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The Clock affecting 1 mutation of Neurospora is a recurrence of the frq^{7} mutation7

Abstract

The *clock affecting-1 (cla-1)* mutation of *Neurospora crassa* increases the period and decreases temperature compensation of the circadian rhythm, and was thought to define an uncloned gene with a possible role in the *Neurospora* clock. This defect, thought to be due to a translocation, was associated with a slow growth rate and a period of about 27 h at 25cla-1 and found the growth rate and period defects to be due to linked independent mutations. The translocation was not the cause of the long period. The *csp-1* mutation, present in the original *cla-1* strain, had a period shortening effect, thus *cla-1* strains lacking *csp-1* had a period length similar to that of *frequency*^{7} (*frq*^{7}). The *cla-1* period defect mapped to the *frq* locus, and sequencing of *frq* revealed *cla-1* to be a re-isolation of *frq*^{7}.

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The *Clock Affecting 1* mutation of *Neurospora* is a recurrence of the *frq*^{7} mutation

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The *clock affecting-1* (*cla-1*) mutation of *Neurospora crassa* increases the period and decreases temperature compensation of the circadian rhythm, and was thought to define an uncloned gene with a possible role in the *Neurospora* clock. This defect, thought to be due to a translocation, was associated with a slow growth rate and a period of about 27 h at 25<C. We mapped *cla-1* and found the growth rate and period defects to be due to linked independent mutations. The translocation was not the cause of the long period. The *csp-1* mutation, present in the original *cla-1* strain, had a period shortening effect, thus *cla-1* strains lacking *csp-1* had a period length similar to that of *frequency*^{7} (*frq*^{7}). The *cla-1* period defect mapped to the *frq* locus, and sequencing of *frq* revealed *cla-1* to be a re-isolation of *frq*^{7}.

The *cla-1* mutation was originally reported as an apparent spontaneous mutation arising from a cross between *csp-1 A; bd* and *a; ufa* (FGSC4441). The *cla-1* strain had a period length of 28 h at 22<, reduced temperature compensation (i.e. as temperature increases the period length decreases, as opposed to the wild type strain where period length remains nearly constant over a range of temperatures) and was associated with a 30% slower growth rate (Brody *et al*. 1988 Genome **30**, **Suppl. 1**: 299). This strain was found to possess a chromosomal rearrangement which was thought to be responsible for the *cla-1* mutation (Lakin-Thomas *et al*. 1990 Crit. Rev. Microbiol. **17**: 365-416; Dunlap 1993 Ann. Rev. Physiol. **55**: 683-728). The rearrangement was reported to be a reciprocal translocation between IL and VIIR, with the breakpoint on IL reported to be near *mat* (3% recombination) and that on VIIR near the *oli* locus (2% recombination, Perkins 1997 Adv. Genet. **36**: 239-398). The *oli* locus is 2 map units from *frequency* (Loros *et al*. 1986 Genetics **114**: 1095-1110), raising the possibility that *cla-1* was a new allele of *frq*. However, Southern analysis of a *cla-1* containing strain revealed no translocation within the 8.7-kb DNA fragment containing the *frq* locus and flanking regions that is sufficient to rescue a null allele of *frq* (Johnson 1993 Ph.D. Thesis, Dartmouth College). The 28 h period length, slow growth rate and translocation outside of the known *frq* locus suggested that the *cla-1* mutation defined a new clock affecting mutation in *Neurospora*.

To confirm the placement of *cla-1* in relation to markers on IL, and obtain a strain of appropriate mating type for further genetic analysis, we crossed strain T(IL;VIIR) *cla-1 csp-1 A; bd* (strain 7035) with a clock wild type strain, *a; bd* (87-3, hereafter referred to as wild type) and scored the progeny for *csp-1,* period length and growth rate at 25<C (cross 189, see Table 1). The presence of the translocation on IL and VIIR in the *cla-1* parent will cause a decrease in recombination on these linkage groups. This analysis agreed in part with previous unpublished data; i.e. the *cla-1* phenotypes were linked to *csp-1* and *mat*. However, we found the 30% reduction in growth rate associated with the *cla-1* period defect was linked to, but separable from, the period length mutation (7 map units). Hence the period length, and growth rate defects were caused by independent mutations. We named the slow growth mutation *slg*. Additionally, progeny which were *csp-1*$^{+}$ had a slightly longer period than those which were *csp-1*$^{-}$ as previously reported (Dharmanada and Feldman 1979 Pl. Physiol. **63**: 1049-1059). Thus those progeny with a long period fell into 3 classes (Table 1): (1) Those which possessed the phenotype of the parental 7035 *cla-1* strain (that is a period length +/- SEM of 27.2 +/- 0.1 h, a slow growth rate and the *csp-1* spore separation defect); (2) one strain (189-33) which had a period length of 29.2 h +/- 0.3 h, a slow growth rate, and was *csp-1*$^{+}$; and (3) two progeny (189-24 and 189-52) which had period lengths of 28.6 h +/- 0.2 h and 28.8 h +/- 0.4 h respectively, a wild type growth rate, and were *csp-1*$^{+}$. As the growth rate defect and period length defect from the original *cla-1* strain were separable, and in the absence of the *csp-1* mutation the period associated with *cla-1* was almost 29 h, which is close to what has previously been reported for strains carrying the *frq*^{7} mutation, we directly compared the period of strains carrying *cla-1* and lacking *csp-1* to a strain bearing the *frq*^{7} mutation. The period length of the long period *csp-1*$^{+}$ progeny (i.e. *cla-1; bd*, *slg*$^{+}$ or *slg*$^{-}$) was quite similar to the period of a strain carrying the *frq*^{7} allele (strain 585-7 from the Feldman lab, UC Santa Cruz, CA) (period 29.6 h +/- 0.1 h).

We next mapped the *cla-1* period length mutation in relation to VIIR markers by crossing a strain *slg a; bd; cla-1* (189-33) with *A; bd; oli*^{R} *frq*^{2} *for* (strain 922-131, cross 208, Table 1).
This mapping placed *cla-1* between *oli* and *for*, and in this cross inseparable from *frq*. This suggested for the first time the possibility that *cla-1* was an allele of *frq*. As the *oli*^{R} mutation alters growth rate we were unable to accurately map *slg* in this cross.
To determine whether the *cla-1* or *slg* mutations from the original *cla-1* isolate were associated with the translocation we tested progeny from cross 189 that were recombinant for *cla-1, slg* and *csp-1* for the presence of the translocation. This was done by crossing these progeny to tester strains *fl; a* or *fl; A* (FGSC 4347 and 4317 respectively). Unordered asci from these crosses were collected and analyzed (Table 2). This method allows one to determine the presence of a translocation in the strain being tested. A cross of *translocation* X *normal* leads to a fraction of the ascospores from the cross being unpigmented (Awhite@), in contrast to ascospores from structurally homozygous crosses, which are almost all black. Failure of pigmentation results from genetic deficiencies generated by meiotic chromosomal reassortment which results in lethality in these ascospores. Additionally, the frequency and pattern of white and black spores characterizes different kinds of translocations. For a reciprocal translocation one typically sees a 50:50 distribution of ascus classes centered around the 4:4 class, although exceptions to this are possible, in particular white ascospores are sometimes under represented. (For a detailed description see Perkins 1974 Genet. **77**: 459-489.)
This analysis (Table 2) demonstrated that all strains with *slg* that were analyzed also possessed a chromosomal translocation; however, not all strains with the long period had the translocation, and all strains which possessed the growth rate of the wild type

parent lacked a detectable translocation. The distribution of the ascus classes in the 189-33 strain was skewed from the 50:50 black:white ratio expected for the presence of a reciprocal translocation, so whether this represents the presence of a translocation is uncertain. However, 189-38, which has a wild type period length but possesses *slg* clearly has a translocation. Strains 189-24 and 189-52 which both have a wild-type growth rate and the *cla-1* period defect lack a detected translocation.

Thus the mutation resulting in increased period length in the *cla-1* strain was not due to a chromosomal translocation. Given these data it seemed likely that the chromosomal translocation was the cause of the slow growth rate; however, as our interest was focused on the clock phenotype we did not pursue this possibility further.

Since the clock-affecting mutation in *cla-1* was separable from the translocation, mapped to *frq* on linkage group VII, and when separated from *csp-1* the long period was similar to that known for the frq^7 allele, we sequenced a region of *frq* spanning the frq^7 mutation (corresponding to nucleotides 2643 to 3147 of the *frq* sequence in GenBank, accession number U17073) from strains 189-19, 189-24, 189-33, 7035 and wild-type. All strains with the long period (189-24, 33 and 7035) possessed the G to A point mutation that defines frq^7 (Aronson *et al*., 1994 Proc. Natl. Acad. Sci. USA **91**: 7683-7687) resulting in a Gly to Asp mutation at position 433 of the FRQ protein, while those with wild type period (189-19 and wild type) had the wild type sequence. This was the only sequence difference detected between long period and wild type strains. This sequence data, in addition to the period length similarity and genetic data thus demonstrate that the *cla-1* period phenotype is caused by the frq^7 mutation.

This is not the first instance of re-isolation of clock mutations. Mutations originally reported as frq^2, frq^4 and frq^6, all of which were known to have the same period length (Feldman and Hoyle, 1976 Genetics **82**: 9-17; Gardner and Feldman, 1980 Genetics **96**: 877-886), were later shown by sequence analysis to be the result of identical G **6** A mutations resulting in an Ala **6** Thr mutation at position 869 in the FRQ protein; the frq^7 and frq^8 alleles were similarly shown to be the result of identical G **6** A mutations resulting in a Gly **6** Asp mutation at position 433 of FRQ (Aronson *et al*., 1994 Proc. Natl. Acad. Sci. USA **91**: 7683-7687). Likewise, the per^{01}, per^{02} and per^{03} mutations were shown to be due to identical mutations in the *period* gene of *Drosophila* (Hamblen-Coyle *et al*., 1989 J. Neurogenet **5**: 229-256), and the *Clock* mutation of *Drosophila* was originally believed to define a novel genetic locus, but later mapped and shown to be an allele of *per* (Dushay *et al*., 1992 J. Neurogenet **8**: 173-179).

In light of the molecular basis of this clock affecting allele, one possibility for the origin of *cla-1* is that a frq^7 conidiospore inadvertently found its way into a cross wherein a translocation occurred, resulting in the A*cla-1*@ strain. Alternatively, although the G **6** A mutation of frq^7 is only one of a wide variety of different mutations of *frq* that result in a long period lengths, the formal possibility also exists that *cla-1* represents a truly independent occurrence of the frq^7 change.

Stuart Brody, UC, San Diego, provided us with strain 7035, *T(IL; VIIR)SB332 slg A csp-1; bd; cla-1*. We thank Keith Johnson for initial characterization of the *cla-1* mutation at Dartmouth. This work was supported by grants from the National Institutes of Health (GM 34985 to J.C.D., MH44651 to J.C.D. and J.J.L.), the National Science Foundation (MCB-0084509 to J.J.L.) and the Norris Cotton Cancer Center core grant at Dartmouth Medical School.

Table 1. Mapping the *cla-1* mutation.

Cross Number	Zygote Genotype and Percent Recombination	Number of Progeny: Parental	Single Cross-Overs: Region I	Single Cross-Overs: Region II	Single Cross-Overs: Region III	Double Cross-Overs Regions I & II	Total
189	*cla-1* *slg* *A* *csp-1*	24	2	1	0	0	57
	I II III						
	$cla\text{-}1^+$ slg^+ a $csp\text{-}1^+$	27	1	0	1	1	
	7 3.5 1.8						
208	oli^R frq^2 for^-	46	0	1		0	101
	I II	52	1	1		0	
	oli^S *cla-1* for^+						
	1.0 2.0						

Table 2. The translocation associated with the long period length in the *cla-1* strain is independent of the period length phenotype. The table shows the number of unorded ascus types containing various ratios of black and white ascospores arising from different strains crossed with either *fl; A* or *fl; a*. Strains without a translocation are expected to have almost exclusively asci which have black ascospores, i.e. almost all 8:0 asci, those strains which possess a reciprocal translocation are expected to have 50:50 black:white ascospores, with a symmetrical distribution of ascus classes around the 4:4 class.

Male Strain Number	Genotype	Ascus class (black : white)				
		8:0	6:2	4:4	2:6	0:8
189-24	*slg*$^{+}$ *a csp-1*$^{+}$*; bd; cla-1*$^{-}$	32	4	2	0	0
189-33	*slg*$^{-}$ *a csp-1*$^{+}$*; bd; cla-1*$^{-}$	50	3	1	0	18
189-38	*slg*$^{-}$ *A csp-1*$^{-}$*; bd; cla-1*$^{+}$	24	1	22	2	28
189-52	*slg*$^{+}$ *a csp-1*$^{+}$*; bd; cla-1*$^{-}$	25	0	0	0	0
	fl; A x *fl; a*	14	1	0	0	0