STAT5A (signal transducer and activation of transcription), previously known as MGF (mammary gland factor), is a central mediator in the lactogenic hormone response. Moreover, STAT5 transcription factors are mediators of growth hormone (GH) and prolactin (PRL) actions on target genes. The DNA binding activity of STAT5A is hormonally regulated by PRL and essential for β-casein promoter activity. Since both lactation and growth performance are important production traits in farm animals, we began to investigate possible associations between nucleotide sequence polymorphism in the bovine STAT5A gene and its function with regard to production traits in cattle. Analysis of the DNA of 102 animals using SSCP (single strand conformation polymorphism), led to the identification of two alleles (A and B) and three genotypes (AA, AB, BB). The frequency of the genotypes of the STAT5A gene was as follows: AA - 0.73; AB - 0.23; BB - 0.04; the frequency of genotypes was very different in the cattle breeds studied. Upon sequencing of the genomic DNA derived from animals of different genotypes, we found a stretch of highly variable nucleotides located around position 12000 in the STAT5A gene. It included three nucleotide substitutions - A→G, G→A, G→C, and the deletion of G. The variable region lies within exon 16 coding for the DNA binding domain of the STAT5A protein. Therefore, we used EMSA (the electrophoretic mobility shift assay) to study the relationship between STAT5A gene polymorphism and the DNA-binding properties of the STAT5A protein. This study showed that proteins extracted from the mammary gland cell nuclei of cows carrying the AA genotype of the STAT5A gene bind DNA with a higher affinity then those of the other genotypes. Our study showed that polymorphism within the bovine STAT5A gene might influence the expression of genes regulated by this transcription factor, including the genes coding for milk proteins.