Heat Shock Proteins and poultry - an introduction

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A little history

1800's
- Gregor Mendel - 1856 - 63
- Theodor Boveri - mid 1880's
- Friedrich Miescher - 1869

< 1950's
- Phoebus Levene - 1919
- Nikolai Koltsov - 1927

> 1950's
- Alfred Hershey & Martha Chase - 1952
- James D Watson & Francis Crick - 1953

Prof. Ferruccio Ritossa – 1960’s
The Heat Shock Response

“many scientists were considering the work with fruit flies as not very important, secondary to phage research, which was the vogue of the time.”

“I cannot remember whether it was John Pulitzer or Inge or Clara Ghini or Giordano who shifted the temperature (higher) of my incubator, but one day I noticed a different (chromosome) puffing pattern!”

Published in Experientia in 1962 (Ritossa 1962)
Heat Shock Proteins

12 years later (Tissières et al. 1974)

• Correlated with the manufacture of specific proteins with the appearance of the chromosomal puffs.

• They were called Heat Shock Proteins because they were only present when cells were grown at higher than normal temperatures.
Heat Shock Proteins

- Also known as – Chaperonins, HSPs, Stress Protein (Sps)
- Inducible HSPs
- Cognitive, constitutive chaperones, heat shock cognates (Hscs)
- Induced by (Heat Shock Factors) HSFs
Heat Shock Proteins

- Highly conserved proteins MW 16 – 100 kDa
- Produced in all cells exposed to stress
- Both intracellular and extracellular
- Found in cell membranes
- Immune signalling and antigen recognition
The heat shock protein Hsp60 is an intercellular signalling molecule.

Expert Reviews in Molecular Medicine © 2001 Cambridge University Press

Figure 2. The heat shock protein Hsp60 as an intercellular signalling molecule. Hsp60 has been shown to have several immunological effects, including the induction of pro-inflammatory cytokine secretion from, and adhesion molecule expression on, a number of myeloid and vascular cell types, including smooth muscle cells. Abbreviations: ICAM-1, intercellular adhesion molecule 1; IL, interleukin; TNF-α, tumour necrosis factor α; VCAM-1, vascular cell adhesion molecule 1 (fig002gps).
Heat Shock Proteins

- Present in unstressed cells
- Maintain the integrity of the nuclear matrix and DNA
- Maintenance of the cytoskeleton
- Folding of newly synthesised proteins
- Stabilise and refold proteins
Heat Shock Proteins

- Refold partially damaged proteins
- Elimination of badly damaged proteins
- Protein transportation - unfold and refold
- Conformation of hormone receptors
- Important in embryonic development
# Mammalian HSP’s - Groups and activity

<table>
<thead>
<tr>
<th>Major family and members</th>
<th>Cellular localisation</th>
<th>Cellular function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Small</strong> Hsp27, Hsp32 18 -40 kDa</td>
<td>Cytoplasm/nucleus</td>
<td>Cytoskeletal stabilisation antioxidant</td>
</tr>
<tr>
<td><strong>Hsp60 or chaperonins</strong> 558 – 65 kDa</td>
<td>Mitochondria, Cytoplasm, Chloroplast</td>
<td>Assist correct folding, assemble multimeric complexes</td>
</tr>
<tr>
<td><strong>Hsp70</strong> Hsc70 67 – 76 kDa</td>
<td>Cytoplasm, Nucleus, ER, Mitochondria, Chloroplast</td>
<td>Bind to extended polypeptides; prevent aggregation of unfolded proteins; regulation of HSF1 activity and repression of HSP gene transcription</td>
</tr>
<tr>
<td><strong>Hsp90</strong> 82 – 96 kDa</td>
<td>Cytoplasm, ER</td>
<td>Correct assembly and folding of newly synthesised protein, HSF1 monomeric</td>
</tr>
<tr>
<td><strong>Hsp110</strong> 80 – 110 kDa</td>
<td>Nucleolus, Cytoplasm Nucleus, chloroplast</td>
<td>Thermal tolerance, Protein refolding</td>
</tr>
</tbody>
</table>
HSP’s - Production and control

- in the normal cell
- in the stressed cell following stresses such as:
  - heat, cold, UV radiation
  - anoxia, hypoxia, ischaemia
  - microbial damage, toxins
  - acidosis, nutritional deficiency
- Common factor
  oxidative stress  →  protein degradation
The function of HSP in stressed cells

Stressor → Protein damage → Increase HSP production → Repair damaged protein → Homeostasis returned
Figure 1. Regulation of transcription of heat shock protein genes by heat shock factor. Heat shock factor (HSF) is present in the cytoplasm as a latent monomeric molecule that is unable to bind to DNA. Under stressful conditions, the flux of non-native proteins (which are non-functional, prone to aggregation, protease-sensitive, and bind to chaperones) leads to phosphorylation (P) and trimerisation of HSF. The trimers translocate to the nucleus, bind the promoter regions of heat shock protein (hsp) genes and mediate hsp gene transcription. The activity of HSF trimers is downregulated by hsps (e.g. Hsp70) and the heat shock binding protein 1 (HSBP1) that is found in the nucleus. Diagrams are based on those included in Refs 11 and 14 (fig001gps).
Experimental work shows that HSP levels in pre-conditioned stressed cells (yellow line) rise more quickly (about 20 minutes) than untreated (blue line) stressed cells (about 2 hours). Significant cell damage can occur in that time.
Diving in a hyperbaric chamber.

Diving in a hyperbaric chamber (35 meters, for 30 minutes). These conditions are sufficient to increase the level of HSPs in peripheral blood.

Before

After

Fatigue!
HSPs blood levels during a simulated dive in a hyperbaric chamber.

- TEX-OE preconditioned (n=4)
- Non Treated

![Graph showing HSPs blood levels over time.](image)
The effect of heat stress on 3 consecutive days - man

1. The HSP level is the same in both groups before heat stress.
2. The HSP level achieved is the same for both pre-conditioned and untreated groups.
3. TEX-OE preconditioning - the increase in HSPs is observed within 15 minutes from the onset of stress, and remains stable for 3 days.
4. Without preconditioning the increase is observed after 120 minutes from the onset of stress. Further stress on days 2 and 3 uses up the available HSP.
HSP’s - HSP stimulators

- Non-lethal heat shock
- Feeding heat shock stimulated bacteria as an exogenous source of HSPs
- TEX-OE
Production of TEX-OE

1. Prickly Pear  
   (*Opuntia ficus indica*)

2. Harvest the Fruit

3. Dry the skin of the Fruit

4. Extract the TEX-OE

5. Prepare Formulations
Observations on HSP levels

1. Salmon
2. Racing Pigeon
3. Broiler Breeder
Vaccination of S0 Parr in Summer.
(Temperture 21°C.)

- Mortalities due to temperature stress make vaccination of S0 pre-smolts problematical.
- In 2006 summer temperatures were particularly high.
- With Protex exposure beforehand, losses were negligible over 500,000 parr vaccinated between 19°C and 21°C.
- Attempts to vaccinate without Protex, as controls, were abandoned.

Slide courtesy of Prof. R.J. Roberts
Racing Pigeons & HSP’s

- 18 pigeons were selected.
- They were divided into two groups of 9
- One group was treated with TEX-OE
- The other group was the untreated control
Racing Pigeons – The Stresses

1. Being confined to one half of the loft on Sunday.
2. Being kept in a basket overnight on Sunday.
3. Being transported to and from Langford.
4. Being handled and examined by the vet students.
5. Being blood sampled.
Racing Pigeons – Conclusion

- The HSP levels were not increased in the absence of stress (Sunday evening sample)
- No rise in HSP was seen in the untreated controls.
- High levels of HSP were demonstrated in the treated birds
- Both groups of birds looked normal
- However, the detection of HSPs in the preconditioned birds indicates that the transport and handling was stressful to the racing pigeons.
Broiler Breeders & HSP’s

2 houses of 10,000 pullets, 16 week old, from the same parent flock.

1 house was pre-conditioned with TEX-OE in the drinking water for 3 hours.

The birds in both houses were vaccinated with three 0.5ml doses of vaccine i/m.
Broiler Breeder – the Stresses

- Being fed early
- Limited access to water before being penned up.
- Vaccinated and then released back into the pen.
- Being disturbed for the whole of their day.
- Increased day length for one day.
Broiler Breeder - conclusions

- Marked beneficial behavioural observations following vaccination
- Easier catching prior to transport
- Quieter flock on laying farm
- Into lay 2 to 3 days earlier
- Improved early body weights
- Improved early egg production
- No effect on the NDV ELISA titres
Summary and the Future

• Nutrition – Salmon HSP 70 elevated when fed GM maize but not wild type maize
• Semen extenders
• Vaccine production and development
• Welfare monitoring
• As we learn more about which HSP’s, I believe that specifically stimulating or repressing HSP’s will become essential to ensure health and welfare of our flocks
References


3. R J Roberts, C Agius, C Saliba, P Bossier and Y Y Sung Heat shock proteins (chaperones) in fish and shellfish and their potential role in relation to fish health: a review Journal of Fish Diseases 2010

4. A. Graham Pockley, Reader in Immunobiology, Division of Clinical Sciences (North), Clinical Sciences Centre (University of Sheffield), Northern General Hospital, Herries Road, Sheffield, S5 7AU, UK. Tel: +44 114 271 4450; Fax: +44 114 261 9246; E-mail: g.pockley@sheffield.ac.uk. Heat shock proteins in health and disease: therapeutic targets or therapeutic agents?

