Centre for Molecular and Cellular Genetics (CGMC), Lyon, France

June 2009

PhD PROJECT: Clearance of protein aggregates by NF-κB-induced autophagy

Doctoral fellowships for European mobility
The International Doctoral College of Université de Lyon offers doctoral fellowships for the next academic year. This College gathers 17 doctoral schools covering all scientific areas within laboratories of 8 universities, « Grandes Ecoles » and engineering schools: among them is Université Claude Bernard Lyon I and its 340 research laboratories.

Fellowship gross: 1658 €/month for 3 years

Eligibility criteria

- Fellowships can be awarded to any European Union citizens or to any citizens from country signatory of the agreement on European Higher Education Area (*link*)
- Students having completed a Master's degree (2 years: first and second year) outside of France.

Thesis director:

Carole KRETZ-REMY, assistant professor, Centre for Molecular and Cellular Genetics, Claude Bernard Lyon I University. Email: carole.kretz@univ-lyon1.fr

Research laboratory:

Stress, chaperones and cell death laboratory - Centre for Molecular and Cellular Genetics (CGMC) - Bât. G. Mendel - 16 Rue Dubois – Claude Bernard Lyon I University, 69622 Villeurbanne Cedex, France.
Head: Pr André-Patrick ARRIGO- Email: arrigo@univ-lyon1.fr

General themes: cellular biology

Key words: NF-κB, heat shock, autophagy, protein aggregation

PhD project:
Clearance of protein aggregates by NF-κB-induced autophagy.

Beginning: September 2009

Scientific background:

The heat shock response is a ubiquitous cell defense mechanism [1]. In mammals, it is induced when the cell environment becomes deleterious and alters protein folding (as for example during heat shock, recovery from ischemia, UV irradiation or after exposure to heavy metals or oxidants). Many processes are affected by heat shock (HS); among them are protein folding [2], membrane fluidity [3], intracellular redox state [4] and transcription [5]. Indeed during a heat stress, heat shock genes are preferentially transcribed [6]; the resulting heat shock proteins (Hsps) protect cells from thermally induced injuries by acting as molecular chaperones that help the cell to cope with aberrantly folded proteins [7]. Several years ago, we reported that NF-κB transcription factor displays an intense activation during the recovery period subsequent to a heat challenge [8].

NF-κB is an inducible transcription factor, composed of two subunits (p50 and p65), that is inactivated by binding to inhibitory subunits of the IκB family. Most of IκB proteins inhibit NF-κB by blocking both its DNA binding and transactivation ability [9]. NF-κB targets numerous genes and is essential in many biological processes such as inflammation [10], immunity [11], stress response [12], apoptosis and cancer [13, 14]. Recently, a direct cross talk between NF-κB and autophagy was demonstrated [15]. Indeed, in some circumstances NF-κB activation was shown to repress autophagy [16]. On the other hand, autophagy was described to regulate NF-κB activity. [17]

Autophagy is an evolutionary conserved mechanism whereby cells self-digest cellular components in the cytoplasm [18]. During macroautophagy process (herein referred as autophagy) expanding-membrane structures called phagophores are generated, isolate parts of the cytoplasm and form double-membrane autophagosomes. Once formed, autophagosomes fuse with lysosomes, forming autophagolysosomes where autophagic cargos are degraded by lysosomal hydrolases; the resulting degradation products are released in the cytoplasm for recycling. Autophagy is basally active in most cell types and is involved in the regulation of long-lived proteins turnover and also in the clearance of damaged organelles [19]. Several studies indicate a dual role of autophagy in cell survival and cell death. Indeed, when cells are in starvation conditions, autophagy is fundamental to cell survival by maintaining cellular ATP energy production and macromolecules synthesis [20]. Autophagy is also involved in the protection against muscular disorders and neurodegeneration through its ability to degrade protein aggregates [21, 22]. In contrast, autophagy can represent a non-apoptotic type of cell death [23] and be involved in tumor suppression [15] or tissue degradation [24].

Team's groundwork:

We have previously reported that NF-κB transcription factor is activated during the recovery period after heat shock [8]. Our team is interested in the consequences of NF-κB activation during the recovery period after heat shock. To this end, we compared the heat shock response of NF-κB competent and incompetent (p65/RelA-depleted) cells.

We demonstrated for the first time that NF-κB plays a major and crucial role during the heat shock response by activating autophagy, which increases survival of heat-treated cells. Indeed, we observed that autophagy is not activated during heat shock recovery and cell death is

strongly increased in NF-κB incompetent cells. Moreover, if autophagy is artificially induced in these cells, the cytotoxicity of heat shock is turned back to normal.

We showed that despite a post-heat shock increase of Beclin 1 level in NF-κB competent cells, neither Beclin 1/class III PI3K complex, Bcl_2/Bcl_{XL} nor mTOR kinase (which are classical autophagy regulators) are NF-κB targets whose modulation of expression could be responsible for NF-κB activation of autophagy during heat shock recovery.

In contrast, we demonstrated that aberrantly folded/aggregated proteins are prime events in the signaling pathway leading to NF-κB mediated autophagy after heat shock. Hence, our findings demonstrate that NF-κB-induced autophagy during heat shock recovery is an additional cell response to HS-induced protein denaturation/aggregation [25]; we postulate that this mechanism increases cell survival, probably through a modulation of the clearance of irreversibly damaged proteins.

PhD project:

Our team project is to characterize NF-κB-induced autophagy and its role in the clearance of protein aggregates. To this end three major approaches are planned and/or under investigation.

1. analysis of the protein aggregation status in NF-κB depleted cells
2. determination of the nature of the signal leading to NF-κB activated-autophagy
3. characterization of the NF-κB target genes responsible for the activation of autophagy by protein aggregates. We suggest that this third approach would be the central topic of this PhD project. This study has been initialized by performing differential analysis of microarrays hybridized with cDNA from control and p65-depleted cells grown in control conditions or submitted to a heat shock treatment in order to induce protein aggregation. The PhD student will be involved in the determination of genes of interest, that are genes showing a differential expression (after heat shock) in control cells but not in p65-depleted cells and that could be involved in autophagy regulation. The differential expression of these genes after heat shock or protein aggregation will have to be confirmed by qPCR and, at the protein level, by western blot or ELISA. Thereafter, the role of these genes in the regulation of the autophagic process by NF-κB, in the cell, will have to be checked (specific inhibitors).

This project will allow our team to determine the mechanisms involved in the NF-κB induction of autophagy by heat shock or protein aggregation. These mechanisms stimulate the clearance of protein aggregates and therefore cell survival to protein aggregation. Hence, this work will probably have repercussion in the field of therapeutic research for protein conformational diseases such as myopathies or neurodegenerative diseases and for cancer therapy since hyperthermia is use as anticancer treatment.

How to apply

Prerequisites:

Having completed a Master of Science in biology outside of France.
Expertise in molecular and cellular biology; expertise in microarrays, qPCR would be well appreciated.

Please contact Carole Kretz-Remy; carole.kretz@univ-lyon1.fr

References:

1. Lindquist S.; Annu Rev Biochem 1986; 55:1151-91.
2. Pinto M, Morange M, Bensaude O.; J Biol Chem 1991; 266:13941-6.
3. Balogh G, Horvath I, Nagy E, Hoyk Z, Benko S, Bensaude O, Vigh L.; The FEBS journal 2005; 272:6077-86.
4. Davidson JF, Schiestl RH.; Molecular and cellular biology 2001; 21:8483-9.
5. Park HG, Han SI, Oh SY, Kang HS.; Cell Mol Life Sci 2005; 62:10-23.
6. Morimoto RI, Santoro MG.; Nature biotechnology 1998; 16:833-8.
7. Young JC, Agashe VR, Siegers K, Hartl FU.; Nature reviews 2004; 5:781-91.
8. Kretz-Remy C, Munsch B, Arrigo AP.; J Biol Chem 2001; 276:43723-33.
9. Ghosh S, Karin M.; Cell 2002; 109 Suppl:S81-96.
10. Tak PP, Firestein GS.; J Clin Invest 2001; 107:7-11.
11. Hayden MS, West AP, Ghosh S.; Oncogene 2006; 25:6758-80.
12. Pahl HL.; Oncogene 1999; 18:6853-66.
13. Dutta J, Fan Y, Gupta N, Fan G, Gelinas C.; Oncogene 2006; 25:6800-16.
14. Kim HJ, Hawke N, Baldwin AS.; Cell death and differentiation 2006; 13:738-47.
15. Xiao G.; Cytokine & growth factor reviews 2007; 18:233-43.
16. Djavaheri-Mergny M, Amelotti M, Mathieu J, Besancon F, Bauvy C, Souquere S, Pierron G, Codogno P.; J Biol Chem 2006; 281:30373-82.
17. Qing G, Yan P, Qu Z, Liu H, Xiao G.; Cell research 2007; 17:520-30.
18. Levine B, Klionsky DJ.; Developmental cell 2004; 6:463-77.
19. Meijer AJ, Codogno P.; The international journal of biochemistry & cell biology 2004; 36:2445-62.
20. Lum JJ, DeBerardinis RJ, Thompson CB.; Nature reviews 2005; 6:439-48.
21. Martinez-Vicente M, Cuervo AM.; Lancet neurology 2007; 6:352-61.
22. Codogno P, Meijer AJ.; Cell death and differentiation 2005; 12 Suppl 2:1509-18.
23. Tsujimoto Y, Shimizu S.; Cell death and differentiation 2005; 12 Suppl 2:1528-34.
24. Berry DL, Baehrecke EH.; Cell 2007; 131:1137-48.
25. Nivon M, Richet E, Codgno P, Arrigo A-P, Kretz-Remy C.; Autophagy 2009; 5.