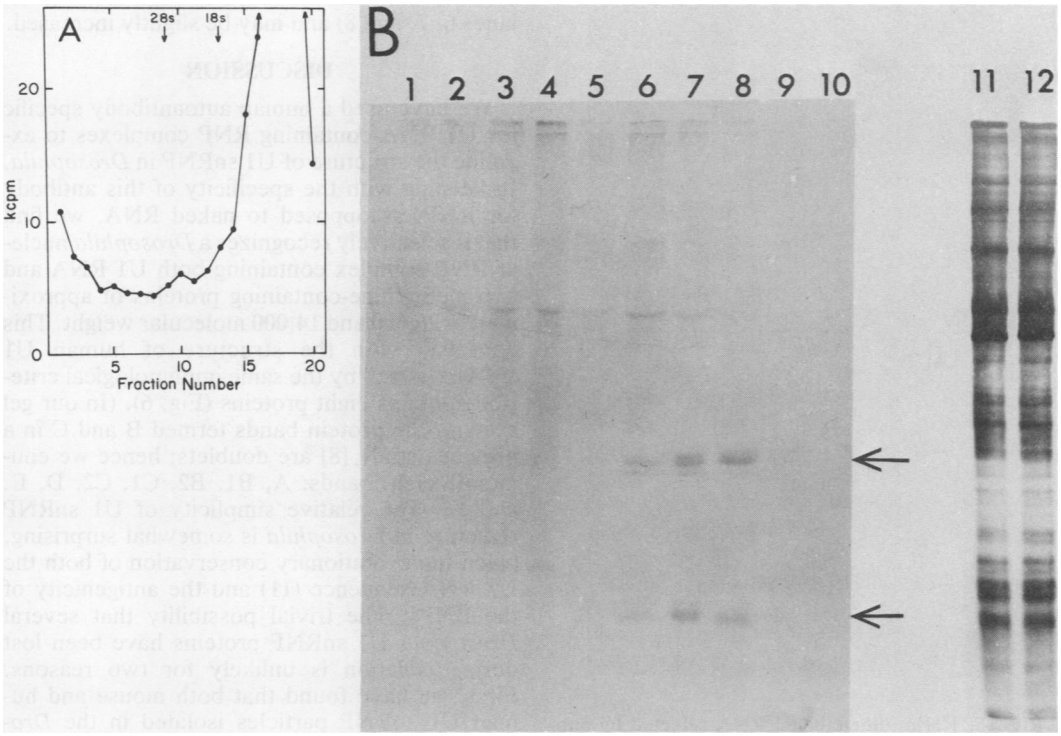

FIG. 4. Sucrose gradient sedimentation of proteins complexed with *Drosophila* U1 RNA. Cells were labeled for 22 h with [^{35}S]methionine (10 μCi/ml), and nuclei were incubated (without sonication) in 0.1 M NaCl–1 mM $MgCl_2$–10 mM Tris-hydrochloride (pH 8.5) for 90 min at 0°C. After centrifugation for 3 min in an Eppendorf microfuge, the supernatant was centrifuged on a 15 to 30% sucrose gradient in the same buffer (Beckman SW41 rotor; 23,000 rpm, 17 h, 4°C). Samples (25 μl) of each gradient fraction were assayed for [^{35}S]methionine incorporation into trichloroacetic acid-insoluble material. The remainders of every two adjacent fractions were pooled and reacted with anti-U1 RNP IgG. In panel B lanes 1 through 10 are anti-U1 RNP IgG-selected proteins across the gradient from every two consecutive fractions (lane 1 contains fractions 1 and 2, lane 10 contains fractions 19 and 20). Lanes 11 and 12 contain proteins not selected by control IgG (lane 11) and anti-U1-RNP IgG (lane 12). Arrows in panel B indicate positions of 26,000- and 14,000-molecular-weight proteins.

snRNP in heat-shocked cells. The antigenic integrity of *Drosophila* U1 snRNP persisted during heat shock, implying that U1 snRNP remains unaltered (Fig. 5, lanes 4 and 5). This was confirmed by the effects of heat shock on the U1 snRNP proteins (Fig. 6). Cells were heat shocked at 36°C for 15 min and then labeled with [^{35}S]methionine for an additional 45 min at 36°C. The characteristic set of heat shock proteins appeared in the nuclear proteins not selected by normal IgG (Fig. 6, lane 3) or by the anti-U1 RNP antibody (Fig. 6, lane 4). The nonselected material represents essentially the total protein in these nuclear extracts. The major heat shock proteins in Fig. 6, lanes 3 and 4, are indicated by arrows, with their estimated molecular weights ($\times 10^{-3}$). (We note in passing that the results shown in Fig. 6, lanes 3 and 4, corroborate previous reports [16, 17] that the heat shock proteins have, at least in part, a nuclear localization.) After heat shock, the human anti-U1 RNP antibody selected the same two proteins as at 25°C (Fig. 6, lane 6). In addition, it was consistently observed that the amount of labeled 14,000-molecular-weight U1 snRNP protein was increased slightly after heat shock. Therefore, neither the synthesis of this protein nor its assembly into antibody-recognizable U1 snRNP is impaired after heat shock. The behavior of the 26,000-molecular-weight U1 snRNP protein after heat shock is more difficult to assess because all the major heat shock proteins, one of which is also 26,000 molecular weight, have a slight affinity for protein A-Sepharose and are therefore recovered in small amounts in both control IgG-selected and anti-U1 RNP-selected material (Fig. 6, lanes 7 and 8). However, the results indicate that the synthesis of the 26,000-molecular-weight U1 snRNP protein is probably not decreased after heat shock (compare Fig. 6, lanes 6, 7, and 8) and may be slightly increased.

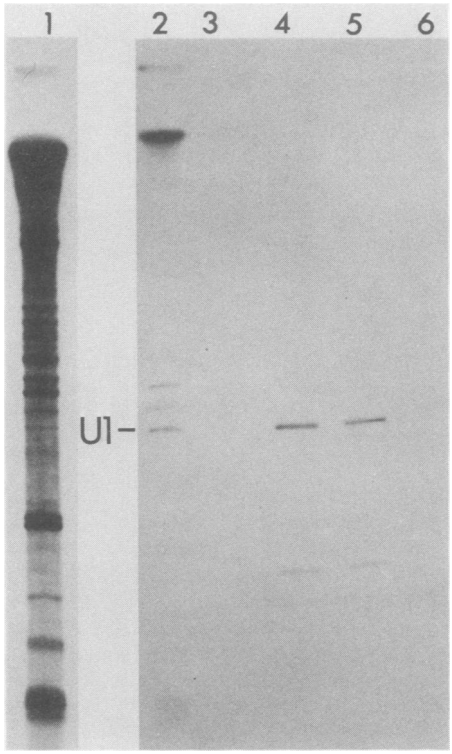

FIG. 5. [^{3}H]uridine-labeled RNA selected by anti-U1 RNP from control or heat-shocked *Drosophila* cells. Cells were labeled for 22 h with [^{3}H]uridine (100 μCi/ml), suspended in minimal medium containing [^{3}H]uridine (100 μCi/ml), and incubated for an additional hour at either 25 or 36°C. Nuclear extracts were then prepared and reacted with antibody as designated. Electrophoresis of RNA was in a 7 M urea–10% polyacrylamide gel. Lanes: 1, total *Drosophila* snRNAs; 2, total HeLa snRNAs; 3, blank; 4, 25°C, anti-U1 RNP IgG; 5, 36°C, anti-U1 RNP IgG; 6, 25°C, control (nonimmune) IgG.

DISCUSSION

We have used a human autoantibody specific for U1 RNA-containing RNP complexes to examine the structure of U1 snRNP in *Drosophila*. In keeping with the specificity of this antibody for RNP, as opposed to naked RNA, we find that it selectively recognizes a *Drosophila* nuclear RNP complex containing both U1 RNA and two methionine-containing proteins of approximately 26,000 and 14,000 molecular weight. This contrasts with the structure of human U1 snRNP, which by the same immunological criterion contains eight proteins (Fig. 6). (In our gel system, the protein bands termed B and C in a previous study [8] are doublets; hence we enumerate eight bands: A, B1, B2, C1, C2, D, E, and F.) The relative simplicity of U1 snRNP structure in *Drosophila* is somewhat surprising, given the evolutionary conservation of both the U1 RNA sequence (11) and the antigenicity of the RNPs. The trivial possibility that several *Drosophila* U1 snRNP proteins have been lost during isolation is unlikely for two reasons. First, we have found that both mouse and human U1 snRNP particles isolated in the *Drosophila* lysis buffer have the complete set of eight proteins. Second, it is clear that the labeling and RNP isolation procedures we have used do permit the recovery of snRNP complexes from *Drosophila* which contain additional proteins. At least four proteins were selected by the Sm antibody which were not recognized by the anti-U1 RNP antibody (Fig. 3). This finding emphasizes the distinct structure of U1 snRNP in *Drosophila* and contrasts sharply with the

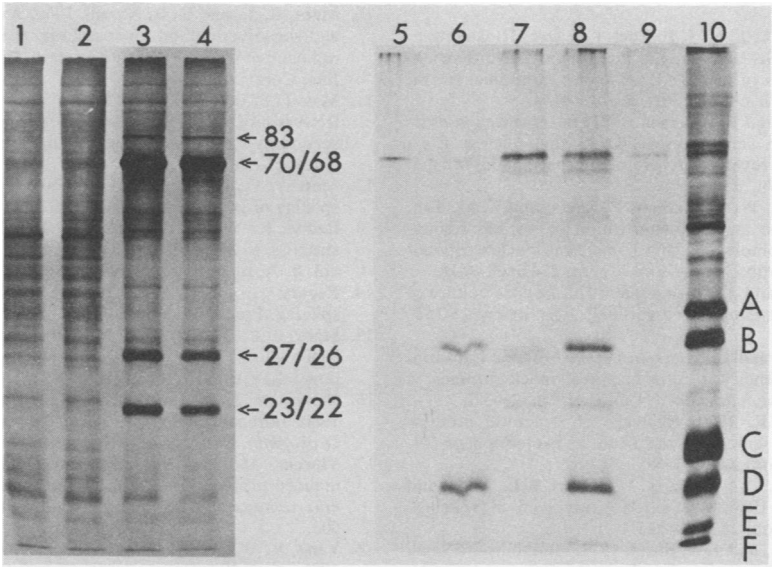

FIG. 6. *Drosophila* nuclear proteins selected by anti-U1 RNP antibody before and during heat shock. *Drosophila* cells were incubated in minimal medium for 15 min at the indicated temperature and then for an additional 45 minutes with [^{35}S]methionine (160 μCi/ml). Nuclear extracts were prepared and reacted with antibody as designated. Lanes 1 through 4, proteins not selected by antibody; 1, 25°C, control IgG; 2, 25°C, anti-U1 RNP IgG; 3, 36°C, control IgG; 4, 36°C, anti-U1 RNP IgG; 5 through 8, antibody-selected proteins from *Drosophila* nuclear extracts; 5, 25°C, control IgG; 6, 25°C, anti-U1 RNP IgG; 7, 36°C, control IgG; 8, 36°C, anti-U1 RNP IgG; 9 and 10, [^{35}S]methionine-labeled HeLa cell nuclear proteins selected by control IgG (lane 9) and anti-U1 RNP (lane 10). The HeLa extracts were prepared from nuclei isolated in hypotonic buffer (0.01 M NaCl, 1.5 mM $MgCl_2$, 0.01 M Tris-hydrochloride, pH 7.2) and then processed exactly as described in the text for *Drosophila* nuclear extracts.

situation in mammals where U1 snRNP contains the same set of proteins found in association with U1, U2, U4, U5, and U6 RNAs as a whole. We cannot exclude the possibility that *Drosophila* U1 snRNP contains additional proteins which lack methionine. Attempts to label *Drosophila* snRNP proteins with a mixture of tritiated amino acids have been unsuccessful. However, it is unlikely that each of several proteins in an otherwise conserved structure would have had amino acid replacements at all of their methionine residues during the evolutionary divergence of insects and mammals, and we therefore favor the interpretation that U1 snRNP in *Drosophila* consists of U1 RNA and the 26,000- and 14,000-molecular-weight proteins. The 9 to 18S sedimentation velocity of *Drosophila* U1 snRNP (Fig. 4) suggests the presence of more than one molecule each of the 26,000- and 14,000-molecular-weight proteins. These results indicate that the structural or functional role of *Drosophila* U1 snRNP (or both) can be fulfilled by only two proteins as compared with the eight found in mammalian systems, thereby implicating that these two proteins are of central importance.

The fact that the synthesis of *Drosophila* U1 snRNP proteins and their assembly into antigenic RNP are both sustained during heat shock suggests that U1 snRNP may be an essential aspect of nuclear structure for the survival of cells under this physiological stress. Perhaps this represents a preparation for the resumption of normal metabolism after heat shock. It is worth noting that these results neither support nor necessarily contradict a role of U1 snRNP in RNA splicing (8, 18), because most of the heat shock genes lack intervening sequences. The results presented here permit the 26,000- and 14,000-molecular-weight U1 snRNP proteins to be included among the relatively few *Drosophila* proteins whose translation is not markedly suppressed during heat shock.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grants GM21595 and GM28274 to T.P. from the National Institutes of Health and by Public Health Service postdoctoral fellowship CA06571 to E.D.W. from the National Institutes of Health.

We thank Patience White and Naomi Rothfield for providing serum, Steve Mount and Joan Steitz for *Drosophila* U1 DNA, Sam Wadsworth for Kc0 cells, and Steve Madore for expert technical assistance.

LITERATURE CITED

1. **Ashburner, M., and J. J. Bonner.** 1979. The induction of gene activity in *Drosophila* by heat shock. Cell 17:241–

254.
2. **Branlant, C., A. Krol, J.-P. Ebel, E. Lazar, H. Gallinaro, M. Jacob, J. Sri-Widada, and P. Jeanteur.** 1980. Nucleotide sequences of nuclear U1A RNAs from chicken, rat and man. Nucleic Acids Res. **8**:4143–4154.
3. **Calvet, J. P., and T. Pederson.** 1981. Base-pairing interactions between small nuclear RNAs and nuclear RNA precursors as revealed by psoralen cross-linking *in vivo*. Cell **26**:363–370.
4. **Douvas, A. S., W. E. Stumph, P. Reyes, and E. M. Tan.** 1979. Isolation and characterization of nuclear ribonucleoprotein complexes using human anti-nuclear ribonucleoprotein antibodies. J. Biol. Chem. **254**:3608–3616.
5. **Echalier, G., and A. Ohanessian.** 1970. *In vitro* culture of *Drosophila melanogaster* embryonic cells. In Vitro **6**:162–172.
6. **Findly, R. C., and T. Pederson.** 1981. Regulated transcription of the genes for actin and heat-shock proteins in cultured *Drosophila* cells. J. Cell Biol. **88**:323–328.
7. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
8. **Lerner, M. R., J. A. Boyle, S. M. Mount, S. L. Wolin, and J. A. Steitz.** 1980. Are snRNPs involved in splicing? Nature (London) **283**:220–224.
9. **Lerner, M. R., and J. A. Steitz.** 1979. Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. Proc. Natl. Acad. Sci. U.S.A. **76**:5495–5499.
10. **Mans, R. J., and G. D. Novelli.** 1960. A convenient, rapid and sensitive method for measuring the incorporation of radioactive amino acids into protein. Biochem. Biophys. Res. Comm. **3**:540–543.
11. **Mount, S. M., and J. A. Steitz.** 1981. Sequence of U1 RNA from *Drosophila melanogaster*: implications for U1 secondary structure and possible involvement in splicing. Nucleic Acids Res. **9**:6351–6368.
12. **Murray, V., and R. Holliday.** 1979. Mechanism for RNA splicing of gene transcripts. FEBS Lett. **106**:5–7.
13. **Reddy, R., and H. Busch.** 1981. U snRNAs of nuclear snRNPs, p. 261–306. *In* H. Busch (ed.), The cell nucleus, vol. 8. Academic Press, Inc., New York.
14. **Rogers, J., and R. Wall.** 1980. A mechanism for RNA splicing. Proc. Natl. Acad. Sci. U.S.A. **77**:1877–1879.
15. **Stark, B. C., R. Kole, E. J. Bowman, and S. Altman.** 1978. Ribonuclease P: an enzyme with an essential RNA component. Proc. Natl. Acad. Sci. U.S.A. **75**:3717–3721.
16. **Velazquez, J. M., B. J. DiDominico, and S. Lindquist.** 1980. Intracellular localization of heat shock proteins in *Drosophila*. Cell **20**:679–689.
17. **Vincent, M., and R. M. Tanguay.** 1979. Heat shock induced proteins present in the cell nucleus of *Chironomus tentans* salivary gland. Nature (London) **281**:501–503.
18. **Yang, V. W., M. R. Lerner, J. A. Steitz, and S. J. Flint.** 1981. A small nuclear ribonucleoprotein is required for splicing of adenoviral early RNA sequences. Proc. Natl. Acad. Sci. U.S.A. **78**:1371–1375.