Genomics 106 (2015) 178–184

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Review

Learning about mammalian gene regulation from functional enhancer assays in the mouse

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ARTICLE INFO

Article history:
Received 12 February 2015
Received in revised form 6 May 2015
Accepted 8 June 2015
Available online 14 June 2015

Keywords:
Mouse
Enhancer
Function
Gene regulation

ABSTRACT

Enhancer biology is emerging as a critical area of research that informs studies of evolution, development, and disease. From early experiments that defined and mapped the first enhancers to recent enhancer models of human disease, functional experiments in the mouse have played a central role in revealing enhancer biology. Three decades of in vivo enhancer studies in mouse have laid the groundwork for the current understanding of mammalian enhancers, demonstrating the developmental and tissue-specific activity of enhancers and illuminating general features of enhancer evolution and function. Recent studies offer an emerging perspective on the importance of chromosomal context, combinatorial enhancer activity, and the functional consequences of enhancer sequence variation. This review describes the basic principles of functional testing in mouse, summarizes the contributions these studies have made to our understanding of enhancer biology, and describes limitations and future outlook of in vivo mouse enhancer studies.

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1. Introduction

Mouse models have advanced our understanding of the in vivo function of enhancer sequences, providing critical insight into how mammalian enhancer sequences function at the whole organism level, from their activity during development across complex tissues to their role in evolution and disease. Despite the significant strides in our understanding of enhancer function, much work remains, and functional studies in the mouse will be critical to our future understanding of the regulatory capacity and functional mechanisms of enhancers in mammalian systems. This review takes on three primary objectives. First, it describes basic principles of enhancer testing in the mouse. Next, it highlights mouse-based studies that have revealed the roles of enhancers in evolution, development, and disease. Finally, it describes

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http://dx.doi.org/10.1016/j.ygeno.2015.06.008
0888-7543/

new approaches and opportunities for using mouse-based approaches to understand the mechanisms of enhancer activity.

2. Transgenic enhancer assays in mouse

2.1. How do enhancer assays work in mouse?

Kothary et al. showed that a construct using a minimal promoter taken from the *Hsp68* heat-shock inducible gene paired to the *Escherichia coli* beta-galactosidase (lacZ) reporter gene could be used to screen for endogenous elements that drive expression [1,2]. This construct can be delivered via microinjection into fertilized mouse eggs and will stably integrate into the genome, enabling image-based activity screening of transgenic mice (Fig. 1). This approach can be used to "trap" endogenous regulatory elements via insertion in *cis*, enabling characterization of regulatory element function. The extension of this assay to enable candidate enhancer screening by fusing an enhancer sequence upstream of the minimal promoter and reporter gene has been used extensively to study the function of enhancers in vivo in mouse. In this assay, the enhancer drives expression of the reporter in the same structures as the endogenous version of the enhancer is active, thus enabling characterization of activity in vivo.

While powerful, this functional enhancer assay is susceptible to position effects driven by insertion site, so multiple independent transgenic individuals must be screened. Furthermore, the rate of genome integration can be relatively low and screening requires many microinjected eggs to be generated. Thus, testing a candidate sequence in this assay requires construct cloning and preparation, the collection and microinjection of tens to hundreds of fertilized eggs, surgical reimplantation, and eventual sample collection and histological preparation and scoring. As such, mouse transgenic assays require high levels of technical expertise, are expensive and require substantial time to perform, and have not been amenable to high-throughput analysis. Furthermore, activity differences between enhancers or alleles can be difficult to identify due to variation driven by position effects, a limiting factor with regards to using this approach to compare subtle quantitative changes in function. Nonetheless, these experiments provide information that cannot be generated via other methods, enabling characterization of enhancer activity from the single cell to whole organism level, maintaining complex developmental context in a mammalian model system.

2.2. Results of mouse transgenic assays and the VISTA database

Functional enhancer testing in the mouse has been critical to discovering and validating individual enhancers, as well as to understanding general principles of gene regulation and enhancer biology. A particular strength is the ability to characterize in vivo function in the context of complexity of heterogeneous tissues and developmental stages. Examples of early applications include studies of *En2* in the central nervous

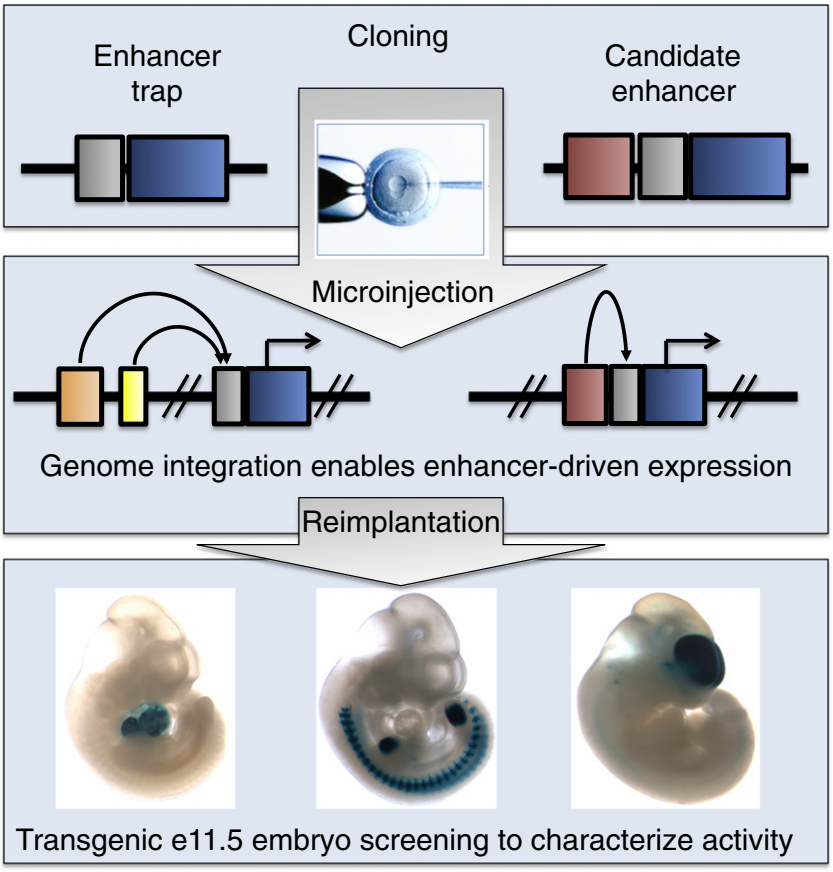

Fig. 1. Schematic of transgenic mouse enhancer assays. Left: Enhancer Trap approach uses a minimal reporter (gray) and a reporter gene (blue) construct that, when microinjected into a fertilized mouse egg, inserts randomly in the genome, "trapping" the activity of nearby enhancers (orange and yellow blocks). Right: Enhancer screening using the same construct except with a candidate enhancer cloned upstream of the minimal promoter (red). When inserted in the genome, the candidate enhancer will drive expression of the reporter gene. Other nearby enhancers may also contribute to reporter gene expression, and multiple transgenic embryos must be screened to identify consistent patterns. After microinjection, eggs are surgically reimplanted, and transgenic mice are screened at desired timepoint. Example e11.5 transgenic embryos with enhancer-driven reporter gene expression selected from VISTA enhancer database (enhancer.lbl.gov).