

Conserved through the Ages: GATA-1 and Friend of GATA-1 (FOG-1) Interactions with DNA

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Abstracts

There are six GATA proteins involved in a variety of developmental processes. GATA and FOG are zinc finger proteins often associated with DNA binding activities.

GATA proteins are highly conserved and recognize the 'GATA' sequence in DNA. Direct interaction between the transcription factor GATA-1 and cofactor FOG-1 is crucial for erythroid and megakaryocyte development.

GATA-1 binds to DNA in the promoter and enhancer regions of all erythroid and megakaryocyte specific genes. These interactions ensure normal erythropoiesis and are involved in the later stages of megakaryopoiesis.

Without these interactions, multiple blood-related diseases result

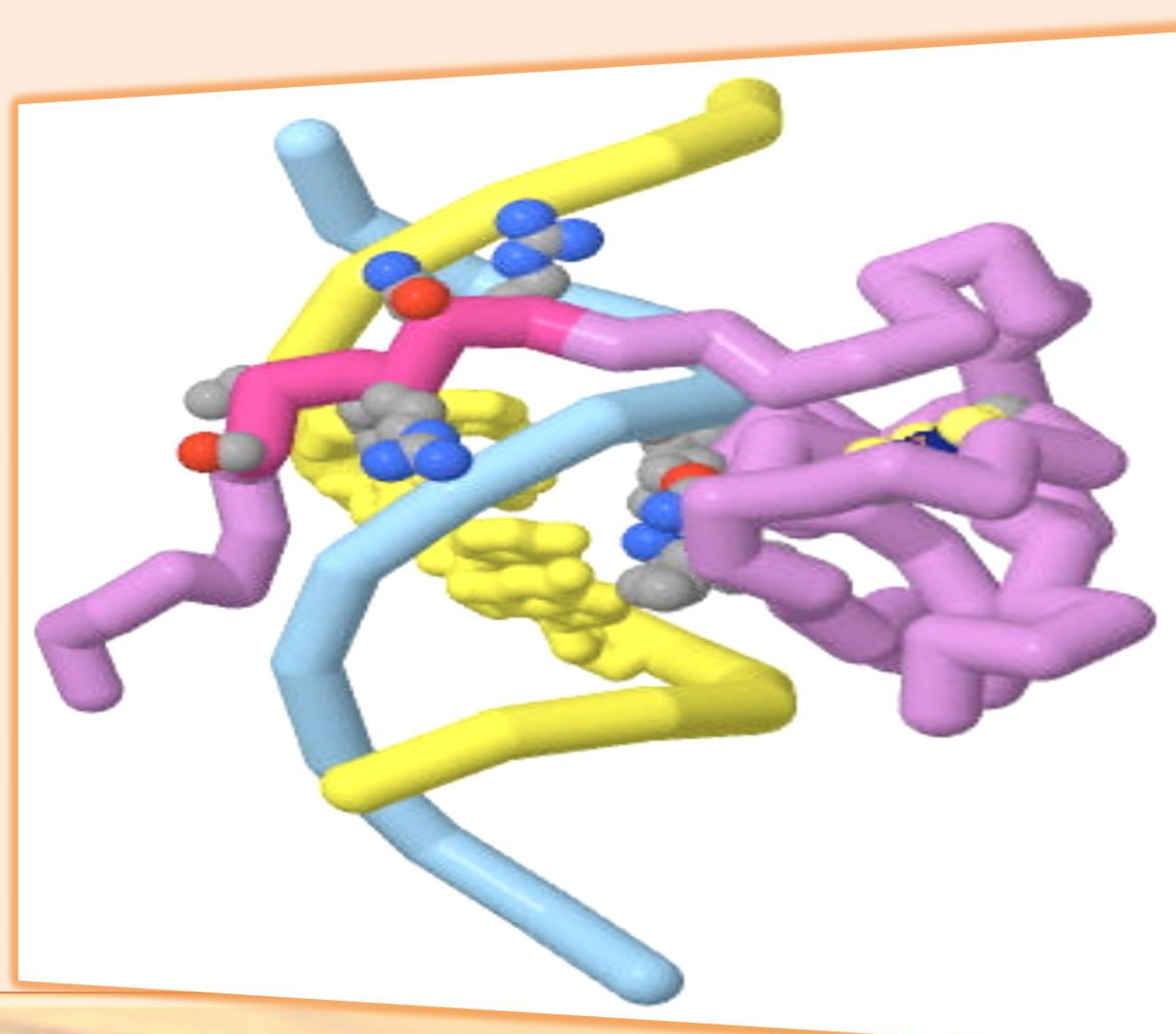
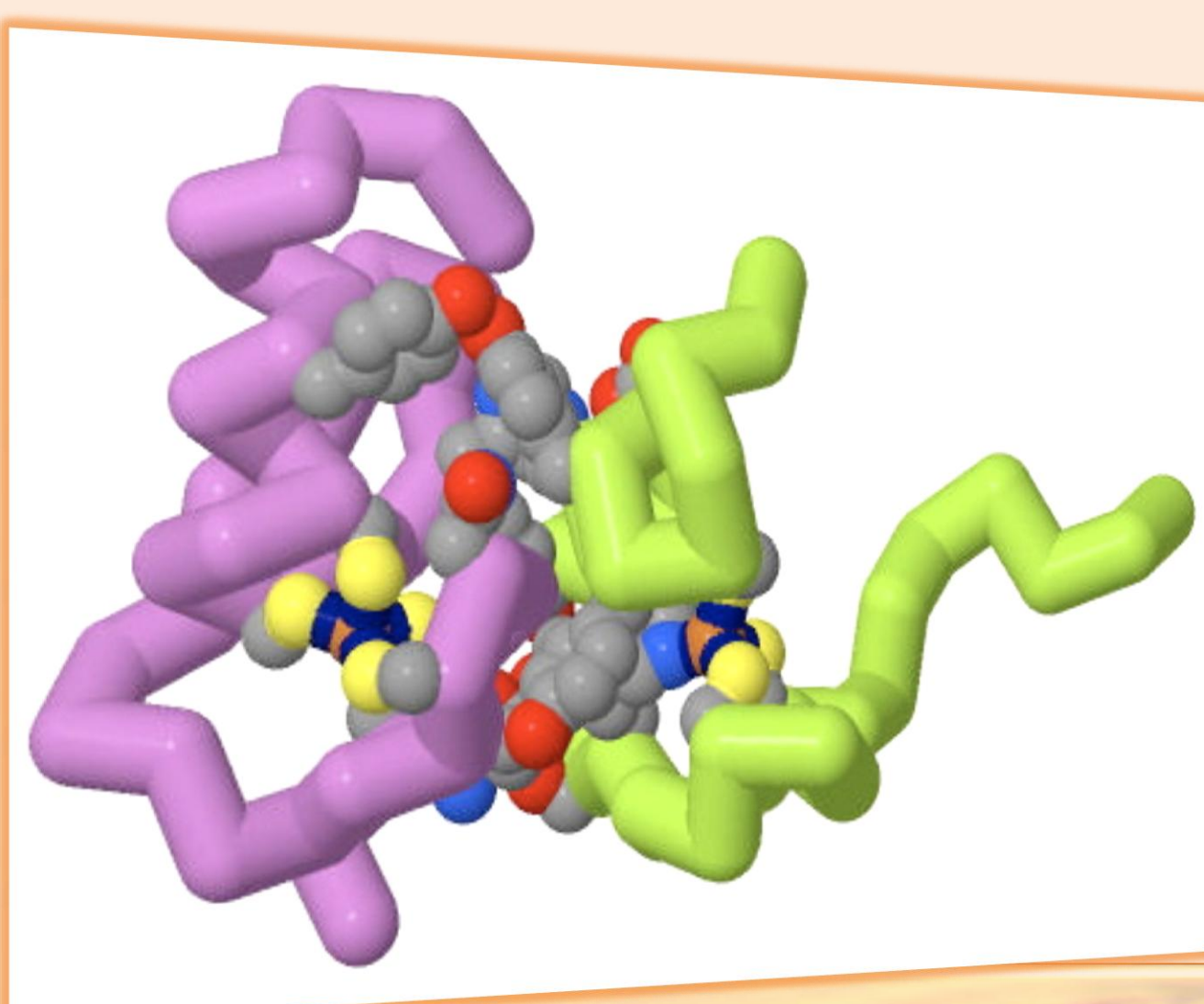
The C-terminal zinc finger of GATA-1 interacts with "GATA" sequence on the minor groove of the DNA. An additional factor, FOG-1 is needed to allow transcription by binding the N-terminal zinc finger of GATA-1, which then interacts with the major groove to help stabilize the DNA.

These interactions ensure normal erythropoiesis and are involved in the later stages of megakaryopoiesis by insuring that transcription of the correct DNA sequences occurs at the appointed time.

Because of the highly conserved nature of the proteins, determining the interactions of GATA-1 and FOG-1 may lead to clarification of other GATA-FOG relationships

Citations:
Cantor, A.B., et al. (2002). Distinct Domains of the GATA-1 Cofactor FOG-1 Differentially Influence Erythroid versus Megakaryocytic Maturation. *Mol. Cell Biol.* 22 (12): 4268-4279.
Liew, C.K., et al. (2005). Zinc fingers as protein recognition motifs: structural basis for the GATA-1 friend of GATA interaction. *Proc.Natl.Acad.Sci.U.S.A.* 102 (3): 583-588.

Citations:
Tjandra, N. Omichinski, J.G., Gronenborn, A.M., Clore, G.M. (1991) Solution structure of the C-terminal domain of Chicken GATA-1 bound to DNA. *NMR, regularized mean structure.* *Nat.Struct.Biol.* 4: 732-738.
Vonderfecht, T.R., et al. (2008). Substitutions of DNA contacting amino acids with functional variants in GATA-1 zinc finger: A structurally and phylogenetically guided mutagenesis. *Biochem. Biophys. Res. Comm.* 269 (4) 1052-1056.

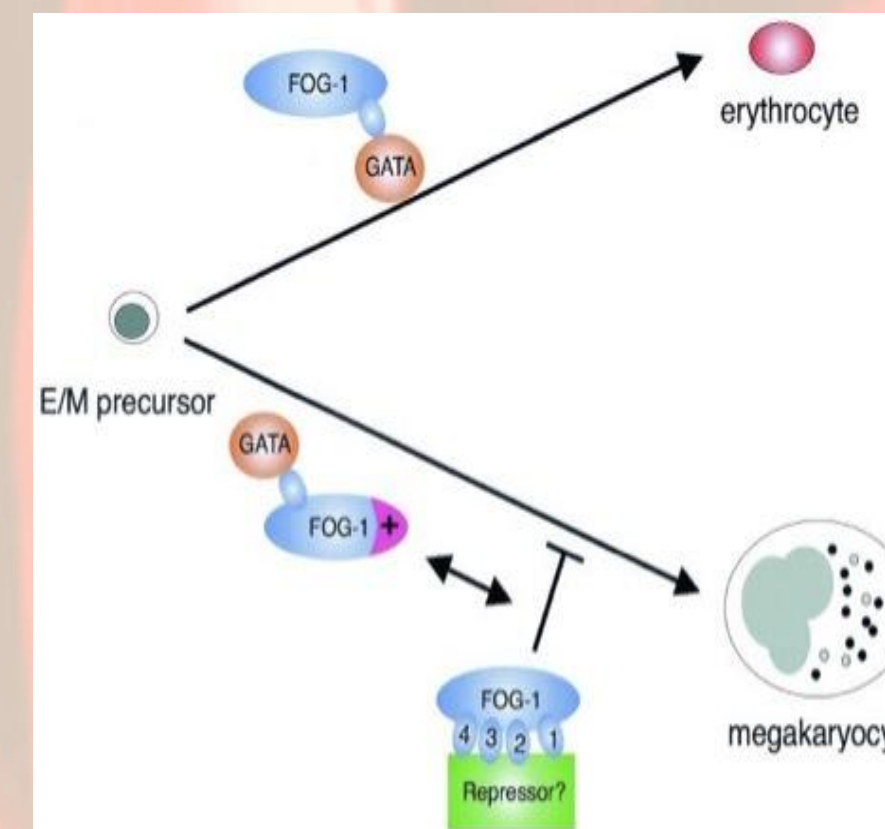


I. Introduction

Proteins that contain zinc fingers constitute about 2-3% of the proteins encoded by the human genome. These proteins have a specific affinity and bind to other proteins, DNA, RNA, or lipids. Differentiation of hematopoietic stem cells to generate mature blood cell types requires the transcription of lineage-specific genes. One important transcription factor directing hematopoiesis is GATA-1. Together with another zinc finger protein, Friend of GATA-1 (FOG-1), they regulate transcription. Both GATA-1 and FOG-1 are known to be essential for normal embryonic erythrocyte and megakaryocyte development. Mutation of GATA-1/FOG-1 genes can lead to human blood related diseases.

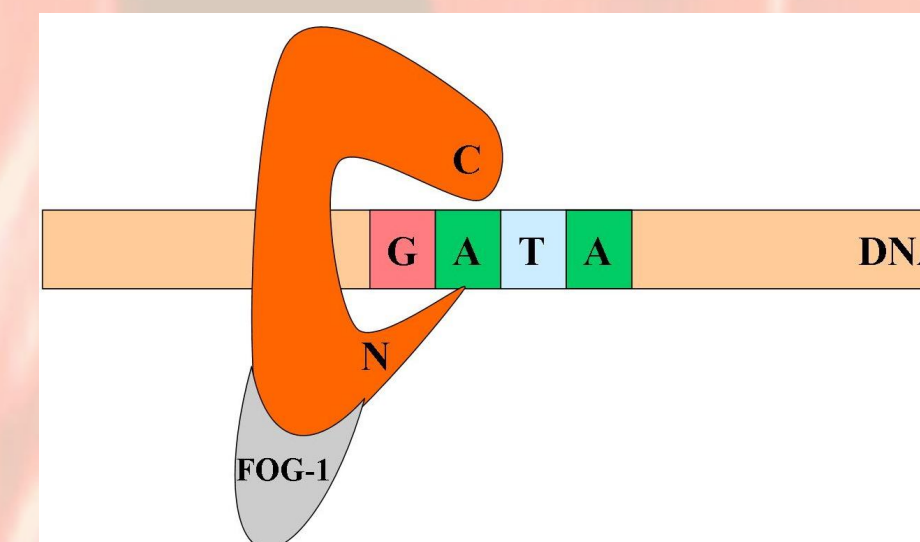
II. Story

Hematopoietic stem cells differentiate into multipotential progenitor stem cells and finally into specific cell lineages. This process is controlled by transcription factors activating or repressing the expression of specific genes.



Distinct Domains of the GATA-1 Cofactor FOG-1 Differentially Influence Erythroid

GATA-1, with cofactor FOG-1, physically interact with the DNA to allow transcription. The N-terminal zinc finger of GATA-1, which stabilizes the DNA molecule during transcription, interacts with zinc fingers 1, 5, 6, and 9 of FOG-1. The specific interactions are between amino acid residues on both proteins and are hydrophobic and polar in nature.



FOG-1 binds to GATA-1 before they will together interact with the DNA (Vakoc, 2005). The C-terminal zinc finger of GATA-1 binds to the DNA in the minor groove at the (T/A)GATA(A/G) motif, causing the DNA to bend to about a 15 degree angle, opening the double helix and allowing RNA polymerase to bind. This then results in transcription of the genes encoding proteins needed for the development of erythrocytes and megakaryocytes (Morceau et. al. 2004; Molkenin, 2000). To aid in the DNA binding, the N-terminus zinc finger of GATA-1 binds to the major groove of the DNA thus stabilizing the GATA-1 and DNA interactions. (Morceau et. al. 2004; Molkenin, 2000)

The entire GATA family is highly conserved in the zinc finger regions across many species. Without these transcription factors, normal hematopoiesis cannot occur. Dr. Battle is interested in the interactions of GATA-4 and FOG-1 and their role in jejunal and duodenal differentiation. Because GATA-4's structure has not been determined, the model is of GATA-1, which is likely very similar.

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III. How do we Know?

Yeast two-hybrid analysis was used to identify proteins capable of interacting with GATA-1. The amino-terminal GATA-1 zinc finger domain was inserted into a yeast two-hybrid bait vector resulting in a plasmid encoding the GATA-1 zinc finger domain inframe with the GAL4 DNA binding domain. This plasmid was transformed into yeast along with the prey cDNA library generated from a mouse blood cell line. The cDNAs are cloned inframe with the GAL4 transcriptional activation domain. If a protein encoded by the prey plasmid interacts with GATA-1, this protein-protein interaction will bring the GAL4 DNA binding domain into complex with its transcriptional activation domain allowing for transcription of reporter genes. A color will show whether or not transcription has occurred.

IV. What's the Next Question?

1. To define the entire cascade of events that occurs between GATA-1, FOG-1, other proteins, and transcription factors that either inhibit or activate the genes that GATA-1 controls
2. Relate the knowledge of GATA-1 to other interactions performed by other GATA family members

Citation for story
Molkenin, J.D. (2000). The zinc finger containing transcription factors of GATA-4, 5, and 6. *Journal of Biological Chemistry* 275 (50) 38949-38952
Morceau, F., Schneckeburger, M., Dicato, M., & Diederich, M. (2004) GATA-1: Friends, brothers, and coworkers. *Annals of New York Academy of Science.* 1030, 537-554.
Vakoc, C.R., Letting, D.L., Gheldof, N., Sawado, T., Bender, M.A., Groudine, M., Weiss, M.J., Dekker, J., Blobel, G.A. (2005) Proximity among distant regulatory elements at the beta-globin locus requires GATA-1 and FOG-1