the mating-type locus. This is consistent with recent findings that Dis3, an exosome-associated ribonuclease, is also required for silencing at centromeres and the mating-type locus (Murakami et al., 2007). Unlike *cid14*, neither of these exosome components is required to generate siRNAs that are homologous to heterochromatin regions, suggesting that the role of *cid14* may be more complex than simply exosome recruitment.

The findings of Bühler et al. (2007) strongly suggest that Cid14 is involved in targeting centromere transcripts for degradation. However, it remains to be determined whether heterochromatin transcripts are bona fide substrates for Cid14 polyadenylation. Centromere transcripts are known to have poly(A) tails, and these tails are unchanged in cells lacking Cid12, so it might be revealing to check their status in a *cid14* mutant (Win et al., 2006b). It would also be informative to examine whether Cid14 or the whole TRAMP complex associates with centromeric transcripts. Another outstanding question is the relationship between Cid14 and Cid12. Based on their observation of a large RNA species associated with Ago1 in cells lacking Cid14, the authors suggest that Cid14 may be required to convert single-stranded precursor RNA into dsRNA. This is a role also proposed for the RDRC complex raising the possibility that Cid12 and Cid14 may have some functional redundancy, analogous to Trf4 and Trf5. Such an effect might explain how Cid14 can be intimately associated with the RNAi pathway despite having a distinct mutant phenotype.

Clearly much remains to be revealed about the mechanisms underlying RNAi-directed heterochromatin formation and silencing. Nevertheless, the analyses by Bühler et al. (2007) reveal that siRNAs are made from transgene insertions at centromeres, and expose intriguing connections between heterochromatin silencing and general RNA turnover mechanisms.

REFERENCES

Bühler, M., Haas, W., Gygi, S.P., and Moazed, D. (2007). Cell, this issue.

Buhler, M., Verdel, A., and Moazed, D. (2006). Cell *125*, 873–886.

Grewal, S.I., and Jia, S. (2007). Nat. Rev. Genet. *8*, 35–46.

LaCava, J., Houseley, J., Saveanu, C., Petfalski, E., Thompson, E., Jacquier, A., and Tollervey, D. (2005). Cell *121*, 713–724.

Motamedi, M.R., Verdel, A., Colmenares, S.U., Gerber, S.A., Gygi, S.P., and Moazed, D. (2004). Cell *119*, 789–802.

Murakami, H., Goto, D.B., Toda, T., Chen, E.S., Grewal, S.I., Martienssen, R.A., and Yanagida, M. (2007). PLoS ONE *2*, e317.

Stevenson, A.L., and Norbury, C.J. (2006). Yeast *23*, 991–1000.

Volpe, T.A., Kidner, C., Hall, I.M., Teng, G., Grewal, S.I., and Martienssen, R.A. (2002). Science *297*, 1833–1837.

Win, T.Z., Draper, S., Read, R.L., Pearce, J., Norbury, C.J., and Wang, S.W. (2006a). Mol. Cell. Biol. *26*, 1710–1721.

Win, T.Z., Stevenson, A.L., and Wang, S.W. (2006b). Mol. Cell. Biol. *26*, 4435–4447.

Immunological Synapses: Breaking Up May Be Good to Do

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DOI 10.1016/j.cell.2007.05.008

Activated T cells form stable immunological synapses with antigen-presenting cells whereas naïve T cells initially engage in more transient interactions. Sims et al. (2007) demonstrate that these transient interactions are due to the kinase PKCθ, which serves to destabilize the synapse thereby permitting T cells to migrate elsewhere. They also show that re-establishment of a synapse involves the actin regulator WASp.

The immunological synapse comprises a series of tight membrane juxtapositions between the contact face of a T cell and an antigen-presenting cell. Studies performed either using T cell clones or activated T cells implied a certain degree of synapse stability. For example, although calcium ion signaling induced by the activated T cell receptor (TCR) begins with the appearance of just a few small TCR clusters in the synapse, these typically coalesce within minutes to form a single mega-cluster (Krummel et al., 2000). Such a distribution, called a cSMAC (central supramolecular activation cluster), is surrounded by a peripheral or pSMAC of clustered integrins (Monks et al., 1998) and may be stable on the order of hours.

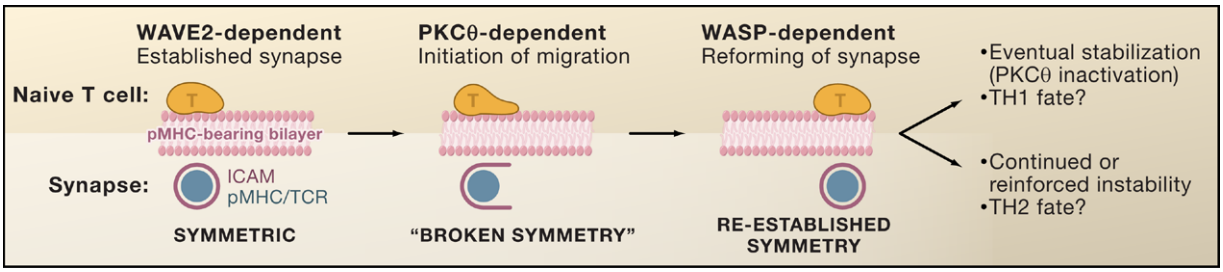

Figure 1. PKCθ and WASp at the Immunological Synapse

A T cell is shown at three separate time points during its interaction with an artificial lipid bilayer that simulates an immunological synapse formed in vivo with an antigen-presenting cell. Initially, the synapse forms as a series of concentric zones of proteins, highlighted by the TCR/pMHC in the cSMAC and ICAM and PKCθ in the pSMAC. PKCθ is required to break the symmetry of these concentric zones and to open up a portion of the ICAM ring, promoting migration of the T cell in that direction. Subsequent closing of the ring, which is dependent on WASp, leads to restabilization of the synapse at a new area of the bilayer.

Even these "experienced" T cells seem to generate dynamic ruffles resembling lamellipodia (actin-rich projections) at their distal edges that continue to move along the surface of antigen-presenting cells. Such ruffles—like their counterparts in synapses between B cells and dendritic cells (Fleire et al., 2006)—may serve to recognize additional signals by exposing the T cell to new regions on the antigen-presenting cell surface. In addition, T cells also may respond to new complexes of peptide nestled in major histocompatibility complex (pMHC) molecules away from the synapse. For example, human T cell clones transfer their synapses from antigen-presenting cells to those bearing higher amounts of antigen (Depoil et al., 2005). When faced with wrinkles in the antigen-presenting cell membrane, TCRs also may encounter pMHC complexes on membrane patches that had not previously made contacts. Such a continuous scanning strategy allows T cells to accumulate the maximum number of pMHCs ensuring the strongest TCR signal possible.

In this issue, Dustin and colleagues (Sims et al., 2007) provide a new molecular explanation for the propensity of naïve T cells to wander from the site of an initial synapse. These authors studied the role of protein kinase C θ (PKCθ) during T cell activation and synapse formation. They examined the structure of T cell synapses using a supported lipid bilayer system to mimic an antigen-presenting cell and observed two new features of naïve T cells. First, PKCθ itself is localized in the peripheral region of the synapse formed by these cells (the so-called pSMAC), whereas most previous reports using experienced T cells found this molecule in the central or cSMAC region. Secondly, the ring of ICAM molecules (integrin ligands) surrounding the synapse formed by these naïve cells periodically breaks open and the T cell then migrates toward this break. Such a "symmetry break" (Figure 1) is followed by re-establishment of another SMAC array at another site on the bilayer, usually within a few minutes. In the absence of PKCθ, these investigators noticed that naive T cells formed unusually long-lived contacts with antigen-presenting cells in vivo and maintained their cSMAC/pSMAC morphology for extensive periods when activated on supported lipid bilayers in vitro. The most profound in vivo phenotype of PKCθ knockout mice reported to date is a defect in Th2-type immune responses in asthma and helminth infection (Marsland et al., 2004).

These results suggest a new role for PKCθ in controlling the T cell cytoskeleton, perhaps through the direct inhibition of actin assembly in the pSMAC. Actin nucleation in T cells is induced by the WAVE2 complex (Nolz et al., 2006), which mediates actin-rich membrane protrusions during basal migration as well as during initiation of synapse formation. Actin nucleation in T cells also is induced by the actin nucleation-promoting factor WASp, which is required to stabilize interactions between T cells and antigen-presenting cells in vitro and TCR clustering on artificial surfaces. Sims et al. (2007) examined immunological synapses in WASp-deficient T cells and found that although WASp was not necessary to establish or break symmetry, it was essential to re-establish it. Notably, blocking PKCθ activity using a small-molecule inhibitor reversed this effect, suggesting that WASp activation may normally help to overcome the synapse-destabilizing activity of PKCθ. The role of WAVE2 in the process remains unresolved.

PKCθ in the pSMAC might also act by regulating the actin-based motor protein myosin IIA, which also accumulates at this site (Jacobelli et al., 2004). Myosin IIa may create cortical tension as a result of forming myosin IIA filaments that crosslink actin filaments. Notably, myosin IIA heavy chain is phosphorylated following TCR stimulation, resulting in disassembly of myosin filaments. Myosin IIA remains phosphorylated only for the first 5 to 10 min of T cell activation and might subsequently establish cortical tension across the synapse and help to generate the symmetric forces that keep the synapse stably in place. It will be interesting to determine whether PKCθ regulates myosin IIA and to examine the role of myosin IIA in breaking and re-establishing symmetry at the immunological synapse.

Given these observations, what is the functional purpose of the continual scan by T cells? On the one hand, it may permit T cells to accumulate

"hits" via sequential encounters with antigen-presenting cells bearing relatively weak stimuli. Such encounters by T cells may result in a decrease in pMHC complexes borne by antigen-presenting cells (Garcia et al., 2007). This strategy may allow T cells to use all of the available pMHC complexes, even when they are not confined to just one antigen-presenting cell. In a similar vein, it may permit T cells to find the antigen-presenting cells with the strongest overall antigen signal, and this might be quite important, for example, when T cells are faced with "waves" of dendritic cell emigrants from inflamed tissue (Itano et al., 2003), for shifting synapses to antigen-bearing B cells, or for moving to a common APC upon which multiple T cells might coaggregate and share cytokines (Beuneu et al., 2006). In this context, Sims et al. (2007) document an increase in interleukin (IL)-2 production when T cells express PKCθ and make multiple synaptic contacts.

Another appealing hypothesis for the function of continual versus stable scanning lies in regulating ultimate T cell effector function. Recent evidence suggests that T cells might hold a memory of their former synapse and use this site in the same way that yeast use their former bud site to center subsequent asymmetric divisions (Chang et al., 2007). Stabilization of synapses may favor a stronger cellular asymmetry and therefore a more robust skewing, perhaps toward the Th1 effector cells that produce IL-2 and γ-interferon. An intriguing aspect is whether T cells continue to accumulate "hits" resulting in synapses being converted from unstable to stable (perhaps by movement of PKCθ to the cSMAC). Alternatively, the memory of short-lived interactions might in some cases promote the formation of short interactions in the future, thereby influencing the nature of T cells effector functions. Clearly, the accumulated history of a T cell's multiple encounters can affect the quality of the ensuing response and PKCθ appears to be critical in regulating this history.

REFERENCES

Beuneu, H., Garcia, Z., and Bousso, P. (2006). J. Immunol. *177*, 1406–1410.

Chang, J.T., Palanivel, V.R., Kinjyo, I., Schambach, F., Intlekofer, A.M., Banerjee, A., Longworth, S.A., Vinup, K.E., Mrass, P., Oliaro, J., et al. (2007). Science *315*, 1687–1691.

Depoil, D., Zaru, R., Guiraud, M., Chauveau, A., Harriague, J., Bismuth, G., Utzny, C., Muller, S., and Valitutti, S. (2005). Immunity *22*, 185–194.

Fleire, S.J., Goldman, J.P., Carrasco, Y.R., Weber, M., Bray, D., and Batista, F.D. (2006). Science *312*, 738–741.

Garcia, Z., Pradelli, E., Celli, S., Beuneu, H., Simon, A., and Bousso, P. (2007). Proc. Natl. Acad. Sci. USA *104*, 4553–4558.

Itano, A.A., McSorley, S.J., Reinhardt, R.L., Ehst, B.D., Ingulli, E., Rudensky, A.Y., and Jenkins, M.K. (2003). Immunity *19*, 47–57.

Jacobelli, J., Chmura, S.A., Buxton, D.B., Davis, M.M., and Krummel, M.F. (2004). Nat. Immunol. *5*, 531–538.

Krummel, M.F., Sjaastad, M.D., Wülfing, C., and Davis, M.M. (2000). Science *289*, 1349–1352.

Marsland, B.J., Soos, T.J., Spath, G., Littman, D.R., and Kopf, M. (2004). J. Exp. Med. *200*, 181–189.

Monks, C.R., Freiberg, B.A., Kupfer, H., Sciaky, N., and Kupfer, A. (1998). Nature *395*, 82–86.

Nolz, J.C., Gomez, T.S., Zhu, P., Li, S., Medeiros, R.B., Shimizu, Y., Burkhardt, J.K., Freedman, B.D., and Billadeau, D.D. (2006). Curr. Biol. *16*, 24–34.

Sims, T.N., Soos, T.J., Xenias, H.S., Dubin-Thaler, B., Hofman, J.M., Waite, J., Cameron, T.O., Thomas, V.K., Varma, R., Wiggins, C.H., et al. (2007). Cell, this issue.

"Wunder" F-BAR Domains: Going from Pits to Vesicles

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DOI 10.1016/j.cell.2007.05.006

Clathrin-mediated endocytosis is a key mechanism by which cells take up extracellular cargo. In this issue, Shimada et al. (2007) reveal the mode of action of the F-BAR domain, which deepens the initial membrane pit that forms during clathrin-mediated endocytosis.

Lipid membranes are highly flexible and can be deformed into a wide range of shapes or broken apart to form smaller entities. In some way, this is what happens in clathrin-mediated endocytosis, a fundamental mechanism by which cells take up liquids and particles from the environment. In clathrin-mediated endocytosis, cells first form hemispherical plasma membrane invaginations around the cargo, such as a ligand-bound receptor (Fig-