FTSZ COUPLES CELL DIVISION TO NUCLEOID PARTITIONING IN A PROKARYOTIC CELL

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FtsZ protein is required for the earliest detectable step of septum formation in eubacteria. It is also the target of several regulators, such as MinC, involved in proper placement of the division site, and SfiA, which prevents division from taking place when replication is halted. We have characterized a 53 nt prophage-encoded division inhibitor, DicF-RNA, and demonstrated that it regulates ftsZ translation by an antisense mechanism (1). This has allowed us to investigate the consequences of a sublethal inhibition of ftsZ gene expression on cell cycle parameters in E. coli (2). Underexpression of ftsZ led to delayed constriction initiation. Surprisingly, we found that nucleoid separation (partition) is similarly delayed when FtsZ is underproduced. Since it was previously shown that FtsZ is not needed for partition, we hypothesized that active FtsZ inhibits partition before the time of division. In agreement with this view, we observed that if de novo synthesis of FtsZ is blocked, partition is prevented at first, and later resumes as FtsZ is further diluted into the elongating filaments. We conclude that FtsZ prevents nucleoid separation during the G2-like period when it has not yet reached a level sufficient to trigger division. This provides an effective means of coupling septation to nucleoid separation. The shift from a partition-inhibiting, septation-deficient, to the partition-permissive, septation-triggering state may rest on post-translational modifications, or on the formation of a septum-associated ring structure of FtsZ (3).

(1) TETART F and BOUCHE J-P (1992) Mol. Microbiol. 6: 615-620.
(2) TETART F, ALBIGOT R, CONTER A, MULDER E, and BOUCHE J-P (1992) Mol. Microbiol. 6: 621-627.
(3) BI E and LUTKENHAUS J (1991) Nature 354: 161-164.

FLUID PHASE ENDOCYTOSIS AND CELL CYCLE ; A STUDY WITH THE FLUORESCENT PROBE TMA-DPH

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Adherent L929 mouse fibroblasts were synchronized by double thymidine block and the phases enrichment controlled by flow cytometry with the fluorescent DNA marker 7-amino-actinomycin D (1). Fluid phase endocytosis was monitored by fluorescence with TMA-DPH (2). In interaction with cells, this probe is rapidly incorporated into the plasma membrane and then follows its internalization-recycling traffic. Besides, thanks to a water (probe not fluorescent) - membrane (fluorescent) partition, a simple cell washing suppresses the peripheral labelling, allowing to quantify the internalized membrane fraction. The results exhibit marked differences between S, G1 and G2 phases. Mitosis could not be studied.

1. RABINOVITCH P.S., TORRES, R.M. & ENGEL D. (1986) *J. Immunol.*, 136, 2769-2775.
2. ILLINGER D., FONTENEAU P., POINDRON Ph., MODOLLEL M. & KUHRY J.G. (1990) *Biochim. Biophys. Acta*, 1030, 73-81.

EVIDENCES FOR AUTOCRINE GROWTH STIMULATION BY A GASTRIN/CCK-LIKE PEPTIDE OF THE GASTRIC CANCER HGT-1 CELL LINE.

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Gastrin has been shown to promote the growth of some colonic tumor cell lines. To evaluate the involvement of this hormone in the proliferation of gastric tumors, we studied the effects of gastrin/CCK-receptor antagonists (L-365,260, L-364,718) and C-terminal-specific gastrin antiserum on the human gastric adenocarcinoma cell line HGT-1. L-365,260, but not L-364,718, dose-dependently inhibited cell proliferation evaluated either by cell counting or by [^{3}H]thymidine incorporation (72% after 4 days at 10 nM L365,260). Similarly, the C-terminal-specific gastrin antiserum, even at low concentration, caused a dramatic decrease in both cell number (IC_{50}= 1:4000 antiserum dilution) and [^{3}H]thymidine incorporation (IC_{50}= 1:400 antiserum dilution) in the HGT-1 cell line. In addition, immunofluorescence data revealed that these antibodies specifically label HGT-1 cells. In contrast, gastrin ([Nle^{15}]HG-17) did not induce cell proliferation or [^{3}H]thymidine incorporation at any concentration tested and no high affinity gastrin binding sites were evidenced on this cell line. These data strongly support the hypothesis of the presence in the surrounding of the cell of a gastrin/CCK-like peptide which is involved in autocrine regulation of this cell line.

ACTIVATION OF MAP-KINASE AND p34^{cdc2}-H1-KINASE BY ONCOGENIC RAS p21 IN XENOPUS OOCYTES.

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Injection of oncogenic lys12-Ras into prophase-arrested *Xenopus laevis* oocytes promoted the activation of Mitogen-Activated-Protein kinase (MAP-kinase) after about 1 h 30 lag. MAP-kinase activity was 10-fold higher 4 h after injection of oncogenic lys12-Ras than after injection of non-oncogenic gly12-Ras. The stimulated MAP-kinase activity remained at a plateau for at least 18 h. Maximal stimulation was obtained with 5 ng of lys12-Ras. MAP-kinase activation by lys12-Ras was associated with tyrosine phosphorylation of p42 related to MAP-kinase.

5 ng of lys12-Ras promoted 50% germinal vesicle breakdown (GVBD) 8h postinjection. p34^{cdc2}-H1-kinase activity remains unchanged 4 h after Ras injection whereas MAP kinase activity is markedly increased. This suggest that MAP-kinase activation did not result from p34^{cdc2}-H1-kinase action since maximum H1-kinase activation was detected much later than MAP-kinase activation.

Lys12-Ras with an additional mutation (glu38) in the effector region that binds GTPase-activating-protein (GAP), promoted neither MAP-kinase and H1-kinase activation nor GVBD.

Our results indicate that Ras-GAP complex promotes MAP-kinase and H1-kinase activation in oocytes.