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Tissue specific glucocorticoid receptor expression, a role for alternative first exon usage?

Jonathan D. Turner [a,1], Andrea B. Schote [a,b,1], Joana A. Macedo [a,b], Laetitia P.L. Pelascini [a], Claude P. Muller [a,b,*]

[a] Institute of Immunology, Laboratoire National de Santé, 20A rue Auguste Lumière, L-1950 Luxembourg, Grand Duchy of Luxembourg
[b] Department of Immunology, Graduate School of Psychobiology, University of Trier, D-54290 Trier, Germany

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Abbreviations:
ALL, acute lymphoblastic leukaemia
EST, expression sequence tag
GC, glucocorticoid
GR, glucocorticoid receptor
HPA, hypothalamic–pituitary–adrenal
NGFI-A, nerve growth factor inducible protein A
RACE, rapid amplification of complimentary ends
UTR, untranslated region
YY1, Yin Yang 1

ABSTRACT

The CpG island upstream of the *GR* is highly structured and conserved at least in all the animal species that have been investigated. Sequence alignment of these CpG islands shows inter-species homology ranging from 64 to 99%. This 3.1 kb CpG rich region upstream of the *GR* exon 2 encodes 5′ untranslated mRNA regions. These CpG rich regions are organised into multiple first exons and, as we and others have postulated, each with its own promoter region. Alternative mRNA transcript variants are obtained by the splicing of these alternative first exons to a common acceptor site in the second exon of the *GR*. Exon 2 contains an in-frame stop codon immediately upstream of the ATG start codon to ensure that this 5′ heterogeneity remains untranslated, and that the sequence and structure of the GR is unaffected.

Tissue specific differential usage of exon 1s has been observed in a range of human tissues, and to a lesser extent in the rat and mouse. The *GR* expression level is tightly controlled within each tissue or cell type at baseline and upon stimulation. We suggest that no single promoter region may be capable of containing all the necessary promoter elements and yet preserve the necessary proximity to the transcription initiation site to produce such a plethora of responses. Thus we further suggest that alternative first exons each under the control of specific transcription factors control both the tissue specific *GR* expression and are involved in the tissue specific *GR* transcriptional response to stimulation. Spreading the necessary promoter elements over multiple promoter regions, each with an associated alternative transcription initiation site would appear to vastly increase the capacity for transcriptional control of *GR*.

1. Introduction

Multicellular organisms with specialised tissues require mechanisms to control differential expression of proteins in a tissue-specific manner. Protein expression is either regulated at a transcriptional, post-transcriptional or a translational level. Chromatin condensation, initiation, and DNA methylation are important for the regulation of expression of many genes at the

* *Corresponding author*. Tel.: +352 490604; fax: +352 490686.
E-mail address: claude.muller@LNS.ETAT.LU (C.P. Muller).
[1] These authors contributed equally to this work.
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transcriptional level. Post-transcriptional mechanisms of gene regulation include, e.g. alternative RNA splicing, and modulation of mRNA stability. Certain genes possess multiple promoter regions each associated with a specific alternative transcriptional start site in a specific, non-coding alternatively transcribed first exon. The alternative use of one of multiple exon 1s has only recently be recognized as another mechanism of transcriptional control of gene expression.

The use of alternative proximal promoters located immediately upstream of known transcription start sites results in RNA polymerase-generated primary transcripts of varying length. In this case, the location of the 5′ 7-methyl-guanylate RNA cap structure may correspond to different positions within the genomic sequence depending upon the promoter used. Each primary RNA transcript is an exact copy of the genomic DNA and includes the first exon adjacent to the promoter region used, as well as all other downstream first exons that are excised only during further nuclear processing to produce mRNA.

Within the human genome more than 3000 genes contain multiple first exons [1]. In the mouse over 2000 genes with multiple first exons were identified. Zhang et al. [1] observed that, except for gene clusters encoding the immunoglobulins and T-cell receptors, only the variable 5′ exons of the neural protocadherin (Pcdh), UDP glucuronosyltransferase 1 (UGT1), and I-branching β-1,6-*N*-acetylglucosaminyltransferase (IGnT) gene clusters displayed intra-gene sequence similarity and their first exons are translated giving a variety of closely related N-terminal protein variants. The variable 5′ exons of all other genes investigated showed no such sequence similarity, nor were they thought to be translated. Although the majority of genes seem to have only a few alternative first exons, some genes, such as the glucocorticoid receptor have a remarkable number of first exons to choose from [2,3]. This profusion of alternate first exons would be conducive to an important role in the transcriptional control of gene expression.

Glucocorticoids (GC) are involved in many physiological processes: they regulate the intermediary metabolism, control cellular differentiation, functions of the nervous and immune system and maintain homeostasis under stress. The activity of endogenous and exogenous GC is mediated by the type II glucocorticoid receptor (GR, *NR3C1* OMIM +138040). The GR is expressed in virtually all cell types. Levels of both *GR* mRNA and protein vary considerably between tissues. Also, within some tissues such as the hippocampus, *GR* expression is subjected to a significant level of plasticity [4]. The specific level of *GR* expression is tightly controlled. An artificial reduction of GR levels by only 30–50% results in major neuroendocrine, metabolic, and immunological disorders [5,6]. It has been shown that post-transcriptional mechanisms such as the stability of different mRNA transcript variants do not play a significant role in controlling GR levels. In particular, the stability of mRNA transcripts was not influenced *in vitro* by the use of different alternatively spliced first and last *GR* exons [7].

2. The *GR* gene structure

The gene coding for the GR has been studied in some detail only in three species, humans, mice and rats. The human *GR* gene covers a region of more than 80 kb. Similarly, the rat and mouse *GR* genes span >80 and >110 kb, respectively. The three species have a similar gene structure with seven constant coding exons (exons 2–8), multiple alternative 5′-non-coding exon 1s, and two exon 9s encoding the alpha and beta protein isoforms [8,9]. The alternative first exons were not designated sequentially but according to their order of discovery, which somewhat complicates the nomenclature. Most of the variable first exons of humans, rats, and mice are located in a CpG rich region of about 3.1 kb, starting some 4.6 kb and finishing 1.5 kb upstream of exon 2, although, a group of corresponding exons (human 1-A, rat 1_1 to 1_3 and mouse 1-A) is located 27–30 kb upstream.

Analysis of the 5′ UTR of the human *GR* has revealed 11 splice variants derived from 7 exon 1s (exons 1-A to 1-H [2]). Similarly, the rat has 11 alternative exon 1s (1_1 to 1_{11} [3]), and the mouse has 4 (1-A to 1-E [10]). With the exception of mouse exon 1-E there is a very good structural similarity and sequence homology between the alternative exons (Fig. 1) as previously described for the rat and the human *GR*s [2]. All of the known exon 1 variants in the human, mouse and rat have unique splice donor sites, splicing to a common exon 2 splice acceptor site. All mRNA splice variants have an in-frame stop codon (mouse and rat TAA; human TGA) before the ATG translation start site in exon 2 that is common to all mRNA variants. Since the translation start codon lies within the exon 2 the 5′-heterogeneity is untranslated and does not affect the sequence of the receptor.

3. *GR* tissue specific expression

Tissue specific differential usage of exon 1s has been examined systematically in a range of human tissue. Our earlier data suggest that no first exon is expressed in all tissues [2]. Conversely, at least the human hippocampus expressed all of the known exon 1 transcript variants, with exon 1-D being unique to this brain region. In addition to their presence in the hippocampus, exons 1-E and 1-F, seem to be biased towards the immune system. Exon 1-E was observed in both CD8+ T cells and CD14+ monocytes, while exon 1-F was best expressed in CD19+ B lymphocytes and BCDA2+ peripheral blood dendritic cells. Exons 1-B and 1-C appeared to be broadly expressed in many tissues, although they were absent from both subcutaneous adipose tissue and heart muscle and from the liver, respectively.

Exons 1_{10} and 1_6 the rat homologues of the latter human exons also showed a broad expression pattern [3]. In the rat, 1_{10} represents at least 50% of the total *GR* transcripts, while exon 1_6 accounts for far less. As in humans, the other rat exons were expressed in a tissue-specific manner. Exon 1_1 was observed in the thymus, but not in the hippocampus and the liver. As in humans, most other exons 1s were expressed in the hippocampus, including significant levels of transcripts containing the minor exons 1_5, 1_7, and 1_{11}. As its human homologue 1-D, exon 1_7 is hippocampus-specific. Exons 1_5-, 1_7-, and 1_{11}-containing transcripts were either low or undetectable in the liver and thymus [3].

Much less is known about the promoter usage and first exon expression in the mouse. Strahle et al. [9] showed that mouse exon 1-A was found in two T-lymphoma cell lines (S49

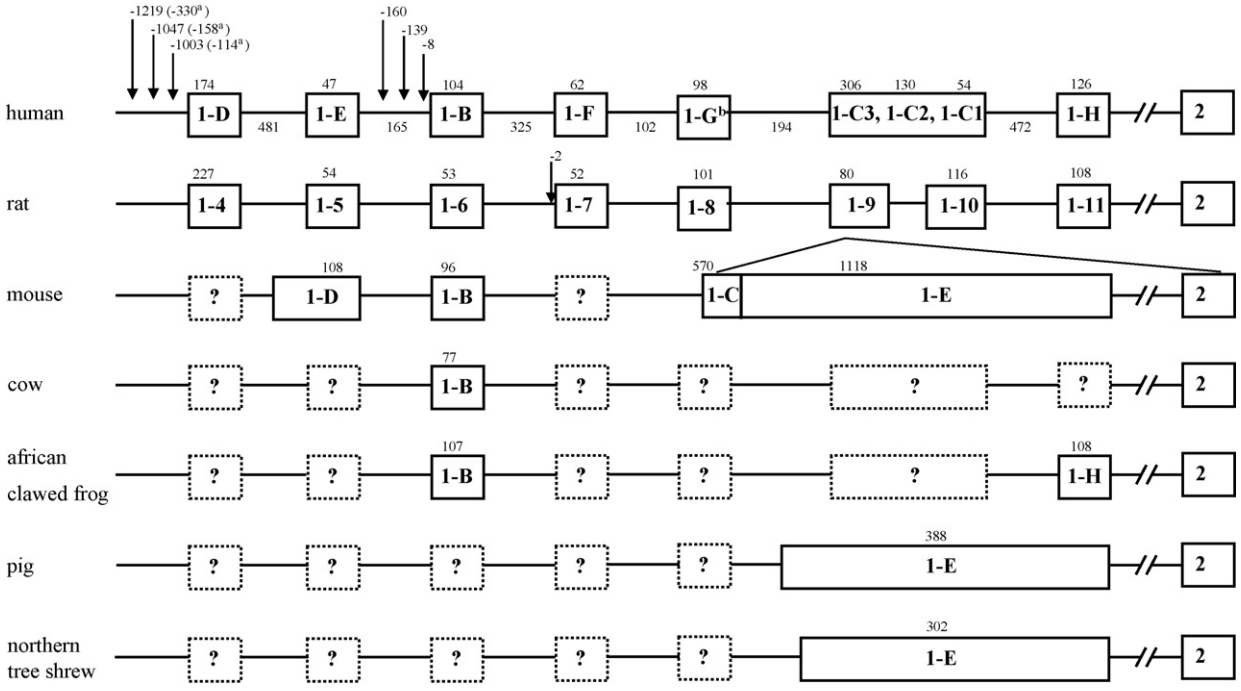

Fig. 1 – Map of the known alternative exon 1s of *GR* genes from all species for which the data is available. Experimentally identified exons (line boxes) and those predicted by genomic homology (dotted boxes) are shown. Incomplete sequencing data is indicated by dashed lines. Drawing is not to scale, exon and intron sizes are given in base pairs.

and WEHI-7 cells) but not in fibroblasts, the liver, or brain. In contrast, transcripts containing exons 1-B and 1-C were found in the latter tissues and at lower levels in T lymphocytes. Exons 1-D and 1-E have only been observed in mouse S49 T lymphoma cells, and AtT-20 pituitary tumour cell lines [10], but otherwise have not been investigated.

4. The *GR* CpG island is common to many species

The human alternative 5′ UTRs in the CpG islands upstream of *GR* exon 2 have been compared to those of the rat based on their high homology [2]. The alignment of the corresponding CpG islands demonstrates that this region is highly conserved among species as shown in Fig. 2 for exon 1 coding stretches. Over the entire CpG island (3.1 kb) the total inter-species homology ranges from 65.5% (mouse versus cow) to 88.1% (mouse versus rat) and even 98.9% (human versus chimpanzee). The cow seems to share a greater homology with the human and the chimpanzee than with either the rat or the mouse (77.3 and 77.0% versus 66.2 and 65.2%, respectively) (Table 1).

5. The *GR* mRNA sequence is conserved between species

Since the human *GR* was first cloned and sequenced in 1985 [8] full length *GR* sequences have been obtained from more than a dozen species, and partial sequences from a dozen other species. As expected, the phylogenetic tree of the *GR* alpha mRNA (Fig. 3), shows that the sequence homology between mammals is high, while length and sequence are less well conserved in other species [11,12]. The DNA binding and ligand binding domains show the greatest sequence homology between species [13]. In our hands, the rat sequence showed a higher homology with the mouse *GR* sequence than with the

Table 1 – Sequence homology of the *GR* 5′ UTR, mRNA and intron sequence between different species

	Cow	Chimpanzee	Human	Rat
500 bp intron[a]				
Mouse	61.9[b]	65.8	65.7	82.5
Rat	61.9	65.3	66.0	
Human	78.0	100.0		
Chimpanzee	76.0			
CpG island[c]				
Mouse	65.2	66.1	66.4	88.1
Rat	66.2	67.5	67.6	
Human	77.3	98.9		
Chimpanzee	77.0			
CDS[d]				
Mouse	85.3	87.8	88.0	92.6
Rat	81.9	86.2	81.5	
Human	90.0	99.7		
Chimpanzee	90.2			

[a] Comparison of 500 bp of intron sequence between the CpG island and exon 2.
[b] Data are expressed as the percentage of identical base pairs.
[c] Comparison of 3.1 kb genomic sequence used.
[d] Comparison of GR alpha isoform RefSeq mRNA from the start of exon 2 to the end of the CDS.

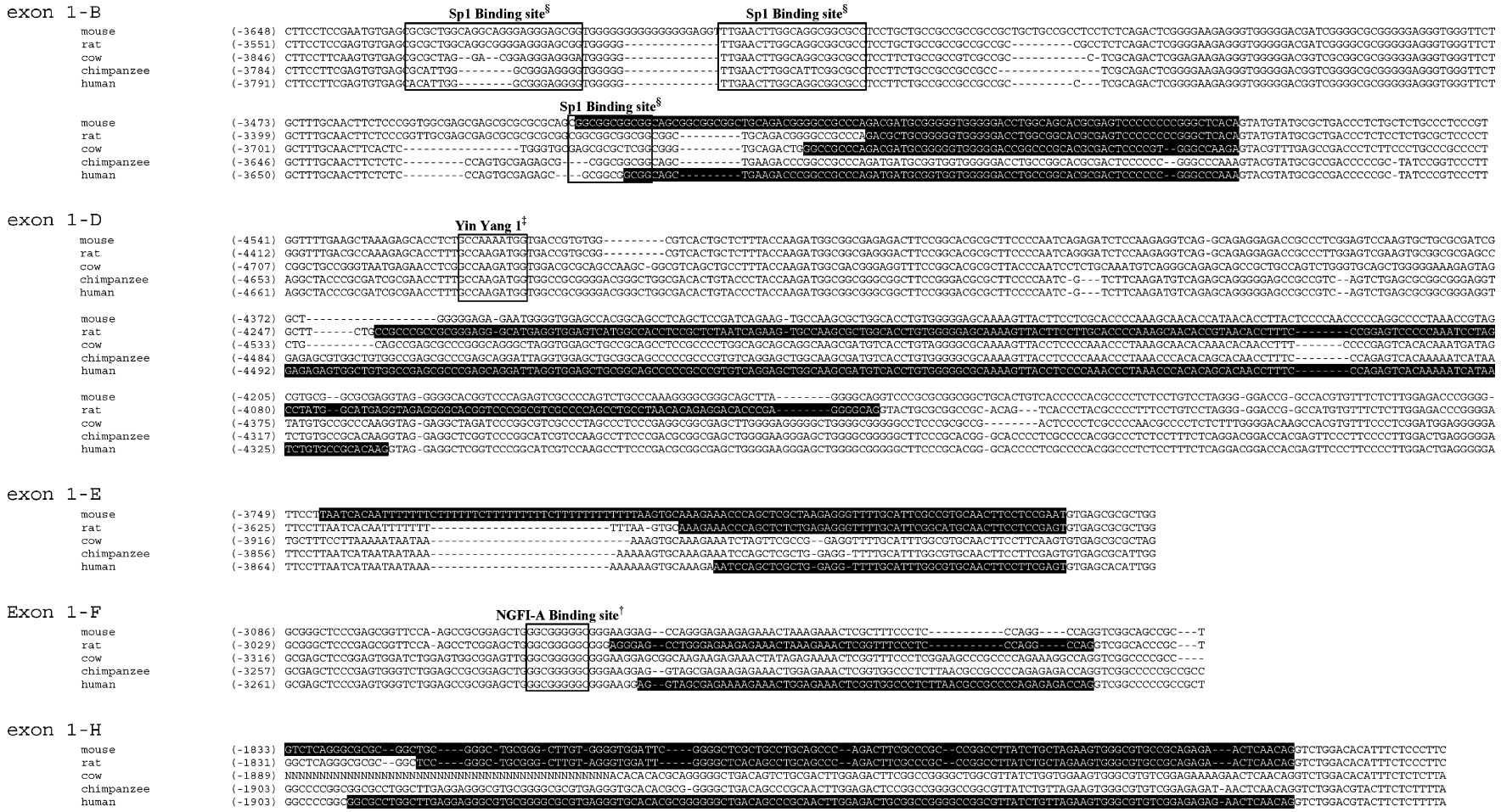

Fig. 2 – Alignment of genomic sequences coding transcribed first exons 1-B, 1-D, 1-E, 1-F and 1-H. Numbering is with respect to the translation start site within exon 2 for each species. The start of the human exon 2 is at −13 bp. Shaded sequences represent previously identified alternative exon 1s. Previously identified transcription factor binding sites are boxed. ‡ from [16]; † from [17]; § from [41]. Alignments of the genomic DNA of the human chromosome 5 (July 2004, UCSC), the rat (AJ271870, NCBI), mouse (NCBIM35:18: 39863309:39869358:1, Ensembl), chimpanzee (CHIMP1A:5: 149403812:149409861:1, Ensembl) and cow (ChrUn.313: 334239:339538:1, Ensembl) upstream of the GR exon 2 was performed using Vector NTi (Invitrogen, Paisley, UK).

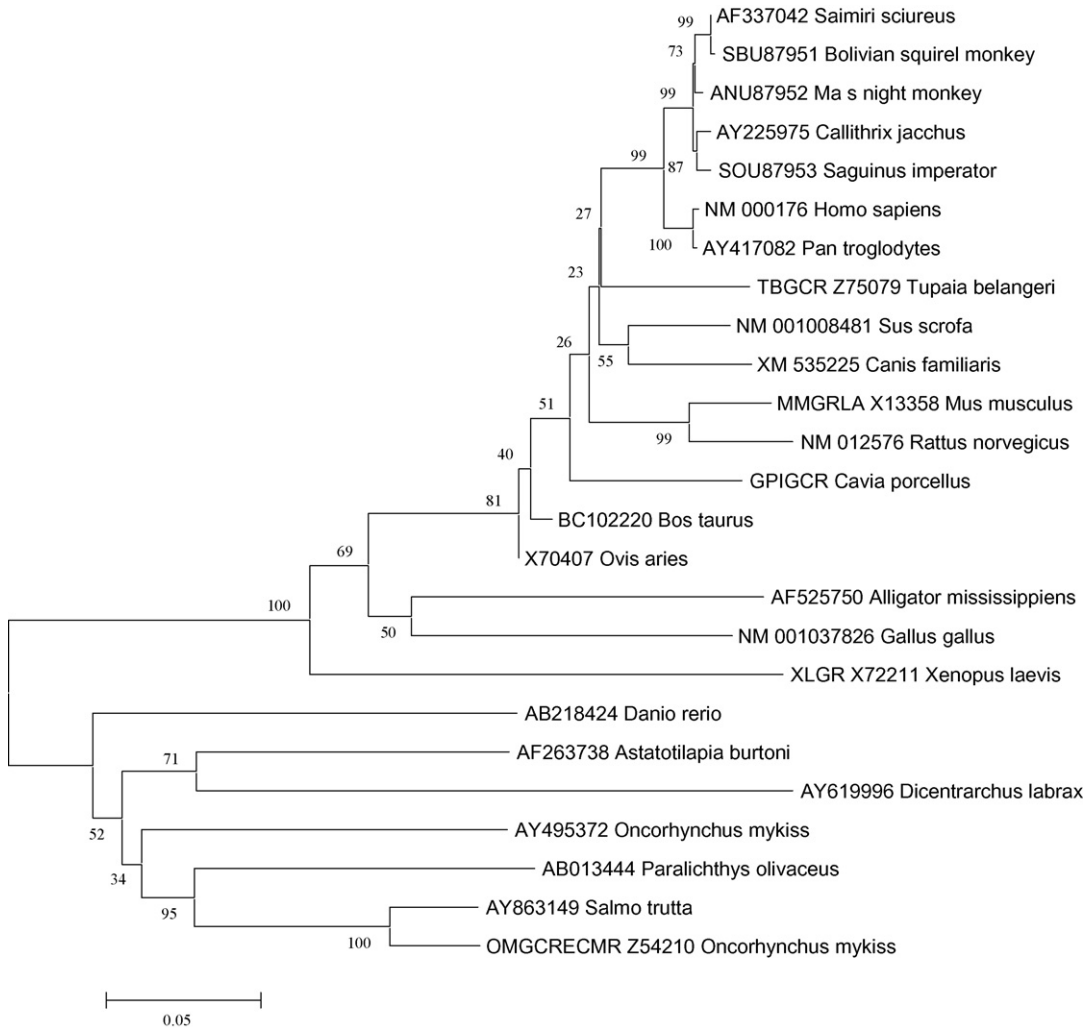

Fig. 3 – Phylogenic analysis of 25 complete NR3C1 coding sequences currently available on GenBank. Numbers at nodes correspond to bootstrap values. Alignment of the mRNA sequences was performed using ClustalX. Phylogenetic analysis was performed using neighbour-joining Kimura 2 parameter method and the tree was constructed using Mega 3.1 [42].

human, although, a higher homology between rat and human was previously reported [14].

6. Alternative GR 5′ UTRs can be identified in many species

Alignment of the known human first exons with the genomic sequence of the cow CpG island, shows that the human exons 1-B, -D, -E and -F have homologies of 82.1, 80.6, 84.0, and 64.2%, respectively with the cow sequence. While the homology with exon 1-H was still significant although including of a small segment (25 bp) of unknown genomic sequence, there was none with 1-G.

The known 5′ UTRs of GR of the species for which they are known align with one of the known rat or human variable exon 1s. The published mRNA sequences suggest that only the African clawed frog (*Xenopus laevis*) has alternative first exons and these correspond to the human exons 1-H and 1-B, only the latter exon being shared also with the cow. The sequences of the pig (*Sus scrofa*) and northern tree shrew (*Tupaia belangeri*) seem homologous to the mouse 1-E, but are significantly different from the corresponding rat and human region. The corresponding human region encodes the multiple intra-exon spliced 1-C as well as 1-H, while in the rat three independent exons, 1_9, 1_{10} and 1_{11} are found here. The mouse exon 1-C, included in the 5′ end of exon 1-E, is spliced to exon 2 similarly to the intra-exon splicing of the human 1-C. This mouse exon 1-C overlaps with the ephimeric human 1-G and the rat exon 1_8.

Fig. 2 shows that exons 1-B, -E, -F, and -H share identical 3′ splice donor sites. As previously noted [2] exon 1-D is slightly longer in the rat than in the human. From these homologies we can predict with reasonable accuracy the 3′ end of exons 1-B, -D, -E, -F and -H in the cow, all five alternative first exons in the chimpanzee, and exons 1-D and -F of the mouse. Although, it is much more difficult to predict the 5′ end of these potential exons, the predicted genomic region covered by these exons can be narrowed down. These comparisons suggest that the 3′ ends of alternative first exons of GR will be predictable in new genomic sequences as they become

available from other species. As the repertoire of alternative first exons grows, it would however seem logical that a unified system of nomenclature be developed. Such a system should allow for the comparison between species and also prevent the situation arising, as currently exists, with homologous exons having different names between species.

7. Alternative 5′ UTRs may have their own promoters and transcription factor binding sites

Assuming that each variable first exon has its own proximal promoter, the high degree of inter-species genomic homology suggests that transcriptional control mechanisms may be similar among different species. The known transcription factor binding sites within the alternate promoter elements are summarised in Table 2 and their location shown in Fig. 1. The human 1-B promoter contains previously identified binding sites for SP1 and Yin Yang 1 (YY1) [15–17]. Detailed analysis showed that SP1 sites are found in all species some 127 and 158 bp upstream of the exon 1-B transcription start site. The YY1 sites were identified 1219, 1047, and 1003 bp upstream of the transcription initiation site of exon 1-B. However, later only 330, 158, and 114 bp downstream of these YY1 sites the initiation site of exon 1-D was discovered (Fig. 2). Therefore, these YY1 binding sites may be part of the proximal promoter region of exon 1-D rather than 1-B. From the latter work [17] it was not possible to establish exactly which elements control the transcription starting at exons 1-B, 1-C, or 1-D.

In the rat, analysis of the proximal promoter region of exon 1_7 showed that the transcription factor NGFI-A binds only 2 bp upstream of the transcription initiation site of this exon. Fig. 2 shows the known SP1 and NGFI-A binding sites upstream of the human exon 1-B and the rat exon 1_7, respectively. Sequence homology in these regions suggests that these transcription factors are also operational in other species such as the chimpanzee, cow and mouse.

The proximal promoter of human exon 1-A has received more attention, because of its role in GR auto-regulation. This promoter is reportedly activated selectively in hematopoietic cells [17,18] and highly sensitive to dexamethasone stimulation. Its sensitivity to dexamethasone may be partially explained by the presence of two distinct GRE-like sequences found in DNA footprinting experiments including a half-site pair [19], and an isolated half-site [20], although neither footprint corresponded to a consensus GRE. In super-shift assays only GR beta bound to the half-site pair footprint, but technical reasons may have prevented the detection of GR alpha binding [19].

The second single GRE-like half-site appears to be paired with a second footprint that binds c-Myb and members of the

Table 2 – Regulatory elements of the GR proximal promoter regions

Associated exon	Transcription factor	Sites	Technique[a]	Location[b]	Comments	Reference
1-A	Ets-1/2[c]	1	RG	−34412 (95)	Steroid induced recruitment	[20]
	PU.1[c]	1			confirmed by ChIP	[20]
	Spi-B[c]	1				[20]
	c-Myb	1	RG	−34416 (91)		[20]
	GR[d]	1	RG	−34432 (75)		[20]
	GR[e]	1	RG, FP, E	−34546 (175)		[19]
	IRF-E	1	RG, E			[43]
1-B	SP1	3	RG, FP, E	−3774 (−160)		[17]
				−3753 (−139)		
				−3622 (−8)		
	YY-1	3	FP, D, E	−4808 (−1219)	−330 relative to 1-D	[16]
				−4636 (−1047)	−158 relative to 1-D	
				−4592 (−1003)	−114 relative to 1-D	
1-C	SP1	Numerous	FP, E		11 footprints observed in the region	[16]
	AP2	1	FP, E		covering 1-C and 1-D promoters	[16]
1-D	n/d[f]					
1-E	n/d					
1–7 (1-F)	NGFI-A	1	ChIP	−3226 (−2)	Identical binding site at −7 bp from	[41]
1-G	n/d				human homologue 1-F	
1-H	n/d					

[a] RG, reporter gene; FP, DNAse protection/DNA footprinting; ChIP, chromatin precipitation; D, deletion analysis; EMSA, electrophoretic mobility shift assay.
[b] Locations—with respect to ATG start codon and to associated first exon in parentheses.
[c] Ets-1/2, PU.1 and Spi-B are members of the Ets family, binding to the same site. Overexpression of all three factors increases exon 1-A expression [20].
[d] FP11 from [20] one non-consensus GRE half site (GTAAAATGCGC) is present and this may not be enough for complete steroid responsiveness by itself.
[e] FP6 from [19] two GRE half sites AGAAAA and TCTTCT.
[f] Not determined.

ETS family. The T and B cell lines differentially express members of these families, explaining the different results upon dexamethasone stimulation.

It is difficult to attribute transcription factor binding sites to specific alternate first exons. Experimentally, it has been shown that the footprints observed display promoter activity using techniques such as luciferase reporter genes. However, examination of promoter elements upstream of exons 1-B and 1-C was performed on approximately 1 kb segments [16,17]. Promoter constructs for exon 1-B included exons 1-D and 1-E, whilst for 1-C both 1-F and 1-G were included, in the test constructs. Therefore these experiments were unable to unequivocally assign the promoter activity observed to the alternative exons.

8. Identification and localisation of alternative 5′ UTRs

Whilst coding sequences can be found in databases such as the NCBI RefSeq database [21] with relative ease, multiple alternative 5′ UTR are more difficult to retrieve. Also experimentally, their identification is complicated.

Expressed sequence tag (EST) represents single pass, partial sequences generated from either the 5′ or the 3′-end of a cDNA clone prepared from various tissues or cell lines. EST databases provide a view of the mRNA species within given cell types and conditions. However, 5′ sequences are under-represented in EST databases. This, together with sequencing errors, frequent insertions and deletions, contaminations by vector and linker sequences, and the non-random distribution of sequence start sites in oligo(dT)-primed libraries reduces their overall usefulness. In addition, the patterns of overlapping sequences of the same gene caused by alternative transcripts are different from those obtained from genomic sequencing [22]. For the identification of alternative exon 1 splice variants the EST database is only partially useful because while only transcribed regions of the genome are represented, the number of tissues included is limited, and expression of the different first exons of the *GR* is tissue specific. BLAST searches for the reported human alternative first exons provided no matches with any EST clones. Similarly, experimental determination of alternative 5′ UTR sequences is tedious as it relies upon techniques such as 5′ RACE PCR applied to each individual tissue.

9. *GR* variable exons and disease

Although differential expression of the *GR* variable first exons has received little attention in various disorders, modulation of their expression has been observed in certain psychobiological conditions.

The hippocampus is well known to be a site of inhibitory feedback of glucocorticoids on the hypothalamic–pituitary–adrenal (HPA) axis. Perinatal manipulations (e.g. postnatal handling) significantly increased *GR* mRNA level in the rat hippocampus and attenuated the stress response of the HPA axis. This increase in hippocampal *GR* mRNA levels was due to the selective upregulation of the rat hippocampus specific exon 1_7. As a result, adult rats that were handled after birth were more sensitive to negative glucocorticoid feedback and had a decreased HPA reactivity to stress throughout their adult life [23,24].

In conditions such as major depression where the HPA axis is hyperactive, because of lower hippocampal *GR* (mRNA) levels resulting in an impaired glucocorticoid feedback, the selective upregulation of exon 1_7 has been considered to play a role in the therapeutic mechanism of certain antidepressant agents. In rats, a 4-week antidepressant treatment with fluoxetine increased hippocampal exon 1_7 expression [25] associated with increased total *GR* expression. *MR* and *GR* expression was also increased in the rat hippocampus after treatment with other antidepressant drugs, but the role of alternative first exon usage was not investigated [26–28]. Similarly, it was shown that prenatal exposure of rats to synthetic glucocorticoids such as dexamethasone increased hepatic *GR* mRNA levels. As a result, the relative expression of the predominant hepatic first exon, 1_{10}, was reduced suggesting an increase of any of the other variable exons, although the responsible exons have not been identified.

In mouse lymphocytes exon 1-A expression was upregulated by GC exposure in a similar manner to the rat, although this effect was dependent on the organ source and the phenotype of lymphocytes [29].

In humans GR regulation in acute lymphoblastic leukemia (ALL) has been investigated in an effort to understand GC resistance, which is associated with a poor prognosis. Chemotherapy of childhood ALL includes GCs, which in GC-sensitive ALL cell lines upregulates GR mRNA and protein levels, and induces lymphoblast apoptosis [30–32]. The GR promoter 1-A has a weak glucocorticoid response element (GRE), whereas promoters 1-B to 1-H had no obvious predictable GRE. It was hypothesised and later confirmed in the human ALL cell line CEM-C7 [19,33] that the upon GC exposure alternative GR promoters are regulated independently and in different ways [34].

In ALL patients baseline promoter usage and transcript variant expression levels for the three exons investigated (1-A, -B, and -C) showed no difference between either GC-sensitive or insensitive ALL patients and with healthy controls. Similarly, after GC stimulation GR expression was induced in both GC-sensitive and insensitive ALL cells in the same manner as in healthy controls. Therefore, GC resistance in primary ALL cells cannot be attributed to the inability of resistant cells to upregulate the expression of the GR upon GC exposure, nor to differences in GR promoter usage upon GC treatment [35]. Thus, in humans *in vitro* and *in vivo* studies seem to give conflicting results, or GR expression is simply regulated differently in both cell types. Although alternative mechanisms may explain the above results, differences in mRNA half-life of the transcript variants have so far not been reported [7].

10. Conclusion

The CpG island upstream of the *GR* is highly structured and conserved at least in all the animal species that have been investigated. Sequence alignment of these CpG islands shows inter-species homology ranging from 64 to 99%. The 3.1 kb CpG

rich region upstream of the *GR* exon 2 encodes 5′ untranslated mRNA regions, organised into multiple first exons and, as we and others have postulated, each with its own promoter region. Alternative mRNA transcript variants are obtained by the splicing of these alternative first exons to a common acceptor site in the second exon of the *GR*. Exon 2 contains an in-frame stop codon immediately upstream of the ATG start codon to ensure that this 5′ heterogeneity remains untranslated, and that the sequence and structure of the GR is unaffected.

Tissue specific differential usage of exon 1s has been observed in a range of human tissues, and to a lesser extent in the rat and mouse. Baseline and stimulated levels of *GR* expression are tightly controlled within each tissue or cell type. We suggest that no single promoter region may be capable of containing all the necessary promoter elements and yet preserve the necessary proximity to the transcription initiation site to produce such a plethora of responses. Thus we further suggest that alternative first exons each under the control of specific transcription factors control the tissue specific *GR* expression and are involved in the tissue specific *GR* transcriptional response to stimulation. Spreading the necessary promoter elements over multiple promoter regions, each with an associated alternative transcription initiation site would appear to vastly increase the capacity for transcriptional control of *GR*.

Transcriptional control via multiple alternate first exons provides new possibilities for control also at the translational level. Many mRNA features control its translation but most translational control elements are located within the UTRs, especially the 5′ UTR (reviewed in [36,37]). In eukaryotes, translation is mostly initiated by a cap scanning mechanism and to a lesser extent through internal ribosome entry. The key features of the 5′ UTR involved in translational control include their length, initiation consensus sequences, together with characteristic secondary structural elements, upstream start codons and micro RNAs, and internal ribosome entry sites (IRES) [38,39]. In addition, 5′ UTRs can contain sequences that function as binding sites for regulatory proteins. So far, the effect of multiple alternate 5′ UTRs on translation of the *GR* mRNA has received little attention, although, the conspicuous and evolutionary conserved diversity in 5′ UTR length and sequence, suggest an important role in translation. For instance it has recently been shown that secondary structure elements within one of the alternative sequences of the breast cancer gene (BRCA1), down-regulate protein production [40]. This gene has a transcriptional control mechanism similar to the *GR*, albeit with only two alternative promoters and associated first exons. Although, the UTR of the *GR* is much more complex, *GR* translation may be controlled in a similar fashion. It is interesting to hypothesise that different first introns in these alternative primary RNA transcripts may also play a role in controlling the subsequent processing of the RNA to mRNA.

In summary, tissue, cell and stimuli specific transcriptional control of the *GR* appears to rely on alternative promoters and the alternative transcription start sites associated with them and this mechanism appears to be conserved across many species. Such a transcriptional control mechanism has significant, although currently unexplored implications for the translation of the *GR* mRNA.

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