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mRNA Modulation and Gene Expression in Yeast

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Immediately after transcription in the nucleus, eukaryotic mRNAs form complexes (mRNP) with RNA binding proteins that affect RNA processing, transport, translation, stability and localization (Hentze et al., 2000). For mRNAs to be actively translated in cytoplasm, a variety of mRNA modulation processes are required. These include unwinding of the secondary structures at the 5'UTR, association with translation initiation factors, modulation of 5' cap structure, etc (Fuller-Pace, 1994; Wilhelm and Vale, 1993; Mathews et al., 1996). Selective translation control of mRNA subsets in response to biological stimuli or during differentiation would employ these mRNA modulation mechanisms. Our current study reveals a certain aspect of this mRNA modulation mechanism during the yeast MAP kinase-associated processes.

In the yeast *Saccharomyces cerevisiae*, the mating-specific MAP kinase cascade, composed of MEKK (*STE11*), MEK (*STE7*), and MAPK (*FUS3/KSS1*) are required for both mating processes and pseudohyphal development (Liu, *et al.*, 1993; Madhani and Fink, 1997). The transcription factor, Ste12p, is a downstream component of this cascade and is required for the expression of both filamentation-specific and mating-specific genes. Pathway specificity at the terminal gene expressions was found to require a second transcription factor. During filamentation and invasion, a transcription factor, Tec1p, forms heterodimers with Ste12p to drive transcriptions of *TEC1* itself and filamentation-specific targets such as the cell surface flocculin *FLO11* (Lo, *et al.*, 1998; Madhani, *et al.*, 1997).

During mating, a mating pheromone induction leads to the cell fusion of opposite mating types, and this is followed by nuclear fusion, called karyogamy (Byers, 1981; Marsh, *et al.*, 1997). Nuclear fusion as well as cell fusion are induced by mating pheromones (Curran, *et al.*, 1986; Marsh, *et al.*, 1997; Rose, *et al.*, 1986). Kar4p is a karyogamy-specific transcription factor that assists Ste12p in the pheromone-induced transcription of *KAR3* and *CIK1* (Beh et al. 1997; Kurihara et al. 1996; Meluh and Rose 1990; Page and Snyder 1992).

During the study of these MAP kinase-driven processes, we have identified the 5'-3' exoribonuclease gene, *KEM1* which is involved in RNA turnover, and the RNA helicase genes, *ROK1* and *FAL1*, which unwind the duplex RNA structure (Kim et al., 1990; Song et al., 1995; Park et al., 2002). The *KEM1/XRN1* gene was originally identified because of its functions in microtubule-mediated processes, and is also known to be a major cytoplasmic 5'-3' exoribonuclease gene, which is involved in RNA turnover (Larimer and Stevens, 1990). Mutations in *KEM1* cause a number of phenotypes including a defect in nuclear fusion, a delay in spindle pole body separation, benomyl-hypersensitivity, and RNA turnover defects. We present here the evidence that *KEM1* plays a role in nuclear fusion by affecting the expression of karyogamy-specific genes.

Rok1p synthesis, as well as mating-pheromone induction of Kar3p and Kar4p, is greatly impaired in *kem1* mutant cells. *ROK1* and *KAR4* transcripts are detected at the same level in the *kem1* mutant cells as in the wild-type, indicating that the effects of the *kem1* mutation are not at the transcription level. The *ROK1* gene has an unusually long 5' untranslated region with multiple ATG initiation codons preceeding the *ROK1* ORF. Replacement of this 5'UTR with that of *GAL1* resulted in the *KEM1*-independent expression of *ROK1*. The *KAR4* gene was already known to have two in-frame ATG intitiation codons. We postulate that Kem1p somehow modulates the 5'UTR of target genes and affects their translations in response to the cellular signals.

We also found the evidence that *KEM1* plays a role in filamentous growth (Kim and Kim, 2002). Both haploid invasive growth and diploid pseudohyphal growth were found to be greatly impaired in *kem1* mutant strains. *KEM1* affected the level of *FLO11* transcripts and the expression of the filamentation-associated reporter genes, *Ty1-lacZ* and *FLO11-lacZ*. Suppression analysis implies that *KEM1* possibly functions downstream of the MAP kinase pathway during filamentation.

Our results imply that some of the transcriptionally induced genes are also regulated at the level of translation. We have searched for genes that are post-transcriptionally regulated. Based on the idea that actively translated mRNAs are associated with polysomes, the polysome mRNAs from yeast and hyphal cultures were, analyzed. We identified three *S. cerevisiae* genes, *GPA2*, *STE12*, and *CLN1*, whose mRNAs are activated for translation during the filamentation growth. To analyze the expressions of the newly identified target genes at the protein level, the HA-epitope of 27 amino acids were inserted into these genes. The expression profiles of these epitope-tagged proteins were induced upon the filamentation growth conditions. We are currently investigating the effect of RNA modulating enzymes such as the 5'-3' exoribonuclease and the RNA helicases on the expression of the target genes *GPA2*, *STE12*, and *CLN1*.

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