Temperature 2:4, 476–482; October/November/December 2015; Published with license by Taylor & Francis Group, LLC

Regulation of brown adipose tissue recruitment, metabolism and thermogenic function by peroxisome proliferator-activated receptor γ

Andressa Bolsoni-Lopes[1], Yves Deshaies[2], and William T Festuccia[1,*]

[1]Department of Physiology and Biophysics; Institute of Biomedical Sciences; University of São Paulo; São Paulo, SP Brazil; [2]Department of Medicine; Faculty of Medicine; and Québec Heart and Lung Institute; Université Laval; Québec, Canada

Keywords: brown adipose tissue, peroxisome proliferator-activated receptor γ, thermogenesis, uncoupling protein 1

Abbreviations: ATGL, adipose triglyceride lipase; cAMP, cyclic adenosine monophosphate; FA, fatty acids; HSL, hormone sensitive lipase; NE, norepinephrine; PERI, perilipin; PKA, protein kinase A; TAG, triacylglycerol; β3, beta 3 adrenergic receptors.

Brown adipose tissue contributes importantly to homeothermy and energy balance in rodents due its ability under demand to produce heat through a process denominated nonshivering thermogenesis. Such thermogenic ability of brown adipocytes relies on the activity of mitochondrial uncoupling protein 1 that, when properly activated, dissipates energy from oxidative metabolism as heat. Brown adipose tissue sympathetic innervation through norepinephrine release not only induces brown adipocyte lipolysis and thermogenesis, but also acts as the major determinant of tissue mass, cellularity and mitochondrial content. Several pieces of evidence gathered over the years indicate that, in addition to tissue sympathetic innervation, the nuclear receptor peroxisome proliferator-activated receptor γ plays an important role in regulating the development, metabolism and thermogenic function of brown adipose tissue. Herein we review the main evidence supporting such key role of peroxisome proliferator-activated receptor γ to brown fat biology and discuss the future directions of this important area of research.

Introduction

Brown adipose tissue, due its ability under demand to dissipate energy from the oxidation of fatty acids and glucose as heat, is an important determinant of rodent body temperature and energy homeostasis.[1] The ability of the brown adipocyte to produce heat depends on the activity of a mitochondrial protein denominated uncoupling protein 1, which upon allosteric activation by fatty acids released from the hydrolysis of intracellular triacylglycerol stores, deviates mitochondrial proton gradient energy from adenosine triphosphate synthesis to heat production (**Fig. 1**). This process termed nonshivering thermogenesis is under complex neurohormonal regulation where the sympathetic nervous system through norepinephrine release by tissue innervation and the thyroid hormone triiodothyronine mainly produced locally by the action of type 2 iodothyronine deiodinase play major roles.[1,2] In addition to triggering thermogenesis, brown adipose tissue sympathetic innervation promotes brown adipocyte proliferation, differentiation, mitochondrial biogenesis and uncoupling protein 1 expression, and is therefore a major determinant of tissue mass and thermogenic capacity.[1,3,4]

Although sympathetic innervation is considered a major regulator of brown adipose tissue recruitment, it has been recognized that there exists a few situations in which brown adipose tissue mass and uncoupling protein 1 content can be increased (recruited) without any major change in brown adipose tissue adrenergic outflow. A good example of such alternative non-adrenergic brown adipose tissue recruitment is the significant increase in brown adipose tissue mass and uncoupling protein 1 content featured by Syrian hamsters exposed to a short photoperiod or fed a high-energy diet, which are not associated with major changes in tissue sympathetic activity.[5,6] Furthermore, it is very unlikely that the brown adipose tissue recruitment displayed by animals preparing for hibernation or by newborn human babies is promoted by brown adipose tissue sympathetic activation, although tissue adrenergic activity was not evaluated in these conditions.[1] Although the mechanisms underlying non-adrenergic brown adipose tissue recruitment are still elusive, recent studies have provided some compelling evidence indicating a likely involvement in this process of the nuclear receptor peroxisome proliferator-activated receptor γ, which is highly expressed in brown and white adipocytes. Indeed,

*Correspondence to: William T Festuccia; Email:william.festuccia@usp.br
Submitted: 12/12/2014; Revised: 01/19/2015; Accepted: 01/21/2015
http://dx.doi.org/10.1080/23328940.2015.1011564

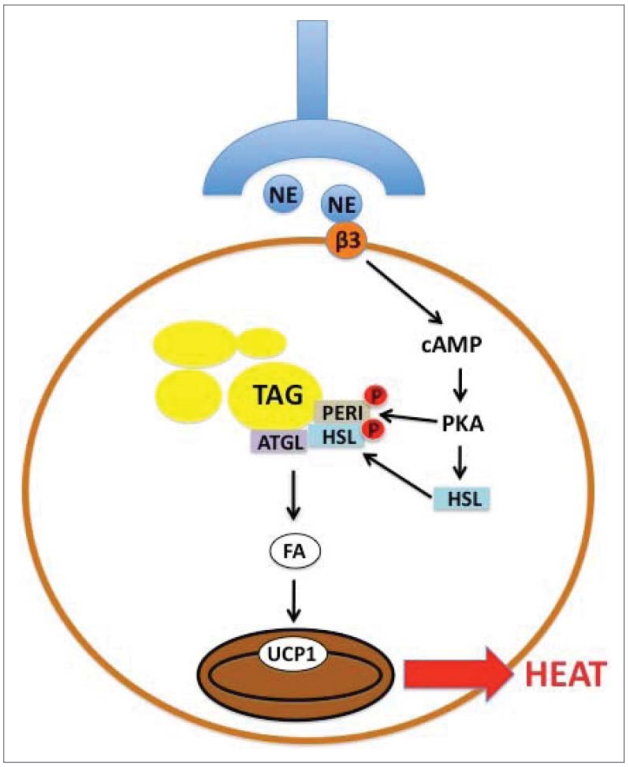

Figure 1. Sympathetic activation of brown adipocyte thermogenesis. Norepinephrine (NE) released by tissue sympathetic innervation interacts with and activates β 3 adrenergic receptors (β3) in brown adipocytes promoting an increase in intracellular levels of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) activation. PKA thus phosphorylates perilipin (PERI) and hormone sensitive lipase (HSL) that translocates to the lipid droplet and along with adipose triglyceride lipase (ATGL) promote triacylglycerol (TAG) lipolysis to fatty acids (FA) that allosterically activates uncoupling protein 1 which deviates mitochondrial proton gradient energy from adenosine triphosphate synthesis to heat production.

pharmacological peroxisome proliferator-activated receptor γ activation with synthetic ligands is associated with a marked increase in brown adipocyte number, diameter and uncoupling protein 1 content in rodents in vivo,[7-10] even in the face of a reduced brown adipose tissue sympathetic activity and thyroid status.[11] In fact, we have recently shown that the peroxisome proliferator-activated receptor γ mediated increase in brown adipose tissue mass, which as detailed below is the result of both brown adipocyte hypertrophy and hyperplasia, is completely independent of brown adipose tissue sympathetic innervation.[12]

Peroxisome proliferator-activated receptor γ can be found in 2 isoforms, 1 and 2, which result from different usage of separate promotors and alternative splicing of a common peroxisome proliferator-activated receptor γ gene.[13] While the isoform 1 of peroxisome proliferator-activated receptor γ can be found in a wide variety of tissues such as heart, pancreas, liver and skeletal muscle, the isoform 2 is expressed exclusively in brown and white adipocytes.[14] Independently of the isoform, proper peroxisome proliferator-activated receptor γ activation requires receptor interaction with a ligand, formation of an obligatory heterodimer with the retinoic acid receptor and recruitment of coregulators, altogether forming a complex that binds to the promoter region of the target genes, either enhancing or inhibiting transcription. Over the last decades, several studies were carried out in an attempt to investigate the biological functions of peroxisome proliferator-activated receptor γ in rodents and humans. In the following sections, we review progress generated by these studies regarding the characterization of peroxisome proliferator-activated receptor γ involvement in the regulation of brown adipose tissue development, metabolism and thermogenic function.

Peroxisome Proliferator-Activated Receptor γ and Brown Adipose Tissue Development

Recent studies have gathered strong evidence indicating that brown adipocytes originate from precursor cells derived from a myogenic cell lineage, indicating a developmental proximity of brown adipocytes to skeletal muscle cells rather than white adipocytes.[15] In agreement with those lineage-tracing studies, brown preadipocytes and adipocytes were shown to feature global gene expression, mitochondrial proteins and microribonucleic acid signatures resembling those found in myocytes, but not in white adipocytes.[16-18]

Subsequent studies attempting to investigate the molecular mechanisms that promote the commitment of myogenic precursor cells to brown preadipocytes identified the transcriptional factor PR domain zinc finger protein 16 as an essential promoter of brown fat phenotype determination.[15] Accordingly, ectopic expression of PR domain zinc finger protein 16 in myoblasts was shown to induce their differentiation into brown fat cells, whereas PR domain zinc finger protein 16 deficiency in brown preadipocytes is associated with a loss of brown fat features.[15] Mechanistically, PR domain zinc finger protein 16 exerts these actions by interacting and forming a transcriptional complex with CCAAT/enhancer-binding proteins β promoting myogenic cell commitment to brown preadipocytes.[19-21] Noteworthy, however, whole-body deletion of PR domain zinc finger protein 16 induces a significant, but only modest impairment in some morphological and molecular brown adipose tissue features indicating that other PR domain zinc finger protein 16-independent pathways may also be involved in the promotion of myogenic cell commitment to brown preadipocytes.[15]

One of the major components of brown adipose tissue recruitment and elevation in tissue thermogenic capacity induced by cold exposure is an increase in brown adipocyte number, or hyperplasia.[22] Brown adipose tissue contains in its stromal vascular fraction a population of brown adipocyte precursor cells with the ability, under demand, to proliferate and differentiate into mature brown adipocytes. Norepinephrine is a major positive regulator of brown preadipocyte proliferation as determined in both in vivo and in vitro studies, through a mechanism that involves the activation of the β1 adrenergic receptor and the intracellular protein kinases A and C.[22-24]

Interestingly, non-adrenergic brown adipose tissue recruitment induced by peroxisome proliferator-activated receptor γ activation is also associated with brown adipocyte hyperplasia indicating the existence of alternative mechanisms regulating brown adipocyte proliferation other than those induced by norepinephrine. Indeed, pharmacological peroxisome proliferator-activated receptor γ activation with troglitazone is associated with an increase in brown adipose tissue mass, such an effect being in part attributed to an enhanced brown adipocyte proliferation as evaluated by bromodeoxyuridine incorporation into brown adipose tissue cells, later identified by electron microscopy as endothelial cells, interstitial cells (pericytes, preadipocytes, and fibroblasts), and mature brown adipocytes.[25] Importantly, pharmacological peroxisome proliferator-activated receptor γ activation was shown to increase brown adipose tissue mass and DNA content (indicative of cell number) independently of tissue sympathetic activity, as evidenced by the similar increase in these variables in both sympathetically innervated and surgically denervated brown adipose tissue fat pads.[11,12] The mechanisms by which pharmacological peroxisome proliferator-activated receptor γ activation promotes brown preadipocyte proliferation in vivo are unknown, but the findings that these cells express peroxisome proliferator-activated receptor γ even in the undifferentiated state[26,27] opens the possibility of a direct action of peroxisome proliferator-activated receptor γ ligands, a hypothesis that remains to be tested in in vitro experiments.

In spite of its unclear role in proliferation, the complete differentiation of brown preadipocytes into mature, fully competent brown adipocytes and their survival require a functional peroxisome proliferator-activated receptor γ, as evidenced by the complete absence of all forms of fat in mice with whole-body or fat-specific peroxisome proliferator-activated receptor γ deficiency.[28,29] The identity of the endogenous ligand that activates peroxisome proliferator-activated receptor γ during differentiation is still unknown. Importantly, in vitro pharmacological peroxisome proliferator-activated receptor γ activation during adipogenesis accelerates brown adipocyte differentiation and enhances cell thermogenic capacity,[30] altogether suggesting that such naturally occurring endogenous ligand does not activate peroxisome proliferator-activated receptor γ to its maximal activity during adipogenesis.

Given that peroxisome proliferator-activated receptor γ is essential for both white and brown adipocyte differentiation, inducing in these cells many common features such as the expression of proteins involved in lipid transport and metabolism, among others, it is of interest to delineate the involvement of this nuclear receptor in the promotion of brown adipocyte-specific uncoupling protein 1 expression. In fact, the uncoupling protein 1 gene not only has in its promotor region a peroxisome proliferator-activated receptor response element,[26,31] but also its transcription is promoted by pharmacological peroxisome proliferator-activated receptor γ activation in vitro and in vivo in both brown and white adipocytes.[10,11,30,32,33] The reasons why peroxisome proliferator-activated receptor γ does not promote uncoupling protein 1 expression in white adipocytes in the absence of pharmacological activation and what is the real contribution of this nuclear receptor to uncoupling protein 1 expression in brown adipocytes under non-stimulated and stimulated conditions are still unknown and clearly deserve to be investigated. Importantly, some key coregulators of peroxisome proliferator-activated receptor γ transcriptional activity that are mainly expressed in brown rather than white adipocytes such as PR domain zinc finger protein 16,[34] CCAAT/enhancer-binding proteins β[20,35] and peroxisome proliferator-activated receptor γ coactivator α [36] may be involved in directing activated peroxisome proliferator-activated receptor γ toward the induction of brown adipocyte-specific features.[37]

Peroxisome Proliferator-Activated Receptor γ and Brown Adipocyte Metabolism

The ability of brown adipose tissue to produce heat is directly related to the maintenance of adequate intracellular stores of triacylglycerol, the hydrolysis of which provides the fatty acids required for uncoupling protein 1 allosteric activation, as well as to mitochondrial oxidation, which generates most of the energy dissipated as heat during thermogenesis (**Fig. 1**). The important role of brown adipocyte triacylglycerol lipolysis in thermogenesis is underlined by both the marked increase in brown adipose tissue triacylglycerol synthesis that occurs upon cold exposure [38] and the complete abrogation of tissue heat production upon deletion of adipose triglyceride lipase, a major enzyme catalyzing triacylglycerol hydrolysis.[39] Mechanistically, cold increases triacylglycerol synthesis by enhancing both glycerol 3-phosphate generation via glycolysis, glyceroneogenesis and glycerokinase, as well as fatty acid availability through endogenous *de novo* lipogenesis and from circulating very-low-density lipoprotein- and chylomicron-bound triacylglycerol via the activity of the endothelium-located enzyme lipoprotein lipase.[38,40-42]

Similarly to cold exposure, in vivo pharmacological peroxisome proliferator-activated receptor γ activation also markedly increases brown adipose tissue triacylglycerol synthesis promoting brown adipocyte hypertrophy and an increase in tissue mass. Such increased triacylglycerol synthesis induced by peroxisome proliferator-activated receptor γ activation was shown to result from an enhanced uptake and esterification of fatty acids from circulating triacylglycerol via lipoprotein lipase, along with a higher generation of glycerol 3-phosphate via glyceroneogenesis and glycerokinase and elevated activities of glycerol 3-phosphate acyltransferase and diacylglycerol acyltransferase, enzymes that catalyze the first and last acylation of glycerol 3-phosphate, respectively.[43–45] Because no thermogenesis can be evoked without proper activation of triacylglycerol lipolysis, the enhanced triacylglycerol accumulation induced by pharmacological peroxisome proliferator-activated receptor γ activation may perhaps indicate that brown adipose tissue is being prepared for periods of intense stimulation resembling what happens in Syrian hamsters exposed to a short photoperiod or in pre-hibernating mammals, for example.[1,5,6] Importantly, such increase in brown adipose tissue triacylglycerol content induced by peroxisome proliferator-activated receptor γ activation is associated with an

upregulation of brown adipose tissue lipolytic machinery and expression of lipases,[12] which however are not translated into higher lipolysis and thermogenesis in vivo due to an impairment in brown adipose tissue sympathetic activity and thyroid status found in this condition.[11]

In addition to fatty acids, brown adipose tissue strongly relies on glucose as a metabolic substrate to support brown adipose tissue thermogenesis as indicated by both the significant amounts of glucose stored as glycogen in brown adipocytes and the marked increase in brown adipose tissue glucose uptake seen upon sympathetically-mediated thermogenesis activation.[1,46,47] Indeed, brown adipose tissue sympathetic innervation through norepinephrine release and activation of glucose transporter 1 is the major inducer of brown adipocyte glucose uptake during cold exposure.[48] Among its many fates, glucose is mainly used in cold-activated brown adipose tissue for the synthesis of either glycerol 3-phosphate and lactate through glycolysis, or fatty acids through *de novo* lipogenesis,[1] or citric acid cycle intermediates (anaplerosis),[49] which is very important for maintaining enhanced levels of fatty acid oxidation.[1] Furthermore, the increased conversion of glucose to lactate seems to be important to generate the energy required to maintain brown adipose tissue function in the presence of heat producing, uncoupled mitochondria.[1]

In contrast to cold exposure, however, in vivo pharmacological peroxisome proliferator-activated receptor γ activation is associated with a marked reduction in brown adipose tissue rates of glucose uptake, glucose transporter 4 and glycogen contents, such a phenotype perhaps being partly attributable to the reduced tissue sympathetic activity found in this condition.[11,42] Thus, in contrast to the above-described changes in brown adipose tissue lipogenic and lipolytic profiles that seem to prepare the tissue for periods of intense thermogenesis, the alterations in glucose metabolism associated with pharmacological peroxisome proliferator-activated receptor γ activation in vivo may be deleterious to brown adipose tissue ability to produce heat.

Peroxisome Proliferator-Activated Receptor γ and Brown Adipose Tissue Thermogenic Function

In addition to its role as a main activator of lipolysis and thermogenesis, chronic sympathetic activation, by upregulating brown adipocyte number, mitochondrial content and function and uncoupling protein 1 levels, is the major regulator of brown adipose tissue thermogenic capacity.[1] Such sympathetically-regulated brown adipose tissue plasticity is a very important adaptative mechanism that allows rodents to adjust rates of heat production according to various environmental conditions and brown adipose tissue sympathetic activity.[1]

Because of the absolute peroxisome proliferator-activated receptor γ requirement for brown adipocyte differentiation and survival, which precludes the execution of studies with complete receptor loss of function, it is unclear whether peroxisome proliferator-activated receptor γ is activated and directly participates in brown adipocyte thermogenesis elicited by sympathetic activation. Therefore, most of the knowledge available regarding peroxisome proliferator-activated receptor γ regulation of brown adipose tissue thermogenic function originated from studies using pharmacological receptor activation. From these studies, the concept emerged that peroxisome proliferator-activated receptor γ acts to recruit and prepare brown adipose tissue for periods of intense heat production, such higher thermogenic capacity needing proper sympathetic activation to be actuated at the functional level.[10,11] According to this concept, peroxisome proliferator-activated receptor γ -mediated upregulation of brown adipose tissue mass, oxidative, lipolytic and thermogenic proteins are translated into higher rates of energy expenditure in vitro and in vivo only upon pharmacological adrenergic activation.[7–10,30] In fact, the higher brown adipose tissue thermogenic capacity induced by peroxisome proliferator-activated receptor γ activation seems to be silenced by the concomitant alterations of the neurohormonal milieu characterized by an impaired brown adipose tissue sympathetic activity and thyroid status (reduced triiodothyronine levels and brown adipose tissue type 2 iodothyronine deiodinase expression).[11]

In contrast to pharmacological elicitation of thermogenesis through treatment with a β3 agonist, however, the higher thermogenic potential of brown adipose tissue of rodents with ligand mediated peroxisome proliferator-activated receptor γ activation did not translate into higher thermogenic activity upon an acute 24 h cold exposure.[50] Surprisingly, such unaltered thermogenic response to cold was associated with an impairment in the upregulation of the expression of the adrenergically regulated genes brown adipose tissue peroxisome proliferator-activated receptor γ coactivator α and type 2 iodothyronine deiodinase,[50] even in the face of the cold-induced increase in tissue sympathetic activity. Altogether those findings indicate that pharmacological peroxisome proliferator-activated receptor γ activation through unknown mechanisms affects intracellular adrenergic signaling in brown adipocytes. Understanding the mechanisms underlying such discrepancy between pharmacological and physiological elicitation of brown adipose tissue thermogenesis are of foremost importance in the determination of the real potency of peroxisome proliferator-activated receptor γ to recruit functional brown adipose tissue.

Conclusions

The recent identification of functional brown adipose tissue in adult humans [51] has opened the possibility of using brown adipocyte thermogenic function as a strategy to increase energy expenditure and counteract obesity development. Furthermore, several studies have also lent strong support for the putative utilization of brown adipose tissue enhanced ability to take up and oxidize circulating lipids and glucose as an alternative approach in the treatment of metabolic diseases such as dyslipidemias, hyperglycemia and insulin resistance.[42,45]

In the face of these many therapeutic possibilities, targeting brown adipose tissue will require the development of pharmacological, nutritional and/or behavioral strategies not only to

recruit and enhance tissue thermogenic capacity, but also to safely turn thermogenic activity on and off. In this regard, pharmacological peroxisome proliferator-activated receptor γ activation has emerged as a putative strategy to enhance brown adipose tissue thermogenic capacity, and thus possibly its efficiency as a therapeutic target (**Fig. 2**). Importantly, efficient pharmacological sympathetic activation is the only means known to date of actuating peroxisome proliferator-activated receptor γ-enhanced thermogenic capacity into increased thermogenesis.

It is worthy of note that the ligands used nowadays to activate peroxisome proliferator-activated receptor γ have many adverse side effects such as fluid retention, weight gain, congestive heart failure [52-54] and osteopenia [55] that currently limit their clinical use. Thus, progress must be made toward the development of selective peroxisome proliferator-activated receptor γ modulators that maintain peroxisome proliferator-activated receptor γ beneficial actions without inducing the above-mentioned health complications. More specifically, such optimal ligand would increase brown adipose tissue thermogenic capacity without reducing tissue sympathetic activity, glucose metabolism, thyroid status and intracellular adrenergic signaling (**Fig. 2**). Discovery of such optimal ligand would allow the recruitment of significant amounts of brown adipose tissue, in which thermogenesis could be turned on either spontaneously (through basal brown adipose tissue adrenergic activity), or by the administration of small doses of a β-adrenergic ligand, or ideally by increasing adrenergic activity in a tissue-specific manner. Further studies are clearly needed to elucidate the best strategy to explore the fascinating therapeutic potential of brown adipose tissue in the treatment of metabolic diseases.

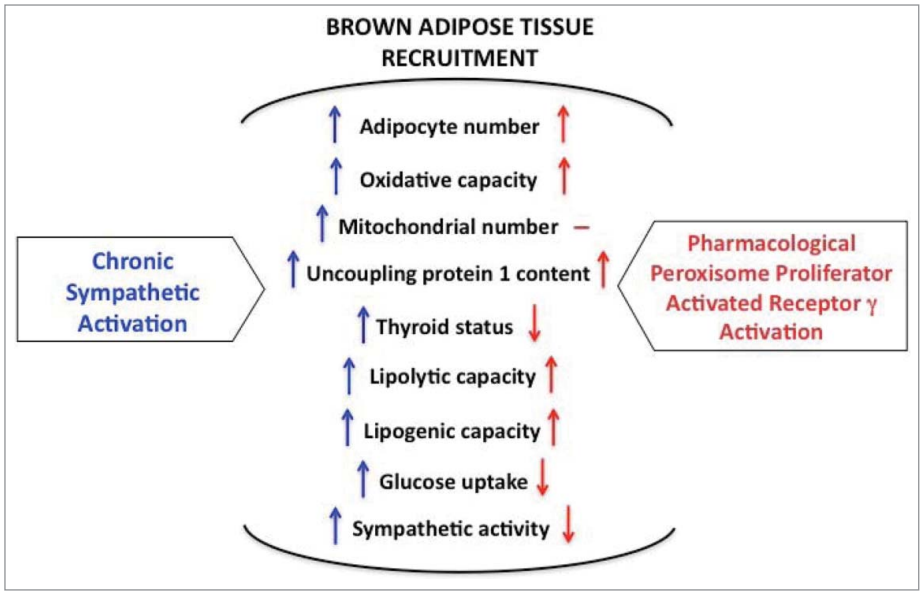

Figure 2. Major differences between brown adipose tissue recruitment induced by chronic sympathetic activation (blue) and pharmacological peroxisome proliferator-activated receptor γ activation (red)., upregulated;↓, downregulated; –, unaltered.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This work was financially supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) to William Festuccia (#2009/15354-7, 2010/19018-9 and 2013/50394-5), the Canadian Institutes of Health Research (CIHR, #MOP-36451) and the Natural Sciences and Engineering Research Council (NSERC, #36-2011) to Yves Deshaies.

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