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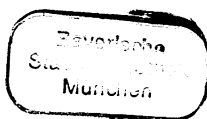
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Transcriptional regulation of the tyrosine amino-transferase gene : structure of a regulatory switch

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INTRODUCTION

To understand the differentiation processes which yield the various cellular phenotypes, it is necessary to elucidate mechanisms by which genes are selectively expressed. Of particular importance for the establishment of a given pattern of gene activity is the interplay between controlling genes and signaling molecules such as hormones. Furthermore, it seems clear that tissue-specific patterns of gene activity depend on both positive and negative regulatory factors. The tyrosine aminotransferase (TAT) gene is an example of a gene whose expression is controlled by positive and negative factors and by hormones. TAT expression is regulated not only by glucocorticoids and by the peptide hormone glucagon acting via the cAMP pathway, but also by two genetically defined, trans-acting loci (Fig. 1).

First, a dominant negative regulator, termed tissue-specific extinguisher (*Tse-1*), has been defined by intertypic somatic cell hybrids and mapped to a small region of human chromosome 17 (Killary and Fournier, 1984; Lem et al. 1989).

Second, a positive regulator has been postulated to account for the lethal phenotype of certain albino mice carrying a homozygous deletion around the albino locus on chromosome 7. TAT as well as several other liver-specific enzymes are not expressed in the liver of these newborn albino mice (Gluecksohn-Waelsch, 1979). Since the structural gene for TAT is not deleted, and has been mapped to a chromosome other than 7, it has been suggested that the deletion at the albino locus removes a regulatory locus (Schmid et al., 1985).

Previously, we have shown that glucocorticoid responsiveness of TAT expression is conferred by a conditional enhancer 2500 bp upstream of the transcription start site (Jantzen et al., 1987). Here we show that a liver-specific enhancer even further upstream of the TAT promoter, mediates the effect of the dominant negative regulator *Tse-1* as well as responsiveness to cAMP. This enhancer has structural and functional characteristics of a signal transducer and we suggest that it works as a regulatory switch which controls the hormone triggered timed onset of expression of the TAT gene in the newborn liver.

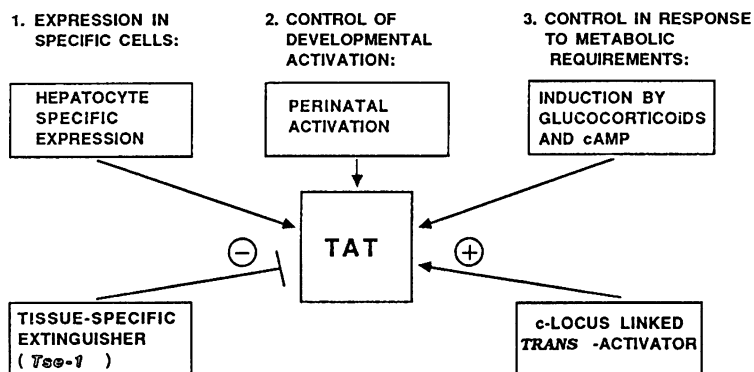


Fig. 1 Control of TAT gene expression

RESULTS AND DISCUSSION

To identify *cis*-acting sequences mediating the complex transcriptional regulation of the TAT gene, we fused the TAT promoter including different portions of its 5'-flanking region to the universal reporter gene chloramphenicol acetyltransferase (CAT) and transiently transfected these constructs into various cell lines. First, we found that a sequence 3600 bp upstream of the transcription start site strongly activated CAT expression in well differentiated rat hepatoma cells (FT02B) but not in rat fibroblasts. A series of 5'- and 3'-deletion mutants defined a sequence of 80 bp absolutely essential for transcriptional stimulation. This sequence conferred hepatoma-specific activation to the heterologous TK promoter. The same sequence also mediated response to hormonal signals transduced via the cAMP pathway. We also investigated whether this sequence was the target for dominant negative regulation by the product of the tissue-specific extinguisher (*Tse-1*) locus. To this end we transfected the same constructs used to define the liver-specific enhancer into a hepatoma microcell hybrid line (Lem et al., 1989) which contains a small segment of human fibroblast chromosome 17, carrying and expressing the *Tse-1* locus. The enhancer was inactive in this cell line. However, induction with cAMP was able to overcome extinction completely, thus revealing a functional antagonism between *Tse-1* and the signal transduction pathway.

As a first step towards understanding the mechanism of extinction of enhancer function by *Tse-1*, we sought to identify the specific sequences required for liver-specific enhancer activity, for induction by cAMP and for extinction by *Tse-1*. A complete set of clustered point mutants covering the enhancer was constructed and transfected into rat hepatoma cells. Within the 80 bp minimal enhancer fragment two short DNA sequences were found, each of which was absolutely essential for enhancer function. Each of the two sequences was inactive on its own, if placed in front of the heterologous TK promoter. However, multiple copies of these sequences conferred strong transcriptional stimulation onto the TK promoter.

A multimer of the distal 26 bp sequence was responsive to cAMP and was subject to negative regulation by *Tse-1*. Again the functional antagonism between *Tse-1* and the cAMP-pathway was observed; cAMP induction completely reversed the

negative regulation by Tse-1. In vivo footprinting revealed characteristic changes in DMS-reactivity at this sequence element which correlated with (i) cAMP induction, (ii) the presence of the Tse-1 carrying chromosome fragment, and (iii) the relief from extinction by cAMP.

A multimer of the proximal 18 bp element behaved as a liver cell specific activator of transcription. In the natural context of the enhancer the distal cAMP- and Tse-1-responsive element must cooperate with the proximal liver-specific element to overcome the requirement for multimerisation. Artificial combination of the 26 bp- and 18 bp-sequences created an element with all the regulatory properties of the entire TAT enhancer.

Thus, the structure of the enhancer exemplifies the concept of the modular architecture of regulatory elements: a cAMP responsive module and a liver-specific module must cooperate to make up a functional unit with regulatory properties unique to the combination of both. The strict cell-type specificity of this combination is guaranteed by a double control and by the absence of redundancy: both modules are absolutely essential and both are inactive in fibroblasts. In the case of the cAMP-responsive element, which might be recognized by an ubiquitous factor, Tse-1 exerts negative control in fibroblasts.

As the balance between Tse-1 activity and cAMP induction is critical for activity of one component which is synergistically interacting with a second component, the TAT enhancer must have the properties of a sensitive switch responding to changes in the Tse-1/cAMP balance with dramatic changes in overall activity. Therefore, one attractive model would give Tse-1 a central role in prenatal repression of TAT. Activity of Tse-1 in the liver would decrease during liver development rendering the repressed TAT gene increasingly responsive to hormonal stimulation towards the end of gestation. The timed onset of TAT expression around birth would then be triggered by the strong release of gluconeogenic hormones as a consequence of neonatal hypoglycemia. In fact, TAT can be induced prematurely, days before birth by administration in utero of glucagon (acting via cAMP) (Greengard, 1970).

REFERENCES

- Gluecksohn-Waelsch, S. (1979): Genetic control of morphogenetic and biochemical differentiation: lethal albino deletions in the mouse. *Cell* 16, 225-237.
- Greengard, O. (1970): The developmental activation of enzymes in rat liver. In *Mechanisms of Hormone Action*, Vol. I, G. Litwack, ed. (New York: Academic Press), pp. 53-85.
- Jantzen, H.M., Strähle, U., Gloss, B., Stewart, F., Schmid, W., Boshart, M., Miksicek, R., Schütz, G. (1987): Cooperativity of glucocorticoid response elements located far upstream of the tyrosine aminotransferase gene. *Cell* 49, 29-38.
- Killary, A.M. and Fournier, R.E.K. (1984): A genetic analysis of extinction: trans-dominant loci regulate expression of liver-specific traits in hepatoma hybrid cells. *Cell* 38, 523-534.
- Lem, J., Chin, A.C., Thayer, M.J., Leach, R.J. and Fournier, R.E.K. (1989): Coordinate regulation of two genes encoding gluconeogenic enzymes by the trans-dominant locus Tse-1. *Proc. Natl. Acad. Sci. USA* 85, 7302-7306.
- Schmid, W., Müller, G., Schütz, G. and Gluecksohn-Waelsch, S. (1985): Deletions near the albino locus on chromosome 7 of the mouse affect the level of tyrosine aminotransferase mRNA. *Proc. Natl. Acad. Sci. USA* 82, 2866-2869.