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Original article

The effect of methylation on some biological parameters in *Salmonella enterica* serovar Typhimurium

Effets de la méthylation sur quelques paramètres biologiques de Salmonella enterica *Typhimurium*

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Abstract

Cell growth is tightly coupled to DNA replication and its methylation [Proc Natl Acad Sci U S A 93 (1996) 12206–12211]. In a culture medium, growing of *Salmonella* Typhimurium (*S.* Typhimurium) mutant cells (dam$^-$) decreased (2.5 fold) relative to the wild type strain (dam$^+$). In this study, we show that the reason for this growth deficiency is due to the DNA methylation. The absence of a Dam methyltransferase protein results in poor growth efficiency and disturbs the synchrony of replication initiation in vivo, as evaluated by flow cytometry. On the other hand, we show that lack of methylation could increase the DNA response to thermal stress (decreasing the DNA melting temperature, T_m), and the reason for this effect is due to the methylation status and not to the number of guanine and cytosine bases (G + C) in the duplex DNA. Our results show that methylation is an epigenetic factor that may play a key role in the cell growth, the synchrony of DNA replication [C R Biologies 330 (2007) 576–580] and the stress protection.

Keywords: Dam methylase; *S.* Typhimurium; Methylation; DNA replication; Bacterial growth; Melting temperature

Résumé

La croissance cellulaire est étroitement liée à la réplication de l'ADN et à sa méthylation [Proc Natl Acad Sci U S A 93 (1996) 12206–12211]. Dans un milieu de culture, la croissance de *salmonella* Typhimurium (dam$^-$) est inférieure (2,5 fois) par rapport au type sauvage (dam$^+$). Dans cette étude, nous avons montré que la raison pour cette différence de croissance est due à la méthylation de l'ADN. L'absence de la protéine Dam méthyltransférase a comme conséquence une déficience de croissance et altère la synchronie de l'initiation de la réplication in vivo, comme cela a été évalué par cytométrie de flux. Par ailleurs, nous avons montré que l'absence de méthylation pourrait augmenter la réponse de l'ADN au stress thermique (en diminuant la température de fusion de l'ADN, T_m), et la raison pour cet effet est due à l'état de méthylation et non pas au nombre de bases de guanine et de cytosine (G + C) dans le duplex d'ADN. Nos résultats prouvent que la méthylation est un facteur épigénétique qui peut jouer un rôle principal dans la croissance cellulaire, la synchronie de la réplication d'ADN [C R Biologies 330 (2007) 576–580] et la protection contre les stress.

Mots clés : Dam méthylase ; *S.* Typhimurium ; Méthylation ; Réplication de l'ADN ; Croissance bactérienne ; Température de fusion

1. Introduction

Much of our research is focused on *Salmonella* which causes diseases ranging from food and blood poisoning to typhoid fever and heart disease [30]. *S.* Typhimurium accounts for approximately 17% of worldwide intestinal *Salmonella* infections reported yearly [1]. It's an important pathogen responsible for many of all the reported cases of enterocolitis and the second most frequent cause of bacterial food-borne disease with an estimated 1.4 million cases per year in the US alone [34]. The rationale for examining *Salmonella* as a model system to study microbial pathogenesis is that it provides a

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well-characterized genetic system to understand its infection mechanism [37]. Methylation is carried out by the *dam* gene product [31]. *dam* mutants have been isolated that contain no detectable DNA methylase activity and yet are viable [20]. However, since the first *dam* mutants of *S*. Typhimurium were isolated [46], the biological functions of these methylated sites have been widely investigated by numerous workers, and several important roles have been deduced [44–45]. The first solitary DNA adenine methyltransferase that was found to play a key role in biological functions was the Dam enzyme of *Escherichia coli* (*E. coli*) [30]. Recently, Dam methylase and its homologs were characterized among bacteria in the gamma and alpha subdivision of Proteobacteria, such as *S*. Typhimurium [17], *Yersinia pseudotuberculosis* and *Vibrio cholera* [24], and *E. coli* [40]. The *Salmonella* gene encoding the dam protein has homologs in almost every prokaryotic organism and cell that has been examined to date, ranging from *E. coli* to many other species. This ubiquity and evolutionary conservation indicate that it may play a fundamental physiological role. The DNA methylation by this dam protein at GATC sites has been shown to have enormous impact on nucleoid stability, replication, the cell cycle, mismatch repair, and gene expression [23]. Many enterobacterial species have a central metabolite: S-adenosylmethionine (SAM) which is synthesized from methionine and ATP by the enzyme SAM synthetase [9], it is the major methyl donor in metabolism. *S*. Typhimurium strain has a DNA adenine methyltransferase (the Dam enzyme) which catalyzes the adenine methylation at N^6 in the sequence GATC in the duplex DNA [41,16,19], a reaction in which SAM is the methyl group donor [22] and also an allosteric effector [4]. The aim of this work was to examine if *salmonella* dam^- could grow equally to *salmonella* dam^+ on a nutrient broth. It was also of interest to see whether the type and the composition of medium (minimal or complete) enhanced the survival and growth of these strain. For the T_m, we decided to examine the relationship between the melting temperature of intracellular DNA and its methylation and to see if the difference observed between dam^+ and dam^- is due to the number of (G + C) or to the absence of methylation. Our future plans are to understand the molecular basis of how Dam controls the bacterial growth and the synchronous initiation of chromosome replication. For the second parameter (T_m) it will be of interest to see if there is a clear correlation between T_m, number of (G + C) and methylation.

2. Material and methods

2.1. Bacterial strains

One isogenic strain of *Salmonella enterica* serovar Typhimurium was used in this study and both strains and their relevant genotype are listed in Table 1. SL1344 reference strains and SV1610 (contains *dam* disrupted gene) were kindly provided by Dr. Francisco Ramos-Morales (Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Spain).

Table 1
Strains used in this study.

Bacterium	Strain	Relevant genotype or alternate designation	Reference or source[a]
S. Typhimurium	SL1344	Wild type	Portillo et al. [13]
S. Typhimurium	SV1610	SL1344 dam-228::Mud*J* Km^r	Portillo et al. [13]

[a] Omitted for strains described in this study.

2.2. Bacterial culture and growth conditions

Cultures were prepared by inoculating liquid media with a single colony from nutrient agar. Bacterial growth were done aerobically in a shaker water bath at 37 °C in Erlenmeyer flasks (250 ml) containing 50 ml volumes of a fresh sterile liquid medium and aerated by shaking during the experimental procedure. SL1344 and SV1610 were grown exponentially in the following media, which yield different growth rates: NB (nutrient broth) and M9 (M9ZB) media were prepared as described by Miller (1972) [37]. The sterile complete medium is prepared with NB (Pronadisa, Hispanlab) 8 g l^{-1} and the sterile minimal medium M9ZB is formed by 200 ml of a saline solution M9 (5 × concentrated) supplemented with 20 ml of a glucose solution 20% as carbon source, with 2 ml of $MgSO_4$ 1 M, and 0,1 ml of $CaCl_2$ 1 M. Media were autoclaved at 120 °C for 30 min. The speed of growth of a bacterial culture can be appreciated by measuring its absorbance (optical density [OD]) at 600 nm using a spectrophotometer. In exponentially growing cultures, the doubling time was calculated for each experiment: samples are taken every 60 min and OD was measured. Spectrophotometric measurements are consigned on a graph expressing the OD_{600} according to time.

2.3. Coulter counter analysis

Coulter counter analysis was done according to Vinella et al., 1992 [51], with some modifications by which we use the new following experimental conditions:

- usual sample dilution: 0.2 ml sample (bacterial culture) in 9.8 ml diluents (NB liquid medium);
- typical settings:
  - lower threshold: 10,
  - upper threshold: 99.9 (out),
  - manometer: 500 µl,
  - current: 100.

Cells/ml in original suspension = number of counts × 2 (for 500 µl) × 50 (200 µl in 10 ml)

2.4. Flow cytometry analysis

An overnight culture of bacteria in M9 supplemented with glucose (0.2%), Casamino acids (0.5%), tryptophan (40 mg ml^{-1}), and vitamin B1 (1 mg ml^{-1}) was diluted 500-fold into the same fresh medium and cultivated at 37 °C until