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Mechanisms Underlying Muscle Protein Imbalance Induced by Alcohol

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Abstract

Both acute intoxication and longer-term cumulative ingestion of alcohol negatively impacts the metabolic phenotype of both skeletal and cardiac muscle, independent of overt protein calorie malnutrition, resulting in loss of skeletal muscle strength and cardiac contractility. In large part these alcohol-induced changes are mediated by a decrease in protein synthesis that in turn is governed by impaired activity of the protein kinase, the mechanistic target of rapamycin (mTOR). Herein, we summarize recent advances in mTOR signal transduction as well as similarities and differences between the effects of alcohol on this central metabolic controller between skeletal muscle and heart in response to acute versus chronic intake. While alcohol-induced alterations in global proteolysis via activation of the ubiquitin-proteasome pathway are equivocal, emerging data suggest alcohol increases autophagy in muscle. Further studies are necessary to define the relative contributions of these bidirectional changes in protein synthesis and autophagy in the etiology of alcoholic myopathy in skeletal muscle and heart.

Keywords

mTOR; translational control; protein synthesis; ubiquitin-proteasome pathway; autophagy; amino acids; alcoholic myopathy

1. INTRODUCTION

1.1 Scope of problem

The consumption of alcohol (i.e., ethanol) containing beverages has continued largely unabated for centuries because of its neurologic effects and its putative medicinal benefits. Today alcohol use and misuse represents a major global concern being directly or indirectly implicated in a range of disease conditions (21). Excessive consumption of alcohol is the fifth leading risk cause of disease and injury worldwide, although considerable variation exists between and within countries as to the amount and pattern of alcohol use. In the United States (US), the per capita consumption averages almost 9 L of pure ethanol per year

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and approximately 75,000 deaths annually are attributed to excessive alcohol consumption (108). These adverse health effects of alcohol result from an increased prevalence of gastrointestinal, hepatic, cardiovascular, infectious and neoplastic diseases (38).

While the effects of excessive alcohol on the brain and hepatobiliary system garner the greatest attention, the impact of alcohol on the structure and metabolic phenotype of striated muscle – both skeletal and cardiac – has been recognized for more than half a century and adversely affects morbidity and mortality. A significant loss of lean body and muscle mass is detected in 40–60% of individuals with prolonged heavy alcohol use, making it more prevalent than the inherited myopathies (89). This skeletal muscle myopathy ultimately leads to a loss of strength that is proportional to the lifetime alcohol consumption (74). Likewise, long duration of heavy alcohol consumption (> 80 g pure ethanol [≈8 standard drinks in the US] per day for > 10 years) often produces a spectrum of ultrastructural changes in the heart leading to the thinning of the ventricular wall, decreased ventricular function, and the development of alcoholic cardiomyopathy (83). The prevalence of this dilated cardiomyopathy varies widely (ranging between 4–40%) reflecting the criteria used for diagnosis, the diverse patient population studied as well as the amount, duration and pattern of alcohol misuse. Because of the difficulty in diagnosis, the prevalence of both skeletal and cardiac myopathy is believed to be underestimated. However, it is noteworthy, that many of alcohol's effects on muscle protein metabolism and strength are not permanent and can be at least partially reversed with abstinence or controlled low-dose alcohol consumption (15, 118).

Early studies on the etiology of chronic alcoholic myopathy have largely eliminated disturbances in electrolytes, peripheral neuropathy, inactivity and a host of other factors as being causative (reviewed in (56, 84)). However, heavy alcohol consumption of long duration in humans can produce a secondary protein calorie malnutrition as well as deficiencies in vitamins and trace minerals (75) that may contribute to the development of alcoholic myopathy (119). While the relative importance of such nutritional effects are difficult to dissect in human studies, they can be largely excluded from tightly controlled animal studies (see Section 1.2). Hence, it is generally held that nutritional factors, while potentially contributory, are not essential for the development of either skeletal or cardiac muscle myopathy. In this regard, as described in subsequent sections, the effect of alcohol on striated muscle is characterized by its ability to alter metabolic processes critical for cellular protein homeostasis under basal conditions and often in response to a nutritional stimulus, such as refeeding or leucine. Thus, the current review focuses on recent advances that have improved our understanding of protein metabolism and the development of alcoholic myopathy. Throughout, we have highlighted gaps in our understanding and potential areas for future research.

1.2 Preclinical models

Although seminal epidemiological data pertaining to the effect of alcohol have been obtained in humans, there is a relative scarcity of human studies in which alcohol's effect on muscle have been investigated and essentially no human studies on cellular mechanisms for the development of alcoholic myopathy. This paucity of human studies originates from the

difficulty in quantitating and controlling the amount, type, pattern and duration of alcohol intake in addition to the inability to tightly control nutritional, genetic and environmental differences that may impact outcomes. Hence, the large majority of the available data in this area are derived from preclinical rodent models that appear to mimic the clinical condition. In general, chronic consumption is most frequently modeled in rats and mice by including alcohol either in the drinking water or as part of a nutritionally complete liquid diet. These models produce clinically relevant increases in the blood alcohol concentration (BAC) (39). While each of these methods has advantages and disadvantages, it is noteworthy that all chronic alcohol models include time-matched control animals that are pair-fed an isocaloric isonitrogenous non-alcohol containing diet. Thus, differences in the metabolic phenotype of muscle between alcohol-fed and control animals are most likely the result of alcohol and/or one of its oxidative metabolites (e.g., acetaldehyde). However, alcohol may also influence the digestion and absorption of select nutrients (66), and as this variable is not routinely monitored its contribution to the development myopathy in animals consuming an alcohol-containing diet for several months cannot be excluded.

Heavy episodic drinking (e.g., ≥ 5 standard drinks [≈14 g ethanol/drink] on an occasion for men and ≥ 4 standard drinks for women) is on the rise in the US (27). This binge drinking behavior is modeled in rodents by the bolus administration of alcohol either by oral gavage or intraperitoneal injection, with the latter approach being used most often despite its lack of clinical relevance. Although direct side-by-side comparisons are rare, the route of administration does not appear to greatly influence alterations in muscle protein metabolism and this may reflect the fact that the peak BAC and area under the BAC disappearance curve is remarkable similar regardless of the route of administration (60). These acute models of alcohol intoxication have provided valuable information related to possible early mechanisms or initiating causes for the metabolic disruption, but data from such models may not be indicative of changes observed with chronic consumption.

2. ALCOHOLIC MYOPATHY

While there are many similarities between the effects of alcohol (both acute and chronic) on protein homeostasis in skeletal and cardiac muscle, there are also clear tissue-specific differences that may be important in disease etiology and progression. In the following sections, we start by describing the effects of alcohol on skeletal muscle, as they are the most thoroughly investigated, and then compare and contrast these to alcohol-induced changes in heart. Table 1 provides a comparison of the key changes in protein hemostasis that have been reported for skeletal and cardiac muscle in response to acute and chronic alcohol intake, and these will be elaborated upon in subsequent sections.

2.1 Muscle mass

Alcoholic skeletal muscle myopathy in humans is characterized by the loss of lean mass that can approach 20% of whole-body mass in severe conditions (110). This atrophic response to chronic excessive alcohol ingestion is often characterized by what is termed proximal wasting and evidenced by a reduction in the mass and cross-sectional area of muscles with a predominance of fast-twitch type II fibers as opposed to type I fibers (16, 26, 77, 85).

Comparable changes have also been detected in rats and mice fed a nutritionally complete diet containing alcohol for at least 6 weeks (62, 87), providing reassurance on the fidelity of the preclinical model used.

In contrast, the development of alcoholic heart muscle disease often requires months of sustained alcohol intake (52). Divergent results exist regarding the effect of chronic alcohol-feeding on heart weight and protein content. Most animal studies report a decrease in ventricular weight or protein content in rodents (57, 116–118) or a thinning of the ventricular wall and septum is typically observed in animals and humans leading to ventricular dysfunction (52, 114). However, there are also now several studies reporting cardiac hypertrophy (i.e., increased heart-to-body weight ratio) in alcohol-fed mice (24, 64), but the mechanism underlying the difference from earlier reports remains unclear.

2.2 Protein synthesis

Early studies demonstrated a relatively specific decrease in in vivo-determined total mixed protein synthesis in fast- versus slow-twitch skeletal muscle of chronic alcohol-fed rats and mice, consistent with the presence of type I atrophy (62, 87). A decrease in global protein synthesis has also been reported in skeletal muscle from humans with chronic alcohol use (78). In rodent models, this inhibitory effect can be observed as early as 7–14 days after initiating feeding (86) and there is no refractoriness for up to at least 4 months (53, 117). Subsequently, alcohol was demonstrated to impair the synthetic rate of both sarcoplasmic and myofibrillar pools in skeletal muscle (62, 85, 88), and this may be causally related to the decrease in contractile proteins such as the Iβ, IIx and IIβ myosin isoforms (91), titin and nebulin (34).

A comparable decrease in skeletal muscle protein synthesis occurs in response to acute alcohol intoxication (85). This alcohol-induced decrease manifests rapidly within the first hour after intoxication and, depending on the initial dose administered, can be maintained for up to 24 hours, a time when the BAC was nondetectable (60, 90). The acute alcohol-induced decrease in synthesis is independent of the route of administration (oral versus intraperitoneal) as well as the sex and age of the rat (55, 60). Independent lines of investigation suggest that alcohol can directly affect skeletal muscle. For example, alcohol decreases protein synthesis in cultured myocytes, incubated whole muscle and the isolated perfused hindlimb (33, 60, 107). A direct action of alcohol on muscle is further supported by studies where animals were pretreated with a chemical inhibitor of alcohol dehydrogenase and the reduction in muscle protein synthesis was equivalent to that seen in vehicle-treated animals (55, 85). However, muscle protein synthesis can be inhibited by acetaldehyde, the key metabolite of alcohol oxidation, when present in high concentrations. For example, protein synthesis is reduced in myoblasts and myotubes cultured with acetaldehyde (33, 107) as well as in animals pretreated with a chemical inhibitor of acetaldehyde dehydrogenase (85).

Acute alcohol intoxication also decreases global protein synthesis in heart as well as the synthetic rate for cardiac myofibrillar, non-myofibrillar and mitochondrial proteins (50, 79, 97, 111). However, in contrast to skeletal muscle, animals must often ingest alcohol for extended periods of time (at least 12–14 weeks) before decreases in myocardial protein

synthesis are detected (112, 116). In general, ribosomal number as estimated by total RNA is not altered by alcohol in either heart or skeletal muscle, suggesting that alcohol impairs protein synthesis by decreasing translational efficiency (50, 62).

2.3 Translational control

2.3.1 Regulation of mRNA translation—The translation of mRNA into protein occurs in three steps: initiation, elongation, and termination (Fig. 1). During initiation, the 40S ribosomal subunit binds to initiator methionyl-tRNA$_i$ (met-tRNA$_i$) to form the 43S pre-initiation complex that subsequently binds to the mRNA, often at the 5' m^{7}GTP cap structure, to form the 48S pre-initiation complex (28). The complex then scans the 5'-untranslated region (5'-UTR) to locate the AUG start codon. The 60S ribosomal subunit joins the complex to form the active 80S ribosome that translates the open reading frame during the elongation step. Assembly of the 43S pre-initiation complex is mediated by a heterotrimeric complex referred to as eukaryotic initiation factor (eIF) 2. During formation of the 43S pre-initiation complex, eIF2 binds to GTP and met-tRNA$_i$ forming a ternary complex that then binds to the 40S ribosomal subunit. During the last step in initiation, the GTP bound to eIF2 is hydrolyzed and the eIF2•GDP complex is released from the complex, leaving behind the met-tRNA$_i$. For eIF2 to re-bind met-tRNA$_i$, the GDP bound to it must be exchanged for GTP, a process catalyzed by the guanine nucleotide exchange factor (GEF), eIF2B. The GEF activity of eIF2B is regulated indirectly through phosphorylation of the substrate, eIF2, whereby phosphorylation of eIF2 on its α-subunit converts the protein from a substrate into a competitive inhibitor of eIF2B.

Binding of mRNA to the 43S preinitiation complex is mediated by a complex of initiation factors referred to as eIF4F that is comprised of eIF4A, eIF4E, and eIF4G. eIF4E binds to both the m^{7}GTP cap and eIF4G (Fig. 1). eIF4A is a RNA helicase whose activity is enhanced by eIF4B, and acts to unwind secondary structure in the 5'-UTR allowing the 40S ribosomal subunit to scan. In addition to binding to eIF4A and eIF4E, eIF4G binds to the 43S pre-initiation complex, thereby localizing it to the m^{7}GTP cap. Assembly of the eIF4F complex is regulated through the interaction of eIF4A with programmed cell death 4 (PDCD4) and the interaction of eIF4E with the eIF4E binding proteins (4E-BP1–3). Binding of PDCD4 to eIF4A and the 4E-BPs to eIF4E prevents them from binding to eIF4G, leading to decreased cap-dependent binding of mRNA to the 43S preinitiation complex. The binding PDCD4 (programmed cell death 4) to eIF4A and 4E-BPs to eIF4E is regulated through phosphorylation of the binding proteins, whereby phosphorylation attenuates the binding of PDCD4 to eIF4A and the binding of 4E-BPs to eIF4E.

2.3.2 Regulation of mRNA translation by mTORC1—The mechanistic target of rapamycin (mTOR) complex 1 promotes the binding of mRNA to the 43S pre-initiation complex, in part, by phosphorylating the 4E-BPs, freeing eIF4E to bind to eIF4G 1 (81). Similarly, by activating the 70 kDa ribosomal protein S6 kinase 1 (p70S6K1), mTORC1 promotes phosphorylation of both PDCD4 and eIF4B thereby enhancing eIF4F assembly and function. Each of these phosphorylation events is necessary for maximal stimulation of protein synthesis by mTORC1 (12). As most mRNAs are translated in a cap-dependent manner, it is not surprising that inhibition of mTORC1, e.g. with Torin1, leads to a greater

than 50% decrease in protein synthesis (109). However, inhibition of mTORC1 has a preferential effect on the translation of certain mRNAs, including those with a terminal oligopyrimidine (TOP) sequence at or near the 5'-cap (109) as well as those with highly-structured 5' UTRs (63).

2.3.3 Regulation of mTOR by hormones and amino acids—An obligate step in mTORC1 activation is its association with the small GTPase referred to as Ras homolog enriched in brain (Rheb, 7, 18, 35, 106) (Fig. 2). Rheb is farnesylated (9) and its localization to the cytoplasmic surface of late endosomal and/or lysosomal (LEL) membranes is necessary for it to activate mTORC1 (5). Although mTORC1 can bind to Rheb in either its GDP- or GTP-bound form, only binding to Rheb•GTP enhances the recruitment of substrates to mTORC1 in a Raptor-dependent manner (96). The GTP loading status of Rheb depends primarily on the activity of a GTPase activating protein (GAP) referred to as tuberous sclerosis complex 2 (TSC2, a.k.a. tuberin) (35, 122). TSC2 functions as part of a heterotrimeric complex to stimulate the GTPase activity of Rheb leading to decreased mTORC1 activation. The GAP activity of TSC2 is inhibited by phosphorylation by Akt (36). Akt is activated by phosphorylation on two key residues, Thr308 and Ser473 (69). Akt Thr308 is phosphorylated by phosphoinositide-dependent protein kinase 1 (PDK1) whereas Ser473 is phosphorylated by mTORC2. Although this might suggest that mTORC2 is an upstream regulator of mTORC1 activity, phosphorylation of Akt on Ser473 is dispensable for Akt-mediated TSC2 phosphorylation (23, 37), suggesting that mTORC1 is regulated by insulin and insulin-like growth factor I (IGF-I) in a PDK1-dependent but mTORC2-independent manner. In addition to Akt, the extracellular regulated kinase (ERK) and the 90 kDa ribosomal protein S6 kinase (p90RSK) also inactivate TSC2 GAP activity toward Rheb through phosphorylation on residues distinct from those phosphorylated by Akt (68, 94).

In contrast to insulin and IGF-I, amino acids activate mTORC1 in mouse embryo fibroblasts lacking TSC2 (93, 99). Moreover, whereas exogenous expression of a Rheb variant that constitutively binds GTP is sufficient to maintain mTORC1 in its active state in serum-deprived cells, it does not prevent the downregulation of mTORC1 activity caused by amino acid deprivation (11). Thus, most studies suggest that amino acids activate mTORC1 in a TSC2- and Rheb-independent manner. Instead, amino acids act through the Ras-related GTP-binding (Rag) proteins to activate mTORC1 (for recent reviews see: 4, 72, 121). The Rag proteins form a heterodimer consisting of either Rag A or B associated with either Rag C or D that binds to mTORC1, but not to mTORC2 (95). Interestingly, it is a complex of the GTP-bound form of Rag A or B and the GDP-bound form of Rag C or D that maximally activates mTORC1 (Fig. 3).

The GTP loading status of Rag A and B depends in part on the GTPase stimulating activity of a protein complex referred to as GAP activity towards Rags (GATOR) 1. By promoting GTP hydrolysis by Rag A and B, GATOR1 acts to repress mTORC1 activity. In contrast, an independent complex, GATOR2, opposes GATOR1 function leading to mTORC1 activation. Recent studies have identified three GATOR2-interacting proteins that act as amino acid sensors to transduce the signal from leucine, arginine, and methionine to GATOR2, and subsequently to mTORC1. Specifically, in cells deprived of leucine, arginine, or methionine, the Sestrin proteins 1, 2, and 3 (82, 120), the cellular arginine sensor for mTORC1

(CASTOR1) (8), and S-adenosylmethionine sensor upstream of mTORC1 (SAMTOR) (22) respectively, bind to and repress the function of GATOR2. Adding back the deprived amino acid results in dissociation of the repressor protein from GATOR2 leading to mTORC1 activation.

2.3.4 mTOR regulation by alcohol—There is considerable inconsistency in the published data pertaining to alcohol-induced changes in signaling upstream of mTORC1 in skeletal muscle (Table 1). For example, while there are minor or no changes in the phosphorylation state of the insulin receptor, IGF-I receptor, and insulin-like substrate-1 in response to alcohol (46, 73), Thr308-phosphorylated Akt has been reported to be decreased (47, 73, 104) or unchanged (46, 47, 105) by either acute or chronic alcohol intake. A dose-dependent effect of alcohol on Akt phosphorylation may account for some of this discrepancy (17). Where assessed, there is no change in the relative phosphorylation of the Akt substrate PRAS40 on Thr246 between alcohol-fed and pair-fed control rats (46). Furthermore, no alcohol-induced change in TSC2 phosphorylation or the association of TSC1•TSC2 in skeletal muscle has been detected (61). As a result of these equivocal changes, many studies have focused on unraveling alcohol-induced changes that negatively impact the activity of mTORC1 and protein-protein interactions within mTORC1 that are illustrated in Figure 1.

As highlighted above, mTORC1 is a central regulator of mRNA translation and there is considerable information pertaining to the role of this kinase in the development of alcoholic myopathy. A decrease in mTORC1 activity is often evidenced in vivo by decreased phosphorylation of 4E-BP1 and S6K1 – two authentic downstream substrates for this Ser/Thr kinase (Fig. 1). In this regard, there is a consistent decrease in 4E-BP1 phosphorylation in skeletal muscle in response to either acute exposure or chronic ingestion of alcohol. However, a coordinate decrease in S6K1 phosphorylation has only been seen in some (45, 73) but not other (49, 54, 60, 61, 100) studies, and the mechanism for alcohol's preferential inhibitory effect on 4E-BP1 phosphorylation remains to be elucidated. The alcohol-induced decrease in 4E-BP1 phosphorylation is physiologically significant, as this change was associated with a relative increase in the binding of the translational repressor molecule 4E-BP1 with eIF4E, and a reciprocal decrease in the formation of the active eIF4E•eIF4G complex (46, 49, 54). Such changes would be expected to decrease cap-dependent translation. Despite the variable effect of alcohol on S6K1 phosphorylation, acute and chronic alcohol has consistently been shown to decrease the phosphorylation of several downstream target proteins, such as S6 (Ser240/244), eIF4G (Ser1108) and eIF4B (Ser422), but not the phosphorylation of PDCD4 (49, 54, 61). All of these changes in protein phosphorylation are independent of changes in the relative amount of the total protein. By placing the alcohol-fed rats on a diet without alcohol for 3 days, all of the above mentioned alcohol-induced changes in mTOR signaling and protein synthesis in skeletal muscle could be reversed (118). Finally, the abundance and phosphorylation of eIF4E, which can enhance cap-dependent translation when increased, do not differ in skeletal muscle of control and alcohol-fed rats (62). A direct inhibitory effect of alcohol on in vitro-determined mTORC1 kinase activity has also been reported in cultured myocytes as have the reduction in the phosphorylation state of downstream target proteins (30).

The suppressive effects of alcohol on mTORC1 activity cannot be attributed to alterations in the total cellular content of the various core and associated proteins (e.g., mTOR, Raptor, mLST8, Deptor, and PRAS40) in the complex, either under in vivo (59, 61) or in vitro (30, 31) conditions. However, alcohol feeding markedly alters various protein-protein interactions within this complex that are internally consistent with the reduction in muscle protein synthesis (59). Specifically, alcohol increases the molecular interaction of the scaffolding protein Raptor with the negative regulatory protein Deptor, while simultaneously decreasing the association of Raptor with 4E-BP1 (45). While the total amount of RagA and RagC in muscle of alcohol-fed rats is unchanged, there is a marked decrease in the binding of Rag A to Raptor that might be expected to impair nutrient-stimulation of protein synthesis (45). Finally, although unexpected, it has been repeatedly demonstrated that the extent of mTOR binding to Raptor is increased by alcohol. Such findings may indicate alcohol promotes a “closed conformation” rendering it less active. Chronic alcohol also increases Ser792-phosphorylated Raptor (45, 59). As AMP-activated protein kinase (AMPK) phosphorylates this reside on Raptor, and thereby inhibits mTORC1 activity, it was therefore unexpected that there is no concomitant increase in AMPK activity in skeletal muscle of chronic alcohol-fed rats. The reason for this disconnect between AMPK activity and Raptor phosphorylation is unknown. Finally, the relative abundance of the inhibitory protein REDD1 (regulated in development and DNA damage responses 1) in skeletal muscle is also unchanged by acute and chronic alcohol consumption (45, 54, 103).

For cardiac muscle, there is consensus that the phosphorylation of both 4E-BP1 and S6K1 is decreased by acute alcohol intoxication (50, 111) and chronic alcohol ingestion (114, 116, 118). It is noteworthy that chronic alcohol feeding has only been reported to decrease protein synthesis and mTORC1 in male, but not female, animals (57, 114). When observed, the decrease in myocardial mTORC1 appears governed in part by the generation of acetaldehyde as overexpression of mitochondrial aldehyde dehydrogenase prevents the alcohol-induced decrease in 4E-BP1 and S6K1 phosphorylation (20). Additionally, while the relative abundance of all proteins in mTORC1 were unchanged in alcohol-fed animals, there was an increased binding of mTOR with Raptor similar to that observed in skeletal muscle (61).

In contrast to skeletal muscle, there is general agreement that acute and chronic alcohol increases AMPK phosphorylation (activity) in heart (25, 40, 57). Results pertaining to the abundance of another negative regulator of mTORC1, REDD1, indicate either an increase (57) or no change (115) in heart in response to alcohol feeding. As these studies were performed in rats and mice, respectively, a species-specific response cannot be excluded. Furthermore, the bulk of the data suggest the phosphorylation of T308- or S473-Akt, a positive effector of mTORC1 activity, is not altered by either acute (14, 47, 58, 111) or chronic alcohol (115, 116). As myocardial mTORC1 activity is simultaneously determined to be decreased in all of the above mentioned studies, collectively these data suggest alterations in the phosphorylation state of AMPK and Akt are not essential for the alcohol-induced decrease in cardiac protein synthesis.

2.3.5 43S Pre-initiation complex—As described in Section 2.3.1, protein synthesis may in part be mediated through the formation of the 43S preinitiation complex (Figures 4

and 5A). In this regard, chronic alcohol decreased eIF2B activity that is required to regenerate the active eIF2•GTP complex to which the met-$tRNA_i^{met}$ binds (62). The underlying mechanism for this change has not been pursued since the original observation, but appears independent of alcohol-induced changes in the expression of the catalytic subunit eIF2Bε as well as the amount of total and phosphorylated eIF2α (62). Furthermore, decreased eIF2B activity is not detected in skeletal muscle after acute alcohol intoxication (51), suggesting a more sustained exposure is necessary to evoke the change. Counter to what is seen in skeletal muscle, eIF2B activity in heart does not differ between alcohol- and pair-fed control rats (62). Furthermore, there are no alcohol-induced changes in the abundance of eIF2Bε or eIF2α (62, 64). There are no reports on the effects of acute alcohol intoxication on the eIF2/2B system and 43S preinitiation complex formation in heart. These acute and chronic effects of alcohol in skeletal and cardiac muscle are summarized in Table 1.

2.3.6 Translation elongation and termination—

Chronic alcohol consumption decreases the RNA content of free nonpolysome-associated 40S and 60S subunits in fast-twitch, but not slow-twitch, skeletal muscle suggesting alcohol also impairs the elongation-termination phase of translation (62). Consistent with this observation, the protein abundance of eukaryotic elongation factor (eEF)1A is decreased in skeletal muscle from alcohol-fed rats (117). Such a reduction would be consistent with impaired translocation of aminoacyl-tRNA to the A-site of the ribosome. Contrary to expectations, the amount of total and Thr56-phosphorylated eEF2, which directs the movement of tRNA to the P-site, is not different in muscle from alcohol-fed and control rats (117). However, acute alcohol intoxication in mice decreases eEF2 phosphorylation, which would be expected to enhance elongation, but does not alter total or Ser1108 phosphorylation of eEF1A (117). In contrast, eEF2 phosphorylation is increased in myocytes incubated with alcohol (29). More research is needed to directly measure the impact of alcohol on elongation per se, and to explore the mechanism underlying these differences between acute and chronic alcohol on translation elongation in skeletal muscle.

There are few data on the effects of alcohol on translation elongation in heart. In contrast to skeletal muscle, chronic alcohol feeding decreases both eEF1A and eEF2 in heart (112, 117, 118). However, similar to muscle, no inhibitory effect is seen in heart in response to acute alcohol intoxication (117). The relative importance of chronic alcohol-induced decreases in elongation versus initiation remain to be determined and represent a gap in our knowledge.

2.4 Leucine resistance

Branched-chain amino acids in general and leucine in particular are fundamental nutrient signals regulating the translational control of muscle protein synthesis (see Section 2.3.3 and Fig.3). In this regard, alcohol markedly impairs the typical stimulatory effects of orally administered leucine. For example, the ability of a maximally stimulating dose of leucine to enhance protein synthesis and increase 4E-BP1 phosphorylation as well as increase the formation of the active eIF4E•eIF4G complex in skeletal muscle is attenuated by acute alcohol intoxication (49). Furthermore, acute alcohol completely blocks leucine's ability to increase the phosphorylation of S6K1 (Thr389 and Thr421/424) and S6. Similarly, alcohol

inhibits mTORC1 activity stimulated by an intravenous infusion of a complete amino acid mixture (100). This alcohol-induced leucine resistance could not be attributed to differences in the prevailing plasma concentration of leucine or insulin (e.g., leucine is an insulin secretagogue) after oral leucine (49). Similarly, culture of myocytes with alcohol for 24 hours antagonizes the ability of leucine to promote phosphorylation of mTOR, S6K1, S6 and 4E-BP1 as well as blunts the interaction between mTOR and Rheb (31). The available literature is consistent with the role of leucine resistance as a contributor to the development of alcoholic skeletal muscle myopathy. In contrast, acute alcohol intoxication does not impair the ability of oral leucine to increase cardiac protein synthesis, the formation of the active eIF4E•eIF4G complex, or the phosphorylation of 4E-BP1 and mTOR (111). No data are currently available on whether chronic alcohol consumption alters the anabolic response of the heart to either leucine or refeeding. Hence, alcohol appears to produce a tissue-specific leucine resistance. Finally, there are large gaps in knowledge related to alcohol's effect on the various regulator protein complexes (e.g., GATOR, CASTOR, Sestrin, Ragulator) under either basal or after nutrient stimulation.

2.5 Circulating hormones and substrates

Alcohol-induced decreases in muscle protein synthesis could be mediated by a decreased concentration of anabolic hormones (e.g., insulin, growth factors and testosterone) or an increased concentration of catabolic hormones (glucocorticoids). Similarly, a change in the prevailing concentration of total or branched-chain amino acids may also govern proportional changes in muscle protein synthesis. The overwhelming number of studies indicate that acute and chronic alcohol either does not significantly alter or slightly increases the plasma insulin concentration in humans and rodents (as reviewed in (102). Likewise, under well-controlled experimental conditions, inhibitor studies have demonstrated that the suppressive effects of alcohol are not due to elevations in the plasma corticosterone concentration (49, 55). Finally, there are no differences in the concentration of total amino acids, branched-chain amino acids or leucine between control and alcohol-fed rats (3, 59). Hence, the consensus from the available literature suggests that changes in the concentration of selected hormones and protein substrates are not causally related to the reduction in muscle protein synthesis.

The exception to this generalization is the decreased concentration of the anabolic hormone IGF-I in blood and muscle of chronic alcohol-fed animals that has been consistently reported by independent laboratories using several preclinical models (48, 101). The alcohol-induced decrease in the plasma IGF-I concentration or the IGF-I mRNA/protein content in skeletal muscle is directly correlated with a reduction in protein synthesis and the formation of the active eIF4E•eIF4G complex (56). Moreover, protein synthesis can be increased back to pair-fed control levels in alcohol-fed rats injected with IGF-I formulated to prolong its circulating half-life (53). In contrast, plasma IGF-I levels are not routinely decreased by acute alcohol intoxication (59) and therefore this potential mechanism does not appear operational under this circumstance. However, acute alcohol intoxication does impair the normal stimulatory actions of IGF-I (and insulin) on S6K1 and S6 phosphorylation in skeletal muscle (46, 59) providing evidence for the presence of an anabolic resistant state in response to short-term alcohol exposure.

2.6 Protein degradation

2.6.1 Ubiquitin-proteasome pathway—The contribution of protein breakdown to the alcohol-induced type II atrophy remains controversial. Studies have reported the urinary excretion of 3-methylhistidine, a biomarker for myofibrillar degradation, is either unchanged (92), decreased (70) or increased (75). Estimates of global muscle proteolysis have also been reported to be unchanged in chronic alcohol infused monkeys (71) and chronic alcohol-fed rats (70). Finally, there is no change detected in global proteolysis of whole muscles or cultured myocytes incubated acutely (4–24 hours) with alcohol (33) or in the isolated perfused hindlimb or incubated epitrochlearis treated with alcohol (113).

Because of its central regulatory role in the degradation of myofibrillar proteins, the ubiquitin-proteasome pathway has also been examined (10). While chronic alcohol consumption has been reported to decrease in vitro-determined 20S proteasome activity in skeletal muscle (44), the majority of studies show no alcohol-induced change (45, 107, 113) in vitro-determined 20S proteasome activity in skeletal muscle. In contrast, there are now several reports that chronic alcohol feeding increases the mRNA content for two muscle-specific E3 ubiquitin ligases, muscle atrophy F-box (MAFbx or atrogin-1) and muscle RING finger-1 (MuRF1) (45, 76, 77), with acute alcohol intoxication increasing both ligases in a dose- and time-dependent manner (113). While these E3 ligases are causally related to muscle atrophy in other catabolic conditions based on data from gene knockout mice, there is also increasing appreciation that elevations in atrogin-1 and MuRF1 mRNA are not consistently associated with enhanced proteasome activity (1). Moreover, treatment of alcohol-fed rats with the antioxidant procysteine attenuated the type II fiber atrophy but exaggerated the elevation in atrogin-1 and MuRF1, thereby questioning their role in the loss of muscle mass (77). Finally, the activity of calpain 1 and 2, that are necessary for the initial cleavage of myofibrillar proteins, as well as their inhibitor calpastatin do not differ in skeletal muscle of control and alcohol-fed rats (44). Hence, there is currently a lack of compelling data to support the hypothesis that alcohol-induced skeletal muscle wasting results from an increase in global proteolysis and activation of the ubiquitin-proteasome pathway. Similarly, there is a paucity of data supporting the activation of this pathway in the etiology of alcoholic cardiomyopathy (57, 113).

2.6.2 Autophagy—Autophagy is an evolutionarily conserved process whereby intracellular macromolecules and organelles are engulfed by a double membrane that forms a structure referred to as the autophagosome (2). Subsequent fusion of the autophagosome with a lysosome results in acidification and digestion of the luminal contents by lysosomal enzymes. The digestion products, e.g. amino acids, can be metabolized to generate ATP or used in the liver for gluconeogenesis (41), thereby helping the organism to survive various stresses. For example, in wild type neonatal mice, a drop in blood amino acid concentrations shortly after birth leads to repression of mTORC1 activity, leading to activation of autophagy in skeletal muscle (13). The amino acids produced by autophagy are used in the liver for production of glucose to prevent perinatal hypoglycemia. In contrast, in mice in which the wild type Rag A gene is replaced with a constitutively active mutant, mTORC1 is not repressed after birth even though blood amino acids fall to a similar extent as in wild type mice, and activation of autophagy and induction of hepatic gluconeogenesis is

impaired, resulting in hypoglycemia and death unless glucose is exogenously supplied. mTORC1 inhibits autophagy by phosphorylating Unc-51 like autophagy activating kinase 1 (ULK1) and UV Radiation Resistance Associated Gene (UVRAG) (42, 43). ULK1 is a protein kinase that activates an early step in autophagy and phosphorylation by mTORC1 represses its protein kinase activity (42). In contrast, UVRAG is part of a complex containing Vps34 that plays a role in autophagosome maturation and fusion with the lysosome (65). Phosphorylation of UVRAG leads to impairment of this step in autophagy (43).

As a second major mechanism central to the degradation and recycling of intracellular proteins and organelles, autophagy has been the focus of recently studies in the development of alcoholic myopathy. Lysosomes are central to this process as they contain a family of proteases, the cathepsins. However, neither chronic alcohol intake nor acute intoxication alters the activity of cathepsins B, D, H and L in skeletal muscle (44). In contrast, emerging evidence, although not entirely consistent, suggests alcohol may increase skeletal muscle autophagy. Specifically, autophagy-related gene (Atg)7, Beclin 1 and the abundance of the lipidated LC3B protein (i.e., LC3B-II) are all increased, while p62 (which is consumed during autophagy) is decreased in skeletal muscle of male alcohol-fed mice (107). Moreover, an increase in LC3B-II is also observed in muscle of alcoholic patients (107). In contrast, in alcohol-fed female mice, LC3B-II, p62 and ULK1 phosphorylation are all unchanged compared to control animals (103). Likewise, little or no change is detected in skeletal muscle from female mice after acute alcohol intoxication (104). As the alcohol-feeding model was similar in the above studies, it is possible that the discrepancies in these studies represent a sexual dimorphic response. However, there is also no apparent increase in autophagy in myoblasts isolated from chronic binge alcohol consuming male rhesus macaques (98).

Despite the uncertain nature of the above mentioned in vivo results from alcohol-treated animals, several studies have provided definitive data that relatively short-term incubation (6 to 24 hours) of C2C12 myotubes and myoblasts in vitro with alcohol increases autophagy (32, 107). For example, alcohol increases autophagy in myotubes as assessed by GFP-LC3B vesicle accumulation and the direct measurement of autophagic flux (107). Moreover, knockdown of Atg7 prevents alcohol-induced increases in autophagy and the decrease in myocyte cross sectional area. Further, chemical inhibitor studies indicate the acute in vitro stimulatory effect of alcohol on autophagy is largely dependent on the generation of acetaldehyde rather than the direct effects of alcohol. This general conclusion has been independently confirmed in alcohol-treated myoblasts where alcohol increases LC3B-II and Atg7, and decreases p62; changes that appear to be regulated by a FoxO1-ULK1-mediated increase in the binding of Atg14 with the BECN1•VPS34 complex (32). Additional studies are required to confirm whether alcohol increases autophagy in vivo in skeletal muscle and under what experimental conditions.

There is a more consistent literature pertaining to the effect of alcohol on cardiac autophagy. Collectively, the data indicate that both acute and chronic alcohol intake increase autophagy in heart as evidenced by an increased LC3B-II/I ratio and Atg7, and a decrease in Ser757-phosphorylated ULK-1 (19, 40). This increase in autophagy is mediated by elevated levels

of acetaldehyde in the heart (19), and appears AMPK-dependent as it is largely prevented in alcohol-treated AMPK knockout mice (40). Similarly, short-term incubation of freshly isolated cardiomyocytes or H9c2 cells with alcohol also increases LC3B-II abundance, an effect that is prevented by pretreatment with the AMPK inhibitor Compound C or the knockdown of ULK-1 (19, 25, 40). Given the leucine resistance produced by alcohol, at least in skeletal muscle, additional studies should be directed to determine whether alcohol also antagonizes the ability of leucine or refeeding to suppress autophagy.

3. CONCLUSIONS AND DIRECTIONS FOR FUTURE DIRECTIONS

To summarize, acute intoxication and long-term heavy alcohol decrease the activity of the key regulator of protein homeostasis mTORC1 in skeletal and cardiac muscle. As a result, a host of downstream protein targets governing translation initiation and elongation are hypo-phosphorylated impairing both sarcoplasmic and myofibrillar protein synthesis. Emerging data indicate that, at least for skeletal muscle, alcohol also produces leucine resistance that minimizes the normal protein anabolic response to refeeding. The data also suggest that despite the similar decrease in mTORC1 and protein synthesis, the underlying mechanisms, as they related to protein-protein interactions with mTORC1 as well as upstream regulators, may differ between heart and skeletal muscle as well as between acute intoxication and chronic consumption. Because of the rapid recent advances in understanding basic nutrient signaling via mTORC1, there is a considerable knowledge gap in how alcohol affects these newly discovered regulatory elements. Finally, there is a paucity of data from humans that is essential to determine the translational nature of the mechanistic data generated from preclinical models and cell culture.

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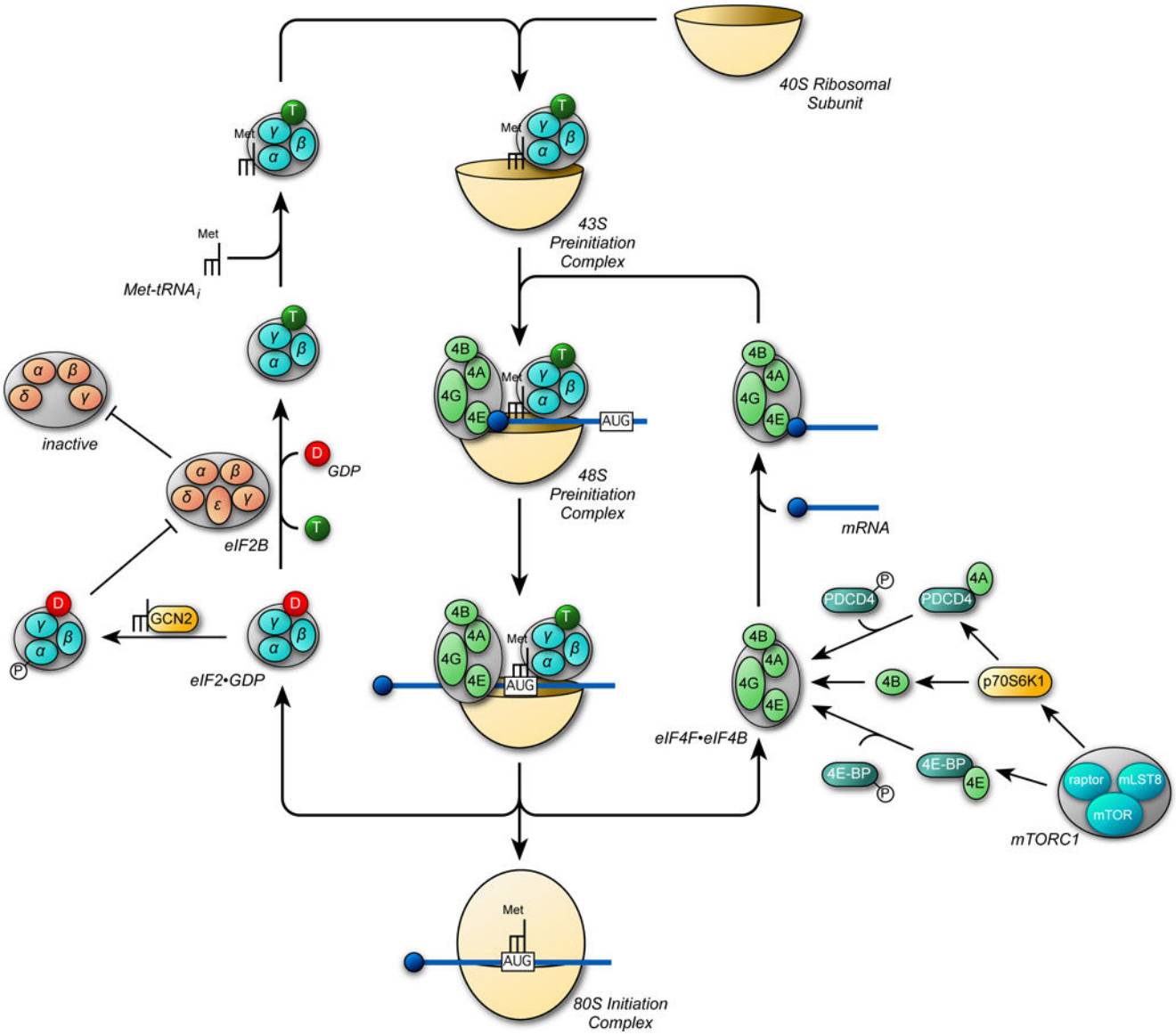

Figure 1.

Key steps in the initiation phase of mRNA translation. The initiation step in translation involves the binding of the ternary complex, consisting of eukaryotic initiation factor 2 (eIF2) associated with GTP and the initiator form of methionyl-tRNA (met-tRNAi), to the 40S ribosomal subunit to form the 43S preinitiation complex. The eIF4F•eIF4B complex in association with mRNA subsequently binds to form the 48S preinitiation complex. The 40S ribosomal subunit then scans along the 5'-untranslated region of the mRNA and stops at an AUG start codon, triggering hydrolysis of GTP bound to eIF2 to GDP leading to release of the eIF2•GDP and eIF4F•eIF4B complexes. The GDP bound to eIF2 is exchanged for GTP by eIF2B and the eIF4F•eIF4B complex binds to another mRNA to re-start the process. GCN2 phosphorylates eIF2 on its α-subunit, converting it from a substrate of eIF2B into a competitive inhibitor, leading to decreased ternary complex formation. mTORC1 promotes assembly of the eIF4F complex through phosphorylation of the eIF4E binding proteins (4E-

BPs) and programmed cell death 4 (PDCD4). Phosphorylation of 4E-BP by mTORC1 releases it from the eIF4E•4E-BP complex, allowing eIF4E to bind to eIF4G. Similarly, phosphorylation of PDCD4 by p70S6K1 (which is also activated by mTORC1) frees eIF4A from the eIF4A•PDCD4 complex, allowing it to bind to eIF4G. In addition, p70S6K1 phosphorylates eIF4B, thereby enhancing its stimulatory activity toward eIF4A.

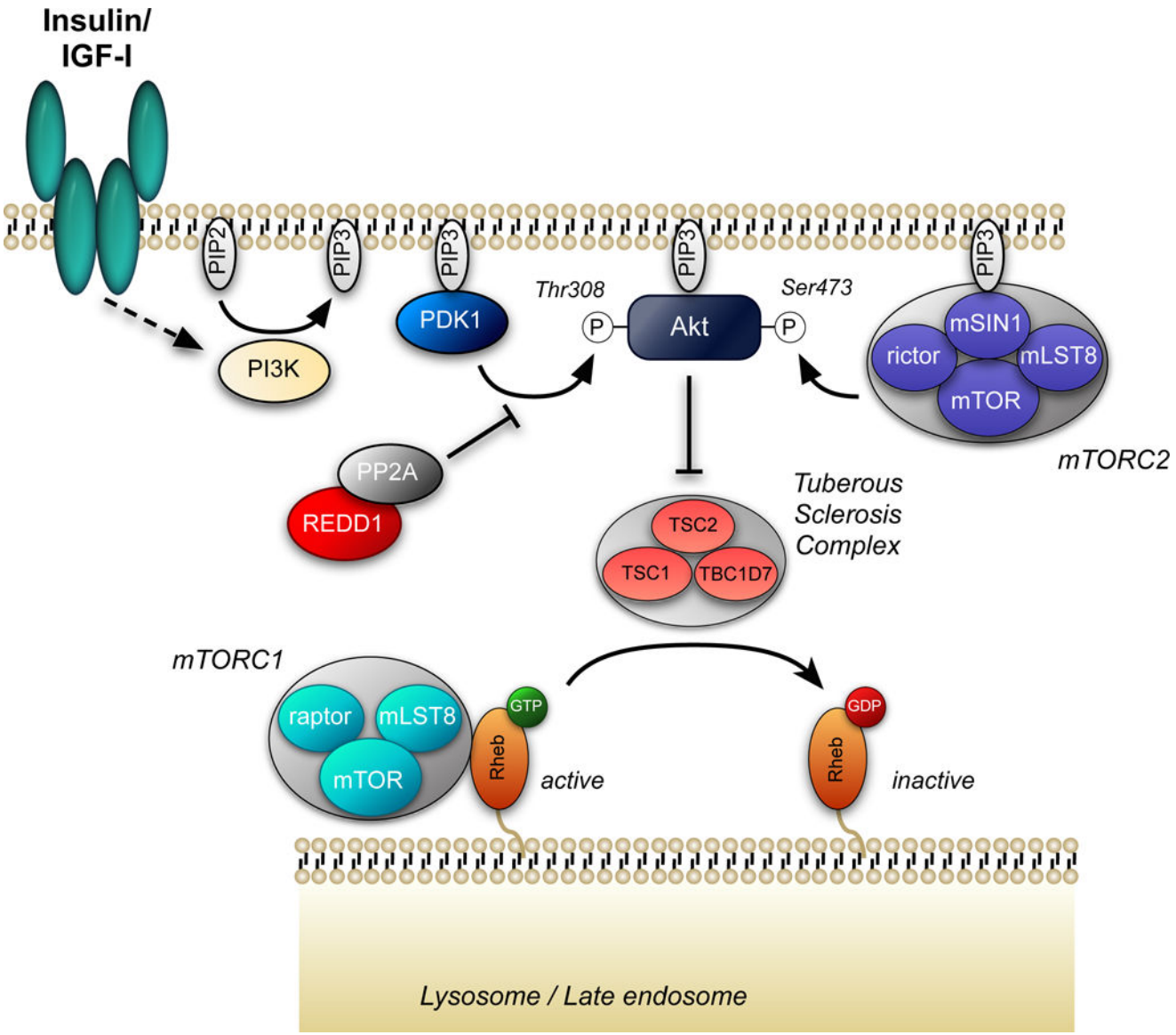

Figure 2.

Insulin/IGF-I signaling to mTORC1. Insulin and IGF-I both activate phosphatidylinositol 3-kinase (PI3K) leading to production of phosphatidylinositol-3,4,5-trisphosphate (PIP3) on the cytoplasmic face of the plasma membrane. PDK1, Akt, and mSIN1 have pleckstrin homology domains that interact with PIP3 resulting in their localization at the plasma membrane (67, 80). In addition, binding of mSIN1 to PIP3 results in activation of mTORC2 (67). Phosphorylation of Akt on Thr308 is sufficient for phosphorylation, and thus inactivation of TSC2 (23, 37). Phosphorylation of TSC2 by Akt leads to dissociation of the TSC complex from the late endosomal/lysosomal membrane, allowing Rheb to accumulate in the active GTP-bound form (6). Whether or not phosphorylation of TSC2 by either ERK- or p90RSK promotes TSC dissociation from the LEL membrane is unknown.

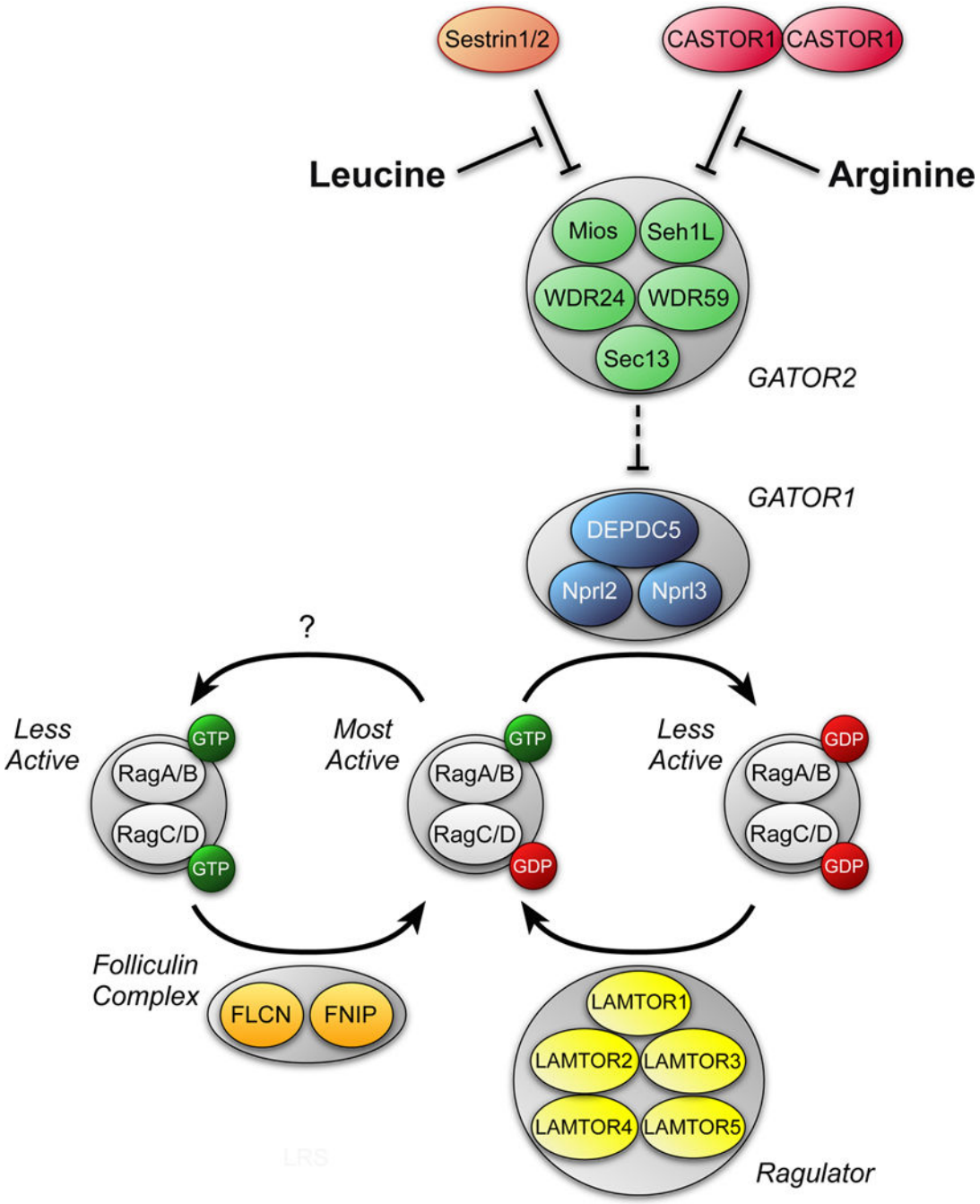

Figure 3.

Regulation of mTORC1 by amino acids. Leucine and arginine activate mTORC1 in part by modulating the GTP loading status of Rags A and B. Leucine binds to Sestrins 1 and 2 and promotes their dissociation from GATOR2 (120), which consists of the proteins meiosis regulator for oocyte development (Mios), WD repeat-domain-containing proteins 24 and 59 (WDR24 and WDR59, respectively, Sec13-like protein (Sec13), and Sec13. Similarly, arginine binds to a protein referred to as cellular arginine sensor for mTORC1 (CASTOR) 1 and promotes its dissociation from GATOR2 (8). Binding of either Sestrin1/2 or CASTOR1

to GATOR2 represses the inhibitory effect of GATOR1 (consisting of DEP domain containing 5 (DEPDC5) and nitrogen permease regulator-like 2 and 3 (Nprl2 and Nprl3)) GAP activity toward Rags A and B. Ragulator acts to oppose the GAP activity of GATOR1 (comprised of LEL adaptor and MAPK and mTORC1 activators (LAMTOR) 1–5), and instead acts to promote exchange of GDP for GTP on Rags A and B. However, the mechanism through which amino acids act to regulate Ragulator GEF activity is unknown. The folliculin complex, composed of folliculin (FLCN) and the folliculin-interacting protein (FNIP), has have been reported to act as GAPs for Rags C and/or D. Neither the mechanism through which folliculin is regulated by amino acids nor its amino acid selectivity are known. Note that the RagA/B-GDP•RagC/D-GTP complex is not shown for simplicity, but would be expected to be less active than the RagA/G-GTP•RagC/D-GDP complex in activating mTORC1.

Table 1.

Acute and chronic alcohol-induced changes in selected proteins regulating skeletal and cardiac muscle protein metabolism

	Skeletal muscle		Cardiac muscle	
	Acute	**Chronic**	**Acute**	**Chronic**
Tissue weight/protein content	—	↓	—	✣
Protein synthesis	↓	↓	↓	↓
Translation efficiency	↓	↓	↓	↓
AKT phosphorylation (T308)	⤭	⤭	—	—
REDD1 total	—	—	—	↑
AMPK phosphorylation (T172)	—	—	↑	↑
4E-BP1 phosphorylation (T47/36)	↓	↓	↓	↓
eIF4G-eIF4E association	↓	↓	↓	↓
4EBP1-eIF4E association	↑	↑	↑	↑
eIF4E phosphorylation (S209)		—	—	ND
S6K1 phosphorylation (T389)	⤭	⤭	⤭	↓
S6 phosphorylation (S240/244)	↓	↓	↓	ND
eIF2B activity	ND	↓	ND	—
eIF2Bε	ND	—	ND	—
eIF2α phosphorylation (T51)	—	—		
eEF1A	—	↓	—	↓
eEF2	—	—	—	↓
eEF2 phosphorylation (T56)	↓	—	—	—
Proteasome activity	—	—	ND	—
Atrogin-1/MuRF1	↑	↑	—	—
LC3B-II/I	—	⤭	↑	↑

Directional changes in pathways and regulators of protein homeostasis in skeletal and cardiac muscle in response to acute alcohol intoxication (hours) and after chronic consumption (≥ 6 weeks) of an alcohol-containing diet. Original citations are contained in the text of the manuscript. Symbols: ↑, increased compared to time-matched control value; ↓ decreased compared to time-matched control value; —, no change compared to time-matched control value; ⤭, reported changes are equivocal and not definitive.