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Effects of Desmin Gene Knockout on Mice Heart Mitochondria

Monica Lindén,[1,2,5] Zhenlin Li,[3] Denise Paulin,[3] Takahiro Gotow,[4] and Jean-Francois Leterrier[1]

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In heart tissue from mice lacking the intermediate filament (IF) desmin, mitochondria show an abnormal shape and distribution (Thornell *et al.*, 1997). In the present study we have isolated heart mitochondria from desmin null (D−/−) and control (D+/+) mice, and analyzed their composition by SDS–PAGE, immunoblotting, and enzyme measurements. We found both *in vitro* and *in situ* that the conventional kinesin, the microtubule-associated plus-end directed motor, was frequently associated with D+/+ heart mitochondria, but not with D−/− heart mitochondria, suggesting that the positioning of mitochondria in heart is a dynamic event involving the IF desmin, the molecular motor kinesin, and, most likely, the microtubules (MT) network. Furthermore, an increased capacity in energy production was found, as indicated by a threefold higher creatine kinase activity in heart mitochondria from D−/− compared to D+/+ mice. We also observed a significantly lower amount of cytochrome *c* in heart mitochondria from D−/− mice, and a relocalization of Bcl-2, which may indicate an apoptotic condition in the cell leading to the earlier reported pathological events, such as cardiomyocytes degeneration and calcinosis of the heart (Thornell *et al.*, 1997).

KEY WORDS: Desmin; intermediate filament; mitochondria; creatine kinase; cytochrome *c*; Bcl-2; apoptosis; kinesin; porin.

INTRODUCTION

The phenotype of mice in which the desmin gene has been knocked out has been reported earlier (Li *et al.*, 1996; Capetanaki *et al.*, 1997). The main observations were that these mice developed normally and were fertile, but were weaker and fatigued more easily. Modifications were observed in skeletal muscles, such as the soleus and diaphragm and in the heart. Morphological abnormalities were observed in the diaphragm of adult mice and the heart exhibited hemorrhagic areas with fibrosis and ischemia. At the ultrastructural level, besides disorganization of fibrils, an abnormal subsarcolemmal accumulation of mitochondria, often in rounded shapes associated together in grape-like aggregates, was observed in the heart (Thornell *et al.*, 1997). Several reports have described drastic alterations in mitochondria distribution and shape upon disruption of cytoskeleton structures such as microtubules (MT) or intermediate filaments (IF) (Soltys and Gupta, 1992; Collier *et al.*, 1993). In the present work, we have studied changes in heart mitochondria associated with the absence of the intermediate filament desmin. Our results show several differences between D−/− and D+/+ heart mitochondria with respect to both mitochondrial composition and function.

MATERIALS AND METHODS

All chemicals were from Sigma. Electrophoresis molecular weight standard proteins were from BioRad. Desmin knock-out mice (D−/−) and control mice (D+/+), 3 months old, were obtained from D. Paulin and Z. Li (Department of Biochemistry, University Paris 7)

[1] Groupe de Biologie des Interactions Cellulaires, UMR CNRS 6558, Batiment de Biologie, 40, Avenue Recteur Pineau, 86022 Poitiers Cedex, France.
[2] Department of Biochemistry, University of Stockholm, S 10691 Stockholm, Sweden.
[3] UFR de Biochimie, Université Paris VII, 2 Place Jussieu, 75005 Paris, France.
[4] Laboratory of Cell Biology, College of Nutrition, Koshien University, 10-1 Momijigaoka, Takarazuka, Hyogo 665-0006, Japan.
[5] To whom all correspondence should be mailed. email address: Monica@dbb.su.se

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(Li *et al.*, 1996; Thornell *et al.*, 1997). Mitochondria were isolated according to Lee *et al.* (1979). A mixture of protease inhibitors were added as in Leterrier *et al.* (1994). Protein determination was performed according to Lowry *et al.* (1951).

Enzyme Measurements

Creatine kinase was measured using SIGMA diagnostic kit, procedure 520. Cytochrome oxidase was assayed by oxidation of reduced cytochrome *c* according to Ades and Cascarno (1977). Mitochondria were incubated 15 min in buffer containing 0.05% Tween 80 (Merck). The intactness of the mitochondrial outer membrane was estimated by measurement of cytochrome oxidase, as above, but in the absence of detergent.

SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting

Immunoblotting was performed according to Towbin *et al.* (1979). Proteins were separated on 10% SDS–polyacrylamide gels (Laemmli, 1970) and transferred to immobilon membranes (Amersham). The blots were probed with specific monoclonal or polyclonal antibodies. The same blot was processed either with several antibodies at the same time if molecular weight were largely different or was reprocessed after release of first antibody according to Gomez-Cambronero *et al.* (1992). HRP-conjugated second antibodies (DAKO, Glostrup, Denmark) were used and the bound antibody was detected by chemiluminescence (Amersham, Buckinghamshire, England).

Antibodies

A monoclonal antibody against kinesin SUK4 (gift from J. M. Sholey, University of California at Davis, Davis, CA) was used. The SUK4 antibody specifically recognizes the head domain of the conventional kinesin heavy chains and inhibits kinesin-dependent microtubules motion (Ingold *et al.*, 1988). The SUK4 monoclonal antibody specifically detects the conventional KHC in molecular motor fractions from central nervous tissues in which all types of kinesin-related molecules are recognized by a pan-kinesin polyclonal antibody (Yabe *et al.*, 1999).

A monoclonal antirat Bcl-2 antibody was used (gift from Y. Uchiyama, Osaka Medical School, Osaka, Japan). In an earlier work (Gotow *et al.*, 2000), we screened several commercial Bcl-2 antibodies and found that the monoclonal antirat Bcl-2 antibody was the best for recognizing Bcl-2 in rat and mouse brain.

Specific polyclonal antibodies against rat liver porin (Lindén *et al.*, 1984), ATP/ADP translocator (ANT) (gift from G. Brandolin, Grenoble, France), β-subunit of ATPsynthetase (gift from Y. Uchiyama, Osaka Medical School, Osaka, Japan), and subunit II of bc_1 complex (gift from B. D. Nelson, Stockholm University, Stockholm, Sweden) were used. Monoclonal antibodies to subunit I of human cytochrome oxidase and cytochrome *c* were from Molecular Probes (USA) and BD PharMingen (USA), respectively.

Quantification of Antibody Staining on Western Blots

Autoradiographs were scanned using videorecording and an imaging system (Candela). Obtained values were plotted and statistically analyzed using Graph Pad Prism 2.

Cryo Immunoelectron Microscopy

Anesthetized mice were perfused with 0.1% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), followed by 4% paraformaldehyde alone in the same buffer. Tissue fragments were immersed overnight in 4% paraformaldehyde in 0.1 M phosphate buffer. Smaller fragments were immersed in 1.89 M sucrose and 20% polyvinylpyrolidone (Sigma) in 0.1 M phosphate buffer and frozen with liquid nitrogen as described elsewhere (Tokuyasu, 1980; Gotow *et al.*, 1996). Thin frozen sections were obtained on a LKB ultramicrotome (Pharmacia, SW) and mounted on formvar carbon-coated nickel grids. Sections were rinsed with PBS, treated with 1% bovine serum albumin (BSA) and 1% goat nonimmune serum (Sigma) for 1 h, and incubated overnight with a mixture of polyclonal and monoclonal antibodies (diluted 1/100) in the same buffer with 1% BSA and goat serum. After extensive washing with PBS, sections were incubated for 1 h with a mixture of 5 nm gold-coupled goat anti-mouse and 15 nm gold-coupled goat anti-rabbit immunoglobulins (British Biocell International, UK) (diluted 1/20) in 1% BSA and goat serum in PBS before extensive washing with PBS. Sections were fixed with 2% glutaraldehyde in PBS, postfixed with 1% OsO_4 in H_2O, stained with 1% uranyl acetate before dehydration with ethanol solutions, and embedded in LR white resin (London Resin, UK) for examination in a Jeol 100 electron microscope (Jeol, Japan) at 80 kV.

Controls for nonspecific staining were done on sections treated following the same procedure, but in which the mixture of primary antibodies were omitted.

RESULTS

The abnormal distribution and shape of mitochondria observed in heart tissue from desmin knock-out mice prompted us to investigate their biochemical properties. Heart mitochondria were isolated from D−/− and D+/+ mice and their compositions analyzed by SDS–PAGE and by Western blot analysis and enzyme measurements. We found that purified heart mitochondria from desmin-null mice lack polypeptides with molecular masses close to 130 kD (Fig. 1). One of these was identified as the conventional kinesin (the microtubules associated plus-end directed motor) using a monoclonal antibody SUK 4 specific for the conventional kinesin heavy chain (Fig. 2). The presence or absence of kinesin on D+/+ and D−/− heart mitochondria, respectively, was also confirmed *in situ* by cryo immunoelectronmicroscopy of mice heart tissue (Fig. 3). Kinesin labeling was rare in D+/+ mice heart tissue, but frequently associated with mitochondria, while no labeling was found in D−/− mice heart (Fig. 3B). Kinesin is known to be associated with mitochondria in brain tissue where it drives mitochondrial motion in the axon. However, this is the first time that kinesin has been shown to be associated with mitochondria in muscle tissue.

The submitochondrial distribution of proteins from D+/+ and D−/− heart mitochondria was analyzed by immunoblotting using antibodies against nuclear and mitochondrial encoded proteins of the different mitochondrial compartments. As shown in Fig. 4, there is a significantly lower content of cytochrome *c* (33–45%) in D−/− relative to D+/+ heart mitochondria. No significant changes were observed in other proteins related to oxidative phosphorylation, such as ANT, subunits of the respiratory chain proteins, or the outer membrane protein porin (Figs. 2 and 4), indicating that neither mitochondria biogenesis nor the relative composition was affected by the subcellular redistribution of mitochondria associated with the absence of desmin (Li *et al.*, 1996; Thornell *et al.*, 1997).

It is possible that the lower content of cytochrome *c* observed in D−/− heart mitochondria is due to damage of the outer mitochondrial membrane. Therefore, intactness of the outer mitochondrial membrane was analyzed by measuring cytochrome oxidase activity with reduced cytochrome *c* in the presence or absence of detergent. No significant difference in cytochrome oxidase activity was observed in the presence of detergent between D+/+ and D−/− heart mitochondria (Fig. 5). However, in the absence of detergent, where holocytochrome *c* cannot enter an intact mitochondria, the cytochrome oxidase activity was higher in D+/+ relative to D−/− heart mitochondria (data not shown). This result suggests that the lower content of cytochrome *c* in D−/− heart

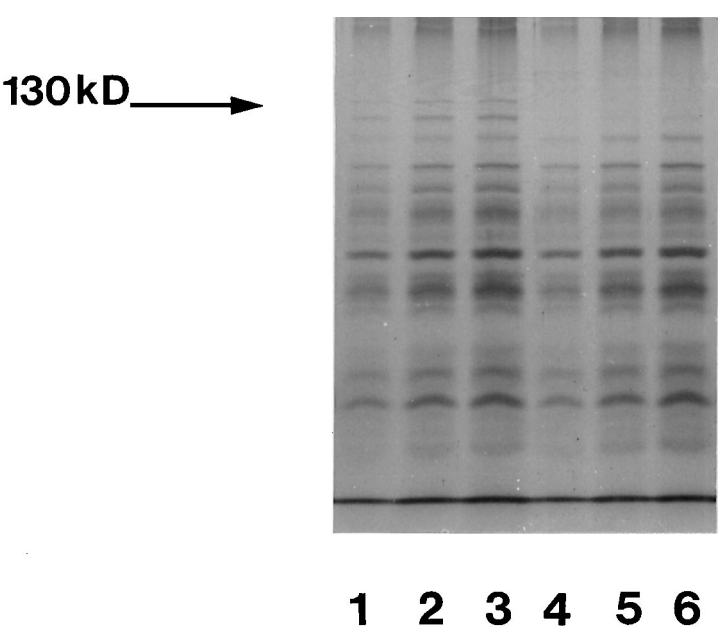

Fig. 1. SDS–PAGE 10% of purified heart mitochondria from D−/− and D+/+ mice mitochondrial proteins 20, 40, and 60 μg were applied. Lanes 1–3, D+/+ heart mitochondria; lanes 4–6, D−/− heart mitochondria. Gels were stained with Coomassie blue.

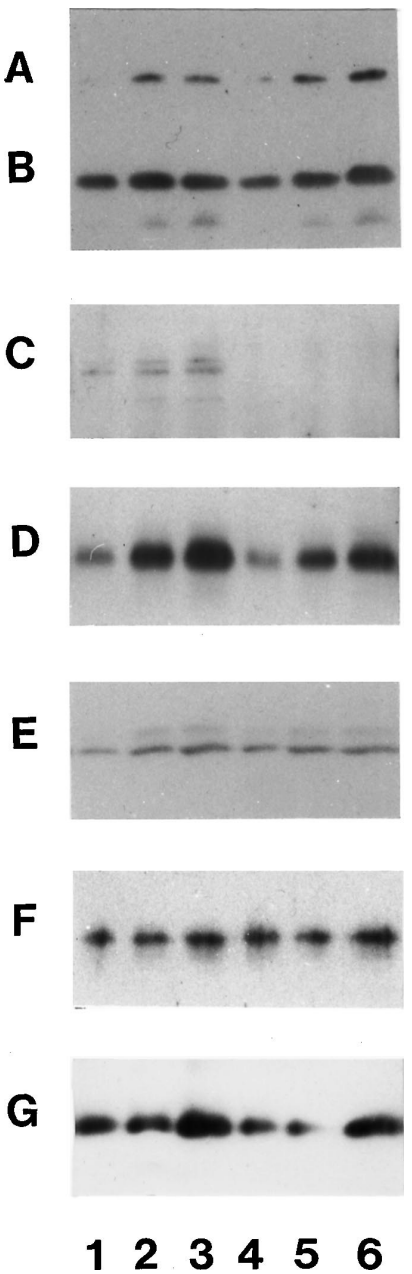

Fig. 2. Western blotting of purified heart mitochondria from D−/− and D+/+ mice. Mitochondrial proteins 20, 40, and 60 μg were applied. Lanes 1–3, D+/+ heart mitochondria; lanes 4–6, D−/− heart mitochondria. Specific antibodies were used together on the same blot or after release of first antibody. (A) β-subunit of ATPase; (B) porin; (C) kinesin; (D) cytochrome *c*; (E) ANT; (F) subunit I of cytochrome oxidase; (G) subunit II of bc_1 complex.

mitochondria is not due to leakage through damaged outer mitochondrial membranes.

Cytochrome *c* release from mitochondria is an early step in apoptosis, which is suggested to involve the proapoptotic proteins (BAX, BAK) and is inhibited by the antiapoptotic proteins of the Bcl-2 family (Shimizu *et al.*, 1999; Cook *et al.*, 1999; Doran and Halestrap, 2000). Our attempts to quantify the antiapoptotic protein Bcl-2 in purified heart mitochondria were unsuccessful. Bcl-2 was not detected by Western blotting, although the same antibody recognizes Bcl-2 in mouse and rat brain mitochondria (Gotow *et al.*, 2000) and rat liver mitochondria (data not shown). Several other commercially available antibodies were also used with the same result. Thus, Bcl-2 is either absent from heart mitochondria, i.e., another protein of the same family may regulate heart mitochondria, or it is rapidly degraded during heart mitochondria purification even in the presence of protease inhibitors. To discern between these possibilities, we performed cryo immunoelectronmicroscopy of fixed heart tissue. Interestingly, we found that Bcl-2 was present in both D+/+ and D−/− heart mitochondria, although labeling was rare, which might explain why we could not detect Bcl-2 by Western blotting. Furthermore, Bcl-2 was localized inside mitochondria in D+/+ heart tissue, but was found adjacent to the outer mitochondrial membrane and in the cytoplasm in D−/− heart tissue (Fig. 6). This is in agreement with our recent findings with mitochondria from wt- and Bcl-2-transfected PC12 cells, where Bcl-2 was localized inside mitochondria, but changed localization toward the outer mitochondrial membrane and the cytoplasm following an apoptotic stimulation induced by serum deprivation (Gotow *et al.*, 2000).

An important energy source in heart is creatine phosphate generated by mitochondrial creatine kinase. Mitochondrial creatine kinase is located in the mitochondrial intermembrane space where it uses ATP produced by mitochondria for phosphorylation of creatine to creatine phosphate (Lipskaya *et al.*, 1995). Creatine phosphate is subsequently exported to the cytosol for regeneration of ATP by the cytosolic isoforms of creatine kinase. As the redistribution of mitochondria in D−/− heart tissue (Li *et al.*, 1996; Thornell *et al.*, 1997) might be expected to influence mitochondria energy production, the activity of mitochondrial creatine kinase was measured. We found a threefold increase in creatine kinase activity in heart mitochondria isolated from D−/− mice when compared to D+/+ mice (Fig. 5). Cytochrome oxidase enzyme activity (Fig. 5) was measured in order to investigate if the high creatine kinase activity of D−/− heart mitochondria was accompanied by a similar increase in the activity of enzymes related to energy production of the respiratory chain and/or a modification in mitochondria protein content. The activity of cytochrome oxidase was identical in both types of mitochondria suggesting a true increase

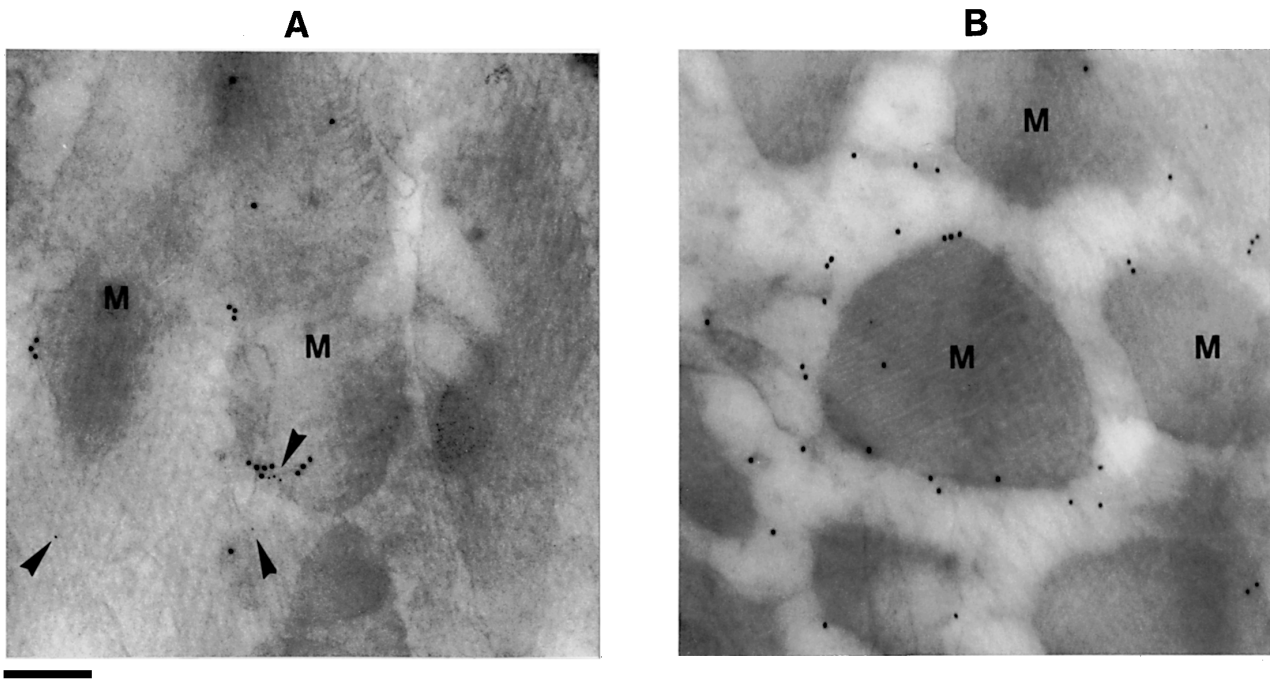

Fig. 3. Immunoelectronmicroscopy on cryosections of fixed mice heart tissue. Gold-labeled (5 and 15 nm) second antibodies were used for kinesin (SUK4) and porin antibodies, respectively. (A) D+/+ heart tissue; (B) D−/− heart tissue. Kinesin labeling was rare in D+/+, but was frequently associated with mitochondria, while no labeling was found in D−/−. In D−/− heart tissue, the ultrastructure of mitochondria is altered. The shape of mitochondria is changed and they are not surrounded by ordered arrays of myofibrils. M, mitochondria, Arrowheads, kinesin-associated labels. Bar = 0.25 μm.

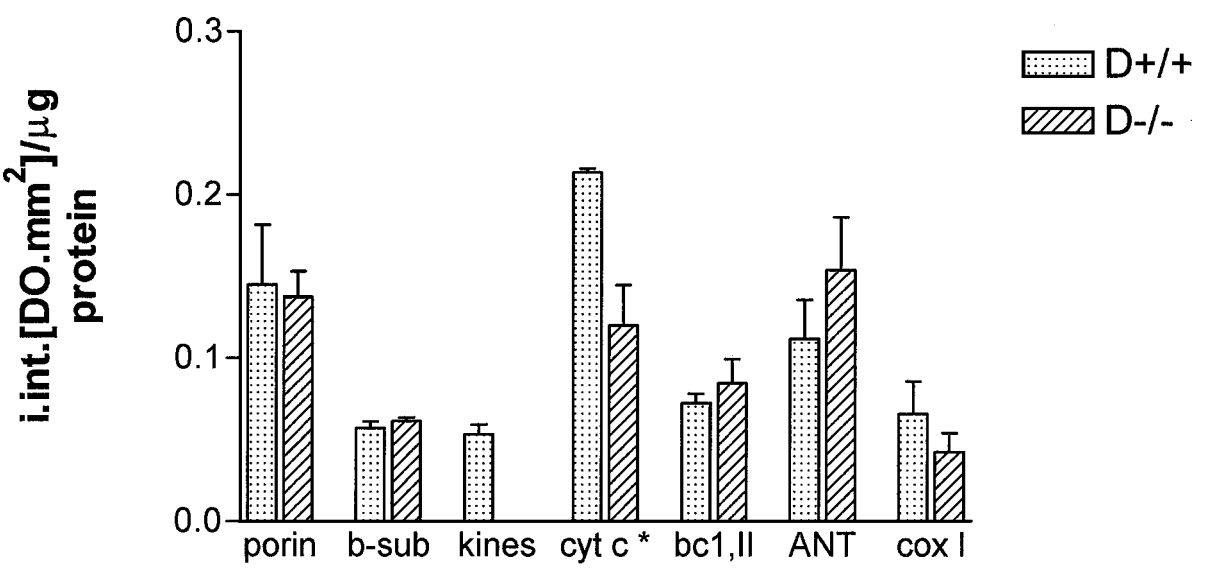

Fig. 4. Quantification of mitochondrial proteins from D+/+ and D−/− heart mitochondria. Autoradiographs from western blotting were scanned and labeling intensities per microgram mitochondrial protein were calculated and plotted. The results are means of three different measurements ± SD. (*) Values statistically different ($p < 0.005$) versus controls.

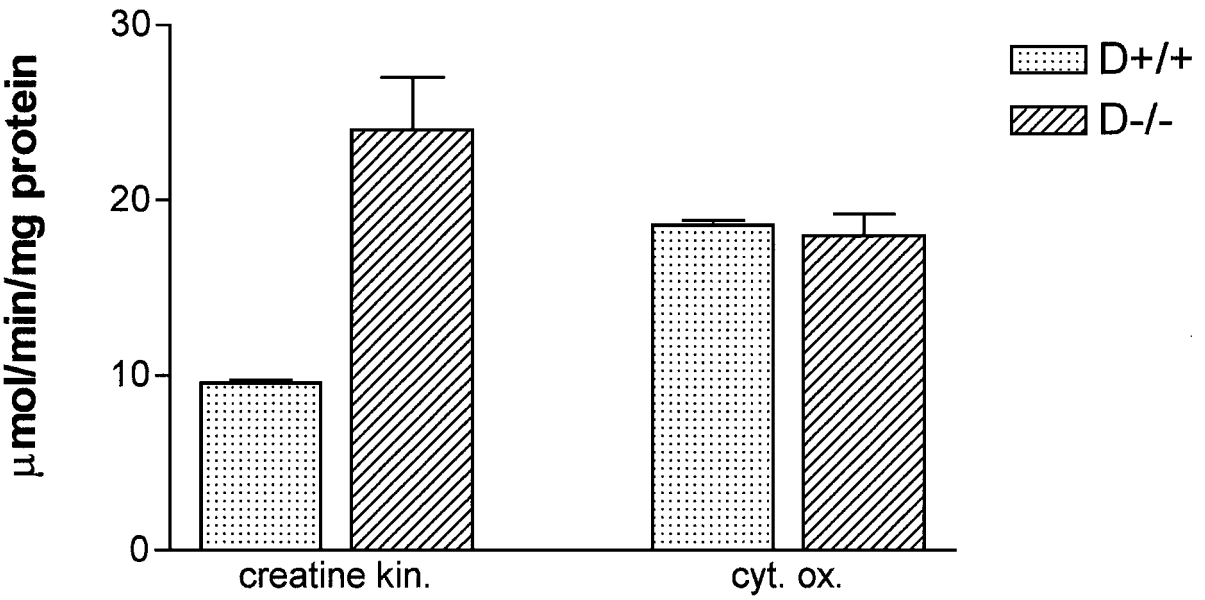

Fig. 5. Creatine kinase and cytochrome oxidase activities in heart mitochondria isolated from D−/− and D+/+ mice. The results are means of three different measurements ± SD. (*)Values statistically different ($p < 0.005$) versus controls.

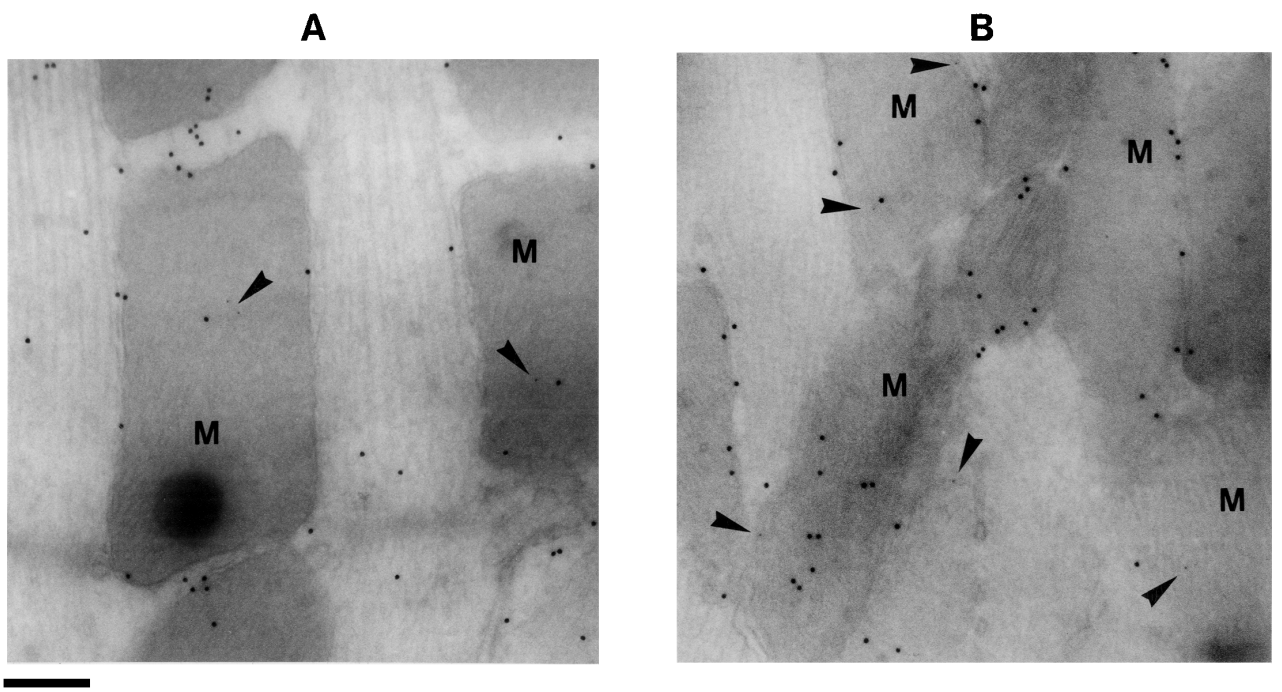

Fig. 6. Immunoelectronmicroscopy on cryosections of fixed mice heart tissue. Gold-labeled (5 and 15 nm) second antibodies were used for Bcl-2 antibodies and porin antibodies, respectively. (A) D+/+ heart tissue. (B) D−/− heart tissue. Bcl-2 labeling was rare but associated with the inner membrane of D+/+ mitochondria, while in D−/− mitochondria, Bcl-2 was found close to the outer mitochondrial membrane and in the cytoplasm. M, mitochondria. Arrowheads, Bcl-2-associated labels. Bar = 0.25 μm.

in creatine kinase activity in D−/− relative to D+/+ heart mitochondria (Fig. 5).

DISCUSSION

Desmin Knockout Alters the Distribution of Two Mitochondria-Associated Proteins

In this study, we found that the knockout of the desmin gene results in modifications of the presence and the distribution of two proteins associated with heart mitochondria, Bcl-2, and kinesin.

The conventional MT-dependent molecular motor kinesin is associated with the outer mitochondrial membrane in heart tissues from control mice (Fig. 3) and remains bound to the purified organelle *in vitro* (Fig. 2). This is, to our knowledge, the first evidence for the association of kinesin with mitochondria in heart tissue. Since heart mitochondria are stably localized between myofibrils, the finding that kinesin is associated with mitochondria from normal mouse heart raises new questions regarding the physiological significance of this localization, among which is the intriguing possibility that the distribution of mitochondria in muscle cells is more dynamic than previously thought. Muscle mitochondria are systematically found associated with specific subcellular domains such as the Z line (Stromer and Bendayan, 1988, 1990; Tokuyasu *et al.*, 1983a,b; Ogata and Yamasaki, 1997), suggesting that they are immobilized through stable linkages to the local cytoskeleton. Translocation of mitochondria mediated by molecular motors occurs along MT or/and MF in all cell types studied. Active transport of mitochondria along MT has been studied in axons of neurons where it involves two types of kinesin motors, including the conventional kinesin which copurifies with purified mitochondria (Elluru *et al.*, 1995; Jellali *et al.*, 1994; Leopold *et al.*, 1992; Nangaku *et al.*, 1994). Thus, it is likely that kinesin associated with heart mitochondria is involved in mediating dynamic interactions between the organelle and MT. However, the present study suggests a link between the absence of IF desmin and the disappearance of bound kinesin from mitochondria *in vitro* and *in situ* (Figs. 2 and 3) and open the possibility that normal IF organization is required for the kinesin-mediated interactions between MT and mitochondria. IF and MT networks are interdependant in most cell types (Prahlad *et al.*, 1998; Yabe *et al.*, 1999; Gyoeva and Gelfand, 1991). Accordingly, the absence of mitochondrially associated kinesin in D−/− mice heart (Figs. 2 and 3) might be the direct consequence of MT disorganization induced by the absence of desmin. Alternatively, it is possible that kinesin mediates interactions between desmin IF and mitochondria in D+/+ heart tissue, which would be disrupted in D−/− heart tissue. However, this hypothesis is unlikely since there is currently no evidence for IF-dependent motility of intracellular organelles. Nevertheless, the present observations suggest that heart mitochondrial positioning to restricted subcellular domains, parallel to the myofibrils, is a dynamic event involving the IF desmin, the molecular motor kinesin and most likely the MT network. The possible interdependence between IF and MT for maintenance of mitochondrial location in heart muscle cells could be approached by analyzing the dynamic behavior of mitochondria in cardiomyocyte cell cultures from D+/+ and D−/− mice.

All attempts to detect the antiapoptotic protein Bcl-2 in purified heart mitochondria from both D−/− and D+/+ mice by immunoblotting were unsuccessful, although the antibodies used detected Bcl-2 by immunoblotting of both mouse and rat brain mitochondria (Gotow *et al.*, 2000), as well as rat liver mitochondria (unpublished data). The lack of staining was found to be most likely due to a small amount of Bcl-2 in heart tissue (Fig. 6). This is in agreement with the findings by Abe-Dohmae *et al.* (1993) who showed that the expression of the bcl 2 gene is tissue specific. They found that Bcl-2 is mainly expressed in the central nervous system and in the immune system. According to Abe-Dohmae *et al.* (1993), the expression of Bcl-2 mRNA in skeletal muscle is not detectable and a fourfold lower level was found in the heart in comparison to the brain. Interestingly, we found by immunoelectronmicroscopy of fixed heart tissue that Bcl-2 was localized inside mitochondria in D+/+ heart tissue and that this localization was modified in D−/− heart tissue in which the gold-labeled epitopes were found at the periphery of mitochondria and in the cytoplasm (Fig. 6B). This situation is similar to our recent findings with preapoptotic PC12 cells, in which the antiapoptotic protein Bcl-2 is relocalized from the inner mitochondrial membrane to the outer membrane and endoplasmic reticulum in serum-restricted PC12 cells (Gotow *et al.*, 2000). Thus, the relocalization of Bcl-2 in D−/− heart mitochondria may indicate a preapoptotic condition. This is further supported by a low content of cytochrome *c* in D−/− mitochondria which does not appear to be associated with outer membrane damage (see below). It is not known if the low cytochrome *c* content is related to a preapoptotic condition, in which case one might expect a release of cytochrome *c* via mechanisms that are not fully understood, although not involving a physical damage of the mitochondrial outer membrane (Shimizu *et al.*, 1999; Cook *et al.*, 1999; Doran and Halestrap, 2000; von Ahsen *et al.*, 2000).

Selective Effects of Desmin Gene Knockout on Mitochondrial Proteins

Knockout of the desmin gene does not affect the composition of markers of the outer or inner mitochondrial membranes. The amounts of the outer (porin) and the inner membrane proteins (ANT, subunits of ATP synthetase, cytochrome oxidase, and bc_1 complexes) were unchanged in heart mitochondria from D−/− mice (Fig. 4). Similarly, cytochrome oxidase activity was unaltered (Fig. 5). This is in strong contrast to the behavior of two soluble proteins of the intermembrane space. Knockout of the desmin gene caused a significant decrease in the amount of cytochrome *c* (weakly associated to the outside of the inner membrane) and a large increase in the activity of creatine kinase (weakly associated to the inside of the outer membrane) (Figs. 4 and 5). From these observations, we can assume that the loss of cytochrome *c* does not reflect physical damage of the outer membrane in D−/− heart mitochondria since the intermembrane soluble enzyme creatine kinase is higher in D−/− than in D+/+ heart mitochondria. This is further supported by the test for intactness of the outer mitochondrial membrane, showing a higher degree of intactness (estimated from cytochrome oxidase activity in the absence of detergent) of D−/− mitochondria in comparison with control, and by an identical amount of the outer membrane marker protein (porin) in the two mitochondrial preparations (Fig. 4).

The present data suggest that different mechanisms may be involved in the decrease of cytochrome *c* and the increase in creatine kinase activity in D−/− heart mitochondria. The lower content of cytochrome *c* in D−/− heart mitochondria could be due to a release triggered by an apoptotic condition, as reported elsewhere (Shimizu *et al.*, 1999; Cook *et al.*, 1999; Doran and Halestrap, 2000; von Ahsen *et al.*, 2000). This possibility receives support from the finding that the redistribution of Blc-2 observed in D−/− heart tissue (Fig. 6) occurs in a manner similar to that of preapoptotic PC12 cells (Gotow *et al.*, 2000). The threefold increase in creatine kinase activity in heart mitochondria from D−/− mice (Fig. 5) might be the consequence of a compensatory local increase in energy production similar to that observed in mitochondria from hypertrophied left ventricles of spontaneously hypertensive rats (Seccia *et al.*, 1998) and in heart mitochondria from rats with chronic anemia (Field *et al.*, 1994). The increase in creatine kinase activity seems neither to be coupled to a change in cytochrome oxidase activity (Fig. 5) nor to a difference in respiratory activity, which was recently reported to be similar in heart mitochondria isolated from D−/− and D+/+ mice (Milner *et al.*, 2000). In contrast, the significantly lower amount of cytochrome *c* (33–45%) in D−/− heart mitochondria may be expected to impair respiration. However, it has been shown that release of as much as 40% of the cytochrome *c* from brain mitochondria caused only a moderate decrease in mitochondrial membrane potential (Andreyev *et al.*, 1998). Whether the low cytochrome *c* content in D−/− mitochondria is due to a lower expression of the protein or to a release mediated by an apoptotic signaling process, needs further study.

Physiological Issues of the Knockout of Desmin in Heart Tissue

The cellular distribution of mitochondria is related to the local energy needs of the cell (Bereiter-Hahn and Vöth, 1994). Several reports show a disorganized distribution of mitochondria and changes in mitochondrial shape following the disruption of the MT and IF network (Soltys and Gupta, 1992; Collier *et al.*, 1993). The abnormal subsarcolemmal accumulation of mitochondria observed in D−/− heart tissue (Thornell *et al.*, 1997; Milner *et al.*, 2000) suggests a similar dependence of mitochondrial distribution on the integrity of the IF-MT cytoskeleton in muscle cells. The anchorage of muscle mitochondria to the IF network of the Z line may involve plectin, recently shown to mediate desmin–mitochondria interactions (Reipert *et al.*, 1999). The lack of desmin in skeletal muscles would result in a weaker link between the remains of the Z line and mitochondria through plectin, as the subcellular distribution of plectin has been shown recently to be unchanged in skeletal muscles of D−/− versus D+/+ mice (Carlsson *et al.*, 2000). The present findings suggest the following cascade of events leading to the drastic morphological changes observed in the heart of D−/− mice (Thornell *et al.*, 1997). In contrast to mitochondria in skeletal muscle, which usually are localized in pairs at the Z lines of the sarcomere, heart mitochondria are packed in rows between myofibrils (Milner *et al.*, 2000) along which MT are aligned perpendicular to desmin IF (Watkins *et al.*, 1987). This suggests a kinesin- and MT-dependent association of mitochondria to the myofibrils. Thus the disorganization of Z lines and myofibrils that take place in the absence of desmin in heart (Li *et al.*, 1996) may result in the lack of a kinesin- and MT-dependent association of mitochondria to the myofibrils, and an insufficent supply of ATP for myofibrils contraction. Furthermore an increase in mitochondrial creatine kinase activity in D−/− heart mitochondria may be a way to compensate for the lack of mitochondria close to the contractile process. Thus, the repositioning of mitochondria away from sites where ATP is used in large amounts could provoke the formation of preapoptotic signals (release of cytochrome *c*

and Bcl-2 translocation) driving cardiomyocyte degeneration and calcinosis of the heart in D−/− mice (Thornell *et al.*, 1997).

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