Restricted Expression of E2A Protein in Primary Human Tissues Correlates with Proliferation and Differentiation

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E2A is a basic helix-loop-helix (bHLH) transcription factor required for B cell lymphopoiesis and implicated in myogenesis and the regulation of insulin expression. As E2A is expressed widely in tissues, tissue-specific downstream effects are thought to result primarily from dimerization with other bHLH proteins. To investigate the degree to which regulation of E2A protein abundance may serve to regulate E2A function, expression of E2A was evaluated using immunohistochemistry on histological sections of primary human tissues. Somewhat surprisingly, nuclear staining for E2A was restricted in all tissues examined, often to a small subpopulation of cells. In some tissues, such as adult liver, expression was absent or limited to rare infiltrating lymphocytes. E2A-expressing cells were most abundant in lymphoid tissues. In tonsil, lymph node, and spleen, expression appeared most abundant and prevalent among rapidly proliferating centroblasts of the germinal center dark zone. Scattered E2A-expressing thymocytes were more numerous in the thymic cortex than medulla. In developing skeletal muscle, E2A was detectable in striated myotubes but not in more primitive mononucleated progenitors or mature muscle. Differential E2A expression was also noted in proliferating periventricular neuroepithelial cells in the developing brain. These results suggest that regulation of E2A abundance complements protein-protein interactions in modulating E2A function. (*Am J Pathol* 1998, 153:165–173)

Embryogenesis and normal tissue homeostasis have in common the requirement for exquisite control of gene expression to generate optimal numbers of specialized, functional cells from pluripotent progenitors. Transcription factors are nuclear proteins that regulate the expression of downstream target genes. The role played by transcription factors as regulators of lineage commitment and cellular differentiation has been studied extensively in hematopoiesis and myogenesis. Protein products encoded by the transcription factor gene *E2A* play important roles in both of these systems.^{1,2}

E2A was cloned through binding of its protein products to DNA elements called E boxes present within *cis*-acting transcriptional enhancers related to a number of cell-type-specific genes.³ *E2A* belongs to a large family of transcription factor genes that encode proteins with basic helix-loop-helix (bHLH) domains, which function in protein-protein dimerization and DNA binding.⁴ bHLH proteins regulate growth and development in many tissues, including hematopoiesis, neurogenesis, development of skeletal and cardiac muscle, mesodermal and dermal cell differentiation, and skeletal development.^{5–11}

E2A encodes three proteins, E12, E47, and E2–5 (hereafter referred to collectively as E2A for simplicity), which differ minimally and are generated through alternative pre-mRNA splicing.^{3,12–14} *E2A* has been implicated in B cell lymphopoiesis, myogenesis, and insulin gene expression.^{15,16} However, the observation that mice carrying homozygous null mutations of *E2A* appear relatively normal except for the inability to generate B cells and a relative impairment of T cell development indicates a particular requirement in lymphopoiesis.^{17–20} This relatively isolated defect seems at odds with the postulated roles of E2A proteins in other cell types and with earlier reports of ubiquitous expression.^{3,21} How might *E2A* regulate differentiation in a tissue-specific manner despite relatively unrestricted expression? bHLH proteins must dimerize to interact with DNA. Therefore, it seems likely that at least some E2A functions require heterodimerization with bHLH proteins the expression of which is restricted to particular tissues.¹² Considerable support for this notion has emerged from studies of myogenesis. For example, in myocyte progenitors, interaction between E2A and MyoD results in induction of differentiation and suppression of proliferation.²² In contrast, B-lymphopoiesis is associated with E-box binding by E47 homodimers.²³ Recent work showing that E2A proteins are degraded rapidly by the ubiquitin-proteasome pathway points to the importance of protein stability in regulating E2A function.²⁴

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Two chromosomal translocations implicated in causing B-lineage acute lymphoblastic leukemia (ALL) involve the *E2A* locus.²⁵ As a result of t(1;19), a chimeric transcription factor is produced in which a carboxyl-terminal portion of the *E2A* protein, including the bHLH module, is replaced by most of the homeodomain-containing protein PBX1. In t(17;19), a similar portion of *E2A* is replaced by a portion of a basic leucine zipper (bZIP) transcription factor called HLF. Both *E2A*-PBX1 and *E2A*-HLF are capable of inducing neoplastic transformation in several experimental assays, and this activity requires *E2A*-encoded transcriptional activation domains.

Given these considerations, it is clear that precise patterns of *E2A* expression in different tissues must be taken into account in developing models of *E2A* regulation and function. Early studies suggesting ubiquitous expression were based on Northern blots or electrophoretic mobility shift assays (EMSAs) on homogenates from established cell lines.^{3,21} More recently, detection of *E2A* protein by Western blot has indicated enhanced expression in B-lineage cell lines relative to lines derived from pancreatic, fibroblastic, myogenic, or myeloid cells.^{26–28} A study using *in situ* hybridization uncovered especially abundant *E2A* transcripts in certain cells undergoing rapid cell division in the rat embryo, highlighting the value of studying primary tissues at the single-cell level.²⁹

The current study is the first to have used immunohistochemistry with a monoclonal antibody to *E2A* to characterize patterns of expression in a wide variety of normal, primary tissues. We find that *E2A* protein is detectable in most human tissues. However, in all of these, the level of expression varies considerably from cell to cell. Particularly intriguing is the observation that in some tissues, high-level expression is restricted to functionally relevant compartments or cell types such as the germinal centers of lymphoid follicles. Therefore, our results indicate that the level of *E2A* expression is regulated in a cell-type-specific manner. Furthermore, these data suggest that modulation of *E2A* abundance may complement more widely discussed post-translational processes in regulating *E2A* function.

Materials and Methods

Sources and Preparation of Tissues

All tissue samples were received in the Kingston General Hospital Pathology Department as routine specimens obtained at surgery or autopsy. Eight human, first- or second-trimester products of conception were collected during routine pathological examination of surgical specimens and ranged in developmental stage from the late embryonic to early fetal periods. Samples of lymphoid tissue containing secondary follicles with germinal centers were obtained from several patients, including hyperplastic tonsil from two children, normal appendix from an adult, post-traumatic spleen from an adult, and hyperplastic lymph nodes from three adults. Samples from hyperplastic tonsil were snap-frozen in embedding me-

dium in an isopentane cryobath and stored at -70°C . The remaining samples were fixed for several hours in 10% neutral buffered formalin and subjected to routine tissue processing and paraffin embedding.

Immunohistochemistry and Immunofluorescence

For paraffin-embedded samples, antigen retrieval was accomplished by subjecting deparaffinized, 5- μm sections to microwaving at a gentle boil in 0.5 mol/L Tris, pH 10, for 10 minutes followed by equilibration to room temperature for a minimum of 20 minutes. All incubations with detection reagents were separated by two 5-minute rinses in Tris-buffered saline (TBS), pH 7.6. The sections were blocked for 10 minutes using 10% goat serum in antibody dilution buffer obtained commercially (Dimension Labs, Mississauga, Ontario, Canada), then covered with diluent containing the primary anti-*E2A* monoclonal antibody Yae.³⁰ Sequential, 20-minute incubations were then carried out with biotinylated goat anti-mouse secondary antibody and streptavidin-alkaline phosphatase conjugate (Zymed Laboratories, South San Francisco, CA). The tissue was then subjected to another cycle of incubations with the goat anti-mouse antibody and streptavidin-alkaline phosphatase reagents followed by a 15-minute incubation with Fast Red chromogen substrate solution (Sigma Chemical Co., St. Louis, MO). Finally, the slides were rinsed in tap water, counterstained with Meyer's hematoxylin, coverslipped in aqueous mounting medium, and examined using a standard light microscope. Immunohistochemistry using other primary antibodies was performed in a similar manner, except that the second round of incubations with secondary antibody and streptavidin-alkaline phosphatase conjugate was omitted and an anti-rabbit secondary antibody was used in the detection of polyclonal primary antibodies. An isotype-matched primary antibody (anti-synaptophysin; IgG1) was used as a negative control for anti-*E2A*. A section of tonsil was included as a positive control during each round of staining with anti-*E2A*. In preparing figures for publication, images from standard 35-mm photomicrographs were digitized to facilitate cropping and alignment.

For staining of tonsil sections by two-color immunofluorescence, histological sections of frozen tissue were fixed in 3.7% formaldehyde in phosphate-buffered saline (PBS) for 5 minutes, blocked in blocking buffer (5% bovine serum albumin (BSA) and 0.5% Triton X-100 in PBS) for 20 minutes, and incubated for 45 minutes with a primary antibody mixture containing the anti-*E2A* monoclonal antibody Yae and a polyclonal, anti-Ki-67 rabbit antiserum diluted in blocking buffer. The sections were then washed twice in PBS/Triton X-100 and incubated for 45 minutes with a secondary antibody mixture containing fluorescein-isothiocyanate-conjugated goat anti-mouse and Texas-Red-conjugated goat anti-rabbit antibodies in blocking buffer. The sections were then washed twice, coverslipped using Slow Fade aqueous mounting medium (Molecular Probes, Eugene, OR), and examined by fluorescence microscopy using a microscope equipped with an appropriate light source and filter sets. Digital

Figure 1. E2A expression in hyperplastic tonsil. **A:** E2A-expressing cells are most prevalent within the germinal centers of secondary lymphoid follicles. **B:** On higher magnification, immunoreactive cells are concentrated in the basal region of the germinal center, furthest from the broadest portion of the mantle zone (MZ), in an area corresponding to the dark zone. Staining is clearly nuclear (inset).

images from 35-mm photomicrographs were aligned and superimposed using the program MCID M2 (Imaging Research, St. Catharine's, Ontario, Canada).

Antibodies

The Yae monoclonal antibody was generated against recombinant E12 protein and recognizes all three splice products of the E2A gene (Santa Cruz Biotechnology, Santa Cruz, CA).³⁰ It was used at a dilution of 1:50. Rabbit polyclonal anti-E47 (Santa Cruz Biotechnology) was also used at 1:50 dilution. The following antibodies were purchased from Dako Corp. (Carpinteria, CA) and used at the stated dilutions: anti-Ki-67 (rabbit, polyclonal), 1:40; anti-CD1, 1:80; anti-CD3, 1:40; anti-CD4, 1:20; anti-CD8, 1:250; anti-CD20, 1:100; anti-CD21, 1:10 to 1:40; anti-CD22, 1:40; anti-CD23, 1:200; anti-CD45R, 1:20; anti-CD68, 1:50; and anti-synaptophysin, 1:25. Mouse monoclonal anti-Ki-67 was purchased from Coulter Immunotech (Miami, FL) and was used undiluted.

Results

Lymphoid Tissues

In tissue from hyperplastic tonsils, nuclear staining for E2A was most intense in the germinal centers of secondary lymphoid follicles (Figure 1). Although occasional E2A-expressing cells were scattered throughout the ton-

sil, including in the follicular mantle zone as well as the paracortex and medullary cords, they were much less prevalent.

As sites of B-cell proliferation and antigen-dependent selection, germinal centers play a crucial role in the secondary immune response. In the dark zone, rapidly proliferating centroblasts give rise to nondividing centrocytes that move apically into the light zone.³¹ Here, they are believed to undergo selection during which cells that fail to interact with antigen presented on the surface of follicular dendritic cells are eliminated by apoptosis. The functional compartments of germinal centers have been defined using antibodies against the surface markers CD21 and CD23 and against Ki-67, a nuclear, nonhistone protein expressed throughout the cell cycle but exclusively in cycling cells.³² To better define patterns of E2A expression within germinal centers, histological sections stained for E2A were compared with consecutive sections stained for Ki-67, CD23, or CD21 (Figure 2 and data not shown). These results confirmed that E2A expression was maximal in the dark zone of the germinal center, as defined by a very high prevalence of cells expressing Ki-67 and a low prevalence of cells expressing CD23. As earlier results in cultured fibroblasts suggested a negative correlation between E2A expression and proliferation, we wished to confirm expression of E2A in proliferating cells at a single-cell level.³³ Therefore, we performed two-color immunofluorescence for the concurrent detection of E2A and Ki-67 (Figure 3). This indicated

Figure 2. Distribution of E2A-expressing cells correlated with germinal center subcompartments. In consecutive histological sections of frozen tonsil tissue, the basal region of the germinal center containing the greatest number of E2A-immunoreactive cells corresponds to the dark zone (DZ), as defined by maximal expression of Ki-67, indicating proliferative cells. Follicular dendritic cells expressing CD23 are most numerous in the apical light zone (LZ) and appear absent from the dark zone.

that most mitotically active cells in the germinal center expressed E2A and confirmed maximal expression in dark zone centroblasts. Findings similar to those in the tonsil were noted in adult spleen, hyperplastic lymph node tissue, and mucosal-associated lymphoid tissue in normal adult appendix (not shown). These findings

were similar to those reported recently in lymph node, appendix, and spleen by Goldfarb et al.³⁴ E2A-expressing cells, which appeared to be of hematopoietic origin, were numerous in embryonic liver and somewhat less numerous in the red pulp of embryonic spleen (data not shown).

Figure 3. Co-localization of E2A with the proliferation marker Ki-67 in a lymphoid follicle. Cells expressing E2A (green) have a similar distribution to those expressing Ki-67 (red) when the two proteins are detected in the same histological section of frozen tonsil tissue. Digital superimposition of the two images causes co-expressing cells to appear as yellow or pale orange. This indicates that the overwhelming majority of E2A-expressing cells in the dark zone (centroblasts) are in cycle.

Figure 4. Distribution of E2A-expressing cells in juvenile thymus. Scattered immunoreactive cells appear somewhat more numerous in the cortex (C) than in the medulla (M).

In two first-trimester thymuses and normal thymus from a 2-year-old child, E2A expression was detectable in scattered cells in the cortex and, less frequently, in the medulla (Figure 4). Although thymic cortex and medulla are distinguishable morphologically, we confirmed the identity of these zones in the juvenile thymus by performing immunostaining on consecutive histological sections for the T-subset markers CD4, CD8, and CD1, the T-lineage marker CD3, and the B-lineage markers CD20, CD22, and CD45R (data not shown). Whereas B-lymphoid cells were quite numerous in the medulla, they were very rare in the cortex, suggesting that cortical cells expressing E2A were not of B lineage. The greater prevalence of E2A-expressing cells in the thymic cortex was interesting as 1) like the germinal center, the thymic cortex is distinguished by rapid proliferation and antigen-driven selection of lymphoid cells and 2) recent transgenic mouse studies implicate E2A as well as the related E-box-binding bHLH protein HEB in T-cell development in the thymus.^{20,35}

Muscle

The embryological process of myogenesis is protracted and asynchronous.³⁶ During most of the embryonic period, developing muscle contains both undifferentiated myoblasts and the more differentiated myotubes, which are multinucleated and have started to express contractile proteins. Further differentiation of these cells pro-

duces mature myofibers. In a Carnegie stage 22 specimen, myotubes with rudimentary cytoplasmic contractile proteins were associated with strong nuclear expression of E2A (Figure 5). Less differentiated myoblasts in the same tissue section were devoid of staining. This pattern was observed in four first-trimester specimens containing skeletal muscle. In contrast, the proliferation marker Ki-67 was expressed in myoblasts but was much less abundant or absent in the more differentiated myotubes. This suggests that initiation of muscle differentiation and associated exit from the cell cycle is associated with increased expression of E2A. Sections of skeletal muscle from second-trimester tissue showed reasonably well formed myofibers, only a small proportion of which expressed E2A. No E2A expression was observed in fetal myocardium, and expression in adult skeletal muscle was minimal or absent (data not shown). Therefore, E2A expression appears to be low in committed but minimally differentiated cells, increases during the early stages of muscle differentiation, and returns to low or undetectable levels in fully differentiated muscle fibers.

Neural Tissues

Expression of E2A in the nervous system was evaluated in three first-trimester specimens and observed within 1) the proliferating, neuroepithelial cells of the subventricular and intermediate zones in the forebrain and spinal cord (Figure 5), 2) scattered cells, possibly immature Purkinje cells, in the internal granular layer of the developing cerebellum (data not shown), and 3) dorsal root ganglion cells in the spinal cord (data not shown). E2A expression in these tissues was less intense relative to lymphoid tissue or muscle. In brain and spinal cord, the zones expressing E2A and Ki-67 overlapped but were not identical; Ki-67 expression was most prevalent and E2A expression least prominent in the subventricular zone, whereas moving away from the ventricle or spinal canal into the intermediate zone, the reverse pattern was seen. As in lymphoid and muscle tissues, these findings in neural tissues suggest a correlation between E2A expression and cellular proliferation or differentiation.

Other Tissues

E2A expression was detected in first-trimester kidney, lung, intestine, and testis. In the kidney, the most intense staining was in epithelioid cells within the glomerular tuft in well developed glomeruli (Figure 6). Many primitive renal tubules in the neogenic zone of the kidney and many renal interstitial cells expressed moderate levels of E2A. In the embryonic and juvenile testis, the strongest staining for E2A occurred in germ cells at various stages of differentiation (Figure 6). Many E2A-expressing cells were present in the bronchial tubes and interstitial cells of the embryonic lung (data not shown). In intestine, scattered basal epithelial cells and many submucosal cells expressed E2A (data not shown). Expression of Ki-67 was also evaluated in these tissues. Although no unequivocal, consistent relationship was uncovered be-

Figure 5. E2A expression in fetal (Carnegie stage 22) spinal cord and muscle. In the spinal cord, partial overlap is observed between cells expressing E2A and the proliferation marker Ki-67. However, whereas E2A expression appears most abundant in the subventricular zone, Ki-67 is most prevalently expressed in the ventricular zone (VZ), immediately subjacent to the spinal canal. In developing muscle, nuclear staining for E2A is restricted to the more differentiated, multinucleated myotubes. In contrast, expression of Ki-67 is not detectable in myotubes but prominent in mononuclear myoblasts.

tween E2A expression and proliferation, E2A was often observed in populations of cycling cells, for example, testicular germ cells.

In the first-trimester pancreas, weak E2A expression was seen in some acinar cells. The second-trimester pancreas contained identifiable islets within which a small minority of cells expressed detectable E2A. The surprisingly weak staining in these cells was comparable in intensity to that seen in occasional, scattered acinar cells and far weaker than that associated with lymphocytes in the same histological section (data not shown). Other neuroendocrine tissues were negative for E2A expression, including the anterior and posterior lobes of the adult pituitary gland, the adult pineal gland, and the fetal and adult adrenal gland (data not shown). No staining was observed in adult liver.

In some tissues, bowel and kidney, for example, weak cytoplasmic staining was visible in some epithelial cells. This staining was nonspecific as it was also seen in association with an isotype-matched primary antibody against CD68. Nonspecific cytoplasmic staining was also visible in mast cells. At least some of this signal was probably related to endogenous alkaline phosphatase activity.

Discussion

The observation of particularly abundant E2A protein in germinal center B cells in primary tissue samples con-

firms and extends earlier reports, based on Western blots, of high-level expression in cultured B-lineage cells.^{26,27} It has been argued previously that differential expression may help explain the observation that E2A homodimers appear to form exclusively in B cells.^{23,27} Furthermore, differential expression or differential homodimer formation may reflect the particular, crucial role played by E2A in B lymphopoiesis, as uncovered by gene targeting in mice.^{17,18}

The germinal centers of secondary lymphoid follicles play a central role during T-cell-dependent antibody responses.³⁷ Activated B cells proliferate in the dark zone of the germinal center as centroblasts and activate a somatic hypermutational mechanism that acts on variable regions of immunoglobulin genes. As nonproliferative centrocytes, they then migrate to the light zone of the germinal center. Here, they appear to undergo selection based on their ability to interact, through surface immunoglobulin, with antigen presented on the surface of follicular dendritic cells; cells that fail to bind antigen with high affinity are eliminated by apoptosis. We have demonstrated abundant, differential expression of E2A in proliferating centroblasts in the germinal center dark zone.

A recent report suggests that E2A expression in the germinal center dark zone reflects the involvement of E2A in immunoglobulin heavy chain class switching, a process that occurs at this site.³⁴ Furthermore, it seems reasonable to speculate that E2A could be involved in somatic hypermutation of immunoglobulin genes, as re-

Figure 6. E2A expression in fetal (Carnegie stage 23) kidney and testis. **A:** In the kidney, the most intense staining is in glomeruli at various stages of development, although more primitive epithelioid elements and interstitial mesenchymal cells are also stained. **B:** In the testis, expression is detectable in germ cells within seminiferous tubules and in scattered interstitial cells.

cent work has shown that the major intronic enhancer, which contains multiple E-box elements capable of interacting with E2A proteins, is required for somatic hypermutation of the immunoglobulin light or heavy chain loci.^{38,39} Finally, the possibility exists that expression of E2A by germinal center cells may reflect processes that occur much earlier in B-cell ontogeny. Recent work has shown that expression of the recombinase activating genes *Rag1* and *Rag2*, which are essential in the initiation of immunoglobulin gene rearrangement, is not restricted to lymphoid progenitors in the bone marrow or thymus but also occurs in germinal centers.^{40–42} Given the absence of *Rag* gene expression in *E2A*($-/-$) mice, one intriguing possibility is that E2A expression in some germinal center cells may function to activate transcription of *Rag1* and *Rag2*. E2A may be playing similar roles in centroblasts and thymocytes; these are both proliferating lymphoid cell populations poised for further differentiation or negative selection by apoptosis. This is consistent with the observation that *E2A*($-/-$) mice show defective T-cell maturation in the thymus.²⁰

Evidence derived from cultured fibroblastic or myogenic cells supports the notion that E2A and the myogenic bHLH proteins inhibit proliferation while promoting differentiation.^{33,43–45} These effects are subject to dominant-negative inhibition by Id proteins and appear to involve direct interaction of MyoD or Id with the retinoblastoma gene product pRB1 and of MyoD with the co-

activator p300.^{45–47} Therefore, our finding of differential E2A expression in populations of cells associated with particularly abundant proliferative activity is somewhat surprising, although an earlier study using *in situ* hybridization for *E2A* transcripts noted a similar association.²⁹ It seems reasonable to speculate that downstream effects of E2A expression on proliferation could differ in a lineage-specific manner and that such differences could be related to the participation of different dimerization partners. For example, whereas heterodimerization between E2A and myogenic bHLH proteins is anti-proliferative in fibroblasts, formation of E2A homodimers in B cells may promote proliferation. Interestingly, the paired box transcription factor *Pax-5*, which has been proposed as a potential target for regulation by E2A, is required for B cell development in mice, is expressed in a tissue distribution that overlaps that of E2A, and is capable of stimulating B cell proliferation in tissue culture.^{18,48–50} Therefore, potential proliferative effects of E2A in B cells could be mediated by *Pax-5*.

Whereas t(1;19) or t(17;19) are associated exclusively with B-progenitor ALL, either of the E2A-chimeric oncoproteins E2A-PBX1 or E2A-HLF can induce neoplastic transformation in nonlymphoid cells, at least as reflected by the results of experimental transformation assays.^{51–53} Furthermore, forced expression of E2A-PBX1 or deficiency of E2A in transgenic mice can induce T-lineage lymphomas.^{20,54} As transcription of *E2A-PBX1* or *E2A-HLF* is probably regulated by *cis*-acting DNA sequence elements linked to *E2A*, these observations are consistent with the notion that the consistent association of E2A-chimeric oncoproteins with B-lineage disease is related to preferential activity of the E2A promoter in these cells.

Preferential expression of E2A in myotubes of developing skeletal muscle is particularly interesting in view of the well documented role of E2A and the myogenic bHLH proteins MyoD, myogenin, Myf5, and MRF4 in regulating proliferation and differentiation *in vitro*.⁵⁵ When growth factors become limiting, myogenic bHLH proteins in cultured myoblasts become activated to induce a G1-specific cell cycle withdrawal and differentiation to myotubes. Activation of myogenic proteins appears to occur by release from a number of inhibitory mechanisms, including phosphorylation or interactions with the bZIP transcription factors Fos or Jun.^{56–58} Particularly noteworthy is the loss of Id expression that occurs under these conditions and contributes to the release of active myogenic proteins.⁵⁹ Thus, the up-regulation of E2A expression that we observe in myotubes could complement these mechanisms in generating increased levels of functional heterodimers formed between E2A and myogenic proteins.

We observed enhanced E2A expression in the subventricular zone of the developing human brain. As with thymocytes and germinal center centroblasts, E2A expression in this area occurs in the context of proliferation, differentiation, and apoptosis.⁶⁰ Mammalian E2A proteins are homologues of the *Drosophila* daughterless proteins, transcription factors with multiple roles that include developmental induction of the peripheral nervous system and sex determination.⁶¹ E2A proteins could be impor-

tant for regulating proliferation in developing neural tissue but seem likely to have broader roles, considering the intricate biology of daughterless proteins.⁶

Our observation of weak or absent expression of E2A in pancreatic islets is somewhat surprising, given the available evidence supporting a role in insulin gene expression. This includes the demonstration of E2A expression, by Western blot, in several β -cell-derived cell lines of rodent origin.²⁶ Based on these earlier results, one might have expected strong staining in pancreatic islets, as β -cells constitute 70 to 80% of islet cells.⁶² Possible explanations for this apparent discrepancy include aberrant expression related to the establishment of immortal cell lines and interspecies differences between rodents and humans. Consistent with the finding of scant E2A expression in primary human islet cells are recent data derived from *E2A* knockout mice indicating that, although *E2A* gene products may play some role in insulin gene expression, their function in pancreatic development, insulin production, and normal glucose metabolism is not essential.⁶³

In a previous study, *E2A* expression was evaluated in normal tissues from fetal or adult rats by *in situ* hybridization.²⁹ In many tissues, the findings were similar to our own. These include germinal centers of lymphoid follicles, cells lining cerebral ventricles, testis, fetal glomeruli, bronchi, enteric mucosa, exocrine pancreas, myocardium, and liver. Expression in pancreatic islets was not described in the rat study. Consistent with our observations, E2A message was more abundant in developing than fully developed skeletal muscle. However, some discrepancies are apparent between the two studies. Whereas ubiquitous expression of message was observed in thymus, pituitary, kidney, and adrenal tissue from adult rat, we detected E2A protein in only scattered cells in the thymus and were not able to detect any convincing expression in pituitary, kidney, or adrenal tissue from adult humans. Although these observations may suggest an element of post-transcriptional regulation of *E2A* expression in these tissues, they could alternatively reflect differences in the sensitivity or microscopic resolution of the two detection methods or, perhaps, species differences.

Perhaps most noteworthy in this study is our observation of the generally restricted nature of E2A expression in primary tissues. Although most of the tissues examined contained at least a few E2A-expressing cells, these almost always constituted a minority population. Consideration of mechanisms by which E2A function might be regulated, particularly those by which the tissue-specific downstream effects of E2A might be achieved, have generally been predicated on the assumption of relatively unrestricted expression. Regulatory models have therefore emphasized the role of protein-protein interactions or post-translational modification in inhibiting E2A function in tissues in which the proteins are present but not apparently functional. Although our results do not contradict such models, they do suggest that modulation of protein abundance may complement post-translational interactions in regulating E2A function.

Acknowledgments

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