

assumed, but instead represents a conserved genetic program of immune cell development.

Although there is still a remote possibility that both highly purified CLP and CMP populations contain a small portion of unrecognized pDCs progenitors, it appears likely that pDCs represent a unique hematopoietic lineage, whose development is much more flexible than both conventional lymphoid (B, T, NK) and myeloid (monocyte and granulocytes) cells. Further delineation of how differentiation of this important immune cell is regulated will provide new insights into both the basic biology of pDC or IPC and may also assist in developing their therapeutic potential.

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## Bidirectional Promoters Regulate the Monoallelically Expressed Ly49 NK Receptors

**Members of the Ly49 gene family of natural killer (NK) cell receptors in mice are expressed in seemingly stochastic combinations such that each NK cell expresses a handful of family members. A transcriptional switch appears to establish this interesting pattern of expression.**

The purpose of natural killer (NK) cells is to attack cells displaying abnormalities including infection or transformation. The decision as to whether to attack a potential target cell is made based on the balance of activation of stimulatory and inhibitory receptors. Individual inhibitory receptors are specific for subsets of the highly polymorphic MHC class 1 molecules (Raulet et al., 2001).

The Ly49 family in mice is a family of more than 10 distinct NK cell activating and inhibitory receptors. In humans, the functions of the Ly49 family are subserved by a distinct family, the *KIR* genes, which have an immunoglobulin-like structure instead of the C-type lectin structure of the *Ly49* genes. The expression of a seemingly randomly chosen handful of the Ly49 family members serves to define each NK cell (Raulet et al., 2001).

The different Ly49 gene family members appear to be regulated largely independently, such that the frequency of cells expressing any given pair (or larger group) is approximated by multiplying the respective frequencies of expression of the individual genes; this pattern of expression has been termed the product rule. Another important observation about the regulation of these genes is that they are subject to random monoallelic expression (Held et al., 1995), similar to the regulation of odorant receptor genes (Chess et al., 1994) and interleukin genes (Bix and Locksley, 1998; Hollander et al., 1998; Rhoades et al., 2000). Random monoallelic expression is of particular interest because it indicates that identical sequences (the two alleles of a given gene) present in the same nucleus may be regulated independently.

Two recent papers have recently examined the expression of *Ly49* genes in vivo and in vitro. Tanamachi et al. (2004) used transgenes to recapitulate the variegated expression of *Ly49a*: of eight *Ly49a* transgenic founder lines, six showed variegated expression similar to the endogenous *Ly49* genes. A striking aspect of these transgenic analyses is that they reveal independent regulation of the transgene and the endogenous *Ly49a* alleles. Normally, a properly functioning transgene is expressed in the exact same cells as express the endogenous alleles of that gene. The normal situation reflects the fact that the transgene is exposed to the same transcription factor milieu as the endogenous alleles. Given

the monoallelic expression of the endogenous *Ly49* alleles, it is perhaps not a total surprise then to observe independent regulation of the *Ly49* transgenes and the endogenous alleles. Transgenes containing odorant receptor genes provide other examples in which properly functioning transgenes are expressed in cells distinct from those expressing the endogenous alleles (Serizawa et al., 2000). The unusual finding of independent regulation of the expression of the *Ly49a* transgene provides support for models of *Ly49* gene regulation that involve either competition for limiting transcription factors or some other mechanism that stochastically renders certain copies of the gene transcriptionally on and other (identical or similar) copies off. This type of model was also suggested by the earlier work demonstrating that *Ly49* genes are expressed monoallelically in most cells (from either the maternal or paternal allele) and biallelically in some.

Similar to observations of the endogenous *Ly49a* gene, the *Ly49a* transgenes are regulated by the interaction of other *Ly49* receptors with MHC class I. Another interesting observation was that the transgenes are expressed in all B cells, which is a cell type where the endogenous *Ly49* genes are not expressed. This suggests that the variegated expression is something actively imposed upon the *Ly49a* gene in NK cells and, that with the aberrant expression in B cells, there is no mechanism to dictate variegated expression.

A manuscript by Saleh et al. in this issue of *Immunity* uncovers another very interesting aspect to the regulation of *Ly49* genes. An upstream promoter, Pro1, which is active early in development, appears to be a bidirectional promoter. Note that this Pro1 promoter is within a region of the *Ly49a* transgene that was shown to be essential for proper expression (Tanamachi et al., 2004). Earlier work analyzed only forward transcription from Pro1 and it was labeled as an early promoter present upstream of many *Ly49* genes, whose function was superseded by the Pro2 promoter later in development (Saleh et al., 2002). It appears that a given Pro1 promoter can either be transcribing in one direction or the other direction, but not in both directions simultaneously; Pro 1 can therefore be seen as a bidirectional switch reminiscent of the famous switch that regulates  $\lambda$  phage genes (Johnson et al., 1981).

Saleh et al. were able to visualize cells transcribing *Ly49* genes in one or the other direction by using a transfection experiment that examined integrations of a single copy of a reporter construct. The construct was designed such that forward transcription turned on destabilized yellow fluorescent protein (YFP) and reverse orientation transcription activated destabilized cyan fluorescent protein (CFP). Analyses of individual cells suggest that a Pro1 promoter transcribing in a given direction is stable in maintaining that direction until DNA replication. Upon DNA replication, it appears that switching can occur; for example, a single CFP-expressing cell can give rise to two cells, one of which expresses YFP. Notably, in cells that will give rise to daughter cells expressing different markers, both colors can be seen in the parental cell after DNA replication but prior to cell division. The details of how this switching occurs are not known and whether or not the two prod-

ucts of DNA replication have the same potential to switch their expression has not been assessed. Saleh et al. also performed extensive characterization of the portions of Pro1 required for forward versus reverse transcription and defined some of the transcription factors that bind these sequences and can influence the balance between forward and reverse transcription.

How is a Pro1 promoter able to switch on and off expression of a given *Ly49* gene in mature cells if it is only active (in either direction) in immature cells? If Pro1 is transcribing toward the *Ly49* gene, this appears to inhibit the formation of a repressed chromatin state on the Pro2 site. Thus, when the appropriate transcription factors become available in a mature NK cell, Pro2 sites that have been spared from repression can begin to drive expression of the given *Ly49* gene. In this manner, the direction of Pro1 promoter driven transcription in the immature cells, immediately prior to maturation, can dictate whether or not a given *Ly49* gene becomes transcriptionally active. By having a switchable Pro1 promoter regulate the nonswitchable Pro2 promoter, the system allows for stability in the maintenance of the *Ly49* expression state. It is readily apparent how such a mechanism would lead to independent regulation of individual *Ly49* genes and indeed of the maternal and paternal copies of a given *Ly49* gene. Thus, this model fits nicely with the observed monoallelic and biallelic expression patterns and random nature of the process. It will certainly be interesting to see if similar bidirectional promoters are involved in the regulation of other genes involved in the specification of individual cell identity, or in the regulation of genes with important roles in development or plasticity.

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