

BIOM 555 Reviews

Daniel F. Simola

22 May, 2005

Summary

1. SWI-SNF
2. TFs and hematopoietic development
3. globin switching, LCR

Common theme: sequence analysis

- determine function of sequence: mutate/delete/insert sequence
- to determine binding: ChIP, DNase digest, restriction digest, 2° structure blocking, homologous recombination, cre-lox, etc
- determine binding protein effects: change concentration, alter binding specificity, remove protein (rna or protein transfections)

SWI-SNF Complex

- large complex of 18 proteins
- SWI-SNF complex alters nucleosome conformation, yielding accessible DNA for TF binding
 - associates with RNA pol II
 - creates sizable opening of DNA with the help of histone acetyl transferases (HAT), allowing transcription
- ATP dependent: antagonizes chromatin-mediated transcriptional repression
- SNF - required for DNA methylation
- Many factors interact with SWI-SNF to regulate transcription (to achieve full functioning)
 - glucocorticoid receptor (GR)
 - methyl CpG binding protein (MeCP2) (chromatin remodeling / transcriptional silencing due to methylation)
 - BRCA1 (mutant BRCA affects transcription)
 - SWI-SNF contain bromodomains for protein protein interactions and nuclear localization

Transcription Factors and Hematopoietic Development

1. red blood cells and globin genes (hematopoiesis)
 - globin - globular protein (cf fibrous protein, eg keratin)
 - typically soluble, functionally either enzymes or molecular transporters
 - hemoglobin - iron carrying, O₂ transporter protein in RBC
 - many globin genes differentially transcribed during development using LCR (,) and silencing factors
 - other hemoproteins and globins
 - transport: hemoglobin, myoglobin, neuroglobin, cytoglobin and leghemoglobin

- catalysis: peroxidase
- active membrane transport: cytochromes
- electron transfer: cytochrome c

Hematopoiesis

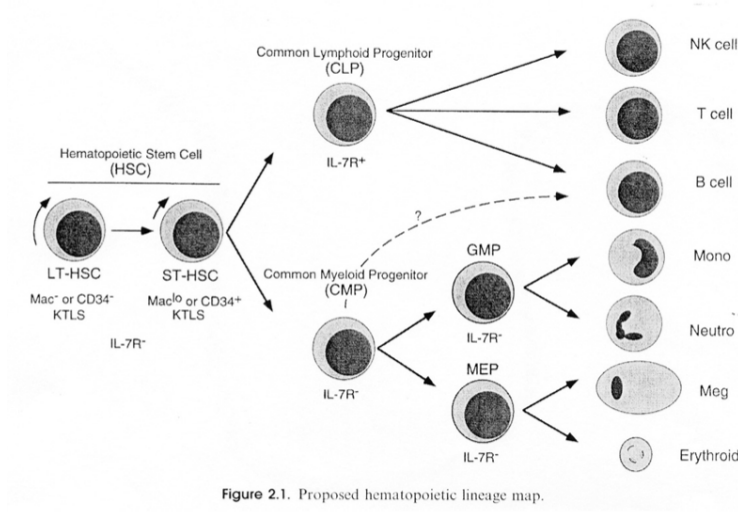


Figure 2.1. Proposed hematopoietic lineage map.

2. Hematopoiesis

RBCs express globins

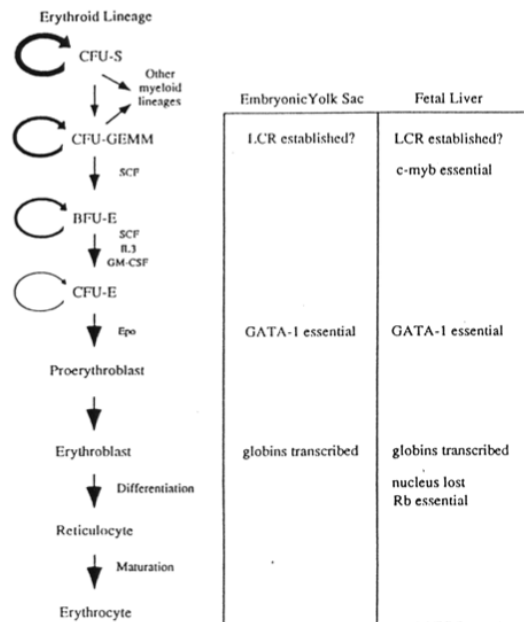


Figure 1. Schematic representation of progenitor cell differentiation and proliferation in the erythroid lineage and the stages at which specific nuclear factors are thought to be important.

3. Globin Expression

4. erythroid differentiation factors

- GATA-1 - factor essential for MEP erythroid differentiation of erythroid cells and megakaryocytes from precursor
- PU.1 - factor essential for lymphoid and myeloid lineages (monocyte/macrophage, neutrophil, T cell, B cell)

- antagonism - lack of one TF influences precursor to become other cell type
- How to regulate level of PU.1 expression to determine that different levels of PU.1 in progenitor cells yields different lymphocyte cells? Homozygote versus heterozygote PU.1?

Locus Control Regions (LCR) and Globin Switching

1. properties of LCR
 - cis-acting element
 - confers high expression
 - regulated by tissue-specific and condition-specific TFs
 - site-of-integration independent (even in heterochromatin) (ie opens chromatin - HAT)
 - works ectopically
 - copy-number dependent
 - early S phase replication
2. finding an LCR (hypersensitive site mapping)
 - hypersensitive sites indicate open chromatin/promoter region
 - purify nucleus of cell
 - DNase I protection digest at varying conc. or time - HS sites will be cut (HS because easily cut in low DNase conc.)
 - extract protein
 - restriction enzyme cleavage to map region of interest (will now cut protein covered regions)
 - Southern blot DNA - run gel at each DNase stage, transfer to filter, probe/blot with HOT probe
3. determine effect of globin expression and order of gene importance in LCR knockouts/deletions/insertions
 - determine effects from TF binding, other required factors, etc
 - some genes expressed at low levels without LCR region (, ,)
 - LCR not required for expression, but for high-level polymerase elongation
4. determine which portions of upstream region confer LCR function
 - Hybrid transformants / gene targeting / chimera (site-specific integration)
 - use stably integrated plasmid/cosmid/YAC/BAC
 - use homologous recombination or cre-recombinase to insert
 - targeting of various TFBS in LCR for insertion/deletion
 - insert contains selectable marker for verification, and is flanked by homologous sites to genomic DNA
 - transgenic mice (random integration) to look at tissue specificity
 - stable integration anywhere, even in heterochromatin, although possible effects include
 - * position effect variegation - HS site deletion in heterochromatin may cause LCR gene repression
 - * cell or stage specific extinction of global expression - due to specific TF/regulatory elements
 - NB LCRs do not work in transient transfections
5. what is LCR currently expressing (S1 nuclease protection assays)
 - expression levels of beta-globin genes throughout murine development
 - plot protection levels of genes over time - indicates gene competition for LCR
6. identify proteins binding to regulatory regions in LCR (ChIP)
7. 3 LCR models
 - looping - LCR loops over to targeted gene region
 - tracking - intergenic transcripts made as LCR protein complex traverses upstream DNA until it reaches target
 - facilitated tracking - looping and intergenic transcripts