J. Biosci., Vol. 9, Numbers 3 & 4, December 1985, pp. 213–221. © Printed in India.

Conditional rifampicin sensitivity of a *rif* mutant of *Escherichia coli*: rifampicin induced changes in transcription specificity

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MS received 24 June 1985

Abstract. A *rif* mutant *of Escherichia coli* that exhibits medium and temperature-dependent sensitivity to rifampicin is described. In the absence of rifampicin, this strain grows in minimal and rich media at 30°C and 42°C. In its presence it is viable in rich medium at both temperatures, but in minimal medium only at 30°C. In minimal-rifampicin medium at the higher temperature, RNA synthesis is decreased. The addition of certain divalent salts ($MgSO_4$, $CaCl_2$, $BaCl_2$) in excess, or chelators (EDTA, EGTA, *o*-phenanthrolein) greatly increase viability in minimal-rifampicin medium at 42°C. Excess $MgSO_4$ (10 mM) also increases the rate of RNA synthesis in the same medium. A model is proposed wherein the *rif* mutation is suggested to cause a structural change in RNA polymerase that allows the binding of rifampicin and other ligands at 42°C. Rifampicin-binding is suggested to alter the conformation of RNA polymerase, impairing its ability to express genes required for growth in minimal medium. Implicit in this view is the assumption that these genes are structurally different from those expressed in rich medium in respect of certain template features recognized by RNA polymerase.

Keywords. Transcription; transcription specificity; rifampicin.

Introduction

Earlier reports from our laboratory described the isolation, genetic mapping and physiological characterisation of a temperature-sensitive transcription mutant of *Escherichia coli* in which the mutation mapped at a locus different from the loci coding for the subunits of RNA polymerase (Jabbar and Jayaraman, 1976,1978; Jabbar, 1979; Jayaraman and Jabbar, 1980). It was suggested that the product of the locus could be an accessory transcription factor. In an attempt to obtain genetic evidence in support of this notion Dass and Jayaraman (1985) isolated a rifampicin-resistant (*rif*), temperature-insensitive derivative from a mutant which originally harboured the temperature-sensitive mutation. Genetic analysis of this derivative (Dass and Jayaraman, 1985) showed (i) the *rif* mutation was not responsible for the temperature-insensitive phenotype of this derivative and (ii) a second mutation mapping in the same gene suppressed the *ts* phenotype of the original mutation. The second mutation, by itself, conferred temperature-sensitivity and caused defects in [^{3}H]-uridine incorporation into RNA (Dass and Jayaraman, 1985). Dass and Jayaraman (1985) mapped the

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locus precisely in relation to other markers in the vicinity and also called the locus *fit* (factor involved in transcription). The present report describes some properties of the *rif* mutation present in the temperature-insensitive derivative. The results suggest that this mutation leads to changes in transcription specificity under certain conditions.

Materials and methods

Media and chemicals

MA minimal medium and LB nutrient medium were used for all experiments (Miller, 1972). Additional salts were used at the indicated concentrations. Chelators were added at 0·1 mM, and rifampicin at 100 μg/ml. Rifampicin and *o*-phenanthrolein were from Sigma Chemical Co., St. Louis, Missouri, USA, while all other chemicals were from local sources. [^{3}H]-Uridine was from Bhabha Atomic Research Centre, Bombay.

Bacterial strains

The bacterial strains used in this study are listed in table 1.

Table 1. List of *E. coli* strains used in this work.

Strain	Relevant genotype	Source/construction
KL16	Hfr *rel*A	B. J. Bachmann
GMS343	F$^-$ *aro*D *arg*E *rps*L	B. J. Bachmann
CSH57	F$^-$ *his trp pur*E *leu met*A *ilv*D *rps*L *arg*G	Cold Spring Harbor collection
BJ240	F$^-$ *his trp pur*E *leu met*A *arg*G *rps*L *fit*76-*fit*24 *rif* (*rpo*B240)	Derivative of CSH57; Dass and Jayaraman (1985)
BJ5701	F$^-$ *his trp pur*E *leu met*A *rps*L *arg*G *rif* (*rpo*B)	Spontaneous Rif^r mutant of CSH57
BJ5702	F$^-$ *his trp pur*E *leu rps*L *arg*G *rpo*B240	Met$^+$ Rif^r derivative of CSH57 obtained from the cross P1/BJ240 × CSH57

Methods

P1 transductions were done as described by Miller (1972). For studying RNA synthesis, cells were grown to mid-log phase at 42°C in minimal medium, with or without rifampicin. Cultures were adjusted to the same turbidity and labelled with [^{3}H]-uridine (12·7 Ci/mmol; 1 μ Ci/ml). At intervals, during a period of 30 min, samples were treated with ice-cold 10 % trichloroacetic acid containing 100 μ g/ml uridine, filtered on membrane filters, washed and counted in a Beckman Liquid Scintillation Counter. Incorporation was linear with time.

Results

*Mapping of the rif mutation in BJ*240

This paper describes the properties of the *rif* mutation present in the strain BJ240 described earlier (Dass and Jayaraman, 1985). First of all, it was necessary to ensure that this mutation maps at the *rpo*B locus (90 min; Bachmann, 1983) as almost all *rif.* mutations do (Yura and Ishihama, 1979). Data presented in table 2 show that the *rif* mutation in BJ240 is cotransducible with *arg*E or *met*A at approximately the same frequency (50 %) which would be expected of mutations in the *rpo*B locus. It can therefore be concluded that the *rif* mutation under study lies in *rpo*B. Accordingly it was designated as *rpo*B240.

Table 2. Transductional mapping of the *rif* mutation in BJ240.

Cross	Donor	Recipient	Selected marker	Unselected marker	Cotransduction frequency (%)
1	BJ240 (*rif*)	GMS343 (*arg*E)	*arg*E$^+$	*rif*	49 (78/159)
2	KL16 (*rif*$^+$)	BJ240 (*met*A *rif*)	*met*$^+$	*rif*$^+$	47 (435/918)

rif—Resistance to rifampicin.
rif$^-$ —Sensitivity to rifampicin.

Viability of BJ 5702

The *rpo*B240 mutation was transduced into an *rpo*B$^+$ strain, CSH57. The resultant transductant, BJ5702, was used for the studies described below. Viability of BJ5702 under different conditions was compared with that of its parent (CSH57) and BJ5701 (a spontaneous *rif* mutant of CSH57). It can be seen from table 3 that in the absence of rifampicin, BJ5702 behaves just like CSH57 and BJ5701. In LB-rifampicin medium, BJ5702 was fully viable at 30°C and 42°C. In minimal-rifampicin medium, however,

Table 3. Viable colony counts of the strains in the presence and absence of rifampicin at 30°C and 42°C in LB and minimal media.

	Minimal medium				LB medium			
	− Rifampicin		+ Rifampicin		− Rifampicin		+ Rifampicin	
Strain	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C
CSH57 (*rpo*B$^+$)	100	100	10^{-7}	10^{-7}	105	108	10^{-6}	10^{-6}
BJ5701 (*rif*)	100	97	94	103	103	99	101	100
BJ5702 (*rpo*B240)	100	101	99	5×10^{-2}	99	98	107	86

Viable counts were normalized to the number (taken as 100) on rifampicin-free minimal plates at 30°.

colony forming ability at 30°C was not affected but viability at 42°C dropped drastically. The few colonies that appeared at the higher temperature (at a frequency of approximately 5×10^{-4}) continued to be similarly non-viable on subsequent plating on minimal-rifampicin plates at 42°C (data not shown). There is thus a medium and temperature-dependent switch in the phenotype of BJ5702 from rifampicin resistance to sensitivity.

*RNA synthesis in BJ*5702

Is the non-viability of BJ5702 under the above conditions a reflection of the sensitivity of transcription to rifampicin? When a culture of the mutant was inoculated in minimal-rifampicin medium at 42°C to an initial density of about 10^7 cells/ml (as is usually done), the culture grew as well as it did in the absence of rifampicin (data not shown). This was in seeming contradiction to the results presented above. In liquid medium, under the usual culture conditions, cells undergo not more than 9 divisions before cessation of growth. On the other hand, a colony on a plate arises from a single cell and is the result ofat least 24 cell divisions. It was therefore decided to increase the duration of growth of BJ5702 in liquid medium such as to mimic growth conditions on plates. The strain was subcultured in rifampicin-minimal medium at 42°C to an initial cell density of 5×10^2 cells/ml. The culture did grow, but at a decreased rate and ceased growth at a smaller cell density, relative to the control (no rifampicin; data not shown). It is not clear how cells that are largely non-viable on plates can grow in liquid medium. A possible explanation is that initially cells grow normally at 42°C. With increasing time of exposure to rifampicin they slacken in their growth rate and ultimately cease to grow altogether, but nonetheless, allowing a small random population to escape the inhibitory action of the antibiotic.

[^{3}H]-Uridine incorporation was studied in cultures grown at 42°C from an initial density of 10^7 cells/ml. There was no difference in incorporation in the presence or absence of rifampicin (table 4). Thus under the usual experimental conditions, RNA synthesis in BJ5702 is resistant to inhibition by rifampicin. This result (coupled with the fact that BJ5702 is viable on LB-rifampicin plates) almost certainly demonstrates that RNA polymerase in this strain is a rifampicin-resistant enzyme. [Under the same conditions, the *rpo*B$^+$ enzyme of the wild-type strain CSH57 is sensitive to rifampicin (table 4)]. BJ5702 was next grown from an initial density of about 5×10^2 cells/ml. In the culture grown in the presence of rifampicin, incorporation was considerably less (table 4). An implicit assumption in correlating [^{3}H]-uridine incorporation and

Table 4. Incorporation of [^{3}H]-uridine at 42°C in cultures grown from different initial densities in the presence and absence of rifampicin.

Strain	Initial cell density (cells/ml)	Incorporation/30 min (cpm)	
		−Rifampicin	+Rifampicin
CSH57 (*rpo*B$^+$)	10^7	3223	105
BJ5702 (*rpo*B240)	10^7	2552	2640
BJ5702 (*rpo*B240)	5×10^2	2287	1212

viability is that incorporation represents all the species of RNA needed for growth under a given set of conditions. This need not necessarily be true with BJ5702 labelled at 42°C in the presence of rifampicin. There could be qualitative differences between RNA synthesized in minimal medium at 42°C in the presence and absence of the antibiotic. Such differences will not show up markedly in labelling experiments, but could be seen as loss of viability. Colony forming ability is therefore a more reliable index of rifampicin-sensitivity/resistance than the rate of [3H] -uridine incorporation.

Effect of cations on rifampicin-sensitivity

Is BJ5702 'conditionally auxotrophic' in minimal-rifampicin medium at 42°C? Addition of various amino acids in different combinations failed to reverse the phenotype of this strain (data not shown). We also wondered if changes in the composition or concentration of media components could reverse its phenotype to rifampicin-resistance. During the course of experiments designed to test this view, it was observed that the addition of excess $MgSO_4$ to minimal-rifampicin medium increased the viability of BJ5702. This observation was followed up by studying the effect of a number of divalent cations. These were: Zn^{2+} (an innate constituent of RNA polymerase); Mg^{2+} (essential for the activity of RNA polymerase); Mn^{2+} (which can substitute for Mg^{2+} in certain transcription reactions); Ca^{2+} (a physiologically important ion) and Ba^{2+} (a randomly chosen ion). Salts of these divalent cations were added to minimal-rifampicin medium at 42°C to test their effect on the viability of BJ5702. It is apparent from table 5 that while Zn S O_4 had no effect and $MnCl_2$ a partial effect, $MgSO_4$, $BaCl_2$ and $CaCl_2$ greatly reversed the rifampicin-sensitivity of this strain.

Table 5. Effect of divalent salts on the viability of BJ5702 in minimal medium at 42°C with and without rifampicin.

	−Salt		+Salt (0·1 mM)		+Salt (1 mM)		+Salt (10 mM)	
Salt	−Rif	+Rif	−Rif	+Rif	−Rif	+Rif	−Rif	+Rif
$ZnSO_4$	100	4×10^{-2}	N.D	N.D	103	7×10^{-2}	99	4×10^{-2}
$MgSO_4$	100	5×10^{-2}	N.D	N.D	97	4×10^{-2}	101	35
$MnCl_2$	100	8×10^{-2}	N.D	N.D	98	3×10^{-2}	98	3
$CaCl_2$	100	4×10^{-2}	102	3×10^{-2}	105	32	N.D	N.D
$BaCl_2$	100	2×10^{-2}	N.D	N.D	100	5×10^{-2}	105	83

Colony counts normalized to the number (taken as l00) on plates without added salt and rifampicin. N. D. —Not determined.

One possibility was that excess of salts/ions in the medium limited the effective concentration of rifampicin per cell, perhaps by complexing with the antibiotic, or by altering the permeability of cells to rifampicin. If this were true, a wild type (*rpo*B+) strain should be less sensitive to rifampicin when $MgSO_4$ is added in excess. However,

the viability of CSH57 was inhibited by rifampicin to the same extent irrespective of the addition of 10 mM $MgSO_4$ (data not shown).

The possible effect of excess $MgSO_4$ on RNA synthesis in BJ5702 was studied. Cells were grown at 42°C, starting from an initial density of about 5×10^2 cells/ml. The culture grown in the presence of rifampicin and 10 mM $MgSO_4$ incorporated more [^{3}H]-uridine (1·5 fold) than the culture grown in rifampicin, but without excess $MgSO_4$. Thus the ability of BJ5702 to grow on rifampicin-plates in the presence of $MgSO_4$ can be accounted for, at least in part, by a positive effect of Mg^{2+} on transcription. In line with the conclusions drawn earlier, Mg^{2+} (and possibly other divalent cations) perhaps delays or prevents an eventual transition of cells to rifampicin-sensitivity in minimal medium at 42°C, allowing for a greater fraction to undergo sufficient divisions and foram colonies.

*Influence of chelators on viability of BJ*5702

If divalent cations can reverse the phenotype of BJ5702 to rifampicin-resistance, would the addition of ion-chelators increase the sensitivity of this strain? Three commonly used chelators were chosen, namely, EDTA, EGTA and *o*-phenanthrolein. These were added to minimal-rifampicin medium at a non-lethal concentration of 0·1 mM. All the 3 chelators greatly restored the viability of BJ5702 in minimal-rifampicin medium at 42°C (table 6).

Table 6. Influence of chelators on the viability of BJ5702 in minimal-rifampicin medium at 42°C.

−Chelator			+EDTA		+EGTA		+*o*-phenanthrolein	
−Rif	+Rif	+Rif+ $MgSO_4$ (10 mM)	−Rif	+Rif	−Rif	+Rif	−Rif	+Rif
100	3×10^{-2}	51	96	29	98	32	95	23

Normalized to the number (taken as 100) on plates without added chelator and rifampicin. Chelators were added to a final concentration of 0·1 mM.

Discussion

We have described in this paper some properties of a strain harbouring a particular *rif* mutation, *rpo*B240. This mutant is conditionally sensitive to rifampicin, depending on the medium, temperature, and the presence/absence of (excess) divalent cations, or metal chelators. Such behaviour can be partially correlated with the pattern of RNA synthesis. Our results can be rationalized by proposing specific conformational changes in the *rpo*B240 RNA polymerase as a result of temperature, and the binding rifampicin, ions, or chelators to the enzyme. There is extensive evidence to suggest that the conformation of RNA polymerase is of crucial importance in transcription (Travers *et al.,* 1981). Mutations in the *rpo*B (*rif*) gene affect promotor selection and termination (Yura and Ishihama, 1979), possibly by altering the conformation of the polymerase

(Ovchinnikov *et al.,* 1983). Conformational changes in the enzyme attendant on rifampicin binding have been proposed to be of importance in its activity (Krakow *et al.,* 1976; Nakamura and Yura, 1976; Doi, 1977; Reisbig *et al.,* 1982). Current evidence suggests that rifampicin may bind to RNA polymerase from *rif* mutants also. Some of these mutants are sensitive to derivatives of the anitibiotic. These derivatives act on the *rif* Polymerase by a mechanism similar to that of rifampicin on the wild-type enzyme, possibly through interaction with the same enzyme site (Nikiforov *et al.,* 1982). Metal ions like Mg^{2+} and Mn^{2+} bind to the substrate-binding site (Goldthwait *et al.,* 1970) located in the *ß*-subunit (Armstrong *et al.,* 1976) and bring about structural changes in the enzyme (Krakow *et al.,* 1976). A Co^{2+}-containing RNA polymerase shows alterations in promotor recognition properties with T7 and *λ plac*5 templates (Speckhard *et al.,* 1977). Various lines of evidence, including enzyme reconstitution studies, suggest that Zn^{2+} in RNA polymerase could be involved in maintaining a required conformation (Krakow *et al.,* 1976; Zillig *et al.,* 1976). Chelators like *o*-phenanthrolein and picolinic acid are believed to inhibit transcription (specifically at the initiation step) by forming a ternary complex with zinc in RNA polymerase (Scrutton *et al.,* 1971; Speckhard *et al.,* 1977; Collins *et al.,* 1979). During a period of transient growth inhibition by chelators, it has been proposed that the proteins synthesized are quantitatively and qualitatively different from those made during normal growth (Collins *et al.,* 1979).

We propose a model that may be quite consistent with the above reports. The *rpo*B240 mutation is suggested to cause a change in RNA polymerase such that it is conformationally altered, but not functionally inactivated, at the higher temperature. Such altered topology allows the binding of rifampicin and other ligands like metal ions and chelators to the enzyme. The rifampicin-bound polymerase is affected in transcription specificity such that it fails to express at 42°C the genes required for growth in minimal medium. Metal ions and chelators possibly revert the conformation of the polymerase-rifampicin complex to one that can express these genes.

An implicit assumption of this model is that genes expressed in minimal medium and those expressed in rich medium are not only functionally different (anabolic *versus* catabolic), but also structurally so. The selective transcription of genes has been partly attributed to the extent of supercoiling of different regions of the chromosome (Smith, 1981). The number of such supertwisted template domains and their degree of coiling may depend on, among other things, the medium of growth (Worcel and Burgi, 1972; Sinden and Pettijohn, 1981). The amino acid biosynthetic operons have characteristic structural features that ensure optimal expression in minimal medium and attenuation in rich medium (Kolter and Yanofski, 1982). Several catabolite sensitive operons have a special promotor sequence recognized by the CAMP-CAP complex (Rosenberg and Court, 1979). A number of operons stringently regulated under amino acid starvation have a sequence called the 'discriminator' that sets them apart from other genes (Travers *et al.,* 1981).

It is possible that the 'minimal medium genes' differ from the 'LB medium genes' in respect of certain template features (sequences, degree of coiling etc.) recognized by RNA polymerase. On the other hand, failure of the *rpo*B240 polymerase-rifampicin complex to express just a few genes (as opposed to a whole group or class of genes) in minimal medium could well lead to non-viability. *Rif* mutants that do not grow as a

result of inability to express one or more genes required for the biosynthesis of any one amino acid have been reported (Morishita and Yura, 1976; Ryu and Takayanagi, 1979). However, this is not likely to be the case with BJ5702 since the addition of different amino acids to minimal-rifampicin medium at 42°C did not restore colony forming ability of this strain (see above).

Our model proposing that rifampicin can alter transcription specificity derives further support from experiments reported by many others. Rifampicin may cause activation of promoters and read-through past terminators (Newman *et al.,* 1982; Fukuda and Fujimori, 1983), and affect varied cellular processes (Buchstein and Hinkle, 1982; Mirkin and Shmerling, 1982; Ito and Ohnishi, 1983; Polaczek and Ciesla, 1983). In addition, we have observed that *rpo*B240 mutation, in combination with different *fit* mutations, leads to changes in the expression of a number of gene groups, including phage genomes and the lactose operon, and also alters the viability of strains in minimal medium. These results will be published elsewhere.

Acknowledgements

This work was supported in part by a grant from the University Grants Commission, New Delhi. S.B.D. was the recipient of Research Fellowships from the Indian Council of Medical Research and University Grants Commission, New Delhi.

Re.ferences

Armstrong, V. W., Sternbach, H. and Eckstein, F. (1976) *Biochemistry,* **15,** 2086.
Bachmann, B. J. (1983) *Microbiol. Rev.,* **47,** 180.
Buchstein, S. R. and Hinkle, D. C. (1982) *Mol. Gen. Genet.,* **188,** 211.
Collins, J. J., Adler, C. R., Fernandez-Pol, J. A., Court, D. and Johnson, G. S. (1979) *J. Bacteriol.,* **138,** 923.
Dass, S. B. and Jayaraman, R. (1985) *Mol. Gen. Genet.,* **198,** 299.
Doi, R. H. (1977) *Bacteriol. Rev.,* **41,** 568.
Fukuda, R. and Fujimori, H. N. (1983) *J. Biol. Chem.,* **258,** 2720.
Goldthwait, D. A., Anthony, D. D. and Wu, C. (1970) in *RNA polymerase and transcription* (ed. L. Silvestri) (Amsterdam: North Holland Publishing Co.) p. 10.
Ito, R. and Ohnishi, Y. (1983) *Biochim. Biophys. Acta,* **739,** 27.
Jabbar, M. A. (1979) *Studies on Transcription,* Ph.D, thesis, Madurai Kamaraj University, Madurai.
Jabbar, M. A. and Jayaraman, R. (1976) *Biochem. Biophys. Res. Commun.,* **72,** 1490.
Jabbar, M. A. and Jayaraman, R. (1978) *Mol. Gen. Genet.,* **166,** 211.
Jayaraman, R. and Jabbar, M, A. (1980) in *Molecular Basis of Host- Virus Interaction* (ed. M. Chakravorty) (Princeton: Science Press) p. 515.
Kolter, R. and Yanofsky, C. (1982) *Annu. Rev. Genet.,* **16,** 113.
Krakow, J. S., Rhodes, G. and Jovin, T. M. (1976) in *RNA polymerase* (eds R. Losick and M. Chamberlin) (New York: Cold Spring Harbour Laboratory, Cold Spring Harbour) p. 127.
Miller, J. H. (1972) *Experiments in molecular genetics* (New York: Cold Spring Harbour Laboratory, Cold Spring Harbour).
Mirkin, S. M. and Shmerling, Zh. G. (1982) *Mol. Gen. Genet.,* **188,** 91.
Morishita, T. and Yura, T. (1976) *J. Bacteriol.,* **125,** 416.
Nakamura, Y. and Yura, T. (1976) *Mol. Gen. Genet.,* **145,** 227.
Newman, A. J., Ma, J. C, Howe, K. M., Garner, I. and Hayward, R. S. (1982) *Nucleic Acids Res.,* **10,** 7409.
Nikiforov, V. G., Trapkova, A. I., Gragerov, A. I. and Maslin, D. N. (1982) *FEBS Lett.,* **150,** 416.

Ovchinnikov, Y. A., Monastyraskaya, G. S., Guriev, S. O., Kalinina, N. F., Sverdlov, E. D., Gragerov, A. I., Bass, I. A., Kiver, I. F., Moiseyeva, E. P., Igumnov, V. N., Mindlin, S. Z., Nikiforov, V. G. and Khesin, R. B. (1983) *Mol. Gen. Genet.,* **190,** 344.
Polaczek, P. and Ciesla, Z. (1983) *Mol. Gen. Genet.,* **190,** 326.
Reisbig, R. R., Woody, A.-Y. M, Woody, R. W. (1982) *Biochemistry,* **21,** 196.
Rosenberg, M. and Court, D. (1979) *Annu. Rev. Genet.,* **13,** 319.
Ryu, J. -I. and Takayanagi, S. (1979) *J. Bacteriol.,* **139,** 652.
Scrutton, M. C, Wu, C.-W. and Goldthwait, D. A. (1971) *Proc. Natl. Acad. Sci. USA,* **68,** 2497.
Sinden, R. R. and Pettijohn, D. E. (1981) *Proc. Natl. Acad. Sci. USA,* **78,** 224.
Smith, G. R. (1981) *Cell,* **24,** 599.
Speckhard, D. C, Wu, F. Y. -H. and Wu, C.-W. (1977) *Biochemistry,* **16,** 5228.
Travers, A. A., Kari, C. and Mace, H. A. F. (1981) in *Genetics as a tool in microbiology,* (eds S. W. Gover and D. A. Hopwood) (Cambridge: Cambridge Univ. Press) p. 113.
Worcel, A. and Burgi, E. (1972) *J. Mol. Biol.,* **71,** 127.
Yura, T. and Ishihama, A. (1979) *Annu. Rev. Genet.* **13,** 59.
Zillig, W., Palm, P. and Heil, A. (1976) in *RNA polymerase* (eds R. Losick and M. Chamberlin), (New York: Cold Spring Harbour Laboratory, Cold Spring Harbour) p. 101.