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## Molecular Biology, Biochemistry, Physiology and Genetics of Extremely Halophilic Archaea (The Haloarchaea)

Regulation of stationary phase gene expression in the haloarchaea. Isolation and characterization of peptide antibiotics ("halocins") from haloarchaea. Improved methods for recovery of haloarchaea from the environment.

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### Research Interests

Introduction: The domain *Archaea* occupies an intermediate phylogenetic position between the domains *Bacteria* and *Eucarya*, and is characterized by three main biotypes: hyperthermophiles, methanogens and extreme halophiles ([unrooted phylogenetic tree](#)). The extremely halophilic *Archaea* (the haloarchaea) require external concentrations of 2 to 4.5 molar NaCl for optimal growth. As a group, they have capitulated evolutionarily to these high salt conditions by maintaining an even higher internal salt concentration, which in the most extreme halophiles is about 5 molar (3 M K<sup>+</sup>, 1 M Na<sup>+</sup> and 1 M Mg<sup>++</sup>). Consequently, haloarchaeal proteins and other macromolecules have evolved to operate in this salty milieu, with many proteins requiring high salt conditions for activity. In addition, the haloarchaea can be slightly thermophilic (e.g., *Haloferax mediterranei* grows optimally between 47 and 54°C) and can grow over a broad range (>50°C) of temperatures ([Arrhenius plot for \*Haloferax mediterranei\*](#)).

The *Archaea* possess a minimalist version of the eucaryotic Pol II basal transcription apparatus: in addition to a TATA box element, factors analogous to the eucaryal system include a multisubunit Pol II-like polymerase, TATA binding proteins (TBPs) and transcription factor B (TFB) proteins.

**Focus 1: Regulation of Gene Expression.** My research has focussed on regulation of gene expression, first in *Salmonella typhimurium* (regulation of the histidine biosynthetic operon by the "alarmone" ppGpp) and then in the haloarchaea (regulation of the bacterio-opsin gene cluster in *Halobacterium salinarum*). Currently, my main focus is stationary phase gene expression in the haloarchaea, using halocin genes as models. Halocin genes are induced as the cells transition between exponential phase and stationary phase. We are in the process of

identifying *trans*-acting regulatory factors that control their expression.

**Focus 2: Isolation and Characterization of Halocins.** Production of protein antibiotics is a nearly universal feature of all living organisms: bacteria produce bacteriocins (e.g., colicins, microcins and lantibiotics); and eukaryotes including protozoans, insects, horseshoe crabs, frogs, mammals (including humans) and plants, produce a wide variety of antimicrobial peptides. A second area of research involves the isolation and characterization of haloarchaeal antimicrobials called "halocins": protein antibiotics that are externalized by the producer cells and kill or inhibit other organisms ([halocin assay picture](#)). Some halocins are small peptides (e.g., the microhalocins S8 and R1; <5 kDa) while others are ten times as large (e.g., halocin H4). Projects in this area include determination of their 3D structure, heterologous expression, mechanism of action, immunity mechanism, and spectrum of activity (which can be very broad) ([halocin inhibition of \*Sulfolobus\* picture](#)).

**Focus 3: Recovery of Haloarchaea from the Environment.** Surveys of both planktonic hypersaline environments and salt rocks utilizing amplification of 16S rDNA by PCR have shown that the diversity of organisms in these environments is far greater than what can be recovered and grown. A third area of research involves the development of new methods for improved recovery of haloarchaea and other extreme halophiles from planktonic hypersaline environments, from surface salt deposits and salt rocks, and from halite (recovery protocol coming soon).



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