Abstract

When cells, from bacteria to humans, are shifted to high temperature, the synthesis of a small number of proteins, called the heat shock proteins (Hsps) is selectively and rapidly induced. Under non-stress conditions many of the Hsps play fundamental roles in normal cellular physiology. This response, the heat shock response, can be induced by various other forms of stressful stimuli, besides heat shock. Stress proteins are also synthesized when an invading pathogen finds itself in a hostile mileu inside the host. The disease cholera in humans is caused by ingestion of the Gram-negative bacterium *Vibrio cholerae*. No information exists on how *V. cholerae* overcomes the hostile conditions like acidic pH, temperature upshift, nutrient diversity *etc.* in its human host. This study was thus carried out with the aim to study and characterise one of these stress responses namely, the heat shock response in *V. cholerae.*

The general properties of the heat shock response in *V. cholerae* were observed. Enhanced or *de novo* synthesis of 24 proteins was observed upon a heat shock from 30°C to 42°C. Of these Hsps, DnaK, DnaJ and GroEL homologues were identified by immunoblotting. Thus, the presence of almost all the proteins which make up the chaperone machine in *E. coli* were observed in *V. cholerae.*

Exposure of organisms, to other stress agents besides heat has also been observed to result in the induction of many of these Hsps. *V. cholerae* cells were exposed to nalidixic acid, ethanol and hydrogen peroxide to observe if any overlap between these stress responses exists. We found that as in *Escherichia coli* and other organisms, there was induction in a set of common stress proteins, the primary among these were DnaK, DnaJ, GroEL and a 16 kDa protein.

The localization of the heat shock proteins DnaK and GroEL in normal and heat shock cells of *V. cholerae*, was investigated by immunogold labelling of ultrathin sections. DnaK, which was found to be localized predominantly at the membrane in unstressed cells, partially relocalized to the cytoplasm upon heat shock, whereas GroEL, in both stressed and unstressed cells was found mainly in the cytoplasm.

The *dnaK* gene, which plays a pivotal role in heat shock response in all organisms, was cloned from *V. cholerae* into *E. coli* and its sequence was analyzed. The sequence of the *dnaK* gene of *V. cholerae* along with its upstream and downstream regions were obtained. The results showed that even though the structure of *V. cholerae dnaK* gene is very similar to that found in other Gram negative bacteria,

the organization of *dna*K, *dna*J and *grp*E genes (*i.e. grp*E-*dna*K-*dna*J) in this organism are more akin to that found in Gram positive bacteria.

Thus, despite some differences like those in the *dna*K operon organization, *V. cholerae* responds to heat shock in a manner similar to that in *E. coli*, though the two organisms diverged some 670 million years ago.