DNA Denaturation

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DNA denaturation refers to the melting of double-stranded DNA to generate two single strands. This involves the breaking of hydrogen bonds between the bases in the duplex. From a thermodynamic point of view, the most important contribution to DNA helix stability is the stacking of the bases on top of one another. Thus, in order to denature DNA, the main obstacle to overcome is the stacking energies that provide cohesion between adjacent base pairs. In general, stacking energies are less for pyrimidine/purine (YR) steps, and for A:T-rich regions. Thus, the sequence TATATA would be expected to melt quite readily, and this is indeed what happens, both in a test tube and inside cells.

There are a variety of ways in which to denature DNA. Perhaps one of the most common (and oldest) methods used in the laboratory is simply to heat the DNA to a temperature above its T_m or melting point. The unstacking of the DNA base pairs can be readily monitored spectrophotometrically. DNA absorbs strongly at 260 nm, and as the DNA melts, the absorbance will increase until all of the DNA is melted, and then remains constant on further heating. (This is called the 'hypochromic effect,' and the absorbance of single-stranded DNA is usually around 50% greater than that of the corresponding duplex DNA.) The process is reversible, and the renaturation time of DNA can be used to estimate its base-composition as well as the presence of repetitive fractions within the sequence. This method was used in the 1960s to monitor differences in the base composition of DNA from different organisms, and also to demonstrate that eukaryotic DNA contained a large fraction of repeated sequences. **Figure 1** shows the melting temperatures of genonic DNA from several different microorganisms as a function of the A:T content of the genome.

The actual T_m of a given piece of DNA will depend on several factors, such as the length of the DNA sequence (shorter pieces of DNA will tend to melt more easily than longer pieces), the base composition of the DNA (in general, regions with alternating pyrimidine/purine steps and A:T-rich regions will melt more readily), the topological condition of the DNA (e.g., whether it is a closed circle that is relaxed or supercoiled, or a linear piece, or is heavily nicked), and the composition of the buffer (in terms of the amount of salt and which ions are present). Given the roles of all of these parameters, it is difficult to predict accurately the exact melting temperature of a given sequence, although it is generally easy to say which region within a long piece of DNA will melt first.

Denaturation of small regions of DNA within a much longer sequence can be estimated by using enzymes or chemicals that modify or cut single-stranded DNA more readily than duplex DNA. Some enzymes, such as methylation enzymes and certain single-strand-specific nucleases, can be used to monitor the denaturation status of a particular region of DNA, either in a test tube or in a living cell. Some chemicals will react preferentially with single-stranded DNA, such as haloacetaldehydes (e.g., chloroacetaldehyde), permanganate, diethyl pyrocarbonate (DEP), or osmium tetroxide. These chemicals can be used in a similar way to the enzymes, and the location of the modified bases can be detected using polymerase chain reactions (PCRs). As an alternative, fluorescently labeled oligomers specifically designed to hybridize to a suspected region of single-stranded DNA can be used both *in vivo* as well as *in vitro*. Another method is to use a cross-linking agent, such as psoralen, to cross-link the single strands together, followed by electron microscopy to monitor the single-stranded regions.

There are at least two major biological reasons for denaturing the DNA within a cell: DNA replication and transcription. In both cases, proteins bind to specific DNA sequences, strongly bend the DNA helix, and then use the localization of torque to force the double-stranded DNA to open (denature) at a specific point. In promoters, this is often at the 'TATA' box, which melts quite readily. In addition, there are specific proteins that bind to single-stranded DNA and stabilize denatured regions; this is important, for example, in DNA replication and transcription. **Figure 2** shows the AT content and stacking energy for the *lac* operon in *E. coli*. Note that the regions that melt most readily are upstream of the genes.

Experimentally, there are times when it is important to keep DNA in a single-stranded state. This can be done by a variety of methods. Single-stranded DNA can be isolated by using a PCR primer that is 'tagged,' and then separating the tagged strand by denaturing gel chromatography. High per cent acrylamide gels (e.g., 12 %) can be used to purify oligomers; these gels have such small pore sizes (around

1.2 nm, depending on the bis: monomer ratio) that double-stranded DNA simply will not fit (the width of double-stranded DNA is about 2 nm). Urea can be added to help stabilize the single-stranded conformation, and running gels at higher temperatures facilitates DNA denaturation. Glyoxal agarose gels can also be used to stabilize single-stranded DNA or RNA. This is particularly important for Southern and Northern blotting methods.

Further Reading

Thomas R (1993) The denaturation of DNA. *Gene* 135: 77–79.

Pedersen AG, Jensen LJ, Stærfeldt HH, Brunak S and Ussery DW (2000) A DNA structural atlas for *Escherichia coli*. *Journal of Molecular Biology* 299: 907–930.

Reference

DNA Genome Atlas. http://www.cbs.dtu.dk/services/GenomeAtlas/

See also: **1098 (Replication), 1299 (Transcription)**

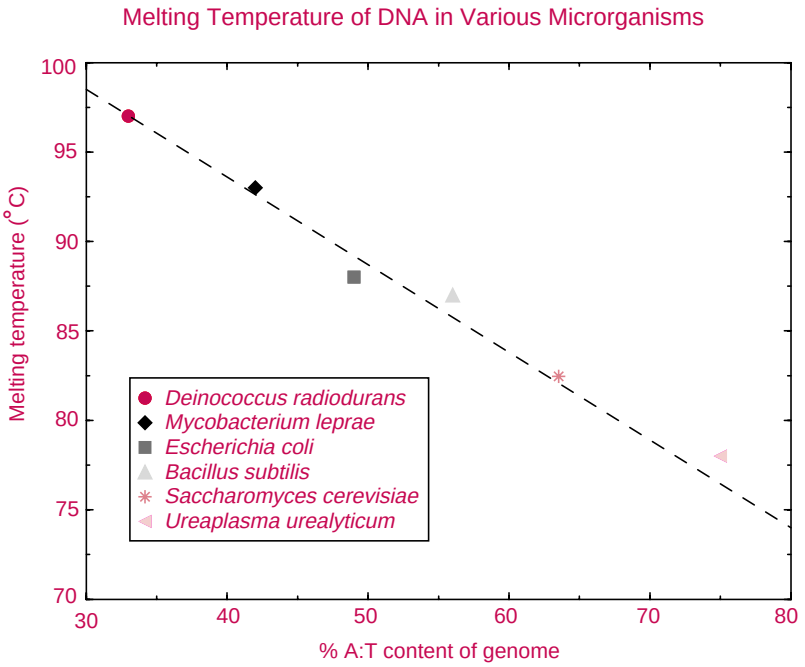

Figure 1 Melting temperatures of the genomic DNA from various organisms, as a function of A:T content, ranging from 33% A:T (*Deinococcus radiodurans*) to 75% A:T (*Ureaplasma urealyticum*). A straight line representing the best fit through the points is also shown.

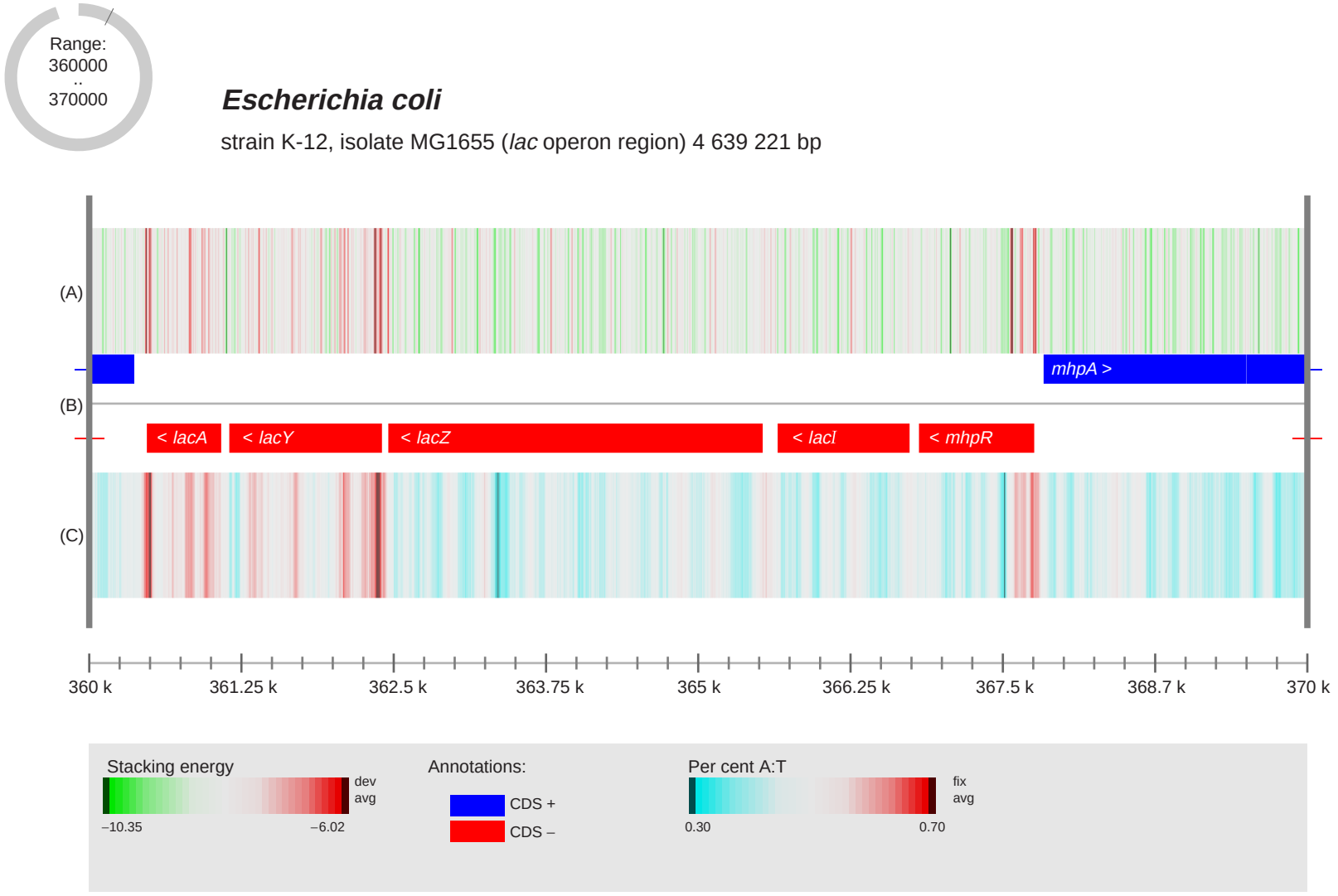

Figure 2 A 'DNA atlas' for the *lac* operon in *E. coli*. Genes oriented in the 'forward direction' are shown in blue, whilst genes in the 'reverse direction' are indicated in red (B). (C) Color-coded bar representing the calculated stacking energy in kcal mol^{-1} of the DNA sequence: green indicates regions that will require more energy to melt (e.g., more negative numbers), and red indicates regions that will melt more readily (that is, the stacking energy values are smaller or closer to zero). Color-coded bar indicating the A:T content of the region: blue represents lower A:T content, and red indicates more A:T-rich regions. Note that near the beginning of the operon there are more A:T-rich regions, which also correspond to regions that will melt more readily. For more information on DNA atlases and melting profiles of regions upstream of genes for whole genomes, see the Silver, 1986