Human–mouse differences in the embryonic expression patterns of developmental control genes and disease genes

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Our understanding of early human development has been impeded by the general difficulty in obtaining suitable samples for study. As a result, and because of the extraordinarily high degree of evolutionary conservation of many developmentally important genes and developmental pathways, great reliance has been placed on extrapolation from animal models of development, principally the mouse. However, the strong evolutionary conservation of coding sequence for developmentally important genes does not necessarily mean that their expression patterns are as highly conserved. The very recent availability of human embryonic samples for gene expression studies has now permitted for the first time an assessment of the degree to which we can confidently extrapolate from studies of rodent gene expression patterns. We have found significant human–mouse differences in embryonic expression patterns for a variety of genes. We present detailed data for two illustrative examples. Wnt7a, a very highly conserved gene known to be important in early development, shows significant differences in spatial and temporal expression patterns in the developing brain (midbrain, telencephalon) of man and mice. CAPN3, the locus for LGMD2A limb girdle muscular dystrophy, and its mouse orthologue differ extensively in expression in embryonic heart, lens and smooth muscle. Our study also shows how molecular analyses, while providing explanations for the observed differences, can be important in providing insights into mammalian evolution.

INTRODUCTION

Although the structural genomics phase of the human genome project is nearing completion, and will shortly provide a complete inventory of human genes, our knowledge of human gene expression is rudimentary (1). High resolution gene expression studies are most conveniently carried out on the miniaturized tissues of the embryo, but the general difficulty in accessing suitable material for studying early human development and the widespread belief that early mammalian development is essentially conserved have prompted widespread studies of rodent models, notably the mouse. The resulting reliance on rodent embryonic expression studies makes it important to know just how far we can extrapolate from them with confidence. The huge emphasis on gene expression in rodent embryos has been justified by strong similarities in the organization and morphology of vertebrate embryos and by the extraordinary evolutionary conservation of many developmental pathways and of key genes that are known or expected to be important in regulating early development. Species differences in development can be represented by an hourglass model (2,3): very considerable differences exist at very early stages in development, such as gastrulation and neurulation, and morphological divergence can be extensive in later development, but at intermediate stages when the body plan is being laid down embryonic development has been widely considered to be highly conserved during vertebrate evolution. However, the idea that there is a conserved embryonic (phylotypic) stage in vertebrates has not been supported by detailed comparative studies: stage-matched embryos from closely related vertebrate and mammalian species were recently shown to exhibit considerable morphological differences (4). Recent molecular studies have also emphasized unexpected species differences and raised concerns regarding the extent to
which we can confidently extrapolate from rodent models. For some human genes, rodent models have not been available simply because orthologues have not been able to be identified in rodent lineages despite intensive zoo-blot and library screening investigations; the same approaches have nevertheless been able to identify clear orthologues in other vertebrates. Examples include known disease genes such as the Kallman syndrome gene whose expression has been studied in human and chick (5; A. Ballabio, personal communication) and the SHOX gene, a locus implicated in Turner syndrome and Weri-Leill syndrome (6–8; G. Rappold, personal communication). For many human disease genes where rodent orthologues have been found, attempts to generate mouse models by gene targeting have often produced phenotypes that do not closely resemble the human disorder (9).

Although the primary structures of products encoded by developmentally important genes, including many homeobox genes, have often been extraordinarily conserved during evolution, there is increasing evidence for some rapidly evolving homeobox genes that may play important roles in speciation (10). Even in the case of genes that are known to encode very highly conserved proteins, species differences in cis-acting regulatory sequences have long been envisaged to be important motors in evolution, resulting in divergent gene expression (11,12). Artificial re-routing of a gene product to an abnormal spatio-temporal location during early development can result in acquisition of different developmental functions (13) and species differences in expression domains have been occasionally noted to arise very rapidly during the evolution of invertebrates, even in the case of genes that are essential in early development (14). Recently, the evolutionary variation anticipated in cis-acting regulatory elements of Hox genes (15) has been validated by experimental data that show that cis-regulatory evolution of the Ultrabithorax gene underpins some morphological differences between Drosophila species (16). It is perhaps not surprising, therefore, that molecular studies have recently suggested significant species differences in what was formerly assumed to be a paradigm of evolutionary conservation: vertebrate limb development (17).

The data from the studies described above now suggest that differences between vertebrate embryos may be more significant than formerly appreciated and that the developmental origins of adult variation can be expected to encompass early embryonic stages (18). With the recent advent of high resolution gene expression studies in human embryos (1,19), detailed comparative human–mouse data are becoming available for the first time. For the most part, the genes that are being studied are known disease genes and/or genes known to be important in early development. The data from these gene expression studies show that heterochrony, species differences in developmental timing, is a common feature but that differences in spatial expression domains are less common. Nevertheless, significant and unanticipated human–mouse differences of the latter kind are being found for some embryonic expression domains. In the present study we present examples of these differences with reference to two types of gene, a very highly conserved gene that is known to play a crucially important role in embryonic development, the WNT7A gene, and a known disease gene, CAPN3, which is a locus for limb girdle muscular dystrophy (20) and encodes the first calpain for which a link with a physiological role has been established.

RESULTS

Human and mouse Wnt7a genes show significant differences in expression in the developing brain

Wnt genes constitute a large multigene family encoding intercellular signalling molecules, which play a variety of crucially important roles in embryonic development (21–23). Like several other mouse Wnt genes, Wnt7a shows discrete expression domains in the embryonic central nervous system (24), and on the basis of the phenotype of the mouse knock-out, appears to be essential in dorsoventral patterning of the limb bud and in permitting sexually dimorphic development of the Mullerian ducts (25,26). Full-length human WNT7A cDNA sequences have been reported recently (27,28) and, consistent with the very high degree of evolutionary conservation of Wnt genes, the human and mouse Wnt7a gene products show 98% sequence identity.

As part of a comprehensive human–mouse comparative study of four Wnt genes (see Discussion), we studied WNT7A expression in a range of human post-implantation embryos, from Carnegie stage 12 to 21 (CS12–CS21, corresponding to 26–52 days post-ovulation). We observed a variety of discrete expression domains that had previously been described in mouse (24), including groups of cells of the dorsal limb ectoderm and ventral hindbrain (data not shown). In addition, however, we identified significant human–mouse differences in spatiotemporal expression patterns in more rostral components of the developing brain; these are summarized schematically in Figure 1. At CS15 (33 days post-ovulation) WNT7A was expressed strongly in the telencephalon, as illustrated by the example of the transverse section shown in Figure 2A. Similar studies on a variety of other CS15 sections showed that WNT7A expression extended from the ventral thalamus in the diencephalon into caudobasal/ventral areas of the future cerebral hemispheres, but not to frontobasal areas such as olfactory eminence/bulb or medial eminence, or dorsal areas (see Fig. 1 for a schematic illustration). The lack of anatomical landmarks makes it impossible to define this area, but it may correspond to the future internal capsule areas. Expression in this area or, indeed, elsewhere in the telencephalon is not evident at later developmental stages examined, from CS17 to CS21 (Fig. 2B, and data not shown); instead, expression does not extend further than the diencephalon boundary (data not shown). In mouse, however, strong expression of Wnt7a is detected at embryonic day (E) 12.5 (equivalent to CS19) in the lateral, ventral and dorsal aspects of the telencephalon (Fig. 3) (29).

Thus, in the telencephalon the differences between human and mouse expression are spatial (caudo-basal/ventral in human versus primarily dorsolateral in mouse), and temporal (expression occurs at CS15 in human but is not detectable by CS17, whereas in mouse expression is strong at E12.5, equivalent to the later stage CS19). At CS15 and E11 (the equivalent stage in mouse) the ventricular layer (which contains dividing neuronal and glial stem cells) is the major layer whereas by E12.5 an intermediate layer of more mature, migrating cells has appeared. In human, WNT7A is never detected outside the ventricular layer, but in the E12.5 mouse Wnt7a expression can clearly be seen both in the ventricular and intermediate layer of the telencephalon (Fig. 3). Thus, not only is there a spatial expression difference in the region of the telencephalon in which the gene is being expressed in mouse and human, but
also temporal differences, and in mouse there is additional expression in the cell layer containing more mature migrating neuronal and glial cells.

A striking human–mouse difference is also apparent in midbrain Wnt7a expression patterns. In the developing mouse midbrain there is a broad band of ventrolateral expression along the length of the mesencephalon at E9.5 (24) and at E12.5 (Fig. 4C). In contrast, in the developing human midbrain, expression of WNT7A is restricted to the dorsolateral regions throughout CS12–CS21 (Fig. 4A and B). In the developing midbrain of both human and mouse, expression is confined to the ventricular layer, but the expression boundaries clearly overlap and are not confined to the alar and basal plates, respectively. Similarly, in the developing hindbrain, where Wnt7a is expressed across most of the basal plate ventricular layer, with the exclusion of the midline sulcus/floor plate area, expression is again not confined by the anatomical boundary of the sulcus limitans (which divides the alar and basal plates), but extends beyond this landmark in both species.

Human and mouse calpain 3 genes show a variety of differences in embryonic expression patterns

The CAPN3 gene encodes the calcium-dependent cysteine protease, calpain 3, and was identified by Richard et al. (20) as the locus responsible for limb girdle muscular dystrophy type 2A, an autosomal recessive progressive myopathy characterized by early-onset, symmetrical weakness and atrophy of the proximal limb and trunk muscles (30). Recently, as part of a wider study of various progressive muscular dystrophy genes, we reported the pattern of calpain 3 expression during early human development (31). Currently, however, little is known about calpain 3 function (32), although Sorimachi et al. (33) reported that it could bind titin (or connectin). To clarify the physiological role of this protein and its contribution to the pathogenesis, and as a resource for devising future therapeutic approaches, we undertook construction of a calpainopathic mouse model. In anticipation of this we examined the spatiotemporal expression patterns of the calpain 3 gene during mouse development. We now report that the mouse calpain 3 expression data, when referenced against the corresponding human data, reveal important species differences in spatiotemporal expression (see below).

Because there are several calpain genes and calpain-like sequences and because of the possibility of differential expression of different calpain 3 (31,34), we used three antisense oligonucleotide probes representing mouse calpain 3-specific sequences. They derived from exon 1 (calpain 3-NS probe), exon 6 (calpain 3-IS1 probe) and exon 16 (calpain 3-IS2 probe) (see Materials and Methods). Sagittal sections of murine embryos were investigated systematically from E9.5 to birth with these probes and, in addition, a parallel study was conducted using an antisense titin probe, as a reference marker for skeletal muscle expression.

The results with the titin probe were analogous to those previously reported (35,36): expression was detected from
expression in the telencephalon at Carnegie Stage 15 (CS15) is seen to be very strong in this transverse section (corresponding at the top to section X marked in Fig. 1) from the junction with dorsal thalamus (DT) across a wide area of the developing cerebral vesicles, though not in the most rostral area near the lamina terminalis (LT). Expression is also seen in this section in the ventral spinal cord. (B) At CS17 expression continues to be seen in the ventral spinal cord, but not in the telencephalon or rostral floor of the diencephalon. LV, lateral ventricle (of telencephalon); V3, third ventricle (of diencephalon).

E9.5 (the first stage studied) onwards, both in skeletal muscles and also in the heart (see below). The initial calpain 3 RNA signals were detected in the lens as early as E12.5 (Fig. 5D) where they persisted at least 6 days after birth (see Fig. 5B for evidence of signal at E16.5). This same tissue failed to hybridize with the titin probe (data not shown). In paraxial muscles, calpain 3 transcription was not detected until E13.5, at the same time as appearance of the M isoform of creatine kinase (37), then at E14.5 in the lower limb, and thereafter calpain 3 activity paralleled muscle development (Fig. 5B). It is also evident from Figure 5B that the intensity of the lens signal is far more dense than in skeletal muscle. Furthermore, detailed histological analyses (data not shown) indicate that lens labelling is confined at E12.5 to primary fibers, and extended at E16 to secondary fibers.

Calpain 3 transcription during skeletal muscle development is therefore similar in human and mouse in that it occurs relatively late in comparison with other muscle proteins and in both species occurs subsequent to muscle innervation. Expression of human calpain 3 in the trunk and limb muscular masses was first detected during the 8th week of human embryonic development (31), which is broadly equivalent to the time of onset of calpain 3 expression in mouse paraxial muscles (E13.5).

In contrast to muscle, some other tissues showed very significant human–mouse differences in calpain 3 expression. First, an unanticipated heart expression domain was found in early human development: all cardiac compartments were labelled at week 4, but expression gradually became restricted to the atria from week 7 where it persisted at least up to week 15, the last stage studied (31). In stark contrast, murine calpain 3 expression was never seen by in situ hybridization in any of the heart compartments (Fig. 6C and F), even in adult heart. However, semi-quantitative RT–PCR experiments revealed that in these same adult mouse hearts, calpain 3 RNA is detectable, but is expressed at very much lower levels (~10 000-fold lower than in skeletal muscle tissue; data not shown). Another system that showed species differences in calpain 3 expression was the developing lens: no signal could be detected with any of the calpain 3 specific probes during human development (31; unpublished data), but in contrast, calpain 3 was expressed strongly in the developing mouse lens (Fig. 5B and D). The three calpain 3-specific probes gave equivalent expression patterns in all positively hybridizing mouse tissues except for the lens where the transcripts seemed to lack sequences corresponding to the calpain 3-3′ probe (data not shown). These results agree with the reported presence in the rodent lens of two calpain 3 protein isoforms, namely Lp82 and Lp85 (38). Finally, the IS2 probe revealed evidence for differential splicing in the smooth muscle of the human digestive tract where expression was detected exclusively with a calpain 3–IS2 probe and a calpain 3-specific 3′-terminus probe (31), whereas in mouse smooth muscle labelling was never observed.

Species differences in promoter and exon usage explain some of the human–mouse differences in calpain 3 expression

Following analysis of murine calpain 3 gene isoforms, we were able to establish a basis for some species differences in calpain 3 expression. In particular, an alternative first exon (39), named exon 1′, was found within intron 1 of the mouse calpain 3 gene, ~2 kb upstream from exon 2. Computer-based analysis of the sequence upstream of this new exon suggested that variant calpain 3 transcripts containing this exon would be transcribed from an alternative promoter (Fig. 7A). In situ hybridization and RT–PCR experiments demonstrated that expression of mRNA containing the alternative exon 1 is restricted to lens tissue throughout murine development (34). The corresponding human sequence showed 83% identity with the mouse exon 1′, and both a potential initiating ATG codon and a donor splicing site [Shapiro’s score of 82.4% (40)] at positions corresponding to equivalent murine sequences. However, alignment of the human and the mouse sequences demonstrate that the former lacks four nucleotides at position 124 downstream of the ATG codon and this frameshifting deletion introduces premature stop codons (Fig. 7A and B). In addition, computer analysis failed to detect any human equivalent of the novel mouse promoter sequence. These two lines of evidence argue against the existence of a similar lens-specific mRNA in human and may explain why during human embryonic development no lens labelling was seen with calpain 3 probes.

Additional computer analyses were also performed on human and mouse calpain 3 genomic sequences to find clues that may explain the other species differences in expression pattern. In particular, we examined whether variation in expression could be due to differential splicing and/or differential promoter usage. No significant differences were evident with respect to cardiac-specific transcription factor sites that could explain the stronger level of calpain 3 expression in the human than in the mouse heart. Nor did we find any evidence...
for alternative promoter that could explain the restrictive IS2 and 3′ labelling in human digestive tract.

The human and mouse calpain 3 gene sequences diverge with respect to at least two other features: in the IS2 region, the presence of splicing and branch sites in mouse intron 16, leads to the formation of a new 137 bp ‘exon’ or to 3′ extension of exon 16 to include the first 308 bp of intron 16. This region can direct the formation of three different isoforms in mice, as validated by RT–PCR and in situ hybridization (34). Despite the fact that the first 430 bp of human intron 16 showed 58% homology with the corresponding murine sequence, no significant consensus splice sites were found at the corresponding positions in the human gene or elsewhere within this intron (Fig. 7C).

The mRNAs for the rat lens 82 and 85 kDa calpain 3 isoforms [Lp82 and Lp85, respectively (38)] have similar structures except that the latter retained, between exons 18 and 19, the 84 bp intron 18. The same event was occasionally observed during the characterization of murine isoforms in myogenic cells (34). As Figure 7D shows, the consequence on the translated protein is the in-frame incorporation in Lp85 of a 28 amino acid peptide (with mice and rats diverging at two amino acids only) showing no homology to database sequences. Although the murine and

Figure 3. Expression of Wnt7a in the mouse forebrain. (A and B) Expression of Wnt7a in coronal sections of mouse forebrain at E12.5 (the dorsal aspect of forebrain being at the upper margin of the images), showing extensive signal in the lateral, dorsal and ventral walls of the telencephalon at this stage. Notice that the signal is not confined to the ventricular layer; some expression can also be detected outside this layer, mainly in the intermediate layer (in contrast, no expression is found in the intermediate layer in human CNS at any site or stage). LV, lateral ventricle (of telencephalon); V3, third ventricle (of diencephalon); V, ventricular layer; I, intermediate layer.

Figure 4. Expression of WNT7A/Wnt7a in the developing midbrain. (A) Sagittal section of a CS17 embryo demonstrating the predominant expression of WNT7A in dorsolateral regions (indicated by arrow) of the midbrain (M), whereas ventral expression is seen in the floor of the hindbrain (and spinal cord). V4, fourth ventricle. (B) WNT7A expression in a transverse section through midbrain (M) at CS17 showing the predominantly dorsolateral midbrain expression domain (sulcus limitans, marked by arrow, divides dorsal upper aspect of image from ventral). V4, fourth ventricle. (C) Wnt7a signal in E12.5 mouse at the caudal end of the midbrain (M), demonstrating the domain to be predominantly ventral to the sulcus limitans area (marked by an arrow; the dorsal aspect of midbrain is at the upper margin of both images). A similar Wnt7a expression signal was seen in the rostral end of the midbrain (data not shown). V4, fourth ventricle.
human sequences of intron 18 show 76.7% homology, the human intron is two nucleotides longer. Thus, in contrast to the mouse or rat sequence, this insertion would lead to a frameshift, followed by a stop codon four nucleotides into exon 19. These results suggest that the corresponding calpain 3 lens isoforms might not be expressed in human.

DISCUSSION

This report provides two illustrative examples of genes that show significant human–mouse differences in embryonic gene expression patterns: a very highly conserved developmentally significant gene in one case and a known disease gene in the other case. As more human expression data become available, however, other examples are also being obtained. The WNT7A/Wnt7a study represented part of a systematic human–mouse comparison of the expression of four Wnt genes, which also included WNT5A, WNT8B and WNT11 and their murine orthologues. Significant human–mouse differences were also identified in the case of WNT5A expression in the developing kidney and hindgut (P. Bullen, S. Lindsay, D.I. Wilson and T. Strachan, manuscript in preparation) but expression of WNT8B and WNT11 appears to have been more conserved (41,42). Significant human–mouse differences in embryonic expression have been reported recently for other developmentally important disease genes, such as the SOX10 gene (43), and the RET proto-oncogene (44), although there are also many conserved expression domains too, as in the WNT5A, WNT7A and CAPN3 studies. Comparative conservation of embryonic expression domains is apparent for other genes, as in the case of Hox genes (45) and the sonic hedgehog gene (46).

However, human–mouse differences in the timing of onset/ extinction of gene expression are often apparent in stage-matched embryos in the published studies and in several other gene studies that we have carried out (unpublished data). Thus, a general consensus appears to be that, although temporal expression differences are quite frequent, spatial embryonic gene expression domains are often conserved in humans and mice but that there are some genes where there are significant species differences in spatial expression. Equivalent studies of genes in other species, including comparative analyses in some closely related species, have also documented some very striking species differences in gene expression (14,47). The data should therefore provide a cautionary reminder of the need to verify rodent models using human tissues, where possible.

To what extent do our findings represent functional differences? The lack of Wnt7a-specific and calpain 3-specific antibodies suitable for immunohistochemistry have precluded any attempt to replicate these species differences in expression pattern at the protein level. In addition, even if the divergent expression domains are manifest at the protein level, functional redundancy could be an issue (48). Heart or smooth muscle abnormalities have not been reported to be associated with LGMD2A and, although Wnt7a/WNT7A differences in embryonic expression are confined to the brain, mouse Wnt7a–/– knock-outs do not appear to exhibit brain abnormalities. Could it be, for instance, that the presence of calpain 3 transcripts in the human heart represents an evolutionary remnant that was lost in mice? The status of this calpain activity in other species may provide clues to this question. There is the possibility that non-orthologous members of a multigene family could be responsible for species conservation of some expression domains (49) and it would be interesting to...
investigate whether Wnt genes other than WNT7A replicate the dorsolateral midbrain expression domain seen with the human WNT7A gene.

One additional and important possibility should be considered in such cases: the possibility that some of the species differences in gene expression could underlie functional differences. When we consider that humans are already known to be uniquely different from the great apes in the expression of some gene products (50), then we must expect to see human–mouse differences in the patterns of expression of some genes. We should therefore exercise considerable caution in extrapolating from mouse models, even though they will undoubtedly continue to be very valuable research tools. Already, there are numerous examples where inactivation of a mouse orthologue results in a phenotype that is substantially different from the human disease that it was intended to model (9). Although the differential contribution of modifier genes and species differences in longevity may explain some differences, species differences in gene regulation could also play a part. Certainly, there are some aspects of rodent development that we can expect to provide rather poor parallels with human development, notably various aspects of brain development. The current work emphasizes the importance and value of comparative studies of developmental expression patterns as a means to validate an experimental animal knock-out model.

MATERIALS AND METHODS

In situ hybridization

In situ hybridization was performed on sectioned paraffin-embedded (Wnt probes) or frozen calpain probe, murine embryos and human embryos as described previously (24,31,41–42). Human embryos were obtained in accordance with accepted ethical guidelines (51) and the approval of the Joint Ethics Committee of Newcastle and North Tyneside Health Authority and the University of Newcastle upon Tyne. Previously reported techniques were used for collection (52) and determining Carnegie Stage (53). The sequences in the 5’→3’ direction for individual calpain 3 antisense oligonucleotide probes were as follows:

calpain 3-NS (exon 1), TTGCGGCTAATGATAGCTGAATAGATGCCACTTGGGT-GTCCACCTCCAGCCTCGGTGGTC;
calpain 3-IS1 (exon 6), GCCCCCTGGGGTCCAGGTCTGAGTCTCTGAG-CAGCGAGTTATCCATATTTCTCACCATCCG;
calpain 3-IS2 (exon 16), TCCTGGTCCACACCCAGCTCCTTGTTTGCTCT-GTCCGAAACGAAGATGATGGGC.

The titin antisense oligonucleotide probe (position 606 from the sequences X64700) is:
TGGTGATTTCTGTGACTTTTAAGTTGACAGGTGGGCTT-GGTGTGTCCAGGACCCTT.

The human WNT7A probe template was a full-length cDNA of 1100 bp, kindly provided by Prof. A. Harris (Oxford, UK) and the mouse Wnt7a probe used was a 400 bp fragment containing coding and 3’-untranslated region sequences as reported by Parr et al. (24). Complementary sense probes were systematically used as controls. They routinely gave no signal and therefore images are not included here.

Quantitative RT–PCR

Expression of the calpain 3 gene was investigated by a quantitative RT–PCR method using TaqMan probes (Perkin Elmer, Foster City, CA) (54). Fluorescence emission was monitored using a sequence detector (Perkin Elmer 7700 instrument). The
Figure 7. (A) Schematic diagram showing the organization of the 5′ part of the mouse and human calpain 3 gene. The first in-frame ATG codons in exon 1 and in exon 1′ or pseudo exon 1′ are indicated. The black box corresponds to the predicted alternative for the mouse gene promoter and the X indicates the lack of prediction in the corresponding human sequence. The first in-frame stop codon is shown in the human pseudo exon 1′. AS and DS, acceptor and donor splice sites, respectively. (B) Alignments of nucleotide sequences of the regions corresponding to exon 1′ from three species: h, human; m, mouse exon 1′ (GenBank accession no. AJ224981); r, rat exon 1′ (38,39). The first in-frame ATG codons and the human in-frame stop codons are boxed. The position of the frameshift is indicated. A period indicates that the same nucleotide is present in the homologous sequence and letters refer to the variant nucleotide. Gaps introduced for alignment are denoted by dashes. (C) Human–mouse comparisons of putative splice site sequences in intron 16, demonstrating the absence of conservation in humans of the putative mouse splice site signals. AS, DS and BS, acceptor, donor and branch sites, respectively. Gaps introduced for alignment are denoted by dashes. (D) Alignments of nucleotide sequence (lower case) and amino acid sequences for regions corresponding to intron 18 of the human (h), mouse (m) and rat (r) genes. The putative mouse splice site signals. AS, DS and BS, acceptor, donor and branch sites, respectively. Gaps introduced for alignment are denoted by dashes.

ubiquitous transcription factor TFIID was used to normalize the data across samples.

Computer analyses

Three programs were used to predict the existence of a promoter: the prediction program, Proscan (55), and TFSEARCH and MatInspector (56) in comparison with the Transfac database (57).

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