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Wnt signalling and the control of cellular metabolism

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Abstract

At the cellular level, the biological processes of cell proliferation, growth arrest, differentiation and apoptosis are all tightly coupled to appropriate alterations in metabolic status. In the case of cell proliferation, this requires redirecting metabolic pathways to provide the fuel and basic components for new cells. Ultimately, the successful co-ordination of cell-specific biology with cellular metabolism underscores multicellular processes as diverse as embryonic development, adult tissue remodelling and cancer cell biology. The Wnt signalling network has been implicated in all of these areas. While each of the Wnt-dependent signalling pathways are being individually delineated in a range of experimental systems, our understanding of how they integrate and regulate cellular metabolism is still in its infancy. In the present review we reassess the roles of Wnt signalling in functionally linking cellular metabolism to tissue development and function.

Keywords

diabetes; metabolic syndrome; metabolism; obesity; Wnt signalling

INTRODUCTION

The name Wnt is derived from a combination of two homologous genes; Wg (the *Drosophila* wingless gene) and Int [the murine homologue MMTV (mouse mammary tumour virus) integration site 1 gene]. Wnts represent a large morphogenic family of secreted lipid-modified glycoproteins that control multiple developmental processes during embryogenesis including cell-fate specification, progenitor-cell proliferation and the control of asymmetric cell division. In adult tissues and organs, Wnts also function to regulate tissue maintenance and remodelling as exemplified by aging bone, adipose tissue plasticity, liver regeneration, muscle regeneration and cancer progression.

Tissue remodelling or plasticity is associated with metabolic and functional adaptations, and is accompanied by titrated recruitment, proliferation and differentiation of specific cell types. This is seen during myogenesis, adipogenesis, liver regeneration and angiogenesis. In the context of diseases such as NAFLD (non-alcoholic fatty liver disease), diabetes and obesity, Wnt signalling has been implicated, in part because many of these diseases involve

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significant remodelling of adult tissues. In all cases, highly regulated yet flexible signalling networks are required to control these complex developmental processes. Such a network should be capable of integrating multiple cues and translating these into the production of a titrated, but co-ordinated response in an otherwise cellular heterogeneous tissue. At the cellular level, Wnt signals are well placed to not only sense alterations in fuel availability and cellular stress, but can themselves co-ordinate changes in cellular metabolism. In the present review we discuss whether the Wnt signalling network could play this integrator role in metabolically relevant organs.

This review comes in three parts. First, we provide an overview of the key components and signalling networks that mediate and integrate with Wnt signals. Secondly, we discuss the molecular links that connect Wnt signalling to cellular metabolic pathways. Finally, we review the role of Wnt signalling in whole-body energy homoeostasis and in diseases associated with metabolic dysregulation.

THE COMPONENTS AND PATHWAYS THAT COMPRISE WNT SIGNALLING NETWORKS

At first glance there is an intimidating array of proteins involved in mediating and modulating Wnt signalling networks [1,2]. In vertebrates, the Wnt ligand family alone comprises up to 19 members of secreted, hydrophobic glycoproteins. There are also more than 12 putative cell-surface receptors and co-receptors. It should, therefore, come as no surprise that Wnts activate more than one type of signalling cascade. That being said, very few (if any) of the intracellular mediators of Wnt signals appear to be unique to Wnt signalling pathways. Indeed, to date only the Wnt ligands and their receptors appear to be selectively involved in Wnt-induced signals. It is this aspect that allows Wnt signals to cross-talk with other signalling pathways and result in integrated, context-dependent cellular responses.

Classification of Wnt signalling pathways

Wnt signalling relies on a sophisticated regulated network of auto-crine and paracrine signalling pathways that are capable of providing titrated responses during development and tissue remodelling. In simple terms, binding of specific Wnt ligands to their cell-surface receptors transduces intracellular signals through either β-catenin-dependent or β-catenin-independent pathways. Wnt/β-catenin-dependent pathways can lead to regulation of TCF/LEF (T-cell factor/lymphoid-enhancer binding factor) transcriptional activity which determines cellular fate such as stem-cell determination, proliferation and differentiation. In contrast, β-catenin-independent pathways have been preferentially implicated in cell movement and polarity and are critical determinants of diverse processes such as gastrulation, inner ear cell polarity, dorsoventral patterning, tissue separation, neuronal migration and oncogenesis [3-5].

While these classifications are helpful in describing the distinct mechanisms of Wnt signal transduction, it is important to note that these signalling pathways are not mutually exclusive and may occur simultaneously (as discussed below). Indeed, the network of Wnt signals is

highly dynamic, and is dependent on the cell-type, tissue and specific developmental stage. Hence it is often referred to as context-dependent. Furthermore, this dynamic flexibility is fundamental to the ability of Wnt signalling networks to adapt in response to changes in local and environmental metabolic cues.

β-Catenin-dependent Wnt signalling—The classical Wnt-signalling pathway (formerly referred to as the canonical Wnt-signalling pathway) has as its main determinant a soluble pool of cytosolic β-catenin, associated with its own degradation complex. This complex comprises the core proteins Axin, APC (adenomatous polyposis coli) and GSK3 (glycogen synthase kinase-3) (Figure 1A). Additional proteins associated with this complex include GBP [GSK-binding protein; also called FRAT1/2 (frequently rearranged in advanced T-cell lymphomas 1/2)], substrate priming kinases CKs (casein kinases) 1α/2 and the catalytic subunit of PP2A (protein phosphatase 2A). In the absence of Wnt ligands (basal unstimulated state) this degradation complex rapidly phosphorylates cytosolic β-catenin and marks it for subsequent ubiquitination-dependent proteasomal degradation [6]. The β-catenin ubiquitination machinery has been elucidated and at least two multimeric complexes have been described. Both are multimeric RING-type E3 complexes: $SCF^{\beta\text{-}TrCP}$ and Siah1–SIP complexes [7-9].

Binding of specific Wnts (such as Wnt1, Wnt10b or Wnt3A), to Fzd (Frizzled)–LRP5 or Fzd–LRP6 receptor complexes (Figure 1B), results in activation of intracellular heterotrimeric G-proteins and Dvl (Dishevelled) proteins, which in turn leads to phosphorylation-dependent recruitment of Axin to the LRP5 (or LRP6) co-receptor [10,11]. The sequestration of Axin to the receptor complex results in disassembly of the β-catenin degradation complex and thereby promotes the stabilization and accumulation of cytosolic β-catenin. This coincides with the nuclear translocation of β-catenin, where it displaces the transcriptional co-repressors Groucho and/or CBP [CREB (cAMP-response-element-binding protein)-binding protein] from the transcription factors of the TCF and LEF families. In so doing, β-catenin acts as a transcriptional co-activator to induce context-dependent Wnt/β-catenin target gene expression. For reviews see [3,5,15].

{An unusual feature of β-catenin is that nuclear import occurs without a NLS (nuclear localization signal). While the mechanism involved remains elusive, studies by our groups and others, suggest that targeting β-catenin directly to the nucleus (through addition of an NLS) is also not sufficient for transcriptional activity. Instead, other factors and/or posttranslational modifications may be a prerequisite for functional nuclear β-catenin [12-14].}

There are more than 80 target genes known to be regulated by the Wnt/β-catenin pathway [1,16,17] (http://www.stanford.edu/~rnusse/pathways/targets.html). Together their expression and activity controls many Wnt-related processes, such as cell fate determination, cell proliferation and self-renewal of stem and progenitor cells. Many, but importantly not all, Wnt/β-catenin target genes have been confirmed to have either LEF- or TCF-responsive promoters or have functionally relevant TCF-binding sites outside the promoter (i.e. in the first intron). Nonetheless Wnt/β-catenin/TCF target genes include those that promote cell-cycle progression [i.e. c-Myc, cyclin D1 and Id2 (inhibitor of DNA binding 2)], cellular

differentiation (i.e. Myf5, Runx2, Dlx5 and osterix), metabolism (see the sections below) and also genes that feedback to modulate both amplitude and duration of active Wnt/β-catenin signalling pathways [i.e. Dkk1 (Dickkopf-related protein 1), Axin2, β-TrCP (β-transducing repeat-containing protein), Dact1 and sFRPs (secreted Fzd-related proteins)] as summarized in Figure 1(C) and at http://www.stanford.edu/~rnusse/pathways/targetcomp.html.

In addition to TCF and LEF, nuclear β-catenin binds other transcription factors, some of which are themselves linked to metabolic regulation. For example, β-catenin interacts with FOXO (forkhead box O) transcription factors particularly under conditions of oxidative stress, and therefore has been implicated in aging and the stress response [18]. It is suggested that FOXO proteins may compete with TCFs for a limited pool of free β-catenin in response to stress [19]. Unsurprisingly, these interactions can be further influenced by growth factors such as insulin and IGF (insulin-like growth factor). β-catenin has also been shown to bind transcription factors that regulate lipid metabolism: PPARγ (peroxisome-proliferator-activated receptor γ) [20,21], RXRα (retinoid X receptor α) [22] and RAR (retinoic acid receptor) [23]. Other factors that bind nuclear β-catenin and are themselves linked to cell-fate determination pathways include MyoD [24], sox3 and sox17 transcription factors [25], and Prop1 (paired-like homeodomain factor 1 or prophet of Pit1) and Pitx2 (paired-like homeodomain transcription factor 2) [26]. Finally, β-catenin also binds chaperone proteins that may play important roles in its activity, nucleocytoplasmic distribution [27] and nuclear export [28].

β-Catenin-independent Wnt signalling—β-Catenin-independent (previously referred to as the 'noncanonical') Wnt signalling pathways do not require the transcriptional activity of β-catenin and represent a diverse array of signalling pathways that are less well-characterized. These can be further subclassed according to their specific receptor usage: (i) Fzd and LRP5/6-mediated, but β-catenin-independent; (ii) Fzd-receptor-mediated, but LRP co-receptor-independent; and (iii) non-Fzd-receptor-mediated.

(i) Fzd and LRP5/6-mediated, but β-catenin-independent, signals: the mTORC1 complex: To date, only one Wnt-stimulated pathway has been reported to be activated independently of β-catenin, but retains the requirement of both Fzd and LRP 5/6 receptors. This pathway links Wnt-receptor binding to activation of the mTORC1 complex. The mTORC1 complex consists of mTOR, Raptor (regulatory associated protein of mTOR) and mLST8 (mammalian homologue yeast LST8). It functions to stimulate ribosome biogenesis and protein synthesis. Wnt-induced activation of this pathway provides the first molecular mechanism to link Wnt signalling to stimulation of protein translation (Figure 2A). The receptor proximal events involve Wnt-induced inhibition of GSK3 activity and require the GSK substrate TSC2 (tuberous sclerosis complex 2 or Tuberin) [29]. TSC2 is a tumour suppressor complex that controls protein translation through Rheb and mTOR. In addition to requiring Fzd, LRP5/6 and GSK3, the receptor-interacting scaffold proteins Dvl and Axin have also been implicated. However, it remains to be formerly established whether the same Wnt–receptor complexes are indeed involved in this pathway as those that activate Wnt/β-catenin signalling [30].

(ii) Fzd-mediated, but LRP5/6-independent, signals: Wnts can signal through Fzd receptors alone to activate multiple second messengers including Ca^{2+}, cGMP and cAMP. Most (if not all) of these signalling pathways regulate microtubule and actin remodelling, and/or activate transcription factors such as NFAT (nuclear factor of activated T-cells), AP-1 (activator protein 1) and CREB.

Both PCP (planar cell polarity) and AB (apical–basal) polarity are regulated by Wnts and involve establishing cellular asymmetry. It is this cellular asymmetry that underlies developmental processes of epithelization, asymmetric cell division and directed cell migration. In vertebrates, these signals are required for processes such as gastrulation and sensory cell orientation. The PCP pathway is arguably the best-characterized β-catenin-independent Wnt-related process. Although the detailed molecular mechanisms that control Wnt-induced redistribution of cellular components remain under debate, activation of this pathway involves the asymmetric redistribution of evolutionarily conserved membrane receptors (and their associated complexes) [31]. These include Fzd (in association with Dvl-Inversin) and the four-pass transmembrane protein Vangl2 (Van Gogh-like 2). Vangl2 interacts with mpk1 (mouse prickle 1) [32] and Dact1 (Dapper1) [33]. Mutations in the components of the PCP pathway in mice have been shown to cause embryonic defects in both axis elongation and neural tube closure.

Among several putative intracellular effectors, PCP signalling is linked to the regulation of two kinases: ROCK (Rho-associated kinase) [34] and JNK (c-Jun N-terminal kinase) [35]. Following Wnt-induced Dvl recruitment to Fzd receptors, two distinct small G-proteins are independently activated: Rho and Rac. Wnt-induced ROCK1 activation requires the interactions between Rho and Daam1. In contrast, Wnt-induced JNK activation, requires Rac and is independent of Daam1 [36] (Figure 2B). Together these signals control the actin remodelling that accompanies cell polarization and motility. It is likely that both cell polarization and motility are energy-consuming processes; however, no evidence currently exists to directly link this pathway to cellular metabolism. Nonetheless, given the potential interactions with other Wnt signalling pathways (as discussed below), it remains an unchartered territory.

The Wnt/Ca^{2+} signalling pathway is defined by Wnt-Fzd-induced PLC (phospholipase C) activation and results in an increase in intracellular cytoplasmic Ca^{2+} levels (Figure 2B). These Ca^{2+} fluxes lead to the activation of Ca^{2+}-sensitive PKCs (protein kinase Cs), CaMKII (Ca^{2+}/calmodulin-dependent protein kinase) and NFAT. In parallel this pathway leads to decreases in intracellular cGMP and can inhibit Wnt/β-catenin/TCF signalling [37,38]. Wnt/Ca^{2+} signalling is involved in regulating cellular adhesion, cytoskeleton rearrangement (e.g. during cell division) and other developmental processes, such as dorsoventral patterning and tissue separation in embryos [39].

Other β-catenin-independent Wnt signals activated by Fzd receptors are less well-characterized and further illustrate the scope of this network. These include: (i) cAMP/PKA (protein kinase A)/CREB to induce expression of MyoD, Myf5 and Pax3 [40]; (ii) CK1e/RAP1 to regulate E-cadherin, integrins and actin cytoskeleton [41]; and (iii) Dvl/aPKC (atypical PKC) activation to regulate microtubules [42]. With this variety in signalling

pathways, it is clear that additional questions remain to be addressed. Are these truly independent signalling pathways? And are they differentially activated in a context-dependent manner (e.g. species-, tissue- and developmental-stage-specific) or by a subset of specific Wnt-Fzd interactions?

(iii) Non-Fzd-receptor-mediated signals: Wnts have also been reported to bind to and activate non-Fzd receptors including the receptor tyrosine kinases ROR2 (receptor tyrosine kinase-like orphan receptor 2) and Ryk (receptor related to tyrosine kinase). Wnt5a-induced ROR2 activation has been described to function in convergent extension and cell migration during skeletal, respiratory and cardiac development. This is substantiated by the fact that murine phenotypes of loss-of-function ROR2 are similar to Wnt5a and characterized by dwarfism, facial anomalies, short limbs and tails, and respiratory dysfunction, leading to neonatal lethality [43]. While the proximal signals transduced by ROR2 in vertebrates remain to be fully validated, in *Xenopus* convergent extension, the ROR2 receptors activate the PI3K (phosphoinositide 3-kinase)-Cdc42 (cell division cycle 42)-MKK7 [MAPK (mitogen-activated protein kinase) kinase 7]-JNK pathway, resulting in activation of AP-1 (ATF2 and c-Jun) and the expression of PAPC (paraxial protocadherin) (Figure 2C). In addition, Wnt binding to ROR2 can also synergize with Wnt/PCP signalling and inhibit Wnt/β-catenin/TCF target gene expression (for reviews see [5,43,44]).

Wnt engagement of Ryk receptors has been linked to axon guidance and differentiation [45]. Ryk has been described to signal alone to activate the Src family of tyrosine kinases and also as a co-receptor with Fzd to activate the Wnt/β-catenin signalling pathway. Wnt stimulation can also promote the protease-dependent cleavage and nuclear localization of the ICD (intracellular domain) of Ryk (Figure 2C). This pathway has been linked to the control of neuronal differentiation [5,46].

Cross-talk within the Wnt signalling network

As mentioned previously, the individual Wnt signalling pathways as described above are not mutually exclusive and should not be regarded as functionally independent linear or unidirectional pathways. There is sufficient evidence suggesting that these pathways have built-in feedback mechanisms for both desensitization and/or amplification. They also antagonize and/or synergize by converging with parallel Wnt-induced pathways. Examples of antagonism between Wnt-induced pathways are provided by the inhibition of Wnt/β-catenin signalling by either Wnt/Ca^{2+} signals or Wnt5a/ROR2 signalling [47,48]. This antagonism is physiologically relevant in tissue regeneration and limb development and for the survival of progenitor thymocytes [5]. Similarly, cross-talk between Wnt signalling pathways may occur in the extracellular space where production of extracellular binding proteins (such as sFRPa and Dkk1) antagonize Wnt action on neighbouring cells. This is particularly relevant when considered in the context of the paracrine signals that occur in cellularly heterogeneous tissues. Furthermore, as our knowledge continues to evolve, it is becoming increasingly clear that it is this level of complexity that confers Wnt signalling networks the capacity to integrate multiple cues into co-ordinated, cell-specific responses.

Cross-talk between Wnts and other signalling networks

Cross-talk between Wnt and cadherin signalling—Many studies have described a complex cross-regulation between Wnt signalling, cell–cell adhesion and cell–matrix adhesion [49]. Indeed, it is this aspect that is often overlooked in studies investigating Wnt/β-catenin signalling. In fact intracellular β-catenin also functions as an important component of the cadherin complexes, which function in cell–cell AJs (adherens junctions), the most common type of intercellular adhesions. Furthermore, type I cadherins form links to the intracellular actin cytoskeleton via β-catenin. Hence these intercellular connections are important for maintaining tissue architecture and cell polarity, and can limit cell movement and proliferation. Activation of the Wnt/β-catenin signalling pathway and promoting accumulation of intracellular β-catenin also increases availability of β-catenin for AJs, thereby altering cell–cell adhesion and cell migration. Conversely, when cytoplasmic β-catenin is limited (e.g. absence of Wnts), cadherin may act as a negative regulator of Wnt/β-catenin signalling as it sequesters soluble β-catenin from the nucleus [50]. Cadherin-bound β-catenin also feeds into the Wnt/β-catenin pathway following AJ dissociation [51]. Hence there is evidence for an intersection between cytoplasmic and cadherin-associated pools of β-catenin [52,53]. In contrast, atypical cadherins or protocadherins that do not bind β-catenin can also play important roles in the Wnt/PCP pathway.

Cross-talk between Wnt, Hh (Hedgehog), Notch and TGFβ (transforming growth factor β)/BMP (bone morphogenetic protein) signalling—Multiple levels and nodes of signal integration have been described between Wnt, Hh [54,55], Notch [56] and TGFβ/BMP [57] pathways. Collectively, they all play important roles in both embryonic development (such as organogenesis) and adult tissue homoeostasis (e.g. during tissue repair). Additionally, these pathways are evolutionarily well-conserved, not only individually, but also at the level of their cross-talk and signal integration.

At the molecular level, the Wnt and Hh pathways have several proteins such as Fzd and Smoothened receptors that are structurally similar and hence provide remarkable parallels in their mechanisms of action and regulation, as reviewed in [54,55]. These pathways also share common intermediates such as GSK and CK1 [58].

Both Wnt and Notch signalling have been implicated in local paracrine signals involved in stem-cell self-renewal and much evidence has been provided from genetic studies to support interactions between these two pathways. In contrast with the complexity of Wnt signalling, the mechanism of Notch signalling is apparently simple: Notch acts as a membrane-bound transcription factor, whose intracellular domain (NICD) is released following an interaction between Notch and its ligands Delta and Serrate. This mechanism of protease-dependent activation of receptor-intracellular domains is strikingly similar to that described above for Wnt-induced activation of Ryk signals. However, unlike Wnts, Notch ligands are themselves transmembrane proteins (members of the DSL family Serrate/Jagged/Delta). Cross-talk between Notch and Wnt signalling occurs at multiple levels and can result in opposing responses. On the one hand, Wnt signalling induces the expression of Notch ligands [59,60]. In contrast, Notch and Wnt signalling can also antagonize each other [61]. This is mediated, at least in part, by Notch ligand-induced β-catenin degradation [62] and

involves a direct interaction between the NICD and β-catenin. Furthermore, Notch has been described to bind Dvl [63,64] and GSK3β [65] thereby providing another level of interaction. In the context of cancer biology, oncogenic Wnt signalling is reported to be Notch-dependent in human breast epithelial cells [66] and colorectal cancer [67].

The most common format of TGFβ/BMP and Wnt cross-talk occurs in the nucleus, where the Smad and LEF/β-catenin (or Smad and TCF/β-catenin) synergistically regulate a set of shared target genes [16,17]. TGFβ/BMP and Wnt can also determine the ligand production of each other. For example, TGFβ can up-regulate Wnt2, Wnt4, Wnt5a, Wnt7a, Wnt10a and the Wnt co-receptor LRP5, and lead to increased nuclear accumulation and stability of β-catenin in human bone marrow stromal cells [68]. Conversely, Wnt target genes include BMP-4, BMP antagonists, Nodal (a TGFβ family member) and Cripto (a TGFβ/Nodal coreceptor) [57]. In addition, protein interactions in the cytoplasm have been demonstrated between Smad7 and Axin and it has been suggested that nuclear localization of β-catenin could be Smad3-dependent in mesenchymal stem cells [69]. Hence there are multiple levels at which these pathways are linked in various settings. Moreover such cross-talk is likely to be physiologically relevant in the regulation of bone mass [70] and myofibroblast differentiation [71].

Cross-talk between Wnt and p53 activity—The p53 pathway is sensitive to cellular stresses, in particular those that can disrupt the fidelity of DNA replication, chromosome segregation and cell division [72]. Such stress signals are transmitted to the p53 protein by post-translational modifications, and its transcriptional activation initiates a programme of cell-cycle arrest, cellular senescence or apoptosis [73]. Hence, the tumour suppressor p53 functions (in part) to antagonize the oncogenic actions of Wnt/β-catenin signalling. Cross-talk between p53 and the Wnt/β-catenin pathway occurs primarily through the actions of their respective target genes. Wnt/β-catenin gene products such as p14/19 ARF and Axin inhibit p53 degradation and stimulate p53 functions respectively [74-76]. Conversely, active p53 down-regulates cytosolic β-catenin levels by inducing Siah-1 expression [77,78], thereby promoting β-catenin degradation via a distinct complex [8,9]. The transcriptional activity of p53 also antagonizes Wnt/β-catenin signalling by repressing TCF4 expression [79]. Taken together, this cross-talk may provide the molecular basis for linking Wnt signalling to cellular stress pathways.

MOLECULAR LINKS BETWEEN WNT NETWORKS AND CELLULAR METABOLISM

It has long been known that proliferating cells exhibit markedly altered metabolism in comparison with non-proliferating and/or differentiated cells. Indeed, the Warburg effect describes enhanced consumption of glucose and production of lactic acid in tumour cells relative to normal cells [80]. Recently, this observation has been revisited and rationalized as evidence of context-dependent partitioning of cellular fuel. Normal nonproliferating or differentiated cells primarily utilize nutrients to fuel basic cellular processes and predominantly mediate catabolic metabolism to efficiently generate ATP. In contrast “metabolism in proliferating cells is adapted to facilitate the (enhanced) uptake and

incorporation of nutrients into biomass (anabolic processes, e.g. nucleotide, protein and fatty acid synthesis) needed to produce a new cell" [81,82]. Hence, Warburg's description of 'respiration' versus 'fermentation' can be replaced by the terms 'quiescent metabolism' and 'proliferating metabolism'.

While the concept of context-dependent partitioning of cellular fuel is an attractive hypothesis, it may be overly simplified. Indeed, much still needs to be elucidated before it can be applied across the board. More information is required to take into account the fact that not all non-proliferating, differentiated cells exhibit the same metabolic profile. For example mitochondrial-dense differentiated cells, such as hepatocytes, myotubes and brown adipocytes, are energetically more active than white adipocytes, osteoclasts and endothelial cells. That being said, it is tempting to speculate that common alterations in metabolic programming may accompany embryonic and/or stem cell differentiation and these may also be involved in adult tissue development and/or remodelling. Moreover, the molecular mechanisms that regulate proliferating versus quiescent metabolism may already be well known to us and involve established regulators of the cell cycle and differentiation. Candidate regulators include oncogenic factors (c-Myc, growth-factor stimulated-PI3K/AKT) and tumour suppressor genes [p53, LKB1/AMPK (AMP-activated protein kinase)].

Here, we propose that the Wnt/β-catenin signalling network is a prime candidate for co-ordinating such metabolic switching during both normal cell physiology and in pathological proliferative states. This is supported by the following facts: (i) Wnt signalling is known to promote cell proliferation, (ii) Wnt signalling is linked to altered expression of key metabolic genes and transcription factors, (iii) Wnt signalling is linked to cellular mechanisms for sensing environmental cues, and (iv) the complexity of Wnt signalling networks makes it ideal for mediating and/or co-ordinating both autocrine and paracrine signals that enable cell-selective events to be appropriately titrated in line with both bioenergetic demands and fuel availability.

Wnt signalling promotes cell proliferation

The Wnt network is comprised of many examples of oncogenes (e.g. Wnt1, β-catenin, FRAT/GBP, Lgs/Bcl9) and tumour suppressors (e.g. APC, Axin, TCF1, Wnt5A, sFRP1). Furthermore, β-catenin/TCF activity includes induction of established oncogenes, such as c-Myc and negative regulation of tumour suppressor proteins, such as p53. The deregulated activation of Wnt/β-catenin signalling has been demonstrated in multiple cancers and is extensively reviewed elsewhere [83-85].

Wnt signalling alters the expression of key metabolic proteins, genes and transcription factors

Wnt-induced inhibition of the GSK3–Axin destruction complex has recently been proposed to alter the stability of multiple target proteins and to control a broad range of cellular activities in addition to β-catenin-mediated transcriptional activation [86]. Among these are a small cluster of 'metabolic enzymes' whose expression is sensitive to inhibition of GSK by both LiCl and more specific GSK inhibitor peptides (composed of a recombinant Axin-interacting domain). These enzymes include aldolase, cytidine deaminase and

dihydrolipoamide *S*-succinyltransferase (Figure 3). The fact that Axin complexes with and activates p53 [75] adds further credence to the concept of post-translational mechanisms mediated by Wnt signalling pathways that acutely impact on diverse cellular machinery.

Many transcript-profiling studies have reported altered mRNA expression of metabolic genes in response to deregulated Wnt signalling. These too support the role of Wnt signals altering metabolic flux to promote cell growth. For example, liver-specific transgenic expression of either wild-type or an oncogenic β-catenin mutant results in changes in genes involved in gluconeogenesis and glutamine metabolism, thereby altering both the synthetic and metabolic function of hepatocytes [87,88]. Similarly, recent proteomic analysis of livers from liver-specific APC knockout mice identified 56 dysregulated proteins. Many of these proteins are associated with mitochondrial dysfunction and carbohydrate metabolism, suggesting that defects in Wnt signalling may also determine a metabolic switch in fuel utilization towards glycolysis and away from fatty acid oxidation [89].

One caveat when interpreting mechanistic insights from genetic *in vivo* studies (e.g. transgenic murine models) is that it is often difficult to separate the direct metabolic actions of Wnt-induced signals from those that are a result of indirect developmental adaptations. However, a more direct approach has been to profile and identify metabolic genes that contain putative TCF/LEF-response elements in their promoters. Schwartz et al. [17] profiled 81 candidate genes with TCF-response elements and identified 67 such genes that were regulated in ovarian endometrioid adenocarcinomas. These included at least eight metabolic enzymes involved in carbohydrate and glutamine metabolism (Figure 3). This is consistent with the notion that proliferative metabolism promotes glutaminolysis to provide a carbon source for biosynthetic precursors [81].

Wnt signals can also indirectly regulate cellular metabolism through their effects on expression and activity of metabolically relevant transcription factors. One such TCF-responsive gene is c-Myc which has been shown to regulate mitochondrial glutaminolysis [90]. Another TCF target gene is the transcription factor, PPARδ, which is highly expressed in metabolically active tissues such as heart, liver and adipose tissue. PPARδ is linked to mitochondrial biogenesis and oxidative capacity and is a regulator of lipid oxidation. PPARδ activation also enhances ES (embryonic stem) cell proliferation [91], keratinocyte proliferation [92] and is implicated in both gastric cancer [93] and azoxymethane-induced colon cancer [94]. Mechanistically, overexpression of PPARδ [or its upstream regulator cPLA2α (cytosolic phospholipase A2α)] in human cholangiocarcinoma cells has been shown to induce the binding of PPARδ to β-catenin and increase their association with the TCF/LEF-response elements [95]. This creates a positive feed-foward loop that potentiates the actions of Wnt/β-catenin signalling.

Wnt/β-catenin signals indirectly suppress lipogenic and adipogenic transcription factors such as PPARγ and C/EBPα through induction of at least four TCF target genes, c-Myc, cyclin D1, Id2 and COUP-TFII (chicken ovalbumin upstream promoter-transcription factor II) (see discussion on white adipocyte differentiation below). Furthermore, direct functionally negative interactions between β-catenin and PPARγ have also been reported. This contrasts with induction of PPARδ expression that negatively regulates Wnt/β-catenin signalling. This

interplay between β-catenin and PPARs is particularly relevant during adipocyte differentiation [21], breast cancer [96] and steatotic tissues [97]. Furthermore, β-catenin interacts with metabolically relevant transcription factors (FOXO, RXR, RAR) indicating that these factors are influenced and/or controlled by Wnt signalling.

Wnt signalling is linked to cellular mechanisms of fuel sensing

Recently we have demonstrated that the expression of key Wnt/β-catenin signalling components is altered in response to nutritional cues *in vivo*. Specifically, in murine models of opposing nutritional states (*ad libitum* feeding, 24 h fasted and refeeding following a 24 h fast) and in models of nutritional surplus (i.e. genetic obesity and high-fat diet-induced obesity) expression of Wnt ligands (Wnt10b, Wnt3a) and Wnt antagonists (Dact1, sFRP1 and sFRP5) are significantly altered in adipose tissue, in a context- and cell-type-specific manner [98]. Similarly, increased β-catenin levels have been reported in liver, muscle and adipose tissue following refeeding of fasted rats (S.H. Anagnostou and P.R. Shepherd, unpublished work cited in the discussion of [99]). Our understanding of the molecular basis for this regulation and the functional consequences, at the level of tissue biology, is still incomplete. However, there is already some evidence suggesting an intimate link between activation of Wnt signalling networks and cellular mechanisms of nutrient sensing.

Wnt and glucose sensing—The HBP (hexosamine biosynthesis pathway) is well established to function in cellular fuel sensing. This biochemical pathway is responsible for shuttling metabolites from glucose to intracellular glycosylation events. At the biochemical level, the enzyme GFAT (glutamine/fructose-6-phosphate amidotransferase) catalyses the conversion of fructose 6-phosphate (from glycolysis) into glucosamine 6-phosphate with glutamine acting as an amino donor. The final product of the HBP pathway is the substrate UDP-GlcNAc, which is the carbohydrate moiety used for O-linked glycosylation of intracellular proteins and also for N-linked glycosylation of cell-surface receptors, transporters and secreted proteins. Since HBP is dependent on substrates that are key metabolites in carbon, nitrogen and energy homoeostasis (fructose 6-phosphate, glutamine, acetyl-CoA and UTP) it is thought to provide a cellular mechanism for sensing changes in metabolic flux and also links these to appropriate responses such as cellular transition between growth and arrest.

An important link between the HBP pathway and activity of Wnt/β-catenin signalling was recently demonstrated in macrophages [99] and relies on the fact that Wnt ligand bioactivity is post-translationally determined. The primary amino acid sequence of all Wnts is remarkably similar and although no crystal structure has yet been solved, Wnts are thought to require N-glycosylation as a prerequisite for proper folding, prior to secretion [100-102]. This aspect is entirely consistent with the observation that Wnts are mostly found associated with cell membranes and the ECM (extracellular matrix) [100]. Anagnostou and Shepherd [99] demonstrated that alterations in glucose availability can induce autocrine activation of the Wnt/β-catenin pathway in macrophages and that this is dependent on the hexosamine pathway. Although much still remains to be addressed [103], the possibility clearly exists whereby Wnt signals may be directly affected by glucose availability.

Wnt and lipid sensing—Another post-translational modification of Wnts links these ligands to the availability of NEFAs (non-esterified fatty acids). Wnts require palmitoylation on the first conserved cysteine residue for proper receptor binding, activation and internalization [102,104]. Furthermore, this modification may be required to tether Wnts to cell membranes. Evidence of modifying enzymes is also available. The ER (endoplasmic reticulum) protein Porcupine (Porcn in mice) has sequence similarity to O-acyltransferases and is physically associated with Wnts [55,101,105]. Conversely the Wnt antagonists, Dickkopf family, might mediate the deacylation of Wnts in opposition to the acylation mediated by Porcupine ([106], and reviewed in [107]). This is supported by the structural similarities with colipases and evidence from structure-function analysis of vertebrate Dickkopf proteins showing that this domain is sufficient for Wnt inhibition [108]. Taken together, this raises the possibility that diminished NEFA availability may lead to reduced Wnt ligand production. Conversely, it can be envisaged that under conditions of excessive lipid availability, enhanced lipid modification may lead to inappropriate Wnt ligand activation. Whether all (or selected) Wnts are palmitoylated is currently unclear, however, Wnt palmitoylation has already been recognized as a putative target for novel anti-cancer treatment via IWP (inhibitors of Wnt production) [109].

Despite being associated with ECM, Wnts may also be transported long distances tethered to the membranes of lipoprotein particles [110,111]. This is an attractive mechanism for transmitting lipophilic endocrine signals from the liver to systemic target organs. This possibility is further supported by the observation that Wnts associate with the chaperone VPS35 (vacuolar protein sorting 35 homologue), a component of the large multimeric complex, termed the retromer complex [112-115]. This complex is involved in retrograde transport of proteins from endosomes to the *trans*-Golgi network. The association of Wnt with lipoprotein particles may occur within the Wnt-producing cells, after endocytosis and the endosomal targeting of extracellular lipoprotein particles [116]. Furthermore, the Wnt co-receptors, Lrp5 and Lrp6 belong to a subfamily of structurally-related LDLR [LDL (low-density lipoprotein) receptor] family [117,118] and these are known to regulate the endocytosis and hepatic removal of LDLs by the liver. Indeed, the regulation of cholesterol homoeostasis is the only known function of the prototypical LDLR. LRP5 and LRP6 (and Arrow in *Drosophila*) are highly homologous in their extracellular domain arrangements, but are distinct from other LDLR family members. Intracellularly, Lrp5 and 6 also differ from other LDLRs in that they each have five copies of PPP(S/T)P (P, proline; S/T, serine or threonine) motifs (which act as Axin-binding sites) and elicit distinct mechanisms of signal transduction [10]. Whether LRP5/6 plays a role in recycling Wnts is not known; however, evidence is emerging to suggest that LRP5 is required for normal cholesterol metabolism and glucose-induced insulin secretion [119]. This implies that Wnts may play a significant role in signalling tissue-specific and whole-body carbohydrate and lipid homoeostasis.

Wnt and amino acid sensing—The availability of amino acids is a fundamental requirement for protein synthesis. Hence, it is possible that synthesis of Wnt ligands and/or secreted antagonists can be directly influenced at the level of protein synthesis, by amino acid availability. Similarly, the fact that β-catenin mRNA is constitutively expressed, but the

protein has a short half-life, suggests that Wnt/β-catenin signals are likely to be highly sensitive to acute reductions in amino acid availability.

As previously mentioned, another well-studied and evolutionarily conserved, intracellular nutrient-sensing pathway is that involving the mTOR protein kinase. The mTOR pathway is activated by nutrient-rich conditions, particularly by high levels of amino acids and by insulin [120-122]. As discussed earlier, there is evidence that Wnt signals can be mediated via pathways involving mTOR. Therefore it is likely that the effectiveness of the Wnt/GSK3/TSC2 pathway on protein synthesis is dependent on cellular amino acid availability.

WNT SIGNALLING NETWORKS AND WHOLE-BODY ENERGY HOMOEOSTASIS

When considering adult tissue remodelling in response to injury, liver regeneration and/or cancer, the Wnt signalling plays fundamental roles in regulating cell-type-specific proliferation, migration and differentiation. Under such circumstances, one can speculate that paracrine signals must exist to direct these events (e.g. angiogenesis) and ensure appropriate fuel availability to those cells that require it most. However, diseases associated with whole-body metabolic deregulation (e.g. the metabolic syndrome, obesity, Type 2 diabetes) are also accompanied by tissue remodelling. In this context new questions are being raised: (i) is Wnt signalling linked to whole-body energy homoeostasis?; and (ii) does Wnt signalling play a role in co-ordinating the metabolic alterations associated with tissue remodelling, obesity, insulin resistance, liver steatosis and/or pancreatic dysfunction?

Wnt signalling in the liver

Normal liver function is crucial for maintaining global metabolic homoeostasis in mammals. The metabolic roles of liver include glycogen storage, gluconeogenesis, decomposition of red blood cells, detoxification, plasma protein synthesis and production of bile acids and lipoprotein particles, to name a few. This wide spectrum of hepatic functions depends on a complex interplay between different hepatic cell types (i.e. hepatocytes, biliary epithelial cells, stellate cells, Küpffer cells and sinusoidal endothelial cells). The repertoire of Wnts and Fzd receptors is quite specific for each one of these cell types [123]. As a dynamic organ able to remodel and adjust to metabolic demands or toxic insults, the components of the hepatic Wnt signalling network have the capacity to co-ordinately change their level of expression under different physiological and pathophysiological conditions. The biological relevance of the cell-type-specific Wnt signalling profile is highlighted by their contribution to the functional and anatomical zonation of the liver [124,125]. The roles of Wnt signalling extend beyond liver zonation, Wnt signalling is involved in diverse aspects, such as its development, regeneration, metabolism, oxidative stress and fibrosis [126]. There is also an extensive literature indicating that β-catenin plays an important role promoting hepatic carcinogenesis including hepatoblastoma, hepatocellularcarcinoma and cholangiocarcinoma [84].

There is evidence that β-catenin is required at each of the different developmental stages and contributes to hepatocyte maturation and biliary differentiation. It is also an important

regulator of postnatal growth and promotes the process of growth and regeneration in the adult liver through its induction of cyclin D1 transcription [127]. Being important, the genetic ablation of β-catenin specifically in liver appears to be compensated by the proliferative effects mediated by IL (interleukin)-6 and activation of the PDGFR (platelet-derived growth factor receptor). The membrane pool of β-catenin may also be important for its proliferative effect through its interaction with E-cadherin modulating the specific cell–cell interactions required for proliferation [127].

Both inducible and tissue-specific genetic studies in rodents suggest that Wnt signalling exerts metabolic effects in adult livers and contributes to xenobiotic, carbohydrate and hepatic glutamine metabolism [87,88,89,128]. It may also prevent oxidative stress through its effects on FOXO, cytochromes P450s and glutathione transferase [129]. Conversely, liver-specific APC-knockout suggests that it may potentially facilitate fat deposition and development of fatty liver under conditions of increased nutrition availability [89].

The role of Wnt signalling in the development of hepatic steatosis and steatohepatitis is less clear. However, we and others have shown that lipid accumulation in liver requires ectopic induction of a lipogenic programme that is normally associated with adipogenesis. Transcription factors such as PPARγ2, SREBPs (sterol-regulatory-element-binding proteins) and C/EBPs are key modulators of the adipogenic programme and are inappropriately induced in the liver in response to nutritional surplus. Their activity is thought to facilitate ‘ectopic’ fat deposition in the liver. Currently, it is not known whether fat deposition in liver is associated with or requires inhibition of Wnt signalling as has been reported for adipose tissue (see below). However, it is well documented that Wnt signalling can be selectively activated in stellate cells, where it promotes transdifferentiation towards a profibrotic type of cells [97,130]. The apparent paradox is that, whereas in the context of obesity and over-nutrition, hepatocytes induce an adipogenic-like programme, under the same conditions, the hepatic stellate cells, activate their Wnt signalling (by increasing Wnt4, Wnt5 and decreasing expression of the Wnt inhibitor, Dkk1) [130]. Initiation of the profibrotic response is also accompanied by loss of PPARγ and PPARδ expression, and loss of vitamin A droplets [97]. This inappropriate activation of Wnt signalling in stellate cells may pose an increased risk of progression towards steatohepatitis with an important fibrotic component.

Wnt signalling in the pancreas

The pancreas plays crucial roles in production of digestive enzymes (from exocrine acinar and ductal cells), and islet-derived endocrine hormones, such as insulin and glucagon that regulate whole-body glucose availability. Wnt/β-catenin signalling has been implicated in the selective development of both exocrine and endocrine pancreas. Conditional gain- and loss-of-function studies have demonstrated that Wnt/β-catenin signalling is required for exocrine pancreas development, during which it promotes proliferation of pancreatic epithelial and acinar precursor cells in part by induction of c-Myc [131-133]. In contrast, conflicting conclusions have been drawn for the role of Wnt/β-catenin signalling in the development of endocrine pancreas [134,135]. One explanation comes from the use of different conditional promoters in these studies and the fact that their expression is regulated

temporally during development rather than solely in a cell-specific manner [136]. Nonetheless, taken together, the role of Wnt/β-catenin signalling in adult islets appears to be time-dependent, wherein early activation of β-catenin prevents β-cell development, while late Wnt activation increases β-cell mass [135,137]. This has potential implications for both pancreatic cancers and regenerative strategies to treat diabetes [138].

In adult pancreatic islets, recent attention has focused on the role of Wnt/β-catenin signalling in β-cell biology. Numerous studies have confirmed that β-cells express Wnts, FRPs, LRPs and DKKs. Most notably, the T-cell transcription factor, TCF7L2 (formerly called TCF4) has also been identified as an important genetic factor involved in insulin secretion, β-cell mass and increased diabetes risk [136]. Subsequent recent studies have gone on to demonstrate that Wnt/β-catenin signalling can modulate mature β-cell biology including glucose-stimulated insulin secretion [139,140], survival [141] and proliferation [137]. Intriguingly, a recent study has suggested that the TSC-mTORC1 pathway is functional in β-cells where it also contributes to β-cell expansion, insulin synthesis and secretion [142]. Whether this pathway is also utilized by Wnt signals (as reported in various other tissues and cell types) [29] seems highly likely, but remains to be formerly demonstrated.

Despite the focused efforts, much remains to be determined with respect to the role of Wnt/β-catenin signalling in β-cell dysfunction. The most widely accepted view is that in dysfunctional β-cells, decreased Wnt signalling, reduces TCF7L2 activity leading to impaired insulin secretion. This is in apparent conflict with Lyssenko et al. [143] showing that mRNA levels of TCF7L2 may be increased in islets of diabetic patients. Although it remains to be confirmed that elevated RNA expression correlates with protein levels and functional activity, it is consistent with reports of elevated expression of multiple other Wnt components including Wnt2b, β-catenin, pGSK3β, TCF3, cyclinD1 and c-Myc, in islets of Type 2 diabetic patients relative to those in healthy donors. These studies suggest that Wnt/β-catenin signals may play a causal role in the development of β-cell dysfunction and diabetes. However, induction of Wnt signalling in islets may be part of an allostatic response attempting to maintain glucose homoeostasis through optimization of β-cell insulin secretion and facilitation of β-cell mass expansion [144] in the context of insulin resistance and/or nutritional load. This may be similar to the observations reported for liver regeneration following toxic insult [128]. However, the regulation of Wnt signalling under conditions of glucolipotoxicity is only recently being recognized [99]. Nonetheless, there is evidence that glucose and adipokines decrease TCF7L2. This, together with increased expression of Wnt-related network genes in diabetics, suggests that Wnt signalling in β-cells may have a biphasic evolution; initially up-regulated in the context of insulin resistance to promote islet hyperplasia followed by secondary failure when hyperglycaemia and dyslipidaemia result in a glucolipotoxic insult.

Additional considerations that are noteworthy in the context of Wnt signalling and insulin secretion are first that the effects of deregulated Wnt/β-catenin signals *in vivo* may be indirect. For instance the link between TCF7L2 and insulin secretion may depend on the enteroinsular axis. More specifically it may be mediated by impaired GLP1 secretion by gut endocrine cells and/or defects in GLP1 receptor activation in β-cells. In this respect the Wnt signalling pathway, and in particular TCF7L2, might mediate the insulinotropic effects of

GLP1 in β-cells. Secondly, Wnt signalling may also contribute to the effect of nutritional programming in β-cells. It is possible the early nutritional defects may promote defective Wnt signalling ultimately resulting in decreased β-cell mass and increased susceptibility to diabetes [145].

Wnt signalling in skeletal muscle

Muscular activity accounts for much of the body's energy consumption to fuel contraction. There are numerous reports to indicate that Wnt signalling, particularly Wnt/β-catenin signalling plays important roles in muscle development, during both embryogenic specification of muscle (in the somites) and also in adult muscle regeneration (in satellite cells). While the detailed mechanisms remain to be fully dissected, it is clear that Wnts are involved in inducing the myogenic-determinant genes Pax3, MyoD and Myf5 during mammalian embryogenesis. In presomitic mesoderm Wnt signalling co-operates with Sonic hedgehog to induce the expression of myogenic bHLH (basic helix-loop-helix) proteins [146,147]. Recent evidence further suggests that Wnt signalling via Lef1 acts to regulate the proliferation of pre-myogenic (Pax3/7-positive) cells in somites via induction of the Wnt/β-catenin target gene, Pitx2 [148]. Intriguingly, the same signals do not regulate myogenic initiation in other locations, consistent with the notion that Wnt signalling in myogenic programmes is context-dependent. Furthermore, in somites, Wnt-induced signalling is temporally active and has also been linked to β-catenin-independent pathways, such as Wnt/PKA/CREB that coincides with the patterns of myogenic gene induction [40].

Adult skeletal muscle contains stem cells (muscle satellite cells) capable of commitment into myoblast lineage. These progenitor cells contribute to muscle growth, and adapt to increased muscle requirements prompted by increased activity demands and/or as part of the repair process of the damaged muscle. To perform this function, skeletal muscle satellite cells require an environment that allows maintenance of quiescence, but may be activated when required by specific nutritional or biophysical external cues. In line with this, there is evidence that components of the Wnt/β-catenin signalling pathway may be nutritionally regulated in muscle (S.H. Anagnostou and P.R. Shepherd, unpublished work cited in the discussion of [99]) and that injured muscle produces several Wnt isoforms [149]. There is marginal evidence that exercise increases the association between Dvl and GSK3β in skeletal muscle and that this is associated with β-catenin dephosphorylation [150]. However, in the context of acute exercise, Wnt-related gene expression does not seem to be altered [151]. Nonetheless, this does not preclude an important contribution of Wnt signalling in muscle adaptation to chronic exercise training. Furthermore, it is the Wnt/β-catenin pathway that is implicated in the Pax7 expression which drives myogenic specification in a subpopulation of muscle-derived SP (side population) cells. These can replenish the satellite cell population during muscle regeneration.

However, while Wnt/β-catenin signalling promotes both myogenic specification and progenitor proliferation, β-catenin gene silencing can promote myogenic differentiation *in vitro* [152]. This effect of β-catenin on myogenesis is likely to be context-dependent as opposing actions on myogenesis have also been documented [24,152,153]. Indeed, it is likely that β-catenin may impact on the myogenic differentiation and myotube fusion

through interactions with MyoD [24] and cadherin proteins [154] respectively. The induction of BCL9, an essential component of Wnt/β-catenin signalling has also been implicated in the differentiation of adult myogenic progenitors during muscle regeneration [155]. Taken together, it appears that Wnt/β-catenin signalling can contribute to the efficiency and regulated titration of myogenic precursor number, myogenic specification and myogenesis.

In light of the potential for Wnt/β-catenin signalling to coordinate metabolic switching, an important aspect worthy of consideration is the potential involvement of Wnt signalling during myofibrillogenesis. It is known that muscle fibre types vary in both their oxidative capacity and glycolytic capacities. Type I fibres have high oxidative and glycolytic capacities and promote oxidative metabolism. They are therefore well suited for endurance and are slow to fatigue. In contrast, Type II fibres have a high glycolytic capacity, but variable oxidative capacity. These fibres are efficient for short bursts of speed and power and are quicker to fatigue. They also use both oxidative metabolism and anaerobic metabolism depending on the particular subtype. Given that the PPARδ-mediated transcriptional pathway is involved in the regulation of the skeletal muscle fibre phenotype, and that this same transcription factor is a classic TCF target gene of Wnt/β-catenin signalling, it is tempting to speculate that Wnt/β-catenin signalling may also play a role in myofibrillogenesis through induction of PPARδ. While this remains speculative, there is some recent evidence to suggest that late hyperactivation of Wnt/β-catenin pathway is linked to aberrant myofibrillogenesis and that a genetic interaction exists between Wnt/β-catenin and myostatin, a member of the TGFβ family that regulates muscle myofibrillogenesis [156].

Metabolic programming of muscle is also an important aspect of whole-body energy dissipation, whereby high oxidative capacity can protect against the metabolic consequences of nutritional overload. The notion that adult myoblasts retain plasticity in developmental potential and can be regulated by Wnts to undergo myogenic, adipogenic or osteoblastogenic differentiation [157], highlights the potential for local Wnt signalling in muscle to impact on whole-body energy balance. This may also be relevant in the context of aging and agerelated diseases. Here, there is evidence that aging is associated with progressive changes in body composition resulting in decreased skeletal muscle mass or sarcopenia, and increased adipose tissue development within the muscle. It is unclear how these changes occur, but recent evidence indicates that muscle satellite cells indeed have adipogenic potential. Hence, the progressive accumulation of adipose tissue within the skeletal muscle characteristically observed in an aging obese population, may involve selective inhibition of Wnt signalling associated with nutrient-induced PPARγ activation, resulting in a pro-adipogenic redirection of satellite cell differentiation [158].

Wnt signalling in BAT (brown adipose tissue)

BAT is another organ highly relevant to whole-body energy homoeostasis. Unlike WAT (white adipose tissue) (see below) brown adipocytes are specialized to function in energy dissipation through oxidative metabolism. The role of Wnt signalling in BAT is relatively unexplored and, until recently, limited by the lack of knowledge regarding the process of

lineage determination. Nonetheless, expression of a number of Wnt-related genes including Wnt10a, Wnt5a and TCF7 inversely correlate with brown adipogenic potential, while Wnt antagonists such as sFRP2 correlate with BAT differentiation [159]. There is also evidence that activation of Wnt/β-catenin signalling early in differentiation blocks brown adipogenesis, whereas activating Wnt signalling in mature brown adipocytes stimulates their conversion into white adipocytes. For instance transgenic expression of Wnt10b under the control of the Fabp4 promoter, blocks development of BAT and results in decreased energy expenditure and failure to maintain core body temperature when challenged by cold exposure [160].

The effects of Wnt signalling on brown fat differentiation and metabolism depends on the transcriptional co-activator PGC1β (PPARγ co-activator 1β) [161] and on insulin stimulation. Interestingly, genetic ablation of IRS1 (insulin receptor substrate 1) results in up-regulation of Wnt10a and prevention of brown fat differentiation [162]. Given the renewed interest in human brown fat in the context of its potential anti-obesity properties, it is likely that the role of Wnt signalling in this area of research will soon become better understood.

Wnt signalling in WAT

Unlike BAT, WAT functions as a major site for storage and liberation of the body's fuel reserves. As such it is also a major endocrine organ and has the additional capacity to expand and retract in response to changes in nutritional surplus [163]. Wnt/β-catenin signalling has been well documented to play a role in preventing stem cell determination down the preadipocyte lineage. However, it is also involved in adipocyte differentiation and in the physiological regulation of adipose tissue plasticity.

From a developmental standpoint, it is thought that active Wnt/β-catenin signals in adult stem cells shifts the balance of transcription factors in favour of osteoblastogenesis and/or myogenesis as exemplified by several genetic studies [160,164-167], and reviewed in [168-170]. However, there is also evidence that Wnt signalling can play a role in committed preadipocytes and in the titrated adipogenic response to nutritional surplus *in vivo* [98]. Although both β-catenin-dependent and -independent Wnt pathways may be active in preadipocytes [167,171-173], it is the Wnt/β-catenin/TCF-dependent pathway that is most well characterized and shown to potently inhibit adipogenesis both *in vitro* and *in vivo* (for recent reviews see [168,169]). Evidence that endogenous Wnt/β-catenin signalling may be relevant to adipogenesis, comes from numerous studies by others and ourselves, demonstrating differential expression of key components of Wnt/β-catenin signalling pathway and TCF target genes in preadipocytes and mature adipocytes. Furthermore, temporal expression profiling studies have confirmed that these genes are also dynamically regulated early during adipogenesis in murine and human and primary preadipocyte cultures and cell lines. Furthermore, recent microRNA profiling studies have identified miR-8 as a candidate regulator of Wnt signalling during adipogenesis [174,175]. Gain- and loss-of-function studies further substantiate the functional impact of key signalling factors and target genes on adipogenic programme.

Recently, we have identified the presence and regulation of the scaffold protein, Dapper1 (Dact1) in preadipocytes and adipose tissue [90]. This protein was previously reported to modulate Wnt/β-catenin signalling in a context-dependent manner [176]. In preadipocytes, Dact1 (and Dvl2) expression is down-regulated during adipogenesis, consistent with loss of Wnt/β-catenin signalling. However, unlike many positive regulators of Wnt/β-catenin signalling, forced overexpression of Dact1 promotes adipogenesis. Furthermore, these effects were mediated by changes in the transcriptional expression of multiple players in the Wnt/β-catenin signalling network and resulted in reduced extracellular (paracrine) Wnt activity [98]. In the context of WAT physiology, we find that Dact1, Wnt ligands (Wnt10b and Wnt3A) and secreted antagonists (sFRPs) are also nutritionally regulated, suggesting that nutritional cues and/or metabolic status may direct the Wnt/β-catenin signalling network and co-ordinate adipose tissue plasticity (Figure 4). Furthermore, cell-specific expression profiles suggest that a functional network exists between Dact1, sFRPs and Wnt ligands which facilitates cross-talk in adipose tissue between preadipocytes and mature adipocytes, thereby ensuring appropriate titration of adipose tissue expansion in response to nutrient availability (Figure 5). More recently, we have added sFRP1 (C. Lagathu, J. Sethi and A. Vidal-Puig, unpublished work) to this network and it is likely that others, such as sFRP4, may soon be added [177].

The mechanism by which Wnt/β-catenin signalling inhibits adipogenesis has been the subject of numerous studies and multiple mechanisms have been proposed. These all converge to inhibit the expression and activity of two adipogenic transcription factors: PPARγ and C/EBPα. It is facilitated by the induction of Wnt/β-catenin/TCF target genes such as cyclin D1 and c-Myc which have been reported to directly inhibit PPARγ and C/EBPα [178,179]. Recently, two new Wnt/β-catenin/TCF target genes have been added to this list, COUP-TFII and Id2. COUP-TFII, is an orphan nuclear receptor recently identified as a potent anti-adipogenic factor which acts downstream of Wnt/β-catenin signal to silence PPARγ and C/EBPα gene expression [180,181]. Surprisingly, genetic ablation studies have linked COUP-TFII to impaired adipogenesis, glucose homoeostasis and energy metabolism *in vivo* [182]. This apparent contradiction is explained by the finding that Wnt10b is itself a direct target of, and is suppressed by, COUP-TFII. Hence, genetic ablation of COUP-TFII results in elevated anti-adipogenic Wnt10b expression. This explanation also supports the notion that COUP-TFII is likely to play an important role in regulating the transient nature of autocrine Wnt/β-catenin signalling. A similar negative-feedback mechanism is also reported for the Wnt antagonist Dkk1, which is itself transiently up-regulated transiently in the early stages of human adipogenesis [183]. Indeed, upon closer inspection, Wnt/β-catenin target gene expression (cyclin D1, PPARδ) is transiently induced within hours following induction of adipogenesis *in vitro*. This presumably coincides with entry into clonal expansion and is subsequently down-regulated as adipogenic transcription factors are expressed [184]. Intriguingly, it is at this time that a switch in metabolic profile is also observed [185].

The second TCF-responsive gene recently linked to adipogenesis is Id2, a dominant negative member of the HLH family of transcription factors that promotes expression of PPARγ and enhances morphological differentiation and lipid accumulation. Conversely, siRNA (small

interfering RNA)-mediated knockdown of Id2 antagonizes adipocyte differentiation [186]. This contrasts with the previous observations which demonstrated that Id gene expression is down-regulated during adipogenesis [187]. Nonetheless, when overexpressed both Id2 and Id3 can interact with the adipogenic factor SREBP1c, and inhibit its transcriptional activity [188]. Future studies using conditional genetic techniques may help to resolve these apparently contradictory findings.

The link between Wnt signalling and adipogenesis may also involve the nuclear envelope. In fact defects in lamin A have been identified as genetic defects associated with human partial lipodystrophy. There is evidence that dynamic complexes of A-type lamins and emerin influence adipogenic capacity of the cell via nucleocytoplasmic distribution of β-catenin [27]. Thus, through the interactions with β-catenin, nuclear-envelope-related partners may influence expression and activation of PPARγ.

Wnt signalling, inflammation and metabolic dysregulation

Although there is clear evidence for a role of Wnt/β-catenin signalling pathways in regulating adipogenesis, and even titrated adipocyte hyperplasia and adipocyte maturation *in vivo*, there is also mounting evidence indicating that Wnt/β-catenin signalling pathways may be dysregulated in obesity and the metabolic syndrome. For instance, in C57BL/6J mice susceptibility to high-fat diet-induced adiposity correlates with a significant up-regulation in multiple adipose tissues of at least two genes associated with Wnt signalling (sFRP5 and Naked1) [189]. In humans, mutations in Wnt10b have been identified in obese subjects [190]. Furthermore, the ability of Wnt signalling networks to modulate metabolic phenotypes is illustrated in mice with transgenic expression of Wnt10b under the control of the FABP4 (fatty acid binding protein 4)-promoter. These mice are resistant to both high-fat diet-induced obesity and genetic obesity, and also exhibit improved insulin sensitivity compared with wild-type mice [160,191]. Similarly, administration with rAAV (recombinant adeno-associated virus) vector encoding murine Wnt10b cDNA, to skeletal muscles of adult DIO (diet-induced obese) rats, decreases adiposity, increases bone mineral density and improves glucose homoeostasis in obese rats [192]. Taken together the prevailing view is that Wnt/β-catenin signalling is required to prevent both adipose tissue expansion and the development of associated metabolic syndrome.

To further delineate the potential physiological and pathophysiological regulation of Wnt/β-catenin signalling, we performed longitudinal studies on rodent models of obesity and insulin resistance, and profiled the relative expression of Wnt/β-catenin signalling molecules in WAT during the development of adiposity and diabetes. As summarized in Figure 5, the expression of anti-adipogenic Wnt ligands decreases with increasing adiposity. Furthermore, the adipose tissue expansion correlates with expression of inhibitors of Wnt/β-catenin signalling: sFRP1, sFPR5 and DACT1. Intriguingly, however, for sFRP1 and DACT1 this relationship with adiposity is not maintained [98]. Instead, both sFRP1 and DACT1 decrease significantly in WAT from obese-diabetic mice. This coincides with a plateauing of adipose tissue expansion and increased adipose tissue deregulation, suggesting that in the pathological setting the Wnt/β-catenin signalling pathway becomes uncoupled and that sFRP1 and DACT1 are potential biomarkers of adipose tissue expandability.

What then are the pathological factors responsible for uncoupling Wnt/β-catenin signalling and adipose tissue plasticity? It has been known for some time that pro-inflammatory cytokines are themselves potent anti-adipogenic factors [193], and that adipose tissue expression of TNFα (tumour necrosis factor α) correlates with obesity-linked insulin resistance [194]. However, the mechanisms have remained incompletely understood. Recently, we and others have demonstrated that the anti-adipogenic action of TNFα also involves β-catenin stabilization and activation of Wnt/β-catenin target genes [184,195]. Although the mechanism by which this is achieved remains elusive, we have shown that it is a direct result of TNF receptor 1 signalling to stabilize β-catenin, and not simply a consequence of the anti-adipogenic actions of TNFα [184]. Hence, it appears that pro-inflammatory cytokines also cross-talk with the Wnt/β-catenin signalling pathway to determine cell fate. We therefore proposed that in inflamed adipose tissue, these pro-inflammatory signals deregulate the normal (physiological) regulation of Wnts by nutritional cues. This 'hijacking' of the β-catenin/TCF signalling pathway may therefore contribute to further limiting adipose tissue expansion despite the prevailing nutritional surplus [184,196,197]. Subsequent fat deposition in tissues that are ill-equipped to manage excessive lipid storage can mediate the lipotoxic consequences that underscore insulin resistance and hyperlipidemia.

CONCLUSIONS

Many studies have firmly established that Wnt signalling lies at the heart of numerous biological processes including embryonic development and patterning, adult tissue regeneration and/or plasticity, and cancer progression. However, we are only just beginning to appreciate/recall that each of these processes must be energetically fuelled through co-ordination of cell-specific changes in cellular metabolism. In the present review we have presented evidence to suggest that the Wnt signalling network is a prime candidate for linking changes in cellular metabolism with these biological events. When considering metabolically relevant tissues, it also becomes apparent that Wnt signalling networks are involved in facilitating tissue adaptation to nutritional challenges, as well as the allostatic adaptations that maintain whole-body energy homoeostasis. Hence, the implications of links between Wnt signalling and cellular metabolism may go beyond the purely metabolic angle.

However, much still remains to be addressed before we can fully understand and harness the therapeutic potential of this complex signalling network. Future studies should shed light on how cross-talk between the Wnt signalling network and other developmental pathways contributes to context specificity and metabolic adaptation. These and additional outstanding questions that can only be addressed by systematic studies include: (i) do Wnt binding proteins such as Fzd receptors, LRP5/6 and sFRPs exhibit selectivity for specific Wnt ligands?; (ii) do all Fzd receptors couple to both the β-catenin-dependent and -independent pathways?; and (iii) is there functional redundancy between the multiple isoforms that exist for many of the intracellular mediators of Wnt signalling pathways? It is hoped that with the application of modern high-throughput '-omic' and systems biology approaches, these and many more insights will soon be available.

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Abbreviations used

AJ	adherens junction
AP-1	activator protein 1
APC	adenomatous polyposis coli
BAT	brown adipose tissue
BMP	bone morphogenetic protein
CK	casein kinase
COUP-TFII	chicken ovalbumin upstream promoter-transcription factor II
CREB	cAMP-response-element-binding protein
Dkk1	Dickkopf-related protein 1
Dvl	Dishevelled
ECM	extracellular matrix
FOXO	forkhead box O
Fzd	Frizzled
GSK3	glycogen synthase kinase-3
GBP	GSK-binding protein
HBP	hexosamine biosynthesis pathway
Hh	Hedgehog
(b)HLH	(basic) helix-loop-helix
ICD	intracellular domain
Id2	inhibitor of DNA binding 2
JNK	c-Jun N-terminal kinase
LDL	low-density lipoprotein
LDLR	LDL receptor
LEF	lymphoid-enhancer binding factor

LRP	LDL (low-density lipoprotein) receptor
mTOR	mammalian target of rapamycin
mTORC1	mTOR complex 1
NEFA	non-esterified fatty acid
NICD	Notch ICD
NFAT	nuclear factor of activated T-cells
NLS	nuclear localization signal
PCP	planar cell polarity
PKA	protein kinase A
PKC	protein kinase C
PI3K	phosphoinositide 3-kinase
Pitx2	paired-like homeodomain transcription factor 2
PPAR	peroxisome-proliferator-activated receptor
RAR	retinoic acid receptor
ROCK	Rho-associated kinase
ROR2	receptor tyrosine kinase-like orphan receptor 2
RXR	retinoid X receptor
Ryk	receptor related to tyrosine kinase
sFRP	secreted Frizzled-related protein
SREBP	sterol-regulatory-element-binding protein
TCF	T-cell factor
TGFβ	transforming growth factor β
TNFα	tumour necrosis factor α
β-TrCP	β-transducing repeat-containing protein
TSC2	tuberous sclerosis complex 2
Vangl2	Van Gogh-like 2
WAT	white adipose tissue

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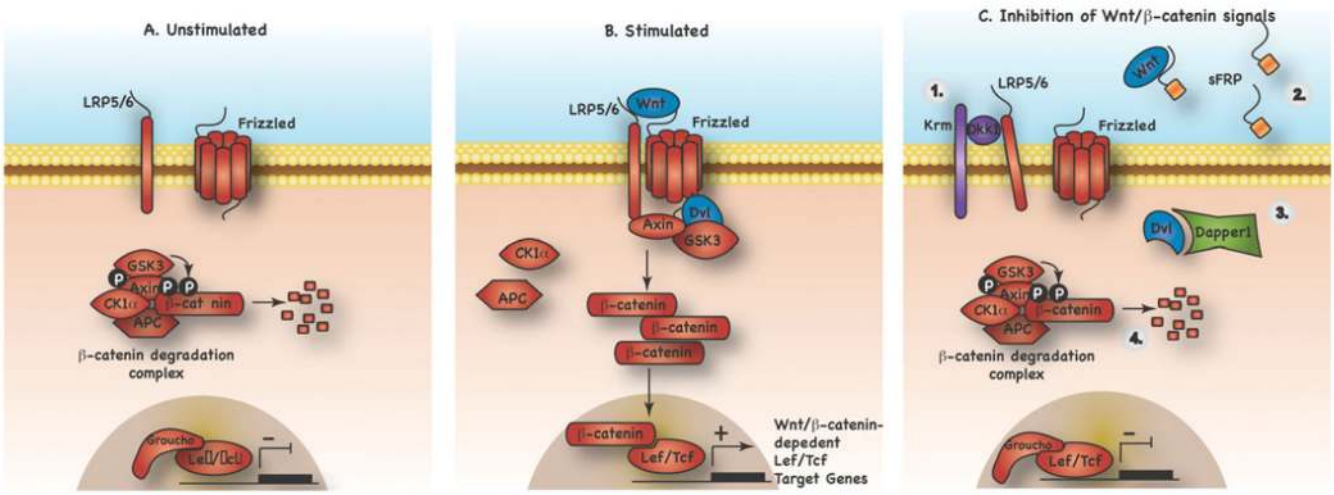

Figure 1. *β*-Catenin-dependent Wnt signalling

Panels show unstimulated (**A**), Wnt-stimulated induction of *β*-catenin/TCF target gene expression (**B**), and commonly reported mechanisms of feedback inhibition (**C**). Wnt/*β*-catenin signalling is inhibited by induction of (1) Dkk1 which sequesters LRP5/6 co-receptors; (2) sFRPs which sequester Wnt ligands; (3) Dapper1 (Dact1) which binds dishevelled proteins; and (4) selective components of the ubiquitin-degradation machinery such as *β*-TrCP. An animated version of this Figure is available at http://www.BiochemJ.org/bj/427/0001/bj4270001add.htm.

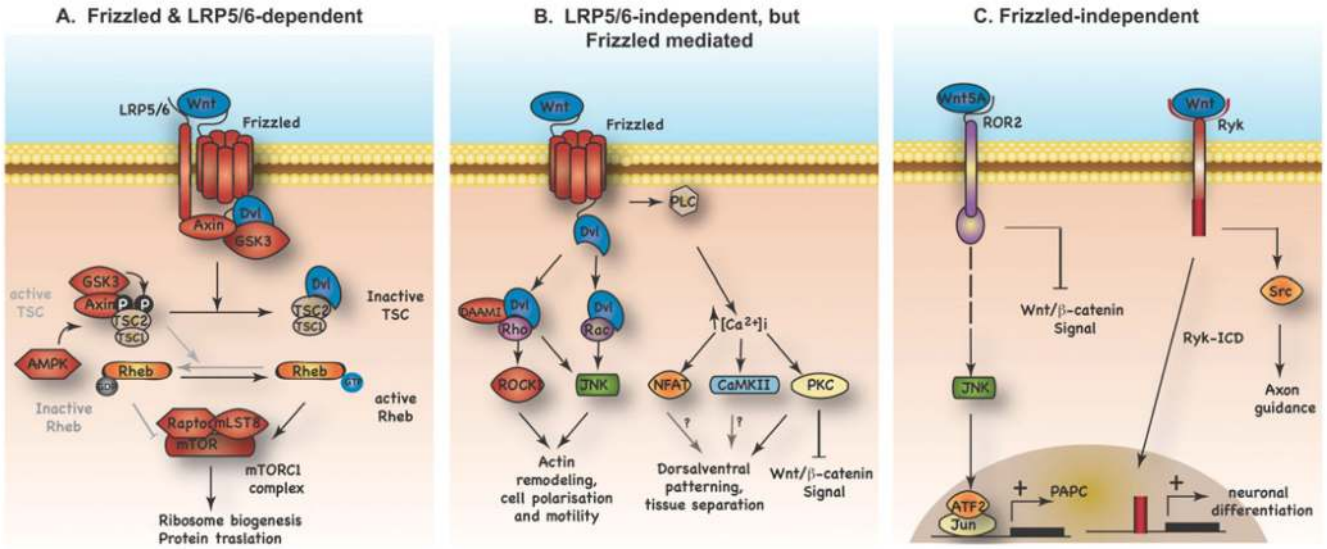

Figure 2. β-catenin-independent Wnt signalling

(**A**) Fzd/LRP-mediated regulation of mTORC1. (**B**) LRP-independent, but Fzd-mediated signals. (**C**) Fzd-independent signalling via ROR2 or Ryk receptors.

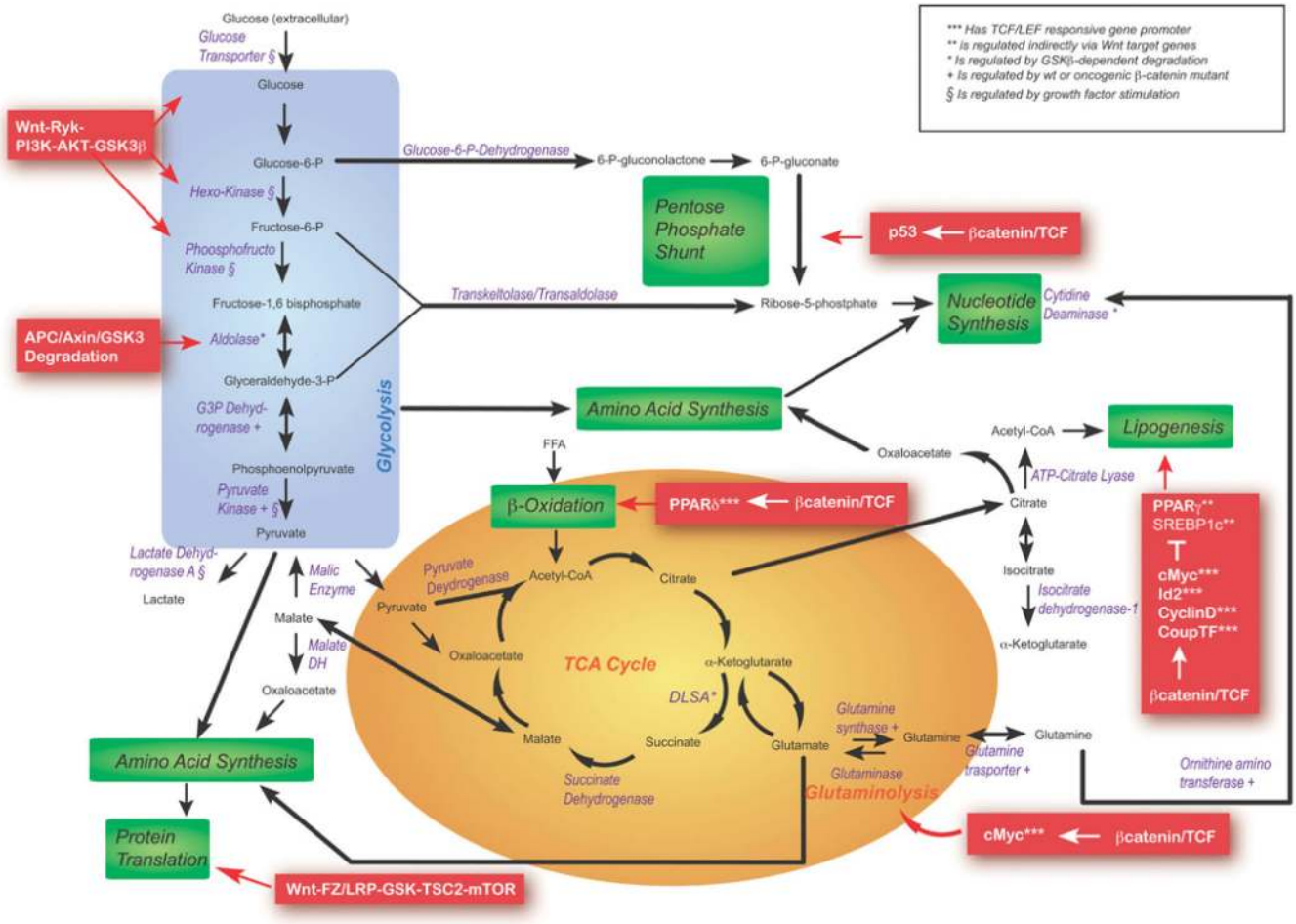

Figure 3. Wnt signalling and Wnt target genes link to cellular metabolic pathways

Activation of Wnt signals can be directly and indirectly linked to increasing flux through glycolysis, the tricarboxylic acid cycle and glutaminolysis. Collectively this promotes lactate production and utilization of glucose and glutamine as a carbon source for biosynthetic processes.

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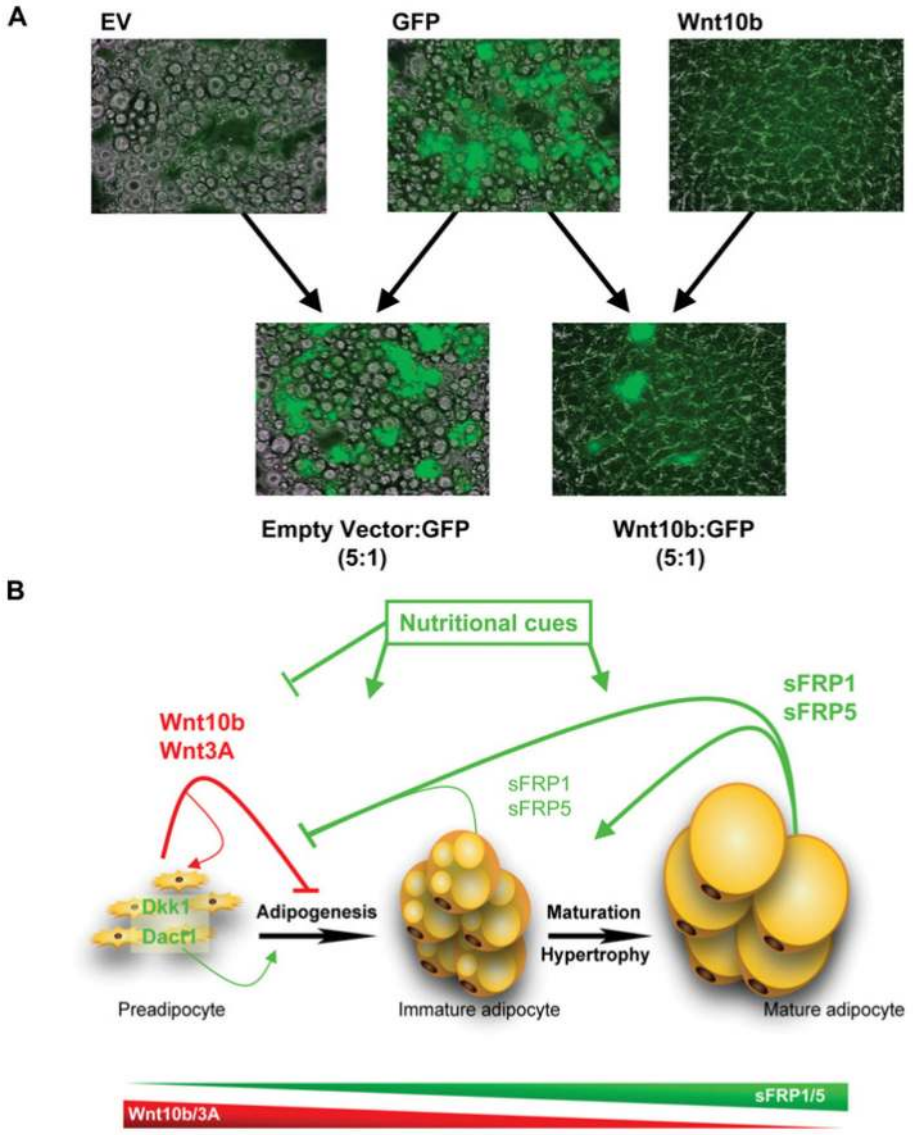

Figure 4. Putative role of Wnt signalling networks in paracrine regulation of titrated adipogenesis

(**A**) Paracrine regulation of adipogenesis by Wnt10b. Co-culture experiments demonstrate that Wnt10b-expressing cells do not differentiate, but can also inhibit the adipogenic potential of neighbouring preadipocytes. Co-cultures of preadipocytes expressing either empty vector (EV), Wnt10b or GFP (green fluorescent protein) only were induced to differentiate into adipocytes. Similar results were observed when Wnt10b-expressing preadipocytes were replaced by preadipocytes in which Dact1 was knocked down [98]. Images were generated using Cell-IQ from Chipman Technologies. (**B**) The paracrine Wnt signalling network mediates cross-talk between preadipocytes and maturing adipocytes. This network is also nutritionally regulated and may regulate titrated adipocyte recruitment *in vivo* [98].

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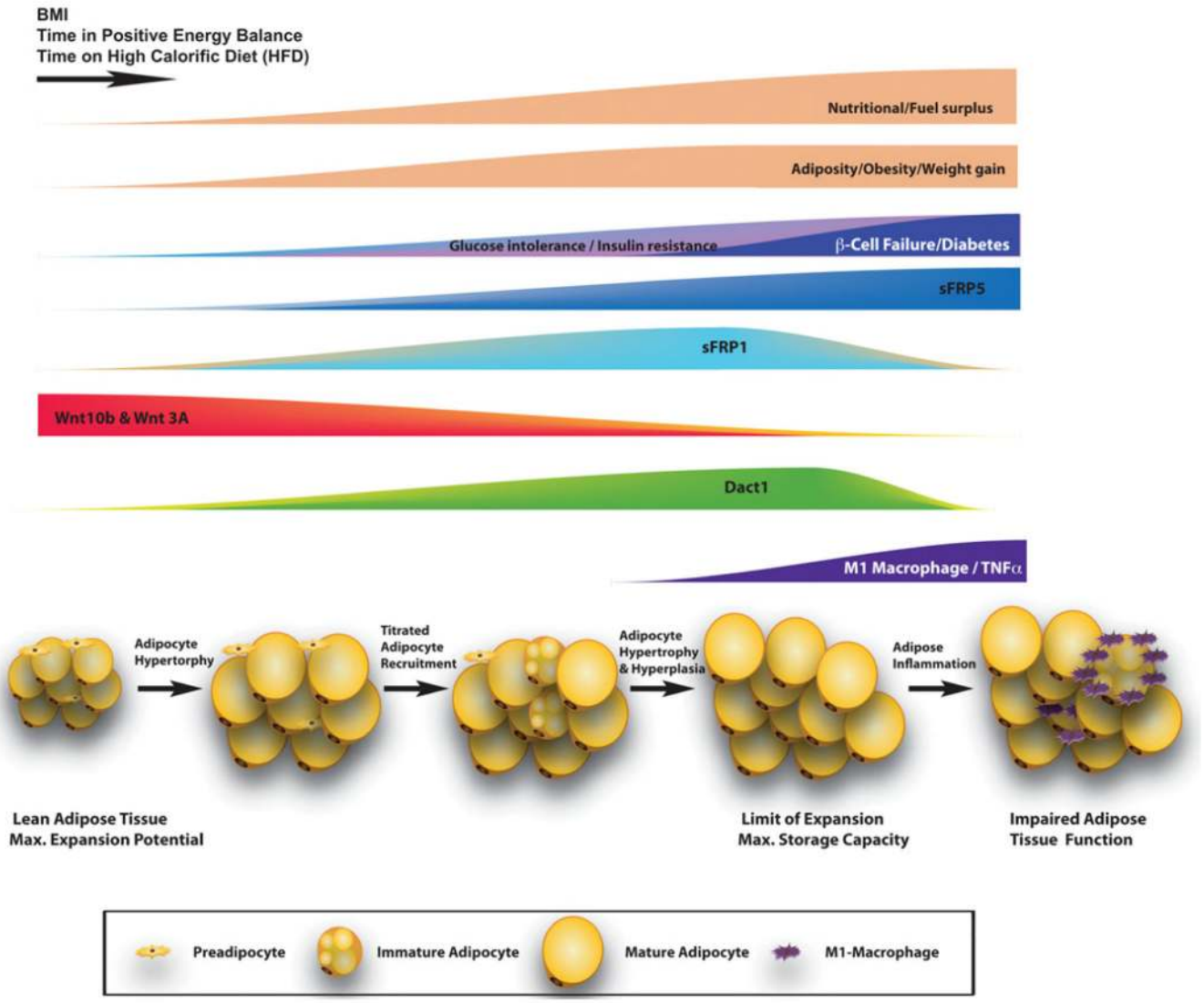

Figure 5. Adipose tissue expansion during the development of obesity and diabetes is accompanied by dynamic alterations in Wnt/β-catenin signalling components

As adipose tissue expands, activators of Wnt/β-catenin signalling are decreased while inhibitors increase. However, this reciprocal regulation appears to be uncoupled in dysfunctional obese insulin-resistant adipose tissue, probably due to increased pro-inflammatory cytokine activity. BMI, body mass index; HFD, high-fat diet.