# *Protein and Amino Acid Synthesis*

This lecture will cover mechanisms and signals of both protein synthesis and amino acid biosynthesis, for the non-essential amino acids. Protein building is important for growth as well as tissue repair. This lecture will also cover in more detail why some amino acids are essential or conditionally essential in our diet.

## *Contents*

## *Learning Objectives*

- Understand the mechanistic differences that explain the difference between dispensable and indispensable amino acids.
- Evaluate the roles of insulin, growth hormone, testosterone and cortisol on protein synthesis and degradation.
- Describe the central roles of glutamate and glutamine as a pool of nitrogen.
- Describe the relationships between the glycolytic and TCA cycle intermediates and amino acid biosynthesis.
- Explain why some amino acids are dispensable only if precursors are available.
- Understand how amino acid biosynthetic rates are controlled by utilization and by negative feedback.
- Understand the role that the indispensable amino acids play in controlling protein synthesis.

## *Protein Synthesis is a Tigthly Regulated Process*

As we will discuss throughout this section protein synthesis involves a complex interplay of detecting the levels of the amino acids[1], integrating a diverse array of hormonal signals and co-ordinating growth with energy demand.

[1] especially the essential amino acids

### *The Rate of Protein Synthesis Depends on the Levels of Available Amino Acids*

### *Several Endocrine Signals Regulate Protein Biosynthesis*

Amino acid levels, particularly essential amino acid levels, are sensed via two systems. One is a slow-acting transcriptional system controlled by GCN2[2]. Short-term regulation is accomplished by the protein kinase mTORC1[3].

[2] This stands for the unhelpful name General Control Non-Derepressable 2 protein.

[3] Mechanistic Target of Rapamycin, again, sorry these names are not exactly easy to remember.

GCN2 REGULATES CHRONIC PROTEIN AND AMINO ACID HOMEOSTASIS.

FGF21[4] IS A LIVER-DERIVED HORMONE THAT RISES IN RESPONSE TO PROTEIN RESTRICTION. Very recent studies have shown that protein restriction results in the production of FGF21, and this has emerged as a signal for restoring amino acid homeostasis [Laeger

[4] Fibroblast Growth Factor 21

et al., 2014]. FGF21 production in response to protein restriction is mediated by GCN2. The mechanisms by which FGF21 might restore protein homeostasis are currently unknown but one hypothesis is that it drives increased appetite[5], as the only way to increase the amount of essential amino acids is to consume them [Solon-Biet et al., 2016].

5 Interestingly this happens in concert with increased energy expenditure, so may be an energy balance neutral adaptation.

Several hormonal signaling systems converge on mTORC1. Several protein sensing mechanisms integrate with mTORC1. Growth Hormone/IGF1[6], insulin and testosterone all activate mTORC1 in protein synthetic tissues such as muscle. Catabolic signals such as Cortisol also funciton in part by reducing mTORC1 activity. In addition to hormonal inputs, mTORC1 can sense the levels of three key amino acids (Leucine, Lysine and Arginine) and energy levels. When these amino acids, energy levels, or the anabolic hormone signaling pathways are elevated, mTORC1 is active. mTORC1 in turn then promotes protein synthesis at several levels, including promoting mRNA translation, ribosome biogenesis and suppressing protein breakdown (both autophagy and proteolysis). More details about mTORC1 action can be found in a recent review by Saxton and Sabatini [2017].

6 Insulin-like Growth Factor

## *Protein Synthesis is Energetically Expensive*

Protein synthesis is the sequential conjugation of amino acids in a series defined by a messenger RNA molecule. Each addition of an amino acid to an elongating chain requires *four ATP molecules*. These are broken down as follows:

1. First a specific tRNA[7] must have a free amino acid added to it. This costs 2 ATP equivalents.
2. Binding of the charged tRNA to the ribosome costs 1 ATP equivalent.
3. The elongation step requires another ATP equivalent.

7 Transfer RNA, which is distinct from a mRNA molecule.

Proteins vary widely in their length, but for one example, Actin a very common protein in humans, has 374 amino acids, which is relatively short in length. This means that for to make a molecule of Actin the ATP cost is:

$$374x4 = 1492 \tag{1}$$

That means that to generate a single Actin molecule, you would need 46 glucose molecules to undergo aerobic glycolysis through the TCA/ETC or 748 glucose molecules to go through anaerobic glycolysis[8]. Thats not even accounting for the energy costs needed if

8 Check the math yourself

any of the essential amino acids need to be made or transported into the cell. This is one major reason why protein digestion has a very high level of diet-induced thermogenesis, and why during growth, energy demands are very high. The flip side of this, is that protein breakdown (which we will discuss next lecture) must be only done under careful control.

## *Synthesis of Non-Essential Amino Acids*

Amino acids contain both a carbon skeleton and at least one amino group. For the non-essential amino acids, five can be generated under most normal conditions[9]. The other non-essential amino acids require at least one precursor[10]. These relationships are summarized in Table 1.

[9] Mnemonic is ADNES using their single letter abbreviations, meaning Alanine, Aspartate, Asparagine, Glutamate, Serine.

[10] Arginine and Proline require Glutamate; Cysteine and Glycine require Serine, Glutamine requires Glutamate, and as we discussed for PKU, Tyrosine requires Phenylalanine

### *Humans Have Lost the Ability to Synthesize Several Amino Acids.*

Some of the more complex amino acid biosynthetic pathways have been lost during human evolution. It is most likely that these amino acids were easier for us to obtain from the diet, and were too evolutionarily costly to continue synthesis[11]. There are some remnants of this process where we can generate an amino acid, but not particularly efficiently. For example, Arginine is synthesized from Glutamate in a eight step pathway. This is why Arginine is nutritionally essential during growth and development, because it is so difficult to synthesize.

[11] Plants, on the other hand are not very effective hunter-gatherers and therefore need to make all of their amino acids.

NON-ESSENTIAL AMINO ACIDS ARE DERIVED FROM GLYCOLYTIC AND TCA CYCLE INTERMEDIATES. As shown in Table 1, Serine, Cysteine and Glycine are all derived from the glycolytic intermediate 3-Phosphoglycerate. Alanine, as we have previously discussed is generated from Pyruvate. Aspartate and Asparagine are eventually generated from Oxaloacetate. Since all amino acids require a nitrogen source, Glutamate and Glutamine are particularly important, not just for Arginine and Proline, but also as a nitrogen source for the remaing amino acids[12].

[12] Except Phenylalanine, which is a special case

| AA | Nitrogen Source | Carbon Skeleton | Conditional |
|---|---|---|---|
| Ser | Glutamate | 3-Phosphoglycerate | Cys, Gly |
| Ala | Glutamate | Pyruvate | |
| Asp | Glutamate | Oxaloacetate | Asn |
| Gln | Ammonia | Glutamate | Glu |
| Glu | Glutamine | | Arg, Pro |
| Tyr | Phenylalanine | | |

Table 1: Summary of biosynthetic pathways of essential amino acids. Amino acids are generally made from a carbon skeleton and a nitrogen source. Conditional indicates that these amino acids are generated by further metabolism of the initial amino acid.

## *The Nitrogen Pool is Key for Amino Acid Synthesis*

Glutamate is a part of several *transaminase* reactions[13]. These are near-equillibrium reactions where an amino group is transfered fom glutamate to another amino acid, or vice versa. Some examples are below:

[13] Transaminases require the cofactor pyridoxal phosphate, derived from Vitamin $B_6$

$$\alpha KG + Ala \rightleftharpoons Glu + Pyr \tag{2}$$

$$\alpha KG + Asp \rightleftharpoons Glu + OAA \tag{3}$$

$$\alpha KG + Val \rightleftharpoons Glu + \alpha Ketoisovalerate \tag{4}$$

Since these are easily reversible reactions, the directionality depends on the concentrations of products and substrates on each side. For example in reaction 2, if there is high levels of Glutamate and Pyruvate, then Alanine and $\alpha$-ketoglutarate will be produced. Because Glutamate and $\alpha$-ketoglutarate are present on both sides of most transaminase reactions, this is one way in which TCA cycle intermediates ($\alpha$-ketoglutarate) and amino acids (*i.e.* Glutamate) are kept in balance.

GLUTAMATE AND GLUTAMINE ARE NON-TOXIC CARRIERS OF NITROGEN. During amino acid breakdown[14], several amino acids can be converted to glutamate via transaminases, then glutamate releases its amino group via the functions of Glutamate Dehydrogenase:

[14] This will be covered in the next lecture

$$Glu + H_2O + NAD^+ \rightarrow \alpha KG + NH_3 + NADH + H^+ \tag{5}$$

In humans this is irreversible, as we cannot re-synthesize glutamate from ammonia. The ammonia released from this reaction is released into the Urea cycle[15].

[15] Also covered in the next lecture

GLUTAMINE IS THE MOST ABUNDANT AMINO ACID IN MOST CELLS. Glutamine is another particularly important amino acid, because it contains two nitrogen atoms, and can be quickly be synthesized to or from Glutamate with the following reactions, catalysed by Glutamine Synthetase:

$$Glu + ATP + NH_3 \rightarrow P_i + Gln \tag{6}$$

and Glutaminase:

$$Gln + H_2O \rightarrow Glu + NH_3 \tag{7}$$

Free glutamine is typically present in muscle cells about 4 fold higher than glutamate, and 8 fold higher than the next highest abundance amino acid (Alanine). This is our mechanism to store nitrogen and make it available for other amino acid biosynthetic reactions[16]. For example, if Aspartate is required, Glutamine is converted by reaction 7 in to Glutamate, which then acts as a nitrogen donor in reaction 3.

[16] Typically the transaminase reactions we described above in Table 1

## *Protein Requirements and Determination*

As we just mentioned, when amino acids are being used, ammonia is generated[17]. This can be measured by urinary nutrogen levels. If dietary nitrogen and urinary nitrogen are equal, then a person is said to be in *Nitrogen Balance*. During periods of protein catabolism, urinary nitrogen is higher than intake. During periods of protein synthesis, urinary nitrogen is lower. *This is because the dietary nitrogen containing amino acids are not being oxidized.*. This is one way by which dietary requirements are determined, since the lack of any essential amino acid causes proteins to be degraded to release the essential amino acids. Now there will be an excess of the non-limiting amino acid, which will then be oxidized and released as urea. Several other methods for determining protein requirements exist, briefly these include:

[17] See reactions 7 and 5 and recall that most amino acids are going to be catabolized via transaminases into Glutamate, which then feeds into reaction 5.

*Nitrogen Balance.* In this method nitrogen intake is compared to nitrogen release, protein synthesis being associated with positive nitrogen balance.

*DIrect Amino Acid Oxidation.* In this method, stable-isotope labelled Phenylalanine, Lysine, Leucine, Isoleucine of Valine are provided. These indispensible amino acids when catabolized release the label to the body's bicarbonate pool which is eventually released as $^{13}CO_2$. The oxidation and release of this amino acid will increase if that amino acid is in excess.

*Indicator Amino Acid Oxidation.* In this method a stable-isotope labelled amino acid is added. If in protein deficiency, that amino acid will be oxidized. As protein intake increases, oxidation will decrease. Therefore the detection of oxidized label (typically $^{13}CO_2$) is inversely proportional to protein levels. More details in this method can be found in Elango et al. [2008].

## *References*

Rajavel Elango, Ronald O Ball, and Paul B Pencharz. Indicator amino acid oxidation: concept and application. *The Journal of Nutri-*

*tion*, 138(2):243–246, 2008. ISSN 1541-6100.

Thomas Laeger, Tara M. Henagan, Diana C. Albarado, Leanne M. Redman, George A. Bray, Robert C. Noland, Heike Münzberg, Susan M. Hutson, Thomas W. Gettys, Michael W. Schwartz, and Christopher D. Morrison. FGF21 is an endocrine signal of protein restriction. *Journal of Clinical Investigation*, 124(9):3913–3922, sep 2014. ISSN 0021-9738. DOI: 10.1172/JCI74915. URL http://www.jci.org/articles/view/74915.

Robert A. Saxton and David M. Sabatini. mTOR Signaling in Growth, Metabolism, and Disease. *Cell*, 168(6):960–976, mar 2017. ISSN 00928674. DOI: 10.1016/j.cell.2017.02.004. URL http://linkinghub.elsevier.com/retrieve/pii/S0092867417301824.

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