

- 6 McEwan, I.J. (2000) Investigation of steroid receptor function in the budding yeast *Saccharomyces cerevisiae*. *FEMS Microbiol. Lett.* 176, 1–9
- 7 Butt, T.R. and Walfish, P.G. (1996) Human nuclear receptor heterodimers: opportunities for detecting targets of transcriptional regulation using yeast. *Gene Exp.* 5, 255–268
- 8 Sitcheran, R. *et al.* (2000) A genetic analysis of glucocorticoid receptor signalling: identification and characterization of ligand-effect modulators in *Saccharomyces cerevisiae*. *Genetics* 156, 963–972
- 9 White, R. and Parker, M.G. (1998) Molecular mechanisms of steroid hormone action. *Endocr.-Relat. Cancer* 5, 1–14
- 10 Garabedian, M.J. and Yamamoto, K.R. (1992) Genetic dissection of the signalling domain of a mammalian steroid receptor in yeast. *Mol. Biol. Cell* 3, 1245–1257
- 11 Wrenn, C.K. and Katzenellenbogen, B.S. (1993) Structure–function analysis of the hormone binding domain of the human estrogen receptor by region-specific mutagenesis and phenotypic screening in yeast. *J. Biol. Chem.* 268, 24089–24098
- 12 Lind, U. *et al.* (1996) Identification of single amino acid substitutions of cys-736 that affect the steroid-binding affinity and specificity of the glucocorticoid receptor using phenotypic screening in yeast. *Mol. Endocrinol.* 10, 1358–1370
- 13 Almlöf, T. *et al.* (1997) Role of hydrophobic amino acid clusters in the transactivation activity of the human glucocorticoid receptor. *Mol. Cell. Biol.* 17, 934–945
- 14 Picard, D. *et al.* (1990) Reduced levels of hsp90 compromise steroid receptor action *in vivo*. *Nature* 348, 166–168
- 15 Fang, Y. *et al.* (1996) Hsp90 regulates androgen receptor hormone binding affinity *in vivo*. *J. Biol. Chem.* 271, 28697–28702
- 16 Yoshinaga, S.K. *et al.* (1992) Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. *Science* 258, 1598–1604
- 17 Henriksson, A. *et al.* (1997) Role of the Ada adaptor complex in gene activation by the glucocorticoid receptor. *Mol. Cell. Biol.* 17, 3065–3073
- 18 vom Baur, E. *et al.* (1998) The yeast Ada complex mediates the ligand-dependent activation function AF-2 of retinoid X and estrogen receptors. *Genes Dev.* 12, 1278–1289
- 19 Gilbert, D.M. *et al.* (1993) Estradiol-inducible squelching and cell growth arrest by a chimeric VP16-estrogen receptor expressed in *Saccharomyces cerevisiae*: suppression by an allele of PDR1. *Mol. Cell. Biol.* 13, 462–472
- 20 Imhof, M.O. and McDonnell, D.P. (1996) Yeast Rsp5 and its human homolog hRPF1 potentiate hormone-dependent activation of transcription by human progesterone and glucocorticoid receptors. *Mol. Cell. Biol.* 16, 2594–2605
- 21 Kralli, A. *et al.* (1995) LEM1, an ATP-binding-cassette transporter, selectively modulates the biological potency of steroid hormones. *Proc. Natl. Acad. Sci. U. S. A.* 92, 4701–4705
- 22 Krobtsch, S. and Lindquist, S. (2000) Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1589–1594
- 23 Knutti, D. *et al.* (2000) A tissue-specific coactivator of steroid receptors, identified in a functional genetic screen. *Mol. Cell. Biol.* 20, 2411–2422
- 24 Kimura, Y. *et al.* (1995) Role of the protein chaperone Ydj1 in establishing Hsp90-mediated signal transduction pathways. *Science* 268, 1362–1365
- 25 Caplan, A.J. *et al.* (1995) Hormone-dependent transactivation by the human androgen receptor is regulated by a dnaJ protein. *J. Biol. Chem.* 270, 5251–5257
- 26 Krstic, M.D. *et al.* (1997) Mitogen-activated and cyclin-dependent protein kinases selectively and differentially modulate transcriptional enhancement by the glucocorticoid receptor. *Mol. Cell. Biol.* 17, 3947–3954
- 27 Gaudon, C. *et al.* (1999) Role of the essential yeast protein PSU1 in transcriptional enhancement by the ligand-dependent activation function AF-2 of nuclear receptors. *EMBO J.* 18, 2229–2240
- 28 Baniahmad, C. *et al.* (1995) Enhancement of human estrogen receptor activity by SPT6: a potential coactivator. *Mol. Endocrinol.* 9, 34–43

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## Epigenetic analysis of kinetochore assembly on variant human centromeres

Peter E. Warburton

**Human centromere formation involves the assembly of the mitotic kinetochore onto chromosomal locations that contain the interphase prekinetochore. Immunofluorescent analysis of two functionally converse human centromere variants, neocentromeres and inactive centromeres, has been used to evaluate the functional significance of over 24 centromere proteins, providing important insight into the epigenetics of centromere formation and kinetochore assembly.**

The CENTROMERE–KINETOCHORE COMPLEX (see Glossary) is located at the primary constriction of mitotic mammalian chromosomes and is responsible for the proper segregation of replicated sister chromatids to daughter cells. This complex consists of the proteinaceous kinetochore assembled onto each sister chromatid, with the underlying centromeric heterochromatin located between these sister kinetochores. There are five known CONSTITUTIVE

CENTROMERE PROTEINS: CENP-A, -B, -C, -G and -H. These are found at centromeres throughout the cell cycle, in the interphase PREKINETOCHORES and in mitotic INNER KINETOCHORE PLATES. FACULTATIVE KINETOCHORE PROTEINS, which function in the spindle assembly checkpoint, microtubule capture and chromosome movement, assemble onto the prekinetochore during mitosis to form the OUTER KINETOCHORE PLATES<sup>1,2</sup>.

Two naturally occurring centromere variants, NEOCENTROMERE-containing chromosomes and stable DICENTRIC CHROMOSOMES, suggest that human centromere formation is an epigenetic process that does not necessarily depend on a specific underlying DNA sequence<sup>3</sup>. Neocentromeres are newly formed centromeres found on rearranged chromosome fragments that have separated from endogenous centromeres, and represent acquisition of the centromeric epigenetic mark in a previously non-centromeric chromosomal

location. Conversely, inactive centromeres are found on stable dicentric chromosomes, and represent loss of the centromeric epigenetic mark from the  $\alpha$ -SATELLITE DNA found at all normal centromeres.

Many human centromere proteins were fortuitously identified by immunolocalization to endogenous centromeres using autoimmune sera or specific antibodies<sup>1,2</sup>. The power of such immunolocalization is greatly extended by analysis of neocentromeres and inactive centromeres, which in effect provides a simple epigenetic assay for the functional significance of centromere proteins. The presence of a protein at normal centromeres and neocentromeres, but not at inactive centromeres, suggests a role in active kinetochore function, regardless of the underlying DNA sequence. By contrast, the presence of a protein at normal and inactive centromeres, but not at neocentromeres, suggests an association with  $\alpha$ -satellite DNA or heterochromatin,

## Glossary

**$\alpha$ -satellite DNA:** The tandemly-repeated DNA family found at every human endogenous centromere.

**C banding (constitutive heterochromatin banding):** A cytogenetic chromosome-banding technique that specifically stains regions of constitutive heterochromatin, including endogenous centromeres.

**Centromere:** The chromosomal location where a kinetochore is formed.

**Centromere-kinetochore complex:** The chromosomal location that contains a centromere with a kinetochore assembled onto it, responsible for the proper segregation of chromosomes during mitosis.

**Constitutive centromere protein:** Proteins that are found at the centromere throughout the cell cycle.

**DAPI (4,6 diamidino-2-phenylindole) brightness:** A fluorescent chromosome counterstain, which shows greater intensity at regions of constitutive heterochromatin, including endogenous centromeres.

**Dicentric chromosomes:** Chromosomal rearrangements that contain two centromeres; in order to be mitotically stable, one centromere must be inactivated.

**Facultative kinetochore proteins:** Proteins that are transiently found at the kinetochore only during mitosis.

**Inner kinetochore plate:** The kinetochore domain that is attached to the chromosome surface and primarily contains constitutive centromere proteins.

**Kinetochore:** The proteinaceous structure that assembles onto centromeres.

**Outer kinetochore plate:** Part of the kinetochore assembled onto the inner plate during mitosis and containing facultative kinetochore proteins.

**Neocentromere:** A fully functional centromere found on rearranged chromosome fragments that have separated from endogenous centromeres and contain no  $\alpha$ -satellite DNA.

**Prekinetochore:** The structure containing constitutive centromere proteins throughout interphase of the cell cycle, marks the position where the mitotic kinetochore will form.

but not a role in kinetochore function. To date, over 24 centromere proteins have been analyzed on normal, neo- and inactive centromeres (Table 1), which has provided important insight into the nature of the interphase prekinetochore and epigenetic centromere determination.

### Variant centromeres

Dicentric chromosomes, which result from the fusion of chromosomal arms, were first exploited for functional analysis of centromere proteins by Earnshaw and Migeon in 1985 (Ref. 4). The presence of two active centromeres on a chromosome can result in individual chromatids being pulled to opposite spindle poles, resulting in anaphase bridging, chromosome breakage and mitotic instability. For a dicentric chromosome to be mitotically stable, one of the two centromeres must be inactivated (unless the two centromeres are very close together<sup>5</sup>). Inactive centromeres usually contain normal arrays of repetitive  $\alpha$ -satellite DNA sequences identical to those at active centromeres (Fig. 1a). Furthermore, they retain properties of centromeric heterochromatin such as C BANDING and DAPI BRIGHTNESS, although they do not have a constriction or form a kinetochore.

Neocentromeres represent a gain of centromeric function of a normally noncentromeric chromosomal domain. The first neocentromere was described in 1993 (Ref. 6). Since then, over 42 examples derived from 16 human chromosomes have been observed<sup>7</sup>. The presence of a neocentromere confers mitotic stability to a chromosome fragment that would

otherwise be acentric and rapidly lost. Neocentromeres have formed in many different chromosomal locations in the genome, apparently with little or no regard for underlying DNA sequence. The majority of neocentromeres are found on inversion duplications of a distal portion of a chromosome arm (Fig. 1b), with others found on chromosome fragments resulting from para- or pericentric deletions. Cells can contain up to four chromosomal regions that are homologous to the neocentromere position, with only one forming an active centromere (Fig. 1b). Neocentromere-containing chromosomes are often observed in less than 100% of cultured or patient cells. However, this mosaicism might not necessarily reflect a compromised mitotic function of neocentromeres, as such mosaicism is frequently seen for chromosomal fragments with normal centromeres. Neocentromeres display primary constrictions and normal kinetochore structure, but do not display properties of centromeric heterochromatin.

### Protein composition at variant human centromeres

Immunofluorescent analysis of normal, neo- and inactive centromeres has become an important criterion in the functional characterization of centromere proteins. Several large studies have examined the presence or absence of known centromere proteins at either neocentromeres<sup>8</sup> or dicentric chromosomes<sup>9-11</sup>, and many newly described centromere proteins were analyzed on both, including CENP-A (Ref. 12), CENP-G (Ref. 13),

CENP-H (Ref. 14), SUV39H (Ref. 15), PARP-1 (Ref. 16), and HZWint-1 (Ref. 17). Overall, these results show that the vast majority of facultative kinetochore proteins are specifically associated with active centromeres; that is, present on normal and neocentromeres and absent from inactive centromeres (Table 1)<sup>8,10,11</sup>. By contrast, only three of the five constitutive centromere proteins associate specifically with active centromeres: CENP-A, a centromere-specific histone H3 homologue; CENP-C, a DNA-binding protein; and CENP-H, a novel coiled-coil-containing protein<sup>9,12,14</sup>. Both CENP-C-null and CENP-A-null mice show severe mitotic defects, confirming their importance in centromere formation<sup>2</sup>. Thus, constitutive CENPs associated with active centromeres are good candidates for establishing the epigenetic mark responsible for the propagation of human centromeres at particular chromosomal locations.

Nonetheless, the essentially identical composition of the mitotic kinetochore in neocentromeres and normal centromeres, and the complete absence of a kinetochore from inactive centromeres, suggests that once the epigenetic mark is in place, kinetochores assemble during mitosis in an 'all or nothing' coordinated cascade of protein interactions, regardless of the underlying DNA sequence<sup>8</sup>. Thus, the identification of 'mutant' neocentromeres that do not assemble complete kinetochores seems unlikely, although only a limited number of neocentromeres have been subjected to extensive immunofluorescent analysis. It is conceivable, for example, that neocentromeres that are incapable of activating the spindle-assembly checkpoint might nonetheless retain sufficient mitotic stability to be observed. Furthermore, examination of the proteins found at artificially created mini-chromosomes with  $\alpha$ -satellite DNA-based *de novo* centromeres<sup>18,19</sup>, and at  $\alpha$ -satellite DNA integrated into ectopic chromosomal locations<sup>20</sup>, could provide further insight into kinetochore assembly.

A few centromere proteins are not specifically associated with active centromeres. A classic example is CENP-B, seen at normal and inactive centromeres but not at neocentromeres or the Y chromosome centromere (Table 1). This pattern is because CENP-B binds specifically to a 9-bp degenerate sequence motif found in  $\alpha$ -satellite DNA from every centromere except the Y chromosome<sup>21</sup>. CENP-B, as

well as  $\alpha$ -satellite DNA and some cytological properties of heterochromatin (Table 1), appear to be neither necessary for centromere formation (as they are absent from neocentromeres), nor sufficient for centromere formation (as they are present at inactive centromeres). The absence of a detectable mitotic phenotype for CENP-B-null mice<sup>2</sup> and the binding of CENP-B to nonfunctional ectopic  $\alpha$ -satellite DNA<sup>20</sup> support a noncritical role for CENP-B. Nonetheless, *de novo* centromere formation has been observed only on  $\alpha$ -satellite DNA that contains CENP-B binding sites, suggesting that CENP-B might facilitate or stabilize centromere formation<sup>18,19</sup>.

Two centromere proteins, CENP-G and mitotic centromere-associated kinesin (MCAK), display another pattern of variant centromere localization, present on normal, neo- and inactive centromeres (Table 1). CENP-G is a 95-kD constitutive centromere protein detected by a human autoimmune serum<sup>13,22</sup>. Assuming specificity of the autoimmune serum, the results presented by Gimelli *et al.* are consistent with CENP-G localizing to centromeres by two mechanisms: (1) binding to the  $\alpha$ -1 subfamily of  $\alpha$ -satellite DNA, explaining the localization to an inactive chromosome 8 centromere; and (2) interacting with other constitutive active centromere proteins, explaining the localization to neocentromeres from chromosome 3 and the Y chromosome, as well as to the endogenous Y chromosome centromere (which does not contain the  $\alpha$ -1 subfamily).

The reduced CENP-G immunofluorescence signal observed at Y, neo-, and inactive centromeres<sup>13</sup> is consistent with localization either by protein interactions or by DNA binding, but not both. The absence of CENP-G from the inactive centromere on a neodicentric Y chromosome (Fig. 1c) is consistent with the absence of both the  $\alpha$ -1 subfamily and active kinetochore proteins on inactive Y centromeres. By bridging between  $\alpha$ -satellite DNA and centromere proteins, CENP-G might stabilize the position of all endogenous centromeres except the Y chromosome, which is the only chromosome observed to form neodicentric chromosomes<sup>7</sup>. Assaying for the presence of CENP-G at ectopic  $\alpha$ -satellite DNA would provide evidence for its DNA-binding activity, although direct testing awaits its cloning and expression. CENP-G-null mice could display phenotypes of intermediate severity between CENP-A/C and CENP-B nulls, reflecting centromere destabilization,

**Table 1. Kinetochore composition at normal, neo- and inactive centromeres**

Normal	Neo-	Inactive	Centromere components	Functional significance	Refs	
+	+	-	CENP-A	Epigenetic centromere determination	12	
			CENP-C		7,9	
			CENP-H		14	
			CENP-E	Motor related, microtubule binding	9,10	
			Dynein		8,10	
			HZW10		8,10	
			p150 <sup>glued</sup>		8,10	
			Arp1		8,10	
			Hzwint-1		17	
			Dynamitin <sup>a</sup>		10	
			TD60 <sup>a</sup>		10	
			clip-170 <sup>b</sup>		8	
			INCENP <sup>b</sup>		8	
			Mad2	Spindle assembly checkpoint, anaphase promoting complex	7,8	
			Bub1		8,11	
			BubR1		8,11	
			p55CDC		8,11	
			ERK1		8,11	
			Tsg24		8,11	
			3F3/2 epitope		8,11	
			CENP-F		8,10	
			PARP-1		Protein modifiers	16
			SUV39H		15	
+	+	+	CENP-G <sup>c</sup>	Kinetochore stabilization	13	
			MCAK <sup>d</sup>		8,10	
+	-	+	CENP-B <sup>e</sup>	$\alpha$ -satellite DNA binding, heterochromatization	21	
			DAPI brightness, C banding, $\alpha$ -satellite DNA			
+	-	-	None detected	(‘All or nothing’ kinetochore assembly)		

+ indicates presence, - indicates absence from normal, neo- or inactive centromere.  
<sup>a</sup>Not tested on neocentromere.  
<sup>b</sup>Not tested on inactive centromere.  
<sup>c</sup>Not present on inactive Y chromosome centromere, present on active Y centromere.  
<sup>d</sup>Present at inactive centromere on unseparated chromatids.  
<sup>e</sup>Not present on active Y centromere.

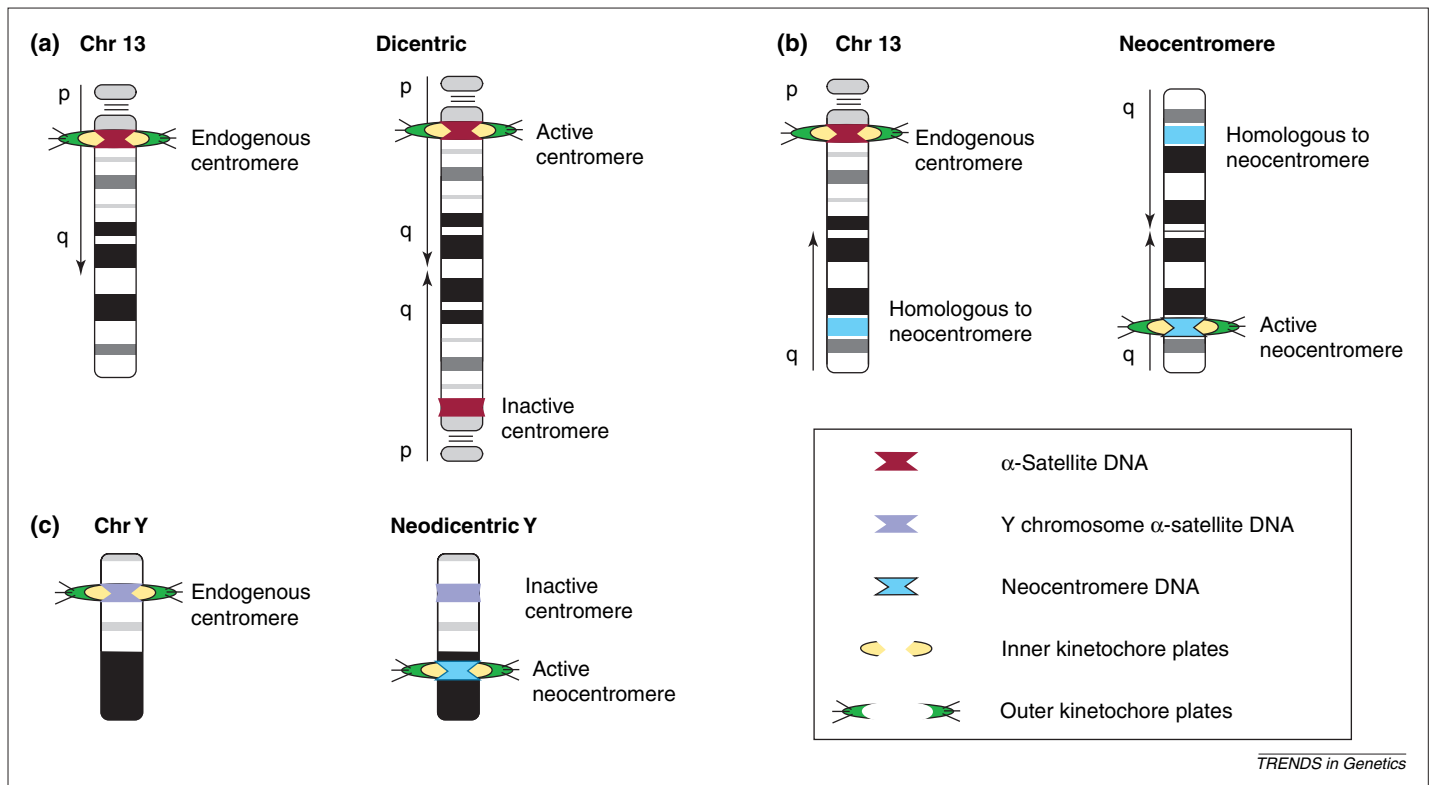
but not complete loss of function. It will be of interest to overexpress CENP-G in the presence of transfected  $\alpha$ -satellite DNA, which could promote *de novo* centromere formation.

MCAK is a facultative kinetochore protein that might function in microtubule depolymerization during anaphase<sup>23</sup>. MCAK localizes diffusely in the centromeric heterochromatin and kinetochore plates on all normal and neocentromeres examined<sup>8</sup>, and at the inactive centromeres of dicentric chromosomes that had not yet separated their sister chromatids<sup>10</sup>. Because MCAK is localized in the heterochromatin, it could be recruited to centromeres during mitosis by a different mechanism than outer kinetochore plate proteins, involving factors that are retained in the heterochromatin of inactive centromeres. However, the premature loss of MCAK from inactive centromeres suggests that MCAK is stabilized by active kinetochore

components such as CENP-H, which interacts with MCAK *in vitro*<sup>14</sup>.

#### Future directions

The cumulative results shown in Table 1 suggest a highly coordinated process of kinetochore assembly, and imply that outer kinetochore plate proteins not specifically associated with active centromeres are unlikely to be identified. The three proteins that do not associate with active kinetochores, MCAK, CENP-B and CENP-G (Table 1), are all located in the centromeric heterochromatin subjacent to the kinetochores. Thus, examination of chromosomal processes that occur in this region at variant centromeres could reveal important insights into centromere function. For example, it is not known whether the cohesin complex, which disassociates from chromosome arms during condensation to remain specifically in centromeric heterochromatin until the



**Fig. 1.** Variant centromeres. (a) Structure of a dicentric chromosome consisting of a long (q) arm fusion<sup>12</sup>, compared with a normal chromosome 13 (Chr 13). The inverted duplicated region is indicated by the arrow. Both the active and inactive centromeres contain  $\alpha$ -satellite DNA (red), but only the active centromere forms a kinetochore (green and yellow). (b) Structure of a neocentromere-containing chromosome consisting of a fusion of the distal portion of the long (q) arm<sup>7</sup>, compared with a normal chromosome 13 (Chr 13). The inverted duplicated region is indicated by the arrow. The neocentromere has formed in a previously non-centromeric region in 13q32 (blue). Regions homologous to the neocentromere are found at the other end of the inversion duplication chromosome as well as on the normal chromosome 13. (c) Structure of a neodicentric Y chromosome compared with a normal Y chromosome. The neodicentric chromosome consists of a Y chromosome containing an inactivated endogenous Y centromere and an active neocentromere in the long arm. The  $\alpha$ -satellite DNA found on Y chromosomes (indigo) is relatively diverged from  $\alpha$ -satellite DNA at other endogenous centromeres, and does not belong to the  $\alpha$ -1 subfamily. Three examples of this type of chromosome have been described<sup>13</sup> (see also Ref. 7).

onset of anaphase, also remains at inactive centromeres or neocentromeres.

Two of the facultative proteins associated with active centromeres, PARP-1 and SUV39H (Table 1), indicate that post-translational modifications of proteins could be important in centromere function. The functional significance of PARP-1 could be confirmed by analysis of poly-ADP ribosylation at centromeres and identification of possible substrates such as histones<sup>2,16</sup>. SUV39H, which is located in the kinetochore plates, specifically methylates histone H3 at lysine 9 *in vitro*, which inhibits histone H3 phosphorylation at serine10 (Refs 15,24). Phosphorylation of histone H3 initiates in the centromeric heterochromatin of normal centromeres, coincident with chromosome condensation, and does not occur in the kinetochore plates<sup>25</sup>. It is not clear whether histone H3 is methylated by SUV39H at kinetochores, perhaps to prevent serine10

phosphorylation and chromosome condensation at the kinetochore. Alternatively, histone H3 might be completely replaced at kinetochores by CENP-A, which was recently shown to be phosphorylated during mitosis as well<sup>26</sup>.

Thus, a distinct centromeric 'histone code'<sup>27</sup> of H3 amino-terminal tail modifications could be crucial in establishing both the underlying centromeric heterochromatin and the specialized kinetochore chromatin. The functional significance of this histone code can be evaluated by examination of variant centromeres, to determine whether, for example, histone H3 phosphorylation also initiates at inactive centromeres, or whether CENP-A is phosphorylated at neocentromeres. Thus, immunofluorescent analysis of variant human centromeres will remain an important part of the characterization of human centromere proteins as they are identified, providing

us with a simple and straightforward evaluation of the functional requirements for centromere formation.

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#### References

- Dobie, K.W. *et al.* (1999) Centromere proteins and chromosome inheritance: a complex affair. *Curr. Opin. Genet. Dev.* 9, 206–217
- Choo, K.H. (2000) Centromerization. *Trends Cell Biol.* 10, 182–188
- Murphy, T.D. and Karpen, G.H. (1998) Centromeres take flight:  $\alpha$ -satellite and the quest for the human centromere. *Cell* 93, 317–320
- Earnshaw, W.C. and Migeon, B.R. (1985) Three related centromere proteins are absent from the inactive centromere of a stable isodicentric chromosome. *Chromosoma* 92, 290–296
- Sullivan, B.A. and Willard, H.F. (1998) Stable dicentric X chromosomes with two functional centromeres. *Nat. Genet.* 20, 227–228
- Voullaire, L.E. *et al.* (1993) A functional marker centromere with no detectable  $\alpha$ -satellite, Satellite III, or CENP-B protein: Activation of a latent centromere? *Am. J. Hum. Genet.* 52, 1153–1161
- Warburton, P.E. *et al.* (2000) Molecular cytogenetic analysis of eight inversion duplications of human chromosome 13q that each contain a neocentromere. *Am. J. Hum. Genet.* 66, 1794–1806
- Saffery, R. *et al.* (2000) Human centromeres and neocentromeres show identical distribution

- patterns of >20 functionally important kinetochore-associated proteins. *Hum. Mol. Genet.* 9, 175–185
- 9 Sullivan, B.A. and Schwartz, S. (1995) Identification of centromeric antigens in dicentric Robertsonian translocations: CENP-C and CENP-E are necessary components of functional centromeres. *Hum. Mol. Genet.* 4, 2189–2197
  - 10 Faulkner, N.E. *et al.* (1998) Localization of motor-related proteins and associated complexes to active, but not inactive, centromeres. *Hum. Mol. Genet.* 7, 671–677
  - 11 Saffery, R. *et al.* (2000) Components of the human spindle checkpoint control mechanism localize specifically to the active centromere on dicentric chromosomes. *Hum. Genet.* 107, 376–384
  - 12 Warburton, P.E. *et al.* (1997) Immunolocalization of CENP-A, a kinetochore-specific histone H3 variant, suggests a distinct nucleosome structure at the inner kinetochore plate of active centromeres. *Curr. Biol.* 7, 901–904
  - 13 Gimelli, G. *et al.* (2000) CENP-G in neocentromeres and inactive centromeres. *Chromosoma* 109, 328–333
  - 14 Sugata, N. *et al.* (2000) Human CENP-H multimers colocalize with CENP-A and CENP-C at active centromere-kinetochore complexes. *Hum. Mol. Genet.* 9, 2919–2926
  - 15 Aagaard, L. *et al.* (2000) Mitotic chromatin association of SUV39H, a novel component of active centromeres, correlates with cell cycle dependent phosphorylation. *J. Cell Sci.* 113, 817–829
  - 16 Earle, E. *et al.* (2000) Poly(ADP-ribose) polymerase at active centromeres and neocentromeres at metaphase. *Hum. Mol. Genet.* 9, 187–194
  - 17 Starr, D.A. *et al.* (2000) HZWint-1, a novel human kinetochore component that interacts with HZW10. *J. Cell Sci.* 113, 1939–1950
  - 18 Ikeno, M. *et al.* (1998) Creation of human artificial chromosomes by introduction of YACs retrofitted with human telomeric DNA. *Nat. Biotechnol.* 16, 431–439
  - 19 Harrington, J.J. *et al.* (1997) Formation of *de novo* centromeres and construction of first-generation human artificial microchromosomes. *Nat. Genet.* 15, 345–355
  - 20 Warburton, P.E. and Cooke, H.J. (1997) Hamster chromosomes containing amplified human  $\alpha$ -satellite DNA show delayed sister chromatid separation in the absence of *de novo* kinetochore formation. *Chromosoma* 106, 149–159
  - 21 Kipling, D. and Warburton, P.E. (1997) Centromeres, CENP-B, and tigger too. *Trends Genet.* 13, 141–145
  - 22 He, D. *et al.* (1998) CENP-G: a new centromeric protein that is associated with the  $\alpha$ -1 satellite DNA. *Chromosoma* 107, 189–197
  - 23 Maney, T. *et al.* (1998) Mitotic centromere-associated kinesin is important for anaphase chromosome segregation. *J. Cell Biol.* 142, 787–801
  - 24 Rea, S. *et al.* (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406, 593–599
  - 25 Van Hooser, A.A. *et al.* (1999) The mammalian centromere: structural domains and the attenuation of chromatin modeling. *FASEB J.* 13, 216–220
  - 26 Zeitlin, S.G. *et al.* (2001) Differential regulation of CENP-A and histone H3 phosphorylation in G(2)/M. *J. Cell Sci.* 114, 653–661
  - 27 Strahl, B.D. and Allis, C.D. (2000) The language of covalent histone modifications. *Nature* 403, 41–45

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### Meeting Report

## Genomics: saviour or millstone?

Sohaila Rastan

**The Keystone Symposium on the Impact of Genomics on Drug Discovery and Development was held in Santa Fe, New Mexico, from 2 to 7 February 2001.**

The opening speaker, Jonathan Knowles of Hoffman La Roche, set the scene in the Keynote Address. The issues facing the drug industry are threefold: first, we have no drugs for certain disorders; second, when we do have drugs many people do not respond to them or suffer adverse side effects; and third, what we do to address the first two issues must be cost effective. Genomics stands in the dock, accused of being expensive and profligate, having greatly inflated R&D spending and having delivered, in terms of drugs on the market, almost nothing. What it has delivered is thousands of potential new targets. The challenge now is to evaluate and validate the myriad of new targets the Human Genome Project identified, and to produce successful therapeutics without bankrupting ourselves in the process. How do we pick the winners from such an embarrassment of riches? Will poorly understood targets fail in the clinic?

What followed was a lively mixed response to the laying down of this gauntlet, but, refreshingly, much of the hype characteristic of earlier genomics meetings had evaporated, being replaced with more realistic views and, sometimes, frank scepticism.

**'Genomics stands in the dock, accused of being expensive and profligate, having greatly inflated R&D spending and having delivered, in terms of drugs on the market, almost nothing.'**

### Transcriptome versus proteome

There was considerable discussion and badinage, mostly good-humoured, about the relative merits of analysis of the transcriptome versus analysis of the proteome. Although the new, lower estimate of the number of genes in the human genome (~32 000) had not been announced at the time of the meeting, the consensus was that eventually analysis of the genome (which represents what could happen) will give way to analysis of the transcriptome (what might be happening) and will finally be superseded by analysis

of the proteome (what is happening). Proteomics faces the most formidable technical obstacles, primarily resolution and reproducibility. Getting away from gel-based methods is likely to hold the key to success.

### High throughput versus low throughput

Miniaturization and automation, to maximize speed and minimize cost, are common themes to all analyses on a genomic scale and are also characteristic of nongenomic companion technologies, such as high-throughput and ultra-high-throughput screening and combinatorial chemistry. But the consensus was clear: all that high-throughput platform technologies have done is move the bottleneck along – the true bottleneck for getting drugs on the market (the only real measure of success) is, more than ever, detailed biology and target validation. One effort at meaningful high-throughput biological screens was described by Jim Piggott of Lexicon Genetics. This was a variation on the SHIRPA protocols<sup>1</sup>. These robust, non-invasive screens are amenable to digital data capture and were