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Evolutionary conservation of alternative splicing in chicken

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Abstract

Alternative splicing represents a source of great diversity for regulating protein expression and function. It has been estimated that one-third to two-thirds of mammalian genes are alternatively spliced. With the sequencing of the chicken genome and analysis of transcripts expressed in chicken tissues, we are now in a position to address evolutionary conservation of alternative splicing events in chicken and mammals. Here, we compare chicken and mammalian transcript sequences of 41 alternatively-spliced genes and 50 frequently accessed genes. Our results support a high frequency of splicing events in chicken, similar to that observed in mammals.

Alternative splicing is the mechanism whereby different transcripts are generated from a single gene. Major alterations in function, regulation, cellular and subcellular localization, and/or abundance, may result from cell-, tissue-and developmental stage-specific alternative splicing events. It is estimated that there are ~30,000 genes in the genomes of higher eukaryotes, including chicken. As many as one-third to two-thirds of mammalian genes are believed to be alternatively spliced resulting in the production of proteins in excess of 100,000 (Croft et al., 2000; Modrek and Lee, 2002; Johnson et al., 2003). Alternatively splicing therefore represents a source of great protein diversity and potential functional diversity in complex organisms.

With the sequencing and analysis of genomes from different species, it has become possible to address the evolutionary conservation of alternative splicing events. Because of the wealth of data available from the human and mouse genomes, most of the studies done to date have involved comparison of these two mammalian species. For example, based on ESTs and mRNAs corresponding to the splice junctions of 2,932 introns from 786 genes, Thanaraj et al. (2003) estimated that 61% of alternative and 74% of constitutive splice junctions are conserved between mouse and human. Analysis of 9,434 human and mouse orthologues by Modrek and Lee (2003) led to the conclusion that exons that are only included in alternative splice forms are poorly conserved in mouse and human, suggesting that they are the products of recent exon creation or loss. In agreement with this study, a survey of 10,818 pairs of human and mouse genes representing 104,103 human splice forms revealed that the majority of human alternative splices are either divergent (49%) or novel (44%) when compared to mouse transcript and genome sequences (Kan et al., 2005). In a recent study, Pan et al. (2005) reported that species-specific alternative splicing of conserved exons is

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relatively common (>11%) in human and mouse, indicating an important role in the evolutionary differences between mammalian species.

Splicing regulatory elements include the 5′ and 3′ splice sites, the adenine-containing branchpoint sequence, and splicing enhancer and suppressor elements (reviewed in Black, 2003). Small nuclear ribonucleoproteins (snRNPs) and related cofactors bind these *cis* splicing regulatory elements which, in turn, associate with each other to form the spliceosome. Alternative splicing involves the binding of *cis* splicing suppressor and enhancer elements by *trans* factors, including heterogeneous nuclear ribonucleoproteins (hnRNPs) and serine-arginine-rich or related proteins. Factors and elements involved in alternative splicing are well-conserved in higher eukaryotes (Boue et al., 2003; Kondrashov and Koonin, 2003; Lareau et al., 2004; Resch et al., 2004).

Comparison of mammals with the phylogenetically distant chicken offers a unique opportunity to address the functional importance of alternatively spliced products and their evolutionary conservation. Here, we carry out a detailed study of 41 relatively well-studied alternatively spliced genes for which information is available in chicken and mammals. Twenty-five of these genes play a role in the retina, while 16 were selected based on reported alternative splicing in chicken. As a more general strategy, we also carry out a survey of the 50 most frequently accessed genes at the GeneCards homesite to assess the frequency of alternative splicing in human versus chicken genes.

Alternative splicing of genes expressed in the retina

Our first strategy was to identify mammalian genes expressed in the retina which have well-documented alternatively spliced forms. We concentrated on genes expressed in the retina as this tissue is remarkably well-conserved both structurally and functionally in chicken and mammals, suggesting the splicing events may be similarly conserved. The retina is composed of six classes of neuronal cells (ganglion, amacrine, interplexiform, bipolar, horizontal, photoreceptor) and one class of glial cells (Müller) derived from multipotent neuroectodermal precursor cells (Turner and Cepko, 1987; Wetts and Fraser, 1988). The orderly appearance of each class of retinal cells, followed by their migration to distinct parts of the retina, culminates in the formation of three nuclear layers (ganglion, inner and outer), separated by plexiform layers.

We used a combination of approaches, including literature survey, and examination of DNA and EST databases to determine which of the alternative splice variants found in mammals were conserved in chicken (Table 1). Coding regions shown to undergo alternative splicing were used to search chicken EST databases using the ‘tblastn’ search criteria. A total of 508,967 ESTs are listed in the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/) which includes >40,000 ESTs from the University of Delaware database (http://www.chickest.udel.edu/) and 339,314 ESTs from the BBSRC database (http://www.chick.umist.ac.uk/). When possible, we have divided the genes into categories; e.g. homeobox genes *(PAX6, SIX3, OTX2, VSX1),* photoreceptor genes *(ARB1, GRK1, PDE6),* cell signaling genes *(RELN, DAB1),* glutamate receptors and transporters *(GLT1, GRM1, GRIN1),* oncogenes and tumor suppressors *(MAF, WT1).*

PAX6, SIX3, OTX2, VSX1

A well-studied gene in the developing retina is the homeobox-containing paired box gene 6 *(PAX6)*. Two alternatively spliced Pax6 isoforms have been identified (Pax6(−5a) and Pax6(+5a)), the longest of which has an exon 5a (14 amino acid) insertion in its paired domain. These two Pax6 isoforms, which are evolutionary conserved in human, mouse and chicken, differ in the DNA binding properties of their respective paired domains (Azuma et al., 2005) (Table 1). Azuma et al. (2005) have shown that ectopic over-expression of Pax6(+5a) in the eyes of developing chick embryos induces a well-differentiated retina-like structure, in keeping with a role for Pax6(+5a) in playing a role specific to a specialized region of the retina called area centralis or fovea where the image of an object is centered.

The sine oculis homeobox homolog 3 *(SIX3)* is another homeobox gene expressed in the retina. Two alternatively spliced Six3 transcripts have been identified in mouse (Kawakami et al., 1996). Only the longer form of Six3 (Six3-beta) has been reported in humans and chicken. Otx2 (orthodendicle homolog 2) controls photoreceptor cell fate in the developing retina (Nishida et al., 2003). Two Otx2 splice variants have been reported, a long form and a shorter form derived from an alternative 5′ UTR and 3′ splice acceptor site in the second coding exon. Both forms are expressed in chicken based on DNA and EST database searches. A fourth homeobox gene expressed in the retina, *VSX1* (visual system homeobox 1), is required for terminal differentiation of cone bipolar cells (Hayashi et al., 2000; Ohtoshi et al., 2004). Of the two Vsx1 splice isoforms reported in human, only one has been identified in chicken.

Arrestin, rhodopsin kinase, PDE6G

In photoreceptors, light activates rhodopsin which in turn activates the G-protein, transducin. Rhodopsin remains active until it becomes phosphorylated by rhodopsin kinase. Arrestin, involved in de-activation of G-protein coupled receptors and cascade inactivation, binds phosphorylated rhodopsin, thus quenching its activity and allowing recovery of the light response. Two alternatively spliced forms of arrestin beta 1 (ARB1) have been identified: p48 and p44. The longer isoform contains 35 amino acids at its C-terminus which are replaced by a single alanine in the truncated form. While both forms of arrestin can bind and quench phosphorylated rhodopsin, the longer form also appears to be able to quench the activity of nonphosphorylated rhodopsin, suggesting a role in dark adaptation (Burns et al., 2006). A search of chicken ESTs resulted in the identification of the long form of arrestin, but not the short form.

Two alternatively-spliced variants of rhodopsin kinase (G-protein receptor kinase 1) have been identified in human: GRK1a and GRK1b (Zhao et al., 1998). The latter includes intron 6 in its coding region. Whereas GRK1a has been described in chicken (Zhao et al., 1999), we were not able to identify cDNAs or ESTs corresponding to GRK1b. Type 6 cGMP-phosphodiesterase (PDE6) is the G-protein-activated effector that regulates levels of cGMP in photoreceptors. The type 6 PDE gamma subunit (PDE6G) has two isoforms generated by alternative splicing, one of which lacks exon 2, resulting in loss of the N-terminal domain required to bind the alpha and beta subunits of PDE (Wistow et al., 2002). We only found evidence for the longer isoform of PDE6G in chicken databases.

DAB1, REELIN

The Reelin-Disabled 1 (Dab1) signaling pathway plays a key role in neuronal cell migration and in the positioning of neurons within laminated structures. *Reelin* and *Dab1* knock-out mice have a number of retinal abnormalities, primarily involving the retinal synaptic circuitry (Rice et al., 2001). We have found that the Disabled-1 *(DAB1)* gene is alternatively spliced in the developing chick retina (Katyal and Godbout, 2004), resulting in early (E) and late (L) isoforms. Dab1-L differs from Dab1-E by the inclusion of two exons containing two Src family kinase phosphorylation sites. We have evidence that both forms of Dab1 are also present in human retina (Katyal and Godbout, unpublished observations). Additional human and mouse Dab1 splicing variants have been reported in the literature (Bar et al., 2003), none of which are found in chicken databases. Two alternatively spliced transcripts encoding the secreted extracellular matrix glycoprotein Reelin have been reported in humans, both of which appear to be expressed in chicken based on DNA and EST database searches.

$GABA_A$ (γ2, β2), $GABA_B$

Gamma aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the retina. Alternative splicing has been observed in a number of protein subunits associated with GABA. For example, two variants of $GABA_A$ receptor γ2 subunits (γ2S and γ2L) which differ by eight amino acids encoded by a 24-bp cassette exon, have been described in human, mouse and chicken (Glencorse et al., 1992). Similarly, two alternatively spliced $GABA_A$ β2 subunits (β2S and β2L) which differ by 38 amino acids, were identified in human but not in rat or mouse (Harvey et al., 1994; McKinley et al., 1995). Two alternatively spliced variants of $GABA_A$ receptor β2 subunit which differ by 17 amino acids rather than 38 amino acids have been reported in chicken (Harvey et al., 1994). There is little similarity in the 17 amino acid insertion found in chicken and the 38 amino acid insertion found in human $GABA_A$ receptor β2 subunit. Although five variants of the $GABA_B$ receptor subunit 1 have been described in the literature (Isomoto et al., 1998; Schwarz et al., 2000; Benke et al., 2002), we found evidence for only one form (GABAB-1α) expressed in chicken based on database searches.

GLT1, mGluR1, NMDAR1

Three glutamate transporter 1 (GLT) isoforms (GLT1a, GLT1b/v, GLT1c) have been characterized in humans and rats (Schmitt et al., 2002; Rauen et al., 2004). In the retina, GLT1b/v is highly expressed in bipolar cells and cone photoreceptors, whereas GLT1c is primarily found in rod and cone photoreceptors (Rauen et al., 2004). Both the GLT1a and GLT1b/v isoforms are found in chicken based on EST and DNA database searches.

Metabotropic glutamate receptors group 1 (mGluR1) induces slowly arising excitation in postsynaptic neurons and control glutamate release presynaptically. At least four alternatively spliced mGluR1 transcripts have been identified in mammals (Conn and Pin, 1997), two of which (mGluR1a and mGlu1b) were found in chicken ESTs. Transfection of these two variants in chicken retinal cultures demonstrates that targeting of mGluR1 to dendrites and axons is controlled by alternative splicing (Francesconi and Duvoisin, 2002).

Human NMDA receptor 1 (NMDAR1, also called nuclear receptor 1 (NR1) or glutamate receptor, ionotropic, N-methyl D-aspartate 1 (GRIN1)) has three well-characterized alternatively spliced transcripts: NMDAR1a, NMDAR1b, NMDAR1c. Alternative splicing of NMDAR1 involves exons 20 and 21, with NMDAR1a being required for the repair of injured retinal ganglion cells. Chicken NMDAR1b and NMDAR1c cDNA sequences have been entered in the DNA database, and we identified ESTs corresponding to NMDAR1a, suggesting that all three NMDAR1 alternatively spliced products are conserved in mammals and chicken (Kreutz et al., 1998).

Maf, WT1

The musculoaponeurotic fibrosarcoma oncogene *Maf* has been shown to be expressed in the retina, although its function in this tissue is unknown. Two alternatively spliced forms of Maf (c-Maf-α and c-Maf-β) have been identified in human, mouse and chicken (Huang et al., 2002).

At least four splice variants have been reported for the human Wilms tumor 1 *(WT1)* gene, involving an alternatively spliced exon 5 and alternative splice donor sites leading to the insertion/deletion of a tripeptide KTS at the end of exon 9 in the C-terminus of the protein (Haber et al., 1991). WT1-beta(−KTS), which lacks the KTS amino acids, is required for retina formation, whereas WT-beta(+KTS) is important for the development of the olfactory epithelium (Wagner et al., 2005). Both the (−KTS) and (+KTS) isoforms of WT1 are found in chicken.

NETO1

Neuropilin- and tolloid-like protein 1 (NETO1) is a retina and brain specific gene encoding a putative transmembrane protein with two extracellular CUB domains. Three alternatively spliced variants are generated through alternate usage of two leader exons (1a and 1b) and exon 5 (Stohr et al., 2002). The soluble isoform 1 which contains only one CUB domain is restricted to the retina. Although isoforms 2 and 3 were identified in the chicken, we found no evidence of isoform 1 expression based on database searches.

Nrl, NR2E3

There are two alternatively spliced Nrl (neural retinal leucine zipper) transcripts in humans: Nrl and Nrl-ins which contains a 59 amino acid alternative exon between exons 2 and 3 (Wistow et al., 2002). To date, there is no evidence of either Nrl isoform in either DNA or EST chicken databases.

Two alternatively spliced forms of nuclear receptor subfamily 2, group E, member 3 (NR2E3) are expressed in different classes of retinal cells: NR2E3-alpha is specifically expressed in photoreceptors, whereas NR2E3-beta is expressed in Müller glial cells as well as in the retinal pigment epithelium (Chen et al., 1999). The chicken NR2E3-beta cDNA sequence has been entered in the DNA database; however, database searches revealed no counterpart to the photoreceptor-specific form in chicken.

ALDH1A1

ALDH1A1 has previously been shown to be specifically expressed in the dorsal retina of chick, mouse and zebrafish during development where it has been postulated to play a role in the generation of a retinoic acid gradient (McCaffery et al., 1991, 1992; Godbout et al., 1996). An ALDH1A1 variant with two additional 5′ untranslated exons, specifically found in the chick retina, gives rise to a retinal ALDH1A1 transcript that is considerably longer than that found in liver (Godbout, 1992; Godbout and Monckton, 2001). We found no evidence of this longer form of ALDH1A1 in either human or mouse.

Agrin

Agrin in a heparin sulfate proteoglycan involved in the formation of acetylcholine receptor aggregates. Agrin isoforms are expressed in the nervous system including the brain, retina, and spinal cord (Kirsch and Kroger, 1996; Kroger, 1997). The agrin transcript is alternatively spliced to include or exclude small exons at three positions. Four agrin splice variants have been identified in rat and chicken: agrin0 (B0), agrin8 (B8), agrin11 (B11) and agrin19 (B19) where the number indicates the number of amino acids inserted as the result of alternative splicing (Ruegg et al., 1992; Rupp et al., 1992; Thomas et al., 1993; O'Connor et al., 1994).

RPGR

Retinitis pigmentosa GTPase regulator (RPGR) is required for the maintenance of photoreceptor viability (Meindl et al., 1996). Mutations in RPGR account for 10–20% of all cases of retinitis pigmentosa (RP) and >70% of X-linked RP. Three main splicing variants of RPGR have been reported in humans: variant a, with multiple alternative exons in the 3′ coding region; variant b, preferentially expressed in the retina, with multiple alternative exons in the 3′ coding region (shortest isoform); and variant c, the longest isoform with an extensive glutamic acid- and glycine-rich domain in its C-terminus. A sequence similar to variant c has been reported in chicken. Furthermore, we have identified ESTs with a low level of similarity to variant a.

VEGF

Multiple splice variants of vascular endothelial growth factor (VEGF) have been identified, some with pro-angiogenic functions, others with anti-angiogenic functions. The defining features of pro-angiogenic VEGF isoforms is inclusion of the six C-terminal amino acids CDKPRR encoded by exon 8, whereas the anti-angiogenic isoforms result from usage of an alternate acceptor site downstream of exon 8 resulting in the six C-terminal amino acids SLTRKD (Bates et al., 2002). Three major pro-angiogenic isoforms have been described: $VEGF_{189}$, $VEGF_{165}$ (the dominant form) and $VEGF_{121}$ (Ferrara and Davis-Smith, 1997). Anti-angiogenic counterparts to these three VEGFs have also been reported: $VEGF_{189}b$, $VEGF_{165}b$, $VEGF_{121}b$ (Woolard et al., 2004). Both the pro-angiogenic and anti-angiogenic forms of VEGF are expressed in the retina where they are believed to play a role in diabetic retinopathy (Perrin et al., 2005). Two chicken pro-angiogenic VEGF isoforms $VEGF_{165}$ and $VEGF_{206}$ have been annotated in the databases to date. EST database searches failed to reveal anti-angiogenic VEGF isoforms.

NUMB

Numb is a phosphotyrosine-binding (PTB) domain protein involved in cell fate determination in *Drosophila*. Four mouse Numb isoforms (p72, p71, p66, p65) are generated as the result of alternative splicing. The two longest isoforms, p72 and p71, are expressed in the early retina, while the p66 and p65 isoforms are found in adult retina (Dho et al., 1999; Dooley et al., 2003). Two numb isoforms have been identified in chicken, corresponding to the p66 and p71 isoforms.

Alternative splicing of genes expressed in chicken brain and other tissues

Our second strategy was to examine well-documented alternative splicing events in chicken and to determine whether similar alternative splicing events occur in mammals based on literature searches and sequence comparisons using EST and DNA databases (Table 2). Many of the genes in this category represent genes expressed in brain, likely reflecting a preponderance of alternative splicing events in brain compared to other tissues.

NGF

Nerve growth factor is a member of the neurotrophin family which consists of functionally and structurally related small basic proteins of ~ 120 amino acids. Analysis of the chicken *NGF* gene and transcripts reveals five alternatively spliced 5′ non-coding exons grouped into two clusters, followed by the coding exon 4. The first cluster consists of three leader exons (1a, 1b, 1c) and the second cluster contains exons 2 and 3. Differential usage of leader and internal exons, alternative transcription start sites, alternative donor and acceptor sites generates at least 21 different transcripts (Bertaux et al., 2004). Comparison of the 6-exon chicken *NGF* with the 3-exon human *NGFB* gene reveals significant nucleotide alignment of chicken *NGF* exons 2 and 4 with human *NGFB* exons 2 and 3, with the chicken and human proteins showing 87% identity. There is no equivalent to chicken exon 3 in either the human or mouse genome (Bertaux et al., 2004).

TERT

Telomerase reverse transcriptase (TERT) adds repeats to telomeres during DNA replication to maintain telomere length. Recent evidence suggests that TERT may have additional roles in the regulation of cell growth and tumor formation. In humans, four insertion variants of TERT have been identified, as well as three deletion variants and various combinations thereof (Kilian et al., 1997; Ulaner et al., 1998; Hisatomi et al., 2003). Nineteen variants have been identified in chicken, involving exon skipping, intron retention, and alternative usage of splice donor and acceptor sites (Chang and Delany, 2006). The number of variants ranged from ten in adult chicken liver to 13 in chicken embryo fibroblasts. One of the variants, found in all tissues examined, is predicted to generate a truncated product lacking telomerase activity.

Somatostatin

The neuropeptide somatostatin was first isolated based on its ability to inhibit growth hormone secretion from the anterior pituitary (Brazeau et al., 1973). Two biologically active forms of somatostatin (SS1, SS2) generated through alternative splicing of precursor

somatostatin (PSS1, PSS2) have been identified in mammals, amphibians, fish and chicken (reviewed in Trabucchi et al., 2003). Whereas PSS1 is widely expressed in the central nervous system, where it functions as a neurotransmitter and a neuromodulator, PSS2 is only expressed in specific regions of the brain.

JNK2-α1

c-Jun N-terminal kinases (JNK) constitute a subgroup of the mitogen-activated protein kinases (MAPK). Alternatively spliced isoforms of mammalian JNK2 which differ in their carboxy-terminus have been identified; however, extensive analysis of JNK2 in chicken revealed only the C-terminus truncated subtype α1 (Ishikawa et al., 1997). JNK2-α1 is predominantly expressed in the developing chick brain, suggesting a role in neuronal cell formation.

GRIA4/AMPA4

AMPA receptors, which mediate rapid excitatory synaptic transmission in the vertebrate CNS, are pentameric assemblies of four glutamate receptors (GIuR1–4). The type and amount of GluR incorporated into an AMPA receptor determines the receptor's properties. Each GluR, including GluR4 (GRIA4), exists in two alternatively spliced forms, called flip and flop that are evolutionarily conserved in chicken and mammals (Ravindranathan et al., 1997). In addition, a C-terminal splice variant called GluR4c, which also exists in flip and flop forms, has been described in mammals and chicken (Ravindranathan et al., 1997; Kawahara et al., 2004). Two additional alternatively spliced variants, GluR4d with a 184-bp fragment inserted at the 4c splice site, and GluR4s, a shortened version of GluR4 that lacks the 4^{th} transmembrane and flip/flop domains have been described in chicken (Ravindranathan et al., 1997). Mammalian GluR4d and Glu4s have not yet been reported.

FGF8, FGFR2

Fibroblast growth factor 8 (FGF8) is a signaling molecule involved in cell proliferation and differentiation. The human genomic structure predicts four alternatively spliced isoforms, three of which have been identified (Ghosh et al., 1996) while the mouse genomic structure predicts eight alternatively spliced isoforms, seven of which have been identified (reviewed in Haworth et al., 2005). Extensive analysis of chicken FGF8 has revealed only two isoforms, equivalent to mouse FGF8-a and FGF8-b (Haworth et al., 2005).

Alternative splicing of FGF receptors (FGFR) results in different ligand binding properties and alterations in biological function. The *FGFR2* gene encodes two well-characterized alternatively spliced products: FGFR2b and FGFR2c. These two isoforms are identical except for a 49 amino acid sequence in the extracellular region (Ornitz and Marie, 2002). These isoforms exhibit different ligand-binding properties as well as distinct expression patterns in the developing embryo (Ornitz and Marie, 2002). Both FGFR2 isoforms have been described in chicken (Havens et al., 2006).

ST2

The ST2 gene encodes receptor-like molecules that are similar to interleukin-1 receptors. The chicken, mouse, rat and human *ST2* genes consist of 13 exons and two promoters

followed by two non-coding exons 1a and 1b. Three ST2 variants, generated by alternative splicing, have been described in humans (Tominaga et al., 1999; Li et al., 2000). Analysis of chicken ST2 revealed three variants, ST2 and ST2L previously described in humans, as well as a novel variant called ST2LV (Iwahana et al., 2004).

PDE5

Three splice variants of cGMP-binding cGMP-specific phosphodiesterase (PDE5) have been described in humans: PDE5A1, A2, A3 (Lin et al., 2000). All three variants have recently been described in chicken dorsal root ganglia (Giordano et al., 2004).

Drebrin

Drebrins (developmentally regulated brain proteins, DBN1) are actin binding proteins believed to play a role in the formation of actin filaments in dendritic spines. Three isoforms, generated by alternative splicing, have been identified in chicken brain: two embryonic types (E1 and E2) and an adult type (A) (Kojima et al, 1993). Two DBN1 isoforms have been reported in human (1a (E2) and 1b (A)), three in mice (A, A2, E2) and two in rat (A and E) (Chew et al., 2005).

Neurofascin

Neurofascin (NFASC) is a neural cell adhesion molecule implicated in cell adhesion, cell migration and neurite outgrowth. The chicken *NFASC* gene has 33 exons, eight of which have been shown to undergo alternative splicing. Analysis of 138 independent NFASC cDNAs derived from chick embryonic brain at day 6 and day 16 of incubation led to the conclusion that there are at least 50 different NFASC splice products expressed at different developmental stages in chicken (Hassel et al., 1997). A large number of different NFASC splice products have also been found in humans (http://www.ensembl.org/index.html).

Prosaponin

Prosaponin is the precursor of the lysosomal activator molecules saponins A, B, C and D. The prosaponin gene has 15 exons, one of which (the 9-bp exon 8) undergoes alternative splicing. Three prosaponin mRNAs have been identified in human and mouse, containing 0,6 or 9 bp of exon 8. In chicken, there are only two prosaponin variants, containing 0 or 9 bp of exon 8 (Cohen et al., 2004). This suggests that the 6-bp exon 8 splice variant may not be biologically important, in keeping with the low levels of this splice product (Cohen et al, 2004).

Tau

Tau is a microtubule-associated protein expressed in brain. Alternative splicing of a single *tau (MAPT)* gene generates five isoforms in adult chicken brain (Yoshida and Goedert, 2002) and six isoforms in human brain (Goedert et al., 1989; Andreadis et al., 1992). The chicken and human tau isoforms differ by the number of repeated microtubule-binding domains that they contain. Isoforms with the highest number of repeats are thus better at promoting microtubule assembly. Whereas human tau adult isoforms consist of three or four repeats, two of the five adult chicken tau isoforms consist of five repeats. Both human and

chicken express an additional isoform, a three-repeat tau without an insert in the N-terminus, which predominates at early developmental stages (Yoshida and Goedert, 2002).

Casein kinase 1

Casein kinase 1 is a family of serine/threonine protein kinases shown to phosphorylate acidic peptides in a variety of substrates. At least two members of the family, α and γ3, undergo alternative splicing. There are four splice variants of chicken CKIα which differ by the presence or absence of two peptides, a 28 amino acid L insert containing a nuclear localization signal and a 12 amino acid S insert with no known motifs (Green and Bennett, 1998; Fu et al., 2001). The four splice variants, CKIα, CKIαS, CKIαL and CKIαLS have specific patterns of expression in chicken tissues (Green and Bennett, 1998). Three of the four splice variants (CKIα, CKIαL, CKIαS) have been reported in mammals (Zhang et al., 1996; Yong et al., 2000).

Myb

Two alternatively spliced forms of c-myb have been reported in chicken and mammals. These splice variants differ by the inclusion/exclusion of an exon (9A) encoding 120 (chicken) or 121 (human, mouse) amino acids (Dasgupta and Reddy, 1989; Schuur et al., 1993). This exon is not present in v-myb. Functional analysis of Myb(+9a) suggests that it has stronger transactivation ability compared to Myb (−9a) although both forms are equally efficient at transforming primary chicken hematopoietic cells (Woo et al., 1998). Alternative splicing of exon 9A has also been described for B-myb (Kamano et al., 1995). Interestingly, B-myb(−9a) lacks transactivation activity as part of the transactivation domain of B-myb is found in exon 9a (Horstmann et al, 2000).

c-Src

Cellular Src is a membrane-associated tyrosine protein kinase with oncogenic potential. c-Src levels are elevated in neuronal tissue suggesting a role in neuronal differentiation. A neuron-specific isoform of c-Src (NI) which contains an 18-bp exon inserted between exons 3 and 4 has been identified in mammals and chicken (Lynch et al., 1986; Levy et al, 1987). Exons 3 and 4 encode part of the domain involved in substrate interaction and regulation of c-Src activity. A second neuronal-specific isoform of c-Src (NII) has been identified in human brain, consisting of a 33-bp exon inserted between exons 3 and 4 (Pyper and Bolen, 1990). The NII isoform has not been reported in chicken. Alternative splicing of c-Src, involving different splice products, has also been described in chicken skeletal muscle cells (Dorai and Wang, 1990).

Alternative splicing in the top fifty genes listed under GeneCards

As a third strategy, we compared alternative splicing in the 50 most frequently accessed genes based on the GeneCards database (http://www.genecards.org/). Table 3 indicates the number of alternatively spliced products listed in the GeneCards and Ensembl databases for the human genes, compared to the number of alternatively spliced products listed in the Ensembl database for the chicken orthologues.

As shown in Table 3, alternative splicing is much more commonly reported for human genes than for their chicken counterparts, likely reflecting the extent of work that has been carried out on analysis of human versus chicken genes to date. Of the 50 genes analysed, only one gene, *CFTR,* had more alternatively spliced variants in chicken compared to human. Alternatively spliced products were reported in 9/50 chicken genes compared to 42/50 human genes, although there was significant variation in the number of alternatively spliced products listed in the GeneCards and Ensembl databases for many human genes. Conservation of splice variants between chicken and human was observed for 12 genes which had more than one human splice variant; however 11 of these genes had only one reported chicken transcript, suggesting that the chicken transcript represented a main or constitutive splice product. Two conserved splice variants in human and chicken were identified for the *VEGF* gene.

Conclusions

A survey of 50 commonly accessed genes reveals little conservation in alternative splicing between chicken and human. However, rather than denoting a penury of splicing variants in chicken, this likely reflects the fact that mRNA sequence analysis is still at an early stage in chicken. Specific analysis of 16 genes known to undergo alternative splicing in chicken reveals as many alternatively spliced products in chicken compared to mammalian species, with a total of 37 splice products in chicken and 38 in humans. Furthermore, for these well-characterized chicken genes, the same alternatively spliced products are commonly found in both chicken and mammalian species (32 out of 38 human splice products were conserved in chicken), suggesting evolutionary conservation of splice variant function. Perhaps the most informative genes are those selected on the basis of expression in the well-conserved retina with well-documented alternative splicing in mammals. Of the 65 mammalian splice variants shown in Table 1, 42 could be documented as conserved in chicken. Although this study is limited in scope because of a general lack of mRNA data in chicken, our results support a high frequency of alternative splicing events in chicken, comparable to that observed in mammals. Brett et al. (2002) have compared the number of alternative splicing events in seven eukaryotic species for which sufficient mRNA/EST data were available (human, mouse, rat, cow, fly, worm, plant). When differences in mRNA and EST coverage were taken into consideration, these investigators found that the amount of alternative splicing was similar in all seven organisms, with 10% of mRNAs having alternatively spliced forms. These results suggest that the complexity between higher and lower eukaryotes may not be reflected in the number of alternative splice events, but rather in the nature of alternative splice events.

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Table 1

Alternative splicing of genes expressed in retina

Gene symbol	Gene name	Isoforms	nt accession #/reference	Chicken isoforms
AGRN	Agrin	agrin 0 (B0)	NM_198576	NM_205527
		agrin 8 (B8)	Rupp et al., 1992	Ruegg et al., 1992
		agrin 11 (B11)	Rupp et al., 1992	Ruegg et al., 1992
		agrin 19 (B19)	Rupp et al., 1992	U07271
ARB1/ARRB1	arrestin beta 1	ARRB 1a	NM_177231	EST
		ARRB 1b	NM_178220	Not found
DAB1	Disabled-1	DAB1-L	NM_021080	AY242123
		DAB1-E	Unpublished	AY242122
FOXN3	Forkhead box protein N3	FOXN3-alpha	AM114794	Not found
		FOXN3-beta	AM114795	EST
GABRB2	gamma-aminobutyric acid A receptor beta 2	$GABA_A\beta 2S$	NM_000813	Harvey et al., 1994
		$GABA_A\beta 2L$	NM_021911	Harvey et al., 1994
GABRG2	gamma-aminobutyric acid A receptor gamma 2	$GABA_A\gamma 2S$	NM_000816	Glencorse et al., 1992
		$GABA_A\gamma 2L$	NM_198904	NM_205345
GABBR1	gamma-aminobutyric acid B receptor 1	$GABA_B1a$	NM_001470	EST
		$GABA_B1b$	NM_021903	Not found
		$GABA_B1c$	NM_021904	Not found
		$GABA_B1d$	NM_021905	Not found
		$GABA_B1e$	Billinton et al., 2001	Not found
GLT1/SLC1A2	glutamate transporter 1	GLT1a	NM_004171	EST
		GLT1b/v	Schmitt et al., 2002	NM_001012899
		GLT1c	Rauen et al., 2004	Not found
GRK1	G-protein receptor kinase 1 (rhodopsin kinase)	GRK1a	NM_002929	NM_204695
		GRK1b	Zhao et al., 1998	Not found
MAF	musculoaponeurotic fibrosarcoma oncogene	c-Maf-alpha	NM_005360	D28596
		c-Maf-beta	NM_001031804	D28596
GRM1	metabotropic glutamate receptor 1	mGluR1a	NM_000838	EST
		mGluR1b	HSU31216/gi:945098	EST
		mGluR1c	Pin et al., 1992	Not found
		mGluR1d	Laurie et al., 1996	Not found
		mGluR1g	Makoff et al., 1997	Not found
NETO1	neuropilin- and tolloid-like protein 1	NETO1 isoform 1	NM_138999	Not found
		NETO1 isoform 2	NM_153181	XM_414109
		NETO1 isoform 3	NM_138966	XM_419104
NMDAR1/ GR1N1/NR1	NMDA receptor 1/ glutamate receptor, ionotropic, N-methyl D-aspartate 1/Nuclear factor 1	NMDAR1a	NM_000832	EST
		NMDAR1b	NM_021569	NM_206979
		NMDAR1c	NM_007327	AAY663373

Gene symbol	Gene name	Isoforms	nt accession #/reference	Chicken isoforms
NR2E3	Nuclear receptor subfamily 2, group E, member 3	NR2E3-alpha	NM_016346	Not found
		NR2E3-beta	NM_014249	NM_204594
NRL	Neural retina leucine zipper	Nrl	NM_006177	Not found
		Nrl-ins	Wistow et al., 2002	Not found
NUMB	numb homologue	numb isoform 1	NM_001005743	Not found
		numb isoform 2	NM_001005744	AF176086
		numb isoform 3	NM_003744	AF213691
		numb isoform 4	NM_001005745	Not found
OTX2	orthodenticle homolog 2	OTX2A	NM_021728	NM_204520
		OTX2B	NM_172337	EST
PAX6	Paired box gene 6	PAX6-alpha	NM_000280	Not found
		PAX6-beta	NM_001604	NM_205066
PDE6G	phosphodiesterase-6 gamma	PDE6G	NM_002602	NM_204445
		PDE6G (–ex2)	Wistow et al., 2002	Not found
RPGR	retinitis pigmentosa GTPase regulator	RPGRa	NM_00328	EST
		RPGRb	NM_001023582	Not found
		RPGRc	NM_001034853	XM_416780
RELN	Reelin	RELNa	NM_005045	XM_425424
		RELNb	NM_173054	EST
SIX3	sine oculis homeobox homolog 3	SIX3-alpha	Kawakami et al., 1996	Not found
		SlX3-beta	NM_005413	AY373324
VEGF	vascular endothelial growth factor	VEGF165a	NM_001025368	AY168004
		VEGF206a	NM_001025366	NM_205042
		VEGF165b	NM_001033756	Not found
VSX1	visual system homeobox 1	VSX1A	NM_014588	NM_204769
		VSX1B	NM_199425	Not found
WT1	Wilms tumor 1	WT1-beta(−KTS)	NM_024424	EST
		WT1-delta(+KTS)	NM_024426	NM_205216

Table 2

Alternative splicing of genes expressed in chicken brain and other tissues

Gene symbol	Gene name	Isoforms	nt accession # (human)/reference	Chicken isoforms
CSNK1A1	casein kinase I	CKIalpha	NM_001025105	NM_205053
		CKIalphaL	Yong et al., 2000	Green and Bennett, 1998
		CKIalphaS	Yong et al., 2000	Green and Bennett, 1998
		CKIalphaLS (chicken only)		Green and Bennett, 1998
DBN1	drebrin	Drebrin E2	NM_004395	NM 205499, Kojima et al., 1993
		Drebrin El (chicken only)		Toda et al., 1993
		DrebrinA	NM_080881	Kojima et al., 1993
FGFR2	fibroblast growth factor receptor 2	FGFR2IIIb/K-SAM	NM_022970	Sato et al., 1992
		FGFR2IIIc/BEK	NM_000141	NM_205319, Sato et al., 1991
GRIA4	ionotropic glutamate receptor 4/AMPA4	GluR4a	NM_000829	NM_205214
		GluR4c	Kawahara et al., 2004	U65992
		GluR4d (chicken only)		U65993
		GluR4s (chicken only)		U65991
IL1RL1/ST2	interleukin 1 receptor-like 1	ST2	D12763	AB041737
		ST2L	AB012701	AB041738
		ST2V	AB029084	Not found
		ST2LV (chicken only)		AB041739
MAPK9	c-Jun N-terminal kinase 2	JNK2-alpha1	NM_139068	NM_205095
		JNK2-alpha2	NM_002752	Not found
		JNK2-beta1	NM_139069	Not found
		JNK2-beta2	NM_139070	Not found
MAPT	microtubule-associated protein tau	MAPT variant 1	NM_016835	Yoshida and Goedert, 2002
		MAPT variant 2	NM_005910	Yoshida and Goedert, 2002
		MAPT variant 3	NM_016834	Yoshida and Goedert, 2002
		MAPT variant 4	NM_016841	Yoshida and Goedert, 2002
MYB	c-myb	c-myb+9a	Dasgupta and Reddy, 1989	Schuur et al., 1993
		c-myb (−9a)	NM_005375	NM_205306
NFASC	neurofascin	NFASC variant 1	NM_001005388	XM_417958
		NFASC variant 2	NM_015090	Hassel et al., 1997
		NFASC variant 3	NM_001005389	Hassel et al, 1997
NGF	nerve growth factor	Multiple	multiple	
PDE5A	cGMP-binding, cGMP-specific 3′, 5′-cyclic nucleotide phosphodiesterase	PDE5A variant 1	NM_001083	Giordano et al., 2004
		PDE5A variant 2	NM_033430	Giordano et al., 2004
		PDE5A variant 3	NM_033437	Giordano et al., 2004

Gene symbol	Gene name	Isoforms	nt accession # (human)/reference	Chicken isoforms
PSAP	prosaposin	hPSAP0	NM_002778	Cohen et al., 2004
		hPSAP9	NM_001042465	Cohen et al., 2004
		hPSAP6	NM_001042466	Absent in chicken, Cohen et al., 2004
SRC	Src	Src	NM_005417	XM_425696
		neuronal Src-NI	Pyper and Bolen, 1990	Martinez et al., 1987
		neuronal Src-NII	Pyper and Bolen, 1990	Not found
SST	somatostatin	SS1/PSS1	NM_001048	NM_205336
		SS2/PSS2	Trabucchi et al., 2003	AY221945
TERT	telomerase reverse transcriptase	multiple	multiple	
TPM2	beta-tropomyosin	TPM2–6a	NM_213674	Libri et al., 1991
		TPM2–6b	NM_003289	Libri et al., 1991

Table 3

Alternative splicing in 50 commonly accessed genes

Gene symbol	Gene name	Number of splicing variants			Conserved transcripts[b]
		Human		Chicken	
		GeneCards[a]	Ensembl	Ensembl[b]	
BRCA1	breast cancer 1, early onset	9	8	1	0/1
TP53	tumor protein p53 (Li-Fraumeni syndrome)	4	2	NA	NA
ALDH2	aldehyde dehydrogenase 2 family (mitochondrial)	4	1	1	0/1
COL2A1	collagen, type II, alpha 1	6	4	1	0/1
CFTR	cystic fibrosis transmembrane conductance regulator, ATP-binding cassette (sub-family C, member 7)	1	1	4	0/4
BTK	Bruton agammaglobulinemia tyrosine kinase	6	7	2	0/2
A2M	alpha-2-macroglobulin	1	1	NA	NA
BRCA2	breast cancer 2, early onset	1	2	1	0/1
ASS	argininosuccinate synthetase	11	10	NA	NA
EGFR	epidermal growth factor receptor	1	3	3	0/3
IGF1	insulin-like growth factor 1	1	2	1	1/1
BCL2	B-cell CLL/lymphoma 2	2	1	1	1/1
APP	amyloid beta (A4) precursor protein	1	7	1	1/1
APOE	apolipoprotein E	4	1	NA	NA
ATM	ataxia telangiectasia mutated	1	2	1	0/1
HEXA	hexosaminidase A	11	3	1	1/1
AKT1	v-akt murine thymoma viral oncogene homolog 1	8	1	1	0/1
HBB	hemoglobin, beta	1	5	3	NA
RBI	retinoblastoma 1	1	3	2	0/2
JAK2	Janus kinase 2	1	1	1	0/1
AR	androgen receptor	1	2	1	0/1
BAX	BCL2-associated X protein	3	1	NA	NA
JAK3	Janus kinase 3	1	4	NA	NA
PTEN	phosphatase and tensin homolog	1	2	1	1/1
GBA	glucosidase, beta; acid	10	14 (12 without translation)	NA	NA

Gene symbol	Gene name	Number of splicing variants			Conserved transcripts[b]
		Human		Chicken	
		GeneCards[a]	Ensembl	Ensembl[b]	
HFE	hemochromatosis	11	9	0	
SOD1	superoxide dismutase 1, soluble	7	1	1	1/1
CREBBP	CREB binding protein	1	3	1	0/1
INSR	insulin receptor	1	2	1	0/1
ACE	angiotensin I converting enzyme	12	2	2	0/2
ADRB2	adrenergic, beta-2-, receptor, surface	1	1	1	1/1
TNF	tumor necrosis factor (TNF superfamily, member 2)	1	4	NA	NA
JUN	v-jun sarcoma virus 17 oncogene homolog (avian)	1	1	1	0/1
PSAP	prosaposin	8	2	2	1/2
ATBF1	AT-binding transcription factor 1	1	1	1	0/1
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (P105)	4	1	1	0/1
ACHE	acetylcholinesterase (YT blood group)	10	3	NA	NA
VEGF	vascular endothelial growth factor	6	9 (2 without translation)	5	2/5
APC	adenomatosis polyposis coli	1	1	1	0/1
ABL1	v-abl Abelson murine leukemia viral oncogene homolog 1	1	2	1	1/1
AHCY	S-adenosylhomocysteine hydrolase	9	2	1	1/1
ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	3	1	2	0/2
ADRB3	adrenergic, beta-3-, receptor	1	1	1	0/1
CASP8	caspase 8, apoptosis-related cysteine peptidase	14	9	1	0/1
FGFR3	fibroblast growth factor receptor 3	1	5	1	0/1
MTHFR	5,10-methylenetetrahydrofolate reductase (NADPH)	8	5	1	0/1
HEXB	hexosaminidase B (beta polypeptide)	1	2	1	0/1
DMD	dystrophin (muscular dystrophy, Duchenne and Becker types)	1	19	1	0/1
PPARG	peroxisome proliferative activated receptor, gamma	7	2	1	0/1
ARSA	arylsulfalase A	8	2	1	1/1

[a] 'Hot genes' are defined genes that have been most frequently requested at the GeneCards home site (http://www.genecards.org/) since Jan 1, 2006 sorted by frequency of access.

[b] 'NA' denotes information not available.