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TRANSCRIPTIONAL PROMOTERS IN A REPLICATION  
REGION OF F PLASMID

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##### ORIGIN REGION

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## ABSTRACT

This thesis describes aspects of genetic regulation within and near a replication origin (ori-1) of the F plasmid. A number of transcriptional promoters were isolated, precisely mapped, and characterized with respect to their strengths and modes of regulation. The principal techniques employed in these investigations were: "shotgun" molecular cloning of restriction fragments into a galactokinase-based promoter selection vector, assays for galactokinase activities, DNA sequencing and S1 nuclease mapping of transcripts. Major findings from this study can be summarized as follows:

- 1). Promoters for the essential replication genes pifC and E were cloned and shown to be autoregulated at the transcriptional level.
- 2). An E.coli protein, integration host factor (IHF), was found to modulate the activity of the pif operon promoter.
- 3). Two promoters which direct transcription in opposite directions from within the minimal ori-1 region were discovered.
- 4). Transcription from both ori-1 promoters was shown to be repressed by the mini-F encoded D protein.
- 5). Precise transcriptional startsites of the pifC gene and the two ori-1 promoters were determined.
- 6). A mini-F protein (D) was shown to resolve dimers of a plasmid which contains a site-specific recombination locus from near ori-1, and a facile assay system for this function was developed.

## ABBREVIATIONS

|       |  |
|-------|--|
| Amp   | ampicillin                               |
| ATP   | adenosine triphosphate                   |
| BCIG  | 5-bromo-4-chloro-3-indolyl-B-galactoside |
| bp    | basepair(s)                              |
| BSA   | bovine serum albumin                     |
| °C    | degrees Celsius                          |
| CCC   | covalently-closed circular               |
| cpm   | counts per minute                        |
| Cm    | chloramphenicol                          |
| cm    | centimetre                               |
| DBM   | diazobenzyloxymethyl                     |
| dATP  | 2'-deoxyadenosine 5'-triphosphate        |
| dCTP  | 2'-deoxycytidine 5'-triphosphate         |
| dGTP  | 2'-deoxyguanosine 5-triphosphate         |
| ddNTP | dideoxynucleoside triphosphate           |
| dNTP  | deoxynucleoside triphosphate             |
| DNA   | deoxyribonucleic acids                   |
| dpm   | disintegrations per minute               |
| DTT   | dithiothreitol                           |
| dTTP  | thymidine 5'-triphosphate                |
| EDTA  | ethylene diamine tetraacetic acid        |
| EtBr  | ethidium bromide                         |
| GalK  | galactokinase                            |
| IPTG  | isopropyl-B-D-thio-galactopyranoside     |
| Kan   | kanamycin                                |
| kb    | 1000 base pairs                          |
| kCal  | kilocalorie                              |

|       |  |
|-------|--|
| kd    | kilodalton                                   |
| kPa   | kilopascal                                   |
| LB    | Luria broth                                  |
| M     | moles per litre                              |
| mA    | milliampere                                  |
| mg    | milligram                                    |
| ml    | millilitre                                   |
| mM    | millimolar                                   |
| mm    | millimetre                                   |
| ng    | nanogram                                     |
| nm    | nanometre                                    |
| O.D.  | optical density (absorbance)                 |
| PIPES | piperazine-N-N'bis [2-ethane sulphonic acid] |
| RNA   | ribonucleic acids                            |
| rpm   | revolutions per minute                       |
| SDS   | sodium dodecyl sulphate                      |
| Sm    | streptomycin                                 |
| SSC   | saline sodium-citrate                        |
| TBE   | Tris-borate-EDTA electrophoresis buffer      |
| Tc    | tetracycline                                 |
| TCA   | trichloroacetic acid                         |
| TE    | 10mM Tris (pH 8.0), 1mM EDTA                 |
| Tris  | Tris (hydroxymethyl) aminomethane            |
| uCi   | microcurie                                   |
| ug    | microgram                                    |
| ul    | microlitre                                   |
| V     | volt   |
| wt    | wild type                                    |
| YT    | yeast-tryptone broth                         |