GATA-3 transcriptional imprinting in Th2 lymphocytes: A mathematical model

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Immunological memory involves the fast recall of cytokine expression by T helper (Th) lymphocytes. Two distinct profiles of cytokine expression, Th1 and Th2, can be induced by antigen and polarizing signals during activation of naive Th cells and can subsequently be reexpressed on stimulation by antigen alone. The transcription factor GATA-3 induces Th2 development. GATA-3 is activated by the Th2-polarizing stimulus, IL-4, and has recently been observed to autoactivate its transcription. Based on these experimental data, we developed a mathematical model of GATA-3 expression that assumes independent activation of GATA-3 transcription by IL-4 and by GATA-3. Cooperativity of GATA-3 transcriptional activation is shown to create a threshold for autoactivation, resulting in the coexistence of two distinct GATA-3 expression states: a state of basal expression and a state of high expression sustained by autoactivation. Suprathreshold IL-4 signals induce a transition from basal to high GATA-3 expression. Thus, GATA-3 autoactivation creates a bistable system that can memorize a transient inductive signal. The model further predicts conditions under which the state of high GATA-3 expression can be abolished, which may extinguish the Th2 cytokine memory.

In a multitude of developmental processes, cell differentiation is initiated by inductive signals (1), and much current research focuses on the underlying genetic control networks. In the immune system, the differentiation of T helper (Th) lymphocytes plays a crucial role in mounting an effective immune response and in establishing immunological memory to a pathogen. Naive Th cells stimulated by antigen and polarizing signals can develop in effector cells with distinct cytokine profiles: Th1 cells express IFN-γ and thereby activate cell-mediated immune responses, whereas Th2 cells activate B cell proliferation and antibody production by expression of IL-4, IL-5, and IL-13 (2). Polarizing signals that induce Th1 and Th2 differentiation are IL-12 and IL-4, respectively (3). Differentiated Th1 and Th2 cells recall their cytokine profiles when stimulated by antigen alone and thus exhibit a "cytokine memory" (4, 5). Initially, Th1 cells can be reprogrammed in Th2 cells by Th2-polarizing conditions, and vice versa (5). This plasticity is lost when the cells are stimulated repeatedly under one type of polarizing conditions. An understanding of the molecular mechanisms underlying the induction and stabilization of cytokine memory is of prime interest for the design of therapeutic strategies for autoimmune disorders and allergy (5–7).

The transcription factor GATA-3 has been found to be pivotal for Th2 cytokine memory by inducing Th2 cytokine expression and inhibiting IFN-γ (8–11). In naive Th cells, GATA-3 occurs in low concentration. A slow and long-lasting up-regulation of its expression is induced by Th2-polarizing conditions [simultaneous IL-4 delivery and T cell receptor (TCR) stimulation by antigen; cf. ref. 12] (9). GATA-3 transcription is activated by Stat6 (signal transducer and activator of transcription 6; ref. 13), which itself is under the control of the IL-4 signal (14). As a further mode of regulation, Stat6-independent autoactivation of transcription has recently been discovered in cells retrovirally transduced with a GATA-3 gene. Expression of this gene triggered endogenous GATA-3 expression, with accumulation of considerably larger amounts of endogenous mRNA than transcript from the retroviral gene (15). In Th1-polarizing conditions (IL-12 and TCR stimulation), however, GATA-3 expression is inhibited (9).

Previously, mathematical models have successfully been used to study the complex cytokine interactions in Th cell populations (16–18). In the present paper, we use mathematical modeling to explore the genetic mechanisms of cytokine memory in Th2 cells. In the model, specific assumptions on the regulation of GATA-3 are made, and their consequences for the GATA-3 dynamics are evaluated. In this way, we aim to establish conditions under which autoactivation supports sustained GATA-3 expression as a basis of Th2 cytokine memory. In particular, we study which mechanisms prevent the triggering of autoactivation by the basal GATA-3 levels in naive Th cells, allow the induction of autoactivation by the Th2-polarizing signal IL-4, and may mediate suppression of autoactivation by signals inhibiting Th2 polarization.

Mathematical Modeling

Several regulatory mechanisms of GATA-3 activity have been identified at the transcriptional and posttranslational levels (ref. 5; Fig. 1*A*). Transcription of GATA-3 is enhanced by two transcription factors that are under the control of external signals: Stat6 (13) and NF-κB (19). NF-κB is activated in both Th1- and Th2-polarizing conditions through TCR stimulation. By contrast, Stat6 is stimulated by IL-4 through the IL-4 receptor and associated Janus kinases (14), and is thus differentially active in Th2 conditions. An internal regulatory loop is established by the autoactivation of GATA-3 transcription (15). A double GATA site in the first intron of the GATA-3 gene (20), in which a transcriptional activator is located (21), could be involved in a direct action of GATA-3 on its gene. At the posttranslational level, regulatory steps may include the modulation of GATA-3's transcriptional activity by phosphorylation (22), acetylation (23), and interaction with inhibitory proteins (24, 25). It is not clear from the outset which role these various processes play in the dynamics of GATA-3, and not all details of the underlying molecular mechanisms have yet been elucidated. We therefore propose a model that accounts for transcriptional regulation by external signals and autoactivation, and, in a simple but general fashion, accounts for the existence of a number (as yet unspecified) of posttranscriptional steps (Fig. 1*B*).

Starting with the synthesis of the primary transcript in the nucleus, R_1, we consider a series of conversions, including splicing, nuclear export of mRNA, etc., that lead, via intermediate mRNA forms, R_i ($i = 2, \ldots, m - 1$), to the functional mRNA associated with ribosomes, R_m. The translated GATA-3 polypeptide chain, G_1, is converted through modification and

Abbreviations: HE, high expression; LE, low expression; Stat6, signal transducer and activator of transcription 6; TCR, T cell receptor; Th, T helper.

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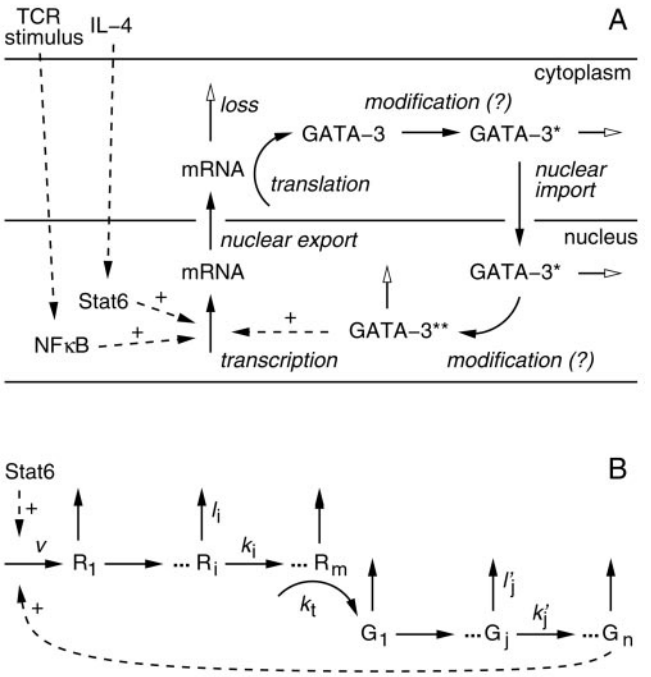

Fig. 1. Model of GATA-3 expression. (*A*) Basic molecular processes involved in the regulation of GATA-3. Solid and dashed arrows indicate reaction steps and regulatory interactions, respectively. Several modification steps (e.g., acetylation, phosphorylation) have been implicated by experimental studies, but their involvement in regulation of GATA-3's transcriptional activity remains to be fully defined. (*B*) Scheme of the mathematical model.

nuclear import steps, with associated protein intermediates G_j ($j = 2, \ldots, n-1$), into active transcription factor in the nucleus, G_n. Assuming that all species occur at sufficiently large concentrations to justify a deterministic description, the scheme of Fig. 1*B* translates into the kinetic equations

$$\frac{dR_1}{dt} = v(G_n, t) - (k_1 + l_1)R_1 \qquad [1]$$

$$\frac{dR_i}{dt} = k_{i-1}R_{i-1} - (k_i + l_i)R_i, \quad i = 2, \ldots, m \qquad [2]$$

$$\frac{dG_1}{dt} = k_t R_m - (k'_1 + l'_1)G_1 \qquad [3]$$

$$\frac{dG_j}{dt} = k'_{j-1}G_{j-1} - (k'_j + l'_j)G_j, \quad j = 2, \ldots n, \qquad [4]$$

where the concentrations of the mRNA and protein species are denoted by R_i and G_j, respectively. Linear rate laws were used for simplicity, assuming that the enzymatic steps are not saturated by their respective substrates (for a related model of biochemical pathways see, e.g., ref. 26). As more experimental data become available, a more detailed description of individual steps may become feasible. The rates of transcription and translation are v and $k_t R_m$, respectively. For GATA-3 mRNA and protein we distinguish between conversion reactions, with rate constants k_i and k'_j, and loss reactions, with rate constants l_i and l'_j. Loss is thought to occur primarily by degradation. Loss rate constants may be zero, except at the end points of the mRNA and protein conversion chains; R_m and G_n are degraded ($l_m > 0, l'_n > 0$) but not further converted ($k_m = k'_n = 0$).

To determine the rate of transcription v, the following assumptions are made. (*i*) In naive Th cells, the GATA-3 gene is transcribed with a small constant rate v_B. (*ii*) Transcription can be activated independently by Stat6 and GATA-3. For simplicity, we assume that mRNA synthesis proceeds with certain constant rates v_S and v_G when Stat6 and GATA-3, respectively, are bound to regulatory sites. [Further required transcription factors that are activated in both Th1 and Th2 conditions, such as NF-κB (19), are assumed to be present, without being considered explicitly.] The assumption of independent action of Stat6 and GATA-3 is corroborated by the observations of Stat6-independent autoactivation of GATA-3 (15) and of Stat6-induced GATA-3 expression in cells with inhibited autoactivation (25). (*iii*) For GATA-3 to activate its transcription, two GATA-3 molecules must bind to a regulatory site of the gene. As will become clear below, this assumption of cooperativity of GATA-3 action is of critical importance for the behavior of the system. For the IL-5 gene, cooperativity of GATA-3 transactivation has been observed (27).

The binding of GATA-3 and Stat6 to their regulatory sites in the GATA-3 gene is subject to thermal fluctuations, and, therefore, the occupancies of these sites are stochastic variables. However, if the fluctuations of binding and dissociation are much faster than the rate of transcription or the rates of the subsequent mRNA processing steps, one can show, by a rapid-equilibrium approximation (e.g., ref. 28), that the transcription rate is predominantly determined by the time-averaged occupancies of the binding sites. This simplifying assumption will be made. To be specific, binding of two GATA-3 monomers with the dissociation constant K_G is assumed, giving rise to the expression for the transcription rate $v(G_n, t) = v_B + v_S\varepsilon(t) + v_G G_n^2/(K_G + G_n)^2$, where G_n is the nuclear concentration of transcriptionally active GATA-3, and $\varepsilon(t)$ describes the as yet unspecified time course of binding of activated Stat6. The activity of Stat factors appears to be transient even in the continued presence of stimulating cytokine, which is likely to be due to cytokine receptor inactivation (29). This transient behavior is modeled by assuming Stat6 activation at time $t = 0$, followed by an exponential decay with characteristic time T,

$$\varepsilon(t) = \begin{cases} 0 & t < 0 \text{ (no signal)} \\ e^{-t/T} & t \geq 0 \text{ (signal with exponential decay).} \end{cases} \qquad [5]$$

Decay times measured for Stat1 are in the range of tens of minutes and will be used as guidance (29). An appropriate concentration scale is chosen by giving the experimentally unknown concentration values in units of the GATA-3 dissociation constant K_G: $R_i \to R_i/K_G$ and $G_j \to G_j/K_G$. Eq. **1** then becomes

$$\frac{dR_1}{dt} = k_B + k_S\varepsilon(t) + k_G\frac{G_n^2}{(1 + G_n)^2} - (k_1 + l_1)R_1, \qquad [6]$$

where $k_B = v_B/K_G$, $k_S = v_S/K_G$, and $k_G = v_G/K_G$ are first-order rate constants. Eq. **6** and Eqs. **2–4** constitute the model of GATA-3 dynamics in a single cell. In the following, we focus on the implications of the model analysis for Th1/Th2 differentiation; mathematical details can be found in *Supporting Text*, which is published as supporting information on the PNAS web site, www.pnas.org.

Results

GATA-3 Can Attain Two Stable Expression Levels. The GATA-3 transcription rate in Eq. **6** consists of the sum of the GATA-3-independent term, $k_B + k_S\varepsilon(t)$, and the GATA-3 autoactivation term. The GATA-3-independent term can be increased by the Th2-polarizing stimulus, IL-4, via the Stat6 pathway. How does GATA-3 expression respond to a Stat6 signal? Insight can be gained by focusing on the amplitude of the Stat6 signal and assuming its duration to be unlimited [experimentally, this could

be realized by a constitutively active form of Stat6 (13)]. For such a permanent signal, the system will approach a steady state in which the rates of generation and loss of each component balance. To be specific, we set $\varepsilon = 1$ in Eq. **6** and measure the Stat6 activity by k_S. We now calculate how GATA-3 expression in the steady state depends on k_S. Setting the left-hand sides of Eqs. **2**–**6** to zero, the concentration of the transcriptionally active form of GATA-3 in the nucleus, $\bar{G}_n$, is found by solving

$$\alpha + k_G \frac{\bar{G}_n^2}{(1 + \bar{G}_n)^2} = \kappa \bar{G}_n. \qquad [7]$$

The concentrations of the other variables are proportional to $\bar{G}_n$. The molecular processes enter via three parameters: the GATA-3-independent transcription rate made up of the sum of the basal rate and the Stat6 activity, $\alpha = k_B + k_S$; the rate constant of GATA-3 autoactivation k_G; and a combined first-order rate constant for the posttranscriptional steps $\kappa = (l_m l'_n/k_t) \Pi_{i=1}^{m-1}(1 + (l_i/k_i))\Pi_{i=1}^{n-1}(1 + (l'_i/k'_i))$. The parameter κ can be interpreted as an effective loss rate constant of the expression chain: it is increased by increasing the individual loss rate constants and by down-regulating the conversion steps of mRNA and protein.

The left-hand side of Eq. **7** expresses the GATA-3 transcription rate. Due to cooperative autoactivation, it is a sigmoidal function of GATA-3 concentration (Fig. 2*A*, sigmoidal solid, dashed, and dot-dashed lines, corresponding to different parameter values). The right-hand side is a measure of the overall loss rate of the expression chain downstream of transcription (Fig. 2*A*, straight line). At the steady states, both rates equal, corresponding to the intersection points of the sigmoidal line with the straight line. For the pair of solid lines, three steady states exist. The system can be either in a stable state of low GATA-3 expression (low-expression state, LE) or in a stable high-expression state (HE). The intermediate state (U) is unstable and will not be attained. In the LE state, the nuclear GATA-3 concentration is too low to induce significant autoactivation, whereas in the HE state autoactivation is "turned on." These states are alternatively available at a single value of Stat6 activity—a phenomenon referred to as bistability. When parameters are changed, either the LE state or the HE state can disappear. A sufficient increase in the Stat6 activity causes the LE state to vanish, whereas the HE state continues to exist (Fig. 2*A*, dashed line). Conversely, the HE can be made to disappear by lowering the rate constant of autoactivation k_G (Fig. 2*A*, dot-dashed line) and by an increase in the effective loss rate constant κ (not shown). This type of disappearance of a stable steady state is referred to as a saddle-node, or fold, bifurcation in dynamical systems theory. Bifurcation analysis allows us to determine the parameter regions for which only one expression state exists and for which the LE and HE states coexist (Fig. 2*B*; also see *Supporting Text*). Bistability requires the GATA-3 autoactivation rate constant to exceed a critical value, which is given, as a function of Stat6 activity, by the line marked with an asterisk in Fig. 2*B*.

When parameters are chosen such that the autoactivation rate constant exceeds the threshold for bistability, the response curve of nuclear GATA-3 to Stat6 activity has two separate branches, one for the LE and another for the HE states (Fig. 3, solid lines). This dose-response curve provides a rationale for the behavior of GATA-3 in naive and in Th2 cells. In naive Th cells, GATA-3 expression is low (9), and accordingly the naive Th state can be located at a basal value of the GATA-3-independent transcription rate on the LE branch (Fig. 3, open circle). The Th2-polarizing IL-4 signal induces Stat6 activity, which can increase the GATA-3-independent transcription rate above a threshold, beyond which the LE branch no longer exists. Then GATA-3 expression switches to the remaining HE state and autoactivation is turned on. GATA-3 expression remains in the HE state even when Stat6 activity subsides and the GATA-3-independent transcription rate returns to its basal value (Fig. 3, filled circle). Thus, the point of disappearance of the LE state defines a threshold for Th2 polarization by Stat6 (Fig. 3, polarization threshold).

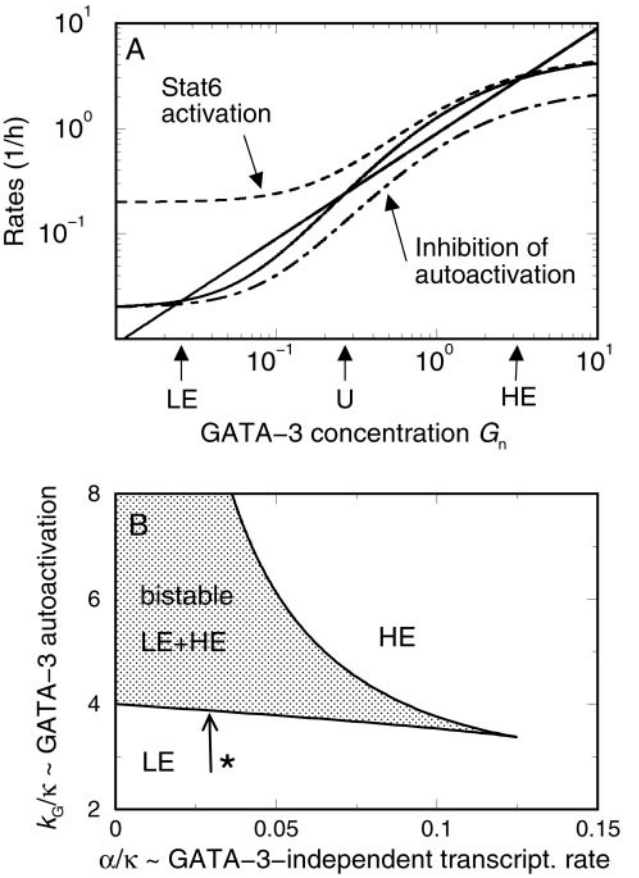

Fig. 2. Bistability of GATA-3 expression. (*A*) Transcription rate (sigmoidal curves) and combined loss rate (straight line) as functions of GATA-3 concentration. Solid sigmoidal line: no Stat6 activity; a stable LE state, a stable HE state, and an unstable steady state (U) coexist ($\alpha = 0.02$/h, $k_G = 5$/h, $\kappa = 1$/h). Stat6 activation ($\alpha = 0.5$/h) increases the transcription rate (dashed line) and the LE state disappears. Inhibition of autoactivation ($k_G = 2.5$/h, dot-dashed line) causes the HE state to vanish. (*B*) Regions of monostable behavior (only LE or HE state) and bistable behavior (LE and HE state). The line labeled with an asterisk gives the lower threshold value of k_G for bistability to exist.

The model further predicts that an LE to HE transition can also be triggered by the addition of a constant, "ectopic" GATA-3 expression term (results not shown). In the experimental report of GATA-3 autoactivation, this phenomenon has been observed in cells transduced with a GATA-3 gene (15).

Transient Stat6 Signals Can Trigger GATA-3 Autoactivation. The activity of Stat factors has been found to decay within tens of minutes (29), whereas GATA-3 expression is up-regulated under Th2 conditions over the course of several days (9, 10). To investigate whether short-lasting Stat6 signals can trigger autoactivation, the temporal behavior of the model was computed numerically. We model Stat6 stimuli by taking $\varepsilon(t)$ as in Eq. **5** with $T = 15$ min. For such transient signals, we again find a threshold behavior: sufficiently large Stat6 activity (Fig. 4, gray line) induces a switch from the LE to the HE state of GATA-3 expression (Fig. 4, thick lines), whereas subcritical Stat6 activity

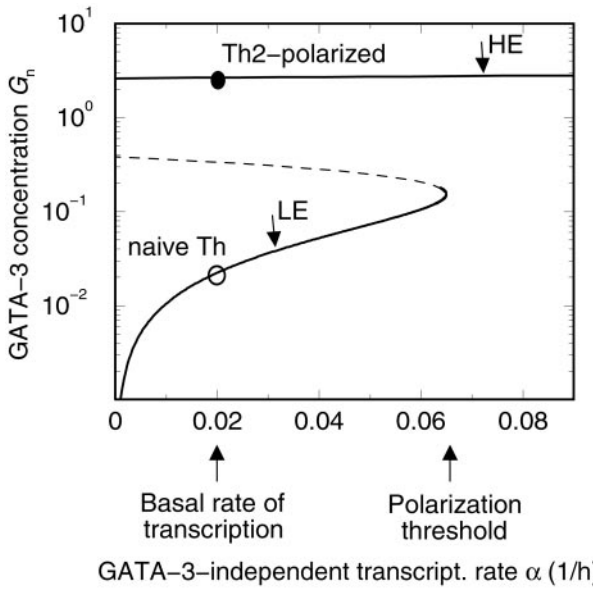

Fig. 3. Dose-response curve of nuclear GATA-3 vs. Stat6 activity. GATA-3 can be in the LE or HE state when Stat6 activity is below the polarization threshold (for completeness, the dashed line indicates the location of the unstable state; see Fig. 2*A*). A stable transition from the naive Th state to the Th2-polarized state can be induced by Stat6 activity raising the GATA-3-independent transcription rate, provided that autoactivation is sufficiently strong ($k_G/\kappa > 4$); here $k_G = 5/h$, $\kappa = 1/h$.

causes only transient GATA-3 expression (Fig. 4, thin lines). The threshold for Stat6 activity for the parameters of Fig. 4 is $k_S/k_G \approx 1.55$; that is, the activation of GATA-3 transcription by Stat6 and saturating GATA-3 concentration is predicted to be of similar magnitude.

In the simulations, the kinetic parameters were chosen such that the time course of GATA-3 agrees with the experimentally observed slow rise. As a consequence, the activation of GATA-3 expression by Stat6 and GATA-3 autoactivation appear as successive events during the LE to HE switch. The parameter

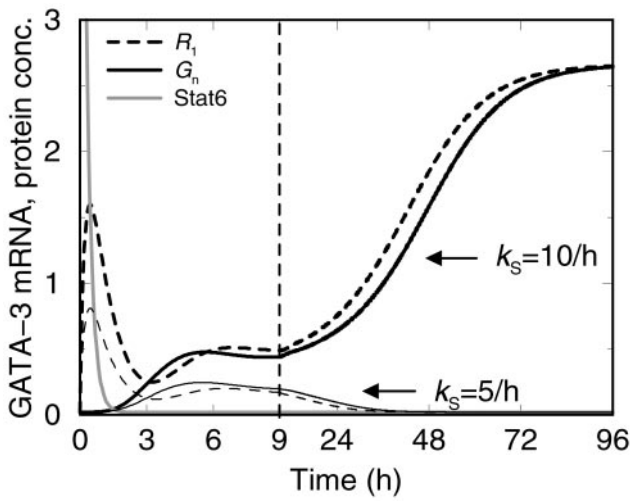

Fig. 4. Kinetics of GATA-3 expression after a transient Stat6 stimulus. Nascent transcript (R_1) and nuclear GATA-3 protein (G_n) are shown in the cases of Stat6 activity being supercritical ($k_S = 10/h$; Stat6, gray line; R_1, thick dashed line; G_n, thick solid line) and subcritical ($k_S = 5/h$; R_1 and G_n, thin dashed and solid lines, respectively). Other parameters: $m = n = 3$, $k_1 = k_2 = k_t = k'_1 = k'_2 = 1/h$, $l_3 = l'_3 = 1/h$, $l_1 = l_2 = l'_1 = l'_2 = 0$, $k_G = 5/h$, $T = 15$ min.

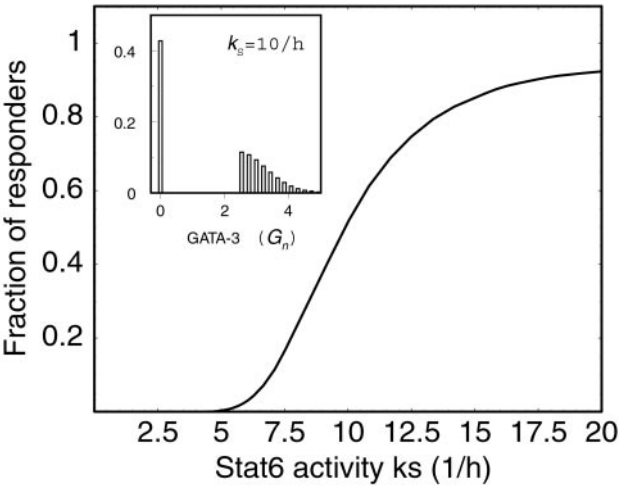

Fig. 5. Response of a cell population with heterogeneously distributed activation thresholds. A log-normal distribution function of the parameter k_G was assumed with mean 5/h and variance $0.5/h^2$. Cells are classified as responders when the Stat6 activity switches GATA-3 to the high-expression state. Inset: distribution of nuclear GATA-3 concentrations across the population at Stat6 activity $k_S = 10/h$. All other parameters as in Fig. 4.

choice results in a delay between the primary mRNA transcript (R_1) and the nuclear protein (G_n) of about 5 h (cf. Fig. 4, thick lines, and *Supporting Text*). Experimental values of the kinetic parameters are presently not known. However, expression delays in the range of several hours have been reported for other proteins (e.g., ref. 30).

Response of a Heterogeneous Cell Population. For an individual Th cell, there is a sharp polarization threshold for Stat6 activity, above which a switch to GATA-3 autoactivation takes place. Within a Th cell population, however, values of kinetic parameters can be expected to vary, such that cells may differ in their individual polarization thresholds. As an example of cellular heterogeneity, we considered a distribution of the GATA-3 autoactivation rate constant k_G in a population and calculated the corresponding polarization thresholds. From the knowledge of the k_G-distribution and the threshold values, the fraction of responding cells can be found for a given Stat6 activity. The resulting dose-response curve for the cell population is a smooth function of Stat6 activity; an example for specific parameters is given in Fig. 5. However, when GATA-3 values are recorded in individual cells, the frequency histogram conveys the bistable behavior, with a clear gap between low-expressing and high-expressing cells (Fig. 5 *Inset*). Experimental data suggest that, in Th2 populations induced with a saturating dose of IL-4 *in vitro*, GATA-3 rises homogeneously in the population (31).

Inhibition of GATA-3 Autoactivation. The basal GATA-3 expression seen in naive Th cells is inhibited in Th1-polarizing conditions (9), and it has also been hypothesized that suppression of GATA-3 expression in short-term Th2-polarized cells can occur in Th1-polarizing conditions (5, 32). The model shows that a decrease in the parameter combination k_G/κ below a threshold (Fig. 2*B*, line marked by asterisk) abolishes the HE state. Under this condition, elevated GATA-3 expression cannot be sustained in the absence of Stat6 activity. The autoactivation rate constant, k_G, will be lowered by repressors of the transactivating capacity of GATA-3. Such a repressor may be the GATA-interacting protein, friend of GATA-1 (FOG-1). FOG-1 has been shown to inhibit the induction of GATA-3 autoactivation mediated by retroviral GATA-3 transduction, but not the induction of

GATA-3 expression by Stat6 (25). Moreover, factors that do not directly act on GATA-3 transcription but inhibit translation and modification steps of GATA-3 [e.g., phosphorylation and acetylation (22, 23)], or activate its degradation, will increase the effective loss rate constant, κ, and can therefore abolish the HE state.

Discussion

The mathematical model of GATA-3 regulation developed here shows that cooperative autoactivation of transcription results in the coexistence of two distinct expression states of GATA-3. This result naturally accounts for the observation of low expression in naive Th cells and sustained high expression in Th2 cells. A threshold for the Th2-polarizing signal, IL-4 acting through the Stat6 pathway, is predicted. If the signal exceeds this polarization threshold, a switch from low to high GATA-3 expression is induced. In this fashion, a transient polarizing signal can be memorized by elevated GATA-3 expression. Given the key role of GATA-3 in Th2 cytokine expression, this process can be viewed as transcriptional imprinting for Th2 differentiation.

The regulatory features of the model that allow the triggering of sustained GATA-3 expression by a transient inductive signal are as follows: (*i*) cooperative autoactivation of transcription, and (*ii*) independent up-regulation of transcription by the inductive signal and by autoactivation. Cooperativity results from the assumption that binding of two GATA-3 molecules is required for transcriptional activation, yielding the sigmoidal shape of the GATA-3 synthesis rate as a function of GATA-3 concentration. The sigmoidicity implies that the effect of an increase in GATA-3 concentration on autoactivation is large at intermediate concentration values (for which the slope of the sigmoidal curve is large; see solid sigmoidal line in Fig. 2*A*), whereas it is comparatively small at the basal values of GATA-3 concentration found in naive Th cells. This behavior results in a threshold for self-amplification of GATA-3 expression and in bistability.

Feature *ii* implies two regulatory properties. First, the parameters of the Stat6 pathway determine the susceptibility of the system for Th2-polarizing conditions, without interfering with autoactivation. This prediction agrees with the experimentally observed autoactivation by GATA-3 transduction in Stat6-deficient cells (15). Second, the model suggests that repressors of GATA-3 transcriptional activity may play a role in regulating the threshold for inducing autoactivation by a transient inductive signal. Such repressors may be repressor of GATA (ROG) and friend of GATA-1 (FOG-1) (24, 25).

In the model, the IL-4 signal is considered as an input, reflecting the set-up of *in vitro* experiments. *In vivo*, IL-4 secreted by the Th cells might participate in Th2 differentiation. However, our analysis demonstrates that Th2 polarization through GATA-3 elevation can be based on positive feedback in an intracellular transcription factor network, and can thus be achieved without autocrine or paracrine IL-4 feedback. It is an interesting open question whether feedback of extracellular IL-4 interacts with these intracellular dynamics. Its study may benefit from combining the modeling approach presented here with the modeling of cytokine-mediated cell interactions, as carried out in refs. 16–18.

GATA-3 is likely to be part of a larger regulatory network of Th1/Th2 differentiation. It has been suggested that signal pathways and/or transcription factors involved in Th1-differentiation, such as the Th1-specific transcription factor T-bet, may inhibit GATA-3 expression (4, 5, 33). Although the molecular mechanisms are not yet clear, the model shows that the HE state of GATA-3 can be abolished by sufficiently strong inhibition of autoactivation. Inhibition can be achieved by repressing the transactivating capacity of GATA-3 and by increasing the effective loss rate constant along the expression chain κ (e.g., by inhibiting putative modification steps of the GATA-3 protein). This result clearly indicates that the GATA-3 expression loop does not merely function as a Stat6-induced on-switch but may also be susceptible to adverse signals that down-regulate GATA-3 expression. Such negative regulation could be involved in the reprogramming of Th2-polarized cells in Th1-polarized cells observed in Th1-polarizing conditions (5).

Autoactivating transcription factors have previously been suggested to participate in lineage commitment (34). Besides GATA-3, several such factors have been identified, among them GATA-1, Pit-1, and MyoD (35–37). The principle mechanisms identified here for GATA-3 could also govern the regulation of other autoactivating transcription factors involved in inductive signaling processes.

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