Centromeric Repositioning of Coreceptor Loci Predicts Their Stable Silencing and the CD4/CD8 Lineage Choice

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Abstract

The differentiation of CD4+ CD8+ double positive (DP) thymocytes requires the irreversible choice between two alternative lineages, distinguished by the mutually exclusive expression of either CD4 or CD8. Differentiating DP cells transiently down-regulate both CD4 and CD8, and this has complicated the debate whether the mechanism of CD4/CD8 lineage choice is instructive, stochastic/selective, or more complex in nature. Using fluorescence in situ hybridization, we show that the stable silencing of coreceptor loci, and ultimately lineage choice, is predicted by the spatial repositioning of coreceptor alleles to centromeric heterochromatin domains. These data provide evidence that lineage-specific developmental programs are established early during the transition from the DP to the single positive stage.

Key words: lineage commitment • FISH

Introduction

As cells make developmental choices, their genomes are modified to reflect their previous developmental history as well as their developmental potential (1). This occurs at an epigenetic level, rather than at the level of primary nucleotide sequence. Epigenetic modifications include DNA (CpG) methylation (2), the packaging of DNA in chromatin, the posttranslational modification of chromatin proteins (3), and the spatial partitioning of gene loci into transcriptionally permissive and repressive domains within the nucleus (4, 5). Silent, but not active, gene loci are often found in close proximity to centromeric heterochromatin in activated B cells (4, 6). In double positive (DP) thymocytes, the spatial repositioning of RAG and Tdt loci to pericentric heterochromatin marks their stable silencing in response to TCR engagement (6) and is associated with the spreading of repressive histone modifications over an extended region of the Tdt locus (7). Centromeric repositioning does not occur during transient RAG and Tdt silencing in the DP cell line VL3-3M2 (6), where repressive histone modifications remain confined to the Tdt promoter region (7). These data suggest that for the Tdt locus at least, centromeric repositioning is part of a concerted program of epigenetic events that stabilize gene silencing. Proximity to centromeres in cis (as a result of chromosomal translocations or the centromeric integration of transgenes) can result in gene silencing (8), and the recruitment of chromatin domains to centromeric heterochromatin in trans has been mechanistically linked to gene silencing by the requirement for polycomb proteins, histone methyltransferases, and heterochromatin binding proteins in Drosophila (9, 10).

The development of T cells in the thymus is understood in considerable detail and provides a useful model for cellular commitment and differentiation in metazoans. Commitment and differentiation of CD4+ CD8+ DP thymocytes to the CD4 or the CD8 lineage is triggered by TCR engagement and proceeds via a series of intermediate (DPlo and CD4+ CD8lo) stages (11, 12). Transiently reduced expression of CD4 and CD8 RNA and protein occurs in thymocytes en route to either lineage and is not predictive of lineage fate (12–19). This has added complexity to the debate as to whether thymocyte lineage choice operates instructive or stochastic/selective mechanisms, or results from cellular computations of signal strength and duration (11, 12).

Here, a functional appraisal of thymocyte lineage commitment and developmental potential is combined with a...
detailed analysis of CD4 and CD8 locus position (4) in transitional thymocyte subsets. We show that lineage choice is anticipated by the repositioning of coreceptor alleles to centromeric heterochromatin domains. Centromeric repositioning is progressive, developmentally regulated, and selectively affects coreceptor loci that are subject to stable silencing. Our results provide evidence that lineage-specific developmental programs are implemented at an early stage of DP to single positive (SP) differentiation.

Materials and Methods

Mouse Strains, Cell Sorting, and Cell and Organ Culture. OT-I (20) and AND (21) TCR transgenic mice on a RAG-1 o/o (22) background were used as sources of MHC class I– (K b) or II– (E k/A b) selected thymocytes, respectively. A B o/o (23), B2m o/o (24), or TAP1 o/o (25) thymi were used as sources of thymocytes with a wild-type TCR repertoire selected by MHC class I or II, respectively. MHC o/o (A b/H9252 o/o/H9252 2m o/o) thymocytes were used as the source of MHC-naive thymocytes. All procedures involving animals were approved by the Home Office, UK. Thymocytes were stained with CD4-PE/Cy5, CD8a-PE (Caltag Laboratories), and in some experiments CD69-FITC (BD Biosciences), and sorted on a FACS DIVA (Becton Dickinson) and subjected to fluorescence in situ hybridization (FISH; see below), or re-aggregated with MHC o/o or C57BL/10 (H-2 b) thymic stroma as described previously (26). Where indicated, CD3/CD4- or CD3/CD3-bispecific antibodies (27) were added to the cultures and thymocyte suspensions were stained with CD4-PE/Cy5 or -APC, CD8-PE or -FITC, and analyzed on a FACSCalibur (Becton Dickinson). Thymocytes were stained with CD4-PE/Cy5, CD5-PE (Caltag Laboratories), and sorted for FISH analysis after 18 h.

FISH. Three-dimensional (3-D) FISH was performed as described previously (4). For FISH involving heat denaturation, cells were fixed to coverslips in 4% paraformaldehyde/PBS for 10 min, permeabilized in 0.2% Triton X-100/PBS for 12 min, heated to 95°C for 8 min in the presence of probes in hybridization mix, and placed on ice for 2 min. Hybridization and probe detection were performed as described previously (4). Probes for γ satellite repeats, CD4 (p7.3.1), CD8 (CD8α-1), and TCRα (32.1w7) were provided by N. Dillon (Imperial College, London, UK), A. Rahemtulla (Imperial College, London, UK), D. Kioussis (National Institute for Medical Research, London, UK), and M. Malissen (Centre d’Immunologie de Marseille-Luminy, Marseille, France). Samples were counterstained with DAPI and analyzed on a Leica TCS-4D or SP2 confocal microscope. Distance measurements were taken with Volocity software (http://www.improvision.com).

Online Supplemental Material. A description of distance measurements (Fig. S1) and data obtained in the coreceptor reexpression assay (Fig. 2) are available at http://www.jem.org/cgi/content/full/jem.20041127/DC1.

Results

Coreceptor Locus Repositioning during Lineage Commitment and Differentiation. To ask if nuclear organization is predictive of lineage choice, we sorted thymocyte populations in transit from the DP to the SP stage from mice where thymocyte differentiation is driven either by MHC class I or II and the ultimate lineage outcome is known. DP hi

Figure 1. Developmental regulation of coreceptor locus position. (A) The position of CD8 alleles (green) relative to γ satellite repeats (red) was analyzed by 3-D FISH and defined as associated, constrained, or not associated as detailed in Fig. S1. Bar, 2 μm. (B) The percentage of cells with centromeric loci with both CD4 and CD8, and the percentage of cells with CD4 or CD8 alone, in thymocyte subsets isolated from MHC o/o, class I-deficient, AND TCR transgenic, and OT-I TCR transgenic mice. For MHC o/o, AND, and OT-I thymocyte subsets, the position of CD4 alleles was also evaluated.
CD69−, DPlo CD69+, CD4− CD8lo, and CD4 SP thymocyte populations from MHC class I–deficient mice (TAP1−/− or B2m−/−) and AND TCR (RAG-1−/− H2b) transgenic mice were subjected to 3-D FISH, which preserves the spatial relationship between proteins and DNA in the nucleus (4). The position of CD4 and CD8 alleles was determined relative to pericentric heterochromatin domains, as defined by γ satellite DNA (4). Alleles were classified either as not associated with γ satellite foci, as associated, or as constrained to the dark area surrounding the γ satellite domains, which appeared to exclude background hybridization (Fig. 1 A). Based on previous work using γ satellite probes together with antibodies against nuclear proteins, this area would correspond to pericentric foci of Ikaros proteins (4, 6, 28). Distance measurements from the center of locus-specific FISH signals to the nearest γ satellite domain substantiated this classification (>0.8 μm for “nonassociated,” 0.55–0.8 μm for “constrained,” and <0.55 μm for “associated” alleles; Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20041127/DC1).

CD8 alleles were not spatially associated with centromeric heterochromatin in the great majority (>90%) of MHC−/− (MHC−/−) DP thymocytes (n = 284), or DPlo cells from class I–deficient and AND mice (83%, n = 100 and 91%, n = 200, respectively). In contrast, centromere-associated or constrained CD8 alleles were recorded in 46% of class I–deficient CD4 SP (n = 101) and in 57% of AND CD4 SP cells (n = 111). Critical, centromeric repositioning was already apparent in class II–selected transitional thymocyte subsets: 38% of class I–deficient CD4+ CD8lo (n = 95) cells had constrained or γ satellite–associated CD8 alleles. In AND thymocytes, repositioning of CD8 alleles was seen in DPlo cells (42%, n = 201) as well as in CD4+ CD8lo cells (48%, n = 115; Fig. 1 B).

In contrast to CD8 alleles, CD4 alleles did not undergo centromeric repositioning in developing AND thymocytes (3% of DPlo cells, n = 82; 6% of DPlo cells, n = 101; 7% of CD4+ CD8lo cells, n = 151; 6% of CD4 SP cells, n = 150; Fig. 1 B), indicating that repositioning was selective for CD8 during class II–driven thymocyte differentiation.

Centromeric repositioning of CD8 did not occur during the development of thymocytes transgenic for the class I–restricted OT-I TCR (7% of OT-I DPlo, n = 101; 7% of CD4+ CD8lo, n = 114; 6% of DPlo, n = 94; 10% of CD8 SP, n = 115; Fig. 1 B), which we examined as an example of MHC class I–driven thymocyte differentiation. Instead, the frequency of cells with constrained or γ satellite–associated CD4 alleles, which was constant throughout MHC class II– driven thymocyte differentiation, increased during OT-I differentiation from 6% in DPlo cells (n = 150) to 20% in DPlo (n = 100), and 21% in CD4+ CD8lo cells (n = 150) to 37% in the CD8 SP (n = 150; Fig. 1 B). Hence, repositioning of CD4 in class I–selected OT-I thymocytes appeared less extensive than that of CD8 in class II–selected thymocytes, but was initiated in transitional thymocyte subsets.

To further evaluate the selectivity of coreceptor locus repositioning during MHC class I– and II–driven thymocyte differentiation, we examined the nuclear position of control loci. TCRα expression continues during the DP to SP transition, and TCRα loci remained noncentromeric during both MHC class I– and II–driven thymocyte differentiation (we detected constrained or centromere–associated TCRα loci in 17% of MHC−/−/DPhil n = 103; 13% of class II–selected [AND] CD4+ CD8lo n = 109; and 2% of class I–selected (AB−/−) CD4+ CD8lo n = 102; Fig. 2 A). In contrast to TCRα, expression of Tdt is terminated by TCR signals at the DP stage (6, 7). Tdt was found in noncentromeric positions in MHC−/− DPlo cells (15% of MHC−/−/DPhil cells had constrained or centromere–associated Tdt loci, n = 106; Fig. 2 A) and was repositioned to centromeric heterochromatin domains during both MHC class I– and II–driven thymocyte differentiation (87% of AB−/− CD4+ CD8lo, n = 102 and 92% of AND CD4+ CD8lo, n = 60; Fig. 2 A).

In addition to centromeric repositioning, the shutdown of coreceptor loci during thymocyte differentiation was accom-
panied by a progressive reduction in locus accessibility to FISH probes under 3-D FISH conditions (not depicted). This was reflected in nonequivalent probe hybridization, which was not due to technical failure because our probes worked with >90% efficiency in DPhi cells (not depicted). To address whether changes in locus accessibility resulted in an underestimate of the actual extent to which silenced coreceptor loci were repositioned, we modified our FISH protocol to include heat denaturation. This compromised the preservation of nuclear structure (not depicted), but improved the detection of CD8 alleles in AND CD4+ CD8lo cells. Under these conditions, we found centromere-associated CD8 alleles in 81% of AND CD4+ CD8lo cells, compared with 48% by 3-D FISH (Fig. 2 B). Hence, denaturation conditions that disrupt protein–DNA interactions revealed previously undetectable CD8 alleles in centromeric positions.

**Developmental Potential of Transitional Thymocyte Subsets.** In parallel to evaluating the position of CD4 and CD8 coreceptor loci in the nucleus, we analyzed lineage commitment and developmental potential of sorted thymocyte subsets. Initial experiments suggested that coreceptor reexpression (14) was not necessarily indicative of lineage commitment (Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20041127/DC1) and we opted for re-

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**Figure 3.** Lineage commitment and loss of CD8 lineage potential during MHC class II–driven thymocyte differentiation. (A) DPhi, DPlo, CD4+ CD8lo, and CD4 SP thymocyte subsets were isolated from MHC class I–deficient (Tap1<sup>−/−</sup>) mice. (B) Isolated MHC class I–deficient thymocyte subsets were cultured in reaggregates with MHCo/o thymic stroma for the indicated number of hours and then analyzed for CD4 and CD8 expression. (C) Isolated MHC class I–deficient thymocyte subsets were cultured in reaggregates with MHCo/o thymic stroma for 96 h. (D) Isolated MHC class I–deficient thymocyte subsets were cultured in reaggregates with H2b stroma for 96 h. (E) Isolated MHC class I–deficient thymocyte subsets were cultured in reaggregates with anti-CD3/CD3 for 96 h. (F) Isolated MHC class I–deficient thymocyte subsets were cultured in reaggregates with AND TCR transgenic mice and cultured in reaggregates with MHCo/o thymic stroma for 72 h.
aggregate cultures with H2b thymic stroma (to allow for sustained TCR–pMHC contact) or MHCo/o stroma (26). The latter monitors the cells’ competence to differentiate without pMHC contact. When combined with bispecific CD3/CD4 or CD3/CD3 antibodies (27), which drive the differentiation of uncommitted thymocytes to the CD4 or the CD8 lineage, respectively (29), the system becomes a powerful tool to probe thymocyte lineage potential.

DPhi CD69+, DPlo CD69+, CD4+ CD8lo, and CD4 SP thymocyte populations were sorted from MHC class I–deficient mice (Fig. 3 A) and subjected to reaggregate culture. The CD4+ CD8lo population readily generated CD4 SP progeny in MHCo/o stroma (67 ± 4% at 96 h, n = 3; Fig. 3 B) as well as a minor CD8 SP population (11 ± 2%, n = 3). To further evaluate residual CD8 lineage potential, we exposed CD4+ CD8lo reaggregate cultures to bispecific CD3/CD4 antibodies, which drive uncommitted thymocytes to the CD4 lineage (29). The CD4 bias of the CD4+ CD8lo population was not reversed by anti-CD3/CD3 (13 ± 8% CD8 SP at 96 h, n = 3), whereas DPhi cells readily generated CD8 SP progeny under these conditions (33 ± 4% CD8 SP at 96 h, n = 3; Fig. 3 C). For comparison, DPhi, DPlo, CD4+ CD8lo, and CD4 SP thymocyte populations were sorted from AND TCR (RAG-1o/o H2b) transgenic mice and placed in MHCo/o reaggregates (Fig. 3 E). AND CD4+ CD8lo differentiated to the CD4 SP stage with similar efficiency as class I–deficient CD4+ CD8lo cells, without generating CD8 SP progeny (Fig. 3, compare B with E). In this experimental system, the CD4+ CD8lo phenotype therefore marks a stage during MHC class II–driven thymocyte differentiation at which cells appear committed to the CD4 lineage and are able to progress to the CD4 SP stage without further pMHC contact. As demonstrated above for AND thymocytes, CD8 but not

Figure 4. Lineage commitment and loss of CD4 lineage potential during MHC class I–driven thymocyte differentiation. (A) OT-I TCR transgenic RAG-1o/o H2b thymocytes were isolated and analyzed as described in Fig. 2 A. (B) The generation of CD4 SP (red), DP (blue), and CD8 SP (green) progeny by MHCo/o and OT-I thymocyte subsets is plotted for control reaggregates with MHCo/o stroma (left). Bispecific CD3/CD4 antibodies were added to drive the differentiation of uncommitted thymocytes to the CD4 lineage (right). Cell cycle analysis showed that the generation of CD4 SP progeny was not due to the expansion of preexisting cells (CD4+ CD8lo in MHCo/o stroma: G1 = 84.3%, S = 2.3%, G2/M = 3.4%; CD4+ CD8lo with bispecific CD3/CD4: G1 = 85.6%, S = 2.8%, G2/M = 2.4%, not depicted). (C) CD4+ CD8lo and DP subsets were isolated from class II–deficient (APo/o) mice and analyzed 24 and 48 h after reaggregation with MHCo/o stroma.
CD4 alleles are repositioned to centromeric heterochromatin at this stage (Fig. 1 B).

In contrast to CD4+ CD8lo cells, the class II–selected DPlo population failed to progress to the CD4 SP stage when deprived of pMHC. Most (77%) returned to a DPhi phenotype within 24 h, whereas others were diverted to a CD8 SP phenotype (5% at 24 h, 13% at 48 h, and 58% at 96 h; Fig. 3 B). CD8 differentiation of DPlo cells could not be ascribed to residual MHC class I expression by class I–β2m or Tap1 deficient cells because a fraction of AND TCR transgenic DPlo cells also became CD8 SP (5%; Fig. 3 E). The yields of CD8 SP progeny generated by class I–deficient DPlo cells increased moderately (4.5 ± 2.1-fold, n = 4) in the presence of bispecific anti-CD3/CD3 (Fig. 3 C), and they generated CD8 SP (15%) as well as CD4 SP (25%) progeny in reaggregates with H2b stroma (Fig. 3 D). By these criteria and in this experimental system, class II–selected DPlo cells are not uniformly or irreversibly CD4 committed. Nevertheless, CD8 alleles were selectively repositioned to centromeric heterochromatin in AND DPlo cells (Fig. 1 B).

Next, we examined DPlo, DPhi, CD4+CD8hi, and CD8 SP subsets from OT-I TCR transgenic (RAG-1−/− H2b) mice as an example for MHC class I–selected thymocytes. DPlo cells generated relatively few CD8 SP progeny in MHC+/- stroma (15 ± 12% at 72–96 h, n = 3), but efficiently differentiated to the CD8 SP stage in H2b stroma (Fig. 4 A). We were surprised by the behavior of DPlo cells, which we expected would up-regulate CD4 to become CD4+ CD8lo (12, 16). Only a minority of DPlo cells became CD4+ CD8lo (6% in MHC+/-, Fig. 3 A; 4% in H2b stroma, not depicted) or DPhi (14% in MHC+/- stroma, Fig. 4 A; 4% in H2b stroma, not depicted). Instead, they quickly progressed to the CD8 SP stage (45% at 24 h, 70% at 48 h, not depicted; 97% at 72 h, Fig. 4 A). Conversely, many CD4+ CD8lo cells became DPlo (44% at 24 h) and then progressed to the CD8 SP stage (14% at 24 and 80% at 72 h, Fig. 4 A). Consistent with residual CD4 potential (see below), CD4+ CD8lo but not DPlo cells reproducibly generated a minor CD4 SP population (13 ± 3%, n = 7; Fig. 4 A).

These results tentatively placed DPlo downstream of CD4+ CD8lo cells, suggesting that the developmental sequence in the OT-I thymus was reversed relative to that of MHC class II–selected thymocytes (Fig. 3 A). Therefore, we examined the developmental potential of OT-I thymocyte populations in reaggregates exposed to bispecific CD3/CD4 antibodies, which drive uncommitted thymocytes to the CD4 lineage (reference 29 and Fig. 4 B). The CD4+ CD8lo population generated a much higher percentage of CD4 SP progeny (61%) than the DPlo population (20%; cell cycle analysis confirmed that the increase of CD4 SP was due to differentiation, not expansion of preexisting cells; see legend to Fig. 4 B for details). Hence, in contrast to class II–selected CD4+ CD8lo cells (Fig. 3 A), the OT-I CD4+ CD8lo subset appears flexible in its lineage choice. Progressive CD8 lineage commitment and the loss of developmental potential for the CD4 lineage place OT-I thymocyte populations in the following order: DPlo > CD4+ CD8lo > DPlo > CD8 SP. To test whether this applies to MHC class I–selected thymocytes in general, we isolated CD4+ CD8lo and DPlo subsets from MHC class II–deficient (AB+/-) mice. DPlo cells differentiated to the CD8 SP stage within 24 (36%) to 48 h (64%) and generated few CD4+ CD8lo or DPlo progeny (Fig. 4 C). CD4+ CD8lo cells became DPlo (75% at 24 h) en route to the CD8 SP stage (81% at 48 h; Fig. 4 C). These data confirm that in our experimental system, the majority of class I–selected DPlo cells behave as progeny, not precursors of CD4+ CD8lo cells.

Discussion

Coreceptor Locus Repositioning Is Predictive of CD4/CD8 Lineage Choice. 3-D FISH analysis revealed marked centromeric association of CD8 loci increased in MHC class II–selected CD4+ CD8lo populations, coincident with the cells’ ability to differentiate to the CD4 SP stage without continued pMHC contact, and with their reduced potential to differentiate toward the CD8 lineage in response to anti-CD3/CD3. In AND TCR transgenic thymocytes, substantial centromeric association of CD8 occurred at the earlier DPlo stage, suggesting that for this TCR specificity at least, CD8 repositioning precedes the ability to differentiate to the CD4 lineage without further pMHC signals. Analysis of OT-I TCR transgenic cells as an example of class I–selected thymocytes indicates that the CD4 locus is repositioned during the differentiation to the CD8 (not the CD4) lineage, if to a lesser extent than CD8 in CD4–committed cells. Robust repositioning of CD4 during CD8 T cell differentiation has been seen in other models of CD8 T cell differentiation (Robey, E., personal communication). Hence, in contrast to CD4/CD8 protein and RNA expression, locus repositioning appears to be predictive of lineage choice before differentiation to the SP stage.

Functional Nonequivalence of MHC Class I– and II–selected Transitional Thymocyte Populations. Coreceptor locus repositioning and differential accessibility demonstrates that transitional thymocyte subsets selected by MHC class I and II are not equivalent, a conclusion that is substantiated by the functional analysis of their developmental potential. First, in contrast to class I–selected DPlo cells, which reach the CD4 SP stage independently of stroma pMHC, class II–selected DPlo cells need pMHC to become CD4 SP in our experimental system. They are not uniformly CD4 committed and can generate CD8 SP progeny when deprived of pMHC or exposed to bispecific anti-CD3/CD3. Second, class II–selected CD4+ CD8hi become CD4 SP without pMHC and appear largely CD4 committed because they are not readily redirected to the CD8 lineage by anti-CD3/CD3. Class I–selected CD4+ CD8hi cells also differentiate to the SP stage in MHC+/- stroma, but in contrast to their class II–selected counterparts, they are not uniformly or irreversibly lineage committed and can be redirected to the CD4 lineage by bi–specific antibodies. Because it takes 24 h for MHC–naive OT-I cells to become CD4+ CD8hi when placed in a selecting environment (not depicted), CD8 lineage commitment appears to be a pro-
tracted process, which can be overridden for many hours after initial pMHC contact. Finally, MHC class I– and II–
selected transitional thymocytes appear to differ with re-
spect to developmental sequence. Our analysis agrees with
models that place DPlo cells between the DPsu and the
CD4+ CD8lo population (12–16) in MHC class II–driven
differentiation. However, three lines of evidence place the
majority of class I–selected DPlo cells firmly downstream
of CD4+ CD8lo: (a) class I–selected DPlo cells transit to
the CD8 SP stage faster than CD4+ CD8lo cells; (b) class
I–selected CD4+ CD8lo cells transit through a DPlo stage en
route to the CD8 SP stage, but not vice versa; and (c) class
I–selected DPlo cells lack CD4 lineage potential in our as-
say, in contrast to CD4+ CD8lo cells. This does not exclude
that some MHC class I–selected thymocytes transit through
a DPlo stage before they become CD4+ CD8lo (16).

Implications for Models of CD4/CD8 Lineage Commitment.
We find that CD8 locus repositioning is indicative of the
fate of CD4–committed CD4+ CD8lo thymocytes before
their differentiation to the SP stage and may precede com-
mitment and/or the competence of MHC class II–signaled
erplo cells to differentiate to the CD4 lineage without fur-
ther pMHC contact. These data provide novel markers (30–
33) of thymocyte lineage choice before overt differentiation
and contribute to a growing body of evidence against purely
stochastic/selective models of CD4/CD8 lineage commit-
ment (for review see reference 12). Our functional data in-
dicate that MHC class I–selected CD4+ CD8lo thymocytes
are competent to progress toward the CD8 lineage, yet re-
tain CD4 lineage potential. This calls into question the con-
ceptual distinction between instructive and stochastic/select-
ive models of lineage choice. It adds to evidence (for
review see reference 12) that the CD4/CD8 cell fate de-
cision can be iterative and is not necessarily made “on the
spot” as had been implied in the original models. Taken to-
gether with data that the specification of CD4 lineage fate is
initially labile (as predicted by kinetic signaling models; ref-
ences 12, 33, and 34), but becomes firmly specified within
hours (34), and evidence that CD4 lineage specification is
promoted by signal strength/lck activity (12, 29, 35), one
could liken thymocyte lineage choice to an auction where
thymocytes will firmly accept a “CD4 bid” within a few
hours, whereas cells receiving “CD8 bids” may remain
open to higher offers (and a CD4 lineage fate) for an ex-
tended period. At a mechanistic level, a cascade of chroma-
tin-based events, including repositioning of coreceptors and
other silenced loci (7), would ultimately result in lineage
progression. The lineage–specific reorganization of the thy-
mocyte nucleus opens new avenues for investigating the
molecular basis of lineage choice.

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