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Transcriptional control of metabolism by interferon regulatory factors

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Abstract

Interferon regulatory factors (IRFs) comprise a family of nine transcription factors in mammals. IRFs exert broad effects on almost all aspects of immunity but are best known for their role in the antiviral response. Over the past two decades, IRFs have been implicated in metabolic physiology and pathophysiology, partly as a result of their known functions in immune cells, but also because of direct actions in adipocytes, hepatocytes, myocytes and neurons. This Review focuses predominantly on IRF3 and IRF4, which have been the subject of the most intense investigation in this area. IRF3 is located in the cytosol and undergoes activation and nuclear translocation in response to various signals, including stimulation of Toll-like receptors, RIG-I-like receptors and the cGAS–STING pathways. IRF3 promotes weight gain, primarily by inhibiting adipose thermogenesis, and also induces inflammation and insulin resistance using both weight-dependent and weight-independent mechanisms. IRF4, meanwhile, is generally pro-thermogenic and anti-inflammatory and has profound effects on lipogenesis and lipolysis. Finally, new data are emerging on the role of other IRF family members in metabolic homeostasis. Taken together, data indicate that IRFs serve as critical yet underappreciated integrators of metabolic and inflammatory stress.

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All authors researched data for the article, contributed substantially to the discussion of content and wrote the manuscript. Z.A. and E.D.R. reviewed and edited the manuscript before submission.

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Introduction

Metabolic physiology can be thought of as a complex symphony of actions and counteractions orchestrated by many different organs and tissues, including the brain, liver, adipose tissue, muscle and immune cells, among others. The purpose of these activities is to ensure that there is adequate nutrition coming into the body and that nutrients are being distributed, utilized and stored appropriately, given ambient conditions. Under favourable conditions, such as when available nutrients are adequate but not excessive, this carefully coordinated set of metabolic activities facilitates growth, reproduction, tissue repair, proper immune functioning and other hallmarks of normal physiology. However, in the setting of starvation or overnutrition, the metabolic symphony becomes discordant, with organ dysfunction and systemic disease being the probable outcomes. Because inhabitants of modern Western societies and those influenced by them generally live under conditions of caloric excess, we find ourselves in the midst of a wave of nutritionally mediated conditions, including obesity, type 2 diabetes mellitus (T2DM), insulin resistance, dyslipidaemia and metabolic dysfunction-associated steatotic liver disease (MASLD; also known as non-alcoholic fatty liver disease).

The cells and tissues that coordinate metabolic physiology use an extraordinary array of signals, which include direct neural inputs as well as extracellular messengers such as hormones, cytokines, metabolites, exosomes, nucleic acid fragments and others. These signals are detected by the appropriate target cells, which activate or inhibit intracellular pathways that ultimately mediate the appropriate response. Notably, the outcomes of cellular signalling events are not fully explained by the cytoplasmic actions of these pathways; there is also extensive nuclear signalling through the activation of key transcription factors. These transcription factors regulate chromatin structure and gene expression in ways that both drive and reinforce the effects of metabolic signalling, typically on a longer timescale (hours to days) than that seen with the upstream cytoplasmic events (minutes to hours). Dozens, if not hundreds, of the roughly 1,500 mammalian transcription factors from all classes have been implicated in metabolic processes, including nuclear hormone receptors (such as glucocorticoid receptor, thyroid hormone receptor, liver X receptor (LXR) and peroxisome proliferator-activated receptors (PPARs)), homeodomain proteins (such as IRX3 and IRX5), basic helix–loop–helix proteins (such as ChREBP) and basic leucine zipper proteins (such as C/EBPs). Another group of transcription factors, the interferon regulatory factors (IRFs), have been well studied in the context of immunity, but there is increasing awareness of their important functions in other contexts, including metabolism.

The IRF family of transcription factors

The first IRF to be discovered (IRF1) was reported in 1988 as a protein that bound to a virally inducible enhancer of the *IFNB* gene (encoding interferon-β)[1]. Subsequent IRF family members were cloned based on homology, although many were given other names at first (for example, PIP or LSIRF for IRF4). The appearance of IRFs coincides with the advent of multicellularity in animals, although insects and roundworms subsequently lost their IRF genes. The IRF family has expanded and contracted several times during

evolution: teleost fish have 11 IRFs and birds have 10, while mammals have 9 paralogs (IRF1–IRF9)[2].

All IRFs bind DNA via a conserved amino-terminal winged helix–loop–helix domain with five regularly spaced tryptophan residues (Fig. 1a). This domain recognizes motifs based on the sequence (A/GNGAAANNGAAACT), where N can be any base and GAAA represents the core repeat element, called an interferon-stimulated response element (ISRE)[3]. ISREs, or similar sequences, are found in the *cis*-regulatory elements of interferon genes as well as in genes regulated by interferons. IRFs can bind DNA as homodimers or they can heterodimerize with a variety of interacting proteins, including other members of the IRF family. Dimerization is mediated by a C-terminal IRF association domain (IAD), which is less well-conserved across family members than the DNA-binding domain. Most IRFs (other than IRF1 and IRF2) share the IAD1 form of this domain, which has structural homology to the Mad-homology 2 domain of SMAD transcription factors[4]. IRF1 and IRF2 share a distinct domain called IAD2.

The choice of dimerization partner dictates which response elements will be bound by the IRF complex, which target genes will be affected, and thus what the biological outputs will be. Another interesting feature of the C-terminal region of IRF3, IRF5 and IRF7 is the presence of an autoinhibitory domain. This region blocks the hydrophobic DNA-binding and dimerization surfaces until it is phosphorylated, after which dimerization and nuclear translocation occurs[5] (Fig. 1b).

Most IRF family members are expressed nearly ubiquitously across cell types and tissues, although some IRFs display tissue selectivity. For example, IRF6 is predominantly, although not exclusively, expressed in skin and mucosal surfaces (GTEx Portal). By far, the best-characterized actions of IRFs involve the antiviral response, so it is no surprise that immune cells have been the focus of most IRF-related studies. IRFs have been implicated in T and B lymphopoiesis, macrophage formation and polarization, apoptosis, cytokine production, and virtually every other aspect of immune cell development and function[6–8]. Evidence for the connection between IRFs and immunity is strengthened by the obvious effects on the immune systems of human patients with mutations in IRF genes, including enhanced susceptibility to viral and mycobacterial infections[8,9]. IRFs are also involved in many other fundamental biological processes, including cellular differentiation, oncogenesis, autophagy and, most relevant to this Review, cellular and organismal metabolism[7,10]. Although most mammalian IRFs have been implicated in metabolism in one way or another, the most data at present relates to IRF3 and IRF4.

The IRF3 pathway

IRF3 is constitutively expressed in virtually all cells, residing in an inactive form in the cytoplasm. In response to stimulation of pattern recognition receptors, such as Toll-like receptors (TLRs), RIG-I or RIG-I-like receptors, and cGAS, IRF3 becomes phosphorylated, dimerizes and then translocates to the nucleus. It then activates genes associated with antiviral activity, including type I interferon response genes (Fig. 2).

Toll-like receptors: TLR3 and TLR4

TLRs respond to a wide variety of ligands, including proteins, lipids, nucleic acids, proteoglycans and polysaccharides, most of which are released in the setting of infection or tissue damage[11]. Of the 13 TLRs, only two activate IRF3: TLR3, located in endosomes, and the cell surface receptor TLR4. TLR3 recognizes double-stranded RNA (dsRNA), which can derive from viral sources, or which can result from cellular injury due to UVB radiation or other insults[12–14]. TLR4 is best known for responding to bacterial lipopolysaccharide (LPS)[15], but other, endogenous ligands have also been identified, including hyaluronan-based oligosaccharides[16], high-mobility group box 1 (ref. 17) and heat shock proteins[18–20]. TLR4 has also been shown to respond to saturated fatty acids, such as palmitate, although there is debate about whether the lipid binds directly to TLR4 or if binding is mediated by fetuin A[21,22]. The role of many of these endogenous ligands in the activation of TLR4 has been studied in the context of diseases such as cardiovascular disease and type 2 diabetes mellitus[23,24].

The response elicited by TLR activation depends on the recruitment of adaptor proteins such as MyD88, TIRAP, TRIF or TRAM[25,26]. Most TLRs (but not TLR3) utilize MyD88 to activate nuclear factor-κB (NF-κB), thus inducing the expression of inflammatory cytokines[26,27]. TLR3 and TLR4 use TRIF and TRAM adaptors to signal through a MyD88-independent pathway, leading to the activation of IRF3 (ref. 28). These adaptors promote activation of IKKε and TANK-binding kinase 1 (TBK1), which phosphorylate several residues in the C-terminus of IRF3 (notably Ser396, Ser398, Ser402, Ser404 and Ser405 of human IRF3). This phosphorylation relieves autoinhibition of the C-terminal region and enables homodimerization or heterodimerization with IRF7 followed by translocation to the nucleus[29,30]; IRF3-containing dimers then bind to ISREs near target genes and induce their expression[31–34]. Moreover, activated IRF3 and IRF7 can assemble an enhanceosome with other pro-inflammatory transcription factors such as NF-κB, activating transcription factor 2 (ATF2) and c-Jun, all of which function cooperatively to drive transcription[35].

RIG-I or RIG-I-like receptors

RIG-I and RIG-I-like receptors belong to a family of DExD/H box RNA helicases and are ubiquitously expressed in the cytoplasm of all innate immune cells, where they serve as important sensors of nucleic acids[36,37]. RIG-I is also expressed by epithelial cells and is upregulated during viral infection[38]. When RIG-I or a RIG-like receptor, such as melanoma differentiation antigen 5 (MDA5), recognizes dsRNA, the adaptor protein MAVS localizes to the mitochondrial membrane and to peroxisomes to mediate downstream events[39]. Signalling through peroxisomal MAVS induces a rapid induction of antiviral protein expression independent of interferon production[40], while mitochondrial MAVS uses slower kinetics to promote the expression of interferon and associated genes. MAVS also interacts with TRAF3 and TBK1, which subsequently phosphorylates IRF3 and promotes type I interferon production, creating an antiviral state[41,42].

cGAS–cGAMP–STING pathway

cGAS is a cytosolic pattern recognition receptor that recognizes double-stranded DNA and then induces a type I interferon response[43–45]. cGAS undergoes a conformational change

upon sensing double-stranded DNA in the cytosol, thereby catalysing the transformation of ATP and GTP into the second messenger cGAMP. cGAMP is subsequently detected by the endoplasmic reticulum-resident protein STING[45]. Upon activation, STING activates TBK1 and IKKε, which then induce the phosphorylation and nuclear translocation of IRF3 (Fig. 2). In addition to its role as a pathogen sensor, cGAS–STING serves an important function in sterile inflammation[46,47]. Under certain conditions (such as mitochondrial stress), genomic DNA and/or mitochondrial DNA can be released into the cytoplasm, where it triggers immune responses through cGAS–STING and IRF3 (refs. 47–49). Importantly, expression and/or activity of various components of the cGAS–STING pathway are upregulated in obese mice[50,51].

Additional activators of IRF3

Phosphoinositide 3-kinase (PI3K)–AKT signalling is a major metabolic effector pathway downstream of the insulin receptor — it is therefore of interest that PI3K–AKT signalling has been implicated in IRF3 activation. Studies in mouse macrophages and human lung epithelial cells have demonstrated that activation of type I interferon genes by viral infection or TLR3 and/or TLR4 signalling can be reduced by PI3K or AKT inhibitors[52–55]. Specifically, RIG-I and MDA5 appear to mediate PI3K activation in response to viral infection[52,55]. Interestingly, loss of PI3K–AKT signalling only partially reduced IRF3 phosphorylation, dimerization and nuclear translocation, but had a much larger effect on cofactor recruitment and target gene transactivation[54]. It is as yet unclear if PI3K or AKT directly phosphorylate IRF3 or whether they modify some other protein critical for the IRF3 response. It should be noted that it has also been suggested that IRF3 is activated by a decrease in AKT signalling, at least in pancreatic cancer cells[56]. In some settings, AKT might also be a downstream target of IRF3; this result is not, of course, inconsistent with an additional role upstream of IRF3 (ref. 57).

Apoptosis signal-regulating kinase 1 (ASK1) is a mitogen-activated protein (MAP) kinase known to respond to multiple cellular stressors, including TLR4 signalling. Increased ASK1 expression was noted in white adipose tissue (WAT) from people with obesity relative to lean control individuals, and ASK1 expression was correlated with whole-body insulin resistance[58]. Moreover, in a study conducted in 2020, ASK1 was shown to act as an upstream kinase for IRF3 in adipose tissue, resulting in decreased thermogenesis (see below in ‘IRF3, body weight and thermogenesis’)[59].

Finally, one study conducted in 2023 reported a CRISPR-based screen of several glucose-related genes to assess their effect on antiviral immunity; this approach identified UDP-N-acetylglucosamine pyrophosphorylase 1 (UAP1) as a key upstream regulator of IRF3 (ref. 60). UAP1 catalyses the pyrophosphorylation of IRF3 at Ser386, that is, phosphorylation of an already existing serine or threonine-linked phosphate group. This ‘double’ phosphorylation was shown to be required for IRF3 action, although there is little understanding as to why a metabolic enzyme used in the hexosamine pathway should be required for viral immunity.

Non-nuclear actions of IRF3

IRF3 is a transcription factor; therefore, most of its actions have been presumed to occur in the nucleus through binding to DNA. However, some actions of IRF3 might not require nuclear translocation. For example, IRF3 blocks the proliferation of colorectal cancer cells by binding to β-catenin in the cytosol[61]. IRF3 can also translocate to the mitochondrion, where it promotes apoptosis through activation of the proapoptotic factor Bcl-2-associated X[62,63]. A role for non-genomic actions of IRF3 linked to metabolic outputs has not been explored.

The role of IRF3 in obesity-associated inflammation

Obesity induces a chronic, sterile inflammatory state, most notably in WAT, characterized by increased numbers of inflammatory macrophages and by elevated levels of pro-inflammatory cytokines relative to the lean state[64,65]. In mice, this inflammatory state appears to play a causal role in the metabolic dysfunction associated with obesity (such as insulin resistance)[64]. Human WAT also displays increased inflammation in obesity, although causal links between inflammation and insulin resistance have been harder to prove in humans[66]. Regardless, there has been substantial interest in understanding how the inflammatory state develops in adipose tissue during weight gain and the mechanisms that, at least in mice, link inflammation to insulin resistance. Several reviews in the past few years have addressed this topic, which involves multiple types of immune cells, dozens of cytokines and complex interactions with metabolically relevant cell types such as adipocytes, hepatocytes and myocytes[66,67]. Here, we focus on the role of IRF3 in obesity-associated inflammation.

First, it is important to note that upstream ligands of TLR4 are elevated in the serum of obese animals compared with lean animals[68,69]. In obesity, the gut becomes ‘leaky’, allowing bacterial products to enter the bloodstream. Blood levels of LPS and fatty acids thus rise because of increased intestinal absorption[70]; levels of fatty acids also rise because of increased lipolysis in obesity. Consistent with this finding, mice lacking TLR4 signalling show greatly attenuated diet-induced inflammation compared with wild-type mice[21,71–74]. Mitochondrial DNA, which activates cGAS–STING, is also elevated in the serum of patients with obesity and type 2 diabetes mellitus, and plasma levels of mitochondrial DNA correlate with insulin resistance[75]. TLR3 ligands include RNA released from dying cells and, since apoptotic adipocytes accumulate in obesity[76], TLR3 activation could also contribute to adipose inflammation. However, although TLR3 stimulation increases markers of inflammation in vitro, *Tlr3*$^{-/-}$ mice do not show reduced inflammation or insulin resistance after a high-fat diet (HFD) relative to wild-type controls[77].

IRF3 is highly expressed in immune cells, especially macrophages, which play a critical role in determining the inflammatory tone of the fat pad, at least in mice[78]. In vitro studies have outlined a paradigm in which macrophages can be polarized towards a pro-inflammatory (M1) or an anti-inflammatory state (M2). Although we now recognize that this oversimplified distinction fails to capture the true diversity of macrophage populations in vivo, there is still utility in recognizing that some macrophage populations have a more

pro-inflammatory profile ('M1-like') than others ('M2-like')[79]. IRF3, in general, drives the expression of pro-inflammatory genes and thus pushes macrophages towards an M1-like phenotype[80,81]. Ablation of IRF3 specifically in myeloid cells (including monocytes, macrophages and neutrophils) resulted in an attenuated type I interferon response after injection of a high dose of LPS[82]. Although it is unclear how this finding translates to the context of HFD feeding, it seems probable that macrophage IRF3, with or without contributions from other immune cells, contributes to obesity-associated inflammation.

Another cell type in which IRF3 might exert pro-inflammatory actions is the adipocyte. Adipocyte TLR4 is activated in obesity, inducing the production of downstream IRF3 targets such as monocyte chemoattractant protein 1 (MCP1), which acts to recruit pro-inflammatory macrophages to the fat pad in mice[83]. MCP1 levels have been found to be higher in the WAT and plasma of obese mice than of lean mice[64,84–87]. Consistent with this finding, IRF3 protein levels are elevated in adipocytes in obese mice, and elevated adipose expression of *IRF3* mRNA is positively associated with BMI in humans[88]. Global *Irf3*$^{-/-}$ mice also show reduced inflammation in their WAT after HFD feeding compared with wild-type mice fed a HFD[88]; however, this result needs to be qualified. Firstly, global *Irf3*$^{-/-}$ mice have inadvertent knockout of a neighbouring gene, *Bcl2l12*, making it hard to ascribe a phenotype to IRF3 specifically[82]. Secondly, *Irf3*$^{-/-}$ mice have a lower body weight on a HFD than wild-type mice because of the repressive actions of IRF3 on thermogenesis (see below in 'IRF3, body weight and thermogenesis')[89]; therefore, the reduced macrophage infiltration into WAT in *Irf3*$^{-/-}$ mice could be secondary to overall reduced adiposity. Consistent with this possibility, mice with an adipocyte-specific knockout of IRF3 that were raised in thermoneutrality have equivalent body weights and appear to have similar levels of macrophage infiltration in WAT than wild-type mice (S. Yan, personal communication). This finding suggests that at least part of the pro-inflammatory actions of IRF3 are encoded in locations other than the adipocyte.

IRF3 and adipogenesis

One of the earliest indications that IRFs might play a role in metabolism came from an unbiased analysis of the changes in chromatin state that accompany adipocyte differentiation or adipogenesis, which identified the ISRE as an over-represented motif in such loci[90,91]. Subsequent overexpression and knockdown studies in cultured adipocytes demonstrated that IRF1, IRF3 and IRF4 were all anti-adipogenic[91]. Later studies by others confirmed that IRF3 is an inhibitor of adipogenesis and further suggested that IRF3 acts by reducing expression of the master regulator of adipogenesis, PPARγ[92]. In macrophages, IRF3 also represses RXRα, an obligate heterodimerization partner of PPARγ[93]; if this were also true in developing adipocytes, it could also account for some of the anti-adipogenic effects noted.

IRF3, body weight and thermogenesis

One way that adipose tissue regulates whole-body homeostasis is through adaptive thermogenesis, in which fatty acids and glucose are oxidized without generating ATP. This uncoupling of electron transport and ATP production creates heat and takes place in specialized mitochondria-rich brown and beige adipocytes. Energy dissipation classically

occurs through the actions of uncoupling protein 1 (UCP1), although several UCP1-independent mechanisms of adipose thermogenesis have also been described[94]. Importantly, adipose thermogenesis is a mechanism by which mammals can diminish the negative impact of a HFD by 'wasting' extra calories in the form of heat, and thermogenesis helps to reduce weight gain and preserve insulin sensitivity, at least in mice[94]. However, in obese mice and people with obesity, adipose thermogenesis is markedly inhibited. In mice, this inhibition occurs, at least in part, because of the influence of local inflammation[95]. In the presence of cytokines, such as TNF and IL-1β, adipogenesis of new beige adipocytes is impaired, brown adipocytes undergo apoptosis, and expression of thermogenic genes in both brown and beige adipocytes is reduced[96–100]. Activation of TLRs and cGAS–STING also decreases expression of *Ucp1* and heat production by brown and beige adipocytes[101–103].

A role for IRF3 in inhibiting adipose thermogenesis was initially suggested by the observation that global *Irf3*$^{-/-}$ mice are protected from HFD-induced obesity despite elevated food consumption[88]. These mice display increased energy expenditure and elevated expression of thermogenic genes, such as *Ucp1*, compared with wild-type mice. Conversely, IRF3 overexpression attenuated the expression of thermogenic genes in cultured adipocytes[88]. Furthermore, loss of IRF3 specifically in adipocytes resulted in a similar effect to that seen in the global *Irf3* knockout mice: elevated energy expenditure, improved cold tolerance and increased thermogenic gene expression[89]. Moreover, mice that overexpress a constitutively active form of IRF3 in adipocytes exhibit the opposite effect to the *Irf3* knockout mice; notably, the increased browning of inguinal WAT elicited by the absence of IRF3 in adipocytes was associated with better glucose tolerance and reduced adipose tissue mass than in wild-type mice[89].

ASK1 was identified as a downstream effector of TLR4 that phosphorylates IRF3 in adipocytes; adipocyte-specific *Ask1* knockout mice phenocopy *Irf3*$^{-/-}$ mice when fed a HFD[59]. Specifically, these mice are protected from diet-induced obesity and display elevated thermogenesis compared with wild-type mice. Conversely, overexpression of ASK1 in adipocytes repressed thermogenic gene expression and energy expenditure. Importantly, however, ASK1 is not the only such kinase with this effect as genetic or pharmacological inhibition of IKKε and TBK1 also enhances adipose thermogenesis[50,51].

In 2021, the small ubiquitin-like protein interferon-stimulated gene 15 (ISG15) was identified as a critical mediator of the effect of IRF3 on thermogenesis[89]. ISG15, which is a direct downstream gene target of IRF3, has potent antiviral activity due to its ability to covalently attach to viral proteins (in a process called ISGylation), leading to their degradation. ISG15 alters adipocyte metabolism through ISGylation and subsequent inhibition of glycolytic enzymes (particularly LDHA) and reduction of lactate production[89]. Interestingly, ISGylation seems to cause functional inhibition of glycolytic enzymes rather than their degradation. *Isg15*$^{-/-}$ mice display increased thermogenesis compared with wild-type mice and are protected from HFD-induced obesity and glucose intolerance, thus phenocopying loss of IRF3. *Isg15* is almost certainly not the only IRF3 target gene that affects thermogenesis; knockout of the IRF3-responsive gene *Rsad2*, also known as viperin, increased heat production, reduced adipose tissue mass and enhanced cold tolerance compared with wild-type mice[104]. It should be noted that there is a report showing that

Irf3$^{-/-}$ mice are heavier than controls on a chow diet, an effect attributed to reduced physical activity[92].

IRF3 and insulin resistance

In mouse models, IRF3 has been implicated in the development of obesity-associated insulin resistance, a major pathological component of T2DM. *Irf3*$^{-/-}$ mice on a HFD are more insulin sensitive than wild-type littermates on the same diet[88]. However, studies on insulin sensitivity can be confounded if there is also an effect on body weight — the fact that *Irf3*$^{-/-}$ mice weigh less than their wild-type littermates on a HFD makes it hard to determine whether IRF3 has an independent effect on insulin action. One way to address this issue is by assessing insulin sensitivity before body weight begins to diverge. When this was done, *Irf3*$^{-/-}$ mice still showed an improved response to insulin as assessed by insulin tolerance testing as well as by hyperinsulinaemic–euglycaemic clamp[88]. These data are consistent with observations that upstream regulators of IRF3 are also required for the development of diet-induced insulin resistance. For example, *Tlr4*$^{-/-}$ mice are protected from developing insulin resistance after lipid infusion[21] or HFD feeding[72]; in neither situation was body weight reduced. Similarly, global loss of STING prevents obesity-associated insulin resistance[105,106] and stimulation of the cGAS–STING pathway exacerbates obesity-induced insulin resistance[107]. Oddly, loss of RIG-I was reported to worsen insulin sensitivity, although this finding was in the context of increased body weight, which makes direct causality difficult to infer[108].

Expression of the IRF3 upstream kinases TBK1 and IKKε is elevated in the WAT of obese mice, and inhibition of these kinases by genetic or pharmacological means has been reported to improve systemic insulin sensitivity[109,110]. A 2013 study reported that amlexanox, a small-molecule inhibitor of TBK1 and IKKε, prevents weight gain and inflammation and increases energy expenditure and insulin sensitivity in mice[50]. Furthermore, a small double-blinded, placebo-controlled study in human participants with T2DM showed that amlexanox improved glycaemic control in a subset of patients; this subset was notable for a more pro-inflammatory gene expression profile in their subcutaneous adipose tissue at baseline than the subset that did not show improvement of glycaemic control[111]. There has been mixed data on genetic loss of TBK1. *Tbk1*$^{-/-}$ mice are somewhat protected from the effects of a HFD[110], but specific ablation of *Tbk1* in adipocytes led to a paradoxical increase in inflammation and insulin resistance relative to wild-type mice[51]. In both cases, the metabolic effect of TBK1 ablation was attributed to downstream targets of the kinase other than IRF3, although a role for IRF3 was not ruled out.

What cell types are likely to mediate the effects of IRF3 on insulin action? One obvious candidate would be immune cells, such as macrophages or lymphocytes, as IRF3 drives pro-inflammatory cytokine expression and many studies have demonstrated that inflammation is a causal factor in the development of obesity-associated insulin resistance, at least in rodents[66,67]. To date, however, the effect of knocking out IRF3 in immune cells on glucose tolerance or insulin sensitivity has not been reported.

IRF3, adipocytes and insulin resistance

Adipocytes represent another cell type in which IRF3 could cause insulin resistance (Fig. 3). IRF3 expression is elevated in the adipocytes of obese mice and there is a linear relationship between body weight and *IRF3* expression in unfractionated human adipose tissue[88]. Cultured human and mouse adipocytes stimulated with LPS or polyinosinic-polycytidylic acid (poly(I:C); a synthetic analogue of dsRNA used in in vitro studies) display increased phosphorylated IRF3 and reduced insulin-stimulated glucose uptake relative to vehicle-treated cells, and knockdown of IRF3 rescues insulin action in these cells[88,112].

Furthermore, expression of a constitutively active allele of IRF3 causes insulin resistance in cultured adipocytes, even in the absence of LPS or poly(I:C) stimulation[88]. Moreover, a study conducted in 2023, currently available as a preprint, showed that adipocyte-specific knockout of IRF3 rescues insulin action in obese mice, while adipocyte-specific overexpression of a constitutively active IRF3 allele causes insulin resistance. These experiments were performed in animals raised at thermoneutrality, thus enabling study at equivalent body weights[112]. A search for gene targets of IRF3 in adipocytes that could account for this effect revealed regulation of androgen-inducible gene 1 (AIG1)[112]. AIG1 encodes a hydrolase for fatty acyl esters of hydroxy fatty acids, which are insulin-sensitizing lipids produced by de novo lipogenesis in adipocytes[113,114]. Knockdown or knockout of IRF3 reduces *Aig1* levels, and the converse effect is seen with IRF3 overexpression. Finally, a chemical inhibitor of AIG1 raises fatty acyl ester of hydroxy fatty acid levels and prevents IRF3-dependent insulin resistance from developing in isolated adipocytes and systemically, establishing *Aig1* as a downstream effector gene of IRF3 in adipocytes[112].

In another study, the Jumonji C domain protein JMJD8 was found to be increased in the adipocytes of obese mice[115]. Loss of JMJD8 rescued insulin action in mice on a HFD, while transgenic overexpression of JMJD8 in adipocytes in vitro and in vivo was sufficient to promote insulin resistance. Because JMJD8 was shown to be required for LPS-induced insulin resistance in cultured mouse adipocytes, a possible role for IRF3 in mediating the effects of JMJD8 was assessed. Indeed, JMJD8 was found to interact with IRF3, and loss of IRF3 prevented JMJD8 from reducing insulin sensitivity. Interestingly, loss of JMJD8 only minimally blocked insulin resistance due to overexpression of IRF3 (ref. 115), suggesting that IRF3 reduces insulin action in both JMJD8dependent and JMJD8-independent ways, with the latter mechanism probably predominant.

IRF3, hepatocytes and insulin resistance

The liver is also a critical node for insulin action and glycaemic control through the careful balance of glucose production and storage. Obese mice have elevated TBK1 levels, increased IRF3 phosphorylation and, concordantly, increased nuclear content of IRF3 (ref. 116). Loss of IRF3 specifically in hepatocytes, either chronically by Cre-lox-mediated gene ablation or acutely by injection of IRF3 antisense oligonucleotides, reduced systemic glucose levels in mice compared with controls without affecting body weight. Similarly, overexpression of a constitutively active allele of IRF3 in cultured mouse hepatocytes drove glucose production[116]. This effect was found to be mediated by direct IRF3 transactivation of the *Ppp2r1b* gene (Fig. 4). PPP2R1B is a liver-selective component of the PP2A protein

phosphatase complex. PP2A is comprised of several subunits and dephosphorylates a wide array of substrates[117]. Consistent with this fact, IRF3 activation and subsequent PPP2R1B induction were associated with reduced serine phosphorylation of hepatocyte AKT and AMP-activated protein kinase (AMPK). Finally, overexpression of PPP2R1B alone was shown to phenocopy expression of the constitutively active IRF3 isoform with respect to the increase in glycaemia[116]. These data are consistent with the finding that knockdown of either STING or IRF3 led to diminished free fatty acid-induced hepatic inflammation, improved glycogen storage and reductions in gluconeogenic enzyme expression[118].

Little is known about the role of IRF3 in the other major insulin-sensitive tissue, skeletal muscle. LPS has been reported to inhibit insulin sensitivity in myocytes, in part by reducing GLUT4 expression[119,120], but it is unknown if IRF3 mediates these effects.

IRF3 and hepatic steatosis

Overnutrition and obesity promote lipid accumulation in hepatocytes, leading to MASLD. Global *Irf3*$^{-/-}$ mice are protected from hepatosteatosis after HFD feeding, even when studied at equivalent body weights to their wild-type littermates[116]. Interestingly, hepatocyte-specific IRF3 knockout mice were not protected from steatosis, indicating that IRF3 must be acting in cells other than hepatocytes to promote lipid accumulation in the liver[116]. This result is not entirely surprising given that liver monocyte and/or macrophage populations can drive steatosis[121]. In human cohorts, phosphorylated IRF3 levels were elevated in the livers of participants with MASLD compared with healthy participants and there was evidence of increased IRF3 transcriptional activity[116].

IRF3 and cholesterol metabolism

Cholesterol metabolites participate in immunity and cholesterol metabolism is altered by infection. For example, viral infection reduces cholesterol synthesis in macrophages and virally induced type I interferons further reduce intracellular cholesterol in several cell types by inducing the expression of cholesterol-25-hydroxylase, which converts cholesterol to 25-hydroxycholesterol[122–125]. 25-Hydroxycholesterol then blocks the entry and replication of a wide range of viruses, including Zika, HIV and Ebola[122,124,126]. Following viral infection, macrophages repress 7-dehydrocholesterol reductase expression. 7-Dehydrocholesterol reductase catalyses the final step in cholesterol synthesis; therefore, infection reduces cholesterol levels in the cell and causes the accumulation of the precursor sterol 7-dehydrocholesterol[57]. 7AKT and AMP-activated protein kinase, in turn, increase levels of AKT3, which phosphorylates and activates IRF3 (ref. 57). In addition, stimulation of TLR3 and TLR4 represses cholesterol efflux from macrophages. This repression is accomplished by inducing IRF3, which prevents LXR from engaging its target promoters, including that of the critical cholesterol transporter ABCA1 (ref. 127). This NF-κB-independent process might underlie some of the atherogenic actions of inflammation. How IRF3 inhibits LXR is still unclear, although IRF3 strongly reduces the expression of RXRα, a critical heterodimerization partner of LXR[93].

IRF3 as a downstream effector of leptin

Leptin is an adipocyte-derived hormone that signals satiety; when leptin binds to its receptor on various types of hypothalamic neurons, food intake is reduced and energy expenditure is increased[128]. Leptin exerts some of its effects through transcriptional mechanisms, and STAT3 and ATF3 have both been identified as leptin-sensitive transcription factors. A 2023 unbiased search for other transcriptional mediators of leptin action, currently available as a preprint, used chromatin state mapping in leptin-sensitive AgRP neurons to identify transcription factor-binding motifs in regions altered by leptin administration — among the top-scoring hits was an ISRE[129]. Consistent with this finding, loss of IRF3 in AgRP neurons in mice conferred resistance to the satiety-inducing effects of leptin, while expression of a constitutively active IRF3 allele in these cells suppressed feeding even in the absence of leptin. Furthermore, IRF3 was shown to undergo nuclear translocation in response to leptin[129]. However, many important questions remain regarding other cell types that might utilize IRF3 as a downstream mediator of leptin action, the upstream signalling pathways that connect the leptin receptor to IRF3, and the specific gene targets of IRF3 in AgRP neurons.

IRF4

IRF4 was originally described as an immune cell-specific member of the IRF family[130]; in fact, it was originally called LSIRF, for lymphocyte-specific IRF. However, over the past decade, data has accumulated indicating that IRF4 is also expressed in adipocytes and myocytes, sometimes to high levels. In immune cells, IRF4 exerts many effects on the development and function of B and T lymphocytes, macrophages, and dendritic cells, and mice and humans bearing germline mutations in IRF4 exhibit combined immunodeficiency[131,132]. Unlike IRF3, which has a complex mode of activation involving multiple kinases, IRF4 is primarily regulated transcriptionally. IRF4 can bind as a homodimer to classic ISREs, and its functionality can expand by binding as a heterodimer with the transcription factor PU.1 to erythroblast transformation-specific IRF composite elements or with Fos–Jun proteins to activating protein 1 (AP1)–IRF composite elements (AICE1 or AICE2)[133–135]. In addition to binding to DNA, IRF4 has also been reported to physically interact with MyD88 in a competitive manner with IRF5 (ref. 136). Some functions of IRF4 might therefore represent inhibition of IRF5 activity rather than direct actions of IRF4 on the genome.

IRF4 in immune cells

T cells, including $CD4^+$ T helper cells, require IRF4 for their formation and activation[137,138]. Interestingly, mice lacking IRF4 also show defective $CD8^+$ cytotoxic T cell expansion and function, an effect tied to direct regulation of oxidative and glycolytic metabolism by IRF4 in these cells[139]. Adipose tissue T regulatory cells, which limit obesity-associated inflammation and insulin resistance[140], also require IRF4. IL-33, secreted by specific subtypes of adipose stromal cells[141], drives differentiation and maintenance of T regulatory cells, an effect accomplished by promoting the binding of IRF4 and its partner BATF to the *Pparg* locus, among others[142].

IRF4 was initially discovered to act as a negative regulator of TLR4-mediated responses in macrophages[136,143]. In adipose tissue, M2-like macrophages promote tissue remodelling and protect against many of the adverse consequences of HFD feeding. Relative to M1-like proinflammatory macrophages, the alternatively activated (M2-like) state of a macrophage is characterized by enhanced glucose utilization, fatty acid oxidation and oxidative phosphorylation, all of which are promoted by IRF4 (ref. 144). In this regard, IRF4 acts downstream of IL-4, mTORC2, STAT6 and JMJD3, all drivers of the alternatively activated state[144–147]. As one might predict, knockout of IRF4 in myeloid cells ($Irf4^{\Delta LysM}$) increases inflammatory cytokine production by adipose tissue macrophages and leads to substantial insulin resistance on HFD despite no difference in body weight relative to wild-type controls[145]. The detrimental effect on insulin signalling seen in $Irf4^{\Delta LysM}$ mice was noted in WAT, muscle and liver[145].

IRF4 in adipocytes

As mentioned earlier in the ‘IRF3 and adipogenesis’ section, the IRF DNA-binding motif emerged as highly enriched in enhancer elements associated with adipogenesis[90,91]. IRF4 was one of several IRFs found to block adipocyte differentiation but the fact that the IRF motif was enriched in adipocytes relative to preadipocytes suggested that it might also play an important role in mature cells[91]. This idea was supported by the observation that IRF4 expression was dramatically increased in human and mouse adipocytes by fasting[148]. FOXO1, which translocates to the nucleus as insulin levels fall during fasting, is a major driver of *Irf4* expression in adipocytes[148]. In adipocytes, IRF4 was shown to mediate the promotion of lipolysis and inhibition of lipogenesis induced by fasting. These effects on lipolysis and lipogenesis might be mediated by IRF4 binding to genes encoding key lipolytic enzymes, such as adipose triglyceride lipase (ATGL; encoded by *Pnpla2*) and hormone-sensitive lipase (HSL; encoded by *Lipe*), although other, unknown gene targets are also probably involved.

Mice lacking IRF4 in adipocytes cannot maximally increase their basal lipolysis in response to cold, β-adrenergic agonist treatment or prolonged fasting[148]. The effect of insulin to repress IRF4 expression via the physical sequestration of FOXO1 in the cytosol is an unappreciated mechanism by which insulin represses lipolysis, albeit on a slower timescale than achieved by processes such as the activation of PDE3B[149]. In a separate set of studies, the bacterial cell wall component muramyl dipeptide was shown to act as an insulin sensitizer in mice, working through RIPK, NOD2 and IRF4, although the dependency of muramyl dipeptide on adipocyte IRF4 to exert its insulin-sensitizing effects was only seen in male mice[150,151].

Interestingly, mice lacking IRF4 in adipocytes also show increased body weight and cold intolerance compared with wild-type mice, effects that were originally ascribed to the defect in lipolysis[148]. However, these effects were subsequently shown to result primarily from the actions of IRF4 in thermogenic brown and beige adipocytes. Cold exposure, which induces sympathetic activation, and cAMP, which is downstream of sympathetic action, induce the expression of *Irf4* in brown adipocytes. Additionally, ablation of IRF4 using a brown and beige adipose-restricted Cre (Ucp1-Cre) leads to the same body weight and cold intolerance

phenotype seen in mice lacking IRF4 in all adipocytes[152]. Loss of IRF4 in brown and beige adipocytes prevented cold from inducing the thermogenic gene programme and, conversely, mice that overexpressed IRF4 in a Ucp1-Cre-dependent manner displayed enhanced energy expenditure and were resistant to diet-induced obesity[152]. At a molecular level, IRF4 was found to serve as the DNA-binding partner of the critical thermogenic coactivator protein PGC1α (encoded by *Ppargc1a*); overexpression of PGC1α was not effective in inducing thermogenic genes in the absence of IRF4 (ref. 152).

Taken together, IRF4 promotes adipose thermogenesis directly in brown adipose tissue (BAT) but also indirectly by providing a substrate for oxidation via lipolysis in white adipocytes (Fig. 5). The effect of IRF4 inducing the M2-like state in macrophages might also be involved as alternatively activated macrophages have been proposed to enhance thermogenesis[153–155], although this suggestion has been disputed[156].

Unexpectedly, loss of IRF4 in BAT was also found to affect skeletal muscle indirectly, with mice showing reduced exercise capacity compared with wild-type controls. Despite no reduction of IRF4 levels in myocytes, the muscles of BAT-specific *Irf4* knockout mice showed numerous defects, including central nuclei (an indicator of muscle dysfunction), reduced mitochondrial number and function, tubular aggregates, and diminished mTOR signalling, the latter causing reduced ribosomal subunit synthesis[157]. The BAT of these mice was characterized by increased expression of many myocyte-specific genes compared to wild-type mice, including myostatin, which was shown to mediate these negative effects on muscle. As one might expect given the role of the sympathetic nervous system in inducing IRF4 expression, placement of mice in thermoneutral conditions (30 °C) phenocopied the effect of IRF4 ablation on exercise capacity, an effect reversed by surgical removal of BAT[157].

IRF4 in skeletal muscle

IRF4 is normally expressed at very low levels in skeletal muscle; however, muscle levels of IRF4 increase in two seemingly opposing conditions, exercise and obesity[158,159]. Mice lacking IRF4 specifically in skeletal muscle have no obvious metabolic phenotype in the chow-fed state[158]. However, these same mice are protected from the adverse consequences of a HFD, showing resistance to weight gain and improved glycaemic control relative to wild-type mice. Conversely, mice that overexpress IRF4 in skeletal muscle showed increased weight gain on HFD. IRF4 was found to directly activate the expression of mitochondrial branched-chain aminotransferase in myocytes, which catalyses the catabolism of branched-chain amino acids[159]. However, it is unclear whether the phenotype of increased weight gain in these mice derives from alterations in branched-chain amino acids or from changes in their breakdown product, branched-chain ketoacids[160].

The same group also demonstrated that mice lacking IRF4 in muscle have increased exercise capacity relative to wild-type controls, while mice that overexpress IRF4 specifically in myocytes have the opposite phenotype. The effect of IRF4 on exercise tolerance was not associated with altered blood levels of glucose or lactate but was instead caused by alterations in muscle levels of glycogen, with lower IRF4 levels associated with increased glycogen stores in muscle. The mechanism was reported to be an indirect effect of IRF4

on the expression of *Ppp1r3c*, a subunit of protein phosphatase 1 (also known as protein targeting to glycogen). *Ppp1r3c* was increased in the muscle of mice lacking IRF4 relative to wild-type controls, while the opposite was seen in mice overexpressing IRF4 in muscle[158]. Mice lacking IRF4 in muscle also display less hepatic steatosis and fibrosis when on a HFD than wild-type littermates, an effect mediated by follistatin-like protein 1 (FSTL1)[161]. IRF4 induces the expression of FSTL1 in myocytes, and lack of IRF4 reduces serum levels of FSTL1. Restoring serum levels of FSTL1 to normal in mice lacking IRF4 in myocytes is sufficient to cause hepatic steatosis[161].

IRF4 summary

Overall, the available data makes it difficult to draw overarching conclusions about how IRF4 fits into the metabolic framework. Is it primarily a driver of catabolism? In adipocytes, IRF4 promotes the breakdown of triglycerides, fatty acid oxidation and thermogenesis. In muscle, however, it drives weight gain. Does IRF4 promote insulin sensitivity? The net effect of IRF4 in macrophages is to produce an M2-like state that is conducive to insulin action in adipose tissue, muscle and liver. In adipose tissue, IRF4 expression is opposed by insulin but its actions on thermogenesis eventually lead to weight loss and improved insulin action. In muscle, however, we see the opposite effect on body weight and glycaemic control. We will need a better understanding of the specific gene targets of IRF4 in these cell types to determine whether there is a conserved metabolic theme.

In some ways, the actions of IRF4 in metabolically relevant cell types are the opposite of IRF3 (Table 1). Thus, IRF3 polarizes macrophages towards an M1-like pro-inflammatory phenotype, while IRF4 is involved in alternative activation to an M2-like state. Furthermore, IRF3 is anti-thermogenic in beige and brown adipocytes, while IRF4 plays a critical role in inducing a thermogenic gene expression profile in those same cells. An obvious counterexample is the fact that both IRF3 and IRF4 are anti-adipogenic. There is no direct evidence that IRF3 and IRF4 alter each other's expression, and it is unclear how crosstalk is mediated between these two factors, if at all.

Other IRFs

Although not as firmly established as IRF3 and IRF4, there are data that point to roles in metabolism for other IRF family members. Specific details are discussed below; in addition, the strength of the genetic evidence for associations between IRFs and metabolic diseases and traits in humans is presented in Fig. 6.

IRF1

Like IRF3 and IRF4, IRF1 is anti-adipogenic[91]. IRF1 promotes a chondrogenic phenotype when overexpressed in adipose-derived stem cells[162], and it is known that differentiation down the osteochondral pathway precludes adipogenesis[163]. In mature adipocytes, IRF1 is pro-inflammatory and has effects that oppose insulin sensitivity and metabolic health. For example, IRF1 was found to be more active in primary adipocytes isolated from human WAT than in mature adipocytes derived from cultured human preadipocytes in vitro, and this higher level of IRF1 activity correlated with a more inflammatory gene expression

portfolio[164]. Concordantly, transplantation of immortalized mouse preadipocyte 3T3-F442A cells into the flanks of mice led to a more inflammatory profile in the newly developed fat pad if the cells had been transfected with a vector expressing human IRF1 compared with a control vector[164].

Interestingly, SARS-CoV-2 infection was associated with an IRF1-dependent gene signature in human lung, liver, WAT and islets, along with reduced expression of insulin signalling components compared with healthy individuals[165]. In vitro modelling suggested that activated IRF1 could be causally linked to reduced insulin action. Finally, a 2017 study found that the thermogenic transcriptional cofactor PRDM16 suppresses the type I interferon response in brown adipocytes, possibly by competing with IRF1 for binding to key target genes[166]. Conversely, type I interferon signalling impaired thermogenic gene expression as well as mitochondrial structure in brown adipocytes[166].

IRF2

IRF2 is generally anti-inflammatory and exerts this effect in macrophages by blocking the actions of HIF1α on glycolytic gene expression[167]. Other work suggests that the effect of IRF2 on macrophage polarization is context dependent[80], but data linking IRF2 to metabolic outcomes are scarce.

IRF5

IRF5 expression is upregulated in the subcutaneous and visceral WAT of patients with obesity and T2DM, correlating with dysglycaemia and inflammatory markers[168,169]. These studies used whole WAT and therefore did not identify which cell type was expressing IRF5; however, mice a HFD also show increased adipose tissue IRF5 levels, found specifically in macrophages[170]. Consistent with this finding, targeted ablation of IRF5 in adipocytes did not affect adiposity or metabolic parameters after HFD feeding, while mice with IRF5 knockout in myeloid cells (*Irf5*$^{\Delta LysM}$) displayed increased inguinal WAT mass and adipocyte hypertrophy on a HFD compared with wild-type controls[169]. Despite increased adipose tissue mass, these mice were partially protected from insulin resistance after HFD feeding. Interestingly, *Irf5*$^{\Delta LysM}$ mice had increased numbers of M2-like macrophages in their visceral adipose tissue along with enhanced TGFβ expression and collagen deposition. Moreover, adipose tissue macrophages lacking IRF5 have increased oxidative metabolism and mitochondrial membrane potential, mediated by transcriptional derepression of the gene encoding growth hormone inducible transmembrane protein (*Ghitm*)[170].

That IRF5 promotes an inflammatory profile in macrophages by driving glycolysis preferentially over mitochondrial oxidation is supported by the fact that gain-of-function polymorphisms at the *IRF5* locus in humans are associated with increased macrophage glycolysis and cytokine expression[171], and by the observation that a human macrophage-like cell line cultured in the presence of repetitive bouts of hyperglycaemia was shown to polarize towards a pro-inflammatory phenotype and that this shift could be blocked by IRF5 knockdown[172]. Of note, *IRF5* variants are associated with human systemic lupus erythematosus (SLE), and loss of *Irf5* protects mouse models from this disease[173]. SLE is associated with higher rates of obesity and insulin resistance than those seen in the general

population[174], although no direct link between IRF5 and metabolic outcomes has been made in the context of SLE.

IRF7

IRF7 is a downstream target gene of IRF3 as well as a frequent heterodimerization partner. Accordingly, to the limited extent that IRF7 has been studied in a metabolic context, its actions mirror those described for IRF3. Thus, IRF7 levels increase in mouse WAT in obesity, and *Irf7*$^{-/-}$ mice on a HFD are protected from the development of obesity, inflammation and insulin resistance[83,175]. In adipose-derived stromal cells, overexpression of IRF7 reduces mitochondrial function as well as branched-chain amino acid degradation[176]. IRF7 also inhibits glycolysis, analogous to the effect seen with IRF3, although it is not known if ISG15 is also a downstream effector of IRF7 (ref. 177).

IRF8

Although IRF8 is most closely related at the sequence level to IRF4, its actions regarding macrophage polarization diverge sharply from those of IRF4. Whereas IRF4 is anti-inflammatory and promotes an M2-like phenotype, IRF8 often works in tandem with IRF1 to induce a pro-inflammatory state in macrophages[178]. Consistent with this idea, mice lacking multiple eIF4E-binding proteins, which inhibit translation, are prone to obesity and their adipose tissue macrophages exhibit an exaggerated inflammatory response associated with elevated IRF8 (and IRF1) levels[179].

IRF9

IRF9 acts downstream of type I interferon signalling as part of a heterotrimeric complex with STAT1 and STAT2, collectively known as ISGF3 (ref. 180). DNA binding of the ISGF3 complex is mediated through IRF9. Interestingly, knocking out the interferon receptor IFNAR1, which acts upstream of ISGF3, does not fully phenocopy knockout of IRF9, suggesting that IRF9 could have transcriptional activity outside of its interactions with STATs[181]. This notion is consistent with observations that IRF9 can interact with PPARα in hepatoma cells[182]. Global loss of IRF9 exacerbates obesity due to HFD in male mice, which can be rescued by treatment with a PPARα agonist. Furthermore, adenoviral overexpression of IRF9 in the livers of obese mice reduces hepatic steatosis and improves glycaemia relative to mice receiving an empty adenovirus[182]. Whether IRF9 binds to the same enhancers as PPARα is not yet known.

Conclusions

IRFs are clearly much more than transcriptional regulators of the interferon response. We should instead consider them as stress sensors perched at the intersection of immunity, cellular differentiation and metabolism. While some of their metabolic effects are probably mediated by cytokines and other inflammatory proteins, IRFs also regulate many non-immune targets such as *Aig1* in adipocytes and *Ppp2r1b* in hepatocytes. Certainly, many open issues remain, including the need for a more complete understanding of which cell types are most relevant to the actions of widely expressed IRFs as well as the development of a comprehensive catalogue of IRF-binding sites and IRF-regulated genes in metabolically

relevant cell types. As new tools emerge and additional studies are published, it is reasonable to expect that the metabolic functions of IRFs will come into sharper focus. This greater understanding of IRFs might allow for novel therapeutic opportunities for conditions such as obesity, T2DM, insulin resistance and MASLD.

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Key points

- Interferon regulatory factors (IRFs) comprise a family of nine transcription factors that evolved coincident with the development of multicellularity in animals and serve to integrate the response to stress, most notably related to infection and inflammation.
- IRFs help to coordinate metabolic physiology and mediate key aspects of metabolic pathophysiology in diseases such as obesity, type 2 diabetes mellitus and metabolic-associated steatotic liver disease.
- There is increasing awareness that IRFs not only affect metabolism through their effects on immune cells but also by altering transcription in parenchymal cells such as adipocytes, hepatocytes, myocytes and neurons.
- IRF3 is generally pro-inflammatory and promotes obesity, insulin resistance, and hepatic steatosis and might also mediate some of the transcriptional actions of leptin in the hypothalamus.
- IRF4 is generally anti-inflammatory and has essential roles in adipogenesis, lipolysis and thermogenesis in adipose tissue.
- Other IRFs are beginning to be implicated in metabolic homeostasis, and more insights are likely to emerge over the next few years.

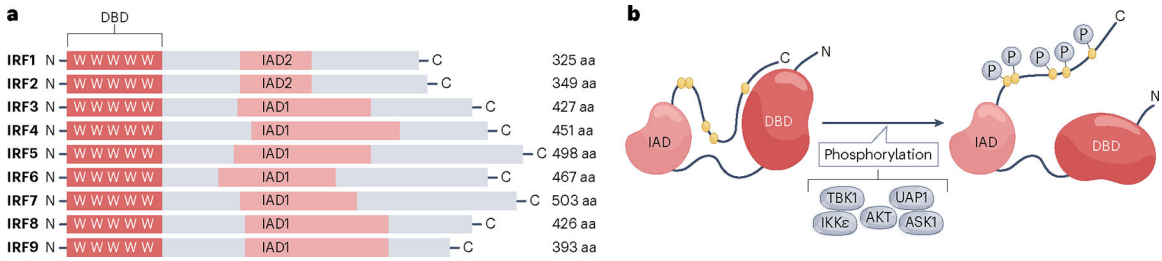

Fig. 1 |. The structure of IRF family members.

a, Domain structure of interferon regulatory factor (IRF) proteins, showing N-terminal DNA-binding domain (DBD), with five tryptophan (W) residues, and the IRF interaction domain (IAD). **b**, Schematic of IRF3 activation showing autoinhibition of the DBD by a C-terminal extension, relieved by phosphorylation. ASK1, apoptosis signal-regulating kinase 1; TBK1, TANK-binding kinase 1; UAP1, UDP-N-acetylglucosamine pyrophosphorylase 1.

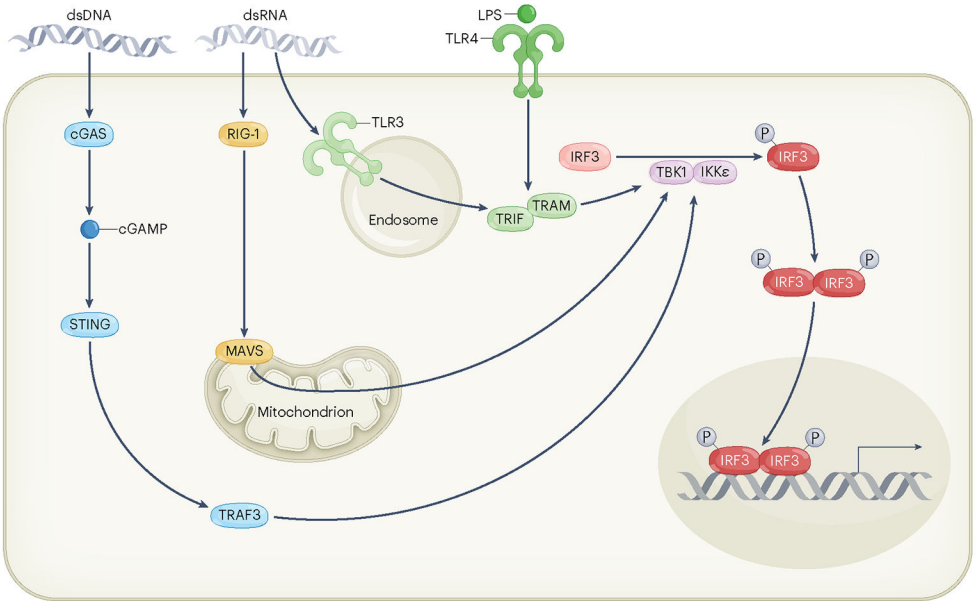

Fig. 2 |. Classic mechanism of IRF3 activation.

Interferon regulatory factor 3 (IRF3) is activated by a variety of signals, including double-stranded DNA (dsDNA) via the cGAS–STING pathway; double-stranded RNA (dsRNA) via the RIG-I–MAVS pathway and/or endosomal Toll-like receptro 3 (TLR3); and lipopolysaccharide (LPS) via TLR4. All of these pathways culminate in the activation of TANK-binding kinase 1 (TBK1) and IKKε, with mediation by various adaptor proteins. Once phosphorylated by TBK1–IKKε, IRF3 dimerizes and translocates to the nucleus.

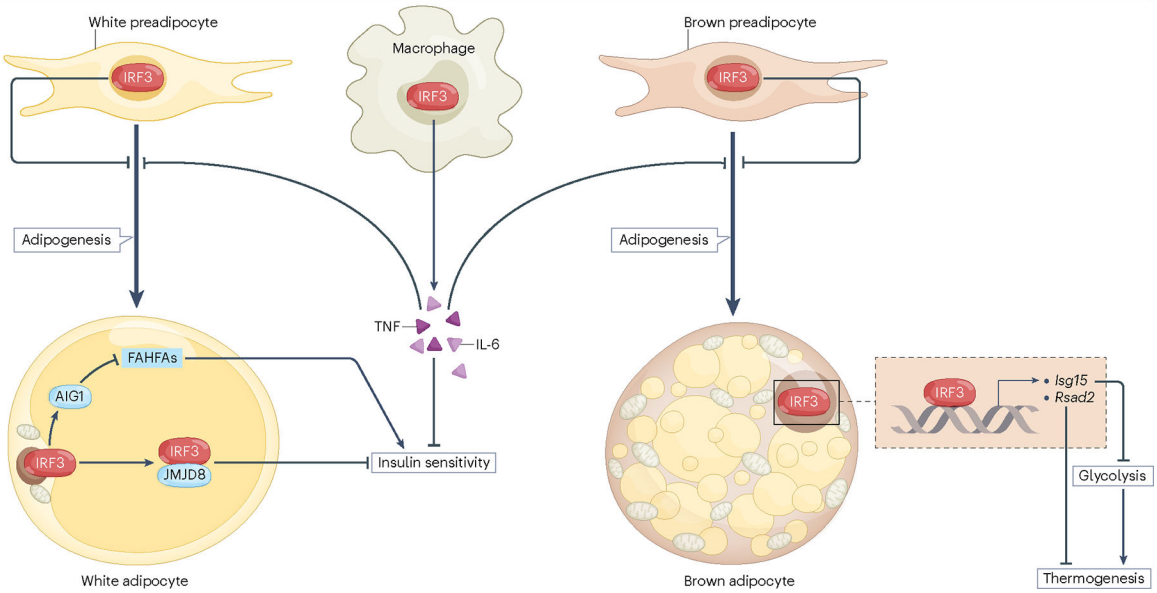

Fig. 3 |. Regulation of adipose insulin sensitivity and thermogenesis by IRF3.

Interferon regulatory factor 3 (IRF3) affects insulin action in white adipose tissue through several mechanisms, including inhibition of adipogenesis in preadipocytes, induction of androgen-inducible gene 1 (AIG1) and interaction with JMJD8 in mature white adipocytes and release of cytokines by macrophages. In brown adipose tissue, IRF3 inhibits the differentiation of thermogenic adipocytes and drives the expression of target genes in mature brown and beige adipocytes. These genes include *Isg15*, which inhibits thermogenesis by repressing glycolysis, and *Rsad2*. FAHFAs, fatty acid esters of hydroxy fatty acids.

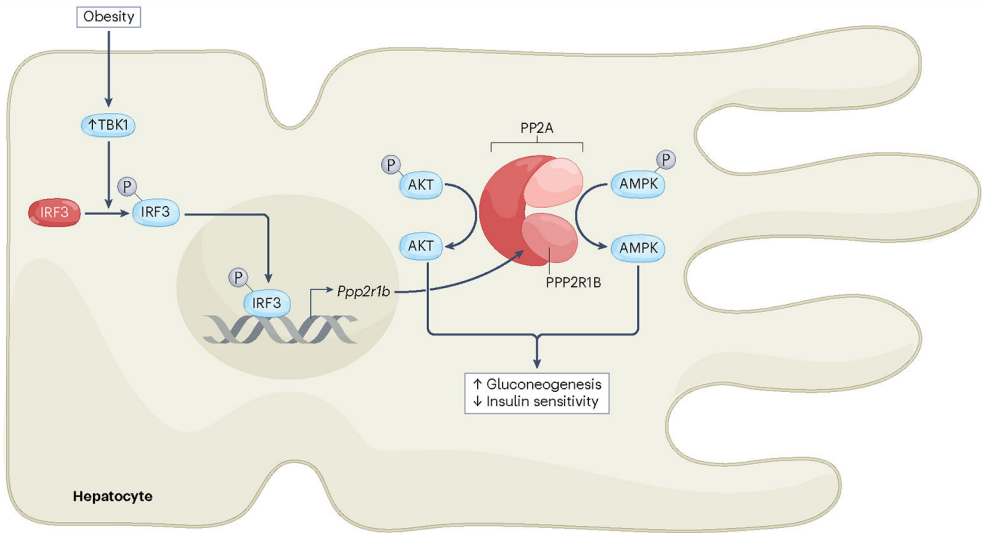

Fig. 4 |. The effect of IRF3 in hepatocytes.

Obesity increases TANK-binding kinase 1 (TBK1) expression and activation in hepatocytes. TBK1 then phosphorylates interferon regulatory factor 3 (IRF3) and induces a transcriptional signature that includes expression of *Ppp2r1b*. The PPP2R1B protein is a component of the PP2A protein phosphatase complex, which dephosphorylates AKT and AMP-activated protein kinase (AMPK) and AKT, leading to increased gluconeogenesis and reduced insulin sensitivity. Other IRF3 gene targets and/or other PP2A protein targets might also participate in the metabolic changes associated with obesity.

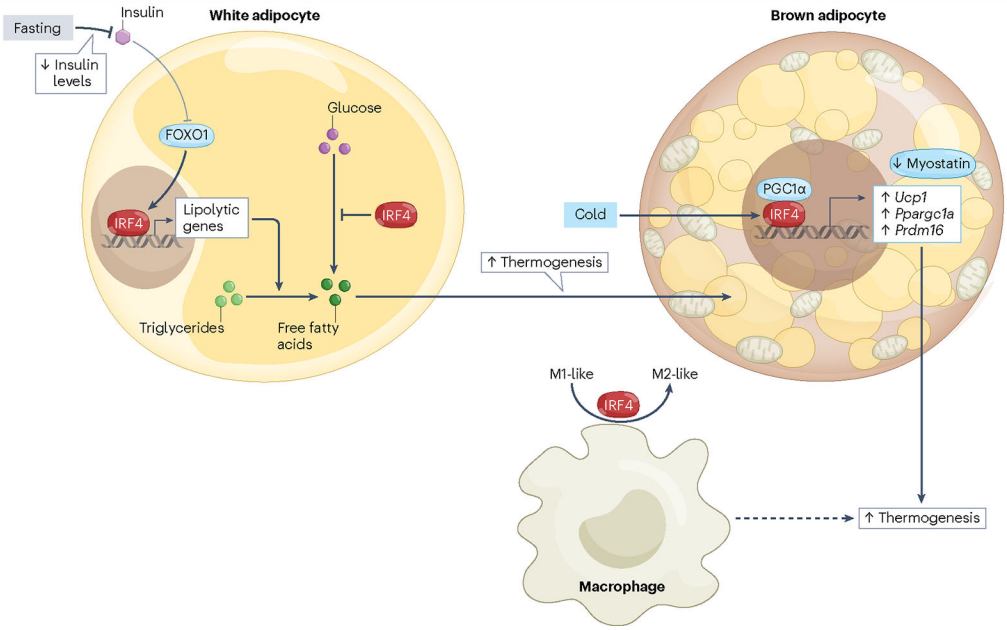

Fig. 5 |. IRF4 and adipose thermogenesis.

Interferon regulatory factor 4 (IRF4) promotes thermogenesis by coordinating the activities of multiple cell types. In brown adipocytes, IRF4 is induced by cold exposure and has direct effects on the thermogenic gene portfolio by acting as the transcription factor partner of the thermogenic cofactor PGC1α. In white adipocytes, IRF4 levels are increased by fasting, which acts through reduction of insulin levels and subsequent activation of FOXO1. IRF4 suppresses lipogenesis and drives expression of lipolytic genes, providing a substrate for thermogenesis. IRF4 also promotes the alternatively activated (M2-like) state of macrophages, which could have additional effects on thermogenesis.

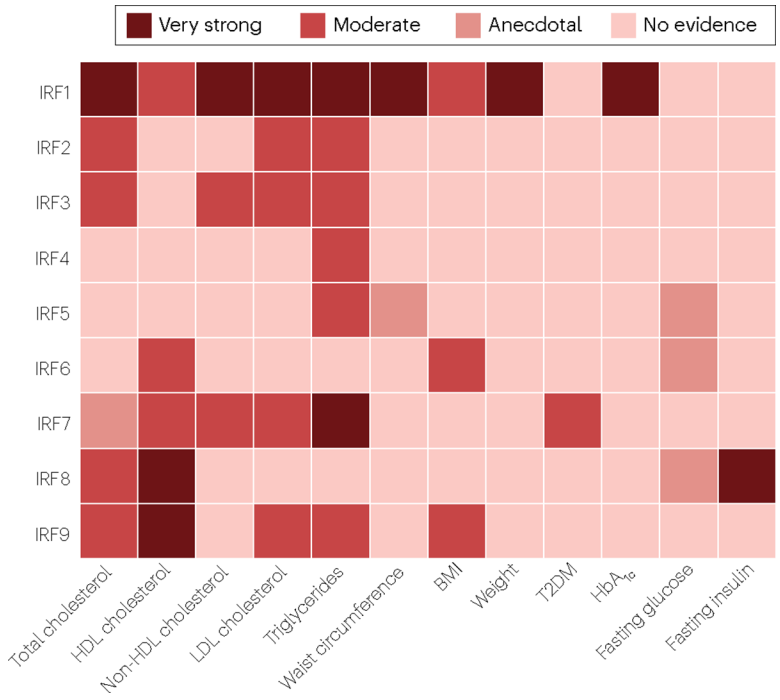

Fig. 6 |. Associations between IRF family members and metabolic traits.

Heat map depicting the strength of various associations between the genes encoding interferon regulatory factors (IRFs) and metabolic traits. The score is based on the Human Genetic Evidence Calculator (HuGE), calculated by Flannick and colleagues[183], available at the Type 2 Diabetes Knowledge Portal. T2DM, type 2 diabetes mellitus.

Table 1

Comparison of metabolic actions of IRF3 versus IRF4

Location	Effect	
	IRF3	**IRF4**
Hypothalamus	Increased satiety	Unknown
Immune cells	Increased levels and activity of M1-like macrophages	Increased levels and activity of M2-like macrophages; T regulatory cell development
Liver	Increased gluconeogenesis; promotion of insulin resistance; increased steatosis (probably indirect)	Unknown
Adipose tissue	Repression of adipogenesis; increased inflammation; reduced insulin sensitivity; reduced thermogenesis	Repression of adipogenesis; promotion of lipolysis; promotion of thermogenesis; indirect enhancement of muscle function by repression of myostatin in brown adipose tissue
Muscle	Unknown	Promotion of weight gain on a high-fat diet; reduced exercise capacity; indirect promotion of hepatic steatosis via induction of follistatin-like protein 1

IRF, interferon regulatory factor.