Suzci, A. and A. Radford

ARS8 sequences in the Neurospora genome.

ARS8 ("Autonomously Replicating Sequence 8") is a Neurospora DNA sequence selected in *Saccharomyces cerevisiae* by its effect on increasing transformation frequency in that organism (Stinchcomb et al. (1980) PNAS 77: 4559-4563). The mechanism is via its role as a DNA replication origin in yeast, permitting autonomous replication of a plasmid containing the sequence.

Transformation of Neurospora by plasmids containing ARS8, i.e. pES155 and its derivatives, have shown no evidence of ARS8-mediated increased transformation frequency, nor evidence of autonomy as judged by the recovery of plasmid DNA or plasmid-encoded genetic markers from Neurospora transformants (Case (1982) in "Genetic Engineering of Microorganisms for Chemicals", Plenum). In fact, the limited number of transformants studied genetically show evidence of chromosomal integration.

Although ARS8 functions in yeast as a replication origin, it is not fulfilling this role in Neurospora. If its physiological role is indeed that of a replication origin, its homology with chromosomal origins in the Neurospora genome may be such that it is integrating at these homologous sites rather than functioning autonomously.

Plasmid pES155 contains pBPR322, the yeast *URA3* gene, the Neurospora *ga-2*$^{+}$ gene in the HindIII site, and the ARS8 sequence in the EcoRI site. Plasmid pAR121 is a derivative in which the *ga-2*$^{+}$ sequence has been removed. pAR121 was further cleaved with HindIII and EcoRI, the restriction fragments separated on an agarose gel, and the isolated ARS8 sequence electro-eluted. ARS8 was then nick-translated, and used as a probe by Southern blotting for any related sequence in Neurospora genomic DNA. Neurospora DNA restricted with either EcoRI or HindIII, and pAR121 as a control, were probed for sequences hybridizing to ARS8 A single band was found on the pAR121 track, but multiple bands of hybridization were seen with genomic DNA cleaved with either enzyme.

While the function of ARS8 in Neurospora has not been elucidated, it has been demonstrated that ARS8 is not present as only a single copy per genome. Its frequency is not incompatible with a role as a DNA replication origin, the role which it fulfills in Saccharomyces, but it has not been observed to fulfill that function in a plasmid transformed into Neurospora. · · · Department of Genetics, University of Leeds, Leeds LS2 9JT, United Kingdom.