

[PS1.40]

**Dynamics of DNA synthesis during the trophozoite to cyst differentiation cycle of
*Entamoeba invadens***

Nishant Singh*, Jaishree Paul, Sudha Bhattacharya
Jawaharlal Nehru University, India

The DNA dynamics which mediate conversion of uninucleate trophozoite into quadrinucleate cyst in *Entamoeba histolytica* is not well understood. Here we have addressed this question in *Entamoeba invadens* (a model system for encystation) through a detailed time course study of the differentiation process. We combined flow cytometric analysis with the change in rate of thymidine incorporation and the number of nuclei per cell. Our data shows that during encystment the cell population passes through three phases- 1. Early phase (0-8h); of rapid DNA synthesis which may correspond to completion of ongoing DNA replication. Bi-nucleated cells increase with concomitant drop in uni-nucleated cells. 2. Commitment phase (8-24h); in which DNA synthesis rate slows down and a broader G0/G1 peak is seen. Possibly new rounds of replication are initiated which proceed slowly, followed by mitosis at 20h, with further increase in bi-nucleated cells. After this the number of bi- and uni-nucleated cells gradually decline and the tri and tetra-nucleated cells begin to increase. 3. Consolidation phase (24-72 h); in which the rate of DNA synthesis shows a small increase till 40h and then begins to decline. The G2/M peak reappears at 48h, showing that more rounds of DNA replication may be getting completed, followed by nuclear division. By 72h the encystment is virtually complete. The bi-nucleated stage could be an intermediate both in the conversion of trophozoite to cyst and back. Our study provides a comprehensive view of DNA dynamics during encystation and excystation of *E. invadens*.

Keywords: entamoeba invadens, DNA replication, Flow Cytometry, Thymidine incorporation