Putting Rac1 on the Path to Glucose Uptake

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In spite of numerous and key advances in our understanding of insulin signaling, the molecular basis for impaired insulin-stimulated glucose uptake underlying insulin resistance remains unclear. Because skeletal muscle accounts for the majority of glucose utilization in the postprandial insulin-regulated state, defects in insulin action in muscle can determine whole-body glucose utilization. In muscle and fat cells, overall similarities exist in the signals emanating from the insulin receptor that mobilize glucose transporter GLUT4 to the membrane. Importantly, defects in the signaling network connecting the activated insulin receptor to GLUT4 vesicles are of particular consequence to insulin resistance in muscle, where, unlike the case of fat cells, GLUT4 expression is not significantly reduced.

The complex structural organization of muscle tissue and its associated cells have limited the molecular scrutiny of glucose uptake regulation. Instead, cell culture systems have been instrumental in revealing intricacies in the signals and traffic machinery mobilizing GLUT4 to the membrane. This signaling relay is initiated by insulin receptor substrate (IRS)-1 (and not IRS-2)–associated activation of class I phosphatidylinositol-3-kinase (PI3K). At this point, a signal bifurcation takes place, one arm leading to Akt2 activation, inhibition of its substrate AS160 (a GTPase-activating protein for Rabs) and consequent activation of its target Rab GTPases, which in skeletal muscle cells are Rab8A and Rab13 (1). The other arm leads to Rac1, a Rho-family GTPase (2,3) that enacts a dynamic cycle of cortical actin filament remodeling through the Arp2/3 complex and cofilin (4). Joint activation of these distinct signaling arms is required for GLUT4 mobilization to the cell surface (Fig. 1), since inhibiting Akt or expressing constitutively active AS160 did not alter insulin-induced actin remodeling (5,6) and, conversely, silencing Rac1 or preventing actin remodeling did not alter Akt activation (7); yet each of those manipulations obliterated GLUT4 translocation.

Lessons learned from cell cultures must, however, be put to the test by mature muscle and whole-body analysis. Although the participation of IRS-1, PI3K, Akt2 and to some extent AS160 in insulin-mediated stimulation of glucose uptake have been mechanistically verified in skeletal muscle, proof of the parallel signaling arm involving Rac1 and the nonsarcomeric, actin cytoskeleton, was virtually lacking. In this issue of *Diabetes*, Sylow et al. (8) show that Rac1 is an obligatory element in the stimulation of glucose uptake by insulin in skeletal muscle. Insulin caused Rac1 activation (GTP loading) in mouse muscle ex vivo, and muscles of mice conditionally lacking Rac1 in skeletal muscle, or treated with Rac inhibitors, showed reduced insulin-simulated glucose uptake. Under these circumstances, insulin-stimulated Akt was not affected. These data complement a previous study with another muscle-specific Rac1-knockout mouse model, in which insulin-triggered GLUT4 translocation was reduced (9).

Which Rac1 effectors are promoting glucose uptake? The studies in cell culture have already revealed the activation of three Rac-effector pathways: The aforementioned Arp2/3 (mediated by nucleating factors of the Wave family and leading to actin remodeling) (4,10); the serine/threonine kinase PAK1 (7,11); and the small G protein Ral (12). Arp2/3-dependent actin remodeling and Ral are important for GLUT4 translocation in muscle cells. Do these three effectors jointly affect similar steps, or do they act on defined echelons leading to the stimulation of glucose uptake? Is actin remodeling the convergence point? Indeed, agents that disrupt nonsarcomeric actin dynamics in muscle cells and tissue prevent GLUT4 translocation and reduce the stimulation of glucose uptake in skeletal muscle (8,10,13,14), and GLUT4 interacts with actin filaments via actinin-4 (10).

A key finding of the Sylow et al. study is that muscle-specific Rac1 deletion lowers insulin-induced PAK1 phosphorylation and causes insulin intolerance, and that 7-h of intralipid infusion in healthy volunteers impaired PAK1 phosphorylation. Other studies in mice show that PAK1-knockout mice display insulin resistance (15). The precise mechanism whereby Rac1-GTP loading causes PAK1 activation in response to insulin will require future study. Similarly, it should be investigated whether PAK1 activation is an exclusive readout of Rac1 activity or whether it reflects defects in other upstream signals. In muscle cells, insulin-dependent PAK1 is sensitive to PI3K inhibition (11) and Rac1 ablation (7). In other systems, PAK1 can signal to Rac1, suggesting a possible feed-forward loop. Future work should explore whether, in the context of obesity and diabetes, Rac1 drives the PAK1 defect or vice versa, or whether either defect feeds into the other. If the low PAK1 phosphorylation observed in high-fat diet–fed mice is indeed a surrogate of diminished Rac1 activity, it may relate to the ability of ceramide or oxidative radicals to reduce the insulin-induced Rac1 activation demonstrated in cultured myotubes (5), given that both are considered to be molecular outcomes of saturated fat excess (16,17).

The study of Sylow et al. further hints to far-reaching consequences of Rac1 activation toward metabolism. Muscle-specific Rac1 depletion caused not only insulin intolerance but also glucose intolerance. Is this a consequence of the insulin intolerance? If insulin physiologically activates Rac1 in other tissues in addition to skeletal muscle, will high-fat feeding diminish such activation? What would be the consequence of these extramuscular defects on glucose homeostasis? As a tantalizing hypothesis, Rac1 defects in

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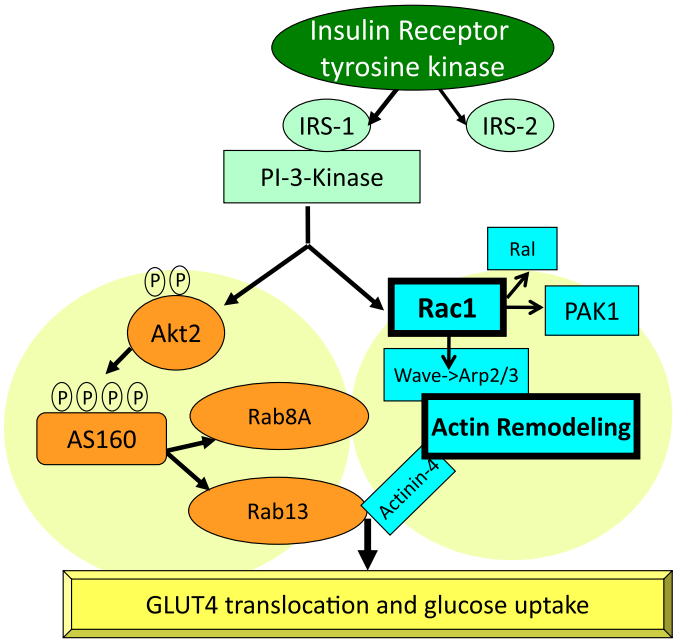

FIG. 1. Rac1 is an obligatory element in insulin signaling in muscle, leading to glucose uptake. In cultured muscle cells, insulin activates Rac1 leading to cortical actin filament remodeling, and this module is required for GLUT4 translocation, in parallel to input by the Akt2 module. Sylow et al. (8) show that Rac1 is similarly required for insulin-stimulated glucose uptake in skeletal muscle and that Rac1 or PAK1 defects are associated with insulin-resistant states in mice and humans.

obesity may lead not only to insulin resistance but also to reduced insulin availability, given the recently demonstrated Rac1 input in glucose-stimulated insulin secretion (18).

Finally, Sylow et al. have found in a related study (19) that the inducible Rac1 knockout mice also display reduced contraction-stimulated glucose uptake into muscle. At first glance this would elude the view that independent signaling pathways govern the stimulation of glucose uptake by insulin and muscle contraction, as insulin resistance does not alter the response to contraction (20). A plausible reconciliation could be if the defect in Rac1 in obesity/diabetes arises from defects in PI3K input toward Rac1 activation, whereas, being PI3K-independent, contraction-induced Rac1 activation would remain intact. This requires experimental verification. As well, it will be exciting to decipher the upstream regulators of Rac1 in each case (whether guanine exchange factors or GTPase-activating proteins). In any case, the combined cellular and animal studies discussed here show that Rac1 activation by insulin can no longer be overlooked.

SUMMARY AND FUTURE OPPORTUNITIES

In summary, the study by Sylow et al. (8) constitutes the required validation of Rac as an obligatory insulin-activated signal contributing to glucose uptake stimulation in mature skeletal muscle and provides compelling evidence for the impact of this process on whole-body glucose homeostasis. Importantly, it also demonstrates the potential relevance of Rac1 to human insulin resistance and dysglycemia. Future studies should unravel whether an insulin resistance–relieving strategy might rely on Rac1 or its downstream effectors to restore GLUT4 translocation and insulin stimulation of glucose utilization, and to what degree might Rac1 stimulation circumvent muscular insulin resistance.

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