S-16-3 Retinoid Status and RARs Expression

S.KATO, H.MANO, R.KOJIMA, Y.YOSHIZAWA, and S.MASUSHIGE

Department of Agricultural Chemistry, Tokyo University of Agriculture, 1-1-1, Sakuragaoka, Setagayaku, Tokyo 156 (Japan)

I. INTRODUCTION

Vitamin A (retinol) and its derivative retinoic acid are well known to have profound effects on a wide range of biological functions such as vision, reproduction, growth and cellular differentiation in adult animals [1]. Moreover, recent studies have shown that retinoic acid is profoundly involved in the vertebrate development and embryogenesis [2].

The discovery of three nuclear retinoic acid receptors (RARα, RARβ and RARγ) has provided an understanding of underlying mechanisms of retinoid transduction [3,4]. As RARs activate the transcription of retinoid-inducible genes as ligand-inducible transcription factors like other members of the nuclear steroid/thyroid receptor superfamily, the RAR-mediated gene expression can at least account for some parts of the retinoid action.

Therefore, the expression of RAR genes themselves has a central role on the target specificity of the tissues in adult animals and embryogenesis. In the latter case, it has already been shown that RARs differ in spatial and temporal patterns of gene expression [5]. In intact animals, although it has been described that the expression of the cellular retinoid binding proteins is tissue-specific and some of them are dependent on retinoid ligand status, there has been no report investigating the influence of retinoid ligand status on RAR expression.

As a first step to know a factor regulating the expression of RAR genes, in this study we investigated the levels of RARα, β and γ mRNAs in the various tissues of retinoid-deficient, retinoid-repleted rats as well as normal animals given excess retinoids.

II. DIFFERENTIAL EXPRESSION OF THE α, β AND γ RAR mRNA IN TISSUES OF RAT.

Using [32P]-labeled mouse RARα, RARβ and RARγ cDNA probes, Northern blot analysis of total RNA or poly(A)+ mRNA extracted from rat tissues was carried out under stringent conditions. As shown in Fig. 1A three receptor genes differentially expressed in various rat tissues. The RARα mRNAs were ubiquitously expressed with two distinct transcripts of 2.8 and 3.8Kb in all tissues examined. In contrast, the transcripts for RARβ (3.1 and 3.6Kb) and RARγ (3.3Kb) were tissue-specific. These results suggest that each RAR may have distinct roles on the retinoid action.
Vitamin A deficiency causes the decrease of the $\text{RAR}\beta$ mRNA.

To investigate whether the $\text{RAR}$ gene expression is affected by retinoid status in whole animals, the levels of $\text{RAR}$ transcripts were investigated by Northern blot analysis of $\text{RAR}$s extracted from various tissues of rats fed with normal or retinol-deficient diets for various periods. Depletion of retinoid in rats was achieved by feeding a retinol-deficient diet, and retinol deficiency was judged by measuring the serum and hepatic retinol content with HPLC.

After 35 days, the level of $\text{RAR}\beta$ was clearly reduced in various tissues of rats in a retinol-deficient state (compare the lanes "C" (control) and "D" (deficiency) in Fig. 1). The significant reduction of $\text{RAR}\beta$ mRNA levels in various tissues was confirmed by the densitometric analysis of autoradiographs (Fig. 1B). In contrast, the levels of $\text{RAR}\alpha$ and $\text{RAR}\gamma$ mRNAs were not affected by retinol-deficiency.

To determine the onset of retinol-deficiency-induced reduction of $\text{RAR}\beta$ transcripts, rats fed experimental diets were killed every 10 days up to 30 days and the $\text{RAR}\beta$ mRNAs and the serum retinol were analysed. After 10 days, the transcripts for $\text{RAR}\beta$ gene in lung were already decreased (30% decrease when compared to day 0), though serum retinol was not significantly reduced. When the serum retinol decreased to 50% of control level on days 20, $\text{RAR}\beta$ transcripts were completely abolished. Thus, it is most likely that the maintenance of the normal level of $\text{RAR}\beta$ mRNAs may require a precise threshold of serum retinol concentrations (approximately 25 $\mu$g/dl).

IV. Administration of retinol and retinoic acid restored the reduced level of $\text{RAR}\beta$ mRNAs caused by vitamin A deficiency.

To rule out the possibility that the decrease of $\text{RAR}\beta$ mRNA might be due to an irreversible malfunction suffered from retinol deficiency, a recover test was performed.
Administration of retinoic acid to retinol-deficient rats rapidly restored the level of RARβ mRNA in various tissues within 4hr. Retinol also induced a transient RARβ gene expression, but in limited tissues. Moreover, the magnitude of the induction was less and the kinetics differed from retinoic acid.

To investigate at which level the induction of RARβ by retinol or retinoic acid is achieved, specific inhibitors of transcription (actinomycin D) or translation (cycloheximide) were injected 1hr before administration of retinoids. Actinomycin D completely blocked the 4hr-induction of RARβ gene expression by retinoids, whereas cycloheximide did not affect the induction. Thus, the induction of RARβ gene expression by retinoid may occur at the transcriptional level without de novo protein synthesis.

V. RETINOIC ACID INDUCES THE OVEREXPRESSION OF RARβ GENE.

From the replenishment study, we addressed the question of whether retinoic acid can enhance the level of RARβ transcripts in the tissues of rats in normal retinoid status. For this purpose, 1mg of retinoic acid and retinol was given intragastrically to normal rats. As a result, we found that only the RARβ transcripts were significantly overexpressed when compared to normal rats (Fig. 2A and 2B).

Thus, the present results clearly demonstrated the existence of autoregulation of RARβ gene in intact animals.

VI. THE INFLUENCE OF RETINOID STATUS ON RAR ISOFORMS.

More recently, each subtype of RAR gene has been shown to transcribe a group of different mRNAs, designated as RAR isoforms [6]. The analysis of the gene structure suggested that the gene expression of the isoforms is controlled by different promoters. To get a better comprehension of retinoid transduction, currently we are investigating how the retinoid ligand status and synthetic retinoids affect the levels of each RAR isoform, as well as retinoid binding protein mRNAs.
From these results, we clearly demonstrated that the retinoid ligand status affects the RARβ mRNA level in intact animals. The altered levels of RARβ by hypo- and hyper-vitaminosis A may affect retinoid-inducible gene expression, thereby affecting a wide range of retinoid action.

REFERENCES