ESRF	**Experiment title:** Structural interactions in the spindle checkpoint: the Mad1/Mad2 complex	**Experiment number:** **LS1933**
Beamline: ID14-3 ID29	**Date of experiment:** from 28-04-2001 to 30-04-2001 from 23-06-2001 to 25-06-2001	**Date of report:** 25-07-01
Shifts to BAG: **12** **6**	**Local contact(s):** Edward Mitchell Andy Thompson	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(*) Andrea Musacchio, Lucia Sironi, Marina Mapelli
Department of Experimental Oncology
European Institute of Oncology
Via Ripamonti 435
20141 Milan
Italy

The spindle checkpoint insures fidelity of chromosome segregation by halting sister chromatid separation in cells with a defective mitotic spindle (Shah and Cleveland, 2000). Mitotic-Arrest Deficient (MAD) mutants impair the ability of Saccharomyces *cerevisiae* to arrest cell cycle progression as a consequence of spindle damage. Homologous proteins in higher eukaryots are involved in functionally equivalent pathways. Vertebrate Mad1 and Mad2 associate with kinetochores in prometaphase, from which they detach in metaphase and anaphase, consistent with a role for these proteins in processes that monitor attachment of sister chromatids to the spindle. Human Mad2a is almost entirely spanned by the Horma domain. Its structure consists of a central helical layer flanked on one side by a large -sheet, and on the other by a long and irregular -hairpin. Several conserved Mad2 residues map to the solvent-exposed face of the large -sheet, and possibly identify the location of a binding site for interacting proteins (Luo et al., 2000). The interaction between Mad2 and Mad1 these proteins is extremely strong, and resilient to chaotropic agents and high salts.

Recently, we have been able to raise crystals of Mad2 in complex with a C-terminal region of Mad1. Structure determination and comparison with the unliganded structure will help us to define the structural bases of activation of Mad2 during spindle checkpoint activation. Although this project was not included in last year's application, we decided to collect data at the ESRF based on its particular scientific interest. Mad1-Mad2 crystallizes in two highly related monoclinic crystal forms, which share two of the cell axes, while the third axis undergoes a doubling in dimensions (we refer to these crystals as short- and long-axis). During our visits at the ESRF we have been able to collect a high-resolution datasets from native short-axis crystals, and several datasets from crystals soaked in heavy atom derivatives belonging to either cell. We have also collected MAD data at the Se edge from a long-axis crystals (we don't seem to be able to obtain Se derivatives with a short axis). There are 56 Se atoms in the AU of these crystals. Data collection was complicated by a combination of relatively high mosaicity of the crystals (0.6°) and the fact that they orient in cryoloops with their long axis perpendicular to the spindle axis. This leads to poor completeness, especially at high resolution. We would now like to collect fresh data with improved freezing conditions, and with crystals oriented differently in the cryoloop, so to obtain a complete a somewhat higher resolution dataset.

Luo, X., Fang, G., Coldiron, M., Lin, Y., Yu, H., Kirschner, M.W. and Wagner, G. (2000) Structure of the mad2 spindle assembly checkpoint protein and its interaction with cdc20 [In Process Citation]. *Nat Struct Biol*, **7**, 224-229.

Shah, J.V. and Cleveland, D.W. (2000) Waiting for anaphase: Mad2 and the spindle assembly checkpoint. *Cell*, **103**, 997-1000.

Table: Summary of Mad1-Mad2 data collection (ID14-3, from 28-04-01 to 30-04-01)

Crystal	native short cell
Space Group	P21
Unit cell	a=111.9 b=63.4 c=139.5 beta=111.6
Resolution	2.05 Å
N° measurements	361702
N° reflections	113032
Completeness	99.8 %
Rsym	4.6 %

Crystal	Pb derivative short cell
Space Group	P21
Unit cell	a=111.6 b=63.1 c=139.5 beta=111.8
Resolution	2.5 Å
N° measurements	168521
N° reflections	53499
Completeness	85.5 %
Rsym	8.5 %

Crystal	Pt short cell
Space Group	P21
Unit cell	a=111.1 b=63.7 c=137.3 beta=111.5
Resolution	3.4 Å
N° measurements	75490
N° reflections	22876
Completeness	91.6 %
Rsym	8.4 %

Crystal	Sm short cell
Space Group	P21

Unit cell	a=111.6 b=63.1 c=139.0 beta=111.8
Resolution	2.8 Å
N° measurements	137543
N° reflections	44369
Completeness	100 %
Rsym	5.5 %

Crystal	Pb long cell
Space Group	P21
Unit cell	a=111.7 b=63.2 c=261.8 beta=90.5
Resolution	3.0 Å
N° measurements	232332
N° reflections	72604
Completeness	97.9 %
Rsym	3.9 %

Crystal	Os long cell
Space Group	P21
Unit cell	a=112.0 b=63.4 c=261.5 beta=90.6
Resolution	3.5 Å
N° measurements	123495
N° reflections	38591
Completeness	77.7 %
Rsym	9.6 %

Crystal	Hg long cell
Space Group	P21
Unit cell	a=111.1 b=63.3 c=259.6 beta=91.0
Resolution	3.7 Å
N° measurements	99647

N° reflections	29308
Completeness	73.0 %
Rsym	7.9 %

Table: Summary of Mad1-Mad2 data collection (ID29, from 23-06-01 to 25-06-01)

Crystal	Se long cell peak
Space Group	P21
Unit cell	a=111.7 b=63.2 c=262.6 beta=90.6
Resolution	2.8 Å
N° measurements	359660
N° reflections	124021
Completeness	70.0 %
Rsym	5.7 %

Crystal	Se long cell inflection
Space Group	P21
Unit cell	a=111.8 b=63.3 c=263.3 beta=90.6
Resolution	3.0 Å
N° measurements	277615
N° reflections	97409
Completeness	70.0 %
Rsym	5.7 %

Crystal	Se long cell remote
Space Group	P21
Unit cell	a=111.8 b=63.4 c=263.69beta=90.6
Resolution	3.5 Å
N° measurements	184186
N° reflections	59415
Completeness	65.0 %

Rsym	7.9 %