Regulation of Tissue Transglutaminase Gene Expression as a Molecular Model for Retinoid Effects on Proliferation and Differentiation

E. Antonio Chiocca, Peter J.A. Davies, and Joseph P. Stein

Department of Internal Medicine, Division of Endocrinology (E.A.C., P.J.A.D., J.P.S.) and Department of Pharmacology (P.J.A.D.) University of Texas Medical School at Houston, Houston, Texas 77225

Retinoids (structural and functional analogs of vitamin A) are potent antiproliferative agents whose mode of action is poorly understood. It has been suggested that the molecular events that underscore their action involve alterations in gene expression, but no gene has yet been shown to be directly regulated by these molecules. Several years ago, we found that retinoic acid caused an accumulation of the enzyme tissue transglutaminase in murine peritoneal macrophages and in human promyelocytic leukemia (HL-60) cells. We now report that this induction is caused by an increase in the mRNA for this enzyme. Retinoic acid is the only mediator of this induction, since its effects do not depend on the presence of serum proteins. The induction of tissue transglutaminase mRNA is not due to an increase in its stability but to an increase in the relative transcription rate of its gene. We present a model to correlate the retinoid induction of tissue transglutaminase with retinoid effects on cellular growth and differentiation.

Important insights into questions related to cellular growth and differentiation have been gained by the isolation of factors and the identification of mechanisms that inhibit cellular proliferation. The fact that retinoids (structural and functional analogs of vitamin A) have profound inhibitory effects on the proliferation of both normal and transformed cells has been demonstrated by numerous investigators. For example, retinoic acid will cause the maturation of human promyelocytic leukemia (HL-60) cells into granulocytes [1], F9 teratocarcinoma cells into parietal endoderm [2], and neuroblastoma cells into neuronal cells [3]. Retinoid deficiency can provoke changes in the state of differentiation of tracheal epithelial cells [4]. Furthermore, retinoic acid has been shown to be one of the morphogenic substances critical to embryonic development [5-7]. Several models of retinoid action have been proposed:

we favor the hypothesis that retinoids act by altering the expression of specific genes in a manner similar to steroid hormones [8]. However, direct experimental confirmation of this model has not been forthcoming, primarily because of the inability to identify genes whose expression is directly regulated by retinoids.

Recently, we have found that the gene for the enzyme tissue transglutaminase is regulated by retinoids in a direct and acute manner [9–11]. Although this effect occurs in a number of normal and transformed cell lines such as Swiss 3T3-C2 or HL-60 cells [12] (Chiocca, Stein, and Davies, unpublished observations) the majority of our experimental efforts have been directed towards understanding this phenomenon in murine resident peritoneal macrophages, primarily because their response to retinoids is very large and extremely rapid. In this report, we review the evidence that led us to concentrate on this particular enzyme in macrophages and we present results that show that retinoic acid regulates the transcription of this gene.

MATERIALS AND METHODS

Isolation of Mouse Macrophage Tissue Transglutaminase cDNAs

The procedures employed in the isolation of macrophages have been described previously [9,10]. In order to isolate tissue transglutaminase cDNAs, a retinoic acid stimulated mouse macrophage cDNA lambda gt-11 library was obtained from Clontech (Palo Alto, CA). This library was screened with an affinity-purified tissue transglutaminase antibody leading to the isolation of three overlapping tissue transglutaminase cDNA clones (TG700, TG1600, and TG3000). The authenticity of these cDNAs was established by hybridization-selected translation and by nucleotide sequencing, as described in detail elsewhere [11]. By comparison with a guinea pig tissue transglutaminase cDNA recently isolated by Ikura et al. [13], the three mouse cDNAs include the majority of the coding sequence and the entire 3'-untranslated sequence of the full-length mouse tissue transglutaminase cDNA.

Measurements of Tissue Transglutaminase Activity, Enzyme, and mRNA Levels

Macrophages were routinely incubated in the presence of 10^{-6} M all-trans-retinoic acid and 1.5% delipidized mouse serum (DLMS), unless otherwise noted. Transglutaminase activity was determined by measuring the Ca^{2+}-dependent incorporation of ^{3}H-putrescine into N,N-dimethyl casein as described previously [9]. Protein concentrations were determined by the method of Bradford [14]. Levels of tissue transglutaminase protein were determined by immunoblot analysis with an affinity-purified antitissue transglutaminase antibody [9]. The tissue transglutaminase cDNAs were subcloned into the EcoRI site of a pGEM3 plasmid vector (Promega Biotec, Madison, WI) and named pgmTG700, pgmTG1600, pgmTG3000, respectively. This site is located between SP6 and T7 promoters, permitting the construction of single-stranded cRNAs labelled to a high specific activity with α-^{32}P-UTP. Levels of tissue transglutaminase mRNA were determined either by Northern or slot blot analysis using ^{32}P-labelled tissue transglutaminase antisense cRNAs, which were transcribed from the T7 promoter of linearized pgmTG700, pgmTG1600 or pgmTG3000 plasmids [11].

Northern blot analysis of total RNA was carried out using published protocols [15,16]. Total RNA (5 μg) was fractionated on 6% formaldehyde/1% agarose gels

and then stained with ethidium bromide to visualize the 28S and 18S rRNA bands. After transferring the RNA to nitrocellulose filters, hybridizations were performed at 65°C in the presence of 50% formamide, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 0.1% SDS, 0.75 M sodium chloride, 0.075 M sodium citrate, 20 mM Hepes (pH = 7), 1 mM EDTA, 200 mg/ml of denatured salmon sperm DNA, and 0.5 mM UTP. Filters were serially washed with $0.1 \times$ SSC/0.1% SDS at 65°C for 30 min, with RNAase A (1–10 μg/ml) at room temperature for 20 min, and with $0.1 \times$ SSC/0.1% SDS at 65°C for 45 min. Autoradiography was performed at -70°C with intensifying screens, and then the tissue transglutaminase mRNA bands were quantitated with laser densitometry. In order to correct artifacts derived from any differences in the loadings of total RNA during the formaldehyde/agarose gel electrophoresis, the 28S rRNA bands from the ethidium bromide gels were also quantitated by laser densitometry. The values of the 28S rRNA band were used to normalize the values obtained from the scans of the tissue transglutaminase mRNA bands.

Tissue transglutaminase mRNA levels were also measured by slot-blot analysis. Total RNA (1 μg) was blotted onto nitrocellulose filters using a slot-blotter apparatus (Schleicher and Schuell). After baking the filter at 80°C for 2 h, the RNA was hybridized to a ^{32}P-labelled tissue transglutaminase cRNA using the same buffers and washes described above for the Northern blots.

The relative transcription rates of the tissue transglutaminase gene were measured by using a transcriptional elongation assay [17,18]. Sense and antisense cRNAs were prepared by transcribing linearized pgmTG1600 from either the SP6 or the T7 promoter. These cRNAs were blotted onto nitrocellulose filters using a slot-blotter apparatus (Schleicher & Schuell). Alternatively, denatured pgmTG3000 (a pGEM3Z vector which contains a 3,000-base-pair tissue transglutaminase cDNA) or denatured pgmTG1600 were blotted onto nitrocellulose filters. Control and retinoic acid stimulated mouse resident peritoneal macrophages were scraped from the culture dishes with a rubber policeman, and nuclei were isolated by homogenization in a buffer containing 0.3 M sucrose, 60 mM potassium chloride, 15 mM sodium chloride, 0.5 mM spermidine, 0.15 mM spermine, 15 mM Tris HCl (pH = 7.5), 5mM DTT, 2 mM EDTA, and 0.2% NP40 [17]. After sedimentation at 500g, the nuclei were washed once in the same buffer without NP40 and then once in nuclear storage buffer (40% glycerol, 4 mM manganese chloride, 4 mM magnesium chloride, 20 mM TrisCl (pH = 7.5) 5 mM DTT, and 0.1 mM EDTA). After counting in an hemocytometer, they were adjusted to a concentration of approximately 5×10^7 nuclei/ml of storage buffer and stored at -70°C.

For transcription reactions 5–10×10^6 nuclei were incubated in the presence of 200 μCi of α-^{32}P-UTP in 25 mM Hepes (pH = 7.5), 142 mM potassium chloride, 2.5 mM DTT, 50 μM EDTA, 1 mM ATP, 0.5 mM CTP, 0.5 mM GTP, 8.8 mM creatine phosphate, and 40 μg/ml creatinine phosphokinase for 30 min at 25°C. Nuclear RNA labelled with ^{32}P was then isolated [17] and hybridized to the nitrocellulose filters that contained the linearized pgmTG3000 and pgmTG1600 plasmids and the antisense and sense cRNAs. The filters were then washed twice at room temperature, three times at 45°C in $5 \times$ SSC/0.2% SDS/0.5 mM EDTA, twice at 45°C in $2 \times$ SSC, once at room temperature in the presence of 20 μg of RNase A, and once at room temperature in $2 \times$ SSC. Autoradiography was conducted at -70°C in the presence of intensifying screens. Laser densitometry of the signals was then subsequently performed to quantitate the autoradiographic signals.

RESULTS

Retinoid Induction of Tissue Transglutaminase

Schroff and others [19,20] had reported several years ago that inflammatory peritoneal macrophages contained high levels of transglutaminase activity. This prompted us to measure levels of tissue transglutaminase in resident, thioglycolate elicited, and Calmette-guèrin bacillus (BCG)-activated macrophages using an affinity-purified tissue transglutaminase polyclonal antibody. We found that thioglycolate-elicited mouse peritoneal macrophages contained at least eight times more tissue transglutaminase than the resident peritoneal macrophages and that the levels of this enzyme were even higher in BCG-activated macrophages [9]. These results suggested that tissue transglutaminase levels increased during the process of macrophage activation.

In an effort to replicate this process in vitro, we obtained resident peritoneal macrophages and cultured them in the presence of 10% mouse serum. We found that an increase in transglutaminase activity was apparent within 90 min of serum stimulation and reached levels that were 150 times greater than basal activity after 30–40 h. This increase was not due to enzyme activation, but rather to an actual increase in enzyme levels [9].

In order to characterize the factors in serum that were responsible for this induction, we depleted the lipid fraction of fresh mouse serum [10] and found that delipidized mouse serum lost its ability to induce tissue transglutaminase in macrophages. We attempted to restore this induction by adding to this serum bacterial lipopolysaccharide, testosterone, stearic acid, lecithin, and cholesterol, without success [10]. However, when 1 μM retinoic acid was added with the delipidized mouse serum, the induction of this enzyme was completely restored. These findings indicated that retinoic acid was the component in serum responsible for the induction of tissue transglutaminase [10].

In order to examine the mechanism of this induction, we obtained transglutaminase cDNA clones by antibody screening of a library constructed in the lambda gt-11 expression vector with mRNA obtained from retinoid stimulated macrophages. Three overlapping transglutaminase cDNA clones of 1,600, 700, and 3,000 bp were obtained. Together they encode the majority of the coding sequence and the entire 3'-untranslated sequence of the full-length tissue transglutaminase mRNA (Fig. 1). These cDNA clones were used as hybridization probes to measure tissue transglutaminase mRNA levels in resident mouse peritoneal macrophages stimulated for 6 h with or without 1 μM retinoic acid. As shown in the Northern blot of Figure 2, there was a large induction of tissue transglutaminase mRNA in these cells.

To characterize this induction further, time-course experiments were performed in which mouse peritoneal macrophages were either left untreated or stimulated with one dose of 10^{-6} M retinoic acid. Total RNA from these cells was then isolated at different time points and blotted onto nitrocellulose with a slot-blot apparatus. Tissue transglutaminase mRNA levels were measured with an antisense tissue transglutaminase cRNA probe (see Materials and Methods). Figure 3 shows that retinoic acid caused a rapid increase in the amounts of tissue transglutaminase mRNA.

The Retinoid Induction of Tissue Transglutaminase mRNA Is Independent of Serum-Retinol Binding Protein (sRBP)

We had previously reported that the induction of tissue transglutaminase in macrophages by nanomolar concentrations of retinoic acid was facilitated by recon-
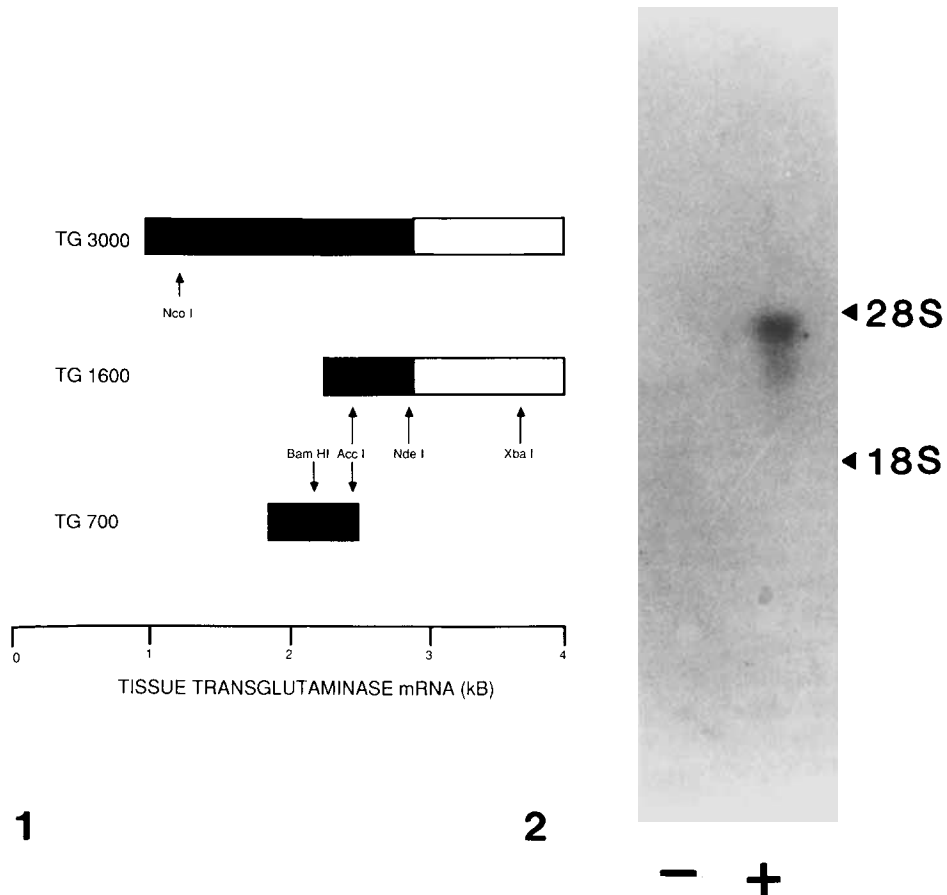
Fig. 1.   Partial restriction maps of tissue transglutaminase cDNA clones. Three cDNA clones were obtained by screening a lambda gt-11 library, as reported in [11]. These cDNAs measured approximately 3,000, 1,600, and 700 nucleotides in length, and were named TG3000, TG1600, and TG700. The black boxes represent coding sequence, while the white boxes represent 3'-untranslated sequence. The positions of several unique restriction sites are indicated. The size of the tissue transglutaminase mRNA is approximately 4,000 nucleotides and it is shown at the bottom of the figure.

Fig. 2.   Northern analysis of retinoid-induction of tissue transglutaminase mRNA. Mouse resident peritoneal macrophages were either left untreated (−) or stimulated with 10^{-6} M retinoic acid for six hours (+). Total RNA was then isolated, fractionated on a formaldehyde/denaturing agarose gel, and transferred to nitrocellulose filter by capillary blotting. The filters were then probed with a nick-translated TG700 cDNA. The positions of the 28S and 18S rRNA bands are indicated.

stitution of the retinoid in medium containing delipidized mouse serum. We suggested that the binding of retinoic acid to the apo-form of sRBP, present in DLMS, improved its ability to induce tissue transglutaminase expression [10]. However, these experiments depended on assays of tissue transglutaminase activity, which meant that the macrophages had to be exposed to retinoic acid for several hours in order to induce changes in enzyme activity significant enough to be detected. The possession of tissue transglutaminase cDNA probes allowed us to determine the requirement for serum
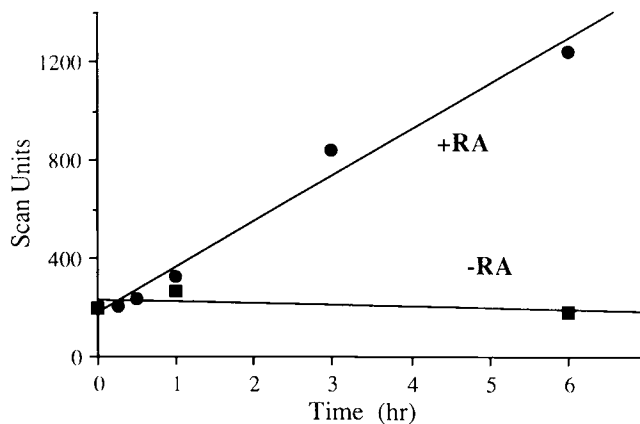
Fig. 3. Time course of tissue transglutaminase mRNA induction by retinoic acid. Resident mouse peritoneal macrophages were either treated with a control solution (squares), consisting of 1.5% DLMS and 0.1% ethanol (the solvent for retinoic acid) or with 1.5% DLMS + 10^{-6} M retinoic acid for 0, 5, 15, 60, 180, and 360 min (circles). Total RNA was then extracted and blotted onto a nitrocellulose filter using a slot-blot apparatus (Schleicher & Schuell). This RNA was probed with the tissue transglutaminase cRNA described in the Materials and Methods section. After autoradiography, the blot was quantitated by laser densitometry, and total scan units were plotted versus hours of retinoic acid treatment.

proteins in the induction of tissue transglutaminase mRNA at much shorter times following the exposure of cells to retinoic acid. To evaluate this requirement, resident mouse peritoneal macrophages were treated either for 40 min or for 7.5 h with 1.5% DLMS alone (control), with 10 nM retinoic acid, or with 10 nM retinoic acid + 1.5% DLMS. Total RNA was prepared and analyzed with the Northern blotting technique. Macrophages exposed to retinoic acid for 40 min showed a twofold increase in the levels of tissue transglutaminase mRNA compared to control cells, and this induction was not affected by the addition of DLMS (Fig. 4). In contrast, at the 7.5-h time point the presence of DLMS made a difference. The cells exposed to 10 nM retinoic acid showed no increase in tissue transglutaminase mRNA levels compared to control cells, whereas the cells exposed to the combination of retinoic acid + DLMS showed a ninefold induction in tissue transglutaminase mRNA levels. These results indicated that the retinoic acid induction of tissue transglutaminase mRNA at short times does not require the presence of serum proteins.

Retinoic Acid Does Not Alter the Stability of Tissue Transglutaminase mRNA

To determine if the induction of tissue transglutaminase mRNA was due to an increase in its stability, we measured the apparent half-lives of tissue transglutaminase mRNA in control and retinoic acid treated macrophages. Actinomycin D (0.1 μg/ml) was added to control cells and to cells that had been stimulated with 1 μM retinoic acid for 10 h and RNA was isolated 1, 2, 3, 4, and 5 h later. Equal amounts of RNA were fractionated by formaldehyde/agarose gel electrophoresis and tissue transglutaminase mRNA was detected by the Northern transfer/hybridization technique with a ^{32}P-labelled antisense cRNA transcribed from pgmTG1600. The levels of the tissue transglutaminase mRNA were quantitated by laser densitometry. In order to give comparable band intensities, longer exposure times for the autoradiograms were

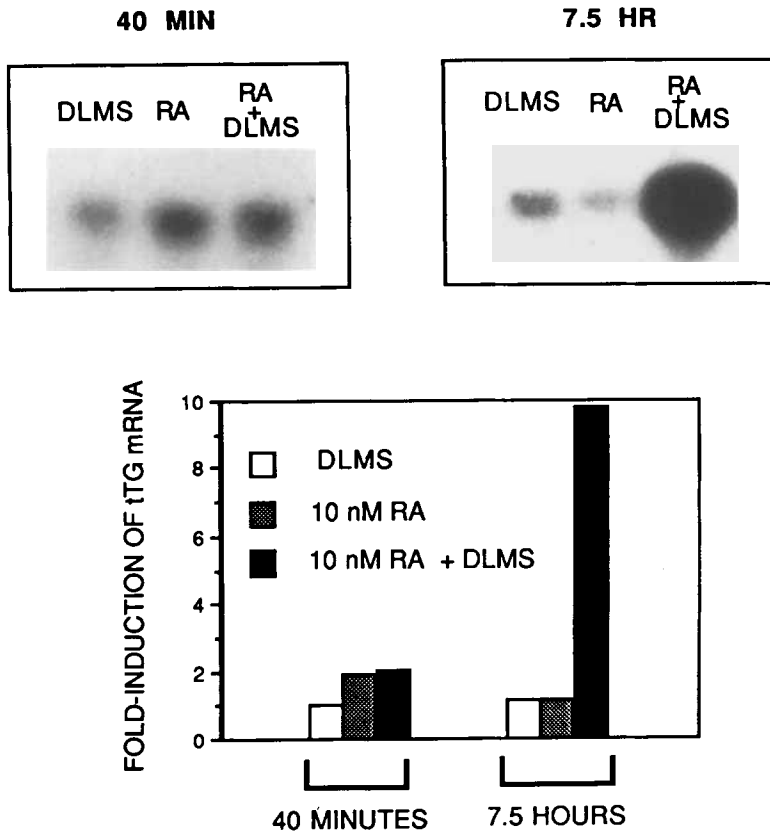**40 MIN**   **7.5 HR**

Fig. 4.   Effect of DLMS on the retinoic acid induction of tissue transglutaminase mRNA. Resident mouse peritoneal macrophages were stimulated with 1.5% DLMS alone, with 10 nM retinoic acid, or with 10 nM retinoic acid + DLMS either for 40 min (**top left**) or for 7.5 h (**top right**). Total RNA was then isolated and fractionated (5 μg per lane) on formaldehyde/agarose denaturing gels. After ethidium bromide staining of the gels, the RNA was transferred to nitrocellulose filters and probed with the antisense RNA probe described in the Materials and Methods section. The autoradiograms were then scanned with a laser densitometer, correcting the values for any loading differences as described in the Materials and Methods section. The scan unit values from three independent experiments were averaged; the average values were converted into fold-induction of tissue transglutaminase mRNA above control and displayed as a bar graph at the bottom of the figure.

required for the untreated macrophages (17 h) than for the retinoic acid treated cells (1 h). Figure 5 shows the actinomycin D induced decline in tissue transglutaminase mRNA in untreated cells and in retinoic acid treated cells. The half-life for the tissue transglutaminase mRNA in retinoic acid treated cells (90 min) was similar to the one in control cells (70 minutes).

Retinoic Acid Induced Increase in the Relative Transcription Rate of the Tissue Transglutaminase Gene

Since retinoic acid did not cause an increase in the stability of tissue transglutaminase mRNA, we tried to determine whether the relative transcription rate of the tissue transglutaminase gene was altered in retinoid-stimulated cells. After resident mouse peritoneal macrophages were stimulated with or without retinoic acid for 5 h,
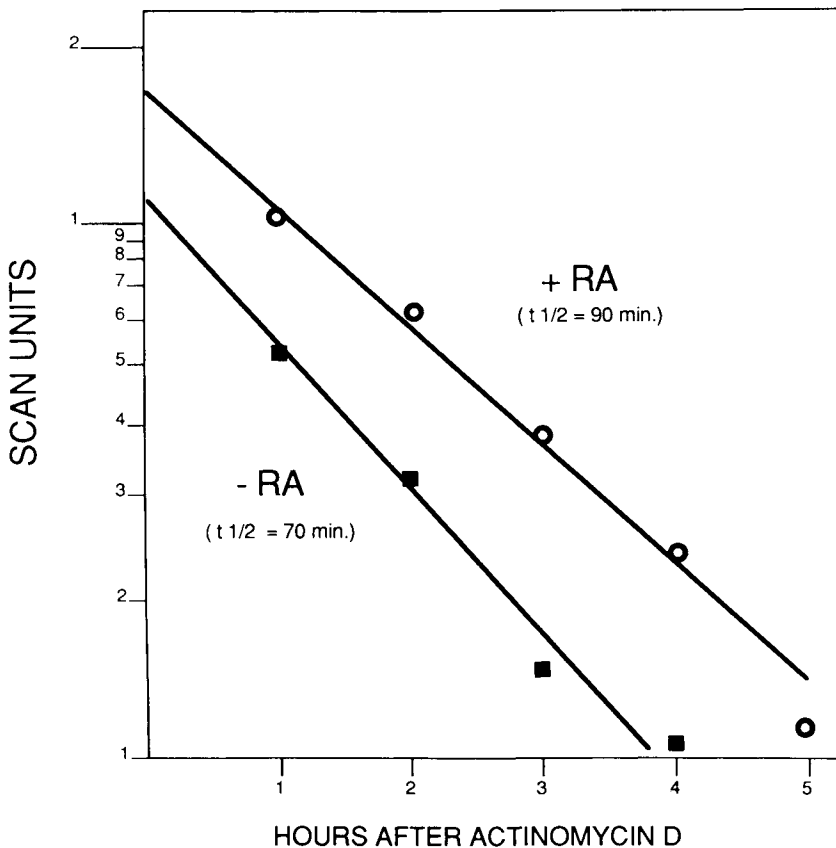
Fig. 5. Effect of retinoic acid on the stability of tissue transglutaminase mRNA. Resident mouse peritoneal macrophages were either left untreated (−RA) or were stimulated for 10 h with 1 μM retinoic acid (+ RA). Cells were then thoroughly washed and treated with actinomycin D (0.1 μg/ml.). Total RNA was isolated hourly after treatment and equal amounts were fractionated on a formaldehyde/agarose denaturing gel. After ethidium bromide staining and laser densitometric scanning of the 28S ribosomal RNA bands to ensure equal RNA loadings, the RNA was transferred to nitrocellulose filters which were then probed with an ^{32}P-labelled antisense tissue transglutaminase cRNA. Autoradiography was performed at −70°C with intensifying screens. To get signals of comparable intensity between the retinoic acid stimulated tissue transglutaminase mRNA and the tissue transglutaminase mRNA from control cells, the autoradiograms' exposure times were varied (1 and 17 h, respectively). The tissue transglutaminase mRNA bands were then scanned. The scan unit values of the 28S ribosomal RNA bands were used to normalize the tissue transglutaminase mRNA bands. These values were plotted onto a semilog graph as a function of time after actinomycin D treatment. The tissue transglutaminase mRNA half-lives for the control cells and the retinoic acid treated cells were found to be 70 and 90 min, respectively.

nuclei were isolated and incubated in a buffer designed to support the elongation of nascent RNA chains in the presence of α-^{32} P-UTP. The ^{32}P-labelled RNA chains were then hybridized to nitrocellulose filters that contained an antisense tissue transglutaminase cRNA. After autoradiography, laser densitometry was performed to quantitate the signals. The results from four transcription run-on experiments are shown in rows 1–4 of Table I. In the retinoic acid treated macrophage there is an induction in the relative transcription rate of the tissue transglutaminase gene com-

TABLE I. Laser Densitometric Scan Units of Transcription Run-On Assays of the Tissue Transglutaminase Gene in Unstimulated and Retinoid Stimulated Macrophages

Row[a]	Control[b]	Retinoic acid
1	100	1220
2	180	1220
3	220	1340
4	140	545
5 (+ α-amanitin)	ND[c]	0

[a]Rows 1–4 represent experiments where macrophages were either left untreated or were stimulated with 1 μM retinoic acid for 5 h (rows 1–3) or for 3 h (row 4). Row 5 represents an experiment where nuclei from retinoic acid treated cells were allowed to transcribe in the presence of α-amanitin, an inhibitor of RNA polymerase II.
[b]Units are laser densitometric scan units.
[c]Not determined.

pared to the untreated macrophage. The magnitude of the induction varied from 4- to 12-fold. To ensure that the transcriptional activity of the nuclei was due to RNA polymerase II, we added α-amanitin (1 μg/ml) to nuclei isolated from cells treated with retinoic acid (row 5). This completely abolished the transcription of the tissue transglutaminase gene.

DISCUSSION
Retinoid Regulation of Tissue Transglutaminase Gene Expression

We have been interested in elucidating the molecular mechanism of retinoid action. These molecules are able to stop proliferation and to induce differentiation in a number of cell lines, primarily by exerting profound changes in the expression of still unidentified genes. One approach towards understanding the basis of retinoid action consists in characterizing the molecular events essential for the retinoid regulation of gene expression.

Our studies began with the observation that activated macrophages contain high levels of tissue transglutaminase [9]. This enzyme belongs to a group of enzymes that cross-link proteins [21,22]. We found that the treatment of mouse resident macrophages with retinoic acid resulted in a pronounced induction of tissue transglutaminase levels [10]. We therefore attempted to define the mechanism responsible for this increase, since it could provide a model for understanding the action of retinoids. Our results indicate that the retinoid induction of tissue transglutaminase is caused by an accumulation of tissue transglutaminase mRNA levels and that the action of retinoic acid in these cells is not mediated through an increase in the stability of the tissue transglutaminase mRNA. Rather, the results presented in this manuscript indicate that this induction is caused by an increase in the relative transcription rate of the tissue transglutaminase gene.

Role of RBP in Retinoid Action

The isolation of tissue transglutaminase cDNA probes has allowed us to evaluate the earliest features of the response of macrophages to retinoic acid. One of these

features is the role of the serum retinol binding protein (sRBP) in retinoic acid induced gene expression in mouse macrophages. Previous work from our laboratory had indicated that the sensitivity of macrophages to low concentrations of retinoic acid (1–100 nM) was greatly increased by reconstitution in medium containing delipidized serum. We showed that this effect could be negated by procedures that removed sRBP from the serum and that it could be reproduced with purified sRBP in serum-free medium [10]. These observations suggested that the macrophage responded preferentially to retinoic acid bound to sRBP rather than to free retinoic acid. However, these experiments depended on the induction of tissue transglutaminase activity, an end-point that took several hours to achieve in cells treated with low levels of retinoic acid. We have now reevaluated these observations using the sensitive hybridization assay for the induction of tissue transglutaminase mRNA. At very early times there was no difference between the ability of low concentrations of free retinoic acid (10 nM) and protein-bound retinoic acid to induce tissue transglutaminase gene expression in macrophages (Fig. 4). These results argue against our previous suggestion that association with sRBP might be an obligatory component of retinoic acid action in macrophages. Rather, they suggest that free retinoids can diffuse into cells and bring about the activation of gene expression directly.

There was no evidence of a response to free retinoic acid after 7.5 h of retinoic acid treatment of macrophages. Nevertheless, both the time-course experiments and the Northern blot of Figure 4 reveal that retinoic acid delivered in serum-containing medium was effective in inducing tissue transglutaminase gene expression. Although there are many possible explanations for these observations, we favor the possibility that they are linked to the stability of the retinoid in culture. There is abundant evidence in the literature that cells can rapidly oxidize retinoids and that retinoic acid itself undergoes non-enzymatic oxidation in aqueous media [23,24]. We believe that low levels of free retinoic acid are able to induce tissue transglutaminase gene expression in macrophages only transiently because free retinoic acid is rapidly destroyed. If the retinoid is presented to the cells in medium containing DLMS, where it is tightly bound to sRBP,[1] it is protected from degradation and it is capable of causing sustained induction of tissue transglutaminase. Thus, even though the binding of retinoic acid to sRBP promotes its activity in cultured macrophages, we believe that free retinoic acid is the proximal mediator of retinoid action in these cells.

A Model of Retinoid Action

The regulation of tissue transglutaminase gene expression by retinoic acid can provide us with a model for understanding broader questions related to retinoid control of cellular growth and differentiation (Fig. 6). On the basis of our findings, we believe that retinoic acid is the primary effector molecule in macrophages. Retinoic acid may be delivered directly to the cell from the serum or it may be generated within the cell by the oxidation of retinol [24]. Little is known of the relative contribution of these two pathways to retinoic action in vivo. In certain cells, particularly F9 teratocarcinoma cells, it has been shown that the association of retinoic acid with a specific intracellular retinoic acid binding protein (cRABP) is critical for

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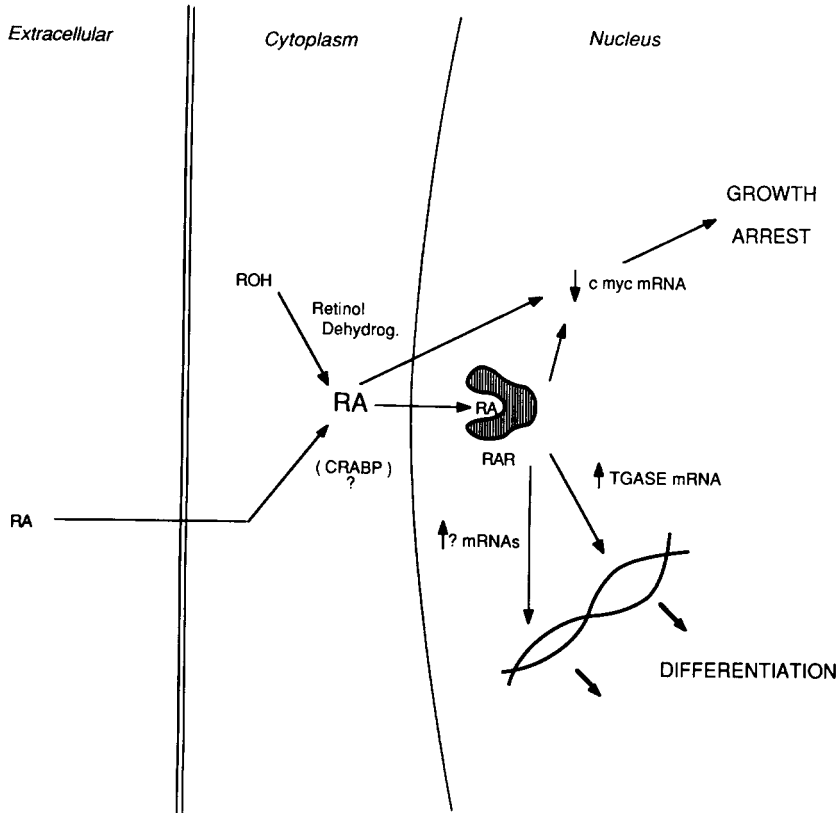
[1]P.J.A. Davies, unpublished observation.

Fig. 6. A model for retinoid effects on tissue transglutaminase, differentiation, and growth arrest.

retinoid action. Chytil and his colleagues have shown that this protein facilitates the delivery of retinoic acid to the nucleus [25–27]. However, no cRABP has been detected in macrophages[2] or HL60 cells [28,29], making it unlikely that this protein plays a critical role in retinoid action in these cells. Nevertheless, we believe that retinoic acid does interact with a retinoid receptor protein, leading to the transcriptional activation of specific retinoic acid-regulated genes. While the identity of the retinoic acid receptor in macrophages is still unknown, it is likely to be identical (or related) to the retinoic acid receptor that has been recently isolated [30,31].

Another effect of retinoids is to suppress c-myc mRNA [32], primarily through some type of post-transcriptional regulation [33,34]. Considerable evidence links the expression of the proto-oncogene c-myc to cellular proliferation [35,36]. The challenge now is to determine how the retinoid-induced changes in the expression of c-myc, tissue transglutaminase, and perhaps other mRNAs, lead to a cessation of cellular growth and to the appearance of a differentiated phenotype. The identification and characterization of other retinoid-regulated genes should help to explain how

[2]J. Johnson and P.J.A. Davies, unpublished experiments.

retinoic acid control of the expression of specific genes is integrated into a general program of antiproliferation and differentiation.

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