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Drosophila GAGA factor-FACT Complex and its Role in *Hox* Gene Expression

Takahiro Nakayama, Tsukasa Shimoj:ma, Masahiro Okada, Hitoshi Ueda and Susumu Hirose

Department of Developmental Genetics, National Institute of Genetics, Mishima, Shizuoka-ken 411-8540, Japan

Chromatin structure plays a critical role in the regulation of transcription. *Drosophila* GAGA factor directs chromatin remodeling to its binding sites. We found that *Drosophila* FACT, a heterodimer of dSPT16 and dSSRP1, is associated with GAGA factor through its dSSRP1 subunit, binds to a nucleosome and facilitates GAGA factor-directed chromatin remodeling. Immunostaining of polytene chromosomes revealed colocalization of GAGA factor and FACT in many specific loci. Mutation in *Trithorax-like* encoding GAGA factor abolished the colocalization, indicating that GAGA factor recruits FACT to specific loci in the genome. Moreover, genetic interactions between *Trithorax-like* and *spt16* implicate the GAGA factor-FACT complex in expression of *Hox* genes *Ultrabithorax*, *Sex combs reduced* and *Abdominal-B*. Chromatin immunoprecipitation experiments indicated presence of the GAGA factor-FACT complex in the regulatory regions of *Ultrabithorax* and *Abdominal-B*. These data illustrate a crucial role of the GAGA factor-FACT complex in the modulation of chromatin structure for regulation of *Hox* gene expression.

Materials and Methods

Fly lines, identification of GAGA factor-associated proteins, GST pull-down assays, electrophoresis mobility shift assays, chromatin remodeling assays, chromatin immunoprecipitation assays, and genetic analyses have been described previously[1].

Result and Discussion

The identity of the body segment of metazoans is determined by expression patterns of *Hox* genes. Expression of these genes is started by actions of many transcription factors during embryogenesis. Curiously, the expression patterns of *Hox* genes are maintained after disappearance of the transcription factors. The active or inactive state is remembered in a chromatin structure and transmitted to daughter cells through cell division. Although the precise mechanism of this epigenetic gene expression is not clear, genetic studies have revealed many genes involved in the process. These genes are classified into two groups. *Pc* group genes mainly govern the maintenance of the inactive state, while *trx* group genes are mainly responsible for the maintenance of the active state. These genes are conserved between insects and vertebrates. To elucidate the mechanism underlying the epigenetic maintenance of the active state, we analyzed the *Drosophila Trithorax-like* (*Trl*) gene product GAGA factor.

When chromatin was reconstituted on a plasmid DNA carrying the *fushi tarazu* (*ftz*) promoter, the template was inactive for transcription. However, when the chromatin template was incubated with

GAGA factor and an embryonic extract of *Drosophila* in the presence of ATP, we observed chromatin remodeling in the promoter region and transcriptional activation[2]. In this system, the chromatin remodeling occurred only around the GAGA factor-binding sites but GAGA factor itself has no chromatin remodeling activity. To investigate the mechanism of the site-specific chromatin remodeling, we searched for GAGA factor-associated proteins. To do this, we made a transgenic fly line expressing Flag-tagged GAGA factor. From an embryonic nuclear extract of the fly line, we isolated GAGA-factor associated proteins by immunoprecipitation with anti-Flag antibody beads and subsequent elution with Flag peptides. Starting from a control line without the transgene, we were unable to find any significant bands. By contrast when we started from the transgenic line, we obtained two proteins dSSRP1 and dSPT16 in addition to Flag-GAGA factor. SSRP1 and SPT16 are conserved across species from yeast to human and form a heterodimer. The human heterodimer FACT has been purified as a factor that is essential for transcription elongation on chromatin template[3].

Using bacterially expressed and purified proteins, we characterized these proteins. GST pull-down assays showed that GAGA factor binds directly to dSSRP1. Gel mobility shift assays using mononucleosome as a probe showed that FACT binds to a nucleosome but not to a half nucleosome consisting of histone H3/H4 tetramer. These data suggest that FACT binds to a nucleosome through histones H2A and H2B. To examine the effect of FACT on GAGA factor-directed chromatin remodeling, we reconstituted chromatin on a plasmid DNA carrying *ftz* promoter in the embryonic extract of *Drosophila* and the chromatin was further incubated with GAGA factor and FACT. These samples showed normal nucleosome ladders indicating that GAGA factor and FACT do not affect the bulk chromatin structure. When the DNA was transferred to a membrane and probed with the GAGA factor binding site in the *ftz* promoter, we observed GAGA factor dependent smearing of nucleosome ladders. Such smearing was barely detectable when the amount of GAGA factor was reduced to one-tenth. However, FACT induced the smearing even in the presence of low concentration of GAGA factor. In the absence of GAGA factor, FACT did not induced the smearing. These results demonstrate that FACT stimulates GAGA factor-directed chromatin remodeling. From these results, we propose a following model for the site-specific chromatin remodeling[1]. GAGA factor binds to a GAGAG sequence on DNA and recruits FACT. FACT binds to a nucleosome and weaken the interactions between histones H2A/H2B and DNA. This facilitates chromatin remodeling in a GAGA factor binding site-dependent manner (Figure 1).

To examine whether the GAGA factor-FACT complexes are present on specific loci within the genome, we carried out immunostaining of these proteins on polytene chromosomes. First, we analyzed dSSRP1 and dSPT16. These proteins occupy many specific loci. Upon merge, two staining patterns coincided completely, indicating that these proteins are present as the heterodimer FACT. In collaboration with John Lis and Danny Reinberg, we have shown that these loci are active sites of transcription by RNA polymerases I and II but not III[4]. As reported by other researchers, GAGA factor was also detected in many specific loci. Upon merge, most of the GAGA bands colocalized with the dSSRP1 or dSPT16 bands. In addition, there were small numbers of strong dSSRP1 and dSPT16 bands

without the GAGA signals. In larvae homozygous for Trl^{13C} mutation, all the bands disappeared except small number of the strong signals of dSSRP1 and dSPT16. These results show that most of FACT are recruited to the specific loci by GAGA factor.

In order to address the biological significance of the GAGA factor-FACT complex, we carried out genetic studies on *Hox* gene expression. Usually, insects have two pairs of wings. However, the posterior wing becomes a tiny structure termed haltere in files like *Drosophila*. This is due to suppression of the wing formation by *Ultrabithorax* (*Ubx*) in the body segment T3. When the *Ubx* function decreases, the haltere becomes larger and more wing-like in structure. This provides a sensitive assay for epigenetic maintenance of *Ubx* expression. In flies doubly heterozygous for *Trl* and *Ubx*, halteres are quite heterogeneous in size and larger than those in *Ubx* single heterozygotes. We observed similar effect in *spt16, Ubx* double heterozygotes. Moreover, *spt16, Trl, Ubx* triple heterozygotes showed stronger phenotype than each double heterozygotes. These data indicate that the GAGA factor-FACT complex contributes to the maintenance of *Ubx* expression.

We then analyzed expression of another *Hox* gene *Sex comb reduced* (*Scr*). Number of teeth per sex comb was about 11 in wild type, about 10 in *Trl* heterozygote, about 9 in *spt16* heterozygote and about 7 in *spt16, Trl* double heterozygote. This effect was reversed by expression of dSPT16 from a transgene. These data show that the GAGA factor-FACT complex is involved in *Scr* expression. We also searched for bristles on the male abdominal segment 6 (A6). No bristle was detectable on the wild type A6. This is due to suppression of bristle formation in A6 by *Abdominal-B* (*Abd-B*). In contrast, we observed bristles on A6 in 11% of *Trl* heterozygotes, 30% of *spt16* heterozygotes, and 67% of *Trl, spt16* double heterozygotes. Moreover, we observed significant increase in the number of bristles in the double heterozygotes. The effect was reversed by expression of dSPT16 from a transgene. These data indicate that the GAGA factor-dFACT complex contributes to the maintenance of *Abd-B* expression.

If the GAGA factor-FACT complex is directly involved in the regulation of *Hox* genes, the complex should be present in the regulatory regions of *Hox* genes. Chromatin immunoprecipitation assays showed the presence of GAGA factor, dSSRP1 and dSPT16 in the *bxd* regions of *Ubx* and the *iab-6* element of *Abd-B*. Based on these data we conclude that the GAGA factor-FACT complex plays a crucial role in the regulation of *Hox* gene expression through chromatin remodeling[1] (Figure 1).

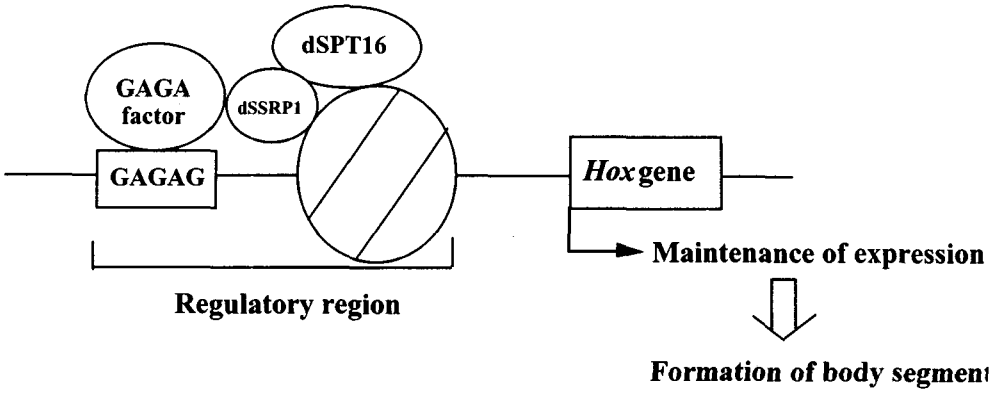

Figure 1. GAGA factor-FACT complex plays a crucial role in the regulation of *Hox* genes through chromatin remodeling

Reference

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