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REVIEW

# Transcriptional regulation and biological significance of the insulin like growth factor II gene

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**Abstract.** The insulin like growth factors I and II are the most ubiquitous in the mammalian embryo. Moreover they play a pivotal role in the development and growth of tumours. The bioavailability of these growth factors is regulated on a transcriptional as well as on a posttranslational level. The expression of non-signalling receptors as well as binding proteins does further tune the local concentration of IGFs. This paper aims at reviewing how the transcription of the IGF genes is regulated. The biological significance of these control mechanisms will be discussed.

The insulin like growth factors I and II (IGF I and IGF II) belong to a family of structurally related polypeptides which also include insulin and relaxin (Blundell & Humbel 1980; Dafgård *et al.* 1985). Studies of the evolutionary pathway of this family have suggested that insulin and insulin like growth factors became distinct molecules only after vertebrates arose. Experimental evidence for this notion was provided by the finding that a single molecule with homology to both insulin and the IGFs exists in *Amphioxus lanceolata*. However, recently two different cDNAs representing a primitive insulin and a primitive IGF were isolated from the tunicate *Chelyosoma productum* indicating that insulin and the IGFs have in fact maintained separate lineages in both vertebrate and prochordate evolution (McRory & Sherwood 1997).

IGFs were discovered on the basis of their ability to stimulate cartilage sulphation and to replace the sulphation factor activity of growth hormone both in *in vivo* and *in vitro* test systems (Salmon & Daughaday 1957). The biological significance of this finding was rapidly expanded beyond the study of cartilage sulphation to include stimulation of DNA replication, proteoglycan synthesis, glucosamine synthesis and protein synthesis and accumulation (Jones & Clemmons 1995). Purification and subsequent amino acid sequence determination revealed the existence of two separate molecules that were denominated by their high degree of homology with insulin, IGF I and IGF II (Rinderknecht & Humbel 1978a and b).

IGF I and IGF II are single chain polypeptides that in most species contain 70 and 67 amino acids, respectively (Daughaday & Rotwein 1989, Ward & Ellis 1992). In both cases,

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the mature peptides consist of four distinct domains: A, B, C and D. The A and B chains show strong homology with pro insulin. Both IGF I and IGF II are produced as pre-propeptides that contain a signal peptide as well as a trailer peptide. In IGF I, there are different signal and trailer peptides that combine to yield different precursor molecules. Hence multistep post-translational processing is required to obtain identical end-products.

The 3D-structure of the IGFs was modelled onto the X-ray crystallographically determined structure of insulin (Blundell *et al.* 1978, Dafgård *et al.* 1985). The predicted IGF structures were essentially confirmed by experiments determining the nuclear magnetic resonance (NMR) solution structures of IGF I and IGF II (Blundell *et al.* 1978, Dafgård *et al.* 1985, Cooke *et al.* 1991, Sato *et al.* 1992, 1993, Terasawa *et al.* 1994, Torres *et al.* 1995).

The IGFs bind to and exert most, if not all, of their biological actions via three membrane receptors; the type I and type II IGF receptors and the insulin receptor (Engström & Heath 1988). The affinities and kinetic properties differ between each of the ligand-receptor interactions. The type I IGF receptor has the highest affinity for IGF I and the type II receptor the highest affinity for IGF II. The insulin receptor binds both IGF I and IGF II with low affinity (Steele-Perkins *et al.* 1988, Nissley & Kiess 1991, Werner *et al.* 1992).

The type 1 IGF receptor, like the insulin receptor, is a heterodimeric transmembrane protein that consists of two alpha and two beta subunits. Ligand binding induces tyrosine specific autophosphorylation of the receptor, as well as of cytoplasmic substrate proteins, which is followed by a pleiotropic biological response. The type I IGF receptor is nowadays considered to be responsible for nearly all biological effects exerted by the IGFs (de Meyts *et al.* 1994, Jones & Clemmons 1995). The functional relationship between IGF II and the insulin receptor was unclear for some time, in particular since it was shown that tumour hypoglycaemia increases the rate of IGF II transcription (Schofield *et al.* 1989). In a key experiment it was demonstrated that the insulin receptor can mediate the mitogenic messages of IGF II but not of IGF I (Morrione *et al.* 1997)

In contrast, the type II IGF receptor is a monomeric protein which consists of a major extracellular portion which contains 15 repeats of a cysteine-rich sequence, as well as a single hydrophobic transmembrane helix and a minor cytoplasmic sequence. The type II receptor was found to be the equivalent of the mannose-6-phosphate receptor (Morgan *et al.* 1987). However, IGF II and mannose-6-phosphate occupy two separate binding sites on this receptor (Braulke *et al.* 1988). Binding of any of the ligands does not induce a phosphorylation response from this receptor. Its role rather appears to be to participate in endocytosis as well as the sorting of lysosomal enzymes. The type II receptor is also involved in membrane trafficking through rapid cycling between cytosolic membrane compartments and the plasma membrane. It induces a redistribution of receptors (Braulke & Mieskes 1992) as well as modulating insulin exocytosis under physiological conditions (Zhang *et al.* 1997). In keeping with this cell biology, mouse genetic experiments indicate that the type II receptor acts primarily as a scavenger for IGF II. Loss or inactivation of the type II receptor gene results in a general overgrowth that is ameliorated in the absence of the IGF II ligand (Filson *et al.* 1993, Wang *et al.* 1994).

The mature IGF I and IGF II molecules that are released into the bloodstream circulate as conjugates with high affinity binding proteins (McCusker & Clemmons 1992). Six different human binding proteins as well as six rodent binding proteins have been isolated and are well characterized. In addition some binding proteins have been purified from porcine, bovine and ovine tissues, but their characterization remains to be completed (Shimasaki & Ling 1991, Rechler 1993). The existence of additional binding proteins in man and rat have been proposed (Chan & Nicholl 1994, Wilson *et al.* 1997). The binding proteins differ in binding

characteristics as well as in their tissue distribution. This points to the binding proteins having a plethora of roles that include prolonging the half-life of IGFs, acting as a main transporter, inhibiting or promoting IGF action or as a storage of presynthesized IGFs (Jones & Clemmons 1995).

## THE ROLE OF IGF II IN GROWTH AND DEVELOPMENT

IGF I and IGF II display a wider range of developmental and tissue-specific expression than any other known growth factors (Schofield 1992, Schofield *et al.* 1993). It is generally implied that they play a pivotal role in promoting embryonic and fetal growth. Although the IGFs were originally believed to act as classical hormones, mediating the action of growth hormone, they are now known to act in a paracrine as well as an autocrine fashion. During development many fetal tissues express IGF II from early post implantation onwards (Scott *et al.* 1985, Hyldahl *et al.* 1986). It is noteworthy that type I IGF receptors are expressed either by the IGF expressing cells or by adjacent cells, which forms a prerequisite for paracrine or autocrine loops (Schofield 1992).

A large variety of normal and neoplastic cells cultured *in vitro* express the IGF II gene. The level of expression can be influenced by a variety of culture conditions including the serum concentration. In addition to the *bona fide* 67 amino acid IGF II protein there are examples of high molecular weight variants produced by cells cultured *in vitro* (Gowan *et al.* 1987, Schofield *et al.* 1990, Granerus *et al.* 1993) that show a different affinity to the IGF receptors (Schofield *et al.* 1994). The biological implications of these variant IGF II molecules are unclear, but it has been suggested that the competition for the type I receptor might modulate the amplitude of the biological response (Schofield *et al.* 1994). IGF II exerts a wide range of biological activities in cells in culture: It promotes cell proliferation by acting on the cell division cycle (i.e. DNA-replication and mitosis) as well as on the cell growth cycle (cellular enlargement) (Zetterberg *et al.* 1984, Dafgård 1990); It induces differentiation *in vitro*, an effect which has been characterized in detail in myoblasts (Florini *et al.* 1991); It profoundly affects cellular survival and counteracts apoptosis in some cell systems (Biddle *et al.* 1988, Granerus *et al.* 1995, Granerus & Engström 1996), whereas in other cell lines there appears to be an apoptosis-inducing effect by IGF II (Granerus *et al.* 1998); Its release also induces a functional modulation, without otherwise altering the differentiated phenotype, in certain cell types; It stimulates hormone synthesis and secretion in ovarian granulosa and theca cells (Giudice 1992); It also binds to the type I receptor and thereby potentiates the release of histamine from basophils in response to immunoglobulin E (Hirai *et al.* 1993); Finally, it has been shown that IGF II can stimulate motility in cultured rhabdomyosarcoma cells (Minniti *et al.* 1992).

The human eye has been a useful model for growth factor activity. By using embryonic eye bulbs, cut open at the limbus, it was possible to assay short-term effects of defined tissue culture media on the proliferation of the different corneal cell layers (Hyldahl 1986). This technology opened up a possible route to assay how individual growth factors affected the sensitive corneal endothelial cells. It was shown subsequently that IGF II (as well as IGF I and basic FGF) leads to an increased proportion of S-phase cells in the corneal endothelial layer (Hyldahl *et al.* 1986, 1990, Storckenfeldt *et al.* 1991). These data became interesting in the light of data that showed that IGF II (along with bFGF) was expressed preferentially in the posterior eye, whereas the cornea displayed a completely silent IGF II gene. Thus it was concluded that IGF II acts in a paracrine fashion, being synthesized at the back of the eye

and then transported via the aqueous humour to the corneal endothelium where it exerts its growth stimulatory action.

It has been known for some time that overproduction of IGF II has been observed in some rare genetic syndromes (e.g. Wiedemann Beckwith syndrome (Engström *et al.* 1988, Schofield *et al.* 1989, Nyström *et al.* 1992a,b, Ekström *et al.* 1992, Ward 1997). Wiedemann Beckwith syndrome leads to overgrowth as well as growth disturbances and increased frequencies of neoplasia (Nyström *et al.* 1992a,b, Schofield & Engström 1992). Clinical evidence accumulated over time has therefore suggested that increased levels of IGF II exert a genuine effect on growth and development *in vivo*. Overexpression of IGFs in transgenic mice has resulted in altered growth properties. Increased expression of an IGF I transgene leads to increased bodyweight and a limited overgrowth. Different tissues responded differently and growth disturbances and tumour formation was sometimes observed (Matthews *et al.* 1988, Coleman *et al.* 1995, Reiss *et al.* 1996, Bol *et al.* 1997). In several experimental situations, prolonged IGF II expression from transgenes using tissue-restricted regulatory elements, has lead to organ overgrowth and tumour formation (Ward *et al.* 1994, Rogler *et al.* 1994, Bates *et al.* 1995, van Buul-Offers *et al.* 1995, Rossetti *et al.* 1996). More generalized IGF II overexpression has been achieved by introducing additional copies of the IGF II gene into embryonic stem cells, which were then used to generate chimaeric mice (Sun *et al.* 1997). An alternative approach to study how increased levels of IGF II can affect overall growth properties was to assay double mutant mice carrying a deletion around the H19 region as well as a targeted IGF type 2 receptor allele. Such mice have extremely high levels of IGF II and display most of the clinical features of the Wiedemann Beckwith syndrome as well as skeletal defects and a cleft palate, which are features of the Simpson-Golabi-Behmel syndrome (Eggenschwiler *et al.* 1997). In both of these models of more general overgrowth, the affected animals die perinatally thus making it impossible to assess their susceptibility to neoplasms.

The development of the transgenic technology has also rapidly made it possible to examine the effects of growth factor deficiency *in vivo*. When a disrupted IGF II gene was introduced into the mouse germ line, the prenatal growth rate decreased and the body weight at term only reached 60% of the normal birth weight. However, the growth rate *post partum* appeared to be normal (de Chiara *et al.* 1990). Likewise, knockout-mice carrying null mutations for the IGF I gene lead to a significantly decreased birthweight, but with otherwise normal body proportions. Unlike the IGF II deficient mice, these transgenic animals had a decreased postnatal growth rate and a high degree of neonatal lethality (Baker *et al.* 1993, Liu *et al.* 1993).

## THE STRUCTURAL ORGANIZATION OF THE IGF II GENE

The initial isolation of and characterization of cDNA clones encoding human (Bell *et al.* 1984) and rat (Dull *et al.* 1984) IGF II enabled further studies of possible evolutionary relationships between IGFs as well as comparisons of the IGFs with other members of the insulin gene family. To date, cDNA cloning and subsequent sequencing of the coding region in a variety of species has revealed a remarkable degree of conservation which has persisted throughout evolution. By comparing the human nucleotide sequence as well as the predicted protein primary structure with the coding sequences of mouse (Bell *et al.* 1986), pig (Catchpole & Engström 1990), sheep (O'Mahoney & Adams 1989), cow (Boulle *et al.* 1993), mink (Ekström *et al.* 1993) and horse (Otte & Engström 1994, Otte *et al.* 1996), it was found that differences occur at a maximum of only six out of 67 amino acids, and these are mostly

**Table 1.** Nucleotide and derived amino acid sequence homology between human and other vertebrate IGF II genes

| | Species homology (%) | Nucleotide sequence homology (%) | Amino acid homology (%) |
|---|---|---|---|
| Human | 100 | | 100 |
| Mink | | 92 | 100* |
| Horse | | 92 | 95 |
| Pig | | 90 | 97 |
| Pig | | 90 | 97 |
| Sheep | 85 | | 86 |
| Rat | | 87 | 85 |
| Mouse | 84 | | 84 |

*Plus one inserted amino acid

conservative changes. The notable exception is mink which has an extra inserted amino acid which yields a 68 amino acid final protein product (Ekström *et al.* 1993) (Table 1).

Complete genomic sequence information is as yet only available for the mouse and rat IGF II genes (Ikejiri *et al.* 1990, Sasaki *et al.* 1996). In addition large parts of the human (de Pagter-Holthuizen *et al.* 1986, 1987), ovine (Ohlsen *et al.* 1994) and equine (Otte *et al.* 1998) IGF II genes have been sequenced. Whereas the mouse and rat genes span over a 12-kilobase distance, the equine IGF II gene only spans a 9-kB distance (Otte *et al.* 1998). The human gene is substantially longer, covering more than 30 kB (de Pagter-Holthuizen *et al.* 1987, van Dijk *et al.* 1992).

The human IGF II gene is located on the distal tip of the short arm of chromosome 11 (11p15.5) where it is closely linked to the loci for insulin and tyrosine hydroxylase (Brissenden *et al.* 1984). This chromosomal region has aroused considerable interest since it contains several disease loci including the Beckwith Wiedemann locus, the insulin dependent diabetes mellitus locus and the Long QT syndrome locus (Junien *et al.* 1991, Higgins *et al.* 1994). The rodent IGF II genes appear to have a similar linkage group, either on chromosome 1 in the rat (Frunzio *et al.* 1986, Soares *et al.* 1986) or chromosome 7 in the mouse (Rotwein & Hall 1990). The recently revealed homology between the HSA11p15.5 and an equine locus ECA12 indicates that the entire region with its linked genes might in itself be a universal phenomenon (Raudsepp *et al.* 1997).

Vertebrate IGF II genes (with mink and chicken as notable exceptions) encode a 180 amino acid precursor protein which consists of a 24 residue amino terminal signal peptide, a 67 amino acid core IGF II and an 89 amino acid trailer sequence. The signal and trailer sequences are proteolytically removed post-translationally.

## DEVELOPMENTAL REGULATION OF IGF II TRANSCRIPTION

In all species hitherto examined, the IGF II gene comprises several exons and multiple promoters, thereby giving rise to multiple transcripts (Schofield & Tate 1987, Hedley *et al.* 1989, Joujou-Sisic *et al.* 1993, Bäcklin *et al.* 1998). It consists of 10 exons in the human, nine exons in sheep and six in rodents and the horse (Figure 1). The exon-intron organization and the structure of regulatory elements are partly conserved between species. In rodents as well as in the horse exons 4, 5 and 6 encode the 180 base pairs (bp) precursor protein. In contrast exons 1, 2 and 3 are non coding and are used to form alternative 5′-untranslated regions of different IGF II transcripts. The six rodent exons have corresponding counterparts in the

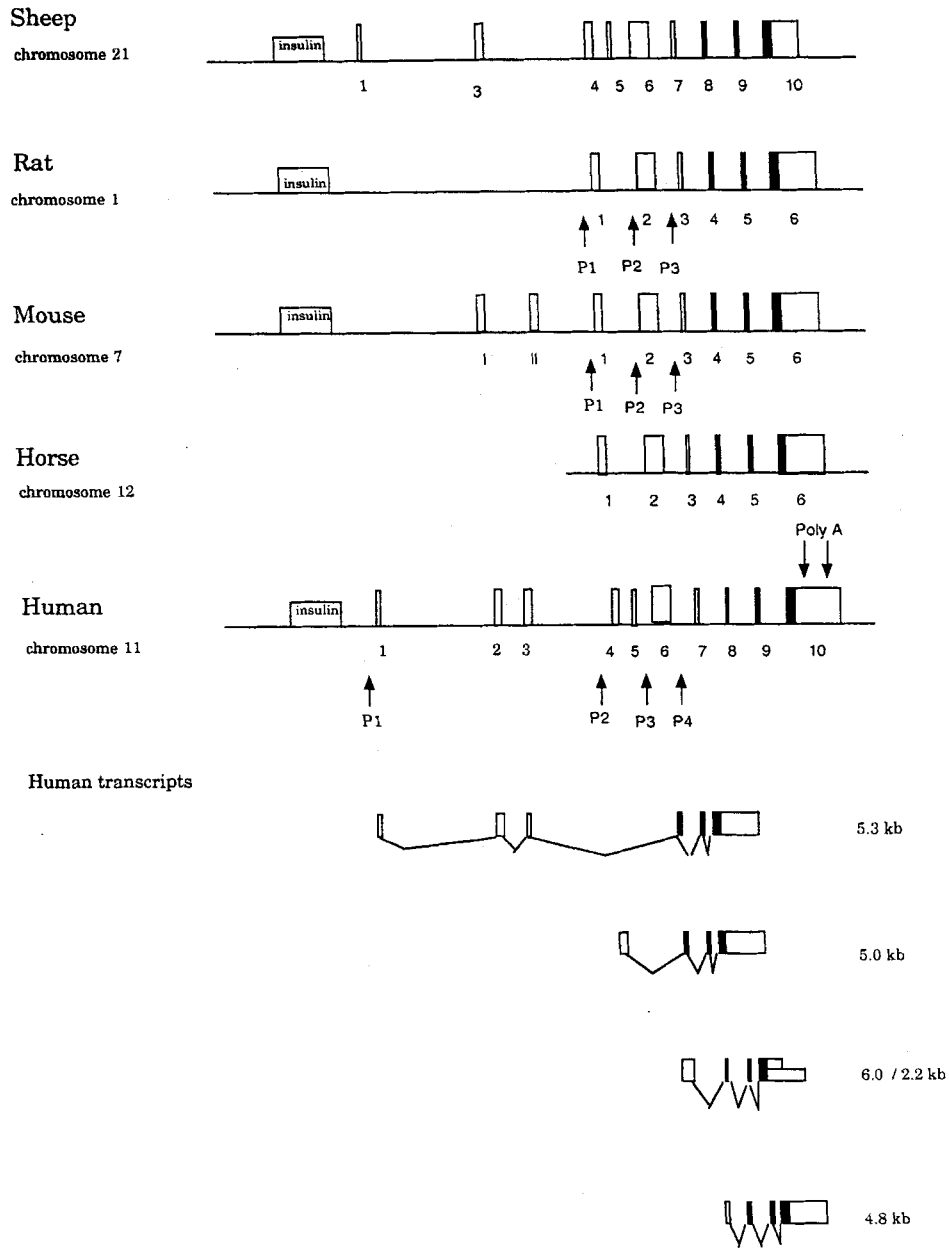


**Figure 1.** An overview of the IGF II gene in different species and different human mRNAs is presented. Exons 1–10 are indicated by boxes. Coding exons are shown as solid boxes and noncoding as open boxes. Promoters (P) and polyadenylation sites are indicated on the human IGF II gene. Transcripts and respective length derived from human IGF II are shown below.

human but in addition the human and ovine IGF II genes have three additional non coding 5′-exons.

The transcription of the IGF II gene is driven by multiple promoters each associated with different 5′-untranslated leader exons, that generate transcripts that differ in length (Figure 1). In addition there are alternative splicing mechanisms that further modulate the 3′-non-translated part of the transcripts. In rodents there are three promoters (P1, P2, P3) that control the transcription of each of the three leader exons. Consensus sequences including a TATA box and GC boxes are present in the region upstream of the P2 and P3 (Kadonaga *et al.* 1986, Frunzio *et al.* 1986, Soares *et al.* 1986). Experimental evidence suggests that the GC boxes within the P2 and P3 promoters function as SP1 recognition sequences (Evans *et al.* 1988, Matsuguchi *et al.* 1990).

The most upstream rodent promoter (P1) lacks TATA and GC elements and the starting sites are heterogeneous (Ueno *et al.* 1987). Recently the P1–P3 promoters have been identified and partly characterized in the horse (Otte *et al.* 1998).

In addition, the human, ovine and baboon genes contain an extra promoter (P1′), located 5′ to the most upstream leader exon (Schofield & Tate 1987, Ohlsen *et al.* 1994, Jin *et al.* 1995). The start of this exon lies only 1.4 kB from the insulin gene in the human genome (Schofield & Tate 1987). The human P1′ promoter contains no TATA or CAAT boxes but includes an SP1 recognition signal and a repeated GC rich motif. It has the capacity to bind the transcription factors C-EBP-a and C-ERB-b as well as LAP (van Dijk *et al.* 1992, Sussenbach *et al.* 1993, Rodenburg *et al.* 1996). This promoter can be downregulated by protein(s) that bind to two inverted repeat elements situated within reach from the major transcript initiation site (Rodenburg *et al.* 1996). Both the human and rodent P1 promoters are relatively weak promoters that are heterogeneous with respect to start sites (Ueno *et al.* 1987, van Dijk *et al.* 1993). In the horse this promoter appears to be silent at least in the tissues examined (Otte *et al.* 1998).

The rodent and human P2 promoters contain TATA and CAAT boxes as well as two SP1 recognition sequences and two Egr 1 binding motifs (Frunzio *et al.* 1986, Soares *et al.* 1986, de Pagter-Holthuizen *et al.* 1987, van Dijk *et al.* 1993). The primary structure of this promoter region is strongly conserved also in the ovine and bovine IGF II genes (O'Mahoney *et al.* 1991, Boulle *et al.* 1993). The P2 promoter is repressed by binding of the WT1 protein and as well as by the p53 protein (Drummond *et al.* 1992, Ward *et al.* 1995, Zhang *et al.* 1996).

The human and rodent P3 promoters contain a single TATA box and several SP 1 recognition sequences. The P3 promoter is activated by binding of the AP1 complex (Caricasole & Ward 1993) and repressed by the binding of the WT1 protein (Drummond *et al.* 1994, Ward *et al.* 1995) and by p53 (Zhang *et al.* 1998).

The IGF II gene is active in nearly all human embryonic and fetal tissues (Scott *et al.* 1985). The quantity of the transcripts differ considerably between organs, but in all cases the transcription is driven from the P1–P3 promoters with P3 being predominantly active. The transcription of the IGF II gene declines rapidly after birth in most tissues. In rodents, in most tissues where IGF II is expressed at high levels throughout embryogenesis and fetal development, all three promoters are downregulated after birth and the transcriptional activity continues only in exchange tissues surrounding the central nervous system (Soares *et al.* 1985, 1986, Brown *et al.* 1986, Frunzio *et al.* 1986, Lund *et al.* 1986, Beck *et al.* 1987, Gray *et al.* 1987, Murphy *et al.* 1987, Ueno *et al.* 1988, Lee *et al.* 1990).

In human adult life, transcripts derived from P1′ are exclusively found in liver and choroid plexus—leptomeninges. These transcripts contain an internal ribosomal entry site in their

leader sequence (Teerink *et al.* 1995, Li *et al.* 1996). P1 derived transcripts are usually found in low quantities in fetal liver and only reach higher levels in transformed cell lines or in neoplastic tissues. P3 transcripts are polysomal and therefore used for protein synthesis (Nielsen *et al.* 1990, Ikejiri *et al.* 1991, de Moor *et al.* 1994, Newell *et al.* 1994). In contrast, P2 and P3 derived transcripts are found in fetal as well as adult tissues with the P3 promoter being predominantly active. In the mouse, transcripts derived from the P2 promoter disengage from polysomes during development (Newell *et al.* 1994) and in a variety of cultured cells (de Pagter-Holthuizen *et al.* 1987, Schofield and Tate 1987, Nielsen *et al.* 1990, de Moor *et al.* 1994, Nielsen *et al.* 1995, Li *et al.* 1996).

Human as well as rodent IGF II mRNAs are degraded by endonucleolytic cleavage downstream from the translation termination codons (3'UTR). The cleavage occurs in a highly conserved motif which contains two large hairpins and an intramolecular guanosine quadruplex (Christiansen *et al.* 1994) which offers a binding site for transacting factors (Scheper *et al.* 1996a). The endonucleolytic cleavage of IGF IImRNAs, like the translational switch-off of P2 derived transcripts, depends on the growth conditions in cultured cells (Scheper *et al.* 1996b), however, this may not be true *in vivo* (Newell *et al.* 1994).

## IMPRINTING OF THE IGF II GENE

Genomic imprinting is a form of developmental gene regulation whereby only one of the parental alleles is expressed. As more examples of imprinted genes are being discovered, it is becoming obvious that these sequences are clustered into chromosomal domains, implying that imprinting may be regulated in a regional fashion. The IGF II gene was one of the first genes shown to be imprinted and it was clearly shown that the paternal IGF II allele is transcribed whereas the maternal allele is silent (de Chiara *et al.* 1991). This principle is persistent in rodents as well as in man (de Chiara *et al.* 1991, Giannoukakis *et al.* 1993, Ohlson *et al.* 1993, Rainier *et al.* 1993, Pedone *et al.* 1994) with some notable exceptions. In adult life both alleles are transcribed in human liver as well as in the central nervous system. In man, there appears to be a fundamental difference between the different promoters, since the fetal promoters (P2, P3, P4) are clearly subject to imprinting whereas the adult P1 promoter is not (Vu & Hoffmann 1994). Moreover, imprinting of the IGF II gene is relaxed in a variety of human neoplastic tissues. Biallelic expression of the IGF II gene has been reported in Ewing sarcoma and rhabdomyosarcoma (Zhan *et al.* 1995a,b) kidney tumours (Wilms tumour, clear cell sarcoma and renal cell carcinoma) (Oda *et al.* 1997, 1998, Okamoto *et al.* 1997, Sohda *et al.* 1997, Zhan *et al.* 1995), Glioma (Uyeno *et al.* 1996) and a variety of gynaecological (Yaginuma *et al.* 1997) and testicular (Nonomura *et al.* 1997) tumours. It has recently been demonstrated that loss of imprinting is a stage specific event during carcinogenesis (Harris *et al.* 1998). Given the evidence that IGF II can be the experimental cause of tumour formation in mice (Christofori *et al.* 1994, Rogler *et al.* 1994), loss ofimprinting mutations may be an important route to increased IGF II expression in many types of tumours.

Parental imprinting, like other transcriptional silencing mechanisms, has been frequently suggested to depend on DNA methylation (Li *et al.* 1993). Evidence in support of this notion was provided by Rudolf Jaenisch's laboratory who were the first to produce mice where the DNA methyltransferase gene had been disrupted (Li *et al.* 1993). Such animals displayed an abberant expression of the IGF II gene as well as of the neighbouring H19 gene. Different methylation patterns on the two alleles have been established on most imprinted genes currently known. This is certainly true for the IGF II receptor gene and the H19 gene, in

addition to the IGF II gene (Bartolomei *et al.* 1991, 1993, Brandeis *et al.* 1993, Stöger *et al.* 1993, Feil *et al.* 1994, Tremblay *et al.* 1995).

However, there is no clear-cut relationship between methylation and inhibition of transcription. In the mouse IGF II gene, two differentially methylated regions (DMRs) have been mapped. One is located 5′ to the first exon whereas the second resides in the 3′ region of the gene. Both DMRs are more heavily methylated on the paternal allele which is transcribed (Sasaki *et al.* 1992, Feil *et al.* 1995). Whereas these regions are clearly involved in the overall transcriptional regulation of the IGF II gene (Dell *et al.* 1997), methylation of the DMRs is not a primary imprinting signal but rather acts by maintaining the established imprint (Razin & Cedar 1994).

By using different inhibitors of DNA methyltransferase it was possible to examine the effects of induced demethylation on the imprinting status. Demethylation leads to increased overall expression of IGF II (Eversole-Care *et al.* 1993, Hu *et al.* 1996). Alternatively, a switch from monoallelic to biallelic expression, or a silencing of the paternal allele and expression of the maternal imprintes allele (i.e. an allelic switch) was observed (Hu *et al.* 1996).

Other epigenetic mechanisms have been suggested as controllers of imprinting. Imprinted genes are sometimes found in clusters, suggesting a possible involvement of higher order regulatory elements controlling expression and imprinting of genes organized in such clusters. The murine IGF II gene is physically linked to five imprinted genes: Mash2, Ins-2, H19, kvLQT and p57 kip2 (Bartolomei *et al.* 1991, Deltour *et al.* 1995, Guillemot *et al.* 1995, Hatada & Mukai 1995, Manniens & Wilde 1997). The H19 gene which is an expressed but not translated gene is imprinted oppositely, i.e. it is only transcribed from the maternal allele with the paternal copy being overmethylated (Bartolomei *et al.* 1991, 1993, Ferguson-Smith *et al.* 1993, Feil *et al.* 1994, Tremblay *et al.* 1995). Rapidly accumulating evidence suggests that the IGF II and H19 genes are under some common control mechanism. On the maternal allele, regional cis-acting elements preferentially activate H19 and are thus unavailable to interact with the distant IGF II gene, leaving it in a silent state. On the paternal allele, however, DNA methylation of regulatory sequences flanking H19 prevents these contacts and the enhancers are free to turn to the IGF II gene (Leighton *et al.* 1995). The location of enhancer elements is essential for the imprinting of both the H19 and IGF II genes (Webber *et al.* 1998). This model has since been modified since it was found that repeat sequences and modifications of chromatin structure influenced the imprinting phenomenon (Banerjee & Smallwood 1995). It was shown that a specific loss of the maternal H19 allele induces changes in IGF II gene methylation both in the expressed sequences and the putative regulatory regions (Forne *et al.* 1997). Also a tandem repeat in the mouse IGF II upstream region was discovered that may participate in the control of tissue-specific methylation dependent expression (Moore *et al.* 1997).

The biological significance of the parental imprinting has been the subject of a great deal of discussion (reviewed in Ward *et al.* 1994). A number of explanations have been offered for the imprinting phenomenon being a mechanistic adaptation which prevents unwarranted parthenogenetic development (Solter 1988). One elegant hypothesis postulates that imprinting reflects an ongoing struggle between maternal and paternal genomes (Haig & Westoby 1989). The finding that the IGF II and the type II receptor genes are oppositely imprinted supports this hypothesis (Haig & Graham 1991). According to these authors, the maternally produced type II receptor acts as a scavenger for paternally expressed IGF II before the growth factor can reach the signal transducing type I receptor. Other recently proposed explanations for the imprinting phenomenon include the result of dominance

modification (Sapienza 1989), a means of restraining placental growth (Hall 1990), a mechanism for providing exact levels of growth factor concentration (Cattanach 1991), a result of host defence mechanisms (Barlow 1993) and finally a protective device against germ cell tumours in females (Varmuza & Mann 1994). Whatever the underlying reason, the existence of imprinting must confer a selective advantage that outweighs the susceptibility of imprinted genes to loss of imprinting mutations.

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