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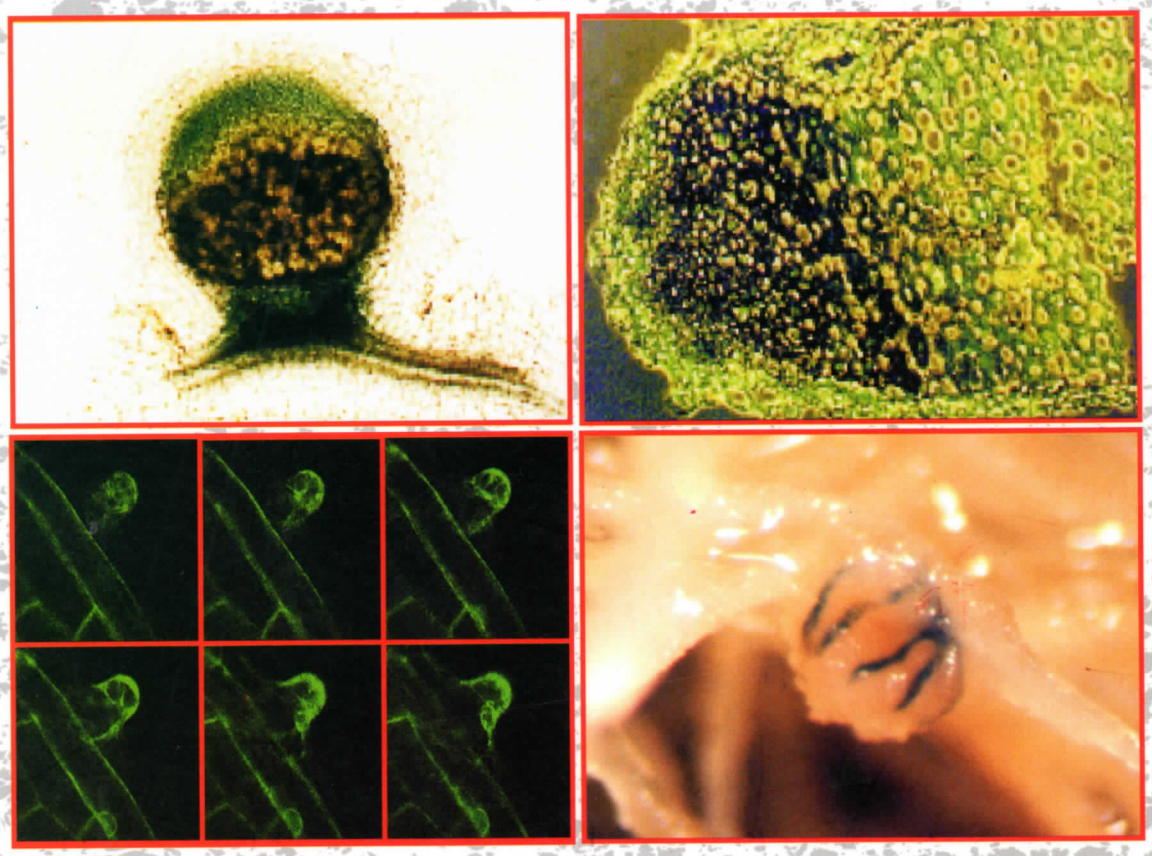

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NITROGEN REGULATION IN *KLEBSIELLA PNEUMONIAE*: PROTEIN-PROTEIN INTERACTIONS AMONGST NTRB AND NTRC DOMAINS.

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In *Klebsiella pneumoniae*, signal transduction in response to nitrogen availability is mediated by the two-component regulators NtrB and NtrC. NtrB, a bifunctional histidine-kinase, modulates the activity of the response regulator NtrC by phosphorylation. NtrB is a tight dimer capable of switching between opposing kinase and phosphatase activities. This switch, mediated by PII according to the N-status, requires the integrity of the amino-terminus of NtrB, which is not conserved among histidine kinase proteins. The carboxy-terminus of NtrB (transmitter module) posses similarity to other histidine kinases and has been shown to be responsible for NtrC regulation. Function of NtrC as a transcriptional activator depends on phosphorylation of its N-terminal domain by NtrB.
To contribute to the understanding of signal transduction by NtrB and NtrC proteins, we have used the yeast two-hybrid system to probe interactions between full length and individual domains of NtrB and NtrC from *Klebsiella pneumoniae*. Protein fusions of NtrB, NtrC, and derived polypeptides to GAL4 activation and DNA-binding domains were generated. Domain boundaries for NtrB truncated derivatives were chosen to separate sensor (S), phosphotransfer (H), N box (N) and kinase (G) domains. Domain boundaries for NtrC truncated derivatives separate receiver (R), catalytic (C), and DNA-binding (D) domains. To determine the ability of two given polypeptides to interact, we determined expression of both *GAL1:lacZ* and *GAL1:HIS3* reporters in strains of *Saccharomyces cerevisiae* Y190.
Results confirm previous data on NtrC association states and indicate that the sensor domain of NtrB provides the dimerisation interface, which is at variance with results in other systems. In addition, our data indicate a strong interaction between NtrB and NtrC, which maps to the phosphotransfer domain of NtrB and to the receiver domain of NtrC, indicating that recognition specificity in this two-component system does not involve interactions between surfaces outside the transmitter and receiver domains. In addition, the data suggest that dimerisation of the receiver domain is important for transmitter recognition.