# Differential gene expression of a human α2,3-sialyltransferase in leukaemic cell lines and leucocytes

Uta Gassen[1], Sørge Kelm, Roland Schauer*

*Biochemisches Institut, Christian-Albrechts-Universität Kiel, Otto-Hahn-Platz 9, 24098 Kiel, Germany*

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**Abstract The gene expression of the human Galβ1,4(3)GlcNAc/Galβ1,3GalNAcα-2,3-sialyltransferase was investigated in the leukaemic cell lines HL60, K-562, MOLT-4, THP-1 and in blood leucocytes. Five different transcripts were identified. In HL60 and THP-1 cells the expression levels of two of these changed during differentiation. Two potential AP1 binding sites were detected in the promoter regions of the gene. THP-1 cells contain proteins binding with higher affinities to these sequences in the sialyltransferase gene than to the AP1 consensus sequence, whereas nuclear extracts from HL60 cells have the opposite affinity.**



*Key words:* Sialyltransferase; Differential gene expression; Leukemic cell line; AP1

## 1. Introduction

The sialyltransferase family consists of more than 15 enzymes with the common function of catalysing the transfer of sialic acid from CMP-sialic acid to oligosaccharide chains of glycolipids and glycoproteins [1,2]. Sialic acids function as key determinants of oligosaccharide structures which mediate a variety of biological phenomena [3,4]. With their negative charge sialic acids mediate the binding of selectins [5,6]. A more specific function is fulfilled in the mediation of cellular interactions by CD22, CD33, MAG and sialoadhesin [6,7].

The different sialyltransferases can be classified enzymatically by their acceptor substrate specificities [1]. As one of the cloned α2,3-sialyltransferases [8–13] the human ST3Gal IV is able to transfer sialic acids to Galβ1,3GalNAc and Galβ1,4GlcNAc. The cDNA of this sialyltransferase has been cloned from the human melanoma cell line WM266-4 [12] and human placenta [9]. The gene is distributed over 14 exons which span at least 25 kb of genomic sequence and its transcription results in five distinct mRNAs which are generated by alternative splicing and alternative promoter utilisation. The expression of different transcripts has also been reported for the ST6Gal I [13,14]. The predicted promoter region of the ST3GAl IV lacks TATA or CCAAT boxes but contains consensus sequence of other regulatory elements such as AP1 and HLH [15]. The AP1 consensus sequence is recognised by Jun and Fos proteins. The interaction between members of the Jun/Fos family generates a diversity of protein complexes which regulate the expression of various genes. Their affinities depend on the formation of homo- compared to heterodimers [16,17]. To date, only the ST6Gal I has been analysed with regard to the involvement of liver-restricted transcription factors in its expression [14].

In this study we investigated the expression of the ST3Gal IV transcripts in leucocytes and the leukaemic cell lines K-562, HL-60, THP-1 and MOLT-4. The presence and the transcription start sites of the five transcripts were determined with primer-extension analysis. Furthermore we investigated whether the identified AP1 recognition sequences in the gene of the ST3Gal IV were bound by nuclear proteins from the different cell types.

## 2. Materials and methods

### 2.1. Materials

Oligonucleotides were purchased from Eurogentec, [γ-$^{32}$P]ATP was obtained from Hartmann and CMP-[$^{14}$C]Neu5Ac from Amersham and the direct-mRNA Kit from Qiagen was used. Polymorphprep was obtained from Nycomed. SuperScript RT was ordered from Gibco BRL. Human placenta was obtained from the Universitäts-Frauenklinik Kiel.

### 2.2. Cell culture

All cells were grown at 37°C in a humidified atmosphere containing 5% $CO_2$ in RPMI 1640 supplemented with 10% FCS and 2 mM glutamine without antibiotics. Differentiation of HL-60 cells to granulocyte-like cells was induced by treatment with 1.35% DMSO and of both HL-60 and THP-1 cells to macrophages with 16 μM PMA. Cell differentiation was monitored by adhesion to culture plates, cell morphology and nuclear staining (Wright).

### 2.3. Preparation of leucocytes

The mono- and the polymorphonuclear fractions of the leucocytes were isolated from peripheral blood by density gradient centrifugation with Polymorphprep (Nycomed). The purity of the leucocyte fractions was about 95% as determined by cell staining (Wright).

### 2.4. Isolation of mRNA, primer-extension analysis and dot blots

The following oligonucleotides specific for the different transcripts, in parentheses, were used: 5′-GCT-GAG-GGC-TTG-ACC-CTG-ATT-GTA-C-3′ (A1), 5′-GAG-CTT-CCA-GCC-CAC-TGT-CCT-TCA-A-3′ (A2), 5′-AGC-GAC-CTT-GCG-ACT-CCT-GCA-CAC-A-3′ (B1), 5′-CAC-TGT-GTG-AGG-GCT-TGA-GTC-CTC-T-3′ (B2), 5′-GGG-ACT-TGC-TGA-CCA-TGT-TTC-TCA-G-3′ (B3). Up to 7 μg mRNA was isolated from $2\times10^7$ cells with Oligotex direct mRNA isolation kit from Qiagen and stored at −80°C. The primer-extension analysis was performed with 0.5 μg mRNA, radiolabelled oligonucleotides and SuperScript RT following the supplied protocol with a 30 min extension step at 37°C. The quantitative analysis of the mRNA was performed by dot blot analysis with 5′-$^{32}$P-oligonucleotides following the protocol from Qiagen.

*Corresponding author. Fax: (49) (431) 880-2238.
E-mail: schauer@biochem.uni-kiel.de

[1]Present address: The Queen's University of Belfast, School of Biology and Biochemistry, 97 Lisburn Road, Belfast BT9 7BL, UK.

*Abbreviations:* EMSA, electrophoretic mobility shift assay; PMA, 4β-phorbol-12-myristate 13-acetate; DMSO, dimethyl sulphoxide; PMSF, phenylmethylsulphonyl fluoride; RT, reverse transcriptase; ST3Gal IV, Galβ1,4(3)GlcNAc/Galβ1,3GalNAc-α2,3-sialyltransferase


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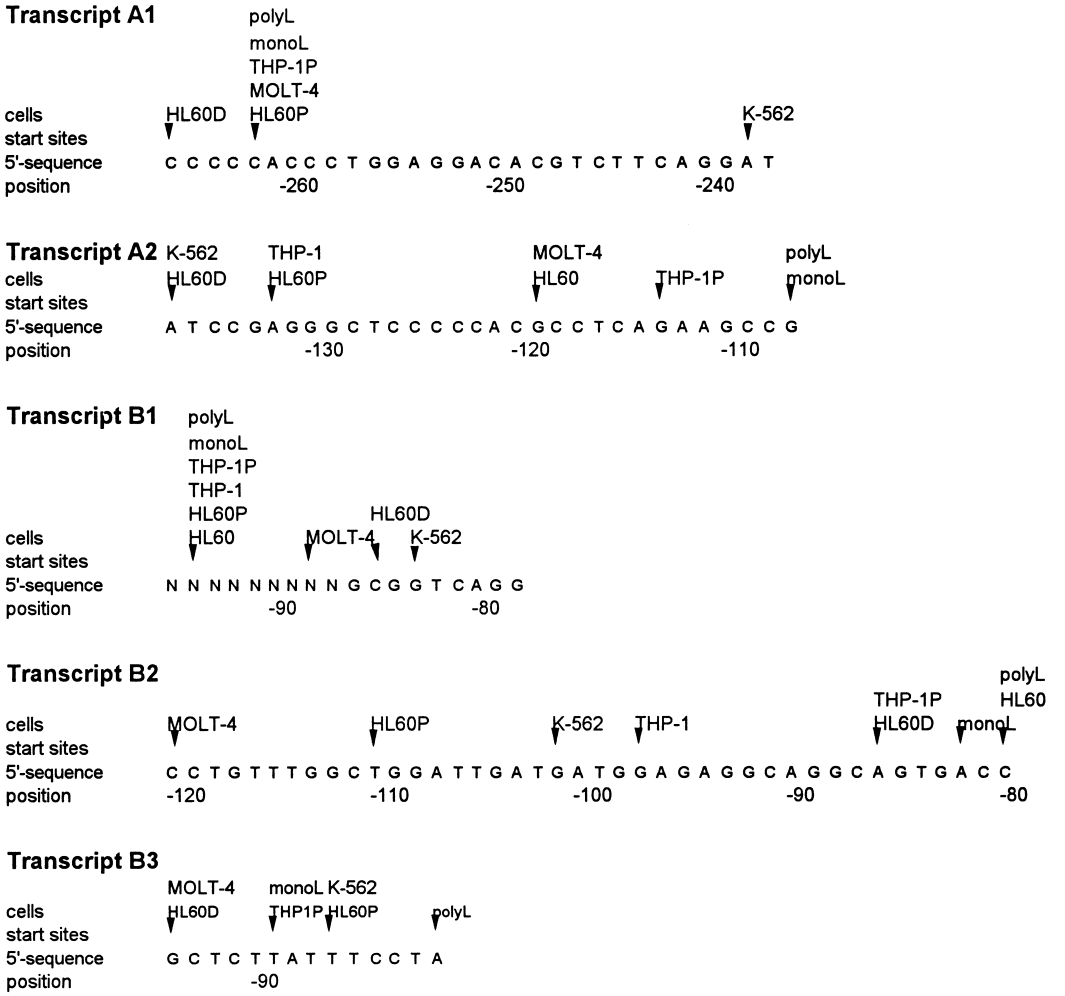


Fig. 1. Transcription start sites of the five transcripts. The transcription start sites were determined by primer-extension analysis with oligonucleotides specific for each transcript and mRNA from the cell lines and leucocytes as described in Section 2. The individual transcription start sites of the indicated transcripts found in the different cell lines are marked with arrows. The sequences of the corresponding 5′-ends are given. HL60 D stands for DMSO-treated HL60 cells, HL60 P and THP-1 P for PMA-treated HL60 and THP-1 cells, respectively. PolyL stands for polymorphnuclear leucocytes and monoL for mononuclear leucocytes.

*2.5. EMSA*

Electrophoretic mobility shift assays were performed with double-stranded oligonucleotides ($AP1_{100}$: 5′-CTA-GGC-CGA-CTG-ACT-AAT-GAT-C-3′, $AP1_{75}$: 5′-TTC-TGC-AGT-GAC-TGC-AGC-GTG-G-3′, $AP1_{68}$: 5′-CGG-ATC-TGT-GAC-CTC-GTC-CTG-T-3′). The indices represent the percentage of homology to the consensus binding sequence for transcription factor AP1 [18]. $AP1_{75}$ was found in the promoter region of B2, $AP1_{68}$ in that of A1, A2, B3. The nuclear extracts were prepared from $5\times10^8$ cells according to a described protocol [19]. Protein concentration was about 2.5 μg/μl. Binding reactions were performed in a total volume of 20 μl containing 1×binding buffer (100 mM NaCl, 4% glycerol, 10 mM Tris pH 7.5), 1 μg dI-dC, 0.4 pmol of $^{32}$P-labelled double-stranded oligonucleotide, 5–10 μg of nuclear protein. Where indicated, up to 100-fold excess of unlabelled competitor oligonucleotide was added. After 20 min incubation on ice, the mixture was loaded on a non-denaturing polyacrylamide gel which was run at 100 V in 1×TGE, dried and subjected to autoradiography for 2 h at −80°C.

*2.6. Sialyltransferase assays*

Membranes were prepared from $10^7$ sonicated cells by ultracentrifugation at 100 000×*g* for 30 min (TLA-45) and resuspended in 100 μl of 50 mM Na-cacodylate (pH 6.5, 5 mM $MnCl_2$) buffer. The assays were performed with lactose as acceptor substrate and contained 5 μl membrane suspension (140–160 μg protein, with 1% Triton X-100, 50 nM Na-cacodylate, 5 mM $MnCl_2$, 0.2 M lactose and 3 μM CMP-[$^{14}$C]Neu5Ac (10.8 Bq/pmol) in a total volume of 50 μl). Reaction mixtures were incubated for 45 min at 37°C. Assays were performed in five parallels and controls without lactose in duplicate. The radioactive sialyllactose products were purified by anion-exchange chromatography on Dowex 1×8, 200–400 mesh (phosphate form) [20] and on Dowex 1×8 (formiate form) and eluted with 1.5 M formic acid and total radioactivity was determined. Radioactive α2,3- and α2,6-sialyllactoses were separated by TLC [21] and quantified on a TLC linear analyser (Berthold).

## 3. Results

### *3.1. Analysis of the presence of the different transcripts and the transcriptional start sites*

Specific oligonucleotides for the five different transcripts A1, A2, B1, B2 and B3 were used together with freshly extracted mRNA from the cell lines HL-60, MOLT-4, K-562 and THP-1 and from peripheral blood leucocytes in a primer-extension analysis. In addition, the two cell lines HL60 and THP-1 were differentiated with DMSO and/or PMA. The existence of the five transcripts and the transcriptional start

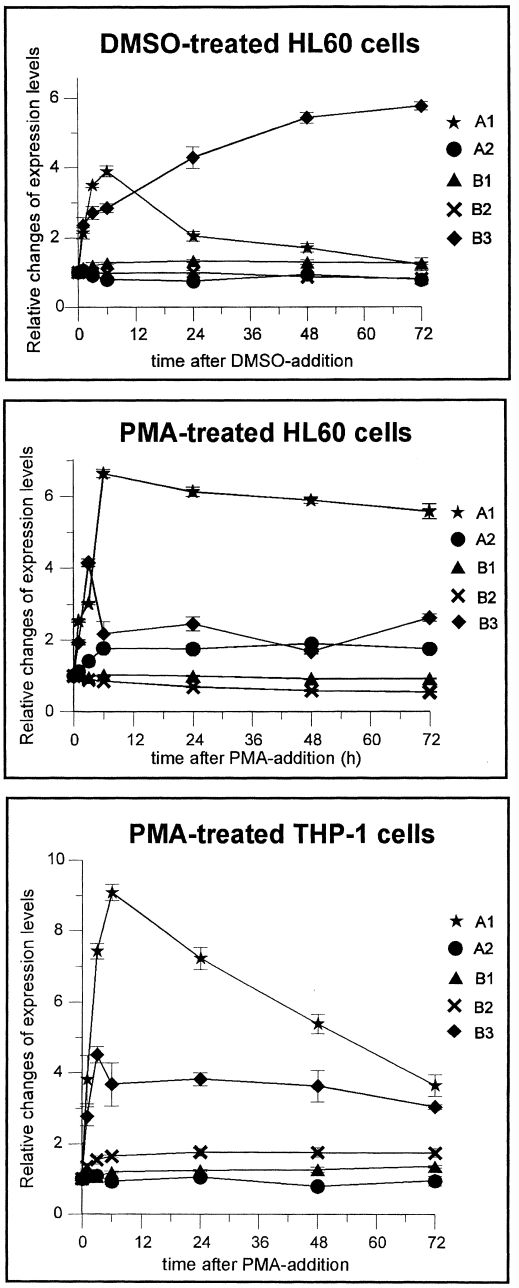


Fig. 2. Time course of transcript expression in HL60 and THP-1 cells. Poly(A)$^+$ mRNA was extracted from differentiated cells after 0–72 h of treatment with DMSO or PMA, 100 ng RNA was applied to nylon membranes, hybridised with probes specific for each transcript and bound radioactivity was quantified. The mean values of triplicates are shown as relative changes of expression levels against time.

sites were then determined by comparison of the product length with the published sequences [15]. The results are shown in Fig. 1.

The three transcripts A2, B1 and B2 are expressed in all different cell types investigated, whereas the transcripts A1 and B3 could not be detected by this method in undifferentiated HL60 and THP-1 cells. After treatment of these two cell lines with PMA or DMSO, they also expressed the transcripts A1 and B3.

The transcription does not seem to start at the same site in the different cells investigated. In Fig. 1 only the start sites at the very 5′-ends are shown.

### 3.2. Investigation on the time course of transcript expression in differentiated HL60 and THP-1 cells

In order to investigate the time course of expression, we determined the relative amounts of the five transcripts in cells harvested after different times of treatment with DMSO or PMA (Fig. 2). The transcripts A1 and B3 which could not be detected by primer extension analysis in undifferentiated HL60 and THP-1 cells appeared already after 1 h of treatment with DMSO or PMA. In DMSO-treated HL60 cells the expression of transcript A1 rapidly increased during the first 6 h of treatment and then decreased, reaching almost background level after 24 h. In contrast to this, the expression of transcript B3 continuously increased during the 72 h of the experiment. Also in HL60 cells incubated with PMA the expression of A1 increased rapidly to a 6.5-fold level within 6 h. In contrast to DMSO-treated cells, the expression was maintained at the higher level. The expression of B3 after 3 h of treatment was only transient and decreased to a 2-fold level. The expression pattern in THP-1 cells treated with PMA was similar to that in HL60 cells treated with PMA. But the expression of A1 decreased slightly faster and of B3 only to a 3.5-fold level. For the other transcripts no relevant changes during the incubation time could be observed. Only the transcript A2 showed an increased expression in PMA-treated HL60 cells.

### 3.3. Sialyltransferase activity

In order to investigate whether the changes of transcript expression levels observed after DMSO or PMA treatment influence the enzyme activity, α2,3- and α2,6-sialyltransferase activities were determined (Fig. 3). The activity of the α2,6-sialyltransferase decreased during DMSO or PMA treatment, whereas the activity of the α2,3-sialyltransferase remained at the same level in THP-1 cells or increased slightly as observed for HL60 cells. This increase in enzyme activity seems to correlate with the elevated expression of A1, A2 and B3 found for these cells.

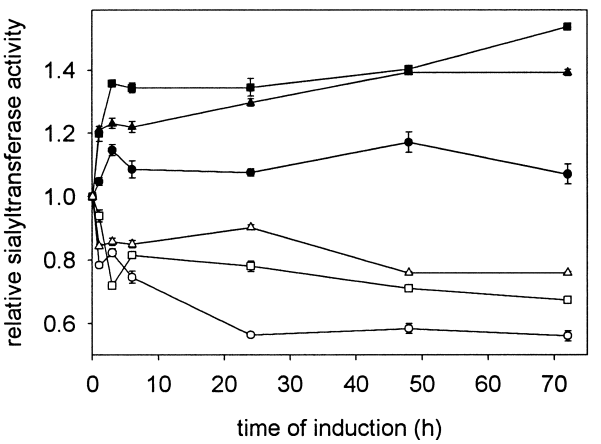


Fig. 3. Sialyltransferase activity in HL60 and THP-1 cells. The α2,3- (filled symbols) and α2,6-sialyltransferase (open symbols) activities were determined in membrane suspensions of DMSO- (circles) or PMA-treated (squares) HL60 and PMA-treated THP-1 cells (triangles) harvested at the time points as described in Section 2.

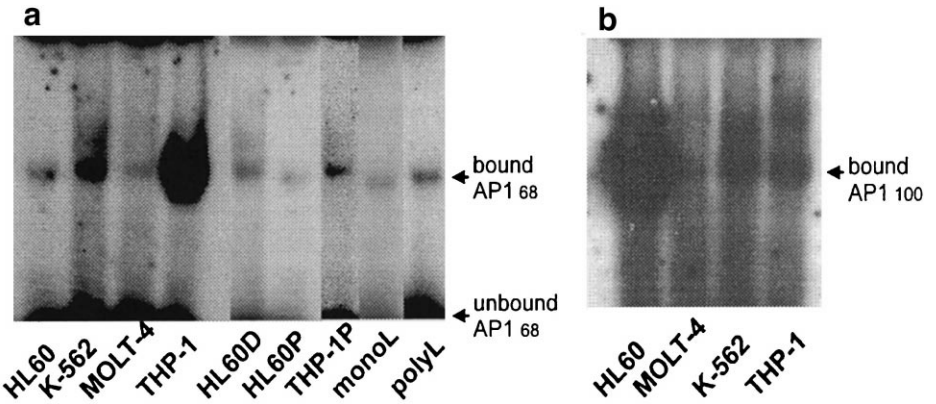


Fig. 4. EMSA with nuclear extracts and AP1 oligonucleotides. EMSAs with nuclear extracts from the different cell types were performed as described in Section 2. Arrows indicate the retarded oligonucleotides bound to protein and unbound oligonucleotides. Abbreviations as in Fig. 1. a: Assays with the oligonucleotide $AP1_{68}$. b: Assays with the oligonucleotide $AP1_{100}$. The EMSAs with differentiated cells and leucocytes were similar to those with $AP1_{68}$.

*3.4. Binding of nuclear proteins to the AP1-like sequences*

The 5′-flanking regions of the transcripts lack TATA or CCAAT boxes, but they contain other well-characterised regulatory elements [15]. At two different positions AP1-like binding sequences were detected. The identified recognition sequences differ in two ($AP1_{75}$) or three bases ($AP1_{68}$) from the consensus sequence [18]. We performed EMSAs with the AP1 consensus sequence and the oligonucleotides $AP1_{75/68}$. Fig. 4a shows the results of incubations of the oligonucleotide $AP1_{68}$ with nuclear extracts from the different cell lines and leucocytes. The highest binding of this oligonucleotide was observed with extracts from THP-1 cells, followed by K-562 cells, whereas extracts from the other cell types showed only weak binding. Strikingly, nuclear proteins from PMA-treated THP-1 cells also bound weakly to $AP1_{68}$. A different situation was observed in EMSAs with $AP1_{100}$ as shown in Fig. 4b. In this case the highest binding was detected with nuclear proteins from HL60 cells. A comparison of the EMSAs with $AP1_{68}$ and $AP1_{100}$ revealed striking differences. Nuclear proteins from THP-1 cells showed a high affinity to $AP1_{68}$ and $AP1_{75}$ (not shown) and low affinity to $AP1_{100}$. For nuclear extracts from HL60 cells the contrary was observed. They exhibited a good binding to $AP1_{100}$ and a lower affinity to the two other oligonucleotides. These differences show that different proteins exist which bind to the oligonucleotides with specific affinities. To further elucidate this finding, cross-competition assays with nuclear extracts from HL60 and THP-1 cells and radioactively labelled $AP1_{100}$ and $AP1_{75}$ were performed. In the case of HL60 cells (Fig. 5a), for the competition of labelled $AP1_{100}$ to almost background level a 10-fold excess of unlabelled $AP1_{100}$ was sufficient to reduce the binding, whereas a 100-fold excess of $AP1_{75}$ was necessary before a reduction of the signal to a background level could be observed. In contrast, nuclear extracts from THP-1 cells showed a stronger affinity to $AP1_{75}$, since this was a more potent competitor than $AP1_{100}$ (Fig. 5b).

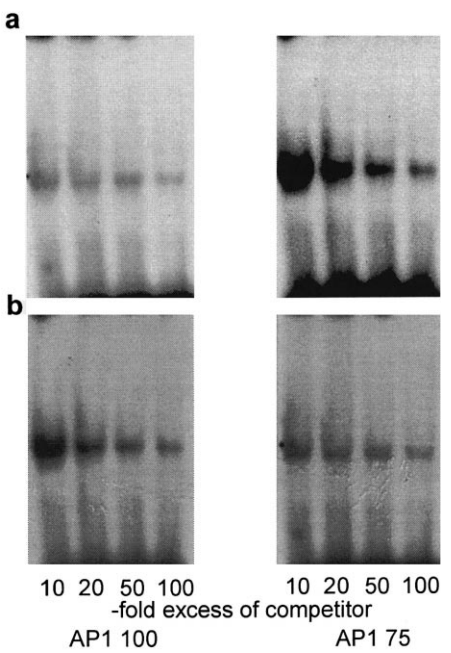


Fig. 5. Cross-competition with nuclear extracts from HL60 and THP-1 cells. EMSAs in the presence of unlabelled competing oligonucleotides $AP1_{100}$ (left) or $AP1_{75}$ (right) were performed as described in Section 2. a: Nuclear proteins from HL60 cells were incubated with radioactively labelled $AP1_{100}$. b: Nuclear proteins from THP-1 cells were incubated with radioactively labelled $AP1_{75}$.

## 4. Discussion

The analysis of transcription start sites by primer-extension analysis revealed more than one start site in leucocytes and leukaemic cell lines for all five transcripts. Similar results were obtained by Kitagawa et al. [15] in human placenta. In Fig. 1 only the start sites at the very 5′-ends are shown. The relevance of several start sites is not yet known. One reason for this phenomenon might be a stuttering of the RNA polymerase II, as suggested by Wang et al. [22] for the ST6Gal I. Another possibility is the formation of secondary structures in the mRNA which result in the interruption of cDNA synthesis by RT. Therefore, in these cases only the start sites at the very 5′-ends are thought to be relevant. However, the variability of biological systems may lead to the usage of more than one start site.

The transcripts A1 and B3 were only expressed at very low levels in undifferentiated HL60 and THP-1 cells. Because of this observation the time course of transcript expression dur-

ing treatment with DMSO or PMA was investigated. After 1 h treatment these transcripts could be detected and expression levels changed during incubation. Except for A2 the other transcripts were expressed more or less at the same level throughout the experiment. This aspect may result from the usage of the same promoter for A2 as for transcript A1. The transcript A2 lacks the exon E5 and therefore encodes a truncated protein without cytoplasmic tail and only a short putative signal anchor domain [9]. This may have an influence on the targeting of the sialyltransferase to Golgi compartments. However, signals which define the localisation of glycosyltransferases within the Golgi have not been definitively determined [23,24]. Transcript A1 possibly also encodes a truncated protein, since three different in-frame start codons were identified and according to Kozak [25] the third start codon represents the strongest one.

In HL60 cells changes in the expression levels of transcripts led to a small increase of the α2,3-sialyltransferase activity. In a previous study [26] no such increase of this enzyme activity was detected for DMSO-treated HL60 cells. This could be due to the use of total cell lysates in those experiments, which may contain endogenous inhibitors. Here we purified membranes as enzyme source and took special care to use conditions under which the product formation was linear dependent on both the amount of membranes and the time of incubation.

The predicted promoter region does not contain TATA or CCAAT boxes [15], which is believed to be typical of so-called housekeeping genes expressed in almost all tissues at low levels [27]. We directed our interest to the involvement of the transcription factor AP1 in the regulation of the gene, since it contains two AP1-like sequences. We observed that nuclear proteins from HL60 and THP-1 cells recognise the AP1 consensus sequence and homologous sequences with different affinities. The gene products of the Jun/Fos family are expressed in HL60 cells treated with PMA for a short period of time and are just detectable after several hours [28]. The fast increase of the transcripts A1/A2 and B3 can possibly be correlated to this finding. Noti et al. [29] observed a cooperation between SP1 and AP1 in myeloid cells. In undifferentiated cells this cooperation was not detectable, whereas in PMA-treated cells the binding of AP1 was facilitated under the influence of SP1. In this respect low amounts of Jun and Fos may guarantee the expression of ST3Gal IV transcripts, whereas at the beginning of differentiation without the support of SP1 higher amounts of Jun/Fos gene products are required.

In conclusion, this study shows the differential expression of ST3Gal IV transcripts in human leucocytes. Strikingly, THP-1 cells contain in contrast to HL60 cells nuclear proteins binding to the AP1-like elements in the promoter regions of this gene with higher affinities than to the AP1 consensus sequence. Therefore, these promoter regions represent a suitable model to identify and compare the constituents of AP1 dimers in THP-1 and HL60 cells.

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