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PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2022
Prot. 2022A4XZA2

PART A

1. Research project title

The structural and functional role of minispryn in striated muscle

2. Duration (months)

24 months

3. Main ERC field

LS - Life Sciences

4. Possible other ERC field

5. ERC subfields

1. LS1_9 Molecular mechanisms of signalling processes
2. LS1_7 Molecular biophysics, biomechanics, bioenergetics
- 3.

6. Keywords

nº

Testo inglese

1. Sarcomeric and cytoskeletal proteins

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2. Cardiac physiology
 3. Striated muscle physiology
 4. Protein-protein interactions
 5. Molecular biology
-

7. Principal Investigator

BANG
(Surname)

MARIE-LOUISE
(Name)

Primo ricercatore
(Qualification)

16/11/1972
(Date of birth)

BNGMLS72S56Z107Z
(Personal identification code)

Consiglio Nazionale delle Ricerche
(Organization)

0282245210
(Phone number)

marie-louise.bang@cnr.it
(E-mail address)

PI - Certified E-mail (CEM)

marie-louise.bang@pec.it

Age limits derogation

The PI and/or the substitute PI are over 40 and they don't intend to benefit from derogations to the age limits for the amount allocated to under 40 PI;

8. List of the Research Units

nº	Associated Investigator	Qualification	University/ Research Institution	Registered office (address)	Operating office (address)	e-mail address
1.	BANG Marie-Louise	Primo ricercatore	Consiglio Nazionale delle Ricerche	Piazzale Aldo Moro, 7 - Roma (RM)	City: Rozzano (MI) Address Via Manzoni 113, 20089 Rozzano (Milan)	marie-louise.bang@cnr.it
2.	CAREMANI Marco	Ricercatore a t.d. - t.pieno	Università degli Studi	P.zza S. Marco, 4 -	City: Sesto Fiorentino (FI)	marco.caremani@unifi.it

(art. 24 c.3-b L. 240/10)	di FIRENZE	FIRENZE (FI)	Address Via Madonna del Piano 6, 50019 Sesto Florentino (Firenze)
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9 - Substitute Principal Investigator (To be identified among one of the associated investigators participating in the project).

CAREMANI

(Surname)

MARCO

(Name)

Ricercatore a t.d. - t.pieno (art. 24 c.3-b L. 240/10)

(Qualification)

08/11/1973

(Date of birth)

CRMMRC73S08A291X

(Personal identification code)

Università degli Studi di FIRENZE

(Organization)

0554754753

(Phone number)

marco.caremani@unifi.it

(E-mail address)

Substitute PI - Certified E_mail (CEM)

marco.caremani@pec.it

10. Brief description of the proposal

Minispryn (encoded by FSD2) is a 90 kDa striated muscle specific protein, recently identified as a homologue of the C-terminal TRIM-like region of the 449 kDa protein myospryn (encoded by CMY45). Both proteins are localized in and around the nucleus as well as at the junctional sarcoplasmic reticulum (jSR) in cardiomyocytes, where they have been found to form a complex with the ryanodine receptor (RyR2) Ca²⁺ release channel. Through its C-terminal TRIM-like region, myospryn has been shown to interact with a variety of proteins, including the regulatory RIIa subunit of protein kinase A (PKA), defining myospryn as an A-kinase anchoring protein (AKAP), a scaffolding protein that recruits PKA to distinct subcellular locations to enable activation of local signaling. Based on the homology of minispryn to the C-terminal region of myospryn, we hypothesize that minispryn shares interaction partners with myospryn and that the two proteins have overlapping roles and/or collaborate in regulating Ca²⁺ handling, PKA signaling, and other important mechanisms in heart and skeletal muscle, thus contributing to the pathogenesis of human disease. Knockout of myospryn in mouse has been shown by others and us to result in left ventricular dilation and systolic dysfunction, associated with severe structural alterations, including mitochondrial, SR, intercalated disc, and nuclear defects, mislocalization of connexin 43 and RyR2, and the presence of amorphous regions containing mitochondrial clusters and convoluted membranes. However, as of yet, nothing is known about the functional role of minispryn in striated muscle. The objective of the proposed project is to provide insights into the role of minispryn in cardiac and skeletal muscle based on the analysis of minispryn knockout mice. In particular, through application of a combination of state-of-the-art molecular, cellular, histological, ultrastructural, advanced imaging, physiological, and biophysical methods, we will determine the structural and functional role of minispryn with particular focus on the role of minispryn in signaling, excitation-contraction coupling, and muscle performance. Furthermore, we will determine the expression and subcellular location of minispryn in health and disease through analysis of heart and skeletal muscle from human and mice as well as myocardial biopsies from heart failure patients. New insights into signaling pathways and mechanisms of Ca²⁺ handling in striated muscle are important for the development of novel therapeutic strategies for the treatment of cardiac and skeletal muscle diseases.

11. Total cost of the research project identified by items

Associated Investigator	item A.1	item A.2.1	item B	item C	item D	item E	sub-total	Total
BANG Marie-Louise	35.880	54.275	54.093	0	0	41.632	185.880	185.880
CAREMANI Marco	10.910	55.000	39.546	0	0	5.454	110.910	110.910
Total	46.790	109.275	93.639	0	0	47.086	296.790	296.790

N.B. The Item B and TOTAL columns will be filled in automatically

- item A.1: enhancement of months/person of permanent employees
- item A.2.1: cost of contracts of non-employees, specifically to recruit
- item B: overhead (flat rate equal to 60% of the total personnel cost, A.1+A.2.1, for each research unit)
- item C: cost of equipment, tools and software products
- item D: cost of consulting and similar services
- item E: other operating costs

PART B

B.1

1. State of the art

In striated muscle, excitation-contraction (EC) coupling involves the conversion of electrical stimuli to mechanical response enabled by the release of Ca²⁺ from the sarcoplasmic reticulum (SR) into the cytosol through the ryanodine receptor (RyR) Ca²⁺ release channel. Subsequent Ca²⁺ sequestration into the SR or extrusion from the cytosol allows muscle relaxation. Abnormal Ca²⁺ handling related to altered RyR function has been associated with cardiac and skeletal myopathies as well as cardiac arrhythmias and sudden cardiac death (1), although the molecular basis for this is largely unknown.

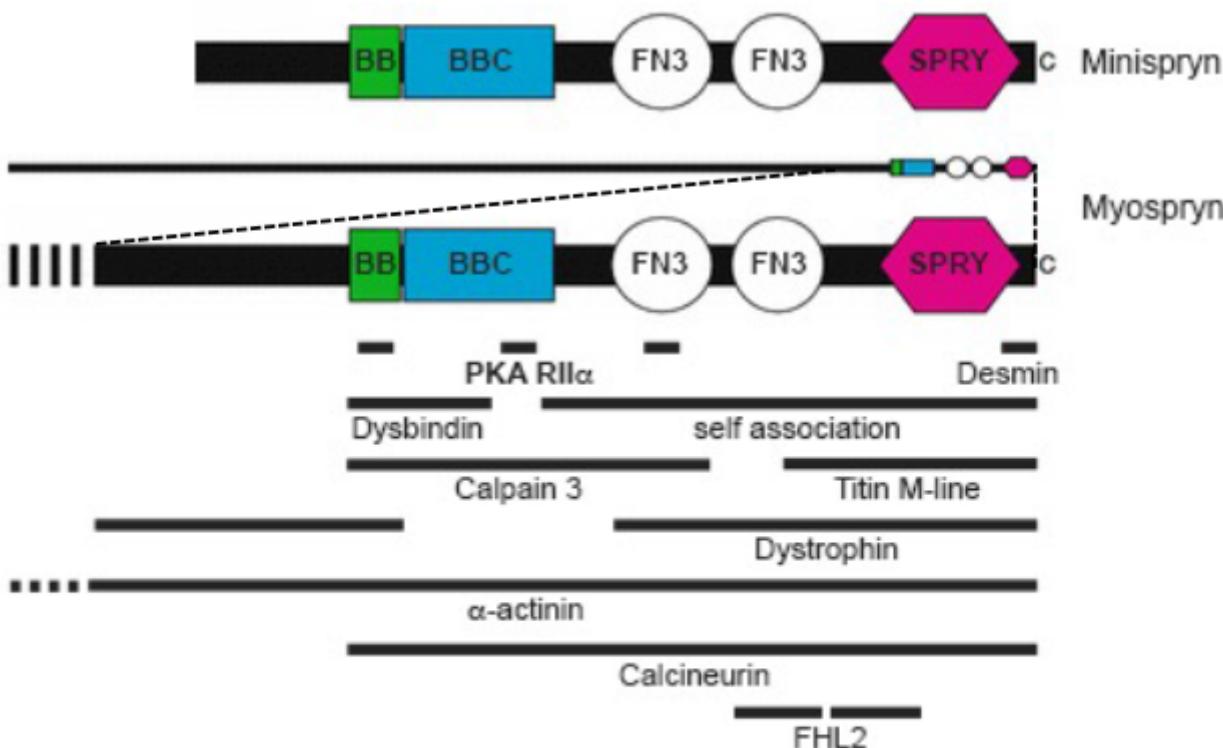


Figure 1: Schematic view of minispryn, myospryn, and myospryn interaction partners. The major part of myospryn is composed of repetitive sequence without predictable domains. Its C-terminus contains a TRIM-like region, including a B-Box' zinc finger domain (BB), a B-box coiled coil (BBC), two fibronectin III repeats (FN3), and a SPRY (SPIA and RYanidine receptor) domain. Minispryn is homologous to the C-terminal TRIM-like region of myospryn. Bars below myospryn indicate binding domains for known interaction partners. Modified from (2).

Minispryn, encoded by the FSD2 (fibronectin type III and SPRY domain containing 2) gene, is an about 90 kDa protein, recently identified as a homologue of the C-terminal TRIM-like region of the 449 kDa protein myospryn, encoded by CMYA5 (cardiomyopathy associated 5) (3). Like myospryn, minispryn contains a B-Box' zinc finger domain, a B-box coiled-coil (BBC), 2 fibronectin type III

(FN3) repeats, and a C-terminal SPRY (SPIA and RYanidine receptor) domain (Fig. 1). However, in contrast to minispryn, myospryn contains an about 300 amino acid N-terminal conserved region as well as a long repetitive glutamate-rich sequence, lacking predictable domains and differing considerably in length and repeat composition between species. Myospryn is principally expressed in striated muscle and brain, while minispryn appears to be specifically expressed in striated muscle, with highest expression levels in cardiac muscle (3). Both minispryn and myospryn are localized in and around the nucleus (3-5) as well as at the junctional sarcoplasmic reticulum (jSR) in cardiomyocytes (CMCs), where they have been found to form a complex with the ryanodine receptor (RyR2) Ca²⁺ release channel (3). Furthermore, myospryn has been detected in the intercalated disc (ICD), Z-line, M-band, costamere, and associated with lysosomes (3-5).

Through its C-terminal TRIM-like region, homologous to minispryn, myospryn has been reported to self-associate (6) and interact with a variety of proteins, including dysbindin (6), desmin (4), α-actinin (7), PKA-RIIa (8, 9), dystrophin (9), calcineurin (10), the titin M-band region (5), calpain 3 (5), and FHL2 (11) (Fig. 1). The binding of myospryn to PKA-RIIa establishes it as an A-kinase anchoring protein (AKAP) (8), which is a scaffolding protein that recruits PKA to distinct subcellular compartments to enable local PKA-mediated phosphorylation. PKA phosphorylates a large number of proteins, including myospryn's interaction partners RyR, dystrophin, desmin, calcineurin, and titin (3-5, 9, 10) as well as proteins involved in EC coupling and transcription (8). PKA signaling plays an important role in modulating cardiac and skeletal muscle function, and altered PKA signaling has been associated with cardiac pathophysiology (12). Furthermore, Duchenne Muscular Dystrophy (DMD) caused by dystrophin deficiency, has been found to be associated with mislocalization of PKA-RIIa and reduced PKA activity as well as reduced myospryn levels, suggesting that myospryn degradation may contribute to the pathogenesis of DMD (9, 13). Furthermore, through binding to calpain 3 and M-band titin, myospryn may be involved in the pathogenesis of tibial and limb girdle muscular dystrophy (5). Myospryn was also shown to inhibit calcineurin, and transgenic mice overexpressing myospryn's TRIM-like domain consequently exhibited impaired skeletal muscle regeneration due to inhibited calcineurin signaling as well as the ability to repress slow fiber transformation in calcineurin transgenic mice (10). Knockout (KO) of myospryn in mouse resulted in left ventricular dilation, atrial enlargement, systolic dysfunction, and fibrosis, associated with ICD alterations, severe mitochondrial defects (intermitochondrial vacuoles, critolysis, blebbing), loss of SR-mitochondrial contact sites, dilated SR and T-tubular membranes, abnormal nuclear shape and positioning, connexin 43 laterization, and RyR2 disorganization (14), consistent with an essential role of myospryn for normal striated muscle structure and function. In human, a CMY45 polymorphism (K2906N) has been associated with cardiac hypertrophy in hypertensive patients (15) and myospryn has been reported to be upregulated under hypertrophy-inducing conditions both in vitro and in vivo (15).

Since, minispryn is structurally similar to the C-terminal TRIM-like region of myospryn, minispryn is likely to interact with at least some of the same interaction partners as myospryn. Furthermore, based on the ability of myospryn to self-associate through its TRIM-like region and the presence of minispryn and myospryn in complex with RYR2, it is possible that minispryn and myospryn can directly interact. Thus, we hypothesize that the two proteins have similar functions and collaborate in regulating Ca²⁺ handling, PKA signaling, and other important mechanisms in heart and skeletal muscle.

2. Detailed description of the project: methodologies, objectives and results that the project aims to achieve and its interest for the advancement of knowledge, as well as methods of dissemination of the results achieved

The present project aims to determine the structural and functional role of minispryn in striated muscle under physiological and pathological conditions. In particular, using a multidisciplinary approach combining state-of-the-art molecular, advanced imaging, and biophysical methods, we will study the effect of minispryn KO on cardiac and skeletal muscle structure and function with particular focus on the role of minispryn in signaling, EC coupling, and muscle performance. A multidisciplinary team with established experience in muscle molecular biology, physiology, and biomechanics will address this aim at different integrated levels.

Preliminary results

Our interest in minispryn derives from our studies of its larger homologue myospryn, whose C-terminal TRIM-like region is structurally similar to minispryn. Based on the high homology between the two proteins and colocalization in striated muscle, we hypothesize that the two proteins have similar functions and the results from our analysis of myospryn KO (MYKO) mice are therefore relevant for understanding the possible function of minispryn.

To study the role of myospryn in vivo, we generated a mouse model in which exon 1 containing its start codon was deleted. Transmission electron microscopy (TEM) studies of MYKO mice revealed severe ultrastructural abnormalities both in heart and skeletal muscle (Fig. 2, 3), including Z-line abnormalities, irregular ICDs, sarcomeric misalignment, and abnormal Ca²⁺ release units (CRUs). Furthermore, amorphous regions were observed under the sarcolemma and between the myofibrils, containing mitochondria and convoluted membranes, possibly deriving from SR membranes and altered T-tubules, phagosomes, and lysosomes.

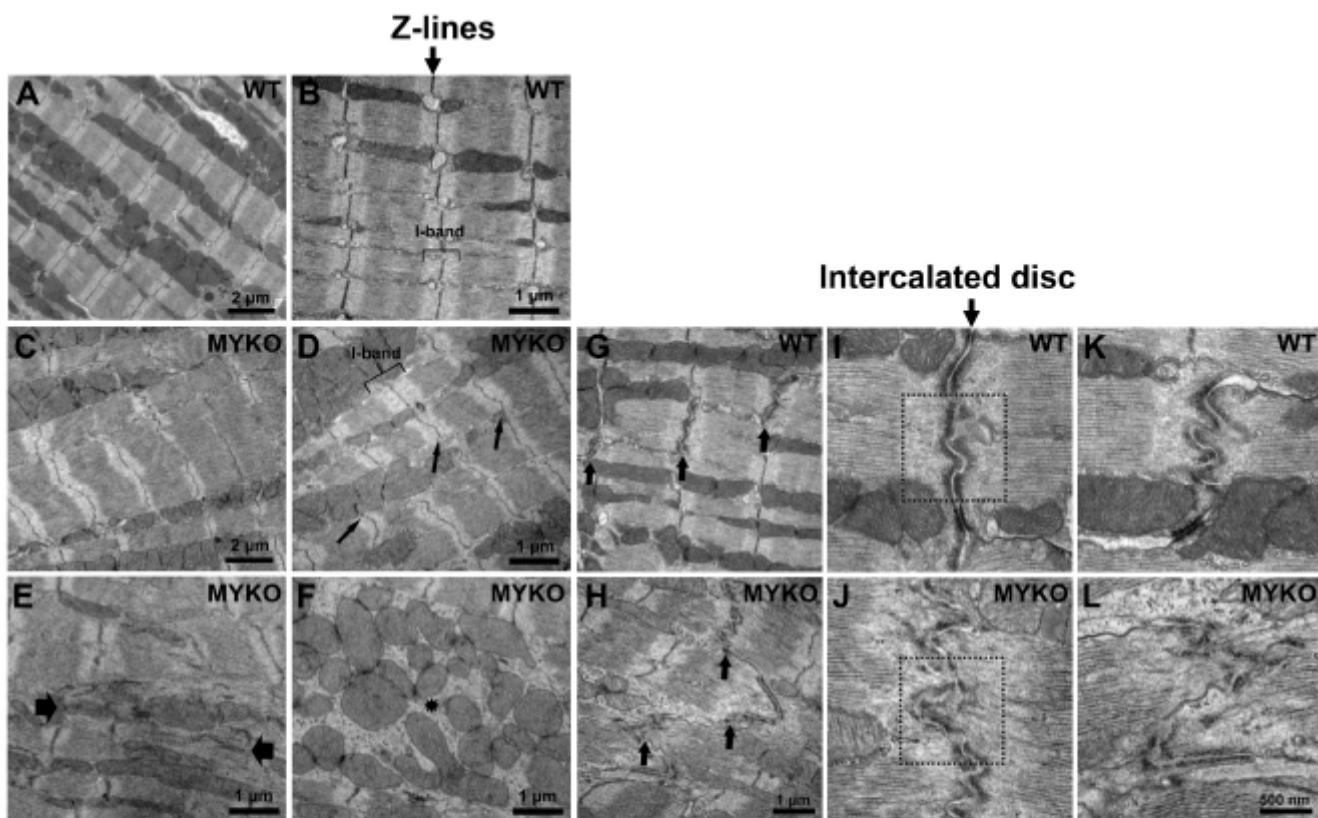


Figure 2: Transmission electron microscopy (TEM) analysis of cardiac left ventricle from 2-month-old WT and MYKO mice. Left (WT: A-B; MYKO: C-F), Jagged Z-lines are indicated by black arrows (D) and Z-line streaming is indicated by big arrows (E). In MYKO mice, Z-lines often present a jagged profile with the I-band unevenly stretched and in some areas barely recognizable (C, D). Some Z-line streaming is also present (E) and in those cardiomyocytes with most compromised Z-lines, myofibrils and Z-lines have lost lateral alignment. Amorphous material between mitochondrial clusters is present in many MYKO fibers (F). Right (WT: G, I, K; MYKO: H, J, L), Intercalated discs (arrows in G and H) have a more irregular appearance in MYKO mice (dotted box in I and J), in particular in the region of the adherens junction. G, H and I-L have the same magnification (performed in collaboration with Prof. Simona Boncompagni).

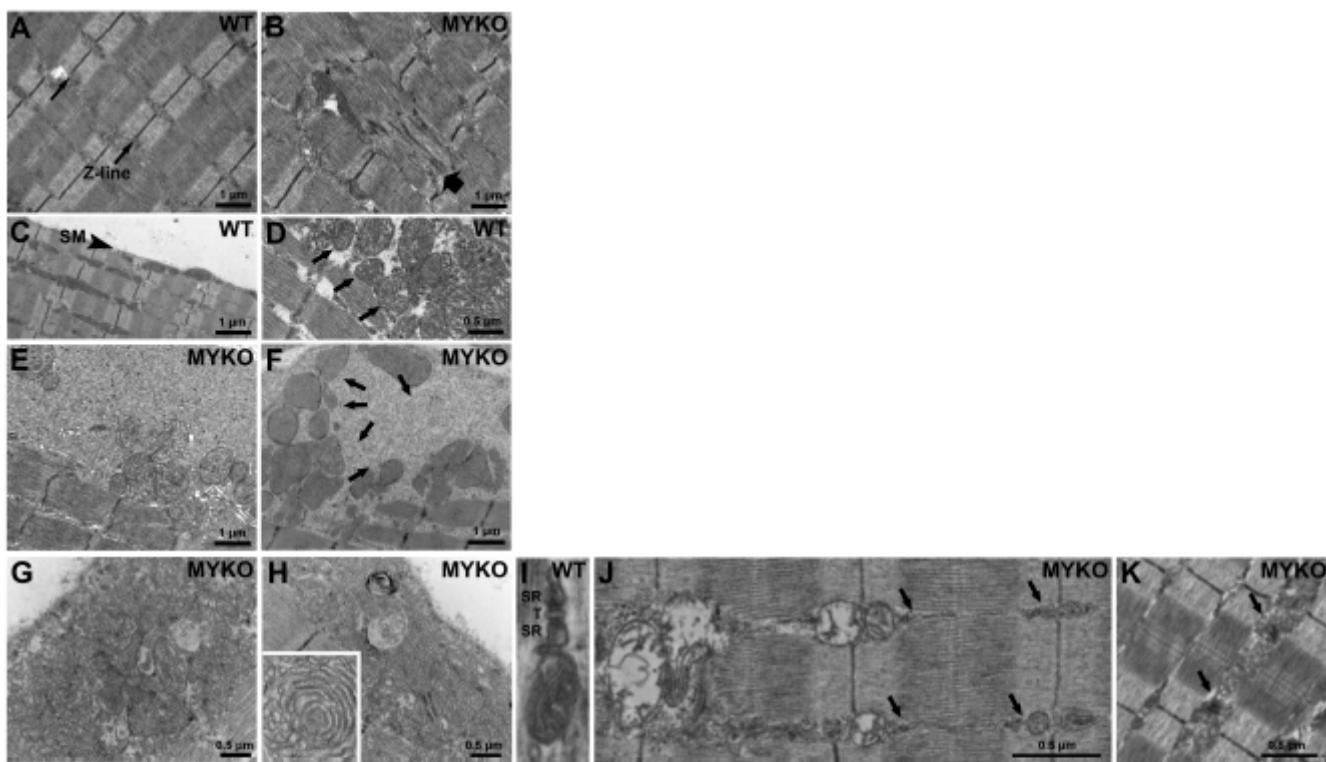


Figure 3: TEM analysis of tibialis anterior (TA) muscle from 2-month-old WT and MYKO mice. In MYKO muscle, Z-line streaming (big arrow) and sarcomeric misalignment is often observed (B). In WT muscle, myofibrils usually fill the intracellular space almost completely and not much space is present between the sarcolemma and the myofibrils (C), that also sometimes contain mitochondrial clusters (D, arrows). In contrast, MYKO muscle contains regions in which the sarcolemma and myofibrils are separated by an abnormally large space, often filled with amorphous electron-dense material, frequently containing mitochondria (F, arrows). Other subsarcommal areas are filled with very convoluted membranes (G, H), possibly including SR membrane, altered T-tubes, phagosomes, and lysosomes. In WT muscle, the Ca^{2+} release unit (CRU) has the typical triad structure with one T-tubule (T) flanked by two SR cisternae (I), while in MYKO muscle the triads are often abnormal (J, K, arrows, abnormal CRUs). Furthermore, abnormal nuclei with stellar shape and nuclear envelope with indentation were frequent (not shown) (performed in collaboration with Prof. Simona Boncompagni).

Echocardiography analysis showed that MYKO mice develop progressive cardiac dilation and systolic dysfunction (Fig. 4) and show a maladaptive response to mechanical pressure overload by transaortic constriction (TAC) (Fig. 5). Furthermore, following activation of the PKA pathway through chronic minipump administration of isoproterenol (ISO), MYKO mice showed reduced contractile function as well as increased dilation and hypertrophy compared to WT mice (Fig. 6).

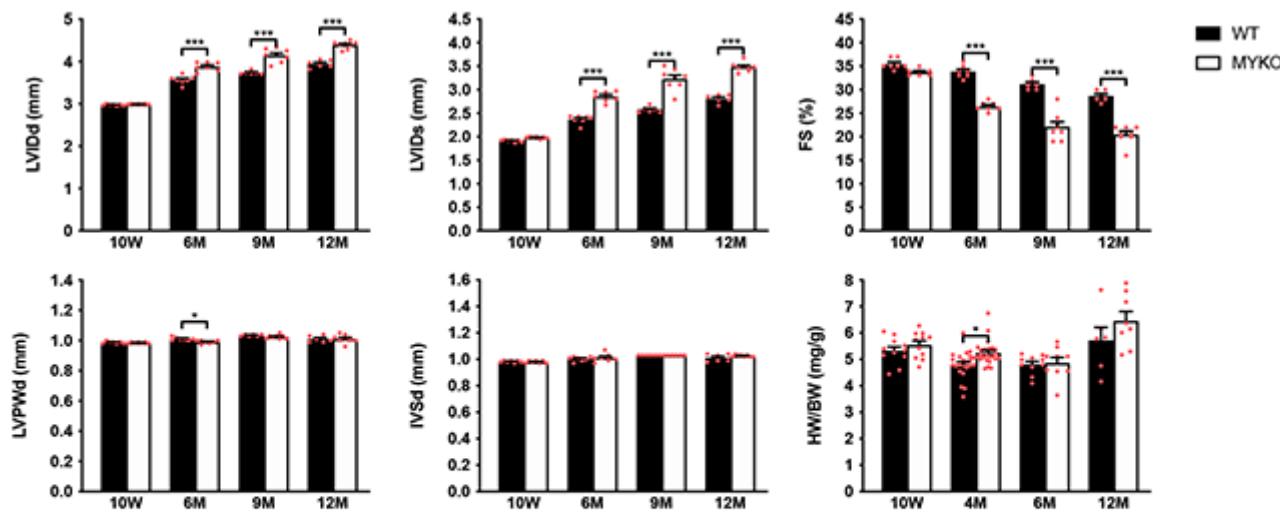


Figure 4: Echocardiographic analysis of MYKO and WT male mice at different ages. W, weeks, M, months. LVID, left ventricular inner diameter; LVPW, left ventricular posterior wall thickness; IVS, interventricular septum thickness; FS, fractional shortening; d, diastole; s, systole. Data are represented as mean \pm SEM ($n = 7-14$ per group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; two-way ANOVA with Bonferroni's multiple comparison test.

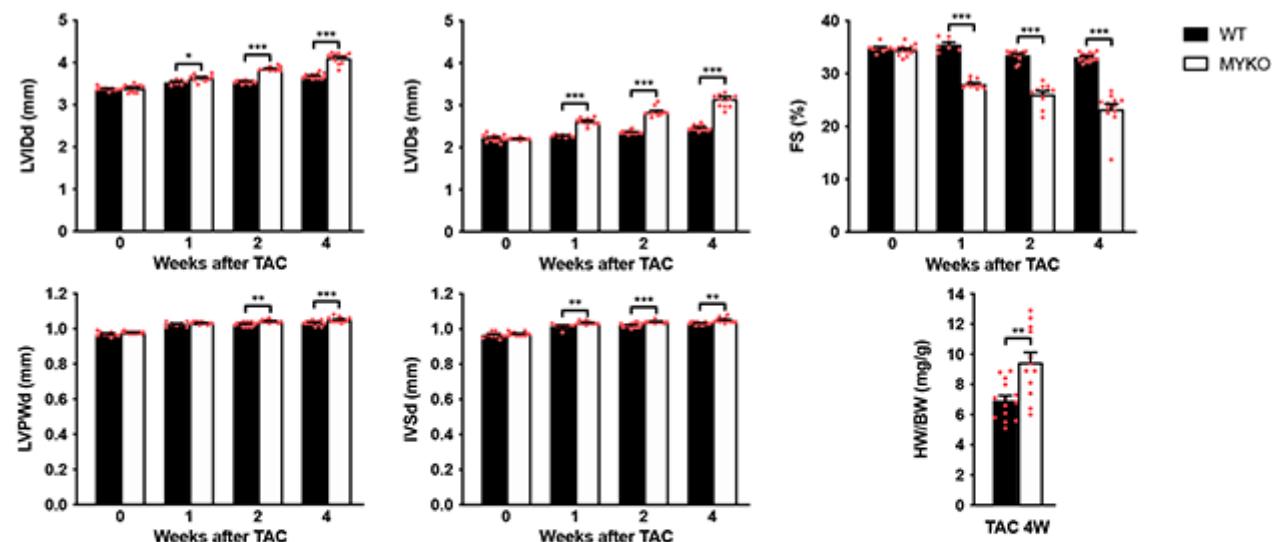


Figure 5: Echocardiographic analyses of 8-week-old MYKO and WT male mice at basal conditions and following transaortic constriction (TAC) for 1, 2, and 4 weeks. Pressure gradient > 70 mmHg. LVID, left ventricular inner diameter; LVPW, left ventricular posterior wall thickness; IVS, interventricular septum thickness; FS, fractional shortening; d, diastole; s, systole. Data are represented as mean \pm SEM ($n = 7-14$ per group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; two-way ANOVA with Bonferroni's multiple comparison test.

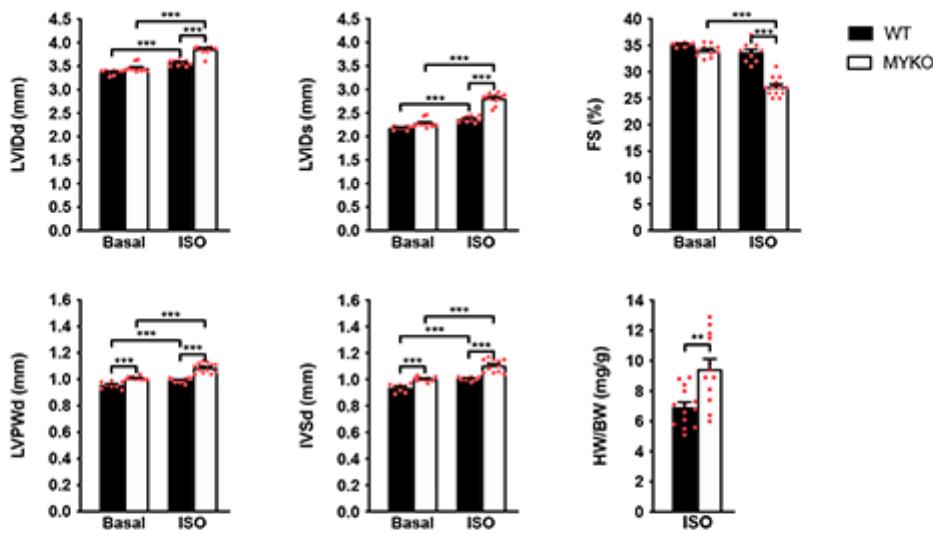


Figure 6: Echocardiographic analyses of 8-week-old MYKO and WT male mice at basal conditions and following chronic minipump administration of isoproterenol (ISO; 30 mg/kg/day) for 14 days. LVID, left ventricular inner diameter; LVPW, left ventricular posterior wall thickness; IVS, interventricular septum thickness; FS, fractional shortening; d, diastole; s, systole. Data are represented as mean \pm SEM ($n = 8-13$ per group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; two-way ANOVA with Bonferroni's multiple comparison test.

Invasive hemodynamic studies of 6-month-old MYKO mice by catheterization in the absence and presence of graded doses of the beta-adrenergic agonist dobutamine showed reduced contractile function at baseline, but a normal response to dobutamine stimulation (Fig. 7).

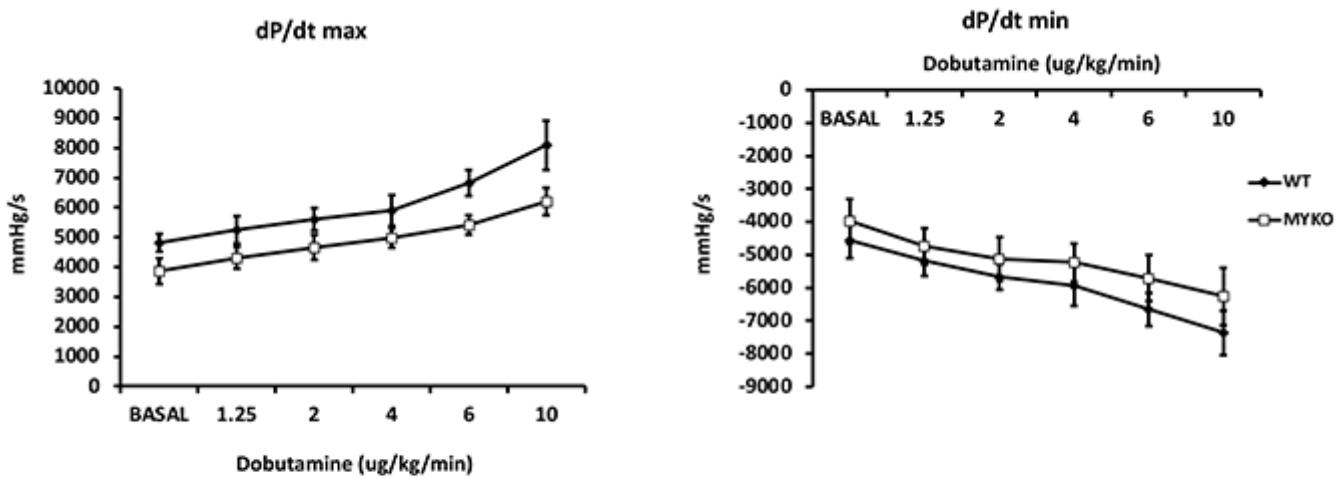


Figure 7: Hemodynamic analysis of 6-month-old MYKO and WT male mice by catheterization in the absence and presence of graded doses of dobutamine. dP/dt max, maximum derivative of change in systolic pressure over time; dP/dt min, minimum derivative of change in diastolic pressure over time. Data are represented as mean \pm SEM ($n = 8$ per group). * $P < 0.05$.

Consistent with a role of myospryn as an AKAP associated with the SR, PKA activity was 22% reduced in the membrane fraction of both heart and skeletal muscle from MYKO mice (Fig. 8A). In addition, WB analysis showed increased RyR1 levels, but reduced PKA-mediated phosphorylation of RyR1 in MYKO muscle (Fig. 8B).

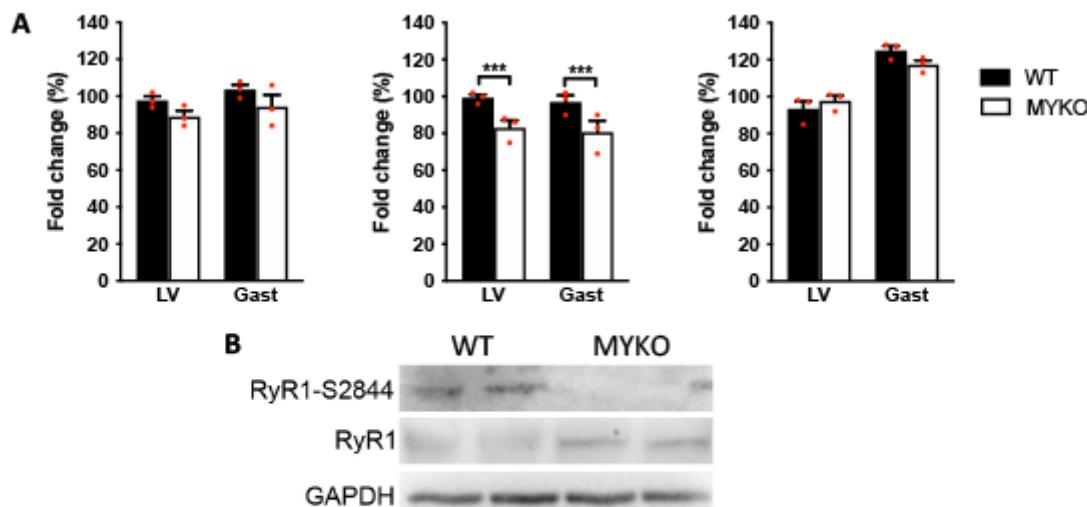


Figure 8: Reduced PKA activity in the membrane fraction and reduced RyR1 phosphorylation in 8-week-old MYKO male mice. A. PKA activity assay (units/ μ l) in subcellular fractions from left ventricle (LV) and gastrocnemius muscle (Gast) from MYKO and WT mice. Data are represented as mean \pm SEM ($n = 3$). ***P < 0.001. **B.** Western blot analysis for RyR1-Ser2844 (PKA phosphorylation site) and RyR1 on tibialis anterior (TA) muscle from MYKO mice compared to WT. GAPDH was used as loading control.

WP1. CHARACTERIZATION OF THE EXPRESSION AND SUBCELLULAR LOCALIZATION OF MINISPRYN IN HEALTH AND DISEASE

Rationale

Immunofluorescence (IF) staining of ventricular CMCs from guinea pig showed localization of minispryn in the jSR, the nucleus, and the perinuclear region (3). However, the location of minispryn in adult heart and skeletal muscle has not been determined. In the present WP, we will determine the location of minispryn in cardiac and skeletal muscle from mouse and human. Furthermore, to investigate whether minispryn is altered during cardiac pathological conditions, the expression and location of minispryn in myocardial biopsies from cardiomyopathy patients vs. non-failing hearts will be determined.

Tasks

1.1 – Minispryn subcellular localization in mouse cardiac and skeletal muscle (RU1)

IF stainings will be performed on cryosectioned heart and skeletal muscle using a commercial minispryn antibody. The presence of a Super resolution Leica SP8 STED microscope at RU1 will allow for high definition imaging.

1.2 – Minispryn expression and subcellular localization in human biopsies from cardiac and skeletal muscle (RU1)

To determine the expression and subcellular localization of minispryn in human during health and disease, qRT-PCR, WB, and IF analyses will be performed on:

- 1) Skeletal muscle biopsies obtained from patients undergoing anterior cruciate ligament reconstruction (obtained with informed consent from Humanitas Research Hospital; Ethical approval number #1102).
- 2) Myocardial biopsies from patients with dilated and hypertrophic cardiomyopathy vs. non-failing hearts (obtained from the Duke Human Heart Repository, North Carolina, USA; see letter of collaboration).

WP2. DETERMINATION OF INTERACTION PARTNERS OF MINISPRYN

Rationale

Through different parts of myospryn's C-terminal TRIM-like region, homologous to minispryn, myospryn has been reported to self-associate (6) and interact with a variety of proteins as indicated in Fig. 1. Furthermore, myospryn and minispryn have been shown to form a complex with RyR2, suggesting their possible direct binding (3). Additionally, in a large-scale Y2H study in skeletal muscle, optineurin, SNAPIN, and myosin binding protein C2 (MYBPC2) were identified as possible interaction partners to the C-terminal TRIM-like region of myospryn (16). Based on the close homology of minispryn to the myospryn TRIM-like region, we hypothesize that minispryn may interact with at least some of the same proteins as myospryn.

Tasks

2.1 – Y2H screening to identify interaction partners of minispryn (RU1)

Binding of minispryn to known interaction partners of myospryn's C-terminal TRIM-like region will be determined by cotransformation in yeast and plating on selective plates with X- α -Gal as previously described (17). The strength of the interactions will be tested by a chorophenol red- β -D-galactosidase (cPRG) assay, as described (18). Furthermore, minispryn will be used as a bait for a Y2H screening by mating with a human heart Mate & Plate Library using the Matchmaker Gold Yeast Two-Hybrid System (Takara Bio).

Cotransformations will be performed to confirm possible interactions and narrow down binding sites.

2.2 – Biochemical and cellular assays for confirmation of minispryn interaction partners (RU1)

Biochemical and cellular assays will be performed to verify the interaction of minispryn with interaction partners identified by Y2H or known to bind to the homologous C-terminal TRIM-like region of myospryn, including: 1) co-immunoprecipitation (Co-IP) in vitro by in vitro transcription and translation of candidate interactors, taking advantage of the c-Myc and HA epitope tags within the Y2H vectors; 2) co-IP in HEK293 cells, following co-transfection with HA- and FLAG-tagged proteins; 3) IP in HL-1 cardiac cells and/or differentiated C2C12 cells using minispryn antibody, followed by WB for potential interaction partners; 3) NanoBRET interaction assay (Promega), a bioluminescent resonance energy transfer (BRET)-based assay to measure interactions in living cells (19); 4) Duolink Proximity ligation assay (Merck) for visualization of endogenous interactions in sectioned heart using antibodies against minispryn and interaction partners of interest (20).

WP3. ROLE OF MINISPRYN IN CARDIAC AND SKELETAL MUSCLE STRUCTURE AND FUNCTION

Rationale

To study the role of minispryn *in vivo*, we will take advantage of minispryn KO (MIKO) mice available from GemPharmatech (B6/JGpt-Fsd2em21Cd5422in6/Gpt; Strain #T046413) in the C57BL/6JGpt background. The mice have been generated by deletion of Fsd2 exon 4-7 using CRISPR/Cas9 technology, resulting in complete KO of minispryn. Another MIKO line (Fsd2em1(IMPC)Mbp) in the C57BL/6NCrl background has been generated by the International Mouse Phenotyping Consortium (IMPC) as part of the Mouse Biology Program at UC Davis (<http://www.informatics.jax.org/allele/MGI:6158496>). Partial basal phenotype information is available from IMPC (<https://www.mousephenotype.org/data/genes/MGI:2444310>), indicating enlarged heart, increased heart weight, abnormal heart morphology, increased heart rate, and shortened RR interval, consistent with a role of minispryn in the heart. We have chosen to focus our studies on the MIKO mice in the C57BL/6J background available from GemPharmatech, since this is the most well characterized and commonly used genetic background for studying cardiac function. The MIKO mouse model has already been ordered and would be available from the beginning of the project. In our analyses we will study both male and female mice, especially since cardiac dysfunction was found to be more severe in female MIKO mice compared to male mice (14).

WP3.1. STRUCTURAL ROLE OF MINISPRYN IN STRIATED MUSCLE

Rationale

Structural analyses of MYKO mice have shown severe structural alterations, including ICD, mitochondrial, SR, T-tubular, and nuclear defects as well as the presence of amorphous regions containing mitochondrial clusters and convoluted membranes ((14) and Fig. 2, 3). Based on the homology and similar subcellular localization of minispryn and myospryn, we expect to observe similar structural changes in MIKO mice. Furthermore, since myospryn was found to interact with the biogenesis of lysosome-related organelles complex 1 (BLOC-1) subunit dysbindin (6) and possibly SNAPIN (16), another BLOC-1 subunit (21), myospryn, and consequently minispryn, may play a role in lysosome function and vesicle trafficking, consistent with the accumulation of abnormal membranous structure in MYKO muscle (Fig. 3). Lysosomes are required for the turnover of intracellular components and degradation of unfolded/misfolded proteins through fusion with autophagosomes, and defective lysosome function thus results in accumulation of autophagosomes and abnormal organelles. Furthermore, impairment of autophagic flux has been associated with the unfolded protein response (UPR), which is associated with SR stress and consequently myofiber breakdown, loss of function, and ultimately apoptosis (22).

Detailed studies of striated muscle structure will be performed in MIKO and WT mice both at histological and ultrastructural levels.

Tasks

3.1.1 – Verification of MIKO mice (RU1)

The absence of minispryn in MIKO mice will be confirmed by qRT-PCR and WB analysis on heart and skeletal muscle.

3.1.2 – Structural analyses of cardiac and skeletal muscle structure in MIKO mice (RU1)

The structural analysis of MIKO mice will include:

- 1) Histological analyses of heart and skeletal muscle (H&E staining, Picro Sirius red staining, TUNEL staining) to evaluate for the presence of myocardial remodeling, signs of myopathy (centralized nuclei), fibrosis, inflammation, necrosis, and apoptosis. For skeletal muscle, ATPase staining for fiber type distribution, SDH staining for oxidative potential, and α -GPD staining for glycolytic potential will be performed. Fiber type distribution will also be determined by SDS-PAGE.
- 2) Determination of heart and muscle weight to body weight and tibia length as well as measurements of adult CMC size and myofiber cross-sectional area in skeletal muscle.
- 3) Assessment of damage to muscle fibers by measurement of serum creatine kinase activity (23).
- 4) Evaluation for the presence of degenerative muscle fibers by intraperitoneal (IP) injection of vital Evans blue dye, which stains cells with damaged plasma membranes (24).
- 5) TEM analysis of heart and skeletal muscle (EDL and soleus muscle) from MIKO mice in collaboration with Prof. Simona Boncompagni (see letter of collaboration and Fig. 2, 3). Special attention will be paid to the structure of the CRUs, ICDs, and mitochondria, which were found to be abnormal in MYKO mice.

3.1.3 – Effect of minispryn KO on protein expression and localization as well as vesicle trafficking (RU1)

WB analysis for myospryn, minispryn-interacting proteins (identified in WP2), and other proteins of interest will be performed on left ventricular (LV) and skeletal muscle lysate from MIKO and wildtype (WT) mice. The localization of the same proteins will be determined by IF analysis by confocal or STED microscopy on cryosectioned heart and skeletal muscle from MIKO and WT mice (25). Since myospryn colocalizes with the jSR, the SR will be visualized by IF stainings for the SR marker KDEL. Furthermore, protein levels of ER stress markers will be determined by WB analyses and transcriptional changes will be checked among the differentially expressed genes identified by RNA-Seq (see Task 5.1). In addition, WB and IF stainings will be performed for the mitochondrial marker TOM20.

To determine whether lysosomes may be mislocalized or altered in size in MIKO mice, we will perform staining with antibodies against the lysosomal proteins LAMP2 and Cathepsin D (tissue), or LysoTracker dye (unfixed CMCs; Molecular Probes). Furthermore, to determine whether autophagy may be affected, WB analyses for autophagy markers (e.g. LC3, P62) on heart and skeletal muscle from fed and starved mice will be performed. If changes are observed, co-IF stainings will be performed for markers of autophagy-related vesicles and lysosomal markers and mice will be treated with chloroquine, an inhibitor of lysosome-autophagosome fusion and lysosome protein degradation, to determine whether the altered autophagy is due to defective lysosome function (26).

WP3.2. FUNCTIONAL ROLE OF MINISPRYN IN CARDIAC AND SKELETAL MUSCLE

Rationale

Based on the development of cardiac dysfunction and severe ultrastructural abnormalities in MYKO mice ((14) and Fig. 2-7) as well as partial phenotype information regarding a MIKO mouse model available from IMPC (see above), we expect that also minispryn will show functional defects in heart and skeletal muscle. In particular, the strong homology to myospryn suggests a possible involvement of minispryn in important processes, such as PKA signaling, EC coupling, vesicle trafficking, cardiac hypertrophy, and muscle regeneration (7-10, 15). We will study this by detailed functional analyses of cardiac and skeletal muscle function in MIKO vs. WT mice.

Tasks

3.2.1 – Effect of minispryn KO on cardiac and skeletal muscle function in vivo (RU1)

1) Basal cardiac function in MIKO mice will be determined by echocardiography (27) at different ages to detect morphological and functional abnormalities. Furthermore, since abnormal RyR2 function has been associated with cardiac arrhythmia (28) and MIKO mice show a shortened RR interval and increased heart rate, surface electrocardiogram (ECG) will be recorded before and after arrhythmia challenge by IP injection of epinephrine (2 mg/kg)/caffeine (120 mg/kg). To determine the response of MIKO mice to cardiac stress, they will be subjected to: i) mechanical pressure overload by transaortic constriction (TAC) (see Fig. 5) for up to 1 month, and ii) chronic minipump administration of ISO for 14 days (see Fig. 6). The effect of TAC or ISO administration will be determined by echocardiography, histological analyses, and qRT-PCR analysis for markers of cardiac remodeling (Nppb, Acta1, Actc1, Myh6, Myh7), fibrosis (Ctgf, Col1a, Col3a, Acta2, Tgfb1), apoptosis (P53, Bax, BclIII), and inflammation (P53, Il1b, Tgfb Tnfa).

2) Skeletal muscle function of MIKO mice will be evaluated in vivo by determination of forelimb grip strength by a grip strength test as well as voluntary running on running wheels, while recording total running time and distance.

3.2.2 – Biomechanical studies of cardiac and skeletal muscle function (RU2)

The following biomechanical studies will be performed on MIKO and WT mice. The optimal time points for the biophysical experiments will be determined based on the ultrastructural analyses performed in Task 3.1.2.

1) Force dependency on sarcomere length in heart. To determine the effect of minispryn on cardiac contractility, the relation between peak twitch force and sarcomere length (SL) will be determined in intact trabeculae and/or papillary muscle using sarcomere level mechanics (29) (Fig. 9).

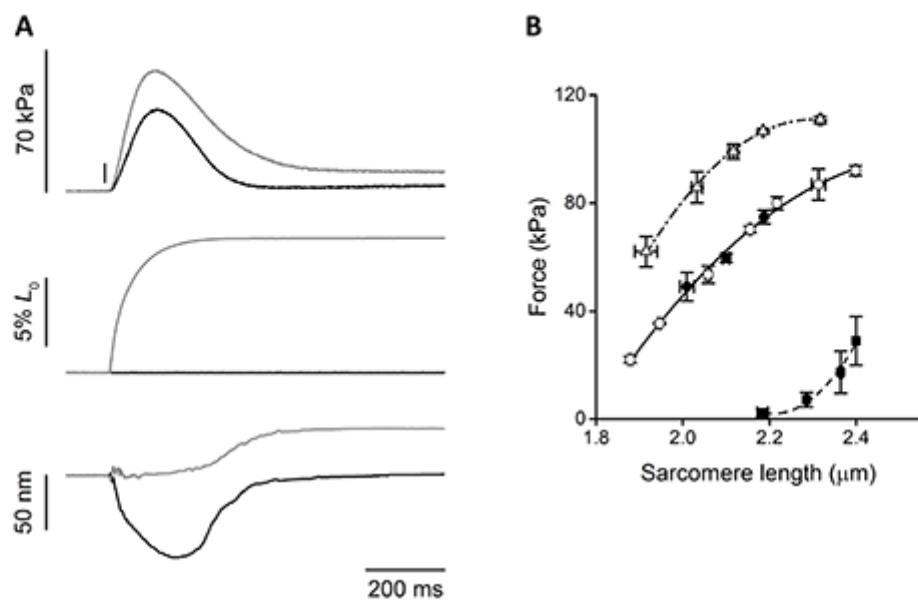


Fig. 9. Force developed in a twitch and its relation with sarcomere length in trabeculae. A. Force development upon electrical stimulation at 0.5 Hz and 1.0 mM $[Ca^{2+}]_o$, during fixed-end (black trace) and isometric-sarcomere conditions (gray trace) at an initial sarcomere length of 2.2 μm . Upper trace, force; middle trace, motor position; lower trace, half-sarcomere length change. The bar on the force trace marks the stimulus start. Length of the trabecula, 3.35 mm; segment length under the striation follower, 1.28 mm; average sarcomere length, 2.18 μm ; CSA, 26,700 μm^2 ; temperature, 27.2°C. B. Relations between peak force and sarcomere length at 1 mM $[Ca^{2+}]_o$ (active force, circles; passive force, squares) and 2.5 mM (active force, triangles). The lines are polynomial fits to data. The relation between active force and sarcomere length obtained in fixed-end conditions (filled circles) does not differ from that under sarcomere-isometric conditions (open circles). Modified from (29).

2) Force-velocity relation and power output. The ability to shorten and the power developed for any given load will be estimated by determining the force-velocity (T-V) relation in intact trabeculae (29, 30) and skeletal muscle (TA, EDL, soleus) (31) (Fig. 10) At each load the power output will be calculated by the product T*V.

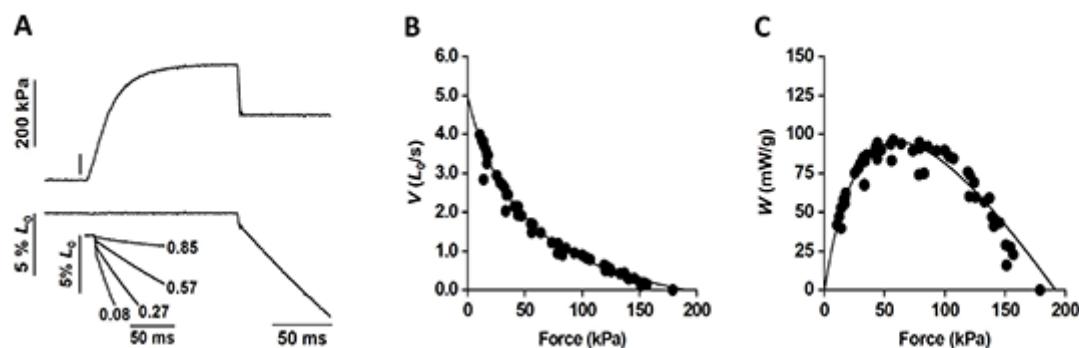


Figure 10. Force-velocity relation and power output in EDL muscle. A. Sample record of length change (lower trace) during isotonic contraction against a load of one-half the isometric force (upper trace) from EDL muscle. The vertical line indicates the stimulus start. Inset, sample records of length changes during isotonic contraction against different loads as indicated by the values close to the traces. B. Force-velocity relation obtained from an EDL muscle. The solid line is Hill's hyperbolic equation fit to the data. C. Force-power relations obtained from the same data as in (B). Temperature, 25.2°C.

3) Energetics and kinetics of the acto-myosin interaction in heart and muscle. The impact of the lack of minispry on sarcomere mechanics and energetics will be investigated in skinned cardiac and skeletal muscle preparations by simultaneously measuring isometric force and ATPase (32, 33) (Fig. 11C, D). The effect of minispry on the kinetics of the acto-myosin interaction will be determined by measuring the rate of force development following a period of unloaded shortening imposed during an isometric contraction (34) and, in the case of isolated myofibrils, by measuring the rate of force activation and relaxation following sudden Ca^{2+} activation and removal by fast solution switching (Fig. 11A, B).

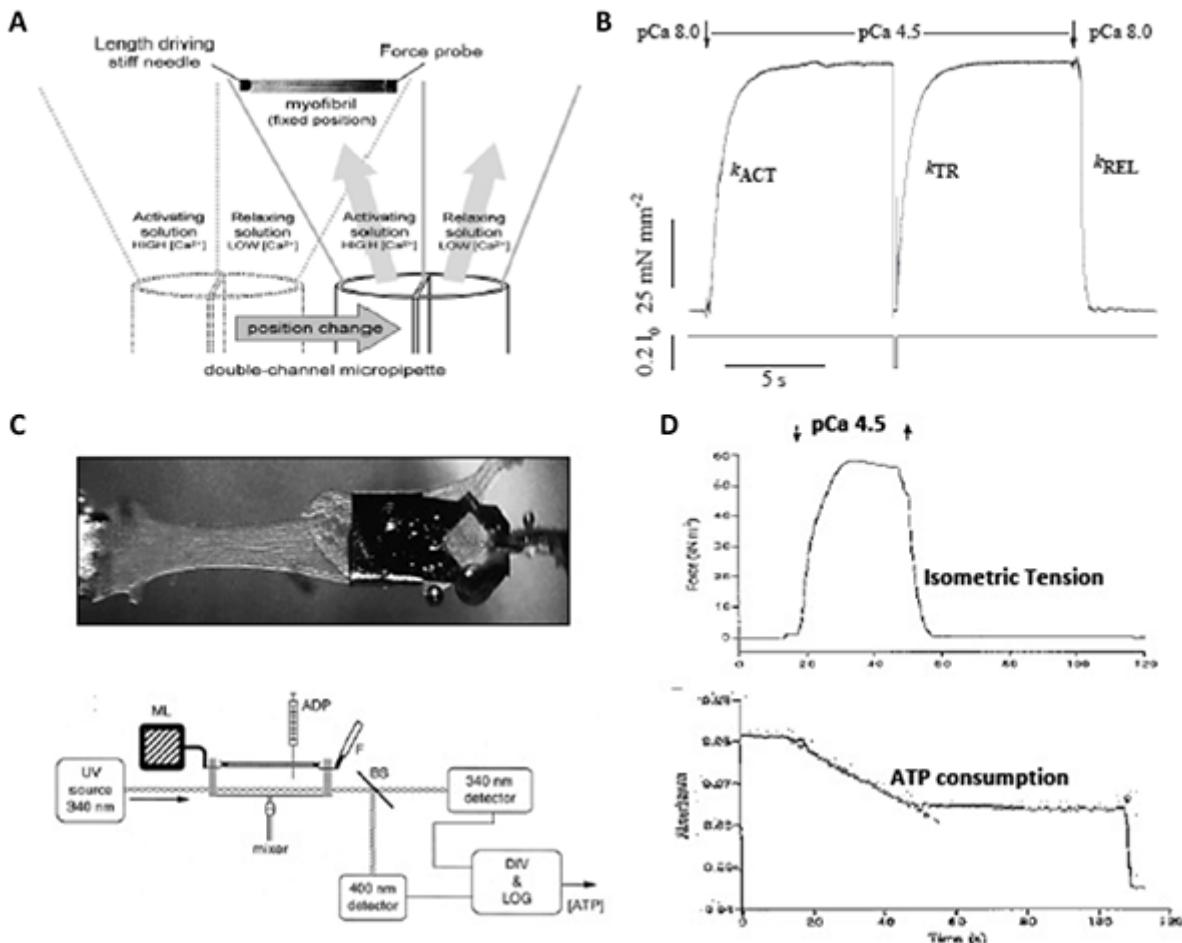


Figure 11. Methods to measure myofibril mechanics and kinetics (A and B) and mechanics and energetics of skinned trabeculae and skeletal muscle fibers (C and D). A. Isolated myofibrils mounted between a cantilever force transducer and a length control motor are maximally Ca²⁺-activated and fully relaxed by fast solution switching between high Ca²⁺ (pCa 4.5) and nominally Ca²⁺ free (pCa 8.0) solutions. **B.** Following sudden Ca²⁺ activation the myofibril develops force with a rate (k_{ACT}) that has been shown to reflect apparent cross-bridge turnover. Under steady-state conditions of activation, a fast release-restretch perturbation is applied to the myofibril to detach force generating cross-bridges and start a force redevelopment process with rate k_{TR} . Following Ca²⁺ removal the myofibril force decays to zero with a biphasic time course that allows us to measure an early slow and a later fast k_{REL} rates (10, 45). **C, D.** Simultaneous measurements of isometric force and ATP consumption can be performed in multicellular skinned cardiac samples (trabeculae or strips of papillary muscle) and single/small bundle of skeletal muscle fibers using a previously published technique (32, 33). Briefly, ATPase activity was measured using an enzyme coupled assay in which ATP regeneration from ADP and phosphoenol-pyruvate by the enzyme pyruvate kinase is coupled to the oxidation of NADH to NAD and the reduction of pyruvate to lactate by L-lactic dehydrogenase. NADH oxidation was photometrically measured from the absorbance at 340 nm of near-UV light. The maximal Ca²⁺-activated ATPase activity was calculated by normalizing the maximal NADH oxidation to the volume of the muscle strip and subtraction of the basal NADH oxidation (basal ATPase activity).

4) Force per elementary functional unit. In collaboration with Prof. Boncompagni, the fractional area occupied by contractile elements in EDL and soleus muscle will be determined by TEM on cross-sectioned muscle. Furthermore, X-ray diffraction experiments will be performed at the European Synchrotron (ESRF, Grenoble) to measure the distance between thick and thin filaments (Fig. 12). Knowing the fractional area occupied by the contractile elements and the interfilamentary distance, the force developed by the elementary unit, the half-thick filament, will be determined by dividing the isometric force per cross-sectional area by the filament density. Furthermore, to evaluate if the energy-saving conformation of the myosin motor at rest (interacting heads motif) (35-38) is altered in MIKO mice, the intensity profile and fine structure of the myosin-based meridional reflections will be compared between MIKO and WT mice (Fig. 12).

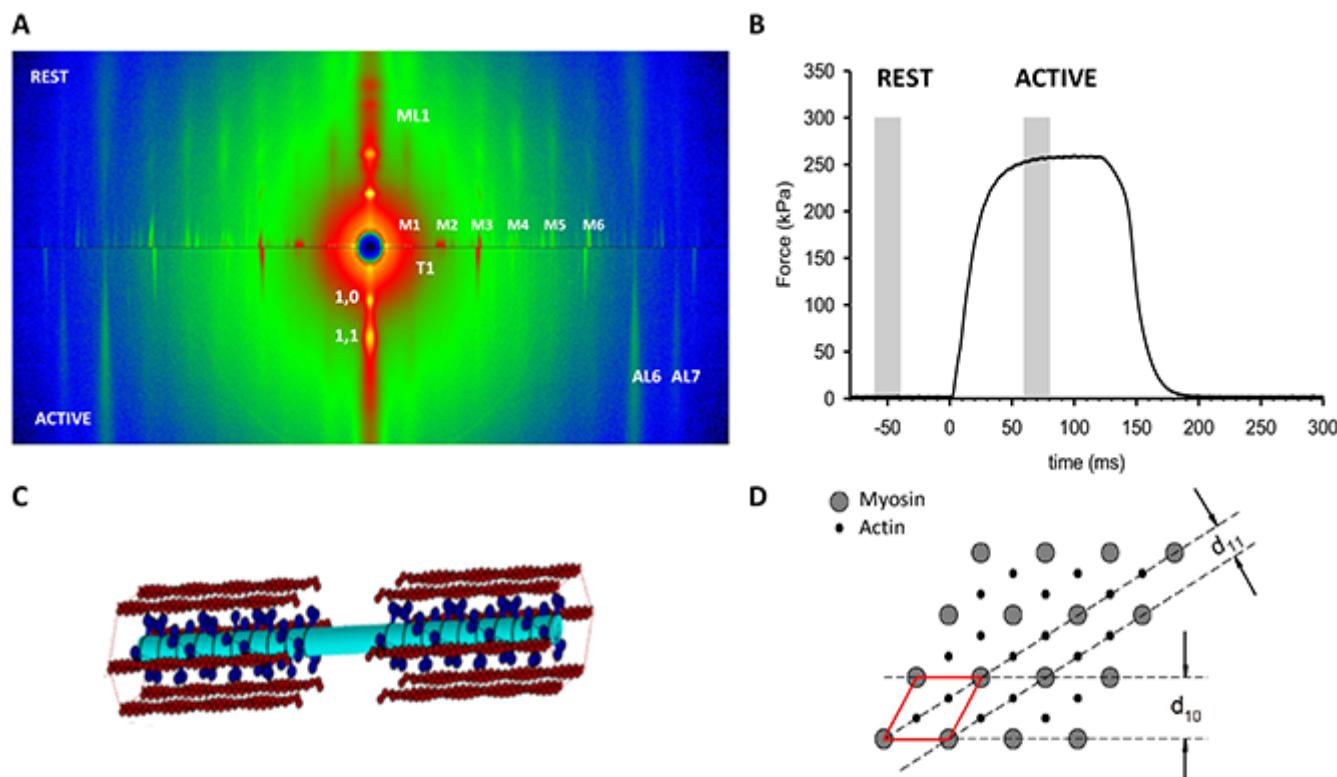


Figure 12. Low-angle x-ray diffraction patterns from EDL muscle. **A.** The upper and lower half are collected from the muscle at rest and at the plateau of the isometric tetanus respectively (30° C). The horizontal (meridional) axis is parallel to the muscle axis. Each pattern is obtained with $2 \times 20 \text{ ms}$ exposure windows. On the meridional axis are indicated the myosin-based (M) and troponin-based (T) reflections. On the vertical (equatorial) axis are indicated the strong 1,0 and 1,1 reflections arising from the filament lattice. The myosin (ML) and actin (AL) layer lines that extend in the radial direction are due to the helical arrangement of the two proteins in the thick and thin filaments. **B.** Force trace with the gray bars indicating the two exposure windows. Stimulus starts at 0. **C.** Schematic of a part of the thick filament (cyan) with the helical arrangement of the myosin motors (blue), surrounded by six thin actin-containing filaments (red). **D.** Schematic cross-section of a sarcomere, showing the regular disposition of the myofilaments and the crystallographic planes that originate the equatorial reflections. Red lines delimit an elementary cell.

5) Muscle endurance. Skeletal muscle endurance (EDL, soleus) will be determined by measuring the decline in isometric force during a fatigue protocol consisting of successive series of isometric tetani (see Fig. 13). Furthermore, the time for isometric force recovery to the value preceding the fatigue protocol will be measured (Fig. 13).

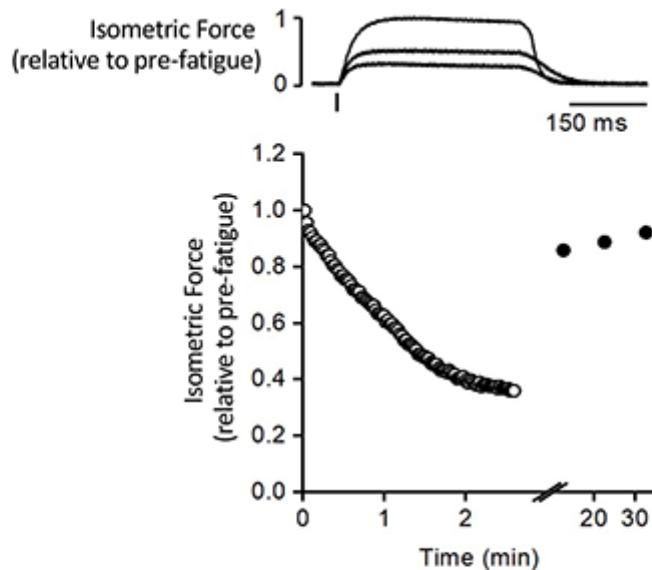


Figure 13. Protocol for evaluating muscle endurance (EDL muscle from WT mouse). Upper panel: time course of the force during the development of an isometric tetanus at different times during a fatigue protocol, consisting of a 400 ms, 100 Hz tetanus every 3 s for 150 s. Force is normalized for the plateau value at the beginning of the protocol. The vertical bar marks the start of stimulation. Lower panel: changes in isometric force during fatigue and recovery. The descending part of the curve shows the decline in isometric force. The ascending part of the curve shows the partial recovery of isometric force up to 35 min from the end of the fatigue protocol.

WP4. ROLE OF MINISPRYN IN CA2+ HANDLING

Rationale

The localization of minispryn at the jSR, where it forms a complex with myospryn and RyR2 (3) as well as the presence of CRU alterations, RyR2 disorganization, reduced PKA-mediated phosphorylation of RyR1, and mitochondrial abnormalities in MYKO mice ((14) and Fig. 2, 3, 8) suggest a role of minispryn in EC coupling. In striated muscle, mitochondria and CRUs are physically linked to one another (39, 40), which is important for Ca2+ signaling between the two organelles during EC coupling as Ca2+ released from the SR enters into the mitochondrial matrix to stimulate ATP production (41). We expect to observe abnormalities in EC coupling and Ca2+ handling in MIKO mice, resulting in defective force generation and possibly cardiac arrhythmia.

Tasks

4.1 – Simultaneous measurements of sarcomere shortening and Ca2+ transients in CMCs (RU1)

Simultaneous measurements of sarcomere shortening and Ca2+ transients will be performed in electrically stimulated CMCs isolated from MIKO and WT mice at basal conditions and following ISO stimulation using Fura-2 AM as Ca2+ probe on an IonOptix system (42).

4.2 – Assessment of SR Ca2+ load and release in adult CMCs (RU1)

RyR2 function will be assessed by measuring the biophysical properties of spontaneous SR Ca2+ release events (Ca2+ sparks) in Fluo-4 loaded CMCs from MIKO and WT mice on a confocal microscope at basal conditions and following ISO administration (43). SR Ca2+ load will be determined at the end of each experiment by rapid delivery of caffeine, resulting in opening of RyR2 channels.

4.3 – Assessment of LTCC biophysical properties (RU1)

An *in vitro* electrophysiological profile of LTCC currents (I-V relations, steady state activation, inactivation curves) will be obtained using the Patch-Clamp technique (44). Furthermore, scanning ion conductance microscopy (SICM; Ionscope) combined with Patch-Clamp will be used for morphological characterization of T-tubules and recordings of single LTCC currents (45).

4.4 – Force-pCa relation (RU2)

The Ca2+ sensitivity of the contractile system will be evaluated by determining the force-pCa relation in skinned skeletal muscle fibers (EDL, TA, soleus) (31, 34) as well as skinned strips from papillary muscle or trabeculae (31, 32).

WP5. ROLE OF MINISPRYN IN SIGNALING

Rationale

Since myospryn has been reported to contain three PKA-RIIa binding sites in its C-terminal TRIM-like region (see Fig. 1) (8), homologous to minispryn, we predict that also minispryn can bind to PKA-RIIa and, like myospryn, function as an AKAP. Consistent with a role of myospryn as an AKAP, in our preliminary studies of MYKO mice we found decreased PKA activity in the cardiac

membrane fraction (Fig. 8A), reduced PKA-mediated phosphorylation of RyR1 (Fig. 8B), and a pathological response to long-term beta-adrenergic agonist stimulation (Fig. 6). We thus expect to find similar alterations in MIKO mice. Myospryn was found to colocalize and interact with several PKA targets, including calcineurin (10), desmin (4), dystrophin (9), titin (5), and RyR (3), suggesting its possible role in the targeting of PKA to those substrates as well as other targets within the compartments (e.g. SR and costamere), where myospryn and PKA-RIIa colocalize. Based on similarity, we expect a similar role of minispryn. Thus, we will determine the effect of minispryn ablation on PKA-mediated phosphorylation of PKA target proteins binding to minispryn (identified in WP2) as well as other PKA target proteins colocalizing with minispryn.

Based on the ability of myospryn to bind and inhibit calcineurin (10), a protein phosphatase involved in pathological cardiac hypertrophy as well as myogenic differentiation and regeneration, we hypothesize that also minispryn can interact with calcineurin (tested in WP2). If this is confirmed, we will determine whether MIKO mice show abnormal calcineurin-NFAT signaling, which may affect myofiber size, fiber type distribution, and muscle regeneration.

Through whole genome transcriptomic analysis under basal conditions and in response to pressure overload, we will determine the possible participation of minispryn in other signaling pathways.

Tasks

5.1 – Whole genome transcriptomic analysis (RU1)

To unbiasedly identify pathways affected by the ablation of minispryn, we will perform RNA-Seq analysis on LV and TA tissue from MIKO and WT mice both at basal levels and 1 week following TAC. Gene oncology and ingenuity pathway analysis will be performed and differentially expressed genes of interest will be confirmed by qRT-PCR and WB analyses.

5.2 – Effect of minispryn on PKA signaling (RU1)

If, as expected, minispryn binds to PKA-RIIa, PKA expression/activity levels and localization of PKA subunits will be determined in total lysate and subcellular fractions from CMCs as well as LV and skeletal muscle tissue from MIKO vs. WT mice at basal levels and following ISO injection. Furthermore, to identify PKA substrates affected by minispryn, WB analysis for phosphorylation of PKA target proteins will be performed on LV and skeletal muscle tissue from MIKO and WT mice at basal levels and after ISO injection. More specifically, PKA phosphorylation of minispryn interaction partners, identified in WP2, known to be phosphorylated by PKA as well as other known PKA target proteins involved in EC coupling (8), will be determined using phospho-specific antibodies or, if not available, by WB analysis for total protein following Phos-tag gel electrophoresis. Furthermore, to identify potential other PKA substrates affected by minispryn, a phospho-(Ser/Thr) PKA substrate antibody will be used and differentially phosphorylated bands in MIKO vs. WT mice will be excited and identified by mass spectrometry. IF stainings on skeletal and cardiac muscle for PKA-RIIa and PKA target proteins affected by the absence of minispryn will be performed under basal conditions and following ISO stimulation.

5.3 – Role of minispryn in calcineurin signaling and muscle regeneration (RU1)

To determine whether the calcineurin-NFAT pathway is affected in MIKO mice, we will perform a calcineurin activity assay on heart and skeletal muscle tissue from MIKO and WT mice. Furthermore, since calcineurin is involved in muscle regeneration, the ability of MIKO muscle to regenerate following injury will be determined by histological analyses and fiber size determination at different time points following muscle damage induced by BaCl₂-injection into the TA muscle (31).

WP6: DISSEMINATION

The results of the project will be presented at national and international congresses and are expected to lead to scientific publications in peer-reviewed high impact international journals. In case of findings of commercial interest, a patent will be filed. We will also seek to communicate the results to the general public through interviews and articles for non-specialists.

3. Project development, with identification of the role of each research unit, with regards to related modalities of integration and collaboration

This project will exploit the scientific synergy between the two research units (RUs), which have internationally recognized experience in experimental research in skeletal muscle and heart physiopathology. Also, as auspicated by the present call for proposals, the two RUs will bring complementary and synergistic expertise to the project through their unique, advanced, and integrated knowledge in the fields of molecular mechanisms of cardiac and skeletal muscle physiopathology due to alterations in sarcomeric proteins (RU1) and muscle contraction (RU2).

RU1 and RU2 have a longstanding collaboration on investigating the role of sarcomeric proteins (31, 46) and have collaborated in several funded projects from national agencies (Ministry of University of Research (MUR), Ministry of Health, Telethon Italy). We have kept the consortium to two partners with well-defined roles to implement efficient and rapid communication and exchange of information. The overall proposal is structured into 6 interconnected work packages (WPs), involving both RUs as indicated in section 2. Therefore, each WP will require continuous exchange of expertise and experimental work between the two RUs.

RU1 is led by Dr. Marie-Louise Bang, who is a principal investigator at IRGB-CNR in Milan. A postdoc with experience in molecular and functional characterization of animal models of cardiac and skeletal muscle disease will be recruited for the project. Furthermore, Simona Nemska, a postdoc in her lab, will assist with echocardiography and mouse surgery. The recruited postdoc will visit RU2 for

biophysical analyses of striated muscles. The research of Dr. Bang's group is focused on studying the role of sarcomeric proteins in cardiac and skeletal muscle structure, signaling, and function to dissect the molecular and physiological pathways leading from alterations in sarcomeric proteins to cardiomyopathies and skeletal myopathies. These studies are based on the analysis of various mouse and cellular models using a multidisciplinary approach including cellular, molecular, biochemical, histological, ultrastructural, physiological, and biophysical methods.

Dr. Bang's group will be responsible for the molecular and functional analysis of the cardiac and skeletal muscle phenotype of MIKO mice. In particular, RU1 will study cardiac and skeletal muscle structure, signaling, and function by molecular, biochemical, cellular, and histological analyses as well as IF studies by advanced microscopical techniques. Furthermore, RU1 will analyze the cardiac functional phenotype of MIKO mice by echocardiography, hemodynamics, animal surgery, and electrophysiological studies.

The lab of Dr. Bang is located at the Humanitas Research Hospital and is part of the cardiovascular research team comprising approximately 40 researchers working on a variety of topics, including genetics of cardiac diseases, molecular mechanisms of myocardial contractility under normal and pathological conditions, non-coding RNAs, epigenetics, cardiovascular physiology, stem cells, and cardiac inflammatory pathways. Thus, RU1 possesses all research facilities and equipment necessary and an excellent environment for the execution of the proposed studies. ICH has about 13.000 m² of laboratory space dedicated to research and education as well as a 400 m² mouse specific pathogen free (SPF) animal facility. The cardiovascular research laboratories are fully equipped for molecular and cellular biology research and in vivo experimentation with complete access to a 500 m² shared molecular cardiology facility equipped with state of the art instrumentation, such as ES cell/BL2/BL3 rooms, a IonOptix system, and a complete setup for electrophysiological studies (Axon Digidata 1440A, pCLAMP, MultiClamp 700B, Scanning Ion Conductance Microscopy, etc.). ICH hosts several core facilities, including: an advanced optical microscopy facility (equipped with a Super-resolution Leica SP8 STED system, TrimScope II two-photon microscope (LaVision BioTec), Olympus Fluoview FV1000 and Leica SP8 confocal microscopes, LEICA DMi8 Wide-Field Microscope for live cell imaging, VS120 Virtual Microscopy Slide Scanning System (Olympus), Hyperion Imaging system (Fluidigm), etc.), a histology facility (equipment for tissue processing for fixed paraffin-embedded and/or cryopreserved samples; Leica ASP300 processor, Cryostats (Leica and Microm), Microtomes (Microm, Histo-line), and Immunostainer IntelliPath FLX (Biocare Medical)), a flow cytometry facility (FACSCalibur, a BD FACSymphony system, a FACSCalibur flow cytometer, a FACSAria II cell sorter (Beckton Dickinson), and the 10XGenomics single-cell sorter), a genetics core (ION TORRENT sequencer, NextSeq 500 Sequencing System, Illumina HiSeq 2000 system, AB 3500 genetic analyzer, IScan System, NGS Express Workstation, TECAN dispenser/DNA extractor, Agilent 4200 TapeStation, LabChip DX, NanoSight NS300, CLARIOstar Plus Microplate Reader), and a bioinformatics core. ICH also hosts a mouse specific pathogen free (SPF) facility with a well-equipped cardiac physiology lab, including surgical equipment, Vevo 2100 Imaging system (Visual sonics), MPVS Ultra Pressure Volume System (Millar Instruments), FMT2000 Fluorescence Tomography and IVIS Lumina Series III in vivo imaging systems (Perkin Elmer), telemetry system, Langendorff apparatus, running wheels, and grip strength meter.

Budget justification RU1		
Item A.1	Permanent employed personnel at IRGB-CNR: Dr. Marie-Louise Bang (6 months).	€35.880
Item A.2.1	One Postdoc (24 months) will be recruited for the molecular, histological, and functional analysis of the cardiac and skeletal muscle phenotype of minispryn knockout mice.	€54.275
Item B (flat rate equal to 60% of the total personnel cost A.1+A.2.1)	General expenses: Overheads, participation in meetings in Italy, travel expenses for combined experiments and meetings among partners of the RUs, open access expenses for publications of the results on international peer-reviewed journals.	€54.093
Item C	Not required	€0
Item D	Not required	€0
Item E	Consumables: Glass and plasticware (€3.000); reagents for molecular biology, biochemistry and histology (chemicals, enzymes, buffers, drugs, etc.) (€4.000); cell culture reagents (buffers, growth medium, serum, transfection reagents) (€4.500); kits (DNA and RNA isolation kits, cDNA synthesis kit, SYBR green kit, etc.) (€8.000); primary and secondary antibodies (€2.907), oligos (€1.000); Sanger sequencing (€500), RNA-Seq analysis, including quality check, library generation, and sequencing (€500 /sample for 12 samples; €6.000). Animal housing and maintenance (estimated average of 12 cages/day for 2 years - cost per cage/day in pathogen free facility managed by Charles River (€0,91 + TAX (22%)/cage/day; €9.725). Participation in one/two international meetings of one/two components of RU1 to present the results related to the project in an international environment (European Society of Cardiology congress (ESC), Heart failure Winter meeting, European Muscle Congress, etc.) (€2.000).	€41.632
Total	A+B+C+D+E	€185.880

RU2 is headed by Dr. Marco Caremani, Researcher at the Department of Biology, University of Florence (UNIFI) and Dr. Nicoletta Piroddi, Associate Professor at the Department of Experimental and Clinic Medicine, UNIFI. The two departments are joined in the PhysioLab Research Unit (UNIFI). A postdoc, with experience in physiology/biophysics of muscle contraction, will be recruited for the project.

Dr. Caremani will coordinate RU2, designing the experiments planned with RU1, perform the experiments on intact and skinned muscle fibers/trabeculae, and analyze data. Dr. Piroddi will be responsible for the execution of the experiments and data analysis on myofibrils from skeletal and cardiac muscle, and together with Dr. Caremani for simultaneous mechanical and energetic measurements in permeabilized skeletal and cardiac preparations. The recruited postdoc will be involved full-time in the execution of the biomechanical and combined mechanical and X-ray diffraction experiments at the ESRF as well as in data analysis on skeletal muscle (intact muscle, skinned fibers) and heart trabeculae/papillary muscle (intact and skinned). The postdoc will visit the lab of RU1 for the molecular and functional analysis.

The lab of Dr. Caremani is located in the Department of Biology, situated in the scientific campus of the University of Florence together with the Departments of Chemistry and Physics. The department, composed of approximately 45 researchers working on several topics (genetics, microbiology, zoology, and ecology), has a biochemistry facility, a high-throughput RNA-Seq system, its own electronic workshop for development of hardware and software as well as access to the mechanical workshop of the scientific campus and the animal house of the University of Florence. RU2 possesses all necessary research facilities and equipment and an excellent environment for the execution of the proposed studies. The department has about 5.000 m² of laboratory space dedicated to research. The laboratory of RU2 is equipped with the instruments and facilities necessary for the proposed experiments, including a setup for intact muscle/trabeculae and skinned muscle fibers/trabeculae, including an optoelectronic system for nm-μs sarcomere length control (striation follower); a force transducer with 50 kHz frequency resonance and loudspeaker motor servo system for length control; a setup for mechanics on single myofibrils, including a cantilever force transducer and a length control motor; and a setup for combined measurements of isometric force and ATP consumption in single/multicellular skinned skeletal muscle and cardiac preparations.

Budget justification RU2		
Item A.1	Permanent employed personnel at UNIFI: Dr. Marco Caremani (1 month) and Prof. Nicoletta Piroddi (1 month)	€10.910
Item A.2.1	One Postdoc (24 months) will be recruited to perform biomechanical studies and X-ray diffraction experiments as well as data analysis.	€55.000
Item B (flat rate equal to 60% of the total personnel cost A.1+A.2.1)	General expenses: Overheads, participation in meetings in Italy, travel expenses for combined experiments and meetings among partners of the RUs, open access expenses for publications of the results in international peer-reviewed journals.	€39.546
Item C	Not required	€0
Item D	Not required	€0
Item E	Consumables: Chemicals (€1.000), electronic and mechanical components for home-made devices (€1.000), animal house expenses (€1.000), participation in one/two international meetings per year for one/two components of RU2 to present the results related to the project in an international environment (European Muscle Conference, EU; Biophysical Society, USA) (€1.500), and for the Postdoc recruited for this research, also participation in biophysical schools in Europe (€954).	€5.454
Total	A+B+C+D+E	€110.910

The two RUs will cooperatively collect and interpret the data, perform statistical analyses, and disseminate the results of their research studies. Furthermore, Prof. Simona Boncompagni will perform the ultrastructural analyses (see letter of collaboration). Based on the expertise of the two RUs and collaborators in their respective fields, we do not anticipate major problems with the proposed experiments and expect to successfully complete the project with in the 2-year time frame and publish our results in high impact journals. We strongly believe to have in our hands the tools and scientific competence to perform the proposed experiments in the expected time frame, as described in the timeline (Table 1).

Table 1: GANTT

WP/Tasks	Title/Months	Year 1				Year 2				RUs involved
		3	6	9	12	15	18	21	24	
WP 1	Characterization of the expression and subcellular location of minispryn in health and disease									RU1
Task 1.1	Myospryn subcellular localization in mouse cardiac and skeletal muscle									RU1
Task 1.2	Minispryn expression and subcellular localization in human biopsies from cardiac and skeletal muscle									RU1
WP 2	Determination of interaction partners of minispryn									RU1
Task 2.1	Y2H screening to identify interaction partners of minispryn									RU1
Task 2.2	Biochemical and cellular assays for confirmation of minispryn interaction partners									RU1
WP 3	Role of minispryn in cardiac and skeletal muscle structure and function									RU1
WP 3.1	Structural role of myospryn in striated muscle									RU1
Task 3.1.1	Verification of MIKO mice									RU1
Task 3.1.2	Structural analyses of cardiac and skeletal muscle structure in MIKO mice									RU1
Task 3.1.3	Effect of minispryn KO on protein expression and localization as well as vesicle trafficking									RU1
WP 3.2	Functional role of myospryn in cardiac and skeletal muscle									RU1, RU2
Task 3.2.1	Effect of minispryn KO on cardiac and skeletal muscle function in vivo									RU1
Task 3.2.2	Biomechanical studies of cardiac and skeletal muscle function									RU2
WP 4	Role of minispryn in Ca2+ handling									RU1, RU2
Task 4.1	Simultaneous measurements of sarcomere shortening and Ca2+ transients in CMCs									RU1
Task 4.2	Assessment of SR Ca2+ load and release in adult CMCs									RU1
Task 4.3	Assessment of LTCC biophysical properties									RU1
Task 4.4	Force-pCa relation									RU2
WP 5	Role of minispryn in signaling									RU1
Task 5.1	Whole genome transcriptomic analysis									RU1
Task 5.2	Effect of minispryn on PKA signaling									RU1
Task 5.4	Role of minispryn in calcineurin signaling and muscle regeneration									RU1
WP 6	Dissemination									RU1, RU2

In addition to the regular contact between the units through e-mail and phone, the progression of the project will be monitored through periodic meetings (every 6 months) between the two RUs, facilitating evaluation of the progress, exchange of ideas, planning of future directions, and implementation of experiments. In addition, regular video conferences will be arranged whenever needed. Within the two collaborating labs, results will be presented and discussed at weekly lab meetings also including other labs in the respective departments, allowing for input and suggestions from senior PIs and other researchers. Furthermore, weekly journal clubs will be held where recent and prominent research findings from published papers in our research area will be discussed. This will keep us up-to-date with the newest research findings in our research fields as well as develop skills in discussion and critical reading of research approaches and results.

Exchange of expertise and transfer of knowledge between labs will be facilitated by exchange of personnel between labs.



Dawn E. Bowles, Ph.D.
Co-director Duke Human Heart Repository
Assistant Professor
Department of Surgery
Division of Surgical Sciences

21 March, 2022

To whom it may concern,

Dear Dr. Bang, Dear Dr. Marco Caremani,

With the present letter, I am writing to express our support of your research project: "*The structural and functional role of minispryn in cardiac and skeletal muscle*", coordinated by Dr. Marie-Louise Bang, to be submitted under the call for proposals to Progetti di Ricerca di Interesse Nazionale (PRIN) 2022 managed by the Ministry of University and Research (MUR).

The Duke Human Heart Repository (DHHR) directed by Drs. Bowles and Milano has amassed a collection of over 50,000 bio specimens from cardiac surgery patients. These specimens (obtained with informed patient consent) include heart samples acquired during surgery, explanted failing native heart, and non-failing heart explants, which were not used for cardiac transplantation. Thorough clinical information (medical history and echocardiogram reports) is available for most hearts. Tissues from non-failing hearts that have not been used for transplantation are available for use as controls or in basic cardiovascular research. Since the early 2000's the DHHR has collaborated in studies both internal and external to Duke in which we contribute by providing quality samples and clinical information. This has given these investigators the opportunity to use highly relevant models to discover and test causes and treatments for human cardiovascular disease.

More specifically, we agree to provide specimens from failing and non-failing hearts for determining whether minispryn is altered during cardiac disease. All specimens are consented and collected under an approved IRB protocol for future research purposes.

I am looking forward to our collaboration and wish you good luck with your proposal.

Sincerely yours,

A handwritten signature in black ink that reads "Dawn E. Bowles".

Dawn Bowles, Ph.D.



March 28th, 2022

Marie-Louise Bang, PhD
Istituto di Ricerca Genetica e Biomedica -
Consiglio Nazionale delle Ricerche (IRGB-CNR) at
Humanitas Research Hospital
Via Rita Levi Montalcini
20072 Pieve Emanuele (Milan), Italy

Dear Marie-Louise,

I am writing to express my strong willingness to serve as a collaborator for your PRIN 2022 research grant submission entitled: *"The structural and functional role of minispryn in cardiac and skeletal muscle"*.

As you know, our laboratory has expertise in studying cardiac and skeletal muscle ultrastructure by transmission electron microscopy, as in the past decade we have been involved in many studies of transgenic mouse models carrying mutations leading to defective cardiac or skeletal muscle function and disease. Thus, my expertise will provide great support for the ultrastructural characterization of cardiac and skeletal muscle of minispryn knockout mice.

I look forward to collaborate with you on this project by performing a detailed assessment of the potential ultrastructural defects of heart and skeletal muscle of minispryn knockout mice by transmission electron microscopy. As we have done previously for the ultrastructural analysis of myospryn knockout mice, you can provide me with fixed tissue samples and we will then perform the embedding and electron microscopy as well as the necessary analysis and image processing, including quantification of possible organelle alterations.

I wish you the best of luck with your application.

Yours Sincerely,

Simona Boncompagni



Ministero dell'Università e della Ricerca
SEGRETARIATO GENERALE
Direzione generale delle istituzioni della formazione superiore

N: 2461

Gent.le
Marco CAREMANI
E-Mail: marco.caremani@unifi.it

Courtesy translation of the original document in Italian language.

We hereby certify that Marco CAREMANI, born in Anghiari (AR) on 08/11/1973 , achieved the National Scientific qualification as associate in the Italian higher education system, in the call 2016/2018 (Ministerial Decree n. 1532/2016) for the disciplinary field of 05/D1 - Physiology. (Academic Recruitment Field 05/D - Physiology, according to the national classification).

The validity of the qualification is nine years, starting from the 03/08/2018 and will expire on the 03/08/2027¹.

Rome, 29/03/2022

La Dirigente
Dott.ssa Maria Giovanna Zilli²

¹The list of qualified candidates are always available on the website <https://abilitazione.miur.it>, "CANDIDATI E RISULTATI" section, by clicking on the year of the session of interest, link "Risultati".

²Firma autografa sostituita a mezzo stampa al sensi e per gli effetti dell'art.3, c.2, D.Lgs n.39/93

Il Responsabile del procedimento: La Dirigente Dott.ssa Maria Giovanna Zilli
Ex DGFIS – Ufficio V "Coordinamento dello stato giuridico ed economico del personale universitario
Via Michele Carcani, 61 – 00153 Roma – Tel. 06 9772 7057
email: dgsfis.ufficio5@mur.gov.it – PEC: dgsfis@postacert.istruzione.it



Ministero dell'Università e della Ricerca
SEGRETARIATO GENERALE
Direzione generale delle istituzioni della formazione superiore

N: 2461

Gent.le
Marco CAREMANI
E-Mail: marco.caremani@unifi.it

OGGETTO: ASN 2016/2018 - Attestazione di avvenuto conseguimento dell'Abilitazione Scientifica Nazionale alle funzioni di professore universitario di Seconda Fascia nel Settore Concorsuale 05/D1 - FISIOLOGIA.

Con la presente si attesta che Marco CAREMANI, nato a Anghiari (AR) il giorno 08/11/1973 , ha conseguito, all'esito delle procedure di Abilitazione Scientifica Nazionale bandite con decreto direttoriale n. 1532/2016, l'Abilitazione Scientifica Nazionale alle funzioni di professore universitario di seconda fascia nel Settore Concorsuale 05/D1 - FISIOLOGIA.

La validità dell'Abilitazione è di nove anni a decorrere dal 03/08/2018 e avrà scadenza il 03/08/2027¹.

Roma, 29/03/2022

La Dirigente
Dott.ssa Maria Giovanna Zilli²

¹Gli elenchi dei candidati abilitati sono sempre consultabili sul sito <https://abilitazione.miur.it> , sezione "CANDIDATI E RISULTATI", cliccando sull'anno della tornata di interesse, link "Risultati".

² Firma autografa sostituita a mezzo stampa ai sensi e per gli effetti dell'art.3, c.2, D.Lgs n.39/93

Il Responsabile del procedimento: La Dirigente Dott.ssa Maria Giovanna Zilli
Ex DGFIS – Ufficio V "Coordinamento dello stato giuridico ed economico del personale universitario
Via Michele Carcani, 61 – 00153 Roma – Tel. 06 9772 7057
email: dgis.ufficio5@mur.gov.it – PEC: dgis@postacert.istruzione.it

Minispryn was only relatively recently discovered as a striated muscle-specific protein highly homologous to the C-terminal TRIM-like region of myospryn with which it colocalizes at the jSR as well as in and around the nucleus (3). As of yet, the functional role of minispryn has remained completely unknown. However, based on its strong similarity to myospryn, we predict that the two proteins have similar functions and may collaborate in regulating EC coupling, PKA signaling, and other important pathways in heart and skeletal muscle. The potential impact of the present study can thus be predicted from what is known about myospryn, although this would need to be determined, which is the aim of the present project. Although analysis of MYKO mice have provided new insights into the role of myospryn in striated muscle, there is still a lot to be learnt about its specific role in Ca²⁺ handling and signaling in striated muscle. Also, it is possible that the role of myospryn may not be fully revealed in MYKO mice due to possible overlapping functions of minispryn that may partially compensate for the absence of myospryn. The same might be true for the MIKO mice, where myospryn may compensate for the lack of minispryn, although partial phenotype information from a MIKO mouse model, i.e. enlarged heart, increased heart weight, abnormal heart morphology, increased heart rate, and shortened RR interval, indicates that myospryn cannot fully compensate for the absence of minispryn. Thus, in the future, beyond the scope of the present project, our aim is to generate and study double KO mice for minispryn and myospryn. In addition to providing insights into the structural and functional role of minispryn in heart and skeletal muscle, the present project will thus provide the basis for future studies on double KO mice for minispryn and myospryn.

Several lines of evidence have suggested a role of myospryn in cardiac pathologies: 1) the identification of a single nucleotide polymorphism (SNP) within the CMY5 gene associated with cardiac hypertrophy in hypertensive patients (15); 2) identification of potential disease-causing heterozygous CMY5 mutations in patients with cardiac hypertrophy (47); 3) upregulation of myospryn during hypertrophy-inducing conditions in response to agonist stimulation (15); 4) the ability of myospryn to inhibit calcineurin (10) and role as a direct transcriptional target of MEF2A (7), both mediators of cardiac hypertrophy (7, 10); and 5) the development of cardiac dilation and systolic dysfunction in MYKO mice ((14) and Fig. 2-7), which we also found show a pathological response to TAC (Fig. 5) or chronic ISO stimulation (Fig. 6). Based on its homology to myospryn, we hypothesize that minispryn may likewise play a role in cardiac pathophysiology. The link of minispryn and myospryn to RyR2 (3) and the established role of myospryn as an AKAP through binding of its C-terminal region to PKA-RIIa (8, 9) suggest that they may target PKA to RyR, thereby affecting SR Ca²⁺ release. Furthermore, in a large-scale Y2H study (16), myospryn was found to interact with dysferlin and junctophilin 1/2 through its N-terminal region and SNAPIN through its C-terminal region, all part of the CRU, although this has not been confirmed by independent assays (16). Confirmation of these interactions would suggest a role of myospryn in tethering together regulatory proteins at the jSR, where it may regulate RyR organization and function, and consequently EC coupling, possibly also affecting the link between mitochondria and CRUs. This hypothesis is supported by the observation of altered CRUs (Fig. 3i-k), RyR2 disorganization (14), reduced PKA-mediated phosphorylation of RyR1 (Fig. 8B), and loss of SR-mitochondrial contact sites (14) in MYKO mice. Minispryn would not be expected to bind to dysferlin and junctophilin 1/2, but would be part of the same complex through its link to myospryn and RyR2. Since altered RyR function has been associated with arrhythmogenesis and heart failure (HF) (1), our study of minispryn may provide new insights into the molecular mechanisms leading to cardiac dysfunction. HF is the leading cause of death in the Western world and has an estimated prevalence of 2% in the general population and over 10% in people over 70 years of age (48). The 5-year mortality rate is over 40% (49) with sudden cardiac death in up to 50% of HF patients (50). Thus, HF represents a huge financial burden and significantly reduces longevity and quality of life of patients with enormous social and emotional impact. More insights into the molecular mechanisms leading to HF are therefore highly needed in order to develop novel therapeutic inventions for prevention, treatment, and cure.

If minispryn, like myospryn, can bind to PKA-RIIa and function as an AKAP, it may play a role in adrenergic signaling. The beta-adrenergic signaling pathway provides an important mechanism for enhancing cardiac performance during increased cardiac demands through stimulation of PKA-mediated phosphorylation of proteins involved in intracellular Ca²⁺ handling and the regulation of acto-myosin interactions, thereby increasing cardiac inotropism and lusitropism. For example the drug Milrinone, a phosphodiesterase-3 inhibitor increasing PKA activity through prevention of cAMP degradation, is used in the clinic as an inotropic drug to increase cardiac contractile function in acute HF patients (51). AKAPs act as scaffolding proteins that target PKA to specific subcellular compartments to enable activation of local signaling cascades by PKA phosphorylation, and play key roles in the modulation of cardiac function both under physiological and pathological conditions (52). In particular, AKAPs have been implicated in several cardiac pathologies, such as cardiac hypertrophy, cardiac dysfunction, and arrhythmogenesis (52). Thus, identification of minispryn as an AKAP as well as of PKA substrates affected by minispryn would provide a better understanding of the pathways contributing to cardiac disease, which could provide the basis for the development of drugs selectively interfering with pathological processes in the heart. One could for example imagine the development of peptides that selectively target selected protein-protein interactions within AKAP complexes, for example by inhibiting the ability of an AKAP to bind to a specific PKA substrate or alternatively to PKA itself. Such interfering peptides could be selectively delivered to CMCs via inhalable nanoparticles, a technology developed at IRGB-CNR and therapeutically validated with proof of concept in mouse and pig models of HF (44).

In skeletal muscle, the relevance of adrenergic control is still only partially known, although evidence points to an important role in physiological and pathological conditions. In particular, adrenergic control has proved important for the regulation of the duration of contraction and thus the movement speed (53, 54) as well as for increasing resistance to fatigue (Orbeli effect) (55). A contribution to control of proteolysis and resistance to atrophy has also been suggested (56). Furthermore, altered PKA signaling was reported in dystrophic muscle (9, 57). In particular, mislocalization of PKA-RIIa from the costamere and reduced myospryn levels were found in dystrophic muscle in which reduced PKA activity was found (8). This suggests a role of myospryn in targeting PKA to dystrophin with which it directly interacts, implicating myospryn in the pathogenesis of DMD. Based on homology, also minispryn may bind to

dystrophin and thus play a similar role. Therefore, our study may provide new insights into the mechanisms underlying DMD, a devastating disease affecting about 1 in 5000 male births, causing lifelong disability and early death (58). It is also possible that myospryn, and consequently minispryn, may be involved in the targeting of PKA to other substrates in skeletal muscle, including myospryn's interaction partners titin (5), desmin (4), and calcineurin (10), which are known targets of PKA.

If minispryn, like myospryn can bind to calcineurin, it may also be involved in the regulation of myofiber size, fiber type distribution, muscle growth, and regeneration (10). In fact, transgenic mice overexpressing myospryn's TRIM-like domain exhibited impaired skeletal muscle regeneration due to inhibited calcineurin signaling as well as the ability to repress the slow fiber transformation in calcineurin transgenic mice (10).

The interaction of myospryn's C-terminal region with the BLOC-1 component dysbindin (6) and possible binding to SNAPIN, another BLOC-1 subunit (21), suggests its possible role in the biosynthesis and position of lysosomes. Furthermore, desmin, a binding partner dysbindin and the extreme C-terminal region of myospryn, has been shown to be important for the proper perinuclear localization of myospryn as well as positioning of lysosomes (4). A potential role of myospryn in lysosome function is supported by our TEM analyses of MYKO mice showing accumulation of abnormal membranous structures in muscle (Fig. 2, 3). Based on homology, minispryn may bind to the same proteins, likewise implicating it in lysosome function. Defective lysosome function is associated with various neuromuscular diseases, characterized by accumulation of autophagic vacuoles and consequent buildup of abnormal mitochondria and damaged organelles, eventually leading to myofiber breakdown and loss of function. Thus, the present study may provide new insights into membrane trafficking, potentially leading to the definition of novel therapeutic strategies.

The impact of the project could be efficiently considered also in relation to the Horizon Europe programme, in particular in the cluster "Health" of Pillar 2: Global Challenges and European Industrial Competitiveness.

5. Financial aspects: costs and funding for each research unit

nº	Total cost (euro)	Co-funding (item A.1) (euro)	MUR funding (other items) (euro)
1. BANG Marie-louise	185.880	35.880	150.000
2. CAREMANI Marco	110.910	10.910	100.000
Total	296.790	46.790	250.000

6. Bibliography

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31. Filomena MC, et al. J Cachexia Sarcopenia Muscle 11, 169-194, 2020.
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B.2

1. Scientific Curriculum of the Principal Investigator

Marie-Louise Bang

Orcid: 0000-0001-8859-5034

Scopus: 7004092153

Research ID: B-5683-2015

Publons: 1261929

EDUCATION

09/10/01: Ph.D. in Natural Sciences (Dr. Rer. Nat.), Heidelberg University/European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.

31/01/97: M.Sc. in Chemical engineering, Biotechnology, Technical University of Denmark, Copenhagen, Denmark.

PROFESSIONAL HISTORY

01/21 - present: Principal investigator (level II) at the Institute of Genetic and Biomedical Research (IRGB), UOS Milan, National Research Council (CNR), UOS Milan, Italy.

04/12 - present: Group leader at Humanitas Research Hospital, Rozzano, Milan, Italy.

11/11 - 12/20: Permanent researcher (level III) at IRGB-CNR, UOS Milan, Italy.

12/10 - 10/11: Permanent researcher (level III) at the Institute of Neurobiology and Molecular Medicine (INMM)-CNR, Milan, Italy.

04/08 - 12/10: Assistant Telethon Scientist, Dulbecco Telethon Institute at the Institute of Biomedical Technologies (ITB) - CNR, Segrate, Milan, Italy.

07/07 - 12/11: Principal investigator at IRCCS Multimedica, Scientific and Technology Pole, Milan, Italy.

07/07 – 03/08: Researcher at ITB-CNR, Milan, Italy.

11/01 – 07/07: Postdoctoral researcher at the University of California San Diego (UCSD), Department of Medicine, La Jolla, USA.

04/98 – 10/01: PhD studies at the European Molecular Biology Laboratory (EMBL) in the Structural and Computational Biology program financed by a TMR Marie Curie Research Training Grant.

Thesis: A molecular genetic approach to the ligands of the titin/nebulin filament system.

08/97 – 09/97: Visiting scientist at Tokyo University, Department of Applied Biological Chemistry, Japan.

08/97 – 03/98: Research fellow at EMBL, Structural and Computational Biology, Heidelberg, Germany.

02/96 – 02/97: Master's degree thesis project at Novo Nordisk A/S, Bagsværd, Denmark.

RESEARCH ACTIVITY

The role of sarcomeric proteins in cardiac and skeletal muscle structure, signaling, and function to dissect the molecular and physiological pathways leading from mutations in sarcomeric proteins to cardiomyopathies and skeletal myopathies. These studies are based on the analysis of various mouse and cellular models using a multidisciplinary approach including cellular, molecular, biochemical, histological, ultrastructural, physiological, and biophysical methods.

SUPERVISION

Supervisor of 3 Ph.D. students and 4 postdoctoral fellows of 4 different nationalities.

AWARDS AND HONORS

30/01/14: National habilitation (“abilitazione scientifica nazionale”) in Physiology (sector 05/D1) for Associate Professorship, MIUR.

09/06/14: National habilitation in Applied Medical Technologies and Biotechnologies (sector 06/N1) for Associate Professorship, MIUR.

04/08 – 03/13: Assistant Telethon Scientist award, Dulbecco Telethon Institute.

02/08 – 01/11: Fondazione Cariplo award for young international researcher in Italy.

11/01 – 10/03: Danish Natural Science Research Council postdoctoral fellowship.

11/01 – 07/03: American Heart Association (AHA) postdoctoral fellowship.

04/98 – 03/01: Training and Mobility of Researchers (TMR) Marie Curie Research Training Grant.

PROFESSIONAL MEMBERSHIPS

Since 2016: Treat-NMD Neuromuscular network

Since 2013: European Society of Cardiology (ESC) Working groups: WG02 – “Cellular Biology of the Heart” and WG04 – “Myocardial Function”

Since 2010: International Society of Heart Research (ISHR), Heart Failure association

EDITORIAL ACTIVITY

2021 – present: Editorial board member of Biomedicines Molecular and Translational Medicine Section.

2016 – present: Review Editor at the Editorial Board of Cardiovascular Biologics and Regenerative Medicine, a specialty of Frontiers in Cardiovascular Medicine.

2008 – present: Reviewer for scientific journals: Circulation, Cardiovascular Research, Science Advances, FASEB J, PLoS One, Communications Biology, Journal of Cell Science, Cell and Tissue Research, Human Molecular Genetics, Developmental Dynamics, Cell Motility, Cytoskeleton, BBA Molecular Basis of Disease, BBA Molecular Cell Research, International Journal of Cardiology, Journal of Muscle Research and Cell Motility, International Journal of Molecular Sciences, and Histochemistry and Cell Biology, Gene, EBioMedicine, Journal of Clinical Medicine, Life Sciences, Journal of Zhejiang University-SCIENCE B (JZUS-B) (Biomedicine & Biotechnology), Molecules.

2008 – present: Reviewer of grant applications: Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR); The Association Française contre les Myopathies (AFM), France; French National Research Agency (ANR); Muscular Dystrophy UK, Netherlands Organization for Scientific Research (MWO), Earth and Life Sciences division; KU Leuven Research Council, The Netherlands; Research Foundation - Flanders (FWO); The Netherlands.

2014: External Reviewer of PhD thesis from University of Vienna, Austria.

DECISIONAL ROLES AT PUBLIC EVENTS

2021: Poster judge at the 18th IIM (Istituto Interuniversitario di Miologia) meeting. Online only. October 22-24.

2021: Member of the young investigator Marcus C. Schaub Award Committee of the European Muscle Conference 2021, Online-only event. September 23-26.

2018: Poster judge at the International Society of Heart Research - European section meeting 2018 (ISHR-2018-ES), Amsterdam, The Netherlands, July 16-19.

PUBLICATIONS

Papers

33 peer-reviewed scientific papers, 4 reviews, 2 editorials, 1 book chapter

H-index: 25 (Scopus)

Citations: 4759

Patents

Patent publication no. WO 98/36056 (20th of August 1998): An enzyme with endo-1,3(4)-beta-glucanase activity.

Inventors: Bang ML, Sandal T (Patent holder: Novo Nordisk A/S).

PRESENTATIONS AT NATIONAL AND INTERNATIONAL CONGRESSES

2018: International Society of Heart Research - European section meeting 2018 (ISHR-2018-ES), Amsterdam, The Netherlands, July 16-19. Abstract published in J Mol Cell Cardiol. 120, suppl. 1, 43, July 2018, Abstract #115.

2018: "EMBL in Italy" event at IFOM, Milan, Italy, May 3.

2016: European Muscle Conference. Montpellier, France, September 2-6.

2016: Conference on structure and dynamics of the sarcomere. Belgrade, Serbia May 4-6.

2015: Myocardial function & Cellular Biology of the Heart Meeting 2015. Varenna, Italy. March 30 - April 3.

2014: EMBO conference: Molecular Biology of Muscle Development and Regeneration. Acaya-Lecce, Italy. May 14-18.

2013: EMBL conference: Myofibrillar Z-disc Structure and Dynamics. EMBL Hamburg, Germany October 14-17.

2012: Workshop in connection with the "Muscle Z-disk Protein Complexes: from atomic structure to physiological function" (MUZIC) consortium under the 7th framework programme of EU. University of Padova, Italy, October 2.

2011: International conference on muscle wasting. Ascona, Switzerland. September 18-23.

2011: HFA Winter Research Meeting 2011. Les Diablerets, Switzerland. January 26-29.

2010: Settimo meeting Istituto Interuniversitario di Miologia (IIM). Certosa di Pontignano, Vagliagli (Siena), October 14-16. Keynote speaker.

2010: Spring Padua Muscle Days. Terme Euganee, Padua, Italy. April 22-24.

2010: "Muscle Z-disk Protein Complexes: from atomic structure to physiological function" (MUZIC) meeting under the 7th framework programme of EU. Vienna, Austria. November 25-26.

2009: European Muscle Conference, Lille, France. September 12-16.

2008: Keystone symposium: Common Mechanisms in Arrhythmias and Heart Failure. Keystone, USA, April 2-7.

"Ablation of myopalladin results in pressure overload-induced dilated cardiomyopathy".

2008: Telethon DTI meeting. Bardolino, Italy, October 21-23.

2008: ESC 2008. Munich, Germany, August 30 – September 3.

2008: Heart Failure 2008 Congress. Milan, Italy, June 14-17.

2007: Quarto meeting Istituto Interuniversitario di Miologia. Scuola dello Sport del CONI, Roma, November 21-24.

PUBLIC ENGAGEMENT ACTIVITIES

2016: Speaker at "Stati Generali della Ricerca Sanitaria 2016" event. Rome, April 27-28.

FUNDING

2022 – 2025: Programmi di Ricerca Scientifica di Rilevante Interesse Nazionale (PRIN) 2020, 20207P85MH (PI of unit) "The structural and functional role of the A-kinase anchoring protein myospryn in striated muscle", €189.351

2019 – 2022: ERAPerMed 2018, ID 271 (PI of unit) "Stratification of heart failure patients for cardiac recovery upon cardiac unloading by left ventricular assist device therapy: addressing the molecular, epigenetic, and proteomic changes associated with reverse cardiac remodelling", €500.000

2015 – 2018: Agenzia Spaziale Italia (ASI), Bando Biomedicina e Biotecnologie in ambito Spaziale – 2012 Bando di Ricerca, 2015-009-R.0 (PI of unit) "Identification and characterization of mechanosensors of gravity. Study of their signaling and physiopathological effects on the myocardium, neural tissue, skeletal muscle, and bone tissue", €51.806

2014 - 2018: MIUR-CNR Progetto bandiera Invecchiamento (PI of unit) "Mouse models of senescence induced by stress and associated with amyloidosis", €79.166,67

2013 - 2016: PRIN 2010-2011, 2010R8JK2X_006 (PI of unit) "Physiological and pathological mechanisms in skeletal muscle", €99.354

2012 - 2016: Fondazione Telethon, GGP12282 (coordinator) "Myopalladin in dilated cardiomyopathy and limb girdle muscular dystrophy" €218.900 (total budget: €430.600)

2008 - 2013: Fondazione Telethon, Dulbecco Telethon institute, TCP07006, Assistant Telethon Scientist award (PI), "The role of nebulin in nemaline myopathy", €517.000

2008 - 2011: Fondazione Cariplò, 2007.5812 (PI) "The role of sarcomeric proteins in striated muscle development, function, and disease", €330.000

2008 - 2011: Ministero della Salute, Progetto Ordinario, RF-MUL-2007-666195 (coordinator) "The role of myopalladin in human dilated cardiomyopathy and limb girdle muscular dystrophy", €207.000 (total budget: €425.000)

2. Scientific Curriculum of the associated investigators

1. CAREMANI Marco

Marco Caremani

Orcid: 0000-0001-9128-7227

Scopus: 23110319600

EDUCATION AND TRAINING

2008: Ph.D. in Physiology Sciences Department of Physiology, University of Florence, Florence, Italy

2003: M.Sc. in Natural Sciences, University of Florence, Florence Italy

PROFESSIONAL HISTORY

2017 - present: Researcher, Department of Biology, University of Florence, Florence, Italy

2009 - 2017: Postdoctoral researcher, Department of Biology, University of Florence, Florence, Italy

2008 - 2009: Research Associate, Imperial College London, London, UK

2007 - 2008: Postgraduate student in the laboratory of Prof. Michael Ferenczi, Imperial College London, London, UK

RESEARCH ACTIVITY

My scientific activity concerns the study of the molecular mechanism of muscle contraction and regulation, i.e. how the energy released by hydrolysis of ATP at the catalytic site of the myosin motor attached to the actin filament is transformed into force and shortening at the sarcomere level as well as how myofilament regulation controls heart and skeletal muscle performance. These goals are pursued through an integrated approach, combining mechanical, biochemical, spectroscopy, and X-ray diffraction with synchrotron radiation on skeletal muscle fibers and cardiac trabeculae.

In collaboration with Dr. Marie-Louise Bang and Prof. Miklos Kellermayer, I have been involved in experiments aimed at investigating the role of cytoskeletal proteins (nebulin, myopalladin, and titin) on muscle performance and, more recently, on the mechanisms of action of small molecules used in clinical trials as cardiac myosin activators.

SCIENTIFIC ACHIEVEMENTS

1. Chemo-mechanical coupling investigated at the sarcomere level in demembranated fibers of skeletal muscle. By synchronising the action of the myosin motors in the half-sarcomere with force steps in the presence of different concentrations of calcium and inorganic phosphate (Pi), we discovered that the release of ATP hydrolysis products (Pi and ADP) from the catalytic site of a myosin motor and the execution of the working stroke are not tightly coupled (Linari et al. Proc R Soc B. 277:19, 2010, Caremani et al. J Physiol. 591:5187, 2013; Caremani et al. J Physiol. 593:3313, 2015). These results have provided the constraints for a mechano-kinetic model able to explain the mechanical, biochemical, and energetical aspects of muscle contraction, including the problem related to the

decrease of ATP consumption during prolonged contraction at low load (Fusi et al. J Physiol 595:1127, 2017). During my period in London, I developed a setup combining the T-jump technique with fluorescence measurements in order to estimate the ATPase rate during isometric and isotonic contraction. With this system, it is possible to measure simultaneous fluorescence signals from two probes in demembranated muscle fibers, ensuring correction for artefacts related to mechanical perturbations (Caremani et al. Biophys J. 98:348A, 2010).

2. Structural dynamics of the molecular motor and myofilaments studied by X-ray diffraction in skeletal muscle. The high collimation of the beam of 3rd generation synchrotrons has made it possible to record the fine structure of the myosin-based reflections and the spacing of the high-order myosin- and actin-based X-ray reflections. In this way, we measured the contribution of myofilaments to half-sarcomere compliance (Brunello et al. J Physiol 592:3881, 2014).

3. Regulation of muscle contraction in skeletal and cardiac muscles. Combining sarcomere level mechanics and X-ray interference, we discovered a novel thick filament-based regulation of muscle contraction in skeletal muscle (Linari et al. 2015 Nature 528:276, 2015). Successively, we demonstrated that in mammalian muscle, unlike amphibian muscle, lowering the temperature traps myosin motors in a refractory state that prevents activation (Caremani et al. J. Gen. Physiol 151:1272, 2019). To investigate the dual filament regulation in the heart, I have developed a setup to apply sarcomere-level mechanics to intact cardiac trabeculae (Caremani et al. PNAS. 113:3675, 2016; Pinzauti et al. J Physiol. 596:2581, 2018). The system, adapted for vertical mounting of trabeculae at synchrotron beamlines, allowed us to reveal the role of thick filament mechanosensing in the regulation of cardiac contractility (Reconditi et al. PNAS. 114:3240, 2017). The interdependency of thin and thick filament regulatory mechanisms in intact trabeculae from rat heart has been tested by recording the X-ray diffraction signals that mark the state of the thick filament during inotropic interventions (increase in sarcomere length and addition of the β -adrenergic effector isoprenaline), which potentiate the twitch force developed by an electrically paced trabecula by up to twofold. During diastole, none of the signals related to the OFF state of the thick filament were significantly affected by these interventions, indicating that recruitment of myosin motors from their OFF state occurs independently and downstream of thin filament activation (Caremani et al. J Gen Physiol. 151:53, 2019).

4. Modulation of contraction by sarcomeric and cytoplasmic proteins. In collaboration with Dr. Marie-Louise Bang (Istituto di Ricerca Genetica e Biomedica - (IRGB-CNR), Milan), we studied the role of nebulin (an skeletal muscle-specific protein associated with the actin filament in the sarcomere) and myopalladin (a striated muscle-specific protein located in the Z-line and I-band of the sarcomere) by comparing the performance of demembranated fibers/intact muscle from knockout and wildtype control mice, revealing that nebulin has a fundamental role in modulating muscle contraction (Bang et al FASEB J. 23:4117, 2009), while myopalladin promotes skeletal muscle growth (Filomena et al. J Cachexia Sarcopenia Muscle 11:160, 2020). In collaboration with Prof. Miklos Kellermayer (Semmelweis University, Budapest, Hungary), we studied the role of titin in the passive force response following stretch by integrating single fiber mechanics and single molecule mechanics, demonstrating that the titin PEVK domain contributes to the force response induced by the sarcomere lengthening (Martonfalvi et al. J Cell Sci. 127:858, 2014).

5. Test of specific drugs acting on the molecular motor. In collaboration with Prof. Coen Ottenheijm (University of Amsterdam, The Netherlands), we studied the mechanism of action of omecamtiv mecarbil (OM), a positive inotropic drug, on the β /slow MHC isoform. The results showed that OM has an inhibitory effect on force generation, which can be reversed by a physiological level of intracellular inorganic phosphate (Governali et al. Nat. Commun. 11:3405, 2020). In collaboration with Prof. Dietmar Manstein (Hannover Medical School, Germany), we investigated the effect of rumenic acid (RA) on the cardiac myosin motor, showing that RA acts as an allosteric effector of cardiac myosin motor domains (Pertici et al. J. Physiol 599:3639, 2021).

SUPERVISION

Supervisor of 3 Ph.D. students and 9 M.Sc. Students

TEACHING ACTIVITY

2015 - present: Comparative Physiology, School of Sciences, University of Florence, Italy

2016 - 2021: General Physiology, School of Sciences, University of Florence, Italy

2021 - present: Physiology, School of Human Health Sciences, University of Florence, Italy

INSTITUTIONAL RESPONSIBILITIES

2021: Member of the traineeship and thesis committee of the Biotechnology degree course, University of Florence,

Italy

ORGANISATION OF SCIENTIFIC MEETINGS

2018: Organiser of the 69th National Congress of the Italian Physiological Society, Italy
2022: Organiser of the 50th European Muscle Conference, Florence, Italy

AWARDS AND HONORS

03/08/2018: National habilitation ("abilitazione scientifica nazionale") in Physiology (sector 05/D1, scientific disciplinary sector BIO/09) for Associate Professorship, MIUR

PROFESSIONAL MEMBERSHIPS

2015 – present: Italian Physiological Society, Italy
2010 – present: Biophysical Society, USA

EDITORIAL ACTIVITY

2017 – present: Reviewer for the Journal of Physiology, Physiology, and Frontiers in Physiology

PUBLICATIONS

Papers (from Scopus)
26 peer-reviewed scientific papers (11 first and 3 last/corresponding author)
H-index: 15
Number of citations: 838

INVITED SPEAKER AT NATIONAL/INTERNATIONAL CONFERENCES

2019: 16th Alpbach Motors Workshop on "Myosin & Muscle, and other Motors", Alpbach (Austria), March 30 - April 5
2018: Europhysiology 2018, London, United Kingdom. Symposium, September 14-16
2016: 67th National Congress of the Italian Physiological Society, Catania, Italy, September 21-23
2015: 66th National Congress of the Italian Physiological Society, Genoa, Italy, September 16-18
2013: Annual Meeting of Young Researchers in Physiology, Anacapri, Italy, May 21-24
2013: Alpbach Workshop on "Molecular motors", Alpbach, Austria, March 18-23
2010: Alpbach Workshop on "Molecular Motors", Alpbach, Austria, ", April 1-5
2007: Alpbach Workshop on "Molecular Motors", Alpbach, Austria, March 24-30
2007: Riunione Nazionale dei Dottorandi di Fisiologia, Siena, Italy, July 18-21
2006: III meeting Istituto Interuniversitario di Miologia (IIM), Rome, Italy, November 9-11

FUNDING

2018-2019: University of Florence, bando per progetti competitivi per Ricercatori a Tempo Determinato (RTD) (coordinator). "The role of upregulated titin compliance on the structure-function relationship of striated muscle"

2018: Ministero della Ricerca Fondo di Finanziamento Attività Base di Ricerca (FFABR)

2018 – present: University of Florence (cofin) (coordinator). "In situ study of the mechano-kinetic properties of different isoforms of the the muscle myosin II"

In collaboration with Dr. Marie Louise Bang (coordinator), I participated in the following research projects:

2012 – 2016: Fondazione Telethon, GGP12282. "Myopalladin in dilated cardiomyopathy and limb girdle muscular dystrophy"

2008 – 2011: Ministero della Salute, Progetto Ordinario, RF-MUL-2007-666195. "The role of myopalladin in human dilated cardiomyopathy and limb girdle muscular dystrophy"

3. Main Principal Investigator's scientific publications (Max. 20)

1. Bang M, Bogomolovas J, Chen J (2022). Understanding the molecular basis of cardiomyopathy. AMERICAN JOURNAL OF PHYSIOLOGY. HEART AND CIRCULATORY PHYSIOLOGY, vol. 322, p. H181-H233, ISSN: 0363-6135, doi: 10.1152/ajpheart.00562.2021 - **Articolo in rivista**

2. Filomena MC, Yamamoto DL, Carullo P, Medvedev R, Ghisleni A, Piroddi N, Scellini B, Crispino R, D'Autilia F, Zhang J, Felicetta A, Nemska S, Serio S, Tesi C, Catalucci D, Linke WA, Polishchuk R, Poggesi C, Gautel M, Bang M (2021). Myopalladin knockout mice develop cardiac dilation and show a maladaptive response to mechanical pressure overload. ELIFE, vol. 10, e58313, ISSN: 2050-084X, doi: 10.7554/eLife.58313 - **Articolo in rivista**

3. Filomena MC, Yamamoto DL, Caremani M, Kadarla VK, Mastrototaro G, Serio S, Vydyanath A, Mutarelli M, Garofalo A, Pertici I, Knöll R, Nigro V, Luther PK, Lieber RL, Beck MR, Linari M, Bang M (2020). Myopalladin promotes muscle growth through modulation of the serum response factor pathway. JOURNAL OF CACHEXIA, SARCOPENIA AND MUSCLE, vol. 11, p. 169-194, ISSN: 2190-5991, doi: 10.1002/jcsm.12486 - **Articolo in rivista**

4. Bang M (2017). Animal models of congenital cardiomyopathies associated with mutations in Z-line proteins.. JOURNAL OF CELLULAR PHYSIOLOGY, vol. 232, p. 38-52, ISSN: 0021-9541, doi: 10.1002/jcp.25424 - **Articolo in rivista**

5. Lange S, Gehmlich K, Lun AS, Blondelle J, Hooper C, Dalton ND, Alvarez EA, Zhang X, Bang M, Abassi YA, Dos Remedios CG, Peterson KL, Chen J, Ehler E (2016). MLP and CARP are linked to chronic PKC α signalling in dilated cardiomyopathy. NATURE COMMUNICATIONS, vol. 7, 12120, ISSN: 2041-1723, doi: 10.1038/ncomms12120 - **Articolo in rivista**

6. Rusconi F, Ceriotti P, Miragoli M, Carullo P, Salvarani N, Rocchetti M, Di Pasquale E, Rossi S, Tessari M, Caprari S, Cazade M, Kunderfranco P, Chemin J, Bang M, Polticelli F, Zaza A, Faggian G, Condorelli G, Catalucci D (2016). Peptidomimetic Targeting of Cav β 2 Overcomes Dysregulation of the L-Type Calcium Channel Density and Recovers Cardiac Function. CIRCULATION, vol. 134, p. 534-546, ISSN: 1524-4539, doi: 10.1161/CIRCULATIONAHA.116.021347 - **Articolo in rivista**

7. Bang M, Chen J (2015). Roles of Nebulin Family Members in the Heart.. CIRCULATION JOURNAL, vol. 79, p. 2081-2087, ISSN: 1346-9843, doi: 10.1253/circj.CJ-15-0854 - **Articolo in rivista**

8. Mastrototaro G, Liang X, Li X, Carullo P, Piroddi N, Tesi C, Gu Y, Dalton ND, Peterson KL, Poggesi C, Sheikh F, Chen J, Bang M (2015). Nebulette knockout mice have normal cardiac function, but show Z-line widening and up-regulation of cardiac stress markers.. CARDIOVASCULAR RESEARCH, vol. 107, p. 216-225, ISSN: 0008-6363, doi: 10.1093/cvr/cvv156 - **Articolo in rivista**

9. Bang M, Gu Y, Dalton ND, Peterson KL, Chien KR, Chen J (2014). The muscle ankyrin repeat proteins CARP, Ankrd2, and DARP are not essential for normal cardiac development and function at basal conditions and in response to pressure overload. PLOS ONE, vol. 9, ISSN: 1932-6203, doi: 10.1371/journal.pone.0093638 - **Articolo in rivista**

10. Bean C, Verma NK, Yamamoto DL, Chemello F, Cenni V, Filomena MC, Chen J, Bang M, Lanfranchi G (2014). Ankrd2 is a modulator of NF- κ B-mediated inflammatory responses during muscle differentiation.. CELL DEATH & DISEASE, vol. 5, e1002, ISSN: 2041-4889, doi: 10.1038/cddis.2013.525. - **Articolo in rivista**

11. Yamamoto DL, Vitiello C, Zhang J, Gokhin DS, Castaldi A, Coulis G, Piaser F, Filomena MC, Eggenhuizen PJ, Kunderfranco P, Camerini S, Takano K, Endo T, Crescenzi M, Luther PK, Lieber RL, Chen J, Bang M (2013). The nebulin SH3 domain is dispensable for normal skeletal muscle structure but is required for effective active load bearing in mouse. JOURNAL OF CELL SCIENCE, vol. 126, p. 5477-5489, ISSN: 0021-9533 - **Articolo in rivista**

12. BANG M, CAREMANI M, BRUNELLO E, LITTLEFIELD R, LIEBER RL, CHEN J, LOMBARDI V, LINARI M (2009). Nebulin plays a direct role in promoting strong actin-myosin interactions. FASEB JOURNAL, vol. 23, p. 4117-4125, ISSN: 0892-6638 - **Articolo in rivista**

13. BANG M, ZHANG J, GOKHIN DS, LU Y, CUI L, LI X, GU Y, DALTON ND, SCIMIA MC, PETERSON KL, LIEBER RL, CHEN J (2008). Syncolin is required for generating maximum isometric stress in skeletal muscle but dispensable for muscle cytoarchitecture. AMERICAN JOURNAL OF PHYSIOLOGY. CELL PHYSIOLOGY, vol.

294, p. 1175-1182, ISSN: 0363-6143 - **Articolo in rivista**

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14. BANG M, SHEIKH F, LANGE S, CHEN J (2007). "Z" eroing in on the Role of Cypher in Striated Muscle Function, Signaling and Human Disease. TRENDS IN CARDIOVASCULAR MEDICINE, vol. 17, p. 259-263, ISSN: 1050-1738 - **Articolo in rivista**
15. CARÈ, A CATALUCCI, D FELICETTI, F BONCI D, ADDARIO A, GALLO P, BANG M, SEGNALINI P, GU Y, DALTON ND, LATRONICO MVG, HØYDAL M, AUTORE C, RUSSO MA, DORN GW, ELLINGSEN Ø, RUIZ-LOZANO P, PETERSON KL, CROCE CM, PESCHLE C... (2007). MicroRNA-133 controls cardiac hypertrophy. NATURE MEDICINE, vol. 13, p. 613-618, ISSN: 1078-8956 - **Articolo in rivista**
16. BANG M, LI X, LITTLEFIELD R, BREMNER S, THOR A, KNOWLTON K, LIEBER R, CHEN J (2006). Nebulin-deficient mice exhibit shorter thin filament lengths and reduced contractile function in skeletal muscle. THE JOURNAL OF CELL BIOLOGY, vol. 173, p. 905-916, ISSN: 0021-9525 - **Articolo in rivista**
17. BANG M, MILLER MK, WITT CC, LABEIT D, TROBITAS C, WATANABE K, GRANZIER H, MCELHINNY AS, GREGORIO CC, LABEIT S (2003). The muscle ankyrin repeat proteins: CARP, ankrd2/Arpp and DARP as a family of titin filament based stress response molecules. JOURNAL OF MOLECULAR BIOLOGY, vol. 7, p. 951-964, ISSN: 1089-8638 - **Articolo in rivista**
18. KNÖLL R, HOSHIJIMA M, HOFFMAN HM, PERSON V, LORENZEN-SCHMIDT I, BANG M, HAYASHI T, SHIGA N, YASUKAWA H, SCHAPER W, MCKENNA W, YOKOYAMA M, SCHORK NJ OMENS, JH MCCULLOCH AD, KIMURA A, GREGORIO CC, POLLER W, SCHAPER J, SCHULTHEISS HP, CHIEN KR (2002). The cardiac mechanical stretch sensor machinery involves a Z disc complex that is defective in a subset of human dilated cardiomyopathy. CELL, vol. 111, p. 943-955, ISSN: 0092-8674 - **Articolo in rivista**
19. BANG M, CENTNER T, FORNOFF F, GEACH A, GOTTHARDT M, MCNABB M, WITT CC, LABEIT D, GREGORIO CC, GRANZIER H, LABEIT S (2001). The complete gene sequence of titin, expression of an unusual ~700 kDa titin isoform and its interaction with obscurin identify a novel Z-line to I-band linking system. CIRCULATION RESEARCH, vol. 89, p. 1065-1072, ISSN: 0009-7330 - **Articolo in rivista**
20. BANG M, MUDRY RE, MCELHINNY AS, TROMBITÁS K, GEACH A, YAMASAKI R, SORIMACHI H, GRANZIER H, GREGORIO C, LABEIT S (2001). Myopalladin, a novel 145 kDa sarcomeric protein with multiple roles in Z-disc and I-band protein assemblies. THE JOURNAL OF CELL BIOLOGY, vol. 153, p. 413-428, ISSN: 0021-9525 - **Articolo in rivista**
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4. Main scientific publications of the associated investigators (Max. 20, for each research unit)

1. CAREMANI Marco

1. Caremani M., Reconditi M. (2022). Anisotropic Elasticity of the Myosin Motor in Muscle. INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES, vol. 23, ISSN: 1661-6596, doi: 10.3390/ijms23052566 - **Articolo in rivista**
2. Caremani M., Fusi L., Linari M., Reconditi M., Piazzesi G., Irving T. C., Narayanan T., Irving M., Lombardi V., Brunello E. (2021). Dependence of thick filament structure in relaxed mammalian skeletal muscle on temperature and interfilament spacing. JOURNAL OF GENERAL PHYSIOLOGY, vol. 153, ISSN: 0022-1295, doi: 10.1085/JGP.202012713 - **Articolo in rivista**
3. Pertici, Irene, Taft, Manuel H, Greve, Johannes N, Fedorov, Roman, Caremani, Marco§, Manstein, Dietmar J§ (2021). Allosteric modulation of cardiac myosin mechanics and kinetics by the conjugated omega-7,9 trans-fat ruminic acid. THE JOURNAL OF PHYSIOLOGY, vol. 599, p. 3639-3661, ISSN: 1469-7793, doi: 10.1113/JP281563 - **Articolo in rivista**
4. Governali, Serena, Caremani, Marco, Gallart, Cristina, Pertici, Irene, Stienen, Ger, Piazzesi, Gabriella, Ottenheijm, Coen, Lombardi, Vincenzo, Linari, Marco (2020). Orthophosphate increases the efficiency of slow muscle-myosin isoform in the presence of omecamtiv mecarbil. NATURE COMMUNICATIONS, vol. 11, p. 3405-3407, ISSN: 2041-1723, doi: 10.1038/s41467-020-17143-2 - **Articolo in rivista**
5. Caremani, Marco, Brunello, Elisabetta, Linari, Marco, Fusi, Luca, Irving, Thomas C, Gore, David, Piazzesi, Gabriella, Irving, Malcolm, Lombardi, Vincenzo, Reconditi, Massimo (2019). Low temperature traps myosin motors of mammalian muscle in a refractory state that prevents activation. JOURNAL OF GENERAL PHYSIOLOGY, vol. 151, p.

- 1272-1286, ISSN: 0022-1295, doi: 10.1085/jgp.201912424 - **Articolo in rivista**
6. Caremani, Marco, Pinzauti, Francesca, Powers, Joseph D., GOVERNALI, SERENA, Narayanan, Theyencheri, Stienen, Ger J. M., Reconditi, Massimo, Linari, Marco, Lombardi, Vincenzo, Piazzesi, Gabriella (2019). Inotropic interventions do not change the resting state of myosin motors during cardiac diastole. *JOURNAL OF GENERAL PHYSIOLOGY*, vol. 151, p. 53-65, ISSN: 0022-1295, doi: 10.1085/jgp.201812196 - **Articolo in rivista**
 7. Filomena, Maria Carmela, Yamamoto, Daniel L, Caremani, Marco, Kadarla, Vinay K, Mastrototaro, Giuseppina, Serio, Simone, Vydyanath, Anupama, Mutarelli, Margherita, Garofalo, Arcamaria, Pertici, Irene... (2019). Myopalladin promotes muscle growth through modulation of the serum response factor pathway. *JOURNAL OF CACHEXIA, SARCOPENIA AND MUSCLE*, p. 1-26, ISSN: 2190-5991, doi: 10.1002/jcsm.12486 - **Articolo in rivista**
 8. Gabriella Piazzesi, Marco Caremani, Marco Linari, Massimo Reconditi, Vincenzo Lombardi (2018). Thick Filament Mechano-Sensing in Skeletal and Cardiac Muscles: A Common Mechanism Able to Adapt the Energetic Cost of the Contraction to the Task. *FRONTIERS IN PHYSIOLOGY*, vol. 9, p. 1-7, ISSN: 1664-042X, doi: 10.3389/fphys.2018.00736 - **Articolo in rivista**
 9. PINZAUTI, FRANCESCA, PERTICI, IRENE, Reconditi, Massimo, Narayanan, Theyencheri, Stienen, Ger J. M., Piazzesi, Gabriella, Lombardi, Vincenzo, Linari, Marco, Caremani, Marco (2018). The force and stiffness of myosin motors in the isometric twitch of a cardiac trabecula and the effect of the extracellular calcium concentration. *THE JOURNAL OF PHYSIOLOGY*, vol. 596, p. 2581-2596, ISSN: 0022-3751, doi: 10.1113/JP275579 - **Articolo in rivista**
 10. Percario, Valentina, Boncompagni, Simona, Protasi, Feliciano, Pertici, Irene, Pinzauti, Francesca, Caremani, Marco (2018). Mechanical parameters of the molecular motor myosin II determined in permeabilised fibres from slow and fast skeletal muscles of the rabbit. *THE JOURNAL OF PHYSIOLOGY*, vol. 596, p. 1243-1257, ISSN: 0022-3751, doi: 10.1113/JP275404 - **Articolo in rivista**
 11. RECONDITI, MASSIMO, CAREMANI, MARCO, PINZAUTI, FRANCESCA, Powers, J. D., Narayanan, T., Stienen, G. J. M., LINARI, MARCO, LOMBARDI, VINCENZO, PIAZZESI, GABRIELLA (2017). Myosin filament activation in the heart is tuned to the mechanical task. *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA*, vol. 114, p. 3240-3245, ISSN: 0027-8424, doi: 10.1073/pnas.1619484114 - **Articolo in rivista**
 12. Caremani, M., Pinzauti, F., Reconditi, M., Piazzesi, G., Stienen, G., Lombardi, V., Linari, M., Spudich, J. A. (2016). The size and speed of the working stroke of cardiac myosin in situ.. *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA*, vol. 113, p. 3675-3680, ISSN: 1091-6490, doi: 10.1073/pnas.1525057113 - **Articolo in rivista**
 13. CAREMANI, MARCO, MELLI, LUCA, DOLFI, MARIO, LOMBARDI, VINCENZO, LINARI, MARCO (2015). Force and number of myosin motors during muscle shortening and the coupling with the release of the ATP hydrolysis products. *THE JOURNAL OF PHYSIOLOGY*, vol. 593, p. 3313-3332, ISSN: 0022-3751, doi: 10.1113/JP270265 - **Articolo in rivista**
 14. LINARI, MARCO, BRUNELLO, ELISABETTA, RECONDITI, MASSIMO, L. Fusi, CAREMANI, MARCO, T. Narayanan, PIAZZESI, GABRIELLA, LOMBARDI, VINCENZO, M. Irving (2015). Force generation by skeletal muscle is controlled by mechanosensing in myosin filaments. *NATURE*, vol. 528, p. 276-279, ISSN: 0028-0836, doi: 10.1038/nature15727 - **Articolo in rivista**
 15. BRUNELLO, ELISABETTA, CAREMANI, MARCO, MELLI, LUCA, LINARI, MARCO, M. Fernandez Martinez, T. Narayanan, M. Irving, PIAZZESI, GABRIELLA, LOMBARDI, VINCENZO, RECONDITI, MASSIMO (2014). The contributions of filaments and cross-bridges to sarcomere compliance in skeletal muscle. *THE JOURNAL OF PHYSIOLOGY*, vol. 592.17, p. 3881-3899, ISSN: 0022-3751, doi: 10.1113/jphysiol.2014.276196 - **Articolo in rivista**
 16. Zsolt Martonfalvi, BIANCO, PASQUALE, LINARI, MARCO, CAREMANI, MARCO, Attila Nagy, LOMBARDI, VINCENZO, Miklos Kellermayer (2014). Low-force transitions in single titin molecules reflect a memory of contractile history. *JOURNAL OF CELL SCIENCE*, vol. 127, p. 858-870, ISSN: 0021-9533, doi: 10.1242/jcs.138461 - **Articolo in rivista**
 17. CAREMANI, MARCO, MELLI, LUCA, DOLFI, MARIO, LOMBARDI, VINCENZO, LINARI, MARCO (2013). The working stroke of the myosin II motor in muscle is not tightly coupled to release of orthophosphate from its active site.. *THE JOURNAL OF PHYSIOLOGY*, vol. 591, p. 5187-5205, ISSN: 0022-3751, doi: 10.1113/jphysiol.2013.257410 - **Articolo in rivista**
 18. M. L. Bang, CAREMANI, MARCO, BRUNELLO, ELISABETTA, R. Littlefield, R. L. Lieber, J. Chen, LOMBARDI, VINCENZO, LINARI, MARCO (2009). Nebulin plays a direct role in promoting strong actin-myosin interactions. *THE FASEB JOURNAL*, vol. 23, p. 4117-4125, ISSN: 0892-6638, doi: 10.1096/fj.09-137729 - **Articolo in rivista**
 19. CAREMANI, MARCO, J. A. Dantzig, Y. E. Goldman, LOMBARDI, VINCENZO, LINARI, MARCO (2008). Effect of inorganic phosphate on the force and number of myosin cross-bridges during the isometric contraction of permeabilized muscle fibers from rabbit psoas.. *BIOPHYSICAL JOURNAL*, vol. 95, p. 5798-5808, ISSN: 0006-3495, doi: 10.1529/biophysj.108.130435 - **Articolo in rivista**
 20. LINARI, MARCO, CAREMANI, MARCO, C. Piperio, P. Brandt, LOMBARDI, VINCENZO (2007). Stiffness and fraction of myosin motors responsible for active force in permeabilized muscle fibers from rabbit psoas. *BIOPHYSICAL JOURNAL*, vol. 92, p. 2476-2490, ISSN: 0006-3495, doi: 10.1529/biophysj.106.099549 - **Articolo in rivista**

5. Main staff involved (max 10 professors/researchers for each research unit, in addition to the PI or

associated investigator), highlighting the time commitment expected

List of the Research Units

Unit 1 - BANG Marie-Louise

Personnel of the research unit

nº	Surname Name	Qualification	University/ Research Institution	e-mail address	Months/person expected
1.	BANG Marie-Louise	Primo ricercatore	Consiglio Nazionale delle Ricerche	marie-louise.bang@cnr.it	6,0

Possible sub-unit

Surname	Name	Qualification	e-mail address	Months/person expected

Unit 2 - CAREMANI Marco

Personnel of the research unit

nº	Surname Name	Qualification	University/ Research Institution	e-mail address	Months/person expected
1.	CAREMANI Marco	Ricercatore a t.d. - t.pieno (art. 24 c.3-b L. 240/10)	Università degli Studi di FIRENZE	marco.caremani@unifi.it	1,0
2.	PIRODDI Nicoletta	Professore Associato (L. 240/10)	Università degli Studi di FIRENZE	nicoletta.piroddi@unifi.it	1,0

6. Information on the new contracts for personnel to be specifically recruited

nº	Associated or principal investigator	Number of expected RTD contracts	Number of research grants expected	Number of PhD scholarships expected	Overall expected time commitment (months)
1.	BANG Marie-Louise	0	1	0	24
2.	CAREMANI Marco	0	1	0	24
	Total	0	2	0	48

7. PI "Do No Significant Harm (DNSH)" declaration, in compliance with article n. 17, EU Regulation 852/2020. (upload PDF)

Upload:



"The data contained in the application for funding are processed exclusively for carrying out the institutional functions of MUR. The CINECA, Department of Services for MUR, is data controller. The consultation is also reserved to universities, research institutes and institutions (each for its respective competence), MUR - Directorate-General Research- Office III, CNVR, CdV, and the reviewers in charge of the evaluation peer review.

MUR also has the right to the dissemination of the main economic and scientific data related to the funded projects.".

Date 31/03/2022 ore 14:00
