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DOES CALORIC RESTRICTION ALTER IL-2 TRANSCRIPTION?

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1. ABSTRACT

Caloric restriction has been the subject of intensive research and is known to be the most efficacious means of increasing longevity and reducing pathology. Caloric restriction has been found to influence a wide variety of age-sensitive immunological parameters such as interleukin-2 (IL-2) gene expression, and overall, the immunological status of rodents fed a caloric restriction diet is superior to the immunological status of the non-restricted animals. IL-2 is a growth promoting cytokine that plays a critical role in immune function. The expression of IL-2 has been shown to decrease with age, and the decrease in IL-2 expression parallels the age-related decrease in immune function. The focus of this review article is to discuss the studies on the influence of caloric restriction on IL-2 expression and the recent findings on the mechanisms by which caloric restriction enhances IL-2 gene expression. A number of studies have demonstrated that caloric restriction alters the expression of the IL-2 gene at the level of transcription. The increase in IL-2 expression correlates with an increase in binding activity of the transcription factor NFAT which plays a predominant role in IL-2 transcription. In addition, preliminary results suggest that activation of the upstream signaling molecules, the mitogen-activated protein kinase (MAPK) signaling cascade, may play a role in the enhancement of IL-2 transcription.

2. INTRODUCTION

The initial experiments in the 1930's by McCay *et al.* (1) showed that severe reduction of food intake in rats increased their life spans dramatically. Subsequent studies in the 1950s and 1960s demonstrated that dietary restriction (i.e., undernutrition, not malnutrition) significantly prolonged the survival of rodents. This prolongation has been observed with a variety of different techniques that reduce the amount of food consumed by rodents (reviewed in 2-4). Over the past decade, it has become apparent that the reduction in total calories is the component of the dietary restriction regimen responsible for the increase in survival (5,6). In other words, reducing the caloric intake of the rodents through any nutritional modification increases survival compared to that of rodents fed the normal calories in the laboratory chow diet (*ad libitum*). All evidence currently suggests that caloric restriction increases the survival of rodents by retarding the aging process (2-6). Therefore, caloric restriction has become a powerful technique for studying the process of aging.

Although it is well established that caloric restriction increases survival of rodents and reduces the pathology of diseases associated with aging, the physiological and biochemical basis for this effect have not been estabilished. The view that caloric restriction alters the aging process at the level of gene expression was first suggested by Barrows (7) in 1972. He proposed that protein synthesis was reduced in tissues of rats fed caloric restricted diets, and that the reduction in protein synthesis resulted in a reduced use of the genetic code. Barrows (7) suggested that caloric restriction increased longevity because the genetic code was used less by cells. The view that caloric restriction alters gene expression was expanded further in 1979 when Young (8) introduced the concept that nutrition might alter the aging process(es) by interacting at the structural and functional level of the gene by specifically altering translation and/or posttranslational processes. In 1982, Lindell (9) suggested that caloric restriction was a "physiological stress" that enhanced gene expression and that the enhancement in gene expression was a significant factor in maintaining cellular homeostasis in the caloric restricted rodents. In 1985, Richardson (10) proposed that caloric restriction retards the age-related decline in gene expression. Thus, over the

past two decades, several investigators have put forward the view that caloric restriction might act through changes in gene expression. In recent years, a number of reviews have been written on the aging immune system (11-15) and the anti-aging (2-6,10) and anti-immunosenescent (16-18) effects of caloric restriction. This review is more specifically focused on the influence of caloric restriction on IL-2 expression. In addition, the role of signal transduction molecules that are known to regulate the activity of transcription factors involved in IL-2 transcription will be discussed.

3. INFLUENCE OF CALORIC RESTRICTION ON IL-2 EXPRESSION

Interleukin-2 (IL-2) is a growth promoting cytokine that has received a great deal of attention over the past decade with respect to aging and cancer. It is produced primarily by helper T cells and regulates the growth and function of various cells that are involved in cellular and humoral immunity (19,20). The expression of IL-2 has been found to decrease with age in humans and rodents (reviewed in 21). The decline in IL-2 production has been shown to parallel the age-related decrease in immunologic function. Because caloric restriction enhances longevity and reduces pathology, there has been a great deal of interest on whether caloric restriction decreases pathology through action on the immune system, i.e., by altering IL-2 expression. The initial observation in this area was reported by Fernandes *et al.* (22); they showed that short-lived autoimmune prone (NZB X NZW) F1 mice fed normal calories (20 calories/day) were deficient in the production of IL-2 after 5 months of age. However, 50% caloric restriction preserved the production of IL-2 by the spleen cells of these animals; at 11 months of age, IL-2 production was approximately 2- to 3-fold higher for the caloric restricted mice. Furthermore, the caloric restricted mice responded vigorously to exogenous IL-2 in the thymocyte proliferation assay, while thymocytes from mice fed a normal diet lost much of their ability to respond to IL-2. In a later study, they showed that IL-2 activity and IL-2 receptor (IL-2R) expression (number of IL-2R sites per cell) were increased significantly in the concanavalin A (Con A)-stimulated spleen lymphocytes isolated from 19-month-old F344 rats fed a caloric restricted diet compared to the rats fed *ad libitum* (23)**.** Since 1982, a number of different laboratories (24-30), including our laboratory (31-33), have shown that induction of IL-2 production by mitogens was greater for animals fed a caloric restricted diet (40% reduction in calories). For example, Hishinuma *et al.* (26) reported that Con A induction of IL-2 production in spleen cells isolated from 4-month-old male C3H/He mice fed a caloric restricted diet for 9 weeks was significantly increased compared to spleen cells from mice fed *ad libitum*. Using a limiting dilution assay, it was found that the proportion of IL-2 producing cells decreased with age. However, this decline was lower in mice fed a caloric restricted diet. For example, 32-month-old mice fed *ad libitum* retained only 15% of their helper T cell function (measured as IL-2 producing cells) compared to 7-month-old control mice (30). In old mice fed a caloric restricted diet, 53% of the helper T cell function was retained. Recently, it was demonstrated that caloric restriction prevents the rise in memory helper T cells ($CD4+Pgp\text{-}1^{high}$) with age in mice and maintains both a higher number of virgin/naive helper T cells ($CD4+Pgp\text{-}1^{lo}$) and a higher level of IL-2 production (34).

Research from our laboratory has also shown that caloric restriction retards/reduces the age-related decrease in IL-2 expression. In an early study, we showed that caloric restriction significantly increased mitogen-induced lymphocyte proliferation and IL-2 production in F344 rats (31). In this study, rats (at 6 weeks of age) were subjected to a caloric restricted regimen. After 5, 12, 21, and 28 months of age, Con A induction of proliferation and IL-2 production (activity) by spleen lymphocytes were measured. We found that the proliferative response of lymphocytes to Con A in both caloric restricted rats and rats fed *ad libitum* declined significantly with increasing age. No differences were observed in mitogenesis and IL-2 production between caloric restricted rats and rats fed *ad libitum* at 5 and 12 months of age. However, the induction of proliferation and IL-2 expression was significantly higher for 21 and 22 month old caloric restricted rats compared to the rats fed ad libitum. In addition, we found that the increase in IL-2 activity was paralleled by an increase in the levels of the IL-2 mRNA transcript. Thus, caloric restriction altered the expression of IL-2 by increasing the transcription of the IL-2 gene. This was the first evidence that caloric restriction could alter the expression of a gene that plays a central role in cellular and humoral immune responses.

Because aging is generally characterized by a reduced ability of an organism to maintain homeostasis in response to stress (35), we were interested in studying the effect of caloric restriction on the expression of the most predominant member of the heat shock protein 70 (HSP70) family, hsp70. Our laboratory had observed previously that the induction of hsp70 synthesis by heat shock was lower in lymphocytes isolated from old rats than from young rats (36). The age-related decrease in the induction of hsp70 synthesis was paralleled by a similar decrease in the induction of hsp70 mRNA. In a subsequent study, the induction of hsp70 expression in response to a mild hyperthermia and IL-2 expression in response to Con A were measured in T cells isolated from 24-month-old caloric restricted rats and ad libitum fed control rats. Figure 1 shows the effect of caloric restriction on hsp70 expression by hyperthermia and the induction of IL-2 expression by Con A. The induction of IL-2 mRNA levels was significantly higher in T cells isolated from old rats fed a caloric restricted diet than in old rats fed ad libitum. However, no differences were observed in the induction of hsp70 mRNA levels in the caloric restricted old rats and the old rats fed ad libitum (32). Thus, our study indicated that the influence of caloric restriction on the levels of mRNA transcripts appears to vary from gene to gene.

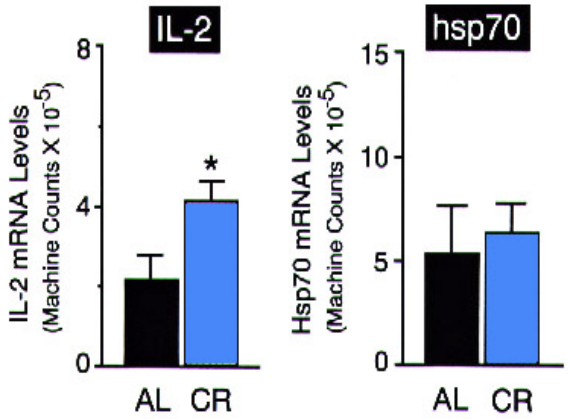

Figure 1. Effect of caloric restriction on the induction of heat shock protein 70 (hsp70) mRNA levels by hyperthermia and IL-2 mRNA levels by Con A in spleen lymphocytes from F344 rats. The splenic T cells were isolated from 24-month-old rats fed *ad libitum* (AL) or 24-month-old rats fed a caloric restricted (CR) diet and were stimulated with Con A for 24 h or exposed to heat shock (42.5°C for 1 h) followed by incubation at 37°C for 1 h. The IL-2 and hsp70 mRNA levels were determined by Northern blot hybridization. The blots were quantified by Molecular Dynamic PhosphorImager, and the data are presented in the graph. Data were taken from Pahlavani *et al.* (32). The values (*) for the caloric restricted rats are significantly different from the values for the rats fed *ad libitum* at $p<0.05$.

4. CALORIC RESTRICTION AND IL-2 TRANSCRIPTION

To gain a better understanding of how caloric restriction affects IL-2 gene expression, our laboratory has focused its attention on transcriptional regulation of the IL-2 gene. Initially, our studies employed a nuclear run-off assay to ask directly if age altered the amount of nuclear transcription of the IL-2 gene. We showed that the induction of transcription by nuclei isolated from T cells was depressed with age; this decrese was proportional to the decline in IL-2 mRNA levels (37). More recently, we measured the nuclear transcription of IL-2 in 24-month-old caloric restricted rats and *ad libitum* fed rats and found that the induction of transcription by nuclei was higher (by approximately 40%) in T cells isolated from caloric restricted rats than in T cells isolated from *ad libitum* fed rats.

How does caloric restriction specifically alter the transcription of genes? Caloric restriction does not appear to alter transcription through a general alteration in the transcriptional apparatus of a cell, since not all genes are affected in the same way by caloric restriction, e.g., the expression of some genes is increased, and the expression of others is decreased or not altered by caloric restriction (reviewed in 38). Thus, caloric restriction must alter transcription through a mechanism that affects only certain genes. It is currently known that transcription requires the recognition of numerous DNA sequences (cis-elements) by a diverse group of proteins, which are termed transcription factors. Transcription factors represent one of the largest and most diverse classes of DNA-binding proteins, and they regulate gene expression at the level of transcription. Over the past decade, it has become evident that these proteins play a critical role in development, differentiation and cellular proliferation (39-41). Transcription factors form a complex with RNA polymerase that initiates the synthesis of RNA. The assembly of the transcription complex requires that the DNA in the chromatin be accessible to the transcription factors, RNA polymerase, and the progression of the RNA polymerase along the DNA. Alteration in the expression and/or localization of transcription factors can result in changes in gene expression that would affect only one gene or a group of genes. Thus, changes in the activities or levels of transcription factors could be a mechanism whereby caloric restriction alters the transcription of genes in a specific manner.

We have postulated that caloric restriction alters a transcription factor that plays a critical role in the regulation of the IL-2 gene. The transcription of the IL-2 gene is regulated by the binding of several transcription factors (NFAT, AP-1, AP-3, NF-κB, and OCT-1) to enhancer sequences within the 300-bp promoter region of the IL-2 gene (reviewed in 42,43). The transcription factors AP-1, AP-3, NF-κB, and OCT-1 are ubiquitous transcription factors; i.e., they are involved in regulation of a variety of genes in various tissues, whereas NFAT is an IL-2-specific transcription factor that is unique to T cells and binds to the NFAT purine-rich sequence in the IL-2 promoter (42,43). The NFAT transcription factor is a multipeptide complex consisting of a cytoplasmic component (NFAT-c) and constitutive and inducible nuclear component (NFAT-n) (44). The constitutive factor consists of members of a family of oncoproteins, i.e., Elf-1 (45). The inducible nuclear component consists of members of the Fos and Jun family of oncoproteins (46-49). Stimulation of T cells with an antigen/mitogen or phorbol ester induces the expression of the nuclear component of the NFAT complex, specifically Fos and Jun, through the protein kinase C (PKC) signaling pathway. In addition, an antigen, mitogen or calcium ionophore stimulates the translocation of NFAT-c from the cytoplasm into the nucleus through the inositol-1,4,5-triphosphate (IP3) signal transduction pathway. The cellular levels of calcium are elevated in the activated T cells, and it is believed that the increase in calcium levels activates the calcium-dependent phosphatase activity of calcineurin, which dephosphorylates NFAT-c (figure 2). The dephosphorylated form of NFAT-c then translocates into the nucleus and forms a complex with the nuclear components (Fos/Jun/Elf-1) of the NFAT complex. Binding of the NFAT complex (NFAT-c + NFAT-n) to the IL-2 promoter then stimulates the transcription of the IL-2 gene.

Using nuclear extracts isolated from mitogen-stimulated T cells from rats fed either *ad libitum* or a caloric restricted diet, we measured the induction of DNA binding activity of the Tcell/IL-2-specific transcription

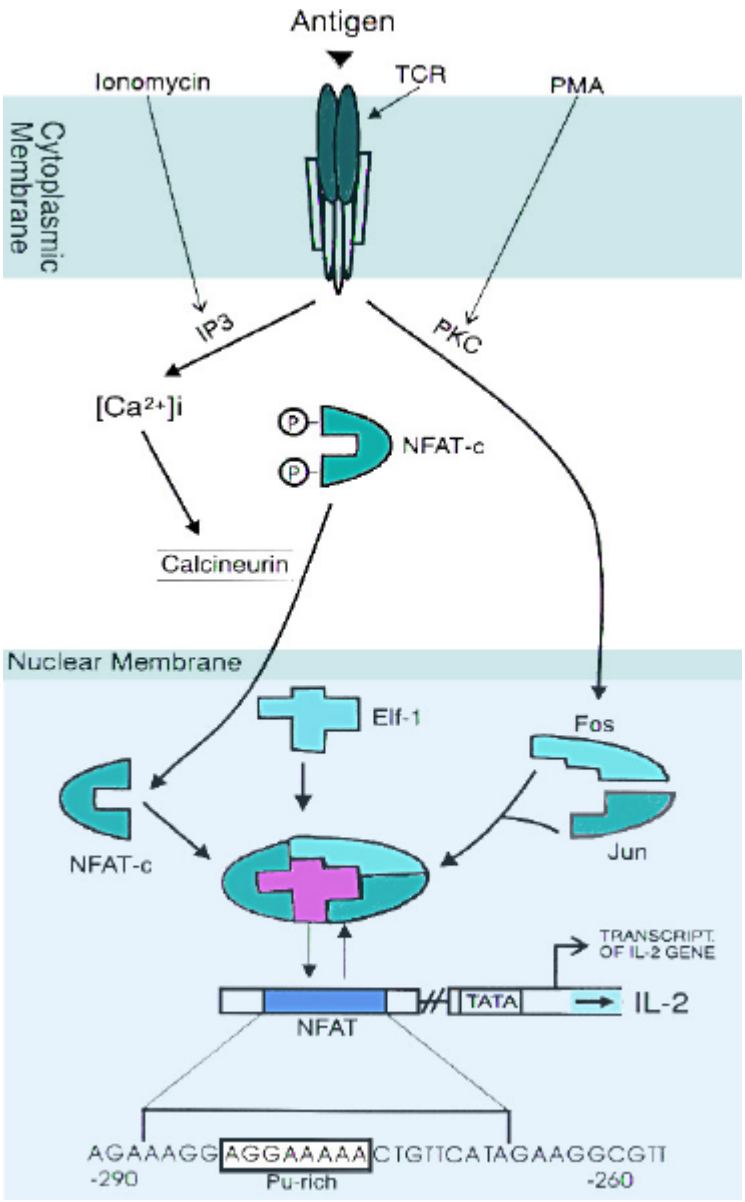

Figure 2. Schematic illustration of NFAT activation by T cell receptor-mediated signal transduction pathways that lead to IL-2 transcription.

factor NFAT and the ubiquitous transcription factor AP-1 by a gel shift assay. As the data in figure 3 show, the binding activity of NFAT was significantly higher in nuclear extracts from T cells isolated from rats fed a caloric restricted diet. In contrast to NFAT, the AP-1 binding activity in the nuclear extracts of T cells isolated from caloric restricted rats and *ad libitum* fed rats was not significantly different (33). In addition, the data in figure 3 also show that the increase in DNA binding activity of the transcription factor NFAT by caloric restriction was closely correlated to the increase in the transcription of the IL-2 gene (IL-2 activity and mRNA levels). Thus, it appears that caloric restriction alters the transcription of IL-2 through changes in the transcription factor NFAT.

Because Fos and Jun proteins are constituents of both the nuclear component of the NFAT protein complex (46-49) and the transcription factor AP-1 (42,43), we focused our attention on determining whether caloric restriction alters c-fos and/or c-jun expression. Figure 4 shows the effect of caloric restriction on the ability of T cells to express c-fos and c-jun after mitogen stimulation. The induction of c-fos expression (protein and mRNA levels) was significantly higher in T cells isolated from caloric restricted rats than from rats fed *ad libitum* (33). In contrast to c-fos, the c-jun expression was similar in caloric restricted and *ad libitum* fed rats. Thus, our study indicated that caloric restriction has a differential effect on c-fos and c-jun expression.

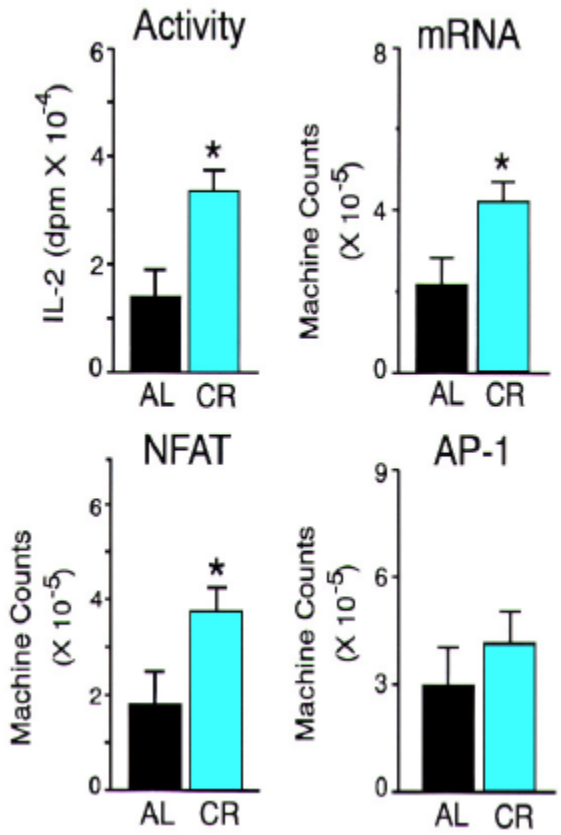

Figure 3. Influence of caloric restriction on the induction of IL-2 activity and mRNA levels and DNA binding activity of the transcription factors NFAT and AP-1 by Con A in T cells from F344 rats. Splenic T cells were isolated from 24-month-old rats fed *ad libitum* (AL) or 24-month-old rats fed a caloric restricted (CR) diet and were stimulated with Con A. IL-2 activity in the culture supernatants was measured by an IL-2-dependent cell line (CTLL-20) and the Con A induction of IL-2 mRNA levels was measured by Northern blot hybridization. The induction of the NFAT and AP-1 binding activity of the nuclear extracts were measured by the gel mobility shift assay. The IL-2 mRNA blots and the autoradiographs of the NFAT and AP-1 binding activities were quantified by Molecular Dynamic PhosphorImager, and the data are presented in the graph. Data were taken from Pahlavani *et al.* (33). The values (*) for the caloric restricted rats are significantly different from the values for the rats fed *ad libitum* at $p<0.05$.

5. SIGNAL TRANSDUCTION AND IL-2 EXPRESSION

The upstream signaling pathways involve a cascade of phosphorylation and dephosphorylation events which lead to augmentation of c-fos and c-jun transcription and ultimately IL-2 expression. Therefore, in order to gain insight into the mechanisms responsible for the changes in NFAT and IL-2 with age and caloric restriction, we have been interested in studying the activation of the upstream signaling molecules. Among various signal transduction molecules, the mitogen-activated protein kinase (MAPK), also known as extracellular regulated kinase (ERK), and the c-jun amino terminal kinase (JNK) have been shown to play an integral role in transduction of receptor-mediated signals in T cells (50-52). The activation of MAPK/JNK has been shown to be an important regulatory signal through which a wide variety of extracellular signals are transduced into the intracellular events. In Jurkat T cells, at least two isoforms of MAPKs, ERK1 ($p44^{mapk}$) and ERK2 ($p42^{mapk}$) are present. These isoforms are transiently activated by various stimuli, including Con A, phytohemagglutinin (PHA), phorbol myristate acetate (PMA), and anti-CD3 mitogenic antibody. It has been shown that over-expression of ERK1 enhances the induction of IL-2, probably through increasing the activity of the transcription factors NFAT and AP-1 (53). Thus, these studies suggest that stimulation of MAPK plays an important role in T cell activation and IL-2 expression.

The current model for MAPK signaling events that leads to regulation of c-fos and c-jun transcription is shown in figure 5. In T cells, TCR (T cell receptor) signaling is mediated through protein tyrosine kinases (PTKs) activity although no tyrosine kinase domain has been identified within the TCR-CD3 structure. The TCR interacts with at least three PTKs (Lck, Fyn, and ZAP-70) through the tyrosine-based activation motif (TAM) contained in the ζ and other CD3 chains (52,54,55). Lck is usually not found physically associated with TCR, but it binds to the co-receptors CD4 and CD8. Lck does, however, interact with the TCR because CD4 and CD8 co-localize with this receptor during antigenic/mitogenic recognition (56). This clustering allows Lck to phosphorylate the TAM on the TCR ζ chain, leading to the recruitment of ZAP-70 (57). In contrast to ZAP-70, Fyn appears to bind directly to the TCR ζ chain without requiring prior receptor ligation (58). The tyrosine kinase activity associated with the TCR-associated molecules is coupled to the activation of downstream signalling molecules (52, 54-58). The tyrosine kinase phospholipase-Cγ (PLC-γ) stimulates its activity, causing the generation of second messengers that stimulate protein kinase C (PKC) activation and trigger an elevation of intracellular calcium. These biochemical events lead to the stimulation of GTP-bound Ras. Ras is activated both as a result of PKC mediated inhibition of Ras-GTPase-activating proteins, and by an additional stimulatory signal that is independent of PKC, and likely involves coupling of TCR-associated protein tyrosine kinase activity to Grb2 and the Ras Grb-exchange SOS molecule (50,51). In the GTP-bound state, Ras stimulates the MAPK pathway, leading to activation of ERKs and JNKs. At the plasma membrane, active GTP-bound Ras directly binds and promotes the activation of the protein kinase Raf-1. Active Raf-1 phosphorylates and activates the MAPKs/ERKs through the activation of MAPK kinase (MEK). Active ERKs phosphorylate and regulate the activity of numerous additional proteins in both the cytosol and nucleus (50-51). Thus, one function of the Ras/Raf-1/MEK/ERK signal transduction pathway is to transmit the stimulatory signal received at the plasma membrane into the nucleus.

Given the potential important role of the upstream signaling molecules, i.e., MAPK in regulation of c-fos and c-jun transcription, and because Fos and

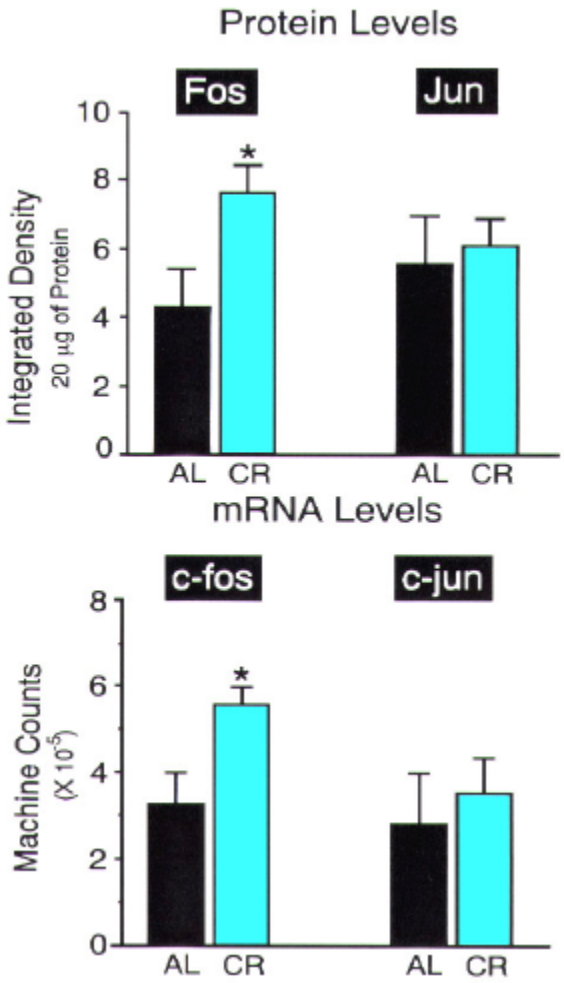

Figure 4. Influence of caloric restriction on the induction of c-fos and c-jun expression by Con A in F344 rats. The splenic T cells were isolated from 24-month-old rats fed *ad libitum* (AL) or rats fed a caloric restricted (CR) diet and were stimulated with Con A. The induction of Fos and Jun protein levels were measured by Western blot analysis and c-fos and c-jun mRNA levels were measured by Northern blot hybridization. The blots were quantified by Molecular Dynamic PhosphorImager, and the data are presented in the graph. Data were taken from Pahlavani *et al.* (33). The values (*) for the caloric restricted rats are significantly different from the values for the rats fed *ad libitum* at $p<0.05$.

Jun proteins constitute the nuclear component of the NFAT protein complex and the transcription factor AP-1, our laboratory has begun to study how caloric restriction affects the upstream signaling molecules. To determine whether aging or caloric restriction affects MAPK and/or JNK activation, purified T cells were isolated from young and old rats fed ad libitum and old rats fed a caloric restricted diet, and the kinase activity of the immunoprecipitated p44 and p42 MAPK, and p46 JNK were measured by the phosphorylation of myelin basic protein and recombinant GST-c-jun peptide substrate, respectively. As shown in figure 6, we found that mitogen induction of MAPK activity decreased with age, and caloric restriction reduced the age-related decrease in MAPK activity. For example, the MAPK activity was 65% higher for T cells isolated from 24-month-old caloric restricted rats compared to T cells isolated from age-matched control rats. On the other hand, the data in figure 6 show that JNK activity did not change significantly with age or with caloric restriction. The changes in MAPK/JNK activities were not associated with changes in their corresponding protein levels as measured using Western blot analysis. Thus, caloric restriction appears to increase the MAPK activity, and this increase was correlated with an increase in c-fos expression.

6. CONCLUDING REMARKS

Caloric restriction is known to be an effective method of prolonging the life-span, delaying immunosenescence, and reducing pathology in various strains of rodents. Because caloric restriction has a profound effect on most physiological systems, it is plausible that the modulation of immune function by caloric restriction occurs through changes in gene expression. Gene expression is a cellular process that is fundamental to all cells, and changes in gene expression can markedly affect cellular processes of the organisms. Therefore, it is logical to hypothesize that changes in the expression of the IL-2 gene play a major role in the biological mechanisms responsible for the immunoenhancing effect of caloric restriction. Research from our laboratory supports this hypothesis. We have shown that the expression of IL-2 decreases with age and this decrease was attenuated by caloric restriction. More importantly, we have shown that caloric restriction can alter the expression of IL-2 at the level of transcription. The changes we have observed in the expression of the IL-2 gene could be physiologically important to an organism, because the protein product of this gene plays a critical role in regulating the growth and function of a variety of cells involved in cellular and humoral immune responses. The increase in expression of IL-2 by T cells from caloric restricted rats in response to antigens would be predicted to result in a more robust immune response, which would provide an organism greater protection from foreign antigens.

Although our research clearly demonstrates that caloric restriction can alter the expression of the IL-2 gene at the level of transcription, the molecular mechanisms responsible for the changes in transcription are currently unknown. Our preliminary experiments indicate that the changes in gene transcription are not simply due to an overall

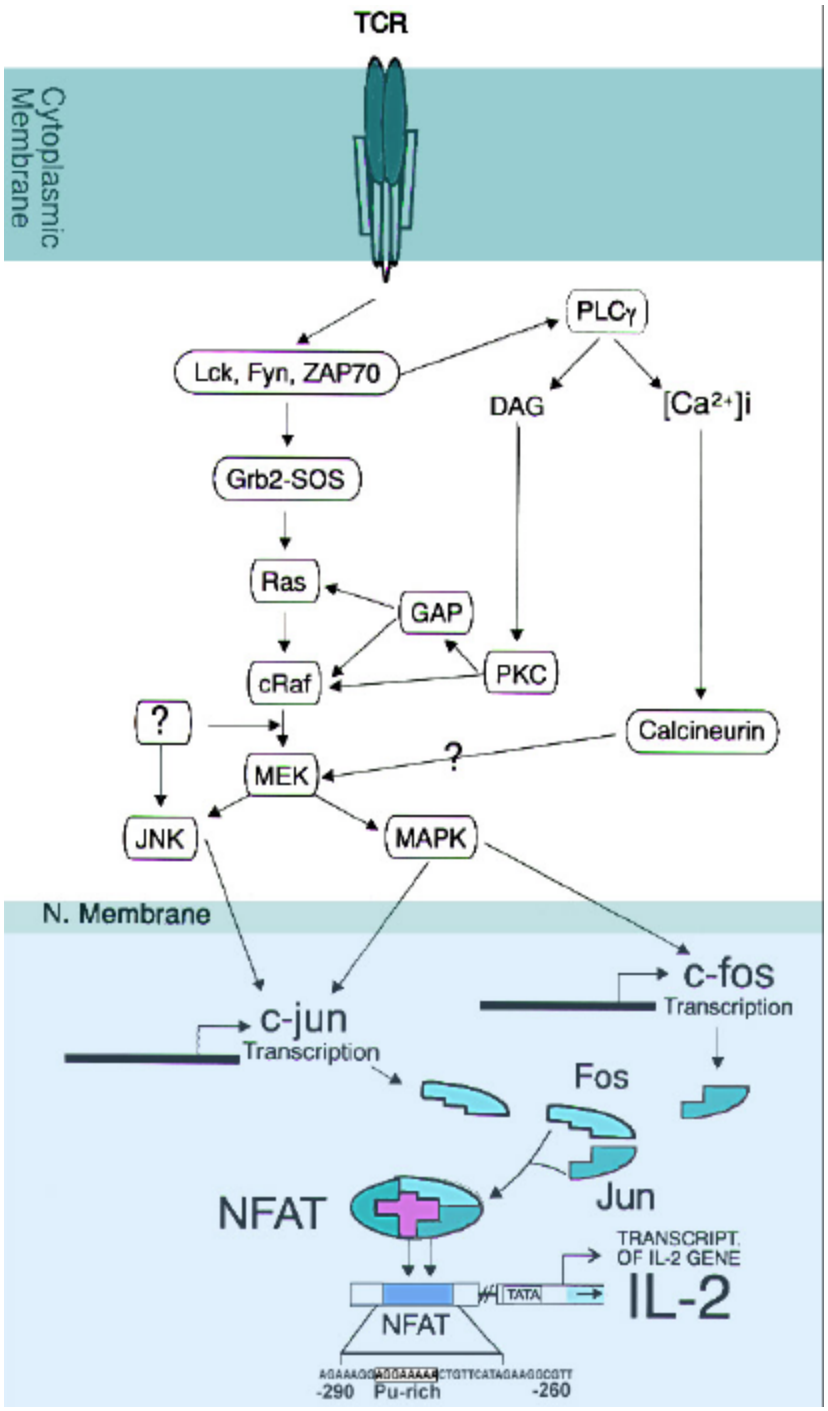

Figure 5. The current model for the intracellular signaling event involving the activation of the MAPK signal transduction pathway acting on c-fos and c-jun transcription and IL-2 expression.

in the transcriptional apparatus of the cell, because not all genes are affected in the same way by caloric restriction. For example, we have observed that while the expression of one gene (IL-2) is affected by caloric restriction, the expression of other gene (e.g., hsp70) is not influenced by caloric restriction. Because the effect of caloric restriction on transcription varies from gene to gene, we hypothesized that caloric restriction alters the transcription of the IL-2 gene through changes in the level and/or activity of the T cell/IL-2-specific transcription factor, i.e., NFAT. We have shown that NFAT binding activity increases with caloric restriction and this increase is correlated with an increase in c-fos expression. Among various signal transduction molecules, the MAPK has been shown to be an important

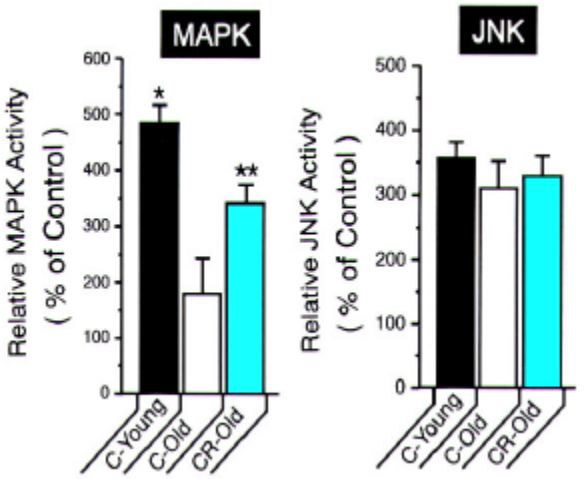

Figure 6. Effect of age and caloric restriction on the induction of mitogen-activated protein kinase (MAPK) and c-jun amino terminal kinase (JNK) activities by Con A in T cells from F344 rats. The splenic T cells from young (6 months) and old (24 months) *ad libitum* fed rats and 24-month-old caloric restricted (CR) rats were stimulated with Con A for 15 min. Protein was isolated and kinase activity in the immunoprecipitated p44 and p42 MAPK or p46 JNK was measured by their ability to phosphorylate a myelin basic protein or a recombinant GST-c-jun exogenous substrate in the presence of ^{32}P-ATP. The ^{32}P-ATP incorporation into the myelin basic protein substrate was measured by scintillation counting. The ^{32}P-ATP incorporation into the GST-c-jun substrate was analyzed by SDS-PAGE and the autoradiographs were quantified and the data are presented in the graph. The kinase activity is expressed as the percent of activity in Con A-stimulated cells over the unstimulated cells. Each value represents mean ± SD of 3 separate experiments. The values (*) for the control young rats are significantly different from the values for the old rats fed *ad libitum* at $p<0.001$. The values (**) for caloric restricted old rats were significantly different from the value for the old rats fed *ad libitum* at $p<0.05$.

alteration regulator of immediate-early genes (e.g., c-fos and c-jun) transcription. Therefore, our current hypothesis is that caloric restriction may alter the levels/activities of the upstream signaling molecules involved in the Ras/MAPK signaling event. This mechanism would logically explain how caloric restriction enhances IL-2 expression because the MAPK signaling cascade is involved in regulating the transcription of immediate-early genes (e.g., c-fos/jun), in which the gene protein products constitute the nuclear component of the NFAT complex. Although our preliminary results suggest that enhancement in MAPK activation with caloric restriction may underlie the increase in c-fos expression, further investigation is needed to determine how caloric restriction alters signal transduction that leads to T cell activation and IL-2 expression.

7. ACKNOWLEDGMENTS

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