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Report

Positional analyses of BRCA1-dependent expression in *Saccharomyces cerevisiae*

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Mutations in BRCA1 account for a significant proportion of hereditary breast and ovarian cancers, but analysis of BRCA1 function is complicated by pleiotropic effects and binding partners (Pol II holoenzyme and transcription factors, chromatin remodelers, recombination complexes and E3 ligases). In vertebrate cells, efforts to elucidate BRCA1 transcriptional effects have focused on specific genes or restricted portions of the genome—limiting analyses of BRCA1 effects on adjoining DNA sequences and along chromosome lengths. Here, we use microarray analyses on the genetically tractable yeast cell system to elucidate BRCA1-dependent genome-wide positional effects on both gene induction and repression. Yeast responses may be of clinical relevance based on findings that BRCA1 severely diminishes yeast growth kinetics but that BRCA1 mutated at sites identified from breast tumors is no longer able to retard yeast cell growth kinetics. Our analysis suggests that BRCA1 acts through both transcription factors to upregulate specific loci and chromatin remodeling complexes to effect global changes in gene expression. BRCA1 also exhibits gene repression activities. Cluster-functional analysis reveals that these repressed factors are required for mitotic stability and provide a novel molecular explanation for the conditional lethality observed between BRCA1 and chromosome segregation genes.

Characteristics of BRCA1-Dependent Gene Upregulation

Mutations in BRCA1 account for a significant proportion of hereditary breast and ovarian cancers.[1] While recent reports have focused on BRCA1-dependent expression effects within specific subsets of genes,[2,3] the role that BRCA1 plays in both a positional and genome-wide context has yet to be performed. Such an analysis would be greatly simplified in yeast—given that yeast gene nomenclature provides for unambiguous positional and strand utilization cues along the chromosome length.[4] Importantly, expression of human BRCA1 in budding yeast appears to provide a clinically relevant readout since BRCA1 severely diminishes yeast growth kinetics but BRCA1 mutated at sites identified from breast tumors is no longer able to retard yeast cell growth.[5,6] In support of this, yeast mutated in *CHL1* (homolog of human BACH1/BRIP1/FANJ DNA helicase that binds BRCA1 and is required for BRCA1-dependent double strand break repair) suppress BRCA1-dependent growth defects.[7-9] Thus, BRCA1-targeted pathways are highly conserved in yeast. To capitalize on this conservation of function and to provide a unique positional context for BRCA1 function along the length of yeast chromosomes, we used human-assisted search methods to assess BRCA1 affects on mRNA levels for both individual genes and extended chromatin domains.

Recent reports document that BRCA1 genetically effects both transcription and chromosome segregation pathways in yeast,[9-12] the latter of which directly produces aneuploidy when mutated. We decided to focus on the C-terminal BRCT domain of BRCA1 because it is both necessary and sufficient to elicit the yeast small colony phenotype and because of its relevance to cancer progression.[5,6,10-14] To elucidate BRCA1 effects on gene expression, vector or vector containing the BRCT domain of BRCA1 (herein termed BRCA1) was transformed into wildtype yeast, RNA extracted from log phase yeast grown at either 23° or 30°C and genome-wide changes in expression levels analyzed by microarray hybridization. We limited our analyses to those genes whose expression was altered two-fold or greater. Results show that mRNA levels of 461 genes were altered beyond this threshold in response to BRCA1 at 23°C relative to vector controls: 307 of which were upregulated and 154 which were downregulated (Suppl. Table 1). mRNA levels of 430 genes were altered two-fold or greater by BRCA1 expression at 30°C relative to vector controls: 350 of which were upregulated and 80 of which were downregulated (Suppl. Table 2).

We identified both discrete genes and contiguous multi-gene domains that were significantly upregulated in response to BRCA1 expression. Of 307 upregulated loci (23°C), 35 instances (11%) were identified in which the affected areas encompassed 2 or more adjacent open reading frames. Of 350 upregulated loci (30°C), 38 instances (11%) were identified in which the affected areas encompassed 2 or more adjacent open reading frames. Independent analyses of both data sets revealed instances in which positively affected areas encompassed 4 adjacent open reading frames to span up to 12 kb of contiguous DNA (Suppl. Table 3). Often, one actively transcribed domain was separated from a similarly upregulated domain by only a single-intervening locus. When we allowed for single locus gaps,

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upregulated regions that encompassed up to 10 loci and spanned over 23 kb were identified (Suppl. Table 4). Under this criterion, a total of 109 genes (roughly 1/3) of all positively affected genes may be attributable to global changes in gene expression. In summary, these results provide novel information that BRCA1 may associate with both yeast transcription factors and chromatin remodeling complexes, similar to those interactions observed in human cells, and that BRCA1-activated complexes elicit global and extensive increases in *Saccharomyces cerevisiae* mRNA levels (Suppl. Fig. 1).

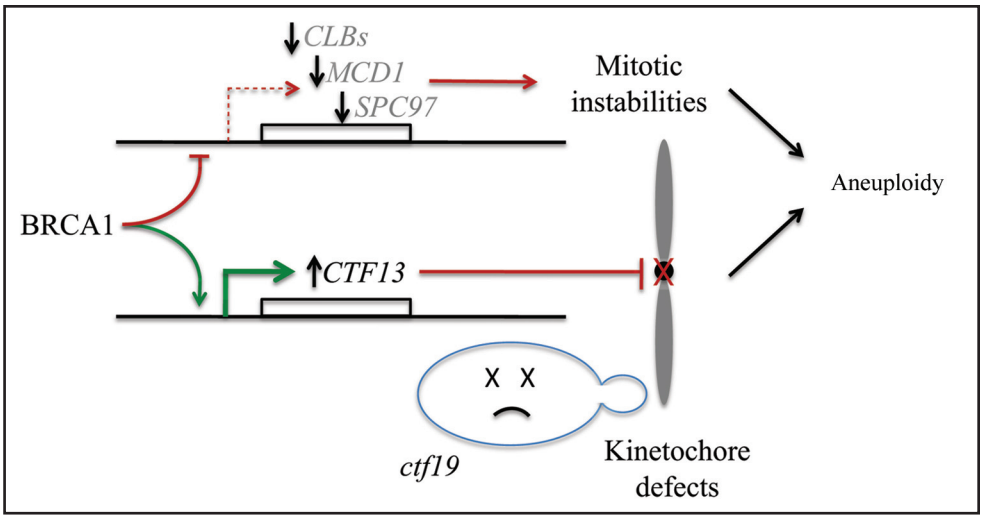

Figure 1. The combination of genetic and microarray analyses indicate that BRCA1 may contribute to cell aneuploidy and chromosomal aberrations via a two-hit mechanism. BRCA1 drives inappropriate elevated expression of *CTF13*, adversely effecting kinetochore assembly (revealed in the context of COMA kinetochore mutants such as *ctf19*). BRCA1 reduces expression of genes required for mitotic stability (numerous B-type cyclins, cohesin factor *MCD1/SCC1* and spindle pole component *SPC97*)—all of which are required for high fidelity chromosome segregation.

Characteristics of BRCA1-Dependent Gene Repression

In human cells, BRCA1 blocks the assembly of pre-initiation transcription complexes—providing one mechanism of gene repression.[12] As noted above, 154 of the 461 BRCA-affected loci were downregulated 2-fold or greater (23°C), revealing a role for BRCA1 in yeast gene repression. This data set also provided an opportunity to quantify extended regions of BRCA1-dependent repressed domains. Thus, we tabulated by hand all incidences in which repressed genes occurred immediately adjacent to one another. 10 instances were identified that encompassed a total of 20 genes (13% of total repressed genes) in which one repressed gene was immediately juxtaposed to another repressed gene (Suppl. Table 5). Independent analyses (30°C) identified two such instances, involving a total of four loci (5%), in which repressed genes were immediately juxtaposed (Suppl. Table 6). No instances of three adjacent repressed loci were observed in either data set. In combination, these findings reveal that while low incidences of multi-gene repression can occur, the role for BRCA1 in repression predominantly occurs in a locus-specific manner and, once established, infrequently spreads to repress adjoining domains.

We next characterized the boundaries between repressed and upregulated BRCA1-affected genes. Of the 5749 verified and expressed yeast genes,[2] genes unaffected by BRCA1 expression (5442) outnumber genes upregulated by BRCA1 (307) roughly 20:1. Thus, the predicted incidence of finding a downregulated locus situated next to an upregulated locus would be at most 5%. We further reasoned that since approximately 1/3 of genes upregulated by BRCA1 appear to occur through a global-acting mechanism, the frequency of finding adjoining but oppositely regulated loci would decrease below 2%. In contrast, however, 18 examples (12%) of the 154 BRCA1-dependent repressed genes (23°C) were positioned immediately adjacent to an upregulated gene (Suppl. Table 7). Similarly, 8 examples (10%) of 80 repressed genes (30°C) were identified in which a downregulated gene was next to an upregulated gene (Suppl. Table 8). In combination, these results reveal that a surprisingly high percentage of repressed genes are situated immediately adjacent to upregulated genes. The boundary elements that establish and then maintain these transcriptional states remain an important but as yet uncharacterized facet of BRCA1-dependent gene regulation.

To better understand these transition states, we tested whether the ability to juxtapose oppositely regulated genes depended on DNA strand context. Out of the 18 adjacent but oppositely affected gene pairs, five were comprised of gene pairs situated on the Crick strand (C), four were comprised of gene pairs situated on the Watson (W) strand and nine involved gene pairs in which one was located on the Watson while the other was located on the Crick strand (including both C→W and W→C orientations). Thus, BRCA1-dependent transition states between adjacent but oppositely affected genes appear to occur independent of strand bias (data not shown).

Functional-Cluster Analyses of BRCA1-Affected Genes

BRCA1 is conditionally lethal when expressed in yeast strains mutated in various kinetochore or cohesion factors.[9] Thus, the second major goal of this study was to elucidate the molecular pathways through which BRCA1 expression promotes lethality in these mutants. Venn analysis was performed to identify, out of the 461 genes (23°C) and 430 genes (30°C) altered by BRCA1 expression, a high confidence level of genes whose temperature-independent regulation depended on BRCA1. The resulting analysis produced a list of 222 genes whose expression was uniformly altered 2-fold or greater in a temperature-independent manner. Of these, 183 genes were upregulated (Table 1) and 39 genes were downregulated (Table 2) in response to BRCA1 expression. For each category, we clustered together genes involved in similar pathways or function.

Repressed genes. BRCA1-deficient human cells exhibit dramatic chromosome segregation defects, inter-sister chromatid gaps and translocations.[1,15] Thus, we first wanted to uncover how BRCA1 might affect pathways that contribute to conditional lethality in yeast chromosome segregation mutants. Of the 39 downregulated loci (Table 2), 13 are termed dubious open reading frames or

Table 1 BRCA-dependent upregulation at both 23° and 30°

Fold change 23°	Up	Fold change 30°	Up	Systematic	Common
3.4433699	Up	2.156699	Up	YMR056C	AAC1
2.840843	Up	2.936136	Up	YJR155W	AAD10
3.4504972	Up	3.541029	Up	YOL165C	AAD15
2.2339227	Up	2.7618916	Up	YER045C	ACA1
2.1536045	Up	2.8568575	Up	YFL055W	AGP3
3.3267636	Up	4.341087	Up	YDR242W	AMD2
2.7140546	Up	2.2674854	Up	YGL156W	AMS1
2.1114006	Up	2.661742	Up	YOL058W	ARG1
3.0350552	Up	2.6912796	Up	YML116W	ATR1
2.1226618	Up	2.1849225	Up	YOR011W	AUS1
4.675042	Up	4.2784038	Up	YNR058W	BIO3
3.1359777	Up	2.564124	Up	YNR057C	BIO4
4.0968795	Up	3.8620148	Up	YNR056C	BIO5
2.5255096	Up	2.0384958	Up	YJR025C	BNA1
7.379563	Up	8.30086	Up	YLR267W	BOP2
2.327485	Up	3.3769863	Up	YML042W	CAT2
4.509865	Up	3.985083	Up	YPR001W	CIT3
2.0578852	Up	3.9770317	Up	YHL048W	COS8
4.398049	Up	2.9582012	Up	YOR100C	CRC1
5.347875	Up	2.6645525	Up	YMR094W	CTF13
7.80064	Up	11.025473	Up	YML054C	CYB2
2.5044303	Up	3.0635834	Up	YIR027C	DAL1
2.550858	Up	5.6318183	Up	YIR028W	DAL4
4.4843516	Up	4.3695993	Up	YDL024C	DIA3
2.1668363	Up	2.9939582	Up	YDR403W	DIT1
6.571231	Up	7.6273603	Up	YDL174C	DLD1
2.6791651	Up	2.0592175	Up	YJR137C	ECM17
2.2003522	Up	2.5504754	Up	YMR062C	ECM40
2.4166193	Up	3.266436	Up	YBR033W	EDS1
3.1554222	Up	2.536958	Up	YOR393W	ERR1
3.7436044	Up	2.9526684	Up	YPL281C	ERR2
2.269241	Up	2.2603095	Up	YLR377C	FBP1
2.2849061	Up	2.2530105	Up	YJL221C	FSP2
7.659798	Up	7.1114554	Up	YIL054W	FYV2
76.735115	Up	14.4041395	Up	YBR020W	GAL1
76.735115	Up	166.01247	Up	YBR019C	GAL10
78.75131	Up	53.76265	Up	YLR081W	GAL2
101.56116	Up	93.95381	Up	YPL248C	GAL4
36.34818	Up	46.29145	Up	YBR018C	GAL7
5.330396	Up	4.0937595	Up	YDR019C	GCV1
2.5090592	Up	2.682127	Up	YPR184W	GDB1
4.222917	Up	3.8256583	Up	YAL062W	GDH3
2.9879272	Up	8.363512	Up	YCR098C	GIT1
2.694075	Up	2.6447406	Up	YDL223C	HBT1
2.7697783	Up	2.462242	Up	YOR202W	HIS3
2.4303727	Up	2.9735975	Up	YCL030C	HIS4
2.7684693	Up	2.5967464	Up	YIL116W	HIS5
3.793316	Up	5.640973	Up	YFL011W	HXT10
2.7209184	Up	2.324241	Up	YHR096C	HXT5
3.2667696	Up	3.6192696	Up	YJL219W	HXT9
2.1079352	Up	2.2038836	Up	YMR108W	ILV2
3.0951784	Up	8.414132	Up	YKL217W	JEN1
2.4908004	Up	2.6344728	Up	YGL009C	LEU1
2.373958	Up	2.3708763	Up	YNL104C	LEU4
2.5583594	Up	2.961486	Up	YIR034C	LYS1
2.2018661	Up	6.01615	Up	YGR292W	MAL12
2.286683	Up	6.6888905	Up	YBR299W	MAL32
3.6775527	Up	2.285604	Up	YKR069W	MET1
3.3087142	Up	3.3314137	Up	YFR030W	MET10
3.7851515	Up	3.1707487	Up	YPR167C	MET16
2.9498475	Up	2.9523308	Up	YLR303W	MET17
12.952447	Up	6.8508286	Up	YNL277W	MET2
4.7936215	Up	3.8631165	Up	YIR017C	MET28
2.9084132	Up	2.3667684	Up	YDR253C	MET32
3.1603968	Up	2.1044753	Up	YNL036W	NCE103
5.837712	Up	11.026299	Up	YDL085W	NDE2
2.7281747	Up	2.2748764	Up	YHR124W	NDT80
2.418558	Up	2.8711798	Up	YIL164C	NIT1
2.4033759	Up	2.2632685	Up	YKL120W	OAC1
3.7699878	Up	4.3708344	Up	YPR194C	OPT2
2.0619988	Up	2.2916145	Up	YAL064W	ORF:YAL064W
4.7190886	Up	5.521752	Up	YBL048W	ORF:YBL048W
4.3965955	Up	5.554456	Up	YBL049W	ORF:YBL049W
4.4264603	Up	5.0889173	Up	YBR047W	ORF:YBR047W
4.103467	Up	6.7295485	Up	YCL048W	ORF:YCL048W
2.192048	Up	4.3749895	Up	YCR099C	ORF:YCR099C
2.638211	Up	2.9866683	Up	YCR101C	ORF:YCR101C
18.36149	Up	5.4998984	Up	YCR105W	ORF:YCR105W
2.1239603	Up	2.6114385	Up	YDL241W	ORF:YDL241W
2.5549884	Up	2.244851	Up	YDR223W	ORF:YDR223W
2.150253	Up	2.8765693	Up	YEL057C	ORF:YEL057C
3.0552046	Up	6.2938676	Up	YEL070W	ORF:YEL070W
2.087863	Up	2.793788	Up	YER185W	ORF:YER185W
3.748168	Up	18.387726	Up	YFL052W	ORF:YFL052W
2.7145524	Up	6.0371685	Up	YFL061W	ORF:YFL061W
3.2444153	Up	2.1592965	Up	YFR012W-A	ORF:YFR012W-A
3.7236464	Up	3.2456906	Up	YGL024W	ORF:YGL024W
2.2985814	Up	2.8179967	Up	YGL059W	ORF:YGL059W
2.628734	Up	3.4248686	Up	YGL117W	ORF:YGL117W
3.6972415	Up	4.12001	Up	YGL230C	ORF:YGL230C
3.283544	Up	2.5214186	Up	YGR043C	ORF:YGR043C
2.307301	Up	2.472971	Up	YGR050C	ORF:YGR050C
4.205944	Up	5.055826	Up	YGR110W	ORF:YGR110W
3.769867	Up	3.490024	Up	YGR161C	ORF:YGR161C
2.0598817	Up	2.2321963	Up	YHL044W	ORF:YHL044W
4.1469936	Up	2.850817	Up	YHR029C	ORF:YHR029C

Table 1 BRCA-dependent upregulation at both 23° and 30°

Fold change 23°	Up	Fold change 30°	Up	Systematic	Common
2.4945712	Up	2.215926	Up	YHR048W	ORF:YHR048W
2.241283	Up	3.735952	Up	YHR209W	ORF:YHR209W
2.6221356	Up	3.084134	Up	YIL121W	ORF:YIL121W
3.1692755	Up	2.6197135	Up	YIL165C	ORF:YIL165C
2.2139091	Up	2.1267798	Up	YIL172C	ORF:YIL172C
2.5514972	Up	5.3436313	Up	YIR043C	ORF:YIR043C
3.59348	Up	2.4726734	Up	YJL045W	ORF:YJL045W
5.3583336	Up	8.838197	Up	YJL160C	ORF:YJL160C
3.1550093	Up	3.1441882	Up	YJL213W	ORF:YJL213W
2.1492753	Up	3.9401343	Up	YKL071W	ORF:YKL071W
6.108961	Up	6.504658	Up	YKL107W	ORF:YKL107W
3.2176416	Up	3.032829	Up	YKL162C-A	ORF:YKL162C-A
2.549582	Up	2.4074943	Up	YKR046C	ORF:YKR046C
2.3823843	Up	3.5281072	Up	YLR054C	ORF:YLR054C
2.1908948	Up	3.9726424	Up	YLR194C	ORF:YLR194C
5.400229	Up	9.768513	Up	YLR311C	ORF:YLR311C
4.7420163	Up	4.786415	Up	YLR312C	ORF:YLR312C
2.472059	Up	2.5937066	Up	YMR007W	ORF:YMR007W
2.1991053	Up	2.0030406	Up	YMR057C	ORF:YMR057C
22.084738	Up	15.958245	Up	YMR107W	ORF:YMR107W
3.4861953	Up	11.092461	Up	YMR118C	ORF:YMR118C
4.8684373	Up	3.8995376	Up	YMR322C	ORF:YMR322C
2.6965623	Up	5.0872335	Up	YMR323W	ORF:YMR323W
2.0763237	Up	2.021945	Up	YNL114C	ORF:YNL114C
4.7190886	Up	5.7443166	Up	YNL335W	ORF:YNL335W
2.286996	Up	4.8582363	Up	YNR064C	ORF:YNR064C
2.2116995	Up	5.510426	Up	YNR066C	ORF:YNR066C
2.7544036	Up	10.333814	Up	YNR073C	ORF:YNR073C
2.2676046	Up	3.1759288	Up	YOL047C	ORF:YOL047C
2.622635	Up	2.360953	Up	YOL157C	ORF:YOL157C
2.3561645	Up	2.3535578	Up	YOL159C-A	ORF:YOL159C-A
2.809437	Up	4.3108993	Up	YOL162W	ORF:YOL162W
2.8775344	Up	4.160822	Up	YOL163W	ORF:YOL163W
2.7927597	Up	5.7056117	Up	YOL166C	ORF:YOL166C
3.3838398	Up	2.3424292	Up	YOR203W	ORF:YOR203W
3.9368253	Up	2.5369966	Up	YOR289W	ORF:YOR289W
3.0312536	Up	2.4283605	Up	YOR338W	ORF:YOR338W
2.6611648	Up	3.1574056	Up	YOR345C	ORF:YOR345C
13.983435	Up	8.293946	Up	YPL033C	ORF:YPL033C
2.1662319	Up	2.6543095	Up	YPL110C	ORF:YPL110C
4.4853578	Up	3.4309742	Up	YPL280W	ORF:YPL280W
2.6383016	Up	3.1795871	Up	YPR015C	ORF:YPR015C
2.6498392	Up	2.718381	Up	YPR061C	ORF:YPR061C
3.1841009	Up	3.0100422	Up	YPR077C	ORF:YPR077C
2.7551093	Up	2.236529	Up	YOR130C	ORT1
2.897362	Up	2.426087	Up	YKR097W	PCK1
3.158231	Up	3.0981598	Up	YHR071W	PCL5
2.2268972	Up	4.8464212	Up	YPR002W	PDH1
3.7822063	Up	7.592838	Up	YBR296C	PHO89
3.041848	Up	8.434918	Up	YKL163W	PIR3
3.034698	Up	4.1046576	Up	YIL160C	POT1
3.4939804	Up	4.4523406	Up	YIL117C	PRM5
2.0202703	Up	2.4520059	Up	YGL062W	PYC1
6.2763557	Up	3.4175785	Up	YGL158W	RCK1
3.5144181	Up	2.9104614	Up	YCR106W	RDS1
4.28649	Up	5.964159	Up	YBR050C	REG2
2.9678848	Up	2.7935257	Up	YBR256C	RIB5
2.1734767	Up	2.2412622	Up	YGL224C	SDT1
2.8915923	Up	4.9005575	Up	YAL067C	SEO1
2.2840748	Up	4.980292	Up	YJL089W	SIP4
8.228353	Up	6.9054456	Up	YMR095C	SNO1
7.2946773	Up	5.99051	Up	YMR096W	SNZ1
2.3738842	Up	2.5341263	Up	YNL012W	SPO1
2.8698087	Up	2.3937123	Up	YMR017W	SPO20
2.821707	Up	3.10122	Up	YOL091W	SPO21
2.4767158	Up	2.5756717	Up	YIL073C	SPO22
2.4217575	Up	3.1003695	Up	YPR007C	SPO69
3.9720883	Up	3.8201165	Up	YKL218C	SRY1
3.1808107	Up	3.0908365	Up	YPL092W	SSU1
2.3064563	Up	2.933995	Up	YKL178C	STE3
2.6517718	Up	3.5276618	Up	YJR130C	STR2
4.557136	Up	4.7047777	Up	YGL184C	STR3
24.49464	Up	10.887441	Up	YBR294W	SUL1
4.3167863	Up	2.0504751	Up	YLR092W	SUL2
5.3514595	Up	7.343781	Up	YJR156C	THI11
4.995868	Up	5.263618	Up	YNL332W	THI12
7.22231	Up	5.4404936	Up	YDL244W	THI13
5.0305867	Up	5.6415343	Up	YFL058W	THI5
3.988496	Up	4.3984036	Up	YER175C	TMT1
2.9507484	Up	3.4170206	Up	YDR059C	UBC5
2.2585113	Up	2.0042167	Up	YLL039C	UBI4
2.1997151	Up	2.295983	Up	YDL170W	UGA3
2.5031931	Up	2.1808121	Up	YGR065C	VHT1
6.214656	Up	2.4561589	Up	YAR035W	YAT1
4.987914	Up	3.2750573	Up	YER024W	YAT2
2.3179839	Up	5.0609155	Up	YNR065C	YSN1
2.4574826	Up	2.6144147	Up	YBR046C	ZTA1

Genes upregulated 2-fold or greater in response to BRCA1 expression at both 23°C (column A) and at 30°C (column B). To facilitate cluster-function analyses, genes are presented alphabetically based on standard gene nomenclature (column F). Systematic gene nomenclature is also provided (column E).

Table 2 **BRCA1-dependent repression at 23° and 30°**

Fold change 23°	Down	Fold change 30°	Down	Systematic	Common
2.6663523	Dn	2.5401378	Dn	YNL141W	AAH1
2.348434	Dn	2.1471424	Dn	YNR044W	AGA1
2.1432571	Dn	2.8746247	Dn	YPL061W	ALD6
3.9368253	Dn	3.039458	Dn	YGR177C	ATF2
2.205411	Dn	3.4943202	Dn	YGR108W	CLB1
2.640706	Dn	2.4160028	Dn	YPR119W	CLB2
2.5200815	Dn	5.1660275	Dn	YGR109C	CLB6
2.0592175	Dn	2.0186563	Dn	YMR215W	GAS3
2.0306427	Dn	2.456279	Dn	YCL036W	GFD2
2.2512314	Dn	2.1754	Dn	YBR244W	GPX2
2.209742	Dn	2.8485	Dn	YOL151W	GRE2
9.850322	Dn	6.3388696	Dn	YOR032C	HMS1
3.0393798	Dn	3.3753817	Dn	YDL227C	HO
2.352046	Dn	2.2479405	Dn	YMR032W	HOF1
18.128693	Dn	12.658796	Dn	YJL153C	INO1
2.695608	Dn	2.2437422	Dn	YDL003W	MCD1
3.2499297	Dn	2.2911003	Dn	YAR047C	ORF:YAR047C
4.06215	Dn	2.2012846	Dn	YDL038C	ORF:YDL038C
2.295059	Dn	3.2390332	Dn	YDR222W	ORF:YDR222W
2.3097382	Dn	2.936668	Dn	YER053C-A	ORF:YER053C-A
6.423311	Dn	2.7086365	Dn	YGR052W	ORF:YGR052W
2.2692592	Dn	2.2693837	Dn	YHL026C	ORF:YHL026C
2.037372	Dn	2.1799862	Dn	YIL158W	ORF:YIL158W
2.5801733	Dn	2.7344072	Dn	YMR088C	ORF:YMR088C
2.198507	Dn	2.792885	Dn	YNL134C	ORF:YNL134C
2.718264	Dn	2.8701663	Dn	YNL194C	ORF:YNL194C
2.1698606	Dn	2.656128	Dn	YNR009W	ORF:YNR009W
3.4301567	Dn	3.1999304	Dn	YOR315W	ORF:YOR315W
3.8612068	Dn	2.2171853	Dn	YPL095C	ORF:YPL095C
2.0609047	Dn	2.051279	Dn	YHR215W	PHO12
2.4203475	Dn	2.2458477	Dn	YBR092C	PHO3
5.1002965	Dn	4.6914625	Dn	YMR006C	PLB2
2.530907	Dn	2.1181576	Dn	YDR501W	PLM2
2.8532867	Dn	2.4002085	Dn	YNL301C	RPL18B
2.2034757	Dn	2.053107	Dn	YHR172W	SPC97
3.815995	Dn	3.740144	Dn	YOR313C	SPS4
2.6739023	Dn	2.9553387	Dn	YHL028W	WSC4
3.9242835	Dn	7.133377	Dn	YNL160W	YGP1
2.2180047	Dn	5.0412126	Dn	YGL255W	ZRT1

Genes downregulated 2-fold or greater in response to BRCA1 expression at both 23°C and 30°C. Column designations are identical to Table 1.

un-annotated and thus are not considered further.[4] Importantly, functional-cluster analysis revealed that the largest class of genes downregulated by BRCA1 (six of the remaining 26) play essential roles in mitosis. *CLB1*, *CLB2* and *CLB6* (all three encode different B-type cyclins) were identified as well as *MCD1/SCC1* (encoding the key structural sister chromatid cohesion factor). We note that BRCA1 ectopic expression in colon cancer cells similarly showed significant reduction of B-type cyclin and cohesion regulators[17,18]—attesting to the efficacy of the current approach. Two other factors downregulated in this class are *SPC97* (encodes a spindle pole body component associated with microtubule nucleation)[19] and *HOF1* (encodes a cytokinesis regulatory factor).[20] While speculative, a plausible model is that BRCA1-expressing yeast cells are deficient in maintaining both a mitotic state and sister chromatid identity—coupling BRCA1 to aneuploidy pathways (Fig. 1). That these yeast pathways are conserved through evolution and of clinical relevance is supported by findings in vertebrate cell studies that BRCA1 regulates numerous aspects of mitosis that include kinetochore, spindle checkpoint, cyclin-dependent kinase, cohesion and cytokinesis pathways.[16-18] In summary, BRCA1 represses a suite of mitotic regulators and structural components that are conserved through evolution, suggesting that the chromosome aberrations and aneuploidy observed in breast/ovarian cancer cells may arise in part through defects in chromosome segregation pathways.

The remaining 20 downregulated genes fall into 6 other functional clusters (Fig. 2), four of which include phospholipid metabolism and phosphate utilization factors (*INO1*, *PHO3*, *PHO12* and *PBL2*), stress responders (*ALD6*, *ATF2*, *GPX2* and *GRE2*), cell wall components and plasma membrane transporter (*AGA1*, *GAS3*, *YPG1* and *ZRT1*) and ribosome subunits and translation factors (*RPL18B*, *WSC4* and *GFD2*). The fifth cluster is comprised of transcription regulators (*HMS1* and *PLMS2*). This latter functional-cluster contains a surprisingly small number of genes, given prior studies that mutations in transcriptional responders suppress BRCA1-induced lethality.[10,11] The sixth cluster is comprised of orphan genes of unrelated functions (*SPS4*, *HO* and *AAH1*).

Upregulated genes. We next performed a functional-cluster analysis on the 183 genes that were upregulated in response to BRCA1 (Table 1). 70 un-annotated loci (and an additional seven genes for which only putative or implied functions exist) were removed from the data set. Functional-cluster analysis of the remaining 106 genes revealed that only a single chromosome segregation gene—encoding the kinetochore factor Ctf13p, was upregulated in response to BRCA1. This observation couples together previously disparate reports that (1) elevated levels of *CTF13* are conditionally lethal in *ctf19* mutant strains and (2) BRCA1 expression is conditionally lethal in strains mutated in COMA kinetochore components including Ctf19p.[9,21,22] Since kinetochore assembly is uniquely sensitive to increased *CTF13* dosage, *CTF13* upregulation by BRCA1 accounts for the conditional lethality of BRCA1 in *ctf19* mutants (Fig. 1)—validating the current study and providing a genetically closed loop of BRCA1 function in yeast through microarray analyses. From these and other results, we posit a two-hit mechanism by which BRCA1 contributes to cell aneuploidy: BRCA1 may drive inappropriate expression of highly dosage-sensitive kinetochore factors and does so in the context of reduced mitotic genes that include B-type cyclins (*CLB*s) and cohesion factors (*MCD1/SCC1*).

Of further interest are the roughly eight genes involved in meiosis and sporulation (including *SPO1*, *SPO20-22* and *SPO69*), suggesting that BRCA1 expression may inappropriately activate recombination or synapsis pathways that contribute to aneuploidy in cancer cells. The bulk of genes upregulated by BRCA1 function either as permeases/transporters (14 loci) or related biosynthetic pathways (59 loci). In many cases, multiple members of a single

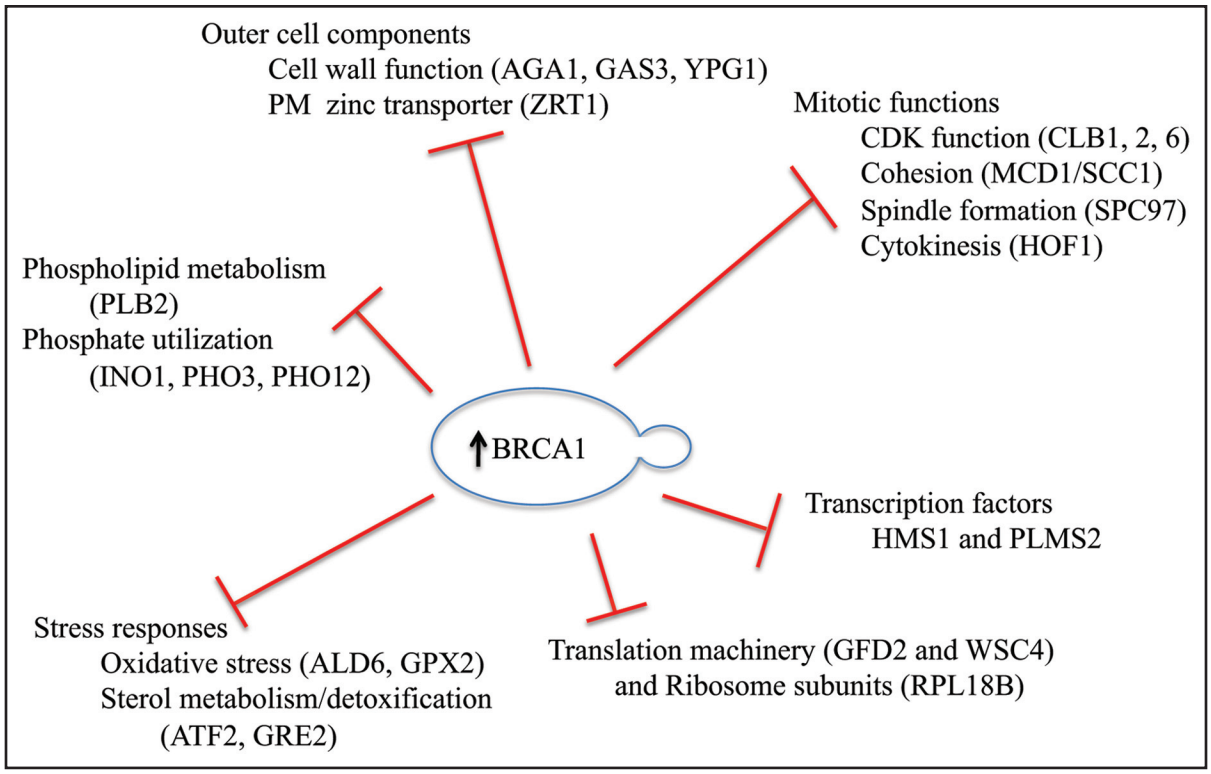

Figure 2. Schematic highlighting cluster-function analyses of genes downregulated in response to BRCA1. Cluster defined as 'other' not shown. See text for details.

pathway were identified (*BIO3-BIO5*; *GAL1*, *GAL2*, *GAL4*, *GAL7* and *GAL10*; *HIS3-HIS5*; *THI5*, *THI11-13* and *MET1*, *MET2*, *MET10*, *MET16*, *MET17*, *MET28* and *MET32*). Our analyses also revealed 13 loci that contribute to mitochondrial function—which may contribute to the small (petite-like) colony phenotype observed in BRCA1-expressing cells.[5,6] We note that many mitochondrial genes are also regulated by BRCA1 in vertebrate cells, while the extent that these genes effect apoptotic responses remains unclear.[17,23] Surprisingly, very few upregulated genes (5 loci) could be classified as transcriptional regulators or in modifying transcript stability. Thus, the BRCA1 affects observed in yeast most likely occur directly through interactions with transcription factors and chromatin remodelers—as opposed to BRCA1 upregulation of transcription factors that contribute secondary and thus indirect effects on gene expression.

In summary, the current study addresses key and novel aspects of BRCA1 function in a genetically amenable and conserved response system. Positional analyses made accessible by yeast gene nomenclature illustrates that both individual loci and large and contiguous multi-loci DNA tracts are positively upregulated in response to BRCA1. In contrast, few adjacent genes are downregulated in tandem, suggesting that BRCA1-dependent transcriptional repression in yeast occurs predominantly (but not exclusively) in a gene-specific fashion. We also found that many more than predicted repressed genes are situated immediately adjacent to upregulated genes. Yeast thus provides a powerful avenue to pursue further the chromatin basis of these transition zones.

Labor-intensive vertebrate cell studies previously demonstrated that BRCA1 alters the expression of mitotic components including kinetochore, checkpoint, CDK and cohesion factors.[1] The current study reveals that BRCA1 affects identical pathways in yeast. Furthermore, our data show that the kinetochore-encoding locus *CTF13* was uniquely upregulated in response to BRCA1. Kinetochore assembly in key mutant backgrounds is highly sensitive to elevated levels of Ctf13p and provides a molecular explanation regarding the conditionally lethality of BRCA1 in kinetochore COMA mutant cells.[9] This finding raises the possibility that inappropriate BRCA1 expression in human cells may similarly induce elevated levels of dosage-critical factors and contribute to aberrant chromosome structures observed in breast cancer cells.

Experimental Procedures

10 ml of log growth cultures harboring vector alone or vector harboring BRCA1 were harvested by centrifugation and RNA extracted from the resulting pellets using either hot phenol or RNeasy (Qiagen) procedures.[24] In all cases, RNA quality was first assessed by A240/A260 ratio (Nanodrop) and further validated by Agilent 2100 Bioanalyzer. Hybridized one-color samples were prepared using Agilent Yeast Oligo Microarrays (V2) 4X44k format (G2519F), which includes >6,200 ORFs with a total of 45,018 features of 60-mer controls and gene probes, according to Agilent instructions and using Agilent reagents. Paired comparisons were made between Control-23°C and BRCA1-23°C and between Control-30°C and BRCA1-30°C RNA extracts. BRCA1 effects on yeast cell growth is temperature independent.[9]

One-color microarrays were scanned with an Agilent Microarray Scanner System, which generated the TIFF images of low and high intensity scans utilized by Agilent Feature Extraction Software (v9.5). Feature Extraction processing of fluorescent data corrected signals

for background noise, foreground intensities, positive and negative spot controls, background subtraction and signal normalization. Tab delimited text files generated for each of the four experimental arrays were then analyzed using Agilent Technologies software GeneSpring GX (v9.0.5). Data were processed in GeneSpring GX (v9.0.5) by first filtering on expression intensities to retain features within the 20.0 to 100.0 percentile range followed by filtering on flags for features either present or marginal in at least one of the two arrays juxtaposed. A fold change threshold of 2.0 was imposed for each pairing. During final manuscript preparations, version GeneSpring (v9.5) was released. Venn re-analyses of our data sets using this updated software identified an additional 54 genes (primarily un-annotated ORFs) common to all data sets with only minor modifications to identified genes.

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Note

Supplementary materials can be found at:
www.landesbioscience.com/supplement/SkibbensCC7-24-Sup.pdf

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