A rheostat tuning thymic selection

Gerald P Morris & Stephen M Hedrick

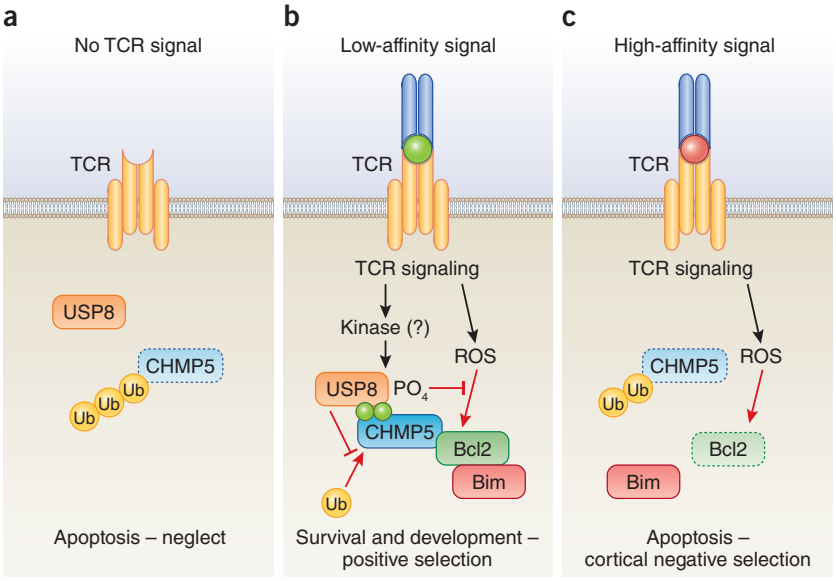
Thymocytes must undergo positive selection to survive and differentiate. This process is regulated by the TCR-sensitive protein CHMPS by preventing Bcl2 oxidation and degradation.

Signals operating through the αβ antigen receptor (TCR) in developing thymocytes can have diametrically opposite consequences depending on the strength of the interaction between the TCR and peptide–MHC ligands. Absence of signal, or induction of a signal strong enough to cause a mature T cell to grow and divide, results in thymocyte cell death, whereas an intermediate 'Goldilocks' signal results in survival and continued differentiation—the process of positive selection. In this issue of *Nature Immunology*, Adoro *et al.* link previously disparate observations to describe a previously unidentified pathway active only in response to signal strength that is 'just right'[1]. The pathway involves TCR signaling, de-ubiquitylation, reactive oxygen species (ROS) and the stability of pro-survival factors such as Bcl2.

Elegant studies have established the kinetics of TCR and peptide-bound MHC interactions that promote positive as opposed to negative selection. Multiple pathways of signal transduction downstream of these interactions decode a continuous spectrum of signal strength to direct cells toward discrete alternate developmental fates, fates that include cell death[2]. This aspect of thymocyte selection appears to be counter-regulated by the expression of pro-survival Bcl2 and, under some circumstances, pro-apoptotic Bim[3–5]; however, the ways that these arbiters of cell viability are themselves regulated during the selection process are not presently understood.

The endosomal sorting complex required for transport (ESCRT) carries out cargo recognition, membrane sculpting and vesicular sorting, often as a means of transporting ubiquitylated proteins to the lysosome for degradation[6]. In this capacity, the ESCRT complexes affect diverse aspects of cell biology that can include finely tuned post-translational regulation of protein abundance[7]. This made the ESCRT machinery a potentially attractive target for probing thymic development, and in particular, previous work showed that CHMP5, an accessory protein in the ESCRT-III complex, has variable effects on EGFR and TGFβRII turnover[8]. In addition, it directly inhibits NF-κB signaling in osteoclasts[9]. In the present study, the authors found that CHMP5 itself can be subject to proteosomal regulation during the progress of thymocyte development, and they went on to show that a lineage-specific deletion of *Chmp5* using *Cd4-Cre* produces an almost complete loss of mature, post-selection $CD24^{lo}TCR^{hi}$ thymocytes. The inference was that CHMP5 is required for thymocyte development past the stage at which positive selection occurs. Similarly to the study in osteoclasts, the authors' work showed no evidence for a defect in ESCRT-dependent trafficking[9]; in contrast to that study, however, there was also no evidence for other defects in receptor-mediated signaling, including NF-κB activation, release of free calcium or phosphorylation of Erk.

The lack of a measurable effect on TCR signaling implied that a block in thymocyte development resulting from *Chmp5* loss of function could be the result of a defect in survival. Indeed, Bcl2 levels were reduced in *Chmp5*-null thymocytes, which is consistent with an increase in apoptotic cells *ex vivo*. Bcl2 stability is regulated through oxidation caused by reactive oxygen species (ROS)[10], and in keeping with this, the authors found that it



Debbie Maizels/Springer Nature

Figure 1 TCR signaling alters post-translational regulation of CHMP5 to modulate double-positive $CD4^+CD8^+$ thymocyte survival. Double-positive thymocytes have increased expression of CHMP5 attributable to increased transcription and decreased ubiquitin (Ub)-mediated protein degradation. (**a**) In the absence of interaction with a positively selecting peptide–MHC ligand, CHMP5 protein is degraded. (**b**) TCR signaling induced by ligation of an intermediate-affinity peptide–MHC ligand (such as in positive selection) promotes stabilization of CHMP5 through phosphorylation (PO_4) via an unknown kinase, and this enhances its physical interaction with the deubiquitinase USP8. CHMP5 is then capable of protecting Bcl2 from reactive oxygen species (ROS) produced during thymocyte stimulation. This enables double-positive thymocytes to continue differentiation and $CD4^+$ as opposed to $CD8^+$ lineage commitment. (**c**) CHMP5 is ubiquitinated and degraded in the presence of strong TCR signals induced by high-affinity ligand binding (such as in negative selection), eliminating its stabilization of Bcl2.

Gerald P. Morris is in the Department of Pathology and Stephen M. Hedrick is in the Departments of Molecular Biology and Cellular and Molecular Medicine of the University of California, San Diego, La Jolla, California, USA.
e-mail: gpmorris@ucsd.edu or shedrick@ucsd.edu

was more highly sulfenylated in the absence of CHMP5. Their key finding was that the stability of CHMP5 itself depended on differential signals through the TCR. Signaling resulting from the recognition of intermediate-affinity peptides, or from low concentrations of PMA and ionomycin, actively promoted stabilization of CHMP5, whereas strong signals, emanating from the recognition of agonist peptides or high concentrations of PMA and ionomycin, did not. Mass spectrometry revealed two phosphorylated serines and a ubiquitylated lysine as key regulators of CHMP5 stability; mutation of the serines resulted in constitutive ubiquitylation, whereas mutation of the lysine resulted in increased CHMP5 stability (**Fig. 1**).

The implication was that CHMP5 is positively regulated by phosphorylation and negatively regulated by ubiquitylation. In an instance of true serendipity, Adoro *et al.* screened a panel of deubiquinating enzymes (DUBs) for activity against CHMP5 and found two, one of which was shown to be nonessential for T cell development, whereas deletion of the gene encoding the other, USP8, almost exactly phenocopied the deletion of *Chmp5* (ref. 11). The authors then demonstrated a physical interaction between CHMP5 and USP8 that was dependent on the identified phosphoserines and occurred only after intermediate TCR stimulation. Finally, deletion of *Usp8* caused a reduction in CHMP5, leading to a model whereby USP8 interacts with and deubiquinates CHMP5 upon signaling that is just right, and this, in turn, stabilizes Bcl2. These results provide the framework for understanding how measured signals allow thymocytes to survive Bim–Bax–Bad-mediated apoptosis[4] and continue on a path to thymic egress and residence in secondary lymphoid organs.

The survival of thymocytes thus involves an ESCRT protein in a new role: not dependent on regulating ubiquitylation or the stability of ubiquitylated proteins, but rather opposing an oxidation reaction that destabilizes Bcl2. The stability of CHMP5 itself appears to be regulated by ubiquitylation that is opposed by serine phosphorylation, and this suggests the existence of one or more CHMP5 kinases or phosphatases that decode the continuum of TCR signals into null, intermediate and strong. A complication to this model is that *Chmp5* mRNA is also reduced in the presence of strong signals, suggesting that phosphorylation and protein stabilization are not the only means by which CHMP5 is regulated. Negative selection may involve a further level of transcriptional or post-transcriptional *Chmp5* control. We see this as a required pathway that supports, but does not drive, positive selection. More directly, this pathway appears to be a key to the enigmatic 'early' or cortical form of negative selection[12]—a process that is invoked in the face of omnipresent antigens that do not require specialized, medullary antigen presentation[13].

Still other questions revolve around USP8 and how its activity in regulating CHMP5 is controlled. Is phosphorylation of CHMP5 the only regulator of USP8 effects on CHMP5, or are there other regulators of USP8 activity? USP8 binds the TCR-proximal signal transducer GADS, as well as 14-3-3, but associates with the TCR signalosome without a requirement for GADS[11]. Does TCR signalosome formation promote the physical interaction between the two molecules described in this report? Does USP8 activation via a cysteine protease[11] provide an additional level of control in determining the survival response to TCR signals?

The CHMP5 control of thymocyte survival is determined by post-transcriptional regulation of signaling components at multiple steps along the pathway. This is yet another illustration of the disconnect between mRNA expression and cellular physiology[14]. It is also an illustration of the means by which science moves forward: that is, by serendipity, or, as Pasteur put it, "In the fields of observation, chance favors the prepared mind."

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Adoro, S. *et al. Nat. Immunol.* **18**, 780–790 (2017).
2. Gascoigne, N.R., Rybakin, V., Acuto, O. & Brzostek, J. *Annu. Rev. Cell Dev. Biol.* **32**, 327–348 (2016).
3. Linette, G.P. *et al. Immunity* **1**, 197–205 (1994).
4. Bouillet, P. *et al. Nature* **415**, 922–926 (2002).
5. Villunger, A. *et al. Proc. Natl. Acad. Sci. USA* **101**, 7052–7057 (2004).
6. Henne, W.M., Buchkovich, N.J. & Emr, S.D. *Dev. Cell* **21**, 77–91 (2011).
7. Rusten, T.E., Vaccari, T. & Stenmark, H. *Nat. Cell Biol.* **14**, 38–45 (2011).
8. Shim, J.H. *et al. J. Cell Biol.* **172**, 1045–1056 (2006).
9. Greenblatt, M.B. *et al. J. Exp. Med.* **212**, 1283–1301 (2015).
10. Li, D., Ueta, E., Kimura, T., Yamamoto, T. & Osaki, T. *Cancer Sci.* **95**, 644–650 (2004).
11. Dufner, A. *et al. Nat. Immunol.* **16**, 950–960 (2015).
12. McCaughtry, T.M., Baldwin, T.A., Wilken, M.S. & Hogquist, K.A. *J. Exp. Med.* **205**, 2575–2584 (2008).
13. Metzger, T.C. & Anderson, M.S. *Immunol. Rev.* **241**, 89–103 (2011).
14. Walley, J.W. *et al. Science* **353**, 814–818 (2016).