Autocrine and Intracrine Signaling for Cardiogenesis in Embryonic Stem Cells: A Clue for the Development of Novel Differentiating Agents

C. Ventura¹,² (✉) · A. Branzi¹,²

¹Laboratory of Molecular Biology and Stem Cell Engineering, National Institute of Biostructures and Biosystems, University of Bologna, Bologna, Italy cvent@libero.it
²S. Orsola-Malpighi Hospital, Institute of Cardiology, Pavilion 21, Via Massarenti 9, 40138 Bologna, Italy

Abstract Cardiogenesis, one of the earliest and most complex morphogenetic events in the embryo, is not fully understood at the molecular level and is typically a low-yield process. Affording a high throughput of cardiogenesis from a suitable population of pluripotent cells is therefore a major assignment in the perspective of a stem cell therapy for heart failure. Analysis of cardiac lineage commitment in mouse embryonic stem cells and in vivo models of cardiac differentiation revealed that a number of crucial growth factors are released from precursor cells, acting in an autocrine fashion on specific plasma membrane receptors to prime a cardiogenic decision. Nevertheless, it is increasingly becoming evident that cell nuclei harbor the potential for intrinsic signal transduction pathways. The term “intracrine” has been proposed for growth regulatory peptides that have been shown to act within their cell of synthesis at the level of the nuclear envelope, chromatin, or other sub-nuclear components. Considerable evidence links known intracrines with transcriptional responses and self-sustaining loops that behave as long-lived signals and impart features characteristic of differentiation, growth regulation and cell memory. This review focuses on a number of autocrine and intracrine systems within the context of cardiac differentiation and emphasizes the identification of cardiogenic mechanisms as a clue for the development of unprecedented differentiating strategies. In this regard, recently synthesized mixed esters...
of hyaluronan with butyric and retinoic acid primed the expression of cardiogenic genes and elicited a remarkable increase in cardiomyocyte yield in mouse embryonic stem cells. This demonstrates the potential for chemically modifying the gene program of cardiac differentiation without the aid of gene transfer technologies and sets the basis for the design of a novel generation of chemicals suited for the organization of targeted lineage patterning in stem cells.

**Keywords** Embryonic stem cells · Cardiogenesis · Gene transcription · Differentiating agents

### 1 Introduction

Mouse embryonic stem (ES) cells have been shown to behave as pluripotent, self-renewable elements that can be committed to multiple lineages, including cardiac myocytes. Due to these features, ES cells proved to be an invaluable model to take a glimpse of the molecular events underlying the specification of a myocardial fate. In this regard, a selected number of lineage-restricted transcription factors, including the zinc finger-containing GATA-4 and the homeodomain Nkx-2.5, has been found to be essential for cardiogenesis in different animal species (Biben and Harvey 1997; Grepin et al. 1995; Heikinheimo et al. 1994; Jiang and Evans 1996; Lints et al. 1993), including humans (Benson et al. 1999; Schott et al. 1998). However, the identification of genes and signaling patterning recruited for the expression of cardiogenic transcription factors is only partially exploited. So far, different cardiogenic growth factors recognized by specific cell-surface receptors have been identified and the signaling mechanisms coupled with receptor activation have been increasingly dissected. A common feature in a number of these growth factors is their potential of being released by a pluripotent cell in the extracellular space, acting in an autocrine fashion onto targeted plasma membrane receptors to elicit a biological effect (Fig. 1). However, there is also increasing evidence to support intracellular sites of growth factor action and a large body of data indicating that such action is not rare (Cook et al. 2001; Re 2000, 2002, 2003). Within this context, the term “intracrine” has been proposed for the action of a peptide hormone either within its cell of synthesis or after internalization (Fig. 1). It is now agreed that an intracrine must retain the potential of being found in the extracellular space producing a response after binding to a membrane receptor such as a traditional endocrine, paracrine, or autocrine. In addition, a putative intracrine factor must be found in association with one or more intracellular organelles not associated with secretory or degradatory structures (these may arguably be viewed as extensions of the extracellular space).

The present review will focus on a number of autocrine/intracrine systems involved in a cardiogenic decision in pluripotent ES cells, and will discuss the