REVIEW ARTICLE

Current concepts of PPAR-γ signaling in diabetes mellitus

M. Balasubramanyam* and V. Mohan

Madras Diabetes Research Foundation, 35 Conran Smith Road, Gopalapuram, Chennai 600 086, India

Peroxisome proliferator-activated receptors (PPARs, α, δ and γ) constitute a distinct subfamily of the superfamily of nuclear receptors that are activated by naturally occurring fatty acids or fatty acid derivatives. Recently, there is an increased interest in PPARγ research because they (a) are key regulators of adipocyte differentiation and energy source and (b) are cellular targets of thiazolidinedione drugs, which are used to treat Type 2 diabetes by decreasing insulin resistance. Additionally, PPARγ has emerged to be a powerful player in general transcriptional control of numerous cellular processes, with implications in diabetes and obesity, cell cycle control, carcinogenesis, inflammation, atherosclerosis and immunomodulation. This review focuses on some of the recent research on the pivotal role of PPARγ in insulin resistance and Type 2 diabetes.

Peroxisome proliferator-activated receptor

The peroxisome is a subcellular organelle whose functions extend well beyond the removal of molecular oxygen and later breakdown of hydrogen peroxide, to include glycerolipid synthesis, cholesterol biosynthesis and breakdown, and fatty-acid oxidation. The fact that proliferation of peroxisomes induced in rodents is associated with a multitude of biochemical changes has been for a long time contrasted with the uncertainty about the underlying mechanisms of peroxisome proliferation. Essentially, the discovery of the first peroxisome proliferator-activated receptor (PPAR) by Issemann and Green[1] was the key to the present understanding of peroxisome proliferation and its growing medical significance. Subsequently, several PPAR isotypes (α, β or δ and γ) have been found in vertebrate species[2], e.g. *Xenopus*, mouse, hamster and human. Based on DNA and protein sequence analyses, PPARs have been assigned to the subfamily of nuclear receptors that include the thyroid hormone receptors and the retinoic acid receptors.

*For correspondence. (e-mail: drmohan@giasmd01.vsnl.net.in)

PPARγ and insulin resistance/Type 2 diabetes

A more pleiotropic role has been recently assigned to PPARγ as it influences multiple fundamental pathways in the cell with wide-ranging biomedical implications[3,4]. In particular, studies looking into the molecular basis of insulin resistance have focused on the PPARγ, as they increase our understanding of the pathophysiology of Type 2 diabetes and also lead to the development of newer anti-diabetic agents. Type 2 diabetes is a major medical problem, the incidence of which is escalating rapidly in developing countries, with India harbouring the largest ever number of diabetics in the world[5]. Insulin resistance is one of the principal defects underlying the development of Type 2 diabetes and Asian Indians are considered to be more insulin-resistant[6–9]. Additionally, the prevalence of micro and macrovascular complications associated with diabetes is also increasing in epidemic proportions[10–14]. There is a general consensus that targetting insulin resistance early in the course of the disease may help achieve optimal glycemic control, halt disease progression, and probably even prevent the diabetic complications. This view has been strengthened by the recent trials of thiazolidinedione group of drugs that treat diabetes by increasing the sensitivity of insulin's action, primarily acting through PPARγ signaling.

Cellular abundance of PPARγ

Although PPARγ expression is detected in the nucleus of many cells, only adipose tissue, large intestine and haemotopoietic cells express the highest levels of PPARγ mRNA and protein[15]. Human muscle tissue expresses only trace amounts of PPARγ under basal conditions. However, PPARγ mRNA has been identified in skeletal muscle and is found to be increased in obese subjects with insulin resistance[16,17]. The expression of PPARγ mRNA or protein or both in adipose tissue changes under the influence of a number of metabolic and hormonal variables. While short-term changes in food intake do not affect the expression of human PPARγ, hypocaloric diets for a longer period result in its down regulation[18]. In rodents, PPARγ is down regulated by fasting and insulin-dependent diabetes melli-

tus[19] whereas its expression is induced by a high-fat diet. Interestingly, PPARγ expression is highly enriched in subcutaneous fat in normal weight subjects and its higher expression culminates in visceral adipose tissue in obese subjects[20]. Additional experiments also point out its regulation by insulin, tumour necrosis factor α(TNFα) and glucocorticoids[21–23]. Moreover, the tissue specific expression of PPARγ in endothelial[24] and vascular smooth muscle cells[25] suggest their causal and additional influence on vascular tone and elevated blood pressure.

While PPARγ seems to have its primary effects on adipose tissue, it is a paradox how PPARγ agonists improve insulin sensitivity in muscle, where glucose uptake maximally occurs. It is important to note that on a whole-body level, adipose tissue is indispensable for glucose homeostasis, as demonstrated by the link between lipoatrophy and insulin resistance[26,27], suggesting that the adipogenic activity of PPARγ contributes to insulin sensitization. As suggested by Martin *et al.*[28], the PPARγ agonists induce a 'fatty acid steal' by the adipose tissue. The resulting decreased systemic availability of fatty acids and diminished fatty acid uptake by muscle will improve insulin resistance. In a nutshell, short-term storage of excess energy, secondary to PPARγ activity, ameliorates insulin sensitivity. Nevertheless, the low abundance of PPARγ mRNA and protein in muscle tissue poses a question. Is PPARγ essential for the normal action of insulin and uptake of glucose? According to Auwerx[29], minute quantities of PPARγ in muscle might, however, be sufficient or alternatively might be induced during treatment with thiazolidinedione, leading to an eventual direct PPARγ-mediated response of the muscle to these insulin sensitizers. Conversely, PPARγ activators may generate an adipocyte-derived signal affecting insulin sensitivity in muscle.

Mechanisms of PPAR activation and regulation of target gene expression

The mechanisms by which PPAR are activated and thus regulate transcriptional expression of target genes are summarized in Figure 1. When PPARγ is bound by natural ligand or synthetic molecules such as a thiazolidinedione, it becomes activated and complexed with another transcription factor known as the retinoid X receptor (RXR). Transcriptional regulation by PPARs is achieved through PPAR-RXR heterodimers which bind to DNA motifs termed peroxisome proliferative response elements (PPREs) in the promoters of target genes. The whole PPRE consensus sequence exhibits a pattern specific for PPAR-RXR heterodimer[30] and is distinguishable from the responsive elements of other nuclear receptors belonging to oestrogen, vitamin D or thyroid hormone. PPAR-mediated transcriptional control of genes is regulated by a new functional class of proteins called cofactors (corepressors and coactivators). SMRT (silencing mediator for retinoid and thyroid hormone receptor) is one such corepressor reported to be involved in down-modulating PPARγ-mediated gene transcription[31]. Interestingly, a number of proteins[29,32,33] have been identified and characterized as coactivators of PPARγ such as CREB binding protein (CBP), P300, steroid receptor coactivator (SRC-1), PPAR binding protein (PBP) and PPARγ coactivator-1 (PGC-1). Zhu *et al.*[34] have recently reported a novel coactivator of PPARγ, designated as PPAR interacting protein (PRIP). It has been postulated that these coactivators act as bridges to transmit the nuclear receptor regulatory signals to the cellular transcriptional machinery. In general, unactivated nuclear receptors are complexed with corepressors, which extinguish their transcriptional activity by the recruitment of histone deacetylases. Activation of the receptor then induces a conformational change which results in the dissociation of corepressors and the recruitment of coactivator complexes that contain proteins with histone acetyl transferase activity, which facilitates target gene transcription[35]. Apart from these cofactors, activation of PPARγ can also be depressed by phosphorylation of a seryl residue in its structural region, mediated by mitogen-activated protein (MAP) kinase[36]. In fact, phosphorylation at Ser114 was proposed as a mechanism by which growth factors and insulin, through MAP kinase, decrease PPARγ activity and adipocyte differentiation[37–40]. The final action of PPARγ depends on a variety of factors such as the abundance of the relevant endogenous ligands/activators, numerous co-activators or co-repressors and the expression and function of RXRs, the companion nuclear receptors essential for formation of the active heterodimeric complex (PPARγ + RXR).

'Glitazones' as pharmacological insulin sensitizers

The thiazolidinedione class of drugs or glitazones as they are called (troglitazone, pioglitazone, ciglitazone, englitazone and rosiglitazone) are specific high-affinity ligands for PPARγ[41], and the order of their receptor-binding affinities *in vitro* mirrors their antihyperglycemic activity *in vivo*[42]. PPARγ enhances the expression of a number of genes encoding proteins involved in glucose and lipid metabolism[43,44]. Particularly, adipocyte differentiation responds well to pharmacological PPARγ ligands. Functional PPREs have been identified in several adipocyte-specific genes (viz. phosphoenol pyruate carboxykinase, aP2, acyl CoA synthase, fatty acid

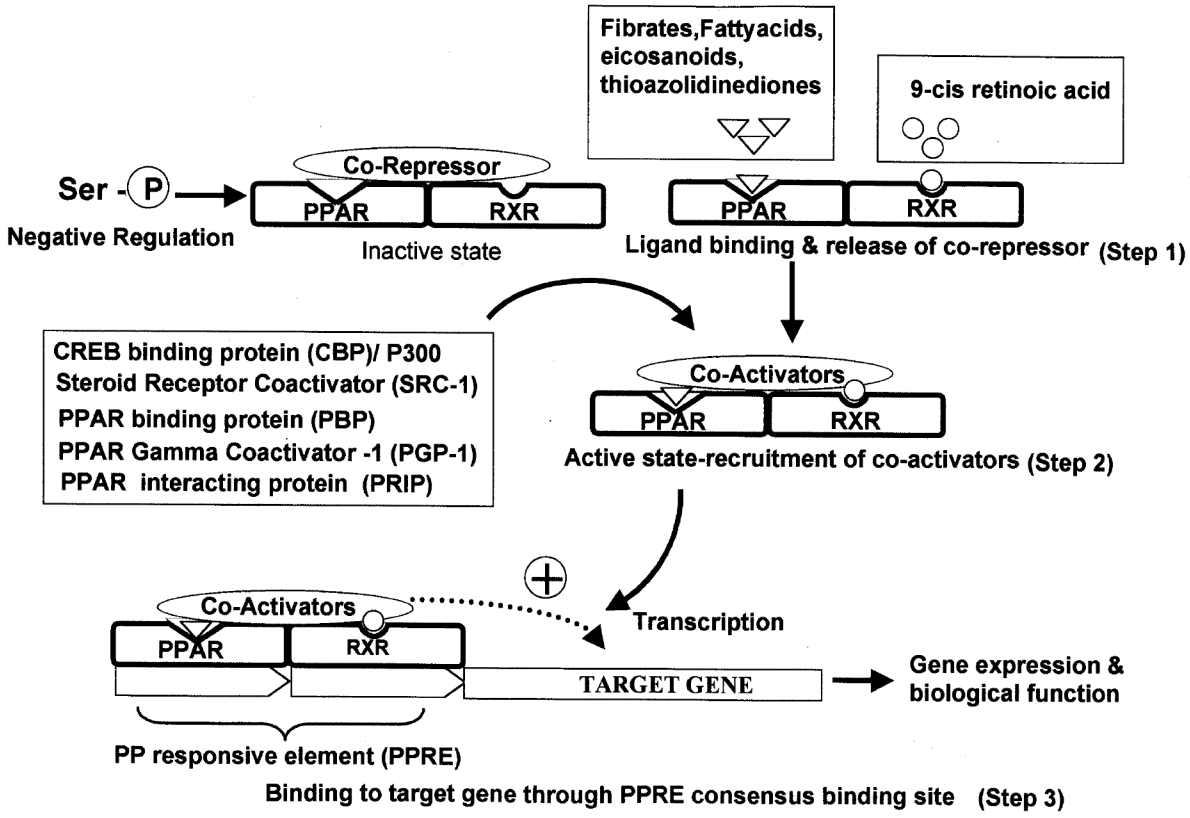

Figure 1. Mechanism of PPAR activation and transcriptional regulation of target genes.

transport protein-1, and lipoprotein lipase) and the fact that all of them regulated by PPARγ provide evidence that PPARγ and its target genes have an interdependent role in adipocyte differentiation[45]. Leptin gene expression is shown regulated by PPARγ[46–48] and the decrease in circulating leptin concentrations after PPARγ activation seem to be associated with an increase in food intake, which provides substrates, subsequently to be stored in the adipocytes. Whereas, TNFα exerts an anti-adipogenic action in part by the down-regulation of the expression of adipogenic factors including PPARγ[22,23,49], activation of PPARγ stimulates adipogenesis and blocks the inhibitory effects of TNFα on insulin signaling[49] as well as the TNFα-induced glycerol and non-esterified fatty acid release[50]. Thus, stimulation of PPARγ may decrease the release by the adipocytes of various signaling molecules, such as free fatty acids, leptin, and TNFα, all of which are able to counteract the hypoglycemic action of insulin[44]. In addition to their role in adipocyte differentiation, glitazones also profoundly affect lipid metabolism. They increase the lipolysis of triglycerides in very-low-density lipoproteins (VLDL) and thereby reduce triglyceride and increase HDL-cholesterol levels[51,52]. Moreover, increased glucose uptake and mRNA expressions of the glucose transporter isoforms (GLUT1 and GLUT4) were induced by glitazones through PPARγ activation[53].

Stimulation of PPARγ with thiazolidinediones in 3T3-L1 adipocytes or in diabetic rodents lead to increased c-Cbl-associating protein (CAP) expression and increased insulin-stimulated c-Cbl phosphorylation that correlates well with increased insulin sensitivity both *in vitro* and *in vivo*[54]. The restricted expression of CAP in cells metabolically sensitive to insulin suggests an important potential role in insulin action. Administration of troglitazone to Zucker (fa/fa) rats markedly increased the expression of the major CAP isoform in adipose tissue. This effect was sustained for up to 12 weeks of treatment and accompanied the ability of troglitazone to prevent the onset of diabetes and its complications. Thus, CAP is the first PPARγ-sensitive gene identified that participates in insulin signaling and may play a role in glitazone-induced insulin sensitization. In support of this, Baumann *et al.*[55] have recently cloned and characterized a functional PPRE in the promoter of the CAP gene. Interestingly, the antidiabetic drug troglitazone has been demonstrated to differentially activate PPAR-γ (either full or as a partial agonist) in a manner dependent on the cellular environment[56]. The observation that ligands can have distinct effects on the receptor raises

the possibility that different PPARγ ligands induce different sets of genes in a tissue specific way to translate distinct downstream biological effects. This explains why thiazolidinediones, besides their metabolic activities, have effects as diverse as the control of host defence, cell proliferation and tumorigenesis[57].

One attractive feature of the thiazolidinedione insulin sensitizers is their synergism with glucose-lowering drugs (metformin, sulphonylurea or insulin) that have a different mechanism of action[51,58]. When added to current treatment in patients whose glycemic control remained unsatisfactory despite sulphonylureas, metformin, insulin, or a combination of these agents, glitazones seem to be very effective, as judged by decreases in serum levels of glucose, insulin and HbA1c[59,60].

While thiazolidinediones more specifically enhance insulin sensitivity, they also potently promote adipocyte differentiation and often increase total fat mass[61]. Because obesity is a major cause of insulin resistance, this presents an apparent paradox. In the absence of controlled long-term studies, it is not clear whether glitazones induce progressive weight increase in patients. However, one should consider the reported observations in humans that treatment with thiazolidinediones results in a redistribution of body fat from visceral to subcutaneous depots[61–63]. Thus, as suggested by Montague and O'Rahilly[64] treatment with thiazolidinediones may induce anatomical distribution of adipose tissue with the redistribution of body fat away from 'dangerous' intra-abdominal sites and toward 'safer' subcutaneous ones.

The arrival of novel thiazolidinediones (KRP-297, JTT-501, NC-2100, NIP-223, MCC-555, L-764486, CS-011) is also promising in that they encounter some of the unfavourable effects of simple agonists like troglitazone[65–67]. Additionally, there is progress in the discovery and development of structurally novel class of tyrosine-based PPAR-γ modulators with antidiabetic activity. The compound GI262570 is, in particular, claimed to be the most potent PPAR-γ agonist reported to date[68,69]. GI262570 is prepared from naturally occurring (L)-tyrosine and does not contain the 2,4-thiazolidinedione structure common to the glitazone class of insulin-sensitizing agents. In addition, unlike the glitazones, GI262570 is a single enantiomer and is not prone to racemization at physiological pH.

Sequence variants of PPARγ and the ultimate phenotypes

Requirement of proper PPARγ signaling for ensuing normal insulin sensitivity is highlighted by recent genetic studies. Barroso *et al.*[70] have reported the identification of two loss-of-function mutations of PPARγ that are associated with severe insulin resistance and Type 2 diabetes in humans. Although such mutations are rare, they have shown that the people affected by loss-of-function PPARγ mutations share common characteristics of the 'insulin resistance syndrome' minus obesity. Typically insulin resistance syndrome is characterized by obesity along with insulin resistance, diabetes, high blood pressure, dyslipidemia and acanthosis nigricans. Interestingly, reduced PPARγ signaling seems to cause insulin resistance in the absence of obesity. This study contrasts sharply with the symptoms of gain-of-function mutation of PPARγ, reported by Ristow *et al.*[71], wherein increased PPARγ signaling was found associated with human obesity. Unexpectedly, the degree of obesity in the study of Ristow *et al.*[71] has no association with Type 2 diabetes or hyperinsulinemia, and possibly defines a specific subclass of obesity.

Insulin resistance is especially likely to occur when excess fat is deposited within the abdominal cavity. This reduces the insulin sensitivity of fat cells and also of other tissues including skeletal muscle and liver. But how might expanding adipose stores impair PPARγ function? Expanding adipose stores may alter the availability of free fatty acids and modify the PPAR ligand binding interactions. The two PPARγ mutations reported by Barroso *et al.*[70] lead to amino-acid substitution in regions of the molecule involved in ligand binding. These changes disrupt the ligand binding process and are associated with insulin resistance and normal body weight in humans. By contrast, the obesity-inducing PPARγ mutation reported by Ristow *et al.*[71] results in an amino-acid substitution adjacent to the serine phosphorylation site. Serine phosphorylation at the site of 114 in the human PPARγ gene suggests a mechanism of negative regulation to limit adipocyte differentiation and lipid accumulation[37,39]. The mutations described by Ristow *et al.*[71] impair this phosphorylation site, increase PPARγ signaling and establish a causal association with obesity.

Though the above studies indicate that 'too much or too little PPARγ signaling is not good', the relation of PPARγ variation to obesity and insulin resistance is not so simple. Recent studies also demonstrate that a much more common Pro12Ala PPARγ2 sequence variant has been variably associated with either increased[72] or decreased[73,74] body mass index (BMI) and improved insulin sensitivity. Additional complexity arose from studies that reveal no association of Pro12Ala substitution with BMI and insulin sensitivity[75]. These results suggest that the physiological consequences of the Pro12Ala polymorphism could be different in the lean and obese states. This has been shown in a Danish study where Pro12 Ala sequence variation was associated with lower BMI among lean subjects and higher BMI among obese subjects[76]. Interestingly, the Pro12Ala polymorphism in PPARγ2 was shown to protect against Type 2 diabetes

in the Japanese[77]. These apparently conflicting results highlight the gene–environment interactions in the determination of the phenotype.

Significance of PPARγ in Indians

Several studies on Asian Indians have shown that they are characterized by higher insulin resistance, early onset Type 2 diabetes and hypertension without having a strong association of obesity[11,78–82]. Will it mean that we could expect more loss-of-function mutations of PPARγ in Indians? Although disease-causing mutations of PPARγ are rare, insulin resistance syndrome may also result from impaired PPARγ signaling in the absence of a mutation. Since a number of free fatty acids are PPARγ ligands[25,83], their alterations in the presence or absence of obesity could reduce PPARγ signaling and lead to insulin resistance. Additionally, the notion that PPARγ is referred to as a 'thrifty genotype'[29] may be very well tested in Indians. The 'thrifty genotype' hypothesis put forward by Neel[84] is stated as follows: 'Among populations exposed to a varying supply of food, it is advantageous to be metabolically thrifty and store a high proportion of energy intake as fat during time of plenty, as insurance against times of famine'. When individuals with the thrifty genotype are confronted with a continuous supply of energy-dense processed foods, coupled with a reduction in physical activity, as is the case with urban Indians now, one could expect to see more prevalence of obesity, impaired glucose tolerance and Type 2 diabetes and indeed this is so[11–14,85]. The enhanced adipocyte differentiation which ensues from PPARγ activation, supports the view that PPARγ coordinates the thrifty response and urges the need for studying PPARγ in Indians as this could explain partly the heterogeneity of insulin resistance and Type 2 diabetes in Indians. It is also important in the context of overwhelming response among patients to the thioazolidinedione and non-thioazolidinedione PPAR agonists in the treatment of insulin resistance and Type 2 diabetes[43,86–88].

Lessons we learn and future directions

Research in PPAR has attained great medical significance because of its multiple effect on metabolic disorders and the fact that developing countries like India are undergoing an epidemiological transition. Combined with genetic predisposition, changes in diet and lifestyle contribute to the huge prevalence of non-communicable diseases and in particular diseases of micro and macrovascular complications of diabetes mellitus[10–13,89]. Dietary modifications play an important role in initiation of insulin resistance syndrome and long chain ω-3 fatty acids in phospholipid of skeletal muscles are important for the action of insulin[90]. There is a competition between ω-6 and ω-3 fatty acids for the enzymes of desaturation and elongation, thus bringing forth high ω-6/ω-3 ratio as a critical factor in development of insulin resistance and atherosclerosis[91]. The overall diet pattern and in particular, the oil preferred for cooking in India is considerably changing with changes in the ratio of ω6/ω3 fatty acids which may play a role in diabetic micro and macrovascular complications. The identification of fatty acids and their derivatives as ligands for PPARγ emphasize lipids as direct modulators of cellular responses. PPARγ is activated by a range of naturally-occurring substances, including polyunsaturated fatty acids, 15-deoxy-delta prostaglandin J2, α-linolenic acid, eicosapentaenoic acid, docohexanoic acid and components of oxidized low-density lipoprotein, such as 13-hydroxyoctadecadienoic acid and 15-hydroxy-eicosatetraenoic acid. However, the identities of endogenous ligands for PPARγ and their means of production *in vivo* in sufficient concentrations have not been fully elucidated. Nevertheless, their local concentrations may rise to a threshold for PPARγ activation via other common metabolites. For example, a role of lipoxygenase has been implicated in the generation of endogenous ligands such as eicosanoids and leukotrienes that in turn act as PPARγ activators[92,93].

No doubt, there is much to be investigated to exploit the modulators of PPARγ for long-term therapeutic use in metabolic diseases. We need to identify novel ways to modulate PPARγ activity without complicating issues such as the enhancement of macrophage foam cell formation[94,95], stimulation of colon carcinogenesis[96,97] and induction of acute liver dysfunction[98,99]. The restricted expression of certain PPARγ isoforms, such as the adipose-restricted PPARγ2 form and macrophage-restricted PPARγ3 form, suggests the feasibility of the development of tissue-specific PPARγ modulators. In fact, PPARγ modulators rather than simple agonists can function better as full or partial agonists or antagonists, depending on cell type and sequence-recognition site. Such agents will have greater medical benefit since they can induce beneficial effects on certain target tissues yet lack activity in other tissues where activation is less desirable. As PPARγ represents an important therapeutic target for the treatment of insulin resistance syndrome, a careful and complete understanding of its exact role in physiology is an absolute requirement. The continued development of pharmacological insulin sensitizers (both new generation thiazolidinediones and non-thioazolidinedione PPAR agonists) also provide us with novel probes to investigate the pathophysiology of Type 2 diabetes with special emphasis on PPAR-γ signaling cascade. The ever-increasing pleiotropic role of PPARγ is certain to initiate a new flurry of research in the coming years.

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Quantum computation using NMR

Kavita Dorai[†], T. S. Mahesh[†], Arvind[‡] and Anil Kumar[†,*,#]

[†]Department of Physics, Indian Institute of Science, Bangalore 560 012, India
[‡]Department of Physics, Guru Nanak Dev University, Amritsar 143 005, India
*Sophisticated Instruments Facility, Indian Institute of Science, Bangalore 560 012, India

This article reviews recent work done by us on some initial steps towards the implementation of quantum computation using liquid state NMR. We describe how special kinds of states required for such computation (called pseudo-pure states) can be created from a thermal ensemble of spins. We demonstrate the implementation of several quantum logic gates through one- and two-dimensional NMR methods, using transition- and spin-selective pulses. Finally, we discuss the implementation of the Deutsch–Jozsa algorithm using NMR.

ALL present-day computations use two-state binary logic and have led to a large revolution in data processing and manipulation. However, several scientists have wondered if quantum mechanical systems could provide a new paradigm for computation. Feynmann in particular, hypothesized that it might be possible to simulate quantum evolution efficiently, provided the simulator was itself quantum-mechanical in nature[1].

The question that arises is: can real quantum systems be used to build computers that operate in the quantum-mechanical regime, and how much more computing power would such devices be able to achieve? These are some interesting issues being addressed by researchers today, and this fusion of ideas from quantum physics and information theory has led to exciting new developments in quantum cryptography[2,3], teleportation[4], error correction[5,7], and quantum computation[8–15].

It was proved early on, that traditional Boolean logic gates can be implemented using a set of modified 'reversible' gates, and that one can find a minimal set of such gates that are sufficient for computation[16,17]. Furthermore, researchers in the early 1980s made a fundamental connection between quantum mechanics and reversible computation by proposing that reversible Boolean computation can be simulated by the time evolution of a quantum system, which is a unitary reversible dynamic[18,19]. Logical operations in quantum computation are implemented on quantum bits (qubits), the basic units of quantum information. A qubit can be visualized as the states of a two-level quantum system, like the two spin states of a spin-1/2 particle or the two different polarization states of a single photon. The realization that, the two eigenstates labelled by $|0\rangle$ and $|1\rangle$ can be mapped onto logical 0 and 1, leads to the possibility of the quantum-mechanical implementation of logic gates and circuits[20–23]. Unlike the classical bit which can exist only in two states, the permitted states for a qubit ($\cos\theta\ |0\rangle + \sin\theta\ e^{i\phi}|1\rangle$), span a 2D complex vector space (Figure 1). A state for n qubits can in general be represented by a 2^n-dimensional complex vector. While all classical computation can be performed using the mapping between eigenstates and logical states, the fact that a qubit can exist in a general coherent superposition of the eigenstates, leads to new possibilities for computation. What is intrinsically different about a quantum computation? The answer lies in the fact that it exploits inherently quantum features like quantum superposition and entanglement to solve problems hitherto deemed intractable, on any classical computer. If two qubits are in a state such as $\frac{1}{\sqrt{2}}\{|00\rangle - |11\rangle\}$, which is not resolvable into the tensor product of the states of the individual qubits, the qubits are said to be entangled[24–26]. Neither qubit by itself has a definite state, in contrast to a classical system which can be completely resolved into the states of each part of the system. The existence of such entangled states is fundamental to the quantum world and leads to counter-intuitive phenomena like the violation of Bell's inequalities. It is interesting that such intriguing states have now found an application in computation and information processing, to reduce the level of complexity of computational tasks.

A measure of computational complexity is how the number of steps required for the computation (denoted s) evolves mathematically as a function of the size of the problem (denoted L). If s is a polynomial function of L, the problem is tractable; if s rises exponentially with L, the problem is thought to be intractable[14,15]. An example of a computationally hard problem is that of the

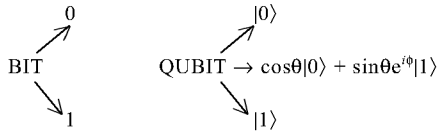

Figure 1. Units of information: bits and qubits. A classical bit can only take the values 0 and 1, whereas a qubit can exist in any coherent superposition of the two.

#For correspondence. (e-mail: anilnmr@physics.iisc.ernet.in)