



## Society for Experimental Biology Annual Main Meeting 28th June – 1st July 2009, Glasgow, UK

### PLENARY – BIDDER LECTURE

#### PL1.1

17:30 Sunday 28th June 2009

#### How does cohesin hold sister DNAs together?

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It has been suggested that cohesin holds sister DNAs together by trapping them inside a huge tripartite ring formed through the interconnection by cohesin's  $\alpha$ -kleisin subunit (Scc1) of its Smc1 and Smc3 ATPase heads at the ends of the two arms of V-shaped Smc1/Smc3 heterodimers. As predicted by this model, site-specific chemical cross-linking of cohesin's Smc1-Smc3 and Smc-kleisin interfaces traps circular sister minichromosome DNAs inside a covalently circularized cohesin ring that is resistant to heating in SDS. This finding is inconsistent with the recent proposal that cohesion between sister chromatids is mediated through an interaction between two separate cohesin rings via a shared Scc3 (SA1/2) subunit, as suggested by a recent variation on a "handcuff" model (Zhang et al., 2008). Another important prediction of the ring model is that stable DNA entrapment should depend on the interaction Smc1 and Smc3 hinge/dimerization domains being very tight. To test this, we have created mutations in Smc1 and Smc3 hinges that weaken but do not eliminate their interaction. Such mutations neither hamper formation of cohesin rings nor prevent their association with chromosomes but they greatly reduce half life of cohesin's association with centromeric chromatin and abolish its ability to mediate sister chromatid cohesion. The ring model raises crucial questions as to how entrapment arises during DNA replica-

tion. If, as seems likely, entrapment is mediated by pre-assembled cohesin rings, then these must transiently open; cohesin must possess an entry gate, which has been proposed to be at the Smc1/Smc3 "hinge" interface. Establishment of cohesion during S phase but not its maintenance during G2 or M phase depends on Eco1 and the Scc2/4 complex. The findings that cohesin still associates with chromosomes in *eco1* mutants but fails to build cohesion during S phase while it fails even to associate with chromatin in *scc2* or *scc4* mutants suggest that Eco1 and Scc2/4 have distinct functions. Eco1 is an acetyl transferase (Ivanov et al., 2002) but how this activity facilitates the establishment of sister chromatid cohesion is not understood. We have identified 219 mutations in genes encoding essential (SMC3, PDS5, and SCC3) and non-essential (RAD61/WAPL) cohesin proteins that bypass the need for Eco1 in *S. cerevisiae*. The *smc3* mutations cluster around and include a highly conserved lysine (K113) close to Smc3's ATP binding pocket, which together with K112 is acetylated by Eco1, as reported by Unal et al., 2008 and Ben-Shahar et al., 2008. Lethality caused by mutating both residues to arginine is suppressed by the *scc3*, *pds5*, and *rad61* suppressor mutations. We show that Scc3, Pds5, and Rad61 form a complex and suggest that it inhibits entrapment of sister DNAs during DNA replication by a process involving the "K112/K113" surface on Smc3's ATPase. According to this model, Eco1 promotes sister DNA entrapment partly by relieving inhibition caused by an anti-establishment activity associated with Scc3, Pds5, and Rad61.

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