Transcription factors involved in the maintenance of stem cells: comparative analysis among eucaryotes

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Motivation

In recent years, somatic stem cells have been heralded as potential therapeutic agents for many degenerative diseases. In order to employ them as effective therapeutic agents, and/or improve treatment of stem-cellassociated malignancies, a better understanding of their basic biological properties is needed. A major limitation in the study of somatic stem cells lies in the difficulty of studying them in vivo. Given the complexity of vertebrate adult somatic stem cell populations and their relative inaccessibility to in vivo molecular analyses, the study of somatic stem cells could then benefit from analyzing their counterparts in simpler model organisms. Animals and plants have almost certainly evolved separately. Thus, the developmental fate of repairing multicellular organs and structures would be expected to have critical differences. However, even though it is likely that pathways used to activate regeneration may be specific to each kingdom, the mechanistic barriers to totipotency are likely to intersect, and basic principles in regeneration could then be distilled from such comparisons. Mature plant cells have the capability of reversing their differentiation state and producing new organs under cultured conditions. In these processes, cells undergo fate switch several times regulated by both extrinsic and intrinsic factors, associated with reentry to the cell cycle, balance between eu- and heterochromatin, gene expression reprogramming. Planarians, free-living representatives of the Platelminthes, are known for their ability to regenerate individuals from fragments of their bodies. Regenerative ability of Planaria is based upon totipotent somatic stem cells, called neoblasts, distributed throughout the body. Planarians serve as models for addressing many problems relevant to human health and biology that are not readily studied in well-established models, such as Drosophila and C. elegans. In this work, a comparative analysis of main transcriptional factors related with the maintenance of totipotency in plants, planaria, drosophila and mammals was undertaken in the context of cellular, developmental and evolutionary processes.

Methods

From the most recent literature, several transcriptional regulators have been found to play a crucial role in the maintenance of totipotency in different metazoans. In order to investigate relationship between their sequences, specific services based on ClustalW and T-coffee have been used. An automated analysis workflow was designed and developed with Taverna Workbench 1.7.1. It implements "parallel" multiple sequence alignments and finally builds a phylogenetic tree. Identifiers of selected proteins or genes are used as input, and a series of alignments with relative plots, phylogenetic distance files, and proteins with their descriptions constitute the output. For plotting alignments, prettyplot, an EMBOSS application provided by Soaplab services, was used. Finally, for an improved visualization and further evaluation of the data, output files have been insert into the Jalview tool.

Results

Several regulator factors involved in the maintaining or restoring of totipotency have been individuated in different eukaryotes. In plants, wus and wox expression, located in shoot apical meristem and root apical meristem respectively, are considered to regulate self-renewal and totipotency of cells. In planarians, neoblasts are the only mitotically active cells, and their division progeny generates the 30-40 different cell types found in these organisms. In intact planarians, these stem cells replace cells lost to normal physiological turnover; whereas, in amputated animals, they give rise to the regeneration blastema, the structure in which missing tissues are regenerated. Two transcriptional regulators have been identified in planarians: DjPum and bruli genes that appear to act in a similar fashion, being both required for stem cell maintenance. Recent studies have also pointed out the ability of some human genes, such as oct and nanog, to restore the totipotency in differentiated mammalian cells. Selected transcriptional factors, specifically expressed in stem cells, were analysed through the Taverna workflow. A dendrogram was produced using genetic distance matrix based on similarity of the main transcriptional regulators involved in the self-renewal of stem cells in plants, planarians, drosophila and

mammals. The elaboration of the workflow has been particularly useful in terms of effectiveness, through automation of repetitive procedures. Moreover, access to both remote services, such as ClustalW, T-coffe and the Sopalab based EMBOSS implementation (emma and prettyplot tools) from EBI, and local processors, including a few Beanshell scripts, has allowed us to display results in different formats, including text, image, .aln and .nj.

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