Involvement of Cell Surface HSP90 in Cell Migration Reveals a Novel Role in the Developing Nervous System*

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Heat shock protein HSP90 plays important roles in cellular regulation, primarily as a chaperone for a number of key intracellular proteins. We report here that the two HSP90 isoforms, α and β, also localize on the surface of cells in the nervous system and are involved in their migration. A 94-kDa surface antigen, the 4C5 antigen, which was previously shown to be involved in migration processes during development of the nervous system, is shown to be identical to HSP90α using mass spectrometry analysis. This identity is further confirmed by immunoprecipitation experiments and by induction of 4C5 antigen expression in heat shock-treated embryonic rat brain cultures. Moreover, immunocytochemistry on live cerebellar rat cells reveals cell surface localization of both HSP90α and -β. Cell migration from cerebellar and sciatic nerve explants is inhibited by anti-HSP90α and anti-HSP90β antibodies, similarly to the inhibition observed with monoclonal antibody 4C5. Moreover, immunostaining with rhodamine-phalloidin of migrating Schwann cells cultured in the presence of antibodies against both α and β isoforms of HSP90 reveals that HSP90 activity is associated with actin cytoskeletal organization, necessary for lamellipodia formation.

The development of the vertebrate nervous system depends on extensive cell migration, which allows different cell types to reach their final destination and establish the composite organization of the adult nervous system. Cell migration is a complex process, which requires the coordination of many molecular and cellular events such as cell-cell recognition, adhesion, transmembrane signaling, and cell motility (1–23). The mechanisms underlying these processes involve orchestrated interactions between a large number of molecules (10, 24). Emerging evidence suggests that many proteins in the nervous system have multiple distinct functions. Thus, molecules involved in migration processes have been shown to participate additionally in other developmental events. These include the cell adhesion molecules (CAMs), which regulate axonal growth and regeneration (25); integrins, which mediate cell proliferation and synaptic plasticity (26); and neuroregulins that promote neuronal differentiation and regulate glial commitment, proliferation, survival, and differentiation (27). We report here that heat shock protein 90 (HSP90) is also a multifunctional protein, processing a novel role in neuronal migration. HSP90 is a highly conserved molecule with a wide distribution in various species. It acts as capacitor of morphological evolution in Drosohila melanogaster (28–30). It also functions as a chaperone in unstressed cells, specifically involved in the folding or conformational regulation of central signal transduction molecules, including steroid hormone receptors and proto-oncogene kinases (31, 32). Moreover, it is a protective agent under stress conditions and is involved in protein renaturation and refolding (33). In the developing mammalian nervous system, HSP90 is highly expressed in the G0 phase of cells during neuroectoderm differentiation, suggesting that it is required in order to maintain cells in this phase (34). Moreover, Loones et al. (35) have reported the differential expression of HSP90 in various regions of the developing nervous system, suggesting involvement of this molecule in specific functions distinct from its chaperone activity. Two isoforms of HSP90 exist, α and β, which share 86% amino acid sequence conservation. However, no isoform-specific functions have so far been attributed to these molecules.

In order to understand the molecular events regulating neuronal migration and development, we have previously characterized a monoclonal antibody (mAb), named 4C5, which was produced after immunization of mice with a brain membrane fraction of 15-day-old rat embryos. As judged by immunoblotting and immunocytochemistry, this antibody recognizes a 94-kDa cell surface peripheral antigen (4C5 antigen) in the developing rat nervous system (36). In the central nervous system, the 4C5 antigen is present in neurons, and its expression is decreased with age (36, 37). Moreover, antibody perturbation experiments, performed in cerebellar explant cultures using mAb 4C5, have indicated that the 4C5 antigen contributes to both the horizontal and the vertical neuron-neuron-mediated migrations of granule cells during cerebellar development (37, 38). In the peripheral nervous system and in particular in the rat sciatic nerve, the presence of the 4C5 antigen was detected not only in neurons but also in myelin-forming and non-myelin-forming Schwann cells (39). Western blot analysis showed that mAb 4C5 recognizes in the sciatic nerve a protein of the same molecular mass as in the brain (39). Expression of the 4C5 antigen decreases with age in both myelin-forming and non-myelin-forming Schwann cells, thus indicating its involvement

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** The abbreviations used are: HSP, heat shock protein; FPLC, fast performance liquid chromatography; HPLC, high performance liquid chromatography; MS, mass spectrometry; NF, neurofilament; mAb, monoclonal antibody; FCS, fetal calf serum; PBS, phosphate-buffered saline; NCAM, neural cell adhesion molecule.
in a developmental process common to all Schwann cells. Moreover, following adult sciatic nerve crush, the molecule was intensely re-expressed in Schwann cells of the distal segment, a few days after injury and during regeneration of the nerve. Antibody perturbation experiments performed in various *in vitro* systems of the peripheral nervous system using mAb 4C5 indicated that the 4C5 antigen participates in Schwann cell migration events during development and regeneration of the peripheral nervous system (40). Finally, data have been presented suggesting the involvement of this protein in actin cytoskeletal dynamics of migrating Schwann cells (40).

In the present work, we show, using a proteomic approach, that the mAb 4C5 recognizes HSP90. Moreover, we demonstrate that the expression of the 4C5 antigen/HSP90 is induced in primary cell cultures derived from rat embryonic brain after heat shock treatment of these cultures. Finally, with the use of polyclonal antibodies against both HSP90 isoforms, we show that these proteins are localized not only in the cytoplasm but also on the cell surface of cells and furthermore that they are involved in cell migration processes in the developing central and peripheral nervous system.

**EXPERIMENTAL PROCEDURES**

**Antibodies and Reagents**—mAb 4C5 was produced in our laboratory, as previously described (36). In the present study, mAb 4C5 was used as concentrated serum-free supernatant in all experiments performed. Rabbit anti-HSP90α and anti-HSP90β were from Chemicon International. Rabbit antibody against BM88, a neuron-specific protein (41), was kindly provided by Dr. Matsas (Hellenic Pasteur Institute). Rabbit anti-NCAM was a kind gift from Dr. Rougon (CNRS/IBDM Marseille). Mouse monoclonal antibody to the cytoskeletal neurofilament (NF) protein, secondary polyclonal fluorochrome-labeled antibodies, rhodamine-conjugated phalloidin, fetal calf serum (FCS), poly-L-lysine, laminin, trypsin, diaminobenzidine tetrahydrochloride, and 5-bromo-2’-deoxyuridine were purchased from Sigma. Dulbecco’s modified Eagle’s medium (DMEM), Ham’s F-12 medium, and CaCl2/MgCl2-free minimum essential medium were obtained from Invitrogen. The anti-mouse IgG-specific ABC complex was obtained from Vector.

**Brain Preparations**—Embryonic day 18 brain extracts were homogenized in at least 10 times their volume of 10 mM Tris-HCl, pH 7.4, in a glass homogenizer on ice. This was centrifuged at 500 × g for 5 min, the pellet was discarded, and the supernatant was treated with 1 M NaCl for 20 min in 10 mM Tris buffer, followed by centrifugation at 15,000 × g for 30 min. The pellet was discarded, and the supernatant was dialyzed in 20 mM Tris, pH 7.8, for 48 h. Protein estimations were performed using the Bradford method.

**Two-dimensional Electrophoresis, Western Blot, and Mass Spectrometry**—The above described brain preparations were separated on a MonoQ column using a fast protein liquid chromatography (FPLC) system (Amersham Biosciences). The column was developed with a linear gradient to 1 M NaCl. Fractions were collected and analyzed by 12% SDS-PAGE followed by Western blotting with mAb 4C5 performed as previously described (36). The immunoreactive fractions were then separated in duplicate two-dimensional gels, with isoelectric focusing as the first dimension (IPG strips; nonlinear pI range 3–10; Amersham Biosciences) and SDS-PAGE (12%) as the second. This was followed by silver staining of one gel and immunoblotting with mAb 4C5 of the other. The spot from the silver-stained gel corresponding to the immunoreactive spot was cut, and after overnight in-gel digestion with sequence grade trypsin (Promega), peptides were extracted from the gel, and a peptide preparation in 2% acetonitrile, 0.1% formic acid was applied to a C-18 reversed-phase high performance liquid chromatography (HPLC) column (75 μm × 15 cm) on an UltimaMate nano-HPLC system (LC Packings; Dionex). The column was developed with a linear gradient to 90% acetonitrile, 0.1% formic acid at a flow rate of 150 nL/min. The eluate was analyzed on an ion trap mass spectrometer with a nanospray source (LCQ Deca, ThermoFinnigan). MS and MS/MS data were used to search protein data bases (SwissProt) with the Turbo-SEQUEST search engine.

**Immunoprecipitation**—For immunoprecipitation, the 4C5-immunoreactive fraction derived from FPLC separation was incubated either with anti-HSP90α or with anti-HSP90β, respectively, coupled to Protein A-Sepharose beads, for 1 h at room temperature. For controls, the 4C5-immunoreactive fraction was incubated with 5 μl of anti-rabbit immunoglobulins coupled to Protein A-Sepharose beads. Samples were mixed for 1 h, and beads were washed three times in PBS. Proteins were eluted from the beads by boiling in sample buffer and analyzed by SDS-PAGE and immunoblotting using mAb 4C5.

**Cell Cultures and Heat Shock Treatment**—Primary cell cultures from embryonic day 18 rat brain were prepared as previously reported (42). Briefly, the brain was aseptically removed from the embryos, dissected, and treated for 45 min at 37 °C with 0.25% trypsin in 10 ml of CaCl2/MgCl2-free minimum essential medium. The digestion was terminated with the addition of 20% FCS, and the cells were dissociated by trituration and passage through a nylon mesh (60 μm). Resulting cells were centrifuged at 500 × g for 10 min, resuspended in DMEM supplemented with 10% FCS culture medium and plated (3.5 × 104 cells/well) on 10-mm diameter glass coverslips, precoated with 1 mg/ml poly-L-lysine in 48-well Costar culture dishes. The dishes were placed in an incubator at 37 °C with a humidified atmosphere of 5% CO2 and 95% air. After 48 h in cultures, cells were heat-shocked for 1 h in a water bath preheated to 43 °C followed by another 24 h in *vitro* before immunostaining. Controls were maintained in the incubator. Cerebellar primary cell cultures from postnatal day 5 rats were prepared as previously described (37).

**In Vitro Migration Assays**—In the two bioassays used, control cultures were grown either in culture medium alone (DMEM, 10% FCS) or in culture medium containing 100 μg/ml polyclonal antibody against BM88.

**Cerebellar Microexplants**—Cerebellum obtained from postnatal day 5 rats was quickly removed from the skull, placed in cold DMEM, and freed from meninges and choroids plexus. The cerebellum was then cut in two, in cross sections which were placed in a 4% (w/v) solution of trypsin, using a surgical blade under a stereoscope. Such prepared micro explants were placed on precoated coverslips with 0.1% (w/v) poly-L-lysine and 20 μg/ml laminin with 100 μl of DMEM, 10% FCS in the presence of 100 μg/ml of anti-HSP90α and anti-HSP90β, respectively, or in control media. After 36 h of culture, granule cells migrating out of the tissue were observed, as identified by peroxidase immunostaining using antibody to the NF protein.

**Sciatic Nerve Explants**—Sciatic nerves were aseptically dissected from postnatal day 1 Wistar rats, and the perineurium was removed. Small pieces, ~2 mm long, of the nerves were placed on poly-L-lysine-coated glass coverslips and cultured in DMEM, 10% FCS in the presence of 100 μg/ml anti-HSP90α and anti-HSP90β, respectively, or in control media. After 36 h of culture, Schwann cells migrating out of the tissue were observed, as identified by immunohistochemistry using antibody to S-100 protein.

**Immunostaining**—Immunolabeling of embryonic day 18 brain cultures for the 4C5 antigen and NF protein was performed by the immunoperoxidase method. The preparations were fixed in 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS) for 20 min and were processed for demonstration of intracellular antigens, the samples were permeabilized with 0.1% Triton X-100. Antibodies were diluted in PBS containing 10% FCS and 0.1 m l lysine. The samples were incubated overnight at 4 °C with the primary antibodies, followed by incubation with the anti-mouse IgG-specific ABC complex. Immunoreactivity was visualized using diaminobenzidine tetrahydrochloride as a substrate with nickel enhancement. The stained preparations were mounted on glass slides in glycerol.

Live cells from primary cerebellar cultures were labeled by indirect immunofluorescence as previously reported (36). Briefly, unfixed cells were incubated with mAb 4C5, anti-HSP90α, anti-HSP90β, anti-NCAM, and anti-NF, diluted in culture medium for 2 h. The cells were then washed in DMEM and fixed in cold acetone for 3 min. Secondary antibodies were fluorescein-conjugated anti-mouse or anti-rabbit immunoglobulins, diluted 1:50 in PBS containing 10% FCS and 0.1 m l lysine.

Immunolabeling of sciatic nerve explants for the S-100 protein was performed by the immunofluorescence method as previously described (38). Briefly, the preparations were fixed in 4% (w/v) paraformaldehyde in PBS for 20 min and washed three times in PBS. Antibodies were diluted in PBS containing 10% FCS and 0.1 m l lysine. The samples were incubated overnight at 4 °C with the primary antibody, followed by incubation with fluorescein-conjugated secondary antibody (anti-rabbit Fab fragments conjugated to fluorescein isothiocyanate, at a dilution of 1:100).

For detection of actin filaments of sciatic nerve explants, the cells were fixed in 4% paraformaldehyde in PBS, permeabilized with 0.1% Triton X-100 in PBS in order to increase phalloidin penetration, rinsed

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twice with PBS, and incubated for 20 min at room temperature with rhodamine-conjugated phalloidin diluted in the same buffer. The stained preparations were mounted on glass slides in CITIFLUOR (CITIFLUOR Ltd.) to prevent fading of the fluorescence. For all experiments, controls were performed by omitting the primary antibodies. All preparations were viewed by a Zeiss microscope.

Quantification of Immunofluorescence—For quantifying the extent of lamellipodia formation, images of Schwann cells labeled with rhodamine-phalloidin were collected using a Leica DM 300 camera connected to a computer. The extent of labeled areas was measured with the help of Image Pro Plus analysis software.

Statistical Analysis—In all of the experimental groups that were statistically analyzed for differences, Student’s t test was used, in which p < 0.05 was defined as statistically significant.
RESULTS

Identification of the 4C5 Antigen as the HSP90 Protein—The functional properties of mAb 4C5 led us to believe that it recognizes an important molecule for cell migration, and we therefore set out to establish its identity. We prepared homogenates from rat embryonic brain and carried out a partial purification of the 4C5 antigen by anion exchange FPLC on a MonoQ column. All fractions were assayed by immunoblotting with mAb 4C5, revealing a peak of the 94-kDa antigen eluting at \(400 \text{ mM NaCl}\). A major band of 94 kDa was present in a silver-stained gel of the three peak fractions, correlating in intensity with that of the immunoreactive band. However, the presence of possible contaminating bands in the complex mixture led us to a further purification using two-dimensional electrophoresis. The MonoQ fraction containing most of the immunoreactive material was separated in two parallel two-dimensional gels, one of which was silver-stained and the other processed for immunoblotting with mAb 4C5. The protein spot that reacted with the antibody was identified and cut from the duplicate gel stained with silver (Fig. 1A). Following in-gel digestion with trypsin, peptides were extracted and separated on a reversed phase C18 nano-HPLC column, coupled to an ion trap mass spectrometer with a nanospray source. Tandem MS data were collected for the entire digest and used to search protein databases. The immunoreactive protein spot was clearly identified as HSP90 (Fig. 1, B and C). Approximately 30 peptides were matched, covering 51.4% of the protein by sequence (Fig. 1D). The same result was obtained with material extracted from the one-dimensional separation of the immunoreactive fractions. No other protein gave a significant match.

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**Fig. 2. Heat shock induction of both cell surface and intracellular 4C5 antigen/HSP90.** A, immunolabeling with mAb 4C5 of primary cell cultures derived from embryonic day 18 rat brains after 3 days *in vitro*. Cells were labeled either without or after treatment with Triton X-100. Immunolabeling with mAb 4C5 was also performed under nonstress conditions (controls) or following heat shock treatment of the cells. The arrows and arrowheads point to neurons and astrocytes, respectively. Bar, 10 \(\mu\text{m}\). B, quantification of the immunoreactivity obtained in the above described cultures. The bars represent the average ± S.E. for each group of five independent experiments. Within a single experiment, peroxidase labeling of -30 cells (neurons and astrocytes) was measured.
Fig. 3. Anti-HSP90α and anti-HSP90β stain the cell surface. A, immunofluorescence labeling of live cells derived from postnatal day 5 rat cerebellum after 3 h in culture using anti-HSP90α, anti-HSP90β, and mAb 4C5. Cell surface immunolabeling with all three antibodies was observed. Antibodies against NCAM and the NF protein were used as positive and negative controls, respectively. Bar, 10 μm. B, quantification of immunolabeling obtained with anti-HSP90α and anti-HSP90β. The decreased immunostaining obtained with anti-HSP90β was not statistically significant.
In order to confirm the above result, we employed specific polyclonal antibodies raised against both isoforms of HSP90, α and β. Western blot analysis of the 4C5 immunoreactive fraction derived from the FPLC separation with mAb 4C5, anti-HSP90α, and anti-HSP90β revealed in all cases a single immunoreactive band (Fig. 1E, lanes 1–3, respectively). The 4C5 immunoreactive fraction derived from the FPLC separation was then incubated with polyclonal anti-HSP90α or -β coupled to protein A beads, and the immunoprecipitated proteins were immunoblotted with mAb 4C5. Whereas in both cases, a single immunoreactive band was observed, the intensity of the band obtained after anti-HSP90α immunoprecipitation was much more intense than that obtained for the β isoform (Fig. 1E, lanes 4 and 5). The two isoforms are closely related in amino acid sequence (86% conservation), and it is therefore not surprising that some cross-reactivity is observed. No bands were observed in controls where protein A beads were incubated with anti-rabbit immunoglobulins and followed by immunoblotting with mAb 4C5 (Fig. 1E, lane 6). The possibility that the commercial anti-HSP90 antibodies and mAb 4C5 recognize two different proteins that interact strongly with each other and have the same molecular weight is highly unlikely given their co-migration in two-dimensional electrophoresis, as shown above. We conclude from these results that the 4C5 antigen and HSP90 are most probably one and the same molecule.

Cell Surface and Intracellular 4C5 Antigen/HSP90 Are Induced by Heat Shock—Our identification of HSP90 as the antigen most likely recognized by mAb 4C5 led us to investigate whether expression of this antigen is induced by heat shock. To this end, cells from embryonic day 18 rat brain were immunolabeled with mAb 4C5 either prior to or following heat shock treatment and in the absence or presence of Triton X-100 to allow cell surface or intracellular labeling, respectively (Fig. 2A). In the absence of Triton X-100 or heat shock treatment, immunoreactivity was observed on cells morphologically identified as neurons (Fig. 2A, arrows). Immunostaining of astrocytes (Fig. 2A, arrowheads) was very weak, close to that obtained in controls where the primary antibody was omitted. When these cultures were treated with Triton X-100, a more intense immunolabeling was observed in both cell types (Fig. 2A, B, and C), indicating the predominantly intracellular localization of the protein in both neurons and astrocytes. Exposure of the cultures to heat shock resulted in a significant increase of 4C5 immunoreactivity both in neurons and astrocytes (Fig. 2A, B, and C). It is interesting to note that increased immunolabeling corresponded both to the cell surface (Fig. 2, A and B) and to the intracellular protein (Fig. 2, A and B) recognized by mAb 4C5. Finally, heat shock treatment of the cultures did not affect the immunostaining observed for the control neuronal NF protein. These results further confirm that the 4C5 antigen and HSP90 are identical. They also demonstrate that whereas HSP90 has a ubiquitous and predominant intracellular localization, in some cell types, such as neurons, it is also present in significant quantities.
amounts at the cell surface, indicating potential novel roles in cellular function.

Anti-HSP90α and Anti-HSP90β Stain the Cell Surface—Our previously published work has shown that the 4C5 antigen is expressed at the cell surface of neuronal cells. In order to confirm cell surface localization of HSP90, unfixed cells from rat cerebellum of postnatal day 5 were incubated after 3 h in culture with anti-HSP90α and anti-HSP90β. The cells were then carefully washed, fixed, and labeled with a fluorescent secondary antibody. Thus, the primary antibody had access only to the external surface of the cells. The observed typical punctate immunostaining confirmed the cell surface localization of both HSP90α and HSP90β isofoms (Fig. 3A). Interestingly, immunolabeling with anti-HSP90α was more intense than with anti-HSP90β; however, the observed difference was not significant (Fig. 3B). In accordance with our previous data, surface immunolabeling of cerebellar cells was also obtained with mAb 4C5 (Fig. 3A). Control experiments using antibodies to cell surface NCAM and to the intracellular NF protein resulted in positive and negative results, respectively (Fig. 3A).

HSP90 Is Involved in Cell Migration in the Central and Peripheral Nervous System—HSP90 has not been associated before with cell migration processes during development of the nervous system. Our finding that this protein is the antigen recognized by mAb 4C5, which has been previously reported to be involved in migration processes in the central (37, 38) and peripheral (39, 40) nervous system, led us to investigate the participation of HSP90 in cell migration events. Thus, we performed antibody perturbation experiments using anti-HSP90α and anti-HSP90β in two in vitro model systems for the central and peripheral nervous system. Control cultures for the two bioassays were performed as described under "Experimental Procedures." It is important to note that no statistically significant difference was observed between the two types of controls used, media alone and a control polyclonal antibody against BM88. The control value illustrated for the two bioassays is the mean value of the two types of control.

When cerebellar and sciatic nerve explants derived from postnatal day 5 and day 1 rats, respectively, were cultured in the presence of polyclonal antibodies against either HSP90 isoform, the migration of neurons as identified by immunolabeling for the S-100 protein, a well documented marker for this cell type (43, 44) (Fig. 5), was significantly reduced compared with control cultures. In accordance with our previously reported data (38, 40), inhibition of cell migration in these in vitro systems was similarly observed when mAb 4C5 was included in the culture medium. It is important to note that in the in vitro assays used, the effect of the anti-HSP90α and anti-HSP90β, is primarily directed toward cell migration and not cell proliferation and/or apoptosis, since very low (<5%) 5-bromo-2′-deoxyuridine incorporation, and no terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling-positive cells were observed in the cells of the explant cultures.

HSP90 Is Involved in Lamellipodia Formation—Previously reported data suggested the association of the 4C5 antigen with F-actin organization during cell migration (40). In order to confirm the involvement of HSP90 in cytoskeletal organization, we examined the localization of F-actin in Schwann cells that
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The present work reveals a novel role for the multifunctional HSP90 protein in cell migration. Surprisingly, this role is mediated by a cell surface-associated pool of HSP90.

We have shown here that the 4C5 antigen, which is expressed on the surface of neuronal cells and is known to be involved in cell migration, is identical to the HSP90 protein. In particular, mAb 4C5 reacts strongly with the α isoform of HSP90, whereas it also cross-reacts weakly with the closely related β isoform. In previous studies, we have demonstrated that the 4C5 antigen is a 94-kDa cell surface peripheral protein present in the central and peripheral nervous system of the rat (36). Moreover and with the use of antibody perturbation experiments, we have shown, using mAb 4C5 in various in vitro models of the central and peripheral nervous system, that the 4C5 antigen participates in granule cell migrations during development of the cerebellar cortex (37, 38) and in Schwann cell migrations during development and regeneration of the sciatic nerve (40). Finally, we have presented data suggesting the association of the 4C5 antigen with actin cytoskeletal dynamics during cell migration (40).

In the present work, we sought to investigate the identity of the 4C5 antigen using a proteomic approach. To this purpose, we performed MS analysis, which revealed that the 4C5 antigen is identical to the HSP90α protein, a member of the heat shock protein family. The identity of the 4C5 antigen was confirmed with immunoprecipitation experiments using mAb 4C5 and polyclonal anti-HSP90 antibodies.

The HSP90 protein is a cytoplasmic molecule widely expressed in cells of various species. Moreover, it is a chaperone molecule that plays an important role in various signal transduction cascades under nonstress conditions and in protein renaturation under stress (33). The intracellular localization of HSP90 was confirmed in brain cultures using mAb 4C5. It is interesting to note that localization of HSP90 on the cell membrane of astrocytes was extremely weak. The differential expression of HSP90 on the cell surface of neurons and astrocytes during the embryonic ages studied in the present work may be related to the fact that these cell types migrate at different stages during development. In particular, neuronal migration in the cerebral cortex takes place mainly throughout embryonic stages (24), whereas astrocyte migration during the perinatal period (45, 46). The stress-inducible nature of the molecule recognized by mAb 4C5 was confirmed after heat shock treatment of the above mentioned cultures.

Cell surface localization of both HSP90 isoforms was demonstrated by immunostaining of unfixed, live cerebellar cells with specific polyclonal anti-HSP90 antibodies. Burt et al. (47) have previously reported cell surface localization of HSP90 in Can-
in particular, they show, using a polyclonal anti-HSP90 antibody developed against a synthetic peptide, localization of an 82-kDa protein on the cell surface of the fungus. Moreover, very recent results (48) show the presence of HSP90 on the cell surface of cultured oligodendrocyte precursor cells derived from embryonic rat brain. Our findings are in accordance with the above and further support cell surface localization of HSP90.

The participation of cell surface HSP90 in migration processes of the central and peripheral nervous system was shown with antibody perturbation experiments using polyclonal anti-HSP90 in cerebellar and sciatic nerve explant cultures, respectively. Moreover, exposure of migrating Schwann cells to polyclonal anti-HSP90 reduced their lamellipodial formation, thus indicating association of HSP90 with actin organization during migration. To our knowledge, this is the first report of involvement of the HSP90 protein with cell migration events during development of the mammalian central and peripheral nervous system. Rousseau et al. (49) have published data demonstrating the involvement of cytoplasmic HSP90 in the actin mediated motility of endothelial cells in vitro. Specifically, the authors showed that vascular endothelial growth factor receptor 2-mediated cell migration relies on HSP90-dependent phosphorylation of focal adhesion kinase. Additionally, the association of HSP90 with cytoskeletal dynamics has been reported by Pai et al. (50), who demonstrated that HSP90 may be essential for protease-activated receptor-1-mediated signaling to the cytoskeleton, which in turn is responsible for astrocyte morphology.

HSP90 exerts its function through the refolding of different proteins and thus contributes to various biological activities under nonstress conditions (51). Our combined data showing association of cell surface HSP90 with migration processes in the developing nervous system attribute a novel biological role to this molecule. Moreover and taking into consideration very recent findings demonstrating expression of HSP90 on the surface of malignant melanoma tumor cells (52), it is tempting to speculate that this protein may be involved in tumor cell invasion processes during cancer metastasis. Indeed, while this manuscript was under review, Eustace et al. (53) showed that HSP90α is localized on the surface of tumor cells and plays an important role in the metastatic process. What are the molecular mechanisms underlying cell surface HSP90 involvement in cell migration processes? We have previously argued that since the antigen recognized by mAb 4C5 is a peripheral protein loosely attached to the cell membrane (36), it is highly unlikely that it mediates transmembrane signaling (38). Moreover, we have suggested (38) that the molecule recognized by mAb 4C5, which in this work is identified as the HSP90 protein, may interact with other cell surface proteins, which through transmembrane signaling will trigger intracellular events necessary for cell migration. Our present findings support this suggestion and prompt us to further hypothesize that cell surface HSP90 may be necessary to maintain a proper conformation of membrane receptor(s), contributing thus to the ligand-receptor interaction, which in turn will activate the cytoplasmic signal transduction cascades, leading to cytoskeletal rearrangement essential for cell motility. However, interaction of HSP90 with extracellular signal molecules, which will lead to the proper orientation of these molecules for binding to membrane receptors, associated with cytoskeletal dynamics, cannot be excluded (Fig. 7). We are currently investigating these hypotheses.

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FIG. 7. Proposed working model concerning the involvement of cell surface HSP90 in cell migration. HSP90 localized on the cell surface either stabilizes the conformation of membrane receptor(s) so that it will interact with its ligand or functions as a scaffold orienting extracellular signals for binding to the membrane receptor. The receptor-ligand complex will in turn activate intracellular signal transduction cascades, leading to actin-based motility.
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