Translocations used to generate chromosome segment duplications in *Neurospora* can disrupt genes and create novel open reading frames†

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In *Neurospora crassa*, crosses between normal sequence strains and strains bearing some translocations can yield progeny bearing a duplication (*Dp*) of the translocated chromosome segment. Here, 30 breakpoint junction sequences of 12 *Dp*-generating translocations were determined. The breakpoints disrupted 13 genes (including predicted genes), and created 10 novel open reading frames. Insertion of sequences from LG III into LG I as translocation *T(UK8-18)* disrupts the *eat-3* gene, which is the ortholog of the *Podospora anserine* gene *ami1*. Since *ami1*-homozygous *Podospora* crosses were reported to increase the frequency of repeat-induced point mutation (RIP), we performed crosses homozygous for a deficiency in *eat-3* to test for a corresponding increase in RIP frequency. However, our results suggested that, unlike in *Podospora*, the *eat-3* gene might be essential for ascus development in *Neurospora*. Duplication–heterozygous crosses are generally barren in *Neurospora*; however, by using molecular probes developed in this study, we could identify *Dp* segregants from two different translocation–heterozygous crosses, and using these we found that the barren phenotype of at least some duplication–heterozygous crosses was incompletely penetrant.

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1. Introduction

In *Neurospora crassa*, 64 insertional and quasiterminal translocations (*IT*s and *QT*s) have been described whose crosses with the normal sequence (*N*) can yield progeny bearing a duplication (*Dp*) of the translocated segment (Perkins 1974, 1997). (The term "duplication" [*Dp*] will be used to designate either a chromosome segment that is present as two non-tandem copies or a strain that contains such a segment.) *IT*s transfer a "donor" chromosome segment to a "recipient" chromosome without any reciprocal exchange, whereas *QT*s transfer a distal donor chromosome segment to the tip of the recipient chromosome distal to any essential gene, and presumably the donor chromosome breakpoint is capped with the tip from the recipient chromosome. The distribution of ascus types produced in translocation by normal sequence crosses can be used to distinguish the *Dp*-generating rearrangements (i.e. *IT*s and *QT*s) from the more common reciprocal translocations (*RT*s, wherein two [or more] chromosomes interchange terminal segments) (Perkins 1974, 1997). In a translocation by normal sequence cross (i.e. $T \times N$), alternate segregation generates asci with parental type ascospores (i.e. four *N*s and four *T*s, which are all viable and blacken [B] with maturation, therefore the resulting ascus is designated 8B:0W), whereas, the equally likely adjacent-1 segregation produces non-parental type ascospores. Adjacent-1 segregation in $IT \times N$ (and $QT \times N$) produces four viable (black) ascospores containing a duplication (*Dp*) of the translocated segment and four inviable (white, W) ascospores with the complementary deficiency (*Df*), therefore the asci are 4B:4W, whereas in $RT \times N$ all eight ascospores contain complementary *Dp*/*Df*

Keywords. Chromosome segment duplications; incomplete penetrance; insertional and quasiterminal translocations; meiotic silencing of unpaired DNA; repeat-induced point mutation

†Dedicated to the memory of our colleague T Bhavani Prasanna.

Abbreviations used: *Dp*, duplication; *Df*, deficiency; *IT*s, insertional translocations; LG, lineage group; OR, Oak Ridge; ORFs, open reading frames; PCR, polymerase chain reaction; *QT*s, quasiterminal translocations; RIP, repeat-induced point mutation

Supplementary figure pertaining to this article is available on the *Journal of Biosciences* Website at *http://www.ias.ac.in/jbiosci/Dec2010/pp539-546/suppl.pdf*

and are inviable, therefore the asci are 0B:8W. Thus, when the ratio of 8B:0W asci to 4B:4W asci is equal, it indicates that there is heterozygosity for a *Dp*-generating translocation, whereas when the ratio of 8B:0W asci to 0B:8W asci is equal, it indicates heterozygosity for an *RT* (figure 1a). Isosequential crosses (i.e. $N \times N$ or $T \times T$) yield mostly 8B:0W asci. Since viable duplication progeny are not recoverable from $RT \times N$, the *RT*s are not regarded as *Dp*-generating rearrangements, although duplications can be obtained from intercrosses of partially overlapping *RT*s whose breakpoints are appropriately staggered on the same two chromosome arms (*see* Perkins 1997).

Dp segregants obtained from crosses of *IT*s or *QT*s with *N* are recognizable by the characteristic barren phenotype of *Dp*-heterozygous crosses (i.e. $Dp \times N$). Barren crosses make normal looking perithecia but produce exceptionally few progeny ascospores. The barrenness of *Dp*-heterozygous crosses is caused, at least in part, by a presumed RNAi-based genome defense process called meiotic silencing by unpaired DNA (Aramayo and Metzenberg 1996; Shiu *et al*. 2001). In *Dp*-heterozygous crosses the *Dp*-borne genes, including those essential for meiosis and ascus formation, presumably fail to pair properly in meiosis, this triggers their RNAi-based silencing and renders the cross barren (figure 1b). The semi-dominant suppressors of meiotic silencing; e.g. *Sad-1*, *Sad-2*, *Sms-2* (Shiu *et al.* 2001, 2006; Lee *et al.* 2003), significantly (> 100-1000 x) enhance the productivity of *Dp*-heterozygous crosses. The suppressor alleles are presumed to disrupt normal pairing of their wild-type homologs (i.e. $sad\text{-}1^{+}$, $sad\text{-}2^{+}$, $sms\text{-}2^{+}$), thereby inducing these genes to silence themselves. Reduction in levels of SAD-1, SAD-2 or SMS-3 proteins is presumed to cause an overall lowering of meiotic silencing efficiency, and, consequently, to lessen the silencing of mispaired *Dp*-borne genes. The general observation that crosses involving progeny from 4B:4W asci were barren had cemented the association of *Dp*s with barrenness. However, we report here that crosses heterozygous for at least some *Dp*s are incompletely penetrant for the barren phenotype. Specifically, using molecular probes developed in this work, we identified *Dp* segregants from $T(UK8\text{-}18) \times N$ and $T(R2394)\ a \times N$, and found that not all *Dp(UK8-18)*- and *Dp(R2394)*-heterozygous crosses were barren. Instead many were fertile. (A fertile cross is defined as one whose productivity is indistinguishable from a wild-type cross [e.g. OR $A \times$ OR a].)

The molecular probes used to distinguish *Dp*, *T* and *N* segregants from $T \times N$ were an outcome of the major focus of this work, namely, the definition of breakpoint junction sequences of 12 *Dp*-generating translocations; *T(EB4)*, *T(UK3-41)*, *T(IBj5)*, *T(UK8-18)*, *T(UK14-1)*, *Tp(T54M94)*, *T(Y112M4i)*, *T(AR173)*, *T(NM177)*, *T(B362i)*, *T(4540)* and *T(39311)*. Prior to this work only one breakpoint junction sequence had been reported, that of the *Dp*-generating rearrangement *T(IR->VIR)UK-T12* (Asch *et al*. 1992). Although Perkins, (1995) had noted that cloned genome segments that cover a breakpoint were known for the *Dp*-generating translocations *AR173*, *TM429 his-3*, *IBj5 cpc-1*, *AR190*, *AR18* and *P2869*, we are not aware that any breakpoint junction sequence was actually obtained from the cloned segments. Thus, the breakpoint junction sequences reported here mark a significant step in our effort to characterize the duplication-generating rearrangements to an unprecedented level of detail on the genome sequence. The identified breakpoints disrupted thirteen genes (i.e. genes with known phenotype, as well as predicted genes), and generated ten novel fusion open reading frames (ORFs). Characterizing the breakpoint and insertion sequences of chromosome rearrangements illustrates that "doing *Neurospora* genetics is not only intellectually rewarding, it is fun" (Perkins 1979).

2. Materials and methods

2.1 N. crassa *strains and their general genetic manipulation*

Neurospora genetic analysis was done essentially as described by Davis and De Serres (1970). *N. crassa* strains were obtained from the Fungal Genetics Stock Center (FGSC), University of Missouri, Kansas City, MO 64110, USA. These included the standard laboratory Oak Ridge (OR) strains 74-OR23-1 *A* (FGSC 987) and OR8-1 *a* (FGSC 988); the wild-isolated strains Agudas Rd-1 *a* (P3974), Agudas Rd-1 *A* (P3975), Bichpuri-1 *a* (P750), Fred *a* (P1138), Madurai *A* (P2539), Mauriceville-1c *A* (FGSC 2225), Mauriceville-1c *a* (FGSC 2226), and Mughalsarai-2 *A* (P0736); and translocation strains *T(VR > VIL)UK3-41, inl A* (FGSC 6869), *T (VR > VII)EB4 A* (FGSC 3046), *T(VIL > I)IBj5, cpc-1 a* (FGSC 4434), *T(IIIR > IL)UK8-18 A* (FGSC 7037), *T(VIR > VL)UK14-1 A* (FGSC 6958), *Tp(IR > IL) T54M94 a* (FGSC 2928), *T(IR > IIIR) Y112M4i, ad-3B A* (FGSC 2637), *T(IR; VR; IR >VII) In(VL;VR)AR173 a* (FGSC 2469), *T(IIR > IL) NM177 A* (FGSC 1610), *T(IVR > I)B362i A* (FGSC 2935), *T(IIL > IVR)R2394 A* (FGSC 2757), *T(IR >IIR)4540, nic-2 a* (FGSC 3209) and *T(IL > IIR)39311 a* (FGSC 1246). The translocation strains will henceforth be referred to as *T(UK3-41) A*, *T(EB4) A*, *T(IBj5) a*, *T(UK8-18) A*, *T(UK14-1) A*, *Tp(T54M94) a*, *T(Y112M4i) A*, *T(AR173) a*, *T(NM177 A)*, *T(B362i) A*, *T(R2394) A*, *T(4540) a* and *T(39311) a*.

2.2 *Nomenclature used for the breakpoint junctions*

The three relevant breakpoint junctions of *IT*s are designated as "A", created by deletion of the translocated segment on

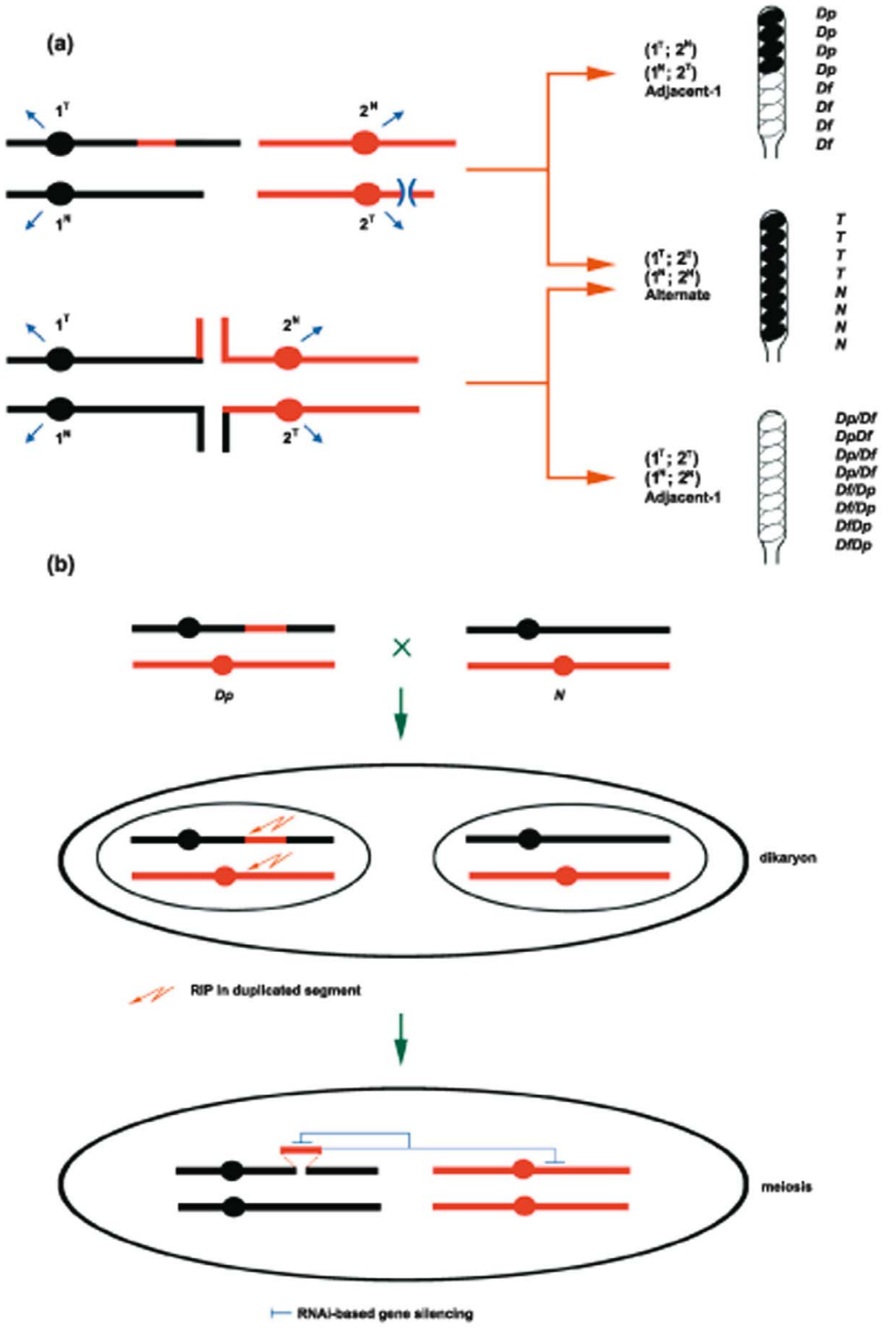

Figure 1. For caption, *see* page No. 542.

the donor chromosome, and "B" and "C" (proximal and distal) created by its insertion in the recipient chromosome. In contrast, *QT*s have only two relevant junctions; "A", marking the boundary between the breakpoint-proximal sequence on the "donor" chromosome and the tip, presumably, from the "recipient" chromosome, and "B", the boundary between the breakpoint-proximal sequence on the recipient chromosome and the "donor" segment grafted onto it. Thus the A breakpoint is *absent* from the *Dp* progeny of both *IT* × *N* and *QT* × *N*. Intrachromosomal transpositions [e.g. *Tp(T54M94)*] are essentially *IT*s in which the same chromosome is both donor and recipient. Genbank accession numbers of the 30 breakpoint junction sequences determined in this work are summarized in table 1.

2.3 *Overview of the method used to define the breakpoint junction sequences*

Strains carrying translocations (*T*) were crossed with one or more wild-isolated strains that served as the normal sequence (*N*) parent (most translocations are derived in the standard OR genetic background). *Dp* progeny were identified by the barren phenotype of their cross with OR strains, and confirmed by demonstrating the presence of both parental (*T* and *N*) alleles of molecular markers (restriction fragment length polymorphisms [RFLPs] or single-nucleotide polymorphisms [SNPs]) linked to a marker known to be covered from previous studies (reviewed by Perkins 1997). *Dp* progeny exhibit both *T* and *N* alleles of markers located within the duplicated segment, but only the *N* allele of markers located just outside this segment. Identification of progressively closer covered (i.e. located within the duplicated segment) and uncovered (i.e. located outside the duplicated segment) markers bracketed the ends of the duplicated (i.e. translocated) segment into progressively narrower intervals (often, < 10 kbp) (Vyas *et al*. 2006; Singh *et al*. 2009). Further delimitation of the junction intervals to 1–3 kbp was done by polymerase chain reaction (PCR) with oligonucleotide primers that anneal within these intervals; failure or success in PCR amplification with translocation DNA template indicated whether or not the breakpoint lay between the primer binding sites (Singh *et al*. 2009). Localizing the ends of a translocated (i.e. duplicated) segment to < 1 kbp intervals implies that sequences immediately flanking it are < 2 kbp apart on the donor chromosome, which makes it possible to isolate the A junction by PCR. The sequence of the resulting amplicon establishes the exact extent of the segment deleted from the donor chromosome. Inverse PCR with translocation DNA template and primers annealing "within" the translocated segment was then used to retrieve the adjoining sequence on the recipient chromosome.

2.4 *Identification of Dp(R2394) and Dp(UK8-18) segregants*

Molecular marker *R3* (covered by *Dp[R2394]*, Singh and Kasbekar 2008) was amplified from genomic DNA of *T(R2394)* or OR using primers 5′CGAGACGGAGAATGGAGAAC and 5′ACCTATGGACTGGACGAGGA. The resulting DNA was digested with *Bam*HI which allowed us to distinguish between the *T(R2394)* and OR alleles of the amplicon. *Dp(R2394)* segregants from *T(R2394)* × OR yield both the *T(R2394)* and OR alleles of *R3* thus making it possible to distinguish them from their *T* and *N* siblings.

To identify the *Dp(UK8-18)* segregants from the cross of *T(UK8-18)* with the Bichpuri-1 strain we performed PCR with two pairs of primers. One pair, 5′GCGCCAACCGACCGAAATAC and 5′CCGAGCACG-AGGCAAAATCAC, amplifies across the "A" junction on the translocation donor chromosome, and the other, 5′AGGACGTCGAACCGGTCTTG and 5′TGCCAGCCAT-CGTGAGCTTG, amplifies across the "B" junction on the translocation recipient chromosome (figure 2, table 1). DNA from the *Dp(UK8-18)* segregants produces an amplicon with the second pair but not the first.

Figure 1. **(a)** Distinguishing between duplication (*Dp*)-generating and reciprocal translocations. The left upper panel shows segregation in a *Dp-generating translocation* x *Normal sequence* cross. Adjacent-1 segregation without crossover produces 4B:4W asci bearing four viable duplication (*Dp*) ascospores and four inviable deficiency (*Df*) ascospores. The left lower panel shows segregation in the cross *Reciprocal translocation* x *Normal sequence*. Adjacent-1 segregation without crossover produces 0B:8W asci bearing eight inviable white ascospores with complementary duplication/deficiency (*Dp*/*Df*) genotypes. In both types of crosses alternate segregation without crossover produces 8B:0W asci bearing eight viable black ascospores with the parental translocation or normal sequence genotypes (right middle). Alternate and adjacent-1 segregation are equally probable, therefore obtaining 8B:0W = 4B:4W is diagnostic of a *Dp*-generating translocation, and obtaining 8B:0W = 0B:8W is diagnostic of a reciprocal translocation. **(b)** RIP and meiotic silencing target duplicated DNA sequences in a sexual cross. The top panel shows a *duplication* x *euploid* cross. Fertilization produces a dikaryon containing the parental *mat A* and *mat a* nuclei (middle panel). The haploid nuclei of the dikaryon eventually fuse to form a diploid zygote nucleus that undergoes homologous chromosome pairing and meiosis (lower panel). RIP occurs during the dikaryon stage and induces G:C to A:T mutation and C-methylation in any sizeable (> 400 bp) duplicated DNA segments. Meiotic silencing by unpaired DNA, occurs following karyogamy and uses RNAi to eliminate transcripts of any gene that does not pair properly with a homolog in meiosis, thereby silencing it, and any genes homologous to it, regardless of whether the homologous genes themselves were paired. *Dp*-borne genes inevitably include some that are essential for meiosis and ascus formation and their silencing renders the *Dp*-heterozygous cross barren.

Table 1. Genbank accession numbers of breakpoint junction sequences of 12 *Dp*-generating translocations

		Breakpoint junction sequence		
	Translocation	A	B	C
1.	*T(VR > VIL)UK3-41*	HM573450	ND	ND
2.	*T(VR > VII)EB4*	GQ504681	GQ504682	GQ504683
3.	*T(VIL > IR)IBj5*	GQ504684	GQ504685	ND
4.	*T(VIL > IR)UK8-18*	GQ504686	GQ504687	GQ504688
5.	*T(VIL > IR)UK14-1*	GQ504703	ND	
6.	*Tp(IR > IL)T54M94*	FJ717709	GQ504689	GQ504690
7.	*T(IR > IIIR)Y112M4i*	GQ504691	GQ504692	GQ504693
8.	*T(IR >VII)AR173*	ND	EU815636	GU053727
9.	*T(IIR > IL)NM177*	GQ504694	GQ504695	GQ504696
10.	*T(IVR > I)B362i*	GQ504697	GQ504698	GQ504699
11.	*T(IR > IIIR)4540*	GQ504700	GQ504701	GQ504702
12.	*T(IL > IIIR)39311*	GQ507751	GQ507752	GQ507753

ND = not determined

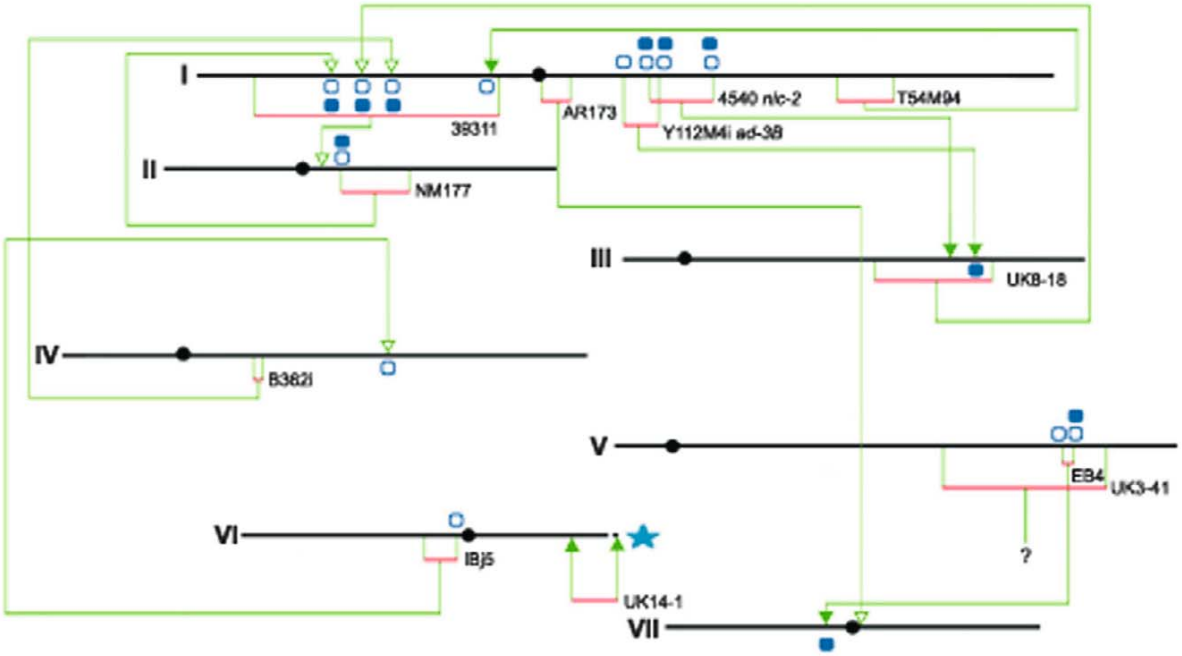

Figure 2. Duplication-generating translocations mapped onto the *N. crassa* genome sequence. Black lines represent the genome sequence supercontig (release 10) assigned to the linkage groups (LGs). Supercontig 10.1 through 10.7, were assigned to LGs I through VII respectively. The break on the donor chromosome of the quasiterminal translocation *T(UK14-1)* is capped by sequence from the unassigned supercontig 10.9 (star), suggesting that supercontig 10.9 might be in distal LG VL (*see* text for details). Arrowheads indicate insertion sites of the donor chromosome segment on the recipient chromosome. Filled arrowheads indicate the translocated segment is inserted non-inverted relative to the centromere, an orientation termed *eucentric* by Darlington (1936), as opposed to *dyscentric* orientation indicated by open arrowheads, that signify a segment that is inverted relative to the centromere. *See* table 1 for accession numbers of the A, B and C junction sequences. Open circles indicate genes disrupted by the translocations and filled circles indicate novel open reading frames (*see* text for details and the Supplementary figure a-i for more on the putative fusion genes).

3. Results and discussion

3.1 *Breakpoint junctions of 12 Dp-generating translocations*

Figure 2 summarizes the extent of the chromosome segments translocated from each donor linkage group (LG) and their insertion sites in the recipient LGs. Table 1 presents the genbank accession numbers of the breakpoint junction sequences. In total eight genes (i.e. genes with known phenotype, as well as predicted genes) were disrupted on donor chromosomes, namely, ncu04223.4 and *tom-7* (**EB4**); *cpc-1* (**IBj5**); ncu03312.4 and *ad-3B* (**Y112M4i**); *gsl-19* (**NM177**); and *nic-2* and ncu09221.4 (**4540**). Five genes were disrupted on the recipient chromosomes, ncu09915.4 (**IBj5**); *eat-3* (**UK8-18**); ncu02735.4 (**T54M94**); ncu02052.4 (**NM177**); and ncu01857.4 (**B362i**,). Figure 2 also shows the locations of putative novel genes created by the fusion of existing genes (*see* Supplementary figure 1a-c for more on the putative novel genes).

In general, the translocated segments had inserted into the linkage groups predicted to be recipients from previous genetic analysis (figure 2). For example, our results confirmed the prediction of Perkins (1972) that in *T(39311)* the LG IL segment is inserted into LG IIR is "in reversed order with respect to the centromere", and of Perkins *et al*. (1995b) that in *Tp(T54M94)* the transposed LG IR segment is inserted in LG IL proximal to *arg-3*, inverted relative to its original orientation but retaining its orientation with respect to the centromere. The one apparent exception was *T(IBj5)*. The translocated LG VIL segment of *T(IBj5)* was transferred to LG IVL, whereas Perkins (1997) had reported it is in LG IR. We hypothesize that *T(IBj5)* could be a complex rearrangement with an additional (uncharacterized) translocation between LG IVL and LG IR that gives the appearance of linkage between LG VIL and LG IR.

Perkins (1997) had described *T(AR173)* as a complex *IT* in which a proximal LG IR segment is inserted into LG VII and the remainder of LG IR is interchanged with LG VR. Additionally, the right-most junction segment in LG I showed linkage with the nucleolus organizer region (NOR) in LG VL but not to markers on LG VL or LG V centromere, as expected if a pericentromeric inversion occurred simultaneously with the LG IR;VR translocation. Our results (summarized in figure 2) show the translocated LG IR segment had inserted into supercontig 10.7 immediately to the right of CEN VII.

T(UK14-1), the only *QT* for which we obtained a breakpoint junction sequence, transfers a LG VIR distal segment to LG VL in exchange for a small terminal segment of the NOR (Perkins *et al*. 1995a). Its A junction (*see* Materials and methods) revealed that the break on the donor chromosome is capped with sequence from the previously unassigned supercontig 10.9. It is possible that supercontig 10.9 is normally located in the NOR region in distal LG VL and that the translocation transfers distal supercontig 10.9 sequences to cap the break on LG VIR, leaving proximal supercontig 10.9 and other NOR sequences behind on LG VL. Another (unlikely) possibility is that Perkins *et al*. (1995a) were incorrect and *T(UK14-1)* in fact is an *IT*, then our results would place supercontig 10.9 in distal LG VIR. A third possibility is the break on the donor chromosome is capped with sequence from the tip of some other chromosome, that is, three or more chromosomes might have exchanged their terminal segments but only the terminal LG VIR segment includes genes, whereas the terminal segments of the other LGs are non-genic (hence LG VIR is designated the donor). We were unable to obtain the B junction because both the breakpoint-proximal sequences on supercontig 10.9 and the breakpoint-distal sequences on supercontig 10.6 are repetitive, making it difficult to verify a true positive result (and distinguish it from potential false positives) in the complementary inverse PCRs.

3.2 Neurospora *crosses lacking an intact* eat-3 *gene show defective ascus development*

Translocation *T(UK8-18)* was found to disrupt the *eat-3* gene (also known as ncu01953). The *eat-3* gene is the *N. crassa* ortholog of the *Podospora anserine* gene *ami1* (*http://www.broad.mit.edu/annotation/genome/neurospora/GeneLocus.html?sp=SNCU01953*; additionally, using the Ami1 protein sequence as query in a Blast search, we verified that the *eat-3*-encoded protein is identified as its most likely *Neurospora* ortholog). Bouhouche *et al*. (2004) have reported that *P. anserine* crosses homozygous for the mutant form of *ami1* show a dramatic increase in the frequency of repeat-induced point (RIP) mutation, a genome defense process of fungi (*see* Selker 1990 for a review on RIP). Our finding raised the possibility to compare gene function between different fungi; specifically, to ask whether *eat-3*-homozygous mutant crosses also increase the frequency of RIP. A gene deletion mutant for ncu01953 (FGSC 13375, designated Δncu01953::*hph mat a*) was available from the *Neurospora* Functional Genomics Program (Colot *et al*. 2006), but only in the *mat a* background. Since ncu01953 and *mat* are closely linked (~23 kbp apart), therefore obtaining a Δncu01953::*hph mat A* strain by cross over was expected to be difficult. However, *T(UK8-18)* strains were available in both mating types and enabled us to perform the cross Δncu01953::*hph a* × *T(UK8-18) A* (i.e. Δ*eat-3 a* × *T(UK8-18) A*).

All crosses of type Δ*eat-3 a* × *T(UK8-18) A* were infertile, and although they formed perithecia they did not contain any developing asci. This result suggested that, unlike in Podospora, the *eat-3* gene might be essential for ascus

development in *Neurospora*. However, a causal relationship between the *eat-3* deficiency and the ascus development defect remains to be established, since *T(UK8-18)* results in alterations to the genome in addition to the disruption of *eat-3* and one or more of these additional alterations might be responsible for the ascus development defect. One way to establish a causal relationship would be to test whether an ectopic copy of *eat-3*, introduced by transformation, can complement the ascus development defect in a cross of type Δ*eat-3 a* × *T(UK8-18) A*.

3.3 *Crosses heterozygous for some duplications are not always barren*

Seventy segregants from the cross *T(R2394) a* × OR *A* were crossed with OR and *Sad-1* strains of opposite mating type. Twelve of these progeny were barren in crosses with OR but their crosses to *Sad-1* mutants were fertile, as expected of *Dp* segregants. By way of contrast, 58 segregants were fertile in crosses to both OR and *Sad-1*, as expected of the non-*Dp* segregants. The twelve "barren" segregants were confirmed to contain duplications using junction specific primers that allow one to distinguish between *N*, *T* and *Dp* (data not shown). Examination of 12 segregants randomly chosen from the 58 that gave fertile crosses with OR revealed that five were *N*, five *T* and two were *Dp*. If this distribution was representative of all the fertile segregants, then we expect that of the 58 fertile segregants 10 would be *Dp*, 24 *T* and 24 *N*. These results suggest that although the barren phenotype is specific for *Dp(R2394)*, it was displayed by only 12/22 (55%) of the *Dp(R2394)* progeny.

The barren phenotype was routinely used to identify putative *Dp* segregants from crosses of *Dp*-generating translocations with the wild-isolated strains. However, no barren segregants were found among the progeny examined from crosses of 17 *Dp*-generating translocations with the wild-isolates, which suggested that barrenness might not be a sensitive marker for duplications of some mixed OR/wild-isolate backgrounds. To evaluate this possibility, we performed a cross of *T(UK8-18)*, with the wild-isolated Bichpuri-1 strain (P750) and used molecular tools to identify its *T*, *N* and *Dp* progeny. We chose Bichpuri-1 because it had failed to give any barren segregants in crosses with several duplication-generating translocations, and *T(UK8-18)* because determination of its breakpoint junction sequences gave us PCR primers to distinguish its *T*, *N* and *Dp* segregants. Of 25 progeny examined, eight were *Dp* as identified by PCR using breakpoint junction specific primers. These eight *Dp(UK8-18)* segregants were further characterized by crossing to OR strains of opposite mating type; five were barren, whereas three gave fertile crosses with OR. This showed that crosses involving *Dp(UK8-18)* segregants from *T(UK8-18)* × Bichpuri-1 are not always barren.

3.4 *Non-barrenness of duplication-heterozygous crosses might have different underlying mechanisms*

Although more research needs to be done to understand why the barrenness of some duplications is incompletely penetrant, different mechanisms might underlie the non-barrenness of different *Dp* heterozygous crosses. For instance, the size of the duplicated region might have a bearing on the penetrance of the barren phenotype. *Dp(R2394)* duplicates only 39 genes (data not shown). It is possible that only a few of them are essential for meiosis and ascus development. Previous results from our laboratory have suggested that the silencing of *Dp*-borne genes might not be complete (Singh *et al*. 2009), therefore the incomplete silencing of these few genes in a *Dp(R2394)*-heterozygous cross might not always result in barrenness.

In contrast, *Dp(UK8-18)* duplicates 362 genes, therefore the incomplete penetrance of its barren phenotype is probably not due to an incomplete silencing of only a few ascus-essential genes. Instead, it is possible that *Dp(UK8-18)* covers at least one gene required for meiotic silencing and that occasionally this gene is *more completely* silenced, at least in crosses heterozygous for *Dp(UK8-18)* segregants from *T(UK8-18)* × Bichpuri-1. Shiu *et al.* (2001, 2006) have suggested that complete (or almost complete) silencing of a meiotic silencing gene can suppress this process and thereby increase the productivity of the cross. Consequently, the identity of genes carried on a duplicated region also may be important in determining whether the cross is barren. Finally, it is possible that the size of the duplication not only affects the chance that a meiosis specific gene might be involved, it also might influence the completeness of silencing.

4. Conclusions

We determined 30 breakpoint junctions of 12 duplication-generating translocations and found that they disrupted 13 genes (or predicted genes) and created 10 new fusion ORFs. Future experiments will use RT-PCR to test whether any of the newly created putative genes are in fact transcribed. If they are, then our findings would have evolutionary implications for gene origin and evolution. Chromosome rearrangements could be a way to generate variation in a genome that has relatively few intergenic sequences and a strong bias against gene duplications. Most translocated segments had inserted into the linkage groups predicted from previous genetic analysis. Our results suggested the *eat-3* gene might be essential for ascus development in *Neurospora*, although this has been shown not to be true for *Podospora*. The barren phenotype of some duplication-heterozygous crosses was incompletely penetrant, apparently, regardless of the duplication size.

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