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Perspectives

A Transcriptional Pathway for Cell Separation in Fission Yeast

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ABSTRACT

Numerous genes are transcriptionally activated and repressed in a cell cycle-dependent manner. We have recently reported the global gene expression program during the cell cycle in fission yeast (*S. pombe*). Among the periodically expressed fission yeast genes, a large proportion shows peak transcript levels during mitosis. Many of these genes are regulated by a transcriptional cascade involving two transcription factors: the forkhead protein Sep1p which activates the zinc finger protein Ace2p. A main function of the Sep1p-Ace2p transcriptional pathway is to trigger the separation of daughter cells after cytokinesis. Absence of Sep1p, Ace2p, or some of their target genes leads to a hyphal-like growth pattern with chains of connected cells. Yeast cells probably evolved from filamentous fungi. It is possible that the Sep1p-Ace2p pathway contributed to the emergence of proliferation through single cells, and that this regulatory pathway can still be modulated to adjust growth modes depending on environmental conditions. Here, various properties of the Sep1p-Ace2p transcriptional pathway and mechanisms for cell separation are discussed in the context of recent findings.

Since the discovery that histone transcript levels oscillate as a function of the cell cycle,[1] nearly forty periodically expressed genes have been described in the fission yeast *Schizosaccharomyces pombe*. We have now identified around 400 cell cycle-regulated genes in this yeast through a genome-wide survey using microarrays.[2] Most of the 'action' with respect to phase-specific gene expression occurs during the M and G_1 phases, which together encompass only ~20% of total cell cycle duration in *S. pombe*. At least three transcription factors or complexes are involved in regulating these genes: Sep1p, a protein of the conserved forkhead family[3] regulates genes during M phase, many of which have known functions in mitosis. One of the Sep1p target genes encodes another transcription factor, Ace2p, which then activates a transcriptional wave that closely follows the Sep1p wave, peaking during cell division at the end of mitosis. Another group of genes, mainly functioning in DNA replication, is transcribed at a similar time as the Ace2p wave, but is regulated by the MBF transcription complex independently of both Sep1p and Ace2p.[2]

Cells containing *sep1* or *ace2* deletions are viable but show defects in cell separation after cytokinesis, leading to filamentous growth with multiple cells remaining attached to each other through division septa (Fig. 1A–C).[4,5] The same phenotype is apparent in *sep1 ace2* double deletion mutants (Fig. 1D). Thus, transcriptional regulation by Sep1p and Ace2p seems most critical for a late step in cell division at the end of the cell cycle. Accordingly, inhibition of protein synthesis by addition of cycloheximide at the G_2/M transition does not interfere with mitosis in *S. pombe*, but new protein synthesis is essential for cell separation.[6,7] This suggests that upon initiation of mitosis, all the proteins required for nuclear division are already present in sufficient amounts. Both Sep1p- and Ace2p-dependent genes show residual weak periodicity in expression levels even in the absence of Sep1p,[2] implicating additional regulators that control these genes. Recent data indicate that a second transcription factor of the forkhead family, Fkh2p, is also involved in transcriptional control during mitosis in *S. pombe*.[2,8,9] Unlike Sep1p, Fkh2p is required for various cell cycle processes, including mitotic functions. Intriguingly, Fkh2p also appears to play a negative role in transcription of Sep1p-dependent genes: these genes are more highly expressed in *fkh2* deletion mutants and show constitutive high expression levels throughout the cell cycle.[2,8] It should be interesting to further explore whether and how Sep1p and Fkh2p interact with each other to bring about periodic expression of genes during mitosis.

Two of the Ace2p target genes encode proteins with known roles in cell separation: (1) the β-glucanase Eng1p that degrades the primary division septum between the new ends of daughter cells,[5] and (2) the α-glucanase Agn1p that hydrolyses the old cell wall surrounding the septum leading to full separation of daughter cells.[10] Cells that constitutively overexpress *ace2* become round (Fig. 1E) and show high transcript levels for both *eng1* and *agn1*.[2] The round shape of these cells could reflect a weakening of cell wall material that is not associated with the division septum, caused by an overdose of glucanases. The *S. pombe* exocyst complex is essential for cell sparation[11] and may function in localized secretion of the glucanases. It is possible that proteins of the septin family[12] function in spatially defining appropriate cortex regions to guide the exocyst and glucanase activity. Deletion mutants of septin genes indicate that septins are required for cell separation: they show very similar phenotypes to *sep1* and *ace2* mutants, with multiple cells remaining connected by septa (Fig. 1F).[12,13] Septins form a double ring structure on either side of the division septum[12] that could provide a barrier between different cortex regions. A role in compartmentalization of the cortex during cytokinesis has recently been described for budding yeast septins.[14] Six of the *S. pombe* septin genes are either constitutively expressed during the cell cycle or are only expressed during meiotic differentiation;[2,15] however, one septin gene (*spn7*) contains a promoter motif recognized by forkhead transcription factors, and its transcript peaks during M phase.[2] Moreover, the anillin Mid2p, which is required for septin ring organization and cell separation,[13,16] is a target of Ace2p and shows strong periodicity in expression levels during the cell cycle.[2,13]

In conclusion, the transcriptional cascade from Sep1p to Ace2p defines a regulatory pathway at the end of the cell cycle, which is required for cell separation. Transcriptional activation of Ace2p by Sep1p is crucial for Ace2p regulation as indicated by the similar phenotypes and overlapping expression signatures of *sep1* and *ace2* mutants, which is true for both single and double mutants (Fig. 1B–D).[2] Sep1p itself is probably regulated by a post-transcriptional mechanism, because its transcript shows no periodicity during the cell cycle.[2,8] Mutants in some genes encoding various components of the transcription machinery are also defective in cell separation and show expression profiles similar to *sep1* mutants,[17] suggesting that 'general' transcription factors can have specialized roles in mediating gene expression that is controlled by the Sep1p-Ace2p transcriptional cascade.

Intriguingly, multi-cellular filamentous structures similar to those seen in *sep1* and *ace2* mutants have been observed in the 19th century by P. Lindner who first described *S. pombe* (Fig. 1).[18] The *S. pombe* strain 132, which is rarely used in the laboratory, grows without cell separation similar to *sep1* and *ace2* mutants under particular culture conditions.[19] Yeasts probably evolved from filamentous fungi,[20] and it is possible that the Sep1p-Ace2p pathway can be modulated in wild *S. pombe* strains to adjust to external factors. Interestingly, the Ace2p target gene *mid2* encodes a protein similar to *Candida albicans* Int1p that is crucial for hyphal morphogenesis and interacts with septins as does Mid2p.[15,16,21,22] Under conditions of starvation, it may be advantageous to grow as a multi-cellular filamentous organism to efficiently forage for nutrients, whereas proliferation as single cells is more efficient in times of plenty.[23] The switch to a filamentous form also plays a crucial role for pathogenesis in *Candida*.[23] It seems possible that the ability to grow as a filamentous fungus has been lost in the *S. pombe* strain propagated for generations in the laboratory under conditions of rich nutrients. However, a novel differentiated state of *S. pombe* has recently been identified that depends on the cAMP signalling pathway and makes cells invade solid medium (Armstrong J, personal communication). While *sep1* mutants are not able to develop hyphae that penetrate solid medium to form a true mycelium, it is possible that downregulation of the Sep1p-Ace2p pathway provides a critical step in this differentiation (ensuring that cells remain attached to each other), while other regulatory inputs such as the cAMP pathway then function in directing hyphal growth towards nutrients. The dimorphic fission yeast *S. japonicus*, a relative of *S. pombe*, has a relatively unstable yeast phase and easily switches to a hyphal phase on solid medium.[24] The transition from unicellular to multicellular growth modes could give a glimpse into the evolutionary past of fission yeast. The Sep1p-Ace2p transcriptional pathway may have been instrumental in the development of the yeast form and proliferation through single cells.

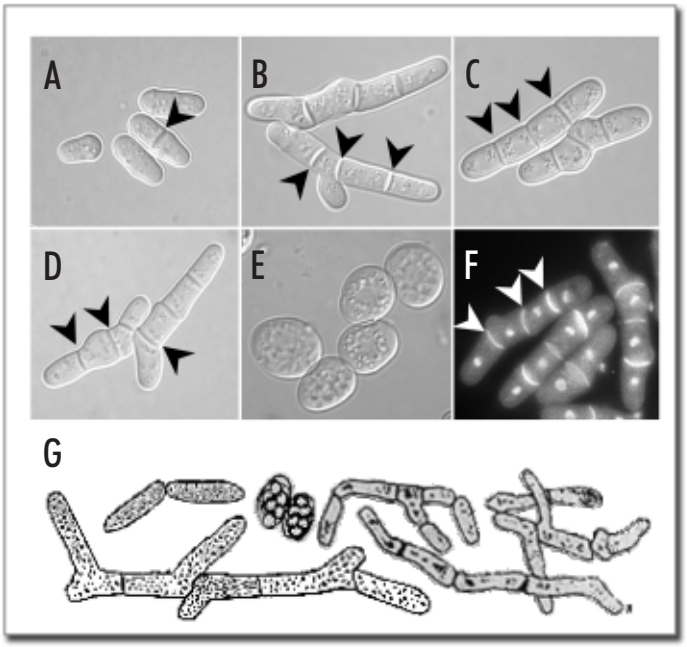

Figure 1. Microscopic images of (A) wild type cells; (B) a deletion mutant in *sep1*; (C) a deletion mutant in *ace2*; (D) a *sep1 ace2* double deletion mutant; (E) cells constitutively overexpressing *ace2*; and (F) a *spn1 spn2 spn3 spn4 spn5* quintuple deletion mutant. Some examples of division septa are indicated by arrowheads. Photographs by G. Rustici (DIC micrographs; A–E) and J.B. (fluorescence micrograph; F). (G) Drawings of *S. pombe* cells showing filamentous growth patterns. Reproduced from the first description of this yeast species.[18]

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