Chapter 7
Gene Regulatory Activity of Vitamin E

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Keywords Vitamin E · Gene expression · Inflammation · Lipid metabolism · MicroRNA · Immune response

Key Points

- Tocopherol and tocotrienols exhibit gene regulatory properties.
- Vitamin E modulates immune response.
- Vitamin E affects hepatic gene expression.
- Vitamin E induces changes in steroidogenesis and affects cholesterol homeostasis.
- Vitamin E influences miRNA levels.

Introduction and Chemistry of Vitamin E

In 1922, Evans and Bishop (University of Berkeley, California) discovered vitamin E to be an essential factor for the reproduction of rats [28]. Other important milestones in vitamin E research include its structural elucidation, synthesis and description of its biological functions which are summarized in Chap. 1 [31, 34, 50, 62, 99]. Furthermore the identification as well as the crystal structures of vitamin E trafficking proteins, including α-tocopherol transfer protein (α-TTP) and supernatant protein factor (SPF), has been solved [19, 70, 96, 97].

Vitamin E naturally occurs in at least eight biologically active isoforms [79]. Research has mainly focused on its antioxidant properties; however, in recent years, the cell signalling and gene regulatory aspects of tocopherols and tocotrienols have also been reported [16]. Through technological advances (e.g. gene chip technology), novel vitamin E-sensitive molecular targets that are controlled through signalling pathways have been discovered [16, 90].

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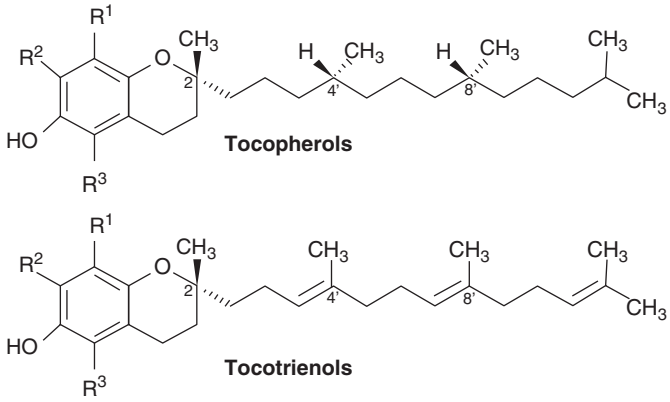

	R1	R2	R3
α-	CH_3	CH_3	CH_3
β-	CH_3	H	CH_3
γ-	CH_3	CH_3	H
δ-	CH_3	H	H

Fig. 7.1 Molecular structure of vitamin E stereoisomers

Tocopherols and tocotrienols are generated by plants from homogentisic acid. All are derivatives of 6-chromanol with an aliphatic 16-carbon side chain attached to the chromanol ring [89]. The four tocopherol isoforms (α-, β-, γ-, δ-) consist of a fully saturated isoprenoid side chain, whereas the isoprenoid side chain of the four tocotrienol homologues (α-, β-, γ-, δ-) contains three double bonds. Accordingly, tocopherols have three chiral centres at positions 2′, 4′ and 8′, while tocotrienols have only one at position 2′ [84]. The individual tocopherols and tocotrienols differ in the number and position of the methyl groups on the phenol ring, with the α-, β-, γ- and δ-isoforms containing three, two, two and one methyl groups, respectively [79, 89] (Fig. 7.1).

These structural characteristics define the biological activity of the isoforms. Each methyl group attached to the chromanol ring confers additional antioxidant capacity. Therefore, the α-isoforms are supposed to be the most biologically active forms of vitamin E, regarding their antioxidant properties [89].

Although humans absorb all forms of vitamin E [79], α-tocopherol represents ~90% of total vitamin E in the body [106]. This is partly due to the higher affinity of α-TTP for α-tocopherol relative to the other tocopherols and tocotrienols [42]. Recently, we have found that self-assembled α-TTP nanoparticles promote the delivery of vitamin E across the endothelial barrier [3]. This offers new strategies to deliver drugs into tissues protected by endothelial barriers (e.g. the brain).

Gene Regulatory Activity of Vitamin E

Overview

Vitamin E is involved in cell signalling processes, independent of its antioxidant properties [5, 10] (Fig. 7.2). It interacts with cell receptors (e.g. low-density lipoprotein receptor (LDL-R) [80]) and redox-regulated transcription factors (e.g. pregnane X receptor (PXR) [55]), thereby driving gene expression (e.g. scavenger receptor (SR), cluster of differentiation (CD) 36 [6, 82, 109]). The first non-antioxidant properties of vitamin E have been described by Azzi and co-workers, indicating that α-tocopherol suppresses the activity of protein kinase C (PKC) and 5-lipoxygenase (5-LOX) and stimulates protein phosphatase 2A and diacylglycerol kinase [11, 12, 24, 62]. Furthermore, α-tocopherol has an influence on the transcription of some genes, including α-TTP [30], α-tropomyosin (TPM1) [4] and matrix metalloproteinase MMP1 [86].

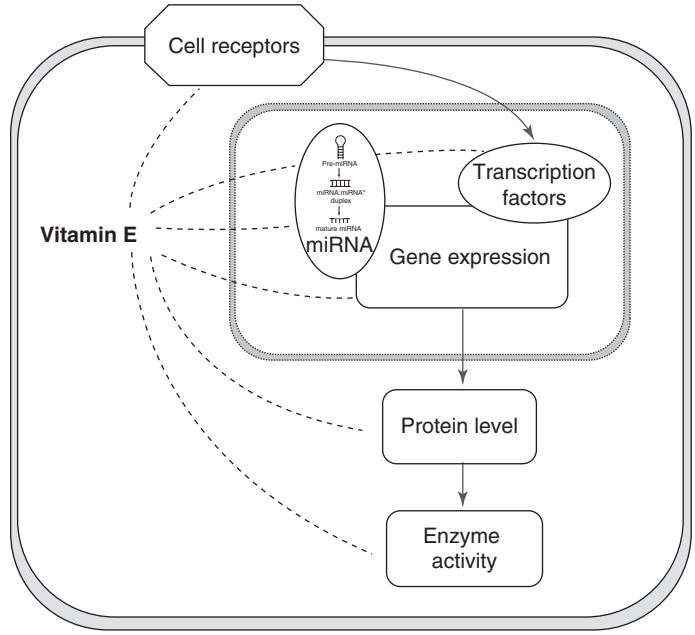

Fig. 7.2 Vitamin E is a cell signalling molecule that affects cell receptors, transcription factors, miRNAs, gene expression, protein levels, and enzyme activities of vitamin E-specific molecular targets

Gene Regulatory Activity of Different Vitamin E Isoforms

Notably, the various isoforms of vitamin E exhibit different gene regulatory activities [26]. In a comparative gene expression profiling study by Berbeé et al. [8], the differences in gene expression between γ-tocotrienol, γ-tocopherol and α-tocopherol in pretreated human endothelial cells after radiation injury were evaluated. γ-Tocotrienol was more potent in inducing gene expression changes than α-tocopherol or γ-tocopherol. Multiple changes in functional pathways, such as the response to oxidative stress, response to DNA-damaging stimuli, cell cycle phase, regulation of cell death and cell proliferation, haematopoiesis and blood vessel development, were affected by γ-tocotrienol. In a study by Zingg et al. [119], the in vivo regulation of gene transcription by α- and γ-tocopherol in murine T lymphocytes was evaluated by gene array analysis. It was shown that dietary supplementation with α- or γ-tocopherol influenced the immune response in a dose- and structure-dependent manner. Genes were found to uniquely respond to either high α-tocopherol (e.g. induction of CD40 ligand, tumour necrosis factor (TNF) ß) or γ-tocopherol (e.g. repression of poliovirus receptor-related-2). In prostate cancer cells, tocopherylquinone, the oxidation product of α-tocopherol, decreased androgen-responsive gene expression, including transmembrane-4-L-six-family-1, kallikrein-2 and kallikrein-3, whereas α-tocopherol did not [29]. γ-Tocopherol supplementation inhibits protein nitration and ascorbate oxidation in rats with induced inflammation by zymosan injection; however, by contrast, α-tocopherol did not significantly affect protein nitration and ascorbate oxidation in response to zymosan treatment [46]. γ-Tocopherol inhibits human prostate cancer cell proliferation to a greater extent than α-tocopherol through the upregulation of transglutaminase 2 (TGM2) and the downregulation of cyclins (CCNs) [105]. Tocopherols, including α-, β-, γ- and δ-tocopherols, inhibited cyclooxygenase-2 (COX2) gene expression in RAW 264.7 macrophages after exposure to macrophage activators. Compared with α-tocopherol, β-, γ- and δ-tocopherols exhibited significantly greater inhibitory properties [72]. Abdala-Valencia et al. [1] showed that the activation of PKCα via intercellular adhesion molecule-1 (ICAM1) is differentially regulated by various vitamin E isoforms in human microvascular endothelial cells. ICAM1 activation of PKCα was inhibited by d-α-tocopherol,

and this inhibition was ablated by the addition of d-γ-tocopherol. These tocopherols regulated ICAM1 activation of PKCα without altering the upstream signal extracellular signal-regulated kinases 1/2 (ERK1/2).

Gene Expression in Vitamin E Deficiency

To gain a global view of the molecular role of action of vitamin E, our group has performed global gene expression profile experiments, both in rat liver in vivo and in hepatocellular liver carcinoma (HepG2) cells in vitro.

To study the influence of a short-term (49 days) [33] or long-term (290 days) vitamin E deficiency in rats [6], animals were maintained on semisynthetic diets either supplemented with or deficient in vitamin E (DL-α-tocopheryl acetate [33]; *RRR*-α-tocopheryl acetate [6]). Furthermore, HepG2 cells were supplemented with vitamin E (*RRR*-α-tocopheryl acetate) concentrations corresponding to those that were obtained in the in vivo study [88]. Differential gene expression in rat liver and in HepG2 cells was monitored by Affymetrix GeneChip technology covering 7000 transcripts. Vitamin E deficiency generated via the diet over a 7-week period did not induce any significant changes in the expression profile amongst the assessed genes. Likewise, in another study, a 7-week vitamin E deficiency did not change the expression pattern of 465 genes evaluated in the liver of rats [33]. A combined vitamin E and selenium deficiency, however, altered the expression of genes encoding proteins involved in inflammation, acute phase response, inhibition of apoptosis, cell cycle and antioxidant defence. Furthermore, long-term vitamin E deficiency in rats induced the expression of hepatic coagulation factor IX (FIX), 5-α-steroid reductase type 1 (5α-R1) and CD36 mRNA levels.

FIX is a blood-clotting protein that plays an essential role in the activity of the intrinsic coagulation pathway [61]. α-Tocopherol is proposed to decrease the levels of FIX by function as an antagonist of the vitamin K-dependent γ-carboxylase, which is necessary to activate enzymes of the blood-clotting cascade (e.g. FIX). As a consequence of the reduced FIX levels, the activated partial thromboplastin (APT) time was increased in our rat study [6] (Fig. 7.3a).

5α-R1, which is widely distributed in numerous tissues, catalyses the irreversible conversion of testosterone to 5-α-dihydrotestosterone [92]. Both testosterone, the primary androgen to be synthesized and secreted by the testes, and 5-α-dihydrotestosterone, which is the major androgen in the prostate, are potent natural androgens that are present in all mammals [118]. It has been demonstrated that dietary vitamin E decreases 5α-R1 mRNA levels, resulting in a changed ratio of 5-α-dihydrotestosterone/testosterone [6] (Fig. 7.3b). In addition, we could show that the rate-limiting enzyme of glutathione synthesis, γ-glutamyl-cysteinyl-synthetase (γGCS), as well as glutathione synthase, was downregulated in the liver of vitamin E-deficient rats. Reduced levels of glutathione in the liver have been associated with reduced tolerance to oxidative stress and xenobiotics [108] and decreased regeneration of vitamin E radicals via ascorbic acid [39] (Fig. 7.3c). Overall, measurement of the corresponding functional endpoints such as APT time, plasma 5-α-dihydrotestosterone and hepatic glutathione confirmed the gene chip data, suggesting that dietary vitamin E exhibits an important part in numerous metabolic processes within the rat liver [6]. Similar to our rat experiment, it was shown in vitro that adding vitamin E to HepG2 cells altered the expression of FIX and CD36 [88] and therefore confirmed partly our in vivo data.

In another set of experiments, two groups of male rats were fed with either a vitamin E-deficient diet or a control diet enriched with *RRR*-α-tocopheryl acetate for 430 days. Differential gene expression in skeletal muscle was controlled at five time-points, with all animals individually profiled [75]. The differentially expressed genes consist of muscle structure and extracellular matrix genes, as well as antioxidative, anti-inflammatory and anti-fibrotic genes. Our data suggest that molecular transcription possibly provides a very initial indicator to identify forthcoming degenerative conditions in vitamin E deficiency. They give additional information into potential molecular

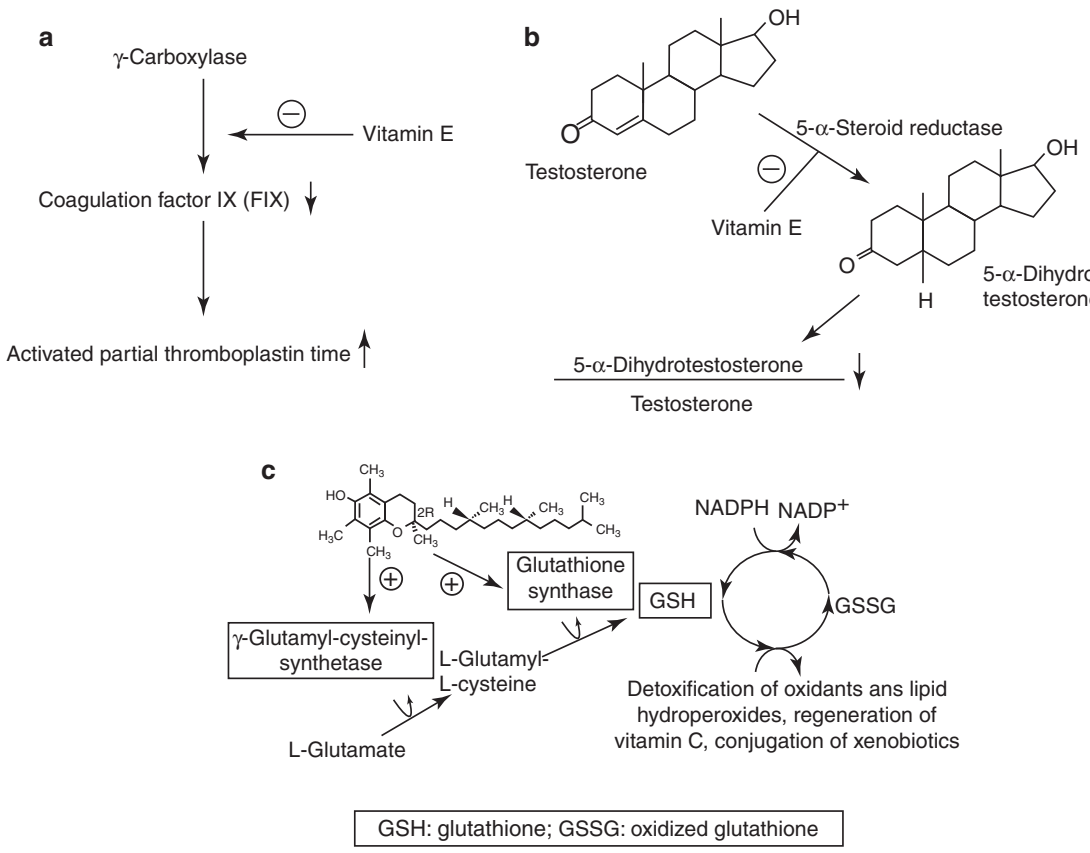

Fig. 7.3 Overview of the effects of vitamin E on (**a**) the inhibition of the activation of coagulation factor IX and the increase in the activated partial thromboplastin time, on (**b**) the inhibition of 5-α-steroid reductase type 1 and on (**c**) the induction of hepatic glutathione synthesis

mechanisms due to vitamin E deficiency in skeletal muscle and display the stimulation of a profound protection programme that may explain the long preservation of muscle structure during vitamin E deficiency.

Lipid Metabolism

Our in vivo experiments in rats also show that vitamin E causes alterations in steroidogenesis, thereby having an effect on cholesterol homeostasis in testes and adrenal glands. Genes encoding proteins regulating the uptake (LDL-R) and de novo synthesis (e.g. 7-dehydrocholesterol reductase (DHCR7), 3-hydroxy-3-methylgluteryl coenzyme A synthase (HMGCS), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), isopentenyl diphosphate δ-isomerase (IPPI) and farnesyl pyrophosphate synthetase (FPPS)) of cholesterol, the precursor of all steroid hormones, have been found to be α-tocopherol sensitive molecular targets [7]. Furthermore, HMGCR and CD141 have also been identified as γ-tocotrienol sensitive molecular targets [81].

Recently, genome-wide expression profiling in the muscle and subcutaneous fat of lambs in response to dietary vitamin E supplementation revealed the upregulation of genes (e.g. sterol regulatory element-binding transcription factor 1 (SREBF1), LDL-R, HMGCS1), encoding proteins centrally involved in

lipid metabolism including sterol, steroid and cholesterol biosynthesis [57]. In muscle, vitamin E supplementation led to the downregulation of genes related to the intracellular signalling cascade and metabolic processes (e.g. cytokine-inducible SH_2-containing protein, insulin-like growth factor 1 receptor and acetyl-CoA acetyltransferase 1 (ACAT1)). In differentiated preadipocytes, tocotrienol modulates crucial lipid metabolism-related genes, e.g. genes that code for lipid biosynthesis (fas cell surface death receptor (FAS), stearoyl-CoA desaturase 1 (SCD1), acetyl-CoA-carboxylase 1 (ACC1), adiponectin receptor 2 (ADIPOR2) and LDL-R) and ß-oxidation (carnitine palmitoyltransferase 1 (CPT1) and uncoupling protein 2 (UCP2)) [18]. In addition, transcription factors such as sterol regulatory element-binding protein (SREBP) 1c and peroxisome proliferator-activated receptor (PPAR) γ were markedly regulated by tocotrienol. The addition of α-tocopheryl phosphate (but not α-tocopherol) to preadipocytes transcriptionally activates a set of genes (tribbles homologue 3, sestrin-2 and insulin-induced gene 1), potentially preventing fat accumulation in these cells [60]. By contrast, the authors could show that, in differentiated adipocytes, α-tocopheryl phosphate is responsible for the transcriptional inhibition of the same genes, possibly facilitating fat uptake and storage. In the aortae of rabbits fed with a cholesterol-rich diet, vitamin E supplementation affords protection by decreasing MMP1 and increasing PPARγ, glutathione S-transferase (GST)-α and ATP-binding cassette transporter 1 (ABCA1) levels [15]. Likewise, using proteomics techniques, the expression levels of apolipoprotein (Apo) AI and ApoE, which are involved in lipid metabolism, and peroxiredoxin (PRDX) 1, PRDX2 and thioredoxin, which are involved in the antioxidant system, were significantly different in the aortae of rabbits fed with a cholesterol- and vitamin E-rich diet, compared with those of control rabbits [48]. In addition, 14-3-3 protein zeta/delta and 14-3-3 protein beta/alpha in cell signalling, and biglycan, vimentin, TPM1 and smooth muscle α-actin (ACTA2) as structural and contractile proteins, were differentially expressed. In ApoE knockout mice, vitamin E conditionally inhibits atherosclerosis by anti-oxidation and regulation of vasculature gene expression [100]. At the ages of 6, 14 and 22 weeks, vitamin E downregulated the vasculature mRNA expression of scavenger receptor CD36 and upregulated the mRNA expression of PPARγ, liver X receptor α and ABCA1, which are involved in reverse cholesterol transportation; however, vitamin E had no significant effects on these genes when given at an older age (30 and 38 weeks). In rat primary hepatocytes, γ-tocotrienol reduces the triacylglycerol level through the regulation of fatty acid metabolism, thereby increasing the expression of CPT1A mRNA and suppressing the gene expression of C/EBP homologous protein (CHOP), SREBP1c and IL1ß [73].

Antioxidative Enzymes and Cellular Ageing, Inflammation and Immune Response

Dietary vitamin E, at various doses, increased the mRNA and protein expression of the antioxidative enzymes glutathione peroxidase 3 (GPX3) and GSTα1 in sheep testes [114] and modulated the gene expression of superoxide dismutase (SOD) 1 and 2, chopper chaperone for SOD (CCS1), PRDX6, catalase (CAT) and forkhead-box-protein (FOXO3A) in vitro [25].

In addition, vitamin E has been suggested to modulate age-associated changes by altering the redox balance, resulting in altered gene and/or protein expression. γ-Tocotrienol has been shown to prevent the cellular ageing of human diploid fibroblasts by the modulation of gene expression [65, 66]. Following the incubation of senescent cells with γ-tocotrienol, global gene expression analysis was performed and revealed 100 genes that were differentially expressed by at least 1.5-fold. Amongst these genes were interleukin 1 receptor-associated kinase 3, selenoprotein S, heat shock protein family A5, homocysteine-responsive ER-resident ubiquitin-like domain 1, DNAJ HSP member B9 and methionine sulfoxide reductase B1. Furthermore, translocase of inner mitochondrial membrane, ADP ribosylation factor 4, RAD50-interacting protein 1, NTF2-related export protein 1, calcium-dependent secretion activator 2, component of oligomeric Golgi complex 6 and glutaredoxin 5 were also differentially expressed.

Enrichment scores revealed that biological processes such as inflammation, protein transport, apoptosis and cell redox homeostasis were affected in these cells treated with γ-tocotrienol. In human lymphocytes from young and old individuals challenged with H_2O_2 and treated with the tocotrienol-rich fraction, 24 proteins were found to be affected. Amongst these were several proteins involved in the stress response (PRDX2, PRDX3 and PRDX6), which were shown to be downregulated with H_2O_2 exposure, and the effect was reversed following treatment with the tocotrienol-rich fraction [22].

Vitamin E modulates the immune response, in part by reducing inflammation. LPS-induced inflammation in chickens was significantly lower in birds fed with vitamin E, as indicated by lower IL6 mRNA steady-state levels [49]. In chickens receiving either α- or γ-tocopherol or both isoforms after inducing oxidative stress by feeding with n-3 polyunsaturated fatty acids, a chicken-specific genome microarray analysis was performed in the liver [53]. The effect of γ-tocopherol was evident in the expression of genes involved in inflammatory processes and the immune response (e.g. interferon regulatory factors (IRFs), lymphocyte antigen 96 encoding gene (LY96), toll-like receptor 2 (TLR2), leukocyte ribonuclease A-2 (RSFR)), while α-tocopherol affected genes involved in lipid and cholesterol metabolism (e.g. SREBP2, PPARα). In old (22–24 month) mice infected with *S. pneumonia*, α-tocopherol supplementation enhanced the resistance of aged mice to bacterial pneumonia and altered the expression of multiple epithelial and neutrophil adhesion molecules involved in migration, including CD55, CD47, CD18/CD11b and ICAM1 [13].

In vitro assays in LPS-stimulated mouse bone marrow cells in the presence or absence of vitamin E revealed enhanced klotho transcript levels and hormone secretion and inhibited IL12p70 protein expression by vitamin E [115]. The TNFα-induced changes in secretion and gene expression of monocyte chemotactic protein 1, IL6, and adiponectin in 3T3-L1 adipocytes were attenuated by γ-tocotrienol [68]. Furthermore, TNFα-mediated IκB-α phosphorylation and nuclear factor κ-light-chain-enhancer of activated B-cell (NF-κB) activation were significantly suppressed by γ-tocotrienol treatment.

In tocotrienol-treated MCF-7 human breast cancer cells, global gene expression analysis revealed an upregulation of genes responsible for modulating the immune response [85]. Interferon-induced transmembrane protein (IFITM) 3 expression was induced by the tocotrienol-rich fraction; IFITM2 was induced by α-tocopherol, tocotrienol-rich fraction, α-tocotrienol and γ-tocotrienol; ferritin heavy polypeptide 1 (FTH1) was induced by γ-tocotrienol; and COL IVα3 was induced by δ-tocotrienol.

Pretreatment of cardiomyocytes with vitamin E under heat stress conditions increased the expression of metallothionein, PPARγ coactivator 1A (PGC1A), nuclear respiratory factor 1 and mitochondrial transcription factor A [110]. γ-Tocotrienol has been shown to inhibit cytokine-triggered activation of NF-κB, and its upstream regulator transforming growth factor ß (TGF-β) activated kinase-1 in murine RAW 264.7 macrophages and primary bone marrow-derived macrophages [111]. In these cells, γ-tocotrienol induced the upregulation of A20, an inhibitor of NF-κB, B-cell lymphoma 2 (BCL2), C-X-C motif chemokine receptor 4, vascular endothelial growth factor and MMP9 [67].

Cytochrome P450

α-Tocopherol is transported to the liver and, through a series of oxidation reactions, converted to α-carboxyethyl hydroxychromane, which involves an initial ω-oxidation step [47]. There is evidence that cytochrome (CYP) P450 enzymes induced after α-tocopherol supplementation perform this ω-oxidation step [9]. Unlike α-tocopherol, other vitamin E forms are significantly metabolized to carboxychromanols via CYP P450 F2-initiated side-chain ω-oxidation (see Chap. 4) [9, 17, 95].

In a study to examine the short- and long-term effects of natural versus synthetic vitamin E on CYP P450-dependent gene expression in vitro, HepG2 cells were incubated with increasing concentrations of *RRR*-tocopheryl acetate (natural vitamin E) or *all-rac*-tocopheryl acetate (synthetic vitamin E). After 1 week, the mRNA levels of several CYP genes were measured applying GeneChip technology.

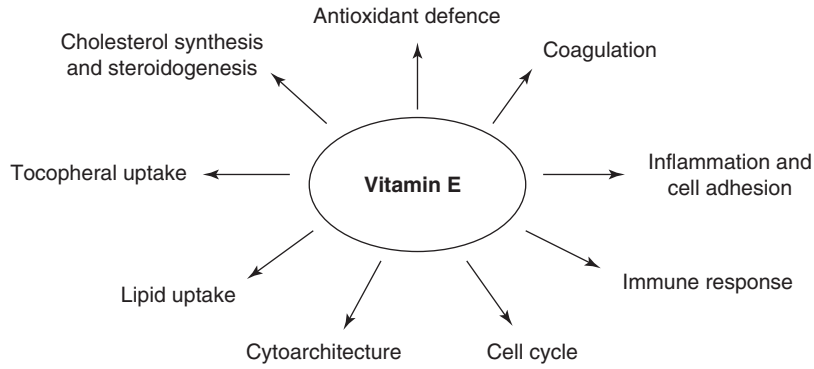

Fig. 7.4 Potential targets of transcriptional regulation by vitamin E

Interestingly, no significant changes in gene regulatory activity were monitored between *RRR*- and *all-rac*-α-tocopheryl acetate. In order to evaluate the function of vitamin E in CYP gene expression in vivo, male albino rats were allocated to either a vitamin E-enriched (*RRR*-tocopheryl acetate) or a vitamin E-deficient semisynthetic diet. However, neither in the vitamin E-supplemented nor in the vitamin E-deficient rats significant changes in CYP mRNA levels occurred in the liver. Likewise, CYP26A1 and its mRNA expression were not affected in vivo by varying vitamin E supplementation in the liver of laying hens and were not affected in hepatocytes in vitro [117]. Hence, it has been shown in mouse melanoma cell line B16 in vitro that γ-tocotrienol upregulates the expression of CYP1A1-sensitive aryl hydrocarbon receptor [116]. Furthermore, γ-tocopherol induced the expression CYP1A1, NAD(P)H dehydrogenase 1 (NQO1), γGCS, heme oxygenase 1 and PPARγ and decreased the tumour volume and multiplicity in a rat model of oestrogen-induced breast cancer [23]. In a rodent model of mammary carcinogenesis, dietary y-tocopherol regulates the expression of oestrogen receptor α, PPARγ and Nrf2 [94].

PXR has been postulated to play a role in the metabolism of α-tocopherol due to the upregulation of hepatic CYP P450, which has been verified in PXR-humanized, wild-type and Pxr-null mouse models [47]. Gene expression analysis revealed significantly increased expression of CYP3a11, as well as that of several other P450s only in wild-type mice. This study revealed that α-tocopherol is a partial agonist of PXR and that PXR is necessary for CYP3a induction by α-tocopherol.

Overall, targets of transcriptional regulation in response to vitamin E induction comprise antioxidant defence, blood clotting, inflammation and cell adhesion. Furthermore, vitamin E regulates genes encoding proteins centrally involved in lipid and cholesterol uptake, as well as those involved in cholesterol synthesis and steroidogenesis (Fig. 7.4).

Tissue-Specific Gene Expression

Vitamin E also seems to influence the expression of several genes in the rat brain. These tissue-specific gene expressions regulated by vitamin E encode proteins associated with hormones and hormone metabolism, nerve growth, apoptosis, dopaminergic neurotransmission and clearance of amyloid-β and advanced glycated end products [91]. In neuroblastoma cells, α-tocotrienol and tocopherols increased the release of amyloid-β by increased amyloidogenic amyloid precursor protein (APP) processing and decreased the degradation of amyloid-β, leading to the formation of neuritic plaques [38]. However, in rats receiving deficient, marginal or sufficient supplements of vitamin E over 6 months, the mRNA concentrations of genes involved in the formation (APP-binding family member 1, a disintegrin and metalloprotease domain 10, β-site APP-cleaving enzyme 1) or degradation (neprilysin, insulin-degrading enzyme, endothelin-converting enzyme) of amyloid-β in the brain [35] were evaluated. In the

cortex and hippocampus of our rats, dietary vitamin E did not affect the mRNA concentrations of the measured genes; hence, the role of vitamin E in brain function remains controversial.

In the kidney of diabetic rats, tocotrienol-rich fraction supplementation downregulated the expression of TGF-β, fibronectin 1 and collagen (COL) IV [93]. Furthermore, the tocotrienol-rich fraction protects against H_2O_2-induced oxidative stress in human skin fibroblast culture by modulating the expression of COL I and COL III genes with a concomitant increase in the rate of total collagen synthesis [63].

In addition, it has been shown that γ-tocotrienol induces cell cycle arrest and apoptosis via activating the BCL2-associated X protein-mediated mitochondrial and AMPK signalling pathways in adipocytes [113]. In human skin melanocytes, the tocotrienol-rich fraction and tocopherol downregulate the expression of tyrosinase (TYR) and TYR-related protein 1 and 2 genes [64].

Likewise, in a study to clarify the distribution of α-tocopherol-associated gene expression in major tissues and change in expression patterns induced by orally administered α-tocopherol to calves, the mean mRNA expression levels for α-TTP, afamin, ABCA1 and tocopherol-associated protein (TAP) were greatest in the liver, whereas scavenger receptor B1 mRNA was greatest in the adrenal gland [41]. The gene for CYP4F2 was most highly expressed in the liver, testes and adrenal gland. In addition, dietary vitamin E influences the expression of the α-TTP gene in the sheep liver, heart, spleen, lung, kidney, *longissimus dorsi* muscle and gluteus muscle in a tissue-specific way [120].

Taken together, vitamin E exhibits significant effects on gene expression with potential downstream effects.

Table 7.1 provides a brief selection of genes differentially regulated by Vitamin E in cultured cells and in vivo as reported in the literature

Vitamin E and DNA Methylation

Research on the function of essential micronutrients, such as vitamin E, in the epigenetic regulation of gene expression is an area of increasingly recognized importance. In this context, we have recently studied the effect of dietary vitamin E deficiency (6 months) on global and specific DNA methylation patterns in the rat liver [32]. 5α-R1 and γGCS were analysed for promoter methylation in putative CpG island regions. We have chosen these two enzymes for promoter methylation analysis because both enzymes were regulated at the mRNA level by α-tocopherol deficiency (see Chap. 2) in our rat study [6].

Under the conditions investigated, vitamin E deficiency was not associated with different CpG methylations of the analysed promoter regions of 5α-R1 and γGCS, respectively. Importantly, global DNA methylation was not significantly different between vitamin E-deficient and vitamin E-supplemented rats. These results suggest that vitamin E may not regulate hepatic DNA methylation patterns in rats. Further studies are needed to test the hypothesis regarding whether vitamin E deficiency may affect the epigenetic modification of histones by acetylation, methylation, phosphorylation, ubiquitination, sumoylation or isomerization. However, recently, in a global gene expression study, the epigenetic influences of parental diet and vitamin E intake on the embryonic zebrafish transcriptome were analysed [71]. Despite overt morphologic consistency, significant differences in gene expression suggested a perturbed energy metabolism and mitochondrial function. Genes affected were the transcript levels of APP, ApoE, nuclear receptor 4A3, cAMP response element-binding (CREB) protein, PPARγ and PGC1A and 1B. Thus, these findings demonstrate that Vitamin E in the parental zebrafish diet has a direct impact on the embryonic transcriptome.

Furthermore, in prostate tumours from the transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse, it was shown that a γ-tocopherol-rich mixture of tocopherols maintains Nrf2 expression via epigenetic inhibition of CpG methylation in the Nrf2 promoter region [43]. The protein expression of DNA methyltransferase 1, 3A and 3B was lower in the prostate of the γ-tocopherol-supplemented group than that in the controls. In addition, TRAMP-C1 cells were treated with

Table 7.1 Selection of genes differentially regulated by Vitamin E in cultured cells and in vivo

Gene name	Gene symbol	Vitamin E form	Tissue/cell	Species	Function of the gene product	Reference
Metabolism						
α-Tocopherol transfer protein	αTTP	α-Tocopherol	Liver	Rat	α-Tocopherol transfer	[30]
Acetyl-CoA acetyltransferase 1	ACAT1	α-Tocopherol	Muscle	Lamp	Energy metabolism	[81]
Transglutaminase 2	TGM2	γ-Tocopherol	Prostate cancer cells	Human	Protein metabolism	[105]
Lipid uptake						
Scavenger receptor CD36	CD36	α-Tocopherol	Liver	Rat	Fatty acid metabolism; receptor for oxidized LDL	[6]
Scavenger receptor class B type 1	SR-BI	α-Tocopherol	Type II pneumocytes	Rat	Receptor for HDL	[52]
Scavenger receptor class A	SR-A I/II	α-Tocopherol	Peritoneal macrophages	Rabbit	Receptor for modified LDL	[103]
Low-density lipoprotein receptor	LDL-R	α-Tocopherol	HepG2 hepatoma cells	Human	Uptake of LDL; key regulator of cholesterol transport	[80]
			Subcutaneous fat	Lamp		[81]
		Tocotrienol	3T3-L1 preadipocytes	Mouse		[18]
Cholesterol, steroid and lipid metabolism						
3-Hydroxy-3-methylglutaryl coenzyme A reductase	HMGCR	α-Tocopherol	HepG2 hepatoma cells	Human	Rate-limiting enzyme for cholesterol biosynthesis	[80]
		γ-Tocopherol	Primary endothelial cells			[81]
3-Hydroxy-3-methylglutaryl coenzyme A synthase	HMGCS1	α-Tocopherol	HepG2 hepatoma cells	Human	De novo cholesterol biosynthetic pathway	[107]
			Subcutaneous fat	Lamp		[57]
Isopentenyl diphosphate delta isomerase	IPPI	α-Tocopherol	HepG2 hepatoma cells	Human	De novo cholesterol biosynthetic pathway	[107]
7-Dehydrocholesterol reductase	DHCR7					
Farnesyl pyrophosphate synthetase	FPPS					
Sterol regulatory element-binding transcription factor 1	SREBF1	α-Tocopherol	Subcutaneous fat	Lamp	De novo cholesterol biosynthetic pathway	[57]
ATP-binding cassette transporter1	ABCA1	Vitamin E	Aortae	Rabbit	Transport of cholesterol	[15]
		α-Tocopherol		ApoE knockout mice		[100]
		α-Tocopherol	Liver	Calves		[41]
Sterol regulatory element-binding protein2	SREBP2	α- and/or γ-tocopherol	Liver	Chicken	Immune response	[53]

Table 7.1 (continued)

Gene name	Gene symbol	Vitamin E form	Tissue/cell	Species	Function of the gene product	Reference
Fas cell surface death receptor	FAS	Tocotrienol	3T3-L1 preadipocytes	Mouse	Lipid biosynthesis	[18]
Stearoyl-CoA desaturase 1	SCD1					
Acetyl-CoA-carboxylase 1	ACC1					
Adiponectin receptor 2	ADIPOR2					
Uncoupling protein 2	UCP2				ß-Oxidation	
Carnitine palmitoyltransferase 1	CPT1					
Carnitine palmitoyltransferase 1A	CPT1A	γ-Tocotrienol	Primary hepatocytes	Rat	ß-Oxidation	[73]
C/EBP homologous protein	CHOP					
5-α-steroid reductase type 1	5α-R1	α-Tocopherol	Liver	Rat	Testosterone metabolism	[6, 87]
Antioxidant defence						
γ-Glutamyl-cysteinyl synthetase (regulatory subunit)	γGCS	α-Tocopherol	Liver	Rat	Glutathione synthesis	[6]
		γ-Tocopherol	Oestrogen-induced breast cancer cells	Rat		[23]
Glutathione peroxidase 3	GPX3	α-Tocopherol	Testes	Sheep	Antioxidative enzyme	[114]
Glutathione S-transferase-α1	GSTα1					
Superoxide dismutase 1 + 2	SOD 1 + 2	Tocotrienol-rich fraction	Diploid fibroblasts	Human	Antioxidative enzyme	[25]
Chopper chaperone for SOD	CCS1					
Catalase	CAT					
Forkhead-box-protein	FOXO3A					
Peroxiredoxin	PRDX6					
Coagulation						
Coagulation factor IX	FIX	α-Tocopherol	Liver	Rat	Intrinsic coagulation pathway	[6]
Thrombomodulin	CD141	γ-Tocopherol	Primary endothelial cells	Human	Receptor for thrombin	[81]
Inflammation, cell adhesion and immune response						
Selectin E	SELE / CD62E	α-Tocopherol	Aortic endothelial cells	Human	Cell adhesion molecule	[112]
Vascular cell adhesion molecule 1	VCAM1	α-Tocopherol	Aortic endothelial cells	Human	Recruitment of monocytes	
Intercellular adhesion molecule-1	ICAM1		Epithelial cells	Mouse		[13]
Integrin, α M	ITGAM	α-Tocopherol	Umbilical vein endothelial cells	Human	Immune response	[102]

(continued)

Table 7.1 (continued)

Gene name	Gene symbol	Vitamin E form	Tissue/cell	Species	Function of the gene product	Reference
Integrin alpha 2b	ITG	α-Tocopherol	Erythroleukaemia cells	Human	Platelet adhesion	[20]
Interleukin 2	IL2	α-Tocopherol	Naïve T cells	Mouse	Proliferation of T and B lymphocytes, immune response	[2]
Interleukin 4	IL4	α-Tocopherol	Peripheral blood T cells	Human	Inflammation and chemotaxis of inflammatory cells	[58]
Interleukin 6	IL6	α-Tocopherol	Spleen	Chicken	Immune response	[49]
5-Lipoxygenase	5-LOX	α-Tocopherol	Activated monocytes	Human	Leukotriene synthesis	[24]
Inducible nitric oxide synthase	iNOS	α-Tocopherol	Gastric mucosa	Rat	Nitric oxide production	[76]
Interferon regulatory factors	IRFs	α- and/or γ-tocopherol	Liver	Chicken	Immune response	[53]
Lymphocyte antigen 96 encoding gene	LY96					
Toll-like receptor 2	TLR2					
Leukocyte ribonuclease A	RSFR				Bactericidal activity	
Cyclooxygenase-2	COX2	α-, β-, γ-, δ-tocopherol	RAW 264.7 macrophages	Mouse	Prostaglandin synthesis	[72]
Interferon-induced transmembrane protein 3	IFITM3	Tocotrienol-rich fraction	MCF-7 breast cancer cells	Human	Immune response	[85]
Interferon-induced transmembrane protein 2	IFITM2	α-Tocopherol, tocotrienol-rich fraction, α-, γ-tocotrienol				
Ferritin heavy polypeptide	FTH1	γ-Tocotrienol				
Cell signalling and cycle regulation						
Cyclin D1	CCND1	α-Tocopherol	LNCaP prostate carcinoma cells	Human	Cell cycle regulation	[40]
Cyclin E1	CCNE1					
Cyclins	CCNs	γ-Tocopherol	Prostate cancer cells			[105]
Fas ligand	CD95L	α-Tocopherol	Peripheral blood cells	Human	Induction of apoptosis	[59]
Peroxisome proliferators-activated receptor γ	PPARγ	α-Tocopherol	SW480 colon cancer cells	Human	Regulator of adipocyte differentiation	[98]
Peroxisome proliferators-activated receptor α	PPARα	α- and/or γ-tocopherol	Liver	Chicken	Immune response	[53]

Table 7.1 (continued)

Gene name	Gene symbol	Vitamin E form	Tissue/cell	Species	Function of the gene product	Reference
Extracellular matrix						
Collagen α1	COL α1	α-Tocopherol	Liver	Mouse	Matrix protein	[21]
Collagen IVα3	COL IVα3	δ-Tocotrienol	MCF-7 breast cancer cells	Human	Matrix protein	[85]
Matrix metallopeptidase 1 (interstitial collagenase)	MMP1	α-Tocopherol	Skin fibroblasts	Human	Breakdown of interstitial collagens, types I, II, III	[86]
Matrix metallopeptidase 19	MMP19	α-Tocopherol	Peripheral blood mononuclear cells	Human	Cellular proliferation, migration and adhesion to type I collagen	[69]
Connective tissue growth factor	CTGF	α-Tocopherol	Aortic vascular smooth muscle cells	Human	Endothelial cell function	[69]
Cytoarchitecture						
ß-Tropomyosin 2	TPM2	α-Tocopherol	Lung	Mouse	Cytoskeleton	[77]
α-Tropomyosin 1	TPM1	α-Tocopherol	Vascular smooth muscle cells	Rat	Actin binding protein; actin-myosin interaction	[4]
Smooth muscle α-actin	ACTA2	α-Tocopherol	Aorta	Rabbit	Cytoskeleton	[48]

γ-tocopherol, which inhibited methylation in the Nrf2 promoter in vitro, and the protein expression levels of Nrf2 and NQO1 were higher than those in untreated controls. Hence, the supplementation of γ-tocopherol leads to higher Nrf2 expression and may contribute to the prevention of prostate tumorigenesis in TRAMP mice.

Vitamin E-Regulated miRNAs

miRNAs are small (22 nucleotides long), noncoding RNAs, which are single-stranded in their mature form. It has been established that miRNA post-transcriptionally influences gene expression by binding at the 3′ untranslated region of mRNA and thereby affecting their translation into proteins [14]. Each miRNA binds many different target mRNAs, allowing the post-transcriptional suppression of many different genes or potentially entire pathways, by a single miRNA [56].

In order to investigate the role of dietary *RRR*-α-tocopherol on miRNA expression in liver, rats were fed with diets deficient or sufficient in vitamin E for 6 months [36]. miRNAs that were earlier presented to participate in processes that were related to vitamin E, in particular lipid metabolism (miRNA-122a) [27], cancer progression and inflammation (miRNA-125b) [78], were used in this study. Vitamin E deficiency significantly lowered the levels of miRNA-122a and miRNA-125b. Besides its role in lipid metabolism, miRNA-122 level was reduced in hepatocellular carcinomas in rodents and humans [54]. As it has been shown that miRNA-125b was downregulated in human prostate cancer tissues [78, 83], lung cancer cell lines [74] and breast cancer [44], a function for miRNA-125b in carcinogenesis has been proposed as well. Results from mouse spleens after whole-body irradiation suggest that γ-tocotrienol pretreatment reverses the expression of several miRNAs that are

involved in postirradiation haematopoiesis. Therefore, in silico cellular pathway analyses have implicated the ERK/P38 mitogen-activated protein kinase pathway as a target signalling pathway [37]. In senescent human diploid fibroblasts, the tocotrienol-rich fraction reduces senescence-associated miR-34a expression and increases miR-20a expression in young human diploid fibroblasts [51]. Furthermore, the tocotrienol-rich fraction increases miR-449a expression in both young and senescent cells. On the other hand, the expression of miR-34a was upregulated in non-small-cell lung cancer cells by δ-tocotrienol, thereby suppressing neurogenic locus notch homologue protein 1 and its downstream targets, including hairy and enhancer of split 1, CCND1, baculoviral inhibitor of apoptosis repeat-containing 5 and BCL2 [45].

A function of miRNA-125b in chronic inflammation is backed up by a study by Tili et al. [104] that described TNFα as a direct target of miRNA-125b. A decrease in miRNA-125b caused increased TNFα generation after LPS stimulation. The authors hypothesized that the decreased miRNA-125b levels, as observed in the liver of vitamin E-deficient rats, may be related to an enhanced inflammatory reaction due to vitamin E deficiency. In the liver of an experimental fish model (*Nile tilapia*), the expression of miRNAs (miR-21, miR-223, miR-146a, miR-125b, miR-181a, miR-16, miR-155 and miR-122) that were reported to be related to oxidative stress was analysed after feeding with a vitamin E-deficient (0 mg/kg) or a vitamin E-excessive (2500 mg/kg) diet [101]. The results showed that vitamin E deficiency decreased the expression of miR-223, miR-146a, miR-16 and miR-122, while excessive supplementation of vitamin E increased the expression levels of all eight miRNAs.

Thus, vitamin E controls cell signalling not only at the mRNA level but also at the miRNA level [36].

Although gene chip and micro-RNA technologies have helped to identify vitamin E-sensitive molecular targets, transcription factors that are specifically regulated by vitamin E have not yet been found [16]. Finally, a receptor that specifically binds to vitamin E is currently unknown and warrants further research.

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