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## Identification of a new gene involved in muscle morphogenesis in vertebrates

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**P22- 337- Preclinical evaluation of tamoxifen and other selective estrogen receptor modulators in mdx5Cv dystrophic mice.**

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We are investigating the effects of selective estrogen receptor modulators (SERMs) in mdx5Cv dystrophic mice (Dys), a model for Duchenne muscular dystrophy (DMD). SERMs display either pro-estrogenic or anti-estrogenic activities in a tissue-dependent manner. Tamoxifen (TAM), the most well characterised SERM, has been used for over 30 years to treat estrogen-sensitive breast cancer in both women and men and has been reported to be also well tolerated in pre-pubertal boys.

In 2013, we published that oral treatment of Dys mice from 3 weeks of age for 15 months with TAM (10 mg/kg/day) improved muscle force and the structure of diaphragm and heart. TAM and its metabolites were present in nanomolar concentrations in plasma and muscles, suggesting signalling through high affinity targets, likely the estrogen receptors alpha and beta that were several-fold more abundant in dystrophic muscle than in normal ones.

Next, we tested TAM in adult Dys mice in order to investigate its efficacy in the low-intensity chronic stage of the disease, which resembles most closely the DMD condition. TAM at doses as low as 0.1 mg/kg/day improved motor performance of active mice and enhanced the contractile characteristics of the triceps surae. At 3 mg/kg/day, TAM corrected most endpoints close to normal values.

We are currently testing other SERMs (all at 3 mg/kg/day): the chlorinated TAM analogues clomiphene and toremifene, the 3-hydroxylated TAM derivative droloxifene, the second generation SERM unrelated to TAM raloxifene (RAL), and the pure anti-estrogen fulvestrant (Faslodex). Overall, the ranked efficacy was as follows: TAM > toremifene > clomiphene > droloxifene ? RAL > Faslodex.

Our data as well as our current understanding of estrogenic signalling in dystrophic muscle suggests that TAM and other SERMs with pro-estrogenic activities on muscle might be beneficial for DMD and maybe also for other muscular dystrophies.

*Dystrophic mouse, pharmacotherapy, tamoxifen, SERM, force*

**P23 – Skeletal muscle development- N° 338 to N° 358**

Skeletal muscle development- #2414

**P23- 338- An efficient RNA interference screening, using C2C12 line, identifies new genes involved in myogenic differentiation.**

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Skeletal muscle is a complex and heterogeneous tissue serving a multitude of functions in the organism. This tissue forms by a highly ordered process: myogenesis, which can be subdivided into a sequence of temporally separable events: myoblast proliferation, cell fusion and myotube maturation into myofibers. Although myogenesis has been widely described, many genes involved in muscle cell proliferation/differentiation are still unknown. To identify novel genes involved in this process, we have developed a functional screening system, based on the use of RNAi technology and C2C12 line (myoblast cell line, which is a very useful tool to study aspects of myogenesis, metabolism and muscle biology). This systematic genetic approach consists in the identification of genes that when knocked-down by RNAi show various phenotypes during proliferation and/or differentiation of C2C12. The knockdown of genes involved in C2C12 proliferation and/or differentiation might modify nuclear number and/or myosin quantity, which were easily detected by immuno-fluorescence staining (myosin antibody) and DAPI. The quantification of such staining indicates if the inactivated gene enhances or blocks proliferation, differentiation or both. In our screen, 100 mouse genes with an unknown function were knocked-down in C2C12 cells. The observed phenotypes were classified according to the following criteria: (i) nucleus counting which reflect the proliferation stage; (ii) myosin quantification, myotube morphology and fusion index which reflect the different steps of the differentiation stage. The screening result shows that among the 100 genes knocked-down, 92 genes display a phenotype. The phenotypic analysis indicates that 4 genes are specifically involved in proliferation stage, 45 genes are essential to the differentiation stage and 43 genes seem to be necessary as well for the proliferation as for the differentiation stages. Our results indicate that RNAi screening appears to be an efficient tool to identify new genes having a role during myogenesis.

*Muscle, myogenesis, RNAi, screening, C2C12*

Skeletal muscle development- #2431

**P23- 339- Distinct branches of Wnt signaling control Neuromuscular Junction formation**

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Understanding the developmental steps shaping the formation of the specialized peripheral synapse connecting motoneurons to skeletal muscle fibers, also called neuromuscular junction (NMJ) is critical. Recently, growing evidences suggest that Wnt morphogens act as key players in the formation of this synapse. Yet, the collaborative function of specific Wnts and downstream signaling at the NMJ remain poorly understood. We demonstrate that Wnt11 is required for the early nerve-independent muscle pre-patterning, a process characterized by acetylcholine receptors (AChR) clustering in discrete domains of the muscle surface. Moreover, both Wnt4 and Wnt11 cooperate to enhance AChR clustering in muscle cell line and in diaphragm in vivo, in part via activation of the canonical pathway. In addition, in utero injections of specific secreted Wnt signaling inhibitors lead to similar pre

and postsynaptic defects suggesting that distinct Wnt signaling branches are required for NMJ formation. We further show that Vangl2, a bona fide core Planar Cell Polarity (PCP) protein, is expressed in both developing NMJ counterparts. Interestingly, we found that both Wnt4 and Wnt11 co-immunoprecipitate with Vangl2 and that mice bearing the Vangl2 looptail mutation exhibit both AChR clustering and motor axon outgrowth defects. Taken together, our results provide genetic and biochemical evidence that the coordinated action of Wnt4 and Wnt11 controls NMJ formation via the activation of both the canonical and Vangl2-dependent core PCP signaling pathways.

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*Neuromuscular Junction, Wnt, MuSK, Planar Cell Polarity, Vangl2*

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Skeletal muscle development- #2474

**P23- 340- Mesodermic pre-patterning optimization for efficient and robust myogenic differentiation from human pluripotent stem cells**

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Myogenic differentiation of pluripotent stem cells remains a challenging question, as far as one tries to achieve without gene surexpression and to obtain a high final myotubes yield. One of the keys may concern the very first days of the induced differentiation, the pre-patterning, that have to lead pluripotent stem cells to the mesodermic lineage in a way that should be the most pronounced and homogeneous as possible, but that also have to preserve a high proliferative rate. While the GSK3-inhibitor use is mainly represented today in the first myogenic step protocols, BMP4 is a central point that divides opinions, wondering whether one should add BMP4, known also as a major mesodermic inducer, or inhibit him, or simply ignore it's impact. We have compared different strategies derived from the literature, either inhibiting BMP4 with LDN or totally ignoring that point, with a personal protocol that combine GSK3 inhibitor chir99021 with BMP4 in a defined medium. All those procedures use GSK3-inhibitor chir99021, but differ by culture media and cytokines used, the combination of which may impact the efficiency of the mesodermic pre-patterning. As our cells are usually maintained in mTesR1 medium, we decided to use defined mTesR- for pre-patterning to reduce the stress and the adaptation step of the cell related to a medium change, thus providing a higher and faster response to pre-patterning conditions. Three days after the initial induction, mTesR-/CHir/BMP4 cells exhibit similar Brachyury expression level as Chir+LDN induced cells, and the higher expression levels of TBX6, Mesogenine, Pax3 and PAX7, without any ectodermic (PAX6) or endodermic (SOX17) contamination of the cell population. Chir+LDN produces a nice mesodermic commitment in DMEM-based media, but failed to enhance TBX6, mesogenine and Pax7 expression in our defined medium. Altogether, those results present Chir+BMP4 in mTesR- as the best combination to initiate pluripotent stem cell in the mesodermic lineage. Under those conditions and after only three days, cells exhibit a presomitic mesodermic gene expression profile, more pronounced than the cells induced by Chir+LDN. On the other hand, our study reminds the impact of the combination of chosen cytokines with the basal culture medium used, moderating at the same time the role of the BMP4 on the basis of the culture medium used.

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*Mesoderm, myogenesis, human, pluripotent, stem, cell, differentiation, pre-patterning*

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Skeletal muscle development- #2478

**P23- 341- BMP signaling controls satellite cell dependent postnatal muscle growth.**

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Postnatal/juvenile muscle growth is achieved by both an increase in myofiber size and the addition of further myonuclei, whereas myofiber number does not increase further. Satellite cells are the resident muscle stem cells which proliferate in growing muscle to supply new myonuclei. Little is known on how satellite cell function is controlled during the postnatal/juvenile growth phase to permit correct muscle mass development. We have previously shown that BMP (Bone morphogenetic proteins) signaling regulates embryonic myogenesis by determining the entry of embryonic muscle precursors into muscle differentiation. Here we demonstrate that this pathway also defines postnatal/juvenile muscle growth. We found that juvenile satellite cells express P-Smad1/5/8 and contain transcripts of BMP signaling components such as BMP4, BMP6 and BMPR1A, showing that BMP signaling is active in these cells. Abrogating BMP signaling in satellite cells in juvenile Pax7CreERT2/+;Rosa26-Lox-Stop-Lox-Smad6-IRES-GFP mice decreased the pool of satellite cells and muscle fibres contained less myonuclei and were smaller than those from control mice. We show that blockade of BMP signaling decreased satellite cell proliferation and diminished the myonuclear recruitment during myofiber growth as the underlying cellular mechanism and this severely retarded muscle growth. In addition, failure of satellite cell proliferation during the postnatal/juvenile growth phase strongly reduced the final satellite cell reservoir in mature muscle. In conclusion, these results show that correct BMP signaling in satellite cells is required for satellite cell dependent myofiber growth and for the generation of the adult satellite cell pool. In the future it will be of large interest to determine whether the BMP signaling pathway is altered in childhood neuromuscular disorders and whether this inflicts satellite cell dependent muscle growth.

Skeletal muscle development- #2487

**P23- 342- Beta-carotene acts as a regulator of skeletal muscle cells**

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The enzyme beta, beta-carotene-15,15'-monooxygenase (BCMO1) cleaves provitamin A carotenoids into active vitamin A principally in liver and intestine. The BCMO1 gene is expressed at low level in the muscle tissue but little is known about its function. In the chicken muscle, we observed that various BCMO1 expression levels are associated with different carotenoids contents. To investigate the potential role of BCMO1 on skeletal muscle, we assessed the impact of beta-carotene (BC, the prototype substrate of the BCMO1 enzyme) supplementation in vitro on proliferative avian myoblasts. Proliferation was evaluated by BrdU incorporation and by flow cytometry. The BrdU incorporation index was reduced and the proportion of G0/G1 cells increased following BC supplementation. Cell differentiation was evaluated by immunolabelling of sarcomeric myosin heavy chain (MHC). In this proliferative environment, the proportion of MHC positive cells increased following BC supplementation. The effects of BC were inhibited in the presence of DEAB, an inhibitor of retinaldehyde dehydrogenase, supporting the hypothesis that the BCMO1 enzyme is active in myoblasts and can contribute to retinoic acid production from BC. Our data suggest that provitamin A carotenoids could be used as nutritional regulators of skeletal muscle growth.

*carotenoids, myogenesis*

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Skeletal muscle development- #2517

**P23- 343- The role of Wnt signalling in embryonic stem cells differentiation into mesoderm**

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Pluripotent stem cells (PSCs) can differentiate into all cell types building mammalian body. There are many indications that Wnt proteins may influence the pluripotency and differentiation of PSCs. It has been shown that canonical Wnt signalling is involved in both- maintaining pluripotent state of PSCs (ten Berge et al. 2011) and their mesodermal specification (Liu et al. 1999). Here we describe the role of Wnt-11, acting through non-canonical signalling pathway, during in vitro differentiation of murine PSCs.

The aim of our research was to design a protocol for directed and efficient differentiation of PSCs, such as embryonic stem cells (ESCs), into skeletal myoblasts. Since Wnt-11 is crucial for the formation of dermomyotome, a source of skeletal muscle precursors during embryogenesis, we assessed the influence of this factor on in vitro myogenic differentiation of ESCs. First, we determined the level of endogenous expression of Wnt-11 in undifferentiated and differentiating ESCs at mRNA and protein level. Next, we checked if ESCs are able to respond to exogenous Wnt-11 by investigating the expression of both Wnt-11 receptors: Frizzled-5 and Frizzled-7. Subsequently, we determined the influence of exogenous Wnt-11 on ESCs morphology, proliferation, and differentiation. We determined the level of expression of germ layers markers: endoderm (GATA-4), ectoderm (Pax6), and mesoderm (Brachyury, Mesogenin) as well as myogenic markers such as Pax3, Pax7, and MRFs. Finally, we assessed the Wnt-11 influence on the ability of ESCs to fuse with skeletal myoblasts. We found that Wnt-11 influences ESCs by promoting their differentiation into mesoderm.

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*ESCs, differentiation, mesoderm, Wnt signalling*

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Skeletal muscle development- #2532

**P23- 344- Molecular control of muscle mass: roles of the proteins GASP/WFIKKN**

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Muscle mass is the result of a dynamic balance between protein synthesis and degradation. In the last decades, myostatin, a member of the transforming growth factor- $\beta$  superfamily, has been shown to be a key molecular element of skeletal muscle development, acting as a negative regulator. Myostatin knockout mice display both hypertrophy and hyperplasia of muscle fibers and a leaner body composition due to reduced fat mass. Several strategies to block myostatin signaling pathway have been carried out to develop therapeutic applications, in particular to increase muscle mass in various diseases including muscular dystrophy. One research focus of our laboratory concerns two myostatin inhibitors, the GASP proteins. GASP-1 and GASP-2 are the only representatives of a unique family of multidomain proteins, with a tissue expression characteristics markedly different, suggesting that these proteins possess a variety of biological roles, in addition to regulation of muscle development.

To better understand the mechanism of action of the GASP proteins, we have generated and characterized mouse lines overexpressing ubiquitously Gasp-1, Tg(Gasp-1) or Gasp-2Tg(Gasp-2). Our results revealed that the Tg(Gasp-1) mice present a muscular phenotype due to hypertrophy without hyperplasia. No reduction in fat mass has been observed. To investigate genetic interaction networks, we initiated an in silico approach combined with the Affymetrix microarray technology. On 20000 genes, more than 3000 were identified as up or down regulated in primary myoblasts overexpressing Gasp-1 compared to wildtype cells. The 10 more relevant genes were selected for validation using quantitative real-time RT-PCR analysis. Phenotypic studies of the Tg(Gasp-2) mouse line are currently in progress.

*Myostatin, GASP/WFIKKN, muscle differentiation, DNA chip*

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Skeletal muscle development- #2579

**P23- 345- Molecular mechanism for nuclear envelope localization of MTOC activity in muscle cells.**

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Microtubules are a major component of the cytoskeleton and mediate important cellular functions including organelle positioning, intracellular trafficking and mitotic spindle formation. In most proliferating cells, microtubules are nucleated radially from a classical microtubule organizing center, the centrosome. By contrast, a variety of differentiated cells nucleate and organize microtubules from other sites than the centrosome, such as the Golgi, the plasma membrane or the nucleus. The latter has been described as major non-centrosomal MTOC during skeletal muscle differentiation, when centrosomal proteins, including pericentrin and  $\gamma$ -tubulin, relocate from the centrosome in myoblasts to the nuclear envelope (NE) in post-mitotic myoblasts and myotubes. However, the molecular mechanisms underlying the recruitment of MTOC activity to the NE and how microtubules are anchored to the NE are still unknown.

Here, we investigated the role of NE components in recruiting MTOC activity by examining pericentrin localization and microtubule nucleation at the NE in a small scale siRNA screen in differentiated C2C12 myotubes. Among the ~300 genes encoding nuclear transmembrane proteins, the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex was found to be involved in these processes. Myotubes depleted of different LINC components showed mislocalization of pericentrin to the cytoplasm and failed to nucleate microtubules from the NE. Using 3D structured illumination microscopy and spectral demixing direct stochastic optical reconstruction microscopy, we demonstrated that pericentrin indeed localizes in close proximity to the LINC complex. Future work aims to uncover the protein network linking the LINC complex to MTOC components in muscle cells and how mutations of LINC components might perturb these processes in muscular disorders, such as Emery-Dreifuss muscular dystrophy.

*LINC, MTOC, nuclear envelope, microtubule*

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Skeletal muscle development- #2623

### **P23- 346- Characterization of slow muscle deficiency in jam mutant Danio rerio embryos**

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The structure and functions of striated muscle are well understood, yet there are gaps in our understanding of the molecular processes involved in the generation of striated muscle. In this study, we characterize the zebrafish jam as a slow muscle mutant with a late-onset phenotype. The expression patterns of sarcomere proteins and chaperones in the developing embryo were observed via qPCR, in situ hybridization, and immunohistochemistry. Jam zebrafish embryos were found to have improper slow muscle structure and fail to develop a swim bladder, leading us to further explore the components of the Hedgehog signaling pathway; a pathway critical in the differentiation between slow and fast muscle fibers and swim bladder development. Expression patterns of the sarcomere proteins, chaperones, and transcription factors will be presented in addition to the analysis of our results. The identification of a novel factor involved in the development or maintenance of muscle structures would strengthen our understanding of muscle development and would create further potential for the development of therapies to target human muscular dystrophies and other diseases in which muscle is affected.

*zebrafish, skeletal muscle development, slow muscle development*

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Skeletal muscle development- #2859

### **P23- 347- Identification of new genes involved in muscle morphogenesis in vertebrates**

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In most animals, myocyte fusion is a cellular process highly regulated during skeletal muscle development, post embryonic muscle growth and regeneration. Myocyte fusion follows an ordered set of cellular events, including myocyte elongation, cell migration, recognition and adhesion, and membrane fusion. However, our knowledge regarding molecular mechanisms controlling this essential step in myogenesis is far from being complete. Our study aimed at identifying new genes involved in myocyte fusion step in vertebrates. First, we performed an in vitro functional screening in C2C12 cell line (murine model) based on siRNA knock-down. Using a selection of genes found to be expressed in proliferating and differentiating muscle C2C12 cells (Moran and al., 2002 ; Tomczak and al., 2004), our functional screening led to the identification of thirteen genes which in vitro knock-down induced a myocyte fusion defect. To identify their roles in vivo, expressional and functional analysis of these genes was further conducted on zebrafish model. Among these thirteen candidates, a gene encoding an N-acetyltransferase enzyme: Naa15, was clearly expressed in somites and has never been related to muscle development. The expression of this gene was highly regulated during muscle regeneration in fish, in accordance with a role in myocyte fusion. The Knock down of the two zebrafish orthologues of this N-acetyltransferase using morpholino micro-injection produce shorter embryos with abnormal curvature of the body and inability to swim after hatching. The first analyses reveal a disorganisation of the myotome structure of these morphants. Deeper observation and controls are currently in progress to characterize the details of the muscle phenotype induced by this gene extinction and the role of the naa15 gene in early muscle development.

*Fish, myocyte fusion, Myotome development, morpholino, naa15*

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Skeletal muscle development- #2934

### **P23- 348- Evolution and function of myomaker in fish**

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Myocytes fusion is a fundamental process for skeletal muscle formation during development, post embryonic muscle growth and regeneration in most animals. Our knowledge regarding molecular mechanisms controlling this essential step in myogenesis is far from being complete. A critical step in fusion is the initial recognition and adhesion between two myogenic cells before they fuse. Among these factors involved in these process, myomaker (also called Tmem8c), a membrane activator of myocyte fusion and muscle formation, was recently discovered in mice. We previously identified the myomaker in zebrafish genome that encode a protein of 220 amino acids as in mouse. Surprisingly, in rainbow trout genome, the unique copy of this gene encode a protein of 434 amino acids although sequence alignment showed a high sequence identity between mice and rainbow trout myomaker protein. Phylogenetic and syntenic analysis clearly demonstrated that the identified trout sequence corresponded to the true ortholog of mouse myomaker. Analysis of the trout myomaker sequence revealed the presence of 14 tandem repeats of 30 nucleotides in the last exon corresponding to minisatellites. Using whole mount in situ hybridization on fish embryos at different stages of embryonic development, we observed that myomaker was expressed at the end of somitogenesis when myocyte fusion started. In agreement with these results, in vitro analysis on trout satellite cells showed that the expression of myomaker increases when the fusion of trout myocytes occurred. In addition, our results from muscle regeneration experiment indicated that myomaker up-regulation is associated with the appearance of new myofibres. Finally, zebrafish embryos injected with morpholino against myomaker showed a marked phenotype characterized by myocyte fusion failure in the fast myotome. These findings reveal an evolutionary conservation of the expression and function of myomaker among fish.

*myomaker, fusion, myocyte, fish, regeneration*

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Skeletal muscle development- #2945

**P23- 349- Myonuclear domains establishment through microtubule regulation during muscle development**

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Myonuclei actively position themselves throughout muscular development. Growing evidences support a direct connection between regulation of nuclear positioning, myonuclear domains establishment, microtubule architecture maintenance and normal function of muscles. The incorrect positioning of nuclei in the center of myofibers is a hallmark of a class of muscular diseases called centronuclear myopathies, which includes myotubular myopathy.

Direct interaction between two microtubule-associated proteins, MAP7 and KIF5B, regulate microtubule architecture and dynamics allowing the correct localization of nuclei in myofibers. Improper nuclear positioning induced by the absence of MAP7 leads to muscle dysfunction. Here, we identify a new adaptator protein, partner of MAP7, that bind to the microtubule domain of MAP7 and contribute to the molecular mechanisms that regulate myonuclear positioning in the early step of muscle formation through MAP7.

We use a combination of cell biology and genetics in cultured and in vivo developing mice models muscles to decipher the implication of this adaptator protein in the regulation of nuclear positioning and microtubules dynamics in developing myofibers. Interestingly, these adaptator protein also impact the later step of maturation of muscle fibers, through t-tubule establishment.

*microtubule, myonuclear domain, CNM, Primary myoblast culture*

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Skeletal muscle development- #3021

**P23- 350- NFATc2, a major regulator of skeletal myogenesis**

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The calcineurin/NFAT (Nuclear Factor of Activated T-cells) signaling pathway plays a regulatory role in skeletal muscle adaptation and muscle regeneration, by its ability to promote myotube differentiation and its role in the establishment of the adult muscle phenotype. Calcineurin dephosphorylates members of NFAT transcription factors allowing NFAT translocation into the nucleus where it cooperates with other transcription factors to induce transcription of target genes. Recently, we demonstrated that NFAT proteins are able to interact with one of the muscle specific transcription factors, MyoD, to control different aspects of skeletal muscle development, depending on the NFAT isoform involved in the interaction: the NFATc3/MyoD complex controls myogenin expression during primary myogenesis whereas the NFATc2/MyoD complex regulates the expression of one specific myosin heavy chain (MyHC), the neonatal MyHC during mouse embryogenesis.

Moreover, we observed that embryonic muscles from *nfatc2*<sup>-/-</sup> mutant mice were characterized by atrophy whereas embryonic muscles from *nfatc2*<sup>-/-</sup>:*myod*<sup>-/-</sup> double mutant presented also severe aplasia. Our ongoing results suggest a defect in myoblast migration in the limb bud of *nfatc2*/*myod* double null embryos. Furthermore, we show that NFATc2 regulates in vitro the expression of a collagenous type II transmembrane protein, collagen XXV alpha 1 (Col25a1), during muscle differentiation.

*NFAT, Col25a1, muscle differentiation, migration*

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Skeletal muscle development- #3024

**P23- 351- Role of the NFAT transcription factors in dystrophin deficient muscle fibers.**

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Nonsense or frame shift mutations in the X-linked dystrophin gene is responsible for the most common fatal muscular dystrophy, Duchenne muscular dystrophy (DMD), in which no functional dystrophin protein is produced. Mutations that retain the reading frame result in production of a protein, which is partially functional and is often expressed at low levels, leading to the milder allelic variant, Becker muscular dystrophy (BMD).

At the molecular level, loss or decrease in sarcolemmal dystrophin proteins destabilizes the large dystrophin-associated protein complex and, therefore, increases Ca<sup>2+</sup> influx and/or disturbs the cytosolic Ca<sup>2+</sup> homeostasis. This increased intracellular calcium affects the cellular processes that are calcium sensitive, and therefore could potentially modulate the activity of the calcium/calmodulin dependent serine/threonine-specific phosphatase, calcineurin. Once activated, calcineurin dephosphorylates members of the NFAT (Nuclear Factor of Activated T-cells) transcription factor family allowing NFAT translocation into the nucleus, where it cooperates with other transcription factors to induce the transcription of target genes.

If it is clear that calcineurin is a key element that is disturbed in DMD, the expression of each NFAT isoform, their activity and function has never been analyzed in details in these muscles.

Here, we clearly identified an intronic NFAT-dependent enhancer of the dystrophin gene, suggesting that dystrophin expression is likely under the control of the NFAT transcription factors. Furthermore, our ongoing results show that the calcineurin/NFAT pathway is activated in dystrophin deficient myofibers. This study may shed light on a potential crosstalk between dystrophin expression and NFAT activity. *NFAT, dystrophin, calcineurin*

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Skeletal muscle development- #3028

### **P23- 352- BMP signalling at the vertebrate neuromuscular junction**

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Innervation is crucial for growth and regeneration of skeletal muscle. Similarly, Bone Morphogenetic Proteins (BMPs) play an important role for skeletal muscle maintenance and growth. BMP signalling is characterized by canonical pathways via Smad transcription factors as well as non-Smad signalling via e.g. MAPK, LIMK and PI3K signalling (Nohe et al., 2002; Foletta et al., 2003; Hiepen et al., 2014). Studies in *Drosophila* have shown that retrograde BMP signalling plays an important role in the formation of the neuromuscular junction (NMJ), an essential structure for the development of skeletal muscle (McCabe et al., 2003). Retrograde BMP signalling at the NMJ may comprise Smad as well as non-Smad pathways, involved in NMJ formation, stability, maintenance and repair. Septins represent a highly conserved family of GTP-binding proteins and have lately been recognized as a novel component of the cytoskeleton as they interact with the actin- and microtubule cytoskeletal networks (Mostowy et al., 2012). Higher ordered septin filaments have recently been acknowledged to mediate signalling, form diffusion barriers for transmembrane proteins and provide scaffolding functions. Non-Smad signalling, emerged to be important for the formation of the NMJ, through regulation of cytoskeletal dynamics. In this study, we show septins as modulators of BMP signalling colocalizing with distinct muscle fiber types and motor neurons. Additionally, we characterized BMP-induced signalling in the motor neuron-like NSC34 cell line. Further, we aim to reconstitute the complex structure of the neuromuscular junction in vitro by developing a motor neuron/muscle co-culture system. Reconstruction of the NMJ in vitro will help to understand the spatiotemporal regulation and segregation of BMP pathways. By application of the CRISPR/Cas genome editing technique to BMP receptors, we aim to evaluate the role of BMP signalling for cytoskeletal dynamics at the vertebrate NMJ.

*BMP, Neuromuscular junction, CRISPR/Cas9, Septin, Cytoskelett*

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Skeletal muscle development- #3037

### **P23- 353- The myotome is necessary for maintenance of Pax7 expression in the dermomyotome and for normal epaxial muscle differentiation in mouse embryo**

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Building skeletal muscles in amniotes is a complex event. Pax3- and/or Pax7-positive myogenic precursor cells (MPCs) in the dermomyotome turn on myogenic regulatory factors, delaminate and differentiate into elongated myocytes, the myotome. MPCs keep entering the myotomal region and either differentiate into myotomal myocytes or continue to proliferate without differentiating and form a pool of MPCs within the muscles masses, which will contribute to muscle development later on and to the pool of adult stem cells.

Here we used the *Myf5nlacZ* line (Tajbakhsh et al. Nature, 1996), which lacks the early myotome to address whether signals from the myotome influences the overlying MPCs during epaxial (deep back muscle) myogenesis. Our data revealed that in these mutants, only one, of the three epaxial muscle masses forms (the transversospinalis), and it differentiates by turning on *MyoD*. Further analyses in mutant embryos showed that Pax7 (but not Pax3) is gradually downregulated from E10.5 onwards and is completely gone from the dermomyotome at E11.5, with no increase in cell death. The myotome expresses several *Fgfs* and we next asked whether blocking *Fgf* signalling in explants of normal embryos phenocopied the mutants. SU5402 treatment for 12 hrs led to a reduction of Pax3, Pax7 and *MyoD* protein expression. Altogether, our experiments suggests that the myotome plays a crucial role in maintaining Pax7-positive MPCs in the dermomyotome and that the loss of this population in the mutants, leads to a defect in epaxial myogenesis.

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*Epaxial muscle morphogenesis, Myotome, Pax7 maintenance, Fgf signalling, Mouse embryo*

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Skeletal muscle development- #3176

**P23- 354- A proteomic analysis of skeletal muscle in HspB1 knock-out mouse**

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Hsp27- encoded by HspB1- is a member of the small heat shock proteins (sHsp, 12-43 kDa) family. This protein is constitutively present in a wide variety of tissues and in many cell lines. There is increasing evidence to substantiate Hsp27 as biomarker in many disease states including renal injury, cancer, cardiovascular disease, and neuro-degenerative disease. Abundance of Hsp27 is the highest in skeletal muscle indicating a crucial role for muscle physiology.

In the context of a research program dedicated to phenotyping of mouse models, we generated an HspB1-null mouse. The mutant mouse is viable, fertile and shows neither apparent morphological, anatomical alterations nor changes in the macroscopic muscle phenotype. However, abnormalities in the myofibrillar structure and ultrastructure of mutant mice have been observed by electron microscopy (Cassar-Malek et al., 2015). A network-basis approach combined to biochemistry enabled predicting Hsp27 targets solely in the m. Soleus (Kammoun et al, 2013).

The aim of this study was to analyse the proteins impacted by HspB1 targeted invalidation in the m. Tibialis anterior and to reveal interactors of Hsp27. Comparative proteomics revealed 22 2-DE spots differentially abundant between HspB1-null mice (n=5) and their controls (n=5) that could be identified by mass spectrometry. Eighteen spots were more abundant in the muscle of the mutant mice and 4 were less abundant. Gene ontology analysis indicated that 60% of proteins were intracellular proteins and 40% were plasma membrane proteins. The biological processes the most represented are the metabolic process (27%), cellular process (11%) system process (11%), cell communication (9%) and cell transport (9%).

The proteins impacted by Hsp27 invalidation belonged mainly to the Hsps family (HspA8, HspA9), apoptotic pathway (Park7), and calcium homeostasis (Sarcalumenin). The information gained by this study will be helpful to predict the side-effect consequences of Hsp27 depletion in muscle development and in cancer or inflammatory pathologies linked to small Hsps.

*Hsp27, muscle proteome, small Hsp, calcium homeostasis, apoptosis*

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Skeletal muscle development- #3183

**P23- 355- IMPACT OF MODIFICATIONS OF MATERNAL NUTRITION FROM EARLY TO MID-GESTATION ON SKELETAL MUSCLE OF BEEF COWS: PROTEOMIC AND BIOINFORMATIC APPROACHES**

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In cattle fetuses, myogenesis has been well documented (Picard et al., 2010). Among the environmental factors affecting myogenesis the maternal nutrition is important. However, there are few studies about its effects on muscle properties in cattle.

We investigated the effects of nutrient restriction and realimentation from early to mid-gestation on myogenesis and adipogenesis. On day 30 of pregnancy, multiparous, non-lactating cows were fed at requirement or restricted. On day 85, cows remained on control (n = 5) or restricted (n = 5) diets, or were realimented to control (n = 5). On day 140, cows were slaughtered, remained on control (CCC, n = 5; RCC, n = 5), or were realimented to control (RRC, n = 5) until day 254. Immunohistochemistry on semitendinosus muscle from 254 days old fetuses showed a lower proportion of small fibers expressing fetal myosin heavy chain in RCC and RRC groups. As these fibers correspond to the third generation giving rise to IIA fibers, this could have consequence on the proportion of IIA fibers of this muscle (Picard et al., 2015).

In the aim to identify proteins that may have contributed to these perturbations we applied a proteomic analysis (two dimensional electrophoresis/mass spectrometry). 28 muscular proteins had abundances modified (P>0.10) by maternal nutrition in 140 days old fetuses. They are mainly involved in glycolysis, tricarboxylic acid cycle, cellular protein metabolic process, and negative regulation of apoptosis. Proteins never reported in muscle, CCT6A, VBP1, UBE2N? were proposed as central proteins for the myogenesis by a bioinformatic study performed with our recently developed webservice ProteINSIDE (<http://www.proteinside.org>). It is an original all-in-one tool allowing from a dataset of genes or proteins from Bovine, Ovine, Caprine, Human, Rat and Murine, a fast overview of biological information, functional annotation, prediction of secreted proteins, and construction of protein networks (Kaspric et al., 2015).

*myogenesis, foetal programming, proteomics, bioinformatics*

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Skeletal muscle development- #3232

**P23- 356- Dissection of the genome-wide transcriptional synergy between Six and Myod during myogenic commitment and differentiation**

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Myogenic regulatory factors of the Myod family are bHLH transcription factors that have the ability to engage pluripotent embryonic cells in the myogenic lineage, and to reprogram differentiated cells to a myogenic fate. In particular, Mouse Embryonic Fibroblasts (MEF) can be reprogrammed by forced Myod expression. Here we show that Six1 and Six4, two members of the Six genes family expressed in MEF, are required during that process. We first show that most Myod targets remain unexpressed after forced Myod expression in Six1/4 mutant MEF. Using microarray experiments, we find 761 genes under the synergistic control of both Six and Myod. Using Myod ChIPseq data along with a genome-wide search for the Six1/4 MEF3 binding sites, we find a significant co-localization of Myod and Six binding on 1,230 1kbp-long Mouse genomic DNA regions. The combination of both datasets yields a set of 82 genes whose expression was synergistically activated by Six+Myod with 96 associated Myod+MEF3 putative enhancers. We tested 19 of these enhancers by luciferase assays and found that 14 of them (74%) reproduced the synergistic behavior of the nearest gene at the reporter level. Finally, we conducted a hybrid binding

site analysis on these enhancers using a de novo search and binding motifs in available databases. We found 15 overrepresented motifs, including known (MEF2, AP1, MEIS1) and new ones. Extensive mutagenesis on two enhancers shows that several of these motifs participate to the Six+Myod transcriptional synergy. Corresponding nuclear proteins were as well found enriched in cells under the control of Six and Myod. Altogether, these results provide an unprecedented regulatory dissection of genetic MEF reprogramming to a myogenic fate relying on synergistic gene activation by Six and Myod including EBF, MEF2 and new transcription factors, in a feedforward manner.

#### *Myod Six reprogramming*

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Skeletal muscle development- #3253

#### **P23- 357- Slow muscle precursors lay down a matrix COLXV-B fingerprint to guide motor axon navigation**

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The extracellular matrix (ECM) provides local positional information to guide motoneuron axons towards their muscle target. Collagen XV is a basement membrane component mainly expressed in skeletal muscle. We have identified two zebrafish collagen XV gene paralogs, col15a1a and col15a1b that display distinct expression patterns. Here we show that col15a1b is expressed and deposited in the motor path ECM by slow muscle precursors also called adaxial cells. We further demonstrate that collagen XV-B deposition is both temporally and spatially regulated prior to motor axon extension from the spinal cord in such a way that it remains in this region after the adaxial cells have migrated towards the periphery of the myotome. Loss and gain of function experiments in zebrafish embryos demonstrate that col15a1b expression and subsequent collagen XV-B deposition and organization in the motor path ECM depend on a previously undescribed two-step mechanism involving Hedgehog/Gli and unplugged/MuSK signaling pathways. In silico analysis predicts a putative Gli binding site in the col15a1b proximal promoter. Using col15a1b promoter-reporter constructs, we demonstrate that col15a1b participates in the slow muscle genetic program as a direct target of Hedgehog/Gli signaling. Col15a1b knockdown or overexpression provokes pathfinding errors in primary and secondary motoneuron axons both at and beyond the choice point where axon pathway selection takes place. These defects result in muscle atrophy and compromised swimming behavior, a phenotype partially rescued by injection of a smyhc1:col15a1b construct. These reveal an unexpected and novel role for COLXV-B in motor axon pathfinding and neuromuscular development.

*extracellular matrix, motor axon navigation, muscle progenitors, zebrafish, signaling pathway*

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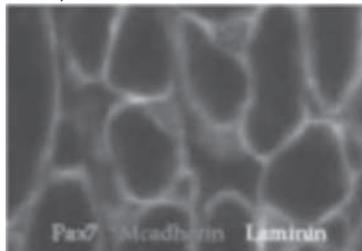
Skeletal muscle development- #3261

#### **P23- 358- Sine oculis homeobox (Six) genes and muscle stem cell environment**

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The environment in which stem cells behave all along their life allow them to retain their stemness properties and to renew their host tissue. In skeletal muscle, this environment is established during the fetal life, assembling all the components that will be essential for muscle stem cell homeostasis: vessels, nerves, extra cellular matrix... This niche is also rebuilt occasionally upon muscle repair after an injury or constantly in the case of muscle degeneration. How skeletal muscles are able to reorganize all the components of the niche is not totally understood. Sine oculis homeobox (Six) genes encodes for transcription factors that are essential for muscle development. We observed that in the absence of Six1 and Six4 proteins, muscle stem cells did not locate correctly in their niche at the end of the fetal life. Upon engraftment in an injured adult muscle, those fetal cells poorly regenerated the host muscle, only forming immature myofibers. We are trying to understand the role of Six homeoproteins in the establishment of the skeletal muscle stem cell niche, with in vivo and in vitro models of myogenic stem cells homing.



*Sine oculis, satellite cells, niche, Pax7, Extra cellular matrix*

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#### **P24 – Spinal muscular atrophy (including variants)- N° 359 to N° 377**

Spinal muscular atrophy (including variants)- #2323

#### **P24- 359- Pharmacologically-induced mouse model of adult spinal muscular atrophy to evaluate effectiveness of therapeutics after disease onset**

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Spinal muscular atrophy (SMA) is a genetic disease characterized by atrophy of muscle and loss of spinal motor neurons. SMA is caused by deletion or mutation of the survival of motor neuron 1 (SMN1) gene and the nearly identical SMN2 gene fails to generate adequate levels of functional SMN protein due to a splicing defect. Currently several therapeutics targeted to increase