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Roles and Regulation of Thy-1, a Context-Dependent Modulator of Cell Phenotype

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Abstract

Thy-1, or CD90, is a glycoposphatidylinositol-linked glycoprotein expressed on the surface of neurons, thymocytes, subsets of fibroblasts, endothelial cells, mesangial cells and some hematopoietic cells. Thy-1 is evolutionarily conserved, developmentally regulated, and often has dramatic effects on cell phenotype; however the effects vary between and in some cases within cell types and tissues, and between similar tissues in different species, indicating that the biological role of Thy-1 is context-dependent. Thy-1 exists in soluble form in some body fluids; however the mechanisms of its shedding are unknown. In addition, Thy-1 expression can be regulated by epigenetic silencing. Because Thy-1 modulates many basic cellular processes and is involved in the pathogenesis of a number of diseases, it is important to better understand its regulation.

Keywords

Thy-1; glycoposphatidylinositol; shedding; epigenetics

Introduction

Thy-1 (Thymocyte differentiation antigen 1), also known as CD90 (Cluster of Differentiation 90), is a highly conserved but somewhat enigmatic molecule that can exist in membrane-bound and soluble forms. Although it is most often used as a marker of certain cell types or of “stem-ness,” its presence or absence has significant effects on cellular biology, and its dysregulation is associated with malignancy and fibrotic diseases. Previous reviews have focused on its immunologic and non-immunologic roles, and mechanisms and consequences of Thy-1-associated signaling [5, 17, 41, 42]. Here we will consider the regulation of Thy-1 in the context of its pathogenic alterations.

Thy-1 was initially discovered in an attempt to raise antiserum against leukemia-specific antigens from the CH3 strain of mouse in the AKR strain and vice versa [43]. It was

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originally designated theta (Θ), but later renamed Thy-1 [51]. In addition to the aforementioned thymocytes and T-cells, a number of other cell types are known to express Thy-1, specifically neurons, retinal ganglion cells, subsets of fibroblasts, vascular pericytes, activated endothelial cells, mesangial cells, and hematopoietic and mesenchymal stem cells. There are important species-specific differences in expression. For instance, Thy-1 is expressed on both peripheral T cells and thymocytes of mice; whereas in humans, Thy-1 is absent in the former and developmentally regulated in the latter [59]. Thy-1 modulates the phenotypes of cells implicated in several disease states including neuronal injury [30, 50, 68], pulmonary fibrosis [42, 47, 48], certain cancers [1, 14, 31], Graves' disease ophthalmopathy [22], and glomerulonephritis [34].

Pathogenic Alterations in Thy-1

Several observations associate Thy-1 with the resolution of neuronal injury. Thy-1 is either not expressed on [67] or restricted to the somatodendritic membranes of growing rodent neurons [67], yet accounts for 2.5% to 7.5% of total protein on axon membranes of mature rat neurons [6]. Thy-1 expression in the nervous system is predominantly neuronal, but some human glial cells also express Thy-1 especially at later stages of their differentiation [21]. Neurite outgrowth is inhibited in Thy-1 (-) neurons made to express either human Thy-1 or mouse Thy-1.2 when grown on a monolayer of astrocytes [57]. In similar conditions, antibodies against Thy-1 or soluble Thy-1 allow neurite outgrowth to occur, presumably by blocking the interaction of Thy-1 with a ligand on astrocytes [57]. Injury to the sciatic nerve in young adult rats causes an initial decline of Thy-1 expression followed by an increase on dorsal root ganglion neurons that coincides with recovery of sensory function [12].

However, presence of Thy-1 on neuron membranes is not sufficient in itself to inhibit neurite outgrowth, but requires correct localization of Thy-1 to its native membrane microdomain to exert an inhibitory effect [58]. Taking these all into account, the prevailing thought is that Thy-1 expressed in neurons, when localized to its native membrane microdomain, inhibits neurite outgrowth and its continued expression in mature neurons likely plays a role in stabilizing them and their junctions. The phenotype of Thy-1 deficient mice is remarkably devoid of major abnormalities involving the nervous system. However, thorough examination revealed subtle phenotypes, including inhibition of hippocampal long-term potentiation in the dentate gyrus, failure to transmit social cues regarding food selection, and an impaired cutaneous immune response [33, 37].

Pulmonary fibrosis is characterized by the development in the lung of fibrotic tissue characterized by excessive and abnormal extracellular matrix [52]. The cell type often implicated in pulmonary fibrosis is the fibroblast, specifically its differentiated phenotype, the myofibroblast [52]. Individual fibroblasts can have vastly differing phenotypes, especially when they originate from different tissues [25, 39, 40, 54, 60]. Even within a particular tissue, fibroblasts are still heterogeneous [42]. For pulmonary fibroblasts, differential expression of Thy-1 is a well-characterized paradigm for distinguishing polar phenotypes with respect to potential for differentiation into myofibroblasts, response to pro-fibrotic cytokines, and localization in areas of active fibrosis [42]. Specifically, rat Thy-1 (-) pulmonary fibroblasts at baseline as well as in response to fibrogenic mediators have greater

myofibroblastic differentiation as evaluated by myogenic gene expression and enhanced contractility. Furthermore, they are resistant to apoptosis in a contracting collagen matrix [47]. TNF- α , IL-1 β , and PDGF-AA all elicit a unique or greater fibrotic response in Thy-1 (-) fibroblasts, in particular with regard to activation of latent TGF- β [42]. The hallmark feature of IPF (idiopathic pulmonary fibrosis) is aggregates of proliferating fibroblasts and myofibroblasts called fibroblastic foci [52], increased numbers of which are associated with poor prognosis [24]. Within these foci are predominantly Thy-1 (-) myofibroblasts, whereas the majority of normal lung fibroblasts express Thy-1. Intra-tracheal bleomycin induces more severe lung fibrosis in Thy-1 knockout mice as evidenced by greater accumulation of myofibroblasts, collagen and increased activation of TGF- β [18]. The relationship of Thy-1 expression to pathogenic alterations in lung fibroblasts in pulmonary fibrosis is depicted in Figure 1.

Thy-1 is thought to act as a tumor suppressor in several types of cancer, including nasopharyngeal and ovarian cancer [31]. In ovarian cancer, LOH (loss of heterozygosity) at 11q23.3-q24.3 is associated with poor prognosis [11] and Thy-1 is mapped to this region [64]. Inducing Thy-1 expression in the tumorigenic ovarian cancer cell line, SKOV-3, by either microcell mediated chromosome 11 transfer [11] or a Thy-1 expression inducible system [1] suppressed tumorigenicity. Concurrently, induced Thy-1 expression in SKOV-3 mediates up-regulation in TSP-1 (Thrombospondin-1) and FN (Fibronectin). These genes are differentially expressed in tumorigenic and non-tumorigenic hybrid clones as well as being associated with cell differentiation and angiogenesis inhibition [2]. In neuroblastoma tumors, lack of Thy-1 expression correlates with reduced patient survival [14].

Patients with GO (Graves' Ophthalmopathy) display an increase in volume of the extraocular muscles and/or the intraorbital adipose tissues. As in IPF, fibroblasts are implicated in the pathology of this disease [15] and Thy-1 expression delineates differentiation potential. Unlike in IPF, only Thy-1 (+) orbital fibroblasts appear capable of differentiating into myofibroblasts, while Thy-1 (-) are incapable of doing so but are unique in their ability to differentiate into mature adipocytes [27]. Intraorbital adipose tissue from patients with GO has a greater proportion of Thy-1 (+) fibroblasts relative to that taken from healthy patients [22]. This seemingly conflicting modulation of differentiation potential by Thy-1 and its involvement in GO is poorly understood and requires further study. Thy-1 fibroblast heterogeneity has also been noted in the human myometrium, in which the Thy-1 (+) subset also differentiates in to myofibroblasts, and in which there are differences in expression of cyclooxygenase isoforms and MCP-1 [25-27].

Psoriasis is a common chronic inflammatory skin disease in which the infiltration of neutrophils is an important feature of pathogenesis. Thy-1 is involved in the adhesion of neutrophils and monocytes to activated microvascular endothelial cells via interaction with the β 2-leukocyte integrin Mac-1 (CD11b/CD18). The enhanced adhesion of psoriatic neutrophils to Thy-1-expressing endothelial cells, via Mac-1/Thy-1 interaction, suggests that this may be an important mechanism of attachment and migration into psoriatic lesions [63].

Given the possible role of Thy-1 in multiple pathogenic alterations, as well as the contrasting phenotypes and activities exhibited between Thy-1 (+) and (-) cells in different

tissues, it is useful to consider carefully the mechanisms by which Thy-1 is regulated in evolution and development, and dysregulated in certain disease states.

Thy-1 Structural Evolution and Species Differences

Thy-1 belongs to the immunoglobulin superfamily [12] and is evolutionarily conserved; significant homology exists among multiple species including squid, chicken, frogs, mice, rats, and humans. Additionally, it has been proposed that the immune system is evolutionarily related to the nervous system, and Thy-1, which is an important molecule in both, possibly represents a primordial domain of the immunoglobulin superfamily ancestry [11]. As the mouse is the predominant *in vivo* mammalian biological model, there is considerably more information about genetic regulation and structure of murine Thy-1. The *thy1* locus is mapped to mouse chromosome 9, at which there are two alleles that encode the proteins designated Thy-1.1 and Thy-1.2. The two are distinguished by a single amino acid (a.a.) at position 89, arginine and glutamine respectively. In humans, *THY1* is mapped to chromosome 11q22.3 and initially expressed in a 161 a.a. pro form but undergoes several post translational modifications [64]. The first 19 a.a. of Thy-1 act as a signal peptide that targets it to the cell membrane and is later removed (Fig. 2). Thy-1 is also *N*-glycosylated at 2 [53] to 3 sites [3]; carbohydrate content accounts for nearly 30% of its mass, which ranges from 25 kDa to 37 kDa [3]. Between different tissues, the carbohydrate moiety composition may vary dramatically [4, 19]. Thy-1 is initially kept at the cell surface by a.a. 132 – 161, which embed into the membrane. However this c-terminal transmembrane domain is cleaved away and a GPI (glycophosphatidylinositol) moiety is added at residue 131. The GPI moiety is composed of two fatty-acyl groups that tether Thy-1 to the cell surface and participate in targeting to lipid rafts [53].

Thy-1 is differentially expressed and distributed among many species and among tissues of the same species. In mice, it is expressed on the surface of various cells including thymocytes, T-lymphocytes, bone marrow stem cells and in high levels in neurons and some fibroblasts. In the thymus, Thy-1 is the most abundant glycoprotein expressed on the surface, covering 10-20% of thymocyte surface area [23]. In humans, Thy-1 is absent from mature T cells, but expressed on a subset of CD34+ bone marrow cells, and umbilical cord blood- and fetal liver-derived hematopoietic cells. The highest expression of Thy-1 in humans is found on (primarily fetal) thymic stromal cells and in most fibroblasts. In bone marrow and in circulating leukocytes, Thy-1 is present in a small proportion of cells, primarily in a subset of CD34+ and CD3+ CD4+ lymphocytes. Thy-1 is also expressed in endothelial cells, smooth muscle cells, some leukemic and lymphoblastoid cells, such as THP-1 [69].

Thy-1 is one of the most highly glycosylated membrane proteins with a carbohydrate content up to 40% of its molecular mass [17]. The composition of Thy-1 carbohydrate moieties varies between different tissues or cells of the same lineage and among cells in different stages of differentiation. For example, in rats sialic acid in thymic Thy-1 far exceeds that found in brain Thy-1 and galactosamine is found only in brain Thy-1 [17]. In contrast to the Thy-1 antigen of most other species, guinea pig Thy-1 has a much higher molecular weight, which is due to a more extensive N-linked glycosylation, bringing the molecular radius up to 36 kDa [49].

Soluble Thy-1

Thy-1 does not exist solely in a membrane bound form. A soluble form of Thy-1 has been detected in serum, cerebral spinal fluid (CSF), wound fluid from venous leg ulcers, and synovial fluid from joints in rheumatoid arthritis [3, 45]. Speculated methods by which cells produce soluble Thy-1 include an alternative mRNA splice variant omitting addition of the GPI anchor, or an enzyme that cleaves Thy-1 away from the cell surface. Interestingly, the glycosylation pattern of soluble Thy-1 can differ from that of its presumptive source. For example, soluble Thy-1 detected in CSF has slightly higher MW than Thy-1 in cerebral cortex membranes. This differential size is attributed to unique glycosylation patterns; specifically, soluble Thy-1 is resistant to Endo H, which indicates low mannose content, whereas Thy-1 in cerebral cortex membranes is Endo H susceptible. However, both the soluble and membranous forms are identical in size when all the *N*-glycosylated groups are removed [3].

Soluble Thy-1 in CSF could possibly originate from a region of the brain other than the cerebral cortex. In rats, the carbohydrate moiety of brain Thy-1 has considerably less sialic acid content than that of thymocytes [4], though variation between regions of the brain is not well characterized. These findings suggest that the GPI anchor is severed in close proximity to the protein moiety, e.g., within the GPI moiety itself or at a protease target just up stream. As for targeting within the GPI moiety, both GPI-specific phospholipase D (GPI-PLD) and phospholipase C (PLC) can do so. The former is produced in mammals whereas the latter is of bacterial origin. These enzymes have distinct enzymatic requirements to cleave Thy-1 from the cell surface. Detergents or saponins are needed by exogenous GPI-PLD to cleave Thy-1, while PLC does not have such a requirement [36]. Although detergents are not required by PLC, the susceptibility of Thy-1 to cleavage varies from one cell type to another [36]. This is relevant given that serum contains GPI-PLD and as a consequence Thy-1 positive endothelial cells as well as circulating T-cells are susceptible, yet the concentration of serum Thy-1 is only 251 ± 105 ng/ml [45]. Localization of Thy-1 to cholesterol rich lipid rafts is thought to protect it from GPI-PLD present in serum [8]. Release of Thy-1 could also result from proteolysis, which may be an important mechanism in fibroblasts given the large number of proteolytic enzymes produced by fibroblasts [13, 38] and the aforementioned protection from GPI-PLD activity afforded by its location within its native membrane microdomain. Shedding of cell surface receptors is a common means of modulating their activity. For example, the ADAMs (a disintegrin and metalloproteases) are known to release a plethora of surface proteins including those tethered by GPI anchors [35]. The exact mechanism(s) of Thy-1 shedding and possible roles of shedding in normal biology and in disease have yet to be determined. Regardless of the means, shedding of Thy-1 could very likely play an important role in facilitating its complete removal from the cell surface, given its unusually slow turnover rate [29].

Cultured lung fibroblasts shed Thy-1 into the media when treated with various profibrotic cytokines, such as IL-1 β and TNF- α [18], yet the soluble and membrane bound Thy-1 have indistinguishable migration speeds through an acrylamide gel (unpublished observation). In contrast to fibroblasts, ECs (endothelial cells) increase their expression of Thy-1 when stimulated with profibrotic cytokines [20, 32]. The leukocyte integrin Mac-1 interacts

specifically with Thy-1 expressed on activated ECs, which suggests a role for Thy-1 in recruiting leukocytes into areas of inflammation [62]. Additionally, $\alpha_v\beta_3$ integrin expressed on melanoma cells interacts specifically with Thy-1 expressed on activated ECs. This is of particular relevance given that $\alpha_v\beta_3$ integrin expression by melanoma cells is closely associated with tumor progression and metastases formation in melanoma [44]. Thy-1^{-/-} mice have an impaired cutaneous immune response. Because wild type thymocytes express high levels of Thy-1 and Thy-1^{-/-} thymocytes do not properly mature, it was suggested that the impaired cutaneous immune response was a consequence of defective fine-tuning of T cell effector functions [7]. Alternatively, or in conjunction with this postulated mechanism, absence of Thy-1 on activated endothelial cells may account for the impaired cutaneous immune response. Regardless, it is evident that regulating Thy-1 expression at the sites of inflammation is of vital importance to resolving tissue injury. As of yet, it is unknown whether Thy-1 in itself has a role in its own regulation.

The biological functions of soluble Thy-1 remain unclear. It is important to note that Thy-1 lacking the GPI anchor very often becomes unrecognizable by antibodies against its membranous form [28]. Shedding of Thy-1 could potentially elicit a number of effects. Fewer Thy-1 molecules at the cell surface could directly affect the cell by limiting ligand binding and signal transduction. Soluble Thy-1 could also retain a comparable affinity with its ligand to that of its membranous form, making ligand unavailable to Thy-1 at the surface of the cell from which it was shed as well as on neighboring cells. In the human uterine cervix, Thy-1-expressing vascular pericytes appear to secrete Thy-1+ intercellular vesicles which communicate with basal epithelial cells as part of the “tissue control unit” of mesenchymal-epithelial interaction [9]. The biological significance of this intriguing phenomenon is uncertain.

Transcriptional Regulation of Thy-1 mRNA

The unique expression profile of Thy-1 is reflective of unusual regulatory elements that govern it (Fig. 3). The Thy-1 promoter has two elements that are traditionally attributed to “housekeeping” genes; specifically it is located within an area of high G/C content and has no classical TATA box [16, 56]. In addition, Thy-1 transcription initiates at multiple sites, and distribution of these sites differs in murine brain and thymus [55]. Transgenic mice with a hybrid or deletion construct of the Thy-1 transcriptional unit were found to have tissue-specificity control elements exclusively downstream of the cap site. Thy-1 expression in the mouse thymus and brain requires sequences located in intron 3 and at the 3′ end of intron 1, respectively. These downstream elements function independently of each other, as deletion of intron 3 eliminates expression in the thymus while levels in the brain are unaffected. Within the third intron of murine *thy1* is a 36 bp region that is capable of specifically binding an Ets-1-like nuclear factor expressed by both mouse thymocytes and splenocytes. Accordingly, Thy-1 is expressed in both mouse cell types. In the rat, however, the corresponding region differs by only three nucleotides and is incapable of binding a similar Ets-1-like nuclear factor in rat thymocytes. However, the region does recognize another nuclear factor expressed by rat thymocytes but not splenocytes. Unlike in mice, rat splenocytes do not express Thy-1. These data suggest that Thy-1 expression in rat thymocytes and not splenocytes is due to the conserved 36 bp recognizing a nuclear factor

found in the former and not in the latter [59]. This differential tissue expression between species as closely related as rat and mouse exemplifies the unique context-dependency of Thy-1 regulation and the difficulty in making broad inferences regarding its biology as it relates to disease states.

Deletion of intron 1 eliminates expression in the brain while levels in the thymus are unaffected. Tissue-specific transcription of Thy-1 requires those cis-acting sequences within the introns to cooperate with at a minimum 300-bp (-270 to +36) of the promoter. However, replacement of the Thy-1 promoter with another heterologous promoter does not abolish the tissue-specific or developmental expression profile of Thy-1 [61]. A murine *thy1.2* genomic expression cassette has been designed to drive expression in the nervous system. The cassette is void of all Thy-1.2 coding sequences and the thymus enhancer in intron 3, but retains the neural enhancer element in the first intron [10].

The endogenous Thy-1 promoter in itself is not sufficient to elicit transcription or tissue specificity without the downstream elements [61]. This makes the promoter unique for the “monogamous” relationship with its endogenous gene. Two transcription factors, Sp1 and CP1, are known to bind the Thy-1 promoter and are indispensable to its transcription *in vivo*. Three additional proteins, distinct from Sp1 and CP1, bind the promoter and were given the designation R1, R2, and R3 [56]. As of yet, the identity of these proteins remains unknown.

Posttranscriptional Regulation of Thy-1 mRNA

Though a far less examined mechanism, early evidence suggests that posttranscriptional regulation of Thy-1 mRNA determines the temporal expression of the Thy-1 protein in specific areas of the developing mouse nervous system. Expression of Thy-1 mRNA in these areas can precede detection of Thy-1 protein by several days. As mentioned previously, transcription of Thy-1 mRNA can occur at different initiation sites. However, the onset of protein expression does not coincide with any change to the size or transcription initiation site of Thy-1 mRNA, rather it appears to be the result of a yet to be elucidated posttranscriptional mechanism [66].

Heterokaryons generated from the fusion of mature Thy-1.1 expressing neurons with immature Thy-1.2 negative neurons became Thy-1 negative within 16 hr of fusion. However, these heterokaryons behave in a similar manner as cultured immature Thy-1.2 negative neurons in that Thy-1.2 expression becomes evident within 3-4 days in culture and also coincides with the re-expression of the Thy-1.1. As the nuclei of the Thy-1.1 and Thy-1.2 neurons are distinct within the heterokaryon, the inhibition of Thy-1.1 expression was concluded to be the consequence of a developmentally regulated diffusible suppressor molecule [46]. This lends support to developmental regulation of Thy-1 in the nervous system being, at least in part, a posttranscriptional event. Therefore unlike fibroblast and endothelial cells for which there is evidence for cytokine involvement in Thy-1 regulation, Thy-1 regulation in developing neurons seems to be the consequence of intrinsic factors.

Genetic and Epigenetic Regulation of Thy-1

The field of epigenetics encompasses regulation of gene expression that is often heritable but does not involve changes to DNA sequences. Two mechanisms by which this occurs include post-transcriptional modifications to histones and methylation of DNA within CpG islands [65]. As stated previously, Thy-1 acts as a tumor suppressor in nasopharyngeal cancer and can be down regulated by methylation of its promoter [31].

In both rat and human primary lung fibroblasts, CpG (cytosine-guanine) islands in the Thy-1 gene promoter are hypermethylated in the Thy-1 negative fibroblast subpopulation but not in the positive. The absence of Thy-1 results in part from promoter hypermethylation. In keeping with this finding, AZA (5-aza-2'-deoxycytidine), a DNA methyltransferase inhibitor, induces Thy-1 expression in Thy-1 (-) cells. Moreover, methylation-specific PCR-in situ hybridization (MSP-ISH) in lungs of patients with IPF demonstrated Thy-1 promoter hypermethylation within fibroblastic foci, which are populated with predominantly Thy-1 (-) myofibroblasts [48]. These findings suggest that epigenetic silencing of Thy-1 may be a pathogenic mechanism in IPF, as has been suggested for nasopharyngeal carcinoma.

Concluding Remarks

Thy-1 as a biofactor is remarkable both in the number of cell types expressing it and the diversity of phenotypes associated with its expression. It is expressed in hematopoietic and stromal stem cells in a relatively undifferentiated state, whereas in neurons, Thy-1 is associated with maturation and cessation of neurite outgrowth. In fibroblasts, Thy-1 expression accompanies specific phenotypes which depend upon the tissue from which they originate. Absence of Thy-1 in lung and synovial fibroblasts indicates a more fibrotic myofibroblast phenotype, whereas the opposite may be the case for orbital and myometrial fibroblasts. As of yet, much is still unknown regarding the exact mechanisms by which Thy-1 modulates these phenotypes and by which Thy-1 itself is regulated. What is clear is that both the roles and regulation of Thy-1 are context-dependent. The regulation of Thy-1 is a promising area of research; increased understanding of the mechanisms of Thy-1 regulation may lead to the possibility of therapeutic manipulation of cellular phenotypes in such diverse fields as nerve injury, cancer and fibrosis.

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Figure 1.

Thy-1 and fibroblast phenotype in pulmonary fibrosis. Fibroblasts in fibroblastic foci, the hallmark lesion of idiopathic pulmonary fibrosis (IPF), lack Thy-1 expression. Evidence exists for shedding of soluble Thy-1 (sThy-1) and epigenetic silencing of Thy-1 expression. Thy-1 (-) lung fibroblasts demonstrate increased migration, myofibroblastic differentiation, activation of latent TGF- β , and resistance to apoptosis. The absence of Thy-1 expression in pulmonary fibroblasts thus promotes a fibrogenic phenotype.

Figure 2.

Thy-1 molecule and proposed soluble forms. Thy-1 is initially generated as a 161 a.a. pro form. The initial 19 a.a. signal peptide is removed, and the terminal 29 a.a. are replaced with a GPI anchor, generating the mature form, which is anchored to the outer leaflet of the cell membrane by the diacyl group of the GPI anchor. Post-translational modifications are not shown. Shed Thy-1 could be generated either by cleavage of the GPI anchor by GPI-PLD, or by undefined proteases acting at as-yet undetermined cleavage sites.

Figure 3.

Murine Thy-1 gene structure and control elements. There are four exons and three introns. There are Sp1 and CP1 binding sites in the promoter region. Methylation of a CpG island in the first intron (*) can result in transcriptional silencing in rat and human fibroblasts. There are sequences conferring tissue specificity for brain and thymus in the first and third introns, respectively.