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Dosage-dependent rescue of definitive nephrogenesis by a distant *Gata3* enhancer

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Abstract

Human *GATA3* haploinsufficiency leads to HDR (hypoparathyroidism, deafness and renal dysplasia) syndrome, demonstrating that the development of a specific subset of organs in which this transcription factor is expressed is exquisitely sensitive to gene dosage. We previously showed that murine GATA-3 is essential for definitive kidney development, and that a large YAC transgene faithfully recapitulated GATA-3 expression in the urogenital system. Here we describe the localization and activity of a kidney enhancer (KE) located 113 kbp 5′ to the *Gata3* structural gene. When the KE was employed to direct renal system-specific GATA-3 transcription, the extent of cell autonomous kidney rescue in *Gata3*-deficient mice correlated with graded allelic expression of transgenic GATA-3. These data demonstrate that a single distant, tissue-specific enhancer can direct GATA-3 gene expression to confer all embryonic patterning information that is required for successful execution of metanephrogenesis, and that the dosage of GATA-3 required has a threshold between 50% and 70% of diploid activity.

Keywords: Gata3; Kidney; Rescue; Enhancer; Allelic series; Haploinsufficiency

Introduction

Mammalian kidney morphogenesis progresses through three sequential stages with the first two embryonic kidneys, the pronephros and mesonephros, being transitory structures (Dressler, 2002; Gilbert, 2000; Kuure et al., 2000). By late embryonic day (e) 9, the nephric duct has fully extended posteriorly. The definitive kidney begins to develop at e11 when the ureteric bud (UB) sprouts from the nephric duct. This initial sprouting is triggered by glial cell line-derived neurotrophic factor (GDNF) secreted from the metanephric blastema (Davies and Fisher, 2002; Sainio et al., 1997). A variety of additional morphogenetic signals lead to further outgrowth and dichotomous branching of the UB to form the mature renal collecting system (Davies and Fisher, 2002; Yu et al., 2004). The UB in turn signals to the surrounding nephrogenic mesenchyme, inducing it to undergo epithelial differentiation to ultimately form much of the nephron.

Of the germline mutations that have been analyzed in mice, loss-of-function alleles for the genes encoding transcription factors Lim1, Pax2, Pax2 plus Pax8 and GATA-3 most often lead to extension failure of the nephric duct (Bouchard et al., 2002; Lim et al., 2000; Shawlot and Behringer, 1995; Tsang et al., 2000). GATA-3 belongs to a six-member family of transcription factors that share conserved zinc fingers, which recognize the consensus sequence (A/T)GATA(A/G) (Ko and Engel, 1993; Merika and Orkin, 1993; Yamamoto et al., 1990). GATA-3 is expressed in many tissues and cell types and has been shown in several cases to play essential roles in their differentiation (George et al., 1994; Hendriks et al., 1999; Kaufman et al., 2003; Lakshmanan et al., 1998; Lim et al., 2000; Ting et al., 1996; van der Wees et al., 2004; van Doorninck et al., 1999; Zheng and Flavell, 1997). Germ line deletion of murine *Gata3* was initially reported to result in grossly normal *Gata3* heterozygous mutant mice (Pandolfi et

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al., 1995), while *Gata3* homozygous mutants die at midgestation of noradrenergic insufficiency leading to secondary cardiac failure (Lim et al., 2000). Pharmacologic rescue of GATA-3-deficient embryos (to late gestation) with synthetic noradrenaline intermediates revealed an array of mutant phenotypes, including renal agenesis, that were previously masked by the early *in utero* lethality (Lim et al., 2000).

To facilitate systematic and rapid isolation of regulatory *cis*-elements within any extended genomic domain, we previously reported the use of YACs (Lakshmanan et al., 1999; Zhou et al., 1998) and BAC-traps (Khandekar et al., 2004) to assess large swaths of the genome for enhancer activity *in vivo*. This led to the identification and crude localization of a *Gata3* kidney enhancer lying within a 662-kbp YAC. To evaluate the *in vivo* contribution of an enhancer to a given biological process, we proposed to establish the necessary and sufficient contribution of a regulatory protein under the control of any enhancer to a discrete developmental event. Here, we describe the localization of a *Gata3* kidney enhancer (KE) that lies 113 kbp 5′ to the structural gene, and the *in vivo* interrogation of its contribution to generation of the definitive kidney.

To evaluate the capacity of the enhancer to fully delineate GATA-3 functions during nephrogenesis, we generated transgenic lines that expressed tissue-specific GATA-3 at various abundances relative to its endogenous level under the direct transcriptional control of the isolated KE. KE-directed GATA-3 transgenes ($Tg^{KE\text{-}G3}$) were then examined in *Gata3* null (*lacZ* knock-in) mice ($Gata3^{z/z}$:$Tg^{KE\text{-}G3}$) in order to assess their ability to specifically rescue metanephrogenesis. In the rescued compound mutants, the degree of renal development is directly proportional to the transgene-derived GATA-3 abundance, reflecting GATA-3 gene dosage dependence in executing the nephrogenic program as seen in HDR patients. Compound mutants with Tg expression of greater than 50% of endogenous GATA-3 levels undergo normal nephrogenesis, while equal to or less than 50% levels of GATA-3 result in a spectrum of intermediate defective nephric duct and ureteric bud phenotypes in midgestation as well as various deficiencies in the definitive kidneys of late gestation embryos. The renal phenotypic variations seen in the partially rescued compound mutants are in some ways reminiscent of the spectrum of highly variable clinical nephric defects encountered in *GATA3* haploinsufficient HDR syndrome patients, suggesting that *Gata3* compound mutant mice might represent a unique experimental model to study kidney deficiencies observed in the human condition.

Results

Localization of a distant Gata3 kidney enhancer

Previously, we generated transgenic lines harboring the B125Z mouse *Gata3* YAC (the genome sequence-revised endpoints are −451 to +211 kbp, with respect to the GATA-3 translational start site; Lakshmanan et al., 1998, 1999), which was tagged with a *lacZ* reporter gene (Lakshmanan et al., 1999). Detailed analyses of B125Z transgenic lines indicated that β-galactosidase staining was detected throughout early and definitive nephrogenesis, consistent with previous *in situ* hybridization studies (George et al., 1994; Labastie et al., 1995; Lakshmanan et al., 1999). Detailed pulsed-field gel electrophoresis analyses indicated that one line (#71) harboring a 5′ truncated YAC [with a breakpoint that mapped imprecisely between −70 and −116 (±20) kbp] still retained urogenital *lacZ* expression (data not shown, and Lakshmanan et al., 1999). Previous transgenic analyses of two other smaller YACs indicated that B124Z (spanning −451 to +69 kbp), but not C4Z (−40 to +69 kbp of the *Gata3* locus), could recapitulate urogenital *lacZ* staining (Lakshmanan et al., 1998, and unpublished). These and other observations allowed us to conclude deductively that a kidney enhancer (KE) must reside in the interval between −40 and −116 kbp 5′ to the *Gata3* structural gene. We therefore performed end fragment rescue to retrieve terminal genomic sequences from *Gata3* YACs that had 3′ endpoints lying within that interval (B143 and B157; Fig. 1A). A rescued 9.2-kbp *Eco*RI genomic DNA fragment was recovered from the YAC B143 3′ terminus, which localized to −110 kbp (Fig. 1B1; data not shown and Lakshmanan et al., 1998).

To determine whether the rescued B143 3′ end fragment contained kidney enhancer activity, we subcloned it into pG3*lacZ* (formerly named −308p*lacZ*; see Materials and methods; Lieuw et al., 1997) for founder (F_0) transgenic analysis (Fig. 1B). X-gal staining was detected in the nephric duct and the UB of all (6/6) e11.5 transgenic embryos (Fig. 1C1); the urogenital β-galactosidase staining pattern recapitulated that observed in whole mount X-gal-stained B125Z transgenic (Lakshmanan et al., 1999) as well as heterozygous *Gata3 lacZ* knock-in embryos (first panel from left, Fig. 1D, $Gata3^{+/z}$; Hendriks et al., 1999; van Doorninck et al., 1999). Hence, we concluded that the 9.2-kbp end fragment recovered from YAC B143 contains a *Gata3* KE. Further analysis resulted in the experimental refinement of this KE activity to a 1.3-kbp fragment that recapitulated the urogenital expression pattern in the majority of e11.5/e12.5 transgenic F_0 embryos (Figs. 1B2–B5 and C2–C5).

To ascertain if the 1.3-kbp KE-directed *lacZ* reporter gene expression recapitulated the temporal profile of *Gata3*, we generated stable lines of this reporter construct. When examined during early- to mid-embryogenesis, transgene expression was strictly confined to the developing nephric ducts, and later in the sprouting ureteric buds, of e9.0–10.5 transgenic embryos (Supplemental Fig. 1). This mirrored the temporal staining profile in $Gata3^{+/z}$ embryos with similar numbers of somites, which however displayed a more extensive X-gal staining pattern in tissues in addition to nephric derivatives. Thus, the 1.3-kbp KE was capable of activating reporter gene expression when endogenous GATA-3 expression was initiated. By birth, the transgene is expressed in the epididymis, seminal vesicles and vas deferens of transgenic males in addition to the kidneys and ureters (second panel from left, Fig. 1D), as do YAC B125Z transgenic and $Gata3^{+/z}$ neonates (Lakshmanan et al., 1998; below).

To verify coincident expression at the cellular level, we replaced the reporter gene in pG3*lacZ* with eGFP and generated