The International Journal of Biochemistry & Cell Biology 40 (2008) 2544–2552

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Coordinate expression of the 19S regulatory complex and evidence for ubiquitin-dependent telethonin degradation in the unloaded soleus muscle

A.E. Heng[a,b,1], S. Ventadour[a,1], M. Jarzaguet[a], M.N. Pouch-Pélissier[a], C.Y. Guezennec[c], X. Bigard[d], D. Attaix[a], D. Taillandier[a,*]

[a] *INRA and Human Nutrition Research Center of Clermont-Ferrand, UMR 1019, 63122 Ceyrat, France*
[b] *Service de néphrologie et réanimation médicale, CHU Clermont-Ferrand, Hôpital G. Montpied, 63003 Clermont-Ferrand, France*
[c] *CNR, 91460 Marcoussis, France*
[d] *CRSSA, Département des Facteurs Humains, 38702 La Tronche, France*

ARTICLE INFO

Article history:
Received 28 February 2008
Received in revised form 16 April 2008
Accepted 18 April 2008
Available online 15 May 2008

Keywords:
Skeletal muscle
Ubiquitin
Proteasome
Hindlimb suspension
Telethonin

ABSTRACT

Catabolic stimuli induce a coordinate expression of the 20S proteasome subunits in skeletal muscles. However, contradictory data have been obtained for the 19S regulatory complex (RC) subunits, which could reflect differential regulation at the transcriptional and/or translational level. To address this point we used a well-established model of muscle atrophy (hindlimb suspension) and determined the mRNA levels for 19S subunits belonging to both the base (non-ATPase S1, ATPases S7 and S8) and the lid (S14) of the 19S RC. Concomitant increased mRNA levels were observed for all studied subunits in rat soleus muscles after 9 days of unloading. In addition, analysis of polysome profiles showed a similar proportion of actively translated mRNA (50%) in unloaded and control soleus muscle. Furthermore, the repressed pool of messenger ribonucleoparticles (mRNPs) was low in both control (14%) and unloaded (15%) animals. Our data show that representative 19S subunits (S7 and S8) were efficiently translated, suggesting a coordinate production of 19S RC subunits. The 19S RC is responsible for the binding of polyubiquitin conjugates that are subsequently degraded inside the 20S proteasome core particle. We observed that soleus muscle atrophy was accompanied by an accumulation of ubiquitin conjugates. Purification of ubiquitin conjugates using the S5a 19S subunit followed by deubiquitination identified telethonin as a 26S proteasome substrate. In conclusion, muscle atrophy induces a concomitant expression of 26S proteasome subunits. Substrates to be degraded include a protein required for maintaining the structural integrity of sarcomeres.

1. Introduction

The ubiquitin–proteasome system (UPS) is involved in most catabolic states and is the main actor of muscle wasting. The 26S proteasome is formed by the 20S proteolytic core capped by two 19S regulatory complexes (RCs). The 19S RC confers the recognition specificity of ubiquitin conjugates and the ATP dependence of the 26S proteasome. We and others previously reported that most of the components of the UPS pathway were up-regulated at the mRNA

Abbreviations: UPS, ubiquitin–proteasome system; mRNP, messenger ribonucleoparticle; HS, hindlimb suspension atrophy; Ub, ubiquitin; NEM, *N*-ethyl maleimide.

* Corresponding author at: INRA, Human Nutrition Unit, 63122 Ceyrat, France. Tel.: +33 4 73 62 48 44; fax: +33 4 73 62 47 55.
E-mail address: daniel.taillandier@clermont.inra.fr (D. Taillandier).

[1] These authors contributed equally to this work.

1357-2725/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocel.2008.04.013

levels in skeletal muscles during highly catabolic situations (ubiquitin, 14 kDa-E2, muscle-specific E3s and 20S proteasome subunits) (Bodine et al., 2001; Taillandier et al., 1996). However, while the 20S proteasome subunits are coordinately elevated during muscle atrophy, studies reported either a concomitant elevation of mRNA levels of 19S subunits (Combaret et al., 2004) or an independent regulation (Dawson et al., 1995; Combaret et al., 2002; Tilignac et al., 2002; Lecker et al., 2004; Arlt et al., 2007). These discrepancies could be related to mild vs. strong situations of muscle wasting and/or not optimal measurement schedule. Another possibility could be that the different 19S subunits are independently controlled at different levels (transcriptional vs. translational), thus implicating differential translational efficiency for mRNAs. The 19S RC can be separated into two entities, the base and the lid, and possesses ATPase and non-ATPase subunits that are believed to accomplish specific roles. It is thus possible that different functions could implicate different levels of recruitment during atrophy processes. We wanted in this study to verify in a well-established model of severe muscle atrophy whether the 19S RC subunits were coordinately regulated at the mRNA levels. To further determine whether the 19S RC subunits are differently regulated, the translational efficiency of different components of the UPS pathway was also studied in both basal and atrophy states, and compared to another proteolytic system also involved in the decrease in muscle mass. Finally, using a 19S subunit, we purified and analyzed substrates of the 26S proteasome and found that at least a protein involved in muscular dystrophy was polyubiquitinated.

2. Materials and methods

2.1. Animals and experimental design

The experiments were conducted in accordance with the National Research Council *Guide for the Care and Use of Laboratory Animals*. In the present study, muscle atrophy was induced by hindlimb suspension, a well-known model of muscle deconditioning that mainly affects slow-twitch skeletal muscles. Forty male Wistar rats (Charles River) of an average body weight of 120 g were randomly assigned to either a control (CT) or a hindlimb suspended (HS) group. After 4 days of standard housing, rats of the HS group were suspended by the tail as previously described (Taillandier et al., 1993). All animals were maintained in a temperature-controlled room ($22 \pm 1\,^{\circ}C$) with a 12:12-h light:dark cycle. After 9 days of hindlimb suspension, rats from both CT and HS groups were killed by cervical dislocation and soleus muscles were carefully dissected, frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ until analysis.

2.2. Rates of protein turnover

Protein synthesis and breakdown were determined as previously described (Taillandier et al., 2003). Briefly, protein synthesis was measured by incorporation of [U–^{14}C]L-phenylalanine into proteins (Taillandier et al., 1996; Combaret et al., 2002; Voisin et al., 1996). Rates of phenylalanine incorporation were multiplied by 0.77, i.e. the ratio of tyrosine to phenylalanine in rat muscle proteins, to obtain tyrosine equivalents (Voisin et al., 1996). Total rates of protein breakdown were measured by following the rates of tyrosine release into the media in the presence of cycloheximide. Since muscle can neither synthesize nor degrade this amino acid, tyrosine release reflects the breakdown of proteins. Tyrosine was assayed by the fluorimetric method of Waalkes and Udenfriend (1957). The contribution of the UPS pathway to total proteolysis was estimated in the presence of 10 mM methylamine and 50 μM E-64c in a Ca^{2+}-free medium to inhibit lysosomal and Ca^{2+}-dependent proteolysis.

2.3. Northern blot analysis

Total RNA was extracted as described by Chomczynski and Sacchi (1987). Twenty micrograms of total RNA was separated in 1% agarose gels containing formaldehyde. RNAs were electrophoretically transferred to a nylon membrane (GeneScreen, PerkinElmer) and covalently bound to the membrane following UV crosslinking. The membranes were hybridized with cDNA probes encoding the human regulatory complex subunits S1, S7, S8 and S14 (generous gift from Pr. Martin Rechsteiner). The hybridizations were performed at 65 °C with [^{32}P]-cDNA fragments labeled by random priming (Ready to go GE Healthcare), as previously described (Taillandier et al., 1996). Following washings at the same temperature, the filters were autoradiographed for 3–48 h at −80 °C with intensifying screens on Hyperfilm MP films (GE Healthcare). Autoradiographic signals were quantified in arbitrary units using digital image processing and analysis (ImageJ 1.34s NIH), and normalized using the corresponding 18S rRNA signals to correct for variations in RNA loading. rRNA to mRNA ratio did not change with the treatments (data not shown).

2.4. Polysome fractionation and dot blot analysis

Polysome isolation on sucrose gradients (15–45%), fractionation and dot blot analysis were performed as previously described (Taillandier et al., 2003) using pooled frozen soleus muscles from control and unloaded animals ($n = 4$–6). cDNA hybridization and autoradiographic signal quantification were performed as described above. Membranes were hybridized with cDNA probes encoding for the ATPase subunit S7 of the 19S regulatory complex, the C9 20S proteasome subunit, ubiquitin (Agell et al., 1988) and m-calpain (Imajoh et al., 1988). Using the rapid decay of [^{32}P] radioactivity, each blot was hybridized in turn with all the probes without stripping off the previous probe.

2.5. Immunoblotting

Soleus muscles were homogenized on ice in 4 ml/100 mg fresh muscle of buffer (5 mM Tris pH 7.5, 5 mM EDTA, 1 mM PMSF, 0.25 mM TLCK, 5 mM NEM, 5 μg/ml leupeptin, 5 μg/ml STI) and fractionated by serial centrifugation at 4 °C for 5 min at $1500 \times g$, 10 min at $10{,}000 \times g$ and then 3 h at $100{,}000 \times g$. The last supernatant contains soluble proteins. The combined pellets containing