# Contrasting models of promiscuous gene expression by thymic epithelium

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**Medullary thymic epithelial cells (mTECs) express a broad spectrum of tissue-restricted self-antigens (TRAs), which are required for the development of central tolerance. A new study suggests that TRA expression is a specialized property of terminally differentiated mTECs. However, as discussed here, an alternative model—whereby TRA expression is regulated by conserved developmental programs active in developing mTECs—may be equally plausible.**

## TRAs and central tolerance

Multiple studies have shown that expression of TRAs by mTECs is critical for establishing self-tolerance to these antigens in the periphery (1). In mice that express transgenes controlled by peripheral tissue-specific promoters, a scattered subset of mTECs express the transgenic antigen, and this expression is sufficient to tolerize the developing T cell repertoire to the antigen. The known mechanism of tolerance induction involves deletion of antigen-specific thymocytes (2–4); the effect of TRA expression on other tolerance mechanisms, such as regulatory T cell development, remains to be determined. These studies also showed that the patterns of endogenous antigen expression in mTECs (insulin, somatostatin [2]; C-reactive protein [CRP], serum amyloid P component [SAP] [3]) is similar to the expression of transgenic antigens under the corresponding tissue-specific promoters (RIP-Tag [2]; human CRP [3]). This shows clearly that TRA expression by mTECs is a physiological phenomenon that is under endogenous transcriptional control.

Building on these early observations, a study by Derbinski et al. (5) showed that TRA expression by mTECs encompasses a large set of antigens characteristic of a broad range of tissues, leading to the formulation of the notion of "promiscuous gene expression" as both a phenomenon and a mechanism for generating tolerance. When it was found that mutations in the gene encoding the transcriptional regulator AIRE (autoimmune regulator) were responsible for the development of a multiorgan autoimmune syndrome (APS-1 or APECED) in humans, it was suggested that AIRE might mediate TRA expression in mTECs (for review see reference 6). Indeed, analysis of *Aire*$^{-/-}$ mice, which have autoimmune activity targeted to multiple endocrine tissues, has shown that expression of some TRAs is reduced or absent in *Aire*-deficient mTECs and that negative selection of thymocytes is impaired (7). However, Aire cannot account for all promiscuous gene expression, as expression of many TRAs is unaffected in *Aire*$^{-/-}$ mice and some of the thymocyte selection defects observed in these mice are independent of TRA expression levels (8). The mechanism of Aire activity has been attributed to direct transcriptional regulation, ubiquitin ligase activity, or unspecified derepression of chromatin (6). However, a definitive molecular function for Aire has not been determined yet, and therefore, the manner in which it controls thymic expression of a subset of TRAs remains speculative.

## mTEC differentiation: knowns and unknowns

The mechanism(s) that may account for promiscuous gene expression by mTECs is difficult to define because mTECs themselves are difficult to define (9). Regarding mTEC development, it can be said conclusively that mTECs are clonally derived, as shown by Rodewald and colleagues (10); that development and maintenance of the highly heterogeneous medullary compartment of the thymus is dependent on reciprocal signals between mTECs and developing thymocytes (referred to as cross-talk [9, 11]); and that the lymphotoxin β receptor–NF-κB–inducing kinase–relB pathway is involved in both the induction of Aire and the generation and/or maintenance of mTEC heterogeneity, although whether this pathway regulates maturation, proliferation, or survival of mTECs is not clear (12–14). The identity and relationship of mTEC subsets is also poorly defined (9). Although some molecules are broadly expressed by most mTECs (such as CD80, MHC class II, Ep-Cam), others (recognized by antibodies and lectins such as 8.1.1, UEA-1, A2B5, etc.) are differentially expressed by mTEC subsets and can be used to subdivide the broader mTEC populations (5, 9). There has been no demonstration of precursor/progeny relationships among the various mTEC subsets, and thus the maturation state, developmental potential, and homogeneity of these subsets is undetermined and open to speculation.

## Refining the promiscuous gene expression model

In this issue, Derbinski et al. (see page 33) provide important clues to the regulation of TRA expression by mTECs (15). They describe a hierarchy of TRA expression by subsets of mTECs that are defined by levels of expression of the costimulatory molecule CD80, with the level of CD80 expression increasing as a function of mTEC

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maturity. This correlation was noted in both *Aire*-deficient and wild-type mTECs, emphasizing that Aire-independent mechanisms play an important role in regulating TRA expression. The results pertaining to the role of Aire in this process will be addressed in more detail later. They also provide evidence that some of the genes encoding TRAs expressed by the CD80$^{hi}$ mTEC population are clustered together on chromosomes, suggesting potential epigenetic regulation of this process. Whereas expression of TRAs in peripheral tissues is regulated by tissue-specific transcription factors, the authors propose that mature mTECs open regions of chromatin and express the clusters of genes in these open regions (Fig. 1 A). Based on their new data, the authors propose a modified version of the promiscuous gene expression model—termed the "terminal differentiation" model—in which promiscuous gene expression by mTECs is a specialized property that is attained upon terminal differentiation.

This model is cogent and provides a reasonable explanation for their observations. However, because of the large gaps in our understanding of thymic epithelial differentiation, the model proposed by Derbinski et al. is based on a number of assumptions (15). We wish to point out that these data can be interpreted differently when alternative but equally valid assumptions are made regarding thymic epithelial differentiation. For instance, by attributing TRA expression to mature mTECs this model implies that thymic epithelial progenitor cells have a restricted transcriptional profile and would express mTEC-specific molecules before activation of TRA expression. At this time, we are not aware of many genes, structural or regulatory, that can be used to define a unique thymic epithelial "identity," nor is there evidence that these progenitor cells are committed to the mTEC lineage. As will be discussed in An alternative to… section, there are grounds to consider the potential of epithelial progenitor cells or immature thymic epithelial cells to express TRAs.

Some have assumed that the program of mTEC differentiation resembles that of dendritic cells, where high levels of CD80, CD86, and MHC class II expression are considered to be markers of maturation. However, several observations suggest that expression of these molecules by nonhematopoietic cells may be inducible and not constitutive, and independent of maturation state (16, 17). For example, mTECs and mTEC cell lines can be induced to express MHC class II at high levels by exposure to IFN-γ (18). In mice lacking promoter IV (pIV) of the class II transactivator (CIITA), hematopoietic antigen-presenting cells constitutively express MHC class II, whereas nonhematopoietic cells do not express MHC class II constitutively (cortical thymic epithelial cells) or in response to systemic IFN-γ treatment

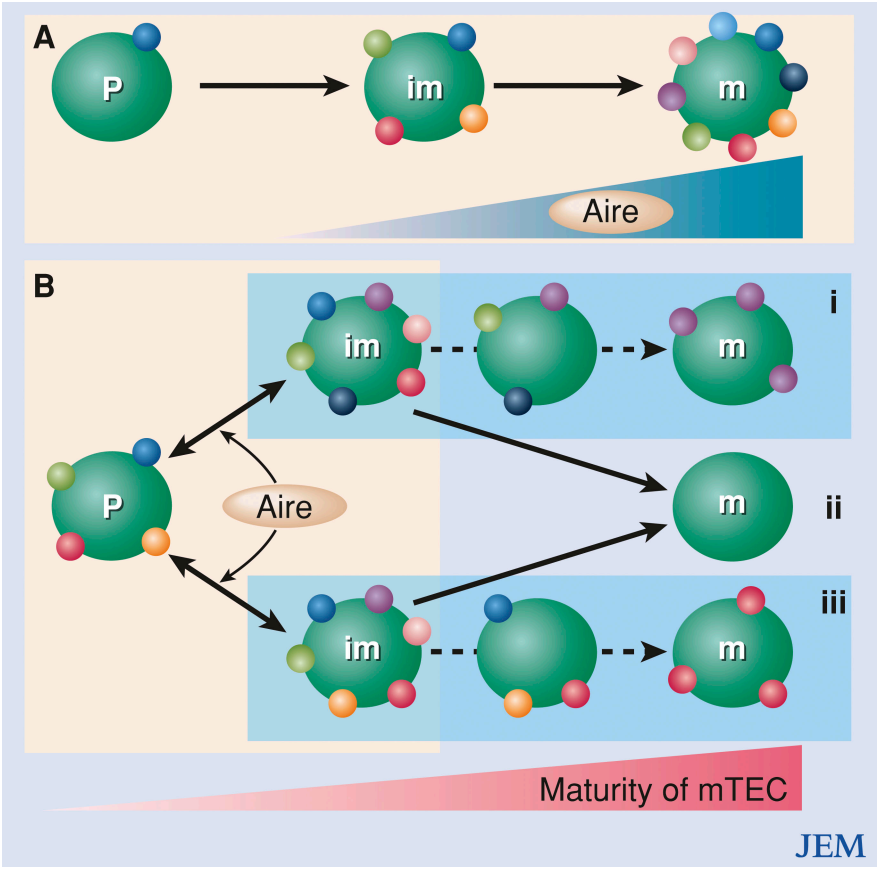


**Figure 1. Models of the mechanisms resulting in TRA expression by mTECs.** (A) The terminal differentiation model. As a committed mTEC progenitor (p) cell differentiates, it progressively expresses an increasing number of TRAs, starting at an immature (im) stage and culminating in a terminally differentiated (mature [m]) cell that is characterized by high level expression of MHC class II, CD80, Aire, and a broad spectrum of TRAs (represented by colored circles). The role of Aire in this model is undefined but is progressively manifested during mTEC maturation. (B) The developmental model. Progenitors have access to a wide variety of lineage-specific transcriptional programs (and the TRAs regulated by those programs) before differentiation. As part of the differentiation process, some of the transcriptional networks are silenced as individual cells mature. Each of these mature cells might express a peripheral lineage program (i and iii), or might extinguish all peripheral regulators and express an undefined terminal mTEC program (ii). In the developmental model, MHC class II and co-stimulatory molecules like CD80 would be inducibly expressed on mTECs throughout differentiation in response to cell–cell or other extrinsic signals. We speculate that Aire acts at early stages of mTEC differentiation, perhaps by regulating lineage decisions or by controlling temporal patterns of the differentiation process.

(multiple cell types; reference 17). Therefore, the CD80$^{hi}$ subset of mTEC may reflect a state of epithelial activation in response to local stimuli rather than a subset of thymic epithelial cells at a particular stage of maturation. Derbinski et al. further assume that the CD80$^{hi}$ subset of mTECs represents a homogenous and mature population, even though several molecules characteristic of embryonic stem cells (Dppa3, Utf-1) and stem cell populations (SCA-1) as well as early regulators of endodermal tissue development (Cdx1, Foxa1) are enriched in this "mature" CD80$^{hi}$ mTEC population (15).

### An alternative to the "terminal differentiation" model

We suggest an alternative interpretation of the data presented in the study by Derbinski et al. (15) that is based on several different underlying assumptions regarding the development and character of the medullary thymic epithelium. We propose that conserved developmental mechanisms regulate TRA expression by mTECs, possibly in one or several ways. First, the absence of a well-defined population of thymic epithelial progenitor cells or well-defined markers that are unique for thymic epithelium suggests that the earliest thymic epithelial progenitor cells could be multipotent and not initially restricted to a thymic lineage. According to this scenario, multiple conserved transcriptional programs that reflect the multipotential nature of these progenitor cells would result in the display of a broad spectrum of TRAs (Fig. 1 B). As cells differentiate (defined here as a progressive restriction of developmental potential or a narrowing of developmental fate choices), there would be sequential silencing or inactivation of some of these transcriptional programs, leading to terminally differentiated cells. Although the duration of the expression profile for peripheral transcriptional networks may vary, the products specified by these networks would contribute to the set of tissue-restricted structural and regulatory genes that are expressed by mTECs. This process might increasingly restrict the expression of discrete sets of tissue-restricted genes as the cells develop (19–21), and allow individual cells to terminally differentiate into either a peripheral lineage fate (2, 22) (Fig. 1 B, i and iii) or lead to the silencing of peripheral transcription networks after commitment to a terminal—as yet undefined—mTEC fate (depicted in Fig. 1 B, ii). We suggest that expression of these peripheral transcriptional programs in various individual cells might account for the molecular mosaic of peripheral self-antigens that are represented within medullary thymic epithelium. If progressive silencing of gene expression is associated with differentiation, individual immature mTECs should express a wider array of TRAs than mature mTECs, regardless of the terminal fate of the mature cell. In addition, the mechanisms that regulate expression of specific TRAs by mTECs should be similar to those active in native tissues. The terminal differentiation model proposed by Derbinski and colleagues predicts the opposite, with terminally differentiated mTEC expressing the highest levels of TRAs as a result of epigenetic derepression or other regulatory mechanisms that are not recapitulated in the periphery (15).

### Arguments in favor of progressive restriction

Although the progressive restriction of transcriptional programs as a mechanism of differentiation has received little attention in epithelial cells, such mechanisms have been described in hematopoietic cells. Studies using either isolated early hematopoietic progenitors (19, 23) or more mature hematopoietic cells (24) have shown that immature cells can express genes characteristic of lineages that are distinct from their terminally differentiated fates. For example, pro–B cells deficient in the transcription factor Pax5, which are incapable of commitment to the B cell lineage, can differentiate into mature T cells (24). By examining lineage-specific gene expression patterns in early hematopoietic populations, Miyamoto et al. (23) concluded that multiple lineage-affiliated differentiation programs are activated at the transcriptional level before commitment. In this context, what has been described in the past as promiscuity of gene expression or lineage commitment could simply reflect the ability of immature cells to utilize multiple developmental programs. This interpretation differs significantly from the promiscuous gene expression of the terminal differentiation model, whereby genes are expressed nonspecifically as long as their chromosomal "neighborhood" is accessible.

The components necessary for a developmental, progressive restriction model are all present in the thymus: a cycling population of epithelial cells (1) in an environment replete with the mechanisms and signaling pathways that are involved in the development of peripheral epithelial (and nonepithelial) tissues. These include the Notch, Hedgehog, bone morphogenic protein (BMP), fibroblast growth factor (FGF), and Wnt signaling pathways (9). A recent study demonstrated that transplanted fetal pharyngeal pouch endoderm can generate thymus and other endodermally derived structures, such as pharyngeal and gut tissues, and that transplanted embryonic day 9 fetal pharyngeal arches (which normally give rise to the thymus) can generate a wide range of tissue types, including skin, hair, cartilage, bone, muscle, and adipose tissue (25). Therefore, it seems reasonable that the ability of mTECs to express a remarkably wide range of TRAs may reflect a general developmental process that occurs in a tissue with high levels of cellular turnover, broad developmental potential, and complex environmental cues that can direct individual mTEC precursors into various developmental pathways. Although the plasticity of the medullary compartment has been shown in multiple experimental contexts (9), the identity and differentiation potential of mTEC progenitors remains to be determined.

The TRA expression data (3, 4, 5, 26) and chromosomal clustering analyses presented in previous studies (27) are consistent with either model. The clustered pattern of TRA expression noted by Derbinski et al. (15) was interpreted to reflect derepressed loci in a homogeneous, terminally differentiated epithelial population that is capable of promiscuous gene expression. But this clustering could also result from immature populations of precursor cells that have retained a broad transcriptional profile, or could represent the summed expression by a heterogeneous mixture of cells, each of which expresses a specific set of peripheral lineage genes. The same can be said for the data showing biallelic expression of the insulin-like growth factor 2 (*Igf2*) gene by mTECs, which Derbinski et al. propose as evidence for a loss of normal gene imprinting (15). This biallelic expression pattern might instead reflect a lack of imprinting in immature progenitors, or a heterogeneous mixture of cells that individually express *Igf2* either mono- or biallelically in a developmental or lineage-specific manner (28).

### The role of Aire in regulating TRA expression

The data presented in the current study indicate that Aire does not regulate TRA expression by random derepression of loci or chromatin remodeling mechanisms as previously proposed (1, 5, 27, 29). For example, within the casein locus the authors show that casein γ is not expressed by $CD80^{hi}$ $Aire^{-/-}$ mTECs. However, this cannot be attributed to a locus that is "closed" by the absence of Aire, as the ability of $CD80^{hi}$ $Aire^{-/-}$ mTECs to express the casein α and κ genes (which flank casein γ; reference 30) demonstrates that the locus must be open in some cells. Therefore, the mechanism by which Aire affects casein γ expression appears to be independent of wholesale chromatin remodeling, run-on transcription of open loci, or direct transcriptional regulation of specific genes. By extension, that Aire is required for casein γ expression in wild-type mTECs (which have an accessible locus) suggests that for both Aire-dependent and Aire-independent antigens epigenetic mechanisms alone are not sufficient for TRA expression.

### Outstanding questions

The data and model put forth by Derbinski et al. highlight the complex and ill-defined nature of mTEC differentiation and advocate an intriguing model to account for the observed TRA expression (15). However, it remains to be determined whether TRA expression reflects a novel derepression mechanism that is unique to mTECs, as they have proposed, or whether it results from developmentally conserved mechanisms that are active in a permissive environment. In either case, the results presented delineate important functional parameters of this phenomenon and help define key issues that need to be resolved before the mechanisms underlying TRA expression can be understood. These include the identity, developmental potential, and plasticity of resident mTEC progenitors, the precursor–progeny relationships between the various subsets of mTECs, and the means to isolate defined, homogeneous subsets of mTECs to test these models directly. It seems likely that the mechanisms controlling thymic epithelial cell differentiation and those that control the expression of TRAs will be found to be highly convergent.

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