Quote Ref: LS/80759

THE UNIVERSITY OF MANCHESTER

FACULTY OF LIFE SCIENCES

PARTICULARS OF APPOINTMENT OF
POST DOCTORAL RESEARCH ASSOCIATE

- The University invites applications for the above post, which is tenable for 36 months and is available immediately.

- Salary will be £27,466 per annum.

- Informal enquiries can be made to: Dr Lisa Swanton, Tel: (44) 161 275 1554, Email: lisa.swanton@manchester.ac.uk

- Applications should be returned by 11 August 2008 to:

  Dr Lisa Swanton
  Faculty of Life Sciences
  Michael Smith Building
  The University of Manchester
  Oxford Road
  Manchester, M13 9PL

  Email: lisa.swanton@manchester.ac.uk

- **A properly constituted application must include a completed University application form obtainable from http://www.manchester.ac.uk/aboutus/jobs or requested from lifesciences-hr@manchester.ac.uk, Tel: 0161 275 8836. You may attach a CV or additional sheets to provide further information if necessary**

- Staff currently employed by the University are advised that on appointment to this post the following terms and conditions will apply:
  - 1 August Incremental date / with a qualification period of 6 months in post (if appropriate)
  - Up to 3 months notice

- Please quote reference LS/80759 on all correspondence

Unfortunately it is not possible for the University to acknowledge applications or contact all unsuccessful applicants. If you have not been contacted within 4 weeks of the closing date, you should assume that, on this occasion, your application has not been successful. We would, however, like to take this opportunity to thank you for your interest in the University of Manchester.

WITH THE COMPLIMENTS OF THE DIRECTOR OF HUMAN RESOURCES

The University of Manchester

Job Description

Job Title: Post Doctoral Research Associate

Reports To: Dr Lisa Swanton

Organisation Unit: Faculty of Life Science

Date: July 2008

Project title:

Insights into the molecular basis of torsion dystonia: understanding the role of torsinA at the endoplasmic reticulum.

Research Environment:

You will join a newly established laboratory investigating protein biogenesis and quality control at the endoplasmic reticulum of mammalian cells. The laboratory is well-equipped to the highest standards, and is located within the recently constructed Michael Smith Building. Access is also available to state-of-the-art facilities for the biophysical and structural analysis of protein complexes, mass spectrometry, and a range of microscopical techniques. The project will also take advantage of the strong collaborative links between laboratories in the Organelle Function research grouping (http://www.ls.manchester.ac.uk/research/themes/organellefunction/). Scientific discussion is strongly promoted, and is facilitated by a vibrant seminar program together with internal symposia, joint laboratory meetings, and journal clubs.

The Faculty of Life Sciences places considerable emphasis on the development of personal and transferable skills, and a postdoctoral training programs includes a series of workshops encompassing presentation, writing, interview/interviewing, and appraisal skills.

Background to the project:

TorsinA and early onset torsion dystonia

TorsinA is the founding member of a novel family of proteins that belongs to the AAA^+ superfamily of ATPases (Neuwald et al., 1999). TorsinA was identified as the protein mutated in early onset torsion dystonia (EOTD), a dominantly inherited neurological disorder characterised by uncontrollable movements and twisted postures (Ozelius et al., 1997). Most cases of EOTD (~80%) are caused by a 3 bp deletion in the gene that encodes torsinA, resulting in the loss of one of a pair of glutamate residues near the C-terminus of the polypeptide (Ozelius et al., 1997) (ΔE, Fig 1). The biological role of torsinA is not known, and it is unclear how the ΔE mutation perturbs cellular function, or why it acts in a dominant manner. The mutant protein appears to be non-functional (Goodchild et al., 2005), and induces formation of membranous inclusions in cultured cells (Hewett et al., 2000; Kustedjo et al., 2000)

and in the brainstem of EOTD patients (McNaught et al., 2004). In the brain, torsinA expression is high in the dopaminergic neurons of the basal ganglia (Augood et al., 1999; Konakova and Pulst, 2001), and there is some evidence that defects in the dopamine system of the basal ganglia underlie the clinical symptoms of EOTD (Augood et al., 2002; Eidelberg, 1998; Pisani et al., 2006).

TorsinA is a 37kDa glycoprotein containing a single AAA+ domain, extending from residues 70-332. The human genome encodes three additional torsinA related proteins, including the highly similar torsinB, and orthologues have been identified in mouse, rat, pig, zebrafish, fruitfly and nematode (Breakefield et al., 2001; Ozelius et al., 1999). TorsinA is expressed in most tissues (Ozelius et al., 1997) and knockout mice die within 48 hours of birth (Goodchild et al., 2005), suggesting that it performs an essential and ubiquitous cellular function. Torsins are related to the Hsp100/Clp AAA+ 'unfoldase' enzymes, noted for their ability to actively drive the disassembly of higher order proteins complexes (Burton and Baker, 2005; Schirmer et al., 1996). Structure and sequence based analyses place the torsins in a family with the C-terminal AAA+ domains of ClpA and ClpB (the ClpAB-C/torsin family) (Iyer et al., 2004). Within this group, torsinA is most closely related to the bacterial chaperone ClpB. ClpB and its yeast orthologue Hsp104 are cytosolic chaperones that have the unique ability to dissolve protein aggregates (Schirmer et al., 1996). TorsinA may also possess a chaperone-like activity *in vivo*, since overexpression of wt but not ΔE torsinA prevents the accumulation of cytosolic α-synuclein aggregates in mammalian cells (McLean et al., 2002), and of polyglutamine-containing proteins in *C. elegans* muscle cells (Caldwell et al., 2003). These studies proposed an active role for torsinA in inhibiting protein aggregation through a mechanism similar to that used by the Hsp100/Clp chaperones.

Protein folding and quality control at the endoplasmic reticulum

Wt torsinA is predominantly localised within the ER (Kustedjo et al., 2000), where we have shown it is peripherally associated with the inner face of the ER membrane (Callan et al., 2007). The ER is the major site for synthesis and structural maturation of membrane and secretory proteins. The lumen of the ER contains a high concentration of molecular chaperones and folding enzymes that assist the folding and oligomeric assembly of newly made proteins. A quality control system monitors protein folding at the ER, and ensures that only correctly folded proteins are allowed to proceed along the secretory pathway (Hebert and Molinari, 2007). Proteins that do not attain their native state are retained by the ER quality control machinery and eliminated by ER-associated degradation (ERAD), whereby the misfolded protein is 'retrotranslocated' out of the ER into the cytosol and degraded by the proteasome (Romisch, 2005). The folding and degradation pathways are intimately linked, and molecular chaperones play a key role in both processes (Brodsky, 2007; Wahlman et al., 2007). By virtue of their ability to bind non-native determinants on polypeptides, chaperones not only promote proper folding, but may also facilitate degradation of misfolded proteins by preventing aggregation and preparing them for retrotranslocation (Forster et al., 2006; Nishikawa et al., 2001). The ER contains a variety of classical chaperones, including abundant members of the Hsp70 and 90 families. However, torsinA and its relative torsinB, are the only potential Hsp100 chaperones within this cellular compartment (Hebert and Molinari, 2007).

A role for torsinA at the endoplasmic reticulum

Although torsinA is localised within the ER, very few studies have addressed its function at this compartment. Overexpression of wt but not ΔE torsinA appears to inhibit the cell-surface expression of several plasma membrane proteins (Cao et al., 2005; Torres et al., 2004), whilst co-expression of wt torsinA was shown to reduce the levels of a mutant membrane protein, ε-sarcoglycan (Esapa et al., 2007). These

results indicate that excess torsinA can promote degradation of immature or misfolded proteins at the ER. In addition, secretion of a soluble reporter protein was recently shown to be impaired in fibroblasts from EOTD patients and torsinA knockout mice (Hewett et al., 2007), suggesting that torsinA function is required for maximum folding efficiency in the ER. Thus, although the function of torsinA has not yet been established, several lines of evidence support a direct or indirect role for this AAA+ ATPase in the control of protein folding and degradation at the ER.

This project follows on from the work of a current PhD student in the laboratory, and aims to test the hypothesis that torsinA plays a role in regulating protein folding at the endoplasmic reticulum. The specific aims are to:

1. Define the oligomeric state of torsinA and determine whether this is regulated by nucleotide binding or hydrolysis.
2. Examine the role of torsinA in protein folding and degradation at the endoplasmic reticulum.
3. Identify torsinA binding partners.

References

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Key Responsibilities, Accountabilities or Duties:

You will be responsible for performing research to address the objectives above, the subsequent compilation of data, and presentation of results.

You will be expected to:

- Design and perform experiments.
- Keep accurate records of your research methods and results.
- Analyse and interpret data.

- Contribute to the academic development of the project.
- Produce work of a suitable quality for publication in peer-reviewed journals.
- Actively read the scientific literature relating to the project.
- Present research findings at relevant meetings.
- Take part in laboratory meetings and internal seminars.
- Contribute to the general running of the laboratory.

Essential Knowledge, Skills and Experience:

- You should have relevant experience in biochemistry or cell biology research, and hold (or expect to hold shortly) a PhD in a biological science.
- Be a highly motivated individual with a genuine enthusiasm for scientific research.
- Be able to work independently and as part of a small team.
- Have good communication skills.

Desirable Knowledge, Skills and Experience:

Experience in one or more of the following areas:

- Biophysical techniques:
    - Light scattering techniques
    - AUC
    - CD

- Protein biochemistry:
    - Recombinant protein expression
    - Protein purification
    - Mass spectrometry
    - Immunoprecipitation, SDS-PAGE, immunoblotting

- Cell biology & molecular biology:
    - Culture of mammalian cells
    - Transfection of mammalian cells
    - Real Time PCR

Please Note: The above particulars are intended as a general guide to the duties of the post and the conditions of service. They do not constitute a contract of employment between the University and the person appointed. The successful applicant will, however, receive a full set of conditions of service on appointment.