REVIEW

Peroxisome proliferator-activated receptor-γ: from adipogenesis to carcinogenesis

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ABSTRACT

Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors, initially described as molecular targets for synthetic compounds inducing peroxisome proliferation. PPAR-γ, the best characterized of the PPARs, plays a crucial role in adipogenesis and insulin sensitization. Furthermore, PPAR-γ has been reported to affect cell proliferation/differentiation pathways in various malignancies. We discuss in the present review recent advances in the understanding of the function of PPAR-γ in both cell proliferation and adipocyte differentiation.
Journal of Molecular Endocrinology (2001) **27,** 1–9

INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor family, the largest family of transcription factors (Mangelsdorf *et al*. 1995). Three distinct members of the PPAR subfamily have been described: α, δ (also called β, NUC-1 or FAAR) and γ, all of them being activated by naturally occurring fatty acids or fatty acid derivatives. PPARs heterodimerize with the retinoid X receptor and regulate transcription of target genes through binding to specific response elements or PPREs, which consist of a direct repeat of the nuclear receptor hexameric DNA core recognition motif spaced by one nucleotide. A number of studies have addressed the important role that PPAR-γ plays in glucose homeostasis and insulin sensitivity. However, in this review we discuss recent data on the function of PPAR-γ in adipogenesis and carcinogenesis.

PPAR-γ AND ADIPOGENESIS

Adipocyte differentiation is a highly regulated process taking place from birth throughout adult life. Adipose tissue is composed of adipocytes, which store energy in the form of triglycerides and release it as free fatty acids (for reviews see Spiegelman & Flier 1996, Fajas *et al*. 1998). Together with muscle, adipose tissue is the major regulator of energy balance of the body. Excessive accumulation of adipose tissue leads to obesity, whereas its absence is associated with lipodystrophic syndromes. PPAR-γ is highly expressed in the adipose tissue and is required for its development. During adipocyte differentiation, which ensues from PPAR-γ activation, expression of numerous genes specific for fatty acid metabolism is induced. In fact, functional PPREs have been identified in several genes implicated in adipocyte differentiation, most of them involved in lipid storage and control of metabolism. Good examples are aP2 (Tontonoz *et al*. 1994*a*), phosphoenol pyruvate carboxykinase (Tontonoz *et al*. 1995), acyl CoA synthetase (Schoonjans *et al*. 1993, 1995), fatty acid transport protein-1 (Martin *et al*. 1997, Frohnert *et al*. 1999), and lipoprotein lipase (Schoonjans *et al*. 1996), which are all regulated by the PPAR-γ protein. However, the conclusive demonstration of the crucial role that PPAR-γ plays in adipogenesis comes from recent observations in

0952–5041/01/027–001 © 2001 Society for Endocrinology *Printed in Great Britain*

PPAR-γ knock-out (KO) mice. PPAR-γ −/− mice are completely devoid of adipose tissue and PPAR-γ +/− mice are characterized by a decreased adipose tissue mass (Kubota *et al*. 1999, Miles *et al*. 2000). Injection of PPAR-γ −/− embryonic mouse cells into wild-type blastocytes produces chimeric mice in which adipose tissue is composed exclusively of PPAR-γ +/+ cells, demonstrating that PPAR-γ is necessary to ensure development of this tissue (Rosen *et al*. 1999). These *in vivo* results are further supported by *in vitro* data showing that embryonic stem (ES) cells lacking both copies of PPAR-γ fail to differentiate into adipocytes after appropriate treatment, whereas ES cells expressing PPAR-γ readily differentiate. Moreover, when fibroblastic cell lines (Tontonoz *et al*. 1994*b*) or muscle precursor cells (Hu *et al*. 1995) were infected with retrovirus expressing PPAR-γ, cells differentiated into adipocytes after the appropriate stimuli, supporting an important pro-adipogenic role of PPAR-γ. It seems that this adipogenic property is not unique since a whole range of transcription factors affects adipogenesis when overexpressed (see below).

In humans, genetic studies have further contributed to determine the role of PPAR-γ in fat metabolism. Several mutations in the PPAR-γ gene have so far been described (Yen *et al*. 1997, Beamer *et al*. 1998, Deeb *et al*. 1998, Ristow *et al*. 1998, Vigouroux *et al*. 1998). A rare Pro115 Gln mutation in the NH_2-terminal ligand-independent activation domain of PPAR-γ was found in four very obese subjects (Ristow *et al*. 1998). This mutation, which inhibits the phosphorylation at Ser112, resulted in a permanently active PPAR-γ and led to increased adipocyte differentiation and obesity (Ristow *et al*. 1998). Phosphorylation at Ser112 was proposed as a mechanism by which growth factors and insulin, through MAP kinase, decrease PPAR-γ activity and adipocyte differentiation (Hu *et al*. 1996, Adams *et al*. 1997, Camp & Tafuri 1997). Furthermore, a much more common Pro12 Ala substitution in the PPAR-γ2-specific exon B (Yen *et al*. 1997, Beamer *et al*. 1998, Deeb *et al*. 1998, Vigouroux *et al*. 1998, Hara *et al*. 2000), resulting in a less active PPAR-γ form, is associated with a lower body mass index (BMI). These results, together with the observations made on the Pro115 Gln substitution, provide strong evidence for a role of PPAR-γ in the control of adipogenesis *in vivo*, such that a more active PPAR-γ (Pro115 Gln) results in increased BMI (Ristow *et al*. 1998), whereas the opposite is seen with a less active PPAR-γ (Pro12 Ala) (Deeb *et al*. 1998).

Despite the fact that it plays a critical role in adipogenesis, PPAR-γ is not the only factor regulating the complex mechanisms that control adipocyte differentiation. Numerous other positive and negative signaling pathways contribute to this process. During the first phases of adipogenesis, the CCAAT enhancer binding proteins (C/EBP), C/EBP-β and -δ are induced in response to adipogenic hormones such as insulin or glucocorticoids (Wu *et al*. 1995, 1996, Yeh *et al*. 1995). Both C/EBPs will induce directly the transcription of PPAR-γ (Clarke *et al*. 1997). However, other transcription factors might also be in part responsible for triggering PPAR-γ expression early in adipogenesis, since studies in C/EBP-α, -β or -δ KO mice show that PPAR-γ expression and adipocyte differentiation are still occurring, although at a lesser extent. Another protein also induced early during adipocyte differentiation is the basic helix-loop-helix protein ADD-1/SREBP-1 (Tontonoz *et al*. 1993, Kim & Spiegelman 1996). This transcription factor plays a pivotal role in cholesterol homeostasis and also regulates the expression of several genes in fatty acid metabolism, and hence it is suggested that ADD-1/SREBP-1 might control the generation of PPAR-γ ligands that in their turn enhance the transcriptional activity of PPAR-γ (Fajas *et al*. 1999). Furthermore, a recent study showed that both ADD-1/SREBP-1 and the related SREBP-2 can induce PPAR-γ transcription through binding to response elements in the PPAR-γ1 and -γ3 promoter regions (Fajas *et al*. 1999). Finally, terminal adipocyte differentiation requires the concerted action of PPAR-γ and C/EBP-α (Tontonoz *et al*. 1994, Hu *et al*. 1995, Wu *et al*. 1999). PPAR-γ controls not only the expression of C/EBP-α, but C/EBP-α, in response, also induces PPAR-γ gene expression, via interaction with C/EBP response elements present in the human (Saladin *et al*. 1999) and mouse (Zhu *et al*. 1995, Wu *et al*. 1999) PPAR-γ promoter. This interdependence or cross-regulation between C/EBPs, PPAR-γ and ADD-1/SREBP-1 is not only required to induce adipocyte differentiation but also to sustain the fully differentiated adipocyte phenotype. Although all the above transcription factors stimulate adipocyte differentiation, evidence in favor of negative regulation of adipogenesis is also accumulating. It has been suggested that PPAR-γ expression and adipogenesis are inhibited by several transcription factors of the GATA family. In particular, constitutive expression of GATA-2 and GATA-3 resulted in a decrease in PPAR-γ expression and a consequent inhibition of adipocyte differentiation (Tong *et al*. 2000).

In addition to these transcription factors which modulate adipogenesis, several secreted factors are involved in the control of adipogenesis. This is the

case for two cytokines produced by the adipocytes: leptin and tumor necrosis factor-α (TNF-α). Leptin is considered to be an adipocyte-derived signaling factor and is thought to have autocrine, paracrine and endocrine actions mediated by specific cytokine-like receptors. Its pleiotropic action includes control of body weight and energy expenditure (reviewed in Auwerx & Staels 1998). Leptin gene expression is regulated in an opposite fashion by PPAR-γ and C/EBP-α, the first reducing its expression (De Vos *et al*. 1996, Kallen & Lazar 1996, Zhang *et al*. 1996), whereas the second induces its expression (He *et al*. 1995, Miller *et al*. 1996, Hollenberg *et al*. 1997). The decrease in circulating leptin levels upon PPAR-γ activation is associated with an increase in food intake, which will provide substrates, subsequently to be stored in the adipocytes. Consistent with this hypothesis, PPAR-γ +/− mice receiving a high-fat diet have a higher circulating leptin level than normal mice (Kubota *et al*. 1999). Leptin expression is probably less attenuated, due to the weaker expression of PPAR-γ in these PPAR-γ +/− mice.

A similar hypothesis can be formulated in relation to adipose tissue TNF-α production. TNF-α is a potent inhibitor of adipocyte differentiation and exposure of 3T3-L1 adipocytes to TNF-α results in lipid depletion and a complete reversal of adipocyte differentiation (Torti *et al*. 1985, reviewed in Beutler & Cerami 1988). TNF-α exerts this anti-adipogenic action in part by the down-regulation of the expression of adipogenic factors such as C/EBP-α (Ron *et al*. 1992, Williams *et al*. 1992) and PPAR-γ (Hill *et al*. 1997, Peraldi *et al*. 1997, Xing *et al*. 1997). Interestingly, obesity characterized by increased adipose tissue mass is associated with increased TNF-α expression in adipose tissue. Although the exact role of high TNF-α levels in obesity is unclear, it might constitute a regulatory mechanism to limit further increase in adipose tissue mass. This increase in TNF-α levels in obesity also interferes with the insulin signaling pathways (Hotamisligil *et al*. 1993, 1995) contributing to the insulin resistance characteristic of the obese state (Hotamisligil *et al*. 1994, 1996). Consistent with the opposing effects of PPAR-γ and TNF-α in adipose tissue, treatment of obese animals with PPAR-γ agonists reduces adipose tissue expression of TNF-α, contributing to weight gain (Hofmann *et al*. 1994, Okuno *et al*. 1998). PPAR-γ activation furthermore blocks the inhibitory effects of TNF-α on insulin signaling (Peraldi *et al*. 1997) as well as the TNF-α-induced glycerol and free fatty acid release (Souza *et al*. 1998).

Very recently, a family of signaling factors, Wnts, which play a major role in the regulation of cell growth and development, have been implicated in the inhibition of adipocyte differentiation. Ross *et al*. (2000) demonstrated that forced expression of Wnt-1 in 3T3-F442A cells inhibited the formation of adipose tissue when these cells were grafted into nude mice. Furthermore, 3T3-L1 cells ectopically expressing a dominant negative form of TCF4, a transcriptional mediator of the Wnt pathway, undergo adipogenesis without any hormonal induction (Ross *et al*. 2000). Repression of PPAR-γ and C/EBP-α expression was suggested as the mechanism by which activation of the Wnt signaling inhibited adipogenesis.

CELL CYCLE REGULATION DURING ADIPOGENESIS

Cell proliferation and differentiation are considered to be mutually exclusive events. However, a close relationship has been established between both cell processes during the adipocyte differentiation program. One of the first events occurring during adipogenesis is re-entry into cell cycle of growth-arrested preadipocytes following hormonal induction. After several rounds of clonal expansion, cells arrest proliferation again and undergo terminal adipocyte differentiation. In the first hours of adipocyte differentiation, an increase in the E2F activity has been observed (Richon *et al*. 1997). E2Fs are transcription factors which regulate the expression of genes involved in DNA synthesis (for reviews see Nevins 1992, Sardet *et al*. 1997, Helin 1998). Consequently, expression of these genes, such as cyclin D1, c-Myc, or cyclin E, is increased in the early stages of adipogenesis (Reichert & Eick 1999). Interestingly, blocking cell cycle re-entry with a DNA synthesis inhibitor, prevents adipocyte differentiation, suggesting that an active cell cycle machinery is required for the differentiation process (Richon *et al*. 1997). Similar results were obtained when degradation of p27, a cyclin-dependent kinase inhibitor, was prevented using a protease inhibitor. As a consequence of p27 protein accumulation, cell cycle re-entry was blocked, and thus differentiation of preadipocytes was inhibited (Patel & Lane 2000).

The role of the retinoblastoma protein (RB) family members, or the pocket proteins pRB, p130, and p107, in adipocyte differentiation seems more complex. The negative role of pocket proteins in cell cycle progression, repressing the expression of the E2F target genes has been demonstrated in several settings. Pocket proteins are inactivated by phosphorylation by the cyclin-dependent kinases, resulting in the activation of the E2F target genes. Consistent with an active cell cycle in the early stages of adipogenesis, pocket proteins have been

found to be hyperphosphorylated following hormonal induction of preadipocytes (Richon *et al*. 1997). However, an apparent paradox arises from the finding that pRB inactivation, by SV40 large T antigen, inhibits adipogenesis (Higgins *et al*. 1996). Moreover, pRB-deficient fibroblasts fail to differentiate into adipocytes when properly stimulated (Chen *et al*. 1996). This apparent paradox was explained by the participation of RB in the growth arrest following clonal expansion. This suggests that RB is involved in two phases of adipocyte differentiation. First, inactivation of RB enables clonal expansion, whereas growth arrest after this expansion phase requires active RB, which positively influences adipocyte differentiation. Interestingly, this function of RB can be compensated by overexpression of both C/EBP-α and PPAR-γ, which would mediate the cell cycle arrest after clonal expansion (Classon *et al*. 2000). A different role, independent of the control of cell cycle, has also been attributed to RB in another aspect of the regulation of adipogenesis such as the enhancement of the transactivation capability of C/EBP, via direct protein–protein interaction (Chen *et al*. 1996). In contrast to RB, the other members of the retinoblastoma family, p130 and p107, have been reported to negatively regulate adipogenesis. Indeed, fibroblastic cells deficient in both p130 and p107 differentiate into adipocytes whereas the wild-type cells do not (Classon *et al*. 2000). Furthermore, reintroduction of p130 and p107 into these cells inhibits adipocyte differentiation. These effects of p107 in adipogenesis have been suggested to be mediated through down-regulation of PPAR-γ activity.

Undoubtedly there is a cross-talk between the cell cycle and the adipocyte differentiation machinery. How the shift in gene expression observed during the transition between preadipocyte proliferation and adipocyte differentiation is regulated needs, however, further investigation.

ROLE OF PPAR-γ IN THE CONTROL OF CELL CYCLE

Studies based on tumor cell lines have implicated PPAR-γ in cell cycle withdrawal. One of the first pieces of evidence implicating PPAR-γ in the control of cell cycle came from the observation that PPAR-γ activation decreased the binding of the E2F/DP heterodimers to its target genes. This decrease in E2F/DP activity is in part mediated by PPAR-γ through the down-regulation of the PP2A protein phosphatase (Altiok *et al*. 1997). Inhibition of E2F/DP activity can also be achieved via activation of RB. Interestingly, PPAR-γ ligands were shown to inhibit phosphorylation of RB in vascular smooth muscle cells (Wakino *et al*. 2000), therefore contributing to maintain RB in its active form. Consequently, the G1/S transition in these cells was abrogated. Another suggested mechanism involving PPAR-γ in the mediation of cell cycle arrest was provided by the study of Morrison & Farmer (1999), who suggested a role of PPAR-γ in up-regulating the cyclin-dependent kinase inhibitors p18 and p21 during adipogenesis. PPAR-γ hence could control the expression not only of genes involved in the acquisition of a differentiated phenotype but also of genes involved in the negative regulation of cell cycle.

The anti-proliferative effects of PPAR-γ go further than participation in the cell cycle arrest during the adipocyte differentiation process. PPAR-γ expression is not restricted to adipose tissue, being expressed in several other cell types. Furthermore, it has been reported that PPAR-γ expression is increased in several epithelial cancer cells. Whereas the physiological function of PPAR-γ in normal epithelial cells is largely unknown, PPAR-γ activation was reported to inhibit the proliferation of malignant cells from different lineages such as liposarcoma (Tontonoz *et al*. 1997), breast adenocarcinoma (Elstner *et al*. 1998, Mueller *et al*. 1998), prostate carcinoma (Kuboto *et al*. 1998), colorectal carcinoma (Brockman *et al*. 1998, Sarraf *et al*. 1998, Kitamura *et al*. 1999), non-small cell lung carcinoma (Chang & Szabo 2000), pancreatic carcinoma (Motomura *et al*. 2000), bladder cancer cells (Guan *et al*. 1999), and gastric carcinoma cells (Sato *et al*. 2000). In adipocytes, macrophages, breast, prostate and non-small cell lung cancer cells, thiazolidinediones are reported to induce apoptosis (Chinetti *et al*. 1998, Elstner *et al*. 1998, Kuboto *et al*. 1998, Mueller *et al*. 1998, Okuno *et al*. 1998, Chang & Szabo 2000). These observations suggest that induction of differentiation by activation of PPAR-γ may represent a promising novel therapeutic approach for cancer as already demonstrated for liposarcoma (Demetri *et al*. 1999) and in xenograft models of prostate (Kuboto *et al*. 1998) and colon cancer (Sarraf *et al*. 1998). Going along with this hypothesis is the observation that somatic mutations in the PPAR-γ gene are present in certain colon cancers (Sarraf *et al*. 1999). The medical relevance of this last observation is at present unclear, until more biopsies have been analyzed. In addition, treatment of patients with advanced prostate cancer with the PPAR-γ agonist troglitazone, resulted in a high-incidence stabilization of prostate-specific antigen levels (Mueller *et al*. 2000), an effect mediated at

least in part by the inhibition of the androgen receptor activation (Hisatake *et al*. 2000). Other evidence for the involvement of PPAR-γ in tumorigenic processes comes from the identification in a subset of thyroid follicular carcinomas of a chromosomal translocation resulting in a fusion protein PAX8-PPAR-γ (Kroll *et al*. 2000). This fusion protein behaves as a PPAR-γ dominant negative, which abrogates the effects of ligand activation of the wild-type PPAR-γ protein.

In sharp contrast with this, however, are the studies showing that activation of PPAR-γ promotes the development of colon tumors in C57BL/6J-APC^{Min}/+ mice (APC is the tumor suppressor protein in adenomatous polyposis coli) (Lefebvre *et al*. 1998, Saez *et al*. 1998), a clinically relevant model for both human familial adenomatous polyposis and sporadic colon cancer (Groden *et al*. 1991, Nishisho *et al*. 1991, Miyoshi *et al*. 1992, Su *et al*. 1992, Powell *et al*. 1993). Even if the exact role of PPAR-γ in the development of colorectal cancer is not yet elucidated, several observations support the idea that this receptor is involved in these pathologies. First, PPAR-γ is highly expressed in the colon (Considine *et al*. 1996, Mansen *et al*. 1996, Lefebvre *et al*. 1998). Secondly, the development of colorectal cancer is influenced by prostaglandins (Kinzler & Vogelstein 1996), which are potential ligands of PPAR-γ. Indeed, in mice with mutations in the cyclooxygenase (COX)-2 gene or in animals and humans treated with COX inhibitors, decreased production of prostaglandins prevents or attenuates colon cancer development (Thun *et al*. 1991, Jacoby *et al*. 1996, Oshima *et al*. 1996). Finally, there is a strong correlation between the intake of fatty acids from animal origin (potential activators of PPAR-γ) and colon cancer (Giovanucci & Willet 1994, Wasan *et al*. 1997). Activation of PPAR-γ by two different synthetic agonists increased the frequency and size of colon tumors in C57BL/6J-APC^{Min}/+ mice (Lefebvre *et al*. 1998, Saez *et al*. 1998). Tumor frequency was only increased in the colon (by 425% for rosiglitazone-treated and by 183% for troglitazone-treated animals), whereas the frequency did not change in the small intestine, coinciding with the colon-restricted expression of PPAR-γ. A similar increase in the frequency of colon tumors was observed previously when these mice were fed with a diet high in saturated fats (Wasan *et al*. 1997), suggesting that PPAR-γ could be involved in establishing the link between a high-fat diet and colon cancer (Giovanucci & Willet 1994). Treatment with PPAR-γ agonists furthermore increased β-catenin levels both in the colon of C57BL/6J-APC^{Min}/+ mice and in HT-29 colon carcinoma cells (Lefebvre *et al*. 1998). These observations seem at odds with the above anti-proliferative properties associated with PPAR-γ activation. It is, however, most likely that the differences are mainly due to the differences in model systems used. In fact, the C57BL/6J-APC^{Min}/+ mouse studies are an adequate model to study the effects of PPAR-γ on the spontaneous development of colon cancers, whereas the xenograft model is better suited to study the anti-proliferative capacity of PPAR-γ activation in cancerous cells. Hence, the action of PPAR-γ on cell cycle, proliferation, differentiation and apoptosis seems to depend on the cell type and/or the mutational events that predisposes tissues to cancer development.

Interestingly, a recent study also showed the involvement of another isoform of PPAR, PPAR-δ, in the development of colorectal cancer (He *et al*. 1999). PPAR-δ like PPAR-γ is expressed in the colon and can be activated by fatty acids. He *et al*. (1999) showed that PPAR-δ is a target gene for the β-catenin/Tcf-4 transcription complex, which is formed when the tumor suppressor protein APC is mutated (Kinzler & Vogelstein 1996). These investigators propose that PPAR-δ can mediate the pro-tumorigenic effects of fatty acids on colon cancer formation. Non-steroidal anti-inflammatory drugs, which perturb the production of endogenous PPAR ligands, were suggested to inhibit PPAR-δ activity. At present it is unclear how these observations on PPAR-δ articulate with the above discussed involvement of PPAR-γ in cell proliferation and colon cancer. One hypothesis that definitely merits further exploration relates to an eventual role of PPAR-δ in the control of PPAR-γ gene expression. Indeed, PPAR-γ expression is in part controlled by a PPRE in its own promoter (Saladin *et al*. 1999). Therefore it is possible that part of the pro-tumorigenic effects of PPAR-δ are mediated by PPAR-γ.

All these *in vivo* and *in vitro* data related to the effects of PPAR-γ on cell cycle, apoptosis and carcinogenesis definitely warrant follow-up. Careful monitoring of type 2 diabetes patients chronically treated with PPAR-γ agonists is indicated. In addition, these data dictate the need for additional laboratory studies to address the role of PPAR-γ in tumorigenesis. Finally, it will be of interest to evaluate a potential role of cofactors in these phenomena and to determine if mutations or modulation in expression of coactivators or co-repressors, affecting their function, could be involved in PPAR-γ-dependent tumor formation. A possible precedent for such a role of cofactors was highlighted in estrogen receptor-dependent breast cancers in which cofactors such as the coactivator

amplified in breast cancer-1, a member of the steroid receptor coactivator-1 family (Anzick *et al*. 1997), or nuclear corepressor were mutated or down-regulated (Lavinsky *et al*. 1998, Takimoto *et al*. 1999).

CONCLUSIONS

Differentiation of preadipocytes into adipocytes is part of a metabolic response to nutritional and hormonal signaling. This differentiation process requires a cascade of changes in gene expression. Both *in vivo* and *in vitro* data have substantiated the important role of PPAR-γ in mediating such changes during terminal adipocyte differentiation. Despite a large body of knowledge about the role of PPAR-γ in this differentiation process, a lot needs to be learned. For instance, little is known about the molecular mechanisms preceding PPAR-γ expression and activation. The identification of transcription factors triggering the expression of PPAR-γ and the onset of differentiation are of utmost importance. Furthermore, we expect that studies in genetically modified animals and of factors interacting with PPAR-γ will be helpful for a better understanding of PPAR's function. We also predict that characterization of new PPAR-γ modulators and ligands might address some of the scientific problems in the PPAR-γ field. Although a wide variety of fatty acids have been reported to be capable of activating PPAR-γ *in vitro*, it is at present unknown which fatty acids are activating PPAR-γ *in vivo*. One important remaining question is whether in tissues where PPAR-γ is expressed, adequate levels of natural ligands are present. Finally, the observation that PPAR-γ expression is elevated in several human malignancies merits further investigation to elucidate the role of PPAR-γ in such proliferative disorders.

ACKNOWLEDGEMENTS

The various members of the laboratory of J A are acknowledged for support and discussion. The research in the laboratory of the authors was supported by grants from CNRS, INSERM, CHU de Strasbourg, ARC (contract #9943), the Juvenile Diabetes Foundation (1–1999–819), the European community RTD program (QLG1-CT-1999–00674), Ligue Nationale Contre le Cancer, and the Human Frontier Science Program (RG0041/1999-M).

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RECEIVED 2 February 2001
ACCEPTED 14 March 2001