Cluster Dissection And Analysis: Theory, FORTRAN Programs, Examples

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Research Article

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Measurement of apoptosis by SCAN©, a system for counting and analysis of fluorescently labelled nuclei

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ABSTRACT **Apoptosis-like programmed cell death (A-PCD) is a universal process common to all types of eukaryotic organisms. Because A-PCD-associated processes are conserved, it is possible to define A-PCD by a standard set of markers. Many of the popular methods to measure A-PCD make use of fluorescent ligands that change in intensity or cellular localization during A-PCD. In single cell organisms, it is possible to quantify levels of A-PCD by scoring the number of apoptotic cells using flow cytometry instruments. In a multicellular organism, quantification of A-PCD is more problematic due to the complex nature of the tissue. The situation is further complicated in filamentous fungi, in which nuclei are divided between compartments, each containing a number of nuclei, which can also migrate between the compartments. We developed SCAN©, a System for Counting and Analysis of Nuclei, and used it to measure A-PCD according to two markers – chromatin condensation and DNA strand breaks. The package includes three modules designed for counting the number of nuclei in multi-nucleated domains, scoring the relative number of nuclei with condensed chromatin, and calculating the relative number of nuclei with DNA strand breaks. The method provides equal or better results compared with manual counting, the analysis is fast and can be applied on large data sets. While we demonstrated the utility of the software for measurement of A-PCD in fungi, the method is readily adopted for measurement of A-PCD in other types of multicellular specimens.**

***doi**: 10.15698/mic2014.12.180*
***Received** originally: 14.09.2014;*
in revised form: 21.10.2014,
***Accepted** 21.10.2014,*
***Published** 26.11.14.*

***Keywords**: apoptosis, PCD, fungi, DNA condensation, TUNEL.*

Abbreviations:
A-PCD - apoptosis-like programmed cell death,
FACS - fluorescence activated flow cytometry,
TUNEL - terminal dUTP nick end-labeling,
DAPI - 4',6-diamidino-2-phenylindole.

INTRODUCTION

Apoptosis was initially described in animals, where it plays central roles in development and homeostasis [1-2]. Activation of apoptosis, by external or internal stimuli, drives a set of coordinated intracellular processes, which terminate with the formation of apoptotic bodies and cell death. The most significant processes that define apoptosis include ions and protein leakage from the mitochondria, phosphatidylserine externalization from the inner to the outer cell membrane, chromatin condensation, DNA fragmentation, membrane blabbing, increased caspase activity and appearance of apoptotic bodies [3]. Following the discovery of apoptosis in animals, programmed cell death with similar characteristics was described in additional systems, including single-celled organisms [4-5]. The similarities in the sequence of events in animals and low eukaryotes promoted the use of simple systems to study apoptosis and apoptosis-related processes. In particular, *Saccharomyces cerevisiae* has become a model system for evaluation of human apoptotic genes and apoptosis-related processes such as autophagy and diseases [6-8]. Additionally, fungi are used to study the role of apoptosis and apoptosis-like programmed cell death (A-PCD) in aging, pathogenicity and stress responses [9-10].

Because apoptosis-associated processes are conserved, it is possible to identify apoptosis and A-PCD by a universal set of assays. A wide range of methods have been developed for this purpose, which measure cytological, molecular and biochemical parameters that are typical of apoptotic cells [11-15]. In this respect, it is important to distinguish between qualitative methods, such as detection of membrane blabbing by electron microscopy, or detection of

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