Green fluorescent proteins (GFP) are widely used in biology for tracking purposes. Although expression of GFP is considered to be innocuous for the cells, deleterious effects have been reported. We recently demonstrated that expression of eGFP in muscle impairs its contractile properties (Agbulut, O., Coirault, C., Niederlander, N., Huet, A., Vicart, P., Hagege, A., Puceat, M., and Menasché, P. (2006) Nat. Meth. 3, 331). This prompted us to identify the molecular mechanisms linking eGFP expression to contractile dysfunction and, particularly, to test the hypothesis that eGFP could inhibit actin-myosin interactions. Therefore, we assessed the cellular, mechanical, enzymatic, biochemical, and structural properties of myosin in the presence of eGFP and F-actin. In vitro motility assays, the maximum actin-activated ATPase rate ($V_{max}$) and the associated constant of myosin for actin ($K_m$) were determined at 1:0.5, 1:1, and 1:3 myosin:eGFP molar ratios. At a myosin:eGFP ratio of 1:0.5, there was a nearly 10-fold elevation of $K_m$. As eGFP concentration increased relative to myosin, the percentage of moving filaments, the myosin-based velocity, and $V_{max}$ significantly decreased compared with controls. Moreover, myosin co-precipitated with eGFP. Crystal structures of myosin, actin, and GFP indicated that GFP and actin exhibited similar electrostatic surface patterns and the ClusPro docking model showed that GFP bound preferentially to the myosin head and especially to the actin-binding site. In conclusion, our data demonstrate that expression of eGFP in muscle resulted in the binding of eGFP to myosin, thereby disturbing the actin-myosin interaction and in turn the contractile function of the transduced cells. This potential adverse effect of eGFP should be kept in mind when using this marker to track cells following transplantation.

Green fluorescent protein (GFP) and its variants are widely used to visualize organs, cell populations, protein localizations, and subcellular processes in in vitro and in vivo studies. In particular, in cell transplantation studies, these reporter proteins are used to quantify engraftment of transplanted cells, monitor their phenotypic changes and correlate these findings with their functional effects (2, 3). The GFP reporter proteins emit fluorescence without any external treatment, which allows visualization of gene expression within living cells. Although expression of GFP is considered to be innocuous for the cells, cytotoxic effects involving apoptotic mechanisms have been demonstrated in several in vitro studies (4, 5). It has also been reported that transgenic expression of GFP caused a dilated cardiomyopathy in two independent transgenic mouse lines (6) and dose-dependent co-expression of eGFP and β-galactosidase in the cytoplasm of forebrain neurons resulted in growth retardation, weakness, and premature lethality (7).

Recently, we reported that cytoplasmic expression of eGFP in muscle cells impairs their contractile function (1). Briefly, we demonstrated that although eGFP-transduced (using a lentiviral vector, ppt-PGK-eGFP-WPRE) rat myoblasts actively proliferate, differentiate, and fuse to form multinucleated myotubes in culture, the percentage of spontaneously contracting myotubes in eGFP-transduced cells was two times lower compared with their non-transduced counterparts. In addition, using a rat model of myocardial infarction, after myocardial transplantation of eGFP- or non-transduced myoblasts, we found that eGFP-transduced skeletal myoblasts yielded a significantly poorer echocardiographic recovery of left ventricular function than their non-transduced counterparts. These data were deemed to be consistent with previous findings of Huang et al. (6) that the transgenic expression of GFP can cause a dilated cardiomyopathy.

The present study was designed to identify the molecular mechanisms linking eGFP expression to contractile dysfunction and, more specifically, to test the hypothesis that eGFP could inhibit actin-myosin interactions. To gain further insights into potential interactions between GFP and myosin, we assessed the cellular (electromechanical coupling), mechanical (in vitro motility assay), enzymatic (actin-activated ATPase rate), biochemical (co-precipitation), and structural properties of myosin in the presence of eGFP and F-actin.

**EXPERIMENTAL PROCEDURES**

Isolation, Preparation, and Transduction of Skeletal Myoblasts—Myoblasts were expanded from newborn Sprague-Dawley rats, as previously described (8) and transduced with a third-generation lentiviral vector (ppt-PGK-eGFP-WPRE) expressing eGFP cDNA under control of a phosphoglycerate kinase promoter by using a multiplicity of infection of 25 in the
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presence of 2 μg/ml Polybrene (Sigma). After a 10-h incubation at 37 °C and 5% CO₂, the medium was replaced with fresh medium. Two days later the cells were harvested by trypsination. The percentage of eGFP-expressing myogenic cells was assessed by fluorescence-activated cell sorter using anti-CD56 (BD Biosciences) and desmin (Dako) antibodies.

Electromechanical Coupling of Cultured Myotubes—Myoblasts were differentiated into myotubes in 2% fetal calf serum. The cells were then loaded with 5 μM fluo-4/AM (Molecular Probes, Eugene, OR) for 10–15 min, transferred to the stage of a fast-acquisition epifluorescence confocal microscope, and superfused with HEPES-buffered Hanks’ solution supplemented with 2 mM Ca²⁺. The field was illuminated at 488 nm (Argon laser). Fluo-4 emission fluorescence was recorded through a dichroic mirror (cutoff 510 nm) and a long pass emission filter (cutoff 520 nm) set on a motorized filter wheel. The images were recorded every 30 ms using a confocal PerkinElmer Ultraview microscope coupled to a CCD camera (Coolsnap, Princeton) and digitized on line by a computer (Metamorph software, Universal Imaging, Downingtown, PA). To calculate the frequency and synchronization of Ca²⁺ spikes in the myotubes, regions of interest were selected in cells and the average pixel intensity was plotted as a function of time. To estimate cell shortening, a region of interest was set at the border of the cell so that each contraction resulted in a negative fluorescence signal. Cells featuring shortening were also counted in the culture dish on the stage of an inverted microscope. All experiments were performed at 35 ± 2 °C and were repeated three times.

In Vitro Motility Assays—In vitro motility assays were performed using purified rabbit heavy meromyosin (HMM) and phalloidin-labeled rabbit F-actin (9, 10). HMM (containing two myosin heads that are the functional motor domain of myosin) was obtained by a proteolytic digestion with α-chymotrypsin of skeletal myosin according to the method of Margossian and Lowey (9). G-actin was extracted as described by Pardee and Spudich (10) from acetone powder prepared from the residues of rabbit muscles after myosin extraction. After polymerization, F-actin was labeled by incubation with phalloidin FluorProbes 547 (Interchim, France) as described by Kron and Spudich (11). Motility assays were recorded at 29 °C as previously described (12). In brief, HMM was diluted to 0.1 mg/ml in myosin buffer (300 mmol/liter KCl, 25 mmol/liter imidazole, 4 mmol/liter MgCl₂, 1 mmol/liter dithiothreitol, and 1 mmol/liter EGTA, pH 7.4). eGFP (Clontech) was infused into the flow cell along with myosin at the following molar ratios: 1:0.5 myosin:eGFP (≈0.5), 1:1 myosin:eGFP (≈1), and 1:3 myosin:eGFP (≈3). Control samples had no added eGFP. Unbound myosin was washed out with myosin buffer supplemented with 0.5 mg/ml bovine serum albumin. Actin buffer consisting of 25 mmol/liter KCl, 25 mmol/liter imidazole, 1 mmol/liter EGTA, 4 mmol/liter MgCl₂, 10 mmol/liter dithiothreitol, and 1 mg/ml glucose oxidase, and 3 mg/ml glucose). The movement of actin filaments was observed after a stabilization period of 5 min under a Zeiss epifluorescence microscope (Axiovert 200, 100/1.30 lens, Jena, Germany) with an intensified camera (Hamamatsu C 2400, Hamamatsu City, Japan) and recorded on videotape. The percentage of moving filaments and the mean velocity of each filament were analyzed using N. J. Carters freeware RETRAC program. This protocol was chosen after preliminary experiments showing that the timing of eGFP infusion did not influence the results.

Actin-activated ATPase Assays—Actin-activated ATPase activity was determined at 29 °C as previously described (14). Briefly, myosin diluted to 0.1 mg/ml in actin buffer was mixed with eGFP at the molar ratios specified. Actin was added to a final concentration of 0, 5, 10, and 30 μmol/liter and the reaction was started with 2 mmol/liter MgATP. Malachite green reagent was used to determine Pᵢ production. Observed ATPase rates were corrected for ATP hydrolysis in the absence of actin. The maximum actomyosin ATPase activity (Vmax, s⁻¹) and the association constant of myosin for actin (Kₘₐₜ, μmol) were determined from a double-reciprocal Lineweaver-Burk plot of the ATPase rate versus actin concentration.

Co-precipitation Assay—Six histidine (His₆)-tagged recombinant eGFP, skeletal muscle myosin (HMM), cardiac muscle myosins or different myosin:eGFP mixtures (1:1) were incubated with nickel-coated agarose beads (HIS-Select Nickel Affinity assay, Sigma) in reaction buffer (50 mM sodium phosphate, 0.3 M sodium chloride, 10 mM imidazole, pH 8). The presence of His₆ residues in recombinant eGFP allows to interact specifically with nickel-coated agarose beads, whereas myosin alone does not. Following centrifugation (3,000 × g for 30 s) and two washings with reaction buffer, the binding proteins were eluted with 250 mM imidazole. After removing the nickel-coated agarose beads by centrifugation, the proteins were separated on 10% SDS-PAGE. Following migration, the gels were silver stained. Western blot analysis was carried out using anti-myosin heavy chain antibody (MHC-s, Novocastra, Newcastle, United Kingdom). Antibody reacting bands were visualized following development with peroxidase-labeled horse anti-mouse Ig (Vector Labs, CA) and a chemiluminescent detection system (Super Signal, Pierce Biotechnology). Rat cardiac muscle myosin was prepared by standard methods (15).

Docking of Myosin-GFP Complex—The crystal structure of the skeletal muscle myosin (Protein Data Bank 2MYS, 16), GFP (1EMA, 17), actin (1ATN, 18), and the model of actin-myosin complex obtained from electronic microscopy (1ALM, 19) were downloaded from the Protein Data Bank. Electrostatic potential was calculated with GROMOS96 implementation of the Swiss PDB viewer. Models of association of eGFP with myosin were generated by the fully automatic ClusPro protein-protein docking server (20). First, a rigid body docking is performed, using ZDOCK (21) based on the fast Fourier transform correlation techniques. ZDOCK uses a scoring function based on shape complementarities, electrostatic potentials, and desolvation terms. Second, filtering is performed using empirical free energy functions and pairwise root mean square deviation clustering. The ligand with the most neighbors is the cluster center, which is then minimized by the CHARMM algorithm in the presence of the
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FIGURE 1. Electromechanical coupling of cultured wild-type (a) and eGFP-expressing (b) myotubes. Myotubes were loaded with 5 μM Fluo-4. Ca2+ transients were monitored using fast acquisition confocal microscopy. Recordings show Ca2+ spikes within selected regions of interest and are expressed as ΔF(F – F0) in arbitrary units (A.U) where F0 is the lowest basal fluorescence recorded at the beginning of the experiment. c, cell shortening was recorded from the changes in fluorescence in the rectangular regions of interest. d, the percentage of spontaneously contracted myotubes in wild-type and eGFP-expressing myotubes.

FIGURE 2. Myosin-eGFP interaction: in vitro motility assays. Percentage of sliding actin filaments (a) and sliding velocity (b) after myosin was incubated with eGFP at 1:0 (Control), 1:0.5 (0.5x), 1:1 (1x), and 1:3 (3x) myosin:eGFP molar ratios. Values are mean ± S.E., *p < 0.01.

RESULTS

To determine the contractile properties of eGFP-transduced and non-transduced skeletal myotubes, we performed electromechanical recordings of excitation-contraction coupling in an in vitro setting (Fig. 1). Ca2+ transients were recorded in both Fluo-4-loaded non-transduced and eGFP-expressing myotubes. We found that the same percentage of both eGFP-transduced and non-transduced cultured myotubes featured spontaneous calcium transients. However, whereas calcium transients were translated into contractions in 39 ± 1% of the non-transduced cells, this percentage was lower (21 ± 1%) in the eGFP-transduced subset, thereby providing evidence, at the single cell level, for an eGFP-induced impairment of electromechanical coupling (n = 9/group, analysis of variance, p < 0.001). We postulated that this excitation-contraction impairment could be due to an inhibition of actin-myosin interactions by the nonspecific sarcemeric binding of the fluorescent protein. To explore this hypothesis, we analyzed both the mechanical (in vitro motility assay) and enzymatic (actin-activated ATPase rate) properties of myosin in the presence of eGFP.

In vitro motility assays were performed using skeletal myosin (HMM), phalloidin-labeled rabbit F-actin filaments, and recombinant eGFP. Fig. 2 illustrates the effect of increasing amounts of eGFP on actin filament sliding over myosin. As eGFP concentration increased relative to myosin, the percentage of moving filaments decreased by 27 ± 5% compared with that seen in the group with no added eGFP (controls) (Fig. 2a) (from 91 ± 1% at baseline (control) to 66 ± 4% moving filaments at 1:1 myosin:eGFP ratio, p < 0.05). At 1:3 myosin:eGFP ratios, only 9 ± 3% of actin filaments were still in movement (Fig. 2a). In the presence of a myosin:eGFP ratio of 1:1, the actin filament velocity was 28% slower than the control rate (3.3 ± 0.1 μm s⁻¹ versus 2.4 ± 0.1 μm s⁻¹ in control and 1:1 myosin:eGFP ratio, respectively, p < 0.05). Increasing the myosin:eGFP ratio to 1:3 induced further decreases in sliding velocity (1.6 ± 0.1 μm s⁻¹, p < 0.05 versus control). No significant changes in filament velocity and percentage of moving filaments were observed at a myosin:eGFP ratio of 1:0.5 (Fig. 2a and b). Lineweaver-Burk plots were used to determine the maximum actin-activated ATPase rate ($V_{\text{max}}$, in s⁻¹) and the associated constant of myosin for actin ($K_m$, in μM) values (Fig. 3, a–c). The actin-activated ATPase rate was determined using purified myosin, F-actin, and eGFP. The presence of a myosin:eGFP ratio of 1:0.5 was associated with a nearly 10-fold elevation of $K_m$ for actin compared with control myosin (80 ± 9.0 versus 8 ± 3 μM, p < 0.05), whereas $V_{\text{max}}$ did not significantly differ between these 2 groups (16.5 ± 2.0 s⁻¹ in control versus 23.2 ± 3.0 s⁻¹ in 1:0.5). As the eGFP concentration increased relative to myosin, $V_{\text{max}}$ decreased dramatically, reaching 6.2 ± 1.8 s⁻¹ and 0.5 ± 0.1 s⁻¹ at 1:1 and 1:3 myosin:eGFP ratios, respectively (p < 0.05). Compared with control, there were no significant changes in $K_m$ at myosin:eGFP ratios of 1:1 and 1:3 (17 ± 6 and 3 ± 1 μM, respectively) (Fig. 3b).

We also used a histidine-select nickel affinity assay to determine whether myosin co-precipitated with eGFP (Fig.
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Our results demonstrated that when mixed with eGFP, the myosin was detected by gel electrophoresis and Western blot analysis (Fig. 4). In contrast, when myosin interacts with nickel-coated agarose beads without eGFP, no myosin was detected. It should be noted that these results are identical with skeletal (HMM) and whole cardiac myosin monomers. These results indicate that myosin co-precipitated with eGFP and also underlined that eGFP bound preferentially to the HMM part of myosin.

Finally, crystal structures of myosin (2MYS), actin (1ATN), and GFP (1EMA) obtained from the Protein Data Bank were analyzed to gain further insights into myosin and GFP interactions. We first found that GFP and actin proteins exhibited similar electrostatic surface patterns (Fig. 5, a and b). It is important to note that actin exhibits mainly negative electrostatic surface potential patterns at the myosin-binding site, whereas myosin exhibits a positive electrostatic surface pattern at the actin-binding site. Therefore, the rigid-body docking program ZDOCK filtered and ranked by ClusPro was used to establish docking models of actin-myosin and GFP-myosin. ZDOCK takes into account the electrostatic potential as well as shape complementarities. To confirm our docking model, we first compared our actin-myosin docking model with the model of actin-myosin complex constructed from electron microscopy images (19) (Fig. 5c). Our results showed that the docking model was close to the electron microscopy model with a root mean square deviation value of about 17 Å. After validation of our docking model, the same protocol was used to establish the model of myosin-GFP complex that is presented in Fig. 5d.

Energetic and surface analysis of the complex myosin-GFP and actin-myosin are given on Table 1. Interface ASA of the complex is slightly higher for actin-myosin complex than for the myosin-GFP (Table 1). In particular, residues 538–561 of myosin are involved in interaction with both actin and GFP. Knowing the residues involved in the interaction, we calculated the energy associated to electrostatic interactions compared with other non-bonded interactions for these residues. Our results showed that the main stabilizing forces of both complexes are the electrostatic ones that confirm the importance of these forces for actin-myosin and myosin-GFP complex formation.

**DISCUSSION**

In the present study we have examined how eGFP reporter gene expression in muscle cells impaired muscular contractile function. To identify the molecular mechanisms involved, we analyzed (i) the *ex vivo* contractile performance and calcium signals in eGFP expressing skeletal myoblasts, (ii) the *in vitro* effects of purified recombinant eGFP on myosin function, and (iii) the crystal structures of myosin, actin, and GFP. Taken together, we showed that excitation-contraction uncoupling and impaired contractile function of the muscle cells were related to eGFP competition with actin for the myosin-binding site.

GFP and its variants are widely used in a variety of experiments in cell biology. When the GFP gene is co-transfected with a gene of interest, it serves as a marker of co-transfection. In addition, transfected cells expressing GFP can be easily identified in host animals. GFP has also been used as an intracellular indicator. When the GFP gene is used as a tag by fusing it with a gene of interest, the localization of the passenger protein can be determined. Although GFP and its variants are particularly attractive, in each situation care must be taken and the cellular and molecular effects of these reporter proteins must be carefully investigated. In fact, some studies have reported deleterious effects of GFP expression in *in vitro* (4–5) and *in vivo* (1, 6, 7). Liu et al. (4) demonstrated cytotoxic effects of GFP expression in NIH3T3, BHK-21, Huh-7, and HepG2 cell lines. In their study, different cell lines were transfected with three GFP-containing plasmids, under the control of the SV40 promoter, and the cells expressing GFP were found to die within a few days. Moreover, it has been reported that transgenic overexpression of GFP caused a dilated cardiomyopathy in...
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The crystal structure of the skeletal muscle myosin (2MYS), GFP (1EMA), actin (1ATN), and the model of actin-myosin complex obtained from electron microscopy (1ALM) were downloaded from the PDB. These models were generated by the fully automatic ClusPro protein-protein docking server and manually selected on the basis of biological knowledge. Models were visualized using Visual Molecular Dynamics for docking models and Deep View for electrostatic potential. The electronic potentials are those of the protein-protein interaction site and were calculated using Deep View with GROMOS96 implementation of Swiss PDB viewer. Electrostatic potential surfaces of actin (a) and GFP (b) at the myosin binding interface. Blue represents positive potential; red, negative; and white, neutral. Docking models of actin-myosin (c) and myosin-GFP (d) complex. Red represents myosin head; green, GFP protein; blue, actin for the model of actin-myosin complex obtained by electron microscopy; yellow represents actin for the docking model of actin-myosin complex.

TABLE 1

| Interface ASA and energy of electrostatic and others in the models of myosin-GFP and actin-myosin complexes |
|---------------------------------------------------------------|
|                                                                                                          |
| After determination of residues involved at the interface of the complexes, the energy associated to these residues was calculated using the GROMOS96 implementation of Swiss PDB viewer. |
|                                                                                                          |
| | Interface ASA | Non-bonded | Electrostatic |
| |                  | $\AA^2$ | KJ/mol | $\AA^2$ | KJ/mol |
| Complex myosin-GFP | GFP 795 | $-417$ | $-734$ |
| Complex myosin-GFP | Myosin 762 | $-22$ | $-192$ |
| Complex actin-myosin | Actin 1070 | $-420$ | $-1009$ |
| Complex actin-myosin | Myosin 1027 | 96 | $-165$ |

Under control of the chicken $\beta$-actin promoter and cytomegalovirus enhancer without any detected abnormality (25). However, it should be noted that only heterozygous mice were presented in this study and it can thus be speculated that the number of copies in this case was insufficient to cause any deleterious effects. In our study, the skeletal myoblasts were transduced with a third generation lentiviral vector expressing eGFP cDNA under control of a ubiquitous phosphoglycerate kinase promoter. Lentiviral vectors are highly effective vectors for transgene integration and expression, suggesting that high expression of eGFP was obtained in our myoblasts. Zielske et al. (26) demonstrated that in an human immunodeficiency virus-based vector, transgene expression and number of copies per cell were highly variable from one cell to the next. Taken as a whole, these observations strongly suggest that the deleterious effects of GFP are manifest in a dose-dependent fashion.

GFP fusion proteins modulate the expression and subcellular localization of GFP within muscle cells. On the one hand, GFP fused to myosin heavy chain or myosin light chain has been extensively used to examine the contractile cytoskeleton in living striated muscle cells (27–30). Interestingly, when these fused proteins are overexpressed in the C2C12 muscle cell line, no disturbance of the contractile machinery is reported. GFP-myosin purified from these cells exhibits the same ATPase activities and in vitro motility as wild-type myosin (27, 28). These data suggest that myosin function is not modified provided that GFP is placed away from the actin-binding site and nucleotide-binding regions of the molecule. On the other hand, we demonstrated that the presence of eGFP markedly affected myosin function, as evidenced by severe changes in both the mechanical and enzymatic properties of myosin. It is important to remind that acto-myosin interaction involves different steps, whose regulations are not necessarily coordinated (31–33). The rate of ATPase is inversely correlated to the overall time cycle duration, whereas the filament velocity, analogous to the unloaded shortening velocity seen in muscle fibers (32, 34), is thought to be limited by the rate of actin-myosin dissociation (33, 35). In addition, thin filaments move over a motility surface at similar speeds over a wide range of myosin densities, providing that enough myosin heads are attached at any time to give con-
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Continuous movement (32). Thus, modifications of myosin properties can be associated with a lack of correlation between ATPase measurements in solution and the myosin velocity in \textit{in vitro} motility assays (31, 36). In the presence of a myosin:eGFP ratio of 1:0.5, our results indicated an apparent reduction in the binding affinity to actin (increased $K_m$) associated with normal sliding velocity and maximum ATPase activity. Thus, at a myosin:eGFP ratio of 1:0.5, eGFP competitively inhibited the apparent binding affinity of myosin to actin, thereby suggesting that actin and eGFP competed for binding to the same myosin active site, \textit{i.e.} the actin-binding site. It was likely that at the low myosin:eGFP molar ratio, reduction of functional myosin, \textit{i.e.} myosin available for acto-myosin interactions was low enough so that myosin density remains sufficient to ensure continued movement of the thin filament. As the eGFP concentration increased relative to myosin, $V_{max}$ decreased dramatically with no significant changes in $K_m$. This behavior reflected non-competitive inhibition of actin and eGFP at higher eGFP concentrations, thereby suggesting that several eGFP molecules nonspecifically bind to myosin and inactivate it. As the number of myosin heads available to ensure continual movement has become insufficient, reduced velocity in \textit{in vitro} motility assays occurred. It was also possible that the slow cycling and noncycling myosins inhibited motility of the fast cycling myosin by exerting a viscous drag force on actin filaments (32, 34).

Structural modeling of myosin-GFP and actin-myosin complexes shows that docking takes place preferentially on the same binding site of the myosin head, which confirmed that GFP may compete with actin for the same site of myosin. Our results showed that the main stabilizing forces of both complexes are the electrostatic ones. In addition, our structural results are consistent with our actin-activated ATPase assay that confirmed that eGFP competes with actin for the active site of myosin. Addition of eGFP in the reaction decreases binding of actin to myosin, which results in an impairment of excitation-contraction coupling in the muscle cells. These findings are consistent with those of Huang \textit{et al.} (6) showing that overexpression of soluble GFP in the cytoplasm of cardiac cells induced dilated cardiomyopathy. In this earlier study, GFP expression was driven by an $\alpha$-myosin heavy chain gene promoter. It is thus possible that in the absence of fusion of GFP with intracellular proteins, GFP, a soluble protein, was expressed and distributed freely throughout the cytosol. This situation could have a potentially toxic effect at high GFP expression levels, whereas GFP fused with other proteins restricted its localization to a cellular compartment, thereby limiting its toxicity.

Taken together, our \textit{in vivo} and \textit{in vitro} experiments, involving electromechanical coupling, \textit{in vitro} motility assays, determination of the actin-activated ATPase rate, and co-precipitation and structural analysis, provide compelling evidence that eGFP markedly affects myosin function via competition of eGFP with actin for myosin-binding sites, thereby causing excitation-contraction uncoupling and impaired contractile function of the muscle cells. Our results do not intend to detract from the use of GFP transgenic models where the number of DNA copies is expected to be smaller than after stable cell transfection and thus to have less impact on the contractile machinery of the cells. They simply raise a cautionary word about the interpretation of the functional outcomes of cell transplantation using eGFP-transduced grafts for the purpose of cell tracking.

Acknowledgments—We thank the Genethon, which is supported by the AFM, in the frame of the Gene Vector Production GVPN network for providing lentiviral vectors. We thank Dr. G. Butler-Browne, Dr. J. L. Samuel, and Prof. D. Paulin for helpful discussions.

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