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A condensed view of chromatin during T cell development

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In cell-mediated immunity, specific T cell subsets proliferate in response to cytokines. But the mechanisms that limit clonal expansion to only those T cells that have recognised foreign antigen via their T cell receptor (TCR) are incompletely understood. In this issue of *The EMBO Journal*, Rawlings *et al* (2011) identify one such mechanism that acts, unexpectedly, through chromatin compaction and involves a subunit of condensin—a complex better known for restructuring chromosomes in preparation for cell division.

Chromosome compaction changes dramatically during the cell cycle to coordinate genome replication, segregation and transcription, but also occurs as specialised transcription programs are implemented during differentiation. The molecular basis of these changes is not well understood, but is thought to include covalent modifications to DNA and histones. It has recently become clear that Structural Maintenance of Chromosome (SMC) complexes, which were initially identified for their roles in altering chromosome structure during M phase, also function at interphase. The condensins are one such group of SMC complexes, and are required for the timely compaction and segregation of mitotic and meiotic chromosomes (Hudson *et al*, 2009). Vertebrates have two condensin complexes (I and II) differing in subunit composition and localisation during the cell cycle. In addition to mitotic functions, condensins have been shown to function in transcription-related phenomena such as dosage compensation in *Caenorhabditis elegans* and transvection in *Drosophila melanogaster* (Wood *et al*, 2010). The work of Rawlings *et al* adds gene silencing in the mammalian immune system to this list.

After TCR rearrangement in the thymus, T cells cease proliferation and become insensitive to cytokines such as IL-2—partly but not completely explained by a down-regulation of cytokine signalling. Mature, but naive-to-antigen, quiescent T cells then emerge out of the thymus and into the periphery. During a normal immune response, TCR activation via antigen-specific recognition leads peripheral T cells to proliferate. This occurs via IL-2 production, and consequent tyrosine phosphorylation of the transcription factor (TF) Stat5 at the IL-2 receptor (IL-2R) complex. Phosphorylated Stat5 dimers translocate to the nucleus, bind their target promoters and activate a transcriptional program that induces naive T cells to exit quiescence (Rawlings *et al*, 2004) (Figure 1).

This raises the question of how the majority of cells, where TCR engagement by antigen has not occurred, ignore the effects of circulating IL-2 and so do not proliferate inappropriately. Surprisingly, Rawlings *et al* show that nuclear translocation of phosphorylated Stat5 still occurs in peripheral T cells exposed to IL-2 in the absence of TCR activation. However, chromatin immunoprecipitation (ChIP) showed that, in these cells, Stat5 was unable to engage the promoter of target genes, and hence activate transcription.

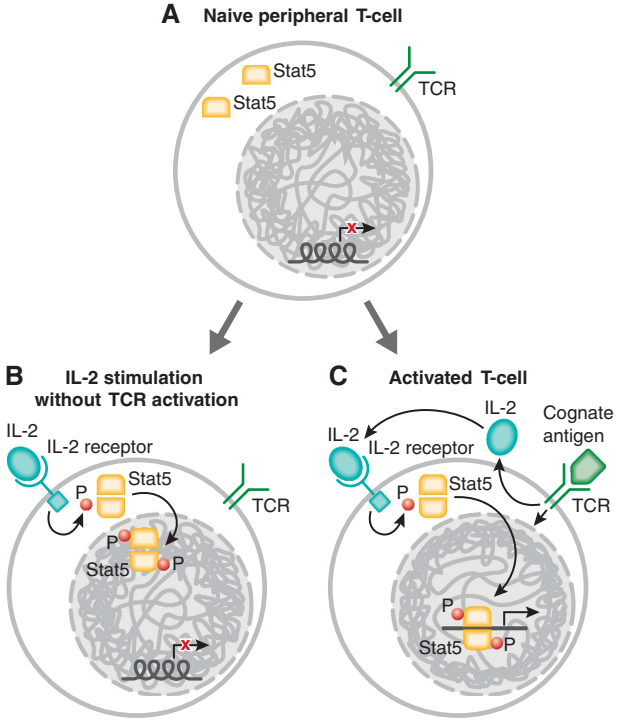

Figure 1 Chromatin condensation during T cell activation. (**A**) In naive (quiescent) peripheral T cells, IL-2 signalling is off, Stat5 remains in the cytoplasm and condensin causes chromatin compaction. (**B**) IL-2 stimulation of naive peripheral T cells, in the absence of TCR-activation, causes IL-2R-mediated phosphorylation of Stat5 and its re-location to the nucleus. However, Stat5 cannot access its nuclear transcriptional targets due to chromatin condensation. (**C**) Following T cell receptor activation, chromatin decondenses, IL-2 signalling leads to Stat5 phosphorylation and re-localization to the nucleus, where it can now access its target promoters to induce transcription.

This suggested that a chromatin-based mechanism limits cytokine responsiveness in cells that have not encountered their specific antigen. Using transmission electron microscopy and chromatin sonication assays, the authors provide compelling evidence that correct T cell activation is associated with global changes in chromatin compaction. The nuclei of naive T cells appeared almost full of condensed chromatin, which dispersed in response to TCR activation, but not to IL-2 treatment alone.

Having excluded that promoter DNA methylation underlies these changes in chromatin structure, the authors turned their attention to histones. Remarkably, they found that, regardless of any post-translational modifications, histone H3 was undetectable by immunofluorescence in the nuclei of naive T cells, but became detectable during TCR activation concomitant with the visible change in chromatin compaction. Reassuringly, histones and modified histones were present in naive T cell chromatin, as revealed by western blotting of acid-extracted histones. Indeed global levels of H3 modifications associated with either active or inactive chromatin states were not changed upon activation. However, the very same antibodies were barely able to detect histones, or their modification, by western blot of solubilised proteins prior to TCR activation. Rather, the histones remained insoluble. These observations may demand a reassessment of published large-scale ChIP studies in unstimulated T cells, as the perceived lack of histone modifications at particular loci might reflect inaccessibility/insolubility rather than absence *per se*.

To identify factors required to compact chromatin in naive T cells, the authors searched the literature for mouse mutants with defects in T cell differentiation post-TCR rearrangement—the stage at which chromatin condensation is initiated. The *nessy* mouse, homozygous for a single amino acid change within the kleisin-β (CAP-H2) subunit of condensin II (Gosling *et al*, 2007), fulfilled these criteria. Rawlings *et al* demonstrate that chromatin compaction is compromised in *nessy* peripheral T cells, which also precociously express Stat5 target genes and other markers of T cell activation (Gosling *et al*, 2007; Rawlings *et al*, 2011).

These findings identify a novel role for condensin during a specialised form of chromatin compaction that regulates access of TFs to their target loci and so maintains quiescence in the immune system. Do similar mechanisms operate elsewhere in differentiation? Intriguingly, mammalian erythroid maturation is associated with chromatin condensation prior to enucleation, and the condensin II subunit CAP-G2 has been implicated in repressing transcriptional activation and promoting terminal differentiation of erythroid cells (Xu *et al*, 2006).

Unanswered questions remain about the exact mechanism, and control, of kleisin-β-mediated chromatin compaction. As not all genes in naive T cells are refractory to IL-2 stimulation, there must be a targeting mechanism for the chromatin condensation and a means by which it is relieved upon TCR activation. Finally, the exact nature of the inaccessible compacted chromatin state remains to be determined. It is noteworthy that on mitotic chromosomes, of which condensin is a key architect, histone epitopes are readily accessible to antibody staining (Terrenoire *et al*, 2010), unlike the case during T cell quiescence (Rawlings *et al*, 2011).

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Conflict of interest

The authors declare that they have no conflict of interest.

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