strongly augment the saturating effects of the positive violet flashes. The abscissa has been expanded.

The results suggest that the response saturation of the blue-sensitive pathways is largely controlled by spectrally opponent neural mechanisms that treat signals from blue-sensitive cones and those from green- or red-sensitive cones in an opposite manner (*13*). The physiological site for this opponent interaction might be the blue-sensitive cones themselves if the sensitivity of these cones is controlled by inhibitory signals originating in other classes of cones. Inhibitory interactions between different spectral classes of cones have been shown in the turtle (*14*). Our results add to the growing support of the hypothesis that the detection of signals from the blue-sensitive cones is through a color-opponent pathway (*15*).

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13. The hypothesis that the saturation is partly controlled by spectrally opponent mechanisms is also supported by several observations. J. D. Mollon and P. G. Polden (paper presented at the meeting of the Association for Research in Vision and Ophthalmology, Sarasota, Fla., 25 to 29 April 1977) observed that the increment threshold curve for a parafoveal violet (420 nm) flash on a steady blue (473 nm) field ascends slightly faster than Weber's law, thus showing partial saturation. The threshold on this steep branch could be lowered as much as 0.4 log unit by adding intense yellow light to the steady blue field to make the mixture field appear white. Both we and Mollon and Polden (paper presented to the 20th Tagung experimentell arbeitender Psychologen, Marburg, Germany, 20 to 22 March 1978) also observed that the threshold for a violet flash on a steady field composed of intense yellow light and violet light of sufficient intensity to induce saturation of the blue pathways is lowest when the fields are first viewed, and the threshold rises considerably within 30 seconds thereafter.
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Physiologically Important Stabilization of DNA by a Prokaryotic Histone-like Protein

Abstract. *The thermophilic mycoplasma* Thermoplasma acidophilum *has tightly bound to its DNA a protein that closely resembles the histones of eukaryotes. DNA associated with this protein is more stable than free DNA against thermal denaturation by about 40°C, as shown in both native nucleoprotein and in hybrid nucleoprotein reconstituted in vitro with calf DNA. Since only about 20 percent of the DNA in this organism is associated with the histone-like protein, we suggest that its physiological function is to prevent complete separation of the DNA strands during brief exposures of the organism to denaturing conditions, and thus to facilitate rapid renaturation when normal environmental conditions return.*

Thermoplasma acidophilum is a nonparasitic mycoplasma that grows in extreme conditions of heat and acidity, its optimal culture conditions being 59°C and *p*H 1.0 to 2.0 (*1*). It was discovered in a spontaneously burning refuse pile, its only known natural environment (*2*).

This organism has associated with its DNA a basic protein that closely resembles eukaryotic histones (*3*). The protein is acid-soluble with a strong net positive charge at neutral *p*H and is small, composed of 89 amino acid residues of which 14 are lysine and 6 are arginine. Neither tryptophan, methionine, nor cysteine is present (*4*). The electrophoretic mobility of the protein in various conditions is similar to that of histone 4 of calf thymus. The protein is tightly bound to DNA in salt concentrations up to about 0.6*M*, which is well above the physiological range (*5*), and reconstituted nucleoprotein is relatively insoluble in 0.1 to 0.2*M* NaCl. Both native nucleoprotein and reconstituted protein–DNA complexes are partly protected from digestion by micrococcal nuclease. Also, DNA fully complexed with this protein is inactive as a template for *Escherichia coli* RNA polymerase. We now report that the protein strongly stabilizes DNA against thermal denaturation. Thus, although this protein shares several characteristics with eukaryotic histones, it does not appear to be closely homologous to any particular class of histone (*3*).

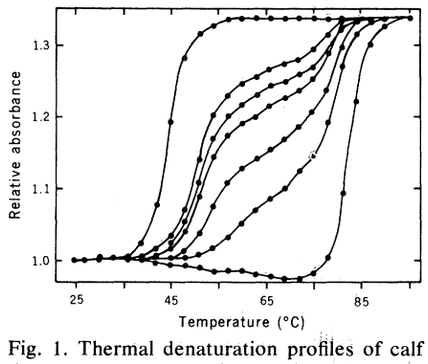

Fig. 1. Thermal denaturation profiles of calf thymus DNA reassociated with increasing amounts of the *T. acidophilum* histone-like protein. The ratio of protein to DNA by weight was (left to right) 0, 0.61, 0.80, 0.97, 1.34, 1.82, and 2.43. The hyperchromicity decreased from 1.34 to 1.29 as the protein content was increased, but in this figure has been normalized to a constant value. The DNA-protein mixtures were reconstituted by gradient dialysis (*9*) and then exhaustively dialyzed against 0.25 m*M* sodium ethylenediaminetetraacetate (EDTA), *p*H 8.0 (*7*). The samples were diluted to an absorbance at 260 nm (A_{260}) of 0.5, degassed, and centrifuged before thermal denaturation. The A_{260} was continuously recorded with a Gilford spectrophotometer as the temperature was raised 0.5 C per minute. Protein was assayed (*17*) with purified histone-like protein as a standard.

Eukaryotic histones stabilize DNA against thermal denaturation, especially under the conditions of low ionic strength that must be used in vitro to prevent precipitation (*6*). Since *T. acidophilum* exists in a high-temperature environment and has an unusually low intracellular ion concentration (*7*), one possible function of the histone-like protein might be to protect the DNA from thermal denaturation.

The protein and the purified calf

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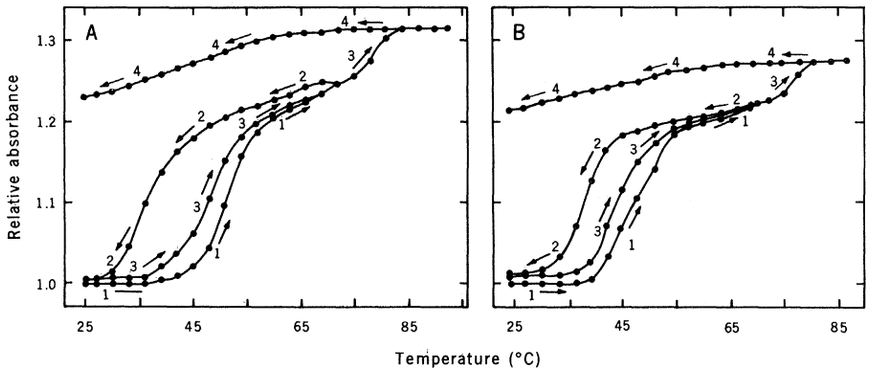

Fig. 2. Heating and cooling profiles of nucleoprotein in 0.25 m*M* EDTA, *p*H 8.0. Nucleoprotein was thermally denatured at a rate of 1.0°C per minute up to 70°C (profile 1), cooled at about 1.4°C per minute to room temperature (profile 2), heated a second time up to 95°C (profile 3), and then cooled again to 25°C (profile 4). (A) Hybrid nucleohistone reconstituted from *T. acidophilum* histone-like protein and calf thymus DNA (0.80:1.00 by weight). (B) Native *T. acidophilum* nucleoprotein. The nucleoprotein was isolated without shearing by lysis of whole cells at *p*H 6.4 in 75 m*M* NaCl with 25 m*M* EDTA, digested with 100 μg of pancreatic ribonuclease per milliliter at 38°C for 30 minutes, and purified by chromatography on Sepharose 2B, taking the excluded fractions (*4*, *6*). Nucleoprotein prepared in this way had the following composition by weight: 1.0 part DNA: 0.58 part histone-like protein: 0.07 part RNA: 1.18 part other proteins. The yield was between 12 and 20 percent based upon the DNA originally present in the cells.

thymus DNA were combined by the reconstitution technique of gradient dialysis (*8*). This method was used in order to vary the ratio of protein to DNA, to increase the amount of DNA stabilized over that found in native nucleoprotein, and to substitute calf DNA for *T. acidophilum* DNA, which is often badly damaged by depurination (*9*). Thus, a well-defined artificial nucleoprotein was obtained to test the stabilizing effect of the histone-like protein.

Thermal denaturation profiles were obtained for DNA reconstituted with increasing amounts of the histone-like protein (Fig. 1). When the DNA was fully complexed with the protein, it melted 38°C higher than pure DNA. However, in nucleoprotein with a lower content of the histone-like protein, the DNA denatured in two distinct phases, the first phase representing free DNA and the second phase representing DNA complexed with the basic protein. The fraction of stabilized DNA increased in proportion to the ratio of protein to DNA. In addition, the protein raised the melting temperature of the unprotected DNA as well, probably because short DNA segments located between stabilized regions tend to melt at a slightly higher temperature than do long segments of entirely unprotected DNA; this effect has already been described for eukaryotic histones (*10*).

The denaturation profiles obtained with native *T. acidophilum* nucleoprotein were also biphasic and resembled those obtained with the reconstituted material. However, only about 20 percent of the DNA was stabilized, consistent with the low ratio of basic protein to DNA, about 0.6 in these preparations. Thus, the function of the protein cannot be to protect the entire length of the DNA strand from separation. However, even if only occasional short regions of the DNA are stabilized, they could facilitate the renaturation of the DNA after the organism has been removed from denaturing conditions. That is, if the complementary strands of DNA were joined together at one site, they could rapidly reanneal upon restoration to more normal physiological conditions. A similar phenomenon has already been studied in vitro with covalently cross-linked reversible DNA (*11*), and also in eukaryotic histone-DNA complexes (*10*).

To test whether the *T. acidophilum* protein could similarly facilitate instant renaturation, a reconstituted nucleoprotein sample was thermally denatured to a point midway between the two melting transitions and cooled to room temperature (Fig. 2A). The nucleoprotein renatured very rapidly; at 25°C the initial absorbance was almost completely regained. However, the renaturation of these helices was not entirely faithful, as revealed by a reduction in the melting temperature (T_m) of the renatured material (Fig. 2, profiles 1 and 3). In contrast, nucleoprotein melted to the end of the second phase did not renature. In this case the complementary strands were presumably fully separated and, therefore, lost from each other.

Almost exactly the same results were obtained with native nucleoprotein isolated from *T. acidophilum* (Fig. 2B). The native nucleoprotein renatured almost completely on rapid cooling, and when denatured a second time the T_m was only 2.8°C below the initial temperature. The reduced T_m may have resulted from in vitro artifacts such as degradation of the DNA at sites of depurination. In addition, the renaturation of the nucleoprotein might have been more complete if physiological conditions of temperature and ionic strength could have been used, or if certain other proteins that are known to promote renaturation were present (*12*).

The *T. acidophilum* nucleoprotein renatured more rapidly or at higher temperatures than did the reconstituted hybrid nucleoprotein. This may partly be due to the slightly higher G + C (guanosine plus cytidine) content of the *T. acidophilum* DNA, or it may reflect some additional stability found in isolated *T. acidophilum* chromatin that was not present in the reconstituted nucleohistone complexes.

Any hypothesis of histone function must demonstrate the proposed effect at the physiological ratio of histone to DNA. For example, when reconstituted with DNA at a ratio of about 2 parts of protein to 1 part of DNA, the histone-like protein almost entirely inhibited the template activity of the DNA to *E. coli* RNA polymerase. However, in both native nucleoprotein (*5*) and in DNA reconstituted with a physiological amount of the protein, the RNA polymerase template activity was not detectably affected. Thus, it seems unlikely that the protein has a role in genetic regulation. Similarly, even though the intracellular environment is relatively hot and acidic, the histone-like protein appears to be ineffective in protecting the DNA from depurination (*5*, *7*). These observations are not surprising, since only about 15 to 20 percent of the DNA is directly associated with the protein. However, even a small amount of the protein is sufficient to prevent complete strand separation of the DNA at temperatures up to 75°C, and we suggest that this is probably an important function of the histone-like protein.

This function could contribute to the survival of *T. acidophilum* in at least two natural types of environmental events. First, during transient periods of high temperatures, the unprotected DNA might be denatured, but upon restoration to lower temperatures the renaturation of the DNA would be facilitated. This hypothesis correlates with the biology of the organism. For example, the intracellular potassium concentration can be as low as 17 m*M* (*7*), and under these

conditions the DNA should be half denatured at about 72°C (*13*). Thus, the primary cause of thermal death in *T. acidophilum* could be DNA denaturation, which is not usual with other thermophilic organisms (*14*). Nevertheless, our cultures have remained viable even after having been heated up to 80°C, providing they were then returned to the culture temperature of 59°C. In contrast, at a constant temperature higher than 60°C it is difficult to obtain growth (*1*). Secondly, another natural situation where the protective effect of the histone-like protein could be important is during periods of osmotic shock in fresh water that might occur during a rainstorm or during dispersal to a new environment. *Thermoplasma acidophilum* lacks a cell wall, and, under normal conditions, is in osmotic equilibrium with its environment (*7*). Nevertheless, it can withstand suspension in distilled water and remain viable (*1*), apparently by releasing intracellular potassium ions and other small solutes. Similar mechanisms have been described in other prokaryotes (*15*). Since very low ionic concentrations destabilize DNA (*13*), the histone should also help protect the DNA when the organism is subjected to osmotic shock. This protein can be of significant survival value to *T. acidophilum*, and a similar function might have accounted for the original evolution of eukaryotic histones (*16*).

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β-Endorphin Is Not Detectable in Plasma from Normal Human Subjects

Abstract. *β-Endorphin is not detectable in plasma from normal human subjects when measured under baseline conditions or after the subjects have received vasopressin, an agent that elevates β-lipotropin and adrenocorticotropic hormone (ACTH). Significant amounts of β-endorphin are present in plasma of patients with endocrine disorders associated with increased ACTH and β-lipotropin production. Highly purified, natural β-lipotropin is not peripherally converted to β-endorphin in vivo in normal subjects.*

Adrenocorticotropin (ACTH) and β-lipotropin (β-LPH) are derived from a common precursor glycoprotein molecule (*1*) of approximately 31,000 daltons, and β-LPH may be an obligatory intermediate for the synthesis of β-endorphin (*2*). Immunocytochemical studies have demonstrated the presence of ACTH and β-LPH or β-endorphin (or both) in the same pituitary cells (*3*). The major opioid peptide of rat pituitary extracts is β-endorphin (*4*), and concentrations of this peptide rise in the plasma during acute stress. However, others have reported a higher content of β-LPH than of β-endorphin in rat anterior pituitary (*5*), and we have previously reported that β-LPH is the major opioid (*6*) peptide in human and rat anterior pituitary (*7*).

We now report that β-LPH is the major opioid peptide in normal human plasma. However, β-endorphin is present in high concentration in the plasma of patients with abnormal ratios of ACTH to β-LPH.

We measured β-endorphin (*8*) and β-

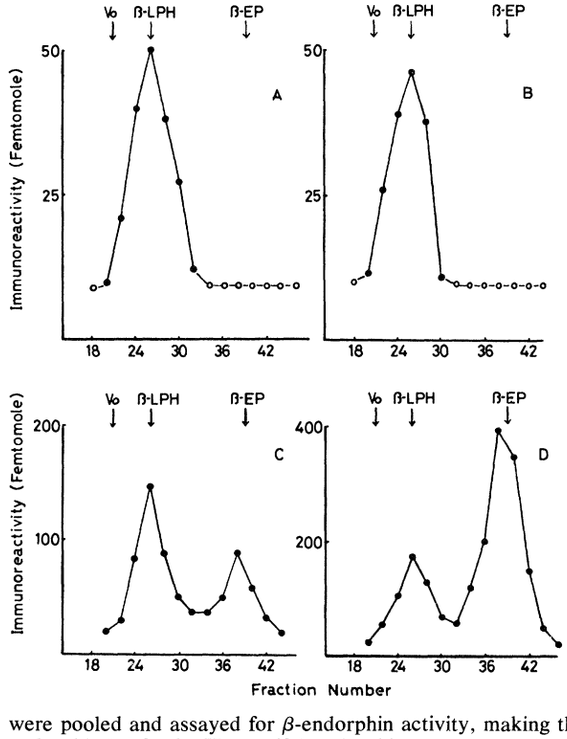

Fig. 1. Sephadex G-50 gel filtration of human plasma extracts from (A) normal subjects at baseline conditions (110-ml pool); (B) normal subjects at time of peak β-LPH levels after vasopressin administration (30 to 60 minutes; 50 ml); (C) a patient with Cushing's disease associated with macroscopic pituitary tumor; and (D) a patient with Nelson's syndrome. Method of quantification of immunoreactivity is described in the legend to Table 1. Arrows indicate the void volume (V_0) (bromophenol blue–bovine serum albumin), and the elution volume peaks of ^{125}I-labeled human β-LPH and ^{125}I-labeled human β-endorphin (*β-EP*). Immunoreactivity is expressed as femtomoles per fraction. The limits of detection for β-endorphin activity in (A) and (B) was 9 fmole per fraction and is indicated by the open circles. Fractions that eluted in the area of the synthetic β-endorphin marker were pooled and assayed for β-endorphin activity, making the effective detection limit for β-endorphin 2.5 fmole. Hence, if β-endorphin were present it would comprise less than 6 percent of the β-LPH activity.

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