Jennifer Villaseñor
Christophe Benoist
Diane Mathis

AIRE and APECED: molecular insights into an autoimmune disease

Authors' address
Jennifer Villaseñor, Christophe Benoist, Diane Mathis
Department of Medicine, Section on Immunology and Immunogenetics, Joslin Diabetes Center, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.

Correspondence to:
Diane Mathis
Department of Medicine
Section on Immunology and Immunogenetics
Joslin Diabetes Center
Brigham and Women's Hospital
Harvard Medical School
Boston, MA 02215
USA
Tel.: +1 617 264 2781
Fax: +1 617 264 2744
E-mail: diane.mathis@joslin.harvard.edu

Immunological Reviews 2005
Vol. 204: 156–164

Immunological Reviews
0105-2896

Summary: Mutations in the autoimmune regulator (AIRE) protein are the causative factor in development of the human disease autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). In mice, the absence of the analogous protein aire influences ectopic expression of peripheral tissue antigens in thymic medullary epithelial cells (MECs), resulting in the development of an autoimmune disorder similar to APECED and establishing aire/AIRE as an important player in the induction of central tolerance. However, the molecular mechanism of AIRE's function, in particular its ability to specifically control the expression of peripheral tissue antigens in MECs, is still unclear. Here, we review current evidence relating to the molecular mechanism of AIRE.

AIRE and APECED

Understanding the etiology and pathogenesis of autoimmune diseases present a major challenge for the development of effective diagnostics and therapies, mostly because of the genetic and phenotypic variability characteristic of such disorders. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) or autoimmune polyendocrinopathy syndrome type-1 provides a powerful means of studying the biological events leading to autoimmunity and autoimmune diseases, given its monogenic nature. Only a few other autoimmune diseases result primarily from genetic defects within a single gene (1–3).

APECED's first manifestation is typically and somewhat surprisingly mucocutaneous candidiasis (4). It progresses to autoimmune destruction of a variable combination of endocrine organs. Diagnosis of APECED requires at least two of the following: candidiasis, hypoparathyroidism, and adrenocortical failure (4). In addition to these common presentations, a variety of other ailments, differing from individual to individual, have been described, e.g. autoimmune hepatitis, intestinal malabsorption, alopecia, and vitiligo. APECED is a rare disease, but it has an elevated frequency in certain populations such as Finns (1 : 25 000), Iranian Jews (1 : 9000), and Sardinians (1 : 14 500) (5, 6).

The causative locus for APECED was mapped by two independent groups to chromosome 21p22.3 (7–9). The protein it encodes, autoimmune regulator (AIRE), is highly conserved between humans and mice (AIRE in the former and aire in the latter) and shares 73% homology (10). The 55-kDa AIRE protein localizes in the nucleus and contains several motifs found on proteins involved in transcriptional regulation: a conserved nuclear localization signal; a homogenously staining region that mediates homodimerzation of speckled protein 100 kDa (Sp100) (11, 12); the DNA-binding SAND [named after Sp100, AIRE, nuclear DEAF-1 related (NUDR), and DEAF-1] domain; two plant homeodomain (PHD)-type zinc fingers (13); and four LXXLL motifs predicted to function as nuclear receptor interaction domains. Currently, at least 50 different mutations of the *AIRE* gene have been identified, with many of the APECED-causing mutations clustered within putative DNA binding and transactivation domains, suggesting the importance of each of these domains in the function of *AIRE* (5).

Arguably, the most intriguing aspect of *AIRE*'s function is its required role in the expression of a variety of peripheral tissue antigens in the medullary epithelial cells (MECs) of the thymus, a function that seems to be required for purging the immune system of autoreactive T cells (14, 15). However, the precise molecular mechanism by which *AIRE* exerts its influence remains an enigma. It remains unknown how a nuclear factor can specifically regulate transcription of a variety of peripheral tissue transcripts within thymic MECs, although each of the transcripts has a unique pattern of expression within tissues. Furthermore, it is not known at what level *AIRE* functions: does it operate on individual promoters or enhancers, or does it orchestrate long-range effects such as chromatin remodeling or gene imprinting? This review highlights some of the major findings related to the molecular mechanism of AIRE's function and synthesizes these observations into a coordinated view of AIRE's transcriptional regulatory functions in thymic MECs.

DNA-binding activity and nuclear localization

It has been hypothesized that AIRE may act as a direct transcriptional regulator on the basis of its subcellular localization and its predicted structural characteristics. Intriguingly, transgenic mice express the SV40 T antigen behind the rat insulin promoter as well as the endogenous insulin gene in the thymus, suggesting that AIRE may act directly at this small promoter region (16). However, given that AIRE mediates the transcription of a variety of genes encoding peripheral tissue antigens, each expressed at considerably different levels in the periphery, it seems more or less unlikely that AIRE may act directly at the promoters or enhancers of these genes. Nevertheless, the presence of a SAND domain in AIRE has been interpreted as an important clue to its function (12). This notion has been reinforced by the observation that R257X, a mutation within a CpG dinucleotide affecting the SAND domain, is the most common APECED-causing mutation amongst the Finnish, Northern Italian, and Eastern European populations (17–19). The SAND domain is a conserved 80-amino acid sequence found in several nuclear proteins. In addition to AIRE, the Sp100 family (20, 21), NUDR protein (22, 23), and glucocorticoid modulatory element-binding protein-1 (GMEB-1) (24, 25) all contain a SAND domain and have been associated with chromatin-remodeling activities.

The crystal structure of the SAND domain of Sp100b has revealed a unique α/β folded structure containing a conserved KDWK sequence present within a positively charged surface patch of the α-helix (26). Using electrophoretic mobility shift assays, the SAND domain of NUDR sufficed to bind the DNA sequence containing nucleotides 52–69 of the NUDR 5′-untranslated region, which was previously characterized as a binding site for NUDR (26). Moreover, Bottomley *et al.* (26) demonstrated that the conserved KDWK sequence motif within the NUDR SAND domain was critical for its DNA-binding activity. Likewise, Surdo *et al.* (25) showed that the SAND domain from GMEB-1 was sufficient to bind the GME DNA sequence. The DNA-binding surface of GMEB-1 was also mapped to an α-helical region encompassing the conserved KDWK motif (25). Based on the crystal structure of the SAND domain of Sp100b, Halonen *et al.* (27) used homology modeling to identify a similar positively charged surface-exposed area composed of an NKAR motif present in the SAND domain of AIRE and predicted it to be important for DNA-binding. Evidence for the direct binding of AIRE DNA remains sparse, confined to only one study (28). Using a randomly generated library of oligonucleotide sequences, Kumar *et al.* (28) claimed that AIRE binds to two consensus sequences: (i) TTATTA, a sequence similar to the TATA box and (ii) a tandem repeat of the ATTGGTTAA sequence. However, the significance of these data has been questioned (29, 30), and it remains to be seen whether AIRE binds to these sequences *in vivo*.

In view of these findings, it is interesting to note the presence of two distinct subcellular localization patterns of AIRE in nuclear dots and cytoplasmic tubular structures (11, 17, 31, 32). The localization of AIRE into nuclear dots has

been compared with but is really distinct from that of promyelocytic leukemia (PML) protein, which functions somehow in transcriptional regulation (33). However, it is not known whether the localization of AIRE within nuclear bodies is functionally significant or whether AIRE-mediated transcription of peripheral tissue antigens occurs within them. More recently, the use of stably transfected green fluorescence protein-tagged AIRE has provided additional insights into its subcellular localization (34). Via biochemical fractionation studies, Akiyoshi *et al.* (34) observed the presence of AIRE associated with a fraction after treatment with detergent, DNase I, ammonium sulfate, and high salt, representing material containing the nuclear matrix, as confirmed by the presence of lamin A/C (a core nuclear-matrix-associated protein). Interestingly, the characteristic speckled pattern of AIRE remained detectable after this treatment. Notwithstanding the difficulties in interpreting forced expression studies, an association of AIRE with the nuclear matrix represents an important finding and may provide an explanation for its ability to regulate the transcription of the diversity of transcripts encoding peripheral tissue antigens in MECs. One might speculate that matrix-associated regions (MARs) or scaffold-attachment regions, both DNA sequences of 150–200 bp that bind to the nuclear matrix to create chromatin loops (35), are present within the intergenic regions of genes encoding peripheral tissue antigens and that they attach to the nuclear matrix via AIRE to create domains of actively transcribed genes. In support of this view, several studies have ascribed functions to the chromatin loops created by the binding of MARs to the nuclear matrix, such as transcriptional activation via chromatin remodeling and insulation of genes from position effects (36).

Transcriptional transactivation properties of AIRE

Demonstration of the transcriptional transactivation properties of Aire has relied on the use of heterologous DNA-binding domains and transient-transfection assays. Nonetheless, these studies do show that AIRE has the potential to activate transcription (17, 37). Of note, Pitkanen *et al.* (32) developed an assay incorporating a luciferase-reporter gene driven by the interferon-β (IFN-β) minimal promoter transiently transfected into COS cells in combination with wildtype or various mutants of AIRE. Interestingly, full-length, wildtype AIRE was capable of inducing transcription via the minimal IFN-β promoter, a finding that has several implications for the immunological modulation of AIRE-mediated transcription. In addition, missense mutations within the two PHD-type zinc fingers abolished AIRE's ability to induce transcription in this assay, in accordance with previous studies (17, 27). More recently, Matsumoto and colleagues (38) localized the transactivation potential of AIRE to the second PHD-type zinc finger, using a GAL4-based reporter assay, showing that APECED patients' mutations within the second but not the first PHD-type zinc finger reduced its transcriptional transactivation potential.

Cyclic adenosine monophosphate-responsive element-binding protein (CBP) is known to function as a transcriptional co-activator for a variety of transcription factors including Jun, Fos, nuclear receptors, nuclear factor (NF)-κB, and the signal transducer and activator of transcription (STAT) proteins (39). CBP contains three cysteine-histidine-rich conserved domains (CH1, CH2, and CH3) that are thought to mediate protein–protein interactions with factors such as STAT2, hypoxia-inducible factor-1α, p300/CBP-associated factor, steroid receptor coactivator-1 (SRC-1), TIFII (SRC-2), and steroid receptor coactivator-3 (SRC-3) (39, 40). The finding that AIRE associates with CBP (37) lends further support towards its involvement in transcriptional regulation. Through a series of co-precipitation GST-pull-down assays using the various domains of CBP, interaction of full-length AIRE with the CH1 and CH3 domains of CBP was revealed, an interaction thought to link AIRE to the basal transcriptional machinery. Again, though, these strictly *in vitro* results should be viewed with caution.

An exciting observation concerning the role of AIRE as a transcriptional regulator was made using AIRE-deficient mice. Those animals developed leukocytic infiltrates into selected areas of several endocrine organs, as well as autoantibodies of similar specificities (14, 41). Using wildtype and AIRE-deficient mice as donors and hosts in a set of radiation/bone-marrow chimera and thymus transplantation experiments, Anderson *et al.* (14) demonstrated a requirement for AIRE expression in the thymic stroma for the induction of tolerance to tissue-specific antigens and for the prevention of APECED. Based on previous observations showing the expression of peripheral tissue antigens along with the high expression of AIRE in thymic MECs (42, 43), it seemed reasonable to hypothesize that AIRE might be directly or indirectly responsible for the transcription of peripheral tissue antigen genes in MECs. Indeed, DNA-microarray analyses showed that MECs lacking aire exhibited downregulation of a subset of peripheral tissue antigens, strongly implicating this molecule in the regulation of peripheral tissue antigen expression in MECs and in the imposition T-cell tolerance centrally (14).

E3 ubiquitin ligase activity?

PHD domain specificity and function

The presence of the two PHD zinc fingers in AIRE, coupled with the presence of such domains in chromatin-associated proteins (13), prompted the speculation that AIRE might be involved in transcriptional regulation. In particular, proteins with the PHD-type zinc finger domain exhibit a range of regulatory functions such as acetylation of histone proteins, ubiquitination, and chromatin remodeling. For example, CBP/p300 contains a PHD finger required for acetyltransferase activity (44), as part of its CH2 domain. Other proteins containing a PHD-type zinc finger include KRIP-1 (45), the Mi-2 autoantigen (46, 47), the chromatin-remodeling protein ACF (48, 49), and the ING (inhibitor of growth) family of putative tumor suppressors (48, 49).

There is much discourse in the literature regarding the specificity and general function of PHD fingers (50–52), especially in light of a recent finding showing the ability of the PHD domain of ING2 (a member of the ING family) to bind phosphoinositide species (53). Although the primary sequence of the PHD-type zinc finger is distinct from the RING finger (Cys4-HisCys3 vs. Cys3-HisCys4), the PHD domain does share a similar structural feature with the RING finger: binding of two zinc atoms in a cross-braced/interleaved topology (54, 55). Given this similarity and observations that RING finger proteins have demonstrated ubiquitin ligase activity (56, 57), it is not too surprising that a subset of PHD-containing proteins, such as Kaposi's sarcoma-associated herpes viral proteins (58, 59) and mitogen-activated protein kinase/ERK kinase 1, have shown E3 ubiquitin ligase activity (60). Consequently, it has been postulated that the PHD domain of AIRE might have ubiquitin-ligase activity.

Ubiquitin ligases and gene transcription

During ubiquitination (*Fig. 1*), an adenosine triphosphate-dependent reaction involving an E1 ubiquitin-activating enzyme leads to the activation of the C-terminal Gly residue and formation of the initial thiol-ester bond between the Cys residue of E1 and ubiquitin. The activated ubiquitin is then transferred to another Cys residue on the active site of an E2 enzyme, the ubiquitin-carrier protein. The last step involves the E3 ubiquitin ligase, which catalyzes the attachment of the C-terminus of ubiquitin to the ε-amino group of the substrate's lysine residue, forming an isopeptide bond. Substrate specificity is achieved by the large number of specific E3 ligases, which are capable of mono-ubiquitination or polyubiquitination of their substrate (57, 61).

A correlation between ubiquitination and transcriptional regulation via proteasome-dependent destruction of transcription factors as well as proteasome-independent mechanisms is supported by a large body of evidence (62, 63). Ubiquitin-mediated transcriptional regulation occurs at several levels: (i) mono-ubiquitination of histone species, followed by methylation; (ii) regulation of RNA polymerase II; (iii) regulation of the location of transcriptional activators; (iv) control of the activity of transcriptional regulators; and (v) control of the levels of transcriptional regulators (reviewed in 62, 63–65). Although the relationship between ubiquitin and transcriptional regulation is a recently recognized one, an association between ubiquitin and chromatin has been appreciated for quite some time. Indeed, the first ubiquitinated protein to be described was histone H2A (reviewed in 63), whose levels were found to vary in response to various stimuli. Additionally, in *Drosophila*, the TATA-binding-protein-associated factor II250 subunit of the transcription factor TFIID was shown to mono-ubiquitinate histone H1, and it is essential for the expression of genes in the correct order during *Drosophila* development (66). Other examples include regulation of the tumor suppressor protein p53 by the ubiquitin ligase MDM2, which catalyzes the addition of a single ubiquitin moiety to a cluster of six COOH-terminal lysines (67, 68), the poly-ubiquitination of p53 by p300 (69), and the ubiquitination of the transactivation domain-containing transcription factor VP16 by the Met30 ubiquitin ligase (70). More recently, the ubiquitination of phosphorylated c-Jun by the SCF^{Fbw7} E3 ubiquitin ligase was found to be important for antagonizing activator protein-1 (AP1) transcriptional activity and apoptosis via c-Jun N-terminal kinase signaling (71).

Adding to the list of ubiquitin ligases important for transcriptional regulation, the most N-terminal PHD-type zinc finger of AIRE was essential for an E3 ubiquitin ligase activity found to be associated with the full-length, recombinant protein using an *in vitro* ubiquitination assay (38). The AIRE E3 ubiquitin ligase activity preferentially employed ubiquitin c4 (Ubc4) an E2 ubiquitin carrier protein predominantly expressed in the thymus. Importantly, APECED-causing mutations residing in PHD1 (C311Y and P326Q) resulted in the loss of E3 ubiquitin ligase activity, suggesting the importance of this activity in preventing autoimmunity by promoting immunological tolerance activity. Although a seemingly disparate function, the E3 ubiquitin ligase function of AIRE does not divorce it from a role in directly or indirectly regulating the transcription of peripheral tissue antigens in MECs. As mentioned, ubiqutin ligase activity commands a prominent position at several levels of transcriptional

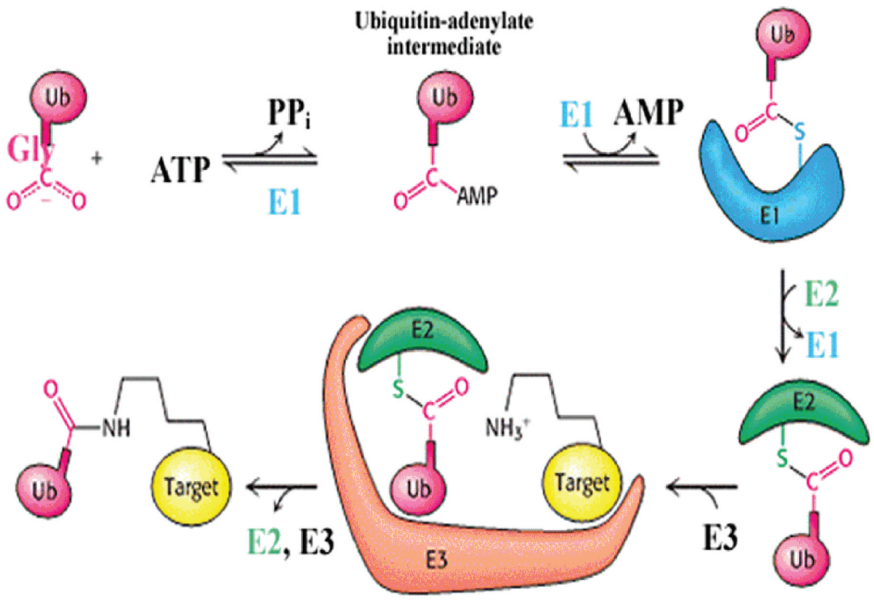

Fig. 1. The ubiquitination pathway. The ubiquitination process generally requires the presence of adenosine triphosphate (ATP), an ubiquitin-activating enzyme (E1), an ubiquitin-carrier protein (E2), and an ubiquitin ligase (E3). The C-terminal Gly residue of free ubiquitin (Ub) is activated via the formation of an ubiquitin-adenylate intermediate. This intermediate subsequently forms the initial thiol-ester bond between the Cys residue of E1 and ubiquitin. The activated ubiquitin is transferred to another Cys residue on the active site of one of a number of E2s. Finally, an E3 ubiquitin ligase catalyzes the attachment of the C-terminus of ubiquitin to the ε-amino group of the substrate's lysine residue, forming an isopeptide bond.

regulation, including histone ubiquitination and degradation of a transcriptional co-factor to allow for the elongation of transcripts by RNA polymerase II. AIRE's ubiquitin ligase activity could play a role at several levels of transcriptional regulation and might provide an explanation for the ability of this factor to mediate the transcriptional regulation of a large variety of peripheral tissue antigens in MECs. In addition, the E3 ubiquitin ligase activity could also be independent of any role for AIRE in transcriptional regulation. Indeed, when the ligase activity mapped to AIRE's most N-terminal PHD-type zinc finger, transactivation potential seemed to reside in the other PHD domain (38). Other potential functions of E3 ubiquitin ligase activity in the context of AIRE and APECED include targeting a subset of peripheral tissue antigens in MECs for degradation and presentation on major histocompatibility complex (MHC) to thymocytes and degradation of immunomodulatory molecules important for activation of thymocytes.

Immune function and ubiquitin

AIRE's function as an E3 ubiquitin ligase is not unique among the variety of immunomodulatory molecules possessing ubiquitin ligase activity. One of the most well-known transcriptional regulators important for immune function is inhibitor of NF-κB (IκB), which prevents the nuclear localization of NF-κB and retains it in the cytoplasm. Upon phosphorylation during inflammation, IκB is ubiquitylated and degraded by the proteasome, allowing NF-κB to enter the nucleus (72). T-cell activation and anergy induction are also subject to ubiquitin-mediated regulation. The RING finger E3 ubiquitin ligase Cbl-b regulates T-cell activation via the downregulation of phosphatidylinositol 3-kinase in lymphocytes (73). Intriguingly, Cbl-b-deficient mice develop spontaneous autoimmunity, manifested in the form of autoantibodies, lymphocyte infiltration of various organs, and hyperresponsiveness of T cells (74). Similarly, the Fathman group (75) identified the gene related to anergy in lymphocytes (*GRAIL*), an E3 ubiquitin ligase, as a highly expressed gene in anergic T cells and demonstrated the role of *GRAIL* in the downregulation of activation-induced interleukin-2 (IL-2) and IL-4 production. More recently, the Rao group (76–78) showed an increase at the mRNA and protein levels of at least three different types of E3 ubiquitin ligases (Itch, GRAIL, and Cbl-b) during ionomycin-induced anergy. In addition, the ubiquitinated substrates phospholipase C-γ1, protein kinase C-θ, and Ras-GTPase-activating protein were reduced in anergic T cells, and as a result, the generation of a stable supramolecular activation complex, which is required for prolonged T-cell activation, was disrupted. Undoubtedly, as more of the relevant ubiquitin ligases and members of this cascade are discovered, new roles in gene control and in immunomodulation will be revealed.

Gene clusters – hidden clues in the genome?

Recent whole-genome studies and microarray expression data have revealed the existence of gene clusters or 'expression neighborhoods' characterized by a non-random correlation between genomic location and expression level. The literature now includes multiple examples of gene expression clusters in a variety of organisms, from yeast to humans. For example,

microarray expression data from *Saccharomyces cerevisiae* demonstrated adjacent and non-adjacent pairs of genes with correlated expression patterns, using correlation maps for yeast chromosomes (79, 80). Similarly, Spellman and Rubin (81) used *Drosophila* gene expression arrays to identify approximately 200 clusters of adjacent and similarly expressed genes spanning more than 10–30 genes or approximately 125-kb of DNA, and they suggested that these patterns were most consistent with regulation of chromatin structure. Analyses of the human transcriptome map identified several regions of increased gene expressions (RIDGEs), each containing clusters of highly expressed genes along each chromosome, including a prominent RIDGE corresponding to MHC (82).

Analyses of the levels of transcripts encoding peripheral tissue antigens via a combination of reverse trascriptase-polymerase chain reaction and microarray experiments with human MECs have revealed chromosomal clustering (83). Gotter *et al*. (83) found several sets of gene clusters arranged as triplets and quadruplets over a window of 10 consecutive genes. Some of the clusters included genes encoding members of the S100 gene family and genes that specify epidermal cell differentiation. Intriguingly, AIRE-controlled genes that express peripheral tissue transcripts within MECs are arranged in chromosomal clusters (Johnnidis, Venanzi, Mathis and Benoist, manuscript in preparation).

Such clustering begs the question of how the expression of specific genes in clusters is achieved. Several explanations have been provided, the most attractive of which include the following: (i) *cis*-acting elements that affect the transcription of surrounding genes, (ii) histone modifications that demarcate areas of open and closed chromatin domains that regulate transcriptional activity, and (iii) *cis*-acting elements (e.g. matrix-attachment regions) that come together to form 'chromatin loops' to target genes to specific areas of the nucleus containing concentrations of transcriptional machinery (84, 85).

Regulators of Aire

Soluble mediators

Because AIRE regulates the transcription of many peripheral tissue antigens in MECs and as it would seem dangerous for it to exert this function in most other cell types, it is not surprising that evidence that AIRE is subject to several levels of regulation has recently been uncovered. Several molecules have been identified as upstream regulators of AIRE expression. Two studies have highlighted a potential interaction between the lymphotoxin (LT) pathway and AIRE expression (86, 87). Chin *et al*. (87) noted that mice deficient for either LT-α or LTβ receptor (LTβR) exhibited a defect in AIRE expression in the thymus. Moreover, when LT-α-deficient mice were treated acutely with an agonist monoclonal antibody directed against LTβR, there was a transient induction of AIRE and insulin-2 expression, in the absence of a change in keratin-14, suggesting a direct effect of the lymphotoxin pathway on AIRE. However, Boehm *et al*. (86) did not detect any defect in AIRE or genes encoding peripheral tissue antigens in LTβR-deficient MECs. LTβR-deficient mice also had a disturbed thymic architecture and reduced numbers of MECs and developed a high level of autoimmune disease that was attributed mostly to a defect in thymic development. Mice deficient in NF-κB-inducing-kinase (NIK), an enzyme in the same signaling pathway, also had decreased levels of AIRE expression and peripheral tissue antigen transcripts such as salivary protein-1. However, NIK-deficient mice also exhibited defective differentiation of MECs and an overall disturbed medullary epithelial architecture (88). So, whether the lymphotoxin pathway is involved in directly affecting the expression of AIRE or, rather, indirectly as the reflection of perturbed thymic development is currently unresolved. *In vitro* studies have shown that human thymic stromal lymphopoietin also appears to induce expression of AIRE in human dendritic cells (89). However, the functional significance and *in vivo* relevance of this finding requires elucidation.

Regulation of AIRE by nuclear localization

The existence of functionally distinct nuclear compartments and their potential for imposing higher order transcriptional regulation have been increasingly appreciated. Transcriptionally inactive areas of the yeast genome tend to be localized in the periphery of the nucleus (90), whereas gene-dense chromosomal regions tend to reside in the center (91). These observations suggest a three-dimensional architecture within the nucleus, capable of promoting the formation of active or inactive transcriptional domains. The nucleus is now considered a complex and dynamic organelle consisting of several intranuclear structures, such as the nucleolus, nuclear speckles, coiled or Cajal bodies, and PML bodies or PODs, each attached directly or indirectly to the nuclear matrix or to chromatin compartments of heterochromatin or euchromatin (92–94). Observations of proteins shuttling in and out of the nucleus and various intranuclear structures in response to different environmental signals have suggested an additional level of complexity (92).

AIRE has a nuclear localization signal within amino acids 101–141 (7, 9), which is sufficient for nuclear transport

into the nucleus (32). Additionally, it has a functional nuclear export signal present within the N-terminus. Treatment of cells transiently transfected with a recombinant *AIRE* gene with Leptomycin B, a specific inhibitor of nuclear export, resulted in a twofold concentration of AIRE within nuclear bodies (32). These observations hint at a regulatory mechanism involving the shuttling of Aire between nuclear and cytoplasmic compartments. The regulation of cell-cycle progression and control of cellular proliferation, circadian clocks, and cytokine and stress responses are among a growing number of cellular processes now known to be mediated by the shuttling of proteins into or out of the nucleus (92). Further, insight into the regulation of AIRE by nuclear localization came from the demonstration that its localization within nuclear bodies is cell-cycle dependent (34). However, the biological significance of this finding remains unclear. Also intriguing and still unexplained is the observation of an increase in the number and size of nuclear bodies formed by Aire upon proteasome inhibition.

Concluding remarks

The molecular mechanism of AIRE in the induction of central tolerance and the prevention of APECED still remains unexplained. Critical to unraveling the molecular mechanism of AIRE is to determine the manner by which it specifically targets a small subset of genes encoding peripheral tissue antigens. Does AIRE bind directly or indirectly to specific sequences present in the genes encoding peripheral tissue antigens? Are these sequences promoters/enhancers or MARs? Or does AIRE acts at the level of chromatin, 'decoding' an epigenetically specified histone arrangement of transcriptionally active domains composed of genes encoding peripheral tissue antigens? Also interesting to consider is whether AIRE mediates the transcription of peripheral tissue antigens within a small population of MECs or all MECs. In addition, Aire's newly discovered ubiquitin ligase activity provides an interesting clue into its mechanism and opens up several possibilities. Further work exploring the role of Aire's ubiquitin ligase activity remains an area open for future research. It is also worth noting that Aire's activity may not be limited to the regulation of transcription of peripheral tissue antigens (30). Anderson *et al.* (14) have noted the expression of transcripts distinct from those that encode peripheral tissue antigens, the significance of which is not clearly understood. However, this observation does provide insights into potentially novel functions of Aire, such as regulating the differentiation of MECs and antigen processing and presentation.

References

1. Rieux-Laucat F, et al. Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. Science 1995;**268**:1347–1349.
2. Bennett CL, et al. The immune dysregulation, polyendocrinopathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 2001;**27**:20–21.
3. Wildin RS, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet 2001;**27**:18–20.
4. Ahonen P, Myllarniemi S, Sipila I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. N Engl J Med 1990;**322**:1829–1836.
5. Vogel A, Strassburg CP, Obermayer-Straub P, Brabant G, Manns MP. The genetic background of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy and its autoimmune disease components. J Mol Med 2002;**80**:201–211.
6. Rosatelli MC, et al. A common mutation in Sardinian autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients. Hum Genet 1998;**103**:428–434.
7. Nagamine K, et al. Positional cloning of the APECED gene. Nat Genet 1997;**17**: 393–398.
8. Aaltonen J, Bjorses P. Cloning of the APECED gene provides new insight into human autoimmunity. Ann Med 1999;**31**:111–116.
9. The Finnish-German APECED Consortium. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. Nat Genet 1997;**17**:399–403.
10. Blechschmidt K, et al. The mouse Aire gene: comparative genomic sequencing, gene organization, and expression. Genome Res 1999;**9**:158–166.
11. Bjorses P, Pelto-Huijjo M, Kaulonen J, Aaltonen J, Peltonen L, Ulmanen I. Localization of the APECED protein in distinct nuclear structures. Hum Mol Genet 1999;**8**:259–266.
12. Gibson TJ, Ramu C, Gemund C, Aasland R. The APECED polyglandular autoimmune syndrome protein, AIRE-1, contains the SAND domain and is probably a transcription factor. Trends Biochem Sci 1998;**23**:242–244.
13. Aasland R, Gibson TJ, Stewart AF. The PHD finger: implications for chromatin-mediated transcriptional regulation. Trends Biochem Sci 1995;**20**:56–59.
14. Anderson MS, et al. Projection of an immunological self shadow within the thymus by the aire protein. Science 2002;**298**:1395–1401.
15. Liston A, Lesage S, Wilson J, Peltonen L, Goodnow CC. Aire regulates negative selection of organ-specific T cells. Nat Immunol 2003;**4**:350–354.
16. Jolicoeur C, Hanahan D, Smith KM. T-cell tolerance toward a transgenic beta-cell antigen and transcription of endogenous pancreatic genes in thymus. Proc Natl Acad Sci USA 1994;**91**:6707–6711.

17. Bjorses P, et al. Mutations in the AIRE gene: effects on subcellular location and transactivation function of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protein. Am J Hum Genet 2000;**66**:378–392.
18. Scott HS, et al. Common mutations in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients of different origins. Mol Endocrinol 1998;**12**:1112–1119.
19. Cihakova D, et al. Novel AIRE mutations and P450 cytochrome autoantibodies in Central and Eastern European patients with APECED. Hum Mutat 2001;**18**:225–232.
20. Bloch DB, et al. Sp110 localizes to the PML-Sp100 nuclear body and may function as a nuclear hormone receptor transcriptional coactivator. Mol Cell Biol 2000;**20**: 6138–6146.
21. Bloch DB, de la Monte SM, Guigaouri P, Filippov A, Bloch KD. Identification and characterization of a leukocyte-specific component of the nuclear body. J Biol Chem 1996;**271**:29198–29204.
22. Gross CT, McGinnis W. DEAF-1, a novel protein that binds an essential region in a Deformed response element. EMBO J 1996;**15**:1961–1970.
23. Michelson RJ, Collard MW, Ziemba AJ, Persinger J, Bartholomew B, Huggenvik JI. Nuclear DEAF-1-related (NUDR) protein contains a novel DNA binding domain and represses transcription of the heterogeneous nuclear ribonucleoprotein A2/B1 promoter. J Biol Chem 1999;**274**:30510–30519.
24. Oshima H, Szapary D, Simons SS Jr. The factor binding to the glucocorticoid modulatory element of the tyrosine aminotransferase gene is a novel and ubiquitous heteromeric complex. J Biol Chem 1995;**270**: 21893–21901.
25. Surdo PL, Bottomley MJ, Sattler M, Scheffzek K. Crystal structure and nuclear magnetic resonance analyses of the SAND domain from glucocorticoid modulatory element binding protein-1reveals deoxyribonucleic acid and zinc binding regions. Mol Endocrinol 2003;**17**: 1283–1295.
26. Bottomley MJ, Collard MW, Huggenvik JI, Liu Z, Gibson TJ, Sattler M. The SAND domain structure defines a novel DNA-binding fold in transcriptional regulation. Nat Struct Biol 2001;**8**:626–633.
27. Halonen M, et al. APECED-causing mutations in AIRE reveal the functional domains of the protein. Hum Mutat 2004;**23**:245–257.
28. Kumar PG, et al. The autoimmune regulator (AIRE) is a DNA-binding protein. J Biol Chem 2001;**276**:41357–41364.
29. Peterson P, Pitkanen J, Sillanpaa N, Krohn K. Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED): a model disease to study molecular aspects of endocrine autoimmunity. Clin Exp Immunol 2004;**135**:348–357.
30. Mathis D, Benoist C. Back to central tolerance. Immunity 2004;**20**:509–516.
31. Rinderle C, Christensen HM, Schweiger S, Lehrach H, Yaspo ML. AIRE encodes a nuclear protein co-localizing with cytoskeletal filaments: altered sub-cellular distribution of mutants lacking the PHD zinc fingers. Hum Mol Genet 1999;**8**:277–290.
32. Pitkanen J, Vahamurto P, Krohn K, Peterson P. Subcellular localization of the autoimmune regulator protein. Characterization of nuclear targeting and transcriptional activation domain. J Biol Chem 2001;**276**:19597–19602.
33. Zhong S, Salomoni P, Pandolfi PP. The transcriptional role of PML and the nuclear body. Nat Cell Biol 2000;**2**:E85–E90.
34. Akiyoshi H, et al. Subcellular expression of autoimmune regulator (AIRE) is organized in a spatiotemporal manner. J Biol Chem 2004;**279**:33984–33991.
35. Laemmli UK, Kas E, Poljak L, Adachi Y. Scaffold-associated regions: cis-acting determinants of chromatin structural loops and functional domains. Curr Opin Genet Dev 1992;**2**:275–285.
36. Glazko GV, Koonin EV, Rogozin IB, Shabalina SA. A significant fraction of conserved noncoding DNA in human and mouse consists of predicted matrix attachment regions. Trends Genet 2003;**19**:119–124.
37. Pitkanen J, et al. The autoimmune regulator protein has transcriptional transactivating properties and interacts with the common coactivator CREB-binding protein. J Biol Chem 2000;**275**:16802–16809.
38. Uchida D, et al. AIRE functions as an E3 ubiquitin ligase. J Exp Med 2004;**199**:167–172.
39. Giles RH, Peters DJM, Breuning MH. Conjunction dysfunction: CBP/p300 in human disease. Trends Genet 1998;**14**:178–183.
40. Chan HM, La Thangue NB. p300/CBP proteins: HATs for transcriptional bridges and scaffolds. J Cell Sci 2001;**114**: 2363–2373.
41. Ramsey C, et al. Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. Hum Mol Genet 2002;**11**:397–409.
42. Derbinski J, Schulte A, Kyweski B, Klein L. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. Nat Immunol 2001;**2**:1032–1039.
43. Kyewski B, Derbinski J, Gotter J, Klein L. Promiscuous gene expression and central T-cell tolerance: more than meets the eye. Trends Immunol 2002;**23**:364–371.
44. Kalkhoven E, Teunissen H, Houweling A, Verrijzer CP, Zantema A. The PHD type zinc finger is an integral part of the CBP acetyltransferase domain. Mol Cell Biol 2002;**22**:1961–1970.
45. Kim S-S, Chen Y-M, O'Leary E, Witzgall R, Vidal M, Bonventre JV. A novel member of the RING finger family, KRIP-1, associates with the KRAB-A transcriptional repressor domain of zinc finger proteins. Proc Natl Acad Sci 1996;**93**:15299–15304.
46. Seelig HP, Moosbrugger I, Ehrfeld H, Fink T, Renz M, Genth E. The major dermatomyositis-specific Mi-2 autoantigen is a presumed helicase involved in transcriptional activation. Arthritis Rheum 1995;**38**:1389–1399.
47. Ge Q, Nilasena DS, O'Brien CA, Frank MB, Targoff IN. Molecular analysis of a major antigenic region of the 240-kD protein of Mi-2 autoantigen. J Clin Invest 1995;**96**:1730–1737.
48. Fyodorov DV, Kadonaga JT. Binding of Acf1 to DNA involves a WAC motif and is important for ACF-mediated chromatin assembly. Mol Cell Biol 2002;**22**:6344–6353.
49. Feng X, Hara Y, Riabowol K. Different HATS of the ING1 gene family. Trends Cell Biol 2002;**12**:532–538.
50. Scheel H, Hofmann K. No evidence for PHD fingers as ubiquitin ligases. Trends Cell Biol 2003;**13**:285–287.
51. Coscoy L, Ganem D. Reply to Scheel and Hofmann: Progressing towards a better definition of PHD domains. Trends Cell Biol 2003;**13**:287–288.
52. Aravind L, Iyer LM, Koonin EV. Scores of RINGS but no PHDs in ubiquitin signaling. Cell Cycle 2003;**2**:123–126.
53. Gozani O, et al. The PHD finger of the chromatin-associated protein ING2 functions as a nuclear phosphoinositide receptor. Cell 2003;**114**:99–111.
54. Capili AD, Schultz DC, Rauscher III FJ, Borden KL. Solution structure of the PHD domain from the KAP-1 corepressor: structural determinants for PHD, RING and LIM zinc-binding domains. EMBO J 2001;**20**:165–177.
55. Pascual J, Martinez-Yamout M, Dyson HJ, Wright PE. Structure of the PHD zinc finger from human Williams–Beuren syndrome transcription factor. J Mol Biol 2000;**304**:723–729.
56. Joazeiro CAP, Hunter T. Ubiquitination – more than two to tango. Science 2000;**289**:2061–2062.

57. Hershko A, Ciechanover A. The ubiquitin system. Annu Rev Biochem 1998;**67**: 425–479.
58. Coscoy L, Ganem D. PHD domains and E3 ubiquitin ligases: viruses make the connection. Trends Cell Biol 2003;**13**:7–12.
59. Boname JM, Stevenson PG. MHC class I ubiquitination by a viral PHD/LAP finger protein. Immunity 2001;**15**:627–636.
60. Lu Z, Xu S, Joazeiro CA, Cobb MH, Hunter T. The PHD domain of MEKK1 acts as an E3 ubiquitin ligase and mediates ubiquitination and degradation of ERK1/2. Mol Cell 2002;**9**:945–956.
61. Pickart CM. Mechanisms underlying ubiquitination. Ann Rev Biochem 2001;**70**:503–533.
62. Conaway RC, Brower CS, Conaway JW. Emerging roles of ubiquitin in transcription regulation. Science 2002;**296**:1254–1258.
63. Muratani M, Tansey WP. How the ubiquitin-proteasome system controls transcription. Nat Rev Mol Cell Biol 2003;**4**:192–201.
64. Strahl BD, Alli CD. The language of covalent histone modifications. Nature 2000;**403**: 41–45.
65. Zhang Y. Transcriptional regulation by histone ubiquitination and deubiquitination. Genes Dev 2003;**17**:2733–2740.
66. Pham AD, Sauer F. Ubiquitin-activating/ conjugating activity of TAFII250, a mediator of activation of gene expression in Drosophila. Science 2000;**289**:2357–2360.
67. Rodriguez MS, Desterro JM, Lain S, Lane DP, Hay RT. Multiple C-terminal lysine residues target p53 for ubiquitin-proteasome-mediated degradation. Mol Cell Biol 2000;**20**:8458–8467.
68. Lai Z, et al. Human mdm2 mediates multiple mono-ubiquitination of p53 by a mechanism requiring enzyme isomerization. J Biol Chem 2001;**276**:31357–31367.
69. Grossman SR, et al. Polyubiquitination of p53 by a ubiquitin ligase activity of p300. Science 2003;**300**:342–344.
70. Salghetti SE, Caudy AA, Chenoweth JG, Tansey WP. Regulation of transcriptional activation domain function by ubiquitin. Science 2001;**293**:1651–1653.
71. Nateri AS, Riera-Sans L, Costa CD, Behrens A. The ubiquitin ligase SCFFbw7 antagonizes apoptotic JNK signaling. Science 2004;**303**:1374–1378.
72. Palombella VJ, Rando OJ, Goldberg AL, Maniatis T. The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. Cell 1994;**78**: 773–785.
73. Fang D, Wang HY, Fang N, Altman Y, Elly C, Liu YC. Cbl-b, a RING-type E3 ubiquitin ligase, targets phosphatidylinositol 3-kinase for ubiquitination in T cells. J Biol Chem 2001;**276**:4872–4878.
74. Bachmaier K, et al. Negative regulation of lymphocyte activation and autoimmunity by the molecular adaptor Cbl-b. Nature 2000;**403**:211–216.
75. Anandasabapathy N, et al. GRAIL: an E3 ubiquitin ligase that inhibits cytokine gene transcription is expressed in anergic $CD4^+$ T cells. Immunity 2003;**18**:535–547.
76. Heissmeyer V, Rao A. E3 ligases in T cell anergy – turning immune responses into tolerance. Sci STKE 2004, 2004: PE29.
77. Scharschmidt E, Wegener E, Heissmeyer V, Rao A, Krappmann D. Degradation of Bcl10 induced by T-cell activation negatively regulates NF-kappa B signaling. Mol Cell Biol 2004;**24**:3860–3873.
78. Heissmeyer V, et al. Calcineurin imposes T cell unresponsiveness through targeted proteolysis of signaling proteins. Nat Immunol 2004;**5**:255–265.
79. Velculescu VE, et al. Characterization of the yeast transcriptome. Cell 1997;**88**:243–251.
80. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 1998;**95**:14863–14868.
81. Spellman PT, Rubin GM. Evidence for large domains of similarly expressed genes in the Drosophila genome. J Biol 2002;**1**:5.
82. Caron H, et al. The human transcriptome map: clustering of highly expressed genes in chromosomal domains. Science 2001;**291**:1289–1292.
83. Gotter J, Brors B, Hergenhahn M, Kyewski B. Medullary epithelial cells of the human thymus express a highly diverse selection of tissue-specific genes colocalized in chromosomal clusters. J Exp Med 2004;**199**:155–166.
84. Hurst LD, Pal C, Lercher MJ. The evolutionary dynamics of eukaryotic gene order. Nat Rev Genet 2004;**5**:299–310.
85. Oliver B, Parisi M, Clark D. Gene expression neighborhoods. J Biol 2002;**1**:1–4.
86. Boehm T, Scheu S, Pfeffer K, Bleul CC. Thymic medullary epithelial cell differentiation, thymocyte emigration, and the control of autoimmunity require lympho-epithelial cross talk via LTbetaR. J Exp Med 2003;**198**:757–769.
87. Chin RK, et al. Lymphotoxin pathway directs thymic Aire expression. Nat Immunol 2003;**4**:1121–1127.
88. Kajiura F, et al. NF-kappa B-inducing kinase establishes self-tolerance in a thymic stroma-dependent manner. J Immunol 2004;**172**:2067–2075.
89. Watanabe N, et al. Human thymic stromal lymphopoietin promotes dendritic cell-mediated $CD4^+$ T cell homeostatic expansion. Nat Immunol 2004;**5**:426–434.
90. Andrulis ED, Neiman AM, Zappulla DC, Sternglanz R. Perinuclear localization of chromatin facilitates transcriptional silencing. Nature 1998;**394**:592–595.
91. Cremer T, Cremer C. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat Rev Genet 2001;**2**:292–301.
92. Carmo-Fonseca M. The contribution of nuclear compartmentalization to gene regulation. Cell 2002;**108**:513–521.
93. Gall JG, Bellini M, Wu Z, Murphy C. Assembly of the nuclear transcription and processing machinery. Cajal bodies (coiled bodies) and transcriptosomes. Mol Biol Cell 1999;**10**:4385–4402.
94. Lamond AI, Earnshaw WC. Structure and function in the nucleus. Science 1998;**280**:547–553.