Antarctica (*24*) raise the prospect that the two events might be connected.

A consequence of the timings observed here is that the LD ACR is shorter than that at EDC or Vostok. Byrd has similarly been noted (*9*) for its short ACR, but this could be connected to uncertainties in ice flow and dating rather than effects that are climatic in origin. The similarity of the Byrd and LD records from East and West Antarctica tend to support the view that the climatic event was shorter than previously thought. Whether the high East Antarctic Plateau had a different climatic signature is less clear. The relative proximity of LD to the oceanic moisture source during deglacial changes (*25*) could also lead to greater sensitivity and a differing isotopic signature to inland sites.

Another feature of note in the LD record, in the context of north-south asynchrony, is the small cooling jump at about the end of the YD. A close inspection of the record suggests weak antiphase behavior through the YD superimposed on the general warming trend.

Perhaps the largest difference between the LD record and the others is in the period after ~11 ka, during which LD shows prolonged warming before reaching an early Holocene maximum at about 9.5 ka. Timing constraints through this period as determined by $\delta^{18}O$ of O_2 (*13*) do not permit modification to match the early Holocene temperature maxima of Vostok and EDC, which come much earlier, around 11 to 11.5 ka. Even without the O_2 ties, distortion of the time scale to match Vostok and EDC would lead to unreasonable inferred ice-flow or accumulation rate changes. The Byrd record through this period is also considerably different from the East Antarctic records. The difference in the LD record at this time may be connected with the near-coastal core location and the more rapid response of the local ice cap to the deglaciation.

The observations regarding the timing of the ACR relative to North Atlantic changes have implications for models of hemispheric connections and millennial-scale changes. Most consideration has been given to the situation in which North Atlantic thermohaline shutdown, or slowdown, triggers a subsequent warming in the Southern Hemisphere. Precisely how rapidly this change might be propagated is unclear, although modelling (*6*) suggests that the delay would be less than ~100 years. Clearly, this does not accommodate the phasing observed here, with Antarctic cooling or warming occurring before the reverse changes in the north. Our data do not completely rule out essentially synchronous change at the BA transition (to within ~100 years with the LD_{min} chronology), but we emphasize that this is an extreme scenario. Certainly, the second southern trend reversal at the end of the ACR leads the YD onset by ~1 ky.

In most models, control of thermohaline overturning modes is through Northern Hemisphere processes that influence freshwater balance in the North Atlantic; however, one alternative (*26*) proposes control by Antarctic sea ice and associated oceanic salinity changes. In this case, changes in Antarctic temperatures and hydrology, especially for a near-coastal site like LD, could conceivably be detected ahead of the more dramatic shifts that follow thermohaline circulation changes.

Data from both Taylor Dome (*8*) and EDC (*9*) have already been used to show that a bipolar seesaw in temperatures is too simplistic to describe deglacial changes; temperatures decreased simultaneously in the north and south during the Bølling and ACR. The LD results alter the duration of overlap but essentially confirm this observation. The identification of a cause originating in one hemisphere or the other may not be appropriate. A recent examination of the timing statistics for the glacial millennial changes has shown that the south can be equally interpreted as leading or lagging the north (*10*). Further insight may come from alternative interpretations, such as the recently invoked mechanism of "stochastic resonance" (*27*), in which noise in the climate system serves to trigger amplified changes of weak forcing signals; even if the amplifier can be geographically isolated, the noise source(s) essential for crossing thresholds in the system might not.

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22. Exactly how much earlier depends on the adopted Δage and the unconstrained potential for earlier placement of this last gas tie. As an extreme interpretation, recalling that we believe the "young" chronology underestimates Δage, and given the noise on the isotope curve, the ACR commencement may be approximately synchronous with the Bølling transition, but it is difficult to place later.
23. The CH_4 changes in this region do not preclude a younger dating, with the ties in Fig. 2 bunched up toward the YD; however, this leads to anomalous inferred shifts in either ice flow or accumulation through the Bølling-Allerød period that are much less plausible.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/297/5588/1862/DC1
Materials and Methods
SOM Text
Fig. S1
Tables S1 to S3

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Instruction of Translating Ribosome by Nascent Peptide

Feng Gong and Charles Yanofsky*

Expression of the tryptophanase operon of *Escherichia coli* is regulated by catabolite repression and tryptophan-induced transcription antitermination. An induction site activated by L-tryptophan is created in the translating ribosome during synthesis of TnaC, the 24-residue leader peptide. Replacing the *tnaC* stop codon with a tryptophan codon allows tryptophan-charged tryptophan transfer RNA to substitute for tryptophan as inducer. This suggests that the ribosomal A site occupied by the tryptophanyl moiety of the charged transfer RNA is the site of induction. The location of tryptophan-12 of nascent TnaC in the peptide exit tunnel was crucial for induction. These results show that a nascent peptide sequence can influence translation continuation and termination within a translating ribosome.

Recent structural studies with bacterial ribosomes have revealed the features responsible for catalysis of protein synthesis and for antibiotic action. The ribosomal locations of the E, P, and A sites; template RNA; the decoding center; the exit tunnel for the nascent peptide; and the peptidyltransferase center have all been determined (*1–4*). The exit tunnel (*5*), for example, passes through the middle of the 50S subunit and is paved mainly by RNA loops (*1*). Certain nascent peptides when within the peptide exit tunnel can act in cis to alter the

progress of translation (*6–8*). TnaC, the nascent leader peptide of the tryptophanase (*tna*) operon of *E. coli*, falls in this group.

Tryptophanase is a catabolic enzyme that degrades tryptophan to indole, pyruvate, and ammonia, allowing tryptophan to serve as a carbon or nitrogen source (*9*). The *tna* operon of *E. coli* consists of a 319–base pair transcribed leader regulatory region, containing a coding region, *tnaC*, for the 24-residue leader peptide. This leader region is followed by two structural genes, *tnaA* and *tnaB*, that encode tryptophanase and a tryptophan permease (*10*). Initiation of transcription of the operon is regulated by catabolite repression (*11*). Once initiated, transcription of the structural genes of the operon is subject to Rho factor–mediated transcription termination at transcription pause sites located immediately after *tnaC* (*12*). Rho action is prevented by high concentrations of tryptophan.

Tryptophan induction requires synthesis of TnaC, with its crucial tryptophan residue at position 12 (*13*, *14*). Recent in vitro studies have shown that induction is due to tryptophan inhibition of release factor 2 (RF2) action at the *tnaC* stop codon (*15*). The nascent TnaC-peptidyl-tRNA^{Pro} remains uncleaved; its retention stalls the translating ribosome at the *tnaC* stop codon (*15*). This blocks Rho factor's access to its binding site located adjacent to the *tnaC* stop codon, thereby preventing transcription termination (*16*). Inducing amounts of tryptophan inhibit both TnaC-peptidyl-tRNA^{Pro} cleavage and TnaC transfer to puromycin (*17*). Previous studies did not detect the recognition site for tryptophan in the translating ribosome. Here we identify the likely induction site by showing that the tryptophanyl moiety of tryptophanyl-tRNA^{Trp} can replace tryptophan as inducer. We also demonstrate the importance of the location of Trp^{12} of nascent TnaC-peptidyl-tRNA^{Pro} in the peptide exit tunnel.

We reasoned that the most likely site of tryptophan binding/induction in the translating ribosome was the region of the ribosomal A site that accepts the incoming aminoacyl-tRNA. The following observations are consistent with this assumption. A specific DNA template was constructed (W25UGA) in which the *tnaC* stop codon, UGA, was replaced by a tryptophan codon, UGG; this tryptophan codon was followed by a UGA stop codon. This template was examined with standard S-30 preparations (*18*). We expected that, if the template's coding region was translated to completion, TnaC would have an additional residue, tryptophan, at position 25. However, if the tryptophanyl moiety of tryptophanyl-tRNA^{Trp} replaced tryptophan as inducer, the 24-residue TnaC peptidyl-tRNA^{Pro} would accumulate in the translating ribosome in the absence of added tryptophan. We observed accumulation of [^{35}S]methionine-labeled TnaC peptidyl-tRNA^{Pro} (*19*) in the absence and presence of added tryptophan (Fig. 1, W25UGA) (*20*). With the wild-type (WT) template (P24UGA), TnaC-peptidyl-tRNA^{Pro} accumulation occurred only in the presence of added tryptophan (Fig. 1A, WT); that is, in the absence of added tryptophan TnaC-peptidyl-tRNA^{Pro} was cleaved, producing free TnaC (*15*). Placing 12 codons between the W25 UGG codon and the UGA stop codon (Fig. 1A, W25+12AA) also led to TnaC peptidyl-tRNA^{Pro} accumulation in the absence of added tryptophan. These results show that tryptophanyl-tRNA^{Trp} can inhibit TnaC-peptidyl-tRNA^{Pro} elongation, in agreement with our previous finding that tryptophan inhibits TnaC-peptidyl transfer to puromycin (*17*). A template bearing a proline codon after the codon for W25 behaved like the W25UGA template (Fig. 1A, W25P26UGA). In contrast, inserting a proline codon between the codons for P24 and W25 eliminated peptidyl-tRNA accumulation (Fig. 1A, P25W26P27UGA). Replacing the codon for P24 with a tryptophan codon (Fig. 1A, W24UGA) also eliminated peptidyl-tRNA accumulation.

We identified the tRNAs associated with the accumulated peptidyl-tRNAs with appropriate primers and reverse transcriptase–polymerase chain reaction (RT-PCR) (*15*). The peptidyl-tRNA accumulated with templates TnaCW25UGA (−/+Trp) and TnaCW25+12AA (−/+Trp) (Fig. 1A) was tRNA^{Pro}, not tRNA^{Trp} (Fig. 1B). Thus, the presence of Trp-tRNA^{Trp} in the ribosomal A site during attempted translation of tryptophan codon 25 inhibits elongation of TnaC beyond proline codon 24.

Inhibition of peptidyl transfer by Trp-tRNA^{Trp} depended on features of the nascent TnaC peptide; for example, replacing the W12 codon of *tnaC* with an arginine codon eliminated peptidyl-tRNA accumulation (Fig. 1A, R12W25UGA, R12W25+12AA). Moreover, Trp-tRNA^{Trp} did not promote peptidyl-tRNA accumulation when proline codon 24 was deleted (Fig. 1A, W24UGA). Because Trp-tRNA^{Trp} was also inactive with the WT template, in which codon 25 of *tnaC* is a stop codon, UGA (Fig. 1A, WT), it appears that a tryptophan codon is required at position 25 for Trp-tRNA^{Trp} to function as inducer. Introducing a phenylalanine (Fig. 1A, F25UGA) or a methionine (Fig. 1A, P24+12AA) codon at position 25 did not lead to peptidyl-tRNA accumulation, with or without added tryptophan. Excess phenylalanine was present in the reaction mixture. These findings suggest that after synthesis of TnaC-peptidyl-tRNA^{Pro} with W25UGA and W25+12AA templates, a tryptophan codon and Trp-tRNA^{Trp} must occupy the A site of the translating ribosome for Trp-tRNA^{Trp} to inhibit peptide elongation.

Addition of tryptophan to S-30 reaction mixtures with templates that contained a tryptophan codon at position 25 increased TnaC-peptidyl-tRNA^{Pro} accumulation slightly, as did addition of 1-methyltryptophan (1MT), an analog of tryptophan that is an effective inducer both in vivo and in vitro (Fig. 1A) (*15*). 1MT is at best poorly aminoacylated onto tRNA^{Trp} (*21*); therefore, uncharged 1MT must serve as inducer. Added tryptophan had little or no effect on TnaC-peptidyl-tRNA accumulation with templates F25UGA, P24+12AA, and P25W26P27UGA (Fig. 1A). These findings suggest that, although synthesis of TnaC-peptidyl-tRNA^{Pro} may create a tryptophan induction site in the translating ribosome, entry of a tRNA other than Trp-tRNA into the ribosomal A site can compete with tryptophan and prevent induction.

We determined the stability of accumulated TnaC-peptidyl-tRNA^{Pro} by measuring its decay after cessation of synthesis. We added kasuga-

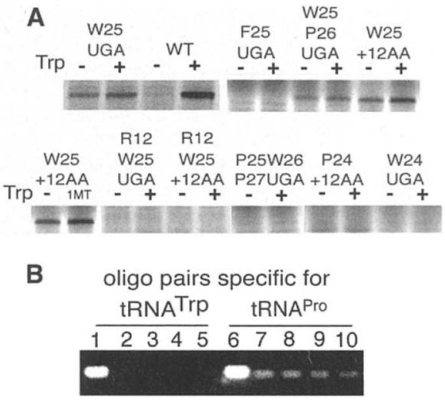

Fig. 1. (**A**) Trp-tRNA^{Trp}–induced TnaC-peptidyl-tRNA^{Pro} accumulation. S-30 reaction mixtures were programmed with circularized DNA templates bearing the changes indicated (*15*). Incubation was in the absence (−) or presence (+) of 1 mM tryptophan. +12AA templates encode a 12-residue segment (MHTQKPTLELLT) added to the COOH terminus of an existing peptide. Radiolabeled products were separated by SDS-PAGE (*15*). Accumulated [^{35}S]methionine-labeled peptidyl-tRNA is shown. (**B**) RT-PCR identification of the tRNA of the accumulated peptidyl-tRNA. Oligonucleotide (oligo) pairs specific for tRNA$_2^{Pro}$ and tRNA^{Trp} were used in RT-PCR, as indicated. Lanes 1 and 6, *E. coli* total tRNA was used as template (positive controls). The peptidyl-tRNA in (A) was recovered from each lane in the gel and used as RT-PCR template (*15*). Lanes 2 and 7, peptidyl-tRNA from reaction W25UGA +Trp was used as template; lanes 3 and 8, that from reaction W25UGA −Trp was used. Lanes 4 and 9, peptidyl-tRNA from reaction W25+12AA +Trp was used as template; lanes 5 and 10, that from reaction W25+12AA −Trp was used.

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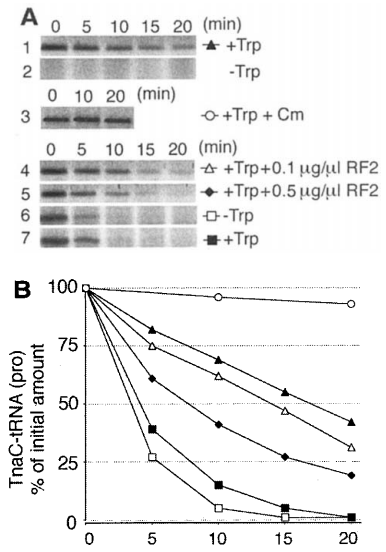

Fig. 2. (**A**) Decay of accumulated [^{35}S]methionine-labeled TnaC-tRNA^{Pro}. Rows 1 to 5: S-30 reaction mixtures ([^{35}S]methionine-labeled products) with the WT *tnaC* template were incubated without (row 2) or with (rows 1, 3, 4, and 5) 1 mM tryptophan at 37°C for 15 min, and then 1 mM unlabeled methionine and kasugamycin (200 μg/ml) were added. Reaction mixtures without added tryptophan were subjected to time course analysis directly. A reaction mixture with tryptophan was distributed into four tubes, and immediately H_2O (row 1, control), chloramphenicol (Cm 500 μg/ml, row 3), or RF2 (rows 4 and 5) was added. Time course samples were taken as indicated. Rows 6 and 7, an S-30 reaction mixture directed by the W25+12AA template was incubated at 37°C for 15 min without tryptophan. After addition of 1 mM unlabeled methionine and kasugamycin (200 μg/ml), one half of each reaction mixture was mixed with tryptophan (1 mM, row 6); the other half was mixed with the same volume of water (row 5). Time course samples were taken as indicated. (**B**) TnaC-peptidyl-tRNA^{Pro} decay curves based on the data shown in (A). No line is shown for row 2.

mycin to inhibit translation initiation, and we added unlabeled methionine to dilute the labeled methionine. We used WT or W25+12AA templates to direct peptide synthesis in S-30 preparations incubated with and without tryptophan (Fig. 2). With the WT template, we detected TnaC-peptidyl-tRNA^{Pro} accumulation only with added tryptophan; upon continued incubation, this peptidyl-tRNA disappeared (Fig. 2A, rows 1 and 2; Fig. 2B.) Addition of chloramphenicol to inhibit peptidyltransferase activity stabilized the peptidyl-tRNA (row 3), whereas adding increasing amounts of RF2 decreased its stability (Fig. 2A, rows 4 and 5; Fig. 2B). With the W25+12AA template, TnaC-peptidyl-tRNA^{Pro} was more labile (Fig. 2A, rows 6 and 7; Fig. 2B). Tryptophan addition may have increased the initial amount of this peptidyl-tRNA or delayed its decay slightly; we cannot distinguish between these possibilities.

Table 1. Features of the TnaC peptide required for cis action. Circularized DNA templates bearing the changes responsible for the amino acid alterations listed below were tested in S-30 reaction mixtures with or without tryptophan, as described in Fig. 1A, and the radiolabeled products were separated by SDS-PAGE and quantified (*15*). The amount of [^{35}S]methionine-labeled TnaC-tRNA indicated is relative to that obtained with a WT *tnaC* template in the presence of 1 mM Trp (+++++). Scoring ranged from no accumulation (−) to strong accumulation (+++++). Δ = deletion; Ins = insertion.

TnaC alteration	Accumulation of TnaC-tRNA^{Pro}	
	− Trp	+ Trp
WT	−	+++++
Crucial Trp12		
W12R	−	−
Alteration between M1 and W12		
N2F, I3S	−	+++++
T9I	−	++++
ΔN2I3L4	−	++++
ΔC7	−	+++++
ΔN2I3L4H5I6	−	+++
Ins after T9; A10	−	++++
Ins after T9; G10G11	−	+++
Ins after L4; H5H6H7H8H9	−	+++++
Ins after I6; N7I8L9H10I11	−	+++++
Ins after T9, H10I11C12V13T14	−	++++
Alteration between W12 and P24		
ΔK18	−	+
K18R	−	+++++
ΔV20D21	−	−
ΔH22	−	+
ΔP24	−	−
ΔR23P24	−	−
P24A	−	±
P24S	−	±
Ins after D21; A22	−	±
Ins after P24; A25	−	±
Ins after P24; W25	++	+++
Ins after P24; M25H26T27Q28K29P30 T31L32E33L34L35T36	−	±
Ins after P24; W25M26H27T28Q29K30 P31T32L33E34L35L36T37	++	+++
Ins after P24; R25P26	−	±
Ins after P24; V25D26H27R28P29	−	±

On the basis of these results and previous data (*14–16*), tryptophan residue 12 of TnaC appears to be essential for creating the tryptophan induction site in the translating ribosome. To further explore the features of TnaC required for induction, we introduced additional changes in TnaC templates (Table 1). The crucial role of W12 in permitting induction was confirmed; the W12R alteration eliminated induction. The NH_2-terminal portion of TnaC was relatively unimportant; additions, deletions, and replacements in this region had no significant effect on peptidyl-tRNA^{Pro} accumulation with added tryptophan. In contrast, features of the COOH-terminal portion of TnaC were essential. P24 could not be deleted or replaced (ΔP24, ΔR23P24, P24A, P24S); however, K18 could be replaced by arginine (K18R) without affecting tryptophan induction. The spacing between W12 and P24 appeared to be crucial; insertions, duplications, and deletions decreased or abolished the effect of added tryptophan. The presence of the stop codon UGA after COOH-terminal residue P24 gave the greatest sensitivity to added tryptophan. This observation is consistent with the conclusion that termination is a slower process than elongation (*22*); thus, RF2 may be less effective than most tRNAs in competing with tryptophan in the A site of the ribosome. These findings suggest that the translating ribosome must recognize the crucial residue W12 at a specific location in the exit tunnel for TnaC-peptidyl-tRNA^{Pro} to create an effective tryptophan induction site.

We believe nascent TnaC-peptidyl-tRNA^{Pro} creates an L-tryptophan–specific induction site in the ribosomal A site. Bound tryptophan apparently rearranges or displaces some element of the ribosome essential for catalysis of peptidyl transfer and peptidyl-tRNA cleavage. This conclusion is supported by our finding that Trp-tRNA^{Trp} inhibits aminoacyl-tRNA addition to TnaC-peptidyl-tRNA^{Pro}. Moreover, Trp-tRNA^{Trp} is active as an inducer at very low concentrations (~0.1 μM) relative to the tryptophan concentration required for induction (>100 μM) (*15*). The tryptophanyl moiety of bound Trp-tRNA^{Trp} presumably occupies the tryptophan induction site (*14*). However, it is likely that elon-

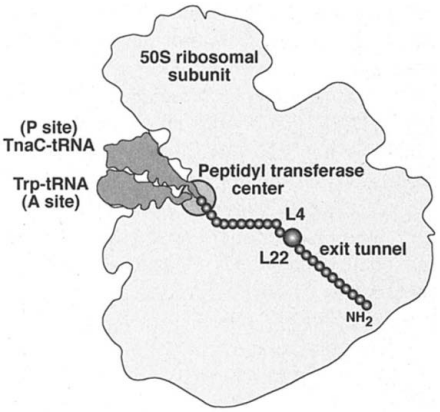

Fig. 3. Schematic representation of the 50S ribosomal subunit with TnaC-peptidyl-tRNA^{Pro} in the P site and a decoding Trp-tRNA^{Trp} in the A site. A segment of the peptidyl portion of TnaC-peptidyl-tRNA^{Pro} and the tryptophanyl moiety of Trp-tRNA^{Trp} are placed in the peptidyltransferase center. We assume the narrowest part of the exit tunnel formed with ribosomal proteins L4 and L22 (*1*, *28*, *30*) responds to the segment of TnaC containing the crucial residue W12 (enlarged circle) by altering features of the peptidyltransferase center, creating a tryptophan induction site.

gation factor Tu, guanosine triphosphate, and/or the associated tRNA^{Trp} contribute to Trp-tRNA^{Trp} binding in the ribosomal A site; this would account for the effectiveness of Trp-tRNA^{Trp} at lower concentrations than free tryptophan. The induction site could be a newly created site that specifically recognizes tryptophan. Alternatively, the peptidyltransferase center could be displaced or altered during synthesis of TnaC-peptidyl-tRNA^{Pro}; thus, when tryptophan enters the center, peptidyltransferase activity is inhibited. It is unlikely that the induction site is formed in the ribosomal D site, a newly identified site proposed to participate in the initial step in decoding (*23*). Our findings strongly suggest that information inherent in the sequence of the nascent TnaC peptide chain is communicated to the translating ribosome and that this information is used to mediate a response to tryptophan binding at the ribosomal A site. Peptide-ribosome interactions of this type could regulate the rate of peptide chain elongation, facilitate cotranslational protein folding (*24*), or, as in the *tna* operon, allow tryptophan to compete with a release factor and force ribosome stalling at a transcript site required for Rho factor binding.

Our findings and those of other investigators therefore attribute to the translating ribosome the ability to sense features of a nascent peptide and of responding by altering one or more events in ribosome action (Fig. 3). Inhibition of peptidyl-tRNA transfer or cleavage may also occur during translation of the uORF (upstream open reading frame) preceding an *arg* gene of fungi (*25*), the uORF2 preceding a gene of the human cytomegalovirus (*26*), and the uORF preceding the coding region for mammalian *S*-adenosylmethionine decarboxylase (*27*). Nascent peptides have also been described that inhibit translation elongation (*6*, *28*, *29*). These examples illustrate ribosomal versatility in mediating regulatory decisions.

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18. S-30 reaction conditions have been described (*15*). A circularized DNA fragment bearing the intact *tna* promoter and the *tnaC* coding region, followed by the *rpoC* terminator sequence, was the template (*15*). Mutations in the *tnaC* coding region were introduced by PCR.
19. This radiolabeled peptidyl-tRNA is ~25 kD judged by SDS–polyacrylamide gel electrophoresis (SDS-PAGE). Its position is distinct from that of the free peptide; it migrated comparably to TnaC-peptidyl-tRNA^{Pro} previously identified by ribonuclease treatment and RT-PCR (*15*). Labeled TnaC-peptidyl-tRNA^{Pro} is shown in Fig. 1B.
20. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Potential Regulatory Function of Human Dendritic Cells Expressing Indoleamine 2,3-Dioxygenase

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Antigen-presenting cells (APCs) can induce tolerance or immunity. We describe a subset of human APCs that express indoleamine 2,3-dioxygenase (IDO) and inhibit T cell proliferation in vitro. IDO-positive APCs constituted a discrete subset identified by coexpression of the cell-surface markers CD123 and CCR6. In the dendritic cell (DC) lineage, IDO-mediated suppressor activity was present in fully mature as well as immature CD123^{+} DCs. IDO^{+} DCs could also be readily detected in vivo, which suggests that these cells may represent a regulatory subset of APCs in humans.

Professional APCs, in particular DCs, are key regulators of the choice between tolerance and immunity (*1*). It has been proposed that immature or resting (steady state) DCs may present antigen in a tolerogenic fashion, whereas mature (activated) DCs drive T cell immunity. Immature DCs may promote tolerance in part by presenting antigens without the costimulatory signals required for full T cell activation (*2*). Alternatively, it has been hypothesized that a specialized subset of mature DCs might actively divert T cell responses toward tolerance (*3*). However, in humans no such subset has been defined, and the molecular mechanisms by which such cells might function remain unclear. We and others have recently shown that