Rapid Capping in α-Spectrin-deficient MEL Cells from Mice Afflicted with Hereditary Hemolytic Anemia

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Abstract. A spectrin-based membrane skeleton is important for the stability and organization of the erythrocyte. To study the role of spectrin in cells that possess complex cytoskeletons, we have generated α-spectrin-deficient erythroleukemia cell lines from *sph/sph* mice. These cells contain β-spectrin, but lack α-spectrin as determined by immunoblot and Northern blot analyses. The effects of α-spectrin deficiency are apparent in the cells' irregular shape and fragility in culture. Capping of membrane glycoproteins by fluorescent lectin or antibodies occurs more rapidly in *sph/sph* than in wild-type erythroleukemia cells, and the caps appear more concentrated. The data support the idea that spectrin plays an important role in organizing membrane structure and limiting the lateral mobility of integral membrane glycoproteins in cells other than mature erythrocytes.

THE erythrocyte membrane skeleton is a network of proteins that defines, in part, the structure and organization of the red cell plasma membrane (for reviews see Marchesi, 1985; Elgsaeter et al., 1986; Goodman et al., 1988; Bennett, 1990). Genetic defects in the structural proteins of the membrane skeleton often manifest themselves as hereditary hemolytic anemias. In both humans and mice, the erythrocytes from affected individuals are irregularly shaped and have unstable or aberrant plasma membranes (for reviews see Palek, 1987; Agre et al., 1988; Lux and Becker, 1989).

The major component of the membrane skeleton is spectrin, a flexible, rod-shaped molecule composed of α and β subunits which self-associate into tetramers (Ungewickell and Gratzer, 1978; Morrow and Marchesi, 1981). While it is clear that the spectrin-based membrane skeleton is critical to the stability of mammalian red cells, the role of this scaffold in cells where spectrin coexists with networks of actin, tubulin, and intermediate filaments, has not been elucidated. To begin an analysis of spectrin's function in such cells, we have used a variant of the Friend erythroleukemia virus (FV)[1] to generate murine erythroleukemia (MEL) cell lines from erythroblasts of the α-spectrin-deficient mouse strain, *sph/sph* (Lux et al., 1979; Bodine et al., 1984).

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1. *Abbreviations used in this paper*: EPO, erythropoietin; FV, Friend erythroleukemia virus; MEL, murine erythroleukemia.

Methods and Materials

Generation and Maintenance of Cell Lines

Sph/sph mice (WBB6F1-*sph/sph*) (generous gift of Dr. Jane Barker, Jackson Labs, Bar Harbor, ME) were injected with a 1:6 dilution of a 20% spleen lysate prepared from C57BL/6 mice infected with the BB6 variant of FV (Steeves et al., 1970). The infected mice were maintained for 3 mo at which time the spleens were removed and macerated with a razor edge to disperse the cells (a heterogenous population) into Iscove's medium (GIBCO BRL, Gaithersberg, MD and Bio-Whittaker, Walkersville, MD) supplemented with 30% heat-inactivated fetal bovine serum (Hyclone, Ogden, UT and Intergen, Purchase, NY), 100 U penicillin/streptomycin (GIBCO BRL), 2 mM glutamine (GIBCO BRL), and 20 μM 2-mercapto-ethanol (Sigma Chemical Co., St. Louis, MO). Some cultures received erythropoietin (EPO) at 0.5 U/ml (Amgen, Thousand Oaks, CA). All cells were maintained at 37°C in 5% CO_2. Cells were fed twice a week with 50% fresh medium and maintained until hematopoietic layers were observed—sometimes 6–12 mo after initiation of culture. Hematopoietically active cultures were then reinfected with 10–50 μl of 20% spleen lysate (as above) in the presence of 8 μg/ml polybrene (hexadimethrine bromide; Aldrich Chemical Co., Milwaukee, WI) for 3 h. After this incubation, fresh medium was added to dilute the reinfection mixture by half. Nonadherent cells from these cultures were saved and grown in the absence of EPO. EPO-independent MEL cells were apparent in these supernatants within weeks of reinfection. These suspension cell cultures were maintained in the medium described above, but were slowly weaned to 20% heat-inactivated fetal bovine serum. Although the cells were originally grown in the presence of 20 μM 2-mercapto-ethanol (Mager et al., 1981), the mercapto-ethanol did not appear to be essential for maintaining the MEL cells, and was withdrawn. Wild-type +/+ MEL cells were derived from normal C57BL/6 mice infected with the BSB variant of FV (Steeves et al., 1970; Geib et al., 1987) and were cultured in conditions identical to the *sph/sph* cells.

Immunofluorescence/Capping

Spectrin was localized by immunofluorescence with an affinity-purified an-

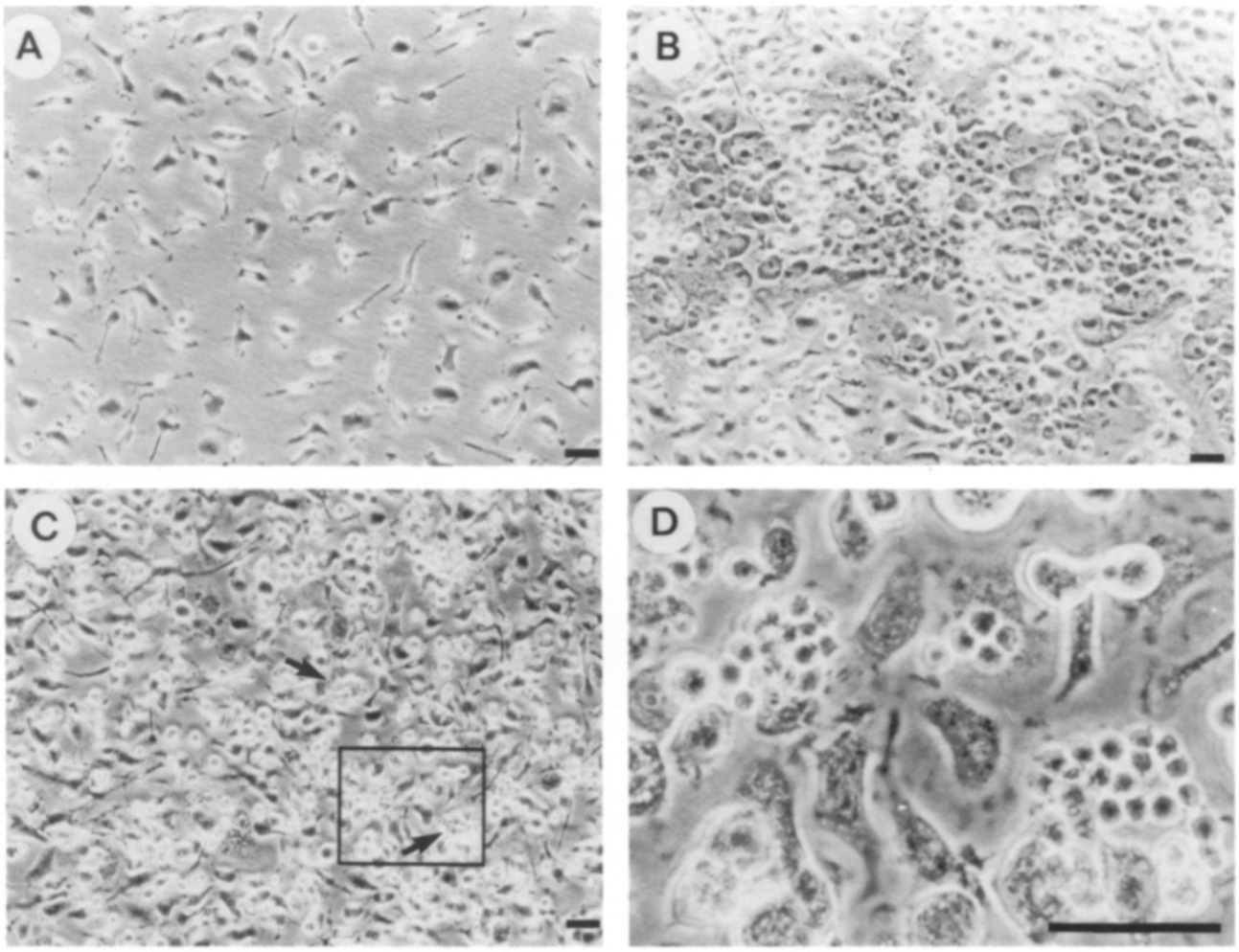

Figure 1. Development of spleen-derived stromal layers for in vitro infection with FV. Spleens from previously infected *sph/sph* mice were macerated to disperse the cells and cultured as described in the text. Substrate-adherent cultures from this heterogeneous cell population initially appeared fibroblastic (*A*). After a number of weeks, these cultures formed a confluent layer of cells (*B*). Hematopoietically active cultures (*C*) took many months to develop. Their activity was marked by the appearance of grapelike cell clusters growing off the stromal cell layer (*C*, *arrows*). (*D*) An enlargement of the boxed area in *C* to show greater detail. Bar, 50 μm.

tiserum to human erythrocyte spectrin that cross-reacts with mouse α- and β-spectrin (generous gift of Dr. Athar Chisti, St. Elizabeth's Hospital, Boston, MA). Cells were washed in PBS, allowed to attach to glass coverslips, fixed for 10 min in 3.7% formalin in PBS, and then extracted for 5 min in TBS (20 mM Tris, pH 7.5, 150 mM NaCl) containing 0.1% Triton X-100 (Sigma Chemical Co.). After extraction, the cells were incubated for 30 min in a blocking buffer containing TBST (TBS supplemented with 0.1% Tween 20; Sigma Chemical Co.) and 5% new born calf serum (Hyclone), and then incubated for 30 min at 37°C with a 1:100 dilution of primary antiserum. The cells were then washed three times in TBST and incubated for 30 min at 37°C in a 1:100 dilution of rhodamine-conjugated goat anti-rabbit antiserum (Southern Biotechnology Associates, Birmingham, AL). All antibodies were diluted into blocking buffer. After incubation in secondary antibody, the cells were washed twice in TBST, twice in TBS, once in distilled water, and then mounted in Gelvatol/PBS (Gelvatol was a generous gift from Monsanto Corp., St. Louis, MO and Air Products and Chemicals, Allentown, PA). Cells were observed with a Leitz Laborlux S microscope equipped for epifluorescence illumination.

To localize filamentous actin, cells were washed and fixed as described above, stained for 20 min in PBS containing 5 U/ml of rhodamine-conjugated phalloidin (Molecular Probes, Eugene, OR), washed twice in PBS, and mounted in PBS. All manipulations were performed on cells in suspension. To better resolve the stained material, the cells were allowed to flatten slightly before sealing the coverslip edges with nail polish.

For studies of antibody-induced capping, cells were washed and attached to coverslips as above and incubated on ice for 30 min with monoclonal antibody 28-14-8 which recognizes H-2D^b MHC-I molecules (Ozato et al., 1980). Cells were then washed three times in ice-cold PBS and incubated in a 1:100 dilution of rhodamine-labeled goat anti-mouse antibody (Southern Biotechnology Associates) in blocking buffer for 30 min. After this incubation, the cells were washed three times in ice-cold PBS and warmed to 37°C. At selected times the cells were fixed in ice-cold 4% formaldehyde (freshly prepared from paraformaldehyde), washed once in PBS, and mounted in Gelvatol/PBS.

To study Con A-induced capping, cells were washed in 4°C PBS and allowed to attach to glass coverslips. The coverslips were then washed in PBS to remove nonadherent cells and incubated on ice with 50 μg/ml rhodamine-conjugated Con A (Sigma Chemical Co.) for 30 min. After this incubation, the cells were washed three times with the ice cold PBS and warmed to 37°C. The cells were fixed at specific time points and processed as above.

Electrophoresis and Immunoblots

Whole cell lysates were prepared by washing 5×10^6 cells in PBS and lysing in 100 μl of electrophoresis sample buffer (50 mM Tris, pH 6.8, 2% sodium dodecyl sulfate, 10% glycerol, 2% 2-mercapto-ethanol, 40 μg/ml bromophenol blue) and boiling immediately for 3 min. Triton-extracted cell lysates were prepared by washing 5×10^6 cells in PBS and lysing for 30

min in 100 μl of ice cold lysis buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 14.6 mM BME, 2 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 10 μg/ml pepstatin A (Sigma Chemical Co.). Lysates were then centrifuged at 12,000 *g* for 10 min. The detergent-soluble supernatant material was transferred to new tubes and the insoluble pellet was washed in lysis buffer, centrifuged as above, and resuspended in 100 μl PBS to equal the supernatant volume. 25 μl of 5× electrophoresis sample buffer was added to the lysates which were then boiled for 3 min and separated on polyacrylamide gels (Laemmli, 1970). To best resolve the spectrin subunits, 0.75-mm-thick mini-gels were made with 8% acrylamide monomer and 0.08% *bis*-acrylamide (Dubreuil et al., 1987). The resolved proteins were electroblotted onto nitrocellulose sheets (Towbin et al., 1979) (Schleicher & Schuell, Keene, NH) and the resultant blots incubated in blocking buffer for 1 h to reduce nonspecific protein binding sites. After blocking, the blots were incubated for 1 h in a 1:1,000 dilution of affinity-purified anti-erythroid spectrin antibody, washed three times in TBST, and incubated for 1 h in a 1:1,000 dilution of alkaline phosphatase–conjugated goat anti-rabbit antiserum (Zymed, San Francisco, CA). Blots were then washed twice in TBST, twice in TBS, and developed in 150 mM Tris, pH 8.8, 75 ng/ml nitro blue tetrazolium, and 50 ng/ml 5-5-bromo-4-chloro-3-indolyl-phosphate. All antibodies were diluted into blocking buffer, and all incubations were performed with continuous rocking of the blots.

Northern Blots

Northern blots were performed as described for formaldehyde gels (Ausubel et al., 1989; Fourney et al., 1988). Total RNA was isolated by a modified protocol of Chomczynski and Sacchi (1987) supplied as Ultraspec RNA (Biotecx Laboratories, Houston, TX). 25 μg of total RNA was loaded onto a 1% agarose gel. The gel was transferred to a nylon membrane (GIBCO BRL) in 10× SSC. After transfer, the blots were washed once in 5× SSC, and then cross-linked by ultraviolet light (Stratalinker; Stratagene Corp., La Jolla, CA). The blots were subsequently processed essentially as described by Church and Gilbert (1984). α-spectrin RNA was detected with a 3.7-kb fragment of mouse α-spectrin cDNA. β-spectrin was detected with a 1.5-kb fragment of β-spectrin cDNA (gifts of Connie Birkenmeir and Michael Bloom, Jackson Laboratories, Bar Harbor, ME). Probes were purified from agarose gels, labeled with ^{32}P (Prime-it II Random Primer Labeling Kit, Stratagene Corp.), and boiled for 5 min immediately prior to use. The blots were prehybridized at 37°C for 30 min in hybridization buffer (0.5 M Na[PO_4], 0.25 M NaCl, 1 mM EDTA, and 1% BSA) and incubated overnight at 42°C with gentle agitation in hybridization buffer containing the boiled probe and 250 μg/ml salmon sperm DNA (Stratagene Corp.). After incubation, the blots were washed twice in room temperature 2× SSC, and twice in 0.1× SSC/0.1% SDS at 50°C. Autoradiograms were prepared by exposing the blots to Kodak X-O-MAT/AR X-ray film with two Dupont Cronex screens (Dupont, Wilmington, DE) for 2–5 d at −70°C.

Results

The pathogenesis of FV-induced erythroleukemia is a two-step process. The first step is an erythroproliferative phase which occurs during the first 4 wk of infection. The second step requires viral integration into an appropriate site, frequently the *spi* locus or *p53*, for transformation (Ben-David and Bernstein, 1991). 2–3 mo are usually required after infection before continuous cell lines can be isolated from infected mice (Wendling et al., 1981). Few *sph/sph* mice survived longer than a month after infection, probably because the additional erythroproliferative load of FV infection overwhelmed the already stressed hematopoietic system of the anemic animal. Cultures prepared from spleens of these moribund *sph/sph* mice never yielded MEL cell lines, but some cultures did develop stromal cell layers that spontaneously produced hematopoietic colonies (Fig. 1). Frequently, this hematopoietic activity could be maintained by the addition of 0.5 U/ml of erythropoietin. Once a hematopoietically active culture was established, reinfection in vitro with FV resulted in the generation of MEL cell lines.

The MEL cell lines obtained from infection of *sph/sph* mice were readily distinguished from +/+ MEL cells by their morphology (Fig. 2). The +/+ MEL cells maintained an essentially spherical shape in culture with occasional membrane projections (Fig. 2 *A*). In contrast, *sph/sph* MEL cells were fragile, irregular in shape, and frequently blebbed (Fig. 2 *B*). Spontaneous cell lysis was common in *sph/sph* cultures. Benzidine staining detected hemoglobin (data not shown) confirming the erythroid origin of the *sph/sph* cells.

Immunofluorescence was used to localize cytoskeletal components in the cells. Erythroid spectrin in +/+ and *sph/sph* MEL cells was both membrane associated and localized to aggregates (Fig. 3). +/+ cells (Fig. 3 *A*) always had a greater overall level of fluorescence than *sph/sph* cells (Fig. 3 *C*). In addition, multiple spectrin aggregates were common in +/+ cells while *sph/sph* cells usually contained a single spectrin aggregate. Cells stained only with secondary

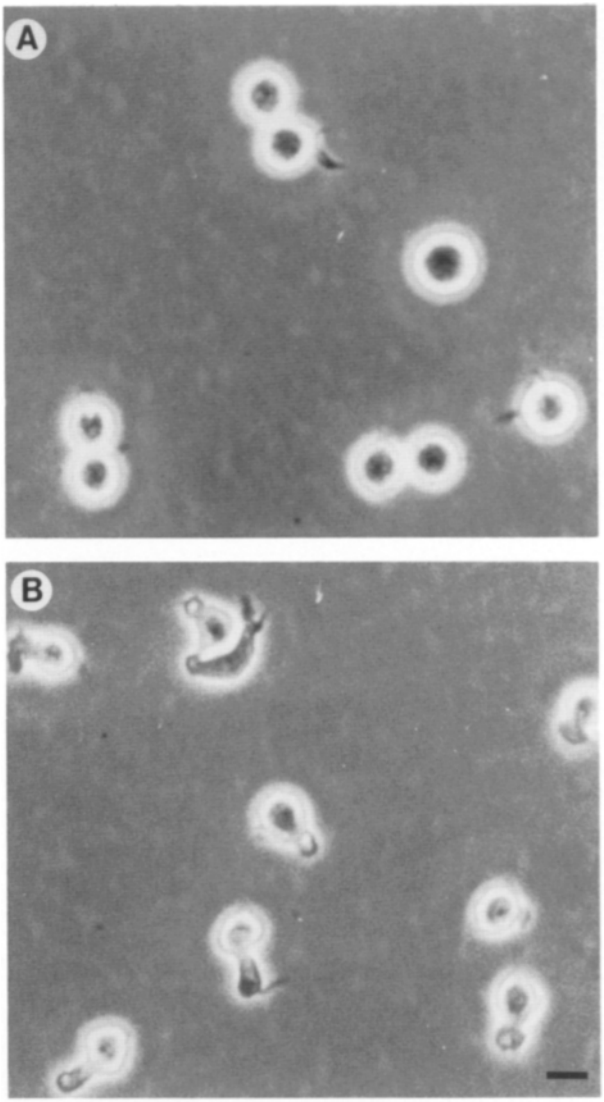

Figure 2. Morphological differences between +/+ and *sph/sph* cells in culture. MEL cells generated from +/+ normal mice were essentially spherical with few membrane projections (*A*). MEL cells generated from *sph/sph* α-spectrin deficient mice were irregular in shape with frequently blebbed membranes (*B*). Bar, 10 μm.

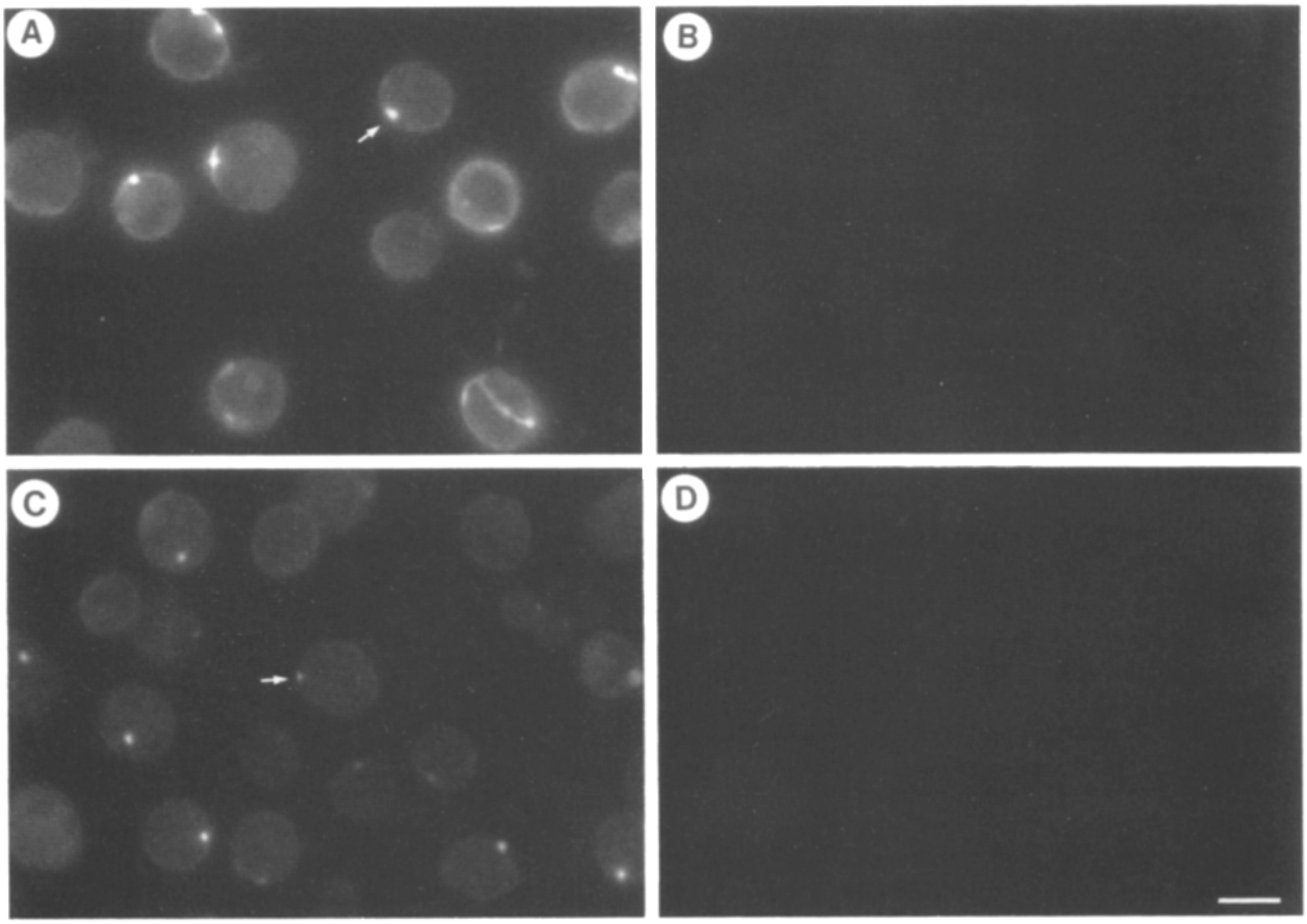

Figure 3. Immunofluorescent localization of spectrin in +/+ (*A* and *B*) and *sph/sph* (*C* and *D*) MEL cells. (*A* and *C*) Cells stained with affinity-purified antibody to erythroid α- and β-spectrin followed by rhodamine-conjugated secondary antibody. (*B* and *D*) Cells stained with secondary antibody only. Exposure-matched micrographs show spectrin aggregates (*A* and *C*, *arrows*) and diffuse membrane staining in both cell types. +/+ cells (*A*) frequently contained multiple spectrin aggregates and stained more intensely than *sph/sph* cells (*C*). A single spectrin aggregate was usually observed in *sph/sph* cells. Bar, 10 μm.

antibody showed minimal background staining (Fig. 3, *B* and *D*). Localization of actin with rhodamine-conjugated phalloidin resembled that reported for FV-infected erythroblasts (Koury et al., 1989). Cells fixed and stained in suspension (Fig. 4) had diffuse, punctate patterns of actin staining that showed little difference between +/+ cells (*A*) and *sph/sph* cells (*B*). Cells fixed and stained after attachment to coverslips showed actin filament bundles at sites of attachment (data not shown). Spectrin staining was not affected by fixation before or after attachment to coverslips.

No α-spectrin was detected in immunoblots of *sph/sph* MEL cells (Fig. 5). α- and β-spectrin were present in +/+ erythrocyte ghosts and MEL cells (Fig. 5, lanes *1* and *2*). β-spectrin was detected in the *sph/sph* cells (Fig. 5, lane *3*). In addition, a variable amount of an immunoreactive 230-kD protein was detected in both +/+ MEL cells (Fig. 5, lane *2*) and *sph/sph* cells (lane *3*). Spectrin breakdown products were always seen in *sph/sph* cells. α- and β- spectrin as well as the 230-kD protein were evenly distributed between Triton X-100–soluble (Fig. 5, *lane 4*) and –insoluble (lane *5*) fractions in +/+ cells. In *sph/sph* cells the Triton X-100–soluble pool of β-spectrin (Fig. 5, lane *6*) was always greater than the insoluble pool (lane *7*). Both +/+ and *sph/sph* cells showed faint immunoreactive bands when stained for nonerythroid α-spectrin (data not shown).

Northern blots confirmed the absence of α-spectrin in the *sph/sph* MEL cells (Fig. 6). The α-spectrin probe detected a single band of 10 kb in the +/+ cells (Fig. 6, lane *1*). No message was detectable in *sph/sph* cultures (Fig. 6, lane *2*), even at low-stringency hybridizations and long autoradiographic exposures (data not shown). When the same blot was stripped and reprobed for β-spectrin, both +/+ (Fig. 6, lane *3*) and *sph/sph* (lane *4*) MEL cells were found to contain approximately equal amounts of β-spectrin message. The predominant band was 10 kb in size. In addition, a larger 11.5-kb band was present only in +/+ cells and a faint band at 8.5 kb was seen in both cell types. Similar patterns of staining for α- and β-spectrin message were observed in Northern blots of poly A–enriched RNA from +/+ and *sph/sph* cells (data not shown).

The spectrin-deficient *sph/sph* cells exhibited altered membrane structure (Fig. 7). +/+ MEL cells labeled with monoclonal antibodies to the MHC-1 molecule, H-2D^b (Fig. 7, *A–D*) required 10 to 15 min (*C* and *D*) to progress through

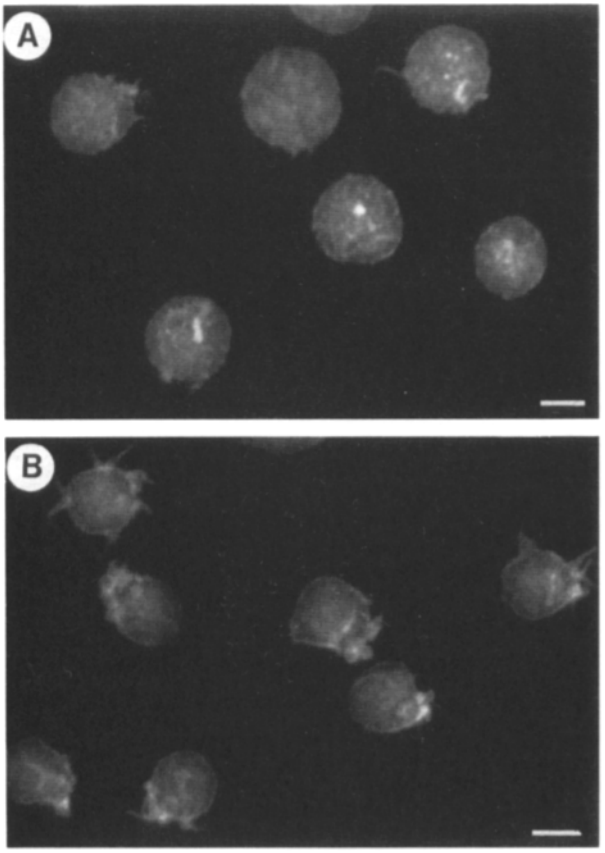

Figure 4. Localization of filamentous actin in +/+ cells (*A*) and *sph/sph* cells (*B*). Cells were fixed and stained in suspension with rhodamine-conjugated phalloidin. A diffuse, punctate pattern of actin staining was observed in both cell types. Bar, 10 μm.

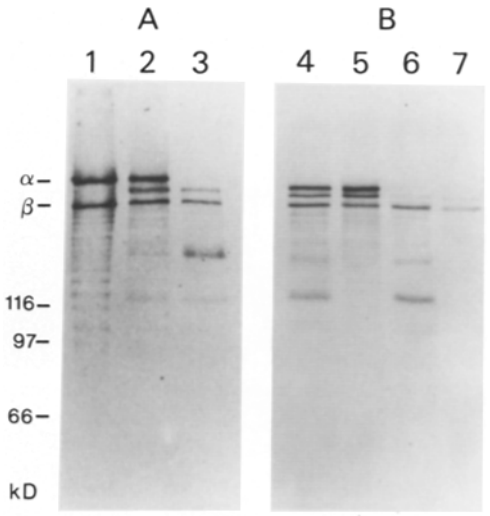

Figure 5. Western blot of cell lysates with affinity-purified antibodies to erythroid α- and β-spectrin. (*A*) Total cell lysates of +/+ murine erythrocyte ghosts (lane *1*), +/+ MEL cells (lane *2*), and *sph/sph* MEL cells (lane *3*). Protein bands were visualized with an alkaline phosphatase–conjugated antiserum. The position of α- and β-spectrin from the ghosts indicates the location of the spectrin subunits. These two proteins as well as an immunoreactive 230-kD band were present in +/+ cell lysates. *sph/sph* cell lysates stained only for β-spectrin and the 230-kD protein. (*B*) +/+ MEL cells (lanes *4* and *5*) and *sph/sph* MEL cells (lanes *6* and *7*) extracted in Triton X-100 and separated into soluble (lanes *4* and *6*) and insoluble (lanes *5* and *7*) fractions.

patching and begin to form caps. Only 15% of the +/+ cells were capped after 15 min of incubation at 37°C. In contrast, capping in *sph/sph* MEL cells (Fig. 7, *A'–D'*) was rapid. 80% of the cells formed a tight cap of fluorescent material within 5 min (Fig. 7 *B'*) of warming the cells to 37°C. In both cell types, H-2D^b capping required cross-linking of the molecules with secondary antibody. Cells labeled with monoclonal antibody to H-2D^b, warmed to 37°C, fixed, and then incubated with fluorescent secondary antibody did not cap (data not shown). Cells labeled with concanavalin A capped in a manner similar to that of H-2D^b (Fig. 8). Caps of fluorescent material were observed in *sph/sph* cells (Fig. 8, *A'* and *B'*) after 5 min (Fig. 8 *B'*) of warming. +/+ cells (Fig. 8, *A* and *B*) were predominantly patched at this time point (*B*).

To more precisely determine the time for cap formation in *sph/sph* MEL cells, capping of H-2D^b was studied at smaller time intervals (Fig. 9). After warming the cells to 37°C, the antibody/antigen complex was capped by the *sph/sph* MEL cells within 60–90 s. Thus, capping of H-2D^b in *sph/sph* MEL cells is 5–10× faster than in +/+ MEL cells.

Discussion

We have shown that MEL erythroblast cell cultures obtained through infection of *sph/sph* mice with FV retain the cytoskeletal defects of the host's erythrocytes. Immunoblots confirm the spectrin deficiency at the protein level, and Northern blots detect no discernable α-spectrin message in the *sph/sph* MEL cells. The absence of message extends previous studies that reported α-spectrin could not be translated from the total mRNA pool of *sph/sph* mouse reticulocyte lysates (Bodine et al., 1984). Although the molecular basis of the *sph* lesion has yet to be identified, the absence of detectable message in blots of total RNA suggests a deletion, a defect in transcription, or the formation of a highly unstable transcription product.

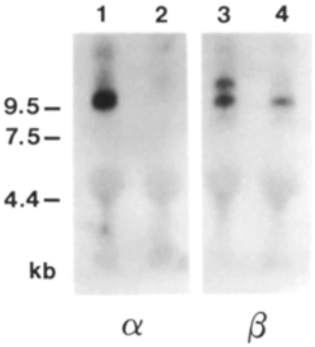

Figure 6. Northern blot analysis of α- and β-spectrin mRNA in +/+ and *sph/sph* MEL cells. 25 μg of total RNA isolated from +/+ (lanes *1* and *3*) and *sph/sph* (lanes *2* and *4*) MEL cells were separated on a 1% agarose gel, blotted onto a nylon membrane, and probed with a 3.7-kb cDNA fragment of erythroid α-spectrin (lanes *1* and *2*). The blot was then stripped and reprobed with a 1.5-kb cDNA fragment of erythroid β-spectrin (lanes *3* and *4*). No α-spectrin message was detected in *sph/sph* MEL cells.

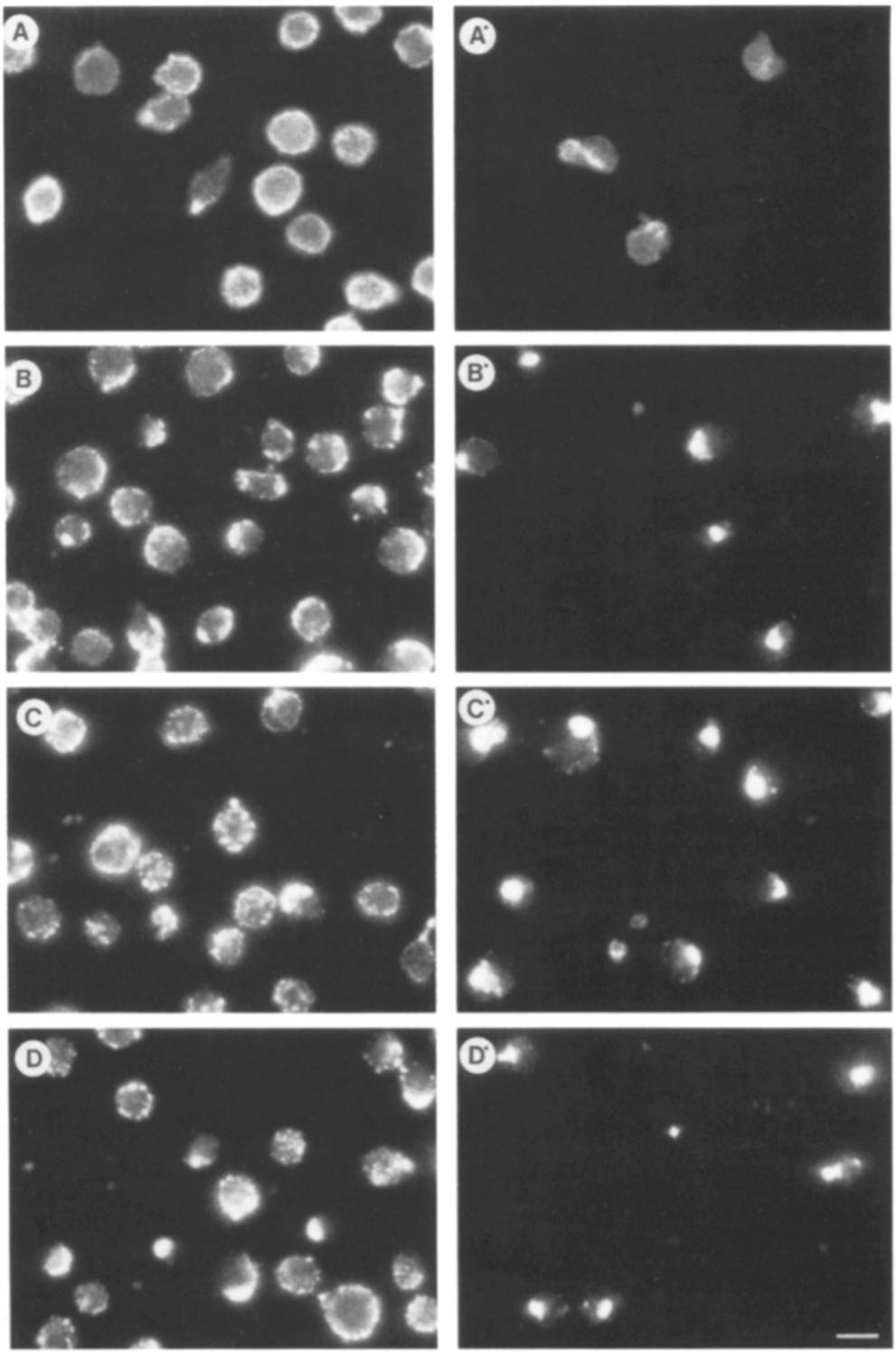

Figure 7. Capping of the MHC class I molecules, H-2D^b in +/+ and *sph/sph* MEL cells. Capping of H-2D^b was induced by incubation with monoclonal antibody 28-14-8 followed by a rhodamine-conjugated secondary antibody. All labeling and washing was done at 0°C. The cells were then warmed to 37°C. for 0 (*A* and *A'*), 5 (*B* and *B'*), 10 (*C*, *C'*), and 15 (*D* and *D'*) min to allow capping to occur, fixed at each time point, and observed by fluorescence microscopy. *sph/sph* MEL cells (*A'–D'*) were tightly capped within 5 min of induction. +/+ MEL cells (*A–D*) required 10–15 min to cap. Bar, 10 μm.

Sph/sph mice are C57BL6/WBF1 heterozygotes (also known as WBB6 *sph/sph*). Both C57BL/6 and WB mice are inherently resistant to FV infection. Resistance in C57BL/6 mice is due to Fv2^r suppression. Resistance in WB mice is thought to be related to *c-kit* receptor defects since *steel* (*Sl*) mutants are also FV resistant (for recent review see Ben-David and Bernstein, 1991). The BB6 variant of FV virus can overcome Fv2^r resistance, and we experienced no problems in obtaining infection and a subsequent erythroproliferative response in *sph/sph* mice. The main difficulty was keeping the animals alive for the two to three month period postinfection required for generating cell lines. This problem was overcome by infecting hematopoietically active spleen-derived stromal cultures in vitro. The +/+ MEL cells used in these experiments were previously generated in homozygous C57BL/6 mice with BSB, a virus similar to

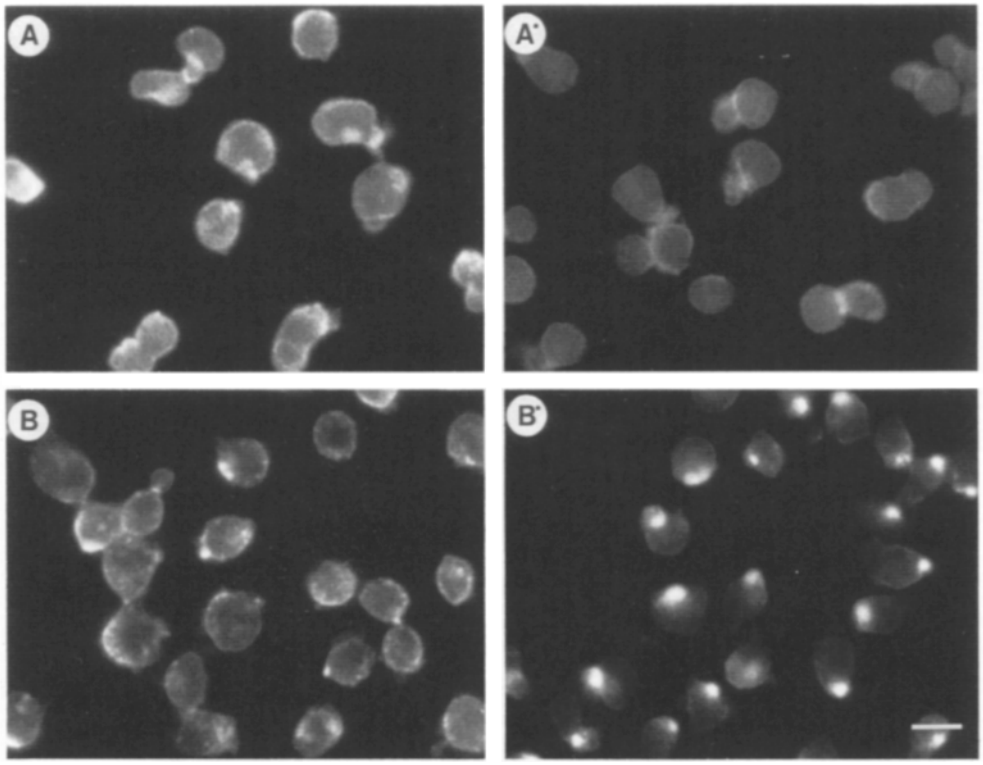

Figure 8. Concanavalin A–induced capping in +/+ (*A* and *B*) and *sph/sph* (*A'* and *B'*) MEL cells. Cells on coverslips were cooled to 0°C, incubated with rhodamine-conjugated concanavalin A, washed with ice cold PBS, and warmed to 37°C. for 0 (*A* and *A'*) and 5 (*B* and *B'*) min to allow capping to occur. The cells were fixed at each time point and observed by fluorescence microscopy. *sph/sph* MEL cells were tightly capped after this time interval. +/+ MEL cells were patched. Bar, 10 μm.

BB6 (Steeves et al., 1970; Geib et al., 1987; Majumdar et al., 1992). Both the *sph/sph* and the +/+ cell lines expressed FV envelope proteins indicating they were generated by similar transformation events (data not shown).

In both our *sph/sph* and +/+ MEL cell lines, spectrin was localized at the cell membrane and in aggregates. The difference in anti-spectrin labeling of the two cell lines was greater than anticipated from immunoblots and may be due to a weaker interaction of the antibodies with β-spectrin in solution than on blots. The difference in the number of aggregates between +/+ and *sph/sph* cells may reflect differences in the amount of spectrin or in the subunit composition of the spectrin that was present. The presence of spectrin aggregates has previously been reported in DMSO-induced MEL cell cultures (Glenney and Glenney, 1984) and in lymphocytes (Pauly et al., 1987). In lymphocytes, activation of the cells with lectin, phorbol esters, ionophore, or hyperthermia mobilizes spectrin from the aggregate to the plasma membrane (Lee et al., 1989). Similar treatments of our MEL cultures did not appreciably alter the spectrin localization (Dahl, unpublished data).

The identity of the immunoreactive 230-kD band identified in the MEL cells is not known. Since polyclonal antibodies to erythroid spectrin from two different laboratories recognize this protein (data not shown), and since anti-β–spectrin antibodies immunoprecipitate a similar protein from differentiating MEL cells (Lehnert and Lodish, 1988), the 230-kD protein may be a stage-specific β–spectrin isoform. A number of β-spectrin isoforms have been identified in non-erythroid cells (Riederer et al., 1986; Bloch and Morrow, 1989; Winkelmann et al., 1990) and non-erythroid spectrins have been reported in MEL cells (Glenney and Glenney, 1984). We are currently investigating whether similar proteins are expressed in our MEL cultures.

Spectrin deficiency affects membrane architecture and membrane integrity. Transmembrane molecules cap more rapidly in spectrin-deficient *sph/sph* MEL cells than in spectrin-normal +/+ MEL cells. This increase in mobility is consistent with previous work on erythrocytes showing lateral diffusion coefficients of membrane proteins 50 times greater in red cells derived from *sph/sph* mice than in red cells from +/+ animals (Sheetz et al., 1980). The irregular shape of the *sph/sph* MEL cells and their fragility in culture also suggest that spectrin is vital for membrane stability in nucleated cells. Similar conclusions have recently been reached for α-spectrin in *Drosophila* where homozygous α-spectrin deficiency results in membrane and developmental abnormalities that are lethal (Lee et al., 1993).

The role of spectrin in membrane organization has been likened to a meshwork of fences that restrict the mobility of transmembrane molecules within domains defined by the spectrin lattice (Sheetz et al., 1980; Edidin, 1992). This idea is supported by experiments that suggest a cytoplasmic matrix on a scale commensurate with a spectrin meshwork regulates short- and long-range interactions at the cell surface in fibroblasts and other cells (Edidin et al., 1991; Kusumi et al., 1993). The α-spectrin–deficient MEL cells described here and similar cell lines to be generated from β-spectrin and ankyrin-deficient mice will be useful in testing these hypotheses and dissecting questions of structure and function through transfection of engineered constructs.

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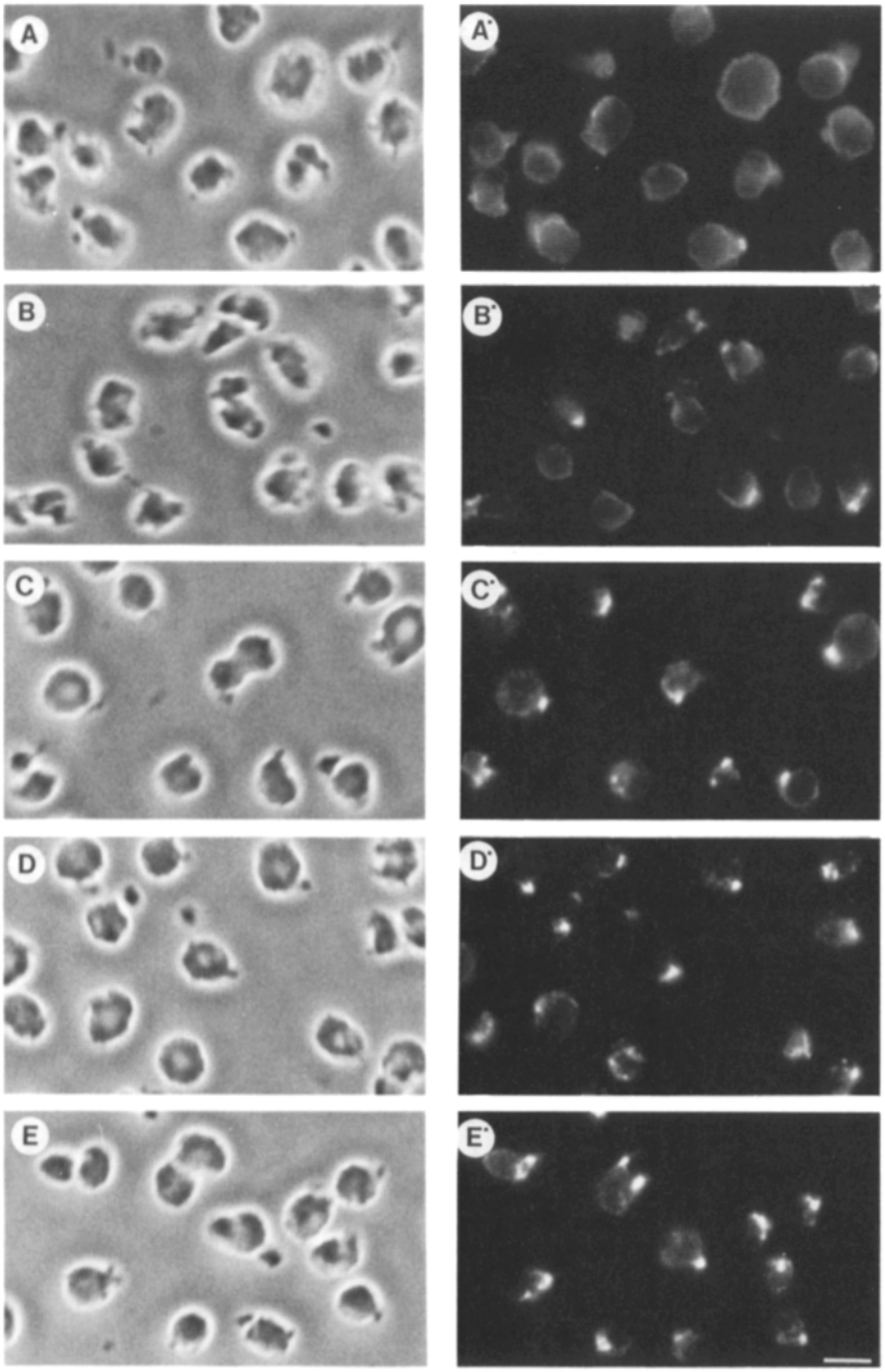

Figure 9. Time course for capping of H-2D^b in *sph/sph* MEL cells. Cells on coverslips were treated as in Fig. 7 and warmed to 37°C. for 0 (*A* and *A'*), 30 (*B* and *B'*), 60 (*C* and *C'*), 90 (*D* and *D'*), and 120 (*E* and *E'*) s, fixed, and observed with fluorescence microscopy. The beginnings of cap formation were apparent within 30 s of warming to 37°C and the cells were essentially capped in 60–90 s. Bar, 10 μm.

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