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The HMG-box protein Lilliputian is required for Runt-dependent activation of the pair-rule gene *fushi–tarazu*

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Abstract

lilliputian (*lilli*), the sole *Drosophila* member of the FMR2/AF4 (Fragile X Mental Retardation/Acute Lymphoblastic Leukemia) family of transcription factors, is widely expressed with roles in segmentation, cellularization, and gastrulation during early embryogenesis with additional distinct roles at later stages of embryonic and postembryonic development. We identified *lilli* in a genetic screen based on the suppression of a lethal phenotype that is associated with ectopic expression of the transcription factor encoded by the segmentation gene *runt* in the blastoderm embryo. In contrast to other factors identified by this screen, *lilli* appears to have no role in mediating either the establishment or maintenance of *engrailed* (*en*) repression by Runt. Instead, we find that Lilli plays a critical role in the Runt-dependent activation of the pair-rule segmentation gene *fushi–tarazu* (*ftz*). The requirement for *lilli* is distinct from and temporally precedes the Runt-dependent activation of *ftz* that is mediated by the orphan nuclear receptor protein Ftz-F1. We further describe a role for *lilli* in the activation of *Sex-lethal* (*Sxl*), an early target of Runt in the sex determination pathway. However, *lilli* is not required for all targets that are activated by Runt and appears to have no role in activation of *sloppy paired* (*slp1*). Based on these results we suggest that Lilli plays an architectural role in facilitating transcriptional activation that depends both on the target gene and the developmental context.

Keywords: Runx; FMR2; *Sex-lethal*; *Engrailed*; *Sloppy-paired*; Ftz-F1

Introduction

Runx proteins comprise a relatively small family of conserved DNA-binding transcription factors that are of widespread importance in animal development and human disease. These proteins are identified through a highly conserved 128-amino acid motif termed the Runt domain that is responsible for DNA-binding as well as for mediating dimerization with the unrelated CBFβ/Brother partner proteins (Bushweller, 2000; Kagoshima et al., 1993; Warren et al., 2000; Yan et al., 2004). Runt domains between vertebrates and *Drosophila* share nearly 70% sequence identity, and almost all of the amino acid substitutions are conserved, indicating that this domain is central to the regulatory functions of these proteins. Runx genes have been identified in all animal species examined including: *runt* and *lozenge* (*lz*) in *Drosophila*, and three different Runx genes in mammals (for review see Coffman, 2003; Levanon and Groner, 2004). Mutations in all three human *Runx* genes are associated with genetic disease (Lund and van Lohuizen, 2002) (Bowcock, 2005; Otto et al., 2002; Yamashita et al., 2005) and targeted mutagenesis experiments in the mouse indicate that these genes have vital roles in several developmental pathways, including hematopoeisis, neurogenesis and osteogenesis (Komori et al., 1997; Levanon et al., 2003; Otto et al., 1997; Wang et al., 1996). A unifying aspect of Runx protein function in these many different developmental pathways is a role in cell fate specification, presumably by transcriptional regulation.

Our work has focused on using the tools available in the *Drosophila* system to investigate the function of Runt, the

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founding member of the Runx protein family. Runt is best characterized for its role in the embryo where it plays vital roles in several developmental pathways, including sex determination, segmentation, and neurogenesis. In the early blastoderm embryo, Runt works in cooperation with other sequence-specific DNA-binding proteins in a dose-dependent manner to trigger the sex-specific transcriptional activation of the *Sex-lethal* (*Sxl*) gene (Kramer et al., 1999). At a slightly later stage, *runt* regulates the transcription of numerous downstream target genes in the developmental pathway of segmentation (Aronson et al., 1997; Manoukian and Krause, 1993; Tracey et al., 2000; Tsai and Gergen, 1994, 1995; Tsai et al., 1998; Vander Zwan et al., 2003; Wheeler et al., 2002). An intriguing aspect of transcriptional regulation by Runt, as well as by the vertebrate Runx proteins, is that they function both as activators and repressors, depending on the specific target gene and the developmental context. The mechanisms used to achieve this context-dependent specificity are not understood.

We have developed a genetic approach that allows us to identify factors that have dose-dependent roles in Runt-dependent transcriptional regulation. This strategy utilizes *Drosophila* lines that maternally express the yeast transcriptional activator GAL4 to drive expression of *UAS-runt* transgenes concomitant with the onset of zygotic transcription in the blastoderm stage embryo. The level of ectopic Runt is quantitatively manipulated in a reproducible manner by varying the strengths of the *NGT* (*nos-GAL4-tubulin*) drivers and the responding *UAS-runt* transgenes (Li and Gergen, 1999; Swantek and Gergen, 2004; Tracey et al., 2000; Wheeler et al., 2002). Different expression levels give reproducible regulatory effects on the expression of different downstream targets of Runt. The odd-numbered stripes of the segment-polarity gene *en* are extremely sensitive to *NGT*-driven Runt (Tracey et al., 2000). Importantly, the threshold level of Runt required for *en* repression coincides with the level that is associated with embryonic lethality. Experiments with different *NGT* and *UAS-runt* lines indicate that a 50% reduction in the level of Runt leads to a substantial increase in viability (Tracey et al., 2000). Based on these observations we screened a collection of chromosomal deficiencies to identify genomic intervals that dominantly suppress the lethality associated with *NGT*-driven Runt expression. Further dissection of some of the deficiency intervals led to the identification of several maternally expressed factors that contribute to Runt-dependent repression and further revealed two distinct steps in this process (Wheeler et al., 2002).

The results presented here emerge from our characterization of one of the other intervals identified in this initial deficiency screen. The lethality associated with *NGT*-driven Runt is greatly reduced in embryos from females that have reduced dosage of polytene interval 23C1-2. Further analysis of this region identifies *lilliputian* (*lilli*) as a factor that contributes to Runt's potency in the blastoderm embryo. *lilli* is the sole *Drosophila* homologue of the mammalian Fragile-X-Mental Retardation (FMR2)/Acute Lymphoblastic Leukemia (AF4) family of transcription factors (Su et al., 2001; Tang et al., 2001; Wittwer et al., 2001). There are four members of this gene family in humans. *lilli* has been described as a novel maternal-effect pair-rule gene that is widely expressed during *Drosophila* development. In addition to a role in segmentation, *lilli* has roles in cellularization and gastrulation during embryogenesis (Tang et al., 2001) with additional roles at later stages involving both MAPK and Dpp signaling pathways (Su et al., 2001; Wittwer et al., 2001).

The characterization of the role of *lilli* in Runt-dependent transcriptional regulation described here reveals that *lilli* does not have a role in *en* repression, but instead plays a critical role in mediating Runt-dependent activation of the pair-rule gene *fushi–tarazu* (*ftz*). We find that Lilli's role is temporally distinct from that of Ftz-F1, another maternally expressed factor that mediates Runt-dependent *ftz* activation. The differential requirements for Lilli and Ftz-F1 reveal new insights on the dynamics of *ftz* regulation in the early embryo. Evidence is presented that Lilli is also required for the initial activation of *Sxl*, a target of Runt in the sex determination pathway. In contrast, Lilli appears to have no role in the Runt-dependent activation of *sloppy-paired* (*slp1*). A consideration of the overlaps in the requirements for *runt* and *lilli* in the context of what is known about the dynamics of different transcriptional regulatory programs and the proposed biochemical function of Lilli suggest that Lilli's role in the early blastoderm embryo involves modulation of developmentally dynamic chromatin architecture.

Materials and methods

Fly strains and crosses

The deficiency chromosomes *Df(2L)JS32* and *Df(2L)C144*, and the P element insertion lines $lilli^{05431}$ and $lilli^{00632}$ were obtained from the Bloomington Stock Center. The $lilli^{XS407}$ and $lilli^{XS575}$ mutations were provided by Arno Müller. The temperature sensitive $lilli^{7F1}$ and $lilli^{2L\text{-}193\text{-}35}$ mutations were generated by Barrett et al. (1997) and Luschnig et al. (2004) and provided by Arno Müller. The Sxl_{PE}*:lacZ* reporter gene line is as described by Kramer et al. (1999). The second chromosome-linked GAL4 driver *P{GAL4-nos.NGT}40* (*NGT[40]*), and the *P{UAS-runt.T}232* (*UAS-runt[232]*) and *P{UAS-runt.T}15* (*UAS-runt[15]*) transgenic lines have been described previously (Tracey et al., 2000). The third chromosome-linked GAL4 driver *P{GAL4.nos.NGT}A* (*NGT[A]*) is described in Wheeler et al. (2002). The transgenic lines for GAL4-dependent expression of *odd-paired* (*opa*), *P{UAS-opa.VZ}10* (*UAS-opa[10]*) and *P{UAS.opa.VZ}14* (*UAS-opa[14]*) are described in Swantek and Gergen (2004). The genetic crosses used to assess the dose-dependent maternal effects of different mutations on the lethality associated with *NGT*-driven Runt expression as well as the assays used to measure levels of *NGT*-driven β-galactosidase activity using the *P{UAS-lacZ.B}4-1-2* (*UAS-lacZ[4-1-2]*) transgene are as described in Tracey et al. (2000). The β-galactosidase levels reported are the averages and standard errors in absolute light units ($\times 10^{-3}$) obtained in assays using extracts from single, carefully staged embryos (N=number of embryos). As noted previously, there is an intrinsic biological variability in this assay of approximately plus or minus 20% of the total.

Germline clone and temperature shift experiments

Mitotic recombination using the *FLP/FRT/ovo[D]* system (Chou and Perrimon, 1996) was used to generate clones of female germ cells homozygous for different *lilli* and *Ftz-F1* mutations. For *lilli*, females homozygous for the X-linked *y w P{hsFLP}22* chromosome and heterozygous for $lilli^{XS407}$ *P{neoFRT}40A* over *CyO* were mated to *P{OvoD1-18}2La P{OvoD1-18}2Lb P{neoFRT}40A/Cyo* males. Progeny from this cross were heat-shocked at 37°C for 2 h on 2 consecutive days starting 24 AEL (after egg-laying). Virgin female progeny from this cross that were heterozygous for the $lilli^{XS407}$ *P{neoFRT}40A* and *P{OvoD1-18}2La P{OvoD1-18}2Lb P{neoFRT}40A* chromosomes were collected and then mated to various males: (1) *y w / Y* (representing wild-