2.3 The Role of cAMP-Dependent Signal Transduction for Cell-type-specific Gene Expression

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Differentiation and development can be described as patterns of gene expression defined in time and space. Differential gene expression largely results from regulation of transcription. Multiple regulatory processes are initiated and coordinated by extracellular signals. In multicellular organisms, signals mediated by cell-cell contacts play an important role. Furthermore, hormonal signals coordinate differentiation processes at the level of the organism. A close relation between signal transduction and cell-type-specific transcriptional control is exemplified by the tyrosine aminotransferase (TAT) gene which is expressed exclusively in liver cells and which is switched on briefly after birth. This switch is part of a terminal differentiation process by which the liver acquires gluconeogenic capacity as a response to specific metabolic requirements of the newborn organism.

The identification of cell-type-specific regulatory elements (promoters and enhancers) in a large number of genes, and cloning of cell type-specific transcriptions factors which act via these elements were prerequisites to understand cell-type-specific transcriptional control. Combination of different transcription factor binding sites in one regulatory element broadens the repertoire of regulatory specificities by synergistic interactions. Several enhancers and the promoter of a gene cooperate and determine the initiation frequency of RNA polymerase and thereby determine the transcription rate of the gene.

The role of trans-acting factors for control of cell-type-specific gene expression had been postulated in the 1960s and 1970s based on genetic experiments with somatic cell hybrids. In hybrid cells generated by fusion of different cell types the expression of cell-type-specific gene products of both fusion partners is usually repressed. This phenomenon termed "extinction" is best characterized in hepatoma X fibroblast hybrids. Killary and Fournier had shown that extinction of liver-specific genes in these hybrids has a specific genetic basis. They have identified a genetic locus on human fibroblast chromosome 17, the tissue-specific extinguisher 1 (TSE1) which repressed the expression of tyrosine aminotransferase (TAT) in hepatoma cells. The TSE1 locus maps to a chromosome distinct from the one carrying the TAT structural gene demonstrating regulation in trans. TSE1 specifically represses a group of liver genes including the TAT gene. Nothing

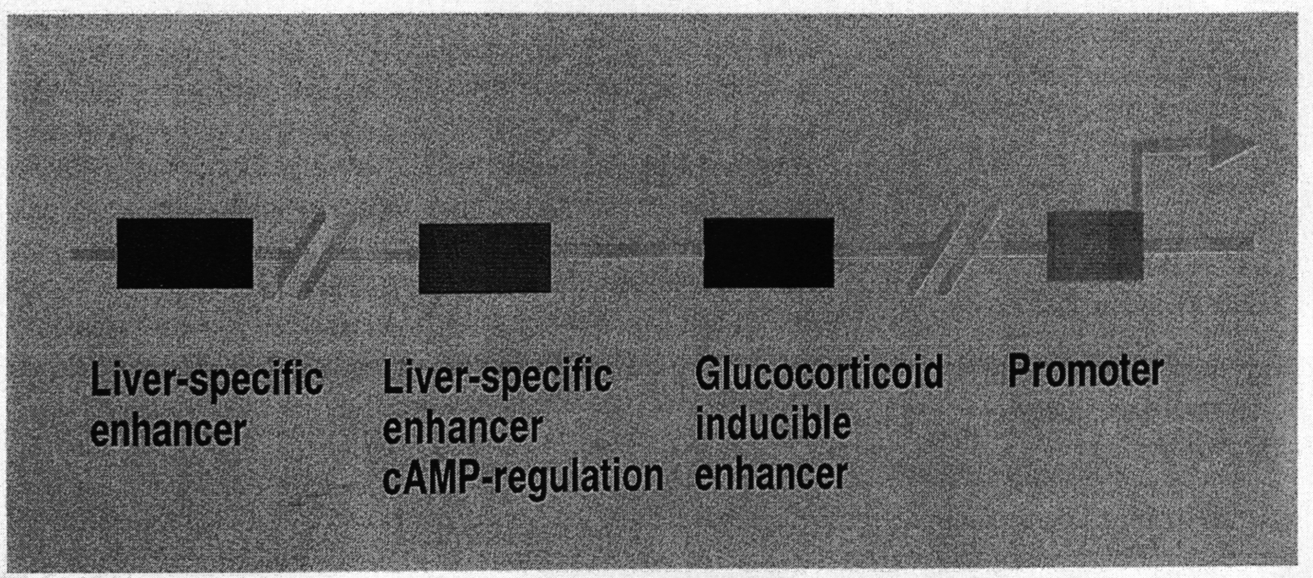


Fig. 18
Shown are the regulatory elements characterized so far, which control the transcription of the tyrosine aminotransferase (TAT) gene. The TAT gene may be induced either by glycocorticoids or by cyclic adenosine monophosphate (cAMP), which activates the protein kinase A. All distances are given in kilobases (kb) and refer to the starting point of the TAT gene transcription (arrow).

was known about the mechanisms of action of extinguisher loci when we started this project. As extinguishers are defined by genetic experiments, it was unknown whether they interact directly with DNA as repressors or whether they would indicate novel mechanisms of cell type-specific control.

The TAT gene is particularly suitable as a model to analyze the role of hormones in cell type-specific gene expression, since it is expressed exclusively in liver cells, is turned on at a defined timepoint during development, and is regulated by two signal transduction pathways. As other key enzymes of the gluconeogenesis pathway, the TAT gene is inducible by glucocorticoids as well as by glucagon acting via the second messenger cAMP. For induction via the cAMP pathway the catalytic subunit of protein kinase A is essential.

The TAT gene is only activated shortly after birth. The transcription rate is maximal 8–12 hours after birth, decreases slightly within 1–2 days, and increases again in adult liver. The postnatal hypoglycemia and the resulting release of glucagon and glucocorticoids seem to play a role for activation of the gene. Therefore, the TAT gene is suitable as a model to analyze the role of signal transduction pathways for developmental-specific transcriptional control.

Control Elements for Cell Type-Specific and cAMP-Dependent Expression of the Tyrosine Aminotransferase (TAT) Gene

The different regulatory elements which control transcription of the TAT gene are located within 10 kb upstream of the transcription start site. Specific regulatory functions could be assigned to individual elements (Fig. 18). To identify regulatory elements, parts of the TAT upstream sequence were placed in front of an indicator gene whose product can easily be determined and the transcriptional activity of these constructs was measured after gene transfer into several cell lines.

We could identify three enhancers which are important for expression of the TAT gene and localized them 11 kb, 3.6 kb, and 2.5 kb upstream of the transcription start site. The elements at –11 and –3.6 are liver-specific enhancers, the latter mediating cAMP induction in addition. The region at –2.5 kb is crucial for glucocorticoid response.

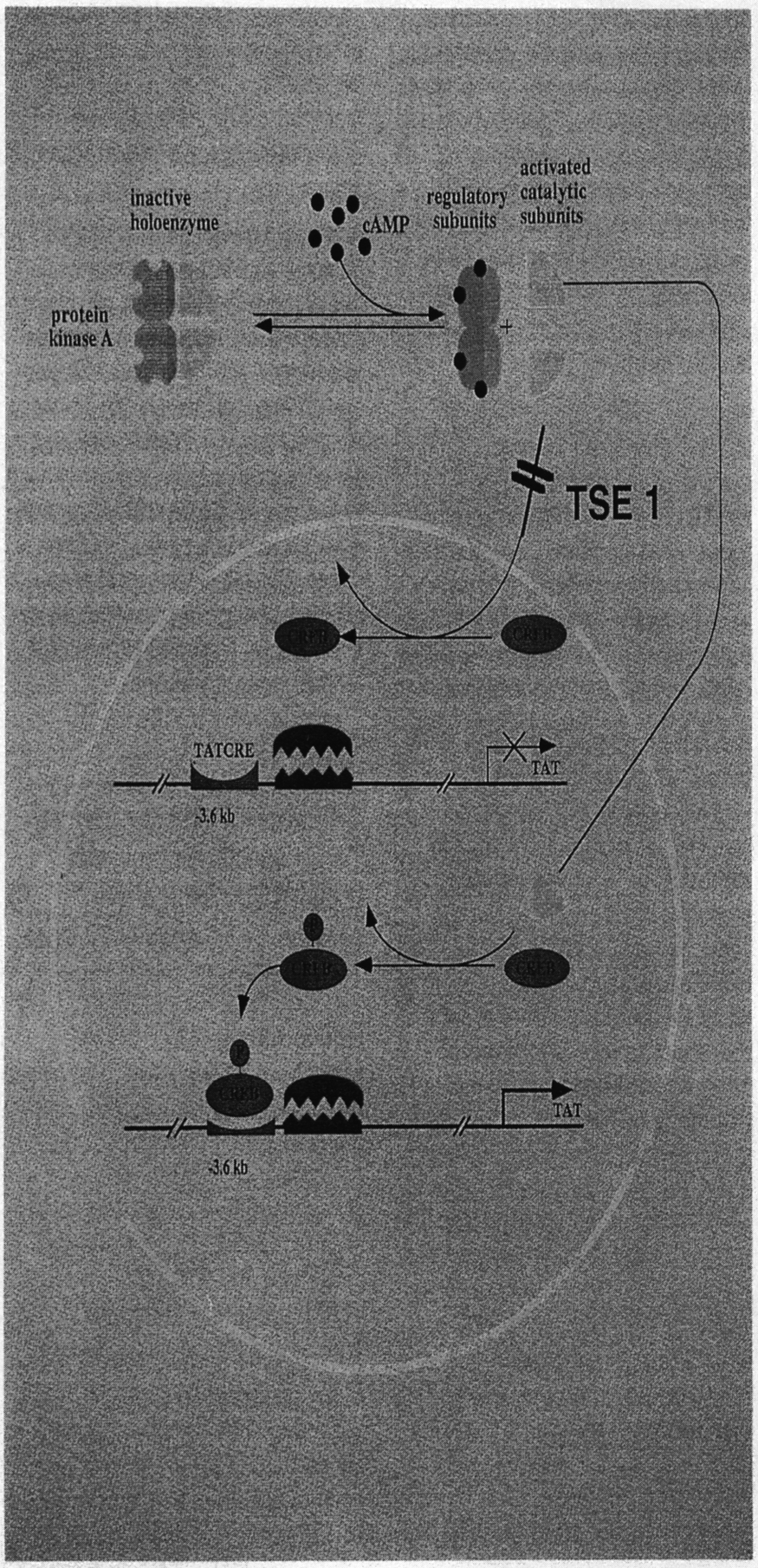
A detailed functional analysis by gene transfer experiments of the enhancer at –3.6 kb upstream of the transcription start site has defined a minimal fragment of 80 bp which mediates all properties of the enhancer. Using a series of point mutations, we found that two short sequence motifs within these 80 bp are absolutely essential, since their mutation leads to total loss of function of the enhancer. The mutual dependence of these elements indicates that they cooperate. The sequence of one of these elements is similar to cAMP-regulated elements (CREs) in other cAMP-inducible genes and mediates a strong response to increased intracellular concentrations of cAMP in highly differentiated hepatoma cells. The second essential element of the enhancer is constitutive and liver-specific and its activity correlates with the degree of differentiation of the respective hepatoma cell line. The high degree of specificity of the enhancer –3.6 kb therefore relies on cooperation of two essential elements with overlaping specificity. Therefore, the activity is restricted to highly differentiated hepatoma cells and in vivo to adult hepatocytes.

Analysis of the enhancer at –3.6 kb demonstrates that the modular structure of regulatory elements provides a safe and economical way to generate specificities. A large number of regulatory patterns can be generated by combinatorial use of a much smaller number of regulatory proteins which interact synergistically. Cooperation of several essential elements allows regulation of the activity of the enhancer over several orders of magnitude in response to much smaller changes in activity of individual regulatory factors.

Cell-specific Repression by the Tissue-specific Extinguisher Locus TSE1: Antagonism between TSE1 and cAMP Signal Transduction

Genetic analysis of extinction in somatic cell hybrids allows identification of trans-acting factors which are most likely part of mechanisms which usually control cell type-specific gene expression. Therefore, we analyzed the mechanism of action of the TSE1 locus to understand the control of cell-type-specific transcription in the liver.

A first hint to the mechanism by which TSE1 represses TAT transcription in somatic cell hybrids came from the identification of a group of liver-specific genes which are coordinately regulated by TSE1. All seven genes are expressed in hepatoma cells and show a



dramatically reduced repression in microcell hybrids generated by fusion of a hepatoma cell with a fragment of human chromosome 17 derived from a fibroblast which carries the TSE1 locus. We found that all TSE1 regulated genes are inducible by cAMP and that in microcell hybrids, cAMP is able to antagonize TSE1-mediated repression.

a) The Mechanism of TSE1-Mediated Extinction

By gene transfer experiments into the aforementioned microcell hybrid cell lines we could show that the cell type-specific enhancer at –3.6 kb mediates repression by TSE1. This repression of the enhancer by TSE1 can be reversed by cAMP. We could show that the cAMP-regulated element (CRE) is required and is solely sufficient for repression of the enhancer by TSE1. The functional antagonism between TSE1 and cAMP is well explained, since the CRE is the target of TSE1.

Furthermore, we found that the repression of TAT gene transcription by TSE1 results from inhibition of the cAMP/ protein kinase A signal transduction path-

Fig. 19
Suppression mechanism of the TAT transcription by the cell-specific extinction factor 1 (TSE1). This increase of cAMP concentration in the cell causes an activation of the protein kinase A. The activated subunit of the enzyme then changes the transcription factor CREB so that it can bind to the cAMP-regulated region of the TAT gene (TATCRE). CREB now causes, together with a cell-specific regulator, the transcription of the TAT gene. In contrast, TSE1 suppresses the gene expression by inhibiting the activated subunit of the protein kinase A.

way. This explains why all TSE1-regulated genes are inducible by cAMP. Since the intracellular cAMP concentration is not affected by TSE1, protein kinase A or one of its substrates should be the target for TSE1. Indeed, we could find a reduction of the basal protein kinase A activity in cells in which TSE1 is active.

b) The TSE1 Gene Product

The latter observation led to a systematic search for a protein kinase A inhibiting activity which might be identical with the TSE1 gene product. The holoenzyme of protein kinase A is a tetramer of two regulatory and two catalytic subunits which are inactive in absence of cAMP. Binding of two cAMP molecules to each regulatory subunit leads to a conformational change and subsequent release and activation of the catalytic subunit. One regulatory subunit gene (RIa) of PKA colocalizes with TSE1 on human chromosome 17. Furthermore, the expression of RIa mRNA perfectly correlates with the TSE1 phenotype in different cell lines and hybrids. High- resolution mapping of RIa and TSE1 on chromosome 17 revealed colocalization on a small segment of the chromosome within less than 500 kb. These data suggested that TSE1 and RIa were identical.

For proof of identity, an expression vector encoding the regulatory subunit was stably introduced into hepatoma cells and the resulting phenotype was analyzed. Increasing expression of the regulatory subunit led to inverse proportional changes of basal protein kinase A activity, binding of CRE binding protein to its cognate element, and finally, expression of the TAT gene. Expression of the regulatory subunit leads to a perfect phenocopy of the TSE1 phenotype. Thus, we could identify the regulatory subunit RIa as the product of the TSE1 locus. This is the first molecular characterization of an extinguisher factor. Figure 19 illustrates the action of TSE1 on TAT gene expression.

Long before cell-type-specific transcription factors had been identified, the analysis of extinction in somatic cell hybrids has already shaped the concept that cell-type-specific gene expression is controlled in trans. TSE1 is the first example of such a genetically defined factor whose mechanism of action has been elucidated. Since regulatory mutants cannot easily be obtained in mammalian cells, somatic cell hybrids are valuable tools to analyze cell-type-specific gene expression.

Perinatal Activation of the TAT Gene

A number of independent observations concerning the increase of TAT expression within the first hours after birth can be combined into a mechanistic concept with our knowledge of the role of cAMP signal transduction for cell-type-specific control of TAT expression: a) The raise in TAT transcription parallels a raise of gluconeogenic hormones due to the postnatal hypoclycemia. b) In utero administration of cAMP leads to premature activation of fetal TAT expression. c) TSE1 activity and RIa mRNA are high in hepatoma cells which express fetal liver markers, but are low in adult hepatocytes.

The model derived from these observations postulates a dominant role of glucagon and other gluconeogenic hormones acting via the cAMP pathway for the properly timed activation of the TAT gene. RIa/TSE1 could repress basal TAT expression in fetal liver. Activation by cAMP shortly after birth is mediated by the cell-type-specific enhancer at –3.6 kb and is mechanistically equivalent to reversal of extinction by cAMP in hepatoma microcell hybrids. In any case, perinatal activation seems to be due to activation of protein kinase A and to be mediated by the cAMP response element (CRE) of the TAT gene.

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