Chapter 10

Detection of RNA Polymerase II in Mouse Embryos During Zygotic Genome Activation Using Immunocytochemistry

Irina O. Bogolyubova and Dmitry S. Bogolyubov

Abstract

Mammalian pre-implantation embryos represent a highly dynamic experimental model for comparative studies of nuclear structure and functions in the context of gradual reactivation of transcription. Here, we present details of the methods that allow localizing RNA polymerase II in mouse pre-implantation embryos with specific antibodies, using fluorescent/confocal and electron microscopy. We stress the special aspects of immunolabeling protocols in respect to the embryonic material. We made a special emphasis on the essential steps preceding the immunocytochemical experiments. In particular, we consider the procedures of female hormonal stimulation and embryo collection. The described approaches are also applicable to study other nuclear proteins.

Key words Mouse embryos, Nucleus, Immunocytochemistry, Confocal microscopy, Immunogold electron microscopy, RNA polymerase II

1 Introduction

The nuclei of mammalian early embryos represent a peculiar experimental model providing a good opportunity to study nuclear structure and functions [1]. They are characterized by a specific nuclear ultrastructure [2] that obviously reflects the unique functional state of the nucleus during embryonic early development. At the initial period, the nucleus of mammalian embryos is transcriptionally silent, whereas transcription is reactivated at the appointed stage later, and the nucleus undergoes a series of successive structural and transcriptional changes known as zygotic gene activation (ZGA) [3]. As a result, the mammalian early embryos allow studying dynamics of the nuclear apparatus at both transcriptionally active and inert stages in natural conditions. In particular, significant alterations of the pattern of RNA polymerase II (RNAPII)-dependent transcription occur during the 1- and 2-cell stages of mouse development.

Kiho Lee (ed.), *Zygotic Genome Activation: Methods and Protocols*, Methods in Molecular Biology, vol. 1605,
DOI 10.1007/978-1-4939-6988-3_10,