JOURNAL OF VIROLOGY, Aug. 1989, p. 3368–3375
0022-538X/89/083368-08$02.00/0
Copyright © 1989, American Society for Microbiology
Vol. 63, No. 8

The Expression of Heat Shock Protein hsp27 and a Complexed 22-Kilodalton Protein Is Inversely Correlated with Oncogenicity of Adenovirus-Transformed Cells

ALT ZANTEMA,* ERIK DE JONG, RENÉ LARDENOIJE, AND ALEX J. VAN DER EB

Laboratory for Molecular Carcinogenesis, Sylvius Laboratoria, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands

Received 7 February 1989/Accepted 17 April 1989

We isolated a monoclonal antibody that immunoprecipitated two proteins of 22 and 27 kilodaltons (kDa) from nononcogenic adenovirus type 5 early region 1 (E1)-transformed rat cells but not from oncogenic adenovirus type 12 E1-transformed rat cells. In a variety of adenovirus-transformed cells including cells transformed by E1A and the c-H-*ras* oncogene, we found a perfect, inverse correlation between the presence of these two proteins and the oncogenicity of these cells in syngeneic immunocompetent rats. Characterization of the two proteins revealed that they occur in a large (700-kDa) complex and that the 27-kDa protein is identical to the already known 27-kDa (28-kDa) heat shock protein hsp27. The suppression of the hsp27 protein in oncogenic cells is further demonstrated by the fact that its mRNA is absent even after heat-shock induction.

Early region 1 (E1) of human adenovirus DNA can transform rodent cells in vitro to an oncogenic phenotype (for reviews see references 8 and 9). Region E1 consists of two transcriptional units, E1A and E1B, each of which specifies two proteins in transformed cells. Complete oncogenic transformation requires the cooperation of both E1A and E1B, but region E1A alone can partially transform cells (17). In immunodeficient nude mice, region E1A-transformed cells are not oncogenic, whereas all E1-transformed cells are (6). On the other hand, the oncogenicity of adenovirus-transformed cell lines in immunocompetent animals depends on the adenovirus serotype used. For example, adenovirus type 12 (Ad12) E1-transformed cells are oncogenic, whereas Ad5 E1-transformed cells are nononcogenic. Our group's previous experiments with hybrid E1 regions, consisting of the E1A region of Ad5 and the E1B region of Ad12 and vice versa, showed that the oncogenicity in syngeneic immunocompetent rats is determined by the origin of the E1A region. When E1A is derived from Ad12 the cells form tumors in syngeneic immunocompetent rats, with the same efficiency as in nude mice (7), whereas when E1A is from Ad5 the transformed cells do not form tumors in these rats, even when they are highly oncogenic in nude mice. An immunological search for differences in protein expression between oncogenic and nononcogenic adenovirus-transformed cells has revealed that the class I major histocompatibility complex (MHC) antigens are suppressed by the Ad12 E1A region (29). This down-regulation of class I MHC antigens might result in an escape of the transformed cells from cytotoxic T-cell lysis, explaining, at least in part, why the cells can grow out to form a tumor. However, other immunological differences between the oncogenic and nononcogenic cells may also play a role, such as differences in the susceptibility to NK cell lysis (27). Furthermore, baby rat kidney (BRK) cells transformed by a combination of the E1A region of either Ad5 or Ad12 and an activated *ras* oncogene are oncogenic, although in these cells the MHC class I antigens are not or only slightly suppressed (19).

* Corresponding author.

The present article reports on a further search for differences in protein expression between oncogenic and nononcogenic adenovirus-transformed cells. We describe a monoclonal antibody, 2C8, which immunoprecipitates a complex of a 22- and a 27-kilodalton (kDa) protein only from cells which are nononcogenic in syngeneic immunocompetent animals. It is shown that the 27-kDa protein is identical to the previously described heat shock protein hsp27, also termed hsp28 (15, 16, 31). This 27-kDa heat shock protein is one of the proteins whose expression has shown to be increased in response to various types of stress, such as heat or arsenite treatment. In general, heat shock proteins induce an increased thermotolerance, but little is known about their precise functions (28). The significance of our observation that the hsp27 gene is suppressed in oncogenic adenovirus-transformed rat cells, even after a heat shock, is still unclear.

MATERIALS AND METHODS

Cells and cell lysates. Cell lines, cell culturing, labeling of cell lines, and immunoprecipitations were performed as described previously (32). The E1A + *ras*-transformed BRK cell lines were described by Jochemsen et al. (19). The hybridoma producing monoclonal antibody 2C8 was isolated from the same fusion, as described previously (32). Heat-shocked labeled cell lysates were prepared by incubating the cells for 12 min at 43.5°C and subsequently labeling them for 2 h at 37°C.

2D gels and gel filtration. Two-dimensional (2D) gel electrophoresis was performed as described by O'Farrell (26). The resulting pH gradient in the first dimension was determined by slicing a control gel, incubating it with water, and measuring the resulting pH. LKB Ampholines from pH 5 to 7 yielded a gradient from pH 5 to 7. A mixture of LKB Ampholines of pH 3.5 to 10, pH 4 to 6, pH 5 to 8, and pH 9 to 11 in a ratio of 10:1:1:1 yielded a pH gradient from pH 4 to 8. Immunoprecipitates were suspended in 0.3% sodium dodecyl sulfate (SDS)–4% Nonidet P40–2% Ampholines–0.1 M dithiothreitol–9.9 M urea, centrifuged, and loaded on the first-dimension polyacrylamide gel.

Gel filtration was performed in E1A buffer (0.1% Nonidet

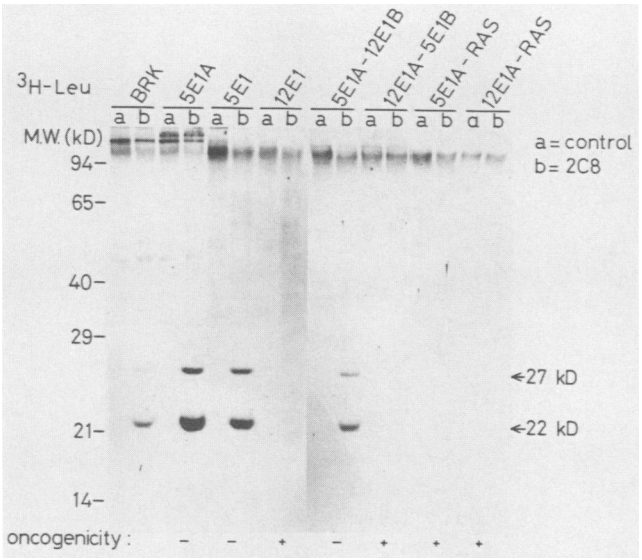

FIG. 1. SDS-polyacrylamide gel electrophoresis (PAGE) of immunoprecipitates of [^{3}H]leucine-labeled cell lysates with a control antibody (lanes a) and the monoclonal antibody 2C8 (lanes b) from primary BRK cells or BRK cells transformed with the indicated fragments. Below the lanes the ability (+/−) to form tumors in syngeneic immunocompetent rats is indicated.

P40, 50 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], pH 7.0, 250 mM NaCl) on a Pharmacia fast-protein liquid chromatography Superose-6 column. The lysate was also made in this buffer (14).

Northern blotting. The isolation of total cytoplasmic RNA and Northern (RNA) blotting were performed as described previously (29). RNA from heat-shocked cells was isolated after the cells were incubated for 3 h at 42°C. Filters were washed to a final stringency of 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 65°C. The fragments used for nick translation were the 420-base-pair *Pst*I fragment from pHS208 (16), carrying a human hsp27 cDNA; the 1.5-kilobase *Eco*RI-*Hin*dIII fragment from UMH213 (a pUC12 derivative of MHS213) (24), carrying a mouse hsp70 cDNA; and the 1.3-kilobase *Pst*I fragment from pRGAPDH-13 (13), carrying a rat glyceraldehyde-3-phosphate-dehydrogenase cDNA.

RESULTS

Monoclonal antibody 2C8. Monoclonal antibody 2C8 was isolated from a previously described fusion of Sp2/0 cells

TABLE 1. Relationship of 22- and 27-kDa proteins with oncogenicity and MHC class I antigen expression[a]

BRK cell lines transformed by:	No. of clones tested	22/27-kDa proteins	Oncogenicity		MHC class I antigens
			Nude mice	Syngeneic rats	
Ad5-E1A + Ad5-E1B	4	+	+	−	+
Ad12-E1A + Ad12-E1B	3	−	+	+	−
Ad5-E1A + Ad12-E1B	2	+	+	−	+
Ad12-E1A + Ad5-E1B	2	−	+	+	−
Ad5-E1A + Ad12-E1A + Ad12-E1B	1	+	+	−	+
Ad5-E1A + EJ-*ras*	2	−	+	+	+
Ad12-E1A + EJ-*ras*	2	−	+	+	+

[a] Presence of the 22- and 27-kDa proteins, immunoprecipitated with the monoclonal antibody 2C8, as related to oncogenicity (6, 7, 19) and expression of the MHC class I antigens (29).

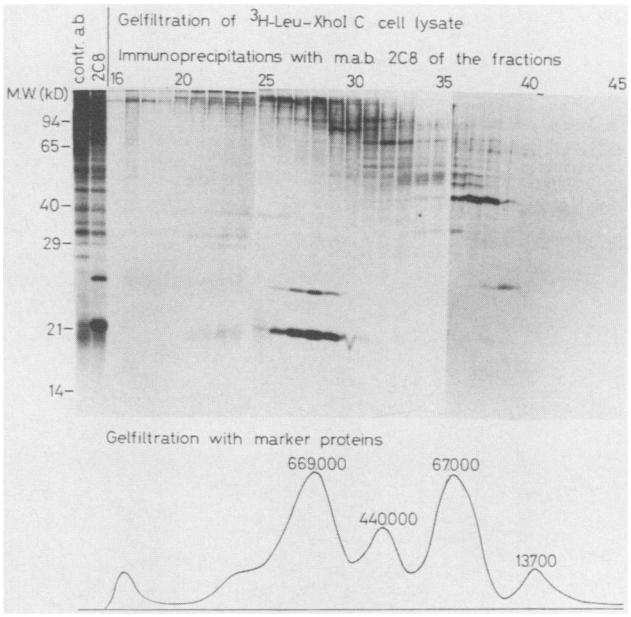

FIG. 2. Gel filtration studies on a fast-protein liquid chromatography Superose-6 column. Top, SDS-PAGE of immunoprecipitates with antibody 2C8 of fractions 16 to 45 from a [^{3}H]leucine-labeled Ad5 E1-transformed cell lysate. The first two lanes are immunoprecipitations from the total [^{3}H]leucine-labeled Ad5 E1 cell lysate with a control antibody and monoclonal antibody 2C8. Bottom, Absorbance profile of the separation of thyroglobulin, ferritin, bovine serum albumin, and RNase A. Molecular weights are indicated above the peaks. The separation conditions are the same as for the top panel, and the fraction position corresponds to the immunoprecipitation lanes above.

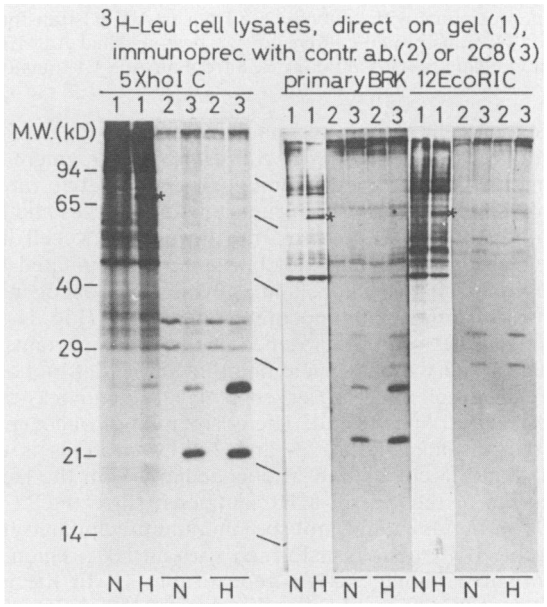

FIG. 3. Heat shock experiments with Ad5 E1-transformed (5XhoI C), primary, and Ad12 E1-transformed (12EcoRIC) BRK cells. [^{3}H]leucine-labeled lysates of cells grown at 37°C (N) or cells heat shocked for 12 min at 43.5°C and labeled at 37°C immediately afterwards (H) were separated by SDS-PAGE directly (200,000 cpm, lanes 1) or after an immunoprecipitation with a control antibody (lanes 2) or with antibody 2C8 (lanes 3). Lanes 1 of primary BRK and 12EcoRIC are from a shorter exposure to demonstrate the appearance of the hsp70 (*).

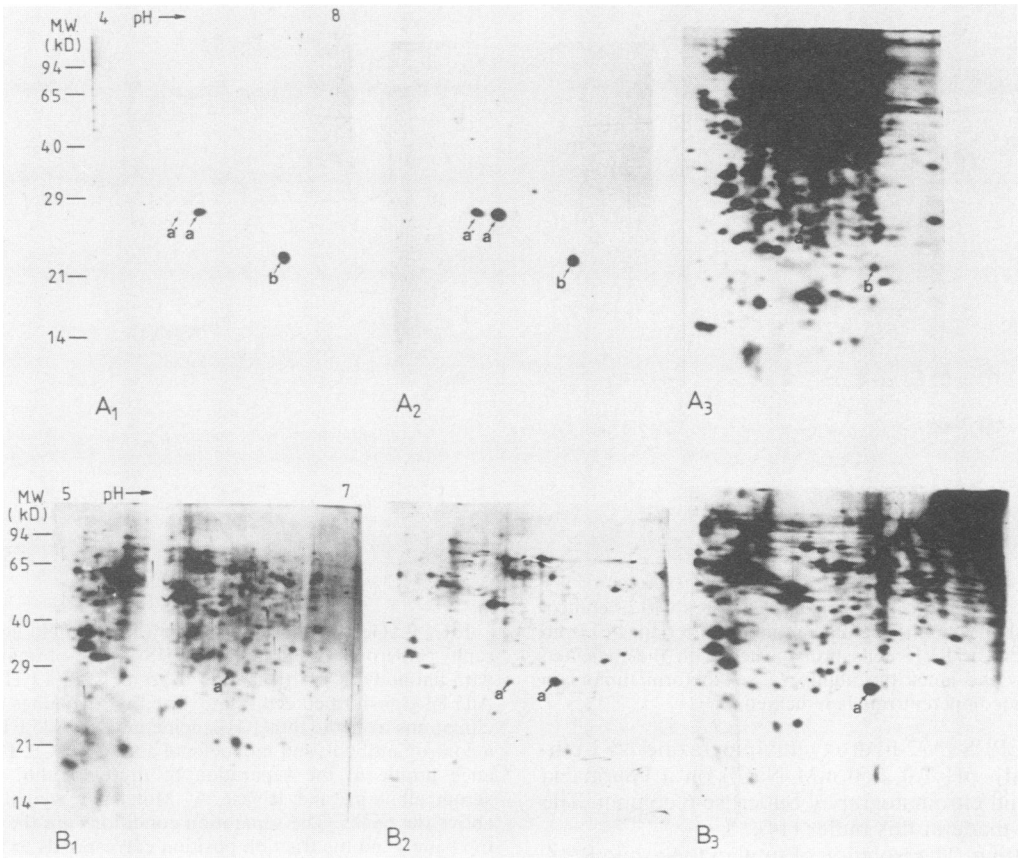

FIG. 4. 2D gels of [^{3}H]leucine-labeled cell lysate or immunoprecipitates. The first dimension has a pH gradient from 4 to 8 (panels A) or 5 to 7 (panels B); the second dimension is SDS-PAGE. a, The 27-kDa protein; a', its phosphorylated form; b, the 22-kDa protein. A_1, Immunoprecipitate with antibody 2C8 from an Ad5 E1-transformed cell lysate; A_2, as for A_1 except that the cells were heat shocked; A_3 and B_1, Ad5 E1-transformed cell lysate; B_2, heat-shocked Ad5 E1-transformed cell lysate; B_3, mixture of Ad5 E1-transformed cell lysate and immunoprecipitate with antibody 2C8 from an Ad5 E1-transformed cell lysate.

with spleen cells from a rat immunized with a syngeneic rat cell line transformed with the Ad5 *Hin*dIII G fragment (32). This monoclonal antibody immunoprecipitates two rat proteins of 22 and 27 kDa from primary BRK cultures and from the nononcogenic adenovirus-transformed BRK cell lines. These two proteins could not be immunoprecipitated from adenovirus-transformed cell lines which are oncogenic in newborn syngeneic immunocompetent animals (Fig. 1). This relationship between the absence of these two proteins and the oncogenicity in syngeneic immunocompetent rats of the transformed cells was observed with all adenovirus-transformed rat cell lines tested. The correlation of oncogenicity with the absence of the 22- and 27-kDa proteins is even better than the correlation of oncogenicity with the lack of expression of the class I MHC antigens, since the 22- and 27-kDa proteins could not be immunoprecipitated from oncogenic BRK cells transformed with an E1A region plus the activated human EJ-*ras* gene (Table 1). In the latter category of transformed cells, the class I MHC antigens are not or are only slightly suppressed (19). The strict correlation with oncogenicity was also indicated by the expression of these proteins in BRK cells transformed with Ad5 E1A plus an EJ-*ras* oncogene under the control of the dexamethasone-inducible mouse mammary tumor virus promoter (19). In the absence of dexamethasone, the *ras* oncogene is not expressed, which correlates with the presence of the 22/27-kDa proteins. Under these conditions, the cells exhibit a nontransformed phenotype, and although the oncogenicity could not be tested (due to presence in the experimental animals of glucocorticoids which would activate the mouse mammary tumor virus promoter), it seems likely that the cells would be basically nononcogenic. When dexamethasone was added the *ras* protein was expressed and the expression of the 22/27-kDa proteins was discontinued (results not shown).

To investigate whether the 22/27-kDa proteins would also be suppressed in cells transformed by a mutant Ad12 E1 region, a BRK cell line transformed by the Ad12 *Hin*dIII G fragment was analyzed with monoclonal antibody 2C8 and was found to express the 22/27-kDa proteins. The Ad12 *Hin*dIII G fragment comprises the entire E1A region and approximately 50% of the E1B region, allowing expression of the 19-kDa E1B protein and an N-terminal fragment of the 55-kDa E1B protein. Cells transformed by this Ad12 DNA fragment had been found previously to be nononcogenic in athymic nude mice (20). We confirmed this observation by injecting an Ad12 *Hin*dIII G fragment-transformed BRK cell line into six nude mice; after 4 months, no tumors had yet appeared in any of the six injected animals. However, when immunocompetent syngeneic rats were injected with this transformed cell line, tumors were formed in two of eight animals after a latency period of 4 to 5 months. When a cell line isolated from one of the tumors was analyzed with the monoclonal antibody 2C8, it was found that the 22/27-kDa

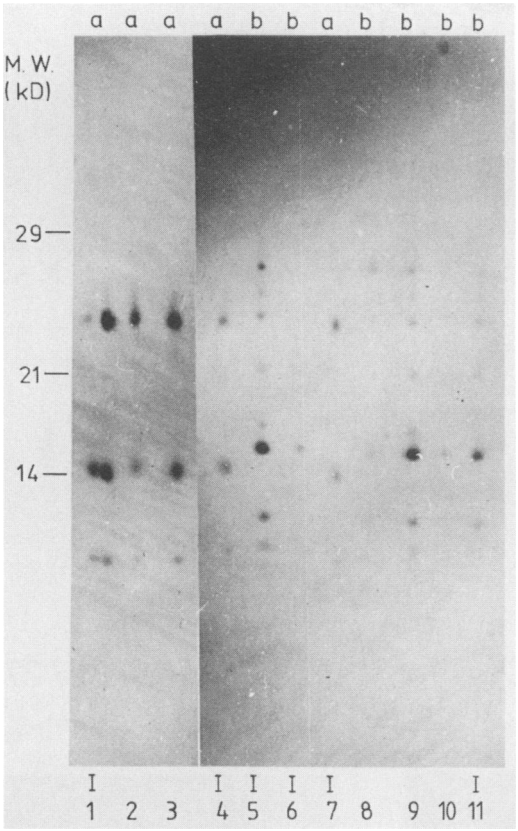

FIG. 5. One-dimensional peptide maps with 100 ng of V8 protease on the 22-kDa, 27-kDa, or hsp27 protein cut from 2D gels, except for lane 1 which is from a one-dimensional gel. Cleveland digestions; a, 22 kDa; b, 27 kDa. Proteins cut from a 2D gel are either from a total cell lysate or from an immunoprecipitate with monoclonal antibody 2C8 (I on figure). Lane 1, 22-kDa protein, immunoprecipitate; lane 2, 22-kDa protein (b in Fig. $6B_1$); lane 3, 22-kDa protein; lane 4, 22-kDa protein, immunoprecipitate (b in Fig. $4A_1$); lane 5, 27-kDa protein, immunoprecipitate (a in Fig. $4A_2$); lane 6, phosphorylated 27-kDa protein, immunoprecipitate (a′ in Fig. $4A_2$); lane 7, 22-kDa protein, immunoprecipitate (b in Fig. $4A_2$); lane 8, hsp27 (a in Fig. $4B_1$); lane 9, hsp27 (a in Fig. $4B_2$); lane 10, phosphorylated hsp27 (a′ in Fig. $4B_2$); lane 11, 27-kDa protein, immunoprecipitate.

proteins were no longer expressed (results not shown). This result shows that adenovirus-transformed cells selected in vivo for an acquired tumorigenic potential, possibly as a result of additional mutations, also lose the expression of the 22/27-kDa proteins.

Characterization of the 22- and 27-kDa proteins. In our initial studies with monoclonal antibody 2C8 and [^{35}S]methionine-labeled cell lysates, we detected only the 22-kDa protein. When $^{32}P_i$ labeling was used, we found very little phosphorylation of the 22-kDa protein but a strongly phosphorylated 27-kDa band (results not shown). Both proteins were detected when [^{3}H]leucine-labeled cell lysates were used (Fig. 1). Apparently, the mature 27-kDa protein does not contain methionine and is strongly phosphorylated. The immunoprecipitation of two proteins with this antibody can be explained either by an antigenic relationship between the two proteins or by the circumstance that the 22- and 27-kDa proteins form a complex. As this monoclonal antibody does not detect any protein in Western blotting (immunoblotting), it is hard to exclude an antigenic relationship. However, results from one-dimensional peptide mapping (see also Fig. 5) and the fact that the 27-kDa protein does not contain methionine, whereas the 22-kDa protein does, all suggest that the two proteins are not related. To investigate whether the proteins form a complex, a gel filtration study was performed. Immunoprecipitations with monoclonal antibody 2C8 showed the presence of both the 22- and 27-kDa antigens in the fractions where proteins with an apparent size of 700 kDa were expected (Fig. 2). Immunoprecipitations with another antibody showed the same background proteins (results not shown); therefore, it seems likely that the complex consists exclusively of 22- and 27-kDa proteins. Furthermore, the immunoprecipitations of gel fractions did not show any monomeric 22- or 27-kDa proteins. Although it cannot be excluded that there were no free 22- or 27-kDa protein molecules in the cells, a more plausible explanation is that monoclonal antibody 2C8 can immunoprecipitate only the complex between these two proteins. This would be consistent with the observation that we could not detect the proteins with the monoclonal antibody 2C8 in Western blotting and that we failed to immunoprecipitate the proteins from in vitro-translated RNA (results not shown).

Identification of the 27-kDa protein as hsp27. The 27-kDa protein does not contain methionine, is strongly phosphorylated, and is present in a large complex. These characteristics have a striking similarity with the properties of a previously described rat protein, hsp27 (22, 31). This prompted us to investigate whether the 27-kDa protein might be encoded by the heat shock gene. Figure 3 shows the results of heat shock experiments for primary BRK cells and Ad5- and Ad12-transformed cells. It can be seen that an increased 27-kDa protein band was immunoprecipitated with antibody 2C8 when cells that expressed the 22- and 27-kDa proteins were labeled after a heat shock. In the oncogenic Ad12-transformed cells, however, these proteins were still absent even after a heat shock. That the heat shock conditions worked in all types of cells was indicated by the increase in the hsp70 proteins observed in a one-dimensional separation of the total labeled cell lysate (asterisks in Fig. 3). Although these data strongly suggest that the 27-kDa protein is indeed hsp27, it should be realized that this antibody seems to recognize only the complex between the 22- and 27-kDa proteins. Therefore, it is also possible that only the amount of the complex is changed upon heat shock, whereas the absolute quantity of the 27-kDa protein is unaltered. As the hsp27 protein is defined by its heat shock characteristics on a 2D gel, we performed similar analyses to establish the identity of the 27-kDa protein.

In Fig. 4A a 2D gel analysis (pH gradient 4 to 8) of the immunoprecipitates with the monoclonal antibody 2C8 of a normal (Fig. $4A_1$) and a heat-shocked (Fig. $4A_2$) Ad5-transformed cell lysate is shown, together with the positions of these proteins in the 2D gel of the total non-heat-shocked cell lysate (Fig. $4A_3$). The positions of the 27-kDa protein (a and a′) and the 22-kDa protein (b) on the 2D gel of the total cell lysate (Fig. $4A_3$) were determined by mixing experiments. To establish whether the 27-kDa protein is identical to hsp27, we used a pH gradient from 5 to 7 to obtain a better resolution for the individual proteins. With this gradient only the 27-kDa protein was detected. Mixing of the 27-kDa protein obtained from an immunoprecipitation with antibody 2C8 from Ad5-transformed cell lysate with the total Ad5-transformed cell lysate (Fig. $4B_1$) localized the 27-kDa protein exactly at the hsp27 position (Fig. $4B_3$), as defined by the increase in intensity found upon heat shock (compare Fig. $4B_2$ and $4B_1$). Also after heat shock, a more acidic hsp27

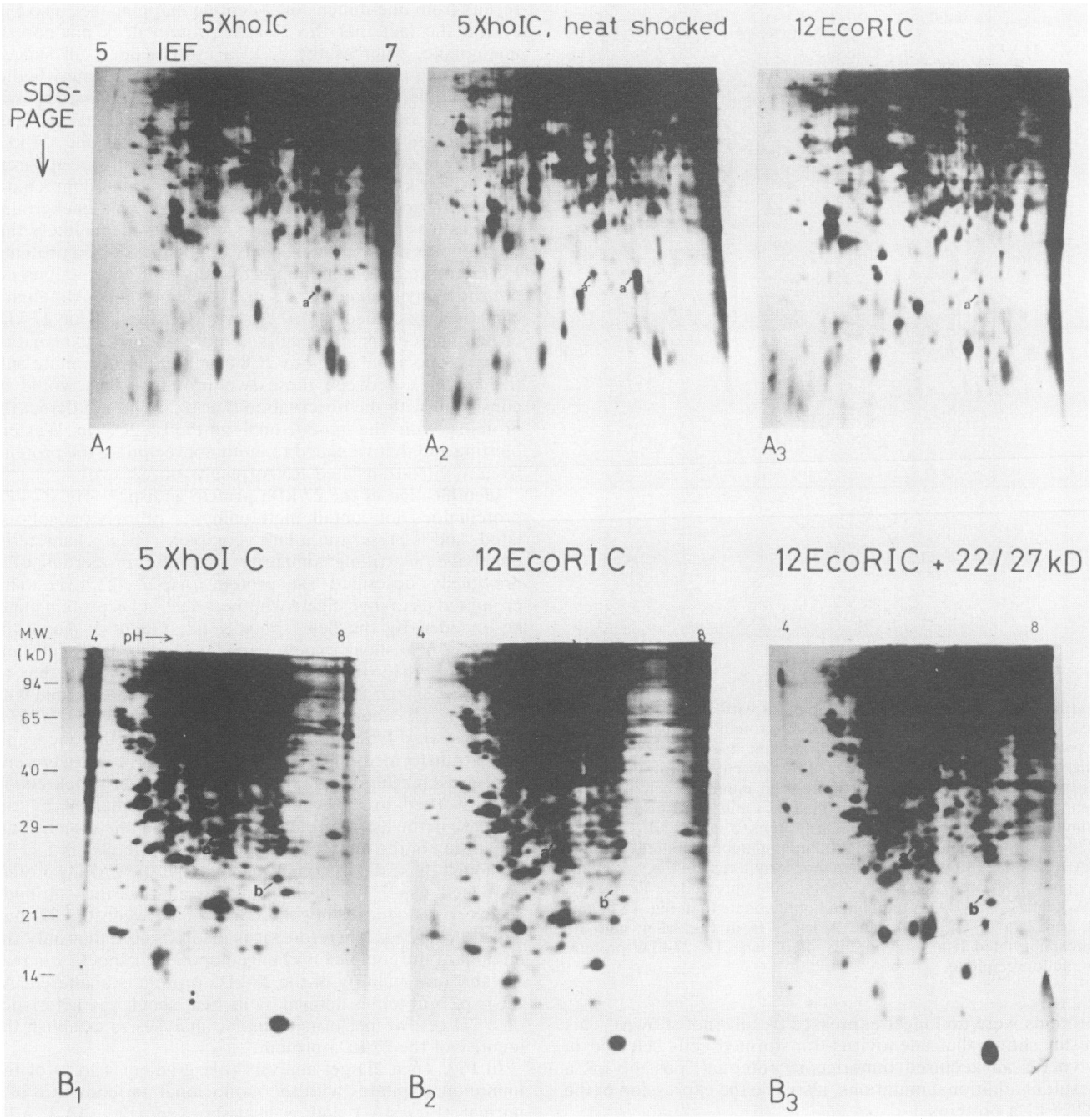

FIG. 6. 2D gels of [^{3}H]leucine-labeled cell lysate which show that the oncogenic Ad12 E1-transformed BRK cells do not express the 27-kDa protein (a and a′) (panels A) or the 22-kDa protein (b) (panels B). The pH gradient in the first dimension is either from 5 to 7 (panels A) or from 4 to 8 (panels B). A_1, Ad5 E1-transformed (5XhoIC) cell lysate; A_2, heat-shocked Ad5 E1-transformed (5XhoIC) cell lysate; A_3, Ad12 E1-transformed (12EcoRIC) cell lysate. a, Position where the 27-kDa protein is expected. B_1, Ad5 E1-transformed (5XhoIC) cell lysate; B_2, Ad12 E1-transformed (12EcoRIC) cell lysate. b, Position where the 22-kDa protein is expected. B_3, Ad12 E1-transformed (12EcoRIC) cell lysate mixed with a small amount of 2C8 immunoprecipitate from Ad5 E1-transformed cell lysate.

protein appeared (a′) which likewise was immunoprecipitated by the antibody 2C8. The differently charged proteins are caused by additional phosphorylation (22, 31). To confirm the localization of the 27- and 22-kDa proteins on the 2D gels, the proteins were cut out from 2D gels of total cell lysate or of immunoprecipitates and were subjected to a partial digestion with V8 protease, followed by separation on a one-dimensional gel (10). The result (Fig. 5) shows that we correctly identified the 22- and 27-kDa proteins on the 2D gel. Therefore, the 27-kDa protein immunoprecipitated by 2C8 indeed must be identical to hsp27. In addition, it can be deduced from the degradation patterns that there is no obvious relationship between the 22- and 27-kDa proteins.

The 22- and 27-kDa proteins are not expressed in the

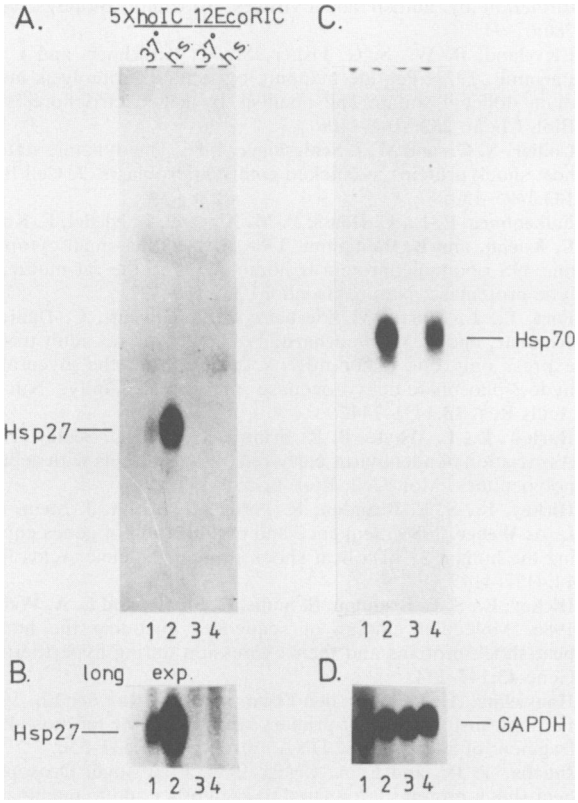

FIG. 7. Northern blot with RNA from control and heat-shocked Ad5 E1- and Ad12 E1-transformed BRK cells. (A) Hybridization with human hsp27 cDNA pHS208. (B) Long exposure of relevant part of panel A. (C) Hybridization with mouse hsp70 cDNA UMH213. (D) Relevant part of the control hybridization with a glyceraldehyde-3-phosphate-dehydrogenase clone. RNA from Ad5 E1-transformed cells grown either at 37°C (lanes 1) or at 42°C (lanes 2) for 3 h and from Ad12 E1-transformed cells grown at 37°C (lanes 3) or at 42°C (lanes 4) for 3 h.

oncogenic adenovirus-transformed cells. Analysis with 2D gels also provides the opportunity to study whether these proteins are indeed not synthesized in the oncogenic adenovirus-transformed cells, as suggested by the immunoprecipitation experiments with monoclonal antibody 2C8. No 27-kDa protein (a) could be found in the Ad12 E1-transformed cell lysate (Fig. $6A_3$) at the position of hsp27 in the Ad5 E1-transformed cell lysate (Fig. $6A_1$ and $6A_2$). Likewise, the 22-kDa protein was not found at the position where it occurs in the nononcogenic cell lysates (Fig. $6B_1$ and $6B_2$). After the Ad12-transformed cell lysate was mixed with a small amount of the 2C8 immunoprecipitate from an Ad5-transformed cell lysate, the 22-kDa protein (b) was observed at the correct position (Fig. $6B_3$).

Oncogenic adenovirus-transformed cells do not have hsp27 mRNA. The human hsp27 cDNA and its gene have recently been cloned by Hickey et al. (15, 16), and we have used a partial cDNA probe from their clone pHS208 to detect the rat hsp27 RNA. Figure 7A and a longer exposure of the relevant part of Fig. 7A (Fig. 7B) show that in the Ad5 E1-transformed cells hsp27 mRNA could be detected (lanes 1) and that the expression was indeed strongly increased upon heat shock (lanes 2). In agreement with the protein results, the hsp27 RNA was not detected in oncogenic Ad12 E1-transformed BRK cells, even after heat shock (lanes 3 and 4). In both cases the heat shock conditions worked well, as indicated by hybridization of the same filter (Fig. 7C) with a mouse cDNA clone for hsp70 (24).

DISCUSSION

BRK cell lines transformed as primary cell cultures by the E1 region of Ad5 or Ad12 or the corresponding hybrid E1 regions are tumorigenic in syngeneic immunocompetent rats when the E1A region is derived from Ad12, whereas they do not form tumors when the E1A region is from Ad5 (alone or together with Ad12 E1A) (7). Previous studies showed that the Ad12 E1A region inhibits expression of the class I MHC antigens (29), which can explain, at least in part, why transformed cells expressing the Ad12 E1A region are oncogenic in immunocompetent animals. On the other hand, experiments with BRK cells transformed with an E1A region together with an activated *ras* oncogene have shown that such cells are oncogenic, even when the E1A region is derived from Ad5 (19), indicating that other factors also play a role. The Ad12 E1A + *ras*-transformed cells still express a fair amount of class I MHC antigens, and the Ad5 E1A + *ras*-transformed cells express normal levels of class I MHC antigens, indicating that in these cases tumorigenicity cannot be explained by reduced expression of class I antigens. In the present study we describe results obtained with monoclonal antibody 2C8, which recognizes a complex between a 22- and a 27-kDa protein. This complex is immunoprecipitated only from the nononcogenic adenovirus-transformed BRK cells as well as from untransformed primary BRK cultures. The various cell lines, their oncogenicity, and the immunoprecipitation results for the 22/27-kDa proteins, as well as the expression of the class I MHC antigens, are summarized in Table 1. As can be seen, the correlation between the presence of immunoprecipitable 22/27-kDa proteins and the lack of oncogenicity holds for all adenovirus-transformed BRK cell lines.

We have also shown that the 27-kDa protein present in the complex immunoprecipitated by monoclonal antibody 2C8 is identical to the previously described hsp27, also called hsp28 (15, 31). Heat shock proteins are preferentially expressed during stress conditions like heat or arsenite addition (28) and can be divided into three classes: the small heat shock proteins (20 to 30 kDa), the hsp70 proteins, and the hsp90 proteins. A relationship with adenovirus transformation has only been reported for the hsp70 gene (25). In viral infection E1A causes a transcriptional activation of this hsp70 and also of an hsp89 (21, 30). The small heat shock proteins occur as multiple polypeptides, for example, in *Drosophila melanogaster*, where four proteins of 22, 23, 26, and 27 kDa have been found (18), whereas in rat cells only one protein of about 27 kD has been described, albeit with multiple phosphorylation states (22, 31). This mature rat protein does not contain methionine, while the human homolog contains one such residue (15). The small heat shock proteins are probably located in the cytoplasm, where they associate with the intermediate filament cytoskeleton (11, 23). Although nuclear localizations have also been reported (2, 22), the presence in the nucleus can possibly be explained by a collapse of the intermediate filament skeleton on the nuclear surface during heat shock (11, 23). The function of the small heat shock proteins is unknown. One possibility is that they play a role in the recovery of collapsed intermediate filaments, since it has been observed in chicken cells that the synthesis of the heat shock proteins is required for this recovery (11).

Small heat shock proteins have also been mentioned in relation to a 19S small cytoplasmic ribonucleoprotein parti-

cle called a prosome (3). One of the suggested functions of this prosome is the regulation of mRNA translation (1), but recently it has been proposed that the prosome is identical to the large cellular multifunctional protease complex (4, 12) which contains less RNA than the about 20% published previously (3), if any. Whether the small heat shock proteins are localized in prosomelike particles is still controversial (3, 5). From the results of Fig. 2 it is clear that the hsp27 protein is part of a 700-kDa complex. Whether this complex contains other components next to the hsp27 and 22-kDa proteins has to be studied. The identity of the 22-kDa protein is not yet known. Some experiments have shown that this protein is synthesized only in small amounts during the heat shock itself, but is expressed afterwards to form a complex with the hsp27 protein (our unpublished observations). Therefore the 22-kDa protein might play a role during the recovery period after a heat shock.

As long as the functions of these two proteins in the cell are not understood, the possible significance of their absence in oncogenic cells is difficult to assess. Experiments involving the introduction of the genes under transcriptional control of a heterologous promoter into oncogenic cells and studies of the oncogenic behavior of these cells may possibly answer this question.

ACKNOWLEDGMENTS

We thank A. Davis-Olivier, C. Tops, and T. van Laar for their contribution to the initial experiments with this antibody; E. Hickey and L. A. Weber for plasmid pHS208; and H. Pelham for plasmid UMH213. We also thank our colleagues in the laboratory for critically reading the manuscript.

This work was supported by grant IKW84-38 from the Dutch Cancer Society.

LITERATURE CITED

1. **Akhayat, O., A. A. Infante, D. Infante, C. Martins de As, M.-F. Grossi de Sa, and K. Scherrer.** 1987. A new type of prosome-like particle, composed of small cytoplasmic RNA and multimers of a 21-kDa protein, inhibits protein synthesis in vitro. Eur. J. Biochem. **170**:23–33.
2. **Arrigo, A.-P., and C. Ahmad-Zadeh.** 1981. Immunofluorescence localization of a small heat shock protein (hsp 23) in salivary gland cells of Drosophila melanogaster. Mol. Gen. Genet. **184**:73–79.
3. **Arrigo, A.-P., J.-L. Darlix, E. W. Khandjian, M. Simon, and P.-F. Spahr.** 1985. Characterization of the prosome from Drosophila and its similarity to the cytoplasmic structures formed by the low molecular weight heat-shock proteins. EMBO J. **4**: 399–406.
4. **Arrigo, A.-P., K. Tanake, A. L. Goldberg, and W. J. Welch.** 1988. Identity of the 19S 'prosome' particle with the large multifunctional protease complex of mammalian cells (the proteasome). Nature (London) **331**:192–194.
5. **Arrigo, A.-P., and W. J. Welch.** 1987. Characterization and purification of the small 28,000-dalton mammalian heat shock protein. J. Biol. Chem. **32**:15359–15369.
6. **Bernards, R., P. I. Schrier, J. L. Bos, and A. J. van der Eb.** 1983. Role of adenovirus types 5 and 12 early region 1b tumor antigens in oncogenic transformation. Virology **127**:45–53.
7. **Bernards, R., P. I. Schrier, A. Houweling, J. L. Bos, A. J. van der Eb, M. Zijlstra, and C. J. M. Melief.** 1983. Tumorigenicity of cells transformed by adenovirus type 12 by evasion of T-cell immunity. Nature (London) **305**:776–779.
8. **Bernards, R., and A. J. van der Eb.** 1984. Adenovirus: transformation and oncogenicity. Biochim. Biophys. Acta **783**:187–204.
9. **Branton, P. E., S. T. Bayley, and F. L. Graham.** 1985. Transformation by human adenoviruses. Biochim. Biophys. Acta **780**:67–94.
10. **Cleveland, D. W., S. G. Fisher, M. W. Kirschner, and U. K. Laemmli.** 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. **252**:1102–1106.
11. **Collier, N. C., and M. J. Schlesinger.** 1986. The dynamic state of heat shock proteins in chicken embryo fibroblasts. J. Cell Biol. **103**:1495–1507.
12. **Falkenburg, P.-E., C. Haass, P.-M. Kloetzel, B. Niedel, F. Kopp, L. Kuehn, and B. Dahlmann.** 1988. Drosophila small cytoplasmic 19S ribonucleoprotein is homologous to the rat multicatalytic proteinase. Nature (London) **331**:190–192.
13. **Fort, P., L. Marty, M. Piechaczyk, S. Sabrouti, C. Dani, P. Jeanteur, and J. M. Blanchard.** 1985. Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. Nucleic Acids Res. **13**:1431–1442.
14. **Harlow, E., P. Whyte, B. R. Franza, Jr., and C. Schley.** 1986. Association of adenovirus early-region 1A proteins with cellular polypeptides. Mol. Cell. Biol. **6**:1579–1589.
15. **Hickey, E., S. E. Brandon, R. Potter, G. Strein, J. Stein, and L. A. Weber.** 1986. Sequence and organization of genes encoding the human 27 kDa heat shock protein. Nucleic Acids Res. **14**:4127–4145.
16. **Hickey, E., S. E. Brandon, S. Sadis, G. Smale, and L. A. Weber.** 1986. Molecular cloning of sequences encoding the human heat-shock proteins and their expression during hyperthermia. Gene **43**:147–154.
17. **Houweling, A., P. J. van den Elsen, and A. J. van der Eb.** 1980. Partial transformation of primary rat cells by the leftmost 4.5% fragment of adenovirus 5 DNA. Virology **105**:537–550.
18. **Ingolia, T. D., and E. A. Craig.** 1982. Four small Drosophila heat shock proteins are related to each other and to mammalian α-crystallin. Proc. Natl. Acad. Sci. USA **79**:2360–2364.
19. **Jochemsen, A. G., R. Bernards, H. J. van Kranen, A. Houweling, J. L. Bos, and A. J. van der Eb.** 1986. Different activities of the adenovirus types 5 and 12 E1A regions in transformation with the EJ Ha-*ras* oncogene. J. Virol. **59**:684–691.
20. **Jochemsen, H., G. S. G. Daniels, J. J. L. Hertoghs, P. I. Schrier, P. J. van den Elsen, and A. J. van der Eb.** 1982. Identification of adenovirus-type 12 gene products involved in transformation and oncogenesis. Virology **122**:15–28.
21. **Kao, H.-T., and J. R. Nevins.** 1983. Transcriptional activation and subsequent control of the human heat shock gene during adenovirus infection. Mol. Cell. Biol. **3**:2058–2065.
22. **Kim, Y.-J., J. Shman, M. Sette, and A. Przybyla.** 1984. Nuclear localization and phosphorylation of three 25-kilodalton rat stress proteins. Mol. Cell. Biol. **4**:468–474.
23. **Leicht, B. G., H. Biessmann, K. B. Palter, and J. J. Bonner.** 1986. Small heat shock proteins of Drosophila associate with the cytoskeleton. Proc. Natl. Acad. Sci. USA **83**:90–94.
24. **Lowe, D. G., and L. A. Moran.** 1986. Molecular cloning and analysis of DNA complementary to three mouse M_r=68,000 heat shock protein mRNAs. J. Biol. Chem. **261**:2102–2112.
25. **Nevins, J. R.** 1982. Induction of the synthesis of a 70,000 dalton mammalian heat shock protein by the adenovirus E1A gene product. Cell **29**:913–919.
26. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
27. **Sawada, Y., B. Föhring, T. E. Shenk, and K. Raska, Jr.** 1985. Tumorigenicity of adenovirus-transformed cells: region E1A of adenovirus 12 confers resistance to natural killer cells. Virology **147**:413–421.
28. **Schlesinger, M. J.** 1986. Heat shock proteins: the search for functions. J. Cell Biol. **103**:321–325.
29. **Schrier, P. I., R. Bernards, R. T. M. J. Vaessen, A. Houweling, and A. J. van der Eb.** 1983. Expression of class I major histocompatibility antigens switched off by highly oncogenic adenovirus 12 in transformed rat cells. Nature (London) **305**: 771–775.
30. **Simon, M. C., K. Kitchener, H.-T. Kao, E. Hickey, L. Weber, R. Voellmy, N. Heintz, and J. R. Nevins.** 1987. Selective induction

of human heat shock gene transcription by the adenovirus E1A gene products, including the 12S E1A product. Mol. Cell. Biol. **7**:2884–2890.

31. **Welch, W. J.** 1985. Phorbol ester, calcium ionophore, or serum added to quiescent rat embryo fibroblast cells all result in the elevated phosphorylation of two 28,000-dalton mammalian stress proteins. J. Biol. Chem. **260**:3058–3062.
32. **Zantema, A., J. A. M. Fransen, A. Davis-Olivier, F. C. S. Ramaekers, G. P. Vooijs, B. DeLeys, and A. J. van der Eb.** 1985. Localization of the E1B proteins of adenovirus 5 in transformed cells, as revealed by interaction with monoclonal antibodies. Virology **142**:44–58.