TOLERANCE

How AIRE wakes up sleepy genes

Immune tolerance to peripheral tissue antigens begins in the thymus, where medullary thymic epithelial cells (mTECs) give differentiating thymocytes a 'sneak preview' of the self antigens they will encounter in the periphery — a process that partially depends on the transcriptional regulator protein autoimmune regulator (AIRE). By looking at the binding partners of AIRE, this study puts forward a new model for how AIRE regulates the transcriptional 'waking' of peripheral tissue antigen genes in mTECs.

Several features of transcriptional regulation by AIRE indicate that it does not function as a classical transcription factor, so Benoist, Mathis and colleagues identified molecules that associate with AIRE and affect its activity. Using mass spectrometry screening of proteins that co-immunoprecipitate with AIRE in transfected cell lines, they highlighted

STOCKBYTE

45 AIRE-interacting proteins that could be clustered into four functional groups: nuclear transport proteins, proteins involved in chromatin binding or structure, proteins involved in the transcription process — such as DNA-dependent protein kinase (DNA-PK) and topoisomerase 2α (TOP2α) — and proteins involved in pre-mRNA processing. They showed that the association with AIRE of at least 21 candidate partners (representing each of the four functional groups) was functionally relevant by observing the effect of knockdown of these partners on the expression of AIRE-dependent transcripts.

The modulation of AIRE activity by nuclear transport proteins is not surprising, probably reflecting an indirect effect through nuclear shuttling of AIRE. A link between AIRE and chromatin binding has been suggested by previous work showing that AIRE can bind unmethylated histone 3 lysine 4 (H3K4) residues (see Further reading). The authors therefore focused on the other two functional groups.

The *in vivo* relevance of DNA-PK–AIRE interactions was tested using NOD.CB17-*Prkdc*^{scid} mice, which have an inactive form of DNA-PK. These mice lack mature thymocytes and therefore mTECs (which depend on thymocytes for maturation), so they were reconstituted with wild-type bone marrow to generate chimeric mice with wild-type lymphocytes and DNA-PK-mutant mTECs. Reconstituted NOD.CB17-*Prkdc*^{scid} mice — but not reconstituted control mice — had decreased transcription of AIRE-dependent genes in mTECs, despite similar levels of AIRE protein and nuclear localization. DNA-PK is known to promote transcription in a complex with TOP2α. Etoposide, which blocks the religation of double-stranded DNA breaks introduced by TOP2α to relax the torsional tension introduced during transcription, had a strikingly similar effect to AIRE on the transcription of AIRE-dependent genes. AIRE also increased the frequency of double-stranded breaks created by TOP2α, indicating that it might function in a similar manner to etoposide by binding to TOP2α and inhibiting its religation function.

In terms of pre-mRNA processing, the authors showed that whereas AIRE increased spliced mRNA levels of AIRE-dependent genes, it had a much smaller effect on the levels of unspliced pre-mRNAs. This could not be explained by an effect on the stability of spliced mRNAs.

These results indicate that AIRE — which might be targeted to weakly transcribed genes by binding to hypomethylated H3K4 — promotes TOP2α-mediated double-stranded DNA breaks, which in turn recruit DNA-PK. TOP2α, DNA-PK and associated proteins then 'wake up' the silent chromatin by linking to chromatin remodelling, transcriptional elongation and pre-mRNA processing complexes.

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ORIGINAL RESEARCH PAPER Abramson, J. *et al.* Aire's partners in the molecular control of immunological tolerance. *Cell* **140**, 123–135 (2010)
FURTHER READING Peterson, P. *et al.* Transcriptional regulation by AIRE: molecular mechanisms of central tolerance. *Nature Rev. Immunol.* **8**, 948–957 (2008)