Promoter expression landscape in skeletal muscle in hindlimb suspension and recovery model in rat

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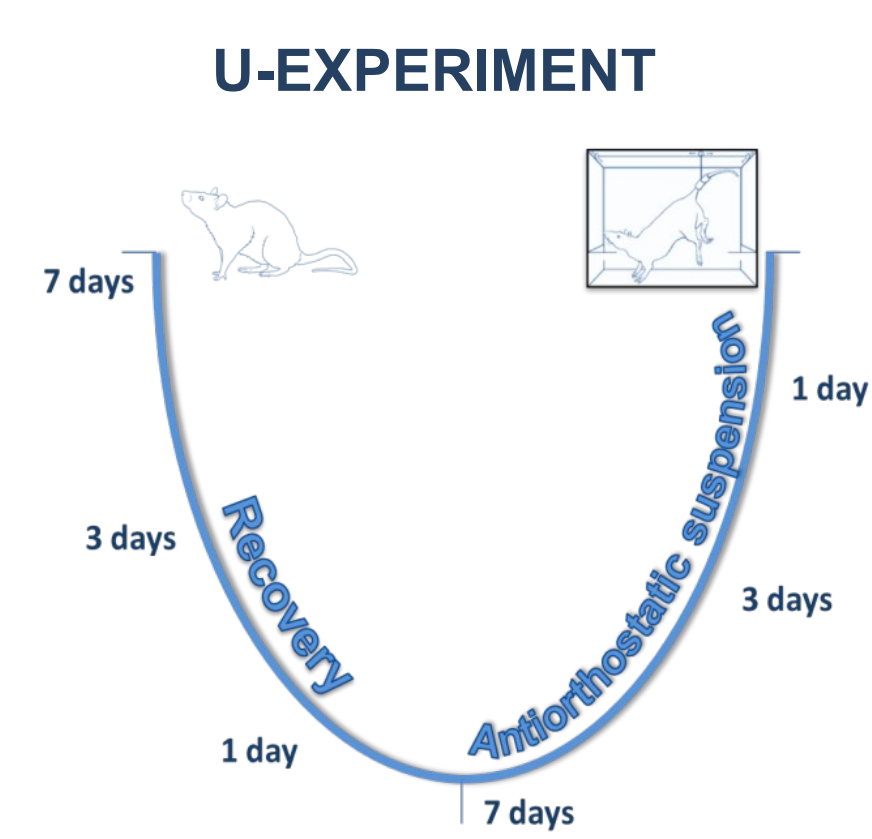
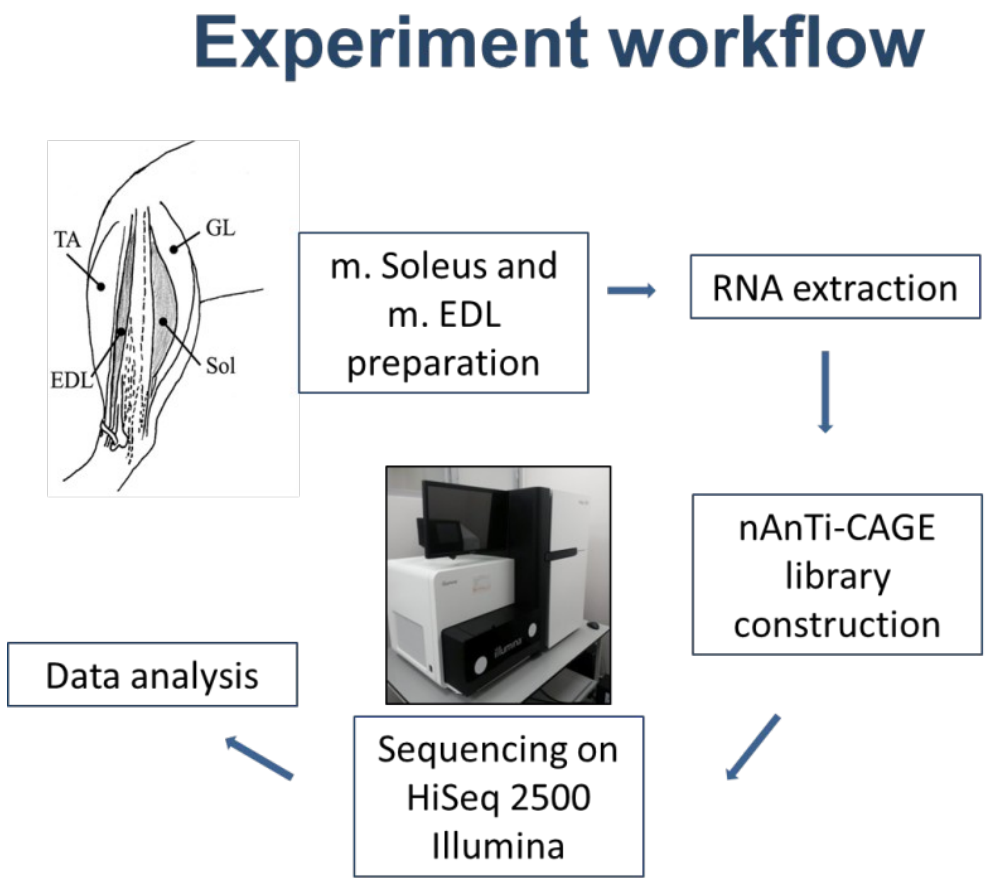
Background:

Skeletal muscle represents adaptive system characterizing by plasticity. It is capable to remodeling in response to different external and internal stimuli. Mechanical unloading causes muscle atrophy but at the same time physical activity induces hypertrophy. Loss of muscle mass and function during long period of physical inactivity still remains a clinical problem for humanity because the significant reducing of life quality and increasing mortality. For resolving this task understanding molecular regulatory mechanisms of disuse muscle atrophy and recovery are required. In recent studies global gene expression in atrophied and recovered skeletal muscles on different animal models was analyzed. However, previously whole-genome regulation of atrophy and following recovery at promoter and enhancer level has not been studied. Here, for the first time we perform analysis of regulatory genome elements in skeletal muscles in hindlimb suspension-recovery rat model. One of the most sensitive approaches for such analysis is Cap Analysis of Gene Expression method (CAGE), which allows determine transcription initiation sites (TSS) with up to one nucleotide precise, thus, evaluate the transcriptional activity of genes at promoter and enhancer level.

Purpose:

In current study we aimed to identify transcription initiation sites (TSS), and evaluate full-genome RNA expression at the level of individual promoters and enhancers during unloading and subsequent recovery in rats.

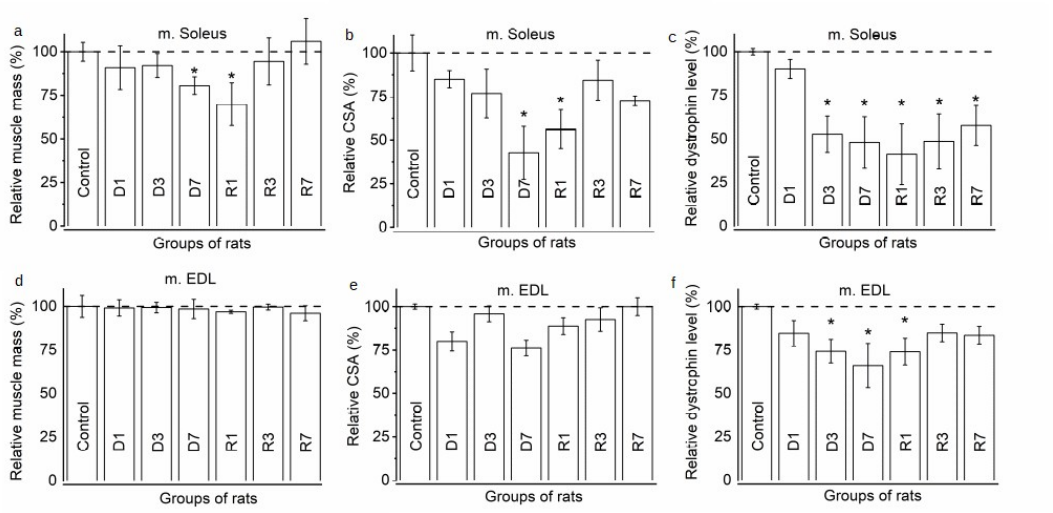




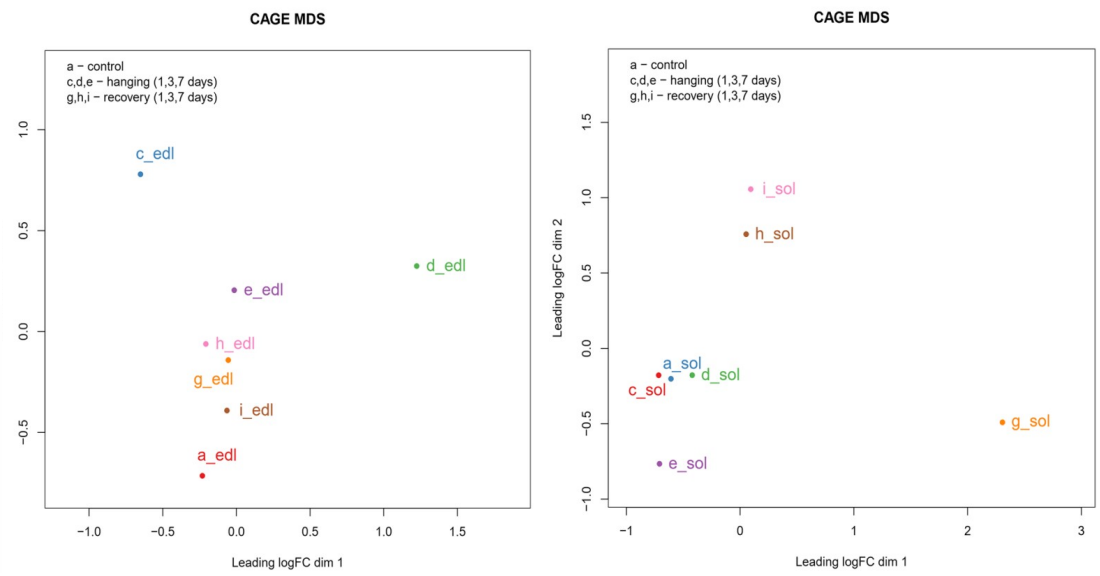
MATERIALS AND METHODS:

Two types of muscles, "slow" (m. Soleus) and "fast" (m. EDL), were examined in rats in normal conditions, after 1, 3 and 7 days of hindlimb suspension and following 1, 3 and 7 days of recovery using CAGE (Cap Analysis of Gene Expression) method followed by Illumina HiSeq 2500 sequencing. After quality check and filtration, CAGE reads were mapped to the current rat genome assembly rn6 (2014) by using bwa and then clustered by python script. Further annotation of CAGE peaks, analysis of differential expression, and functional terms enrichment were proceeded through R environment.

Morphometric analysis demonstrates varying degree of muscle atrophy during disuse in different muscle types



TSS peaks expression analysis



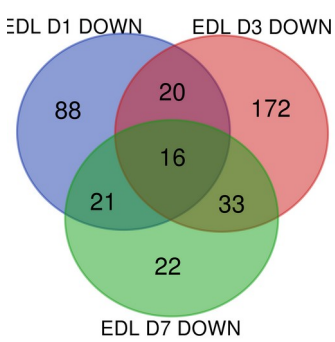
Differential expression of gene-centric TSS

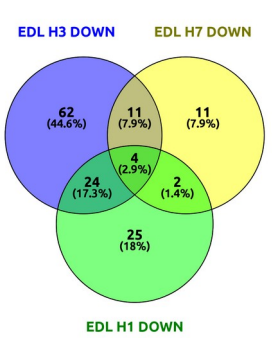
	EDL		Sol	
	UP	DOWN	UP	DOWN
HS1	214	145	204	239
HS3	286	265	140	80
HS7	156	93	131	106
R1	26	10	**2693**	**980**
R3	163	**22**	590	135
R7	8	7	335	118

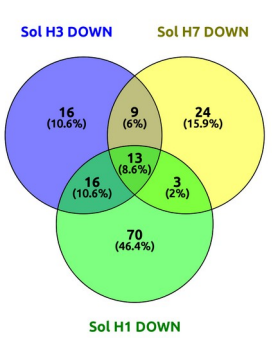
Differential expression of enhancers

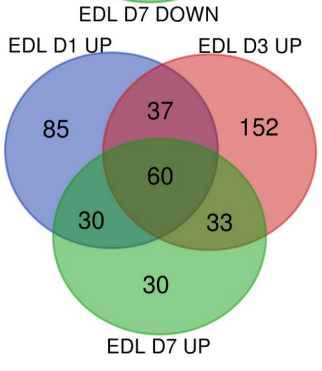
	EDL		Sol	
	UP	DOWN	UP	DOWN
HS1	43	55	65	102
HS3	65	101	51	54
HS7	32	28	45	49
R1	29	54	150	347
R3	32	23	64	68
R7	16	20	90	67

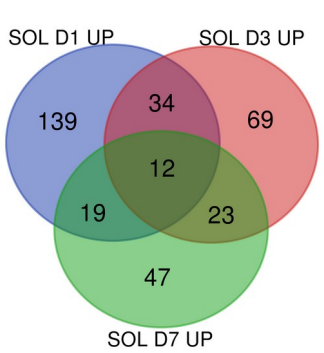
Most of differentially activated promoters and enhancers are unique for each time period of experiment

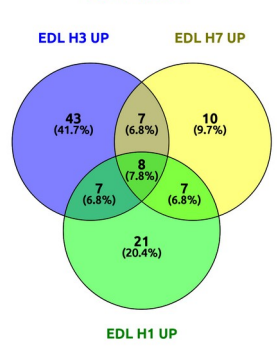


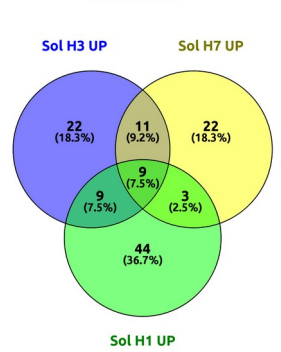


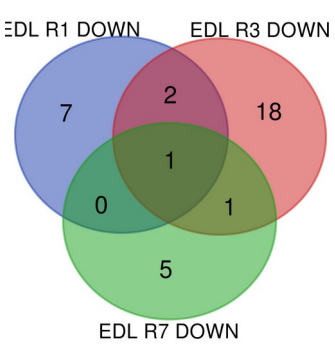


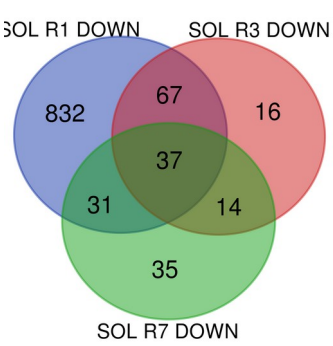


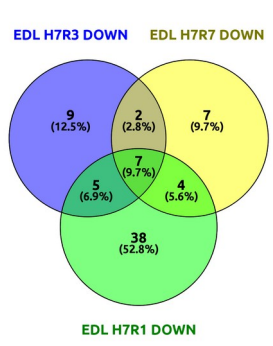


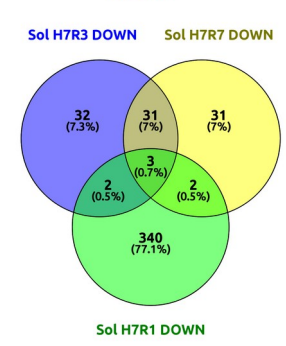


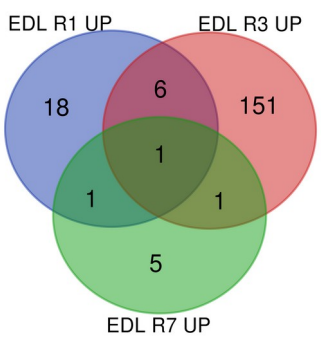


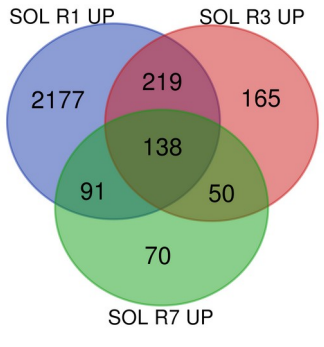


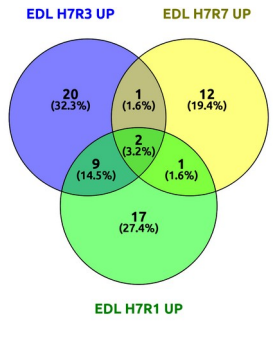


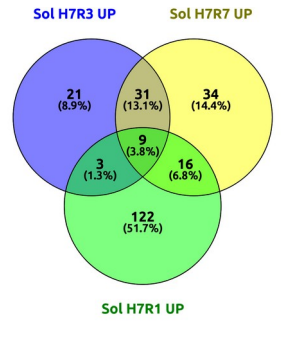












SOLEUS GENE ONTOLOGY TERMS ANALYSIS

Parent	Term	D1 DOWN	D3 DOWN	D7 DOWN	R1 DOWN	R3 DOWN	R7 DOWN
actin cytoskeleton organization	DNA packaging			3.76			
	chromatin assembly or disassembly			3.95			
	mitochondrion organization				5.96		
	cellular component organization	2.71		2.66	2.93		
	actin cytoskeleton organization	4.02	3.59	2.89	4.23	3.69	
	chromosome organization			2.55			
	protein-DNA complex subunit organization			2.69			
	supramolecular fiber organization	4.82		4.64	4.40	4.58	
	actin filament-based process	3.90	3.52	2.82	4.39	3.60	
muscle cell differentiation	muscle system process			2.81	7.59	2.47	
	muscle contraction			2.84	7.69	2.49	
	cellular component assembly involved in morphogenesis			2.09	5.64	5.82	
	cardiac muscle cell differentiation				3.63	7.71	2.90
	muscle tissue development	2.43			2.62	5.82	2.09
	muscle structure development	2.50			6.79	5.71	
cellular component organization or biogenesis	cellular component organization or biogenesis	2.56		2.69	2.35		
energy derivation by oxidation of organic compounds	purine ribonucleotide metabolic process				10.34		
	energy derivation by oxidation of organic compounds				13.60		
	organophosphate metabolic process				7.80		3.65
	small molecule metabolic process				7.21		3.11
	oxidation-reduction process				13.05		2.31
enzyme linked receptor protein signaling pathway	mitochondrion organization				5.96		
	G-protein coupled receptor signaling pathway	2.10			6.56		
generation of precursor metabolites and energy	generation of precursor metabolites and energy				13.74		
	phosphorus metabolic process				5.48		2.29
hydrogen ion transmembrane transport	hydrogen ion transmembrane transport				9.41		
T cell mediated immunity	immune effector process			2.62	2.04		
	T cell mediated immunity	5.58		6.08	2.92		

Parent	Term	D1 UP	D3 UP	D7 UP	R1 UP	R3 UP	R7 UP
actin cytoskeleton organization	DNA packaging					5.49	4.53
	chromatin assembly or disassembly					5.90	6.89
	mitochondrion organization	2.70					
	cellular component organization				5.41	6.67	7.84
	actin cytoskeleton organization				11.61	4.05	3.38
	collagen fibril organization						5.57
	macromolecular complex subunit organization		2.05		3.17	7.61	4.37
	chromosome organization					5.39	4.51
	protein-DNA complex subunit organization					5.75	4.25
	supramolecular fiber organization				9.47	5.25	6.67
	actin filament-based process				11.32	4.36	3.26
cardiac muscle cell differentiation	muscle contraction						
	sensory perception of chemical stimulus				7.28	2.12	
	cellular component assembly involved in morphogenesis						2.05
	muscle structure development						2.81
cellular component organization or biogenesis	cellular component organization or biogenesis				6.44	7.08	7.87
cellular process	cellular process				7.29	3.70	3.12
energy derivation by oxidation of organic compounds	purine ribonucleotide metabolic process	2.62					
	organophosphate metabolic process	3.44					
	small molecule metabolic process	2.08					
	enzyme linked receptor protein signaling pathway				5.51		
	G-protein coupled receptor signaling pathway	2.66			6.03	2.56	
protein folding	protein folding				5.81		
T cell mediated immunity	immune effector process		2.13	6.45			
	T cell mediated immunity		5.50	10.99		2.73	3.74

CONCLUSIONS:

This study provides the first systematic annotation of promoters and enhancers landscape and genes activated in "fast" and "slow" muscle types under induced disuse atrophy and following recovery in rats.

Based on quantity of differential expression of genome regulatory elements in soleus muscle's response to muscle atrophy is more explicit than in m. EDL.

Most of differentially activated promoters and enhancers are unique for each time period of experiment.

According to gene ontology terms analysis in soleus muscle, the major promoters involved in disuse and recovery process are associated with cytoskeleton organization, muscle differentiation, oxidation and biogenesis. Downregulation of promoters controlling actin cytoskeleton and mitochondrion organization during disuse period and extension of this trend up to several days of the recovery demonstrates an inertia of the atrophic process.

Acknowledgements:

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