Hot Topic Review

Intracellular compartmentalization of skeletal muscle glycogen metabolism and insulin signalling

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The interest in skeletal muscle metabolism and insulin signalling has increased exponentially in recent years as a consequence of their role in the development of type 2 diabetes mellitus. Despite this, the exact mechanisms involved in the regulation of skeletal muscle glycogen metabolism and insulin signalling transduction remain elusive. We believe that one of the reasons is that the role of intracellular compartmentalization as a regulator of metabolic pathways and signalling transduction has been rather ignored. This paper briefly reviews the literature to discuss the role of intracellular compartmentalization in the regulation of skeletal muscle glycogen metabolism and insulin signalling. As a result, a hypothetical regulatory mechanism is proposed by which cells could direct glycogen resynthesis towards different pools of glycogen particles depending on the metabolic needs. Furthermore, we discuss the role of skeletal muscle transverse tubules as potential modulators of tissue insulin responsiveness.

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The importance of the structural organization of biological systems

The human body is an extremely complex biological system. Such complexity is only possible thanks to specialization. Without differentiation, structural and/or functional specialization of cells, multicellular organisms would not exist. Different tissues are specialized to perform different physiological functions and, in a tissue, different cell lines have specialized to serve a specific function. Moreover, cells are highly compartmentalized systems, where different organelles and cellular compartments have specialized to serve different cellular functions. Accordingly, our body is a hierarchical integration of complex biological systems based on functional specialization, just as human society is spatially organized in our planet. If I am allowed to put forward such exaggerated but pedagogic metaphor (Fig. 1), researchers for many decades have been trying to understand a system at least as complex as our society by taking a biopsy of a certain tissue, a piece of a country, and destroying its morphology and spatial organization by homogenizing it. As we all can understand, we are missing crucial information without which we will never get a full understanding of the system. Cellular processes can only be understood as several pathways interacting with each other in a dynamic multicompartmentalized system.

In the present Hot Topic Review, we would like to collect and discuss some of the accumulated evidence in the literature that demonstrates a key role of intracellular compartmentalization in skeletal muscle glycogen metabolism and insulin signalling.

Intracellular organization of skeletal muscle fibres

Muscle fibres are long cylindrical multinucleate cells organized with intracellular subdomains. It is believed that each myonucleus synthesizes proteins for a local domain in the vicinity; however, it is still not clear (Gundersen & Bruusgaard, 2008). Skeletal muscle fibres have a highly compartmentalized and specially packed cytoplasm. They contain hundreds to thousands of myofibrils, which consist of compactly packed bundles of contractile myofilaments. Interconnecting tubules of sarcoplasmic reticulum and a complex tubular system of sarcolemma invaginations, the transverse tubules (T-tubules), surround each myofibril. The reduced space

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left between these cellular compartments is packed with mitochondria, vesicles, lysosomes, lipid droplets and glycogen particles, among other cellular structures. As early as 1967, Paul Srere (1967) postulated the idea of a crowded intracellular environment. In such crowded cytoplasm, enzymes of metabolic pathways do not meet by chance; they meet and interact with each other in a regulated and organized way in order to efficiently co-ordinate their action. Therefore, to fully understand any cellular event and its regulation, we need to have information on its intracellular spatial topology. Measurements of intracellular protein content and enzymatic activity in a tissue homogenate are often not directly related to changes in protein action. The *in vivo* action of an abundant and active enzyme can be negligible if the enzyme is found spatially isolated from its substrate.

Compartmentalization of skeletal muscle glycogen metabolism

Glucose is stored inside muscle fibres as glycogen particles. Glycogen particles are not homogeneously distributed throughout muscle fibres, but are mainly found in three topographical regions: subsarcolemmal, intermyofibrillar and intramyofibrillar (Friden *et al.* 1989; Marchand *et al.* 2002). Several studies have reported preferential use of specific glycogen pools to fulfil the energetic requirements for different cellular functions (Friden *et al.* 1989; Lees *et al.* 2001; Marchand *et al.* 2007; Nielsen *et al* 2009). This can only be possible if glycogen metabolism is regulated locally inside the cell. Little is known about the qualitative differences between glycogen particles in the different subcellular pools, and even less is known about the local regulatory mechanisms of glycogenesis/glycogenolysis. What determines where and when a glycogen particle needs to be synthesized or which pool of glycogen particles will be preferentially used for individual energetic demands remains elusive.

Glycogenin (GN) is the proteic core of glycogen particles, and catalyses the addition of the first 7–11 glucosyl units of the particle (Roach & Skurat, 1997), which serve as substrate for glycogen synthase (GS) and branching enzyme (BE) to synthesize glycogen particles in a co-ordinated manner. Overexpression of GN in cultured cells leads to an increased number of glycogen particles, but of smaller size (Skurat *et al* 1996, 1997), resulting in no net change in muscle levels. A very detailed analysis of human skeletal muscle electron micrographs (Marchand *et al* 2002) revealed that most glycogen particles in resting conditions are filled only up to around 20% of their maximal storage capacity. Overall, it seems as if GN expression determines the number of glycogen particles but does not regulate intracellular glycogen content. What determines the basal glycogen levels of a muscle fibre remains a mystery.

A large number of proteins have been reported to associate directly or indirectly with glycogen particles; among them are glycogen phosphorylase (Ph), debranching enzyme (DE), glycogen phosphorylase kinase (PhK), laforin, malin, protein phosphatise 1 glycogen targeting regulatory subunits (PP1G) and AMP-activated protein kinase (AMPK; Fig. 2; for review see Roach,

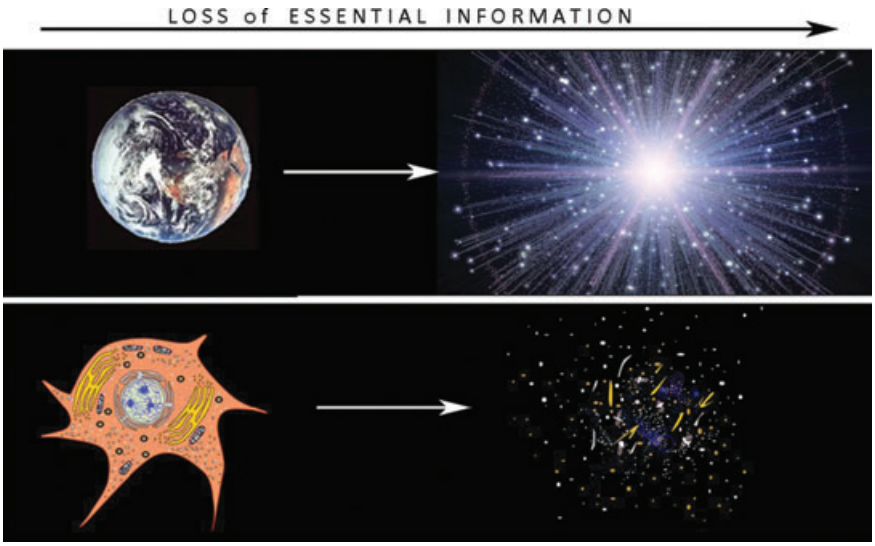

Figure 1. Knowledge of the spatial compartmentalization of a system is essential for understanding its organization and functionality
This figure illustrates a metaphor, where the scientific strategy used for many decades to unravel cellular metabolic pathways by studying *in vitro* tissue homogenates is compared to attempting to understand human society by destroying the globe and studying the pieces.

2002; Graham, 2009; Graham *et al* 2010). Dynamic binding and dissociation of these proteins to certain glycogen particles or multiproteic complexes associated to them regulate the net balance between glycogenesis and glycogenolysis rates locally. Already in the 1960s, glycogen particles were compared to a cellular organelle (Himes & Pollister, 1962; Scott & Still, 1968). Later on, Rybicka (1996) reviewed the field and concluded that glycogen is associated with the enzymes involved in its metabolism and that the glycogen–protein complex forms morphologically distinct cell organelles called glycosomes. Using chronic low-frequency stimulation (CLFS) of rabbit tibialis anterior muscle, a novel intracellular compartment needed in order to start glycogen resynthesis after severe glycogen depletion was reported (Prats *et al* 2005). This compartment was the product of actin cytoskeleton rearrangement, forming some spherical structures with a diameter around 0.4 μm. After 30 min of contraction-induced glycogen depletion, GS was highly activated, while Ph was strongly inactivated; however, net glycogen resynthesis was first detectable only after 3 h of stimulation (Prats *et al* 2002). At this same time point, GS and Ph were associated with the spherical compartments. Such a compartment was not present in rested contralateral muscle. Thus, these results add a new dimension to the complexity of intracellular compartmentalization: the dynamic formation, dissolution and rearrangement of cellular compartments. We believe that when glycogen levels reach a critical minimum, a cellular safety mechanism activates actin cytoskeleton rearrangement to form the reported compartment. Such a compartment would gather glycogen enzymes, in order to start glycogen

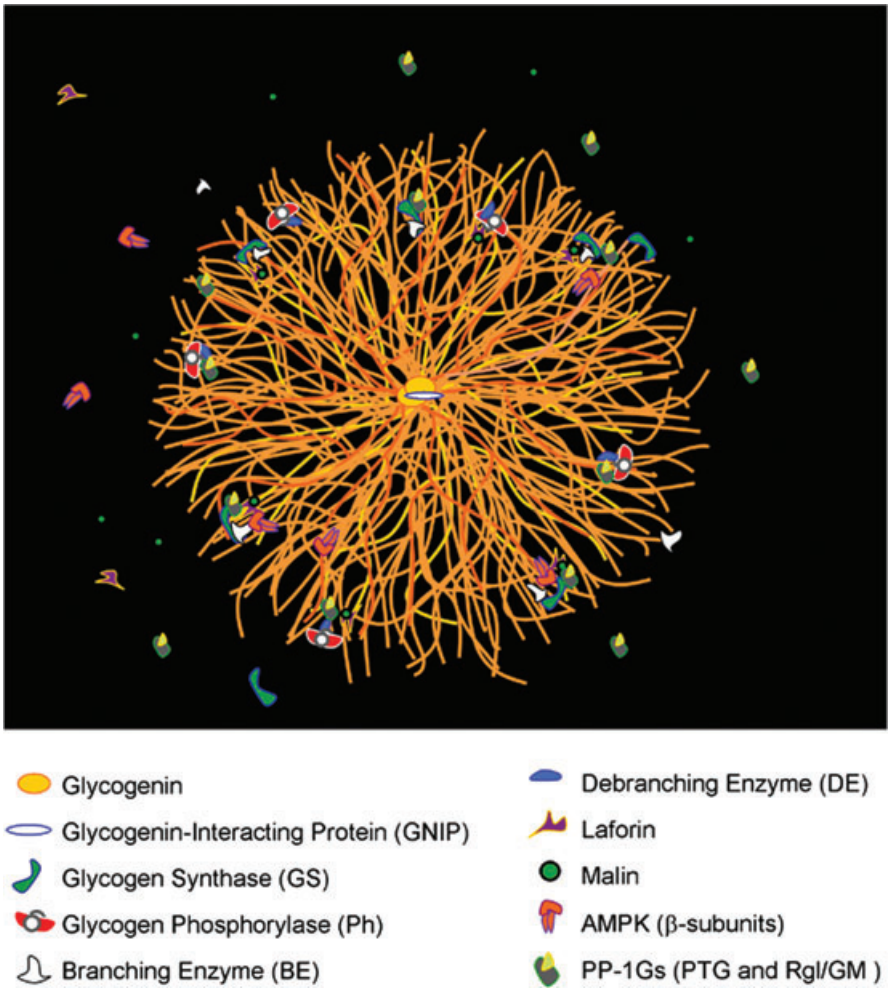

Figure 2. The glycosome
Intracellular carbohydrate energy supplies in mammalian cells are mainly stored as glycogen particles. The cellular mechanisms that determine when, where and how a glycogen particle is to be synthesized *de novo* or used remain unclear. An increasing number of proteins that directly or indirectly bind to glycogen particles have been identified; however, further studies are needed to integrate biochemical, proteomic and morphological data to unravel the role of each of these proteins and how they co-ordinate to regulate glycogen metabolism.

resynthesis efficiently. The same cellular compartment has been observed in human vastus lateralis muscle, after exhausting exercise-induced glycogen depletion (Prats *et al* 2009). Further studies are needed to fully understand the exact metabolic role of such a compartment and how its formation and dissolution are regulated.

Glycogen synthase is inactivated by a complex, hierarchal multisite phosphorylation mechanism involving several protein kinases (Roach, 1991). Dephosphorylation of sites 2, 2a, 3a and 3b increases GS activity, while the exact role of sites 4, 5, 1a and 1b remains unclear (Hardy & Roach, 1993). In both rabbit and human skeletal muscle, GS presents a phosphorylation-dependent intracellular distribution (Prats *et al* 2005, 2009). When phosphorylated on site 1b, GS associates with intramyofibrillar glycogen particles, which are preferentially used during muscle contraction (Wanson & Drochmans, 1968; Meyer *et al* 1970). When phosphorylated at sites 2 + 2a, GS is preferentially associated with subsarcolemmal and intermyofibrillar glycogen particles. By phosphorylating GS on site 1b or 2 + 2a, glycogen synthesis could be directed towards different intramuscular glycogen pools (Fig. 3). This hypothesis is supported by our results showing that during exhausting exercise, increases in plasma adrenaline directly correlate with increases in GS phosphorylation at site 1b, which is phosphorylated by protein kinase A (PKA). During exhausting exercise, intramyofibrillar glycogen particles are preferentially used. Consequently, the cell could target GS action towards the depleted glycogen pool through adrenaline-induced activation of PKA, leading the GS phosphorylation at site 1b. Site 2 can be phosphorylated by PKA and by AMPK, becoming a recognition site for creatine kinase 1 to phosphoryate site 2a. It has recently been shown that AMPK is differentially inhibited by glycogen particles with different branching degrees, leading the authors to hypothesize that AMPK could act as a sensor of cellular glycogen levels (McBride & Hardie, 2009). We believe that AMPK could act as a sensor of intermyofibrillar glycogen particles, phosphorylating GS at site 2 when these are low. Phosphorylation of GS at sites 2 + 2a would inactivate the enzyme and target it to intermyofibrillar glycogen particles, where insulin-induced activation of GS and increase in glucose uptake would lead to local glycogen resynthesis in the depleted glycogen pool. Supporting our hypothesis, chronic activation of skeletal muscle AMPK has been shown to lead to intermyofibrillar glycogen accumulation (Barre *et al* 2007).

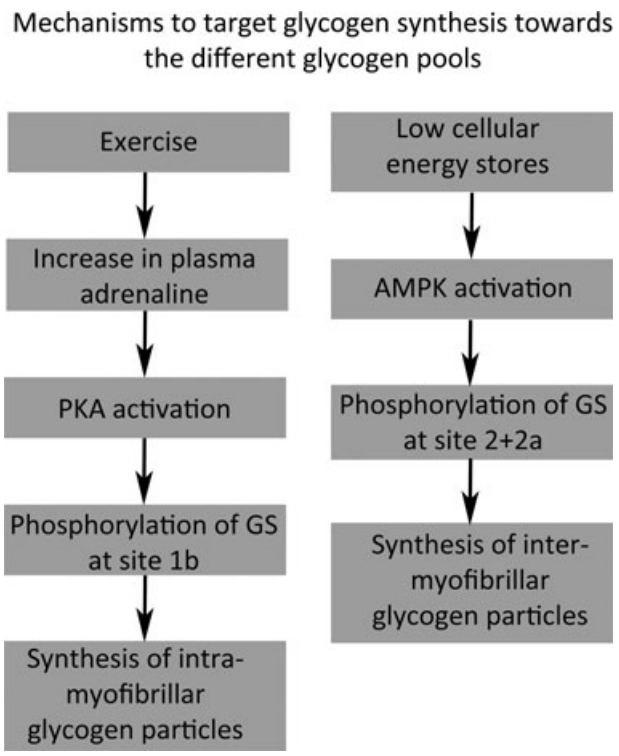

Figure 3. Hypothetical model for a cellular mechanism directing glycogen synthesis towards different glycogen pools in the cell depending on the needs
GS, glycogen synthase; PKA, protein kinase A; AMPK, AMP-activated protein kinase.

Adding importance to GS intracellular distribution, impaired regulation of GS phosphorylation at sites 2 + 2a in skeletal muscle from obese subjects, and hyperphosphorylation of at least GS site 1b in diabetic muscle have been reported (Hojlund *et al* 2009). The question arises of whether increased phosphorylation of site 1b is linked to overaccumulation of glycogen particles in the intramyofibrillar region in diabetic muscle and, if so, what effect this could have on insulin signalling. Further studies are needed to fully understand how the regulatory mechanisms of GS intrinsic activity and intracellular location are integrated to regulate cellular glycogen metabolism.

Co-ordinated activity of Ph and DE leads to glycogenolysis. Entman *et al* (1980) presented evidence for the existence of a sarcoplasmic reticulum glycogenolytic complex, a highly specific, functionally defined compartment for phosphorylase regulation. In such a complex, phosphorylase activation would be controlled by sarcoplasmic reticulum (SR) calcium flux, explaining the well-documented rapid burst of glycogenolysis with the onset of muscle contraction. Later on, Cuenda *et al* (1994) reported that 95% of the Ph associated with SR is in its inactive form and that other enzymes related to carbohydrate metabolism are associated with SR membranes (e.g. creatine kinase, myokinase, PhK, glycosidase, AMP-deaminase and phosphoglucomutase). Our group has evidence of intracellular redistribution of the active form of Ph when muscle glycogen levels are critically low, indicating a hypothetical safety mechanism

to stop further glycogenolysis. Research efforts focused on the metabolic regulation of Ph should expand to unravel the cellular mechanisms involved in regulation of glycogenolysis during exercise and during resting conditions.

Compartmentalization of insulin signalling transduction

Insulin and muscle contraction are key regulators of glycogen metabolism. Apart from its effect on glycogen metabolism as an individual stimulus, muscle contraction is also known to have a positive effect on muscle insulin responsiveness (for review see Wojtaszewski *et al* 2003). The exact mechanism behind such interaction remains elusive; however, muscle transverse tubules could play a key role.

Skeletal muscle fibres have a complex membranous system of T-tubules, which are thought to be interconnected and open tubules. One could compare a muscle fibre to a long cylindrical sponge. The T-tubules play a key role in insulin signal transduction (Lauritzen *et al* 2006; Lauritzen *et al* 2008). Insulin receptor is present not only at the muscle fibre surface, or sarcolemma, but also at the membrane of the T-tubules, which serves as a propagation vehicle for insulin signalling to reach the inside of the muscle fibres. Furthermore, phosphoinositide 3-kinase activity is mainly found in the T-tubule membranes and not in the sarcolemma, conducting insulin-induced Phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) production along the T-tubules into the muscle fibres (Lauritzen *et al* 2006). The T-tubule network is highly irregular, and the diameter of the T-tubules is ∼18 nm (Ploug *et al* 1998). It has been shown that the effective diffusion coefficient for insulin into the T-tubules is ∼1000 times slower than in free solution (Lauritzen *et al* 2006). It takes 10 min for insulin to diffuse into the inner regions of mouse muscle fibres, which has been almost perfectly matched with the time course of PIP_3 production towards the inner areas of muscle fibres and the time course of local glucose transporter 4 (GLUT4) translocation to T-tubules (Lauritzen *et al* 2006). In order to understand the limiting factors for insulin diffusion into muscle T-tubules, Shorten *et al* (2007) constructed a mathematical model, which takes into account insulin diffusion, insulin binding to insulin receptors, T-tubule network tortuosity, interstitial fluid viscosity, hydrodynamic wall effects and insulin receptor internalization and recycling. According to this mathematical model, depending on fibre type there will be a 2–15 min delay in the arrival of insulin into the inner lumen of T-tubules, confirming Lauritzen's experimental data. Furthermore, muscle denervation and a high-fat diet have been shown to reduce PIP_3 production in the T-tubules and local GLUT4 translocation to the T-tubules (Lauritzen *et al* 2008). Overall, in order to have a healthy insulin-induced glucose clearance, glucose and insulin need to reach the surface of muscle fibres and diffuse into the lumen of the T-tubules, where insulin receptors are activated, inducing local translocation of GLUT4. Considering that the membrane surface of T-tubules is two to three times the surface of the plasma membrane (Knudson & Campbell, 1989), it seems clear that reduced accessibility into the lumen of the T-tubules would result in reduced insulin responsiveness of the cell and decreased glucose uptake. This model can also explain why a single exercise bout can increase insulin sensitivity. Muscle contractions could act as a mechanical pump, increasing the flow of interstitial fluid through the T-tubule network and, consequently, increasing glucose and insulin diffusion into their lumen. This idea is supported by Papadopoulos *et al* (2000), who showed that muscle contraction increases protein diffusion speed into the T-tubules. Moreover, the increase in interstitial fluid flow through the T-tubules of contracting skeletal muscle fibres during exercise could also in part explain the increase in muscle blood flow during muscle contraction reported as long ago as 1917 (Krogh & Lindhard, 1917). Further studies are needed to fully understand how insulin signal transduction through the T-tubules is regulated at rest and during exercise and to investigate the potential role of T-tubules in the transduction of other cell signalling cascades.

Concluding remark

Future studies need to recognize the importance of intracellular compartmentalization and the dynamics of their structural organization in order to reach full understanding of cellular processes, such as glycogen metabolism and insulin signalling.

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