Proteome Profiling of C2C12 Myogenesis

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Abstract

C2C12 muscle progenitor cells replicate, fuse, and develop into spontaneously contracting muscle fibers *in vitro*, a process emulating the activity of satellite cells in *vivo* (1,2). Proteomic analyses of these developing myocytes will shed light into the molecular mechanisms of myogenesis. Cells were harvested at three time-points representing three distinct stages of development: myoblasts (Day 0), early myotubes (Day 4), and late myotubes (Day 9). Whole cell extracts of each stage (n=5) were separated by 2D SDS PAGE and were analyzed to determine significant changes in protein expression. A combined total of 301 spots significantly changed intensity during development. Due to experimental limitations, 56 of these proteins were successfully identified and categorized into euKaryotic Orthologous Groups (3). The organized groups represent the functional changes observed during myogenesis and create a more developed foundation for future research endeavors.

Introduction

C2C12 cells change significantly, morphologically and functionally, during myogenesis. Initially the mononucleated cells are actively sensing the environment, migrating, and undergoing replication; all processes requiring specific metabolic and structural proteins (4). As myoblasts fuse together to form early myotubes and eventually late myotubes, the cell's function shifts. Cells exit the cell cycle and acquire many more mitochondria, glycogen stores, and filamentous proteins (4). Further inquiries into protein expression patterns during myogenesis may elucidate mechanisms of disease and expand the current understanding of protein expression within developing muscle progenitor cells.

Materials and Methods

Cell Culture
C2C12 murine cell line
cells harvested at Day 0, Day 4, and Day 9

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Protein Concentration Estimation
Sample Preparation
Lowry assay, acetone precipitation

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Two-Dimensional SDS Polyacrylamide Gel Electrophoresis
horizontally by isoelectric points (pH 5-8)
vertically by apparent molecular weight (250-10 kDa)

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Imaging, Analysis, and Spot Excision
QuantityOne, PDQuest, EXQuest Spotcutter (BioRad)

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Protein Identification
tryptic digestion, microscale desalting,
HPLC-MS, bioinformatics

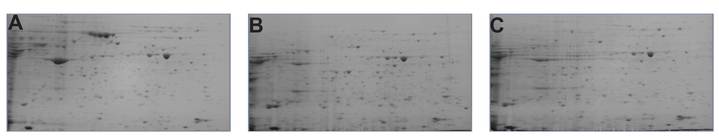

Figure 1: Two-dimensional SDS PAGE gels representing the proteomes of three distinct stages of myogenesis. The pH ranges from 5 to 8, left to right, while the mw ranges from 80 to 10 kDa, top to bottom, on each gel. (A) myoblasts (Day 0), (B) early myotubes, (Day 4), and (C) late myotubes (Day 9).

Results

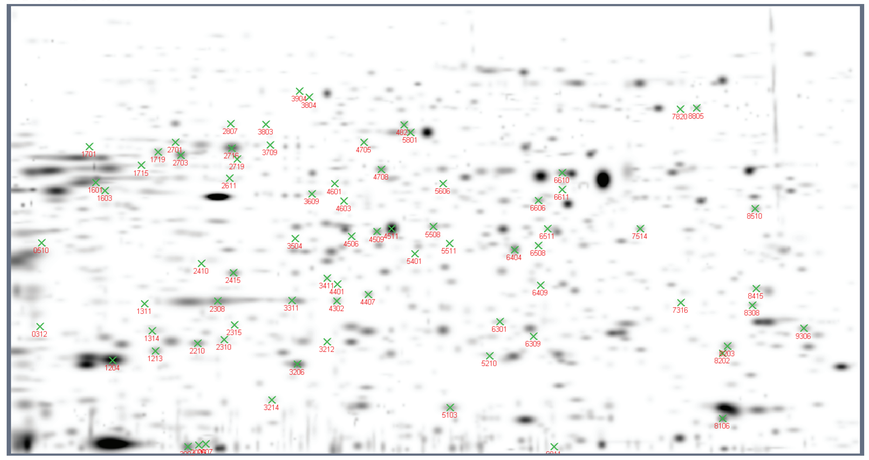

Figure 2: Master gel created by PDQuest that includes proteins present on at least 3 of 5 gels from each time point. Identified proteins are marked with x and tagged with the associated SSP number.

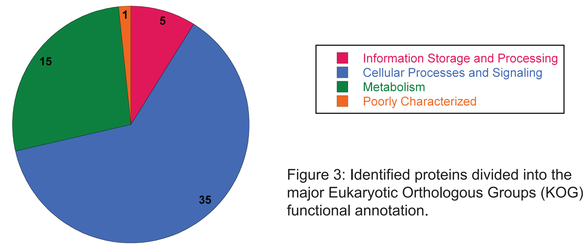

Figure 3: Identified proteins divided into the major Eukaryotic Orthologous Groups (KOG) for functional annotation.

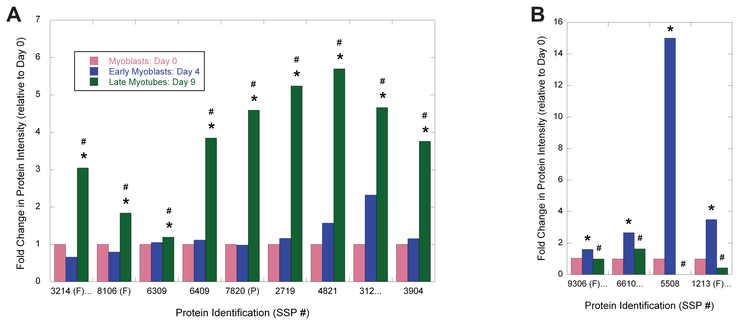

Figure 4: The two most common protein expression patterns observed during myogenesis. (A) KOG subgroup O contains the greatest number of proteins whose expression increases significantly from Day 0 and Day 4 to Day 9. (B) KOG subgroup G contains the greatest number of proteins whose expression increases significantly from Day O to Day 4 before decreasing significantly from Day 4 to Day 9. (* indicates a significant expression change from Day 0, # indicates a significant expression change from Day 4, significant at $p < 0.05$ determined by one-way ANOVA.)

Conclusions

-The proteome of C2C12 cells changes over the course of myogenesis.

-Of the 56 identified proteins that significantly changed in expression during myogenesis, the most common pattern (48%) was an increase from both Day 0 and Day 4 to Day 9. 70% of these proteins are involved in cellular processing and signaling.

-The second most common expression pattern (12.5%) was a significant increase from Day 0 to Day 4 followed by a significant decrease from Day 4 to Day 9. 71% of these proteins are involved in metabolism.

KOG Group		
KOG Subgroup	SSP Number(s)	Protein Identification
Information Storage and Processing		
A: RNA processing and modification	4506 (F)	heterogeneous nuclear ribonucleoprotein K
	8510	poly(rC)-binding protein 1
J: Translation, ribosomal structure and biogenesis	1311	40S ribosomal protein SA
	510, 5511, 6508	60S acidic ribosomal protein P0
	3504	eukaryotic translation initiation factor 3 subunit 1
Cellular Processes and Signaling		
D: Cell cycle control, cell division, chromosome partitioning	2310 (P)	kinetochore-associated protein DSN1 homolog
	2703 (P)	dynactin subunit 2
O: Posttranslational modification, protein turnover, chaperones	312, 1311, 2308 (F)	14-3-3 protein zeta/delta isoform 1
	1715	26S protease regulatory subunit 6A
	3803, 2807, 4705	60 kDa heat shock protein, mitochondrial
	5210	glutathione peroxidase 1
	6011(F)	glutathione S-transferase P1
	2315, 5103	heat shock protein 25
	8106 (F)	peptidy-prolyl cis-trans isomerase A
	1204 (P)	peroxiredoxin-2
	6301	peroxiredoxin-4, precursor
	3311	prohibitin
	6409	proteasome subunit alpha type-1
	6309	proteasome subunit beta type-3
	3214 (F), 3212	proteasome subunit beta type-4
	5801, 4407 (P)	protein disulfide-isomerase A3 precursor
	2719	protein disulfide-isomerase A6 precursor
	3904	stress-70 protein, mitochondrial
	7820 (P)	stress-induced-phosphoprotein 1
	4821	T-complex protein subunit epsilon
	2210 (P)	ubiquitin-conjugating enzyme E2 K
T: Signal transduction mechanisms	312	rho GDP-dissociation inhibitor 1
	2310 (P)	adaptor molecule crk isoform 2
	4601	endophilin-A2 isoform 1
	1311	ran-specific GTPase-activating protein
U: Intracellular trafficking, secretion, and vesicular transport	1314 (F), 6511, 7514	annexin A1
	2719	perilipin-3
W: Extracellular structures	2005, 2004 (P), 2007 (P)	galectin-1
Y: Nuclear structure	8805 (P)	prelamin-A/C
Z: Cytoskeleton	1601, 1719, 2701, 2716, 8202 (F), 3709 (P)	desmin
	4401	F-actin capping protein subunit beta
	5401 (F)	keratin, type II cytoskeletal 1
	1204	myosin light chain 1/3, isoform 1f
	1701	tubulin beta-5 chain
	1701	vimentin
Metabolism		
C: Energy production and conversion	8203 (F)	malate dehydrogenase, cytoplasmic
	2611	creatine kinase B-type
	1601, 8202 (F)	ATP synthase subunit beta, mitochondrial
	3206	ATP synthase subunit D, mitochondrial
F: Nucleotide transport and metabolism	8415	S-methyl-5'-thioadenosine phosphorylase
	2210 (P)	5' (3')-deoxyribonucleotidase, cytosolic
G: Carbohydrate transport and metabolism	312	triosephosphate isomerase
	4708 (P), 2807, 3804, 6511 (F), 5606 (P)	pyruvate kinase isozymes M1/M2 isoform 1
	1213 (F), 4511 (F)	phosphoglycerate kinase 1
	8308	phosphoglycerate mutase 1
	9306 (F), 7316 (P)	fructose-bisphosphate aldolase A
	5508	beta enolase
	6610, 2415, 3411 (P), 4509 (P), 6404 (P)	alpha enolase
I: Lipid transport and metabolism	5801	phospholipase-C alpha
P: Inorganic ion transport and metabolism	3311	chloride intracellular channel protein 4
Poorly Characterized		
R: General function prediction only	4302	N(G),N(G)-dimethylarginine dimethylaminohydrolase 2

Table 1: Protein identifications classified into Eukaryotic Orthologous Groups. All proteins changed either by +/- 2-fold, appeared, or disappeared and were significant at $p < 0.05$ (Student's t-test). (F) indicates fragmented protein. (P) indicates predicted protein.

Acknowledgements

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References

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